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

S. Pisa[1, 8]*, E. M. Biersma[2, 3]*, P. Convey[2, 4], J. Patiño[5, 6, 7], A. Vanderpoorten[5, 7], O. Werner[1] and R. M. Ros[1]

[1] Departamento de Biología Vegetal (Botánica), Facultad de Biología, Universidad de Murcia, Campus de Espinardo 30100 Murcia, Spain

[2] British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge CB3 0ET, United Kingdom

[3] Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA, United Kingdom

[4] Gateway Antarctica, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand

[5] Institute of Botany, University of Liège, 4000, Liège, Belgium

[6] Department of Plant Biology, La Laguna University, Tenerife, Spain

[7] Azorean Biodiversity Group (CITA-A) and Platform for Enhancing Ecological Research & Sustainability (PEERS), Universidade dos Açores, Dep. Ciências Agrárias, Terceira, Portugal

[8] Author for correspondence (e-mail: sergiopisa@um.es; phone: +34 868 884989; fax: +34 868 883963)

***** contributed equally to this paper

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

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

Introduction

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

Two main hypotheses have been proposed to explain how the contemporary terrestrial Antarctic biota endured the glacial events of the Pleistocene (Convey and Stevens 2007; Convey et al. 2008). The first, and long-held but generally untested, view is that the large majority of the Antarctic terrestrial biota was eradicated from both the Antarctic continent and the associated offshore islands and archipelagos of the Scotia Arc (including sub-Antarctic South Georgia). The considerably expanded and thickened ice sheets would have caused a complete loss of terrestrial exposures and habitats during the LGM, as is consistent with current glaciological model reconstructions. As a consequence most or all current Antarctic terrestrial biota would have had to (re-)colonise the continent after the LGM, either from disjunct populations or from refugia..However, while the long-distance dispersal (LDD) capacity of bryophytes would potentially have facilitated (re)colonisation on such a timescale, the ability to disperse over long distances is apparently more limited for many other groups of Antarctic organisms. An additional weakness of the hypothesis is that many elements of the contemporary Antarctic biota show high levels of endemism (Øvstedal and Lewis Smith 2001; Pugh and Convey 2008), which could not have arisen after the LGM. The persistence of such an endemic biota would require refugia beyond the current confines of Antarctica (for instance in the more distant sub-Antarctic islands or the other Southern Hemisphere continents), which would have been situated beyond the current distributions of many species. Therefore, this hypothesis would imply multiple colonisation events out of Antarctica when refugia were required, and subsequent extinction from them once Antarctica was recolonised. The alternative hypothesis is that species have survived *in situ*, in multiple refugia that must have been present in different regions across Antarctica. This hypothesis has received increasingly strong support in recent years from both molecular and classic biogeographical studies, as well as from geological and geomorphological evidence demonstrating the diachrony of ice-sheet expansions around Antarctica, and refining both the thickness and timing of previous episodes of maximum ice sheet extent (Convey et al. 2008, 2009; Pugh and Convey 2008; Vyverman et al. 2010; Fraser et al. 2012).

Bryophytes (mosses and liverworts) are the dominant land plant flora in Antarctica, reaching their greatest diversity and extent in the Antarctic Peninsula and Scotia Arc (Ochyra et al. 2008; Convey 2013). As a group, they are generally regarded as possessing strong LDD capacities, supported by both direct (Lönnel et al. 2012; Sundberg 2013; van Zanten 1978, 1981) and indirect (see Szövényi et al. 2012 for review) evidence. These characteristics would, in principle, equip them well for recolonisation of Antarctica following any episode of regional extinction. . Elsewhere, recent evidence points to the major role of oceanic islands as glacial refugia for the subsequent (re-)colonisation of continents (Laenen et al. 2011, Hutsemékers et al. 2011). However, the geographic scale of Antarctic isolation from other landmasses, along with protection from direct north-south transfer by atmospheric and ocean currents, give the continent considerable geographic isolation (Barnes et al. 2006). Alternatively, a feature common among most bryophytes is their ability to grow at low (sub-optimal) temperatures. More than half of the 40 temperate species investigated by Furness and Grime (1982) showed a growth reduction of less than 50% at 5°C compared to growth at their optimal temperature, and this feature has also been described in Arctic and Antarctic bryophytes (Longton 1988). Indeed, many species, including some from the tropics, seem to be physiologically pre-adapted to cold and can survive temperatures ranging from -10 to -27°C (Glime 2007). Recently, La Farge et al. (2013) have provided evidence for totipotent capacity (the ability of a cell to dedifferentiate into a meristematic state, and subsequently regrow) in Arctic bryophyte tissue buried by ice for 400 years, and Roads et al. (2014) have reported regrowth from gametophytes in moss banks preserved in permafrost for over 1.5 Ka. Furthermore, population genetic data for the temperate moss *Homalothecium sericeum* (Hedw.) Schimp. support persistence of the species in micro-refugia within the extensively glaciated northern Europe during the LGM (Désamoré et al. 2012). These examples suggest that bryophytes may be viable candidates to have survived Antarctic glacial cycles *in situ*.

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

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

Materials and Methods

Specimen sampling and molecular protocols

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

Total genomic DNA was extracted following the protocol described in Werner et al. (2002), or using the DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany). PCR amplification and sequencing of the nuclear internal transcribed spacer (ITS) region were performed using the protocol described in Pisa et al. (2013). Most of the amplifications were carried out using universal primers AB101 and AB102 (Douzery et al. 1999), with some nucleotide modifications to adapt these primers to *B. argenteum*. In some cases, sequences were generated using universal primers ITS-A and ITS-B as described in Blattner (1999), employing a similar PCR step as in Pisa et al. (2013), with exceptions being the use of the Taq PCR Core Kit (Qiagen GmbH, Hilden, Germany) and an annealing temperature of 50°C. Forward and reverse sequence fragments for both ITS1 and ITS2 were edited and assembled using Bioedit 7.05 (Hall 1999) and every polymorphism was checked from the chromatograms. The sequences were aligned by eye, adding gaps where necessary to conserve homology between sequences (Appendix 2). Gaps were counted with SeqState (Müller 2005) using complex indel-coding. The number of polymorphic sites was calculated with DnaSP (Librado and Rozas 2009).

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

Population genetic analyses

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

Phylogeny, molecular dating, and ancestral area reconstructions

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

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

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

Results

The complete alignment had a total length of 928 bp after the exclusion of the 5.8S rRNA gene. No further region was excluded from the alignment. There were 328 sites with gaps, corresponding to106 indels and 111 polymorphic sites. The alignment excluding outgroup sequences had a total length of 844 bp. There were 173 sites with gaps, corresponding to 78 indels and 81 polymorphic sites, allowing for the identification of 77 haplotypes (Appendix 1). Haploid diversity unbiased by population size was lowest in Antarctica (uh=0.62) as compared to other regions (0.79 - 0.94) (Table 1). The frequency of endemic haplotypes exhibited the reverse trend, reaching its highest value (x=0.90) in Antarctica. None of Tajima's D statistics differed significantly from 0.

