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New insights into the species diversity of *Bartramia* Hedw. (Bryophyta) in Antarctica

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Abstract: In Antarctica, the genus *Bartramia* has been restricted to a single polymorphic species, *B. patens*. Its status as a separate species or a subspecies of the Northern Hemisphere *B. ithyphylla* was debated. In the present paper, we combine analyses of chloroplast (*trn*S–*rps*4–*trn*T–*trn*L–*trn*F region) and nuclear *ITS* sequences with a reinvestigation of morphological characteristics to infer the identity of Antarctic *Bartramia*. Phylogenetic and Automatic Barcode Gap Discovery (ABGD) species delimitation analyses indicate that the species diversity of *Bartramia* in Antarctica has been underestimated, since two species were identified, both belonging to *Bartramia* sect. *Pyridium*. Of these, *B. subsymmetrica* is a new record of the species for Antarctica, as it has previously only been recorded from Livingston Island, South Shetlands. The other species is *B. patens*, which is separated from *B. ithyphylla* by newly inferred morphological characteristics and is a sister species to the latter in the molecular phylogenetic analyses. Consequently, we consider *B. ithyphylla* to be a Northern Hemisphere instead of a bipolar species. The suggested conspecificity of both taxa into one species in the ABGD analysis is considered to result from overlumping by this species delimitation method. The delimitation of the three species of section *Bartramia* (*B. halleriana*, *B. mossmaniana* and *B. pomiformis*) and the circumscription of the genus *Bartramia* are discussed.

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Introduction

Only two native and one invasive species compose the flowering plant vegetation of Antarctica, whereas its bryophyte flora includes at least 111 species of mosses (Bryophyta) (Ochyra *et al.* 2008). Despite the comprehensive treatment by Ochyra *et al.* (2008), the species diversity of mosses in Antarctica may remain incompletely known. Bryophyte species, particularly in the polar regions, are often difficult to identify due to their generally small size, relatively few and inconspicuous morphological characteristics, frequent absence of sporophytic characteristics, morphological plasticity in response to environmental factors (especially the harsh polar climates) and as yet unclear species delimitations and taxonomies in many groups (e.g. Hassel *et al.* 2005, Lewis *et al.* 2017). DNA sequence data have been increasingly employed to better understand species delimitations and relationships, evolutionary histories and patterns of geographic variation in polar mosses. In the Antarctic, molecular studies have helped to clarify the circumscription, relationships and intraspecific variation of both endemic species and species with a wider, particularly bipolar, distribution (e.g. Biersma *et al.* 2018a, 2018b, Câmara *et al.* 2018a, 2018b).

The moss family Bartramiaceae ('apple mosses') is represented in Antarctica by three genera and four species: *Conostomum* Sw. with two species, *Philonotis polymorpha* (Müll. Hal.) Kindb. and *Bartramia patens* Brid. (Ochyra *et al.* 2008). *Bartramia* is a cosmopolitan genus with 60 species (Frey & Stech 2009) that is difficult to circumscribe due to its high morphological diversity (Ochyra *et al.* 2008). Three main morphologically distinct groups have been described as sections: sect. *Bartramia* has strongly crispate leaves with recurved margins in the distal portion and transparent distal cells in the limb; sect. *Pyridium* Müll. Hal. (the correct name for sect. *Vaginella* Müll. Hal.; Ochyra *et al.* 2008) has straight and rigid leaves with an abruptly expanded sheathing base, plane or weakly recurved leaf margins and obscure upper

laminal cells; and sect. *Strictidium* Müll. Hal. has neither of these sets of characteristics (Fransén 2004a, 2004b, Ochyra *et al.* 2008). Molecular data indicated that *Bartramia* might in fact not be monophyletic (Virtanen 2003, Damayanti *et al.* 2012), with sections *Bartramia* and *Pyridium* resolved as being closer to the genus *Leiomela* (Mitt.) Broth. than to sect. *Strictidium* (Damayanti *et al.* 2012), but further analyses are necessary.

