# ON THE MORPHOLOGICAL DELIMITATION OF *SPHAGNUM HENRYENSE* О МОРФОЛОГИЧЕСКОЙ ОТГРАНИЧЕННОСТИ *SPHAGNUM HENRYENSE* ANNA V. Shkurko<sup>1</sup>, Yuriy S. Mamontov<sup>1,2</sup> & Vladimir E. Fedosov<sup>3,4</sup> Ahha B. Шкурко<sup>1</sup>, Юрий С. Мамонтов<sup>1,2</sup>, Владимир Э. Федосов<sup>3,4</sup>

Abstract

Sphagnum henryense (subg. Sphagnum) differs from S. palustre in having abundant small pores on the dorsal surface of branch leaves. Recently studies usually consider it as a doubtful species or even merely a synonym of S. palustre. We revealed S. palustre-like plants, with numerous small round pores very similar to those of S. henryense in collection from Russian Far East, South Kuril Islands. The morphometric studies of these plants, along with S. henryense from other regions of Asia and North America and S. palustre from the same areas and Europe revealed statistically reliable differences in three traits of pores in the hyaline cells on abaxial branch leaf surface: (1) number, (2) length, (3) length to width ratio, and significant differences in (4) branch leaf breadth and (5) number of pores in stem cortex cells, distinguishing the henryense-morphotype. Such morphotypes are illustrated, discussed, and likely deserve a further studies.

Резюме

Sphagnum henryense (subg. Sphagnum) отличается от S. palustre наличием многочисленных мелких пор на дорсальной поверхности веточных листьев. В последнее время его часто рассматривают как сомнительный вид или даже синоним S. palustre. Недавно нами были обнаружены растения, похожие на S. palustre, с многочисленными мелкими круглыми порами, очень похожими на таковые у S. henryense в коллекции с острова Кунашир, Южные Курилы. Морфометрические исследования этих растений, наряду с S. henryense из других регионов Азии и Северной Америки, а также S. palustre из различных регионов, выявили статистически достоверные различия по трем признакам пор гиалиновых клеток на абаксиальной поверхности веточного листа: (1) количество, (2) длина, (3) отношение длины к ширине, а также значимые отличия по (4) ширине веточного листа и (5) количеству пор в клетках гиалодермиса стебля, отличающие "henryense-морфотип". Такие дальневосточные морфотипы проиллюстрированы, обсуждены и, на наш взгляд, заслуживают дальнейшего изучения.

KEYWORDS: Sphagnum palustre, Sphagnum henryense, morphological variability, species delimitation, pore patterns

## INTRODUCTION

Mosses from genus *Sphagnum* are considered as one of the most difficult objects in terms of species delimitation. Since the *Sphagnum* species tend to hybridization (Ricca & Shaw, 2010; Meleshko *et al.*, 2018), polyploidization (Såstad *et al.*, 1999a; Shaw *et al.*, 2012) and also are proved to bear signs of incomplete lineage sorting (Shaw & Goffinet, 2000; Meleshko *et al.*, 2021), the molecular phylogenetic analyses are often complicated. The morphological delimitation of the closely related species is also problematic due to a noticeable variation in their morphology, which depend on the environmental conditions (Stenøien *et al.*, 1997; Såstad *et al.*, 1999b; Yousefi *et al.*, 2019). Although several genetically sepa-

rated *Sphagnum* species can overlap morphologically (*S. medium* Limpr. vs. *S. divinum* Flatberg & K. Hassel, *S. divinum* vs. *S. alaskense* R.E. Andrus & Janssens, *S. fallax* (H. Klinggr.) H. Klinggr. vs. *S. pacificum* Flatberg, *S. girgensohnii* Russow vs. *S. tescorum* Flatberg, *S. arcticum* Flatberg & Frisvoll vs. *S. olafii* Flatberg etc.; Shaw *et al.*, 2005; Shaw *et al.*, 2012; Hassel *et al.*, 2018; Duffy *et al.*, 2020; Shaw *et al.*, 2022), in the other groups, where prominent morphological distinctiveness was suggested to be used for species delimitation (*S. fallax* var. *fallax* and *S. fallax* var. *isoviitae* (Flatberg) Lönnell & K. Hassel, *S. majus* (Russow) C.E.O. Jensen subsp. *majus* and *S. majus* subsp. *norvegicum* Flatberg, *S. rubellum* Wilson and its synonyms *S. andersonianum* R.E. Andrus and

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*S. bartlettianum* Warnst.), it was not confirmed by molecular phylogenetic analyses (Shaw *et al.*, 2005; Duffy *et al.*, 2020; Nieto-Lugilde *et al.*, 2022).