There was a significant difference in genotype frequency among geographic regions (Global 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) providing evidence that, on average, the genotypes from the same region were more closely related than the genotypes from different regions. This geographic structure was largely due to the significant genetic isolation of Antarctica. The phylogeographic signal between Antarctica and any of the other six regions was consistently significant, whereas a significant phylogeographic signal could not be detected among any other pairs of regions (Table 2).

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

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

Discussion

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

In agreement with our finding of long-term persistence of *B. argenteum* in Antarctica, a significant phylogeographical signal was found in all pairwise comparisons between Antarctica and each of the six other global regions, while no such signal was identified amongst the latter. This indicates that extant patterns of genetic diversity of Antarctic *B. argenteum* populations are better explained in terms of *in situ* diversification than recruitment of migrants from other areas, resulting in the highest proportion of endemic haplotypes as compared to other regions of the world. Such an interpretation is consistent with recent developments in biogeographical knowledge of much of the contemporary terrestrial biota in Antarctica. Evidence for long-term history *in situ* has been reported in all major groups except the bryophytes, with timescales ranging from mid-Pleistocene (e.g. diatoms, rotifers, cladocerans) to Pliocene, Miocene and Gondwana-breakup (e.g. springtails, chironomid midges, mites, copepods, microorganisms) (see Convey et al. 2008, 2009, and references therein; Vyverman et al. 2010). McGaughran et al. (2010), in a comparative phylogeographic study of different springrail (Collembola) species in Victoria Land and along the Scotia Arc and Antarctic Peninsula, identified analogous evidence of intraregional differentiation and hence colonisation patterns on timescales dating back to at least the earliest Pleistocene.

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

Conclusion

This study demonstrates for the first time *in situ* persistence of bryophytes in Antarctica throughout previous glacial cycles and contradicts the hypothesis of post-glacial recruitment from extra-regional locations..Although *B. argenteum*, like other moss species, exhibits the lowest levels of genetic diversity worldwide in Antarctica, ITS variation is substantial within and among Antarctic populations, making it a highly suitable model for investigating fine-scale patterns of genetic structure and diversity at continental scale in order to reconstruct its biogeographic history. In particular, detailed phylogeographic information at the Antarctic scale would allow for testing Fraser's et al. (2014) hypothesis that refugia indeed correspond to areas of high geothermal activity and contribute, together with information from other terrestrial organisms (Terauds et al. 2012), to enhancing the identification of bioregions that are not fully represented in the current Antarctic Specially Protected Area network.

Acknowledgments

The authors thank the curators of the herbaria AAS, ACHE, CAS, CONN, E, EGR, MHA, MW, NY, S, and also the private herbaria of D.T. Holyoak and B. Goffinet for the loan of material and to B. Albertos, R. Garilleti, B. Goffinet, J. M. González-Mancebo, F. Lara and M. Stech for collecting material used in this work. We also thank Oliva Martin-Sanchez for producing Fig. 1. This study has been supported financially by the Spanish Ministry of Science and Innovation (Projects CGL2008-00275/BOS and CGL2011-22936/BOS) and by the European Regional Development Funds. E. M. Biersma is supported by a Natural Environment Research Council PhD studentship (ref NE/K50094X/1), and P. Convey by Natural Environment Research Council core funding to the British Antarctic Society programme 'Environmental Change and Evolution'. This paper also contributes to the Scientific Commity on Antarctic Research 'State of the Antarctic Ecosystem' programme. J. Patiño and A. Vanderpoorten gratefully acknowledge financial support from the Belgian Funds for Scientific Research and the University of Liege. We thank three reviewers for helpful comments.

References

Aigoin DA, Devos N, Huttunen S, Ignatov MS, González-Mancebo JM, Vanderpoorten A (2009) And if Engler was not completely wrong? Evidence for multiple evolutionary origins in the moss flora of Macaronesia. Evolution 63:3248–3257

Anderson JB, Warnyb S, Askinc RA, Wellnerd JS, Bohatye SM, Kirshnera AE, Livseyf DN, Simmsf AR, Smitha TR, Ehrmanng W, Lawverh LA, Barbeaui D, Wisej SW, Kulhenekj DK, Weavera FM, Majewskik W (2011) Progressive Cenozoic cooling and the demise of Antarctica's last refugium. Proc Natl Acad Sci USA 108:11356-11360.

Baker PE, McReath I, Harvey MR, Roobol MJ, Davies TG (1975) The geology of the South Shetland Islands: V. Volcanic evolution of Deception Island. Br Antarct Surv, Sci Rep 78:1-8.

Barnes DKA, Hodgson DA, Convey P, Allen C, Clarke A (2006) Incursion and excursion of Antarctic biota: past, present and future. Global Ecol Biogeogr 15:121-142.

Benjamini Y, Yekutieli D (2001) The control of false discovery rate under dependency. Ann Stat 29:1165–1188.

Blattner FR (1999) Direct amplification of the entire ITS region from poorly preserved plant material using recombinant PCR. BioTech 27:1180-1185.

Cannone N, Convey P, Guglielmin M (2013) Diversity trends of bryophytes in continental Antarctica. Polar Biol 36:259-271.

Clarke LJ, Ayre DJ, Robinson SA (2008) Somatic mutation and the Antarctic ozone hole. J Ecol 96:378-385.

Clarke LJ, Ayre DJ, Robinson SA (2009) Genetic structure of East Antarctic populations of the moss *Ceratodon purpureus*. Antarct Sci 21:51-58.

Convey P (2013) Antarctic Ecosystems. In: Levin SA (ed) Encyclopedia of Biodiversity Vol. 1, 2nd edn. Elsevier, San Diego, pp. 179-188.

Convey P, Smith RIL (2006) Geothermal bryophyte habitats in the South Sandwich Islands, maritime Antarctic. J Veg Sci 17:529-538.

Convey P, Stevens MI (2007) Antarctic Biodiversity. Science 317**:**1877-1878.

Convey P, Smith RIL, Hodgson DA, Peat HJ (2000) The flora of the South Sandwich Islands, with particular reference to the influence of geothermal heating. J Biogeog 27:1279-1295.

Convey P, Gibson J, Hillenbrand C-D, Hodgson DA, Pugh PJA, Smellie JL, Stevens MI (2008) Antarctic terrestrial life – challenging the history of the frozen continent? Biol Rev 83:103-117.

Convey P, Stevens MI, Hodgson DA, Smellie JL, Hillenbrand C-D, Barnes DKA, Clarke A, Pugh PJA, Linse K, Cary SC (2009) Exploring biological constraints on the glacial history of Antarctica. Quaternary Sci Rev 28:3035-3048.

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9:772.

Désamoré A, Laenen B, Stech M, Papp B, Hedenäs L, Mateo RG, Vanderpoorten A. (2012) How do temperate bryophytes face the challenge of a changing environment? Lessons from the past and predictions for the future. Global Change Biol 18:2915–2924.