In addition to the problematic generic circumscription, morphological plasticity hampers establishing species boundaries in *Bartramia* in the (sub-)Antarctic (Ochyra *et al.* 2008). From southern South America and the sub-Antarctic islands, many species have been described, but only five or six species are well known (Matteri 1984). Ochyra *et al.* (2008) considered four species to occur in the cool-temperate zone and the sub-Antarctic, namely *B. ithyphylloides* Müll. Hal., *B. patens*, *B. robusta* Hook.f. & Wilson and *B. subsymmetrica* Cardot, but they acknowledged that taxonomic problems remain. Cardot (1907, 1908, 1911a, 1911b, 1913) reported four *Bartramia* species and one variety from Antarctica, but Robinson (1972) and later Ochyra *et al.* (2008) suggested that there is only a single variable and widespread species, *B. patens*.

Bartramia patens (sect. *Pyridium*) has an amphiatlantic south-temperate distribution, including Juan Fernández, Patagonia, Tierra del Fuego, Isla de los Estados, the Falkland Islands (Malvinas), South Georgia, South Sandwich Islands, Prince Edward Islands, Kerguelen, Tristan da Cunha and Maritime Antarctica, where it is reported from South Orkney Islands, South Shetland Islands (Elephant, King George, Nelson, Robert, Greenwich, Livingston and Deception islands) and the Antarctic Peninsula (Matteri 1984, 1985, Virtanen 2000, Ochyra *et al.* 2008). It is one of the most conspicuous and common moss species in Antarctica that is easily recognizable in the field by its glaucous green colouration and rigidly erect leaves that are abruptly subulate, forming a white, sheathing base (Ochyra *et al.* 2008). However, the species is highly polymorphic and variable concerning the shape and size of its leaves, which has led to the recognition of various phenotypes as separate species (Ochyra *et al.* 2008). *Bartramia patens* is morphologically close to *B. ithyphylla* Brid., a species considered to be bipolar in distribution, occurring in the Northern Hemisphere and in southern South America (Schofield 1974, Matteri 1984, 1985). Ochyra (1992) doubted the status of *B. ithyphylla* as a bipolar species due to its much wider occurrence in the Southern Hemisphere, including also New Zealand, Australia and eastern Africa. Fransén (2004b) reduced *B. patens* to a subspecies of *B. ithyphylla*, which comprised all Southern Hemisphere populations as opposed to a strictly Holarctic *B. ithyphylla* s.str. Both subspecies would thus only be distinguished by their geographic distribution, but not by morphology. Ochyra *et al.* (2008), in contrast, kept *B. patens* as a separate species and pointed out that it could be distinguished by the tristatose condition of its median leaf section and the stereid band of the costa with fewer than 15 cells, while *B. ithyphylla* has a bistratose median portion of the leaf and 20–30 stereid cells at the costa.

The aim of this study is to carry out a first molecular analysis of the genus *Bartramia* in Antarctica to infer: 1) whether Antarctic *Bartramia* specimens belong to a single species, *B. patens*, or to more than one species, and 2) how the Antarctic specimens are related to the Northern Hemisphere species *B. ithyphylla*.

Materials and methods

Sampling

DNA sequences were obtained from fresh material of 20 *Bartramia* specimens collected during Antarctic expeditions under the Brazilian Antarctic Program (PROANTAR) and field trips to the Falkland Islands and Tierra del Fuego. Additional sequences belonging to seven *Bartramia* species and two *Leiomela* species were downloaded from the online databases GenBank and European Nucleotide Archive. One specimen of *Philonotis fontana* (Hedw.) Brid. was selected as an outgroup representative based on the availability of sequences of the employed markers and the phylogeny of Damayanti *et al.* (2012). Specimen data of the newly sequenced *Bartramia* specimens (deposited in herbaria SP and UB) are given in Table I.

