The Evolutionary History of the Antarctic Flora

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This PhD studentship is between the University of Cambridge and the British Antarctic Survey (BAS).

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"There is grandeur in this view of life, with its several powers, having been originally breathed into a few forms or into one; and that, whilst this planet has gone cycling on according to the fixed law of gravity, from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved."

Charles Darwin

Last sentence of On the Origin of Species

(First edition, published: 24 November 1859)

Declaration

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text.

It is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. I further state that no substantial part of my dissertation has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text.

It does not exceed the prescribed word limit (60,000), in accordance with the Department of Plant Sciences guidelines.

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Acknowledgements

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Summary

How long has the extant flora been present in the Antarctic? Glaciological reconstructions propose that most areas in Antarctica were covered by thick ice sheets throughout the Last Glacial Maximum (LGM; ~22-18 kya) as well as previous glaciations, suggesting terrestrial life must have been extremely limited during these periods. In contrast, recent biogeographic and genetic studies support most extant groups of Antarctic terrestrial fauna having survived past glaciations *in situ*. However, studies on the origin and age of the Antarctic flora remain sparse.

Applying population genetic, phylogeographic and divergence time analyses I assessed the global biogeography, origin and age of several abundant Antarctic moss species, including: four Polytrichaceae mosses, characterised by having bipolar distributions, the most common (~45% of species) distribution pattern amongst Antarctic mosses; the globally widespread moss *Ceratodon purpureus*; the bank-forming moss *Chorisodontium aciphyllum*, also known for its old sub-fossils in Antarctica and long-term viability from revival experiments; and, lastly, the genus *Schistidium*, the most species-rich moss genus in Antarctica, including many endemic species.

Genetic analyses revealed evidence of long-term (multi-million year) survival of plants in Antarctica (several species of *Schistidium*, *Ceratodon purpureus*, and possibly *Polytrichum juniperinum*). However, evidence for a likely more recent (<100 ky) arrival of *Chorisodontium aciphyllum* was also found. Some species revealed multiple separate dispersal events to the Antarctic, suggesting the region may be less isolated for spore-dispersed organisms than previously thought. Evidence for increased genetic diversity in the northern maritime Antarctic compared to other regions point at it including potential refugial areas. Furthermore, genetic patterns revealed geographic features that enable and limit the connectivity of bryophytes globally as well as in Antarctica. This study suggests that, despite the harsh polar climate during glaciation periods, many bryophytes have had a much longer presence in Antarctica than previously thought.

This thesis is based on the following manuscripts:

- **Biersma E.M.**, Jackson, J.A., Hyvönen, J., Koskinen, S., Linse, K., Griffiths, H., and Convey, P. (2017) Global biogeographic patterns in bipolar moss species. *Royal Society Open Science*, 4(7), 170147.

- **Biersma E.M.**, Convey, P., Wyber, R., Robinson, S.A., Dowton, M., van de Vijver, B., Linse, K., Griffiths, H., and Jackson, J.A. Chloroplast DNA reveals latitudinal biogeographic structuring in the globally-distributed moss *Ceratodon purpureus*. Submitted to *Journal of Biogeography*, August 2016.

- **Biersma E.M.**, Jackson, J.A., Bracegridle, T.J., Griffiths, H., Linse, K., and Convey, P. Low genetic variation between South American and Antarctic populations of the bank-forming moss *Chorisodontium aciphyllum* (Dicranaceae). Submitted to *Polar Biology*, February 2017.

- **Biersma E.M.**, Jackson, J.A., Griffiths, H., Linse, K., and Convey, P. Genetic variation within Antarctic *Schistidium* mosses, with emphasis on the endemic species *Schistidium antarctici*. In preparation for submission to *PLoS One*.

During the course of this PhD I have also been involved as equal first author in the following PhD-related publication:

- Pisa, S. & **Biersma, E. M.,** Convey, P., Patiño, J., Vanderpoorten, A., Werner, O., & Ros, R. M. (2014). The cosmopolitan moss *Bryum argenteum* in Antarctica: recent colonisation or *in situ* survival? *Polar Biology*, 37 (10). 1469-1477.

Throughout my PhD I have also been involved in various publications related to other aspects of polar ecology, often including similar research questions related to other organisms, but not directly connected to my PhD:

- Lewis, L.R., Ickert-Bond, S., **Biersma, E.M.**, Convey, P., Goffinet, B., Hassel, K., Kruijer, J.D., La Farge, C., Metzgar, J., Stech, M., Villarreal, J.C., & McDaniel, S.F.

(2017) Future directions and priorities for Arctic bryophyte research. Future directions and priorities for Arctic bryophyte research. *Arctic Science*, (ja).

- Dabert, M., Coulson, S. J., Gwiazdowicz, D. J., Moe, B., Hanssen, S. A., **Biersma, E. M.,** Pilskog H. E. & Dabert, J. (2015) Differences in speciation progress in feather mites (Analgoidea) inhabiting the same host: the case of *Zachvatkinia* and *Alloptes* living on Arctic and Long-tailed Skuas. *Experimental and Applied Acarology*, 65. 163-179.

- Convey, P., Abbandonato, H., Bergan, F., Beumer, L. T., **Biersma, E. M.,** Bråthen, V. S., D'Imperio, L., Jensen, C. K., Nilsen, S., Paquin, K., Stenkewitz, U., Svoen, M. E., Winkler, J., Müller, E. & Coulson, S. J. (2014). Survival of rapidly fluctuating natural low winter temperatures by High Arctic soil invertebrates. *Journal of Thermal Biology*, 54. 111-117.

- Coulson, S. J., Convey, P., Aakra, K., Aarvik, L., Ávila-Jiménez, M. L., Babenko, A., **Biersma, E.M.**, Boström, S., Brittain J. E., Carlsson, A. M., Christoffersen, K., De Smet, W. H., Ekrem, T., Fjellberg, A., Füreder, L., Gustafsson, D., Gwiazdowicz, D. J., Hansen, L. O., Holmstrup, M., Hullé, M., Kaczmarek, Ł., Kolicka, M., Kuklin, V., Lakka, H. -K., Lebedeva, N., Makarova, O., Maraldo, K., Melekhina, E., Ødegaard, F., Pilskog, H. E., Simon, J. C., Sohlenius, B., Solhøy, T., Søli, G., Stur, E., Tanasevitch, A., Taskaeva, A., Velle, G., Zawierucha, K. & Zmudczyńska-Skarbek, K. (2014). The terrestrial and freshwater invertebrate biodiversity of the archipelagos of the Barents Sea; Svalbard, Franz Josef Land and Novaya Zemlya. *Soil Biology and Biochemistry*, 68. 440-470.

- Gilg, O., Moe, B., Hanssen, S.A., Schmidt, N.M., Sittler, B., Hansen, J., Reneerkens, J., Sabard, B., Chastel, O., Moreau, J., Phillips, R.A., Oudman, T., **Biersma, E.M.**, Fenstad, A.A., Lang, J. and Bollache, L. (2013) Trans-equatorial migration routes, staging sites and wintering areas of a high-arctic avian predator: the Long-tailed Skua (*Stercorarius longicaudus*). *PLoS One,* 8 (5), e64614. 10, pp.

Data accessibility:

All molecular data in this thesis will be made accessible following publication procedures of the different chapters: DNA sequences (as listed per chapter in Appendix 8.2) will be uploaded to GenBank (https://www.ncbi.nlm.nih.gov/genbank/), and phylogenetic matrixes will be uploaded to TreeBASE (https://treebase.org/).

Glossary

ABGD	Automatic Barcode Gap Discovery; a species delimitation program
ACBR(s)	Antarctic Conservation Biogeographic Region(s)
ASPA(s)	Antarctic Specially Protected Area(s)
EAIS	East Antarctic Ice Sheet
LGM	Last Glacial Maximum
SNP	Single Nucleotide Polymorphism
RAPD	Random Amplified Polymorphic DNA (method explained in section 1.1.3.2)
PCR	Polymerase Chain Reaction; a method to amplify a specific DNA fragment
WAIS	West Antarctic Ice Sheet

1 Introduction

1.1 Evolutionary history of Antarctic bryophytes

1.1.1 Glaciological and biological evidence for the history of Antarctic biota

The Antarctic continent provides little habitable terrain for terrestrial life. The estimated area currently not permanently covered by ice or snow on the continent ranges from only 0.18% (Burton-Johnson *et al.*, 2016) to 0.32% (Terauds & Lee, 2016). It is clear that during previous, colder, periods even less land was ice-free. Ice sheet reconstructions of past climate based on glaciological models and geomorphological field data provide strong evidence that during previous glaciations ice sheets were both much more extensive and thicker than at present, with many of the models implying that most of the currently ice-free ground would have been covered during these glacial periods (Denton *et al.*, 1984; Huybrechts, 1993; Nakada *et al.*, 2000; Denton & Hughes, 2002; 2002; Denton & Sugden, 2005; Lewis *et al.*, 2006; Pollard & DeConto, 2009). Significant ice sheet extension events occurred throughout the Miocene (23-5 Ma), Pliocene (5-2.6 Ma) and Pleistocene (2.6 Ma-10 ka), culminating in the comparatively recent Last Glacial Maximum (LGM; ~33-14 kya; Clark *et al.*, 2009). (For a more in-depth description of the glaciological and paleontological history of Antarctica see Section 1.1.2.1). Although high mountain summits and the Dry Valley region of Victoria Land are known to have remained ice-free (the latter for at least 12-13 million years; Lewis *et al.*, 2008), their biotas are clearly distinct and do not provide a refuge or source for the contemporary biodiversity of most regions of Antarctica (Convey & Stevens, 2007; Convey *et al.*, 2008). This background led to a widely held view that most pre-Miocene (older than 23 Ma) terrestrial life of Antarctica must have been driven extinct, and underlies the long-held view that virtually all terrestrial diversity occurring today must have (re-)colonised the continent after the LGM.

However, recent studies based on both modern molecular and classical biogeographic analyses have led to a paradigm shift, providing evidence that strongly suggests a long-term presence of much of the contemporary Antarctic terrestrial biota. Examples are drawn from across many geographically dispersed regions in Antarctica (Fig. 1.1), and include most invertebrate groups present, e.g. chironomid midges, mites, cladocerans, rotifers, copepods, nematodes and springtails, as well as diatoms, lichens, and even microbial species (Stevens & Hogg, 2003; Convey & Stevens, 2007; Convey *et al.*, 2008; Convey *et al.*, 2009a; Convey *et*

al., 2009b; De Wever *et al.*, 2009; Vyverman *et al.*, 2010; Fraser *et al.*, 2014; Pisa *et al.*, 2014; Chong *et al.*, 2015; Iakovenko *et al.*, 2015; Bennett *et al.*, 2016). These examples provide evidence for most of the main groups of contemporary Antarctic terrestrial biota having been continuously present *in situ*, with colonisation and diversification ranging from the Pleistocene, Pliocene, Miocene until pre-Gondwana break-up timescales (Fig. 1.2). A comprehensive synopsis of the biological groups and the relevant studies is given in Convey et al. (2008; 2009b). The strengthening evidence for a long-term history of Antarctic terrestrial biota provides a challenge to our current understanding of the glacial history of Antarctica, as well as the function of Antarctica in the Earth's climate system.
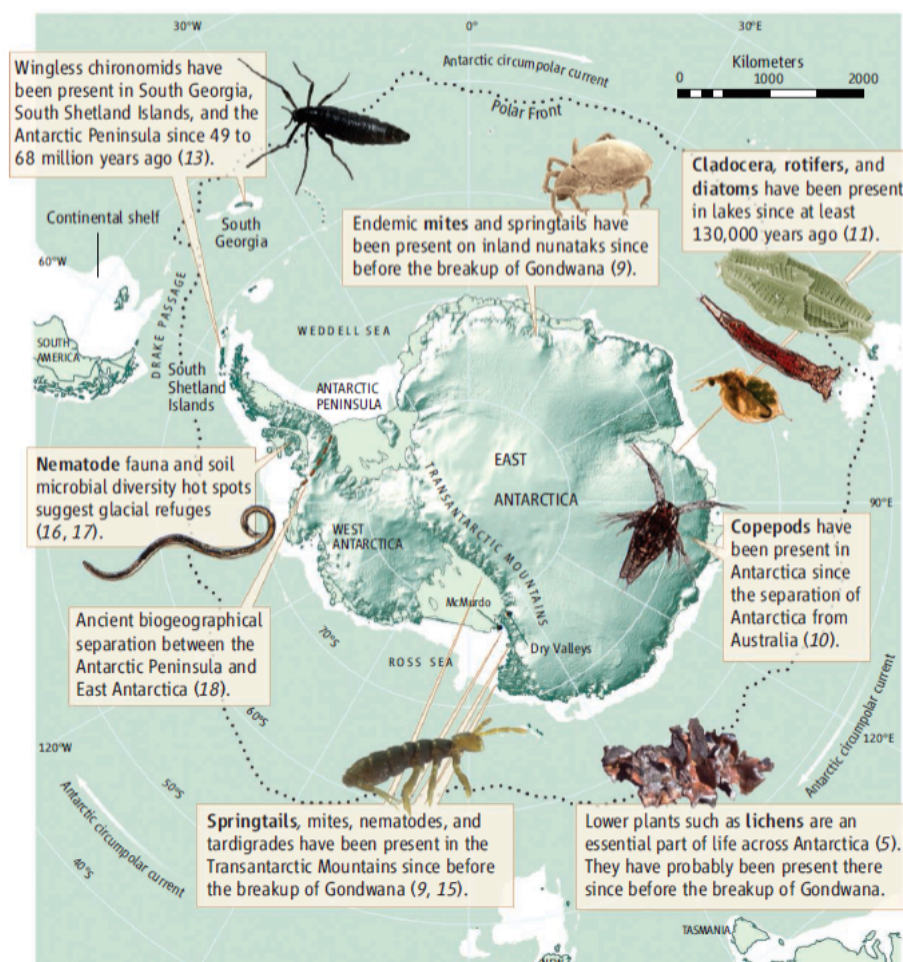
Fig. 1.1 The geographically dispersed regions in Antarctica from which evidence of an ancient (hundreds of thousands to multi-million year timescale) origin in Antarctica is found in various groups of Antarctic terrestrial biota (reproduced from Convey & Stevens, 2007).
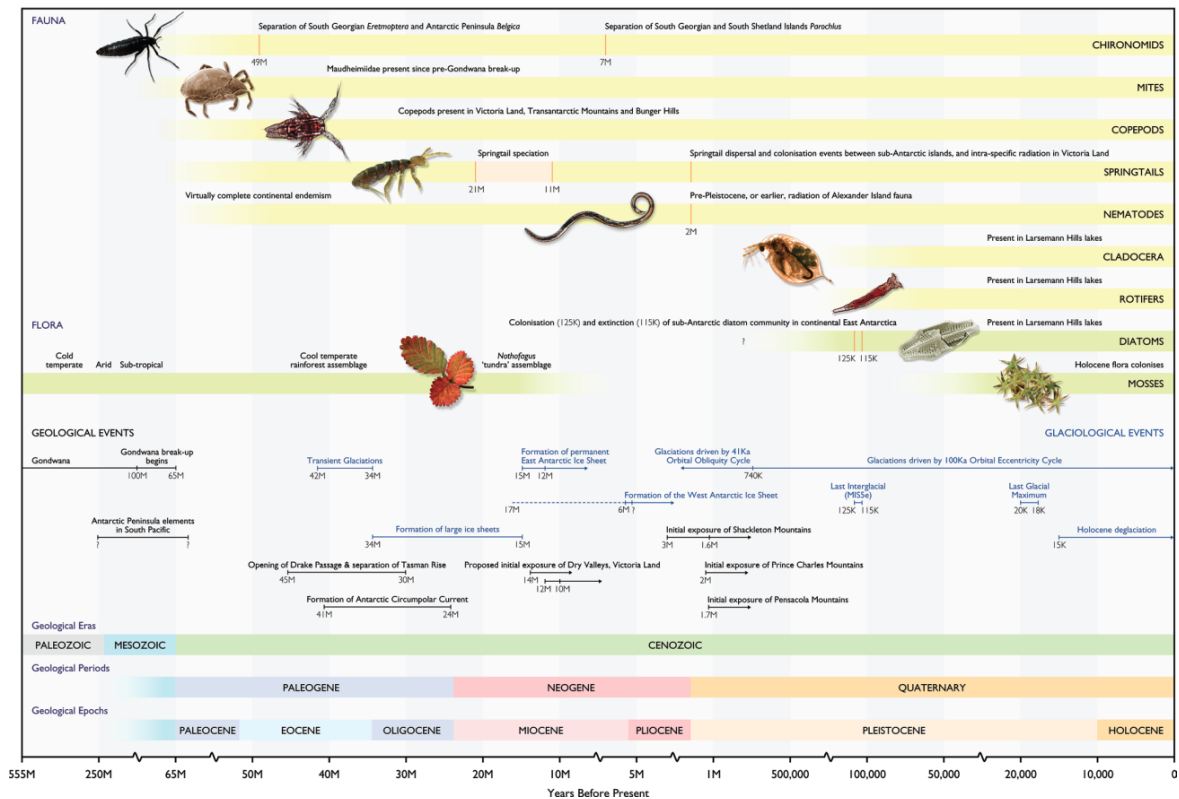
Fig. 1.2 The relative time of arrival of different Antarctic biota on the continent based on biogeographic and molecular studies. The persistence of mosses illustrated in this study is based solely on biogeographic patterns, not including molecular evidence (reproduced from Convey *et al.*, 2008).

1.1.2   Non-molecular evidence of the history of Antarctic bryophytes

Although a long-term history now seems to be the norm rather than the exception in many groups of Antarctic biota, this pattern may not apply to Antarctic bryophytes (e.g. Peat *et al.*, 2007; Convey *et al.*, 2008; Ochyra *et al.*, 2008). This section provides a brief outline of the various general lines of evidence that may be used to infer the history of Antarctic bryophytes. First, a summary of the botanical history of Antarctica from paleontological records is provided in context with the glacial history since the break-up of Gondwana. Second, an overview is given of the general biodiversity and biogeographic patterns of contemporary Antarctic mosses, along with indications of their origin and age in Antarctica. This is followed by a description of studies on the long-distance dispersal abilities of mosses, to start to explore the accessibility of Antarctica for mosses and other spore-dispersed organisms, and to investigate dispersal as a possible post-LGM (re-)colonisation mechanism.

Last, a review is provided of the various possible hypotheses for long-term persistence, which may have relevance to Antarctic bryophytes.

1.1.2.1 *Paleontological evidence in context with glacial history*

From fossil evidence it is clear that Antarctica harboured lush terrestrial ecosystems with a rich angiosperm-dominated flora throughout the Cretaceous (Francis *et al.*, 2008). These ecosystems gradually disappeared as the Earth's climate cooled, most likely caused by declining atmospheric CO_2 (DeConto & Pollard, 2003; Liu *et al.*, 2009; Pagani *et al.*, 2011; Goldner *et al.*, 2014), as well as, to a lesser extent, by the separation of West Antarctica from South America, giving rise to the opening of the Drake Passage and development of a cold circumpolar current (Kennett, 1977; Katz *et al.*, 2008, 2011; Cramer *et al.*, 2009). The period was marked by three stages with particularly high rates of cooling: the Eocene-Oligocene transition (~34 Mya), the Mid-Miocene (~14 Mya), and the late Pliocene (~3 Mya) (Anderson *et al.*, 2011). Over the course of the Oligocene and Miocene, the progressive cooling of the Antarctic region was accompanied by considerable alterations in its terrestrial vegetation (Anderson *et al.*, 2011). Towards the end of the Oligocene (~23 Mya) the vegetation decreased and was replaced by southern beech and conifer-dominated woodlands and tundra. By the mid-Miocene, during the Langhian (15.97-13.82 Mya), there is evidence that the edges of Antarctica were vegetated by low- and high-shrub tundra and prostrate dwarf-shrub tundra (Pound *et al.*, 2012). Evidence from palynological assemblages dated to ~15.7 Mya ago suggest a short period of warming during the Mid-Miocene, with land temperatures reaching up to ~10 °C in the summer in the Dry Valleys sector of the Transantarctic Mountains (Warny *et al.*, 2009). Unique fossil assemblages reveal that a rich tundra community still inhabited the mountains in this region during the Mid-Miocene (Lewis *et al.*, 2008), containing various bryophyte species, including a fossil of *Drepanocladus longifolius* (Mitt.) Paris. After the Mid-Miocene a second cooling transition brought a full polar climate to Antarctica (Lewis *et al.*, 2008). Most tundra assemblages were thought to have been driven extinct in the Antarctic by the Seravallian (13.82-11.62 Mya; Lewis *et al.*, 2008) with the exception of a few localized pockets of tundra on the Antarctic Peninsula (Anderson *et al.*, 2011). Ashworth and Cantrill (2004) reported fossils of tundra vegetation, small dwarf trees and other plants, as well as community of insects (flies and weevils), found in the Sirius Group in the Beardmore region of the Transantarctic Mountains. The exact age of this material is yet to be confirmed, but it likely originated in the Miocene or Pliocene

(Francis & Hill, 1996; Barrett, 2013). It is thought that similar ecosystems may have survived on the Antarctic Peninsula possibly until ~5.3 Mya (Anderson *et al.*, 2011).

Little or no vegetation is thought to have persisted through the late Miocene, which was a time of increased glaciation (Warny *et al.*, 2009; Pound *et al.*, 2011). The Antarctic Peninsula is considered to be the last region of the continent to have become fully overridden by ice sheets, with northern-most Peninsula estimated to be fully glaciated in the early Pliocene (~5.3-3.6 Mya) (Anderson *et al.*, 2011). In the early Pliocene the East Antarctic Ice Sheet (EAIS) and WAIS are thought to have been fully established, although it is estimated that both showed considerable dynamism during Pliocene warm periods (Naish *et al.*, 2009; Dolan *et al.*, 2011; De Schepper *et al.*, 2014). Various lines of evidence suggest that the Antarctic Peninsula experienced several warmer-than-present intervals during the early Pliocene, particularly between ~4.5-4.4 Mya and ~3.6-3.4 Mya (De Schepper *et al.*, 2014). Similarly, the region experienced several warmer-than-present interglacials throughout the Pleistocene, potentially with several full or partial collapses of the WAIS, particularly at ~1.07 Mya, and possibly ~400 kya and ~125 kya (Scherer *et al.*, 2008; Naish *et al.*, 2009; Pollard & DeConto, 2009; DeConto & Pollard, 2016). Over the last ~5 Myr, the WAIS is thought to have undergone rapid transitions between glacial and interglacial conditions (taking one to several thousand years), with dramatic retreat during interglacials, even allowing for the presence of seaways across the base of the Antarctic Peninsula (Pollard & DeConto, 2009).

However, the period also included several intense glaciations, culminating in the Last Glacial Maximum (LGM; for estimated maximum extend during the LGM see Fig. 1.3), with ice sheets advancing as far as the continental shelf margin in most parts of Antarctica (Anderson *et al.*, 2002; Livingstone *et al.*, 2012). The WAIS is estimated to have reached its maximum extent in this period between 33-29 kya, with glacial retreat then commencing ~15-14 kya (Clark *et al.*, 2009). The East Antarctic is also thought to have experienced significant increase in ice extent in this period (Mackintosh *et al.*, 2014; see Fig. 1.3). However, in some regions of East Antarctica, such as in parts of Victoria Land, climatic conditions are known to have remained relatively stable, with limited change in glacial extent or thickness and the distribution of ice-free areas throughout the Pleistocene and Pliocene (Sugden *et al.*, 1993; Oberholzer *et al.*, 2003). The Dry Valleys are thought to have remained largely ice-free for the past ~14 Myr (Sugden *et al.*, 1993). Other areas in East Antarctica currently thought to have remained ice-free during the LGM include the peaks of large mountain ranges: the Transantarctic, Ellsworth and Prince Charles Mountains in particular (Fink *et al.*, 2006;

Sugden *et al.*, 2006; Hodgson *et al.*, 2012), as well as parts of the Larsemann Hills (Hodgson *et al.*, 2001), Bunger Hills (Gore *et al.*, 2001; Rignot *et al.*, 2011) and, perhaps, Vestfold Hills (Gibson *et al.*, 2009; see also Mackintosh *et al.*, 2014).
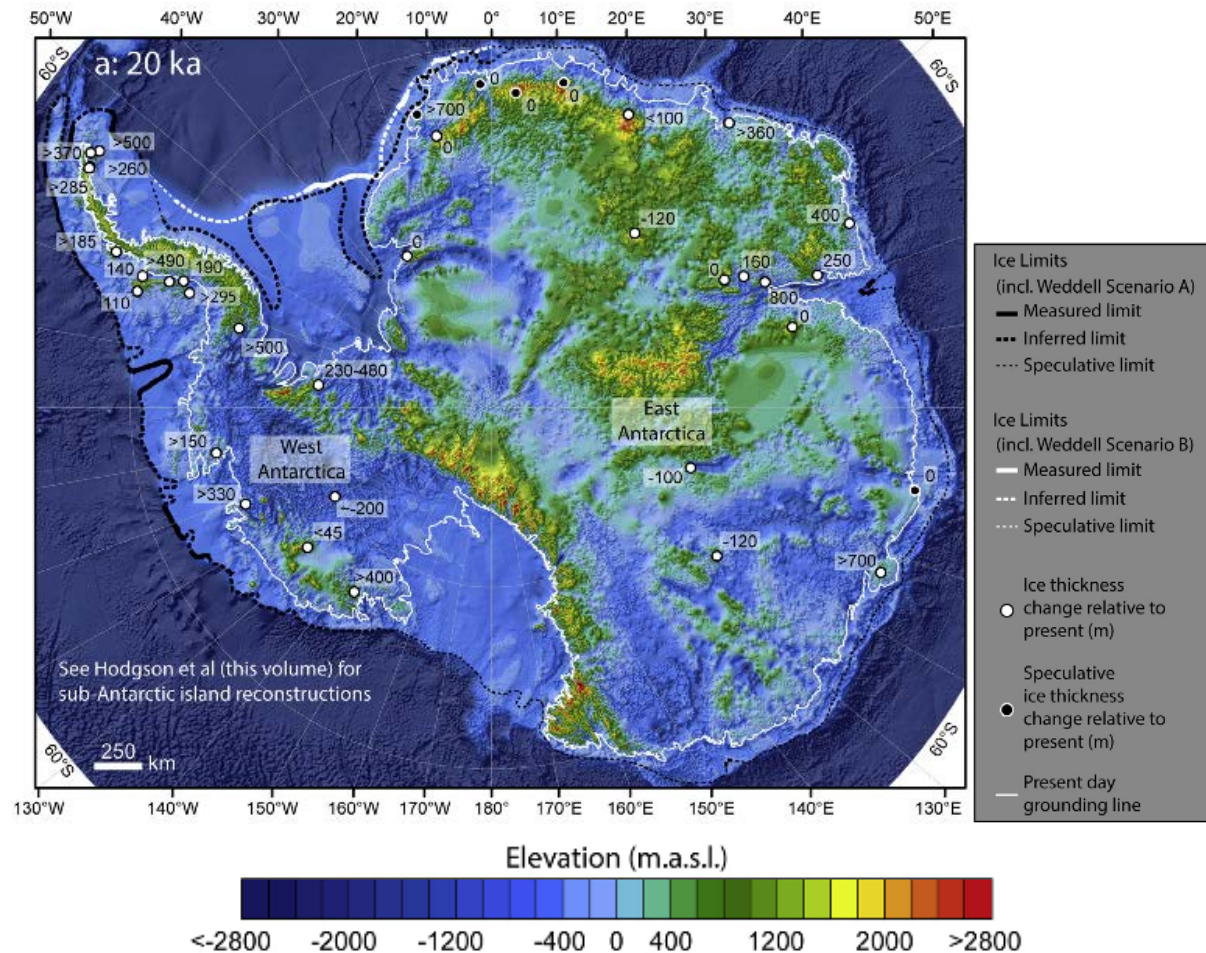
Fig. 1.3 Estimated continental ice extent (black line) during the LGM (reproduced from Bentley *et al.*, 2014, under a Creative Commons Attribution license).

1.1.2.2 *Biodiversity and biogeographic patterns*

With only two native vascular plant species and ~27 liverwort species (Bednarek-Ochyra *et al.*, 2000), mosses are the most diverse group of plants in the Antarctic (Ochyra *et al.*, 2008). Despite this, in total only ~111 species (and two varieties) of moss are found in the Antarctic (Ochyra *et al.*, 2008). This is an extremely low species richness compared to both the nearest continental landmasses and to the general moss richness worldwide (Geffert *et al.*, 2013), as well as in comparison to other polar regions, e.g. an estimated 288 and 535 moss species that are thought to be present in Svalbard (Frisvoll & Elvebakk, 1996) and Greenland (Goldberg, 2003), respectively. However, species richness of bryophytes is often underestimated, as is

shown by the discovery of cryptic speciation in many bryophytes (e.g. Shaw, 2001; McDaniel & Shaw, 2003; Fernandez *et al.*, 2006; Lang *et al.*, 2014). This may be particularly the case in polar environments, where mosses often show very reduced morphology (e.g. small, compact growth forms, lack of sporophytes), making morphological identification very challenging or even impossible (Frisvoll & Elvebakk, 1996; Hesse *et al.*, 2012; e.g. Buryová & Shaw, 2013; Lang *et al.*, 2014), particularly in taxonomically complex genera such as *Schistidium* or *Bryum* (Ochyra *et al.*, 2008), which contribute a considerable proportion of Antarctic bryophyte diversity (Ochyra *et al.*, 2008). Therefore, biodiversity patterns based on morphology alone might give a misleading indication of the true richness, speciation, and subsequently, time of isolation of polar bryophytes.

The majority of Antarctic moss species is either restricted to or only found in the milder and wetter maritime Antarctic (see Fig. 1.4 for commonly used geographic definitions in the Antarctic). Only 24 species of mosses are known from the fringes of the much drier and colder continental Antarctic (see Fig. 1.4). At present, only ~10% of mosses are thought to be endemic (Ochyra *et al.*, 2008) and none of these are endemic to continental Antarctica (Peat *et al.*, 2007). The majority of moss species have a bipolar distribution (~45%), occurring in higher latitudes of both Hemispheres (Ochyra *et al.*, 2008). The second largest group (~23%) has a Southern Hemisphere temperate distribution (with strong affinity to their nearest continental landmasses; South America, south-eastern Australia, Tasmania and New Zealand). The third group has an overlap with sub-Antarctic regions (~16%). And lastly, after the ~10% of mosses that are thought to be endemic, about five species (making up 4.5% of the Antarctic bryoflora) have a cosmopolitan/worldwide distribution (Ochyra *et al.*, 2008).

These general observations (low endemism levels, low richness and overall distribution patterns) lead to an assumption that most mosses did not persist through the LGM and previous glaciations within Antarctica, and that the contemporary Antarctic moss diversity is derived from (re-)colonisation of Antarctica since the LGM. However, the existence of Antarctic endemic species and the fact that the polar bryoflora might harbour considerable levels of cryptic speciation may indicate that this assumption can reasonably be questioned, with existing information not being sufficient to identify signs of long-term persistence.

Fig. 1.4 Geographic extent of the commonly used definitions of maritime Antarctic, continental Antarctic and sub-Antarctic (reproduced from Convey, 2013).

1.1.2.3 *Long-distance dispersal abilities*

The hypothesis of Holocene (re-)colonisation of Antarctica is consistent with the good long-distance dispersal characteristics of mosses, particularly using wind as an important vector for spore dispersal (Winkworth *et al.*, 2002; Muñoz *et al.*, 2004). In the 1970s and 1980s an extensive body of work established strong baseline knowledge of various factors influencing effective long-distance dispersal in brypophytes, with an emphasis on the Southern Hemisphere regions (Van Zanten, 1976, 1978, 1983, 1984; Van Zanten & Pócs, 1981; Longton & Schuster, 1983; Van Zanten & Gradstein, 1987). Although the chances of effective long-distance dispersal and establishment are limited by many factors (e.g. successful release into the air column, survival during transit, deposition in a suitable location at a suitable time of season, and successful establishment), the extensive period of time which has been available for this mechanism to operate (at least 15 ky since LGM ice retreat

commenced) may support a scenario where the current species number of mosses could have been achieved by trans-oceanic post-LGM aerial dispersal (Ochyra *et al.*, 2008) (although no data on this are available). There is some support for such dispersal happening at a continuous rate from spore trapping and particle deposition experiments finding non-native diaspores and pollen in the Antarctic (Kappen & Straka, 1988; Smith, 1991; Linskens *et al.*, 1993; Marshall, 1996). Regional aerobiology studies also suggest that moss propagules can travel hundreds of meters at least (Rudolph, 1970; Marshall & Convey, 1997). Recent phylogeographic studies elsewhere in the world have also demonstrated long-distance dispersal in various moss (Huttunen *et al.*, 2008; Szövényi *et al.*, 2008, 2012; Hedenäs, 2009; Pokorny *et al.*, 2011; Stenøien *et al.*, 2011; Karlin *et al.*, 2012; Piñeiro *et al.*, 2012; Lewis *et al.*, 2014a; Pisa *et al.*, 2014; Kyrkjeeide *et al.*, 2016) and liverwort species (Feldberg *et al.*, 2007; Devos & Vanderpoorten, 2009).

Next to wind dispersal, dispersal *via* animal vectors, such as migratory birds, is often considered as a possible agent for trans-oceanic dispersal. The possibilities of this are limited as there are very few birds that migrate between land areas in the Antarctic and other lower latitude regions. Most Antarctic seabirds come ashore only to breed and, although they could potentially carry moss diaspores, if they do they are likely to originate from the same area in which they breed. However, in the Antarctic winter some birds migrate to other continents at lower latitudes; including skuas, gulls, terns and sheathbills that migrate from the Antarctic Peninsula to southern South America and the Falkland Islands, or even further (Ochyra *et al.*, 2008). Particularly, Arctic Terns (S*terna paradisaea*) are known for their long migration to the Arctic each summer, although they generally do not come to land in the Antarctic, and stay in the Southern Ocean (Egevang *et al.*, 2010). Similarly, South Polar Skuas (*Catharacta maccormicki*) from the maritime Antarctic are known to migrate to various regions across the Atlantic and Pacific (Kopp *et al.*, 2011), while those from the east Antarctic are known to migrate to various locations across the Indian Ocean, New Zealand, Australia and even regions off Japan and northern Russia (Weimerskirch *et al.*, 2015). The ability of moss propagules to survive the journey to the Antarctic *via* birds (potentially including exposure to seawater, although partially protected in the bird plumage) is unknown.