However, there are still a numerous ambiguous cases where morphospecies remain in use despite lacking support from genetic methods. *Sphagnum palustre* L. and *S. henryense* Warnst. apparently represent such case.

Sphagnum henryense was described from Virginia, USA by Warnstorf (1900), who considered presence of the worm-like ridges on internal walls of hyaline cells where they contact with chlorophyllous cells as its main distinction of this species from S. palustre. Subsequently some authors (Andrews, 1913; Allen, 2014) mentioned these characters as the only ones differentiating these two species. Warnstorf (1900)' protologue of S. henryense mentioned also the numerous small round pores in hyaline cells on the branch leaves convex side. This character was used as the second important character by Crum (1984); Anderson & Amman (1991); McQueen & Andrews (2007). These authors also mentioned that the ornamentation on internal walls of hyaline cells is not always present. Andrus (1980) also showed that the smallpored specimens, i.e. S. henryense, mostly have such ridges, but not always. Anderson & Amman (1991) demonstrated that the ridge pattern is quite variable even in the better morphologically circumscribed species such as S. papillosum Lindb. and S. affine Renauld & Cardot. Additionally, the authors mentioned a plant which combined a pore pattern typical for S. palustre with a strong ornamentation on the cell wall, and suggested that S. palustre has the potential to produce papillae but they are mostly entirely or only mildly suppressed.

Karlin & Andrus (1988) according to their observations in New Jersey noticed that overlap of different pore patterns between *S. palustre* and *S. henryense* might be phenotypic rather than genotypic. This hampers plant identification, so not all specimens can be identified with certainty. Later Karlin *et al.* (2010) demonstrated that these species are also impossible to separate by microsatellite analysis. Therefore they supported the synonymizing *S. henryense* with *S. palustre* by Anderson *et al.* (2009). The continuous variation in porosity pattern was also noted by Karlin *et al.* (2010).

Recently, in the collections from Kunashir Island (South Kurils) we found robust plants which largely resemble *S. palustre* due to having the triangular chlorophyllous cells in transverse branch leaf section, lingulate-spatulate stem leaves and dark brown stem. However, these plants differ from the most common and widespread phenotype of *S. palustre* in Russia in having two rows of numerous small nearly round pores on the convex surface of the branch leaves, which are characteristic to *S. henryense*. In Kunashir island we found two localities, in one it forms extensive tufts in a mediumrich fen, in another it grows mixed within *S. rubiginosum* forming a hummock in a swampy coniferous forest. Thus we undertook a special search for small pore morph of *S. palustre* s.l. in herbaria.

### MATERIALS AND METHODS

We examined numerous collections of S. palustre s.l. in MHA, LE, MW, and partially in IRK and VGBI and selected 42 specimens for morphometric studies. All measurements were made on mature portions of stems i.e. starting from two cm below a capitulum with one shoot per specimen. We studied five branch leaves randomly chosen from the several leaves from the middle part of each spreading branch from three different fascicles; five stem leaves and three pieces of stem cortex stained in the mix of methylene blue and gentian violet for further measurements. Nine traits were measured with the light microscope (Table 1), the width of stem and branch leaves was measured at the half of their length; the number of pores was counted in five adjacent central cells of three leaves/pieces of stem cortex; length (maximal measure of pore which is parallel to the cell wall) and width (maximal measure of pore which is perpendicular to the cell wall) of central commissural pores (one per cell, 15 per specimen in total) were measured from the same cells where number of pores were counted. Additionally we used five calculated metrics which reflect the relative characteristics of morphological traits (Table 1).

To illustrate the abaxial branch leaf pore pattern we prepared SEM images for two specimens of S. henryense from Kunashir Island and North Carolina, and one specimen of S. palustre s.str. from the Czech Republic. Measurements of S. henryense and S. palustre s.str. were averaged per specimen and visualized by box plots with GraphPad Prism 8 application for Windows (GraphPad Software, 2020) and tested for difference using Mann-Whitney U-criterion in PAST ver. 4 (Hammer et al., 2001). To visualize an overall morphological differentiation of the two explored morphospecies, we prepared PCA scatterplot using PAST ver. 4 for seven (excluding the stem leaf length and width which appeared to be noninformative for their delimitation due to sufficient variation within each morph and very close mean values between in two morphs) metrics assessed during morphological survey.

#### RESULTS

In course of measuring we referred the specimens with an average number of pores per hyaline cell of branch leaves 10 or more to *S. henryense* and those with lesser number of pores to *S. palustre* s.str. Thus, we compared 22 specimens of *S. palustre* s.str. from the U.S.A., Japan, Russia and Europe with 20 specimens of *S. henryense* from the U.S.A., Canada, Japan, and the Russian Far East.