DNA extraction, polymerase chain reaction amplification and sequencing

Total genomic DNA was extracted using the CTAB protocol (Doyle & Doyle 1987). We amplified and sequenced the chloroplast *trn*S–*rps*4–*trn*T–*trn*L–*trn*F region except for the *trn*T–*trn*L spacer (Hernandez-Maqueda *et al.* 2008) using the primers trnS, rps5', rps4-166F and A-Rbryo from Hernandez-Maqueda *et al.* (2008), as well as C and F from Taberlet *et al.* (1991), and the nuclear ribosomal *ITS* (*ITS1*–5.8S–*ITS2*) region using the primers from Pisa *et al.* (2013). The polymerase chain reaction (PCR) amplification mixture contained 5 µl of 5× thermophilic buffer, 5 µl of 50 mM $MgCl_2$, 0.5 µl Taq (Promega), 2 µl of BSA (10 mg ml^{-1}), 4 µl of 1 mM dNTPs, 2.5 µl of each primer (10 µM) and 2.0 µl of DNA, filled up to a total volume of 50 µl with distilled water. The PCR profile was: 1 min at 94°C, 1 min at 52–58°C and 1 min at 72°C for 35 cycles, preceded by an initial melting step of 2 min at 94°C and with a final extension of 7 min at 72°C. PCR products were purified and bi-directionally sequenced by Macrogen, Inc. (Seoul, Korea).

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Table I. Voucher information and GenBank accession numbers of *Bartramia* specimens newly sequenced for the present study.

Specimen	Voucher number	Geographic origin	Accession number *rps*4–*trn*T	Accession number *trn*L–*trn*F	Accession number *ITS*
Bartramia mossmaniana Müll. Hal. 1	Peralta 19790	Tierra del Fuego	MK948563	MK948583	MK948554
Bartramia mossmaniana Müll. Hal. 2	Peralta 19761	Tierra del Fuego	MK948562	MK948585	MK948553
Bartramia mossmaniana Müll. Hal. 3	Peralta 20054	Tierra del Fuego	MK948570	MK948580	MK948556
Bartramia patens Brid. 1	Carvalho, 57	Deception Island	MK948574	MK948588	MK948549
Bartramia patens Brid. 2	Carvalho 63	Deception Island	MK948573	MK948587	MK948550
Bartramia patens Brid. 3	Dantas & Kitaura 156	King George Island	MK948572	MK948586	MK948551
Bartramia patens Brid. 4	Carvalho 53b	Deception Island	MN010594	-	MN010591
Bartramia patens Brid. 5	Oliveira 2493	Antarctic Peninsula	MK948581	MK948564	MN010592
Bartramia patens Brid. 6	Knop Henriques 260	King George Island	MK948571	MK948585	MK948552
Bartramia patens Brid. 7	Bordin 2841	Elephant Island	MK948575	MK9488589	MK948547
Bartramia patens Brid. 8	Bordin 2836	Elephant Island	MK948582	MK948569	MK948546
Bartramia patens Brid. 9	Carvalho 53	Deception Island	MN010593	-	MN010590
Bartramia patens Brid. 10	Peralta 19856	Tierra del Fuego	MK948576	MK948590	MK948555
Bartramia patens Brid. 11	Câmara *et al.*	Falklands/Malvinas	-	MK948578	MK948548
Bartramia patens Brid. 12	Bordin 3324	Tierra del Fuego	-	MK948479	MN010589
Bartramia subsymmetrica Cardot 1	Valente 2166	Livingstone Island	-	MK948577	MK948561
Bartramia subsymmetrica Cardot 2	Valente 1469	Livingstone Island	MK948565	-	MK948559
Bartramia subsymmetrica Cardot 3	Valente 1466	Livingstone Island	MK948566	-	MK948558
Bartramia subsymmetrica Cardot 4	Valente 1465	Livingstone Island	MK948567	-	MK948557
Bartramia subsymmetrica Cardot 5	Valente, D.V.2036	Livingstone Island	MK948568	-	MK948560