1.1.2.4 *Possible mechanisms for long-term persistence*

Direct evidence for *in situ* survival of mosses in the Antarctic through the Pliocene and Pleistocene does not exist, although a growing body of evidence suggests this to be the case for most Antarctic terrestrial faunal groups, as well as some diatoms, lichens, and even

microbial species (see Section 1.1.1). This may suggest mosses might also have had a long-term presence on the continent, also supported by the fact that they provide suitable habitat for a range of invertebrate groups that could not otherwise survive (Convey *et al.*, in press). Endemic species are currently regarded as strong candidates for survival *in situ* (Ochyra *et al.*, 2008). According to Ochyra *et al.* (2008) this may be especially true for Antarctic and Antarctic-South Georgian endemic mosses, a very distinct and taxonomically isolated group of species which, unless they are discovered in more northerly regions, should be interpreted as pre-Pleistocene survivors (Ochyra *et al.*, 2008).

Several hypotheses have been proposed that provide possible mechanisms for the long-term persistence of Antarctic biota. One of the most recently explored hypotheses is that of survival in geothermally-influenced regions. Fraser *et al.* (2014) showed that within Antarctica significant gradients of decreasing biodiversity exist with increasing distance from geothermally-active areas, particularly around Deception Island in the South Shetland Islands and several volcanoes in Victoria Land. This supports the suggestion that these areas could have acted as regional refugia, although clearly the hypothesis can only apply in those parts of Antarctica with geothermal activity. It should, however, also be noted that the regions in which most geothermally-active areas are present (northern Antarctic Peninsula and Scotia Arc, and Victoria Land) are generally also the regions where most ice-free land is currently located (generally ice-free today regardless of the influence of the geothermally-active areas), making it difficult to differentiate whether the current gradient of decreasing biodiversity truly correlates with geothermal activity or merely with the ice-free area available.

A recent study by La Farge *et al.* (2013) in the Canadian Arctic revealed that some mosses can survive being covered by glacial ice for long periods of time. The study documented the finding of fresh green shoots on several species of 400-year-old moss, which were newly exposed from a retreating glacier, although it did not confirm directly that this was regrowth from existing preserved shoots. The finding both underlies the potential persistence of mosses in extreme environments, as well as broadened the concept of Ice Age refugia, previously and largely implicitly confined to ice-free sites. A unique regrowth observation by Roads *et al.* (2014) showed that the survival abilities of polar mosses in a frozen state can extend even further back in time. In the northern maritime Antarctic the bank-forming moss *Chorisodontium aciphyllum* (Hook. f. & Wils.) Broth, often growing in combination with *Polytrichum alpinum*, develops extensive moss banks often around 1-2m in depth, with some reaching up to 3 m, for instance on Elephant Island in the South Shetland Islands (Smith, 1972b; Collins, 1976b, a; 1979; Fenton, 1980, 1982b; Fenton & Smith, 1982; Björck *et al.*,

1991; 1996; see Fig. 3.2 for an example of *C. aciphyllum* banks on Signy Island, South Orkney Islands). The banks are typically frozen in permafrost below the active layer depth (~30-50 cm deep). As a consequence, the moss shoots deeper in the bank are extremely well preserved. By thawing and exposing a peat core to light and water, new shoots started to grow even in the deepest parts of the core, which were radiocarbon dated to 1697-1533 kya. Microscopic examination revealed that many of the new shoots grew directly from existing preserved gametophyte shoots, making this the longest example of survival and viability of any moss - or indeed multicellular eukaryote - known. New regrowth was also observed in a liverwort species (most likely *Cephaloziella* sp.), with new shoots also found growing at the deepest layer of the core alongside those of *C. aciphyllum*, suggesting that other species growing within the moss bank can become successfully preserved as well. The moss banks produced by *C. aciphyllum* are the oldest known (sub-)fossils in the Antarctic of any extant moss species known. The oldest moss material is found at the base of the banks, with radiocarbon dates obtained from the bases of 1.5 m deep peat banks on Signy Island (South Orkney Islands) and Elephant Island estimated to be ~5,000 and 5,500 years old, respectively (Fenton & Smith, 1982; Björck *et al.*, 1991), and deeper cores may potentially be older. Although the report of Roads *et al.* (2014) and the existence of several thousand year old moss banks suggest that intact bryophytes have the potential to survive through short (century to millennium) periods of ice extension, such as the Little Ice Age, whether this mechanism of survival may allow for survival though entire glacial cycles is unknown.

A possibility often suggested for *in situ* persistence of Antarctic terrestrial biota is survival on ice-free nunataks, which are the summits of mountain peaks rising above the surface of glaciers and ice sheets (Ochyra *et al.*, 2008). Some species of moss can be found growing on nunataks today, providing a starting point for considering candidates for nunatak survival. However, nunataks are today extremely challenging environments, and would only have been more extreme during periods of glacial maxima, and they certainly cannot account for the survival of most moss species or other terrestrial biota, most of which are restricted to the moister coastal areas of the continent and especially the Antarctic Peninsula (Convey & Stevens, 2007; Convey *et al.*, 2008). The same is likely applicable for ice-free areas such as the Dry Valleys in Southern Victoria Land, which, although they are estimated not to have been overridden by glaciers during the LGM and previous glaciations (Sugden *et al.*, 1993; Oberholzer *et al.*, 2003), currently host a very low diversity of bryophytes. Additionally, the fact the fauna in the Dry Valleys is almost entirely endemic and does not overlap with other

regions (Adams *et al.*, 2006), makes the Dry Valleys an unlikely refuge for general *in situ* survival of Antarctica's terrestrial biota.

Submerged habitats are another possible environment for *in situ* persistence of mosses. Although there is no current evidence for this hypothesis, there is evidence for a long-term persistence from aquatic faunal groups such as cladocerans and rotifers, which have been present in lakes in the Larsemann Hills since ~130 kya (Cromer *et al.*, 2006). Several moss species are known to grow (exclusively or non-exclusively) in aquatic environments in Antarctica, including in Alexander Island in the south maritime Antarctic (Heywood, 1977; Convey & Smith, 1997), and several lakes in continental Antarctica in the Schirmacher Oasis in Dronning Maud Land (with mosses growing at depths of up to 32.5 m; Savicz-Lyubitskaya & Smirnova, 1964, 1965; Richter, 1990; Tewari & Pant, 1996; Bednarek-Ochyra *et al.*, 1999), and near the Japanese Sôya station in Enderby Land (at depths of 3-5 m; Imura *et al.*, 1999). The moss *Bryum pseudotriquetrum* (Hedw.) P.Gaertn. B.Mey. & Scherb.*,* frequently found growing in submerged as well as terrestrial habitats in Antarctica, has also been found growing in the permanently ice-covered Lake Vanda in the Dry Valleys, at depths of 9-31 m (Kaspar *et al.*, 1982), as well as at a depth of 81 m in Radok Lake in Amery Oasis, MacRobertson Land (Wagner & Seppelt, 2006). The species is also thought to grow at Bunger Oasis at at least 70-90 m and possibly 100 m depth (D. Anderson, personal communication note quoted by Ochyra *et al.*, 2008). A Mid-Miocene fossil of the aquatic moss *Drepanocladus longifolius* reported by Lewis *et al.* (2008) from the Sirius Formation in the Dry Valleys represents an extant species also recently found growing in a lake on King George Island, South Shetland Islands (Li *et al.*, 2009). However, it is unlikely this species persisted in Antarctica throughout the late Miocene until the present *in situ*, as its current occurrence is far distant and on the opposite side of the continent from the Dry Valleys, and it is very rare. Nevertheless, whether its aquatic lifestyle could potentially have offered this species the opportunity to persist through glaciation periods is unknown, and future work on the phylogeography of this species, as well as other Antarctic aquatic species, would be of interest.

Another possibility for *in situ* survival, although less likely, is on glacier surfaces, with mosses growing in association with cryoconite holes, or as so-called "glacier mice" - clumps of unattached moss balls, also called globular mosses, rolling moss, moss pollsters, unattached mosses, and solifluction floaters (see Perez, 1991 and references therein). Glacier mice have been recorded on glaciers from every continent except Australia (Perez, 1991). In the Antarctic they have been recorded near Mawson station in MacRobertson Land (Seppelt

& Ashton, 1978; Longton, 1985; 1988a) as well as many sub-Antarctic islands (see Perez, 1991 and references therein). The species found growing in MacRobertson Land were *Bryum algens* (Card.) and *Grimmia lawiana* (J.H.Willis), now known as *Bryum pseudotriquetrum* and *Coscinodon lawianus* (J.H.Willis) Ochyra, respectively. In the Arctic, several mosses are reported to occur in this form, including common species also found in the Antarctic such as *Pohlia nutans* (Hedw.) Lindb., *Ceratodon purpureus* (Hedw.) Brid., and *Polytrichum juniperinum* Hedw., as well as species of the genera *Andreaea*, *Ditrichum* and *Drepanocladus* (Heusser, 1972). Glacier mice have even been found to contain relatively rich communities of invertebrates (Coulson & Midgley, 2012). Whether mosses can use this mechanism as a permanent life-style or mode of survival is unknown.

Lastly, burial of spores or other propagules in soil or ice may provide a possible survival strategy. Soil composition and incubation studies from various locations in the maritime (Smith & Coupar, 1986; Smith, 1987, 1991, 1993, 1997) and continental Antarctic (Smith & Ochyra, 2006) have confirmed moss growth from soil after incubation. Most of these were species commonly found in the surrounding area, and were presumed to have derived from the local flora (Ochyra *et al.*, 2008, and references therein). However, exotic species have also been found, e.g. cultured soil from Coulman Island in northern Victoria Land revealed growing shoots of a moss unknown in the Antarctic (Smith & Ochyra, 2006) and a fern was cultured from cryoconite material from Signy Island, South Orkney Islands, while no ferns are known to occur here naturally (Smith, 2014). How long a spore can remain viable while buried in polar soils is not known, however there have been plentiful reports of successful revival of bacteria and fungi entrapped in ice and soils with ages ranging from up to 20 ky old, including from locations in Antarctica (Gilichinsky & Wagener, 1995; Christner *et al.*, 2000), and even revival of bacterial spores from 25-40 My old Dominican amber (Cano & Borucki, 1995). Glacial survival by burial of spores or other propagules in soil or ice in Antarctica may therefore not be an entirely unrealistic scenario for bryophytes.

1.1.3 Molecular studies on Antarctic bryophytes

1.1.3.1 *Isozymes and allozymes*

In the last two decades researchers have started to apply molecular tools, particularly to investigate genetic structure within and between moss populations in the Antarctic and sub-Antarctic. Amongst the first of these studies were those studying the genetic variation of isozymes (Melick *et al.*, 1994; Adam *et al.*, 1997) and allozymes (Selkirk *et al.*, 1997). Both isozymes and allozymes represent different types of enzymes: the former are different forms

of the same enzyme (catalysing the same reaction) encoded by different gene loci, while the latter are enzyme variants encoded by different alleles of the same gene locus. Melick *et al.* (1994) studied 15 isozyme forms in three moss species from the Windmill Islands, East Antarctica. The species *Ceratodon purpureus* and *Schistidium antarctici* revealed no variation, while variation for two allozyme loci was found in *Bryum pseudotriquetrum*. It was therefore suggested that *C. purpureus* and *S. antarctici* populations were likely to have originated from one colonization event, while it was likely that the *B. pseudotriquetrum* populations originated from several independent colonisations. However, each species was represented by a limited number of samples (3-16), which might also explain the observed low variation. A study by Selkirk *et al.* (1997) investigated the variation of allozymes (which is simplified in mosses as the dominant gametophyte stage is usually haploid) of the moss *Sarconeurum glaciale*. This study had a larger sample size (n=60 across two regions, Vestfold Hills and southern Victoria Land, separated by 2700 km) and tested five enzyme systems, with variation found in one allozyme locus. These studies provided useful data, although of limited utility in assessing the true extent of genetic variation through either the small numbers of loci or specimens examined or the resticted source geographic distributions.

1.1.3.2 *RAPD markers*

A second molecular tool to be applied was the use of RAPD (random amplified polymorphic DNA) markers (e.g. Adam *et al.*, 1997; Selkirk *et al.*, 1997, 1998; Skotnicki *et al.*, 1997, 1998a, b, 1999, 2000, 2001, 2002, 2004, 2005; Dale *et al.*, 1999; Seppelt *et al.*, 1999). RAPD is a technique which utilises the Polymerase Chain Reaction (PCR) and several short primers to amplify random regions within the genomic DNA of a sample, generating unique profiles per species or population when separated by electrophoresis. The technique provides a rapid assessment of genetic variation, as no previous knowledge of the targeted genome is required; however, it has limitations when applied to degraded samples or samples from the field (see below). Most studies applying RAPD found extremely high rates of variation between populations, even within a single moss shoot (Adam *et al.*, 1997; Skotnicki *et al.*, 1997, 1998a, b, 2000, 2001, 2004; Dale *et al.*, 1999), a remarkable finding which had never been observed in mosses before. The high variability found in Antarctic mosses was hypothesised to derive from high levels of somatic mutation; as shoots in mosses grow from a single apical cell, mutations in different parts of a single colony would result in genetically different branches within a moss clump ("genetic mosaicism") (e.g. Adam *et al.*, 1997; Skotnicki *et al.*, 1999, 2000, 2004). However, these findings and conclusions were subsequently

questioned, and the extremely elevated levels of genetic variation in Antarctic mosses detected with RAPD were found to have been the result of contamination by biota naturally associated with the mosses (e.g. fungi or protozoa; Stevens *et al.*, 2007). Since the RAPD technique shows all genetic variation in a sample and does not differentiate between DNA derived from the study species or contamination, the technique should only be applied on clean samples. Although RAPD is a quick and easy method and can be used, for example, as an aid in taxonomy (e.g. as applied by Skotnicki *et al.*, 2002, 2004, 2005), it is now evident that RAPD does not provide sufficient information for understanding genetic variation, evolution and dispersal, particularly in the context of potentially contaminated environmental samples (Skotnicki *et al.*, 2005; Stevens *et al.*, 2007).

1.1.3.3 *Microsatellites*

More recently, microsatellite markers have been used to estimate and compare the genetic diversity within and between continental Antarctic, sub-Antarctic and temperate populations of the moss *Ceratodon purpureus* (Clarke *et al.*, 2008, 2009). These studies found that populations from continental Antarctica possess lower genetic diversity than temperate and sub-Antarctic populations. These studies also found no evidence of intra-individual variation or elevated mutation rates in the Antarctic, such as found in RAPD marker studies (see Section 1.1.3.2). It should, however, be noted that the different regions were sometimes only represented by a single population, with a maximum of only five populations, and the continental Antarctic samples originated from only one area in the Windmill Islands, East Antarctica.

1.1.3.4 *Sequence data*

As mentioned above (see Section 1.1.3.2), Stevens *et al.* (2007) investigated whether the extremely high genetic variation found in studies implementing RAPD markers reflected true genetic variation. Using a combination of sequence data (nuclear Internal Transcribed Spacer; *ITS*) and RAPD markers they provided evidence that the unusually elevated levels of genetic variability reported in RAPD studies were artefacts from contamination, and concluded that RAPD markers are inadequate for detecting genetic variation between different organisms or populations in environmental samples. Later, the same research group also examined DNA sequences of the nuclear markers *ITS* and *phy2* from several different populations to investigate the genetic variation and the persistence of *Bryum argenteum* in Antarctica, also including several locations from Australia and New Zealand (Hills *et al.*, 2010). The study

found that Antarctic specimens formed one monophyletic group, and that there was less genetic diversity amongst Antarctic samples than between non-Antarctic samples. The authors proposed that it was likely that Antarctic populations experience a much lower DNA substitution rate due to a lack of sexual reproduction and the possibility of isolation in refugia. They also suggested that the low genetic variation between Antarctic populations was a clear indication of isolation throughout the Holocene and most probably into the Pleistocene. However, this low level of variation between Antarctic populations could also be explained by a relatively recent dispersal of the species to the Antarctic. It should also be noted that the samples used in the study were obtained from relatively adjacent populations within Antarctica, while being compared with non-Antarctic populations from highly dispersed locations, which could also contribute to the low genetic variation found between the populations in Antarctica.

A recent study used sequence data to investigate the identity of an unknown species of aquatic moss growing in lakes of the Sôya Coast region, East Antarctica (Kato *et al.*, 2013). The species was challenging to identify as it had a very different morphology when growing in this aquatic environment. Using two chloroplast regions (*rps4* and *trnL-F*) and the nuclear *ITS* region the moss was identified as *Leptobryum wilsonii* (Mitt.) Broth., a species described from South America. The study found almost no genetic variation between samples from the Antarctic and Chile, and the authors suggested that the species was a post-LGM arrival in the Antarctic (although it should be noted that the Sôya Coast is far isolated along the East Antarctic coastline from southern South America, and the species is currently known from no other geographically intermediate location in Antarctica).

During the course of this PhD project I was involved as joint first author in a phylogeographic study on *Bryum argenteum*, with a particular aim of investigating the age and dispersal patterns of this moss in Antarctica (Pisa *et al.*, 2014). Following up on the work of Hills *et al.* (2010) this study used the nuclear *ITS* marker and increased the spread and number of samples examined (*n*=154), in order to adequately cover the cosmopolitan range and ruderal nature of the species. The study provided a first suggestion of a multi-million year persistence of *B. argenteum*, and therefore of any moss, in the Antarctic. It also proposed evidence of at least three separate dispersal events of this moss to the continent. Conservative molecular dating analyses (based on published molecular clock rates for what are expected to be much more rapidly evolving flowering plants) suggested that the arrival of the different lineages in the Antarctic ranged from four million years ago for the oldest to half a million years ago for the youngest lineage, timings that fall within the Pleistocene, Pliocene

and possibly late Miocene. This study provided a first intriguing indication that other bryophytes may also have had a longer and previously unappreciated persistence in Antarctica.

1.2 Objectives and project outline

This project aims to increase the understanding of the origin, age and general evolutionary history of Antarctic bryophytes. Despite their crucial role within the terrestrial ecosystem, and the apparent conflict between glaciological understanding of Antarctic history and the overall patterns of long-term persistence being revealed increasingly within Antarctic terrestrial biota, very few studies to date have addressed the evolutionary history of Antarctic bryophytes. While several characteristics of Antarctic mosses can be argued to be consistent with recent (post-LGM) arrival on the continent (e.g. low endemism levels, low species richness, distribution patterns, good dispersal abilities), there are also features consistent with long-term persistence in at least some species (e.g. the occurrence of endemic species, possibility of previously un-noticed cryptic speciation, various hypotheses for *in situ* survival, as well as the general implication drawn from the now strong evidence of long-term persistence in members of nearly all other groups of Antarctic terrestrial biota). Increased knowledge of the age and origin of the dominant Antarctic vegetation group is important for increasing our understanding in several key areas:

- how past climate has affected the Antarctic flora
- the provision of biological evidence for the past extent of glaciation in different parts of Antarctica
- the general abilities of bryophytes to survive in extreme environments
- the capability of Antarctic bryophytes to respond to past and future climate change scenarios

In this study I aimed to test the following hypotheses:

1. **Recent dispersal hypothesis**
   Bryophytes were driven extinct during Pleistocene glacial maxima and modern Antarctic bryophytes are recent colonists.

2. **Long-term Antarctic presence hypothesis**
   Bryophytes have a long-term history on the Antarctic continent and have survived through at least one glaciation period *in situ.*

3. **Combination of hypotheses 1 and 2**

   Some bryophyte species show a long-term history in Antarctica, and others are recent colonists.

While several molecular studies have investigated genetic variability in Antarctic mosses, as might be expected in a rapidly developing methodological field these often used inadequate or now inappropriate genetic methods, or insufficient sampling, to assess the relative timescale over which mosses could have been present on the Antarctic continent. In this thesis, I set out to test the hypotheses outlined by combining wide geographic sampling with cutting-edge phylogeographic and population genetic methods, where possible also incorporating molecular divergence time estimates. These approaches have not been applied to Antarctic bryophytes on such a scale before (see 1.1.3). These molecular tools can also provide valuable insights into patterns of population history, such as population connectivity or isolation, bottlenecks, dispersal and gene flow.

By studying multiple moss taxa I aimed to generate a general overview of the evolutionary history of bryophytes in the Antarctic. I decided to select species that had different or contrasting regional or global geographic ranges so that various phytogeographic elements of the Antarctic were represented. Thus, in Chapter 2 I chose to study four common bipolar species of the family Polytrichaceae, as they represent the most common (~45% of species) biogeographic pattern of mosses in the Antarctic. Additionally, I was interested in the origin, direction and timescale of inter-hemispheric movements in bryophytes that have this particularly disjunct distribution. Chapter 3 focuses on the global biogeographic patterns of a very common and globally widespread moss, *Ceratodon purpureus* (Hedw.) Brid., that is both a model organism representing non-vascular plants in laboratory and applied studies, and also one of the most widespread mosses in the Antarctic, including the harsh continental Antarctic, and therefore a suitable study species to investigate possible long-term persistence. Chapter 4 in contrast focuses on genetic variation in the regionally-restricted bank-forming moss *Chorisodontium aciphyllum*, based on material obtained throughout its restricted southern South American and Scotia Arc geographic range; the species was also selected as it has the oldest moss sub-fossils of any extant Antarctic plant, and has demonstrated an extreme case of viability over approaching two millennia from permafrost revival experiments. Finally, in Chapter 5, I set out to study the diversity, richness and relative age divergences within Antarctic species of the genus *Schistidium*, which is the moss genus that

is the most species-rich in the Antarctic, as well as that containing the most Antarctic endemic species, again strong candidates for long-term persistence in the Antarctic.

2 Global biogeographic patterns of four bipolar Antarctic Polytrichaceae mosses

Fig. 2.1 A member of the Polytrichaceae (likely *Polytrichastrum alpinum*) growing on Livingston Island in the South Shetland Islands. (Photo: E.M. Biersma)

This chapter is based on the following manuscript:

Biersma E.M., Jackson, J.A., Hyvönen, J., Koskinen, S., Linse, K., Griffiths, H., and Convey, P. (2017) Global biogeographic patterns in bipolar moss species. *Royal Society Open Science*, 4(7), 170147.

2.1   Abstract

A bipolar disjunction is an extreme, yet common, biogeographic pattern in non-vascular plants, yet its underlying mechanism (vicariance or long-distance dispersal), origin and timing remains poorly understood. Here, combining a large-scale population dataset and multiple dating analyses, we examine the biogeography of four bipolar Polytrichaceae mosses, common to the Holarctic (temperate and polar Northern Hemisphere regions) and the Antarctic region (Antarctic, sub-Antarctic, southern South America) and other Southern Hemisphere regions. Our data reveal contrasting patterns, for three species were of Holarctic origin, with subsequent dispersal to the Southern Hemisphere, while one, currently a particularly common species in the Holarctic (*Polytrichum juniperinum*), diversified in the Antarctic region and *from* here colonised both the Holarctic and other Southern Hemisphere regions. Our findings suggest long-distance dispersal as the driver of bipolar disjunctions. We find successful inter-hemispheric colonizations are rare, occurring on multi-million year timescales. High altitude tropical populations did not act as trans-equatorial "stepping-stones", but rather were derived from later dispersal events. All arrivals to the Antarctic region occurred well before the Last Glacial Maximum and previous glaciations, suggesting that, despite the harsh climate during these past glacial maxima, plants have had a much longer presence in this southern region than previously thought.

2.2   Introduction

Since the 19th Century, scientists have been puzzled by the origin and evolution of plants with disjunct distributions, and particularly with the most extreme pattern of all - bipolar disjunctions (Von Humboldt, 1817; Darwin, 1859; Wallace, 1880). Bipolar distributions characterise species occupying high latitudinal areas of both the Northern (NH) and Southern Hemispheres (SH), with or without small intermediate populations at higher elevations in the tropics (Du Rietz, 1940). The distribution pattern could originate from: i) long-distance dispersal, either in one event or gradually, *via* high altitude intermediate latitude 'stepping-stone' populations, or ii) vicariance, with a large ancestral distribution split into smaller units by environmental barriers such as past climate change (e.g. glaciations, sea level change) or tectonic events.

As bipolar disjunctions in mosses are common (e.g. ~45% of all mosses currently occurring in the Antarctic are bipolar; Ochyra *et al.*, 2008) they have received much attention in

descriptive studies (Du Rietz, 1940; Schofield & Crum, 1972; Smith, 1972a; Schofield, 1974; Ochyra *et al.*, 2008). The disjunction has been suggested to be of post-Pleistocene Holarctic origin, resulting from dispersal along tropical mountainous chains across the tropics (Ochyra *et al.*, 2008), from where the taxa were able to colonise many high latitude SH areas left barren by receding glaciers. However, few molecular studies have addressed the question to date. Two recent molecular phylogeographic studies of bryophytes with disjunct distributions reaching as far south as Tierra del Fuego have suggested the distribution to be due to dispersal events, either recent (e.g. *Cinclidium stygium* Sw., no molecular dating but very low variation between hemispheres; Piñeiro *et al.*, 2012) or in the more distant past (e.g. the dung-moss genus *Tetraplodon* Bruch & Schimp. dispersed to South America ~8.6 Mya; Lewis *et al.*, 2014a). However, the lack of variation in disjunct populations of *C. stygium* makes it difficult to distinguish whether the disjunction is natural or caused by anthropogenic vectors (Piñeiro *et al.*, 2012), and the dung-associated lifestyle of *Tetraplodon* makes this moss a likely candidate for adventitious dispersal *via* migrating birds (Lewis *et al.*, 2014a) (e.g. becoming attached when birds forage for insects attracted to dung), which might not be a typical characteristic of the majority of bipolar moss species. In-depth investigations into global patterns of dispersal of bipolar mosses are clearly needed, including species that are more widespread and have a bryophyte-representative ecology.

We here obtained the first large-scale global population dataset (n=255 data source locations) to explicitly explore the biogeographic history of several common bipolar mosses; we examined whether their distributions result from recent inter-hemispheric dispersal events or long-term separation, and assessed the underlying drivers explaining their distributions. Our study focuses on four common bipolar species of the Polytrichaceae, an old and distinct group of mosses, including three species from the genus *Polytrichum* Hedw. (*P. juniperinum* Hedw., *P. strictum* Brid., and *P. piliferum* Hedw.) plus one species of a closely related genus, *Polytrichastrum alpinum* Hedw. We particularly focussed on *P. juniperinum,* due to its previously observed phenotypic variation throughout its global range (Ochyra *et al.*, 2008). All species occur in higher latitude areas in both Hemispheres, with the bulk of their distributions in the NH. Their SH distributions are more restricted (in absolute area) to the Antarctic region (southern South America, the Atlantic sub-Antarctic islands, and the Antarctic Peninsula), with some species having additional populations in other SH locations (Fig. 2.2; Ochyra *et al.*, 2008). Although some of the species are sometimes described as cosmopolitan, according to the most recent global assessment (Ochyra *et al.*, 2008) all are bipolar. *P. strictum* is strictly bipolar, whereas the other species also have restricted

intermediate populations in high altitude equatorial regions, a feature valuable for assessing whether these intermediate populations have acted as stepping-stones, are remnants of a once wider distribution (vicariance), or the result of separate colonisation events.
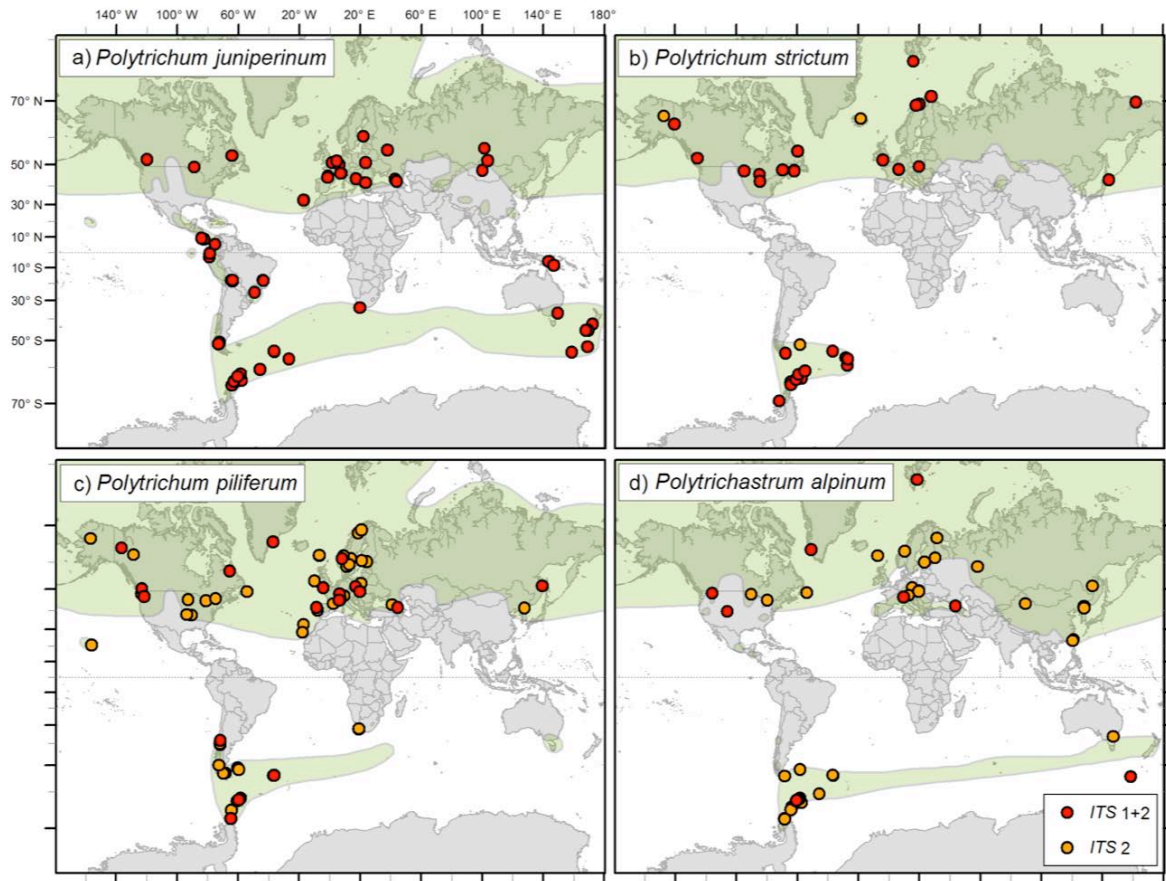
Fig. 2.2 Locations of *ITS* 1+2 (red) and *ITS* 2 only (orange) samples of *Polytrichum juniperinum* (a), *P. strictum* (b), *P. piliferum* (c) and *Polytrichastrum alpinum* (d). Known global distributions of the different species (shown in green) are reproduced from Ochyra *et al.* (2008).

2.3   Materials and Materials

2.3.1   Sampling and molecular methods

We sampled 71, 59, 73 and 52 individuals of *P. juniperinum*, *P. strictum, P. piliferum* and *P. alpinum*, respectively, representing their worldwide distributions (see Table 8.2.1.1 in the Appendix for sample information, and Section 8.1.1-8.1.4 for in-detail laboratory techniques

and protocols). Total genomic DNA (gDNA) was extracted using the DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany), using liquid nitrogen and a mortar and pestle. PCR amplification was performed using the Taq PCR Core Kit (Qiagen GmbH, Hilden, Germany) with addition of Bovine Serum Albumin (BSA), and results were checked using gel electrophoresis. Internal Transcribed Spacer (*ITS)* regions 1 (636-1007 bp) and 2 (386-441 bp) were amplified separately using primers ITS-A and ITS-C (Blattner, 1999) for *ITS* 1, and 5.8S-R (Shaw, 2000) and 25R (Stech, 1999), or ITS3 and ITS4 (White *et al.*, 1990) for *ITS* 2. The plastid spacer *trnL-F* (455-545 bp) was amplified using primers *trn*LF-c and *trn*LF-f (Taberlet *et al.*, 1991). An annealing temperature of 60°C was used for all amplifications. Forward and reverse sequencing was performed by LGC Genomics (Berlin, Germany).

2.3.2 Sequence editing and alignment

Forward and reverse sequences were manually examined and assembled using Codoncode Aligner v.5.0.2 (CodonCode Corp., Dedham, MA). *ITS* and *trnL-F* sequences were aligned using PRANK (Löytynoja & Goldman, 2008) using default settings, with obvious misaligned sequences re-aligned manually. Short partially incomplete sections at the ends of each alignment were excluded. In the *trnL-F* fragment a previously identified hairpin-associated inversion known to be highly homoplastic (Quandt & Stech, 2004) was excluded. The *ITS* 1 and 2 fragments were combined, and hypervariable regions were identified and removed using NOISY (Dress *et al.*, 2008) using default settings, resulting in a reduced alignment with 1614 bp (96.82% of the original 1667 bp). The number of variable and parsimony informative sites was calculated using MEGA7 (Kumar *et al.*, 2016).

2.3.3 Phylogenetic analyses

Polytrichastrum tenellum (Müll. Hal.) G.L. Sm. and *Meiotrichum lyallii* (Mitt.) G.L. Merr. (Genbank accessions GU569750 and AF545011*,* respectively) were chosen as outgroups for *trnL-F*. Based on the prior phylogenetic analyses (Bell & Hyvönen, 2010; Bell *et al.*, 2015) which have established sister-group relationships for this family, *P. alpinum* was used as an outgroup in the *ITS* 1+2 phylogenetic analyses.

Models of sequence evolution were selected for each locus using jModeltest-2.1.7 (Darriba *et al.*, 2012) using the SPR tree topology search operation and the Akaike Information Criterion (AICc). Maximum likelihood (ML) analyses were performed for each locus using RAxML-GUI v1.3.1 (Silvestro & Michalak, 2012), applying GTR and GTR+G for *trnL-F* and *ITS* 1+2, respectively, applying default settings and estimating support values using 1000

bootstrap iterations. Bayesian analyses were performed for *trnL-F* and *ITS* 1+2 separately using MrBayes 3.2 (Ronquist *et al.*, 2012), and were run for 1×10^6 and 2×10^7 generations respectively, sampled every 1.0×10^3 generations, discarding the first 25% as burn-in. Convergence was assessed by checking split frequencies had an average standard deviation of <0.01, and by using Tracer v.1.6 (Rambaut *et al.*, 2014) to check all parameters had effective sample sizes (ESS) >200. Maximum clade credibility trees were visualised using FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

2.3.4   Species delimitation

We explored possible species clusters in *ITS* 1+2 within the currently-described species by testing for intraspecific divergence based on pairwise genetic distances using the Automatic Barcode Gap Discovery (ABGD) web server (Puillandre *et al.*, 2012), using default settings. ABGD uses a genetic distance-based approach based on non-overlapping values of intra- and interspecific genetic distances, sorting the sequences into hypothetical candidate species.

