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Sphagnum diabolicum sp. nov. and *S. magniae* sp. nov.; morphological variation and taxonomy of the “*S. magellanicum* complex”

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ABSTRACT. Until a few years ago, *Sphagnum magellanicum* was understood to be a single widespread species with an intercontinental range. Recent work by Norwegian sphagnologists showed that *S. magellanicum* s.str. is restricted to southern South America and plants known as *S. magellanicum* in Europe should be referred to *S. divinum* and *S. medium*. In a separate publication, we showed that there are two additional major clades in eastern North America, and we describe them herein as *S. diabolicum* and *S. magniae*. These species are very hard to distinguish morphologically (and also from *S. divinum* and *S. medium*) but are distinct phylogenetically, ecologically and geographically, and are important units of biodiversity. Morphological variation within and between species is photographically documented.

KEYWORDS. Cryptic speciation, mosses, peatmoss, *Sphagnum divinum*, *Sphagnum magellanicum*, *Sphagnum medium*.



Sphagnum magellanicum Brid. has generally been interpreted as one of the most geographically widespread and ecologically abundant species of peat moss on the planet. The species was described by Bridel (1798) from a collection made in southern Chile by Philibert Commerson, and transmitted to Bridel by Adres de Jussieu. The type was collected by Commerson in 1767 while he accompanied Louis Antoine de Bougainville on his voyage of circumnavigation, 1766–1769. The red color of *S. magellanicum*, unusual in *Sphagnum* subgenus *Sphagnum* (Shaw et al. 2016), was considered diagnostic and it was not long before European bryologists assigned similar plants found in Europe and across the other northern continents to *S. magellanicum*. Indeed, *S. magellanicum* is often considered one of the most easily recognized species of *Sphagnum*, and has been

included in numerous ecological studies of Northern Hemisphere peatlands.

Kyrkjeeide et al. (2016) first showed using microsatellite markers that *Sphagnum magellanicum* s.l. exhibits marked geographic structure with both (geographically) allopatric and sympatric genetic groups. These authors identified five genetic groups within *S. magellanicum* s.l. Three of the five groups were reported from North America. Hassel et al. (2018), based on genetic/phylogenetic analyses of RADseq data (Yousefi et al. 2017), limited the taxonomic species, *S. magellanicum* s.str., to southern South America, and recognized two species among Northern Hemisphere plants formerly referred to as *S. magellanicum*: *S. medium* Limpr. and *S. divinum* Flatberg & Hassel. These correspond to two of the three groups resolved among North American plants by Kyrkjeeide et al. (2016). Their third group, endemic to North America, was

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unclear. Hassel et al. (2018) showed that while morphologically similar, *S. medium* and *S. divinum* can generally be distinguished by differing growth forms and anatomical traits. In particular, *S. medium* usually has shorter, less tapered sub-capitular branches, and has larger hyaline cell pores at the bases of branch leaves. In Norway, *S. divinum* was most commonly encountered along mire margins and in surrounding forests, whereas *S. medium* is largely restricted to open and mainly ombrotrophic mires. *Sphagnum divinum* often forms less dense, low hummocks, and has longer, more tapered branches than *S. medium*.

While *Sphagnum divinum* and *S. medium* can sometimes be separated morphologically and ecologically, albeit with some subjectivity, distinguishing other species in the complex is even more difficult. When trying to identify species based on field and microscopic traits, we found it extremely difficult to confidently identify many or even most collections. As a strategy for identifying diagnostic morphological characters to distinguish them, we took advantage of the fact that all the species are unambiguously resolved by molecular data (Shaw et al. 2022). We therefore examined specimens that had been independently assigned to species by those data. This paper describes our morphological observations of samples that had been identified in this way.

Our molecular data were subjected to phylogenetic analyses of the *Sphagnum magellanicum* complex, with global sampling, using a combination of whole genome sequencing and RADseq analyses (Shaw et al. 2022). Whole genome sequencing maximizes data per sample and because we had a chromosome-level genome assembly for *S. divinum*, we could deeply investigate genomic variation and differentiation within and among species. RADseq analyses allowed for much broader sampling of populations, including from dried herbarium material. While the RADseq method yields less intensive coverage of the entire genome for each sample, RADseq data were still sufficient to investigate phylogenetic relationships at the individual chromosome as well as the whole-genome level (Shaw et al. 2022).

We herein describe two new species in the *Sphagnum magellanicum* complex, both as presently understood endemic to eastern North America. We first briefly summarize the results of our previous

phylogenetic work pertinent to the nomenclatural proposals herein.

RADseq analyses revealed seven monophyletic groups within the *Sphagnum magellanicum* complex (Fig. 1). *Sphagnum medium* and *S. divinum* occur in both North America and Europe whereas, at present, the species described here, *S. diabolicum* and *S. magniae*, are known only from eastern North America. They both together appear to correspond to one of the genetic groups, endemic to North America, resolved as the “purple group” within *S. magellanicum* s.l. by Kyrkjeeide et al. (2016). Our analyses confirm that *S. medium* occurs in Europe and eastern North America whereas *S. divinum* occurs in Europe, eastern North America, and also western North America westward across Siberia and southward at least to Hokkaido, Japan. *Sphagnum magellanicum* s.str. is recorded only from Tierra del Fuego of Chile and Argentina, whereas plants from northern South America and Central America belong to a clade distinct from *S. magellanicum* s.str., in agreement with the microsatellite data presented by Kyrkjeeide et al. (2016). Asian plants from China and Taiwan form another monophyletic group that appears to be sister to the rest of the complex (Fig. 1). Whole genome sequencing resolved the same groups as the RADseq data, but as fresh collections were not available from Tierra del Fuego (*S. magellanicum* s.str.) nor from China/Taiwan, those taxa were not included in phylogenetic analyses of the whole genome data. Otherwise, results from RADseq and genome sequencing were congruent, with all the samples included in both data sets resolved in the same clades (species) and with congruent phylogenetic relationships between data sets. We show the RADseq results here as they include all seven clades/species (Fig. 1A).

Our inferences about the species and their relationships are robust to data set (amount of allowed missing data) and analytical method (maximum likelihood reconstructions from concatenated data and the coalescent-based SVDquartets method). The only ambiguity with regard to analytical method pertained to whether *Sphagnum divinum* and the clade containing plants from northern South America and Central America are sister taxa within a clade (Fig. 1A, right), or whether they form a grade leading to *S. magellanicum* s.str., *S. diabolicum* and *S. magniae* (Fig. 1A, left). Inferences that are consistent across all analyses