Superficially the plants are rather similar in shape, however the most specimens of *S. henryense* are rather robust plants and have rather flat capitulum while *S. palustre* s.str. vary from medium to robust in size, and

Character	S. henryense	S. palustre	MW
Ν	20	22	
Stem leaf length (SLL), µm	1876±329	1889±326	215
Stem leaf width (SLW), µm	$1101 \pm 207$	1122±221	204
Branch leaf length (BLL), µm	2345±425	2435±402	191
Branch leaf width (BLW), µm*	1557±296	$1715\pm248$	131
BLW at 3/4 leaf length (BLW3/4), µm*	$1076 \pm 200$	$1186 \pm 171$	140
SLL:BLL	$0.81 \pm 0.15$	$0.79{\pm}0.14$	188
SLW:SLL	$0.59{\pm}0.08$	$0.6 {\pm} 0.08$	207
BLW:BLL*	$0.67 \pm 0.06$	$0.71 \pm 0.07$	127
BLW3/4: BLW	$0.69 \pm 0.07$	$0.69 \pm 0.04$	199
Pore N in stem cortex (PSCN)**	3.9±1.2	$2.5 \pm 0.8$	73
Pore N in branch leaves (PBLN)***	11.7±1.4	5.4±1.3	0
Pore length in branch leaves (PBLL), µm***	16.5±1.9	20.3±2.4	18
Pore width in branch leaves (PBLW), µm*	$10.7 \pm 1.4$	9.5±1.5	125
PBLW:PBLL***	$0.7{\pm}0.07$	$0.47 {\pm} 0.05$	0

Table 1. The mean and standard deviation of 14 parameters measured/calculated in *Sphagnum palustre* s.str. and *S. henryense* and the results of the Mann–Whitney U test (MW); \*\*\*p<0.001, \*\*p<0.01, \*p<0.05.

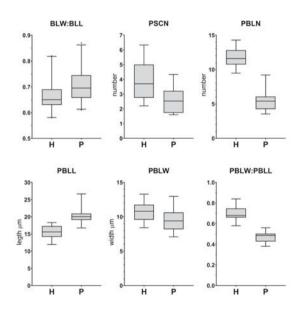


Fig. 1. The box plots of six metrics, where H is *S. henry-ense* and P is *S. palustre*; BLW:BLL is the the ratio of width at the middle part of branch leaf to its length; PSCN: the number of pores on the outer stem cortex cells; PBLN, PBLL, PBLW: the number, length and width of cell pores on the convex side of 1/4 part of the branch leaves accordingly; PBLW:PBLL: the width/length ratio of pores on the convex side of 1/4 part of the branch leaf.

demonstrate rather hemispherical inner part of capitulum. Moreover, the coloration of *S. "henryense*" plants is often pale pinkish-green and sometimes slightly brownish resembling those of *S. alaskense* R.E. Andrus & Janssens or *S. papillosum* Lindb., while *S. palustre s.str.* has mostly pale-green coloration and brighter salmonpink inner part of capitulum.

Microscopically all-twenty plants referred to *S. henryense* have multiple (10-15) pores on the convex side of branch leaves which typically are somewhat distant from

commissures, while those of S. palustre s.str. have less number of such pores (3-9) which are remarkably adjacent to commissures (Figures 1 and 3). The size and shape of pores in these two sets differ statistically (Table 1): S. henryense has rather short round to elliptic pores, in contrast to S. palustre s.str. with its mostly strongly elliptic, thin and longer pores (Figures 3 and 4). The number of pores in the external stem cortex layer also looks as perspective trait differentiating these two. Sphagnum henryense typically has (1-)4-6(-8) pores per cortex cell while in S. palustre s.str. the average number of such pores is lower, 1-4(-5) per a cortex cell (Table 1). However due to remarkable overlap, this difference may only be assessed based on extensive strips of hyalodermis. Except for pore pattern, several differences can be pointed in the branch leaves shape, since leaves of S. palustre s.str. tend to be more spherical in shape and its median width, width at 3/ 4 leaf length part and width/length ratio of branch leaf are significantly higher than those of S. henryense (Table 1, Fig. 1). The scatterplot obtained from the Principal Component Analysis based on the seven traits shows clear separation of the two forms (Figure 3), where the first two components (PC1 - 46.6%, PC2 - 24.0%) explain 70.6% of the total variation in two forms (Table 1).

# DISCUSSION

The results of our morphometric study show good separation of the two morphs according to the pore patterns and rather well distinctiveness in branch leaf shape in *S. palustre* s.l. Only two Japanese plants have the worm-like ornamentation on the inner walls of hyaline cells. However, correlation between the worm-like ridge presence and pore number and size remains indefinite, variously assessed in the special studies by e.g. Andrus (1980) and Anderson & Amman (1991). Likewise, Karlin *et al.* (2010) in their analysis also relied on the pore patterns rather than on ornamentation of branch leaf hyaline cells.