2.3.5   Population diversity analyses

To examine the phylogeographic structure within species, TCS networks (Clement *et al.*, 2000) were produced using *ITS* 1+2 for each species with Popart (Leigh & Bryant, 2015), using default settings. Because of a greater number of *ITS* 2 sequences available for *P. piliferum* and *P. alpinum,* we calculated additional haplotype networks for *ITS* 2 only for these species. Genetic diversity indices, pairwise Kimura-2P distances, demographic and spatial models and neutrality tests Tajima's D (Tajima, 1989) and Fu's Fs (Fu, 1997) were calculated for *ITS* 1+2 for each species with 10000 permutations, using Arlequin v3.5.1.2 (Excoffier & Lischer, 2010). We also performed statistics on various monophyletic clusters within *P. juniperinum.*

2.3.6   Molecular dating

Although *ITS* is a useful marker for investigating population- or species-level variation, it is too variable to be used directly in a larger dating analysis including more distantly related species in which informative fossils can be incorporated. Therefore, to investigate the divergence times of the different species and populations in the *ITS* dataset, we used several different calibration approaches. For all dating analyses we used BEAST v2.2.1 (Drummond *et al.*, 2012), and Tracer v1.6 (Rambaut *et al.*, 2014) to examine stationarity and effective sampling (ESS>100) from the posterior distribution. To investigate the divergence times of

the different species and populations in the *ITS* dataset, we used the following different calibration approaches:

<u>(I) Two-step analyses:</u>

(I1) In the first step of the two-step age estimations, we assessed the age of the split between *P. piliferum*/(*P. juniperinum* + *P. strictum*), using a larger dataset, which spans all major clades of the Polytrichales and six gene compartments: *rbcL*, *trnL-F, rps4, rps4-trnS* (plastid) and *nad5* (mitochondrial) (Bell *et al.*, 2015). All BEAST settings followed Bell *et al.* (2015), excepting the addition of *P. strictum* to all gene regions of the dataset, so that all four study species were represented. We used the same priors as Bell *et al.* (2015), which included 1) a normal (230.53, stdev 22) prior on the age of the MRCA of the Polytrichales (Newton *et al.*, 2007; prior 1 in Fig. 8.2.1.4 in the Appendix); 2) a uniform prior (37.0, 500) on the *Psilopilum*/*Steereobryon*/*Atrichum*/*Delongia* clade (Ritzkowski, 1999; prior 3 in Fig. 8.2.1.4 in the Appendix); and 3) a uniform prior (83.64, 500) on the stem lineage of *Polytrichum* sect. *Polytrichum* + *P.* sect. *Juniperifolia* (prior 2 in Fig. 8.2.1.4 in the Appendix) based on the fossil *Eopolytrichum antiquum* Konopka et al. (Konopka *et al.*, 1997; Hyvönen *et al.*, 2004; Bell *et al.*, 2015). Following the most recent palynological analyses of the source material of *E. antiquum* (Christopher, 1979; Huddlestun & Hetrick, 1991; Gradstein *et al.*, 2012), we used a lower bound of 83.64 Mya (instead of 80 Mya as in Bell *et al.*, 2015).

Similar to Bell *et al.* (2015), as the precise taxonomic placement of *E. antiquum* is not fully resolved (Konopka *et al.*, 1997; Hyvönen *et al.*, 2004; Bell *et al.*, 2015), we performed two analyses: one with (I1a) and one without (I1b) *E. antiquum.* Runs (I1a) and (I1b) were run for 1.2×10^9 and 2.0×10^9 generations, respectively, sampling every 1.0×10^4 generations, with a burn-in of 40% and 10%, respectively. Fig. 8.2.1.4 in the Appendix shows the outcome of the (I1a) run with all priors and the key node of interest (*P. piliferum*/(*P. juniperinum* + *P. strictum*)).

(I2) As a second step in the two-step dating analyses we applied the resulting divergence times and corresponding 95% quantiles of the split between *P. piliferum*/(*P. juniperinum* + *P. strictum*) (as calculated from I1), as a secondary prior on the same node in the *ITS* 1+2 dataset (I2). This was done for both analyses with and without *E. antiquum* (in analyses I2a and I2b, respectively).

<u>(II) Molecular rate analysis</u>

We also performed a dating analysis (II) based on a defined *ITS* substitution rate $(1.35\times10^{-3}$ subst./site/Myr) previously applied in bryophytes (Hartmann *et al.*, 2006; Lang *et al.*, 2015), but originally derived from angiosperms (Les *et al.*, 2003, and references therein).

All BEAST analyses based on the *ITS* dataset (Models I2a, I2b and II) had the same settings, except for the particularities of comparing a rate vs. a fossil-based analysis: therefore, we used a Yule Model and a clock rate of 1.35×10^{-3} subst./site/my in method II, whereas we used a Calibrated Yule Model and a 1/X distribution on the ClockRate variable in I2a and I2b. In all runs we used a Strict clock, a GTR+G site model with empirical frequencies, a 1/X distribution for the BirthRate parameter, and MCMC chains were run for 1.0×10^{7} generations, sampling every 1.0×10^{3} generations. Maximum clade credibility trees were constructed in TreeAnnotator v1.8.2 (Drummond & Rambaut, 2007), with 10% burn-in removed, and visualised using Figtree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

2.3.7 Ancestral range distribution

We used the R-package BioGeoBEARS (Matzke, 2014; R Core Team, 2015) to estimate the probabilities of ancestral area ranges at each node. This package estimates the maximum likelihood of the geographical range as well as models for evolution of geographic range along a time-calibrated phylogeny. We tested different models of dispersal, extinction and/or founder-event speciation (+J; Matzke, 2014) implemented in the script, selecting the best model using the AICc criterion and the likelihood ratio test. The maximum number of areas per node was set to five, the same as the number of regions specified in this study.

2.4   Results

2.4.1 Molecular sequence data

Samples were obtained from a broad range of locations for each species within their global distribution (for *ITS* 1+2 samples see Fig. 2.2; for *trnL-F* samples see Fig. 2.3). Alignments of *ITS* 1+2 and *trnL-F* consisted of 448-1007, 386-426 and 455-545 bp, respectively. The nuclear regions had more genetic variation (after treatment with NOISY (Dress *et al.*, 2008): *ITS* 1 = 244 and 220 variable and parsimony informative (PI) sites, respectively; *ITS* 2 = 72

and 66 variable and PI sites, respectively) than the *trnL-F* region (26 variable sites, 25 PI sites), reflecting the fact that *ITS* is faster evolving than *trnL-F*. Within *P. piliferum*, most intra-species variation occurred within a large indel (~427bp) which was found in *ITS* 1 and is unique to this species. AICc favoured the TPM3uf (nst=6) model for *trnL-F* and TrN+G (nst=6, rates=gamma) model for *ITS* 1+2. A relatively high proportion of double peaks within *ITS* 1+2 chromatograms of several *P. strictum* specimens suggested multiple copies of *ITS* were present within some individuals of this species, possibly the result of a past hybridization event (see section 2.4.3).
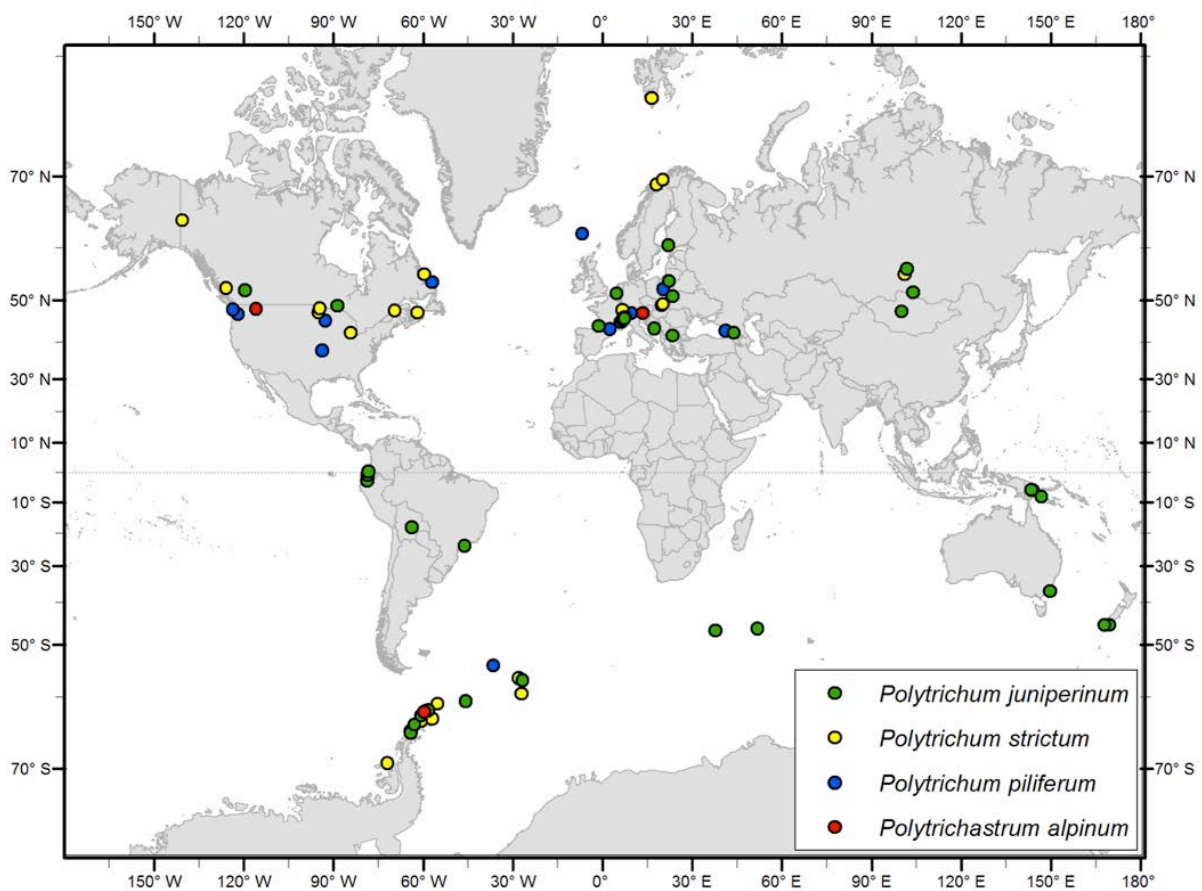
Fig. 2.3 Locations of *trnL-F* samples of *Polytrichum juniperinum* (green), *Polytrichum strictum* (yellow), *Polytrichum piliferum* (blue) and *Polytrichastrum alpinum* (red).

2.4.2 Phylogenetic relationships

Bayesian phylogenetic trees based upon analyses of *trnL-F* and *ITS* 1+2, are shown in Fig. 2.4a and 2.4b, respectively, including posterior probabilities (PP) and bootstrap support (see Figs. 8.2.1.2 and 8.2.1.3 in the Appendix for ML phylogenies of *trnL-F* and *ITS* 1+2,

respectively). The phylogeny revealed a topology consistent with current species definitions and relationships (Bell & Hyvönen, 2010; Bell *et al.*, 2015); *P. alpinum* was the most distantly related and, within *Polytrichum, P. piliferum* was more distantly related than the sister species *P. strictum* and *P. juniperinum.* No topological conflicts were found between Bayesian and ML analyses at key nodes (only PP are mentioned hereafter).

The *ITS* 1+2 tree (Fig. 2.4b) provided well resolved clades with high support values (PP=1.00) for all species. The *trnL-F* topology (Fig. 2.4a) showed high support values (PP=1.00) for all species except for *P. strictum* (PP=0.66). The species delimitation method ABGD (Puillandre *et al.*, 2012) revealed two significant "barcoding gaps" in *ITS* 1+2 at Prior 'maximum divergence of intraspecific diversity' (P_{max}) values 0.0017 and 0.0077 (Fig. 2.4b). At P_{max}=0.0077 four groups were identified, consistent with current morphological species definitions. At P_{max}=0.0017, five and four distinct groups were identified within *P. alpinum* and *P. juniperinum*, respectively, suggesting greater phylogenetic structure within these two species than currently recognized in their taxonomy.

Fig. 2.4 (next page; for larger version see Fig. 8.2.1.1, Appendix 8.2.1) Bayesian phylogenies constructed with (a) plastid marker *trnL-F* and (b) nuclear marker *ITS* (1+2) for *Polytrichastrum alpinum, Polytrichum piliferum*, *P. strictum* and *P. juniperinum*. Posterior probabilities and bootstrap support are shown next to branches (* = conflict between topologies of Bayesian and ML tree; see Figs. 8.2.1.2 and 8.2.1.3 in Appendix 8.2.1 for ML phylogenies). Colours refer to different geographical regions (see map); outgroups are indicated in black. The scale bar represents the mean number of nucleotide substitutions per site. ABGD species delimitation clusters with different P_{max}-values are shown in grey next to (b).
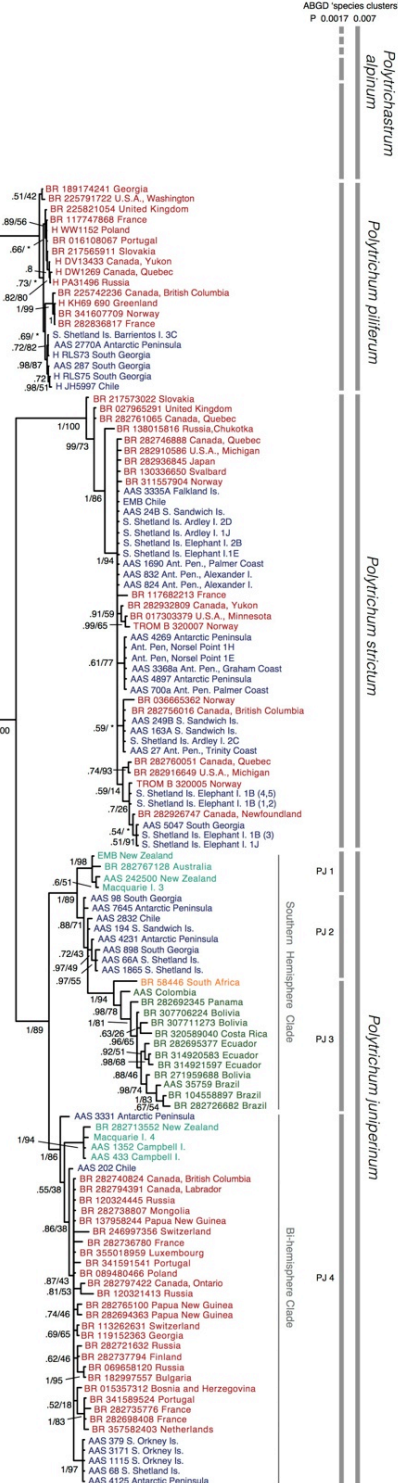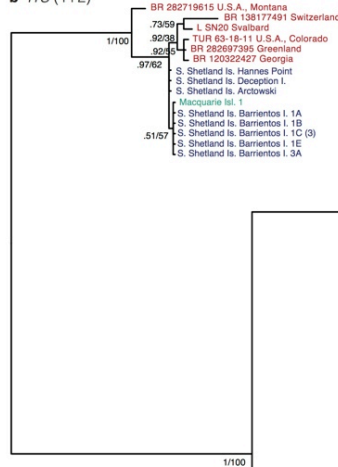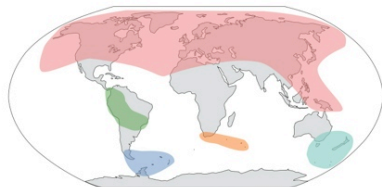
a *Polytrichum juniperinum* (*ITS* 1+2) (n=61)
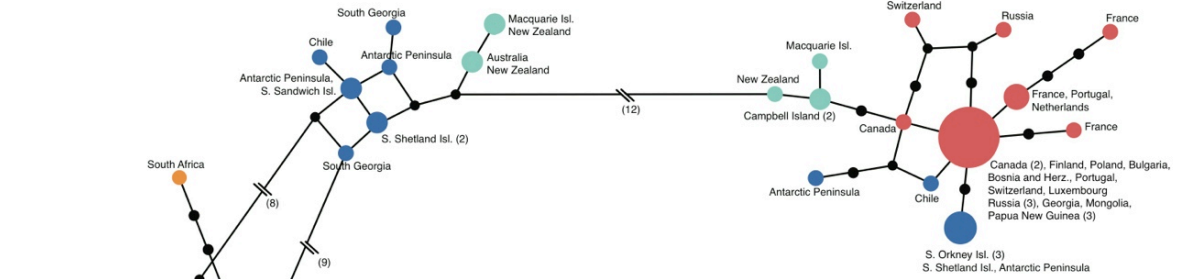
b *Polytrichum strictum* (*ITS* 1+2) (n=44)
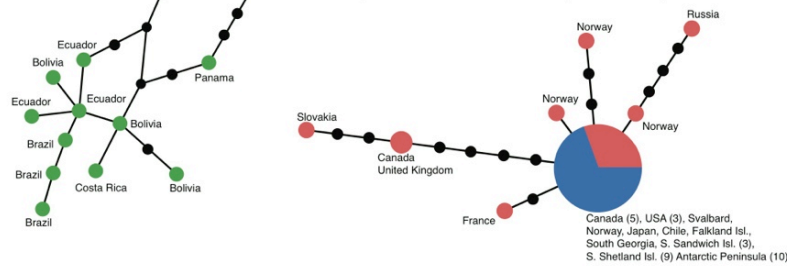
c *Polytrichum juniperinum* and
Polytrichum strictum (*ITS* 1+2) (n=105)

Polytichum juniperinum

(scale 25%)

Polytichum strictum

d *Polytrichum piliferum*

(*ITS* 1+2) (n=20)

(*ITS* 2) (n=63)

e *Polytrichastrum alpinum*

(*ITS* 1+2) (n=15)

(*ITS* 2) (n=47)

Holarctic
Antarctica, Sub-Antarctic + Southern South America
Australasia
Central and North South America
South Africa

10 samples
1 sample

32
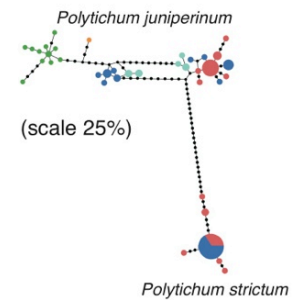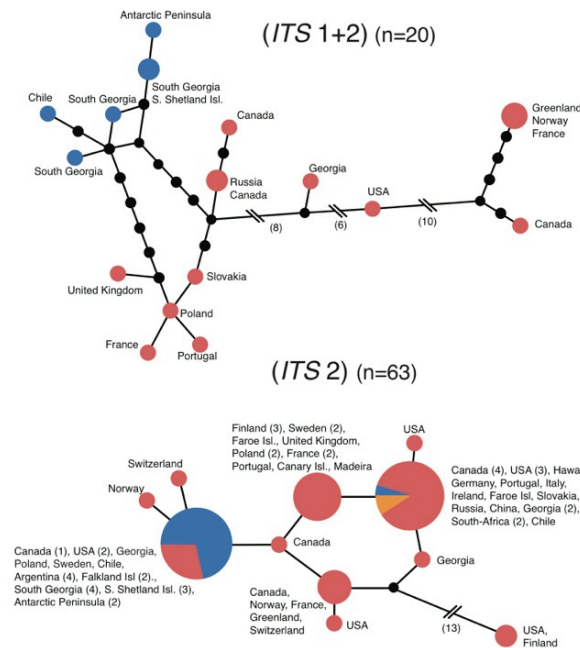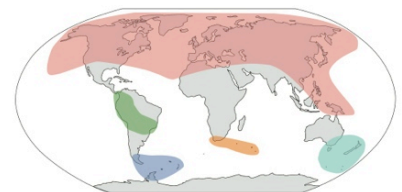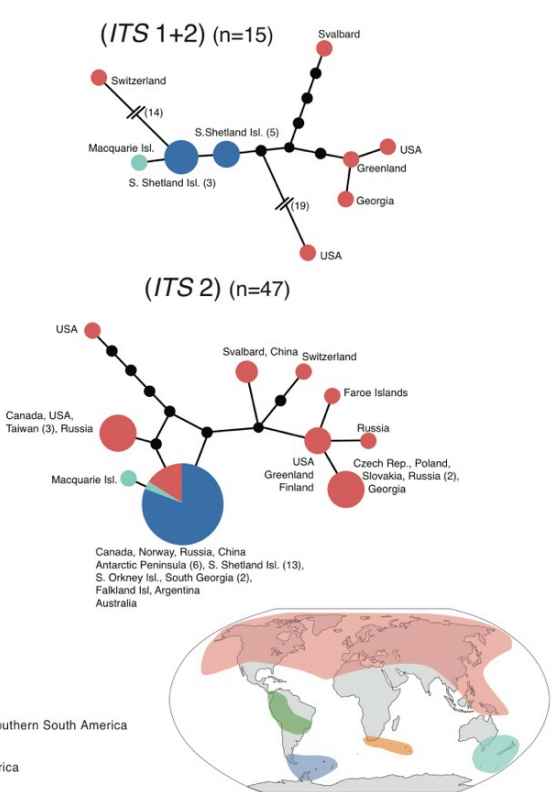
Fig. 2.5 (former page) Haplotype networks of *ITS* 1+2 of (a) *Polytrichum juniperinum*, (b) *P. strictum*, (c) sister-species *P. juniperinum* and *P. strictum* together, (d) *P. piliferum* and (e) *Polytrichastrum alpinum*. Separate haplotype networks of *ITS* 1+2 (d and e, higher panels) and *ITS* 2 only (d and e, lower panels) are shown for the latter two species. Haplotype circle sizes correspond to numbers of individuals with the same haplotype (see legend). Branches represent mutations between haplotypes, with mutations shown as 1-step edges or as numbers. Colours refer to the different geographical regions (see map).

2.4.3   Biogeographic patterns within species

Biogeographic patterns within species were interpreted based within the phylogenetic tree topologies (*trnL-F* and *ITS* 1+2; Fig. 2.4) and structure of haplotype networks (*ITS* 1+2 and *ITS* 2; Fig. 2.5).

Within *P. juniperinum*, two strongly supported clades (PP=1.00) with different geographical distributions were apparent in the *ITS* 1+2 topology (Fig. 2.4b). The first clade consisted of SH regions (hereafter the 'SH clade') with several sub-clades: a monophyletic Australasian sub-clade (PP=1.00; ABGD-cluster PJ 1), a monophyletic sub-clade including one South African specimen and several low latitude South American specimens (PP=1.00; ABGD-cluster PJ 3), and a non-monophyletic group of lineages including specimens from Antarctica, the sub-Antarctic and southern South America (with PP varying from 0.72 to 0.97; defined by ABGD as cluster PJ 2). The second clade (hereafter the 'bi-hemispheric' clade) included multiple early-diverging lineages from the SH (including Australasia and the Antarctic region; PP=0.55-1.00), and a large monophyletic sub-clade (PP=0.87) with specimens from the NH as well as a distinct monophyletic group composed of Antarctic and sub-Antarctic specimens (PP=1.00). A similar pattern was apparent in the haplotype network (Fig. 2.5a), where the two main ('SH' and 'bi-hemispheric') clades diverged by 12 mutational steps, with SH specimens on either side.

In contrast to *P. juniperinum*, the *ITS* 1+2 region within *P. strictum* revealed multiple chromatogram peaks in multiple samples, likely due to a duplication of *ITS* within the species. As ambiguous positions are not taken into account in the phylogenetic or haplotype analyses, this resulted in an underrepresentation of the genetic variation and no strong biogeographic patterns could be inferred (see Fig. 2.4b). However, we found that, despite the exclusion of ambiguous sites, several NH specimens were placed as sister groups to a monophyletic clade of all other (NH and SH) specimens (Fig. 2.4b). The *P. strictum* haplotype network revealed the highest genetic variation in NH specimens, showing a single

haplotype including both SH and NH specimens, with several NH haplotypes diverging by 1-6 mutational steps from the main haplotype (Fig. 2.5b). We further explored the genetic diversity within *P. strictum* by phasing ambiguous haplotypes into different haplotypes within individuals using Phase v2.1.1 (Stephens *et al.*, 2001; Stephens & Donnelly, 2003), applying default options, followed by the same downstream analyses, however this did not improve phylogenetic resolution (data not shown). Although the phylogeographic history of *P. strictum* needs further assessment, the phenomenon of multiple chromatogram peaks is noteworthy in itself, possibly representing the second known case of *ITS* paralogy in mosses (Košnar *et al.*, 2012). The phenomenon is possibly the result of a past hybridization event in *P. strictum*, as previously suggested by (Bell & Hyvönen, 2010). Similar patterns were not observed in the other study species.

As a greater number of *ITS* 2 sequences were available in *P. piliferum* and *P. alpinum*, we analysed *ITS* 1+2 and *ITS* 2 in separate haplotype networks for these species (Figs. 2.5d and 2.5e). As described, most intra-specific variation in *P. piliferum* was located in a 427bp insertion, which was not found in the other species. This diversity was therefore masked when it was aligned with the other species for analysis in a Bayesian phylogenetic framework (Fig. 2.4). The *ITS* 1+2 phylogeny (Fig. 2.4b) revealed three weakly resolved monophyletic clusters of NH specimens and placed all SH specimens together in a fourth monophyletic group. Similarly, the *ITS* 1+2 and *ITS* 2 networks of *P. piliferum* (Fig. 2.5d) revealed multiple clusters, with most genetic variation between NH specimens. In the *ITS* 1+2 haplotype network (Fig. 2.5b, upper panel) all SH haplotypes clustered closely together. The *ITS* 2 haplotype network (Fig. 2.5d, lower panel) identified several distinct haplotypes. One of the main haplotypes contained individuals from the NH and most SH individuals, including all Antarctic and sub-Antarctic specimens. However, a separate common haplotype included individuals of the NH as well as a specimen from Chile and two from South Africa.

The *ITS* 1+2 phylogeny of *P. alpinum* revealed one genetically divergent NH specimen from North America at the base of the clade. Remaining specimens were broadly clustered into SH and NH groups, with the NH group monophyletic and containing more phylogenetic structure than the paraphyletic cluster of SH specimens (Fig. 2.4b). A greater diversity of NH haplotypes was also visible in both *ITS* 1+2 and *ITS* 2 networks (Fig. 2.5e), which revealed distinct regional NH clusters, while all SH specimens were grouped closely together.

2.4.4 Population expansion analyses

Population expansion and neutrality tests to infer the demographic history of each species, as

well as particular monophyletic and ABGD-defined clusters (see Fig. 2.4b) within *P. juniperinum*, are shown in Table 2.1. Demographic and spatial expansion tests did not reject a null hypothesis of population expansion for any species or population within *P juniperinum* (all P-values were non-significant), supporting possible demographic and spatial expansion in all clusters. An excess of low-frequency polymorphisms over that expected under neutrality was inferred from significantly negative Fu's Fs values (Fu, 1997) for all groups within *P. juniperinum*. Considering these data in relation to the haplotype network patterns, the species clade(s) most likely to reflect a past population expansion are the *P. juniperinum* "bi-hemisphere clade" and/or "Holarctic + recent Antarctic dispersal event" clades, which have significant Fu's Fs and large negative Tajima's D values (and low though non-significant p-values), and a star-shaped haplotype network topology. Mismatch distribution patterns are also consistent with this possibility (see Fig. 8.2.1.5 in the Appendix). Another species reflecting a possible past expansion was *P. alpinum*, the only species with a significant and large negative Tajima's D value.

2.4.5   Geographic range probabilities and molecular dating

The ancestral range estimates and molecular dating provided estimates of the diversification, timing and spatial origins of the inter-hemispheric distribution in each species (Fig. 2.6). The ancestral range estimates under the R-program BioGeoBEARS (Matzke, 2014) selected the DEC+J model of species evolution (dispersal-extinction-cladogenesis (DEC), implementing a founder-effect component (+J) (Matzke, 2014)). The ancestral area reconstruction suggested the earliest lineages within *P. alpinum, P. piliferum*, and the ancestor of *P. strictum* and *P. juniperinum*, were of Holarctic origin (Fig. 2.6), and that their SH populations were the result of NH to SH movements. However, as *P. strictum* and *P. juniperinum* diverged, while the ancestor of *P. strictum* remained in the Holarctic for several million years further, the ancestor of *P. juniperinum* dispersed to the Antarctic region (Antarctic, sub-Antarctic, and/or southern S. America). From here, *P. juniperinum* diverged into two different clades, and dispersed into Australasia (from both clades), South Africa and low latitude regions in South America, as well as the entire Holarctic region. Subsequently, a separate trans-equatorial dispersal event occurred from the Holarctic back to the Antarctic region.

Results of all dating analyses are shown in Table 2.2 and Fig. 2.6 (only showing two-step dating analyses with (I2a) and without (I2b) the fossil *E. antiquum*). Applying the nuclear rate (Method II) resulted in considerably older age estimates than those using

Table 2.1 Genetic diversity indices, demographic and spatial expansion model test and neutrality tests (Tajima's D and Fu's Fs) for *ITS* 1+2 within the species *Polytrichum juniperinum*, *P. strictum*, *P. piliferum* and *Polytrichastrum alpinum*. Parameters were calculated only for populations with *n*>10. The different ABGD-inferred clusters PJ1-4 of *P. juniperinum* are shown in Fig. 2.4. None of the SSD or HRI p-values were significant.

Clade	*n*	bp[a]	π	S	*h*	Demographic expansion		Spatial expansion		Neutrality tests:	
						SSD	HRI	SSD	HRI	Tajima's D (*P*)	Fu's Fs (*P*)
Polytrichastrum alpinum (overall)	15	782	0.010 ± 0.005	33	0.876 ± 0.070	0.062	0.080	0.052	0.080	-1.574 (0.047*)	0.115 (0.529)
Polytrichum piliferum (overall)	20	1304	0.017 ± 0.009	46	0.990 ± 0.019	0.013	0.013	0.014	0.013	0.177 (0.622)	-3.062 (0.093)
Polytrichum strictum (overall)	44	979	0.006 ± 0.003	30	0.986 ± 0.009	0.001	0.005	0.001	0.005	-0.846 (0.217)	-2.915 (0.153)
Polytrichum juniperinum (overall)	61	993	0.035 ± 0.017	88	0.996 ± 0.005	0.007	0.002	0.007	0.002	0.263 (0.682)	-13.365 (0.006*)
"SH Clade" (PJ1+PJ2+PJ3)	25	994	0.024 ± 0.012	54	0.997 ± 0.013	0.006	0.008	0.011	0.008	-0.336 (0.694)	-7.060 (0.011*)
S. Africa, N./Centr. S. America (PJ3)	13	896	0.012 ± 0.007	34	1.000 ± 0.030	0.006	0.013	0.007	0.013	-1.005 (0.156)	-5.444 (0.009*)
"Bi-hemisphere Clade" (PJ4)	36	961	0.011 ± 0.006	40	0.991 ± 0.011	0.002	0.004	0.003	0.004	-1.351 (0.069)	-11.525 (0.002*)
Holarctic + recent Antarctic dispersal event	30	963	0.009 ± 0.005	26	0.986 ± 0.016	0.002	0.005	0.003	0.005	-1.100 (0.133)	-9.031 (0.003*)

n: no. of sequences; bp[a]: no. of usable base pairs (loci <5.0% missing data); π: nucleotide diversity (average over locus); S: No. of sites with substitutions; *h*: gene diversity; SSD: Sum of Squared Deviations; HRI: Harpending's Raggedness Index. For Tajima's D and Fu's Fs a *P*<0.05 is significant (*).

the two-step approach (Method I), with ages more than twice those of the oldest two-step approach (I2a; including the fossil). Following the dating analysis that provides the most recent divergence time estimates (I2b; without *E. antiquum*), all SH migrations occurred within the Pleistocene (*P. alpinum*, *P. piliferum*), late Pliocene/early Pleistocene (initial SH arrival *P. juniperinum*) and late Pleistocene (recent Holarctic to Antarctic dispersal within *P. juniperinum*). Ages calculated under (I2a; with *E. antiquum*) suggested SH migrations to have occurred during the late Pliocene/early Pleistocene (*P. alpinum, P. piliferum*), late Miocene/early Pliocene (initial SH arrival *P. juniperinum*) and late Pleistocene (recent Holarctic to Antarctic dispersal within *P. juniperinum*). Following the rate analysis (Method II), SH migrations occurred within the late Pleistocene (*P. alpinum*, *P. piliferum*), late Oligocene/early Miocene (initial SH arrival *P. juniperinum*) and Pleistocene (recent Holarctic to Antarctic dispersal within *P. juniperinum*).