Douzery EJP, Pridgeon AM, Kores P, Linder HP, Kurzweil H & Chase MW (1999) Molecular phylogenetics of Diseae (Orchidaceae): a contribution from nuclear ribosomal ITS sequences. Am J Bot 86:887-899.

Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol Biol Evol 29:1969-1973.

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evol Bioinform Online 1: 47-50.

Fraser CI, Nikula R, Ruzzante DE, Waters JM (2012) Poleward bound: biological impacts of Southern Hemisphere glaciation. Trends Ecol Evol 27:462-471.

Fraser CI, Terauds A, Smellie J, Convey P, Chown SL (2014) Geothermal activity helps life survive ice ages. Proc Natl Acad Sci USA 111:5634-5639.

Furness SB, Grime JP (1982) Growth rate and temperature responses in bryophytes II. A comparative study of species of contrasted ecology. J Ecol 70:525-536.

Glime JM (2007) *Bryophyte Ecology*. Volume 1. Physiological Ecology. Ebook sponsored by Michigan Technological University and the International Association of Bryologists. http://www.bryoecol.mtu.edu/ . Accessed 6 February 2014.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95-98.

Hardy OJ, Vekemans X (2002) SPAGeDI: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol Ecol Notes 2:618-620.

Heled J, Drummond A (2008) Bayesian inference of population size history from multiple loci. BMC Evol Biol 8:289.

Hills SFK, Stevens MI, Gemmill CEC (2010) Molecular support for Pleistocene persistence of the continental Antarctic moss *Bryum argenteum*. Antarct Sci 22:721-726.

Hutsemékers V, Szövényi P, Shaw AJ, González-Mancebo JM, Muñoz J. Vanderpoorten A (2011) Oceanic islands are not sinks of biodiversity in spore-producing plants. Proc Natl Acad Sci USA 108:18989-18994.

Huttunen S, Hedenäs L, Ignatov MS, Devos N, Vanderpoorten A (2008) Origin and evolution of the northern hemisphere disjunction in the moss genus *Homalothecium* (Brachytheciaceae). Am J Bot 95:720-730.

Kay KM, Whittall JB, Hodges SA (2006) A survey of nuclear ribosomal internal transcribed spacer substitution rates across angiosperms: an approximate molecular clock with life history effects. BMC Evol Biol 6:36.

Košnar J, Herbstová M, Kolář F, Koutecký P, Kučera J (2012) A case study of intragenomic ITS variation in bryophytes: Assessment of gene flow and role of polyploidy in the origin of European taxa of the *Tortula muralis* (Musci: Pottiaceae) complex. Taxon 61:709-720.

Laenen B, Désamoré A, Devos N, Shaw AJ, González-Mancebo JM, Carine MA, Vanderpoorten A (2011) Macaronesia: A source of hidden genetic diversity for post-glacial recolonisation of western Europe in the leafy liverwort *Radula lindenbergiana*. J Biogeogr 38:631-639.

La Farge C, Krista H, Williams KH, England JH (2013) Regeneration of Little Ice Age bryophytes emerging from a polar glacier with implications of totipotency in extreme environments. Proc Natl Acad Sci USA doi:10.1073/pnas.1304199110.

Lewis AR, Marchant DR, Ashworth AC, Hedenäs L, Hemming SR, Johnson JV, Lengh MJ, Machlus ML, Newton AE, Raine JJ, Willenbring JK, Williams M, Wolfe AP (2008) Mid-Miocene cooling and the extinction of tundra in continental Antarctica. Proc Natl Acad Sci USA 105: 10676-10678.

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451-1452.

Longton R-E (1988) The Biology of Polar Bryophytes and Lichens. Cambridge University Press, Cambridge.

Lönnel N, Hylander K, Jonsson BG, Sundberg S (2012) The fate of the missing spores - Patterns of realized dispersal beyond the closest vicinity of a sporulating moss. Plos One 7:e41987.

Mackintosh AN, Verleyen E., O'Brien PE, White DA, Jones RS, McKay R, Dunbar R., Gore DB, Fink D, Post A.L, Miura H, Leventer A, Goodwin I, Hodgson DA, Lilly K, Crosta X, Golledge NR, Wagner B, Berg S, van Ommen T, Zwartz D, Roberts SJ, Vyverman W, Masse G (2013) Retreat history of the East Antarctic Ice Sheet since the Last Glacial Maximum. Quat Sc Rev. doi: 10.1016/j.quascirev.2013.07.024

McGaughran A, Toricelli G, Carapelli A, Frati F, Stevens MI, Convey P, Hogg ID (2010) Contrasting phylogeographic patterns for springtails reflect different evolutionary histories between the Antarctic Peninsula and continental Antarctica. J Biogeog 37:103-119.

Müller K (2005) SeqState. Primer design and sequence statistics for phylogenetic DNA datasets. Appl Bioinform 4:65-69.

Nagy LG, Kocsubé S, Csanádi Z, Kovács GM, Petkovits T, Vágvölgyi C, Papp T (2012) Re-mind the gap! Insertion - deletion data reveal neglected phylogenetic potential of the nuclear ribosomal internal transcribed spacer (ITS) of Fungi. PloS One 11:e49794.

Nieto Feliner G, Rosselló JA (2007) Better the devil you know? Guidelines for insightful utilization of nrDNA ITS in species-level evolutionary studies in plants. Mol Phylogenet Evol 44:911-919.

Ochyra R, Smith RIL, Bednarek-Ochyra H (2008) The illustrated moss flora of Antarctica. Cambridge University Press, Cambridge.

Øvstedal DO, Smith RIL (2001) Lichens of Antarctica and South Georgia. A guide to their identification and ecology. Cambridge University Press, Cambridge.

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288-295.

Pisa S, Werner O, Vanderpoorten A, Magdy M, Ros RM (2013) Elevational patterns of genetic variation in the cosmopolitan moss *Bryum argenteum* (Bryaceae). Am J Bot 100:2000-2008.

Pons O, Petit RJ (1996) Measuring and testing genetic differentiation with ordered versus unordered alleles. Genetics 144:1237-1245.

Pugh PJA, Convey P (2008) Surviving out in the cold: Antarctic endemic invertebrates and their refugia. J Biogeogr 35:2176-2186.

Rambaut A, Drummond AJ (2009) Tracer. Version 1.5. Molecular evolution, phylogenetics and epidemiology Web. http://tree.bio.ed.ac.uk/software/tracer/. Accessed 10 December 2013.

Ree RH, Smith SA (2008a) Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. Syst Biol 57:4-14.

Ree RH, Smith SA (2008b) Lagrange: likelihood analysis of geographic range evolution. Version 2.0. http://code.google.com/p/lagrange/. Accessed 11 December 2013.

Ree RH, Moore BR, Webb CO, Donoghue MJ (2005) A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. Evolution 59:2299-2311.