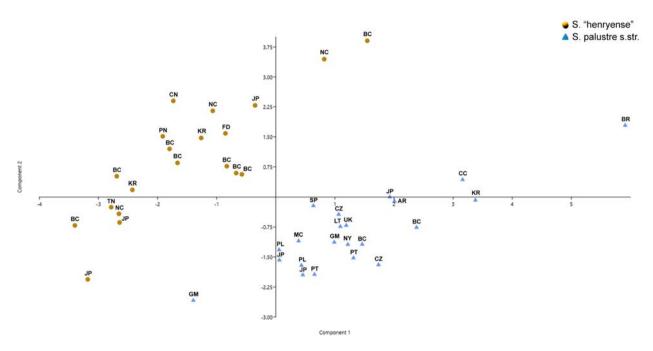


Fig. 2. The PCA scatterplot based on the seven metrics, where orange dots correspond to the specimens of *Sphagnum henryense* and blue triangles to the specimens of *S. palustre* s.str.; specimens are annotated by their geographical origin as follow: AR – Arkhangelsk Province, Russia; BC – British Columbia, Canada; BR – Republic of Belarus; CC – Caucasus: CN – Connecticut, U.S.A.; CZ – Czech Republic; FD – Florida, U.S.A.; GM – Germany; JP – Japan; KR – Kuril Islands, Russia; LT – Lithuania; MC – Michigan, U.S.A.; NC – North Carolina, U.S.A.; NY – New York, U.S.A.; PL – Poland: PN – Pennsylvania, U.S.A.; PT – Portugal; SP – Spain, TN – Tennessee, U.S.A.; UK – United Kingdom.

In the studied species the pore patterns differ conspicuously. The random specimen selection of *S. palustre* from different regions shows no overlap in pore traits, neither any transitional states. The habitat data of the measured samples of "*S. henryense*" do not indicate that the variation can be explained by environmental factors. Likewise, Andrus (1980), Crum (1984), and Anderson & Amman (1991) mentioned that there is no ecological difference between *S. palustre* and *S. henryense* and they can even grow in the mixed stands, so distinct pore pattern cannot be considered as ecologically induced.

Actually, pore pattern is an essential trait for separating Sphagnum species, especially in sect. Cuspidata. However pore sometimes are remarkably distinct within the genetically uniform taxa. Nieto-Lugilde et al. (2022) observed in S. majus subsp. majus rather small pores (less than 1/3 of a hyaline cell width) at the convex surface of the branch leaf, occurring mostly by 2 per fibril interval. At the same time S. majus subsp. norvegicum has larger pores by 1 per fibril interval. These differences of two subspecies were not consistent with genetic data inferred from the results of the DNA RAD-sequencing data. Therefore the authors concluded that the morphological differences between them are either plastic responses to environmental heterogeneity or segregating genetic variation within a single taxon, which however do not merit any taxonomic recognition.

Based on the clear-cut morphological differentiation demonstrated above, we suggest a need for taxonomic rec-

ognition of *S. henryense*, however, pending the establishing taxon at infraspecific level until more suggestive phylogenetic data appear. For now, *Sphagnum henryense* deserves recognition at least in the identification keys for progressive collection of data on its distribution and ecology, and potentially for a further deeper studies of the background for such rather contrasting differentiation.

Despite of the unclear taxonomic status of *Sphagnum henryense*, we provide the description for the purposes of further comparison.

*Sphagnum henryense* (description bases on the East Asian plants, Fig. 5)

Plants medium-sized to robust, pale green with brownish-pinkish tint. Capitulum typically flattered sometimes with aggregated branches of the inner part, terminal bud slightly visible. Stem brown to dark brown, hyalodermis well differentiated, superficial cortical layer with distinct spiral reinforcing fibrils, and usually with 4-6 pores per cell. Sclerodermis well delimited, consisting of small thick-walled cells, dark-yellow to brown. Stem leaves lingulate-spatulate with broad rounded apex, 1.7-2.2 (-2.4) mm long and 0.9-1.2 mm wide, border fringed, hyaline cells rhomboid, efibrillose, typically resorbed or have the rests of cell walls with fibrils and pores. Branch fascicles with 2(-3) spreading and 2 pendent branches. Spreading branches quite tapering with spreading to moderately imbricate leaves, which are sometimes squarrose when dry. Branch leaves in the middle part of spreading branches ovate, 1.9-2.8 mm long and 1.4-1.9(-2.2) mm wide,