Fig. 2.6 (next page) Historical biogeography of four Antarctic Polytrichaceae mosses, highlighting the population history of *Polytrichum juniperinum*. The maximum clade credibility tree shows the median divergence time estimates calculated with two 2-step dating analyses, with (a) or without (b) including the taxonomically uncertain fossil *Eopolytrichum antiquum* as a prior. Median ages and 95% height posterior distributions associated with major nodes are presented in Table 2.2. Coloured pie-charts represent ancestral range probabilities at each node as recovered by the best BioGeoBEARS model. Colours refer to the different geographical regions (see map). Arrows below the figure represent the time and direction of inter-hemispheric movements of all species excluding *P. strictum*. NH and SH represent Northern and Southern Hemisphere, respectively. The black line below each arrow is the branch and therefore timeframe over which the inter-hemispheric movement (according to ancestral range probabilities) was estimated to have occurred (note that 95% height posterior distribution of these branches is not presented here).
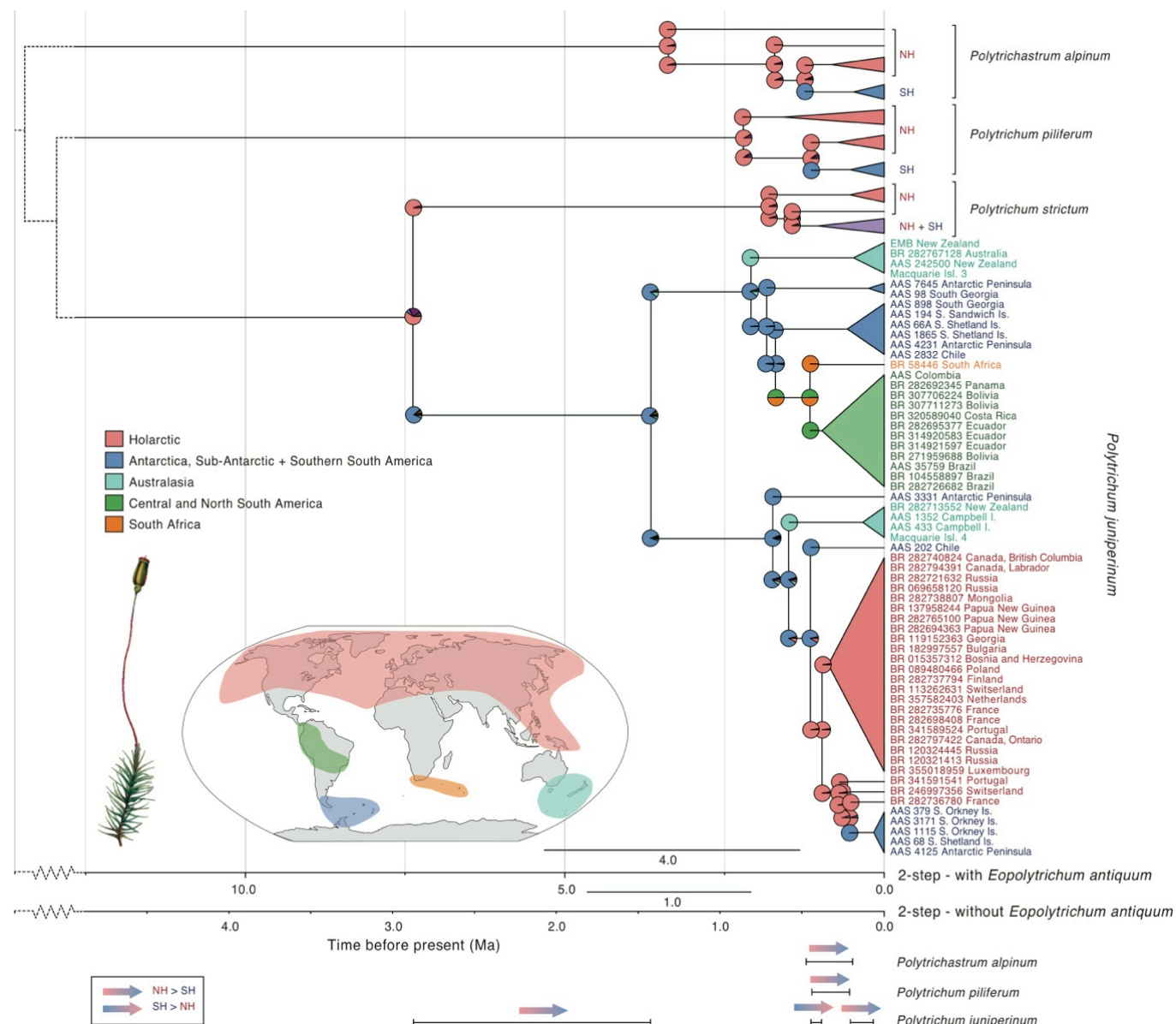
Table 2.2 Median ages (95% HDP lower - upper) (Myr) for the different study species and key clades within *Polytrichum juniperinum* as calculated in BEAST. Dating methods are based on two 2-step dating analyses (Method I), with (I2a) or without (12b) including the taxonomically uncertain fossil *Eopolytrichum antiquum* as a constraint, as well as applying a previously defined *ITS* rate (Method II). The different ABGD-inferred clusters PJ1-4 of *P. juniperinum* are shown in Fig. 2.4. TMRCA: Time to the Most Recent Common Ancestor; SH: Southern Hemisphere; NH: Northern Hemisphere. The mean clock rate in subst./site/my (95% HDP intervals) is provided for Method (12a) and (12b).

| Lineage TMRCA | Method I (two-step) | | Method II (rate) |
| | *Eopolytrichum antiquum* used as constraint: | | |
	(I2a) Yes	(I2b) No	Rate Analysis
Polytrichastrum alpinum + Polytrichum spp.	36.65 (10.74-60.62)	14.13 (5.24-23.09)	77.02 (62.73-91.51)
P. piliferum + P. strictum/P. juniperinum	36.29 (10.49-59.84)	13.99 (5.11-22.70)	65.75 (53.57-78.46)
P. strictum + P. juniperinum	7.57 (2.05-13.38)	2.92 (0.96-5.03)	23.42 (18.41-28.98)
P. alpinum	3.51 (0.76-6.23)	1.36 (0.38-2.41)	11.51 (7.60-16.81)
SH Clade	0.54 (0.07-1.12)	0.21 (0.04-0.44)	2.00 (0.70-3.47)
P. piliferum	2.28 (0.59-4.08)	0.87 (0.28-1.55)	7.16 (5.20-9.41)
SH Clade	0.58 (0.12-1.09)	0.22 (0.06-0.43)	1.93 (0.98-2.98)
P. strictum	1.86 (0.43-3.32)	0.71 (0.22-1.24)	6.32 (4.48-8.47)
P. juniperinum	3.77 (0.97-6.69)	1.45 (0.44-2.51)	12.67 (9.49-15.81)
SH Clade - Australasia (PJ1)	0.53 (0.08-1.10)	0.21 (0.03-0.42)	1.88 (0.65-3.26)
SH Clade - Antarctic, sub-Antarctic, S. S. America (PJ2)	1.91 (0.53-3.44)	0.74 (0.21-1.25)	6.61 (4.89-8.28)
SH Clade - S. Africa, N. and Centr. S. America (PJ3)	1.22 (0.31-2.17)	0.47 (0.14-0.82)	4.25 (3.09-5.51)
NH + SH Clade (PJ4)	1.83 (0.45-3.32)	0.71 (0.19-1.24)	6.40 (4.30-8.60)
Recent dispersal Holarctic → Antarctic	0.19 (0.01-0.42)	0.07 (0.01-0.16)	0.65 (0.13-1.28)
Mean clock rate	5.01×10^{-3} (1.77×10^{-3}-9.77×10^{-3})	1.3×10^{-2} (5.15×10^{-3} - 2.39×10^{-2})	N.A.

2.5   Discussion

2.5.1   Long distance dispersal as driver of species-level disjunctions

Even with the differences between the different dating analyses, all analyses identified similar outcomes indicating that the main inter-hemispheric movements occurred on hundred-thousand to multi-million year timescales, from the Pleistocene, Pliocene and/or late Miocene. These time-scales are too young to be explained by continental vicariance. Climatic vicariance could be a possibility - however we find the inter-hemispheric dispersal events have occurred over much longer timescales than might be expected with the last major glaciation (i.e. the Last Glacial Maximum; LGM). These divergence times therefore support the hypothesis that long-distance dispersal is the underlying driver for the bipolar disjunctions considered in these species.

Such long-distance dispersal could have taken place *via* spores (generally <10µm (Convey & Smith, 1993; Ochyra *et al.*, 2008) in these genera) or other propagules, either *via* wind currents or animal vectors, such as migratory birds. The patterns observed here clearly illustrate the dispersal abilities of bryophytes yet, even so, major trans-equator dispersal events (followed by successful colonization) are extremely rare. In *P. juniperinum* successful inter-hemispheric colonizations appear to have occurred only three times: first, at the split which separated *P. juniperinum* from the ancestor of *P. strictum* + *P. juniperinum*; second, the SH to NH dispersal event; and third, the final more recent NH to SH migration. In *P. piliferum* two or more independent trans-equatorial dispersal events occurred (Fig. 2.5d, lower panel: SH specimens found in two separate clusters; Antarctic/sub-Antarctic in one, South African in one, and Chilean in both). In *P. alpinum* all SH specimens were clustered closely together, suggesting just one NH to SH dispersal event, however sampling is limited for this species. Trans-equatorial dispersals occurred from north to south in all species, and from south to north in *P. juniperinum*. Analyses of *P. strictum* also revealed higher levels of genetic variation in the NH than the SH, with biogeographic patterns indicating that the species likely originated in the NH, and subsequently dispersed to the SH.

2.5.2   Within-species variation in *Polytrichum juniperinum* and *Polytrichastrum alpinum*

The species delimitation analysis identified several clusters within *P. juniperinum* and

P. alpinum, with genetic differentiation consistent with species-level differentiation (Fig. 2.4). Both species are known to be phenotypically variable throughout their range, prompting classification of several infraspecific taxa or subspecies (*P. juniperinum* (Papp, 1933; Walther, 1934) and *P. alpinum* (Yli-Rekola, 1980; Ochyra *et al.*, 2008, and references therein)). Although not currently recognized through assumed phenotypic plasticity (Ochyra *et al.*, 2008), these distinctions regain credence here based on the variability in the *ITS* region. How genetic variation in *P. juniperinum* and *P. alpinum* is correlated with phenotypic variation and whether the species' current classifications should encompass several subspecies or taxa of higher status requires further study integrating morphological and genetic approaches.

2.5.3 No 'stepping-stone' dispersal in *Polytrichum juniperinum*

We found no evidence that intermediate high elevation populations in the South American tropics or South Africa in *P. juniperinum* have acted as 'stepping-stones' for inter-hemispheric dispersal. Rather, these intermediate populations are the result of separate northwards colonisation events from the Antarctic region. Such northward movements could have been facilitated by a temporary lowering of vegetation zones and treelines during interglacial periods, as has been suggested as a mechanism to explain the presence of several members of Polytrichaceae in high elevation areas in tropical South America (e.g. *Polytrichadelphus* Müll. Hal (Mitt.) (Smith, 1972a)). Evidence for northward dispersal into the lower latitudes of South America has only been reported before in five families of angiosperm (Chacón *et al.*, 2012), a liverwort species (Meißner *et al.*,1998; see also Frey *et al.*, 2010) and hornwort genus (Villarreal & Renner, 2014), but never before in mosses.

2.5.4 Dispersal out of the Antarctic region

Phylogeographic analyses suggest all the contemporary and disjunct populations within *P. juniperinum* originate from dispersal from the Antarctic region, including populations in the South American tropics and South Africa, Australasia and the Holarctic (see Fig. 2.6). Two separate migrations from the Antarctic region to Australasia were apparent, revealing a relatively strong connection between these regions, possibly assisted by the strong circumpolar 'westerly wind' belt, a link also implied in an SH aerobiology modelling study (Muñoz *et al.*, 2004) and descriptions of bryophyte biogeographic regions (Schofield, 1992). Very little differentiation was

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identified across the NH distribution of *P. juniperinum* ('Holarctic + recent Antarctic dispersal event' clade; see Table 2.1 and Fig. 8.2.1.5 in the Appendix). This, together with a significantly negative Fu's Fs in this clade and star-like haplotype network, suggests a rapid NH colonisation from a single or limited number of northward dispersal events from the SH. Favourable conditions for this could have been facilitated by the harsh Pleistocene glacial periods in the Holarctic, which, on ice retreat, left extensive barren areas available for colonization for cold-adapted mosses (Smith, 1972a).

2.5.5 Persistence in Southern Hemisphere glaciated regions

Our divergence time analyses imply *P. juniperinum, P. alpinum* and *P. piliferum* all arrived in the Antarctic, sub-Antarctic and/or southern South America well before the LGM. All these regions are thought to have experienced extensive glaciations throughout the LGM and previous glacial cycles, although biological evidence supports the existence of glacial refugia in both southern South America (Sersic *et al.*, 2011), and the Antarctic (Convey *et al.*, 2008; Pugh & Convey, 2008; Convey *et al.*, 2009a). Whether Antarctic and sub-Antarctic populations of our study species are of recent (post-LGM) origin through repeated dispersal events from southern South America, or have persisted in the far south *in situ* requires further investigation.

Recent modelling studies (Pollard & DeConto, 2009; DeConto & Pollard, 2016) have highlighted considerably greater dynamism in ice extent throughout glacial cycles in the Antarctic Peninsula region over the timescales of interest here than has previously been suspected. Several warmer-than-present interglacials occurred throughout the Pleistocene (Scherer *et al.*, 2008; Naish *et al.*, 2009; Pollard & DeConto, 2009; DeConto & Pollard, 2016) and early Pliocene (De Schepper *et al.*, 2014), while the increased dynamism apparent in these models may provide a foundation allowing the persistence of previously unconsidered ice-free regional refugial areas. Additionally, both *P. juniperinum* and *P. alpinum* can often be found growing in geothermally-influenced areas on volcanic Antarctic and sub-Antarctic islands (Convey & Smith, 2006; Ochyra *et al.*, 2008), which are suggested as possible regional refugia (Fraser *et al.*, 2014). Furthermore, recent studies of polar mosses have shown sub-glacial or within permafrost survival over several hundred year (La Farge *et al.*, 2013) to millennial timescales (Roads *et al.*, 2014). Although such timescales still fall short of those required for persistence through entire glacial cycles, these studies suggest that

mosses have the potential to survive through at least shorter periods (several centuries) of ice expansion, and possibly longer periods of unfavourable conditions. Recently the weedy, cosmopolitan moss *Bryum argenteum* Hedw. was suggested to have a multi-million-year Antarctic persistence (Pisa *et al.*, 2014), providing a first intriguing suggestion that long-term persistence might be a more general feature of today's Antarctic flora, and one that is at least consistent with the data presented in this study.

2.6   Acknowledgements

3 Chloroplast DNA reveals latitudinal biogeographic structuring in the globally-distributed moss *Ceratodon purpureus*

Fig. 3.1 *Ceratodon purpureus* (reddish colonies) growing amongst moss banks in Wilkes Land, East Antarctica. (Photo: S. Robinson)

3.1   Abstract

Ceratodon purpureus one of the most widespread moss species found across the Antarctic continent, including in the harsher continental Antarctic. The species is also one of the most widespread ruderal mosses in the world. To investigate how long *C. purpureus* has been present in the Antarctic, as well as its overall global biogeographic structure, a study was undertaken with large-scale worldwide sampling (n=148), investigating the level and drivers of population structure, connectivity, and timescales of population isolation. Since high levels of recombination and a duplication of the nuclear genome have been identified in this species, we focussed on three chloroplast loci (*atpB-rbcL*, *trnL-F*, *rps4*) to obtain an understanding of the matrilineal population structure of *C. purpureus*. The cpDNA data revealed several distinct lineages, generally corresponding to latitudinal bands of population structure across the globe, implying connectivity is strongly influenced by global atmospheric circulation patterns, and that dispersal beyond these latitudinal bands is uncommon. Divergence time analyses indicate that the contemporary matrilineal population structure in *C. purpureus* developed throughout the late Miocene, Pliocene and Quaternary. Several dispersal events into the Antarctic were apparent as well as one old and distinct Antarctic clade, possibly isolated on the continent since the Pliocene. The biogeographic structure identified here provides a useful framework for future genetic and developmental studies on the species, as *C. purpureus* is also commonly used as a model organism for developmental research in non-vascular plants. The global biogeographic structuring corresponding to general wind patterns identified in this study is also relevant to understanding influences on the global biogeography of other small or spore-dispersed organisms.

3.2   Introduction

Bryophytes tend to show extensive distributions, often spanning multiple continents and/or both hemispheres (Schofield & Crum, 1972). 'Cosmopolitan' species can even show an extreme worldwide distribution, similar to that described for some microbial groups (Fontaneto, 2011). From recent phylogeographic studies it is increasingly clear that the majority of bryophyte distribution patterns are formed by relatively frequent short-distance, and more infrequent long-distance, dispersal events (see Heinrichs *et*

al., 2009 and refs. therein). We would therefore expect the global biogeography of cosmopolitan species to reflect a degree of regional biogeographic structuring as well as some long-distance dispersal. However, the levels of biogeographic structure and connectivity between populations in species with global distributions are still poorly known.

The fact that cosmopolitan bryophyte species have good long-distance dispersal abilities is well known, and illustrated by the frequent occurrence of widespread bryophyte species on oceanic (and often geologically young) islands (e.g. Convey *et al.*, 2000; Vanderpoorten *et al.*, 2007). Bryophytes generally disperse *via* spores or asexual specialized leaf tips and other propagules (Longton, 1988a). Extremely prolific spore production (Longton, 1997) combined with small spore size (generally ~10-20 μm in diameter in mosses; Frahm, 2008) gives bryophytes strong potential for long distance dispersal. Indeed, mounting evidence from recent experimental (e.g. Sundberg, 2013) and phylogeographic studies (e.g. Huttunen *et al.*, 2008; Pokorny *et al.*, 2011; Stenøien *et al.*, 2011; Karlin *et al.*, 2012; Piñeiro *et al.*, 2012; Szövényi *et al.*, 2012; Lewis *et al.*, 2014a; Pisa *et al.*, 2014; Kyrkjeeide *et al.*, 2016) has demonstrated the occurrence of long-distance dispersal in mosses with disjunct or widespread ranges. In light of these studies, our aim was to assess the level of biogeographic structuring and connectivity between populations of a moss species with a global distribution. We also sought to evaluate the timescales at which such widespread populations have been isolated, with a particular interest in those in remote regions (e.g. Antarctica).

Ceratodon purpureus (Hedw.) Brid., is one of the most well-known and widespread ruderal moss species known, found in an exceptionally wide range of habitats from the polar areas to the tropics and generally described as having a worldwide distribution (Ochyra *et al.*, 2008). The species is also commonly used as a model organism in genetic, physiological and developmental studies, particularly for studies on the evolution of developmental processes in early land plants (e.g. McDaniel *et al.*, 2007, and references therein). For this type of developmental research, good baseline knowledge on the evolutionary history and global biogeography of *C. purpureus* is fundamental, for instance, for underpinning interpretation of crossing experiments, trait mapping and marker discovery, and controlling for demographic or population effects.

The global genetic diversity in natural populations of *C. purpureus* was initially investigated by McDaniel and Shaw (2005) using a worldwide dataset generated from 34 samples, and sequencing the chloroplast *atpB-rbcL* spacer and nuclear genes adenosine kinase (*adk*) and phytochrome 2 (*phy2*). They found two distinct Northern Hemisphere clades, a Southern Hemisphere clade including some Northern Hemisphere specimens, and several more distantly related, distinct lineages from equatorial regions, suggesting that migration between Australasian and Holarctic regions was more common than among equatorial regions. However, markers differed in implied population structure, with nuclear markers showing evidence of recombination and multiple divergent sequences within individual samples.

Recent studies of the nuclear genome of *C. purpureus* have revealed several further features, which complicate the use of nuclear markers for population genetic analyses of the species. Szövényi *et al.* (2014) revealed an ancient duplication of the nuclear genome in *C. purpureus*; multiple paralogous copies of each gene therefore exist, which complicates sequencing of homologous loci for population genetic studies. Furthermore, non-Mendelian patterns of genetic segregation were also reported for this species by McDaniel *et al.* (2007). These findings in concert complicate the use of nuclear markers to assess the phylogeography of *C. purpureus*, and highlight the need for a comprehensive study with organellar markers to infer the global geographic structure of the maternal line of species.

In the current study, we set out to assess the evolutionary history and population structuring of the maternal line of *C. purpureus* using a large-scale global sampling dataset (148 individuals), including three non-recombining chloroplast markers: the *rps4* gene and the *trnL-F* and *atpB-rbcL* spacers. By using multiple linked markers from a maternally inherited, non-recombining organelle, we aim to reconstruct the population history of the chloroplast marker in this species and illuminate population structure patterns and timings of population isolation (particularly of remote locations, e.g. Antarctica) as revealed by the maternal line. We also included one nuclear marker for comparison (the Internal Transcribed Spacer; *ITS*), to check for biogeographic patterns in line with those reported by McDaniel and Shaw (2005).

3.3   Materials and Materials

3.3.1   Sampling and molecular methods

Moss samples for molecular analyses were obtained from the herbaria at the British Antarctic Survey (AAS), Botanic Garden, Meise (BR), British Museum of Natural History (BM), Royal Botanical Gardens Edinburgh (E), the University of Wollongong (WOLL) and the New York Botanical Garden (NY), augmented by fresh collections during expeditions of authors (EB, RW, PC, SR) (see text and Table 8.2.2.1, Appendix 8.2.2, for sample information). We included sequences available on GenBank, and unpublished sequences from the Honours thesis of RW (Wyber, 2013). All specimens were examined and confirmed as *C. purpureus* by specialist bryologists. Total genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany), using mortar and pestle and liquid nitrogen, following the manufacturer's instructions. As *C. purpureus* has very small gametophytes, multiple stems were extracted per sample to ensure sufficient DNA quantity. The *trnL-F* spacer, the *rps4* gene plus *trnS-rps4* intergenic spacer (both hereafter referred to as *rps4*), *atpB-rbcL* spacer and *ITS* were amplified using the primers trnLF-c and trnLF-f (Taberlet *et al.*, 1991), trnS (Souza-Chies *et al.*, 1997) and rps 5' (Nadot *et al.*, 1994), atpB1 and rbcL1 (Chiang *et al.*, 1998) and ITS1 and ITS4 (White *et al.*, 1990), respectively. PCR reactions were performed using the Taq PCR Core Kit (Qiagen GmbH, Hilden, Germany) with addition of $MgCl_2$ and Bovine Serum Albumin (BSA), according to the manufacturer's instructions and using annealing temperatures of 60°C, 55°C, 53°C and 55°C for *trnL-F*, *rps4*, *atpB-rbcL* and *ITS*, respectively. The number of cycles varied from 35-40. PCR products were checked using gel electrophoresis. Double bands were amplified for many samples, so for a small selection of samples double bands were excised and purified using the Wizard SV Gel and PCR clean up kit (Promega, USA), respectively. Forward and reverse sequencing was performed by LGC Genomics (Berlin, Germany) and the University of Wollongong sequencing facility.

3.3.2   Sequence editing and alignment

Forward and reverse sequences were manually checked using Codoncode Aligner v.5.0.2 (CodonCode Corp., Dedham, MA). A total of 74, 94, 80 and 56 concatenated sequences of *trnL-F* (433-435 bp), *rps4* (589 bp), *atpB-rbcL* (623-627 bp) and *ITS* (674-816 bp) were generated, respectively. All original sequences were deposited in GenBank as listed in Table 8.2.2.1, Appendix 8.2.2. Following McDaniel and Shaw (2005), and based on Hedderson *et al.* (2004), we selected available sequences of

Cheilothela chloropus (Brid.) Lindb. for *atpB-rbcL* and rps4 and *Trichodon cylindricus* (KX446935) and *Pleuridium acuminatum* (EU186546) for *trnL-F* from GenBank as outgroups for the phylogenetic cpDNA analyses. All cpDNA regions were aligned using the Geneious aligner within Geneious 9.0.4 (Biomatters, LTD, Auckland, NZ). *ITS* was aligned using PRANK (Löytynoja & Goldman, 2008), using default settings. Obvious misalignments were adjusted by eye. In the case of partially incomplete data, short sections at the ends of the alignments were excluded. The number of variable and parsimony informative sites of all cpDNA regions were calculated using MEGA7 (Kumar *et al.*, 2016). As McDaniel and Shaw (2005) found evidence for a possible selective sweep, we tested for positive selection in the coding gene *rps4* using the Z-test for synonymous *vs*. non-synonymous mutations, applying the Nei-Gojobori method with Jukes-Cantor correction within MEGA7 (Kumar *et al.*, 2016).

Three alternative alignments of *ITS* were created; (i) a full PRANK alignment, and with removal of ambiguously aligned or hypervariable regions using (ii) NOISY (Dress *et al.*, 2008) and (iii) GBLOCKS (Castresana, 2000), using default settings. Treatment with NOISY and GBLOCKS resulted in alignments of length 588-678 and 514 bp, respectively. To test for recombination we applied all recombination detection methods within the program RDP v4.71 (Martin *et al.*, 2015) to the original *ITS* alignment, using default settings.

3.3.3 Phylogenetic and population genetic analyses

Best-fitting models of evolution were investigated by locus, and by codon for *rps4*, with jModeltest-2.1.7 (Darriba *et al.*, 2012) using the SPR tree topology search operation and AICc calculations. This resulted in the models TIM1+G for *atpB-rbcL* and HKY for *trnL-F*. The *rps4* marker was partitioned by codon position with model TPM1uf selected for the first two codon positions and JC for the third codon position. For all datasets indels were coded according to the simple indel coding (SIC; Simmons & Ochoterena, 2000) in SeqState v1.0. Bayesian analyses were performed using MrBayes 3.2 (Ronquist *et al.*, 2012) on i) each cpDNA locus separately (including indels as binary characters, where present), ii) a concatenated dataset including individuals for which all of *rps4* and *atpB-rbcL* loci were complete, called the '*rps4+atpB-rbcL*' dataset hereafter, and iii) a concatenated dataset including individuals for which all cpDNA loci had been sequenced, called the 'concatenated

cpDNA' dataset hereafter. This dataset was analysed with and without indels included. We also explored iv) a 'total evidence analysis' using all samples and cpDNA loci which had been sequenced, including those samples for which only one or two loci were available.

All MrBayes runs were continued for 1.5×10^6 generations, sampling every 1.0×10^3 generations, and discarding the first 25% as burn-in. Convergence was assessed by confirming that split frequencies had an average standard deviation of <0.01, and by using Tracer v.1.6 (Rambaut *et al.*, 2014) to confirm that all parameters exceeded effective sample sizes (ESS)>200. Maximum clade credibility trees were visualised using FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). Maximum likelihood (ML) analyses were performed on each dataset (i-iv) using RAxML-GUI v1.3.1 (Silvestro & Michalak, 2012), using the 'bootstrap + consensus' option (1000 iterations), applying the model of evolution most similar to the best fitting jModeltest results in each case (e.g. GTR with or without the Gamma model of rate heterogeneity), and applying default settings .

We investigated possible species clusters within the 'concatenated cpDNA' dataset using the pairwise genetic distance-based approach Automatic Barcode Gap Discovery (ABGD; Puillandre *et al.*, 2012), using the web server, applying default settings. ABGD groups samples into hypothetical candidate species based on non-overlapping values of intra- and interspecific genetic distances. We investigated Prior 'maximum divergence of intraspecific diversity' (P_{max}) values over a range of 0.001-0.05.

To examine phylogeographic structure, TCS networks were produced for all cpDNA and nuclear loci, as well as the 'concatenated cpDNA' dataset within the program Popart (Leigh & Bryant, 2015) using default settings. Within *ITS*, haplotype networks were constructed using the original, NOISY and GBLOCKS alignments.

3.3.4   Population diversity and demographic analyses

To evaluate the demographic history indicated by cpDNA, we calculated genetic diversity indices, pairwise Kimura-2P distances, demographic and spatial expansion models and Tajima's D (Tajima, 1989) and Fu's Fs (Fu, 1997) neutrality tests for each cpDNA locus with 10000 permutations, using Arlequin v3.5.1.2 (Excoffier & Lischer, 2010). Analyses were performed on the complete dataset for each individual cpDNA marker, as well as on the total 'concatenated cpDNA' dataset. The former were

analysed to investigate population processes using the maximum available sample sizes; the latter using the maximum available co-segregating sites. In the analyses on the 'concatenated cpDNA' dataset we also considered clade substructure within *Ceratodon*, and performed analyses on the overall dataset (clades I-VII), and the large ABGD-inferred cluster as defined with P_{max}=0.0017-0.0046 (clades III-VII) and P_{max}=0.001 (clades IV-VII), as shown in Fig. 3.3a.

To investigate worldwide latitudinal geographic structure we divided the 'concatenated cpDNA' dataset into three partitions based on geographical areas of >30° N, between 30° S and >30° N, and >30° S. We conducted hierarchical AMOVA analysis of the partitioning of genetic variation within and between these regions in Arlequin v3.5.1.2, using F_{ST} and Φ_{ST} measures of differentiation. We also calculated pairwise differentiation between regions, using 10000 permutations in both cases. F_{ST} estimates reflect differences in lineage frequency between regions, Φ_{ST} estimates reflect levels of genetic differentiation between lineages in different regions.

3.3.5 Divergence time estimation

We investigated divergence times of the different clusters within *C. purpureus* in BEAST v2.4.1 (Bouckaert *et al.*, 2014). Due to the above-mentioned complications with *ITS*, divergence time analyses were performed exclusively on cpDNA loci, on the 'concatenated cpDNA' dataset. In the absence of suitable fossils for our dataset we used a relaxed log normal clock with nucleotide substitution rate of 5.0×10^{-4} and standard deviation of 1.0×10^{-4} substitutions/site/my (subst./site/my), respectively, which has previously been applied in bryophyte studies (see Villarreal & Renner, 2014, and references therein), and corresponds to the average absolute substitution rate of cpDNA across a wide range of land plants and algae (e.g. Villarreal & Renner, 2014). We included the same outgroups, models of evolution, and partitioning as above, and implemented a Yule tree prior. MCMC chains were run for 1.0×10^8 generations, with parameters sampled every 10^3 generations. We combined log and tree files of three runs using LogCombiner v2.3.1 with 10% burn-in. Tracer v.1.6 (Rambaut *et al.*, 2014) was used to assess ESS>200 for all estimated parameters with 10% burn-in. A maximum clade credibility tree with median node heights was visualized using TreeAnnotator v1.8.2 (Drummond & Rambaut, 2007) and FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). We also conducted the same

analysis as above and implemented a coalescent tree prior, to investigate the impact of tree prior choice on divergence times.

3.4   Results

3.4.1   Molecular sequence data

The final sample set comprised a total of 148 individuals with a wide geographic spread across the globe (distribution shown in Fig. 3.2; Table 8.2.2.1, Appendix 8.2.2). The 'concatenated cpDNA' alignment comprised a minimum total combination length of 1645 bp. Of the three cpDNA markers, *atpB-rbcL* was the most variable, followed by *rps4* and *trnL-F* (18, 18, and 10 variable sites, and 17, 12, and 7 parsimony informative (PI) sites, respectively). We detected no evidence for positive selection within the *rps4* gene ($p > 0.05$), but note that the low number of variable sites limits our ability to draw conclusions from this result.

ITS had much greater genetic variation than the cpDNA markers, reflected in the number of variable sites (115) and PI sites (61), even after treatment with NOISY or GBLOCKS (83 and 41 variable sites and 60 and 20 PI sites, respectively). Many PCR-amplified samples provided a 'clean' single band during electrophoresis (Table 8.2.2.1). Other samples yielded double or messy bands, and were not sequenced. Two sets of double bands were excised and sequenced, and revealed multiple copies of *ITS* in the same specimens (samples from Hawaii and Australia). We did not investigate the occurrence of *ITS* copies further (e.g. through cloning) as *ITS* amplification of other contaminants such as fungi and algae is common, and this approach would be better pursued through future studies with fresh rather than herbarium material. The recombination tests revealed recombination signals in five of the seven tests performed (GENECONV, BOOTSCAN, MAXCHI, SISCAN and 3SEQ; Martin *et al.*, 2015).
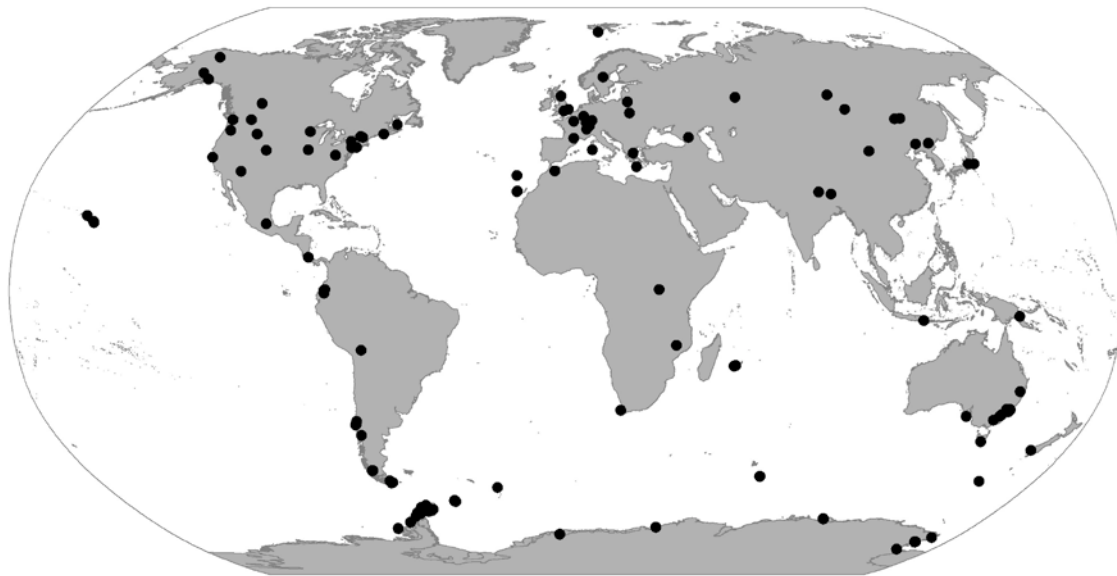
Fig. 3.2 Geographical distribution of the obtained samples of *Ceratodon purpureus*.

3.4.2   Phylogenetic and population genetic analyses

Phylogenetic trees and haplotype networks of the 'concatenated cpDNA' dataset are presented in Figs. 3.3a and b, respectively (for trees and haplotypes of individual loci and the '*rps4 + atpB-rbcL*' dataset see Figs. 8.2.2.1 and 8.2.2.2, Appendix 8.2.2). No topological conflicts were found between Bayesian and ML analyses. Phylogenetic relationships were similar for analyses of the 'concatenated cDNA' dataset with and without indel information included (the former not shown).

The phylogenetic analyses of the 'concatenated cpDNA' dataset revealed seven clades with high support (posterior probability (PP) >0.97 and bootstrap values >90) (Fig. 3.3a). These included, in order, an Antarctic clade (I), a tropical clade (II), a single-specimen lineage originating from Greece (III), and a polytomy consisting of an East African clade (IV), a bipolar clade (V), a Holantarctic clade (VI), and a Holarctic clade (VII). All single-locus analyses also resolved the first three clades (I-III) (see Figs. 8.2.2.1a-c, Appendix 8.2.2), while the latter four clades were resolved only by *atpB-rbcL* and/or *rps4*. The 'total evidence' phylogeny showed very limited resolution by comparison, with most samples falling as a single large polytomy (data not shown).

