

1       **The cosmopolitan moss *Bryum argenteum* in Antarctica:**  
2                               **recent colonisation or *in situ* survival?**

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19 **Abstract**        Since the onset of glaciation following the Oligocene (30–28 Ma), the prevalence of  
20 increasingly cold conditions has shaped the evolution of the Antarctic biota. Two hypotheses, post-  
21 glacial recruitment from extra-regional locations and *in situ* persistence, have been proposed to explain  
22 the biogeography of the contemporary species-poor terrestrial Antarctic biota. Bryophytes, which form  
23 a major group of the Antarctic flora, exhibit a strong, inherent ability to survive cold conditions but  
24 also have high long-distance dispersal capacities, which are compatible with both hypotheses. Here, we  
25 test these hypotheses by means of population genetic and phylogeographic analyses of the  
26 cosmopolitan moss *Bryum argenteum*. We find evidence for at least three independent colonisation  
27 events of the species in Antarctica. Ancestral area reconstruction coupled with molecular dating  
28 suggest colonisation times of the different Antarctic clades ranging from four million years for the  
29 oldest lineage to half a million years for the youngest lineage. This suggests multiple colonisation  
30 events of Antarctica by this species during several glacial cycles within the Pleistocene, Pliocene and  
31 possibly late Miocene. This is the first study to demonstrate *in situ* persistence of bryophytes in  
32 Antarctica throughout previous glaciations.

33

34 **Keywords**        glacial refugia, dispersal, bryophytes, Antarctica, molecular dating, phylogeography

## 35 **Introduction**

36

37 The prevalence of increasingly cold conditions has shaped the evolution of the Antarctic biota since its  
38 separation from the other Southern Hemisphere continents during the final stages of the breakup of the  
39 supercontinent Gondwana (Convey and Stevens 2007; Convey et al. 2009; Fraser et al. 2012). Climate  
40 cooling was accompanied by a decrease in diversity of the angiosperm-dominated vegetation that  
41 inhabited the Antarctic Peninsula and parts of the continent during the Eocene. A mosaic of southern  
42 beech and conifer-dominated woodlands and tundra, comparable to that in parts of southern South  
43 America (Patagonia) today, characterised the region throughout the Oligocene (approximately 34–23  
44 Ma). By the middle Miocene (approximately 16–11.6 Ma), localized pockets of tundra persisted until  
45 at least 12.8 Ma (Anderson et al. 2011). The transition to cold-based, alpine glacial regimes  
46 characterized by perennially dry and frozen conditions from 13.85 Ma has not subsequently been  
47 reversed (Lewis et al. 2008). Since the late Miocene, the Antarctic ice sheets have repeatedly thickened  
48 and advanced beyond their current limits onto the continental shelf. Ice sheets are believed to have  
49 overrun most currently ice-free areas during glaciations in the Pliocene (5–2.6 Ma) and the Pleistocene  
50 (2.6 Ma–10 ka), including the Last Glacial Maximum (LGM; around 22–17 ka) (Convey et al. 2009,  
51 and references therein; Mackintosh et al. 2013). The terrestrial biota remaining in Antarctica today is  
52 depauperate in terms of species diversity, and is often cryptic, the major groups of organisms  
53 represented including micro-invertebrates, cryptogams and microbial groups (Convey 2013).

54 Two main hypotheses have been proposed to explain how the contemporary terrestrial Antarctic  
55 biota endured the glacial events of the Pleistocene (Convey and Stevens 2007; Convey et al. 2008). The  
56 first, and long-held but generally untested, view is that the large majority of the Antarctic terrestrial  
57 biota was eradicated from both the Antarctic continent and the associated offshore islands and  
58 archipelagos of the Scotia Arc (including sub-Antarctic South Georgia). The considerably expanded  
59 and thickened ice sheets would have caused a complete loss of terrestrial exposures and habitats during  
60 the LGM, as is consistent with current glaciological model reconstructions. As a consequence most or  
61 all current Antarctic terrestrial biota would have had to (re-)colonise the continent after the LGM,  
62 either from disjunct populations or from refugia. However, while the long-distance dispersal (LDD)  
63 capacity of bryophytes would potentially have facilitated (re)colonisation on such a timescale, the  
64 ability to disperse over long distances is apparently more limited for many other groups of Antarctic

65 organisms. An additional weakness of the hypothesis is that many elements of the contemporary  
66 Antarctic biota show high levels of endemism (Øvstedal and Lewis Smith 2001; Pugh and Convey  
67 2008), which could not have arisen after the LGM. The persistence of such an endemic biota would  
68 require refugia beyond the current confines of Antarctica (for instance in the more distant sub-Antarctic  
69 islands or the other Southern Hemisphere continents), which would have been situated beyond the  
70 current distributions of many species. Therefore, this hypothesis would imply multiple colonisation  
71 events out of Antarctica when refugia were required, and subsequent extinction from them once  
72 Antarctica was recolonised. The alternative hypothesis is that species have survived *in situ*, in multiple  
73 refugia that must have been present in different regions across Antarctica. This hypothesis has received  
74 increasingly strong support in recent years from both molecular and classic biogeographical studies, as  
75 well as from geological and geomorphological evidence demonstrating the diachrony of ice-sheet  
76 expansions around Antarctica, and refining both the thickness and timing of previous episodes of  
77 maximum ice sheet extent (Convey et al. 2008, 2009; Pugh and Convey 2008; Vyverman et al. 2010;  
78 Fraser et al. 2012).

79 Bryophytes (mosses and liverworts) are the dominant land plant flora in Antarctica, reaching their  
80 greatest diversity and extent in the Antarctic Peninsula and Scotia Arc (Ochyra et al. 2008; Convey  
81 2013). As a group, they are generally regarded as possessing strong LDD capacities, supported by both  
82 direct (Lönnel et al. 2012; Sundberg 2013; van Zanten 1978, 1981) and indirect (see Szövényi et al.  
83 2012 for review) evidence. These characteristics would, in principle, equip them well for recolonisation  
84 of Antarctica following any episode of regional extinction. . Elsewhere, recent evidence points to the  
85 major role of oceanic islands as glacial refugia for the subsequent (re-)colonisation of continents  
86 (Laenen et al. 2011, Hutsemékers et al. 2011). However, the geographic scale of Antarctic isolation  
87 from other landmasses, along with protection from direct north-south transfer by atmospheric and  
88 ocean currents, give the continent considerable geographic isolation (Barnes et al. 2006). Alternatively,  
89 a feature common among most bryophytes is their ability to grow at low (sub-optimal) temperatures.  
90 More than half of the 40 temperate species investigated by Furness and Grime (1982) showed a growth  
91 reduction of less than 50% at 5°C compared to growth at their optimal temperature, and this feature has  
92 also been described in Arctic and Antarctic bryophytes (Longton 1988). Indeed, many species,  
93 including some from the tropics, seem to be physiologically pre-adapted to cold and can survive  
94 temperatures ranging from -10 to -27°C (Glime 2007). Recently, La Farge et al. (2013) have provided

95 evidence for totipotent capacity (the ability of a cell to dedifferentiate into a meristematic state, and  
96 subsequently regrow) in Arctic bryophyte tissue buried by ice for 400 years, and Roads et al. (2014)  
97 have reported regrowth from gametophytes in moss banks preserved in permafrost for over 1.5 Ka.  
98 Furthermore, population genetic data for the temperate moss *Homalothecium sericeum* (Hedw.)  
99 Schimp. support persistence of the species in micro-refugia within the extensively glaciated northern  
100 Europe during the LGM (Désamoré et al. 2012). These examples suggest that bryophytes may be  
101 viable candidates to have survived Antarctic glacial cycles *in situ*.

102 Using the cosmopolitan moss *Bryum argenteum* Hedw. as a model, Hills et al. (2010) interpreted  
103 the lower genetic diversity observed in Antarctic vs non-Antarctic samples as a consequence of a lower  
104 rate of DNA substitution and isolation in refugia within Victoria Land since the Pleistocene. However,  
105 in the absence of 1) evidence for heterogeneity of DNA substitution rate among lineages, 2) an explicit  
106 time frame, 3) representative sampling across the entire range of the species, and 4) explicit analyses of  
107 population genetic structure, the hypothesis of survival in extra-Antarctic areas with subsequent (re-)  
108 colonisation cannot be excluded.