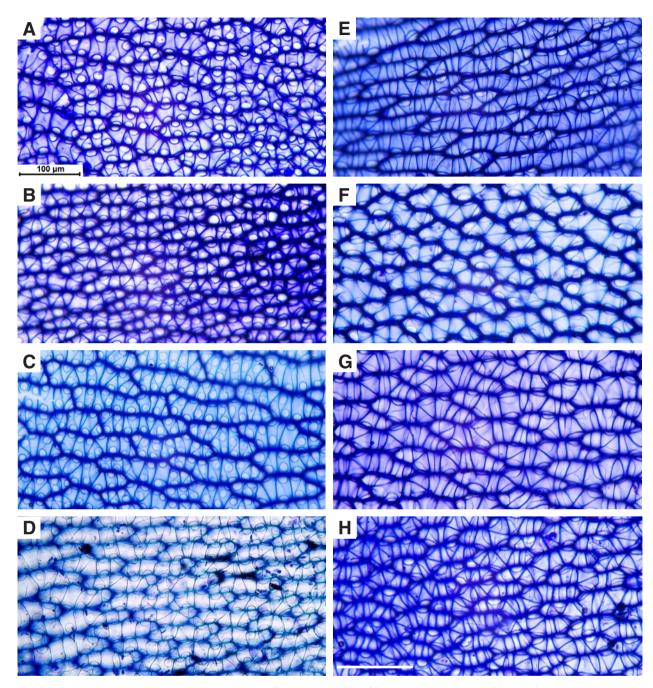


Fig. 3. The pore patterns on 1/4 proximal part of the convex side of branch leaves; A–D: *Sphagnum henryense* (A: Russia, Kunashir Island, *Mamontov 814-1-s10*, MHA9121113; B: Japan, Honshu, *Higuchi*, VLA; C: Canada, British Columbia, *Vitt & Andrus158*, LE; D: USA, Pennsylvania, *Andrus 5562*, MHA9059763); E–H: *S. palustre* s.str. (E: Germany, Brandenburg, *Prager*, MW9013692; F: Russia, Kunashir Island, *Koroteeva 15-6/1-2*, MHA9015882; G: Japan, Honsu, Fukui-ken, *Sakai 31*, MHA9059832; H: U.S.A., New York, *Schrenk*, IRK). Scale bar: 50 μm for all.

apex cucullate; hyaline cells on the internal surface smooth or have worm-like ornamentation; on convex surface with 10–15(–18) round to elliptic pores 12–15 (–17)  $\mu$ m long and 9–13  $\mu$ m wide in two rows along commissures; on concave surface with several large round pores across the lateral leaf margins; chlorophyllous cells in branch leaf transverse section isosceles-triangular to ovate triangular, widely open on concave surface and open or slightly enclosed on convex surface.

# Differentiation

Sphagnum henryense can be confused with S. palustre s.str., S. centrale and S. papillosum in the field. Microscopically S. henryense clearly differs by its triangular chlorophyllous cells of branch leaves in transverse section, while S. centrale has elliptical cells. Sphagnum papillosum typically has a prominent papillosity on internal walls of hyaline cells of the branch leaves which is absent in S. henryense. Sphagnum palustre s.str. is most

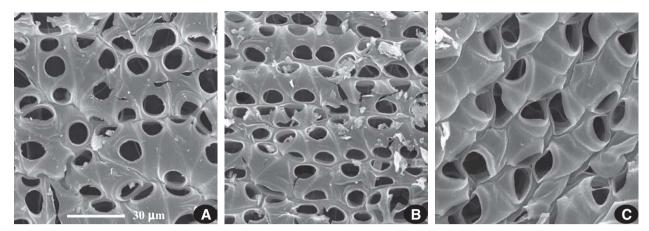


Fig. 4. SEM illustrations of the abaxial side of branch leaves, showing the difference in pore pattern. A: *Sphagnum henryense* (Russia, Kunashir Island, *Mamontov 814-1-s10*, MHA9121113); B: *S. henryense* (North Carolina, Bladen Co., *Tan 93-104*, MHA9059764); C: *S. palustre* (Czech Republic, Karlovy Vary, *Tyuremnov s.n.*, MW9013676). Scale bar 30 µm for all.