The geographic ranges spanned by each clade (I-VII) are shown by locus in Figs. 8.2.2.1 and 8.2.2.2, Appendix 8.2.2, and are visualised in Fig. 3.3c. Clade I included specimens from the Antarctic Peninsula and the nearby South Orkney Islands

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archipelago. Clade II included specimens from equatorial regions in both the Palaeotropics and the Neotropics, including Papua New Guinea, Nepal, Reunion Island, Mexico, Costa Rica and Bolivia. Clade III consisted of specimens from Greece and the Canary Islands. The East African clade (IV) was not resolved by individual loci, but was distinct from the other clades in the concatenated cpDNA dataset (Figs. 3.3a, b), and included specimens from Reunion Island, Malawi and Uganda (the latter was resolved in the concatenated *rps4+atpB-rbcL* dataset; see Fig. 8.2.2.1d, Appendix 8.2.2). The bipolar clade (V) (Figs. 3.3a-c) included specimens from western North America and the sub-Antarctic and Antarctic, as well as one specimen from India. The Holantarctic clade (VI) included specimens from higher latitudes in the Southern Hemisphere (southern South America, Australia, the sub-Antarctic and Antarctica). Finally, the Holarctic clade (VII) included specimens from the Holarctic bryofloristic kingdom, as well as from Hawaii and one specimen from Ecuador.

The species delimitation method ABGD revealed three or four significant "barcoding gaps" in the concatenated cpDNA dataset at P_{max}=0.0017-0.0046 and P_{max}=0.001, respectively. At P_{max}=0.001 clades I, II, III and IV-VII were separated, while at P_{max}=0.0017-0.0046 clades I, II and III-VII were identified as distinct groups (see Fig. 3.3a). Clades I and II were identified as different species clusters from the rest of the dataset in all the P_{max}-values analysed, while support for clade III as a distinct lineage was only present at the lower P_{max}-value.

Haplotype networks were constructed to visualise the genetic variation within *ITS* (based on original, NOISY and GBLOCKS alignments, Fig. 8.2.2.3, Appendix 8.2.2). These revealed that the same samples representing distinct clades I, II and III in the cpDNA (see Fig. 3.3) were also present with long branch-lengths in all *ITS* networks (Fig. 8.2.2.3, Appendix 8.2.2). A sample representing clade IV was also found distinct in the original and NOISY alignments. No distinct clustering of the remaining cpDNA-defined clades (V-VII) could be recognized in the *ITS* networks. Multiple copies within the same samples were placed in widely separated regions in the haplotype networks (indicated with * in Fig. 8.2.2.3, Appendix 8.2.2).
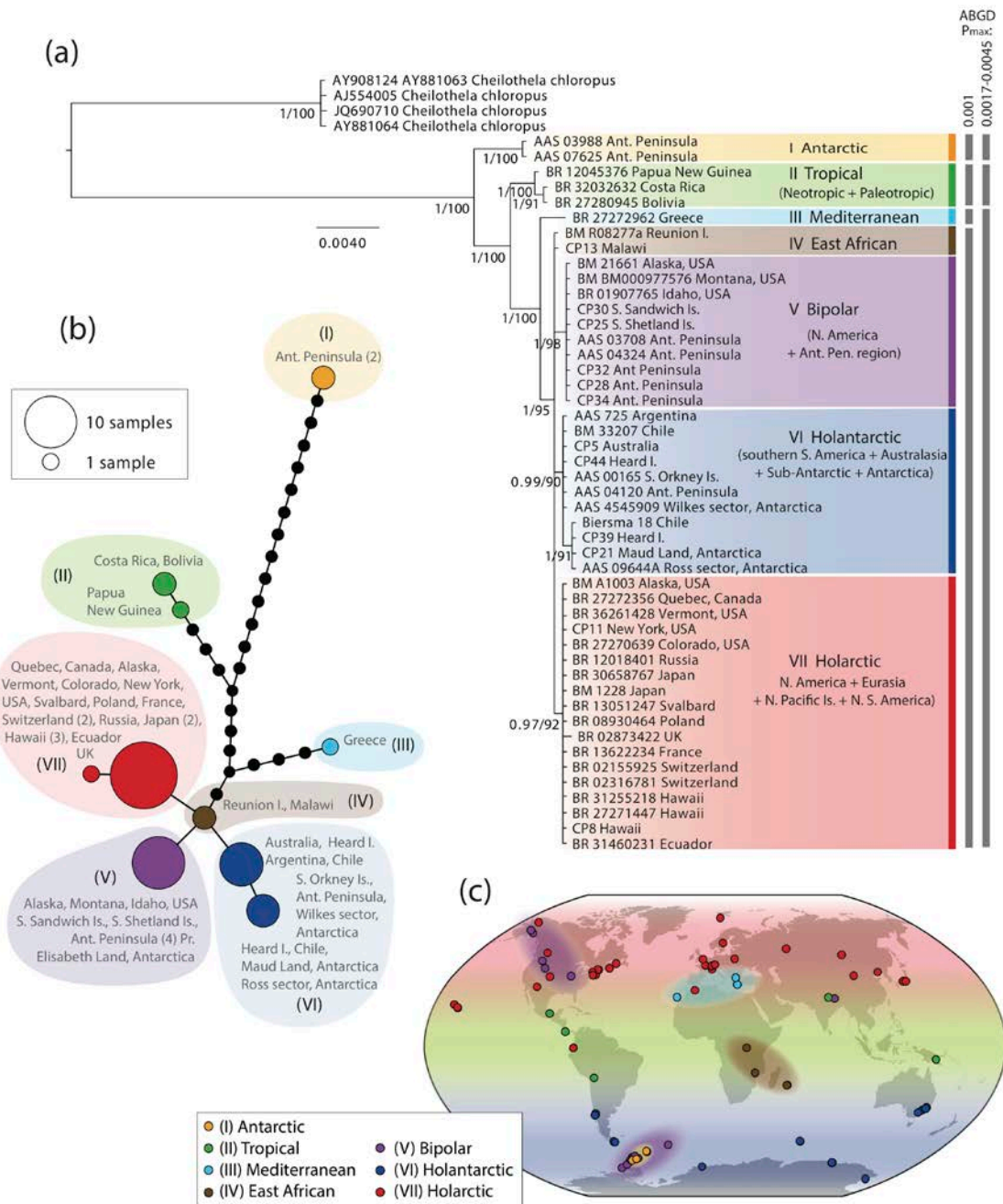
Fig. 3.3 Bayesian phylogeny (a) and haplotype network (b) for *Ceratodon purpureus* constructed with a concatenated cpDNA dataset (*atpB-rbcL+rps4+trnL-F*). Posterior probabilities and bootstrap support are shown next to branches (a). The scale bar represents the mean number of nucleotide substitutions per site. Biogeographic clade descriptions (I-VII) and ABGD species-clusters with different P_{max}-values are shown next to (a). In (b) haplotype circle sizes and colours correspond to the number of specimens and clades I-VII, respectively. Branches represent mutations between haplotypes, with mutations shown as 1-step edges. Figure (c) represents the sample

locations and biogeographic regions of the different clades (I-VII) as interpreted from all well resolved clades in cpDNA datasets (see also Fig. 8.2.2.1a-d, Appendix 8.2.2).

3.4.3 Population diversity and demographic analyses within cpDNA loci

All cpDNA markers had low nucleotide diversity ($\pi>0.01$) (Table 3.1), as would be expected for these markers, which are more commonly used for species- and genus-level rather than population-level studies (Stech & Quandt, 2010). F_{ST} and Φ_{ST} calculations revealed significant genetic differentiation between the latitudinal geographic areas (>30° N, 30° S - 30° N and >30° S; Table 3.2). The hierarchical AMOVA analyses estimated that 16% of haplotypic differentiation (F_{ST}=0.16) and 17% of genetic differentiation (Φ_{ST}=0.17) was found within latitudinal bands, compared to ~83-84% of differentiation between latitudinal bands. For both metrics these results were highly significant ($P<0.01$). The demographic expansion test on the concatenated cpDNA dataset of all clades (I-VII) was significant, rejecting a demographic expansion (Table 3.1). The spatial expansion test was non-significant, indicating that the genetic pattern may be consistent with a spatial expansion. Tajima's D was significantly negative in the full (I-VII) concatenated cpDNA dataset, as well as the full dataset of *rps4* and *trnL-F*, suggesting that rare alleles are present in these loci at lower frequencies than expected. However Tajima's D was not significant when the most divergent lineages (I-II) were removed, suggesting the significant results were mostly influenced by cryptic population structure within *C. purpureus*. Furthermore, Fu's Fs was not significant for any dataset, suggesting that it is not likely that the species has been subject to rapid past expansion.

Table 3.1 Genetic diversity indices, demographic and spatial expansion model tests and Tajima's D and Fu's Fs neutrality tests as performed in using Arlequin v3.5.1.2 (Excoffier & Lischer, 2010) for *atpB-rbcL*, *rps4* and *trnL-F* within *Ceratodon purpureus*. Analyses were performed on total individual cpDNA loci as well as the concatenated cpDNA dataset. Within the latter, analyses were performed on all clades (I-VII; n=47) and the large ABGD-inferred cluster as defined with P_{max}=0.0017-0.0046 (III-VII; n=42) and P_{max}=0.001; (IV-VII; n=41), as shown in Fig. 3.3. SSD or HRI p-values were non-significant except where indicated with *.

Clades	n	bp[a]	π	S	*h*	Demographic expansion SSD	HRI	Spatial expansion SSD	HRI	Neutrality tests: Tajima's D (*P*)	Fu's Fs (*P*)
atpB-rbcL (total)	80	629	0.006±0.003	17	0.793±0.026	0.047	0.092	0.043	0.092	-1.248 (0.092)	2.420 (0.835)
rps4 (total)	94	589	0.003±0.002	18	0.767±0.029	0.007	0.073	0.007*	0.073	-1.525 (0.038*)	-3.290 (0.089)
trnL-F (total)	74	435	0.002±0.002	10	0.319±0.070	0.026	0.385	0.003	0.385	-1.986 (0.002*)	-2.939 (0.066)
Concatenated											
I-VII	47	1660	0.004±0.002	37	0.874±0.029	0.827	0.057	0.028	0.057	-1.452 (0.050*)[1]	2.094 (0.809)
III-VII	42	1658	0.002±0.001	11	0.843±0.033	0.035	0.083	0.037	0.083	-0.873 (0.210)	0.972 (0.708)
IV-VII	41	1657	0.002±0.001	5	0.835±0.033	0.038	0.091	0.040	0.091	0.828 (0.818)	1.247 (0.734)

n: number of samples; bp[a]: no. of usable basepairs (loci <5.0% missing data); π: nucleotide diversity (average over locus); S: No. of sites with substitutions; *h*: gene diversity; SSD: Sum of Squared Deviations; HRI: Harpending's Raggedness Index. For Tajima's D, Fu's Fs and expansion models, *P*<0.05 is significant (*). [1] = The significant Tajima's D of clade I-VII of the concatenated cpDNA dataset had a *P*-value of 0.0499.

Table 3.2 F_{ST} (below diagonal) and Φ_{ST} (above diagonal) of *Ceratodon purpureus* samples of geographical areas >30° N, between 30° S and N and >30° S, including samples from the 'concatenated cpDNA' dataset. The number of samples (n) per geographic area is given in the top row. P values are represented by * for *P<0.05* and ** for *P<0.01*.

$F_{ST} \setminus \Phi_{ST}$	>30° (n=18)	-30° - 30° (n=9)	>-30° (n=20)
>30°	-	0.197 (0.015*)	0.153 (0.002**)
-30°-30°	0.195 (0.001**)	-	0.186 (0.006**)
>-30°	0.244 (0.000**)	0.176 (0.001**)	-

3.4.4   Divergence time estimation

The divergence time analysis indicated that the ancestor of the *C. purpureus* clades originated in the late Miocene ~5.95 Mya (with 95% highest posterior density intervals (95HPD): 9.79-2.72 Mya; see Table 3.3, Fig. 3.4), a time when the Antarctic (I) and tropical (II) clade diverged from the remaining clades (III-VII). Clades I and II diverged not long after this in the mid-Pliocene, ~3.98 Mya (95HPD: 7.11-0.86 Mya). Clade III diverged from the other clades around that same time (~4.64 Mya; 95HPD: 7.84-1.95 Mya), and the remaining clades (IV-VII) diverged, ~3.79 Mya onwards (95HPD: 6.68-1.57 Mya), throughout the late Pliocene to mid-Quaternary. All nodes in the BEAST tree (Fig. 3.4) had high posterior support, except for the node uniting clades IV and VI, and that separating V from IV+VI, relationships which were also not strongly supported in the phylogenetic analyses (see Fig. 3.3a). Using a coalescent tree prior we found older ages (clades I-VII diverged ~2.5 My earlier), with slightly younger clade divergence within clades (data not shown).

Table 3.3 Mean ages (95% HDP lower - upper) (my) for the different clades from the 'concatenated cpDNA' dataset (see Fig. 3.3a) within *Ceratodon purpureus* as calculated in BEAST. TMRCA: Time to the Most Recent Common Ancestor. Ages within clades I-VII reflect ages within clades.

Lineage TMRCA	Mean ages (95% HDP lower - upper) (my)
Cheilothela chloropus + *Ceratodon purpureus*	7.14 (3.84-12.06)
I-VII	5.95 (2.72-9.79)
I-II	3.98 (0.86-7.11)
III-VII	4.64 (1.95-7.84)
IV-VII	3.79 (1.57-6.58)
I Antarctic	0.71 (2.30×10^{-5}-2.20)
II Tropical	1.48 (0.08-3.45)
IV East-African	0.90 (1.01×10^{-5}-2.56)
V Bipolar	1.98 (0.49-3.83)
VI Holantarctic	2.22 (0.59-4.18)
VII Holarctic	2.54 (0.84-4.61)
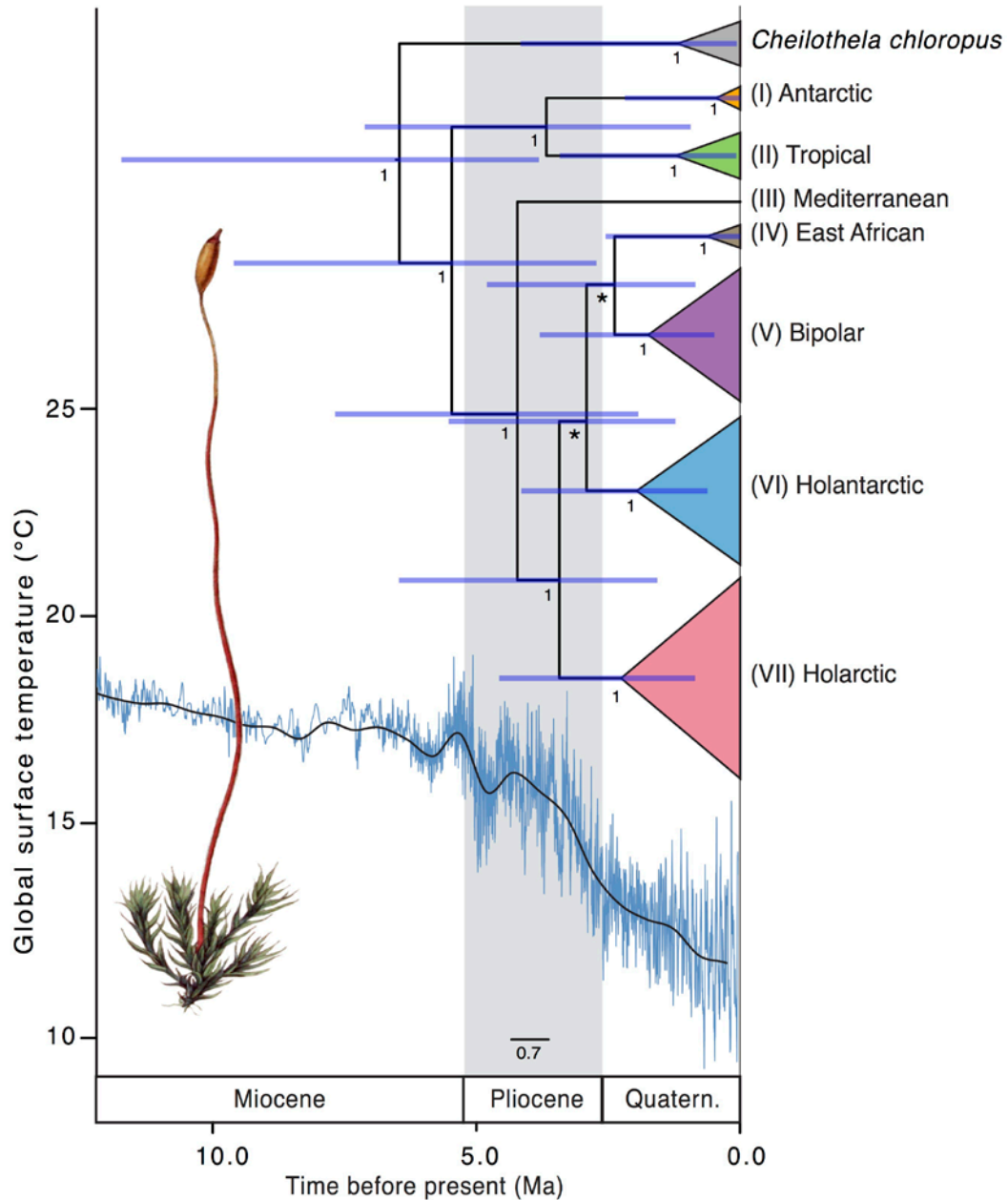
Fig. 3.4 Historical biogeography of *Ceratodon purpureus*. The maximum clade credibility tree presents the median divergence time estimates for major lineages (see Fig. 3.3a) from a concatenated cpDNA dataset (*atpB-rbcL+rps4+trnL-F*). Node bars represent 95% height posterior distribution of age estimates. Posterior support (PP) values are shown below nodes, with PP<0.50 provided as *. Global surface temperature estimates (blue and solid line representing temperature variations and a 500 kyr smoothed resolution, respectively), reproduced from Hansen *et al.* (2013), is provided below.

3.5 Discussion

3.5.1 Distinct biogeographic regions

The analysis of the cpDNA loci within *C. purpureus* resolved well-supported, multi-million-year old lineages (Figs. 3.3, 3.4) derived from distinct biogeographic regions, revealing that the species contains clear phylogeographic structure in its maternal line. The lineages were strongly associated with latitude (Figs. 2c, Table 3.2, AMOVA results, Section 3.4.3). The biogeographic patterns of *C. purpureus* corresponded with those found by McDaniel and Shaw (2005), with the increased sampling and additional cpDNA loci considerably expanding the geographic extent and characterization of several clades (particularly newly-recognised clades I, IV and VI), and providing evidence for the latitudinal structuring of *C. purpureus.*

All cpDNA loci (Fig. 3.3; Figs. 8.2.2.1, 8.2.2.2, Appendix 8.2.2 for individual cpDNA loci) and the *ITS* marker (Fig. 8.2.2.3, Appendix 8.2.2) revealed particularly strong differentiation of the first three clades identified (I-III), with the ABGD species delimitation method supporting a species complex with at least three (I, II and III-VII) and possibly four (also dividing III from IV-VII) species, with the whole complex referred to as *C. purpureus sensu lato (s.l.)* hereafter. Several taxonomic studies have previously also noted differentiation within *C. purpureus* (Burley & Pritchard, 1990; Ochyra *et al.*, 2008, and references therein). Although not currently recognised (Ochyra *et al.*, 2008), the finding that there is significant differentiation amongst *C. purpureus* populations regains credence here based on the variability of the cpDNA and *ITS* regions. Although a strong phylogeographic signal was obtained in the plastid markers of *C. purpureus s.l.*, the nuclear markers studied in the species (*ITS* in this study; *adk* and *phy2* in McDaniel and Shaw, 2005) revealed evidence for recombination as well as multiple copies per sample. This is in line with the previous report of an ancient nuclear genome duplication (Szövényi *et al.*, 2014) and high levels of genetic interactions among nuclear loci (McDaniel *et al.*, 2007, 2008) in *C. purpureus*. The chloroplast, which is inherited maternally in bryophytes (McDaniel *et al.*, 2007), as studied here, provides an evolutionary history for the maternal line in *C. purpureus s.l.*. However, the evolutionary history of species is far more complex than that of the maternal line only, and we highlight the importance of combining different marker systems to unravel evolutionary histories, as all are part of a species' biology. We anticipate future work on the nuclear genome of *C. purpureus* will shed light on the extent, causes and consequences of

recombination and genome doubling on the evolution in *C. purpureus* as well as other non-vascular plants.
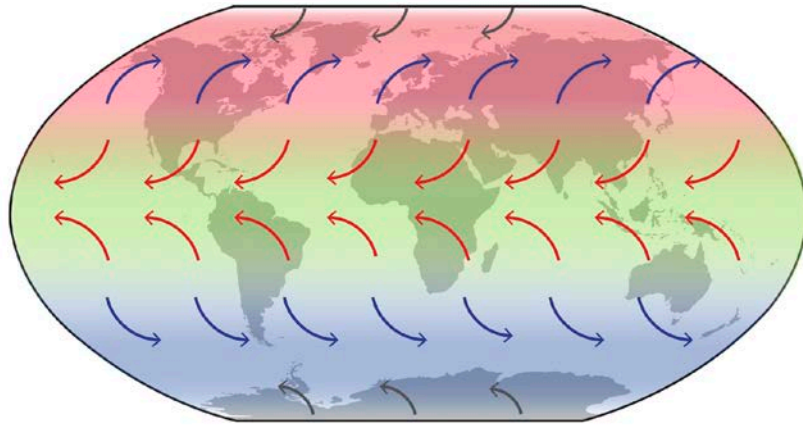
Fig. 3.5 Global wind patterns overlaying the distributions of the Holantarctic (VI), Holarctic (VII), and tropical (II) clades (see Fig. 3.3a). Red arrows: Trade Winds; blue arrows: Westerlies; grey arrows: Polar Easterlies.

3.5.2 Drivers of dispersal

We found clear biogeographic structuring dividing the main global populations of *C. purpureus s.l.* into physically distinct 'latitudinal' geographic areas (see Fig. 3.3c, Table 3.2 and AMOVA results, Section 3.4.3). This structuring is plausibly linked with atmospheric circulation patterns (see Fig. 3.5 for general global wind patterns overlaying the distributions of the Holantarctic, Holarctic, tropical clades). Generally, global air masses, and thus particles that are carried within them, are retained within particular latitudinal bands or geographic regions. At higher latitudes in both hemispheres, the prevailing westerly winds (blue arrows; Fig. 3.5), moving from west to east as well as towards the poles, serve to isolate higher latitudes from equatorial regions. These could therefore be an isolating force for, for instance, the Holantarctic (VI) and Holarctic (VII) clades. Similarly, the easterly trade winds (red arrows; Fig. 3.5) move mostly towards the equator, thus retaining entrained particles within the equatorial band. These could similarly therefore be an isolating force for clade II, found in tropical regions around the globe. Similar isolating patterns *via* wind currents, with extensive latitudinal distributions and lack of dispersal between higher latitudes of both hemispheres, have also been reported in aerobiology modeling studies (Muñoz *et al.*, 2004; Wilkinson *et al.*, 2012). These atmospheric circulation patterns not only restrict the movement of particles within a given hemisphere, latitudinal band or region but could, by

doing so, be the driving isolating force triggering adaptation to particular climatic zones. Such genetic isolation has been shown by McDaniel *et al.* (2007, 2008), who showed that considerable reproductive isolation exists between a tropical and a temperate population (from Ecuador and the northern United States, respectively) of *C. purpureus*, including genetic incompatibilities within hybrids. These patterns are indicative of both genetic isolation and local adaptation.

Global atmospheric circulation patterns strongly influence air movement, and thus dispersal, between hemispheres due to the Intertropical Convergence Zone across the equator. The distinct bipolar group (clade V; Fig 3.3; supported in both *atpB-rbcL* and *rps4*; Figs. 8.2.2.1a, b, Appendix 8.2.2) is nevertheless likely to be a product of at least one long-distance dispersal event across the equator. Bipolar disjunctions are a distribution pattern characteristic of many bryophytes (Ochyra *et al.*, 2008), however recent molecular studies (Lewis *et al.*, 2014a) suggest that trans-equatorial movements are rare events that happen very occasionally over multi-million year timescales. Such trans-equatorial dispersal could have been facilitated by air movements or zoochory (adventitious attachment to other organisms, e.g. birds). As nearly all specimens in clade V belonged to the New World and the Antarctic region directly south of this (excepting a single specimen from India), migratory birds that link parts of these northern and southern regions in their annual migration are plausible vectors (*cf.* Lewis *et al.*, 2014b).

In addition to the influence of wind, several other factors may have aided the spread and dispersal of *C. purpureus s.l.* across the globe. As *C. purpureus s.l.* is a ruderal species, characteristically found in a wide range of dry and disturbed habitats (Nydahl *et al.*, 2015), an increase in environmental (e.g. glacial, fire-influenced and, more recently, anthropogenic) disturbances throughout the Pliocene and Quaternary may have created favourable habitat for the species. The main distribution of *C. purpureus s.l.,* occupying the vast areas of the temperate regions (particularly clades V-VII), became established throughout the late Pliocene and Quaternary. This was a period of high disturbance globally, including global cooling and repeated glacial periods (Hansen *et al.*, 2013; for a comparison with global surface temperature see Fig. 3.4). It is likely that repeated glacial disturbance provided favourable conditions for the spread of *C. purpureus s.l.* in high latitude areas of both hemispheres. Additionally, the rise of modern flammable grass-, shrub- and woodlands (late Miocene onwards with peak origins in late Pliocene; Bond, 2014) could have promoted suitable habitat for *C. purpureus s.l.*, as the species is also frequently found in fire-influenced habitats (Duncan & Dalton, 1982). Furthermore, in the recent post-Quaternary period, the

origin and expansion of urban environments provided major sources of anthropogenically-influenced disturbance, potentially favourable to *C. purpureus s.l.* (Forman, 2014).

The concatenated cpDNA of all clades (I-VII) showed a significant negative Tajima's D (also found in the full datasets of *rps4* and *trnL-F*; see Table 3.1), providing possible evidence of a population expansion or a selective sweep. However Tajima's D was not significant after removal of the most divergent lineages (I-II; Table 3.1), suggesting the significance is mostly influenced by cryptic population structure within *C. purpureus s.l.* (given that clades I and II are regarded as different species clusters in ABGD; see Section 3.4.2 and Fig. 3.3). Additionally, Fu's Fs was not found significant for any dataset, suggesting that it is not likely that the chloroplast lineage has been subject to rapid recent expansion. However, it should be noted that low diversity within clades, particularly within clade V-VII, limits the ability to discern such processes with the data presented here.

3.5.3 Multiple colonisations of the Antarctic, including an ancient lineage

In Antarctica, *C. purpureus s.l.* is, together with *Bryum pseudotriquetrum* (Hedw.) P. Gaertn., the most widespread moss species found across the continent (Ochyra *et al.*, 2008). Ochyra *et al.* (2008) noted considerable diversity in leaf size and morphology between Antarctic *C. purpureus s.l.* plants, suggesting the presence of multiple different lineages within Antarctica, a finding confirmed by the presence of multiple origins of Antarctic populations (clades I, V and VI) in our study. This reveals that Antarctica is not as isolated for spore-dispersed organisms as is often assumed. Recently, Pisa *et al.* (2014) also proposed at least three independent origins of the moss *Bryum argenteum* Hedw. in Antarctica.

According to the dating analysis, Antarctic clade I, whose members consist only of specimens from the Antarctic Peninsula and the South Orkney Islands, has been isolated since the Pliocene (~3.98 Mya; 95HPD: 7.11-0.86 Mya). Although more extensive sampling will be required to fully assess whether clade I is limited to Antarctica, its apparent ancient isolation suggests it may be a remnant lineage that has survived past glaciations in the maritime Antarctic *in situ*. Its arrival could have coincided with relatively warm interglacials on the Antarctic Peninsula during the early Pliocene (particularly between approximately 4.5-4.4 and 3.6-3.4 Mya; De Schepper *et al.*, 2014). Recent climate and glaciological modelling studies have highlighted greater dynamism in glacial extent than previously considered possible throughout the early Pliocene and Pleistocene (Scherer *et al.*, 2008; Naish *et al.*, 2009; Pollard & DeConto, 2009; De Schepper *et al.*, 2014; DeConto & Pollard, 2016), raising the possibility of ice-free local refugial areas persisting throughout these periods. Molecular,

phylogenetic and biogeographic studies also suggest *in situ* survival for many groups of terrestrial fauna in Antarctica throughout the Quaternary, Neogene and even Paleogene (e.g. nematodes, springtails, copepods, mites and diptera; see Convey *et al.*, 2008, 2009a, and references therein). Similarly, in combination with a previous study providing evidence of million-year Antarctic presence of the moss *Bryum argenteum* (Pisa *et al.*, 2014), our study finds that at least one lineage (I) of *C. purpureus s.l.* has survived in Antarctica *in situ* since the Pliocene, suggesting that important elements of the Antarctic flora may also have had a longer-term presence than previously thought.

3.6   Acknowledgements

4 Low genetic variation between South American and Antarctic populations of the bank-forming moss *Chorisodontium aciphyllum*

Fig. 4.1 *Chorisodontium aciphyllum* growing on Robert Island, South Shetland Islands. (photo: E.M. Biersma)

This chapter is based on the following manuscript:

4.1   Abstract

The Antarctic-South American bank-forming moss *Chorisodontium aciphyllum* both holds the record of extreme survival and viability, as well as the oldest known (sub-)fossils in the Antarctic of any extant plant species known, making this a particularly interesting species to investigate for the possible of long-term persistence. Investigating the genetic variability in the plastid markers *trnL-F* and *rps4* and the nuclear Internal Transcribed Spacer (*ITS*) we found very limited genetic variation within *C. aciphyllum* as well as between *C. aciphyllum* and other *Chorisodontium* species. The lack of genetic variation both between and within Antarctic and southern South American populations suggests a relatively recent (likely within the last ~100 kya) colonization of this moss to the Antarctic, as well as a likely severe bottleneck during the last glaciation in southern South America. Simple atmospheric transfer modeling studies also suggest that, for small organisms or spores dispersing aerially, the northern Antarctic Peninsula shows potentially regular connectivity from southern South America, with air masses transferring, particularly southbound, between the two regions. Amongst the Antarctic populations of *C. aciphyllum* examined, we found elevated genetic variation in Elephant Island, the most northern island of the South Shetland Islands as well as the location of the oldest known *C. aciphyllum* banks (>5500 years), suggesting this location to be a genetic hotspot for this species in the Antarctic.

4.2   Introduction

The timing of origin of Antarctic biota and understanding the connectivity of populations between southern South America and the Antarctic Peninsula have increasingly become central questions in Antarctic biogeographic studies (Allegrucci *et al.*, 2006; Convey *et al.*, 2008, 2009b; Allegrucci *et al.*, 2012; Fraser *et al.*, 2012). Ice-sheet modeling studies and glaciological reconstructions suggest the entire Antarctic continent, and in particular the low altitude and generally coastal areas occupied by the better developed terrestrial ecosystems present today, to have been almost fully covered by thick ice-sheets during the Last Glacial Maximum (LGM; ~18-20 ky BP), as well as previous Miocene and Pleistocene glaciations, implying that most contemporary terrestrial life could only have colonised Antarctica since the LGM (see Section 1.1.1). Conversely, recent molecular phylogeographic and classical biogeographic studies have overturned this long-held paradigm, strongly supporting a long-term persistence of Antarctica's extant terrestrial biota, including many faunal as well as

microbial groups, with estimated persistence ranging from hundreds of thousand to multi-million year timescales (e.g Convey & Stevens, 2007; Convey *et al.*, 2008; 2009a; De Wever *et al.*, 2009; Vyverman *et al.*, 2010; Fraser *et al.*, 2014; Chong *et al.*, 2015; Iakovenko *et al.*, 2015).

The origin of the Antarctic bryophytes, the dominant macroscopic flora on the continent, is less well understood. As with the other groups, Antarctic bryophytes have been widely thought to be recent arrivals in the Antarctic, a hypothesis that is consistent with several lines of evidence: their i) low endemicity (see discussion in Convey *et al.*, 2008), ii) low species richness, and iii) distribution patterns, with most species restricted to the relatively mild maritime Antarctic, and very few restricted to the much harsher continental Antarctic (Ochyra *et al.*, 2008). However, a recent population genetic study on the cosmopolitan moss *Bryum argenteum* Hedw. suggested a long-term persistence of this moss in the Antarctic (Peninsula and continent), identifying at least three separate colonisation events on very conservatively estimated multi-million-year timescales (~4.4, ~1.4 and ~0.6 Mya; Pisa *et al.*, 2014). This first direct indication of long-term persistence implies that, perhaps, more extant Antarctic bryophytes have similarly had a long-term (pre-LGM) presence within Antarctica.

The oldest subfossils of any extant Antarctic moss species are of the bank-forming moss *Chorisodontium aciphyllum* (Hook. f. & Wils.) Broth. This moss is therefore a suitable candidate species to examine for evidence of long-term persistence in the Antarctic. *C. aciphyllum* is a common moss in the sub- and maritime Antarctic (Antarctic Peninsula and Scotia Arc archipelagos). Its overall distribution is limited to southern South America, the Falkland Islands, the Scotia Arc, and the Antarctic Peninsula and associated islands, including the South Shetland Islands. *C. aciphyllum* forms banks often up to 1-2 m in depth, with the deepest banks known reaching a depth of up to almost 3 m on Elephant Island in the South Shetland Islands (Smith, 1972b, 1979, 1996; Collins, 1976b, a; Fenton, 1980, 1982b; Fenton & Smith, 1982; Björck *et al.*, 1991; Fig. 4.2). The bases of 1.5 m deep peat banks at Signy Island (South Orkney Islands) and Elephant Island (South Shetland Islands), have been radiocarbon dated at ~5000 and 5500 years old, respectively (Fenton & Smith, 1982; Björck *et al.*, 1991), and deeper cores may potentially be older.

