New Phytologist

Research

Axenic *in vitro* cultivation of 19 peat moss (*Sphagnum* L.) species as a resource for basic biology, biotechnology, and paludiculture

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Received: *23 April 2020*
Accepted: *27 August 2020*

New Phytologist (2020)
doi: 10.1111/nph.16922

Key words: cell cycle arrest, climate change, DNA barcoding, genome size, peat moss, peatland restoration, *Sphagnum* farming, *Sphagnum magellanicum*.

Summary

- Sphagnum farming can substitute peat with renewable biomass and thus help mitigate climate change. Large volumes of the required founder material can only be supplied sustainably by axenic cultivation in bioreactors.
- We established axenic *in vitro* cultures from sporophytes of 19 *Sphagnum* species collected in Austria, Germany, Latvia, the Netherlands, Russia, and Sweden: *S. angustifolium*, *S. balticum*, *S. capillifolium*, *S. centrale*, *S. compactum*, *S. cuspidatum*, *S. fallax*, *S. fimbriatum*, *S. fuscum*, *S. lindbergii*, *S. medium/divinum*, *S. palustre*, *S. papillosum*, *S. rubellum*, *S. russowii*, *S. squarrosum*, *S. subnitens*, *S. subfulvum* and *S. warnstorfii*. These species cover five of the six European *Sphagnum* subgenera; namely, *Acutifolia*, *Cuspidata*, *Rigida*, *Sphagnum* and *Squarrosa*.
- Their growth was measured in suspension cultures, whereas their ploidy was determined by flow cytometry and compared with the genome size of *Physcomitrella patens*. We identified haploid and diploid *Sphagnum* species, found that their cells are predominantly arrested in the G1 phase of the cell cycle, and did not find a correlation between plant productivity and ploidy. DNA barcoding was achieved by sequencing introns of the *BRK1* genes.
- With this collection, high-quality founder material for diverse large-scale applications, but also for basic *Sphagnum* research, is available from the International Moss Stock Center.

Introduction

Peatlands cover $> 4 \times 10^6$ km^2, comprising 3% of Earth's land and freshwater surface (Joosten & Clarke, 2002), and contain about 30% of the global soil carbon (C) (Gorham, 1991; Frolking & Roulet, 2007). Most peatlands in temperate and boreal zones were formed and are dominated by peat mosses; that is, mosses of the genus *Sphagnum* (Clymo & Hayward, 1982; Joosten *et al.*, 2017). They accumulate dead organic matter ('peat') under wet, anoxic, acidic, and nutrient-poor conditions, consequently lowering microbial activity and reducing the decay of organic matter. As a result, pristine peatlands are C sinks; that is, they sequester more C than they emit and function as long-term C stores (Clymo & Hayward, 1982; Joosten *et al.*, 2016). Climate-change scenarios assume that prolonged droughts, elevated temperatures, and increased nitrogen (N) deposition (Galloway *et al.*, 2008) decrease the growth of *Sphagnum* mosses and increase decay, thus reducing the amount of sequestered C (Limpens *et al.*, 2011; Norby *et al.*, 2019). Moreover, changing microbial communities might enhance the functional shift from sink to source (Lew *et al.*, 2019; Juan-Ovejero *et al.*, 2020; Rewcastle *et al.*, 2020). Together, the impact on global C cycling makes *Sphagnum* an important ecological model, attracting a growing number of scientists. Consequently, the first draft genome sequences became available recently (*Sphagnum fallax* v.1.1 and *Sphagnum magellanicum* v.1.1, http://phytozome.jgi.doe.gov/; Weston *et al.*, 2018). Although *Sphagnum* mosses are of growing economic importance for many applications, including wastewater treatment (Couillard, 1994), as sensors of air pollution (Capozzi *et al.*, 2016, 2017; Di Palma *et al.*, 2019; Aboal *et al.*, 2020), and as raw material for growing media (Wichmann *et al.*, 2020), they are not yet analysed in great detail.

The global area of peatlands has been reduced significantly (10–20%) since 1800, particularly by drainage for agriculture and forestry. Moreover, peat serves for energy generation and as a substrate for horticulture (Joosten & Clarke, 2002). Drainage leads to peat mineralization and subsequent emissions of greenhouse gases (GHGs), such as CO_2 and nitrous oxide (Van Den Pol-Van Dasselaar *et al.*, 1999; Boon *et al.*, 2014; Carlson *et al.*, 2017). Whereas drained peatlands cover only 0.4% of the land surface, they are responsible for 32% of cropland and almost 5% of anthropogenic GHG emissions globally (Joosten *et al.*, 2016;

Carlson *et al.*, 2017). Leifeld *et al.* (2019) estimated that in 1960 the global peatland biome turned from a net sink to a net source of soil-derived GHGs. Further, these researchers predict a cumulative emission from drained peatlands of 249 ± 38 Pg of CO_2 equivalent by 2100 if the current trend continues.

Rewetting of drained peatlands decreases these emissions and may even restore the C sink function (Joosten *et al.*, 2016; Wichtmann *et al.*, 2016). Rewetting, however, makes conventional drainage-based land use impossible (Wichmann *et al.*, 2017). Paludiculture, wet agriculture and forestry on peatlands, allows land use to continue and to combine emission reduction with biomass production. It includes traditional peatland cultivation (reed mowing, litter usage) and new approaches for utilization (Abel *et al.*, 2013; Wichtmann *et al.*, 2016).

Sphagnum farming on rewetted bogs is a promising example of paludiculture as it produces *Sphagnum* biomass as a substitute for peat (Gaudig & Joosten, 2002; Gaudig *et al.*, 2014, 2018). It decreases GHG emissions substantially by rewetting drained peatlands, by avoiding the use and oxidation of fossil peat, and by preserving hitherto undrained peatlands as C stores and sinks (Wichtmann *et al.*, 2016; Günther *et al.*, 2017). Potential sites for Sphagnum farming are degraded bogs and acidic water bodies (Wichmann *et al.*, 2017).

Different environmental conditions (e.g. water level or nutrient supply) and different requirements of the produced biomass call for a variety of peat moss species and genotypes as founder material for Sphagnum farms (Gaudig *et al.*, 2018). Lack of sufficient founder material is currently a major bottleneck for the large-scale implementation of Sphagnum farming. In the EU, *Sphagnum* species and their habitats are protected by the Council Directive 92/43/EEC, constraining the collection of founder material from natural habitats. Furthermore, commercial Sphagnum farming requires *Sphagnum* material without unwanted biological contaminations (Gaudig *et al.*, 2018) and of a constitution that is fit for purpose. Moss clones established from a single spore or plant share the same genetic, physiological, and environmental background, allowing the multiplication of selected clones to achieve maximum yields. *Sphagnum* founder material of controlled quality can be produced under aseptic conditions with standard tissue culture methods (Caporn *et al.*, 2017), but probably more rapidly by axenic cultivation in bioreactors.

An important step towards the large-scale production of such founder material was the development of an axenic photobioreactor production process for *Sphagnum palustre*, using monoclonal material generated from a single spore (Beike *et al.*, 2015). Under standardized laboratory conditions, the multiplication rate of the material was up to 30-fold within 4 wk. Initial results with this clone indicated that it can grow in a natural habitat without an intermediate hardening step (Decker & Reski, 2020). Another improvement is the establishment of a protonema-proliferation protocol for *Sphagnum squarrosum* (Zhao *et al.*, 2019). Besides these two species, we found only one report of the establishment of *S. fallax* cultures in a bioreactor (Rudolph *et al.*, 1988). Hence, it remained unclear whether a broader species set can be established as axenic laboratory strains. Moreover, different *Sphagnum* species were described as having haploid, diploid, or even triploid gametophytes, but the evolutionary advantage of polyploidization remains unclear. One testable hypothesis is that polyploid species grow faster, as has been reported for angiosperms (Van Drunen & Husband, 2018; Walden *et al.*, 2020).

Here, we report on the establishment of axenic *in vitro* cultures of 19 *Sphagnum* species from five peat moss subgenera and compare their growth behaviour, their genome size, and their cell cycle. With this collection, high-quality founder material for diverse large-scale applications, but also for basic *Sphagnum* research, is available.