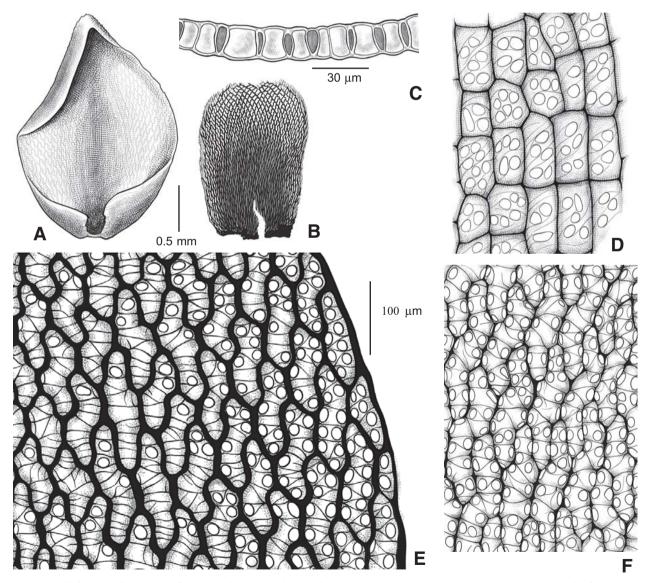


Fig. 5. *Sphagnum henryense* (from: Russia, Kunashir Island, *Mamontov 814-1-s10*, MHA9121113); A: branch leaf; B: stem leaf; C: branch leaf transverse section; D: external cells of the stem cortex; E: adaxial side of the branch leaf margin; F: abaxial side of the branch leaf medium part (from: Russia, Kunashir Island, *Mamontov 814-1-s10*, MHA9121113). Scale bars: 0.5 mm for A, B; 100 µm for D, E, F; 30 µm for C.

similar to *S. henryense*, however the numerous small pores localized in two rows along commissures in convex side of the branch leaves clearly separate the latter from *S. palustre* s.str., which typically has a less number of longer elliptic pores adjacent to commissures.

*Ecology.* In Russia *Sphagnum henryense* occurs in medium to rich fens with *Carex* spp., *Equisetum palustre, Comarum palustre, Menyanthes trifoliata*, and in floodplain swampy forests.

*Distribution in Asia. Sphagnum henryense* is known so far in Japan, Honshu and Shikoku Islands, and in Russian Far East, in Kuril Islands, Kunashir.

Specimens examined: Russia, Sakhalin Province, Kunashir Island, Yuzhno-Kurikl'sk District, Otradnyi Village vicinity, Lake Serebrjannoe, 44.05187°N, 145.85597°E, 20.IX.2020, Mamontov 814-1-s10 (MHA9121113); the same region, Kuril State Nature Reserve, Rudnaja Village vicinity, Severjanka River valley, Broadleaved-high herb floodplain forest with willows, on hummock, 44.32080 N, 145.99403 E, 23.IX.2020, Mamontov 814-1-s35 (MHA9121146); Japan, Honshu, Akita Pref., Yuri-gun, Kisakata-machi, Shishigabana, on wet humus in forest, 250 m, 39.15 N, 140.016667 E, 25.VIII.1992, Higuchi (VGBI); Shikoku, North of Asemi, Motoyma-cho, Nagaoka-gun, Kochi Pref. NI-53-28-6 (Motoyama). Forming a carpet at the magrnal slope of Chamaecyparis forest with Quercus glauca, Sasa borealis, Rhododendron decandrum, Plagiogyra euphlebia, Lycopodium clavatum, Hydragea scandens and Deutzia scabra, 33.75 N, 133.75 E, ca 170 m, 15.V.1953, Suzuki (LE as S. palustre); The same region, Ryujindaira-moor, Mt. Saragamine, Kuma-cho, Kamiukena-gun, Ehime Pref. NI-53-34-2 (Matsuyama-nabu), Forming cusion in the center of a fen characterized by Sasa and Hosta, accompanied by Solidago virgaurea, Scirpus wichurae, Juncus efusus, Eleocharis sp., Allium thunbergii, Ixeris sp. and Lysimachia fortunei, 33.72 N, 133.9 E, ca 1170 m, 2.IV.1952, Suzuki (LE as S. palustre).

## Specimens used for morphometics

Sphagnum "henryense": Russia, Sakhalin Province, Kunashir Island, Mamontov 814-1-s10 (MHA9121113); The same region, Kuril State Nature Reserve, 23.IX.2020, Mamontov 814-1-s35 (MHA9121146): Japan. Honshu. Akita Pref., 25. VIII.1992, Higuchi (VGBI); Shikoku, Kochi Pref. 15. V.1953, Suzuki (LE, as S. palustre); The same region, Ehime Pref., 2.IV.1952, Suzuki (LE, as S. palustre). Canada, British Columbia, Queen Charlotte Islands, Moresby Island, 1.VII.1975, Vitt & Andrus 158 (LE); Graham Island, 26.VI.1966, Schofield, 30105 (LE, as S. palustre); the same place 21.VI.1966, Schofield 29826 (LE, as S. palustre); Vancouver Island, 2.V.1965., Schofield 26519 (LE, as S. palustre); Calvert Island, 11.IV.1970, Schofield 40868 (LE, as S. palustre); Moresby Island, 24.VII.1969, Schofield & Krajina 39505 (LE, as S. palustre); The same place, 6.VI.1966, Schofield 30895 (LE, as S. palustre); Hippa Island, 24. VI. 1967, Schofield 33910 (LE, as S. palustre); USA, Tennessee, Sevier Co., 30.VI.1970, Anderson 20812 (LE, as S. palustre); Connecticut, New London Co., VI-X.1946, Holdridge (LE, as S. palustre); Pennsylvania, Susquehanna, Choconut, 30.VI.1981, Andrus 5562 (MHA9059763); North Carolina, Bladen Co., 7.VII.1992, Tan 93-104 (MHA9059764); North Carolina, Caerteret, 22.II.1952, Blomquist & Anderson 15394 (LE); Jackson Co., 11.V.1973, Anderson 21347 (LE, as S. palustre); Florida, Leon Co., Tallahassee, III.1963, Ruth & Breen (LE, as S. palustre).