Fig. 4.2 Extensive *Chorisodontium aciphyllum* moss bank growing on Signy Island, South Orkney Islands (1 m scale stick in left hand photograph). (Photographs: J. Fenton)

In maritime Antarctic moss banks, the active layer depth is typically 30-50 cm, with depths below that being frozen in permafrost. The moss in these banks is therefore extremely well preserved physically or morphologically, and regrowth studies have revealed that old moss shoots deep within the peat banks are still viable and able to regrow after thawing and supplying with water and light (Roads *et al.*, 2014). New shoots of *C. aciphyllum* grew directly from existing gametophyte shoots (and not spores) at 110 cm depth in the core examined, a depth radio-carbon dated to 1533-1697 yrs BP, revealing the longest survival and viability of any bryophyte (or indeed multicellular eukaryotic organism) known. These observations suggest that mosses such as *C. aciphyllum* have the potential to survive at least through shorter periods of ice extension, such as the Little Ice Age, such as are inferred in various studies of glacial extent over time and through palaeoclimate proxies in the Antarctic (Hodgson & Convey, 2005; Guglielmin *et al.*, 2015). Whether they have the capability to persist similarly through entire glacial cycles appears a considerably greater challenge, but is at present unknown.

These characteristics make *C. aciphyllum* a particularly interesting species to examine for clues of a possible long-term (hundreds of thousand to multi-million year timescales) Antarctic origin. Applying several widely-used genetic markers and Bayesian inference approaches, in this study we investigated the genetic variation between and within populations of *C. aciphyllum* throughout the full extent of its natural distribution in southern South America and Antarctica. Additionally, in order to further assess the connectivity of spore-dispersed organisms between South America and Antarctica we used atmospheric wind modeling techniques to study the relative frequency and direction of atmospheric dispersal

events between the regions. These analyses will increase our general understanding of the likely age of spore-dispersed organisms within Antarctica.

4.3   Materials and Materials

4.3.1   Sampling and molecular methods

Material was sampled throughout the natural range of *C. aciphyllum* from fresh and herbarium sources (see Table 8.2.3.1 and Fig. 4.3; see Section 8.1.1-8.1.4 for in-detail laboratory techniques and protocols). All herbarium samples originated from the British Antarctic Survey (BAS) Herbarium (herbarium code AAS). We also included several closely related species, taxonomically assigned to different *Chorisodontium* species: *C. magellanicum* (Card.) Bartr., *C. lanigerum* (Müll. Hal.) Broth.*, C. spegazzini* (C. Müll.)., *C. dicranellatum* (C. Müll.) Broth., *C. sphagneticola* Roiv.*, C. mittenii* (C. Müll.) Broth. and *C. setaceum* (Bartr.) Bartr. A number of fresh (frozen) samples of *C. aciphyllum* collected recently from locations in the South Shetland Islands (Ardley Island and Elephant Island) and Anvers Island west of the Antarctic Peninsula (Norsel Point), as described in Royles *et al.* (2016) were also included. From these we sampled multiple shoots to investigate within-population variation. These samples were spatially separated by approximately 50-300 m intervals (numbered 1-3), and from each several sub-samples were taken at a finer-scale interval of approximately 5 cm (letters A-E). Several shoots were taken per sub-sample.

DNA was extracted using the DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany), with use of mortar and pestle and liquid nitrogen, following the manufacturer's instructions, and using one gametophyte shoot per sample. We amplified three commonly used markers for phylogenetic inference at the genus to population level (Stech & Quandt, 2010): the nuclear Internal Transcribed Spacer (*ITS*) and the plastid markers *trnL-F* and *rps4* (the latter including the *trnS-rps4* intergenic spacer; with the entire region hereafter referred to as *rps4*). Amplification was performed using the Taq PCR Core Kit (Qiagen GmbH, Hilden, Germany) with addition of Bovine Serum Albumin (BSA), checking the results using agarose gel electrophoresis. *ITS* was amplified using primer combinations ITS1 and ITS4 (White *et al.*, 1990) or ITS-A (Blattner, 1999) and 25R (Stech, 1999). Plastid markers *trnL-F* and *rps4* were amplified using primer combinations *trn*LF-c and *trn*LF-f (Taberlet *et al.*, 1991) and trnS (Souza-Chies *et al.*, 1997) and rps 5' (Nadot *et al.*, 1994), respectively. An annealing temperature of 60°C was used for all amplifications, except for *rps4*, which ranged between

55-60°C. Forward and reverse sequencing was performed by LGC Genomics (Berlin, Germany), using the same primers as mentioned above.
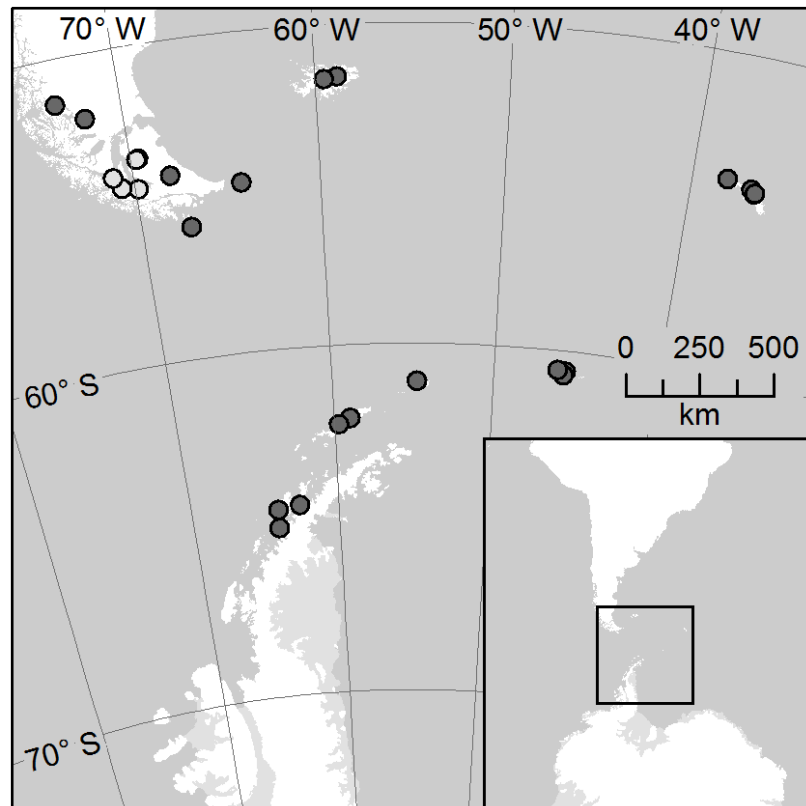
Fig. 4.3 Map showing locations of samples of *Chorisodontium aciphyllum* (dark grey) and other *Chorisodontium* species (*C. magellanicum, C. lanigerum, C. spegazzini, C. dicranellatum* and *C. sphagneticola*; light grey), as used in this study. Specimens from *C. mittenii* and *C. setaceum* are not shown as collection coordinates are unknown or fall outside the map (see Table 8.2.3.1).

4.3.2 Sequence editing and alignment

All sequences were manually examined, with forward and reverse sequences assembled by Codoncode Aligner v.5.0.2 (CodonCode Corp., Dedham, MA). We included several Genbank sequences of *Dicronaloma* species (for which loci were derived from the same original specimens) as outgroups in all alignments: *D. cylindrothecium* (Mitt.) Sakurai (*rps4*: KF423880; *trnL-F*: KF423978; *ITS*: KF423621), and *D. robustum* (Hook.f. & Wils.) Paris (*rps4*: KF423822; *trnL-F*: KF423912; *ITS*: DQ464190). Additionally, as the above mentioned *rps4* outgroup sequences were only partial, we included several other *Dicranoloma* sequences in the *rps4* alignment (AF435280 *D. billardieri* (Brid.) Paris,

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AF435281 *D. blumii* (Nees) Paris, and AY908103 *D. eucamptodontoides* (Broth. & Geh.) Paris), as well as extra *Chorisodontium* sequences (AY908107 and AF435272 *C. mittenii*, and AF435273 *C. setaceum*). For *trnL-F*, we added additional outgroup sequences KF423978 and KF423912 (*D. cylindrothecium* and *D. robustum*, respectively) and two *Chorisodontium* sequences AF435311 and AF435312 (*C. mittenii* and *C. setaceum*, respectively). Loci were aligned for each locus using the Geneious aligner within Geneious 9.0.4 (Biomatters, LTD, Auckland, NZ). Short, partially incomplete sections at the ends of each alignment were excluded. The number of variable and parsimony informative sites were calculated for each locus in MEGA7 (Kumar *et al.*, 2016) using ingroup sequences with *Chorisodontium* species only.

4.3.3 Phylogenetic and population diversity analyses

Bayesian analyses using MrBayes 3.2 (Ronquist *et al.*, 2012) were performed using on each locus separately. Nucleotide substitution models were selected according to the SPR tree topology search operation and AICc calculations as implemented by jModeltest-2.1.7 (Darriba *et al.*, 2012) for each individual marker, resulting in the TIM2, TPM1uf and TPM3uf (n=6, rates=equal for all) for *rps4*, *trnL-F* and *ITS*, respectively. For the MrBayes analysis indels in *ITS* were coded in SeqState v1.0. (Simmons & Ochoterena, 2000) using the simple indel coding. An additional analysis was performed of all loci combined, including only specimens with all markers complete. MrBayes runs of all markers were continued for 1.0×10^6 generations, sampling every 1.0×10^3, ensuring all parameters exceeded effective sample sizes (ESS) >200 and split frequencies reached values >0.01 using Tracer v.1.6 (Rambaut *et al.*, 2014), and discarding the first 25% as burn-in. Maximum clade credibility trees with mean node heights were visualised using Figtree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

We examined phylogeographic structure within ingroup specimens with TCS networks produced for each locus using the program Popart (Leigh & Bryant, 2015), using default settings.

4.3.4 Aerial dispersal modeling

The potential relative frequency of atmospheric dispersal events between different locations was evaluated using a method of following trajectories of air-mass movements from reconstructions of past atmospheric winds. Simplifying assumptions were made that (i) particles are blown by the wind without any independent movement (e.g. fall-out) and that

(ii) there are no thresholds on survival in terms of environmental conditions such as temperature or humidity. For a given location of interest three-dimensional forward trajectories were calculated at daily intervals over a 10 y period from 1979. In other words, for every day, starting at a specified location, a calculation was conducted which estimates the path that a particle released at that location at midnight would follow if it were blown by the wind over the following two days. For the purpose of this study we used two different starting locations in the area of interest: one from southern South America (55°S, 67.5°W) and one from the northern maritime Antarctic South Shetland Islands (62.5°S, 57.5°W).

The atmospheric winds were taken from a reconstruction of past winds available from the European Centre for Medium-Range Weather Forecasts (ECMWF). The specific version used was ERA‑40 (Uppala *et al.*, 2005) and the post-1979 period was chosen, which is known to be more reliable due to the introduction of widespread data from satellites in late 1978 (Marshall, 2003). The three-dimensional air mass trajectories were calculated from ERA-40 data using a service provided by the British Atmospheric Data Centre (BADC) (available at http://badc.nerc.ac.uk/community/trajectory/). Density maps from these trajectories show the proportion (in %) of trajectories from a given location that pass within a 200 km radius of each grid point on the map.

4.4   Results

4.4.1   Molecular sequence data

The *rps4*, *trnL-F* and *ITS* alignments were 649-650 bp, 454-462 bp and 744-777 bp in length, respectively. Variation between *Chorisodontium* species was low in all markers (including only *Chorisodontium* sequences: 9, 3 and 2 variable sites, and 3, 2 and 2 parsimony informative sites in *ITS*, *trnL-F* and *rps4*, respectively).

4.4.2   Phylogenetic and population diversity analyses

The Bayesian analyses resulted in well-supported phylogenic trees, with most ingroup (all *Chorisodontium* specimens) nodes receiving posterior probability (PP) values >0.95, and all had a minimum PP of 0.70 (Fig. 4.4a-c). Haplotype networks of each locus are shown next to each phylogenetic tree in Fig. 4.4. Both phylogenetic and haplotype analyses revealed that in the loci *trnL-F* and *ITS* (Figs 4.4b and c, respectively) *Chorisodontium* species other than *C. aciphyllum* were resolved together with *C. aciphyllum* specimens, suggesting that either very

little variation exists in these markers for these taxa, or that these specimens were initially misidentified. In the *trnL-F* phylogenetic tree specimens of the two neotropical species *C. mittenii* (AF435311) and *C. setaceum* (AF435312; this species is a likely synonym of C. wallisii (D Müll); Frahm, 1989) were identical to *C. aciphyllum*. Similarly, in the *ITS* phylogeny specimens identified as the southern South American *C. spegazzini* (Chile 00523) and *C. dicranellatum* (Chile 00509 and 00511) were resolved together with *C. aciphyllum* specimens. Alternatively, in both *trnL-F* and *ITS* phylogenies (Figs. 4.4b and c, respectively) some specimens identified as *C. aciphyllum* (Chile 00504, 11472A, 02015) were resolved as sister-species or together with other *Chorisodontium* species, suggesting these specimens were initially misidentified and represent different *Chorisodontium* species.

All phylogenetic trees revealed a large polytomy of *C. aciphyllum* specimens, with very little (*ITS*; Fig. 4.4c) or no (*rps4* and *trnL-F*; Figs. 4.4a and b, respectively) genetic variation amongst them. This polytomy included specimens from all populations and the entire geographic range of *C. aciphyllum*, and therefore revealed very little or no genetic variation within the species.

Fig. 4.4 (next page) Bayesian phylogenetic trees and haplotype networks constructed with (a) plastid loci *rps4* and (b) *trnL-F*, (c) the nuclear marker *ITS* and (d) all markers combined (including only specimens with all marker information complete) for *Chorisodontium aciphyllum*. Posterior probabilities are shown next to the relevant branches. Scale bars below the trees represent the mean number of nucleotide substitutions per site. Taxon colours refer to the different locations and/or different *Chorisodontium* species (see legend and map). Outgroup specimens in the trees are indicated in black. Numbers in brackets behind some taxa from the South Shetland Islands and the Antarctic Peninsula represent the number of replicates with identical haplotypes. In the *ITS* phylogeny (c) sample names with a and b represent different haplotypes within Elephant Island samples. Haplotype networks circle sizes correspond to the number of specimens per haplotype (see legend). Different haplotypes are indicated with roman numerals (I-V). Branches represent mutations between haplotypes, with mutations shown as black lines and indel information with double lines (see legend).

a *rps4*
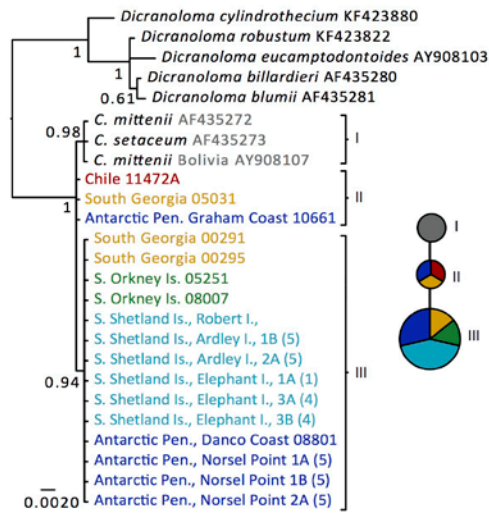
b *trnL-F*
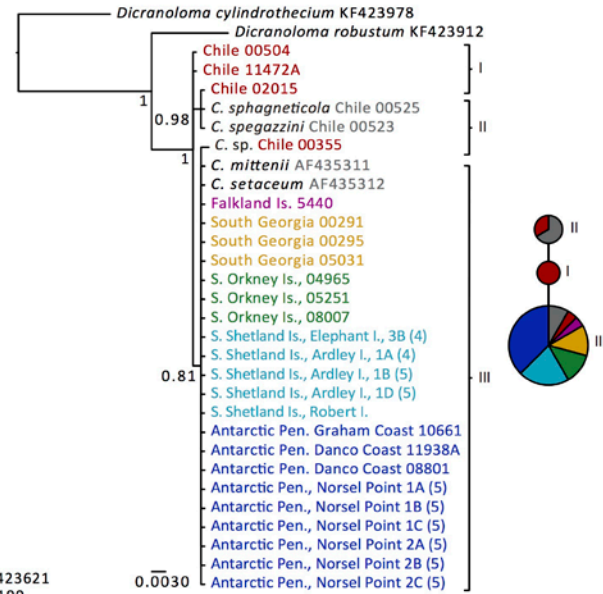
c *ITS*
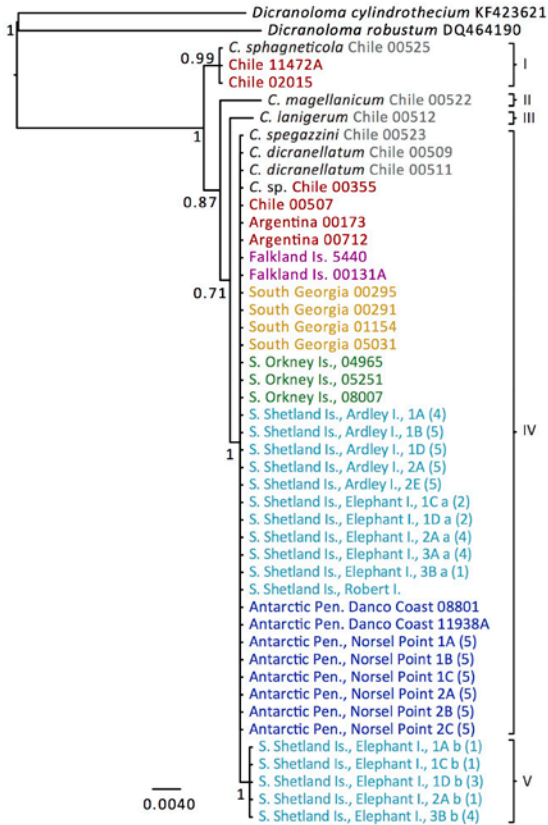
d Combined *rps4 + trnL-F + ITS*
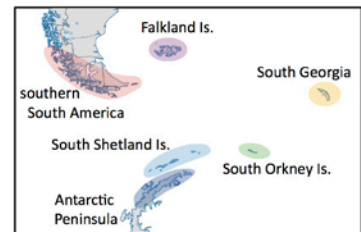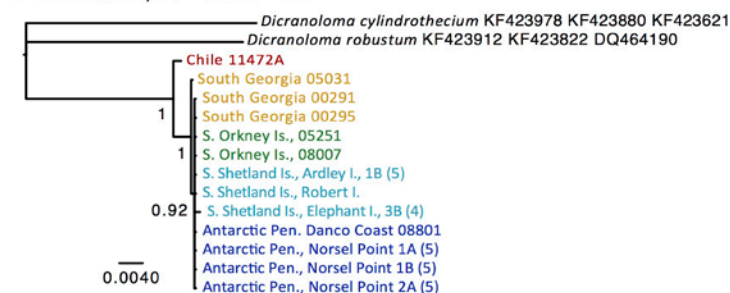
4.4.3 Within-population variation in *Chorisodontium aciphyllum*

The *ITS* marker (Fig. 4.4c) revealed within-population variation in specimens derived from Elephant Island (South Shetland Islands): sample replicates (defined by the numbers between brackets behind samples in Fig. 4.4a-c) revealed variation between specimens sampled from the same 5 cm diameter plots in locations "1C", "1D", "2A" and "3B". The variation between South Shetland Island samples included two nucleotide additions, situated in both *ITS* 1 and *ITS* 2 (for positions of the nucleotide additions in an alignment of Elephant Island samples see Fig. 4.5). The two added nucleotides were only found in Elephant Island samples, and were not present in *C. aciphyllum* specimens obtained from any other locations.

```
                                                130          144          160       460          475          490
                                                 |            |            |         |            |            |
S.Shetland Is., Elephant I., 1C a (2)   ...CCTCCAATATGGAT-GGGGGGAACTCTGCTC...  ...AATCCACTCCCAGCT-CGACTGGGAGTGCGA...
S.Shetland Is., Elephant I., 1D a (2)   ...CCTCCAATATGGAT-GGGGGGAACTCTGCTC...  ...AATCCACTCCCAGCT-CGACTGGGAGTGCGA...
S.Shetland Is., Elephant I., 2A a (4)   ...CCTCCAATATGGAT-GGGGGGAACTCTGCTC...  ...AATCCACTCCCAGCT-CGACTGGGAGTGCGA...
S.Shetland Is., Elephant I., 3A a (4)   ...CCTCCAATATGGAT-GGGGGGAACTCTGCTC...  ...AATCCACTCCCAGCT-CGACTGGGAGTGCGA...
S.Shetland Is., Elephant I., 3B a (1)   ...CCTCCAATATGGAT-GGGGGGAACTCTGCTC...  ...AATCCACTCCCAGCT-CGACTGGGAGTGCGA...
S.Shetland Is., Elephant I., 1A b (1)   ...CCTCCAATATGGATGGGGGGGAACTCTGCTC...  ...AATCCACTCCCAGCTCCGACTGGGAGTGCGA...
S.Shetland Is., Elephant I., 1C b (1)   ...CCTCCAATATGGATGGGGGGGAACTCTGCTC...  ...AATCCACTCCCAGCTCCGACTGGGAGTGCGA...
S.Shetland Is., Elephant I., 1D b (3)   ...CCTCCAATATGGATGGGGGGGAACTCTGCTC...  ...AATCCACTCCCAGCTCCGACTGGGAGTGCGA...
S.Shetland Is., Elephant I., 2A b (1)   ...CCTCCAATATGGATGGGGGGGAACTCTGCTC...  ...AATCCACTCCCAGCTCCGACTGGGAGTGCGA...
S.Shetland Is., Elephant I., 3B b (4)   ...CCTCCAATATGGATGGGGGGGAACTCTGCTC...  ...AATCCACTCCCAGCTCCGACTGGGAGTGCGA...
                                                       1                                  2
```

Fig. 4.5 Partial alignment of *ITS* showing the within-population variation in *Chorisodontium aciphyllum* populations in Elephant Island. The two variable sites between samples are situated in the *ITS* 1 (left; alignment position 144*) and in *ITS* 2 (right; alignment position 475*). Nucleotide differences are marked with number 1 and 2 below the alignment. Sample names with a and b represent samples without and with the extra nucleotide sites, respectively. *= relative position in alignment of Elephant Island specimens only.

4.4.4 Aerial dispersal modeling

Two 95%-probability distribution figures were produced that show the relative connectivity between southern South America and the northern maritime Antarctic (Figs. 4.6a, b). These revealed that, given the assumptions (see methods), small particles transported *via* regional air masses can clearly cover long distances within a 24 h period. The figures also revealed a strong asymmetry in directional probability, revealing that aerial transfer from southern South America to the northern maritime Antarctic (Fig. 4.6a) is more likely than *vice versa* (Fig. 4.6b). Both dispersal density plots show the clear influence of the westerly winds prevailing in the region, and that west-to-east transport is much more likely than east-to-west.
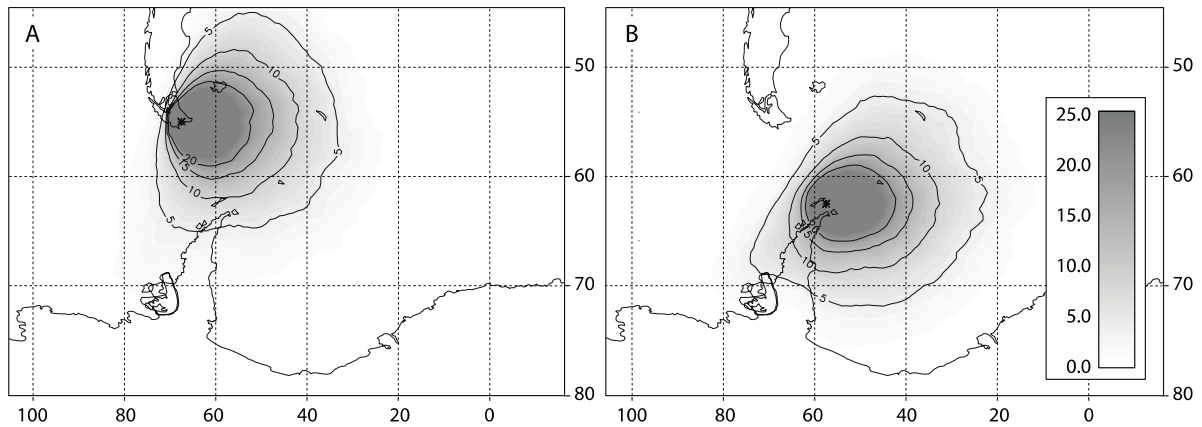
Fig. 4.6 Dispersal density spatial maps expressed as the percentage of times that an air mass from a given initial location passes within a radius of 200 km created from daily air mass movements within a 24 h period. (a) and (b) represent starting locations (shown as *) from southern South America and the northern maritime Antarctic, respectively.

4.5   Discussion

4.5.1   Timing and origin of *Chorisodontium aciphyllum* in Antarctica

Within *C. aciphyllum*, all loci revealed little or no genetic variation between specimens sampled from geographically separate locations throughout the species' natural distribution in southern South America and the Antarctic and/or sub-Antarctic. This suggests the species has been distributed across its current geographic range relatively recently. From dating analyses of peat cores the species is known to have been in the Antarctic for a minimum of ~5.5 kya, the age of the oldest sub-fossil evidence of *C. aciphyllum* in the Antarctic (Fenton & Smith, 1982; Björck *et al.*, 1991). We can therefore dismiss human dispersal as a source of the first arrival of the species in the Antarctic. Exactly how long the species has been present in the Antarctic is uncertain as, because of extremely low levels of variation, molecular dating analyses of the different populations in *C. aciphyllum* were not informative (data not shown). However, theoretically, from a predefined *ITS* substitution rate of 1.35×10^{-3} subst.$^{-1}$ site^{-1} my^{-1}, originally derived from angiosperms (Les *et al.*, 2003, and references therein) we would expect one substitution to occur every 98,241 years in a 754 bp long *ITS* sequence (the *ITS* sequence length of *C. aciphyllum* haplotype IV, Fig 4.4c; 1.35×10^{-3} subst.$^{-1}$ site^{-1} my^{-1} results in 10.179 subst.$^{-1}$ 754 sites^{-1} my^{-1}, which is one mutation every 98,241.48 years). This suggests populations in South America and the Antarctic have likely been separated between: ~100 kya and a minimum of ~5.5 kya, the age of the oldest dated *C. aciphyllum* peat core in

the Antarctic (see above). However, we acknowledge the rate used in this rough estimation does not take into account a rate standard deviation (which is not available), and that this rate might be different in bryophytes compared to angiosperms. From the genetic variation in this study it is not possible to assess the direction of spread, but the species has likely spread from South America to the maritime Antarctic and/or sub-Antarctic, as the extant distribution of sister-species of *C. aciphyllum* only occur in South America. The aerial dispersal modeling studies also suggest transfer is far more likely to have occurred in this direction than *vice versa* (Fig. 4.6).

Even though using three markers that are often variable at species and population level (particularly *ITS*; Stech & Quandt, 2010), there was no genetic variation within South American populations of *C. aciphyllum*, whereas the opposite would be expected of an 'ancestral' population. Further sampling might provide clarification on the genetic variation of *C. aciphyllum* in South American populations (many of the Chilean specimens used in this study identified as *C. aciphyllum* turned out to be misidentified and represent *C. sphagneticola*; see below). It is likely that these southern South American populations experienced a strong bottleneck throughout the LGM, when the region was extensively glaciated (Hulton *et al.*, 2002). Molecular studies on a wide range of terrestrial biota strongly suggest the existence of local refugia in Patagonia throughout the LGM and previous glaciations, rather than recolonisation from northern regions (Sersic *et al.*, 2011, and references therein). This scenario matches the still restricted distribution of *C. aciphyllum*, essentially limited to the far southern latitudes within South America.

Despite the potential in *C. aciphyllum* for regeneration from viable shoots preserved in permafrost (Roads *et al.*, 2014), and therefore a possible survival strategy for long-term persistence in the Antarctic *in situ*, the genetic evidence from this study suggests the species has not been present in the Antarctic on a hundreds of thousands to multi-million year timescale, unlike the suggested Antarctic presence of *Bryum argenteum* (Pisa *et al.*, 2014). If the oldest known bank of *C. aciphyllum* in the Antarctic (~5.500 yrs old, in Elephant I., South Shetland Is.; Björck *et al.*, 1991) represents the approximate arrival date of this species in the Antarctic, such a recent arrival would likely not have generated a strong detectable genetic differentiation, a finding confirmed by the genetic signals in our study. The moss banks on Signy Island on the South Orkney Islands are also estimated to have begun to accumulate approximately 5.59-5.49 kya (Fenton, 1982a; Smith, 1990), suggesting this was one of the earliest periods with suitable conditions for post-glacial colonization. A similar implication of recent (post-LGM) arrival of an Antarctic moss was reported by Kato *et al.* (2013), studying

the moss *Leptobryum wilsonii* (Mitt.) Broth., a species found growing uniquely in lakes of the Sôya Coast region in East Antarctica. Using the same makers as applied here (*rps4*, *trnL-F* and *ITS*) very low genetic variation (one base substitution and three to four indels) was detected between samples of *L. wilsonii* from East Antarctica and Chile, locations separated by a considerably greater distance than those separating *Chorisodontium* populations in the current study. Both Kato *et al.* (2013) and the current study provide examples of species whose genetic diversity is consistent with the widespread but generally untested assumption that Antarctic moss species are post-LGM arrivals (e.g. Peat *et al.*, 2007; Convey *et al.*, 2008; Ochyra *et al.*, 2008). However, other features of the biology of both *C. aciphyllum* and *L. wilsonii*, in particular that neither produce sporophytes in the Antarctic and/or sub-Antarctic (Ochyra *et al.*, 2008) where both rely solely on asexual reproduction, might considerably slow their rates of evolution and hence underestimate the timing of their arrival in the continent.

4.5.2   Elevated genetic variation in Elephant Island

We found evidence of local genetic variation in *C. aciphyllum* within several locations on Elephant Island (Figs. 4.4 and 4.5). Although this genetic variation was only small (two nucleotide additions in *ITS*), it revealed more variation in *ITS* between samples in Elephant Island than between samples from much more geographically divergent locations in South America and the Antarctic. This increase in genetic variation may suggest that Elephant Island, which is also the most northern island in the South Shetland Islands, might possibly have had sufficiently mild environmental conditions to have enabled sexual reproduction in the past. Elephant Island is also the location with the deepest banks of *C. aciphyllum* in the Antarctic, suggesting this is the oldest Antarctic location where the moss has been present. It is possible that Elephant Island represents a genetic 'hot spot' relative to other Antarctic locations and, if so, this may apply to other plant and animal species that occur here. The finding of genetic variation within Elephant Island also highlights the importance of sampling multiple shoots per moss clump/patch to capture the full genetic variation present in a location, a factor overlooked if sampling just single shoots alone.

4.5.3   Taxonomic patterns in *Chorisodontium*

In both *trnL-F* and *ITS* phylogenies (see Figs. 4.4b, c), several Chilean specimens identified as *C. aciphyllum* (11472A, 02015 and 00504) were genetically similar to *C. sphagneticola*, likely due to a misidentification of these specimens. Likewise, several specimens identified as

other *Chorisodontium* species were genetically identical to *C. aciphyllum*. The *ITS* region (Fig. 4.4c) of *C. dicranellatum* was genetically identical to *C. aciphyllum*. Similarly, the *trnL-F* spacer (Fig. 4.4b) of both specimens of the Neotropical *C. mittenii* and *C. setaceum* (i.e. *C. wallisii* (Frahm, 1989)) were genetically identical to *C. aciphyllum*. Frahm (1989) and Hyvönen (1991) distinguish *C. wallisii* and *C. dicranellatum* as different species, and therefore the similarity between these species in our study is likely due to misidentification. This is exemplified by the *rps4* sequences of *C. setaceum* (i.e. *C. wallisii*) and *C. mittenii*, which do differ from *C. aciphyllum* (Fig. 4.4a)*,* while *rps4* is often less divergent between species than *ITS* and *trnL*-F (Stech & Quandt, 2010). Other specimens identified as different *Chorisodontium* species revealing genetic variation relative to the *C. aciphyllum* polytomy were *C. sphagneticola* (*trnL-F* and *ITS*), *C. magellanicum* and *C. lanigerum* (*ITS*), and *C. spegazzini* (00523) (different in the *trnL-F*; no genetic variation in *ITS*), suggesting these specimens indeed represent different species. However, although Hyvönen (1991) identifies *C. sphagneticola* as synonym of *C. aciphyllum*, we find this is likely not the case and the two are distinct species. We highlight here that, while this genus has received attention from systematic morphological studies (Frahm, 1989; Hyvönen, 1991), future taxonomic work on the phylogeny of this genus requires both morphological and phylogenetic approaches, the latter including more variable and/or a larger number of loci. Studies on *Dicranum*, a closely related genus to *Chorisodontium*, also showed that a combination of several variable markers was necessary to delimit species in the genus (Lang *et al.*, 2014; 2015).

4.6   Acknowledgements

5 Genetic variation within Antarctic representatives of the moss genus *Schistidium*, with emphasis on the endemic species *Schistidium antarctici*

Fig. 5.1 A clump of *Schistidium* growing on bare rock in the South Shetland Islands. (Photo: E.M. Biersma)

This chapter is based on the following manuscript:

Biersma E.M., Jackson, J.A., Griffiths, H., Linse, K., and Convey, P. Genetic variation within Antarctic *Schistidium* mosses, with emphasis on the endemic species *Schistidium antarctici*. In preparation for submission to *PLoS One*.

5.1 Abstract

Schistidium is the most-species rich moss genus in the Antarctic, as well as the moss genus containing most Antarctic endemic species. To assess the diversity, richness and relative age divergences within this genus in the Antarctic we applied phylogenetic and molecular dating methods using the nuclear Internal Transcribed Spacer on all species (nine in total) with available samples. We additionally investigated the continent-wide genetic diversity within the most common Antarctic representative of the genus - the endemic species *Schistidium antarctici*. Most previously described Antarctic *Schistidium* species were genetically distinct, confirming their species status. However, we also found a likely overrepresentation of *Schistidium* diversity, as two or possibly three previously described species were genetically identical to other Antarctic species. We also recovered a genetically distinct clade, only represented by samples from Alexander Island (south-west Antarctic Peninsula), potentially representing a new species. All Antarctic *Schistidium* species in this study were separated from each other by ~1 Myr at minimum, revealing that several endemic species have a long history on the continent. Within *S. antarctici* we identified two distinct clades with few overlapping locations, dividing the eastern Antarctic Peninsula and Scotia Arc islands from the western Antarctic Peninsula and continental locations. This suggests that the mountainous spine on the Antarctic Peninsula forms a strong barrier to gene flow in this species. This study provides an important first step towards assessing the diversity and evolutionary history of the largest moss genus in the Antarctic. Additionally, the multi-million year presence of several endemic species contributes to studies on their adaptive potential to climate change over both past and contemporary timescales.