Materials and Methods

Decontamination of sporophytes and spore germination

We collected sporophytes from 19 *Sphagnum* species in the field (Table 1) and stored them at 4°C. The taxonomic status of the *Sphagnum medium/divinum* clones needs clarification to accommodate for taxonomic insights (Hassel *et al.*, 2018) after collection. Spore capsules were surface sterilized and opened with forceps in 1 ml of sodium hypochlorite (NaClO) solution. The solution was freshly prepared with autoclaved water with two drops of Tween 20 per 500 ml water and a final concentration of either 0.6, 1.2, or 2.4% NaClO. The incubation was stopped at different time points between 30 s and 7 min by transferring 100 µl of the suspension to 1 ml autoclaved water. From this dilution, 500 µl were transferred to a sterile Petri dish, which contained one of the following solid media: (1) Knop medium (1.84 mM potassium dihydrogen phosphate, 3.35 mM potassium chloride, 1.01 mM magnesium sulphate, 4.24 mM calcium nitrate, 45 µM iron(II) sulphate) according to Reski & Abel (1985) supplemented with microelements (ME; 50 µM boric acid, 50 µM manganese sulphate, 15 µM zinc sulphate, 2.5 µM potassium iodide, 500 nM sodium molybdate, 50 nM copper sulphate, 50 nM cobalt(II) nitrate) according to Schween *et al.* (2003a), or (2) Sphagnum medium (Knop medium with ME, 0.3% sucrose, and 1.25 mM ammonium nitrate (NH_4NO_3)) according to Beike *et al.* (2015). Petri dishes were sealed with Parafilm (Carl Roth, Karlsruhe, Germany) and cultivated under standard growth conditions: climate chamber, temperature of 22°C, photoperiod regime of 16 h : 8 h, light : dark, and light intensity of 70 ± 5 µmol m^{-2} s^{-1} provided by fluorescent tubes. Light intensities were measured with a planar quantum sensor (Li-Cor 250; Li-Cor Biosciences, Bad Homburg, Germany).

After spore germination, single thalloid protonemata were separated and transferred to new Petri dishes containing either solid Knop ME or solid Sphagnum medium under sterile conditions using needles and a stereomicroscope (Stemi 2000-C; Zeiss, Jena, Germany). Plates contained one of the following media to serve as controls: (1) Knop ME supplemented with 1% glucose and 12 g l^{-1} purified agar (Oxoid Ltd, Basingstoke, UK); (2) LB (10 g l^{-1} Bacto Tryptone (Becton, Dickinson & Co., Franklin Lakes, NJ, USA), 10 g l^{-1} sodium chloride (NaCl), 5 g l^{-1} Bacto Yeast Extract (Becton, Dickinson & Co.) and 15 g l^{-1} Bacto Agar (Becton, Dickinson & Co.)); or (3) tryptic soy agar with 1%

Table 1 *Sphagnum* spp. clones in axenic culture with corresponding International Moss Stock Center (IMSC) numbers, date, and location of spore capsule collection.

			Origin of spore capsule	
Sphagnum sp.	IMSC no.	Six best clones	Date	Location
S. angustifolium	41114	2.1, 2.2, 2.3, **2.4**, 2.5, 7.1	2015–2007	Mālpils (LVA)
S. balticum	41118	1.1, **1.2**, 2.2, 3.3, 8.1, 9.5	2016–2008	Lapland (SWE)*
S. capillifolium	41126	1.2, 1.3, 1.5, **1.8**, 1.9, 1.49	2015–2007	Freiburg, Schauinsland (DEU)*
S. centrale	41129	1.2, 3.3, 6.4, 7.5	2016–2008	Siberia, Surgut Polesye (RUS)
	41134	9.6, **10.2**	2016–2007	Mālpils (LVA)
S. compactum	41137	3.1, 4.6, **5.1**, 5.3, 6.1, 6.2	2018–2006	Bargerveen (NLD)
S. cuspidatum	41146	1.1, 1.4, 3.3, 3.4, 5.1, **5.2**	2016–2007	*Gründlenried - Rötseemoos* (DEU)*
S. fallax	41151	2.1, 3.1, 4.1, 4.4, **4.5**, 4.6	2017–2006	Sphagnum farming pilot: Rastede (DEU) Origin: De Werribben (NLD)
S. fimbriatum	40069	**1.1**, 2.1	2012–2006	Store Mosse (SWE)
	41154	6.1, 6.2, 6.4, 6.5	2015–2007	Mālpils (LVA)
S. fuscum	41158	**1.1**, 2.1, 2.2, 2.3, 3.1, 3.2	2016–2008	Lapland (SWE)*
S. lindbergii	41167	2.1, 2.3, 3.1, **3.2**, 3.3	2016–2007	Lapland (SWE)*
S. medium/divinum	40066	3.1	2012–2007	Store Mosse (SWE)
	41169	4.1, 4.2, 4.3, 5.1, 5.2	2016–2008	Siberia, Yugra (RUS)
S. palustre	40068	2a, **12a**	2012–2008	Lychen-Bohmshof (DEU)
	41175	4.2, 4.3, 5.1, 5.2	2017–2006	Sphagnum farming pilot: Rastede (DEU) Origin: De Werribben (NLD)
S. papillosum	41179	1.1, 2.2, 4.3	2016–2008	Siberia, Potanay Aapa mire (RUS)
	41183	5.2, **6.1**, 7.1	2017–2006	Sphagnum farming pilot: Rastede (DEU) Origin: Ramsloh (DEU)
S. rubellum	40067	**1.1**, 2.1	2012–2006	Store Mosse (SWE)
S. russowii	41191	1.1, 1.2, 3.1, 3.4, 3.5, **4.2**	2016–2008	Siberia, Chistoye Bog (RUS)
S. squarrosum	41193	2.1, **5.2**, 5.3, 6.1	2016–2007	*Gründlenried-Rötseemoos* (DEU)*
	41196	7.1	2017–2005	Buddenhagener Moor (DEU)
	41197	8.3	2017–2009	Steiermark (AUT)
S. subfulvum	41201	4.2, 4.3, 7.2, **7.4**, 8.1, 8.5	2016–2008	Lapland (SWE)*
S. subnitens	40070	1.1	2012–2006	Store Mosse (SWE)
S. warnstorfii	41208	1.3, 1.4, 2.1, 3.3, **5.2**, 5.4	2016–2008	Siberia, Rangetur floating mire (RUS)

The six best-growing clones are listed by the number of spore capsules and of the individual clone. Bold numbers indicate the best-growing clones. The origin of the spore capsule is indicated by date and location of collection. Spore capsules marked with an asterisk were provided by Michael Lüth; all others were provided by the authors. AUT, Austria; DEU, Germany; LVA, Latvia; NLD, the Netherlands; RUS, Russian Federation; SWE, Sweden.

glucose (15 g l^{-1} peptone from casein, 5 g l^{-1} soy peptone, 5 g l^{-1} NaCl) and 12 g l^{-1} purified agar (Oxoid Ltd). These plates were sealed with Parafilm and stored and inspected at room temperature. If no contamination occurred within 4 wk, we considered a culture as axenic. Thus, each line generated derives from a single spore. Clonal material from each line has been established by separating filaments.

In vitro cultivation

Gametophores were cultivated on solid media and in suspension. For cultivation on solid medium, gametophores were transferred to Knop ME or Sphagnum medium. The Petri dishes were sealed with Parafilm and cultivated under standard conditions.

For suspension cultures, gametophores were disrupted with forceps in laminar flow benches and transferred to 35 ml Sphagnum medium in 100 ml Erlenmeyer flasks. Flasks were closed with Silicosen® silicone sponge plugs (Hirschmann Laborgeräte, Eberstadt, Germany) and agitated on a rotary shaker at 120 rpm (B. Braun Biotech International, Melsungen, Germany) under standard conditions. We did not observe gametangia in these cultures.

Light microscopy

Gametophores were analysed with a stereo microscope (SZX7; Olympus Corp., Tokyo, Japan) and a camera (AxioCam ICc 1; Zeiss). Photographs were scaled with AxioVision 4.8 (Zeiss). Stacks of images with different focal points were combined with CombineZ 5.3 (Alan Hadley, https://combinezp.software.informer.com/).

Growth determination

Plant growth was determined on solid media as well as in suspension (Fig. 1). First, growth of up to 16 clones of each species was determined on agar plates with both Knop ME and Sphagnum medium. Clones were randomly selected from all available spore capsules. The uppermost 5 mm from the tip of each gametophore (the capitulum) was cut, transferred to solid media, and cultivated under standard conditions for 4 wk (Fig. 1). Growth was

documented photographically every week. The pictures were transferred into binary images and the area was assessed by counting the pixels (Fig. 1a) using ImageJ v.1.51f (Wayne Rasband, https://imagej.nih.gov/ij/). In addition to the area of growth, the height of the gametophore and the shape and colour were visually assessed to select the six largest clones of each species after 4 wk of growth. These were subsequently assessed for biomass increase in suspensions.

Three gametophores per clone were transferred to 50 ml Sphagnum medium in 100 ml Erlenmeyer flasks and cultivated under standard conditions for 6 wk. Subsequently, the total biomass was harvested by filtering with a Büchner funnel and a vacuum pump. The moss material was transferred to pre-dried (0.5 h at 105°C) aluminium weighing pans (Köhler Technische Produkte, Neulußheim, Germany) and dried for 2 h at 105°C. Subsequently, the DW was determined with an accuracy scale (CPA 3245; Sartorius, Göttingen, Germany) (Fig. 1b). The clone with the highest DW increase of each species was selected as the best-grown clone.