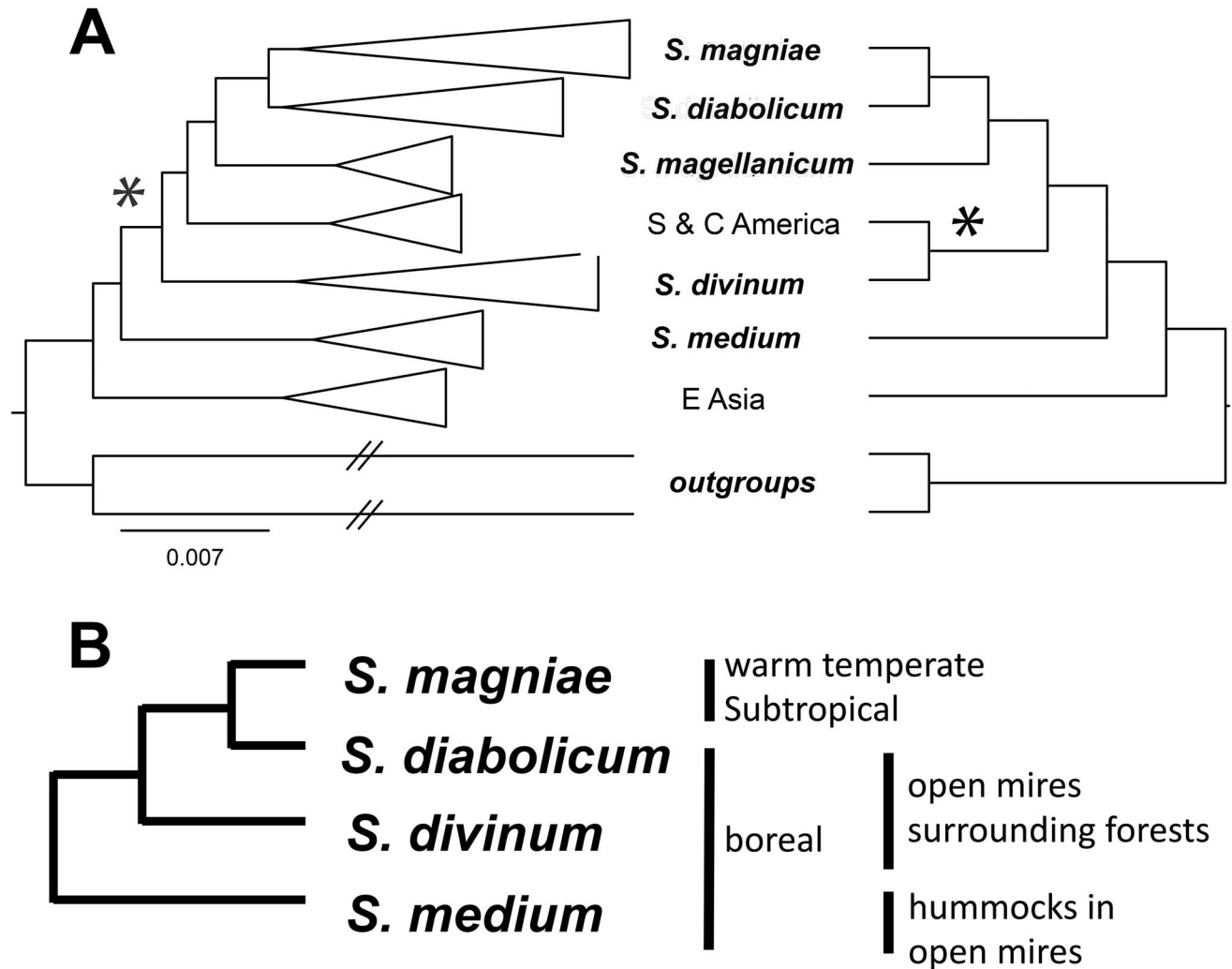


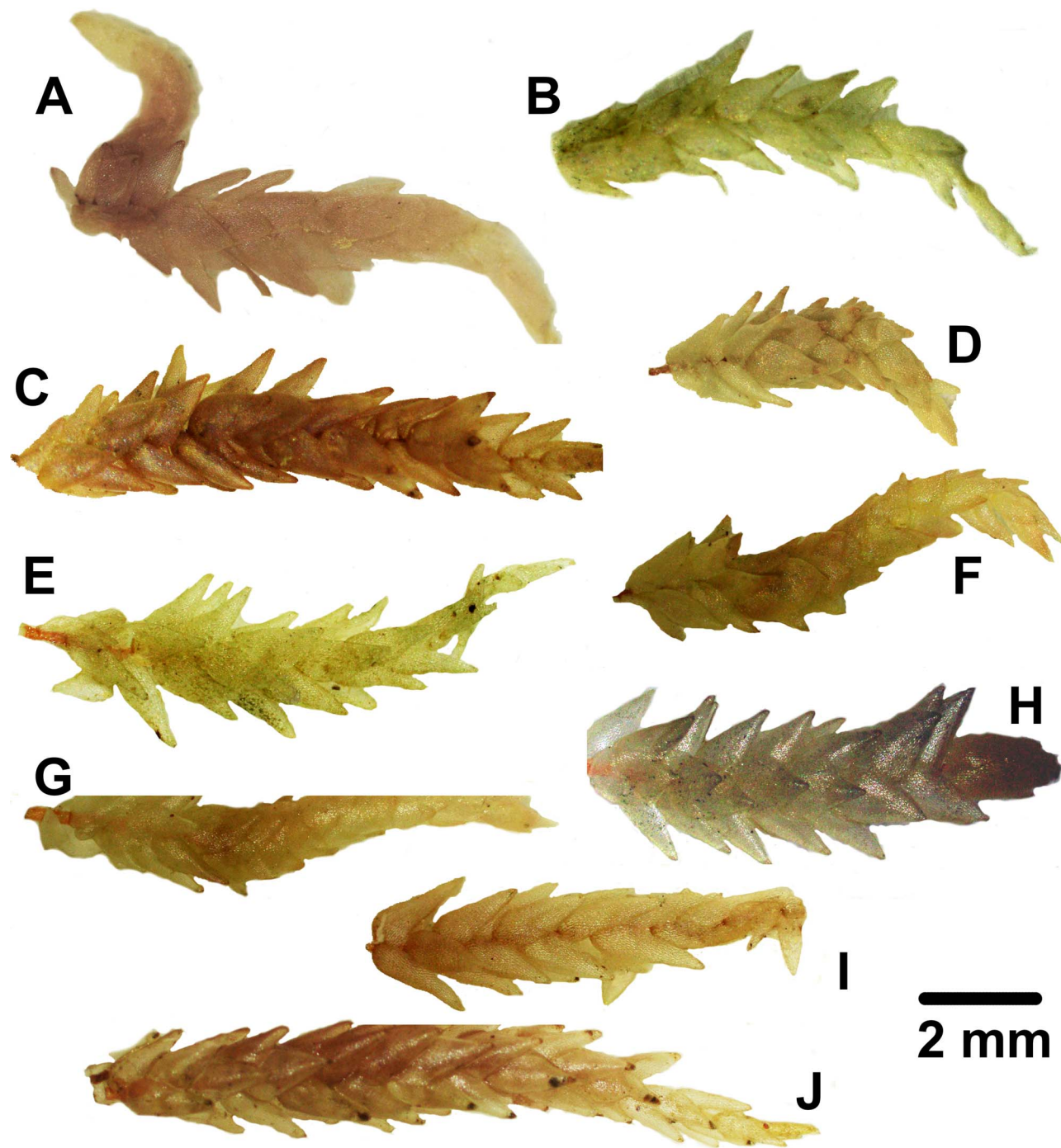
Figure 1. A. Phylogenetic relationships among seven putative species in the *Sphagnum magellanicum* complex based on RADseq data; left= result from concatenated data under Maximum Likelihood, right= results from the coalescent-based method, SVDquartets. B. Relationships among the four species of North America, Europe, and northern Asia, summarizing their biogeographic and ecological ranges. All nodes maximally supported except that marked by asterisks (redrawn from Shaw et al. 2022; see that publication for additional analytical details).

include: (1) *S. medium* diverged early in the diversification of the complex, (2) *S. medium* and *S. divinum* are not sister taxa, (3) *S. magellanicum* s.str. is sister to a clade containing *S. diabolicum* spec. nov. and *S. magniae* spec. nov., (4) *S. diabolicum* and *S. magniae* are closely related and genetically similar sister taxa. Other evidence (Shaw et al. 2022), not presented here, indicates that all four North American-European taxa hybridize, but not so much that species monophyly is in question. *Sphagnum magniae* is the only taxon that occupies warm temperate to subtropical habitats and we infer that this species evolved relatively recently from cold-adapted ancestors.

Our goals in this paper are to (1) assess and illustrate variation in micromorphological traits within and among the four North American taxa, plus *S. magellanicum* s.str. (Figs. 2–12), and (2) provide formal descriptions to validate two new eastern North American species in the complex.

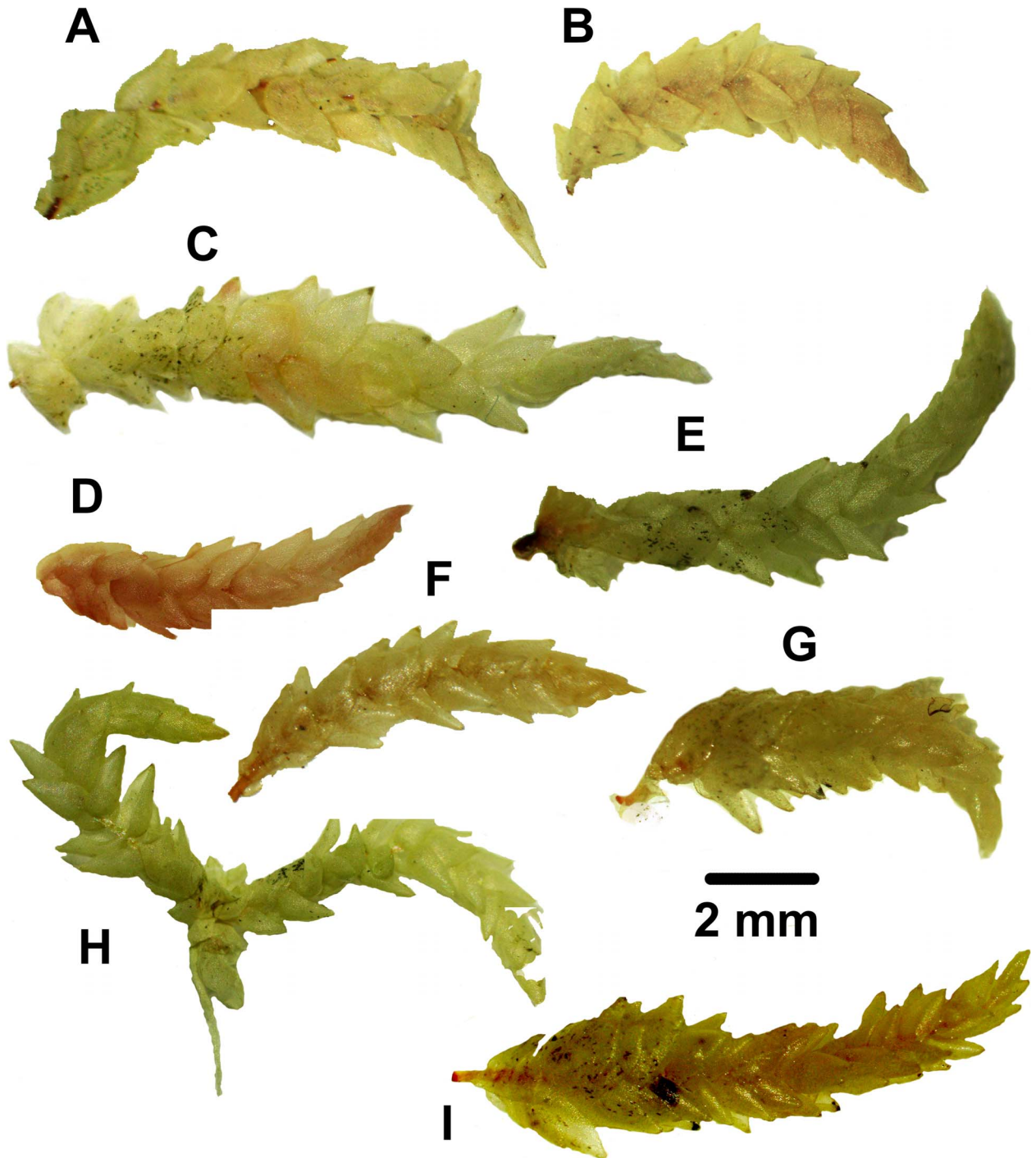
MATERIALS AND METHODS

Plant sampling. The majority of our field work and plant sampling focused on North American plants but our previous phylogenetic paper (Shaw et al. 2022) included nine collections from western Europe and Russia. In addition, over 250 European samples have been included in unpublished ongoing