5.2 Introduction

Only ~0.18% of Antarctica is currently ice-free (Burton-Johnson *et al.*, 2016), of which an even smaller proportion is suitable for colonisation by plants. Most such land is located along the Antarctic Peninsula and associated islands, with smaller areas sporadically placed along the edges of the coastal regions of East Antarctica. Apart from just two species of vascular plants (the Antarctic hair grass, *Deschampsia antarctica* Desv. (Poaceae), and Antarctic pearlwort, *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae)), the Antarctic flora is predominantly composed of bryophytes (mosses and liverworts), particularly mosses (Ochyra

et al., 2008). The ability of mosses to tolerate dehydration and low temperatures makes them particularly well suited to surviving in extreme environments (Longton, 1988b).

With an estimated thirteen species (11.6% of all currently accepted Antarctic moss species), *Schistidium* (Bruch & Schimp) is thought to be the most speciose moss genus in the Antarctic (Ochyra *et al.*, 2008). It also contains roughly two-thirds of known Antarctic endemic moss species (with a total of seven endemic representatives, ~63.6% of the 11 presumed Antarctic endemics) (Table 5.1). Unlike most moss genera in the Antarctic that are often sterile, most species of *Schistidium* produce sporophytes in profusion (Ochyra *et al.*, 2008), making the genus a particularly well-adapted and possibly well-connected genus across the continent.

Despite their relative abundance and large endemic contribution to the Antarctic flora, no studies to date have focused on the phylogeny and genetic diversity within and between species of *Schistidium* in Antarctica. Indeed, globally, *Schistidium* is one of the most taxonomically neglected moss genera, with most genetic work having focused on the Northern Hemisphere, and then particularly on studies of the Russian flora (e.g. Ignatova *et al.*, 2009; Milyutina *et al.*, 2010). The genus is in urgent need of global revision, particularly in the Southern Hemisphere (Ochyra *et al.*, 2008). *Schistidium* is a very widespread and common genus worldwide, particularly in high latitude, polar regions, and cool, high altitude regions at lower latitudes. Many Southern Hemisphere regions still await taxonomic assessment and, judging from preliminary studies, it is likely that the species diversity of *Schistidium* will increase to reach similar levels to that of the Northern Hemisphere (Ochyra *et al.*, 2008, and references therein).

A particularly abundant species within the genus is the Antarctic endemic *Schistidium antarctici* (Card.) L.I.Savicz & Smirnova. It is one of the most widespread and abundant moss species in Antarctica, within the continent as well as on some maritime and sub-Antarctic islands in the South Atlantic region, including the South Shetland Islands, South Orkney Islands, South Georgia, the South Sandwich Islands and Bouvetøya (Ochyra *et al.*, 2008). In the continental Antarctic it is found in nearly all ice-free coastal regions (except for Enderby, Kemp and MacRobertson Lands) of all generally accepted sectors within the Antarctic continent (namely Maud, Enderby, Wilkes, Scott, Byrd and Ronne Sectors; Pugh & Convey, 2008). It is commonly found fruiting (i.e. with mature sporophytes) in the maritime Antarctic (Convey & Smith, 1993; Smith & Convey, 2002), however it is seldom fertile in the dryer and colder continental Antarctic, where it primarily reproduces asexually by means of protonemal gemmae (Ochyra *et al.*, 2008). An early isozyme study on this species revealed no genetic variation between populations in the Windmill Islands, in East Antarctica (Melick

et al., 1994). However, studies with much wider geographic sampling are required to increase the resolution of genetic variation amongst populations of *S. antarctici* on a continent-wide scale.

We here assessed the genetic variation between Antarctic *Schistidium* species within the nuclear Internal Transcribed Spacer (*ITS*), one of the most variable genetic markers known in bryophytes (Stech & Quandt, 2010) and which includes *ITS* 2, one of the most promising barcode markers for mosses (Hassel *et al.*, 2013). The aim of this study was threefold: i) to assess the genetic diversity across Antarctic *Schistidium* species, ii) to investigate the timing of divergences between putative Antarctic endemic and non-endemic species in order to assess their relative age on the continent; and iii) to identify patterns of dispersal, diversity and gene flow within *S. antarctici*, one of the most widespread and common plant species in the Antarctic. Additionally, by including both Southern and Northern Hemisphere specimens of *Schistidium rivulare* (Brid.) Podp. in our analyses, we performed an initial assessment of the genetic variation present between populations across the bipolar distribution of this species. The study catalyses assessment of the phylogeny and genetic variability within and between Antarctic *Schistidium* species, with importance for evaluating the biogeography of the largest plant genus in the Antarctic as well as their adaptive potential to respond to climate change.

5.3   Materials and Materials

5.3.1   Sampling and molecular methods

Herbarium and fresh samples of *Schistidium* species were sampled from most available regions in the Antarctic (see Table 8.2.4.1 in the Appendix for herbarium and location details; see Section 8.1.1-8.1.4 for in-detail laboratory techniques and protocols). All herbarium samples were obtained from the herbaria based at the British Antarctic Survey (BAS) (herbarium code AAS), the Botanic Garden, Meise (BR), and the University of Wollongong (WOLL), and were augmented by fresh collections during expeditions of the authors (EB, PC). The nine *Schistidium* species included here were: *S. falcatum* (Hook.f. & Wilson) B.Bremer, *S. lewis-smithii* Ochyra, *S. rivulare*, *S. andinum* (Mitt.) Herzog, *S. urnulaceum* (Müll.Hal.) B.G.Bell, *S. leptoneurum* Ochyra, *S. amblyophyllum* (Müll.Hal.) Ochyra & Hertel, *S. cupulare* (Müll.Hal.) Ochyra, and *S. antarctici*. We attempted to include representatives of all 13 described Antarctic *Schistidium* species, however samples of four species (*S. deceptionense* Ochyra, Bernarek-Ochyra & Smith, *S. halinae* Ochyra, *S. steerei*

Ochyra and *S. praemorsum* (Müll.Hal.) Herzog) were not available due to lack of material (see Table 5.1). Although not present in the Antarctic continent, we also included three samples of *Schistidium apocarpum* (Hedw.) B.S.G., from southern Chile, and the sub-Antarctic locations of South Georgia and Macquarie Island.

Table 5.1 Information on geographic range, endemic status and occurrence of Antarctic *Schistidium* species (information from Ochyra *et al.*, 2008), and inclusion in the current study. Endemic refers to whether the species is endemic in the sub-Antarctic or Antarctic. Geographic terms: SA = South America; SSI = South Shetland Islands; sub-A = sub-Antarctic; A = Atlantic Ocean; I = Indian Ocean; SG = South Georgia; SSW = South Sandwich Islands; SOI = South Orkney Islands; AP = Antarctic Peninsula; AC = Antarctic continent.

Schistidium species	Occurrence	Endemic	Geographic Range	Included
S. amblyophyllum	Occasional	No	SA and East African high mountains, high latitude SA, A+I sub-A, SSI, AP	Yes
S. andinum	Frequent	No	SA, A sub-A, SSI and AP as far south as Alexander Island.	Yes
S. antarctici	Common	Yes	Endemic to sub-A and AC; SG, SSW, Bouvet I., SOI, SSI, AP and in AC in all sectors except for Enderby, Kemp and Mac.Robertson Land.	Yes
S. cupulare	Very rare	No	SA, A+I sub-A, few localities in SSI.	Yes
S. deceptionense	Very rare	Yes	Only known from one locality in Deception I., SSI	No*
S. falcatum	Very rare	No	SA, A+I sub-A, few localities in SSI.	Yes
S. halinae	Occasional	Yes	Only known from SSI and north-east AP	No*
S. leptoneurum	Rare	Yes	Only known from few localities in SSI	Yes
S. lewis-smithii	Very rare	Yes	Only known from two localities in SSI	Yes
S. praemorsum	Rare	No	SA, in Antarctic only known from three localities in SSI and AP (southernmost in Danco coast)	No*
S. rivulare	Frequent	No	Bipolar with intermediate high altitude populations in tropics; in Antarctic only in SSI and northern AP	Yes
S. steerei	Very rare	Yes	Only known from two localities in SSI	No*
S. urnulaceum	Occasional	Yes	SG, SSI, AP and Marie Byrd Land	Yes

*= Due to a lack of available sample material these species were not included in the analyses of the current study

DNA extraction was performed using the DNeasy Plant Mini Kit (Qiagen GmbH, Hilden, Germany), grinding specimens using a mortar and pestle and liquid nitrogen, following manufacturer's instructions. In most cases, only one gametophyte shoot was included per sample. For amplification of *ITS* we used primer combinations ITS-A and ITS-C for *ITS* region 1, and ITS-E and ITS-B for *ITS* region 2 (Blattner, 1999). We used the Taq PCR Core Kit (Qiagen GmbH, Hilden, Germany), following manufacturer's instructions, with addition of 1 µl of Bovine Serum Albumin (BSA) in all reactions, and using an annealing temperature of 50°C. Sequencing (forward and reverse) was executed by LGC Genomics (Berlin, Germany).

5.3.2 Sequence editing and alignment

For the bipolar species, *S. rivulare*, we also included four herbarium samples from Europe, and four available *ITS* sequences from GenBank from Russia (GenBank nos. HM053934-HM053937; Ignatova *et al.*, 2009). As outgroups we included GenBank sequences of *Schistidium* species *S. sordidum* I. Hagen, *S. sinensiapocarpum* (Müll. Hal.) Ochyra and *S. pulchrum* H.H. Blom (GenBank nos. HM053942, HM053940, and HQ890521, respectively), given their basal position in the genus (Ignatova *et al.*, 2009). The sequence dataset was aligned with PRANK v.140110 (Löytynoja & Goldman, 2008), using default settings. Models of evolution were selected using Jmodeltest v2.7.1 (Darriba *et al.*, 2012) using the SPR base tree search operation, G rate variation option and AICc calculations, resulting in the model TPM1uf+G.

5.3.3 Phylogenetic relationships

The Bayesian analysis was performed in MrBayes v.3.2 (Ronquist *et al.*, 2012), running the analysis for 1.5×10^6 generations, with trees saved every 1.0×10^3 generations, and omitting the first 25% trees as burn-in. Convergence was assessed by checking that split frequencies had an average standard deviation below 0.01 and all parameters exceeded effective sample sizes (ESS) above 200 using Tracer v.1.6 (Rambaut *et al.*, 2014). A maximum clade credibility tree with median heights was visualised using Figtree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

5.3.4 Species delimitation

We used the Automatic Barcode Gap Discovery (ABGD; Puillandre *et al.*, 2012) to explore species clusters within our *ITS* dataset, using the online web server, and applying default

settings. This is an objective species delimitation approach, which uses a pairwise genetic distance-based method to find non-overlapping values of intra- and interspecific genetic distances within the sequence dataset to construct hypothetical candidate species.

5.3.5 Within-species variation in *Schistidium antarctici* and *Schistidium rivulare*

We examined the phylogeographic structure within species with a sufficient sample size (>10 samples; resulting in analyses of *S. antarctici* and *S. rivulare* only, with n=53 and n=12, respectively) by making a TCS network of the *ITS* marker using the program Popart (Leigh & Bryant, 2015), using default settings. We also calculated standard genetic diversity indices using (using Kimura 2P genetic distances; Kimura, 1980) Arlequin v3.5.1.2 (Excoffier & Lischer, 2010) within these species. Within *S. antarctici*, we investigated population structure in different regions of the maritime Antarctic (WAP: West Antarctic Peninsula, including the South Shetland Islands; NEAP: north-east Antarctic Peninsula; SOI: South Orkney Islands) by calculating F_{ST} (using haplotypes frequencies only) and Φ_{ST} (Excoffier *et al.*, 1992) (using Kimura 2P genetic distances; Kimura, 1980) in Arlequin (Excoffier & Lischer, 2010), using 10000 permutations.

5.3.6 Molecular dating

We assessed the relative divergence times within *ITS* between different Antarctic *Schistidium* species, with a particular focus on the relative divergence times between currently-recognized Antarctic endemic and non-endemic species. Divergence times were calculated in BEAST v2.4.1 (Bouckaert *et al.*, 2014). Because of a lack of fossil data suitable for our dataset, we used a lognormal clock with a nucleotide substitution rate of 4.47×10^{-3} (with 95% highest posterior density intervals (95HPD): 1.76×10^{-3}-8.34×10^{-3}), corresponding to the evolutionary rate estimated for *ITS* in Polytrichaceae mosses (Method I2a; Chapter 2). We used the same model of evolution as previously (GTR+G) and applied a coalescent tree prior, as this is both an intra- and inter-species analysis. The MCMC chains were run for a chain length of 4.0×10^7 generations, logging parameters every 10^3 generations. We assessed convergence and to ensure all parameters had ESS>200 with a burn-in of 10% in Tracer v.1.6 (Rambaut *et al.*, 2014). A maximum clade credibility tree with median node heights was constructed using Treeannotator v1.8.2 (Drummond & Rambaut, 2007) and visualized using Figtree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

5.4 Results

5.4.1 Phylogenetic analyses

Nine out of the thirteen Antarctic *Schistidium* species were sampled from most available regions in the Antarctic (see Fig. 5.2 for sample locations of specimens representing different clades of the phylogenetic analysis), with the remaining four species not having material available. The phylogenetic analysis of *ITS* (Fig. 5.3) revealed at least eight strongly-supported clades matching distinct described Antarctic *Schistidium* species (*S. antarctici*, *S. rivulare*, *S. andinum*, *S. falcatum*, *S. lewis-smithii*, *S. leptoneurum*), as well as a clade comprising samples identified as *S. amblyophyllum* and *S. cupulare*, and a new, distinct, clade not matching any previously described species. ABGD revealed a clear 'barcode gap' at P_{max}=0.0046, which included nine putative species clusters (see Fig. 5.3). The more conservative 'initial partition' of ABGD was reached at P_{max}=0.0077, with eight putative species clusters. The only difference between the two partitions was that *S. apocarpum* specimens, sister to *S. andinum*, were grouped together as one cluster (P_{max}=0.0077), forming an *S. apocarpum*/*S. andinum* complex.
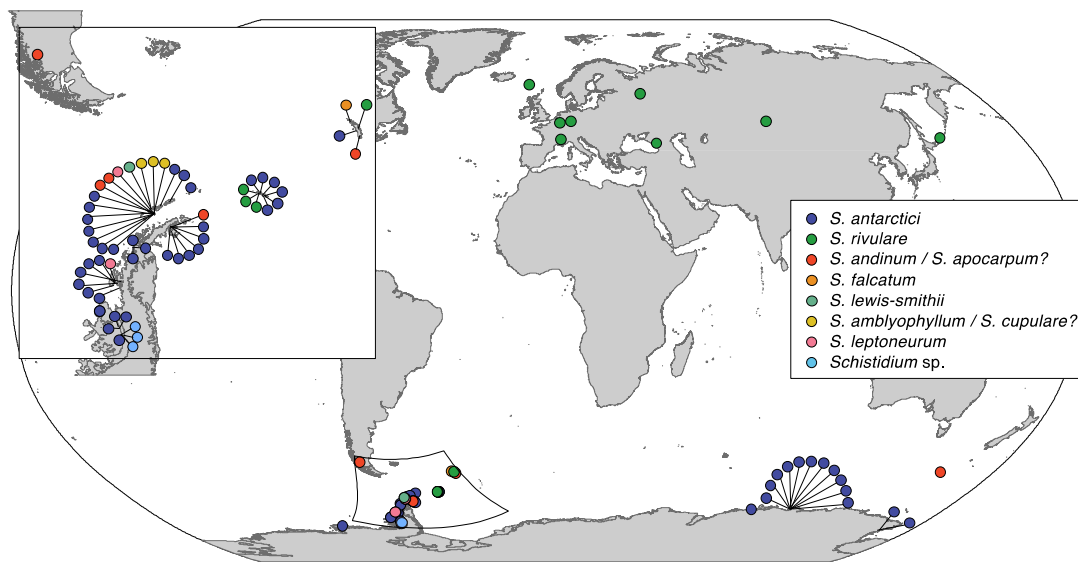
Fig. 5.2 Sampling locations of the different Antarctic *Schistidium* species as found by the phylogenetic analysis (Fig. 5.3), including an enlargement of the northern maritime Antarctic, South Georgia and southern South America. Merged *Schistidium* species clusters (as suggested by the ABGD species delimitation method P_{max}=0.00774; see Fig. 5.3) include specimens identified as *S. antarctici* and *S. urnulaceum* (shown as '*S. antarctici*' in the above figure), *S. amblyophyllum*/*S. cupulare*, and *S. andinum*/*S. apocarpum*.
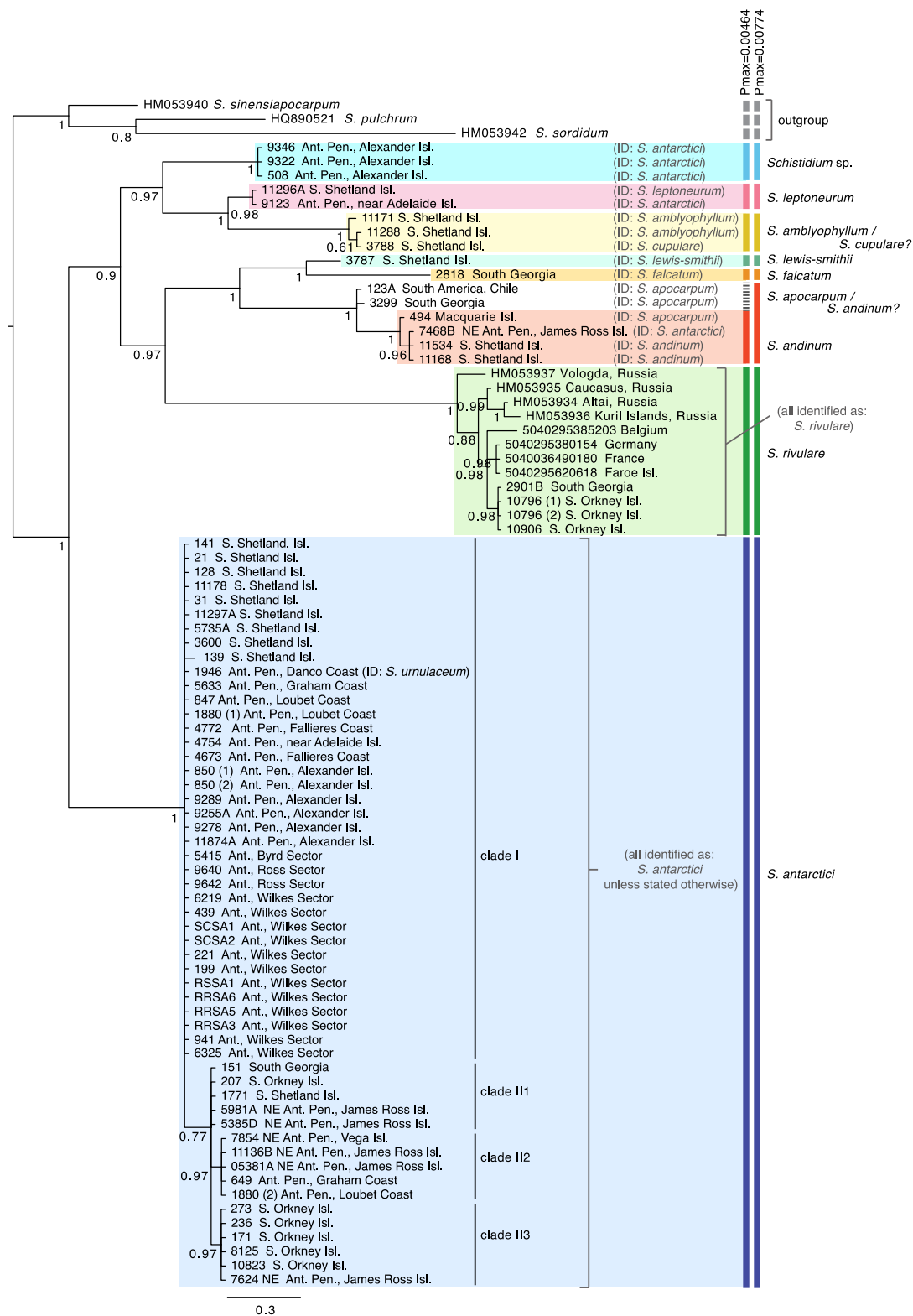
Fig. 5.3 Bayesian phylogeny for Antarctic *Schistidium* species constructed using the *ITS* marker. Posterior probability (PP) is shown below nodes. The scale bar represents the mean number of nucleotide substitutions per site. Sample identification based on morphology is given next to each sample. ABGD species clusters with different P_max-values are shown on the right.

Three samples, with herbarium identifications of *S. antarctici*, were placed in a strongly supported genetically divergent species-level clade, referred to as *Schistidium* sp. *sensu lato* (*s.l.*) hereafter. All samples comprising this cluster were obtained from Fossil Bluff on the east coast of Alexander Island, west of the southern Antarctic Peninsula (for environmental and biological description of this area see Convey & Smith, 1997).

Several samples that were the solitary representative of a particular species examined in this study were grouped into clades of other species, indicating that either i) these species were genetically identical and may belong to the same species, or ii) that these specimens were misidentified. This applied to a single sample representing *S. cupulare*, which was grouped together with two samples identified as *S. amblyophyllum*. Similarly, a single representative of *S. urnulaceum* proved to be identical to *S. antarctici*. Samples of *S. apocarpum* were paraphyletic to *S. andinum*, and a more conservative partition of ABGD (at $P_{max}=0.0077$) grouped these specimens together as one species.

5.4.2 Population genetic analyses *Schistidium antarctici* and *Schistidium rivulare*

A total of 53 samples of *S. antarctici* were sampled throughout the species' geographic range. Fig. 5.4 shows a TCS haplotype network and map of the different haplotypes within *S. antarctici*. Although the total nucleotide diversity within *S. antarctici* was low (nucleotide diversity $\pi=0.002 \pm 0.001$), five genetically- and geographically-distinct haplotypes were present within the species (Fig. 5.4). The main haplotype consisted of clade I1-2 (for samples included in this clade see Fig. 5.3), present in the west Antarctic Peninsula and associated islands (including the South Shetland Islands) and in East Antarctica, including the Byrd, Ross and Wilkes Sectors. Clade II comprised several haplotypes (II1-3; see Figs. 5.3, 5.4), which were predominantly present in the eastern Antarctic Peninsula (including James Ross I. and Vega I.) and more northern Scotia Arc archipelagoes (South Orkney Islands, South Georgia). Three samples within clade II were obtained from the western side of the Antarctic Peninsula and the South Shetland Islands (AAS herbarium nos. 649, 1880 and 1771). The haplotype network (Fig. 5.4) revealed three mutations between clades I and II, and one to two mutations between each of the clade II haplotypes (clade II1-3). The different regions in the maritime Antarctic (WAP: West Antarctic Peninsula, including the South Shetland Islands; NEAP: north-east Antarctic Peninsula; SOI: South Orkney Islands) exhibited highly significant genetic differentiation in *S. antarctici*, with all F_{ST} and Φ_{ST} values being highly significant (see Fig. 5.4B).
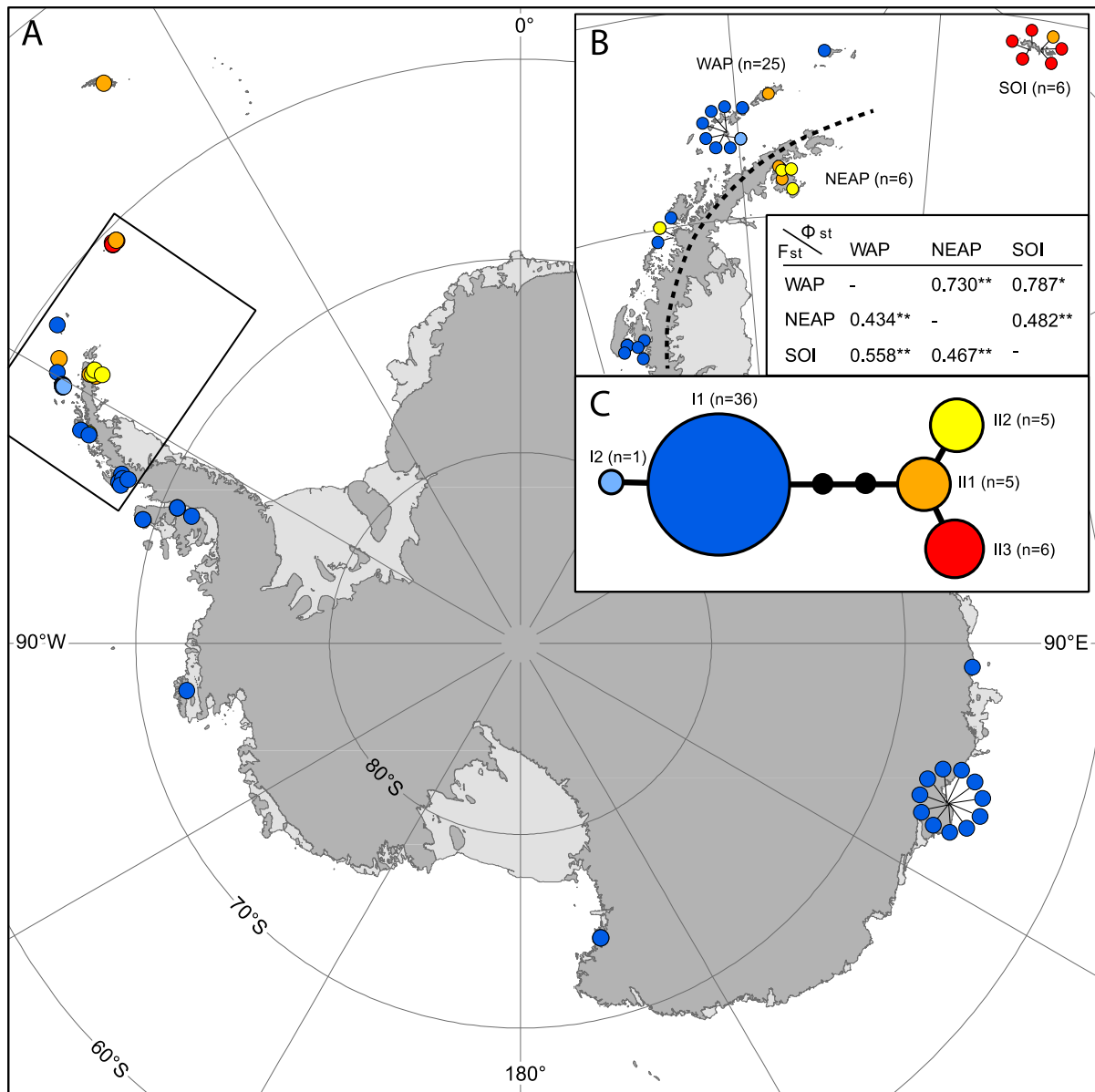
Fig. 5.4 Map showing locations of different haplotypes within *Schistidium antarctici* in the Antarctic and sub-Antarctic (a) with a more detailed map of the northern maritime Antarctic (b). The TCS haplotype network is presented in (c), including clade information (see also Fig. 1) and the number of individuals per haplotype. Section (b) also presents F_{ST} (below diagonal) and Φ_{ST} (above diagonal) of regions of the maritime Antarctic (WAP: West Antarctic Peninsula, including the South Shetland Islands; NEAP: north-east Antarctic Peninsula; SOI: South Orkney Islands). *P*-values are represented by * for *P*< 0.05, and ** for *P*< 0.01. In (a) and (b) some haplotypes are disentangled and have arrows pointing at their original location due to overlapping samples.

The twelve sequences of the bipolar *S. rivulare* revealed higher genetic variation throughout its geographic range (π=0.007 ± 0.004). The phylogenetic analysis (Fig. 5.3) placed specimens from Russia at the root of the clade, followed by more recent clades with samples of European and sub-Antarctic/Antarctic specimens, respectively. The sub-Antarctic and Antarctic specimens formed a distinct clade with high support (PP=0.98; Fig. 5.3). A TCS haplotype network (shown in Fig. 5.5) revealed high genetic variation in specimens from the Northern Hemisphere, with all Southern Hemisphere specimens represented by the same haplotype. Specimens from Eurasia were split into several branches: one with the haplotype from Vologda, Russia; another with the remaining three different haplotypes from Russia; one with a specimen from Belgium; and one with three identical haplotypes from the remaining locations in Europe. The specimens from the latter group and the specimen from the Caucasus were both most closely related to the Southern Hemisphere haplotype, each being separated by two mutational steps from the latter.
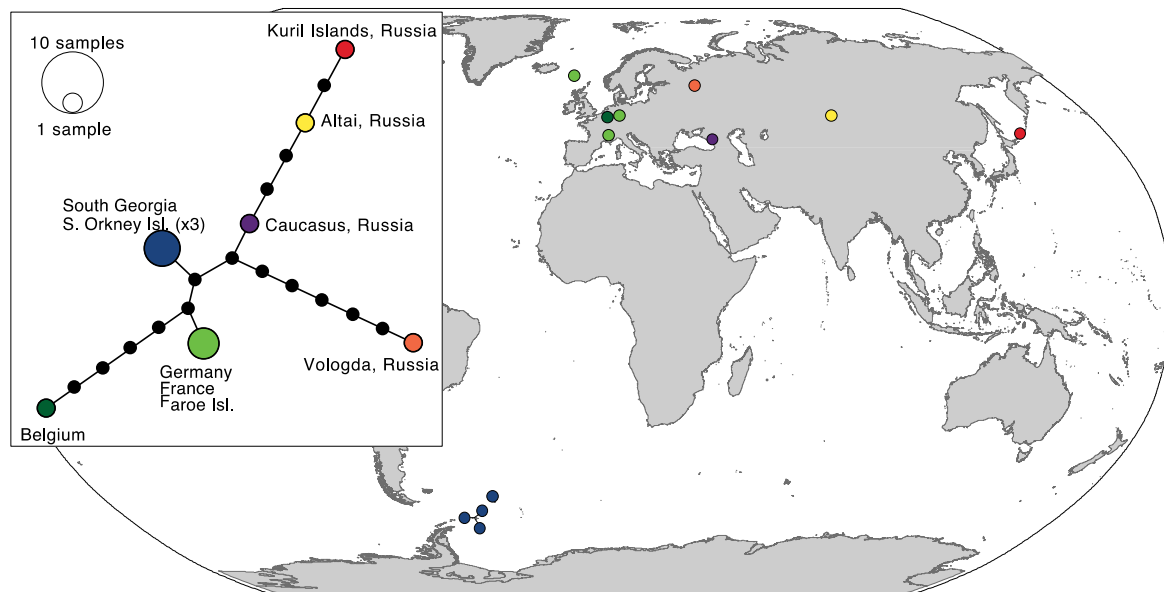
Fig 5.5 Map showing locations of different haplotypes within *Schistidium rivulare*. A TCS haplotype network is provided (see box) with corresponding colours to the haplotypes in the map. A legend with the number of individuals per haplotype is provided next to the haplotype network.
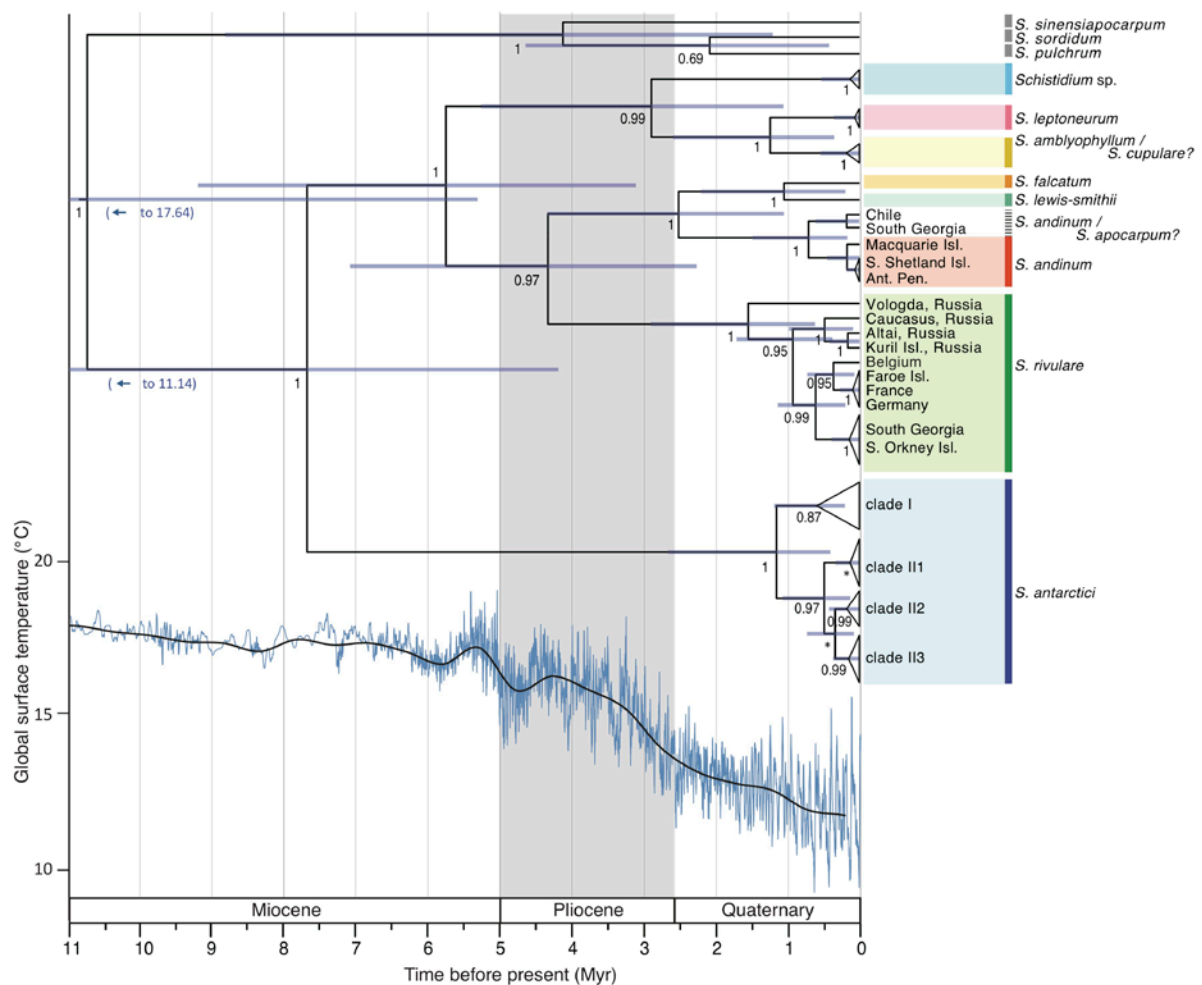
Fig 5.6 Maximum clade credibility tree presenting median divergence time estimates between and within different Antarctic *Schistidium* species as estimated from *ITS*. Posterior probability (PP) is shown below nodes, with PP<0.5 indicated as *. Node bars represent age estimate 95% height posterior distributions. The scale bar shows the mean number of nucleotide substitutions per site. Information on global surface temperature estimates reproduced from Hansen *et al.* (2013) is provided below (blue lines represent global surface temperature variations, and the black line represents a 500 kyr smoothed resolution).

