# The identification and distribution of *Sphagnum balticum* (Russow) C.E.O. Jensen in Britain

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## Abstract

Introduction: The aim of this study is to help clarify the identification of *Sphagnum balticum* in Britain, to review its distribution and to investigate the possible presence of hybrids between it and either *S. cuspidatum* or *S. fallax*.

Methods: All sites from where *S. balticum* has been recorded recently in Britain were visited in 2020, and a search was undertaken for it and other species with which it could be confused, plus possible hybrids. Samples were collected when suitable material was found. DNA was extracted from 31 shoots each representing one field sample. Fifteen microsatellites that have been developed for *Sphagnum* species were amplified and genotyped. The genetic structure of the data was investigated using principal coordinate analysis and cluster analysis.

Results: The genetic analysis supports the recognition of four taxa, which corresponds to four morphologically identified taxa, comprising *S. angustifolium, S. balticum, S. cuspidatum* and *S. fallax*. There is no evidence of hybrids. Over-recording of *S. balticum* has occurred in Britain due to confusion with some forms of *S. cuspidatum* and *S. fallax*, which can exhibit some morphological characters usually used to identify *S. balticum*. An illustrated identification key is provided to help solve this problem.

Conclusions: *Sphagnum balticum* is a very rare species in Britain that has undergone a decline due to habitat destruction and alteration. It is presently known to survive at only three sites, of which two are within protected areas.

Keywords: *Sphagnum fallax, Sphagnum cuspidatum*, microsatellites.



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## Introduction

*Sphagnum balticum* (Russow) C.E.O. Jensen exhibits a broad Boreo-arctic Montane Circumpolar range, mainly occurring in the tundra and northern boreal zone, and widespread in continental interiors (Blockeel et al. 2014; Hill and Preston 1998). Located towards the southern edge of its distribution, it is very rare in Britain and is a high-profile species of conservation concern, being specially protected under Schedule 8 of The Wildlife and Countryside Act 1981 (as amended) and the subject of a national conservation action plan. It is also a designated feature of some of the Sites of Special Scientific Interest (SSSI) where it occurs, requiring conservation agencies to maintain its population at these sites in favourable condition.

Hill (2004), which is the main reference used for *Sphagnum* identification in Britain, makes the following note about *S. balticum*: “Superficially like a strongly coloured, slender form of *S. fallax*. Useful field characters are the ±spreading stem leaves and the smaller number of branches per fascicle. Most British plants have the branches regularly in fascicles of 3, but plants from one site had branches mainly in fascicles of 4.” Identification keys in modern guides that include *S. balticum* have highlighted a wide variety of morphological features important for its identification, sometimes conflicting. The following are generally considered to be the most important features (Daniels and Eddy 1985; Flatberg 2013; Hill 2004; Hölzer 2010; Isoviita 1966; McQueen and Andrus 2007; Laine et al. 2018; Lönnell et al. 2019; Michaelis 2019): (i) stem cortex distinct from the cylinder; (ii) fascicles comprising two spreading branches and one pendent branch; (iii) stem leaves spreading at right angles from the stem; and (iv) stem leaves with fibrillose hyalocysts. All of these features are exhibited by the recently designated lectotype of *S. balticum* (Callaghan and Brinda 2022). Nevertheless, identification of *S. balticum* has sometimes proved problematic in Britain. Unusual morphotypes have been noted (Hill 2004; Maass 1965), and the occurrence of plants that appear to be intermediate with *S. fallax* has caused problems when undertaking monitoring, leading to uncertainty about the population status of *S. balticum* at sites and suggestions that hybrids between these two species may be present (Hodgetts 2006, 2008, 2011; O’Reilly 2012, 2018; Turner 2000, 2002a,b). Confusion with some forms of *S. cuspidatum* is also known to have caused recent identification mistakes in Britain (personal observation), and confusion with *S. angustifolium* has also been highlighted (Laine et al. 2018).