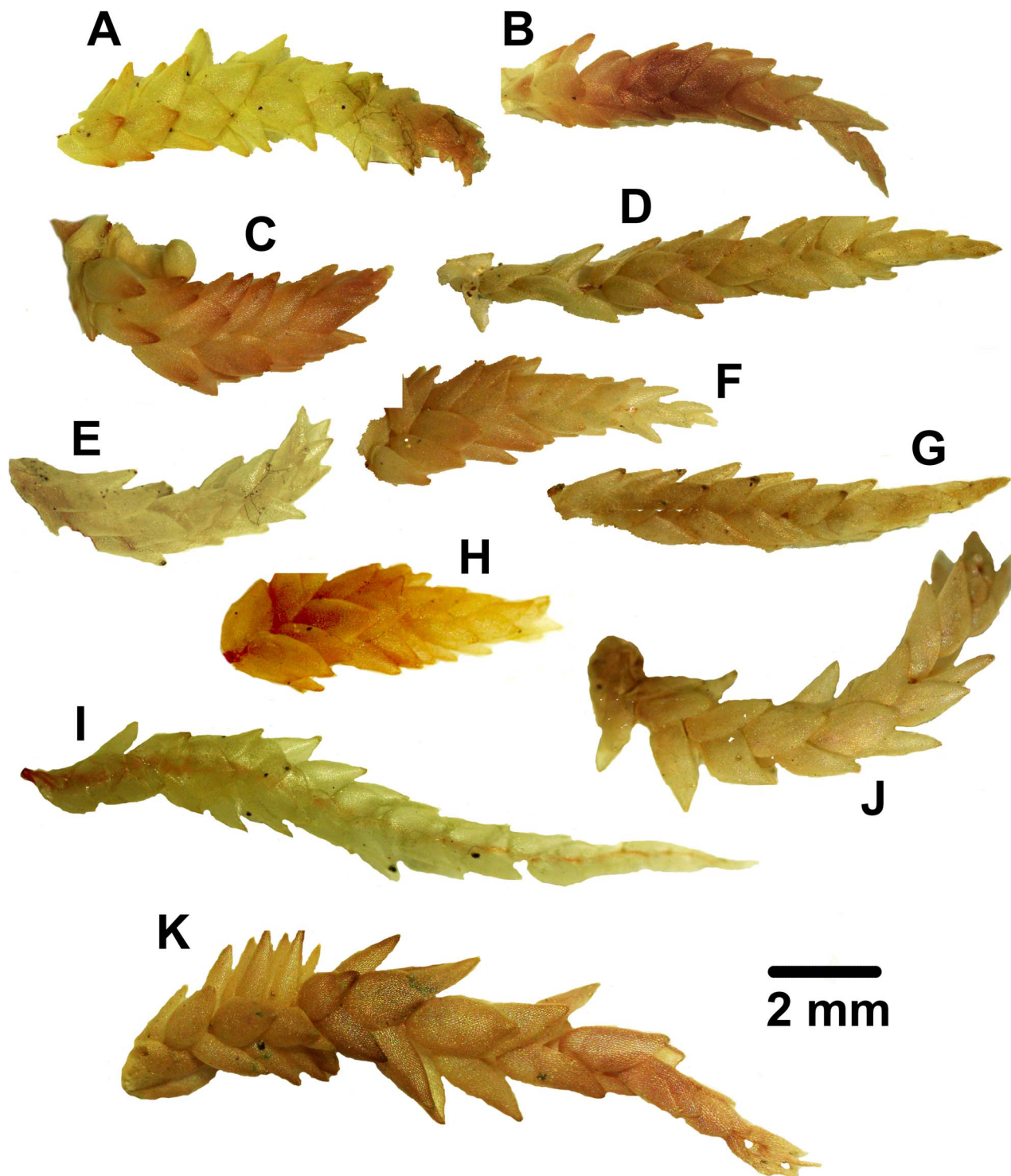
S. magniae

Figure 2. Spreading branch morphology in *Sphagnum magniae*. A. Shaw 2018-79. B. Shaw 2018-82. C. Shaw 2018-41. D. Imwattana 145. E. Shaw 2018-82. F. Shaw 2018-62. G. Shaw 2018-4. H. Shaw 2018-4. I. Shaw 2018-79. J. Piatkowski 2018-41. All vouchers in DUKE. Online pdf in color.



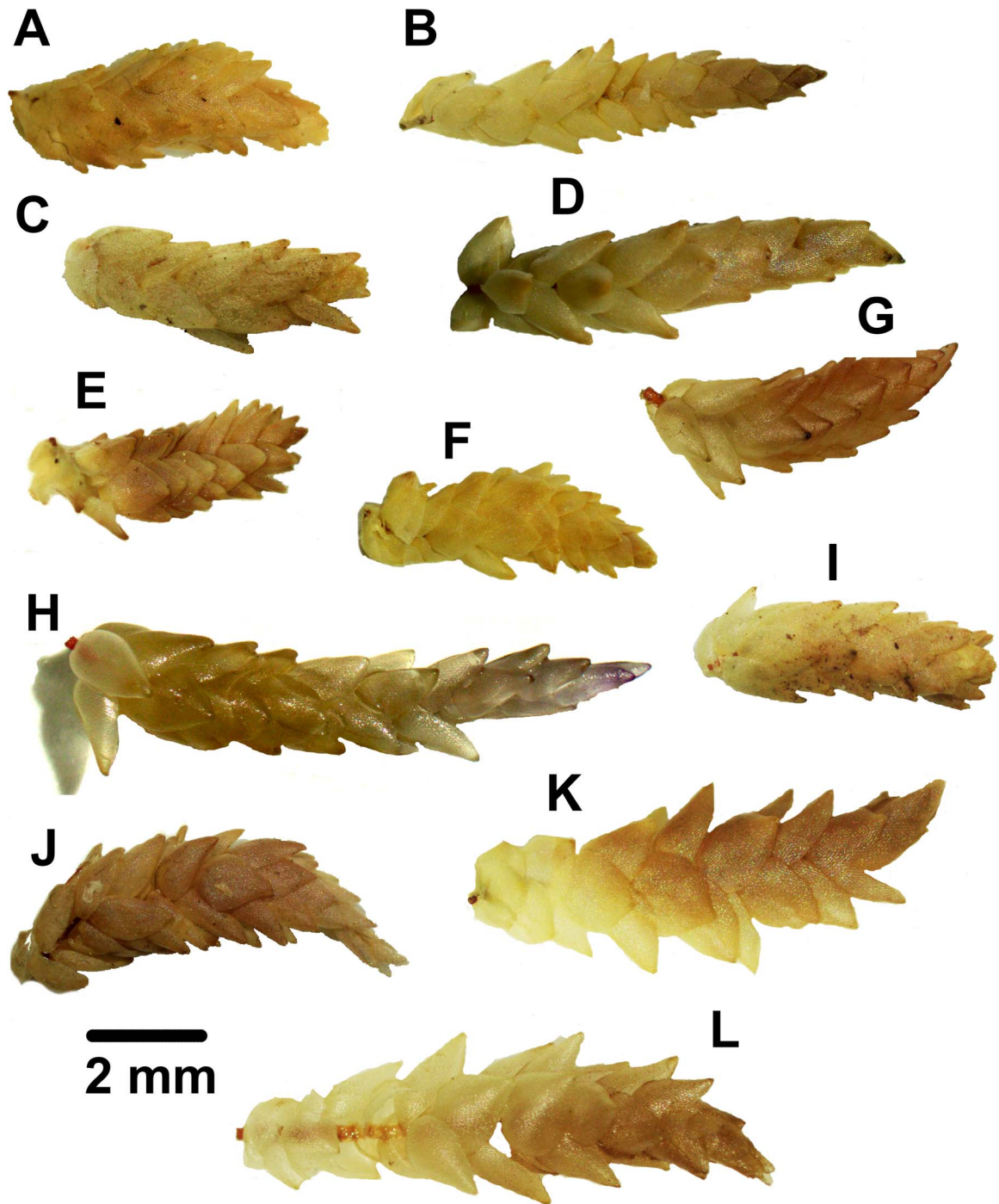
S. diabolicum

Figure 3. Spreading branch morphology in *Sphagnum diabolicum*. A. Shaw 2018-262. B. Shaw 2018-262. C. Shaw 218-334. D. R. Vilgalys 4. E. Shaw 2020-5. F. Shaw 2018-262. G. Shaw 2018-334. H. Shaw 2020-5. I. Shaw 2018-334. All vouchers in DUKE. Online pdf in color.



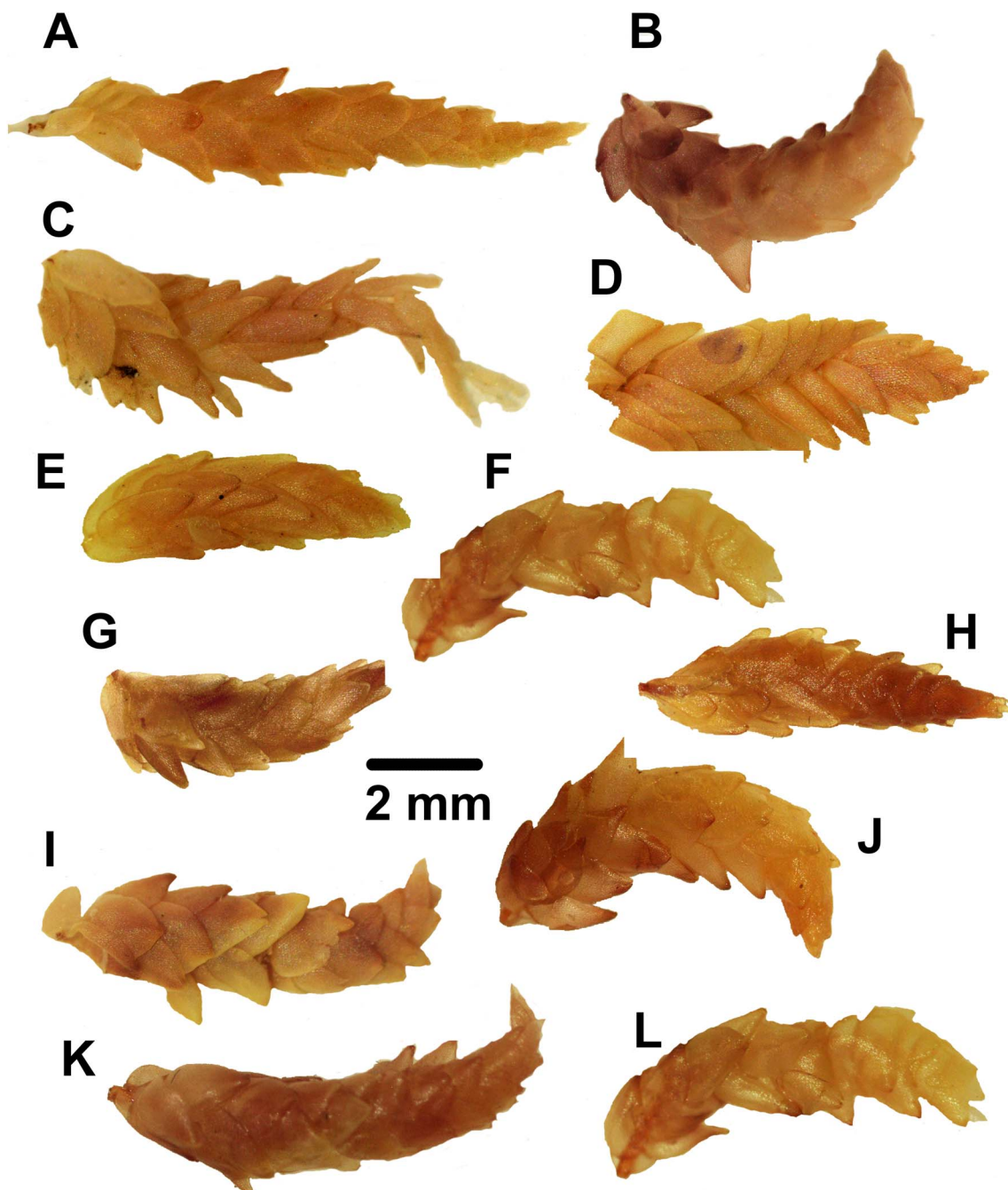
S. divinum

Figure 4. Spreading branch morphology in *Sphagnum divinum*. A. Shaw 2018-245. B. Golinski 2. C. Golinski 2. D. Shaw 2018-262. E. Shaw 2018-293. F. Shaw 2018-280. G. Shaw 2018-262. H. Shaw 2018-245. I. Shaw 2018-245. J. Shaw 2018-262. K. Shaw 2018-310. All vouchers in DUKE. Online pdf in color.