Flow cytometry

Ploidy levels of the six best-grown clones of each species were determined via flow cytometry (FCM). Gametophores were chopped with a razor blade in 0.5 ml 4′,6-diamidino-2-phenylindol (DAPI) solution (Carl Roth) containing 0.01 mg l^{-1} DAPI, 1.07 g l^{-1} magnesium chloride hexahydrate, 5 g l^{-1} NaCl, 21.11 g l^{-1} Tris, and 1 ml l^{-1} Triton X-100. Afterwards, 1.5 ml DAPI solution was added and the material then filtered through a 30 µm sieve and subsequently analysed with a Partec CyFlow® Space flow cytometer (Sysmex Partec, Görlitz, Germany), equipped with a 365 nm UV-LED. *Physcomitrella patens* protonema served as internal standard (modified after Schween *et al.*, 2003b).

Statistical analysis

To determine significance values between the growths of the clones, data were analysed by one-way ANOVA, followed by Fisher's protected least significant difference test, where ***, **, and * denote significance at the 0.1%, 1%, and 5% level, respectively. Statistical analyses were performed with GraphPad Prism®, and diagrams were created with Excel 2016.

DNA extraction

For genomic DNA extraction, gametophores from suspension cultures were vacuum filtrated for 1 min. Up to 100 mg of tissue was disrupted in a tissue lyser (MM 400; Retsch, Haan, Germany) followed by DNA extraction with the GeneJET™ Genomic DNA Purification Kit according to the manufacturer's protocol (Thermo Fisher Scientific, Waltham, MA, USA). The

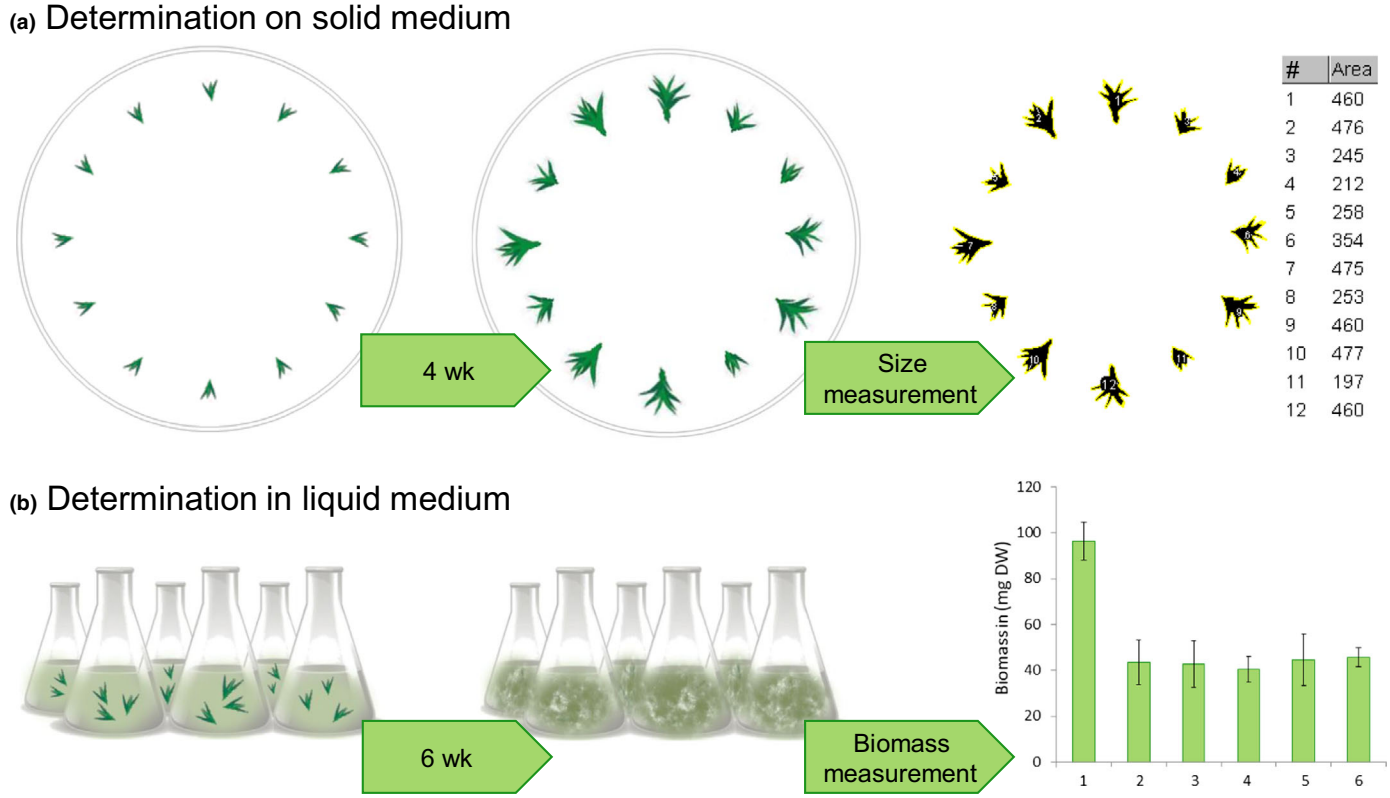

Fig. 1 Schematic representation of the growth determination of *Sphagnum* spp.: (a) on solid medium; (b) in suspension cultures. (a) Gametophores of the same size were transferred to a Petri dish and cultivated for 4 wk. The growth was documented photographically, and the size was analysed by image-processing-supported area measurement using ImageJ. (b) Gametophores were transferred to Erlenmeyer flasks and cultivated for 6 wk. Growth was determined by dry weight (DW) measurement of the biomass.

DNA was dissolved in 50 µl elution buffer, and the concentration was measured using a NanoDrop spectrophotometer (ND-1000; Thermo Fisher Scientific).

Amplification of *SpBRK1* from genomic DNA

For amplification of a part of the nuclear gene *BRK1* (homologue of Pp1s35_157V6.1; Pp3c8_2740V3.1; Lang *et al.*, 2018), the following intron-flanking primers were used: SpBRK1_fwd, TCAATGTGCGCCGTCTCTTTG; and SpBRK1_rev, GTGC TGAATTGGGCTTCCAAG (modified after Beike *et al.*, 2014). The amplification was performed by Phusion DNA polymerase (Thermo Fisher Scientific) or Q5® DNA polymerase (New England Biolabs, Ipswich, MA, USA)-based PCR according to the manufacturer's protocol in 50 µl reaction volume containing 50–250 ng genomic DNA. Expected product lengths were around 530 bp and 1350 bp.

Cloning of PCR products, sequencing, and sequence analysis

After DNA isolation with the GeneJET™ Gel Extraction Kit (Thermo Fisher Scientific) according to the manufacturer's protocol, PCR products were cloned into the vector pJET1.2/blunt using the CloneJET Molecular Cloning Kit (Thermo Fisher Scientific) according to the manufacturer's protocol. After *Escherichia coli* transformation, DNA was extracted using the GeneJET Plasmid Miniprep-Kit (Thermo Fisher Scientific) following the manufacturer's protocol. Sanger sequencing was done by GATC Biotech (Eurofins Genomics, Konstanz, Germany) using primers pJET-FP and pJET-RP.

The sequence chromatograms were analysed with the ChromasPro v.2.1.9 (http://www.technelysium.com.au/ChromasPro.html). Multiple sequence alignments were generated using Clustal Omega (Madeira *et al.*, 2019) and visualized with Jalview (Waterhouse *et al.*, 2009).

Results and Discussion

Induction of axenic *in vitro* cultures

Surface sterilization of spore capsules is an established method to start axenic *in vitro* cultivation (Beike *et al.*, 2015). *Sphagnum* spores are still viable after 13 yr when stored in the cold, and they can form persistent spore banks in nature (Sundberg & Rydin, 2000). We observed spore germination in *Sphagnum angustifolium* and *Sphagnum fimbriatum* after 3 yr storage at 4°C. However, we decontaminated most sporophytes within 2 months after collection. Detergent concentration and exposure time were adjusted individually for each sporophyte. We did not find a correlation between species or sporophyte maturation level and exposure time for successful decontamination and germination of spores. Sugar accelerated spore germination of all species except *Sphagnum compactum*, but all species except *Sphagnum warnstorfii* also germinated on Knop ME. Filaments developed from sterilized spores after 2–20 wk, with high variations within every species. Single gametophores were separated and subsequently cultivated on solid medium as independent clones.

Beike *et al.* (2015) showed that *in vitro* cultivated *S. palustre* plants and plants taken from natural habitats have similar phenotypic characteristics. However, in that study, gametophores grown *in vitro* were smaller and the shoots had more lanceolate leaves than the cucullate, ovate leaves of the thicker and heavier field shoots. We observed such deviating morphological characteristics for all 19 *Sphagnum* species cultivated *in vitro* (Fig. 2). Differences in spore germination, plant development, and plant morphology between axenic moss cultures and field-grown mosses may be due, besides obvious abiotic factors and speed of growth, to effects of the microbiome present only in the latter. Cross-kingdom and cross-clade signalling via small molecules can influence morphology of *Sphagnum* similar to *P. patens* (Kostka *et al.*, 2016; Decker *et al.*, 2017; Vesty *et al.*, 2020).