Cross-breeding between *S. balticum* and either *S. angustifolium*, *S. cuspidatum* or *S. fallax* has not been documented, but molecular evidence shows hybridization occurs frequently within *Sphagnum* and that allopolyploid species are common (Flatberg et al. 2006; Karlin et al. 2009, 2010; Meleshko et al. 2018; Natcheva and Cronberg 2007; Ricca et al. 2011; Ricca



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and Shaw 2010; Shaw et al. 2005, 2012; Shaw and Goffinet 2000). For example, *S. troendelagicum* is of allopolyploid origin, arising from hybridization between female *S. tenellum* and male *S. balticum* (Såstad et al. 2001; Stenøien et al. 2011). Likewise, *S. jensenii* appears to be of allopolyploid origin, with *S. annulatum* and *S. balticum* as progenitors (Såstad et al. 1999).

The aim of the present study is to help clarify the identification of *S. balticum* in Britain, to review its occurrence at sites from where it has been recorded, and to investigate the possible presence of hybrids between it and either *S. angustifolium*, *S. cuspidatum* or *S. fallax*.

## Methods

### *Taxonomy*

Taxonomy follows Blockeel et al. (2021).

### *Field sampling*

All sites from where *S. balticum* has been recorded relatively recently in Britain (*n* = 6) were visited during July – August 2020, including Abernethy Forest (v.-c. 96, Easterness), Black Burn (v.-c. 92, South Aberdeenshire), Cors Caron (v.-c. 46, Cardiganshire), Cors Craig y Bwlch (v.-c. 46), Muckle Moss (v.-c. 67, South Northumberland) and Thorne Moors (v.-c. 63, South-west Yorkshire). A search was undertaken at each site for *S. balticum* and other species with which it could be confused, plus possible hybrids. *Sphagnum balticum* was provisionally determined in the field when plants were found that had fascicles comprising two spreading branches and one pendent branch, plus stem leaves spreading at right angles from the stem. Samples of such plants were collected, checked microscopically and named as *S. balticum* if they also possessed a stem cortex distinct from the cylinder and stem leaves with fibrillose hyalocysts, which amounted to eleven samples. A further 18 samples were collected of possible confusion species, plus seemingly intermediate plants. Two recent collections made by D.K. Reed that had been identified as *S. balticum* from Cors Caron were also included in the study.

### *Genetic analysis*

Details of the 31 field samples included in the genetic analysis are provided in Table 1. DNA was extracted from a single shoot from each sample, using the middle part of the capitulum. We used the NucleoSpin Plant II ‘mini kit for DNA from plants’ from Macherey-Nagel, following the manufactures protocol. Fifteen microsatellites (1, 7, 9, 12, 17, 19, 20, 22, 29,



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30, 56, 65, 68, 78, and 93) that are developed for *Sphagnum* species (Shaw et al. 2008, Stenøien et al. 2011, Shaw et al. 2013) were amplified in four multiplex reactions (Qiagen Multiplex PCR Kit; Qiagen) and genotyped using GENEMAPPER® software (Applied Biosystems). For details regarding multiplex reactions and thermocycling regimes, see Kyrkjeeide et al. (2016).

The genetic structure of the data was investigated using principal coordinate analysis (PCoA) in GenAlEx 6.503 (Peakall and Smouse 2012) and clustering analysis in Structure v.2.3.4 (Falush et al. 2007; Pritchard et al. 2000). The latter was used to explore if the number of genetic groups identified corresponded to the morphologically defined taxa and if there were any admixture between these taxa. We ran the analyses using K = 2–4, with an expectation that the number of genetic clusters equals the four morphologically identified species (*sensu* Meirmans 2015; van Hengstum et al. 2012). The admixture (α = 1/4, Wang 2017) and correlated allele frequency models were applied, with ten replicates per number of *k*, each with 500,000 iterations and a burn-in of 100,000, without specifying any a priori population membership information. The results from Structure were visualised using StructureSelector including Clumpak (Li and Liu 2018).