109 Here, we present a reconstruction of the phylogeography of *B. argenteum* at the global scale, and  
110 use molecular dating techniques to determine whether its presence in Antarctica is the result of recent  
111 (re-)colonisation from Pleistocene refugia outside Antarctica (H1), or whether it survived the  
112 Quaternary and Tertiary glaciations *in situ* (H2). If H1 holds true, we would expect Antarctic  
113 populations to be of recent, post-glacial origin, and therefore to show relatively little genetic  
114 differentiation from populations from other regions. Under that hypothesis, colonisation events might  
115 occur more frequently than under a scenario of long-term *in situ* persistence. Therefore, we would also  
116 expect under H1 a high gene flow from populations outside Antarctica, which could lead to a decrease  
117 in the signature of any founder effect. Conversely, if H2 holds true, we would predict that extant  
118 Antarctic populations derive from ancestors distributed on this continent before the LGM. We would  
119 further expect, provided that gene flow with the sub-Antarctic islands and other Southern Hemisphere  
120 areas has been limited, Antarctic populations to be genetically isolated from other regions and exhibit a  
121 clear phylogeographic signal (*sensu* Pons and Petit 1996).

122

## 123 **Materials and Methods**

124

125 Specimen sampling and molecular protocols

126

127 *Bryum argenteum* is a cosmopolitan, weedy moss species that thrives in disturbed environments. Its  
128 natural occurrence in Antarctica is indicated by its presence in the earliest botanical records for the  
129 continent and its widespread distribution within the regions where it occurs (Ochyra et al. 2008;  
130 Cannone et al. 2013). A total of 154 accessions of *B. argenteum* were sampled from Africa, America,  
131 Asia, Antarctica, the sub-Antarctic islands, Australasia and Europe. From these accessions, 28 samples  
132 were taken from previously published papers (Hills et al. 2010; Pisa et al. 2013; Skotnicki et al. 2005)  
133 available in GenBank. The remaining samples were sequenced for this study, and included 47  
134 specimens collected by the authors and colleagues (all retained at the herbarium of the Universidad de  
135 Murcia) and 70 specimens held at the institutional herbaria of the British Antarctic Survey, California  
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137 Garden of the Russian Academy of Sciences, Moscow State University, New York Botanical Garden,  
138 Royal Botanic Garden Edinburgh, Swedish Museum of Natural History, University of Connecticut, and  
139 the private herbaria of D.T. Holyoak and B. Goffinet. Four of the closely related species to *B.*  
140 *argenteum* (Wang and Zhao 2009) were selected as outgroup species (Appendix 1). The geographic  
141 location of the accessions is detailed in Fig. 1.

142 Total genomic DNA was extracted following the protocol described in Werner et al. (2002), or  
143 using the DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany). PCR amplification and  
144 sequencing of the nuclear internal transcribed spacer (ITS) region were performed using the protocol  
145 described in Pisa et al. (2013). Most of the amplifications were carried out using universal primers  
146 AB101 and AB102 (Douzery et al. 1999), with some nucleotide modifications to adapt these primers to  
147 *B. argenteum*. In some cases, sequences were generated using universal primers ITS-A and ITS-B as  
148 described in Blattner (1999), employing a similar PCR step as in Pisa et al. (2013), with exceptions  
149 being the use of the Taq PCR Core Kit (Qiagen GmbH, Hilden, Germany) and an annealing  
150 temperature of 50°C. Forward and reverse sequence fragments for both ITS1 and ITS2 were edited and  
151 assembled using Bioedit 7.05 (Hall 1999) and every polymorphism was checked from the  
152 chromatograms. The sequences were aligned by eye, adding gaps where necessary to conserve  
153 homology between sequences (Appendix 2). Gaps were counted with SeqState (Müller 2005) using

154 complex indel-coding. The number of polymorphic sites was calculated with DnaSP (Librado and  
155 Rozas 2009).

156 ITS remains the most widely used source of information on genetic variation at the species level in  
157 plants and fungi (reviewed by Nagy et al. 2012), although its use in phylogenetics has been questioned  
158 due to the potential presence of paralogs and pseudogenes (see Nieto Feliner and Rosselló 2007 for  
159 review). In mosses in particular, evidence for ITS paralogy was recently reported (Košnar et al. 2012).  
160 We consider that the use of ITS in *B. argenteum* was justified in the current study as no conflicting  
161 base calls during direct sequencing were observed, suggesting that the presence of intragenomic  
162 paralogous copies is unlikely.

163

164 Population genetic analyses

165

166 The sequences were grouped into haplotypes using DnaSP. For each of the seven geographic regions  
167 defined above, we calculated haploid diversity ( $h$ ), unbiased haploid diversity by population ( $uh$ ) and  
168 frequency of endemic haplotypes ( $x$ ) using GENALEX 6.5 (Peakall and Smouse 2006) and Tajima's  $D$   
169 using Arlequin 3.5 (Excoffier et al. 2005). Genetic differentiation among geographic regions and  
170 presence of phylogeographic signal in the data were assessed by means of comparative analyses  
171 employing fixation index ( $F_{ST}$ ) and  $N_{ST}$ .  $N_{ST}$  is a measure of genetic differentiation among populations;  
172 it is analogous to  $F_{ST}$  but takes the genetic distances among genotypes (here, the pairwise distance  
173 among them) into account (Pons and Petit 1996). When  $N_{ST} > F_{ST}$ , it means that mutation rates are  
174 higher than dispersal rates between geographical regions, generating a phylogeographic pattern. The  
175 significance of  $F_{ST}$  and  $N_{ST}$  were tested by constructing the distribution of the null hypothesis by means  
176 of 9,999 random permutations of individuals among geographic regions, as implemented by Spagedi  
177 1.3 (Hardy and Vekemans 2002). The existence of a phylogeographic signal was tested by assessing  
178 the significance of the observed difference between  $N_{ST}$  and  $F_{ST}$  values by means of 9,999 random  
179 permutations of the allele distance matrix. Global  $F$  and  $N$  statistics among the seven geographic  
180 regions were computed, as well as pairwise statistics among regions. The correction of Benjamini and  
181 Yekutieli (2001) for multiple tests was applied to determine the significance of the pairwise statistics.

182

183 Phylogeny, molecular dating, and ancestral area reconstructions

184

185 The phylogeny and divergence time among ITS genotypes within *B. argenteum* were investigated  
186 using BEAST v1.7.5 (Drummond et al. 2012). In the absence of fossil evidence, a prior on the absolute  
187 rate of molecular evolution was used, following the procedure described in Huttunen et al. (2008) and  
188 Aigoïn et al. (2009). In the absence of a substitution rate for ITS in bryophytes, we used a normal  
189 distribution with a mean and standard deviation of  $4.125e^{-3}$  and  $1.807e^{-3}$  substitutions per site per  
190 million years, respectively, which corresponds to the average absolute substitution rates of ITS across a  
191 wide range of annual herbaceous species (Kay et al. 2006). However, we consider that this rate is  
192 likely to overestimate substitution rates in mosses, which are longer-lived and rely for a large part on  
193 asexual reproduction. This is particularly the case in *B. argenteum*, which is thought to be sterile in  
194 Antarctica (Ochyra et al. 2008). On average, the substitution rate of 18S rDNA, the neighbouring  
195 region of ITS, in mosses is suggested to be less than half that of vascular plants (Stenøien 2008). It is  
196 likely that the substitution rate used here may therefore be an overestimate of the true rate for this  
197 species, and therefore that divergence times derived from this substitution rate may be significantly  
198 underestimated.

199 The Hasegawa, Kishino and Yano (HKY) model with gamma distribution and invariant sites had  
200 the best Bayesian information criterion (BIC) score for the ITS dataset using jModeltest 2.1.4 (Darriba  
201 et al. 2012). A relaxed clock with lognormal distribution was employed for the analysis. Before  
202 running the final dating analysis, the performance of five tree models (i.e. coalescent with constant size  
203 population, coalescent under an extended Bayesian skyline including the two linear and stepwise  
204 models, speciation under a birth-death process and speciation under Yule process) were compared by  
205 using a model selection procedure based on Bayes factors calculated in TRACER v1.5 (Rambaut and  
206 Drummond 2009). Overall, the model using the coalescent under a stepwise extended Bayesian skyline  
207 model (Heled and Drummond 2008) performed best (data not shown). Four independent Markov chain  
208 Monte Carlo (MCMC) analyses were each run for 100,000,000 generations for every model. Parameter  
209 values were sampled every 10,000 generations and convergence and acceptable mixing of the samples  
210 were checked using the program TRACER v1.5. After discarding the burn-in steps (2,000 trees), the  
211 runs were combined to obtain an estimate of the posterior probability distributions of the dates of  
212 divergence.



213 To estimate ancestral areas, we used the maximum likelihood dispersal–extinction–cladogenesis  
214 (DEC) method (Ree et al. 2005; Ree and Smith 2008a) as implemented in the software Lagrange build  
215 20091004 (Ree and Smith 2008b) on the BEAST chronogram. Each genotype was assigned to one or  
216 more of the seven geographic regions defined above. We conducted the analysis in Lagrange based on  
217 an unconstrained model permitting an equal probability of dispersal between all areas at any time.