Selection of the best-growing clones

For subsequent analyses, we reduced the number of clones by preselection on solid medium. Cultivation on solid medium allows long-term storage, whereas suspension cultures yield higher amounts of biomass (Beike *et al.*, 2015).

We describe the selection of the best-growing clone here in detail for *Sphagnum fuscum*, whilst descriptions for the other species are in the supplement (Supporting Information Figs S1–S17). Capitula of eight *S. fuscum* clones were cultivated on solid Knop ME or on solid Sphagnum medium (Fig. 3). Two clones were selected from capsule 1, four clones from capsule 2, and two clones from capsule 3, all collected from the same location in Sweden.

Sphagnum medium comprises Knop ME, sucrose, and NH_4NO_3. Previous studies described growth enhancement of *Sphagnum* by sucrose or other saccharides (Simola, 1969; Graham *et al.*, 2010; Beike *et al.*, 2015), or an N source (Simola, 1975; Beike *et al.*, 2015). Fertilization, especially the addition of N and phosphorus, can affect the morphology of *Sphagnum* (Fritz *et al.*, 2012).

Gametophores on Sphagnum medium were more compact with a darker green colour than gametophores on Knop ME, as depicted for *S. fuscum* in Fig. 3(a II, b II). We found this effect for all *Sphagnum* species in our study.

The six clones covering the largest area were, in descending order, 2.2, 1.1, 3.2, 1.2, 2.3 and 3.1 on Knop ME (Fig. 3a IV) and 1.1, 2.2, 2.3, 2.1, 3.2 and 2.5 on Sphagnum medium (Fig. 3b IV). Clones 1.1, 2.2, 2.3 and 3.2 were among the six best clones on both media, and clones 1.2, 2.1, 2.5 and 3.1 were among the six best clones on one of the plates. In the case of ambiguous results, care was taken that at least one clone of each geographical location remained among the six best clones to maintain the highest possible ecotype variation. In this way, clones 1.1, 2.1, 2.2, 2.3, 3.1 and 3.2 were identified as the six best clones on solid media and subsequently analysed in suspension. Here, clone 1.1 yielded significantly more biomass than the other five clones (Fig. 4).

This high variation in biomass productivity may be due to the fact that *S. fuscum* clone 1.1 originates from another spore

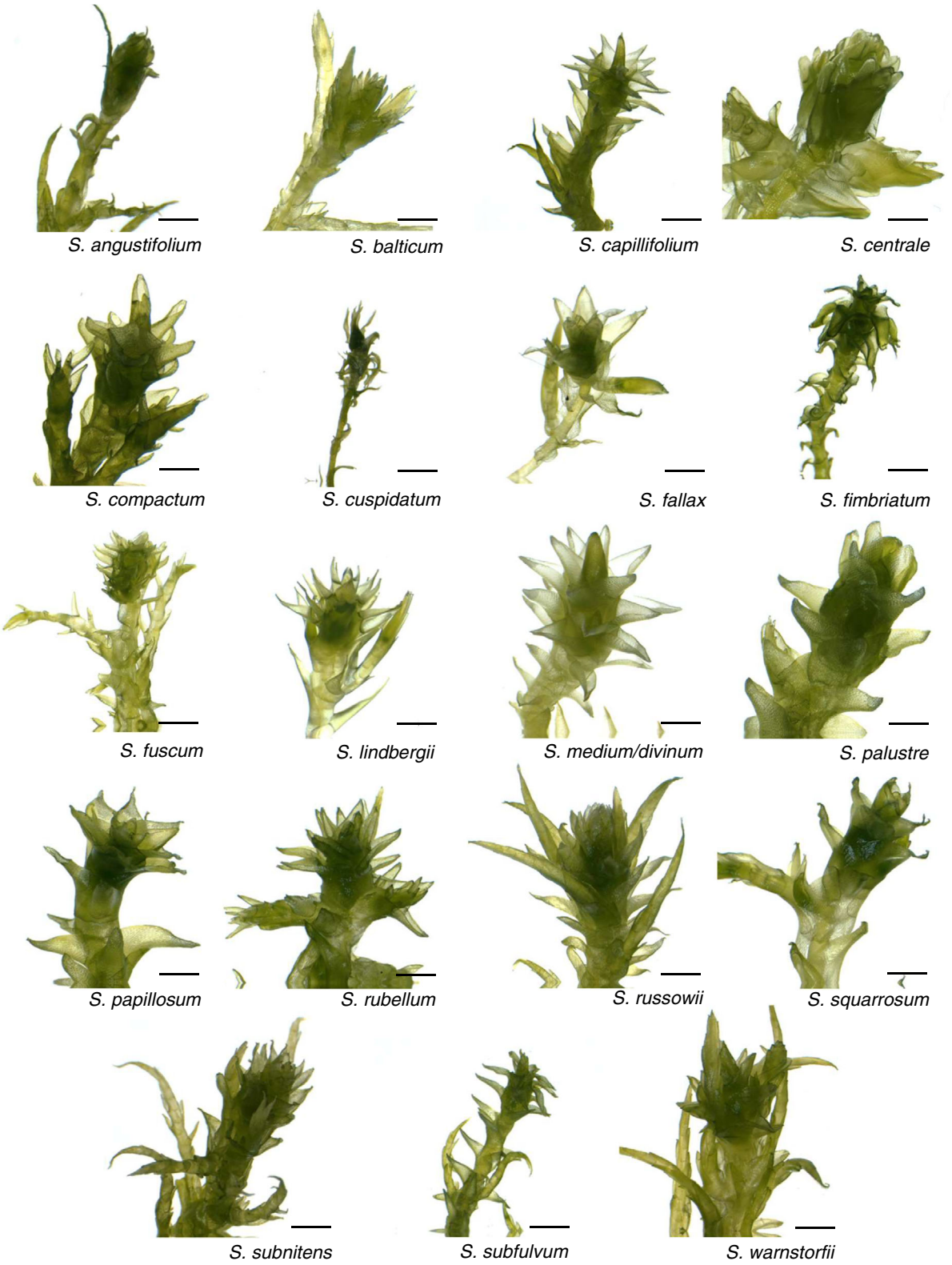

Fig. 2 Light microscopic images of characteristic gametophores of *Sphagnum* spp. after 4 wk of axenic cultivation on solid Sphagnum medium. Bars, 1 mm.

capsule than the other five clones, or because *S. fuscum* is dioecious (Cronberg, 1993). Another dioecious species, *S. angustifolium* (Cronberg, 1993), yielded a high variation in biomass from clones germinated out of one spore capsule, from 34.4 ± 6 mg DW (clone 2.2) to 226.6 ± 7.3 mg DW (clone 2.4). The same was detected for the monoecious species *S. compactum* (Cronberg, 1993), where clone 5.1 yielded five times more biomass than clone 5.3. However, we found no

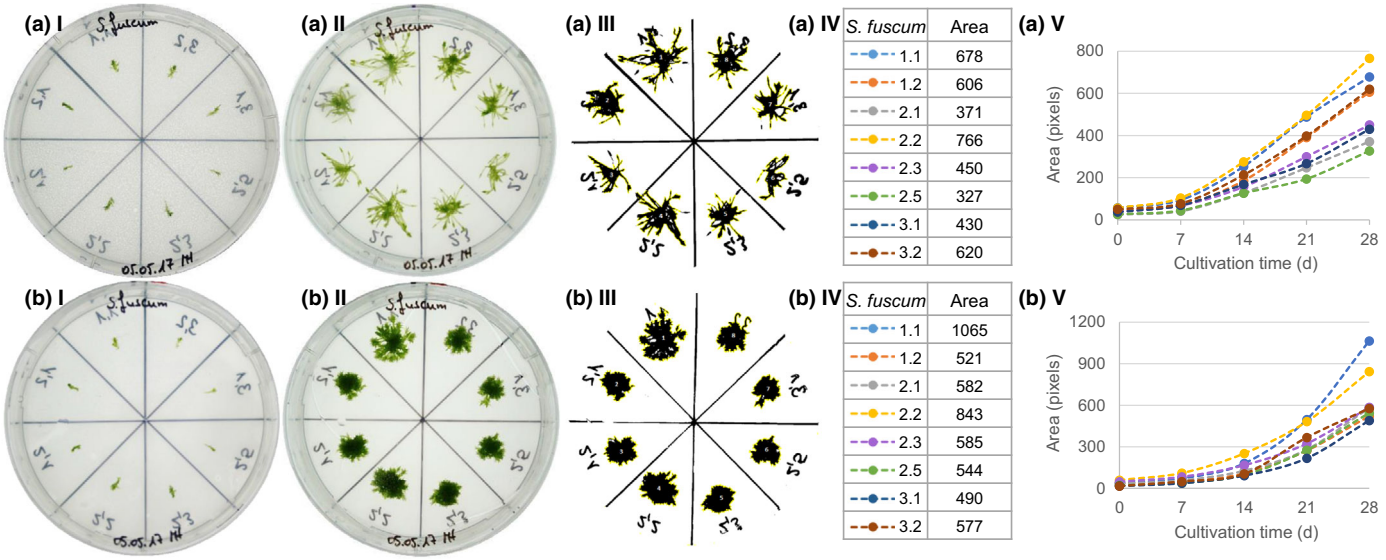

Fig. 3 Growth determination of *Sphagnum fuscum* on (a) solid Knop with microelements and (b) solid Sphagnum medium. (I) Capitula of eight independent clones were cut to 5 mm size and transferred to Petri dishes. (II) Gametophores after 4 wk of cultivation. (III) The size of the gametophores was measured by counting the pixels on binary pictures using ImageJ. (IV) The area (number of pixels) of each gametophore (V) is shown on the *y*-axis; the *x*-axis shows the cultivation time in days.