S. medium

Figure 5. Spreading branch morphology in *Sphagnum medium*. A. Shaw 2018-255. B. Shaw 2019-241. C. Shaw 2018-254. D. Shaw 2018-340. E. Shaw 2018-255. F. Shaw 2019-241. G. Shaw 2018-255. H. Shaw 2018-340. I. Shaw 2018-254. J. Shaw 2018-254. K. Shaw 2018-248. L. Shaw 2018-248. All vouchers in DUKE. Online pdf in color.



S. magellanicum

Figure 6. Spreading branch morphology in *Sphagnum magellanicum*. A. B. Shaw 18233. B. B. Shaw 15405. C. B. Shaw14472. D. B. Shaw18223. E. B. Shaw18223. F. B. Shaw15405. G. Mohlin J7. H. Mohlin J4. I. B. Shaw 15405. J. B. Shaw15405. K. B. Shaw15405. L. B. Shaw15405. All vouchers in DUKE. Online pdf in color.

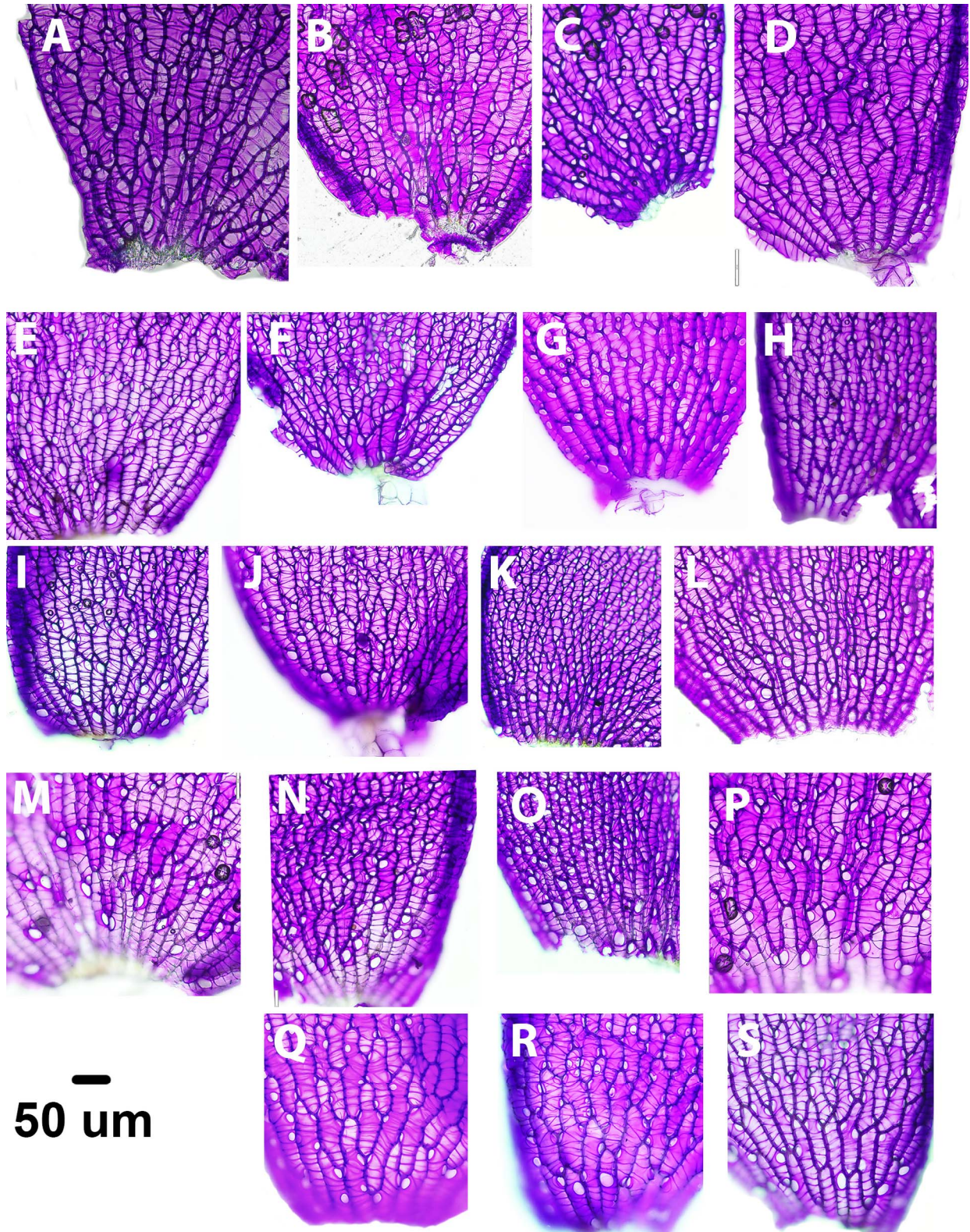


Figure 7. Spreading branch leaf bases showing cell and pore structure in the four North American species of the *Sphagnum magellanicum* complex, and *S. magellanicum* s.str. **A–D.** *S. magniae* (from left to right: Shaw 2018-4, Shaw 2018-79, Piatkowski 2018-41, Imwattana 145). **E–H.** *S. diabolicum* (Shaw 2018-262, Shaw 2018-316, Shaw 2018-334, Shaw 2018-334); **I–L.** *S. divinum* (Golinski 2, Golinski 2, Shaw 2018-262, Shaw 2018-293). **M–P.** *S. medium* (Shaw 2018-340, Shaw 2018-340, Shaw 2018-255, Shaw 2018-340). **Q–S.** *S. magellanicum* (B. Shaw 14472, B. Shaw 15405, Mohlin J4). All vouchers in DUKE. Online pdf in color.

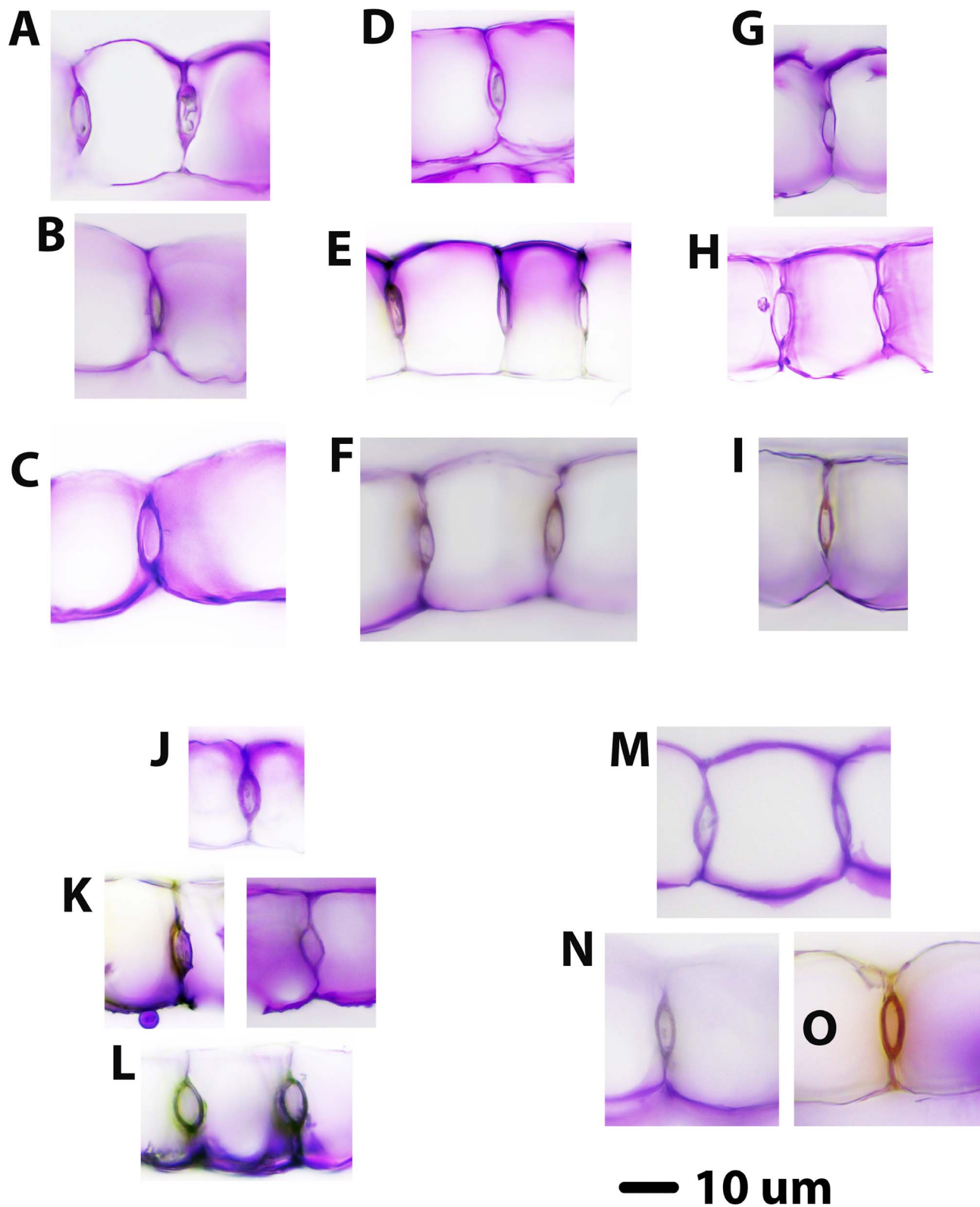


Figure 8. Spreading branch leaf transverse sections in the four North American species of the *Sphagnum magellanicum* complex, and *S. magellanicum* s.str. **A–C.** *S. magniae* (from top to bottom: Imwattana 145, Shaw 2018-4, Imwattana 145). **D–F.** *S. diabolicum* (Shaw 2020-5, R. Vilgalys 5, Shaw 2018-262). **G–I.** *S. divinum* (Shaw 2018-245, Shaw 2018-262, Shaw 2018-280). **J–L.** *Sphagnum medium* (Shaw 2018-254, Shaw 2018-255 [two sections], Shaw 2019-241[two sections]). **M–O.** *S. magellanicum* (B.Shaw 14472, MohlinJ7 [two sections]). All vouchers in DUKE. Online pdf in color.