218

## 219 **Results**

220

221 The complete alignment had a total length of 928 bp after the exclusion of the 5.8S rRNA gene. No  
222 further region was excluded from the alignment. There were 328 sites with gaps, corresponding to 106  
223 indels and 111 polymorphic sites. The alignment excluding outgroup sequences had a total length of  
224 844 bp. There were 173 sites with gaps, corresponding to 78 indels and 81 polymorphic sites, allowing  
225 for the identification of 77 haplotypes (Appendix 1). Haploid diversity unbiased by population size was  
226 lowest in Antarctica ( $uh=0.62$ ) as compared to other regions (0.79 - 0.94) (Table 1). The frequency of  
227 endemic haplotypes exhibited the reverse trend, reaching its highest value ( $x=0.90$ ) in Antarctica. None  
228 of Tajima's D statistics differed significantly from 0.

229 There was a significant difference in genotype frequency among geographic regions (Global  
230  $F_{ST}=0.146$ ,  $P<0.0001$ ). The global  $N_{ST}$  (0.267,  $P<0.0001$ ) was significantly higher than  $F_{ST}$  ( $P<0.0001$ )  
231 providing evidence that, on average, the genotypes from the same region were more closely related  
232 than the genotypes from different regions. This geographic structure was largely due to the significant  
233 genetic isolation of Antarctica. The phylogeographic signal between Antarctica and any of the other six  
234 regions was consistently significant, whereas a significant phylogeographic signal could not be  
235 detected among any other pairs of regions (Table 2).

236 Accessions from Antarctica belonged to three clades (Fig. 2). Clade I was mainly composed of  
237 Antarctic genotypes, with the exception of one European genotype and one common genotype shared  
238 between Antarctica, Europe, Asia and America. Clade II was composed of Antarctic, Sub-Antarctic,  
239 American and Australasian genotypes. Clade III was composed of Antarctic and American genotypes.  
240 The Lagrange analysis indicated that the most recent common ancestor of clade I, which may have  
241 been distributed across Asia, Europe, and America, colonised Antarctica 4.36 Ma (Highest Posterior  
242 Density, HPD, 1.79-14.72). In clades II and III, the earliest colonisation of Antarctica dates back to

243 1.43 Ma (HPD 0.42-4.97) and 0.55 Ma (HPD 0.13-1.97), respectively, from an ancestor most closely  
244 related to American populations. Potential dispersal events from Antarctica to other regions are not  
245 excluded as genotypes in clade II located in Australasia and the sub-Antarctic islands come from  
246 ancestors distributed across Antarctica and America.

247

## 248 **Discussion**

249

250 Evidence for at least three distinct origins of *B. argenteum* in Antarctica was found, with colonisation  
251 times ranging between approximately 4.4 Ma (clade I), 1.4 Ma (clade II), and 0.6 Ma (clade III).  
252 However, as mentioned above, the substitution rate of Kay et al. (2006) is based on studies of annual  
253 herbaceous species, and is likely to be much higher than in mosses. A study on relative substitution  
254 rates amongst major plant groups showed that, on average, the substitution rate of 18s rDNA, the  
255 neighbouring region of ITS, is more than twice as high in vascular plants compared to mosses  
256 (Stenøien 2008). This suggests that the presence of *B. argenteum* in Antarctica may be considerably  
257 more ancient than estimated here. The current study therefore provides the first support for the long-  
258 term persistence *in situ* of a bryophyte species in Antarctica, with time-scales in the order of millions of  
259 years. No evidence supporting strict post-Pleistocene (i.e. recent) colonisation (H1) was found in any  
260 of the lineages. Our results, however, do not exclude potential dispersal events from Antarctica to other  
261 regions and future studies with a larger sample size and obtained from more locations may identify  
262 evidence for recent colonisation events.

263 In agreement with our finding of long-term persistence of *B. argenteum* in Antarctica, a significant  
264 phylogeographical signal was found in all pairwise comparisons between Antarctica and each of the six  
265 other global regions, while no such signal was identified amongst the latter. This indicates that extant  
266 patterns of genetic diversity of Antarctic *B. argenteum* populations are better explained in terms of *in*  
267 *situ* diversification than recruitment of migrants from other areas, resulting in the highest proportion of  
268 endemic haplotypes as compared to other regions of the world. Such an interpretation is consistent with  
269 recent developments in biogeographical knowledge of much of the contemporary terrestrial biota in  
270 Antarctica. Evidence for long-term history *in situ* has been reported in all major groups except the  
271 bryophytes, with timescales ranging from mid-Pleistocene (e.g. diatoms, rotifers, cladocerans) to  
272 Pliocene, Miocene and Gondwana-breakup (e.g. springtails, chironomid midges, mites, copepods,

273 microorganisms) (see Convey et al. 2008, 2009, and references therein; Vyverman et al. 2010).  
274 McGaughran et al. (2010), in a comparative phylogeographic study of different springrail (Collembola)  
275 species in Victoria Land and along the Scotia Arc and Antarctic Peninsula, identified analogous  
276 evidence of intraregional differentiation and hence colonisation patterns on timescales dating back to at  
277 least the earliest Pleistocene.

278 How and where bryophytes and other terrestrial biota could have survived through glaciation events  
279 within Antarctica is not yet well understood. The lowest ITS haplotypic diversity within *B. argenteum*  
280 worldwide was observed in Antarctica, as in the cosmopolitan moss *Ceratodon purpureus* Hedw.  
281 (Brid.) (Clarke et al. 2008, 2009). Although the hypothesis of lower mutation rates in Antarctic *B.*  
282 *argenteum* populations cannot be ruled out (Hills et al. 2010), such a low regional genetic diversity can  
283 also be interpreted in terms of the sterile condition of *B. argenteum* in Antarctica (Ochyra et al. 2008),  
284 either due to the regional absence of one of the sexes in this dioicous species, and/or inhibition of sex  
285 expression due to prevailing cold and dry conditions (Longton 1988). Yet, analyses of patterns of  
286 genetic diversity in Antarctic populations of *B. argenteum* failed to evidence a significant bottleneck.  
287 This observation does not support the hypothesis of a substantial past decrease in population size and  
288 points to the persistence of sufficiently large and numerous populations of the species through time. In  
289 Antarctica, areas of heated ground associated with geothermal activity, where *B. argenteum* is known  
290 to occur (Convey et al. 2000), may be particularly relevant in considering the locations of some  
291 potential refuge sites (Convey and Smith 2006, Fraser et al. 2014). Although individually ephemeral,  
292 the presence of geothermal habitats may have extended over considerable time periods, as volcanism  
293 has been widespread throughout the Tertiary in parts of the northern Antarctic Peninsula and elsewhere  
294 in Antarctica (Baker et al. 1975, Convey et al. 2000; Convey and Smith 2006; Fraser et al. 2014).  
295 Therefore, geothermal habitats might have played a key role in the longer-term regional persistence of  
296 species with rapid colonisation capacities such as bryophytes, allowing survival through periods of  
297 apparently greater environmental extremes than are currently experienced (Convey and Smith 2006).

298

## 299 **Conclusion**

300

301 This study demonstrates for the first time *in situ* persistence of bryophytes in Antarctica throughout  
302 previous glacial cycles and contradicts the hypothesis of post-glacial recruitment from extra-regional

303 locations..Although *B. argenteum*, like other moss species, exhibits the lowest levels of genetic  
304 diversity worldwide in Antarctica, ITS variation is substantial within and among Antarctic populations,  
305 making it a highly suitable model for investigating fine-scale patterns of genetic structure and diversity  
306 at continental scale in order to reconstruct its biogeographic history. In particular, detailed  
307 phylogeographic information at the Antarctic scale would allow for testing Fraser's et al. (2014)  
308 hypothesis that refugia indeed correspond to areas of high geothermal activity and contribute, together  
309 with information from other terrestrial organisms (Terauds et al. 2012), to enhancing the identification  
310 of bioregions that are not fully represented in the current Antarctic Specially Protected Area network.

311

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313

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326

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480

481 **Figure captions**

482 **Fig. 1** Geographic locations of *Bryum argenteum* and outgroup accessions used.