DNA sequence analyses

Sequences were assembled using Geneious v. 6.1.6 (www.geneious.com), initially aligned using Clustal X (Higgins & Sharp 1988), manually adjusted in PhyDE v. 0.9971 (www.phyde.de) and exported as Nexus files. Phylogenetic analyses were carried out under maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) for the chloroplast markers and *ITS* separately, and all of the markers were combined. MP analyses were carried out using PAUP v. 4.0b10 for Macintosh (Swofford 2002). Heuristic searches were performed with 1000 random addition replicates and tree bisection and reconnection branch swapping, saving a maximum of 10 000 trees. All characteristics were unordered and equally weighted, and gaps were either treated as missing data or coded as informative by a simple indel coding strategy (Simmons & Ochoterena 2000) as implemented in SeqState (Müller 2005). ML analyses were carried out using RAxML v. 7.2.6 (Stamatakis 2006). Clade support for MP and ML was assessed from bootstrap analyses with 1000 replicates. For ML and BI analyses, the best-fit model of evolution for each locus was obtained based on the Akaike information criterion using jModeltest 3.06 (Posada 2008). BI analyses were carried out in MrBayes v. 3.2.5 (Ronquist *et al.* 2012). Two runs with four Markov chain Monte Carlo chains were run for 5 000 000 generations. Chains were sampled every 1000 generations and the respective trees were written to a tree file. Convergence of runs was verified by ensuring that the average standard deviation of split frequencies was < 0.01. Tracer 1.5 (http://tree.bio.ed.ac.uk/software/tracer) was used to determine when the tree sampling stabilized. The first 25% of the trees were discarded as 'burn-in'. A majority-rule consensus tree and posterior probabilities were calculated from the resulting trees. In addition to the phylogenetic analyses, we employed the Automatic Barcode Gap Discovery (ABGD) approach (Puillandre *et al.* 2012) to investigate species delimitation within the DNA dataset using the online webserver with the default values.

Morphological analyses

Morphological characteristics from both gametophytes (colour, shoot length, tomentum, leaf base, leaf margins and leaf apex) and sporophytes (capsule surface) in addition to those listed by Ochyra *et al.* (2008) were investigated for all newly sequenced specimens from Antarctica, the Falkland Islands, Patagonia and Tierra del Fuego, as well as for additional specimens of *B. ithyphylla* from herbaria H, S, SP, and UB. Specimens were dissected under a dissecting microscope and examined under a compound microscope.

Results

Of the 20 *Bartramia* specimens from Antarctica, the Falkland Islands, Patagonia and Tierra del Fuego, 17 could be sequenced with *trn*S–*rps*4–*trn*T (of which 3 could be partially sequenced), 16 with *trn*L–*trn*F and all with *ITS*.

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No PCR products could be obtained from additional specimens of *B. ithyphylla* from the Northern Hemisphere. In GenBank, *ITS2* sequences of further specimens from northern Europe and the Arctic are available, which were identical to the *ITS2* sequence of the single included specimen from Switzerland. However, the additional *ITS2* sequences were not included in present analyses because the absence of the chloroplast markers and *ITS1* would decrease the resolution of the phylogenetic reconstructions. Alignment statistics, best-fit models of evolution and tree scores are summarized in Table II. Trees based on the analysis of individual markers and different analysis methods differed only in the degree of resolution, but did not show statistically supported conflicting topologies (data not shown).