Sphagnum palustre s.str.: Portugal, Azores, 26.III.2007, Muñoz (MHA9062312); The same place, 1.IV.2007, Muñoz 40554 (MHA9062316): UK. North Ireland, Craigavon District, 27.VIII.1991, Seregin M-298 (MW9013677); Spain, Guadalajara, 4.XI.2005, Cezon 32317 (MHA9062317); Germany, Brandenburg, Spandau, 26.VII.1902, Prager (MW9013695); The same place, 28.VIII.1902, Prager (MW9013692); Poland, Krakow-Czestochowa Upland, 23.VII.1985, Bendnarek (MHA9059827); Slask Dolny, 20.VII.1972, Berdowski 1077 (MW9013678); Czech Republic, Karlovy Vary, 8.IV.1954, Tyuremnov (MW9013676); Bohmen, 27.VIII.1904, Schiffner (MW9013696); Lithuania, VII.1950, Tyuremnov (MHA9059825): Belarus, Grodno, 13.10.2006, Seregin M-1857 (MHA9059824); Russia, Arkhangelsk, Plesetsky District, 23.VI.2000, Churakova 787 (MW9065382); North Caucasus, Teberda Nature Reserve, 5.VIII.1986, Ignatova (MHA9114614); Kunashir Island, 6.VIII.2015, Koroteeva 15-6/1-2 (MHA9015882); Japan, Honsu, Fukui-ken, 16.IX.1976, Sakai 31 (MHA9059832); Kyyushu, Miyazaki-ken, 29.XI.1962, Mizutani 1394 (IRK); Japan, Shiodani, Hirose, 31.VII.1958, Nishida 897 (IRK); Canada, British Columbia, Vancouver Island, 4.V.1961, Schofield 13720 (LE); Graham Island, 25.VI.1967, Schofield 34152 (LE); USA, Michigan, Oakland Co., 19.X. 1965, Schnooberger 12641 (LE); New York, 10.VII.1894, Schrenk (IRK).

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#### LITERATURE CITED

- ALLEN, B. 2014. Maine mosses: Drummondiaceae-Polytrichaceae. Memoirs of The New York Botanical Garden 111: I-XVI.
- ANDERSON, L.E. & K. AMMANN. 1991. Cell wall ornamentation in the hyaline cells of *Sphagnum. – The Journal of the Hattori Botanical Laboratory* 69: 49–63.
- ANDERSON, L.E., A.J. SHAW & B. SHAW. 2009. Peat Mosses of the Southeastern United States. – Memoirs of The New York Botanical Garden, Volume 102. The New York Botanical Garden Press, Bronx, NY. 111 pp.
- ANDREWS, A.L. 1913. Sphagnales. North American Flora 15: 3-31.
- ANDRUS, R.E. 1980. A large range extension for Sphagnum henryense. – The Bryologist 83(2): 239–242.
- CRUM, H.A. 1984. Sphagnopsida, Sphagnaceae. North American Flora, series II, part 11. NY Bot. Gard., New York.
- DUFFY, A.M., B. AGUERO, H.K. STENØIEN, K.I. FLATBERG, M.S. IGNATOV, K. HASSEL & A.J. SHAW. 2020. Phylogenetic structure in the *Sphagnum recurvum* complex (Bryophyta) in relation to taxonomy and geography. – *American Journal of Botany* 107(9): 1283– 1295.
- HAMMER, O., D.A.T. HARPER & P.D. RYAN. 2001. PAST: Paleontological Statistic software package for education and data analysis. – *Palaeontologia Electronica* 4(1): 9 pp.