483 **Fig. 2** Spatial and temporal dimensions of *Bryum argenteum* evolution. Chronogram of the fifty per  
484 cent majority-rule consensus of the trees sampled from the posterior probability distribution generated  
485 by the BEAST analysis of ITS sequences of the *B. argenteum* genotypes sampled worldwide. The  
486 maximum likelihood reconstruction of geographical range evolution under the unconstrained  
487 dispersal–extinction–cladogenesis (DEC) model (ln L=214.9) implemented in Lagrange is given in  
488 boxes at each node of interest. The geographical areas defined for this study and each haplotype are  
489 provided. The two series of reconstructions at each internal node indicate the ML ancestral range  
490 estimate for the upper and lower branch connecting that node, respectively. Boxes at terminal nodes  
491 indicate the geographic areas occupied by the accessions belonging to each haplotype. The vertical  
492 bars (I, II, and III) indicate the clades with Antarctic genotypes. Thicker branches indicate the support  
493 for the Antarctic clades (PP > 0.9). \* indicates the support for clades with PP > 0.9.

494 **Table 1** Sample size (N), number of genotypes (Na), haploid diversity (h), haploid diversity unbiased by  
 495 population (uh), frequency of endemic haplotypes (x) and Tajima's D (D) in seven geographic regions of the  
 496 worldwide distributed moss *Bryum argenteum* based on the nuclear ITS locus

<b>Continental area</b>	<b>N</b>	<b>Na</b>	<b>h</b>	<b>uh</b>	<b>x</b>	<b>D</b>
<b>Asia</b>	20	17	0.89	0.94	0.71	-0.89 ( 0.19)
<b>America</b>	27	19	0.87	0.91	0.63	- 1.27 (0.09)
<b>Europe</b>	56	22	0.77	0.79	0.68	-0.86 (0.21)
<b>Australasia</b>	7	6	0.80	0.93	0.83	0.35 (0.65)
<b>Antarctica</b>	25	10	0.59	0.62	0.90	-0.93 ( 0.28)
<b>Africa</b>	11	9	0.80	0.88	0.56	0.20 (0.61)
<b>sub-Antarctic islands</b>	8	6	0.75	0.93	0.83	0.08 (0.36)

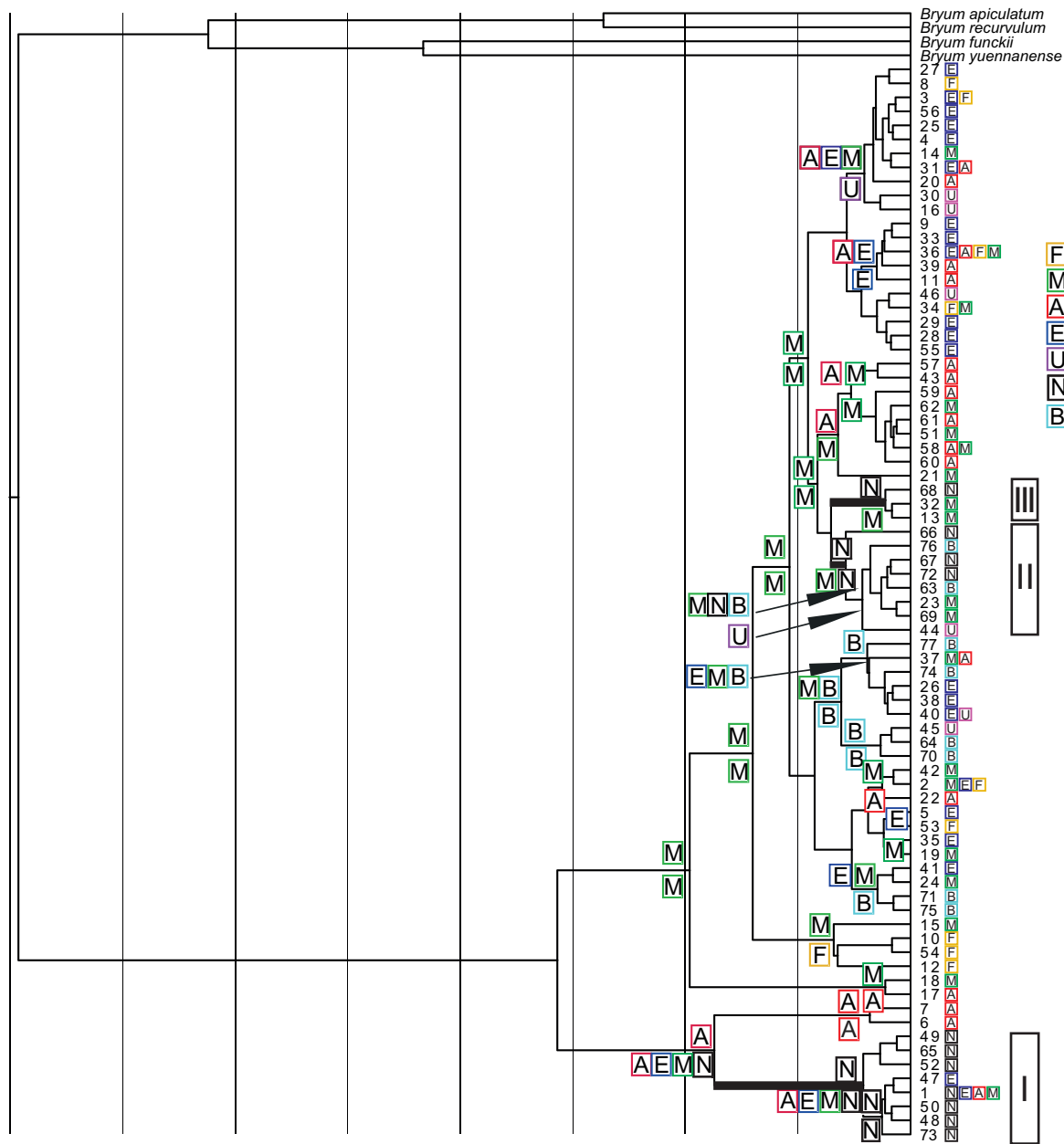
497 **Table 2** Pairwise  $F_{ST}$  values (below diagonal) and  $N_{ST}$  values (above diagonal) among seven geographic  
 498 regions of the worldwide distributed moss *Bryum argenteum* based on the nuclear ITS *locus*. The  $P$ -values (in  
 499 brackets) are associated to the null hypotheses that  $F_{ST}=0$  and that  $F_{ST}=N_{ST}$  respectively

<b>Continental</b>							
<b>area</b>	<b>Asia</b>	<b>America</b>	<b>Europe</b>	<b>Australasia</b>	<b>Antarctica</b>	<b>Africa</b>	<b>sub-Antarctic</b>
<b>Asia</b>	-	0.012 <sup>n.s.</sup>	0.157 <sup>n.s.</sup>	0.124 (0.0358)	0.383 (0.0003)*	0.060 <sup>n.s.</sup>	0.100 <sup>n.s.</sup>
<b>America</b>	0.033 (0.0159)*	-	0.135 <sup>n.s.</sup>	0.049 <sup>n.s.</sup>	0.479 (0.0001)*	0.027 <sup>n.s.</sup>	0.062 <sup>n.s.</sup>
<b>Europe</b>	0.105 (0.0004)*	0.120 (0.0001)*	-	0.180 <sup>n.s.</sup>	0.568 (0.0001)*	0.077 <sup>n.s.</sup>	0.181 <sup>n.s.</sup>
<b>Australasia</b>	0.034 <sup>n.s.</sup>	0.025 <sup>n.s.</sup>	0.122 (0.0085)*	-	0.565 (0.0002)*	0.114 <sup>n.s.</sup>	0.016 <sup>n.s.</sup>
<b>Antarctica</b>	0.186 (0.0001)*	0.210 (0.0001)*	0.272 (0.0001)*	0.251 (0.0001)*	-	0.549 (0.0002)*	0.498 (0.0047)*
<b>Africa</b>	0.056 (0.0082)*	0.044 (0.0334)*	0.107 (0.0031)*	0.066 (0.0466)	0.256 (0.0001)*	-	0.073 <sup>n.s.</sup>
<b>Sub-Antarctic</b>	0.096 (0.0007)*	0.088 (0.0046)*	0.156 (0.0015)*	0.077 (0.0468)	0.298 (0.0001)*	0.127 (0.0025)*	-

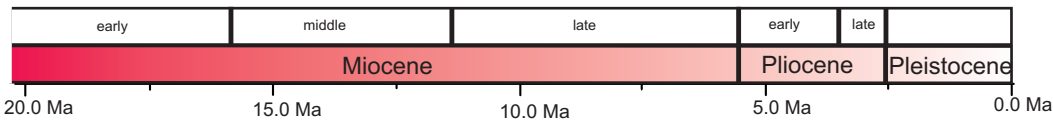
500 <sup>n.s.</sup> indicates that the test is not significant ( $P>0.05$ )