Roads E, Longton RE, Convey P (2014). Millenial timescale regeneration in a moss from Antarctica. Curr Biol 24:R222

Skotnicki ML, Mackenzie AM, Clements MA, Selkirk PM (2005) DNA sequencing and genetic diversity of the 18S–26S nuclear ribosomal internal transcribed spacers (ITS) in nine Antarctic moss species. Antarct Sci 17:377–384.

Stenøien HK (2008) Slow molecular evolution in 18S rDNA, rbcL and nad5 genes of mosses compared with higher plants. J Evolution Biol 21:566–571.

Sundberg S (2013) Spore rain in relation to regional sources and beyond. Ecography 36:364-373.

Szövényi P, Sundberg S, Shaw AJ (2012) Long-distance dispersal and genetic structure of natural populations: an assessment of the inverse isolation hypothesis in peat mosses. Mol Ecol 21:5461-5472.

Terauds A, Chown SL, Morgan F, Peat HJ, Watts DJ, Keys H, Convey P, Bergstrom DM (2012) Conservation biogeography of the Antarctic. Divers Distrib 18: 726–741.

van Zanten BO (1978) Experimental studies on trans-oceanic long-range dispersal of moss spores in the Southern Hemisphere. J Hattori Bot Lab 44:455–482.

van Zanten BO, Pócs T (1981) Distribution and dispersal of bryophytes. Adv Bryol 1:479–562.

Vyverman W, Verleyen E, Wilmotte A, Hodgson DA, Willems A, Peeters K, Van de Vijver B, de Wever A, Leliaert F, Sabbe K (2010) Evidence for widespread endemism among Antarctic micro-organisms. Polar Sc 4:103-113.

Wang C-Y, Zhao J-C (2009) Phylogeny of *Ptychostomum* (Bryaceae, Musci) inferred from sequences of nuclear ribosomal DNA internal transcribed spacer (ITS) and chloroplast *rps4*. J Syst Evol 47:311-320.

Werner O, Ros RM, Guerra J (2002) Direct amplification and NaOH extraction: two rapid and simple methods for preparing bryophyte DNA for polymerase chain reaction (PCR). J Bryol 24:127-131.

Figure captions

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

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

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

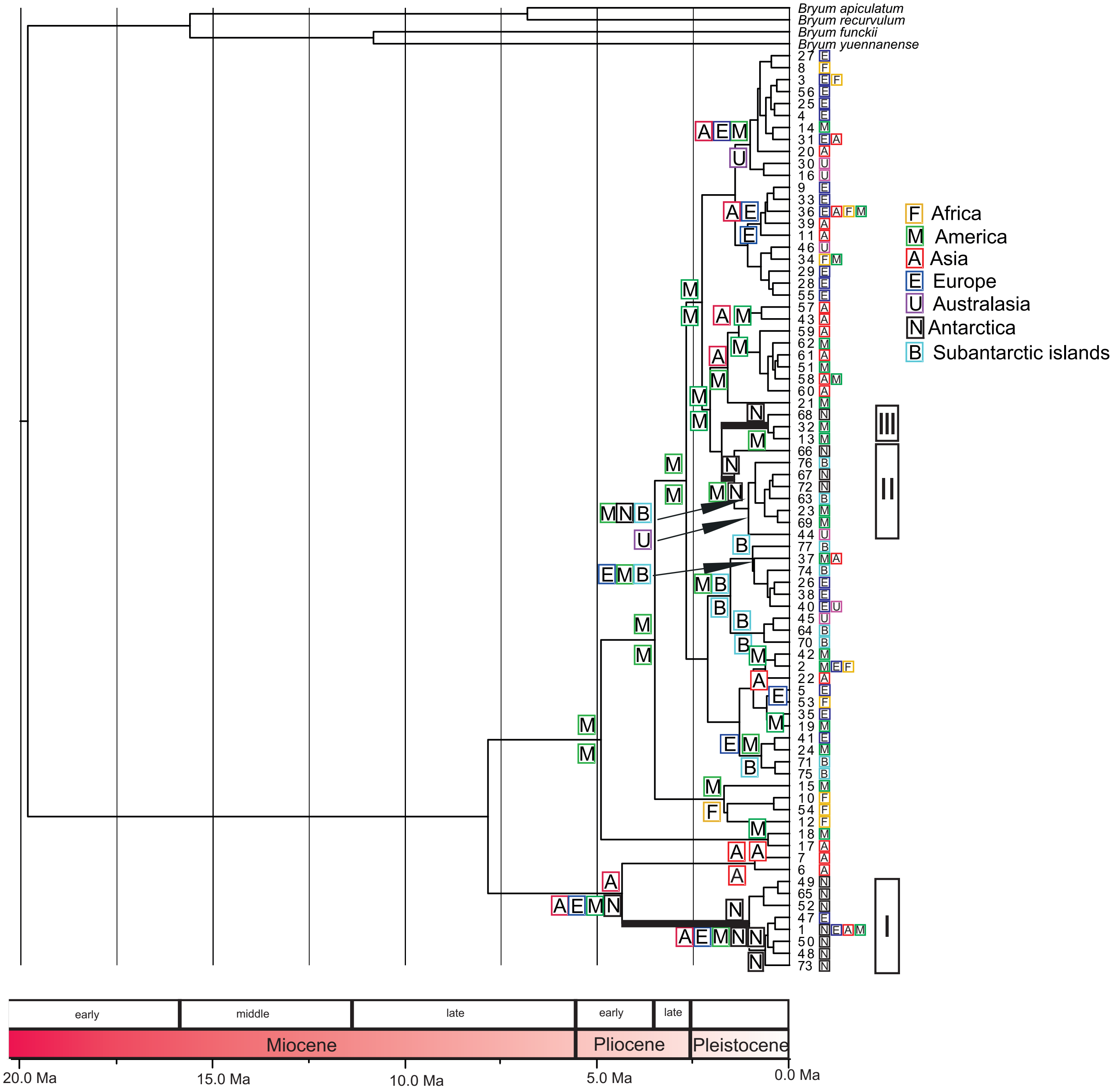
Continental area	**N**	**Na**	**h**	**uh**	**x**	**D**
Asia	20	17	0.89	0.94	0.71	-0.89 (0.19)
America	27	19	0.87	0.91	0.63	- 1.27 (0.09)
Europe	56	22	0.77	0.79	0.68	-0.86 (0.21)
Australasia	7	6	0.80	0.93	0.83	0.35 (0.65)
Antarctica	25	10	0.59	0.62	0.90	-0.93 (0.28)
Africa	11	9	0.80	0.88	0.56	0.20 (0.61)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