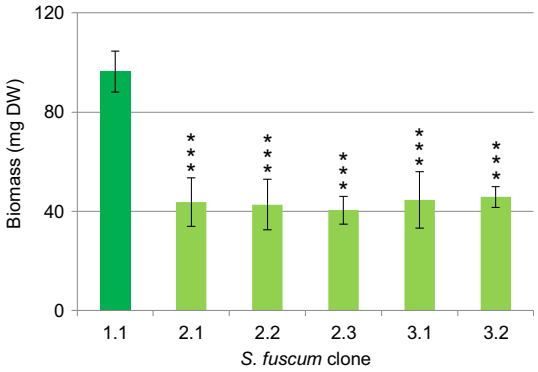

Fig. 4 Biomass (mg DW) of six *Sphagnum fuscum* clones. The growth of the clones was determined in suspension cultures by measuring the DW after cultivation of three capitula in flasks containing 50 ml Sphagnum medium for 6 wk. The *y*-axis shows the biomass in DW; the *x*-axis shows the clone. Data represent mean values with SDs of three biological replicates (ANOVA $P < 0.0001$). Clone 1.1 yielded significantly more biomass than the clones 2.1***, 2.2***, 2.3***, 3.1*** and 3.2***. Asterisks represent results of Student's *t*-test performed in comparison with clone 1.1 (***, $P < 0.001$).

correlation between the variation in productivity of the individual clones of one species and their reproductive morphology.

To capture genetic diversity, up to six best-growing clones per species from different sporophytes were deposited in the International Moss Stock Center (http://www.moss-stock-center.org). All clones deposited, their accession numbers, and the origin of the sporophyte (date of collection, location of collection) are listed in Table 1.

The taxonomic status of clones derived from sporophytes originally collected as *S. magellanicum* needs clarification in future because a new species concept of *S. magellanicum*, *S. medium* and *S. divinum* became available (Hassel *et al.*, 2018) during the course of our study. We cannot group these clones into one of these species based on morphology because they differ slightly between *in vitro* and the field, as described before in the case of *S. palustre* (Beike *et al.*, 2015). However, we can distinguish them by their collection site, as geographical distribution differs. Accordingly, our clones most probably are not *S. magellanicum*, because Hassel *et al.* (2018) suggest its occurrence in Argentina and Chile only. By contrast, we collected those sporophytes in Sweden and Russia. Therefore, our clones are most likely *S. divinum* or *S. medium*, because both are circumpolar in the Northern Hemisphere (Hassel *et al.*, 2018). As both species occur in mixed stands (Hassel *et al.*, 2018), we are currently not able to separate them by collection site either. Therefore, we list these clones here as *S. medium/divinum*. To resolve this uncertainty in future, a detailed analysis with different molecular markers (von Stackelberg *et al.*, 2006; Di Palma *et al.*, 2016; Hassel *et al.*, 2018) is needed. However, we note that these clones are more heterogeneous regarding morphology, colour, and growth rate than clones from the other species in our study. This suggests the existence of two species in our *S. medium/divinum* collection. If this hypothesis is confirmed by molecular genetic analyses in future, our axenic *Sphagnum* collection comprises 20 species instead of 19.

Cell-cycle arrest, genome sizes, and ploidy

The DNA content of the nuclei (ploidy) can affect productivity, at least in animals and seed plants (Dhawan & Lavania, 1996; Paterson *et al.*, 2012; Chen, 2013). Usually, *Sphagnum* species have haploid gametophytes and $n = 19$ chromosomes, but diploid forms with 38 chromosomes exist. Both chromosome numbers exist for populations of some species (Cronberg, 1993), and even triploid peat mosses have been described (Karlin & Smouse,

2019; Kyrkjeeide *et al.*, 2019). Besides chromosome counting, *Sphagnum* genome sizes were estimated by Feulgen absorbance microscopy (Temsch *et al.*, 1998). FCM was applied to determine DNA contents of mosses (Reski *et al.*, 1994), including *Sphagnum* (Melosik *et al.*, 2005). The major peak of the internal standard *P. patens* represents haploid nuclei in the G2-phase of the cell cycle (Schween *et al.*, 2003a). It was set at channel 200, whereas the peak at channel 100 represents nuclei in G1. A peak at 400 indicates diploid nuclei in G2 (Schween *et al.*, 2003a).

Our FCM analysis revealed only one peak for gametophytic cells of all 19 *Sphagnum* species, but at two different positions: either one peak occurred around 100, or a peak occurred near 200 (Fig. 5). As we analysed fast-growing tissue, one might expect that nuclei from one sample were in different phases of the cell cycle and thus would yield two different peaks (G1 and G2) plus intermediary signals for nuclei in the S-phase. Our current findings confirm similar findings of Melosik *et al.* (2005) and suggest that gametophytic cells of all 19 *Sphagnum* species are arrested predominantly either in G1 or G2. A similar cell-cycle arrest occurs in *P. patens* (Reski *et al.*, 1994; Schween *et al.*, 2003a).

In such a situation, FCM cannot clarify if a peak corresponds to G1 or to G2. Thus, a peak at 200 could result from either haploid nuclei in G2 or diploid nuclei in G1. Thus, we considered the published genome sizes that are based on sequencing: the basic nuclear DNA content of *P. patens* is 0.53 pg, with an estimated genome size of 518 Mbp (Schween *et al.*, 2003a), whereas the latest *P. patens* genome assembly yielded 467.1 Mbp (Lang *et al.*, 2018). Based on Feulgen absorbance photometry, haploid *Sphagnum* species have DNA contents between 0.392 pg and 0.506 pg, and diploid species have between 0.814 and 0.952 pg DNA (Temsch *et al.*, 1998), with an average ratio of 1 : 1.92 between DNA content in haploids and diploids (Melosik *et al.*, 2005). Currently, there are two *Sphagnum* genome sequences publicly available. According to this, the *S. fallax* genome comprises approximately 395 Mbp and the *S. magellanicum* genome approximately 439 Mbp (DOE-JGI, http://phytozome.jgi.doe.gov/). Assuming that in our FCM analysis the peaks at 100 and at 200 represent cells in G1, the estimated genome sizes vary between 370 and 460 Mbp for the peak at 100 and between 840 and 890 Mbp for the peak at 200. Although these are only approximations because DAPI binds to AT-rich DNA sequences (Doležel *et al.*, 1992), the values for those *Sphagnum* species characterized by a peak around 100 coincide well with the sizes of both available genome sequences. We therefore conclude that the gametophytic cells of these species are haploid and predominantly arrested in G1.

Although it is an obvious hypothesis that species with a peak around 200 are diploid and arrested in G1, and not haploid and arrested in G2, we tested this hypothesis by comparison with the literature about haploidy and diploidy in *Sphagnum* and compiled the data in Table 2. Our hypothesis is in accordance with data for the 13 haploid species *S. angustifolium*, *Sphagnum balticum*, *Sphagnum capillifolium*, *S. compactum*, *Sphagnum cuspidatum*, *S. fallax*, *S. fuscum*, *Sphagnum lindbergii*, *S. medium/divinum*, *Sphagnum rubellum*, *Sphagnum subnitens*, *Sphagnum subfulvum* and *S. warnstorfii*, as well as the three diploid species *Sphagnum centrale*, *S. palustre* and *S. russowii*. Our *S. fimbriatum* clones, which derive from sporophytes collected in Sweden and Latvia, are haploid. *Sphagnum fimbriatum* was reported to be haploid in the USA (Bryan, 1955), Finland (Sorsa, 1955, 1956), Canada (Maass & Harvey, 1973), and Austria (Temsch *et al.*, 1998), whereas diploid specimens were reported for the UK (Smith & Newton, 1968). Our *Sphagnum papillosum* clones established from sporophytes collected in Russia and Germany are diploid, which is in agreement with material from the UK (Smith & Newton, 1968) and Austria (Temsch *et al.*, 1998), whereas haploid specimens were reported from Canada (Maass & Harvey, 1973). Our *S. squarrosum* clones from Germany and Austria are haploid, like material from Austria (Temsch *et al.*, 1998) and Canada (Maass & Harvey, 1973), whereas diploid specimens were reported from Finland (Sorsa, 1955, 1956).