*Identification*

Informed by the results of the genetic analysis, the morphology and identification of the specimens was reviewed, and an illustrated key was compiled to allow for the discrimination of *S. balticum* and similar forms of *S. cuspidatum* and *S. fallax* in Britain. *Sphagnum angustifolium* was excluded from this key because the sample size in this study was small (*n* = 1) and it appears to be simple to discriminate from *S. balticum* using established criteria (Hill 2004).

*Distribution*

Vouchers labelled as *S. balticum* in BBSUK, BM, MANCH, NMW and various private herbaria were reviewed and an updated distribution map of the species in Britain was compiled.

## Results

*Genetic analysis*

All 31 samples were genotyped for all microsatellites, except *S. fallax* that did not have any alleles in microsatellite 9, seemingly lacking this locus. Otherwise, data was only missing at four loci in three specimens. The samples missing two alleles were excluded from the PCoA



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analysis of all four taxa. The PCoA shows that *S. fallax* is well separated from *S. angustifolium, S. balticum* and *S. cuspidatum* (Figure 1A). One sample identified as *S. cuspidatum* is separated from the other samples at the *y*-axis. This is likely caused by one missing allele. A PCoA excluding *S. fallax* shows that the three other taxa are separated from each other, and whilst *Sphagnum angustifolium* clusters close to *S*. *cuspidatum*, it is only represented by a single specimen (Figure 1B). Like the PCoA, the clustering analysis supports four taxa, with one genetic group identified for each morphologically identified taxon.

*Identification*

The morphological review, informed by the genetic analysis, showed that some terrestrial forms of *S. cuspidatum* and some reduced forms of *S. fallax* can be misidentified as *S. balticum* because they can have a combination of characters usually considered to be characteristic of *S. balticum*, including (i) stem cortex distinct from the cylinder; (ii) fascicles comprising two spreading branches and one pendent branch; (iii) stem leaves spreading at right angles from the stem; and (iv) stem leaves with fibrillose hyalocysts. No other species which could be confused easily with *S. balticum* were found at the sites surveyed. The following provides a key for the discrimination of *S. balticum* from such confusing forms of *S. cuspidatum* and *S. fallax*.

1 Leaves near tips of spreading branches linear lanceolate and mostly >1.5 mm long (Figure 2C).....*S. cuspidatum*

Leaves near tips of spreading branches ovate and mostly <1.5 mm long (Figure 2A–B) ..... 2

2 When compressed beneath a microscope coverslip, tips of most stem leaves with a sharp mucro (Figure 3A) .....*S. fallax*

When compressed beneath a microscope coverslip, tips of stem leaves without a sharp mucro (Figure 3B) .....*S. balticum*

*Distribution*

The review of herbarium material revealed some errors in the identification of *S. balticum* in Britain, involving *S. cuspidatum* from Cors Caron (Cardiganshire; D.K. Reed priv. herb.!), *S. fallax* from Carrington Moss (v.-c. 58, Cheshire; MANCH EM668947! and EM668946!) and Cors Craig y Bwlch (Cardiganshire; NMW C.2011.013.2!), and *S. obtusum* from Netherton (v.-c. 59, South Lancashire; NMW 25.152.14848! and 15.54.78!). Based on the review of



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specimens and fieldwork undertaken in 2020, Figure 4 shows occupancy of Ordnance Survey 10 km grid cells by *S. balticum* in Britain. It has been known from seven sites but has been lost from four due to habitat loss and degradation. It presently survives at three sites, in a small mire at the edge of Abernethy Forest (Easterness), in a mire at the head of Black Burn (South Aberdeenshire) and at Muckle Moss (South Northumberland).