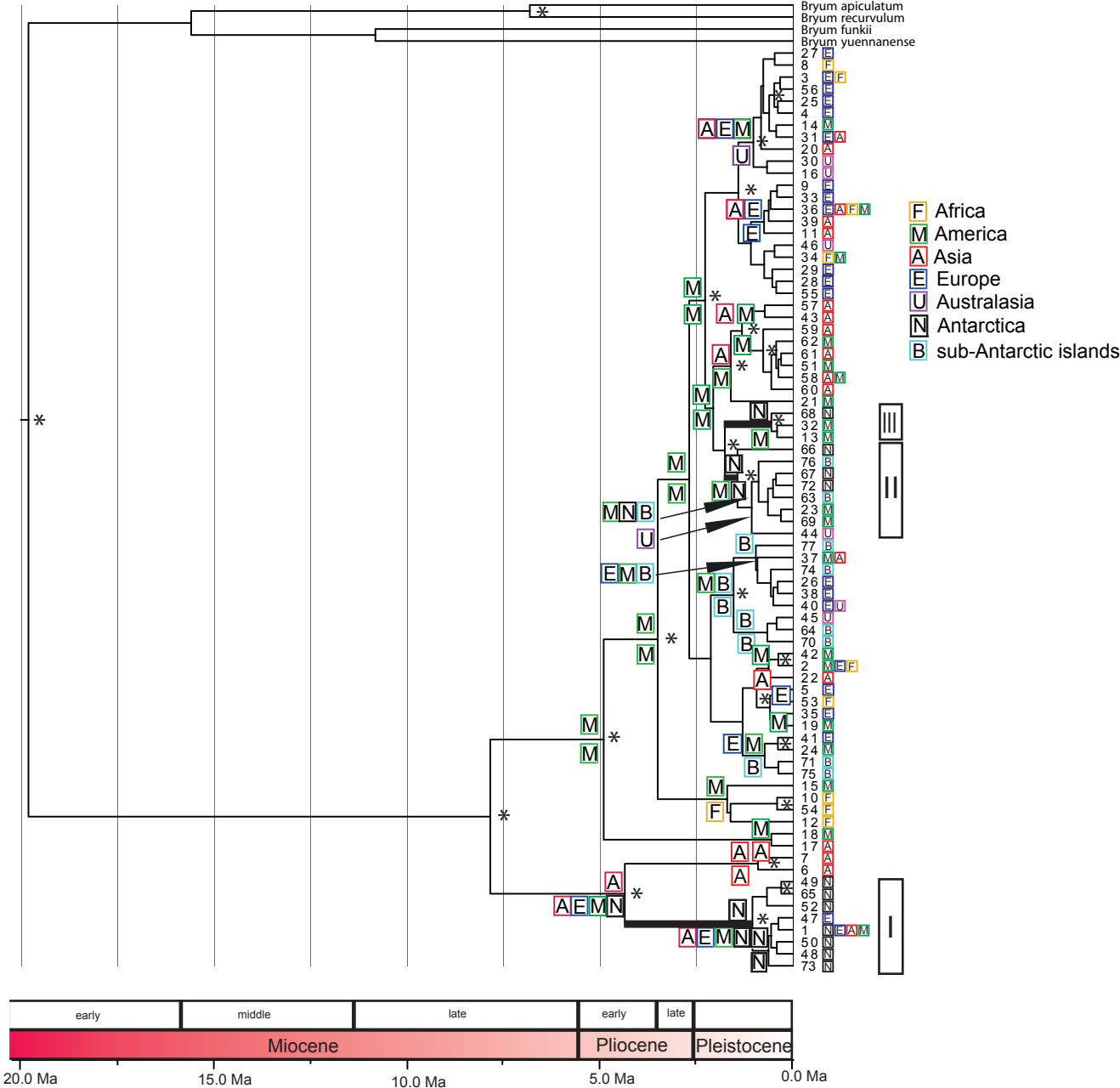
501 \* indicates that the test remains significant after the correction of Benjamini and Yekutieli (2001) for multiple

502 tests at the  $p<0.05$  significance level



- F Africa
- M America
- A Asia
- E Europe
- U Australasia
- N Antarctica
- B Subantarctic islands





**Article title:** The cosmopolitan moss *Bryum argenteum* in Antarctica: recent colonisation or *in situ* survival?

**Journal name:** Polar Biology

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**Appendix 1** Accessions used in this study. For each sample the following information is given: Identification number, *Bryum argenteum* haplotype based on nrITS sequences, or species used as outgroup; geographic origin; herbarium where it is retained or publication source if published previously; collection data for the samples sequenced for this study; geographic coordinates for the ingroup samples; GenBank accession numbers for ITS1 and ITS2 separately, or for the whole ITS region in the case the number is given in the middle of ITS1 and ITS2 columns; and the geographic region from the seven regions considered.

Id no.	Haplotype id or outgroup species	Geographic origin	Herbarium or literature reference if	Collection	Coordinates in decimal degrees	GenBank Accession N.		Geographic region
						ITS1	ITS2	
1	1	Antarctica, Beaufort Island	Hills <i>et al.</i> (2010)	-	S 76.91667 E 166.90000	GU907063		Antarctica
2	1	Antarctica, Beaufort Island	Hills <i>et al.</i> (2010)	-	S 76.91667 E 166.90000	GU907064		Antarctica
3	1	Antarctica, Canada Glacier, Taylor Valley, S. Victoria Land	Skotnicki <i>et al.</i> (2005)	-	S 77.58333 E 163.25000	AY611432		Antarctica
4	1	Antarctica, Cape Hallet	Hills <i>et al.</i> (2010)	-	S 72.30000 E 170.30000	GU907066		Antarctica
5	1	Antarctica, Cape Hallet	Hills <i>et al.</i> (2010)	-	S 72.30000 E 170.30000	GU907067		Antarctica
6	1	Antarctica, Edmonson Point, N. Victoria Land	Skotnicki <i>et al.</i> (2005)	-	S 74.33333 E 164.50000	AY611430		Antarctica
7	1	Antarctica, Granite Harbour	Hills <i>et al.</i> (2010)	-	S 77.00000 E 162.50000	GU907065		Antarctica
8	1	Antarctica, Granite Harbour, S. Victoria Land	Skotnicki <i>et al.</i> (2005)	-	S 77.00000 E 162.50000	AY611434		Antarctica
9	1	Antarctica, Marble Point	Hills <i>et al.</i> (2010)	-	S 76.43333 E 163.83333	GU907069		Antarctica
10	1	Canada, Northwest Territories, District of Fanklin, Ellesmere Island	NY 69323	<i>G.W. Scotter 45657</i>	N 79.71667 W 85.83333	KF952783	KF952892	America
11	1	Norway, Svalbard archipelago, Spitsbergen	MUB 44625	<i>M. Stech &amp; J.D. Kruijer 08-088</i>	N 78.91667 E 11.93333	KF952782	KF952891	Europe
12	1	Sri Lanka (Ceylon), Central province, Nuwara Eliya District	E 00416780	<i>C.C. Townsend 73/1779</i>	N 6.95694 E 80.10889	KF952781	KF952890	Asia
13	2	Spain, Canary Islands, La Gomera	MUB 44654	<i>J.M. González-Mancebo et al. s.n.</i>	N 28.10737 W 17.23866	KF952785	KF952894	Africa
14	2	Spain, Canary Islands, La Palma	MUB 44653	<i>J.M. González-Mancebo &amp; J. Leal</i>	N 28.67562 W 47.11100	KF952784	KF952893	Africa
15	2	Spain, Granada province, Albuñol	Pisa <i>et al.</i> (2013)	-	N 36.80025 W 3.21738	KC493867	KC493893	Europe
16	2	USA, California, Mariposa County, Yosemite National Park	CAS 1083159	<i>J.R. Shevock 29944</i>	N 37.51889 W 119.60000	KF952787	KF952896	America
17	2	USA, California, Tulare County, Sierra Nevada	CAS 1039823	<i>J.R. Shevock 20946</i>	N 36.06111 W 118.59306	KF952786	KF952895	America
18	3	Greece, Prefecture of Iliá, Ráhes	MUB 12158	<i>M.J. Cano, J. Muñoz, R.M. Ros &amp; M.</i>	N 38.90861 E 22.83500	KF952794	KF952903	Europe
19	3	Ireland, E. County Mayo	Herb. D.T. Holyoak	<i>D.T. Holyoak 03-186</i>	N 53.61889 W 9.30639	KF952788	KF952897	Europe
20	3	Morocco, High Atlas, Oukaimeden	MUB 13090	<i>R.M. Ros s.n.</i>	N 31.21667 W 7.86667	KF952793	KF952902	Africa
21	3	Spain, Ávila province, Sierra de Gredos	MUB 44652	<i>R.M. Ros &amp; O. Werner s.n.</i>	N 40.42231 W 5.29667	KF952796	KF952905	Europe
22	3	Spain, Ávila province, Sierra de Gredos	MUB 44653	<i>R.M. Ros &amp; O. Werner s.n.</i>	N 40.42231 W 5.29667	KF952789	KF952898	Europe
23	3	Spain, Ávila province, Sierra de Gredos	MUB 44652	<i>R.M. Ros &amp; O. Werner s.n.</i>	N 40.42231 W 5.29667	KF952795	KF952904	Europe
24	3	Spain, Canary Islands, Tenerife	MUB 44656	<i>J. Patiño s.n.</i>	N 28.35770 W 0.72512	KF952797	KF952906	Africa
25	3	Spain, León province, Abalgas	MUB 44660	<i>S. Pisa s.n.</i>	N 42.89139 W 5.97389	KF952790	KF952899	Europe
26	3	Spain, Murcia province, Campus de Espinardo	MUB 44663	<i>R. M. Ros s.n.</i>	N 8.02056 W 1.16944	KF952791	KF952900	Europe
27	3	Spain, Murcia province, Sierra de las Herrerías	MUB 44664	<i>R.M. Ros &amp; O. Werner s.n.</i>	N 37.58278 W 1.42667	KF952792	KF952901	Europe
28	4	France, Vosges Department, Vosges Mountains	MUB 44641	<i>R.M. Ros &amp; O. Werner s.n.</i>	N 47.90478 E 7.10286	KF952798	KF952907	Europe
29	5	UK, Great Britain, N. Northumberland	Herb. D. T. Holyoak	<i>D.T. Holyoak 08-670</i>	N 52.96667 E 0.55000	KF952799	KF952908	Europe
30	6	Taiwan, Hwalien County, Ta-yu-ling	E 00416777	<i>C.-C. Chuang 5781</i>	N 23.81667 E 121.23333	KF952800	KF952909	Asia
31	7	China, Taiwan, Nantou County, Central	CAS 995161	<i>J.R. Shevock 17888</i>	N 24.12500	KF952801	KF952910	Asia