Taken together, we conclude that the gametophytic cells of 19 *Sphagnum* species are predominantly arrested in G1, at least under our conditions. This contrasts with the G2 arrest of *P. patens* protonemal cells. One prominent feature of *P. patens* is the very high efficiency of homologous recombination (HR) in these cells. This feature facilitates precise gene targeting (GT), and thus genome engineering with outstanding efficiency (Schaefer & Zryd, 1997; Strepp *et al.*, 1998; Hohe *et al.*, 2004). Although it is not yet fully resolved why *P. patens* has such an outstandingly high HR efficiency, two hypotheses were put forward early on: either haploidy or the G2 arrest is a prerequisite (Schaefer & Zryd, 1997; Reski, 1998). These hypotheses can be tested with the collection described here: if haploidy is sufficient, haploid but not diploid *Sphagnum* species should be amenable to efficient GT. If G2 arrest is a prerequisite, none of the species described here is amenable to GT. To our knowledge, no genetic transformation of any *Sphagnum* species has been reported hitherto. Because protoplasts derived from protonemal cells are the preferred target for genetic transformation in *P. patens*, the report about protonema-induction in *S. squarrosum* (Zhao *et al.*, 2019) paves the way for such experiments in the future.

Growth in suspension

Productivity of *Sphagnum* in their natural habitats varies among species, with a biomass production of up to 1450 $g\,m^{-2}\,yr^{-1}$, with an average of 260 $g\,m^{-2}\,yr^{-1}$, depending on phylogeny and microhabitat preferences (Gunnarsson, 2005). To compare productivity without the influence of water level or nutrient supply, we tested cultivation in suspensions under standardized conditions to identify the best-growing clone of each of the 19 species (Fig. 6). To compare the growth behaviour of the species, the inocula have to be normalized. Owing to the variation of the capitula sizes of *Sphagnum* species (Fig. 2), the inoculation material had to be adjusted. *In vitro* cultures facilitate the reproduction due to vegetative growth, because peat mosses regenerate from several parts of the shoot, like capitula, fascicles, branches, and stems, but not from leaves (Poschlod & Pfadenhauer, 1989).

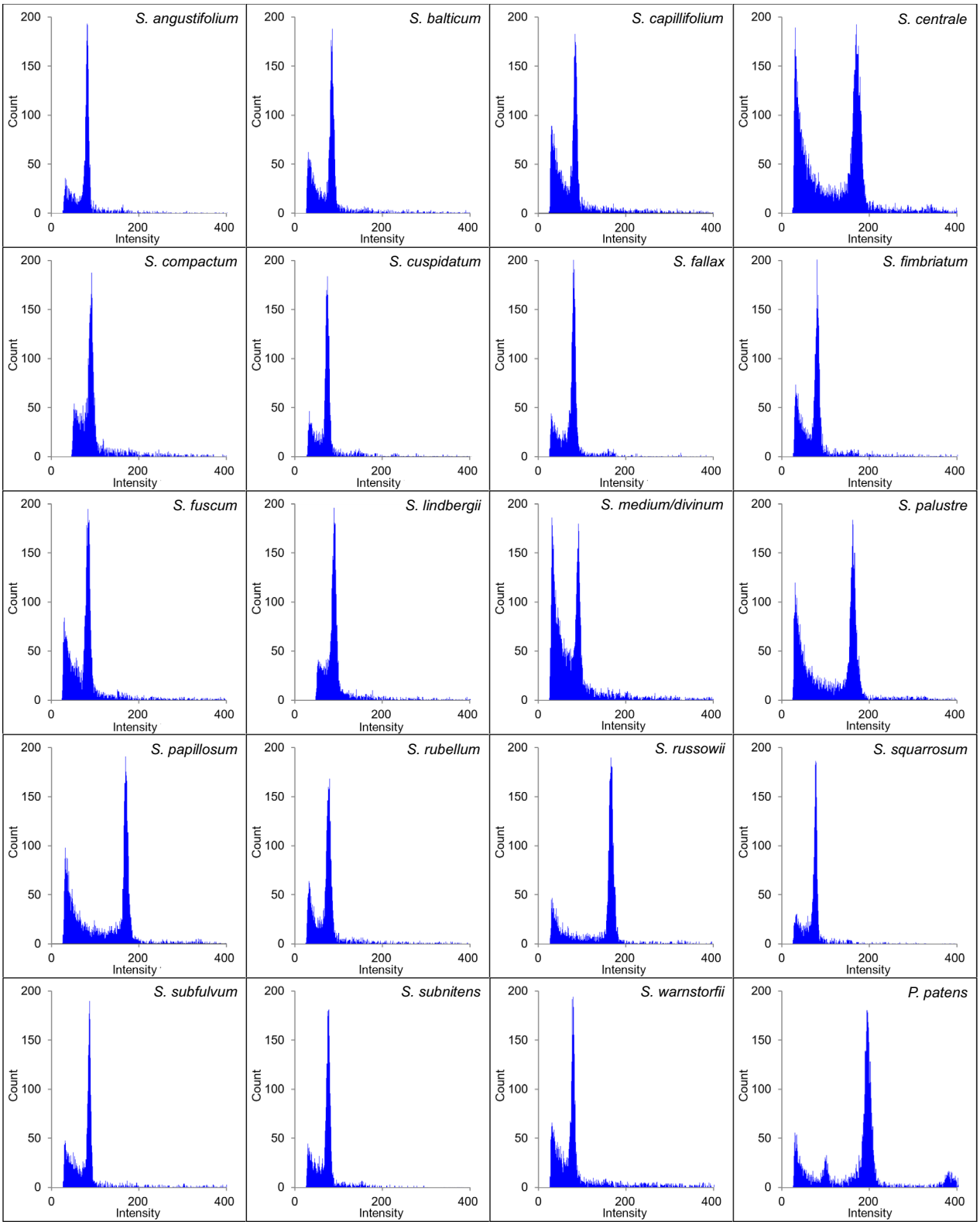

Fig. 5 Flow cytometry signals of 19 *Sphagnum* species and *Physcomitrella patens* after axenic cultivation on solid Sphagnum medium for 4 months. *Physcomitrella patens* was used as internal standard with the major peak set at channel 200. Channel numbers (*x*-axis) reflect the relative fluorescence intensity of the stained nuclei.

We disrupted gametophores with forceps and filled flasks with 50 mg FW (*c.* 3.6 mg DW; see Beike *et al.*, 2015) and 35 ml Sphagnum medium. The nutrient composition was established towards optimized biomass production of *S. palustre*, but it was not proven for the other established axenic *in vitro* cultures of *S. fimbriatum*, *S. magellanicum*, *S. rubellum* and *S. subnitens*

Table 2 The ploidy level of 19 *Sphagnum* species measured by flow cytometry (FCM) in comparison with the literature.

	Level of ploidy						
Sphagnum sp.	This study FCM	Bryan (1955)	Sorsa (1955)	Sorsa (1956)	Smith & Newton (1968)	Maass & Harvey (1973)	Temsch *et al*. (1998)
S. angustifolium	*n*						*n*
S. balticum	n		n	n			
S. capillifolium	*n*						*n*
S. centrale	2*n*					2*n*	2*n*
S. compactum	*n*	*n*	*n*	*n*			*n*
S. cuspidatum	*n*	*n*	*n*	*n*	*n*	*n*	*n*
S. fallax	*n*						*n*
S. fimbriatum	*n*	*n*	*n*	*n*	2*n*	*n*	*n*
S. fuscum	*n*		*n*	*n*		*n*	*n*
S. lindbergii	*n*			*n*		*n*	
S. magellanicum		*n*		2*n*		*n*	*n*
S. medium/ divinum	*n*						
S. palustre	2*n*	2*n*			2*n*	2*n*	2*n*
S. papillosum	2*n*				2*n*	*n*	2*n*
S. rubellum	*n*				*n*	*n*	*n*
S. russowii	2*n*						2*n*
S. squarrosum	*n*		2*n*	2*n*		*n*	*n*
S. subnitens	*n*		*n*		*n*		*n*
S. subfulvum	*n*					*n*	
S. warnstorfii	*n*		*n*	*n*			*n*

Literature data are based on chromosome numbers (Bryan, 1955; Sorsa, 1955, 1956; Smith & Newton, 1968; Maass & Harvey, 1973) or on genome size determination with Feulgen absorbance photometry and a scanning cytophotometer (Temsch *et al*., 1998). *n*, haploid; 2*n*, diploid.