Phylogenetic analyses of the combined matrix resolved *Bartramia* as paraphyletic (Fig. 1) due to the nested position of *Leiomela*. A clade with maximum support (MP bootstrap support (MP-BS) 100%, ML bootstrap support (ML-BS) 100%, BI posterior probability (PP) 1.00) composed of *B. mossmaniana* Müll. Hal. from Tierra del Fuego. *B. halleriana* Hedw., *B. pomiformis* Hedw. and another *B. mossmaniana* specimen from GenBank (sect. *Bartramia*) were resolved as sister species to a clade of the remaining *Bartramia* and *Leiomela* specimens. Among the latter, *Bartramia breutelii* Schimp. ex Müll. Hal. (sect. *Strictidium*) branched off first, followed by *Leiomela* and the species of *Bartramia* sect. *Pyridium*, all with maximum support. Within sect. *Pyridium*, three main clades were resolved: 1) *B. angustifolia* Mitt. and *B. hampeana* Müll. Hal. (maximum support), 2) *B. aurescens* Dixon as a sister species (MP-BS 94%, ML-BS 94%, PP 1.00) to the clade of the *B. subsymmetrica* samples (MP-BS 81%, ML-BS 78%, PP 0.99), and 3) *B. ithyphylla* as a sister species (MP-BS 72%, ML-BS 70%, PP 0.99) to the clade of the *B. patens* specimens (MP-BS 72%, ML-BS 70%, PP 0.96). Within *B. patens*, all but one of the specimens from Antarctica were separated from those from the Falkland Islands and Tierra del Fuego on a clade with MP-BS 70%, ML-BS 74% and PP 1.00.

The ABGD species delimitation method revealed a barcode gap at P_{max} = 4.64e-03, delimitating nine putative clusters (Fig. 1): 1) *Philonotis* (outgroup), 2) *Bartramia breutelii*, 3) *Leiomela ecuadorensis*, 4) *L. bartramioides*, 5) *B. angustifolia* plus *B. hampeana*, 6) *B. aurescens*, 7) *B. subsymmetrica*, 8) *B. ithyphylla* plus *B. patens*, and 9) *B. mossmaniana* plus *B. halleriana* plus *B. pomiformis*.

The results of the morphological comparison between *B. patens* and *B. ithyphylla* are shown in Table III. Shoot length is largely overlapping between both taxa, whereas tomentum, brokenness of leaf tips, leaf dentation and capsule surface differ in the degree to which the respective character states are expressed. Clearly different character states are found in gametophyte colour, involute or plane leaf subula and colour of the leaf base.

Discussion

According to the present data, the species diversity of *Bartramia* in Antarctica, as currently perceived (one single species, *B. patens*; Ochyra *et al.* 2008), is underestimated. The molecular phylogenetic reconstructions (Fig. 1) resolved two species of *Bartramia* in Antarctica, which both belong to the same section, *Pyridium*. Apart from *B. patens*, which we accept at the species level as being separate from the Northern Hemisphere *B. ithyphylla* (see discussion below), we report *B. subsymmetrica* as a new record for Antarctica.

Bartramia subsymmetrica was originally described from South Georgia, and has subsequently been reported from the Falkland Islands, Patagonia, Kerguelen and south-eastern Australia (Fransén 2004b). In Antarctica, the species is so far only known from our collections from Livingston Island, South Shetlands (present data). It might have long been present and more widespread in Antarctica, but misreported as *B. patens*; however, a recent introduction cannot be ruled out either. Fertile plants of *B. subsymmetrica* can be distinguished by the single peristome from the sister species in the molecular

Table II. Alignment statistics, best-fit models of evolution and tree scores (maximum parsimony (MP) and maximum likelihood (ML)) for the separate and combined datasets used in the present study. Number of MP trees 10 000 indicates that the 'maxtrees' limit was reached.