		Mountain Range			E 121.21667			
32	8	Uganda, Western Region, Kabale	Herb. D. T. Holyoak	<i>M.J. Wigginton U5011a</i>	N 1.08333 E 29.80000	KF952802	KF952911	Africa
33	9	Spain, Madrid province, Sierra de Guadarrama	MUB 44668	<i>S. Pisa s.n.</i>	N 40.59030 W 3.98505	KF952803	KF952912	Europe
34	10	Union of the Comoros, Grande Comore (Ngazidja)	EGR	<i>R.E. Magill &amp; T. Pócs 10946</i>	N 11.61222 E 43.33222	KF952804	KF952913	Africa
35	11	Indonesia, Sumatra, Gunung Sinabung	NY 1229461	<i>L. Hoffmann 89-196</i>	N 3.18139, E 98.44111	KF952805	KF952914	Asia
36	12	Kenya, Nyandarua County, Aberdare Mts.	EGR	<i>J. Spence s.n.</i>	N 0.41083 E 36.61667	KF952806	KF952915	Africa
37	13	Bolivia, La Paz, Murillo	NY 1229477	<i>S. Churchill 22828</i>	S 16.61667 W 68.07528	KF952807	KF952916	America
38	14	Chile, Antarctica Chilena province, Isla Navarino, Magallanes	Herb. B. Goffinet	<i>B. Goffinet 6765</i>	S 54.95000 W 67.63333	KF952808	KF952917	America
39	15	USA, California, Tulare County, Sierra Nevada	CAS 989901	<i>J.R. Shevock 17562</i>	N 35.88889 W 118.34583	KF952809	KF952918	America
40	16	Australia, Canberra	Hills <i>et al.</i> (2010)	-	S 35.30000 E 149.13333	GU907059		Australasia
41	17	Thailand, Khao Yai National Park	NY 1229462	<i>C. Charoenphol 4395 with Larsen &amp;</i>	N 14.53333 E 101.36667	KF952810	KF952919	Asia
42	18	Brazil, Bahia state, Municipality of Abaíra, Campo de Ouro Fino	NY 1229480	<i>D.J.N. Hind H50910 with R.F. Queiroz</i>	S 13.25000 W 41.90000	KF952811	KF952920	America
43	19	Dominican Republic, La Vega province, Alto de la Bandera Mountain	NY 635898	<i>W.C. Steere 23098</i>	N 18.81667 W 70.61667	KF952812	KF952921	America
44	20	Russia, NW Caucasus	MW	<i>V. Onipchenko 99/95</i>	N 43.45000 E 41.68333	KF952813	KF952922	Asia
45	21	Bolivia, Cochabamba, Carrasco	NY 1229478	<i>S. Churchill 22558</i>	S 17.75000 W 64.80000	KF952814	KF952923	America
46	22	China, Yunnan province, Gongshan County	E 00477214	<i>D. G. Long 36064</i>	N 27.68539 E 98.30422	KF952815	KF952924	Asia
47	23	Ecuador, Pichinga province	NY 1229481	<i>S.P. Churchill 13518 with I. Sastre-De</i>	N 0.38333 W 78.21667	KF952816	KF952925	America
48	24	USA, California, Fresno County, Sierra Nevada	CAS 989203	<i>J.R. Shevock 13927</i>	N37.16250 W 119.09583	KF952817	KF952926	America
49	25	Sweden, Värmland	S B178200	<i>L. Hedenäs &amp; G. Odelvik s.n.</i>	N 59.78333 E 14.36667	KF952818	KF952927	Europe
50	26	Spain, Granada province, Sierra Nevada	<i>Pisa et al.</i> (2013)	-	N 37.09725 W 3.39753	KC493863	KC493889	Europe
51	27	Spain, Granada province, Vélez de Benaudalla	<i>Pisa et al.</i> (2013)	-	N 36.84465 W 3.50905	KC493873	KC493899	Europe
52	28	Spain, León province, Abalgas	MUB 44661	<i>S. Pisa s.n.</i>	N 42.89139 W 5.97389	KF952819	KF952928	Europe
53	29	Spain, Balearic Islands, Menorca	MUB 44665	<i>R.M. Ros &amp; O. Werner s.n.</i>	N 39.89944 E 4.10389	KF952820	KF952929	Europe
54	30	Papua New Guinea, Chimbu province, Wilhelm Mountain	NY 1229460	<i>J.L. De Sloover 42892</i>	S 5.75667 E 145.03556	KF952821	KF952930	Australasia
55	31	Denmark, Faroe Islands, Streymoy, Thorshavn	S B185204	<i>R. Fargerstén &amp; M. Haapasari s.n.</i>	N 62.01139 W 6.75361	KF952834	KF952943	Europe
56	31	France, Vaucluse Department, commune de Bonnieux, domaine de la Chambarelle	MUB 1097	<i>R.M. Ros s.n.</i>	N 43.81389 E 18.43333	KF952835	KF952944	Europe
57	31	Germany, Baden-Württemberg, Black Forest	MUB 44630	<i>S. Pisa s.n.</i>	N 47.84417 E 8.01917	KF952832	KF952941	Europe
58	31	Germany, Baden-Württemberg, Black Forest	MUB 44637	<i>S. Pisa s.n.</i>	N 47.86444 E 8.02194	KF952824	KF952933	Europe
59	31	Germany, Baden-Württemberg, Black Forest	MUB 44638	<i>S. Pisa s.n.</i>	N 47.85972 E 8.03639	KF952825	KF952934	Europe
60	31	Germany, Baden-Württemberg, Black Forest	MUB 44639	<i>S. Pisa s.n.</i>	N 47.85972 E 8.03639	KF952826	KF952935	Europe
61	31	Germany, Baden-Württemberg, Black Forest	MUB 44640	<i>S. Pisa s.n.</i>	N 47.85972 E 8.03639	KF952827	KF952936	Europe
62	31	Germany, Baden-Württemberg, Eberbach	MUB 44649	<i>R.M. Ros &amp; O. Werner s.n.</i>	N 49.46111 E 8.98778	KF952830	KF952939	Europe
63	31	Germany, Baden-Württemberg, Eberbach	MUB 44650	<i>R.M. Ros &amp; O. Werner s.n.</i>	N 49.46111 E 8.98778	KF952837	KF952946	Europe
64	31	Germany, Baden-Württemberg, Sankt Leon-Rot	MUB 44651	<i>R.M. Ros &amp; O. Werner s.n.</i>	N 49.24333 E 8.65222	KF952831	KF952940	Europe
65	31	Germany, Berlin	MUB 44645	<i>R.M. Ros &amp; O. Werner s.n.</i>	N 52.44028 E 13.58250	KF952828	KF952937	Europe
66	31	Germany, Berlin	MUB 44647	<i>R.M. Ros &amp; O. Werner s.n.</i>	N 52.51861 E 13.39694	KF952829	KF952938	Europe
67	31	Greece, Prefecture of Fokida, Delphi	MUB 12010	<i>M.J. Cano, J. Muñoz, R.M. Ros &amp; M.</i>	N 38.48278 E 22.50444	KF952836	KF952945	Europe
68	31	Norway, Finnmark	S B176550	<i>L. Hedenäs s.n.</i>	N 70.78333 E 23.33333	KF952822	KF952931	Europe
69	31	Russia, Kuril Islands, Kunashir Island	MHA	<i>M.S. Ignatov 06-1810</i>	N 44.35000 E 146.26667	KF952833	KF952942	Asia
70	31	Spain, Granada province, Sierra Nevada	<i>Pisa et al.</i> (2013)	-	N 37.09725 W 3.39753	KC493862	KC493888	Europe
71	31	Spain, León province, Abalgas	MUB 44659	<i>S. Pisa s.n.</i>	N 42.89139 W 5.97389	KF952823	KF952932	Europe
72	32	Spain, León province, Abalgas	MUB 44662	<i>S. Pisa s.n.</i>	N 42.89139 W 5.97389	KF952838	KF952947	Europe
73	32	USA, Connecticut, Hartford County	CONN	<i>D. Les s.n.</i>	N 41.85278 W 72.51611	KF952839	KF952948	America
74	33	Germany, Baden-Württemberg, Heidelberg	MUB 44658	<i>R.M. Ros &amp; O. Werner s.n.</i>	N 49.41111 E 8.70639	KF952841	KF952950	Europe
75	33	Germany, Baden-Württemberg, Heidelberg	MUB 44648	<i>R.M. Ros &amp; O. Werner s.n.</i>	N 49.41111 E 8.70639	KF952840	KF952949	Europe
76	34	Spain, Canary Islands, Tenerife	MUB 44655	<i>J.M. González-Mancebo s.n.</i>	N 28.14167 W 16.65361	KF952844	KF952953	Africa
77	34	USA, California, Monterey County, Los	CAS 1083051	<i>J.R. Shevock 29347</i>	N 36.09639	KF952845	KF952954	America