(Beike *et al*., 2015). Under our conditions, biomass increase ranged from four-fold (*S. rubellum*) up to 80-fold (*S. cuspidatum*) in 6 wk (Fig. 6).

The Sphagnum medium was suitable for axenic *in vitro* cultivation of many *Sphagnum* species, including *S. cuspidatum* (yielding the largest biomass gain), *S. fallax*, *S. papillosum* and *S. squarrosum* (Fig. 6). The medium seems suboptimal for, for example, *S. subnitens*, the species with the highest productivities out of 31 peat moss species from *Sphagnum*-dominated wetlands (Gunnarsson, 2005). However, in our study, *S. subnitens* had

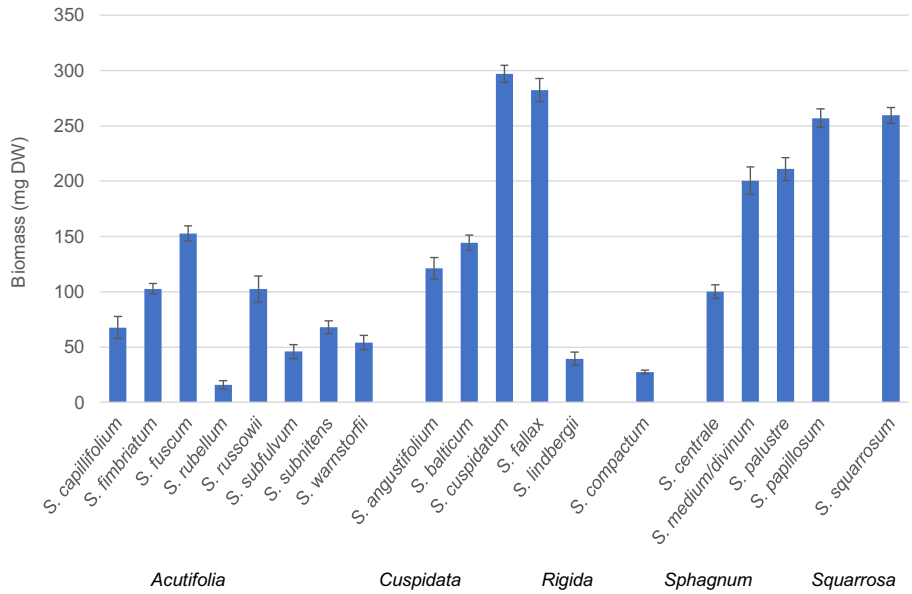

Fig. 6 Biomass increase of 19 *Sphagnum* species sorted by sections after cultivating 50 mg FW (*c*. 3.6 mg DW) of gametophores in flasks containing 35 ml Sphagnum medium for 6 wk. The *y*-axis shows the biomass (mg DW); the *x*-axis shows *Sphagnum* species. Data represent mean values with SDs of three biological replicates ($n = 3$).

only a weak performance. Gaudig *et al.* (2020) reported that *S. papillosum*, *S. palustre*, *S. fimbriatum* and *S. fallax* grow well under nutrient-rich conditions with optimal water supply in a glasshouse experiment. In this study, *S. fallax* had the highest productivity and *S. papillosum* the lowest productivity at high water level. The good productivity of *S. fimbriatum* in the glasshouse experiment contrasts with its comparably low productivity in suspension in our study. In the field, biomass increase is generally largest in pools, less on lawns, and least on hummocks (Clymo, 1970), with species growing in ombrotrophic carpets and lawns, like *S. balticum*, *S. cuspidatum*, *S. magellanicum* and *S. rubellum*, having higher productivities than hummock species, like *S. fuscum* (Gunnarsson, 2005). This corresponds with several studies, which have shown that the growth rate of most *Sphagnum* species is highest at water tables just below the capitula, independent of the species (e.g. Gaudig *et al.*, 2020). Interestingly, we recorded higher growth rates for *S. papillosum* than for *S. palustre*, although the opposite is described for natural habitats (Gunnarsson, 2005; Krebs *et al.*, 2016). Surprisingly, axenic *in vitro* cultivation enhances the productivity of *S. fuscum* but impairs the growth of *S. rubellum*.

We could not correlate productivity and taxonomical subgenus as the four most productive species in our study belong to three different subgenera (*Cuspidata*, *Sphagnum*, *Squarrosa*). Species from the subgenera *Acutifolia* and *Rigida* had a lower average productivity. In natural habitats, species of the subgenus *Cuspidata* are more productive than species of the subgenera *Acutifolia* and *Sphagnum* (Gunnarsson, 2005). To clarify the differences in productivity on a genetic level between the subgenera, a larger number of species from one subgenus should be examined in future. Our inability to detect a correlation between subgenus and biomass gain may reflect the situation in the field. Piatkowski & Shaw (2019) did not detect an influence of phylogeny on the majority of traits in their study on 15 *Sphagnum* species and suggested that the environmental context can obscure the phylogenetic signal. Our novel method creates an artificial but standardized environment for 19 *Sphagnum* species. It facilitates research to gain deeper insights into the ecology of peat mosses, as single parameters like nutrients and light conditions can be changed and tested. Recently, Küttim *et al.* (2020) reported on biomass increases of *Sphagnum* species during boreal winters. Consequently, future studies should also take climate gradients into account.

Another genetic property that influences productivity is ploidy (Otto & Whitton, 2000), as described for many agricultural crops (Henry & Nevo, 2014). In our current study, however, we could not detect a correlation between ploidy and productivity, because the diploid *S. palustre* and *S. papillosum* were among the six best-growing species, but the haploid *S. cuspidatum* and *S. fallax* were most productive. The other diploid species, *S. centrale* and *S. russowii*, yielded an average increase. Because the medium composition may have affected our results, future studies of haploid and diploid populations of the same species using individually optimized media may provide better insights into a correlation between ploidy and yield. Alternatively, polyploidization may have other benefits than pure biomass increase in *Sphagnum*, or even mosses in general.

DNA barcoding of the 19 best-growing clones

To reliably discriminate our *Sphagnum* species, we sequenced parts of the nuclear gene *BRK1*, which is an established marker for phylogenetic analyses and has been identified as a single copy orthologue in many land plants genomes. For example, *BRK1* was used to resolve relationships within the moss family Funariaceae based on its high degree of conservation, including a single intron (Beike *et al.*, 2014). By contrast, we noticed that the genome assemblies of *S. fallax* v.1.1 and *S. magellanicum* v.1.1 (DOE-JGI, http://phytozome.jgi.doe.gov/) each comprise two *BRK1* homologues (Sphfalx09G049800.1 and Sphfalx18G024600.1; Sphmag18G074300.1 and Sphmag09G052500.1), indicating a gene duplication or a polyploidization in the evolutionary lineage leading to the Sphagnaceae. Though Sphfalx09G049800.1 and Sphmag18G074300.1 both harbour only a single intron within their coding sequences, Sphfalx18G024600.1 contains an additional intron in the 5′ untranslated region, and the major transcript isoform Sphmag09G052500.1 exhibits an additional exon of coding sequence. However, the alternative transcript Sphmag09G052500.2, in turn, is annotated only with a single intron. Thus, we designed primers that bind within the conserved coding sequence flanking the introns of both *BRK1* genes (Fig. S18).

We amplified genomic *BRK1* fragments for all the best-growing clones, which resulted in two products: a shorter sequence with individual lengths between 511 and 539 bp, and a longer sequence with individual lengths between 1317 and 1360 bp, as a consequence of different intron lengths of the two *BRK1* loci. For convenience, we named the former fragment '*BRK1* short' and the latter '*BRK1* long' (Table 3). *BRK1* short discriminates 15 of our 19 *Sphagnum* species, but the sequences were identical for *S. balticum* and *S. fuscum* as well as for *S. capillifolium* and *S. rubellum*. The diploid species *S. centrale* and *S. palustre* shared an identical *BRK1* short sequence with a length of 536 bp, yet they both yielded a second sequence of 537 bp, which we used to discriminate between them. The occurrence of two *BRK1* short sequences indicates a hybrid origin rather than a polyploidization of *S. centrale* and *S. palustre*.

The sequence lengths of *BRK1* long are the same for *S. balticum* and *S. fuscum*; however, we found discriminative polymorphisms in two positions. *Sphagnum capillifolium*, *S. compactum*, *S. rubellum*, *S. subnitens* and *S. warnstorfii*, all belonging to the subgenus *Acutifolia*, had a *BRK1* long fragment with 1351 bp, which, with the exception of *S. compactum*, were identical in sequence. Therefore, none of our *BRK1* sequences can discriminate between *S. capillifolium* and *S. rubellum*. However, as *S. capillifolium* is three times more productive than *S. rubellum* under standard conditions in suspension, it is possible to discriminate these two species based on phenotypical characteristics. All sequences were submitted to GenBank (Table 3). Sequence alignments are compiled in Dataset S1 for *BRK1* short and Dataset S2 for *BRK1* long.