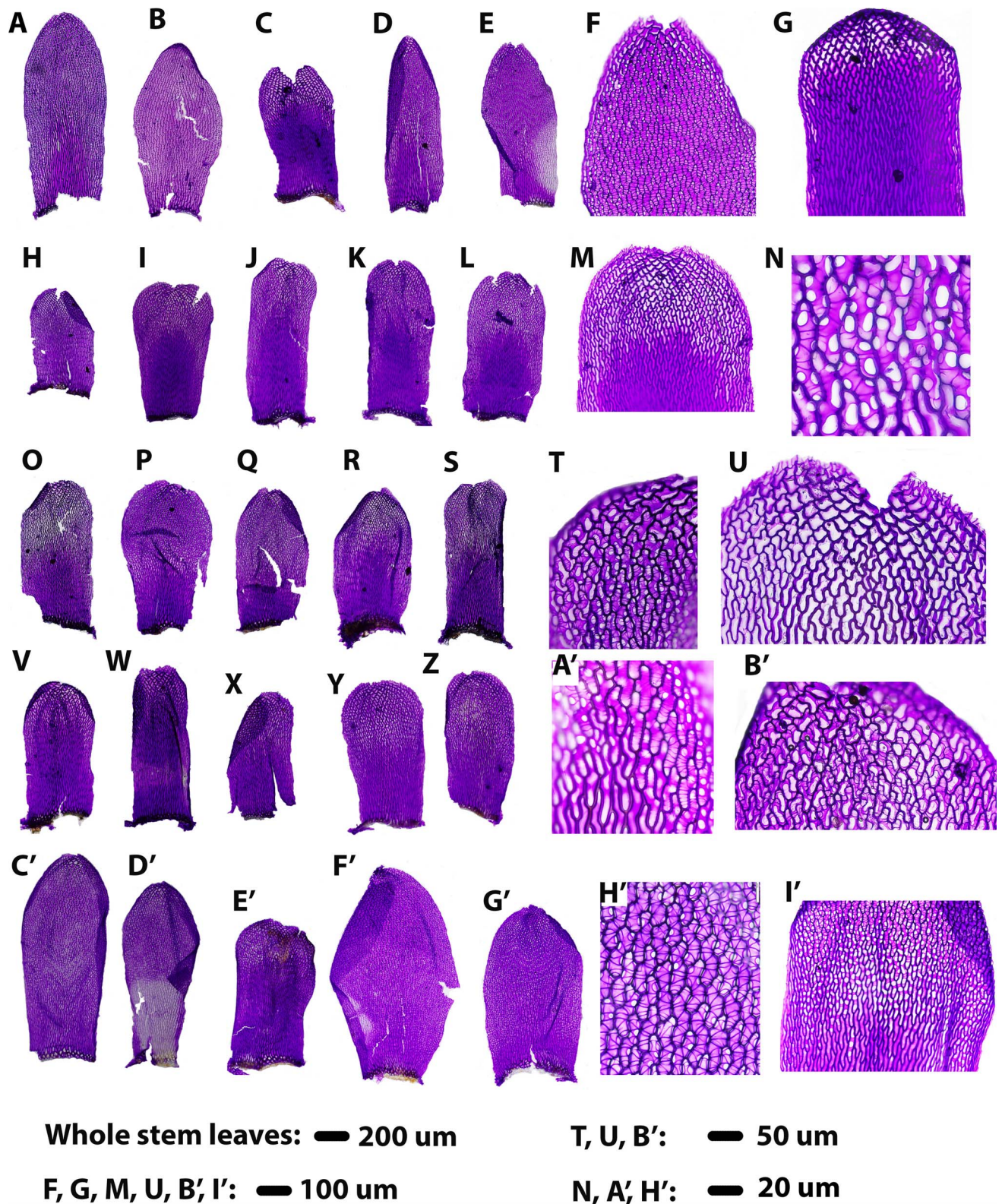


Figure 9. Stem leaves and stem leaf cell structure in the four North American species of the *Sphagnum magellanicum* complex, and *S. magellanicum* s.str. Five columns on left show whole leaf sizes and shapes. Two columns on right are outer stem leaf cells showing various degrees of cell wall resorption. A–G. *S. magniae* (from left to right: Shaw 2018-4, Shaw 2018-82, Piatkowski 2018-41, Imwattana 145, Imwattana 145, Imwattana 145, Piatkowski 2018-41. H–N. *S. diabolicum* (Shaw 2018-262, R. Vilgalys 4, Shaw 2018-316, Shaw 2018-334, Shaw 2020-5, Shaw 2020-5, Shaw 2020-5). O–U. *S. divinum* (Shaw 2018-245, Golinski 2, Golinski 2, Shaw 2018-280, Shaw 2018-293, Golinski 2, Shaw 2018-280). V–B'. *S. medium* (Shaw 2018-340, Shaw 2018-340, Shaw 2018-340, Shaw 2019-241, Shaw 2019-241, Shaw 2018-255, Shaw 2018-255). C'–I'. *S. magellanicum* (B.Shaw 18223, B.Shaw 18223, B.Shaw 15405, B.Shaw 14472, B.Shaw 14472, B.Shaw 18223, Mohlin J4). All vouchers in DUKE. Online pdf in color.

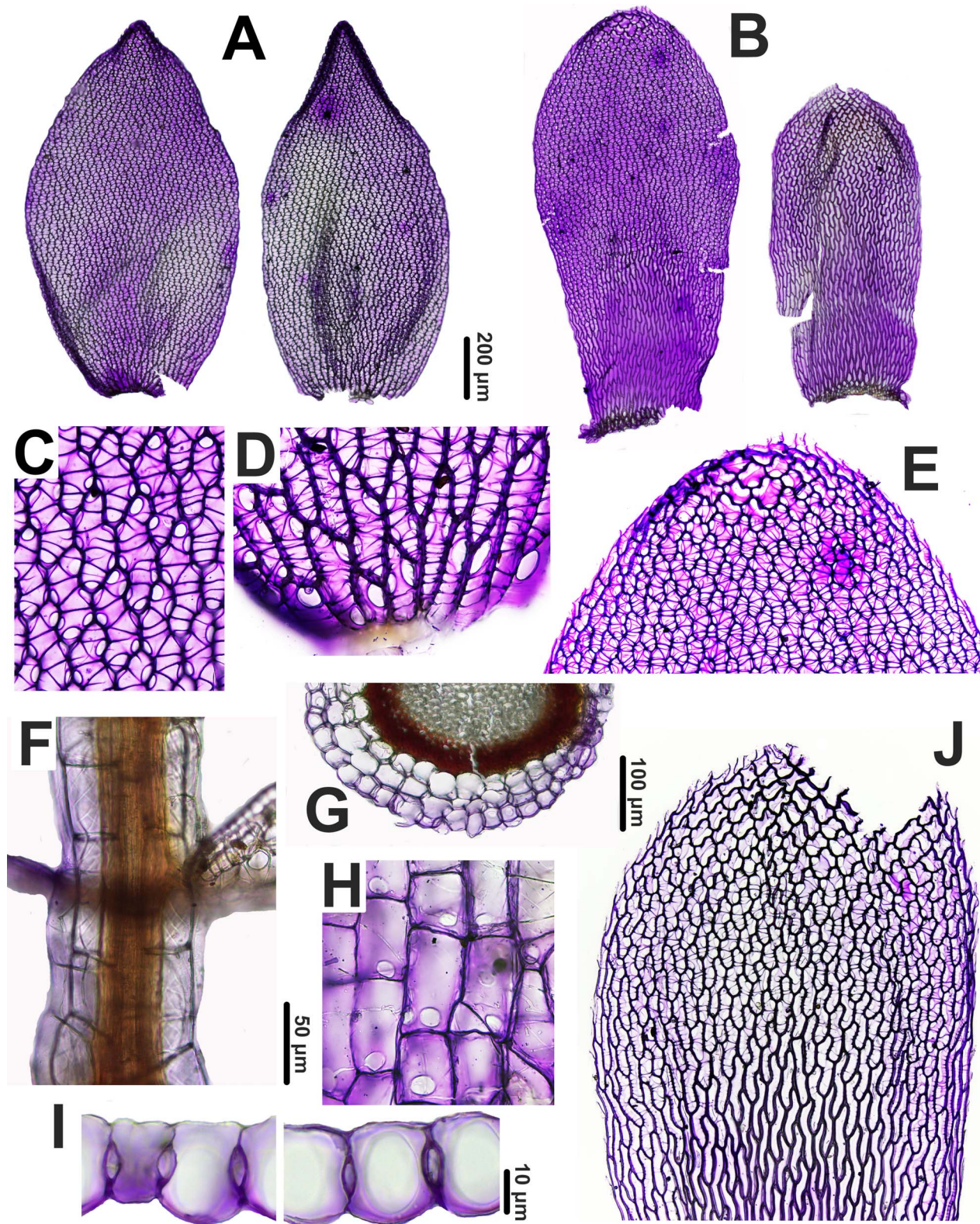


Figure 10. Morphology of the holotype specimen of *Sphagnum magniae* (J. Shaw 2017-320). **A.** Branch leaves from divergent branches. **B.** Stem leaves. **C.** Cell structure on convex surface of divergent branch leaves, middle part. **D.** Cell structure on convex surface of spreading branch leaves, proximal part. **E, J.** Stem leaf, distal part. **F.** Branch in superficial view. **G.** Stem in transverse section. **H.** Stem cortex in superficial view. **I.** Cells of divergent branch leaves in transverse section. Scale bars for (A, B) = 200 µm; (C, D, F, H) = 20 µm; (E, G, J) = 100 µm, (I) = 10 µm. Online pdf in color.