## Discussion

No evidence for the occurrence of hybrids between *Sphagnum balticum* and related species was found during this study. The genetic results agree with recent findings (Shaw et al. 2016, Duffy et al. 2020). Shaw et al. (2016) showed that *S. balticum, S. fallax, S. angustifolium* and *S. cuspidatum* are clearly separated taxonomic entities. Even though hybridization and allopolyploidization are common among species of *Sphagnum* (see Meleshko et al. 2018 for a review), we did not find any sign of admixture in our sampled plants. Likewise, Duffy et al. (2020) were not able to detect significant admixture among *S. balticum, S. fallax* and *S. angustifolium* using genomic data. The same study identified two major genetic clades within the *S. recurvum* complex, and refer to one group, including *S. balticum* and *S. angustifolium,* as having rounded stem leaves and another group, including *S. fallax,* as having pointed stem leaves (Duffy et al. 2020).

The results of this study show that over-recording of *S. balticum* has occurred in Britain due in particular to confusion with some forms of *S. cuspidatum* and *S. fallax*. Similarly, a voucher from East Sutherland (v.-c. 107) of *S. balticum*, which was verified by E.M. Lobley and included in Warburg (1963), was subsequently redetermined as *S. fallax* by Maass (1965). The illustrated key provided by this study should help to alleviate the problem. *Sphagnum balticum* is a very rare species in Britain and has undergone a decline due to habitat destruction and alteration. It is presently known to survive at only three sites. Two of these sites, at Abernethy Forest and Muckle Moss, are within protected areas, where the species receives specific conservation attention. The third site, a mire at the head of Black Burn, has no statutory conservation protection and the *S. balticum* population receives no conservation attention. There is a clear need to safe-guard the Black Burn population, and to undertake surveys of other potential sites to try to locate any further populations that may exist. Significant habitat restoration measures have recently been undertaken at two of the sites where *S. balticum* once occurred, at Cors Caron and Thorne Moors. The potential reintroduction of the species to these sites should be assessed if favourable habitat for the species ultimately redevelops. Populations at both Abernethy Forest and Muckle Moss are reasonably large and could potentially provide source material for trial conservation



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translocations, as has begun recently in England in efforts to conserve another nationally rare boreal peatland moss, *Dicranum undulatum* (Callaghan 2021).

Efforts to assess, monitor and conserve threatened species fundamentally depend on the accurate determination of species. Even in a region such as Britain, which has a long history of bryological study, much work remains to be done to better understand the relationships between similar species and their identification characters. For example, eleven species will be categorised at 'Data Deficient (Taxonomic Uncertainty)' in the forthcoming new IUCN Red List of the bryophytes of Britain because data are so uncertain that both Critically Endangered and Least Concern are plausible categories, and this is mainly because of taxonomic uncertainty (Callaghan Forthcoming). Integrative taxonomic studies, such as undertaken here, are crucial to resolving this problem.

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Figure captions

Figure 1. Principal coordinate analysis of four (A) and three (B) *Sphagnum* species based on microsatellite data. *Sphagnum angustifolium, S. balticum,* and *S. cuspidatum* overlap when analysed together with *S. fallax* (A), but *S. balticum,* and *S. cuspidatum* are well separated when *S. fallax* is excluded from the analysis (B). *Sphagnum angustifolium* is also separated from the rest in (B), but the species is only presented by one sample.

Figure 2. Tips of spreading branches of (A) *Sphagnum fallax*, (B) *S. balticum* and (C) *S. cuspidatum*. Scale bar = 1 mm. Photos: D.A. Callaghan.

Figure 3. Stem leaf tips (convex side) compressed beneath microscope coverslip of (A) *Sphagnum fallax*, (B) *S. balticum* and (C) *S. cuspidatum*. Scale bar = 0.2 mm. Photos: D.A. Callaghan.

Figure 4. Occupancy of Ordnance Survey 10 km grid cells by *Sphagnum balticum* in Britain.