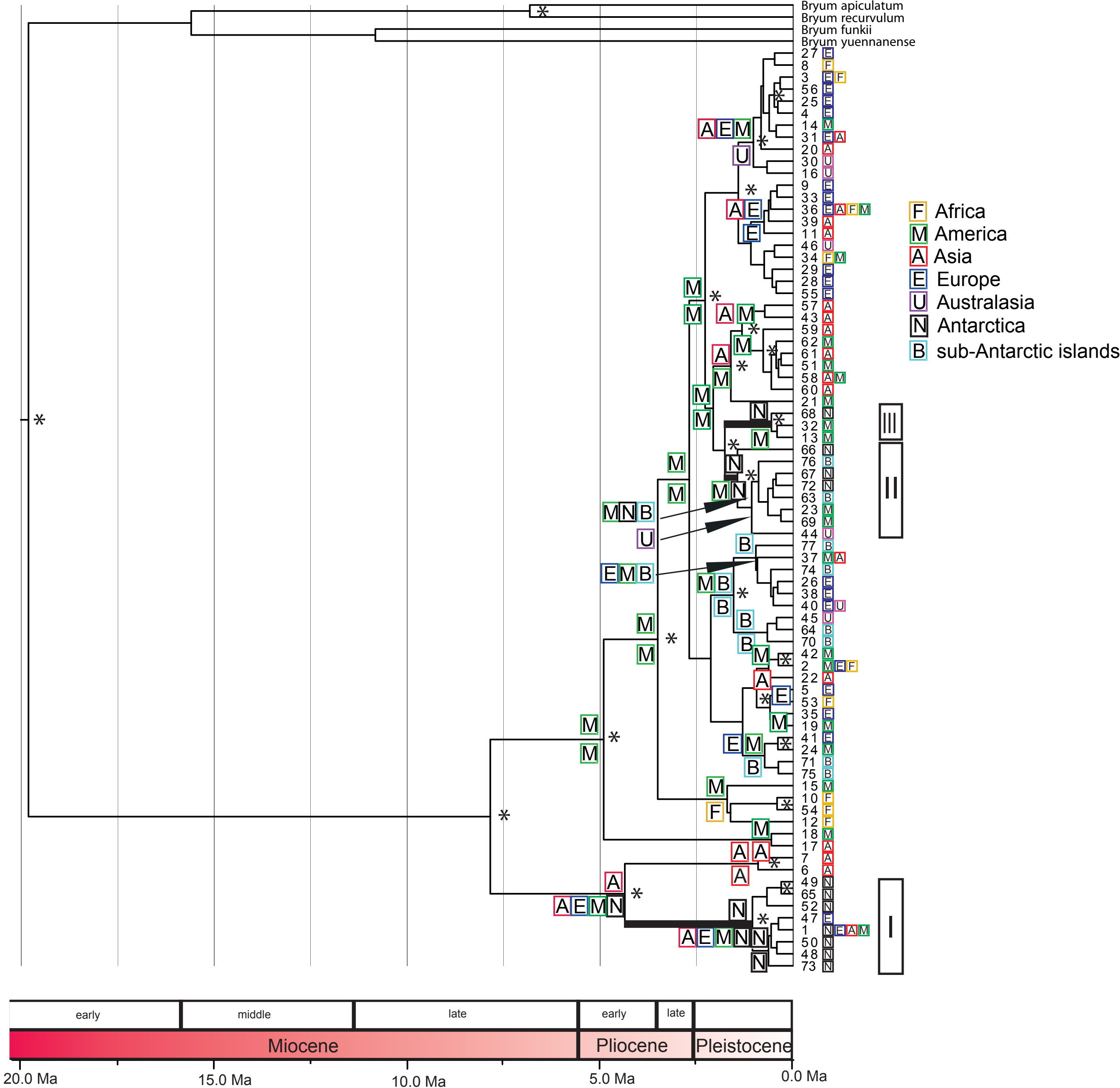
Table 2 Pairwise F_{ST} values (below diagonal) and N_{ST} values (above diagonal) among seven geographic regions of the worldwide distributed moss *Bryum argenteum* based on the nuclear ITS *locus*. The *P*-values (in brackets) are associated to the null hypotheses that $F_{ST}=0$ and that $F_{ST}=N_{ST}$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

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

$^{\text{n.s.}}$ indicates that the test is not significant ($P>0.05$)

* indicates that the test remains significant after the correction of Benjamini and Yekutieli (2001) for multiple tests at the $p<0.05$ significance level


Bryum apiculatum
Bryum recurvulum
Bryum funckii
Bryum yuennanense
27 E
8 F
3 E F
56 E
25 E
4 E
14 M
31 E A
20 A
30 U
16 U
9 E
33 E
36 E A F M
39 A
11 A
46 U
34 F M
29 E
28 E
55 E
57 A
43 A
59 A
62 M
61 A
51 M
58 A M
60 A
21 M
68 N
32 M
13 M
66 N
76 B
67 N
72 N
63 B
23 M
69 M
44 U
77 B
37 M A
74 B
26 E
38 E
40 E U
45 U
64 B
70 B
42 M
2 M E F
22 A
5 E
53 F
35 E
19 M
41 E
24 M
71 B
75 B
15 M
10 F
54 F
12 F
18 M
17 A
7 A
6 A
49 N
65 N
52 N
47 E
1 N E A M
50 N
48 N
73 N
AEM
U
AE
E
M
M
AM
A
M
M
M
M
N
M
N
M
M
MN
MNB
U
B
EMB
MB
B
B
B
M
M
M
A
E
M
EM
B
M
M
M
F
M
A
A
A
A
AEMN
N
AEMNN
N
III
II
I
F Africa
M America
A Asia
E Europe
U Australasia
N Antarctica
B Subantarctic islands
early
middle
late
early
late
Miocene
Pliocene
Pleistocene
20.0 Ma
15.0 Ma
10.0 Ma
5.0 Ma
0.0 Ma


Bryum apiculatum
Bryum recurvulum
Bryum funkii
Bryum yuennanense
27 E
8 F
3 E F
56 E
25 E
4 E
14 M
31 E A
20 A
30 U
16 U
9 E
33 E
36 E A F M
39 A
11 A
46 U
34 F M
29 E
28 E
55 E
57 A
43 A
59 A
62 M
61 A
51 M
58 A M
60 A
21 M
68 N
32 M
13 M
66 N
76 B
67 N
72 N
63 B
23 M
69 M
44 U
77 B
37 M A
74 B
26 E
38 E
40 E U
45 U
64 B
70 B
42 M
2 M E F
22 A
5 E
53 F
35 E
19 M
41 E
24 M
71 B
75 B
15 M
10 F
54 F
12 F
18 M
17 A
7 A
6 A
49 N
65 N
52 N
47 E
1 N E A M
50 N
48 N
73 N
F Africa
M America
A Asia
E Europe
U Australasia
N Antarctica
B sub-Antarctic islands
III
II
I
A E M
U
A E
E
M
M
A M
M
A
M
M
M
N
M
N
M N
M
M
M N B
U
B
E M B
M B
B
B
M
B
M
M
A
E
M
M
E M
B
M
M
F
M
A A
A
A
A E M N
N
A E M N N
N
early
middle
late
early
late
Miocene
Pliocene
Pleistocene
20.0 Ma
15.0 Ma
10.0 Ma
5.0 Ma
0.0 Ma