S. magniae***S. diabolicum***

Figure 11. Scans of dried holotype collections of *Sphagnum diabolicum* (J. Shaw 2022-132) and *S. magniae* (J. Shaw 2017-320) from the moss type collection in DUKE. Online pdf in color.

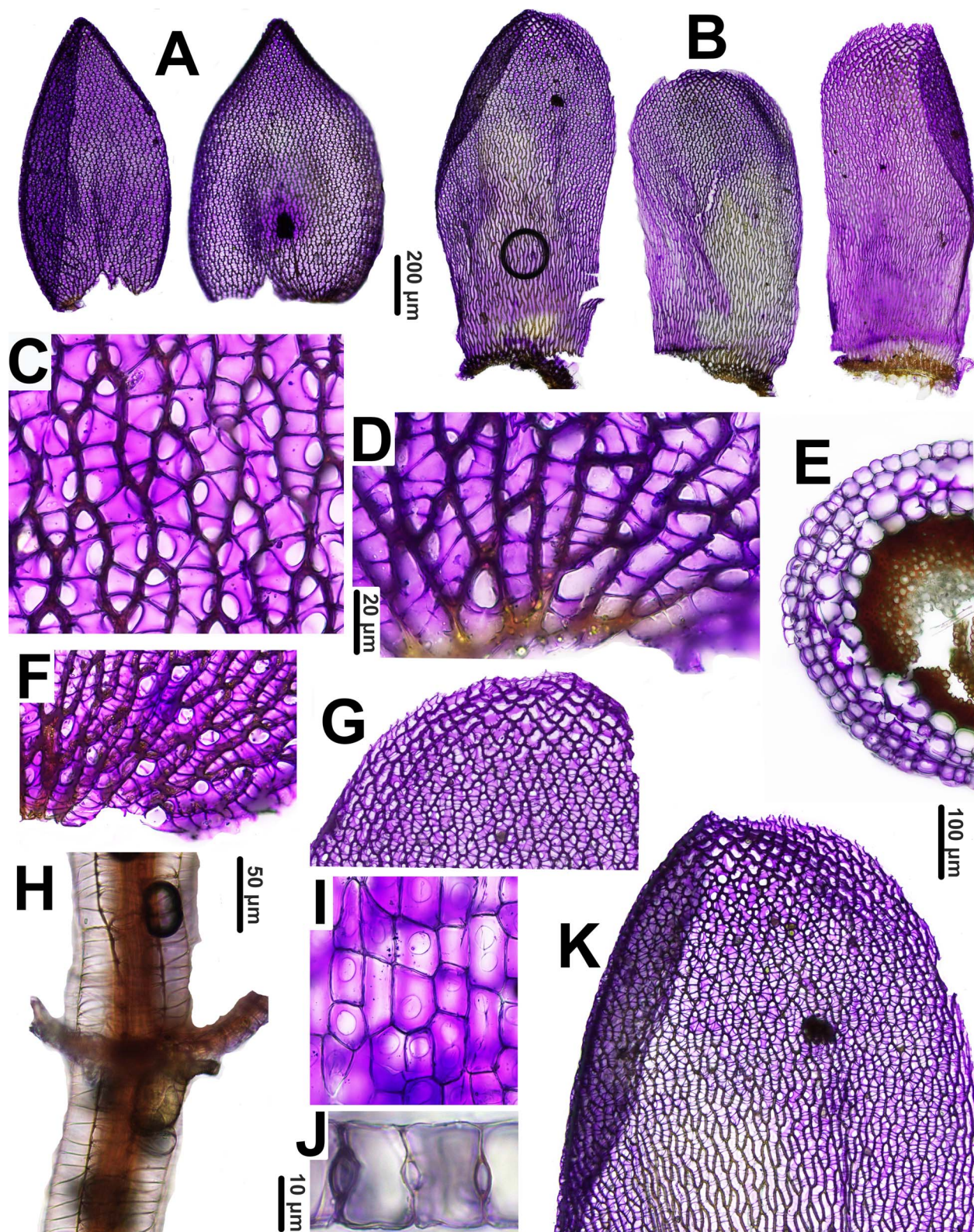


Figure 12. Morphology of the holotype specimen of *S. diabolicum* (J. Shaw 2022-132). **A.** Branch leaves from divergent branches. **B.** Stem leaves. **C.** Cell structure on convex surface of divergent branch leaves, middle part. **D.** Cell structure on convex surface of divergent branch leaves, proximal part. **E.** Stem in transverse section. **G.** Stem leaf, distal part. **H.** Spreading branch in superficial view. **I.** Stem cortex in superficial view. **J.** Cells of divergent branch leaves in transverse section. Scale bars for (A, B) = 200 µm; (C, D) = 20 µm; (E, G, K) = 100 µm, (F, H, I) = 50 µm, (J) = 10 µm. Online pdf in color.

RADseq analyses. The two new species described in this paper (*Sphagnum diabolicum* and *S. magniae*) have not been detected among those European collections, although our sampling to-date has only included relatively few sites in Europe and those two species could eventually be discovered there.

Morphological traits. Hassel et al. (2018) compared *Sphagnum divinum*, *S. medium* and *S. magellanicum* s.str. and described putative morphological differences among them. These authors were not aware of *S. diabolicum* nor *S. magniae*, described herein, so they were not considered explicitly. We examined and photographed traits discussed by Hassel et al. (2018), including (1) spreading branch shape, (2) spreading branch leaf arrangement (degree of spreading versus imbricate orientation), (3) spreading branch basal leaf cell pore sizes and shapes, (4) spreading branch leaf cross sections, including especially chlorophyllose cell wall thickness, (5) stem leaf shape, and (6) stem leaf cell structure. We randomly selected five collections representing each (phylogenetically defined) species and qualitatively documented these morphological traits with photographs. Mature branches were sampled from ca. one centimeter below the shoot apex, below the capitulum. Branch leaves were sampled from the central portions of the branches where they reached their largest mature size. Stem leaves were sampled along a span of ca. 1.0–1.5 cm downward from just below the capitulum.

Our field observations are based on >500 collections of this group made by the authorship team, from Maine to Florida, Arkansas, and Alabama over the last five years. Voucher specimens are deposited in the L. E. Anderson Bryophyte Herbarium at Duke University (DUKE).

General considerations in the examination of morphological traits. It is well known that thorough microscopic examination of *Sphagnum* morphology is tedious compared to many other bryophytes because both stem and branch leaves and their cell structure, often including branch leaf cross sections, are generally necessary. In addition, the appearance of cellular details is impacted by proper staining and the angles from which structures are viewed.

In some *Sphagnum* species, the orientation of branches and leaves differ in moist versus dry plants. Branches of species in this complex were photographed both moist and dry and little or no changes

were observed so these conditional traits are not further considered. Hassel et al. (2018) suggested that *S. medium* and *S. divinum* differ in wall thickness of the branch leaf chlorophyllose cells and this is an especially difficult trait to assess for many collectors, as their microscopic appearance is impacted by the angle at which cross sections are made. Oblique sections can produce walls that appear thickened, at least in part. It is difficult to consistently produce optimal transverse sections for comparison, but we reasoned that when enough sections are made, if there is a consistent tendency for species to differ in wall thickness this will be evident despite a lot of “noise” in the photos resulting from variation in the angles of sections. This approach assumes no nonrandom (among species) tendency for sections to be non-transverse, resulting in recurrent but artifactual differences in wall thickness. There is no reason to suspect that such nonrandomness occurs. Illustrations of branch leaf cross sections provided by Hassel et al. (2018) show differences in chlorophyllose cell wall thickness that do not appear to be artifacts of section orientation. Another trait that can be impacted by microscopic view is the size and shape of hyaline cell pores. Because the hyaline cells are more or less convex on the outside (abaxial) surface, pores near the margins of such cells can appear elliptical when in fact they are round. When pores are being observed in our figures, it is important to note the angle at which the pores are being viewed. Pores near the centers of cells may appear more round than those along the cell margins, but this can be artifactual. Pores in this complex are almost completely restricted to the outer cell surfaces; this can be easily determined on branch leaves because the concavity of the leaves allows the inside versus outside surfaces to be distinguished under a microscope. Because stem leaves are flatter, distinguishing inside and outside surfaces can be more difficult, but repeated observations indicate that as with branch leaves, the pores of stem leaves (when present) are mostly restricted to the outside hyaline cell surfaces.

RESULTS AND DISCUSSION

General morphological features. Many traits of plants in this group are typical of *Sphagnum* subg. *Sphagnum*. Most such traits are illustrated from the

holotype specimens of *S. diabolicum* and *S. magniae* (Figs. 10, 12). Stem cortical cells have 1–2 small circular pores per cell and generally lack fibrils or they are faint. Fibrils typical of subg. *Sphagnum* are more abundant on branch cortical cells. Branch leaves have marginal resorption furrows (not shown) and have extensive wall resorption across the upper leaf such that the leaves appear roughened at the back/outside. Stem leaves are larger than the branch leaves. We found no indication that species in this complex differ in these traits.

Field traits. The four North American species of the *Sphagnum magellanicum* complex are very similar in overall morphology. *Sphagnum medium* is perhaps the most distinctive because of relatively short, blunt branches (Figs. 2–6) that are typically ascending. We nevertheless find morphotypes that are intermediate in growth form and branch shape/orientation between *S. medium* and others in the complex. Our field observations in eastern North America indicate that *S. medium* is by far most common in open mires, as in Norway, and it may form high hummocks, but can occur in lawns closer to the water table. *Sphagnum divinum* and *S. diabolicum* grow abundantly in open mires as well as along mire edges and in surrounding forests. When growing in the open portions of mires they grow in lawns barely above the water table, and in low to moderately raised, rather loose hummocks. Both species can also form denser, higher hummocks that can be similar to those of *S. medium*. In fact, all three species can be extremely difficult to distinguish when they form high, dense hummocks.