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**Table 1. Details of specimens included within the genetic analysis. D.K. Reed collected the samples in 2019 and D.A. Callaghan collected the samples in 2020.**

| Species | Site | Region | GR | Date |
|---|---|---|---|---|
| *Sphagnum balticum* | Abernethy Forest | Easterness | NJ0053612784 | 07/08/2020 |
| *Sphagnum fallax* | Abernethy Forest | Easterness | NJ0054712698 | 07/08/2020 |
| *Sphagnum cuspidatum* | Abernethy Forest | Easterness | NJ0052112763 | 07/08/2020 |
| *Sphagnum cuspidatum* | Black Burn, head of | South Aberdeenshire | NO2759481442 | 06/08/2020 |
| *Sphagnum fallax* | Black Burn, head of | South Aberdeenshire | NO2758781213 | 06/08/2020 |
| *Sphagnum angustifolium* | Black Burn, head of | South Aberdeenshire | NO2767781148 | 06/08/2020 |
| *Sphagnum balticum* | Black Burn, head of | South Aberdeenshire | NO2756381330 | 06/08/2020 |
| *Sphagnum balticum* | Black Burn, head of | South Aberdeenshire | NO2756381330 | 06/08/2020 |
| *Sphagnum fallax* | Cors Caron | Cardiganshire | SN6989263997 | 21/07/2020 |
| *Sphagnum cuspidatum* | Cors Caron | Cardiganshire | SN6987064008 | 21/07/2020 |
| *Sphagnum cuspidatum* | Cors Caron | Cardiganshire | SN6961963573 | 21/07/2020 |
| *Sphagnum cuspidatum* | Cors Caron | Cardiganshire | SN6872261980 | 21/07/2020 |
| *Sphagnum fallax* | Cors Caron | Cardiganshire | SN6987064008 | 21/07/2020 |
| *Sphagnum cuspidatum* | Cors Caron | Cardiganshire | SN6961463581 | 08/11/2019 |
| *Sphagnum cuspidatum* | Cors Caron | Cardiganshire | SN6823061934 | 09/10/2019 |
| *Sphagnum fallax* | Cors Craig y Bwlch | Cardiganshire | SN7136069768 | 24/07/2020 |
| *Sphagnum fallax* | Cors Craig y Bwlch | Cardiganshire | SN7136069768 | 24/07/2020 |
| *Sphagnum fallax* | Cors Craig y Bwlch | Cardiganshire | SN7136069768 | 24/07/2020 |
| *Sphagnum cuspidatum* | Cors Craig y Bwlch | Cardiganshire | SN7136069768 | 24/07/2020 |
| *Sphagnum fallax* | Muckle Moss | South Northumberland | NY8030767149 | 05/08/2020 |
| *Sphagnum fallax* | Muckle Moss | South Northumberland | NY7996467065 | 05/08/2020 |
| *Sphagnum fallax* | Muckle Moss | South Northumberland | NY7996467065 | 05/08/2020 |
| *Sphagnum cuspidatum* | Muckle Moss | South Northumberland | NY8020867108 | 05/08/2020 |
| *Sphagnum fallax* | Muckle Moss | South Northumberland | NY8016967083 | 05/08/2020 |
| *Sphagnum fallax* | Muckle Moss | South Northumberland | NY8030167133 | 05/08/2020 |
| *Sphagnum balticum* | Muckle Moss | South Northumberland | NY7998467011 | 05/08/2020 |
| *Sphagnum fallax* | Thorne Moor | South-west Yorkshire | SE7190415811 | 03/08/2020 |
| *Sphagnum fallax* | Thorne Moor | South-west Yorkshire | SE7245215603 | 03/08/2020 |
| *Sphagnum fallax* | Thorne Moor | South-west Yorkshire | SE7245215603 | 03/08/2020 |
| *Sphagnum fallax* | Thorne Moor | South-west Yorkshire | SE7190215814 | 03/08/2020 |
| *Sphagnum cuspidatum* | Thorne Moor | South-west Yorkshire | SE7240715633 | 03/08/2020 |



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