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

Journal name: Polar Biology

Author names: S. Pisa, E. M. Biersma, P. Convey, J. Patiño, A. Vanderpoorten, O. Werner and R. M. Ros

affiliation and e-mail address of the corresponding author :University of Murcia, sergiopisa@um.es

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
						ITS		
						ITS1	ITS2	
1	1	Antarctica, Beaufort Island	Hills *et al.* (2010)	-	S 76.91667 E 166.90000	GU907063		Antarctica
2	1	Antarctica, Beaufort Island	Hills *et al.* (2010)	-	S 76.91667 E 166.90000	GU907064		Antarctica
3	1	Antarctica, Canada Glacier, Taylor Valley, S. Victoria Land	Skotnicki *et al.* (2005)	-	S 77.58333 E 163.25000	AY611432		Antarctica
4	1	Antarctica, Cape Hallet	Hills *et al.* (2010)	-	S 72.30000 E 170.30000	GU907066		Antarctica
5	1	Antarctica, Cape Hallet	Hills *et al.* (2010)	-	S 72.30000 E 170.30000	GU907067		Antarctica
6	1	Antarctica, Edmonson Point, N. Victoria Land	Skotnicki *et al.* (2005)	-	S 74.33333 E 164.50000	AY611430		Antarctica
7	1	Antarctica, Granite Harbour	Hills *et al.* (2010)	-	S 77.00000 E 162.50000	GU907065		Antarctica
8	1	Antarctica, Granite Harbour, S. Victoria Land	Skotnicki *et al.* (2005)	-	S 77.00000 E 162.50000	AY611434		Antarctica
9	1	Antarctica, Marble Point	Hills *et al.* (2010)	-	S 76.43333 E 163.83333	GU907069		Antarctica
10	1	Canada, Northwest Territories, District of Fanklin, Ellesmere Island	NY 69323	*G.W. Scotter 45657*	N 79.71667 W 85.83333	KF952783	KF952892	America
11	1	Norway, Svalbard archipelago, Spitsbergen	MUB 44625	*M. Stech & J.D. Kruijer 08-088*	N 78,91667 E 11,93333	KF952782	KF952891	Europe
12	1	Sri Lanka (Ceylon), Central province, Nuwara Eliya District	E 00416780	*C.C. Townsend 73/1779*	N 6.95694 E 80.10889	KF952781	KF952890	Asia
13	2	Spain, Canary Islands, La Gomera	MUB 44654	*J.M. González-Mancebo et al. s.n.*	N 28.10737 W 17.23866	KF952785	KF952894	Africa
14	2	Spain, Canary Islands, La Palma	MUB 44653	*J.M. González-Mancebo & J. Leal*	N 28.67562 W 47.11100	KF952784	KF952893	Africa
15	2	Spain, Granada province, Albuñol	Pisa *et al.* (2013)	-	N 36.80025 W 3.21738	KC493867	KC493893	Europe
16	2	USA, California, Mariposa County, Yosemite National Park	CAS 1083159	*J.R. Shevock 29944*	N 37.51889 W 119.60000	KF952787	KF952896	America
17	2	USA, California, Tulare County, Sierra Nevada	CAS 1039823	*J.R. Shevock 20946*	N 36.06111 W 118.59306	KF952786	KF952895	America
18	3	Greece, Prefecture of Ilia, Ráhes	MUB 12158	*M.J. Cano, J. Muñoz, R.M. Ros & M.*	N 38.90861 E 22.83500	KF952794	KF952903	Europe
19	3	Ireland, E. County Mayo	Herb. D.T. Holyoak	*D.T. Holyoak 03-186*	N 53.61889 W 9.30639	KF952788	KF952897	Europe
20	3	Morocco, High Atlas, Oukaimeden	MUB 13090	*R.M. Ros s.n.*	N 31.21667 W 7.86667	KF952793	KF952902	Africa
21	3	Spain, Ávila province, Sierra de Gredos	MUB 44652	*R.M. Ros & O. Werner s.n.*	N 40.42231 W 5.29667	KF952796	KF952905	Europe
22	3	Spain, Ávila province, Sierra de Gredos	MUB 44653	*R.M. Ros & O. Werner s.n.*	N 40.42231 W 5.29667	KF952789	KF952898	Europe
23	3	Spain, Ávila province, Sierra de Gredos	MUB 44652	*R.M. Ros & O. Werner s.n.*	N 40.42231 W 5.29667	KF952795	KF952904	Europe
24	3	Spain, Canary Islands, Tenerife	MUB 44656	*J. Patiño s.n.*	N 28,35770 W 0,72512	KF952797	KF952906	Africa
25	3	Spain, León province, Abelgas	MUB 44660	*S. Pisa s.n.*	N 42.89139 W 5.97389	KF952790	KF952899	Europe
26	3	Spain, Murcia province, Campus de Espinardo	MUB 44663	*R. M. Ros s.n.*	N 8.02056 W 1.16944	KF952791	KF952900	Europe
27	3	Spain, Murcia province, Sierra de las Herrerías	MUB 44664	*R.M. Ros & O. Werner s.n.*	N 37.58278 W 1.42667	KF952792	KF952901	Europe
28	4	France, Vosges Department, Vosges Mountains	MUB 44641	*R.M. Ros & O. Werner s.n.*	N 47.90478 E 7.10286	KF952798	KF952907	Europe
29	5	UK, Great Britain, N. Northumberland	Herb. D. T. Holyoak	*D.T. Holyoak 08-670*	N 52.96667 E 0.55000	KF952799	KF952908	Europe
30	6	Taiwan, Hwalien County, Ta-yu-ling	E 00416777	*C.-C. Chuang 5781*	N 23.81667 E 121.23333	KF952800	KF952909	Asia
31	7	China, Taiwan, Nantou County, Central	CAS 995161	*J.R. Shevock 17888*	N 24.12500	KF952801	KF952910	Asia