	Chloroplast	*ITS*	Combined
Taxa included	32	32	32
Matrix length (positions/indels)	1557 (1528/29)	1585 (1386/199)	3144 (2914/230)
Variable sites	178	353	533
Parsimony informative sites	103	245	349
Number of MP trees	8343	9650	10 000
Tree length	208	420	646
Consistency index	0.894	0.879	0.862
Retention index	0.956	0.942	0.936
Model of evolution	TVM + G	TPM1uf + G	TVM + I
Log likelihood of best ML tree	-3000.741673	-2854.796653	-7379.694613

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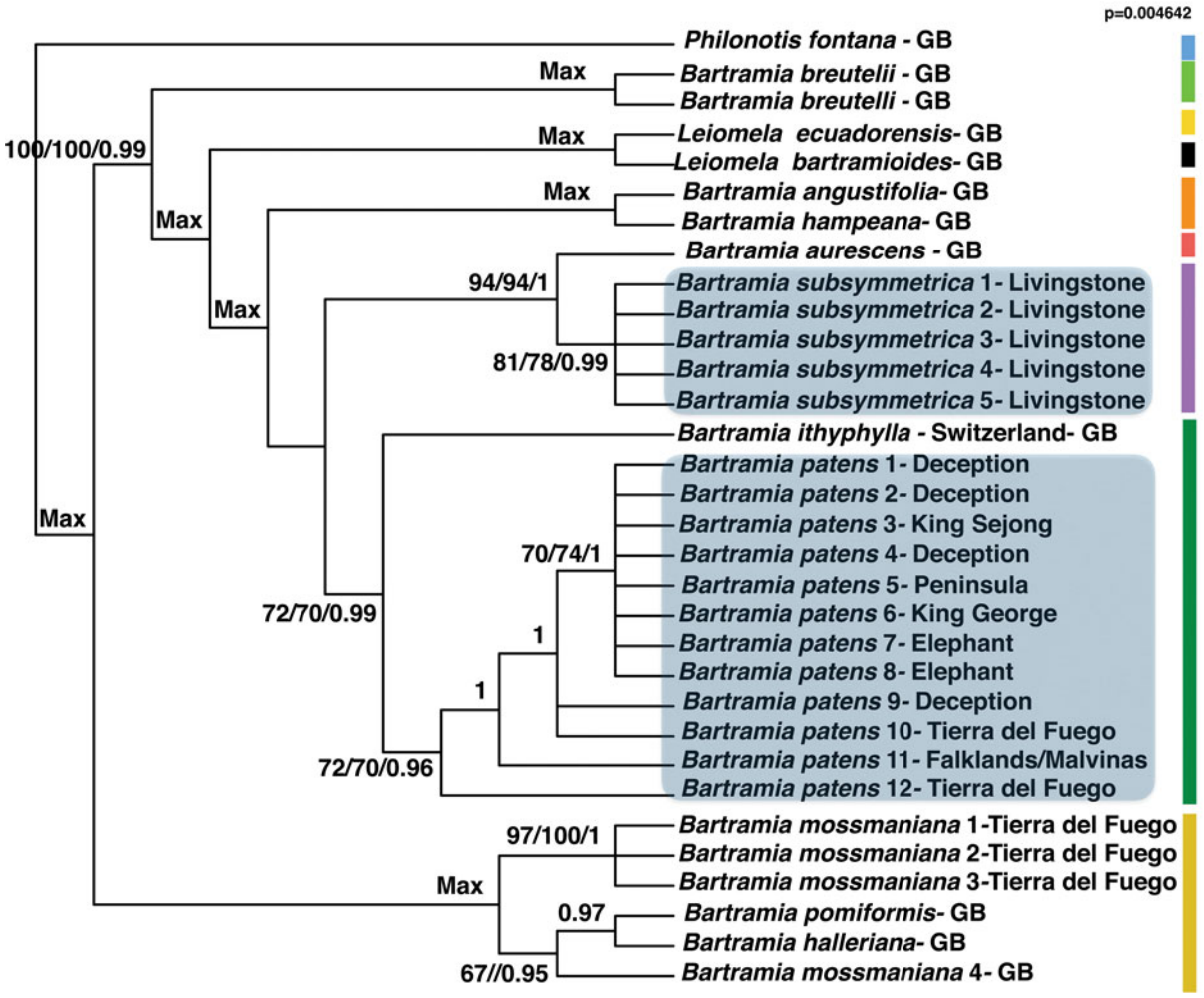