- HASSEL, K., M.O. KYRKJEEIDE, N. YOUSEFI, T. PRESTØ, H.K. STENØIEN, J.A. SHAW & K.I. FLATBERG. 2018. Sphagnum divinum (sp. nov.) and S. medium Limpr. and their relationship to S. magellanicum Brid. – Journal of Bryology 40(3): 197–222.
- KARLIN, E.F., & R.E. ANDRUS. 1988. The Sphagnum species of New Jersey. – Bulletin of the Torrey Botanical Club 168–195.
- KARLIN, E.F., M.M. GIUSTI, R.A. LAKE, S.B. BOLES & A.J. SHAW. 2010. Microsatellite analysis of *Sphagnum centrale*, *S. henryense*, and *S. palustre* (Sphagnaceae). – *The Bryologist* 113(1): 90–98.
- MCQUEEN, C.B. & R.E. ANDRUS. 2007. Sphagnaceae Dumortier. Flora of North America 27: 45–101.
- MELESHKO, O., H.K. STENØIEN, J.D. SPEED, K.I. FLATBERG, M.O. KYRKJEEIDE & K. HASSEL. 2018. Is interspecific gene flow and speciation in peatmosses (*Sphagnum*) constrained by phylogenetic relationship and life-history traits? – *Lindbergia* 41(1).
- MELESHKO, O., M.D. MARTIN, T.S. KORNELIUSSEN, C. SCHRÖCK, P. LAMKOWSKI, J. SCHMUTZ, A. HEALEY, A. PIAT-KOWSKI, J.A. SHAW, D.J. WESTON, K.I. FLATBERG, P. SZÖVÉNY, K. HASSEL & H.K. STENØIEN. 2021. Extensive genomewide phylogenetic discordance is due to incomplete lineage sorting and not ongoing introgression in a rapidly radiated bryophyte genus. – *Molecular biology and evolution* 38(7): 2750–2766.
- NIETO-LUGILDE, M., S. ROBINSON, B. AGUERO, A. DUFFY, K. IMWATTANA, K. HASSEL, K.I. FLATBRG, H.K. STENØIEN, A.V. SHKURKO, V.E. FEDOSOV & A.J. SHAW. 2022. Morphological-molecular incongruence in *Sphagnum majus* ssp. *majus* and ssp. *norvegicum. – The Bryologist* **125**(2): 294–310.
- RICCA, M. & A.J. SHAW. 2010. Allopolyploidy and homoploid hybridization in the Sphagnum subsecundum complex (Sphagnaceae: Bryophyta). – Biological journal of the Linnean society, 99(1): 135-151.
- SÍSTAD, S.M., H.K. STENØIEN & K.I. FLATBERG. 1999. Species delimitation and relationships of the Sphagnum recurvum complex (Bryo-

phyta) – as revealed by isozyme and RAPD markers. – *Systematic bot*any **24**(1): 95–107.

- SHAW, A. J. & B. GOFFINET. 2000. Molecular evidence of reticulate evolution in the peatmosses (*Sphagnum*), including *S. ehyalinum* sp. nov. – *The Bryologist* **103**(2): 357–374.
- SHAW, A.J., C.J. COX & S.B. BOLES. 2005. Phylogeny, species delimitation, and recombination in *Sphagnum* section Acutifolia. – *Systematic botany* **30**(1): 16–33.
- SHAW, A.J., K.I. FLATBERG, P. SZÖVÉNYI, M. RICCA, M.G. JOHNSON, H.K. STENØIEN & B. SHAW. 2012. Systematics of the *Sphagnum fimbriatum* complex: phylogenetic relationships, morphological variation, and allopolyploidy. – *Systematic Botany* 37(1): 15– 30.
- SHAW, A.J., B. PIATKOWSKI, A.M. DUFFY, B. AGUERO, K. IMWAT-TANA, M. NIETO-LUGILDE, A. HEALEY, D.J. WESTON, M.N. PATEL, J. SCHMUTZ, J. GRIMWOOD, J.B. YAVITT, K. HASSEL, H.K. STENØIEN, K.I. FLATBERG, C.P. BICKFORD & K.A. HICKS. 2022. Phylogenomic structure and speciation in an emerging model: the Sphagnum magellanicum complex (Bryophyta). – New Phytologist 326(4): 1497–1511.
- STENØIEN, H., S. BAKKEN & K.I. FLATBERG. 1997. Phenotypic variation in the Sphagnum recurvum complex: a cultivation experiment. – Journal of Bryology 19(4): 731–750.
- YOUSEFI, N., E. MIKULÁŠKOVÁ, H.K. STENØIEN, K.I. FLATBERG, A. KOŠUTHOVÁ, M. HÁJEK & K. HASSEL. 2019. Genetic and morphological variation in the circumpolar distribution range of *Sphag-num warnstorfii*: indications of vicariant divergence in a common peatmoss. – *Botanical Journal of the Linnean Society* 189(4): 408–423.

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