Taken together, we established fast and precise DNA barcoding to reliably identify any of our *Sphagnum* cultivars at any stage of their life cycle, with only two exceptions, which currently require further support by phenotypical characteristics. Further, our *BRK1*

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Table 3 Summary results of axenic cultivation of 19 *Sphagnum* species, including the subgenera, the breeding system based on Cronberg (1993), the ploidy level measured by flow cytometry (FCM), the clones analysed and the number of spore capsules, the identification of the best-growing clone compared with up to five other clones, with detailed information in the figures in Supporting Information, and the sequence lengths and GenBank IDs of *BRK1* introns, where the asterisks mark identical sequences.

				Germination of spores		Best-growing clone out of six best-growing clones			*Sphagnum* barcoding (fragment lengths and GenBank IDs)		
Sphagnum sp.	Subgenus	Breeding system (Cronberg, 1993)	Ploidy by FCM	No. clones	No. spore capsules	Best-growing clone	Significantly more productive than	Not significantly more productive than	*BRK1* short	*BRK1* long	Figure
S. angustifolium	*Cuspidata*	Dioecious	Haploid	6	2	2.4	2.1, 2.2, 2.3, 2.5, 7.1		537 MT900778	1317 MT900755	S1
S. balticum	*Cuspidata*	Dioecious	Haploid	16	6	1.2	1.1, 2.2, 3.3, 8.1, 9.5		537* MT900779	1352 MT900756	S2
S. capillifolium	*Acutifolia*	Polyoecious/ dioecious	Haploid	11	1	1.8	1.2, 1.3, 1.5, 1.9, 1.49		537* MT900780	1351* MT900757	S3
S. centrale	*Sphagnum*	Dioecious	Diploid	14	7	10.2	1.2, 3.3, 6.4, 7.5, 9.6		536*/537 MT900781/ MT900782	1349/1356 MT900758/ MT900759	S4
S. compactum	*Rigida*	Monoecious	Haploid	12	4	5.1	3.1, 5.3, 6.1, 6.2	4.6	534 MT900783	1351 MT900760	S5
S. cuspidatum	*Cuspidata*	Dioecious	Haploid	15	5	5.2	1.1, 3.3, 3.4, 5.1	1.4	538 MT900784	1338 MT900761	S6
S. fallax	*Cuspidata*	Dioecious	Haploid	10	3	4.5	2.1	3.1, 4.1, 4.4, 4.6	537 MT900785	1317 MT900762	S7
S. fimbriatum	*Acutifolia*	Monoecious	Haploid	6	3	1.1	2.1, 6.1, 6.4	6.2, 6.5	539 MT900786	1321 MT900763	S8
S. fuscum	*Acutifolia*	Dioecious	Haploid	8	3	1.1	2.1, 2.2, 2.3, 3.1, 3.2		537* MT900787	1352 MT900764	S9
S. lindbergii	*Cuspidata*	Monoecious/ dioecious	Haploid	5	2	2.1	2.3, 3.3	3.1, 3.2	536 MT900788	1334 MT900765	S10
S. medium/ divinum	*Sphagnum*	Dioecious (*S. magellancium*)	Haploid	8	4	3.1	4.2, 4.3, 5.1, 5.2	4.1	536 MT900789	1356 MT900766	S11
S. palustre	*Sphagnum*	Dioecious	Diploid	9	3	12a	2a	4.2, 4.3, 5.1, 5.2	536*/537 MT900790/ MT900791	1352/1357 MT900767/ MT900768	S12
S. papillosum	*Sphagnum*	Dioecious	Diploid	12	6	6.1	2.2, 4.3, 5.2, 7.1	1.1	511/537 MT900792/ MT900793	1354/1360 MT900769/ MT900770	S13
S. rubellum	*Acutifolia*	Dioecious	Haploid	2	2	1.1		2.1	537* MT900794	1351* MT900771	
S. russowii	*Acutifolia*	Dioecious	Diploid	12	3	4.2	1.1, 1.2, 3.1, 3.4, 3.5		536/536 MT900795/ MT900796	1352/1355 MT900772/ MT900773	S14
S. squarrosum	*Squarrosa*	Monoecious	Haploid	16	6	5.2	2.1, 5.3, 6.1, 8.3	7.1	537 MT900797	1356 MT900774	S15

Table 3 (Continued)

Sphagnum sp.	Subgenus	Breeding system (Cronberg, 1993)	Germination of spores			Best-growing clone out of six best-growing clones			*Sphagnum* barcoding (fragment lengths and GenBank IDs)		
			Ploidy by FCM	No. clones	No. spore capsules	Best-growing clone	Significantly more productive than	Not significantly more productive than	*BRK1* short	*BRK1* long	Figure
S. subfulvum	*Acutifolia*	Monoecious	Haploid	16	6	7.4		4.2, 4.3, 7.2, 8.1, 8.5	535 MT900798	1355 MT900775	S16
S. subnitens	*Acutifolia*	Polyoicous/monoecious	Haploid	1	1	1.1			537 MT900799	1351* MT900776	
S. warnstorfii	*Acutifolia*	Dioecious	Haploid	14	4	5.2	1.3, 2.1	1.4, 3.3, 5.4	537 MT900800	1351* MT900777	S17

data provide hints for a complex evolutionary history of the Sphagnaceae, including polyploidization and hybridization.

Summary results of axenic cultivation of 19 *Sphagnum* species

The results from the 19 *Sphagnum* species, including the subgenera and breeding systems, the ploidy level, the identification of the best-growing clone, the sequence lengths of *BRK1* introns, and their GenBank IDs, are compiled in Table 3.

Conclusion

Apart from *P. patens* as an established model organism, the development of other model mosses is inevitable for ecological and evolutionary genomics, as well as for clarifying open questions, such as the high HR efficiency of *P. patens*. Owing to the large number of peat moss species and their clear patterns of niche differentiation, *Sphagnum* provides an excellent complement (Shaw *et al.*, 2016). Our peat moss collection creates a resource for the increasing interest in *Sphagnum* research to establish new plant model systems. Moreover, the large-scale implementation for diverse applications can rely on the axenic *in vitro* cultivation of *Sphagnum* as fast-growing, high-quality founder material. Scaling up this cultivation method will facilitate a low-cost production process. In particular, Sphagnum farming will benefit, as the lack of *Sphagnum* diaspores is one of the biggest problems and their purchase is the biggest cost factor for establishing Sphagnum farming sites (Wichmann *et al.*, 2017, 2020).

Acknowledgements

This work was supported by the Federal Ministry of Food and Agriculture (BMEL) (MOOSzucht, no. 22007216). Additional support came from the German Research Foundation (DFG) under Germany's Excellence Strategy (CIBSS – EXC-2189 – Project ID 390939984; *liv*MatS – EXC-2193/1 – 390951807). We gratefully acknowledge Anja Kuberski and Richard Haas for expert technical assistance, Michael Lüth for providing sporophytes, Greta Gaudig for discussion, and Anne Katrin Prowse for proofreading of the manuscript. Open access funding enabled and organized by Projekt DEAL.

Author contributions

MAH, VML, ELD and RR planned and designed the research. MAH performed experiments and analysed data. NvG analysed sequence data. M. Krebs, M. Kohl and AP collected sporophytes. MAH, ELD and RR wrote the manuscript. HJ revised this paper. All authors discussed data and approved the final version of the manuscript.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Dataset S1 Multiple sequence alignment of *BRK1* short.

Dataset S2 Multiple sequence alignment of *BRK1* long.

Fig. S1 Determination of the best-growing clone of *S. angustifolium*.

Fig. S2 Determination of the best-growing clone of *S. balticum*.

Fig. S3 Determination of the best-growing clone of *S. capillifolium*.

Fig. S4 Determination of the best-growing clone of *S. centrale*.

Fig. S5 Determination of the best-growing clone of *S. compactum*.

Fig. S6 Determination of the best-growing clone of *S. cuspidatum*.

Fig. S7 Determination of the best-growing clone of *S. fallax*.

Fig. S8 Determination of the best-growing clone of *S. fimbriatum*.

Fig. S9 Determination of the best-growing clone of *S. fuscum*.

Fig. S10 Determination of the best-growing clone of *S. lindbergii*.

Fig. S11 Determination of the best-growing clone of *S. medium/divinum*.

Fig. S12 Determination of the best-growing clone of *S. palustre*.

Fig. S13 Determination of the best-growing clone of *S. papillosum*.

Fig. S14 Determination of the best-growing clone of *S. russowii*.

Fig. S15 Determination of the best-growing clone of *S. squarrosum*.

Fig. S16 Determination of the best-growing clone of *S. subfulvum*.

Fig. S17 Determination of the best-growing clone of *S. warnstorfii*.

Fig. S18 Target genes for PCR screening as encoded by the *S. fallax* and *S. magellanicum* genomes.

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