		Mountain Range			E 121.21667			
32	8	Uganda, Western Region, Kabale	Herb. D. T. Holyoak	*M.J. Wigginton U5011a*	N 1.08333 E 29.80000	KF952802	KF952911	Africa
33	9	Spain, Madrid province, Sierra de Guadarrama	MUB 44668	*S. Pisa s.n.*	N 40.59030 W 3.98505	KF952803	KF952912	Europe
34	10	Union of the Comoros, Grande Comore (Ngazidja)	EGR	*R.E. Magill & T. Pócs 10946*	N 11.61222 E 43.33222	KF952804	KF952913	Africa
35	11	Indonesia, Sumatra, Gunung Sinabung	NY 1229461	*L. Hoffmann 89-196*	N 3.18139, E 98.44111	KF952805	KF952914	Asia
36	12	Kenya, Nyandarua County, Aberdare Mts.	EGR	*J. Spence s.n.*	N 0.41083 E 36.61667	KF952806	KF952915	Africa
37	13	Bolivia, La Paz, Murillo	NY 1229477	*S. Churchill 22828*	S 16.61667 W 68.07528	KF952807	KF952916	America
38	14	Chile, Antarctica Chilena province, Isla Navarino, Magallanes	Herb. B. Goffinet	*B. Goffinet 6765*	S 54.95000 W 67.63333	KF952808	KF952917	America
39	15	USA, California, Tulare County, Sierra Nevada	CAS 989901	*J.R. Shevock 17562*	N 35.88889 W 118.34583	KF952809	KF952918	America
40	16	Australia, Canberra	Hills *et al.* (2010)	-	S 35.30000 E 149.13333	GU907059		Australasia
41	17	Thailand, Khao Yai National Park	NY 1229462	*C. Charoenphol 4395 with Larsen &*	N 14.53333 E 101.36667	KF952810	KF952919	Asia
42	18	Brazil, Bahia state, Municipality of Abaíra, Campo de Ouro Fino	NY 1229480	*D.J.N. Hind H50910 with R.F. Queiroz*	S 13.25000 W 41.90000	KF952811	KF952920	America
43	19	Dominican Republic, La Vega province, Alto de la Bandera Mountain	NY 635898	*W.C. Steere 23098*	N 18.81667 W 70.61667	KF952812	KF952921	America
44	20	Russia, NW Caucasus	MW	*V. Onipchenko 99/95*	N 43.45000 E 41.68333	KF952813	KF952922	Asia
45	21	Boliva, Cochabamba, Carrasco	NY 1229478	*S. Churchill 22558*	S 17.75000 W 64.80000	KF952814	KF952923	America
46	22	China, Yunnan province, Gonshan County	E 00477214	*D. G. Long 36064*	N 27.68539 E 98.30422	KF952815	KF952924	Asia
47	23	Ecuador, Pichinga province	NY 1229481	*S.P. Churchill 13518 with I. Sastre-De*	N 0.38333 W 78.21667	KF952816	KF952925	America
48	24	USA, California, Fresno County, Sierra Nevada	CAS 989203	*J.R. Shevock 13927*	N37.16250 W 119.09583	KF952817	KF952926	America
49	25	Sweden, Värmland	S B178200	*L. Hedenäs & G. Odelvik s.n.*	N 59.78333 E 14.36667	KF952818	KF952927	Europe
50	26	Spain, Granada province, Sierra Nevada	Pisa *et al.* (2013)	-	N 37.09725 W 3.39753	KC493863	KC493889	Europe
51	27	Spain, Granada province, Vélez de Benaudalla	Pisa *et al.* (2013)	-	N 36.84465 W 3.50905	KC493873	KC493899	Europe
52	28	Spain, León province, Abelgas	MUB 44661	*S. Pisa s.n.*	N 42.89139 W 5.97389	KF952819	KF952928	Europe
53	29	Spain, Balearic Islands, Menorca	MUB 44665	*R.M. Ros & O. Werner s.n.*	N 39.89944 E 4.10389	KF952820	KF952929	Europe
54	30	Papua New Guinea, Chimbu province, Wilhelm Mountain	NY 1229460	*J.L. De Sloover 42892*	S 5.75667 E 145.03556	KF952821	KF952930	Australasia
55	31	Denmark, Faroe Islands, Streymoy, Thorshavn	S B185204	*R. Fargerstén & M. Haapasaari s.n.*	N 62.01139 W 6.75361	KF952834	KF952943	Europe
56	31	France, Vaucluse Department, commune de Bonnieux, domaine de la Chambarelle	MUB 1097	*R.M. Ros s.n.*	N 43.81389 E 18.43333	KF952835	KF952944	Europe
57	31	Germany, Baden-Württemberg, Black Forest	MUB 44630	*S. Pisa s.n.*	N 47.84417 E 8.01917	KF952832	KF952941	Europe
58	31	Germany, Baden-Württemberg, Black Forest	MUB 44637	*S. Pisa s.n.*	N 47.86444 E 8.02194	KF952824	KF952933	Europe
59	31	Germany, Baden-Württemberg, Black Forest	MUB 44638	*S. Pisa s.n.*	N 47.85972 E 8.03639	KF952825	KF952934	Europe
60	31	Germany, Baden-Württemberg, Black Forest	MUB 44639	*S. Pisa s.n.*	N 47.85972 E 8.03639	KF952826	KF952935	Europe
61	31	Germany, Baden-Württemberg, Black Forest	MUB 44640	*S. Pisa s.n.*	N 47.85972 E 8.03639	KF952827	KF952936	Europe
62	31	Germany, Baden-Württemberg, Eberbach	MUB 44649	*R.M. Ros & O. Werner s.n.*	N 49.46111 E 8.98778	KF952830	KF952939	Europe
63	31	Germany, Baden-Württemberg, Eberbach	MUB 44650	*R.M. Ros & O. Werner s.n.*	N 49.46111 E 8.98778	KF952837	KF952946	Europe
64	31	Germany, Baden-Württemberg, Sankt Leon-Rot	MUB 44651	*R.M. Ros & O. Werner s.n.*	N 49.24333 E 8.65222	KF952831	KF952940	Europe
65	31	Germany, Berlin	MUB 44645	*R.M. Ros & O. Werner s.n.*	N 52.44028 E 13.58250	KF952828	KF952937	Europe
66	31	Germany, Berlin	MUB 44647	*R.M. Ros & O. Werner s.n.*	N 52.51861 E 13.39694	KF952829	KF952938	Europe
67	31	Greece, Prefecture of Fokida, Delphi	MUB 12010	*M.J. Cano, J. Muñoz, R.M. Ros & M.*	N 38.48278 E 22.50444	KF952836	KF952945	Europe
68	31	Norway, Finnmark	S B176550	*L. Hedenäs s.n.*	N 70.78333 E 23.33333	KF952822	KF952931	Europe
69	31	Russia, Kuril Islands, Kunashir Island	MHA	*M.S. Ignatov 06-1810*	N 44.35000 E 146.26667	KF952833	KF952942	Asia
70	31	Spain, Granada province, Sierra Nevada	Pisa *et al.* (2013)	-	N 37.09725 W 3.39753	KC493862	KC493888	Europe
71	31	Spain, León province, Abelgas	MUB 44659	*S. Pisa s.n.*	N 42.89139 W 5.97389	KF952823	KF952932	Europe
72	32	Spain, León province, Abelgas	MUB 44662	*S. Pisa s.n.*	N 42.89139 W 5.97389	KF952838	KF952947	Europe
73	32	USA, Connecticut, Hartford County	CONN	*D. Les s.n.*	N 41.85278 W 72.51611	KF952839	KF952948	America
74	33	Germany, Baden-Württemberg, Heidelberg	MUB 44658	*R.M. Ros & O. Werner s.n.*	N 49.41111 E 8.70639	KF952841	KF952950	Europe
75	33	Germany, Baden-Württemberg, Heidelberg	MUB 44648	*R.M. Ros & O. Werner s.n.*	N 49.41111 E 8.70639	KF952840	KF952949	Europe
76	34	Spain, Canary Islands, Tenerife	MUB 44655	*J.M. González-Mancebo s.n.*	N 28,14167 W 16,65361	KF952844	KF952953	Africa
77	34	USA, California, Monterey County, Los	CAS 1083051	*J.R. Shevock 29347*	N 36.09639	KF952845	KF952954	America