Fig. 1. Cladogram obtained from Bayesian inference using a combined matrix of chloroplast *trn*S–*rps*4–*trn*T/*trn*L–*trn*F and nuclear ribosomal *ITS* sequences plus indels coded by simple indel coding. Numbers above branches are bootstrap support values for maximum parsimony (MP-BS) and maximum likelihood (ML-BS) and posterior probabilities (PP) based on Bayesian inference, respectively. 'Max' indicates nodes with maximum support (MP-BS 100%, ML-BS 100%, PP 1.00). 'GB' denotes sequences downloaded from GenBank. Coloured bars on the right indicate the species clusters from ABGD species delimitation analysis.

Table III. Morphological comparison of *Bartramia patens* and *Bartramia ithyphylla* as inferred from analysis of herbarium specimens.

Character	*Bartramia patens*	*Bartramia ithyphylla*
Gametophyte colour	Glaucous green	Yellowish–green
Shoot height (cm)	0.5–4.0	1.0–4.5
Tomentum	Stems tomentose only at base	Stems totally tomentose
Leaf tips	Usually broken	Slightly broken, majority unbroken
Leaf margins	Unvolute at subula, dentate only to upper part of the shoulder	Plane at subula, dentate along leaf margin
Capsule surface	Slightly sulcate when dry, appearing to be smooth	Sulcate when dry, with distinct grooves

tree, *B. aurescens* (eperistomate) and the other Antarctic species, *B. patens* (double peristome). Gametophytically, *B. patens* differs from *B. subsymmetrica* by longer cells of the leaf limb with low mamillae and synoicous sexual condition (Fransén 2004b). In addition, *B. subsymmetrica* is characterized by often considerably longer shoots (up to 8 cm; Fransén 2004b) than those observed in other *Bartramia* species, and the costa has greater than 20 stereid cells and being visible at the back, in contrast with the costa having fewer than 15 stereid cells and not being visible at the back in *B. patens*.

Our data indicate that *B. ithyphylla* from the Northern Hemisphere is sister to a clade of the Southern Hemisphere *B. patens*. Unfortunately, no PCR products could be obtained from further specimens of Northern

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Hemisphere *B. ithyphylla*, but the comparison with *ITS2* sequences from northern European and Arctic specimens in GenBank at least indicated that the single specimen included in the present phylogenetic analyses was correctly identified.

There have long been discussions on the similarities of *B. ithyphylla* and *B. patens*. Fransén (2004b) concluded that it is not possible to separate both species based on morphology due to overlap in the traditionally used characteristics. He decided to treat them as subspecies of *B. ithyphylla*, whose sole difference is geographical distribution, meaning that if a specimen comes from the Northern Hemisphere, it would be named as subsp. *ithyphylla*, and as subsp. *patens* if it comes from the Southern Hemisphere. In this circumscription, *B. ithyphylla* is a bipolar species with differentiation at the subspecies level. Ochyra *et al.* (2008), in contrast, considered differences in leaf anatomy, originally reported by Matteri (1985), to be sufficient for distinguishing both taxa as separate species, namely a tristratose limb in the median part and a rather flat costa in *B. patens vs* a bistratose limb and dorsally prominently convex costa in *B. ithyphylla*.

Our analysis of herbarium specimens confirmed the morphological differences in leaf cross-sections, and also revealed several further characteristics that allow, despite some overlap, *B. patens* to be distinguished from *B. ithyphylla* (Table III). Not only the sequenced *B. patens* specimens from Antarctica, but also the three samples from the Falkland Islands and Tierra del Fuego, which we originally identified as *B. ithyphylla*, and all further morphologically studied Southern Hemisphere specimens fit the current morphological concept of *B. patens*.