		Padres National Forest			W 121.44167			
78	34	USA, California, Riverside Co, San Bernardino National Forest	CAS 1047424	<i>J.R. Shevock 24084</i>	N 33.77672 W 116.67814	KF952843	KF952952	America
79	34	USA, California, San Bernardino Co, San Bernardino National Forest	CAS 1047515	<i>J.R. Shevock 24099</i>	N 34.22497 W 117.06186	KF952842	KF952951	America
80	35	Germany, Baden-Württemberg, Black Forest	MUB 44628	<i>S. Pisa s.n.</i>	N 47.84056 E 8.01917	KF952847	KF952956	Europe
81	35	Portugal, Beira Interior, Guarda	MUB 44643	<i>R.M. Ros s.n.</i>	N 40.53250 W 7.26667	KF952848	KF952957	Europe
82	35	Portugal, Beira Interior, Guarda	MUB 44657	<i>R.M. Ros s.n.</i>	N 40.53250 W 7.26667	KF952846	KF952955	Europe
83	36	China, Schuan Province, Muli Co	CAS 1140938	<i>J.R. Shevock 36226</i>	N 28.16667 E 101.22233	KF952849	KF952958	Asia
84	36	Dominican Republic, Independencia province, Sierra de Baoruco	NY 635904	<i>S.A. Thompson 9237</i>	N 18.30000 W 71.70000	KF952851	KF952960	America
85	36	Germany, Berlin	MUB 44646	<i>R.M. Ros &amp; O. Werner s.n.</i>	N 52.51861 E 13.39694	KF952852	KF952961	Europe
86	36	Tanzania, Kilimanjaro	EGR	<i>J. Elia 144</i>	S 4.25722 E 37.99028	KF952850	KF952959	Africa
87	37	Russia, Yakutia	MHA	<i>M.S. Ignatov 00-29</i>	N 61.13333 E 138.05000	KF952855	KF952964	Asia
88	37	USA, New Mexico, Doña Ana County, Las Cruces	NY 710573	<i>R.M. King &amp; R.G. Garvey B315</i>	N 32.31667 W 106.75000	KF952853	KF952962	America
89	37	USA, Utah, Dixie National Forest	NY 53227	<i>J.L. Pawek s.n.</i>	N 37.79667 W 112.80722	KF952854	KF952963	America
90	38	Germany, Baden-Württemberg, Black Forest	MUB 44626	<i>S. Pisa s.n.</i>	N 47.83333 E 8.01667	KF952856	KF952965	Europe
91	38	Germany, Baden-Württemberg, Black Forest	MUB 44627	<i>S. Pisa s.n.</i>	N 47.83333 E 8.01667	KF952857	KF952966	Europe
92	38	Germany, Baden-Württemberg, Black Forest	MUB 44629	<i>S. Pisa s.n.</i>	N 47.84417 E 8.01917	KF952864	KF952973	Europe
93	38	Germany, Baden-Württemberg, Black Forest	MUB 44631	<i>S. Pisa s.n.</i>	N 47.86444 E 8.02194	KF952862	KF952971	Europe
94	38	Germany, Baden-Württemberg, Black Forest	MUB 44632	<i>S. Pisa s.n.</i>	N 47.86444 E 8.02194	KF952863	KF952972	Europe
95	38	Germany, Baden-Württemberg, Black Forest	MUB 44633	<i>S. Pisa s.n.</i>	N 47.86444 E 8.02194	KF952858	KF952967	Europe
96	38	Germany, Baden-Württemberg, Black Forest	MUB 44634	<i>S. Pisa s.n.</i>	N 47.86444 E 8.02194	KF952859	KF952968	Europe
97	38	Germany, Baden-Württemberg, Black Forest	MUB 44635	<i>S. Pisa s.n.</i>	N 47.86444 E 8.02194	KF952860	KF952969	Europe
98	38	Germany, Baden-Württemberg, Black Forest	MUB 44636	<i>S. Pisa s.n.</i>	N 47.86444 E 8.02194	KF952861	KF952970	Europe
99	39	China, Qinghai province, Menyuan County	E 00477222	<i>D.G. Long 27295</i>	N 37.11889 E 102.31639	KF952865	KF952974	Asia
100	39	China, Yunnan province, Degin County	E 00477223	<i>D.G. Long 23999</i>	N 28.63333 E 98.73278	KF952866	KF952975	Asia
101	40	Germany, Berlin	MUB 44644	<i>R.M. Ros &amp; O. Werner s.n.</i>	N 52.50833 E 13.33444	KF952867	KF952976	Europe
102	40	New Zealand, Huntley	Hills <i>et al.</i> (2010)	-	S 37.56667 E 175.15000	GU907062		Australasia
103	41	Spain, Madrid province, Sierra de Guadarrama	MUB 44666	<i>S. Pisa s.n.</i>	N 40.83139 W 3.95278	KF952868	KF952977	Europe
104	41	Spain, Madrid province, Sierra de Guadarrama	MUB 44667	<i>S. Pisa s.n.</i>	N 40.83139 W 3.95278	KF952869	KF952978	Europe
105	42	USA, California, Inyo County, Mojave Desert	CAS 1113251	<i>J.R. Shevock 24458</i>	N 35.85364 W 117.38125	KF952870	KF952979	America
106	42	USA, California, Kern County, Sierra Nevada	CAS 957141	<i>J.R. Shevock 14859</i>	N 35.56250 W 118.95417	KF952872	KF952981	America
107	42	USA, California, Tulare County, Sierra Nevada	CAS 993294	<i>J.R. Shevock 13658</i>	N 36.73750 W 118.85833	KF952871	KF952980	America
108	43	China, Qinghai Province, Yushu Tibetan Autonomous Prefecture	NY 1229472	<i>B.C. Tan 95-1733</i>	N 33.00250 E 97.27611	KF952873	KF952982	Asia
109	43	China, Sichuan Province, Litang County, Hengduan Mountains	CAS 1140922	<i>J.R. Shevock 35853</i>	N 30.17792 E 100.00311	KF952875	KF952984	Asia
110	43	China, Xizang province, SE Tibet	Herb. D.T. Holyoak	<i>G. Miede &amp; U. Wüндisch 94-164-30</i>	N 29.05000 E 93.98333	KF952874	KF952983	Asia
111	44	Australia, Mount Buffalo	Hills <i>et al.</i> (2010)	-	S 36.78333 E 146.05000	GU907057		Australasia
112	44	New Zealand, Christchurch	Hills <i>et al.</i> (2010)	-	S 43.08333 E 172.11667	GU907056		Australasia
113	45	Australia, Mount McKay	Hills <i>et al.</i> (2010)	-	S 36.86667 E 147.25000	GU907061		Australasia
114	46	New Zealand, Hamilton	Hills <i>et al.</i> (2010)	-	S 35.88333 E 175.46667	GU907060		Australasia
115	47	Spain, Granada province, Sierra Nevada	Pisa <i>et al.</i> (2013)	-	N 37.06943 W 3.38662	KC493852		Europe
116	48	Antarctica, Cape Hallet	Hills <i>et al.</i> (2010)	-	S 72.30000 E 170.30000	GU907068		Antarctica
117	49	Antarctica, Cape Bird	Hills <i>et al.</i> (2010)	-	S 77.21667 E 166.43333	GU907070		Antarctica
118	49	Antarctica, Cape Royds, Ross Island	Skotnicki <i>et al.</i> (2005)	-	S 77.58333 E 166.16667	AY611433		Antarctica
119	49	Antarctica, Garwood Valley	Hills <i>et al.</i> (2010)	-	S 78.05000 E 164.16667	GU907072		Antarctica
120	49	Antarctica, Miers Valley	Hills <i>et al.</i> (2010)	-	S 78.08333 E 164.75000	GU907071		Antarctica
121	49	South Shetland Islands, King George Island	AAS 1750	<i>R. Ochyra s.n.</i>	S 62.18000 W 58.58000	KJ409559	KJ409572	Antarctica
122	50	Beaufort Island, Ross Sea	Skotnicki <i>et al.</i> (2005)	-	S 76.91667 E 166.90000	AY611431		Antarctica
123	51	Canada, Northwest Territories, District	NY 69322	<i>G.W. Scotter 45680</i>	N 79.48333	KF952878	KF952987	America