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

		of Fanklin, Ellesmere Island			W 85.26667			
124	51	USA, Alaska, Pribilof Islands, St. Paul Island	NY 321062	*W.B. Schofield 108239*	N 57.15000 W 170.25000	KF952876	KF952985	America
125	51	USA, Alaska, Valdez-Cordova Census Area	MUB 44624	*F. Lara, R. Garilleti & B. Albertos s.n.*	N 61.12583 W 146.35111	KF952877	KF952986	America
126	52	Antarctica, Cape Chocolate, S. Victoria Land	Skotnicki *et al.* (2005)	-	S 77.95000 E 164.50000	AY611429		Antarctica
127	53	South Africa, Cape province, Vredenburg	MUB 5343	*J.M. Egea s.n.*	S 32.76667 E 18.00000	KF952879	KF952988	Africa
128	54	South Africa, KwaZulu-Natal province, Vryheid	EGR	*J. van Rooy 55*	S 27.76667 E 30.78333	KF952880	KF952989	Africa
129	55	France, Department Hautes Pyrénées	Herb. D. T. Holyoak	*D.T. Holyoak 01-553*	N 42.73333 W 0.05000	KF952881	KF952990	Europe
130	56	France, Vosges Department, Vosges Mountains	MUB 44642	*R.M. Ros & O. Werner s.n.*	N 47.90478 E 7.10286	KF952882	KF952991	Europe
131	57	China, Sichuan Province, Jiulong County, Hengduan Mountains	CAS 1141190	*J.R. Shevock 36078*	N 29.22078 E 101.45050	KF952883	KF952992	Asia
132	58	China, Qinghai province, Gonghe County	E 00477221	*D.G. Long 26787*	N 36.97722 E 99.90056	KF952885	KF952994	Asia
133	58	Colombia, Nariño Department , Municipality of Cumbal	NY 1229483	*B.R. Ramirez P. 6489*	N 0.95250 W 77.81944	KF952884	KF952993	America
134	59	Nepal, Taplejung District	E 00477231	*D.G. Long 21500*	N 27.43333 E 87.46667	KF952886	KF952995	Asia
135	60	Russia, Siberia, Taimyr	MHA	*V.E. Fedosov 08-162*	N 71.88083 E 110.78806	KF952887	KF952996	Asia
136	61	Altai Republic	MHA	*M.S. Ignatov 0/111*	N 50.50000 E 89.16667	KF952888	KF952997	Asia
137	62	Canada, Northwest Territories, District of Fanklin, Axel Heiberg Island	NY 69321	*G.W. Scotter 45482*	N 80.03333 W 88.75000	KF952889	KF952998	America
138	63	French Southern and Antarctic Lands, Crozet Islands	Hills *et al.* (2010)	-	S 46.45000 E 52.000	GU907058		sub-Antarctic islands
139	64	French Southern and Antarctic Lands, Crozet Islands, Possession Island	AAS	*B.G. Bell* 1687	S 46.42000 E 51.83000	KJ409558	KJ409571	sub-Antarctic islands
140	65	Antarctica, Antarctic Peninsula, Cockburn Island	AAS	*R.I. Lewis Smith* 7922	S 64.20000 W 56.85000	KJ409560	KJ409573	Antarctica
141	65	Antarctica, Antarctic Peninsula, Jenny Island	AAS	*R.I. Lewis Smith* 4713	S 67.73000 W 68.38000	KJ409561	KJ409574	Antarctica
142	66	Antarctica, Ross Sector, Victoria Land	AAS	*R.I. Lewis Smith* 11794	S 74.33000 E 165.13000	KJ409562	KJ409575	Antarctica
143	67	British Overseas Territories, South Sandwich Islands, Candlemas Island	AAS	*P. Convey* 202B	S 57.07000 W 26.70000	KJ409563	KJ409576	Antarctica
144	68	Antarctica, Antarctic Peninsula, Danco Coast	AAS	*R.I. Lewis Smith* 4176	S 64.68000 W 62.63000	KJ409564	KJ409577	Antarctica
145	68	Antarctica, Antarctic Peninsula, Danco Coast, Cuverville Island	AAS	*R. Weinstein* 8812	S 64.68000 W 62.63000	KJ409567	KJ409580	Antarctica
146	69	British Overseas Territories, Falkland Islands	AAS	*R.I. Lewis Smith* 5437	S 51.70000 W 57.85000	KJ409565	KJ409578	America
147	70	South Africa, Prince Edward Islands, Marion Island	AAS 235	*B.J. Huntley s.n.*	S 46.87000 E 37.85000	KJ409566	KJ409579	sub-Antarctic islands
148	71	South Africa, Prince Edward Islands	ACHE 296	*N.J.M. Gremmen s.n.*	S 46.92000 E 37.75000	KJ409569	KJ409582	sub-Antarctic islands
149	72	British Overseas Territories, South Sandwich Islands, Candlemas Island	AAS	*P. Convey* 207A	S 57.07000 W 26.70000	KJ409568	KJ409581	Antarctica
150	73	South Shetland Islands, Deception Island	AAS	*D. Mason* 40	S 62.9500 W 60.55000	KJ409570	-	Antarctica
151	74	British Overseas Territories, South Georgia Island	AAS	*R.I. Lewis Smith* 8397	S 54.10000 W 36.72000	-	KJ409583	sub-Antarctic islands
152	75	British Overseas Territories, South Georgia Island	AAS	*R.I. Lewis Smith* 3102	S 54.00000 W 37.13000	-	KJ409584	sub-Antarctic islands
153	76	French Southern and Antarctic Lands, Kerguelen island	AAS	*B.G. Bell* 3192B	S 49.35000 E 70.20000	-	KJ409585	sub-Antarctic islands
154	77	French Southern and Antarctic Lands, Amsterdam Island	AAS	*B.G. Bell* 3217	S 37.92000 E 77.67000	-	KJ409586	sub-Antarctic islands
155	*Bryum apiculatum* Schwägr.	China, Yunnan province	Wang and Zhao (2009)	-	-	EU878213		-
156	*Bryum funkii* Mitt.	China, Hunan province	Wang and Zhao (2009)	-	-	EU878209		-
157	*Bryum recurvulum* Schwärg	China, Hebei province	Wang and Zhao (2009)	-	-	EU878217		-
158	*Bryum yuennanense* Broth.	China, Yunnan province	Wang and Zhao (2009)	-	-	EU878211		-

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

Journal name: Polar Biology

Author names: S. Pisa, E. M. Biersma, P. Convey, J. Patiño, A. Vanderpoorten, O. Werner and R. M. Ros

affiliation and e-mail address of the corresponding author : University of Murcia, sergiopisa@um.es

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