Sphagnum magniae and *S. diabolicum* are the two most closely related sister species in this group (Fig. 1) but *S. diabolicum* is most similar to, and difficult to distinguish from, *S. divinum*, and they often grow at the same sites (unpublished data). Both occur in open mires, along mire margins, and in forests. *Sphagnum magniae* can have ranked leaves, sometimes very strongly so. Nevertheless, some plants of *S. magniae* have unranked or very subtly ranked branch leaves. The three other species, *S. divinum*, *S. medium* and *S. diabolicum*, can also sometimes have ranked leaves. Hassel et al. (2018) suggested that *S. medium* but not *S. divinum* has ranked leaves but our observations in eastern North America indicate that both can sometimes exhibit leaf ranking, and it is not a constant feature of either species. A recurrent feature of *S. magniae* is that the

plants are often nearly all green with little red pigmentation. But they are sometimes quite red and other species can be nearly all green on occasion. Hassel et al. (2018) showed that the degree of red pigmentation is impacted by shading (more shaded plants less red). The fact that *S. magniae* is more consistently green than the other species, regardless of microhabitat, suggests that this trait does also have a genetic component.

Branch shapes. As described by Hassel et al. (2018), *Sphagnum medium* (Fig. 5) tends to have short, blunt branches compared to the other species (Figs. 2–6). *Sphagnum magellanicum* s.str. (Fig. 6) also tends to have similar short branches but the other three species have variable, but typically more elongate and tapered branches.

Branch leaf basal cells. Hassel et al. (2018) observed that branch leaf basal cells in *Sphagnum medium* have large hyaline cell pores that often take up half or more the width of those cells whereas *S. divinum* tends to have narrower pores less than one half the hyaline cell width. Hill (2020) noted that while this distinction works to some extent, pores of the basal-most cells in *S. divinum* can also be large and broad, but that they do not extend 2–3 cells upward from the leaf base as they do in *S. medium*. With regard to *S. medium* (Fig. 7M–P) vs. *S. divinum* (Fig. 7I–L), our observations agree with those of Hill (2020). The other two North American species, *S. diabolicum* (Fig. 7E–H) and *S. magniae* (Fig. 7A–D), have pores much like those of *S. divinum*. Unfortunately, differences among the species are “tendencies”; Fig. 7J, for example, shows a sample of *S. divinum* with large pores extending higher than is typical. Interestingly, as with branch shape, *S. medium* is more similar to Fuegian *S. magellanicum* (Fig. 7Q–S), which also tends to have large round pores. We find no consistent distinction between *S. diabolicum*, *S. divinum*, or *S. magniae* in this trait.

Branch leaf cross sections. Hassel et al. (2018) suggested that in transverse view, branch leaf chlorophyllose cells of *Sphagnum medium* have thicker walls than in *S. divinum*. As noted above, this trait can be difficult to evaluate because slight differences in orientation among sections can result in different cell appearances. Nevertheless, based on at least 25–35 sections per species (with representatives shown in Fig. 8A–O), we find no consistent

differences among species. This conclusion agrees with observations reported by Hill (2020), who similarly found no differences between *S. medium* and *S. divinum*. We extend that conclusion to the other three species investigated here.

Stem leaf shape and cell structure. Hassel et al. (2018) suggested that *Sphagnum divinum*, *S. magellanicum* and *S. medium* differ in stem leaf shape and size, albeit with overlapping ranges of variation. We find that stem leaf size and shape vary extensively within species and even within plants. For example, **Fig. 9F' & G'** come from the same stem of *S. magellanicum*. Similarly, leaves shown in **Fig. 9V–X** were attached to a single stem. Each of the five species included here have variably shaped stem leaves and we found no consistent differences in size or shape among them. In each species the stem leaves vary from blunt (**Fig. 9J, Y**) to broadly rounded or more tapered (**Fig. 9A, D**). *Sphagnum magniae* seems to more commonly have bluntly but nevertheless pointed stem leaves than do other species, but this species varies as well (**Fig. 9C, E**). The leaves of each species may be obovate and widened in the apical portion, but less commonly the widest part of the leaf may be near the base or midleaf (**Fig. 9**).

We find tendencies but no consistent differences in cell structure as well. Lower cells in all species can sometimes be more or less conspicuously differentiated in a “basal zone” of elongate cells with minimal wall resorption such that they uniformly stain darkly and are devoid of pores or leaf gaps. Sometimes that basal zone can extend up to 80% of the stem leaf length (e.g., **Fig. 9I**) whereas in other cases it is visible only near the leaf base (**Fig. 9D**) or not at all (**Fig. 9B, F'**). We find no consistent differences among the species. While the basal zones may be difficult to identify in some of our figures, digital versions of this paper can be enlarged such that they are clearly evident.

Upper stem leaf cells can have walls almost completely resorbed and without pores or leaf gaps (e.g., **Fig. 9G, U**), with leaf gaps but no clearly defined pores (**Fig. 9B'**), with both leaf gaps and pores (**Fig. 9I'**), or with pores and fibrils much like those of branch leaves (**Fig. 9F**). Here also, morphology varies not only among plants of the same species, but sometimes within plants as well. In *Sphagnum diabolicum*, for example, different stem leaves from a single plant can have almost complete

cell wall resorption above the basal zone of elongate cells (**Fig. 9M**), or upper cells can have extensive gaps grading into “perfect” pores (**Fig. 9N**). There may be different tendencies among species but intraspecific variation seems to overwhelm any such differences, at least on a practical level.

Conclusions about morphological differentiation. Species in this complex are extremely difficult to distinguish morphologically. They have gametophyte structure that is diagnostic for subg. *Sphagnum* (see Crum 1984; Laine et al. 2018; McQueen & Andrus 2007) but all share the lenticular, completely included branch leaf chlorophyllose cells that distinguish them from other northern species in the subgenus. Our observations suggest that species within the *S. magellanicum* complex differ in morphological “tendencies” but that there is a notable absence of reliable species-specific diagnostic characters. *Sphagnum divinum* and *S. diabolicum* are especially similar and while *S. magniae* can have a different gestalt than the other species, and is often (but definitely not always) weakly red-pigmented or even completely green, we nevertheless find no field or microscopic characters that reliably distinguish it. At present, both *S. diabolicum* and *S. magniae* appear to be endemic to eastern North America, but we cannot eliminate the possibility that one or both occur in Europe. If that is the case, it is possible that some collectors may be calling collections of these species *S. divinum*, because the focus subsequent to the publication by Hassel et al. (2018) has been on distinguishing *S. medium* and *S. divinum*, without considering *S. diabolicum* or *S. magniae*.

Taxonomic considerations. The conceptual issue of species definition and delimitation has received abundant philosophical/theoretical as well as practical attention (e.g., Agapow et al. 2004; De Queiroz 2007; Moritz 1994; Zachos 2018). In the end, taxonomic decisions about delimitation and appropriate rank include substantial subjectivity, but these decisions have significant practical implications for citizen scientists, conservationists and ecologists.

Different criteria for species delimitation can yield alternative taxonomic treatments. Species based on coalescence in molecular phylogenetic studies may or may not correspond to reproductively isolated Biological Species (Campillo et al. 2020). If species are based solely on morphological

differentiation, taxa within the “*Sphagnum magellanicum* complex” might best be treated as conspecific. They represent “cryptic species” (Bickford et al. 2006) that, while often not (readily) distinguishable by morphology, are biological entities that are clearly distinct phylogenetically.

The four North American taxa each have unique ecological and biogeographic characteristics and are significant units of biological diversity. *Sphagnum medium*, and to a lesser extent, *S. diabolicum* and *S. divinum*, occur at both ends of the hydrological spectrum from high hummocks to wet lawns. Our observations suggest that when *S. medium*, *S. diabolicum*, and *S. divinum* occur sympatrically at the same site (which is not uncommon in the eastern United States), *S. medium* tends to occupy open mire areas, whereas *S. diabolicum* and *S. divinum* have wider niches and can occur in open areas, mire margins, or in adjacent forests. At sites where we have observed both *S. diabolicum* and *S. divinum*, the former tends to occur out in the open mires and *S. divinum* is more common in the surrounding forests. Exceptions to these patterns do occur, however, and we have found all three species in close proximity at some sites, mostly in the open parts of mires.

Of the three species, *Sphagnum medium* is the least common in eastern North America, and this itself indicates an ecological difference from the more abundant and widespread species, *S. diabolicum* and *S. divinum*. The ecological niche of *S. magniae* has no counterpart among the other, typically cold temperate-boreal species. *Sphagnum magniae* is biogeographically distinct in being almost completely limited to warm-temperate to subtropical habitats in the coastal plain of the southeastern region and Gulf of Mexico. This species extends as far south as Lake Okeechobee in south-central Florida.

Hassel et al. (2018) observed that *Sphagnum medium* tends to occupy ombrotrophic mires whereas *S. divinum* is more frequent in poor to medium fens. Schwarzer & Joshi (2017) found through experimental studies that *S. medium* grows more robustly than *S. divinum* in the absence of co-occurring species whereas *S. divinum* grows better when mixed with other (vascular plant) species. This variation in response to biotic interactions provides clear evidence of ecologically relevant biological differences between *S. divinum* and *S. medium*.

The question of how to treat plants in the *Sphagnum magellanicum* complex taxonomically creates a practical dilemma – they are ecologically and phylogenetically important units of biodiversity that are difficult and sometimes impossible to distinguish morphologically, especially by non-specialists. Subspecies or varieties within a broadly defined *S. magellanicum* would be a possibility, but many governmental and nongovernmental conservation organizations are obliged to track and monitor such taxa, even at the subspecific level, so assigning them to intra-specific categories does little to help with the practical problem. We conclude that despite difficulties distinguishing these species morphologically, they are too important as units of biodiversity to lump them back into *S. magellanicum* s.l. We therefore provide formal descriptions to validate two new species: *S. diabolicum* and *S. magniae* (and apologize on behalf of the molecular systematics community!).

We have recently developed “barcode” loci that when amplified yield amplicons (visualized as bands on a gel) of different sizes. These barcode loci will be valuable for identifying collections that are difficult to assign based on morphology and are inexpensive and relatively easy to visualize on a gel because their use does not involve nucleotide sequencing. A paper is in preparation where we utilize these barcode loci to document sympatric occurrences and localized niche differentiation among species in this complex. We therein provide primer sequences for amplifying these barcodes, which we hope will provide a valuable resource for collectors.

TAXONOMY

Sphagnum magniae A.J.Shaw, Aguero & Nieto-Lugilde, *sp. nov.* **Figs. 2A–I, 7A–D, 8A–C, 9A–G. Figs. 10, 11 (holotype)**

Plants often (but not always) green and with ranked spreading branch leaves. Growing in warm-temperate to subtropical habitats of the southern United States.