		of Fanklin, Ellesmere Island			W 85.26667			
124	51	USA, Alaska, Pribilof Islands, St. Paul Island	NY 321062	<i>W.B. Schofield 108239</i>	N 57.15000 W 170.25000	KF952876	KF952985	America
125	51	USA, Alaska, Valdez-Cordova Census Area	MUB 44624	<i>F. Lara, R. Garilleti &amp; B. Albertos s.n.</i>	N 61.12583 W 146.35111	KF952877	KF952986	America
126	52	Antarctica, Cape Chocolate, S. Victoria Land	Skotnicki <i>et al.</i> (2005)	-	S 77.95000 E 164.50000	AY611429		Antarctica
127	53	South Africa, Cape province, Vredenburg	MUB 5343	<i>J.M. Egea s.n.</i>	S 32.76667 E 18.00000	KF952879	KF952988	Africa
128	54	South Africa, KwaZulu-Natal province, Vryheid	EGR	<i>J. van Rooy 55</i>	S 27.76667 E 30.78333	KF952880	KF952989	Africa
129	55	France, Department Hautes Pyrénées	Herb. D. T. Holyoak	<i>D.T. Holyoak 01-553</i>	N 42.73333 W 0.05000	KF952881	KF952990	Europe
130	56	France, Vosges Department, Vosges Mountains	MUB 44642	<i>R.M. Ros &amp; O. Werner s.n.</i>	N 47.90478 E 7.10286	KF952882	KF952991	Europe
131	57	China, Sichuan Province, Jiulong County, Hengduan Mountains	CAS 1141190	<i>J.R. Shevock 36078</i>	N 29.22078 E 101.45050	KF952883	KF952992	Asia
132	58	China, Qinghai province, Gonghe County	E 00477221	<i>D.G. Long 26787</i>	N 36.97722 E 99.90056	KF952885	KF952994	Asia
133	58	Colombia, Nariño Department, Municipality of Cumbal	NY 1229483	<i>B.R. Ramirez P. 6489</i>	N 0.95250 W 77.81944	KF952884	KF952993	America
134	59	Nepal, Taplejung District	E 00477231	<i>D.G. Long 21500</i>	N 27.43333 E 87.46667	KF952886	KF952995	Asia
135	60	Russia, Siberia, Taimyr	MHA	<i>V.E. Fedosov 08-162</i>	N 71.88083 E 110.78806	KF952887	KF952996	Asia
136	61	Altai Republic	MHA	<i>M.S. Ignatov 0/111</i>	N 50.50000 E 89.16667	KF952888	KF952997	Asia
137	62	Canada, Northwest Territories, District of Fanklin, Axel Heiberg Island	NY 69321	<i>G.W. Scotter 45482</i>	N 80.03333 W 88.75000	KF952889	KF952998	America
138	63	French Southern and Antarctic Lands, Crozet Islands	Hills <i>et al.</i> (2010)	-	S 46.45000 E 52.000	GU907058		sub-Antarctic islands
139	64	French Southern and Antarctic Lands, Crozet Islands, Possession Island	AAS	<i>B.G. Bell 1687</i>	S 46.42000 E 51.83000	KJ409558	KJ409571	sub-Antarctic islands
140	65	Antarctica, Antarctic Peninsula, Cockburn Island	AAS	<i>R.I. Lewis Smith 7922</i>	S 64.20000 W 56.85000	KJ409560	KJ409573	Antarctica
141	65	Antarctica, Antarctic Peninsula, Jenny Island	AAS	<i>R.I. Lewis Smith 4713</i>	S 67.73000 W 68.38000	KJ409561	KJ409574	Antarctica
142	66	Antarctica, Ross Sector, Victoria Land	AAS	<i>R.I. Lewis Smith 11794</i>	S 74.33000 E 165.13000	KJ409562	KJ409575	Antarctica
143	67	British Overseas Territories, South Sandwich Islands, Candlemas Island	AAS	<i>P. Convey 202B</i>	S 57.07000 W 26.70000	KJ409563	KJ409576	Antarctica
144	68	Antarctica, Antarctic Peninsula, Danco Coast	AAS	<i>R.I. Lewis Smith 4176</i>	S 64.68000 W 62.63000	KJ409564	KJ409577	Antarctica
145	68	Antarctica, Antarctic Peninsula, Danco Coast, Cuverville Island	AAS	<i>R. Weinstein 8812</i>	S 64.68000 W 62.63000	KJ409567	KJ409580	Antarctica
146	69	British Overseas Territories, Falkland Islands	AAS	<i>R.I. Lewis Smith 5437</i>	S 51.70000 W 57.85000	KJ409565	KJ409578	America
147	70	South Africa, Prince Edward Islands, Marion Island	AAS 235	<i>B.J. Huntley s.n.</i>	S 46.87000 E 37.85000	KJ409566	KJ409579	sub-Antarctic islands
148	71	South Africa, Prince Edward Islands	ACHE 296	<i>N.J.M. Gremmen s.n.</i>	S 46.92000 E 37.75000	KJ409569	KJ409582	sub-Antarctic islands
149	72	British Overseas Territories, South Sandwich Islands, Candlemas Island	AAS	<i>P. Convey 207A</i>	S 57.07000 W 26.70000	KJ409568	KJ409581	Antarctica
150	73	South Shetland Islands, Deception Island	AAS	<i>D. Mason 40</i>	S 62.95000 W 60.55000	KJ409570	-	Antarctica
151	74	British Overseas Territories, South Georgia Island	AAS	<i>R.I. Lewis Smith 8397</i>	S 54.10000 W 36.72000	-	KJ409583	sub-Antarctic islands
152	75	British Overseas Territories, South Georgia Island	AAS	<i>R.I. Lewis Smith 3102</i>	S 54.00000 W 37.13000	-	KJ409584	sub-Antarctic islands
153	76	French Southern and Antarctic Lands, Kerguelen island	AAS	<i>B.G. Bell 3192B</i>	S 49.35000 E 70.20000	-	KJ409585	sub-Antarctic islands
154	77	French Southern and Antarctic Lands, Amsterdam Island	AAS	<i>B.G. Bell 3217</i>	S 37.92000 E 77.67000	-	KJ409586	sub-Antarctic islands
155	<i>Bryum apiculatum Schwägr.</i>	China, Yunnan province	Wang and Zhao (2009)	-	-	EU878213		-
156	<i>Bryum funkii Mitt.</i>	China, Hunan province	Wang and Zhao (2009)	-	-	EU878209		-
157	<i>Bryum recurvulum Schwärg</i>	China, Hebei province	Wang and Zhao (2009)	-	-	EU878217		-
158	<i>Bryum yuennanense Broth.</i>	China, Yunnan province	Wang and Zhao (2009)	-	-	EU878211		-

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**Appendix 2** ITS alignment (Fasta file) of the 77 haplotypes of *Bryum argenteum* obtained from 154 accessions sampled worldwide and four outgroup sequences from closely related species. To allow replication of results, the random seed numbers for each of 4 runs for the coalescent under a stepwise extended Bayesian skyline model analyses used in this study are provided below:

- 1) 1389718433023
- 2) 1389718537624
- 3) 1389718538076
- 4) 1389718522438