Consequently, the strict separation of a Northern Hemisphere taxon and a Southern Hemisphere taxon is confirmed. Both options (two species or two intraspecific taxa) are equally supported by the present phylogenetic reconstructions. The ABGD approach suggests that we should treat them as one species. However, several studies have demonstrated that ABGD has the tendency to overlump species (Renner *et al.* 2017, Dellicour & Flot 2018). Considering these observations, and given the re-evaluated morphological differentiation, we agree with Ochyra *et al.* (2008) that we should keep *B. patens* separate from *B. ithyphylla* at the species level. However, further molecular analyses based on extended specimen and marker sampling are desirable in order to study patterns of intraspecific molecular variation in *B. patens* and their possible correlation with morphological variation in this polymorphic species in more detail. Such analyses should reveal, for example, the distribution of the second genotype, now found in only one specimen from Deception Island, which would be important for future conservational measures to protect Antarctica's genetic diversity, especially considering that Maritime Antarctica is facing severe environmental and climate changes.

The present data on the species of sect. *Bartramia* (*B. halleriana*, *B. mossmaniana* and *B. pomiformis*) may present a similar case to the distinction of *B. patens*/*B. ithyphylla*. The ABGD approach suggested that they should all be treated as a single species, which may be supported by the division of the *B. mossmaniana* samples in two subclades and by the fact that, according to Fransén (2004a), the characteristics used to separate those species are length of the seta and geographical range only. The Southern Hemisphere *B. mossmaniana*, in particular, is similar in several morphological characteristics to both Northern Hemisphere species, differing from *B. halleriana* due to the presence of rectangular laminal cells in *B. mossmaniana* and also by its distinct geographic range that differs from that of *B. porniformis* (Fransén 2004a). Under this scenario, the whole clade would represent either *B. mossmaniana* or - if the identification of *B. halleriana* and *B. pomiformis* is correct - *B. halleriana*, the type species of *Bartramia*. In either case, the clade would represent a bipolar species that has not yet been considered as such. However, ABGD may also overlump the species in this clade, and the three newly analysed specimens may represent the true *B. mossmaniana*. In any case, the identification of the GenBank specimens should be thoroughly checked. Considering that *B. pomiformis* includes 16 names in synonymy (Fransén 2004a, 2004b) and that *B. pomiformis* var. *elongata* Turner (included in *B. pomiformis* by Ignatov & Afonina 1992) was characterized as intermediate between *B. halleriana* and *B. pomiformis*, analyses of a larger number of specimens from all three species should be performed.

As discussed by Damayanti *et al.* (2012), further research is necessary to address the circumscription of the genus *Bartramia* due to the nested position of *Leiomela* in the phylogenetic reconstructions. *Leiomela* has formerly been treated as a subsection of *Bartramia* and could be included in *Bartramia* again to keep the latter monophyletic. Otherwise, if *Leiomela* is to be recognized on the genus level, all three currently recognized sections of *Bartramia* should be treated as separate genera as well. Damayanti *et al.* (2012) suggested that a larger taxon sampling may solve this issue. Our study, based on the same marker regions but a different sampling of *Bartramia* species as in Damayanti *et al.* (2012), corroborates the nested position of *Leiomela*, but suggest partly different relationships (e.g. a basal position of sect. *Bartramia*). Consequently, a still more comprehensive analysis in terms of markers and taxa is necessary. Nevertheless, the present results corroborate other recent studies (e.g. Biersma *et al.* 2018b) that a combined morphomolecular approach facilitates species detection, improves species

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delimitation and results in better knowledge of species diversity of mosses in Antarctica.

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Author contributions

PEASC designed the study. PEASC, DKH, DFP and JB carried out the fieldwork. PEASC, AERS, DKH and MCS performed the lab work and sequence editing. PEASC and MS carried out the molecular analyses. DFP and JB carried out the morphological analyses. PEASC and MS wrote the manuscript with contributions from all co-authors.

Details of data deposit

DNA data are available at GenBank and accession numbers are provided in Table I.

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