TYPE: U.S.A., NORTH CAROLINA: Brunswick Co., Green Swamp, 8.4 mi N of Supply, NC 211, 34.12545, –78.32016, *J. Shaw 2017-320*, 19 Nov 2017 (holotype, DUKE; isotypes, CAL, MO, NY, TRH).

Description. Plants robust, often completely green but sometimes ± red. Capitulum well

developed, bushy and hemispherical. Branch fascicles with 2 spreading and 2 pendent branches. Spreading branches relatively long and tapered, spreading widely, horizontal to downward trending. Branch leaves strongly to weakly ranked, sometimes unranked, narrowly to broadly ovate, cucullate, erect-spreading moist or dry; upper hyaline branch leaf cells with elliptical to round pores, sometimes arranged in 3s at cell apices; basal hyaline cells with round to elliptical pores on convex surfaces, especially on basal-most cells, elliptical on cells just above; chlorophyllose cells elliptical and included, not reaching the inner or outer surfaces. Stem leaves narrowly oblong or bluntly lanceolate, occasionally broader or obovate, apices $\pm$ broadly rounded, often with a basal zone of cells not differentiated as hyaline/chlorophyllose, the basal region most often strongly developed and conspicuous; upper cells with well-developed pores and fibrils, conspicuous large membrane gaps, or the inner and outer surfaces almost completely resorbed. Sporophytes observed at two sites in North Carolina, in 2018 and 2021.

Etymology. *Sphagnum magniae* is named for Dr. Magni Olsen Kyrkjeeide (Norwegian Institute for Nature Research), whose work on genetic structure within *S. magellanicum* s.l. led us to this species.

Geographic range. The range of *Sphagnum magniae* is largely allopatric to those of the other three North American species in the complex. We have reliable, genetically confirmed samples from Maryland through South Carolina, Georgia, and Florida south to Lake Okeechobee. The species also occurs west along the Gulf of Mexico; we genetically confirmed samples from Arkansas and Alabama, northward to Tennessee. It is highly likely that all records of “*S. magellanicum*” from warm-temperate to subtropical habitats in the southeastern Atlantic and Gulf coasts belong to this species. We also surprisingly confirmed a sample from Michigan; replicate DNA extractions and reanalyses indicate that the record is not a lab error. In fact, occurrences of this and other species in the complex need to be better understood in the midwestern U.S., including Illinois, Michigan, Ohio and Wisconsin.

We have had poor sequencing success to-date (DNA quality) with several likely samples of *Sphagnum magniae* from north of Maryland, in Delaware and New Jersey, but the species probably

extends at least to New Jersey. It could occur sympatrically with other species in the complex in that region. New Jersey and perhaps southeastern Pennsylvania are for that reason critical areas to explore for sympatric occurrences of *S. magniae* and its closely related sister species, *S. diabolicum* (Shaw et al. 2022).

Sphagnum magniae occurs in the coastal plain (and less commonly in the Piedmont physiographic region) of eastern North Carolina, whereas *S. diabolicum* occurs in the Appalachian Mtns. Genetic analyses (Piatkowski et al. in prep) show that the genomic makeup of mountain plants, despite their unambiguous phylogenetic placement into *S. diabolicum*, contain some 10% *S. magniae* genetic material. North Carolina coastal plain samples of *S. magniae* have a minority representation of *S. diabolicum* in their genomes. In contrast, no such genetic admixture between the species has been demonstrated from areas south of North Carolina; more southern *S. magniae* plants are pure *S. magniae*. Northern plants of *S. diabolicum* are almost all pure *S. diabolicum*. North Carolina northward to New Jersey is a critical area with a history of hybridization between *S. magniae* and *S. diabolicum* (Piatkowski et al. in prep). In addition, unpublished data suggest that a few samples from Pennsylvania and even as far north as New England may be genetically admixed between *S. diabolicum* and *S. magniae*.

The holotype of *Sphagnum magniae*, from eastern North Carolina, may include some introgressed genetic material from *S. diabolicum*, but is a phylogenetically unambiguous collection of *S. magniae*. While it may not be ideal to use a collection of *S. magniae* that could have interspecific hybridization in its history as the holotype, the Green Swamp (North Carolina) population is one of the few for this species where plants are abundant enough to share isotypes with multiple herbaria. Moreover, many collections of all species in this complex exhibit varying degrees of interspecific introgression (Shaw et al. 2022). The paratype of *S. magniae* from Florida is at least close to being genetically “pure.” Holotype collections of both *S. diabolicum* and *S. magniae* are morphologically typical, to the extent that there is a “typical.” Interestingly, the holotype of *S. magniae* has stem leaves that vary from having upper cells weakly fibrillose and extensively resorbed with large irreg-

ular leaf gaps (Fig. 10J), to those much like the branch leaves, with fibrils and elliptical pores (Fig. 10E). These variations on a single plant may reflect different micro-climatic, seasonally related environmental variation, since they matured at different times and under slightly different microenvironmental conditions. Both types of leaves were mature. Sometimes these same variations characterize whole colonies or even populations at a given site. Intraspecific morphological variation is the rule, not the exception, and it is fruitful to focus on variation patterns rather than so-called typical morphs. While the type collections serve an important nomenclatural role, they represent limited examples of morphological variation in the range presented by each species.

Habitat. In its typical warm-temperate to subtropical sites, *Sphagnum magniae* is rarely common and occurs as isolated hummocks under pines (*Pinus elliotii* Engelm., *P. palustris* Mill., *P. taeda* L.) and/or hardwoods (*Acer rubrum* L., *Nyssa biflora* Walter, *N. sylvatica* Walter). In Florida, it occurs in pine-palmetto forests. It also occurs along roadsides with impeded drainage.

Additional specimens examined (paratypes). U.S.A., FLORIDA: Osceola Co., Ecotone Trail along S Poinciana Blvd., 28.181221, -81.456829, M. Nieto-Lugilde 2022-135, 3 May 2022 (DUKE, CAL, MO, NY, TRH). NORTH CAROLINA: Richmond Co., S end of Lake Bagget near dam, ~4 mi WNW of Hoffman, 35.04196, -79.62219, A. Duffy 22001, 5 Nov 2022 (DUKE, CAL, MO, NY, TRH).

Sphagnum diabolicum A.J.Shaw, Agüero, & Nieto-Lugilde, *sp. nov.* Figs. 3A–I, 7E–H; 8D–F;

9H–N. Figs. 11–12 (holotype)

Plants sometimes “oily” in appearance when wet, typically growing in lawns or forming low hummocks in open cold-temperate to boreal peatlands.

TYPE: U.S.A. NEW YORK: Chenango Co., German Township, Jam Pond Bog, N of county rd. 5 (German Road) between Rabbit Path and German Mc Donough Rds., 42.4947, -75.8263333, J. Shaw 2022-132, 15 Jun 2022 (holotype, DUKE; isotypes, MO, NY, TRH).

Description. Plants robust, green to $\pm$ red. Capitulum well developed, bushy and hemispherical or looser and less compact. Branch fascicles with 2

spreading and 2 pendent branches. Spreading branches relatively long and tapered, spreading widely, horizontal to downward trending. Branch leaves sometimes weakly ranked, narrowly to broadly ovate, cucullate, erect-spreading moist or dry; upper hyaline branch leaf cells with elliptical to round pores, sometimes arranged in 3s at cell apices; basal hyaline cells with round to elliptical pores on convex surfaces, especially on basal-most cells, elliptical on cells just above; chlorophyllose cells elliptical and included, not reaching the inner or outer surfaces. Stem leaves oblong with parallel sides or sometimes broadened above, apices broadly rounded to truncate or weakly emarginate, often with a basal zone of cells not differentiated as hyaline/chlorophyllose, the basal region sometimes poorly differentiated; upper cells with conspicuous large membrane gaps or the inner and outer surfaces almost completely resorbed. Reproductively mature male plants with abundant red-pigmented antheridial buds. Sporophytes produced abundantly, at least in wet years.

Etymology. The name *Sphagnum diabolicum* originated in conversations between J. Shaw and H. Rydin (in 2019) and reflects the morphologically confusing (secretive) nature of this species. Levels of morphological variation in the species are almost diabolical at times. Perhaps in opposition to *S. divinum*, *S. diabolicum* can promote a balanced taxonomic treatment for this group.

Geographic range. *Sphagnum diabolicum* has been recorded, with genetic confirmation, from Newfoundland and Nova Scotia southward to North Carolina in the Appalachian Mountains. In the southern part of its range *S. diabolicum* is restricted to higher elevations. This species is common in Pennsylvania, especially in the Pocono Mts. region.

Habitat. This species occurs in wet forests, mire margins, and in open mires where it occurs in low hummocks and in lawns close to the water table. In the southern Appalachians it also occasionally grows along roadsides and on dripping rock walls. There is some indication (needing additional confirmation) that *Sphagnum diabolicum* prefers especially hyper-oceanic sites. In Maine, for example, it occurs most commonly within a few km of the ocean and is largely replaced ecologically by *S. divinum* at sites just 50+ km inland. It does, however, also grow in

Pennsylvania and New York state where the climate is less oceanic.

Additional specimens seen (paratypes). U.S.A. PENNSYLVANIA: Clinton Co., Noyes Township, Sproul State Forest, Cranberry Swamp. *Sphagnum*–Beak Rush Peatland, 41.256881, –77.727266, *J. Shaw* 2022-65, 23 May 2022 (DUKE, MO), *J. Shaw* 2022-66, 23 May 2022 (DUKE, NY, TRH). Tioga Co., Elk Township, Tioga State Forest, Reynolds Spring Natural Area, 41.553883, –77.494627, *J. Shaw* 2022-33, 23 May 2022 (DUKE, NY, TRH), *J. Shaw* 2022-34 (DUKE, MO, TRH). NEW HAMPSHIRE: Cooper Cedar Woods, *J. Shaw* 2018-236, 31 Jul 2018 (DUKE, TRH). MAINE: Hancock Co., Gouldsboro Township., Birch Harbor, between Rice Rd. and Prospect Point Rd. on ME 186, S side of rd. (“Eagle Hill Forest”), *M. Nieto-Lugilde* 2022-250, 26 Jun 2022 (DUKE, MO, NY, TRH). Washington Co., Machias area, wetland along Hadley Lake Rd., 1.4 mi N of jct. with US-1, E side of rd., 44.74154, –67.43991, *B. Aguero* 20128, 25 Jun 2022 (DUKE, CAL, MO, NY, TRH).

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