5.4.3 Divergence time analysis

The divergence time analysis (Fig. 5.6) revealed multi-million year divergences between all Antarctic *Schistidium* species. The outgroup and ingroup were estimated to have diverged ~10.77 (HPD95: 20.51-5.34) Mya. The split of *S. antarctici* from other Antarctic species was estimated at about 7.71 (HPD95: 13.06-4.21) Mya. Divergences between different *S. antarctici* clades (Clades 1 and II1-3; see Figs. 5.3, 5.4, 5.6) were estimated to have occurred around 1.18 (HPD95: 2.69-0.43) Mya. Within the specimens of *S. rivulare* examined,

populations from the Northern and Southern Hemisphere were estimated to have been separated for ~0.63 (HPD95: 1.16-0.22) Mya. The endemic and rare to very rare *S. leptoneurum* and *S. lewis-smithii* were found to be very distinct and old species within the Antarctic *Schistidium* species, estimated to have diverged from their closest Antarctic relatives approximately 1.27 (HPD95: 2.61-0.37) and 1.07 (HPD95: 2.23-0.22) Mya, respectively. Similarly, *Schistidium* sp. *s.l.,* the distinct species-level clade consisting of specimens from Alexander Island, was estimated to have diverged from its nearest Antarctic relative approximately 2.92 (HPD95: 5.30-1.08) Mya. All Antarctic species were found to have diverged throughout the end of the Miocene (split between *S. antarctici* and the remaining species) to the Pliocene and Quaternary, a time when the global climate started to cool (see Fig. 5.6).

5.5   Discussion

5.5.1   Phylogenetic analyses

This is the first molecular phylogenetic study of Antarctic *Schistidium* mosses, the most species-rich moss genus in the Antarctic. Our data confirmed the validity of at least six of the 13 currently recognized Antarctic species (*S. antarctici, S. rivulare, S. andinum, S. falcatum, S. lewis-smithii, S. leptoneurum*). Further analyses with increased sample sizes and other loci are required to confirm the status of *S. urnulaceum* and *S. cupulare*, which were not distinguished from *S. antarctici* and *S. amblyophyllum* with *ITS*, respectively. The high number of endemic species within the genus (relative to other Antarctic moss genera) is of particular interest. Our data show that the endemic species *S. antarctici*, *S. lewis-smithii* and *S. leptoneurum* form genetically distinct clades within the Antarctic *Schistidium* species included in this study. However, our initial results suggest that the endemic *S. urnulaceum* may not be a distinct species, but possibly represents a phenotypic variant of *S. antarctici*.

We found a new and previously undetected species-level clade, *Schistidium* sp. *s.l.*, comprising samples from Fossil Bluff, Alexander Island. This finding complements several classical studies that point to very distinct elements of the fauna of this island, and in particular the ice free areas of the geological region of the south-east of the island (e.g. Greenslade, 1995; Convey & Smith, 1997; Maslen & Convey, 2006). Further morphological and molecular studies will be required to assess the status both of this clade and of the four Antarctic *Schistidium* species not included in this study through lack of available material (*S. deceptionense, S. halinae, S. steerei, S. praemorsum*).

Several Southern Hemisphere samples of the global *S. apocarpum* were closely related to *S. andinum*. *S. apocarpum* is regarded as a problematic taxon due to its phenotypic variability (Ochyra *et al.*, 2008), which has led to the grouping together (e.g. Bremer, 1980b, a) and subsequent differentiation (e.g. Blom, 1996) of distinct species within an *S. apocarpum* complex. With the currently limited availability of taxonomic studies of the genus *Schistidium*, particularly in the Southern Hemisphere, it is possible that *S. apocarpum* specimens in South America need revision and represent a different species. Consequently, specimens included in the entire *S. apocarpum/S. andinum* clade (n=6; as suggested by ABGD P$_{max}$= 0.00774; Fig. 5.3), may in fact represent *S. andinum*, or, *vice versa*, *S. apocarpum*. In the latter case the geographic range of *S. apocarpum* would include the S. Shetland Islands and Antarctic Peninsula. Increased sampling will be necessary to resolve the correct taxonomy of this clade.

5.5.2   Long-term Antarctic persistence of several *Schistidium* species

Our data confirmed the presence of several distinct and old (~1 Mya or older) Antarctic endemic species within *Schistidium* (namely *S. antarctici*, *S. leptoneurum* and *S. lewis-smithii),* supporting a long (certainly well before LGM) persistence of some of these species on the continent. This complements the recently-recognised and recurring pattern of long-term (pre-LGM) Antarctic presence across a range of terrestrial Antarctic biota, suggested from both molecular and classical biogeographic studies of all major extant faunal, floral and even microbial groups (Stevens & Hogg, 2003; Convey & Stevens, 2007; Convey *et al.*, 2008, 2009a; De Wever *et al.*, 2009; Vyverman *et al.*, 2010; Fraser *et al.*, 2014; Pisa *et al.*, 2014; Chong *et al.*, 2015; Iakovenko *et al.*, 2015; Bennett *et al.*, 2016). These findings combine to overturn a long-held but largely untested view that all Antarctic terrestrial life is of recent, post-LGM origin, derived from previous glaciological reconstructions suggesting extensive ice-sheets covered nearly all terrestrial areas and extended far onto the Antarctic continental shelf throughout the LGM and previous glaciations. In part, this divergence of interpretation across different disciplines has been driven by a lack of spatial resolution in earlier glaciological models. However, recent modelling studies reconstructing Antarctica's past climate have proposed considerably greater variations in Antarctica's ice sheets throughout the Pliocene and Quaternary than previously thought (Pollard & DeConto, 2009; DeConto & Pollard, 2016). Although at present precise locations of glacial refugia, where terrestrial life may have persisted *in situ*, remain unknown (Pugh & Convey, 2008; Convey *et al.*, 2009a), the biological evidence requiring such refugia, and at various regional scales, is

increasingly clear (Convey *et al.*, 2008). Our results here suggest the presence of a refugial area in the northern Antarctic Peninsula/South Shetland Islands region of maritime Antarctic, where the diversity of *S. antarctici* is highest. A separate study of the Antarctic Peninsula/South Shetland Islands endemic fly, *Belgica antarctica*, implies a similar conclusion (Allegrucci *et al.*, 2006). Most recently, Carapelli *et al.* (in press) report evidence in three springtail (Collembola) species native to the same region of persistence *in situ* on parts of the South Shetland Islands dating from at least the last interglacial (c. 150 ky, two species), or the previous (c. 500 ky, one species). The age (>2 Mya) of the endemic mosses *S. leptoneurum* and *S. lewis-smithii* documented here, whose geographic range is currently restricted to the South Shetland Islands, provides further support for a regional refugial area to have been present in this archipelago.

Our results suggest the ancestors of the sub-Antarctic and Antarctic populations of *S. rivulare* dispersed from the Northern Hemisphere to the sub-Antarctic and Antarctic. As Northern and Southern Hemisphere populations were estimated to have been separated for ~0.63 (HPD95: 1.16-0.22) Mya, sub-Antarctic and Antarctic populations may have been present at their current locations before the Last Glacial Maximum (LGM; ~20-18 kya). However, more extensive sampling will be required to confirm this, or whether sub-Antarctic and Antarctic populations were derived from other Northern or Southern Hemisphere locations post-LGM.

5.5.3   Diversity patterns within *Schistidium antarctici* and conservation implications

Our data provide valuable indications of where and when the Antarctic endemic species *S. antarctici* must have persisted through repeated glacial periods, and of patterns of dispersal and gene flow across the continent. We found significant genetic differentiation in the northern maritime Antarctic and sub-Antarctic, dividing the regions east of the mountainous spine of the Antarctic Peninsula (eastern Antarctic Peninsula), the South Orkney Islands, and South Georgia from regions on the west of the Peninsula and the rest of the continent (Fig. 5.4B). This reveals that connectivity between the Antarctic Peninsula and Wilkes Land might be stronger than between the two regions on either side of the spine of the Antarctic Peninsula. It also indicates that *S. antarctici* populations in the majority of the sectors of the Antarctic continent are genetically very similar and appear to have been derived from only one clade (clade I). The highest genetic variation was found in the northern Antarctic Peninsula region, suggesting this is likely a region where the species survived the LGM *in situ*. Clades I and II (see Figs. 5.3, 5.4, 5.6) were estimated to have diverged around 1.18

(HPD95: 2.69-0.43) Mya (Fig. 5.6), revealing *S. antarctici* to be an enduring and old species on the continent, originating and persisting there on at least a million-year timescale.

Implications of a pattern of distinct genotypes east of the mountainous spine of the Antarctic Peninsula, are also seen in other taxa including rotifers (Iakovenko *et al.*, 2015) and diatoms (Kociolek *et al.*, in press), possibly providing evidence supporting distinct bioregions on either side. The north-east and north-west Antarctic Peninsula have also been differentiated as distinct Antarctic Conservation Biogeographic Regions (ACBRs), based on multivariate analyses of regional biodiversity patterns (Terauds *et al.*, 2012; Terauds & Lee, 2016). However, at present, the north-east Antarctic Peninsula (ACBR1) is much less well protected than the north-west Antarctic Peninsula (ACBR3). While the latter has 21 Antarctic Specially Protected Areas (ASPAs), covering 1.99% of the region, the eastern side has just one ASPA, covering only 0.03% of the region, Furthermore, no ASPAs in the north-east Antarctic Peninsula have been declared for the purposes of protecting biodiversity (compared to 17 in the north-west Antarctic Peninsula), even though it is the second most visited ACBR by tourists in the Antarctic (Terauds & Lee, 2016). Such observations highlight the conclusion of Hughes *et al.* (2016) about the overall shortage of protection afforded to vegetation in the ASPA system. Given the growing evidence that this area supports unique lineages of multiple terrestrial species we suggest that priority is required towards area protection within the north-east Antarctic Peninsula ACBR1 region.

5.6   Acknowledgements

6   Discussion and concluding remarks

6.1   Introduction

The contemporary terrestrial Antarctic flora and fauna, with their apparent low diversity and species richness, have long been considered as recent post-glacial arrivals on Antarctica (see Section 1.1.1). This is supported by past climate reconstructions suggesting that during past Pliocene and Pleistocene glaciations, including the Last Glacial Maximum (LGM; 20-18 kya), ice sheets were much thicker and more extensive than they are today, and covered most currently ice-free land (with the exception of a few extremely harsh habitats such as nunataks; see Section 1.1.2.1). In contrast to this, biological research over recent decades, particularly after the onset of application of molecular tools, has revealed abundant evidence of the cryptic and ancient nature of all major terrestrial faunal groups, suggesting an *in situ* persistence on hundred thousand to multi-million year timescales. A clear picture is now emerging of the long-term history from most faunal, as well as algal and microbial groups (see Section 1.1.1). However, molecular research focussing on the evolutionary history of the flora of Antarctica have been sparse to date, and in most cases inadequate to reveal its origin and age (see Section 1.1.3). An increased understanding of the origin and age of Antarctica's dominant flora - the mosses - is therefore vital for enhancing our understanding of the general Antarctic biogeography, as well as of cross-disciplinary relevance by informing general regional constraints on climate models. Additionally, such findings are providing important insights into the general survival capacities of mosses through extreme conditions.

In this thesis, several large-scale molecular studies are presented, addressing the genetic variation, phylogeography and estimated date of arrival in the Antarctic of various common and/or widespread moss species typical of the region. With these studies a clearer picture of the evolutionary history of the Antarctic bryoflora is emerging, including not only the timing of arrival in Antarctica within various moss lineages, but also of their geographic origin, frequency of dispersal events, and in some cases, a first basic phylogeny of various moss lineages. The results also provide evidence for various elements that aid or prevent dispersal or gene flow in the Antarctic, and for increased levels of genetic variation in particular regions relative to other regions in the Antarctic.

6.2   Timing of arrival within Antarctic moss species

Fig. 6.1 provides an overview of the long-term persistence of several of the moss species and populations investigated in this study. The results show a varying origin and age of different species, with evidence of long-term persistence in some, and shorter-term histories in others. This suggests that of the three hypotheses (listed in Section 1.2) the third hypothesis, which is a combination of recent as well as long-term Antarctic presence, is the most likely scenario for the Antarctic bryoflora.

Genetic results support the multi-million-year presence of the endemic *Schistidium antarctici* (Chapter 5), estimated to have diverged from all other investigated *Schistidium* species in the late Miocene. The species was genetically very distinct from other Antarctic *Schistidium* species, and is currently only known from the Antarctic and sub-Antarctic. Population divergences within this species are estimated to have occurred in the mid-Quaternary, revealing that even intraspecific divergences are estimated to have occurred on pre-LGM timescales. Other evidence of long-term persistence can be found in several other Antarctic endemic *Schistidium* species: *S. leptoneurum*, *S. lewis-smithii*, and *Schistidium* sp., a newly discovered lineage with specimens only obtained from Fossil Bluff, Alexander Island (although this lineage may also represent an existing *Schistidium* species not sampled in this thesis, though none of these species are known to occur on Alexander Island). These endemic *Schistidium* species were found to be genetically very distinct and are estimated to have diverged from other species in the early to mid Quaternary. The species *S. antarctici*, *S. leptoneurum* and *S. lewis-smithii* are commonly found with sporophytes in the Antarctic (Ochyra *et al.*, 2008), suggesting they are well adapted to the harsh climate. Other *Schistidium* species with ranges outside the Antarctic (e.g. *S. rivulare*) will need future study with more sampling and possibly more variable markers to more precisely estimate the duration of their presence in the Antarctic.

At least one lineage within *Ceratodon purpureus s.l.* (clade I; Chapter 3) has a likely long-term presence in the Antarctic, with dating analyses suggesting the clade split from its most closely related clade (tropical clade II) in the Pliocene. Although this lineage consisted of only three specimens from the maritime Antarctic, no other samples in the large-scale global dataset were assigned to this lineage, suggesting that, before such evidence is found, the lineage is likely restricted to the northern maritime Antarctic. The other Antarctic *Ceratodon purpureus s.l.* clades (bipolar clade V and Holantarctic clade VI) both had geographic ranges including other regions and therefore are likely of more recent origin than clade I, although

future research with more variable markers (particularly organellar markers; see Chapter 3 for issues concerning nuclear markers in *C. purpureus s.l.*) will be necessary to assess the extent of their presence in Antarctica.

Within the Polytrichaceae we found evidence that the bipolar species *Polytrichum juniperinum, Polytrichum piliferum* and *Polytrichastrum alpinum* all arrived in the Antarctic region (which includes Antarctic, sub-Antarctic and/or southern South America) well before the LGM. However, no strong difference was found between populations north and south of the Drake Passage, and the duration that these species have been present in the Antarctic will requires further investigation. Nonetheless, the long-term presence of these species in this southern region is still suggestive of *in situ* survival through glacial refugia in either southern South America or the Antarctic, sub-Antarctic, as all regions of this southern range are thought to have experienced extensive glaciations throughout the LGM and previous glacial cycles. As mentioned in Chapter 2, this finding supports the building body of biological support for the existence of glacial refugia in both southern South America (Sersic *et al.*, 2011), and the Antarctic (Convey *et al.*, 2008, 2009a; Pugh & Convey, 2008). Additionally, in *P. juniperinum,* the high genetic diversity within Antarctic populations is striking, with representatives of various diverse sub-clades of different origin currently occurring sympatrically. It is possible that the lack of sexual reproduction in this region might help support the continued existence of these adjacent different lineages.

Contrasting to these examples of likely long-term presence, was the finding of very low genetic variation in *Chorisodontium aciphyllum* (Chapter 4), which showed no genetic difference between southern South America and the Antarctic. It is clear from ages estimated from peat banks that the species has been present in the maritime Antarctic for at least 5,500 y (Björck *et al.*, 1991), and deeper cores may potentially be older. Nevertheless, the lack of variation in *ITS,* one of the most variable markers known in mosses (Stech & Quandt, 2010), suggests that Antarctic populations are likely not older than ~100 ky (see Chapter 4), and potentially much younger, including the possibility of post-LGM arrival.

Other recent phylogeographic studies (as described in Section 1.1.3.4) also show a combination of short- and long-term presence in Antarctic mosses: while the aquatic moss *Leptobryum wilsonii* growing in lakes in East Antarctica was found to be of likely recent origin (Kato *et al.*, 2013), the cosmopolitan moss *Bryum argenteum* reveals a multi-million year persistence (Pisa *et al.*, 2014). From the growing body of evidence arising from different case studies it can be concluded that the evolutionary history of the Antarctic bryoflora consists of a mixture of long-term survivors and recent arrivals.
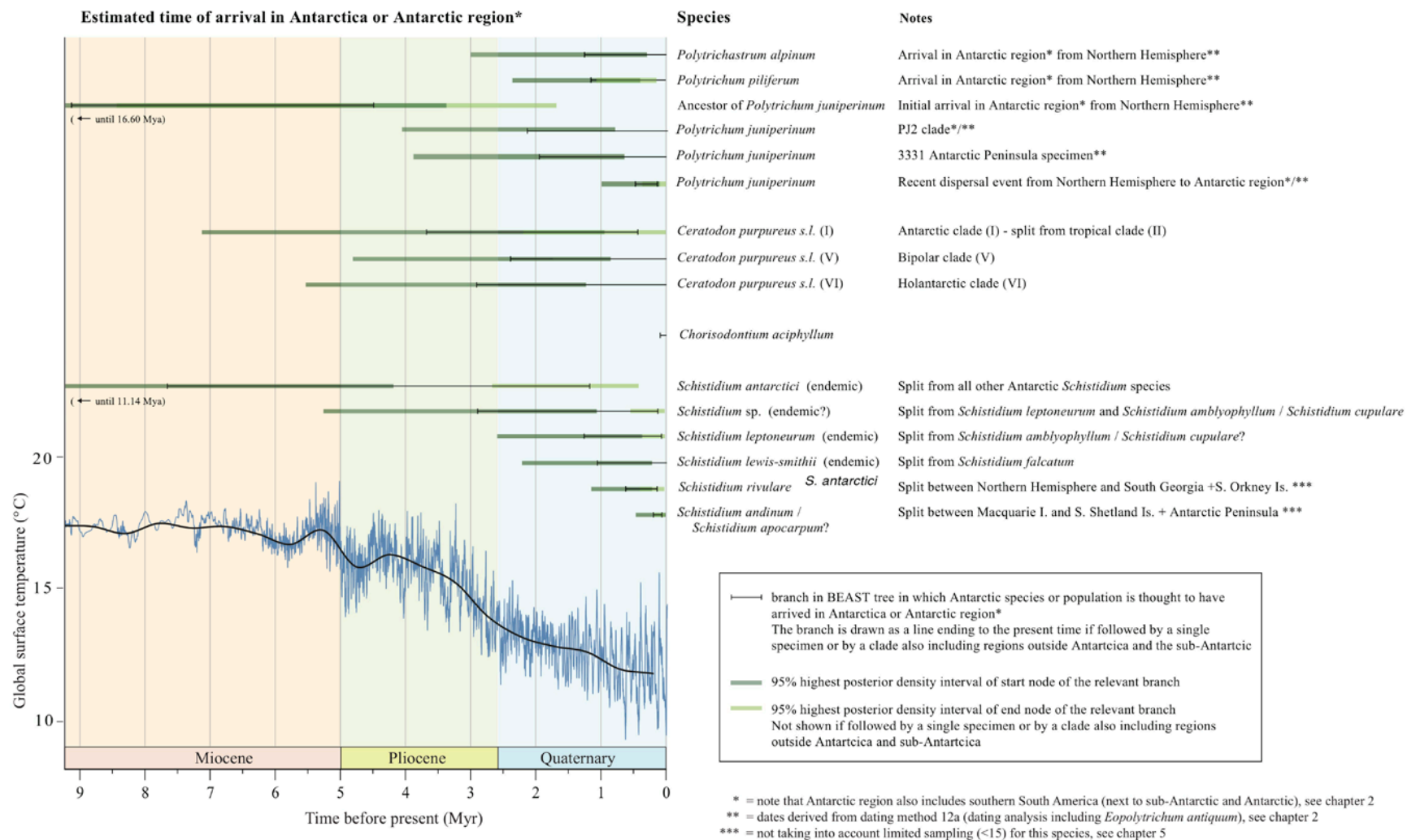
Fig. 6.1 Estimated timing of arrival of moss populations in Antarctica or Antarctic region as described in Chapter 2-5. See legend for figure details.

6.3 Conservative estimation of Antarctic arrival

It is likely that the outcomes of molecular dating analyses in the different studies (Chapters 2-5) represent underestimates of Antarctic persistence rather than overestimates. This is because, due to the lack of suitable fossils in most studies, as well as a lack of data on molecular substitution rates in mosses, many of the molecular dating analyses in this thesis relied on previously defined molecular rates based on angiosperm studies, an approach often used in molecular dating analyses on bryophytes (for examples see Villarreal & Renner, 2014). However, angiosperms and bryophytes are very distantly related and the latter are known to evolve at a much slower rate (Stenøien, 2008; Laenen *et al.*, 2014), with net species diversification rates estimated to be ~30% of those described for angiosperms (Laenen *et al.*, 2014). This implies that the molecular dating analyses in this thesis may considerably underestimate the timing of arrival of the different moss species in the Antarctic.

Additionally, even within moss divergences, mosses in the Antarctic may be expected to evolve at a slower rate than mosses elsewhere. This is due to the low rates or even lack of sexual reproduction in many species (Convey & Smith, 1993; Ochyra *et al.*, 2008) and the slow growth rates and long generation times (with single moss shoots within a *Ceratodon purpureus* colony >40 years old; Clarke et al. 2012). Both factors contribute to reducing the amount of genetic variation that is likely to accumulate through time in Antarctic mosses compared to mosses growing in less harsh climates. It is therefore likely that in dating analyses where populations of both Antarctic and lower latitude are included (thereby excluding Antarctic endemic species), Antarctic populations may be considerably older than estimated.

6.4 Possibilities for *in situ* glacial survival

Two studies in this thesis point towards increased genetic variation in the northern maritime Antarctic region relative to other regions in the Antarctic (*Schistidium antarctici*, Chapter 5; *Chorisodontium aciphyllum* in the South Shetland Islands, Chapter 4). This region also hosts the highest diversity of extant floral and faunal species (Peat *et al.*, 2007; Ochyra *et al.*, 2008). These patterns suggest the northern maritime Antarctic may host a possible refugial area, where species survived throughout the LGM and previous glacial maxima. Although confirmed locations at present are unknown (Pugh & Convey, 2008; Convey *et al.*, 2009a) the growing body of biological evidence clearly requires the presence of refugia, indeed

multiple refugia at a regional scale, to allow for the current regional diversity and endemicity patterns in the Antarctic (Convey *et al.*, 2008). The patterns found in this thesis suggest the northern maritime Antarctic may have hosted at least one such location.

Many of the study species in this thesis (*Ceratodon purpureus s.l.*, *Polytrichastrum alpinum*, *Polytrichum* and *Schistidium* species) are frequently found in fellfield communities dominated by cushion mosses and fruticose lichens, and the latter also include many endemic species (Øvstedal & Smith, 2001). Given the high endemicity of species in these communities, in both the lichens and the now-confirmed long persistence of several mosses (whether this applies to a species, e.g. *S. antarctici*, or a particular species clade, as in *C. purpureus s.l.*), it is likely that an element of such fellfield communities has survived the LGM. However, beyond these communities there may have been possibilities for survival. *Ceratodon purpureus*, *S. antarctici* and *Bryum argenteum* can often be found growing on dry and exposed sites and in rock crevices, and, though more abundant at lower altitudes at <200 m a.s.l. (Ochyra *et al.*, 2008), are also found at high altitude locations (ranging up to 1379 m; Ochyra et al. 2008). These species could therefore be possible candidates for nunatak survival. Additionally, *P. juniperinum*, *P. alpinum*, *C. purpureus* and *B. argenteum* are frequently found growing near geothermally-influenced areas in the Antarctic and sub-Antarctic (Convey *et al.*, 2000; Convey & Smith, 2006; Ochyra *et al.*, 2008), areas which may have acted as regional refugia (Fraser *et al.*, 2014).

6.5   Previously hidden diversity in Antarctic bryophytes

The different case studies revealed various examples of cryptic species and multiple colonisations to the Antarctic. For example, Antarctic populations of *Polytrichum juniperinum* (Chapter 2) and *Ceratodon purpureus s.l.* (Chapter 3) were found to be derived from two to three separate colonisation events, and may possibly even represent different species (see ABGD analyses in the respective chapters). Three separate colonisation events were also found in *Bryum argenteum* (Pisa *et al.*, 2014). In addition to these cases of high population diversity we also found a possible new and previously undetected species-level lineage within *Schistidium*, *Schistidium* sp. *s.l.*, identified in several samples from Fossil Bluff, Alexander Island (see also Chapter 5 and Section 6.1).

In contrast, several previously described *Schistidium* species were found to be identical or very similar to other species genetically. *Schistidium urnulaceum* and *S. cupulare* were found to be undistinguishable (although only based on the sequences used here) from *S. antarctici*

and *S. amblyophyllum*, respectively (Chapter 5). Additionally, populations of *S. apocarpum* and *S. andinum* included in Chapter 5 were found to be very similar and likely represent the same species. Similarly, many species within the genus *Chorisodontium* were found to be genetically similar (Chapter 4).

This work provides a starting point for new morphological studies. They also confirm the notion that relying solely on morphological descriptions to elucidate patterns of diversity, endemism and timing of arrival in bryophytes can be misleading, due to high levels of phenotypic plasticity, and lack of expression or reduction of informative phenotypic features in cold and dry polar environments.

6.6 Geographic features influencing bryophyte biogeography, and implications for other taxa

The findings presented in this thesis provide evidence for geographic features that enable (e.g. westerly winds circulating Antarctica; Chapters 2, 3) and limit (e.g. the Antarctic Peninsula mountain range; Chapter 5) the connectivity of bryophytes in Antarctica. In the phylogeographic studies on a global scale (e.g. Chapters 2, 3), evidence was found for global atmospheric circulation patterns to considerably influence bryophyte biogeography, with ancient biogeographic structuring following particular hemispheres or latitudinal 'bands' (Chapters 2, 3) and the relatively rare occurrences of successful inter-hemispheric movements (these occurring on million year timescales; Chapter 2).

Such regional and global features may be of great relevance to the biogeography of other widespread microscopic or spore-dispersed organisms. However, the practical aspects of studying bioegeographic patterns are extremely difficult for many microscopic organisms, highlighting the unique advantage of studies on spore-dispersed plants such as mosses: as the dispersal of both mosses and microscopic organisms is generally governed by similar physical laws (acting on the micro-sized spore phase; although spore sizes may differ), the (macro-sized) bryophyte gametophyte phase offers practical sampling of a visible, distinguishable organism throughout its global range.

Evidence for endemism and substantial cryptic diversity is now also increasingly emerging in many microbial groups (see Vyverman *et al.*, 2010 and references therein), including rotifers (Iakovenko *et al.*, 2015), green algae (De Wever *et al.*, 2009), diatoms (Kociolek *et al.*, in press) and lichens/fungi (Green *et al.*, 2011). Yet, many of these microbial groups still await detailed genetic studies to estimate their population structure and persistence on the

continent, including studies that focus on only one species or genus, and with extensive sampling of populations and molecular dating analyses.

6.7   Future directions

A major issue that needs resolving is the identification of possible glacial refugial locations. This may be achieved by more fine-scale genetic studies on more taxa, however answering this question also requires integration with new studies in paleontology, glaciology and glacial modeling. The apparent disagreement between current understanding of biological and glacial history of Antarctica (see Section 1.1), which was identified over a decade ago, is in urgent need of a resolution compatible with both biological and glaciological reconstructions. This issue is of great and timely relevance - also beyond the Antarctic - for improving past climate reconstructions, which in turn is required to inform future climate scenarios.

Several related studies could provide important advances in the understanding of the evolutionary history of the Antarctic flora. First, increased understanding of the persistence of the two vascular plant species in Antarctica, *Deschampsia antarctica* and *Colobanthus quitensis*, is required. Additionally, more phylogeographic studies on Antarctic mosses would provide a more solid baseline for the evolutionary history of the Antarctic bryoflora. Of particular interest would be particular moss species with a strong presence in the continental Antarctic. Suitable target species for such studies could include *Syntrichia sarconeurum* Ochyra & R.H. Zander, a very widespread Antarctic endemic species, and *Bryum pseudotriquetrum*, a very common species in Antarctica which also has a bipolar distribution and is found in a multitude of different environments, including lakes (see Section 1.1.2.4). Additionally, to obtain a more comprehensive picture of bryophyte evolution in Antarctica more studies are needed on pleurocarpous mosses (e.g. *Sanionia uncinata* in Hedenäs, 2012), as well as liverworts, the other major bryophyte lineage present (albeit rarer) in the Antarctic. Within moss phytogeography studies, it is clear that there is an urgent need for improved estimates of molecular substitution rates, the use of more variable markers (particularly for population studies), and tools to make more use of herbarium specimens, also including powerful methods such as Next Generation Sequencing (e.g. population genetic approaches based on Restriction site Associated DNA (RAD) markers or exon-capture). Within Antarctic phytogeographic studies, increased sampling from locations outside the maritime Antarctic, Victoria Land and Wilkes Land are essential for understanding how species are distributed

throughout the continent, in particular the many locations that have never been sampled before. Studies using more variable markers and increased sampling will be required to confirm whether Antarctic bryophyte populations demonstrate distinct biogeographic regionalisation in line with the clear general regionalisation of Antarctic biodiversity patterns (as shown by the differentiation of 16 Antarctic Conservation Biogeographic Regions (ACBR's); Terauds *et al.*, 2012; Terauds & Lee, 2016). This information will also be necessary to better inform appropriate protection measures for the Antarctic flora.

The data and analyses presented within this thesis show that a significant element of the Antarctic bryoflora has a long-term persistence on the continent (see Section 6.2), revealing the great resilience of bryophytes to polar stresses throughout glacial periods. This finding is of importance to future physiology studies on mosses in extreme conditions. On a different note, the long-term Antarctic persistence of mosses also implies these mosses must also have coped with past warmer-than-today interglacial periods, of relevance to their capacity to adapt to future climate change. Although this may be expected for species with ranges outside the Antarctic, it is an important observation for Antarctic endemic species. It should, however, be noted that contemporary rates of warming are estimated to be much more rapid than they have been previously (Vaughan *et al.*, 2003; Turner *et al.*, 2009), and the Antarctic flora also faces further stresses such as changes in UV-B exposure maxima associated with the anthropogenic spring ozone hole (Rozema, 1999). Possibly more importantly, competition or ecosystem alteration from the increasing numbers of non-native species recorded in Antarctica (Convey, 2003), is likely to become an increasing influence on bryophyte populations in some regions of the Antarctic. Nevertheless, the data presented in this thesis illustrate the considerable resilience and extreme survival capabilities of bryophytes in polar environments.

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8 Appendices

8.1 Laboratory protocols and analyses

8.1.1 Sampling

The majority of sequence data were generated using dried bryophyte specimens obtained from various herbaria. For the specific herbaria sampled, see the Methods section in each chapter. The main herbaria sampled in my work were the United Kingdom National Antarctic Herbarium based at BAS (herbarium code AAS) and the Herbarium of the National Botanic Garden of Belgium (BR) in Meise, Belgium. In addition to herbarium samples, in January 2015, I collected fresh samples (which were later kept frozen) from the South Shetland Islands, which offered much higher quality DNA. I also made several opportunistic collections on work-related or personal travel trips, which I preserved by drying. Other fresh collections were made by several BAS colleagues; Peter Convey in South Georgia, the Falkland Islands and Gough Island, Dominic Hodgson in Macquarie Island and Jessica Royles in the South Shetland Islands and Antarctic Peninsula. In some studies existing sequences from publicly available databases (e.g. GenBank) were added as suitable out- or in-groups. Additionally, combining my dataset with previously sequenced data from other researchers resulted in increased sample size in the following species: *Polytrichum piliferum* and *Polytrichastrum alpinum* in Chapter 2 (collaborators Jaakko Hyvönen and Satu Koskinen - all samples starting with "H" in Table 8.2.1.1); and *Ceratodon purpureus* in Chapter 3 (collaborators Rhys Wyber, Sharon A. Robinson and Mark Dowton).

8.1.2 DNA extraction

Genomic DNA was extracted using the Qiagen Plant Mini DNA extraction kit (Qiagen GmbH, Hilden, Germany) using liquid nitrogen and a mortar and pestle. DNA was extracted from single shoots, where possible. However, some specimens, such as those of *Ceratodon purpureus* and some *Schistidium* mosses, have such small individual shoots that several shoots were used. In most cases only one sub-sample was taken per herbarium sample. In cases where several DNA extractions were made per sample, this was to investigate inter-shoot variability within a sample, since different shoots in a moss clump can theoretically belong to different individuals. However, several shoots in a moss clump can also represent the same individual, which has expanded vegetatively.

8.1.3 PCR protocol

PCR amplification was performed using the Taq PCR Core Kit (Qiagen GmbH, Hilden, Germany) with addition of $MgCl_2$ provided by the kit. In most reactions bovine serum albumin (BSA) was also added (Sigma-Aldrich, Poole, UK). An overview of the quantities and concentrations used in the PCR reactions is given in Table 8.1.1. All reactions were prepared on a cool-block and quickly spun down using a centrifuge before the reaction.

Table 8.1.1 Volumes and concentrations of components used per PCR reaction.

Component	Quantity (µl)
Q solution (unknown concentration)	5
dNTP (10 mM)	0.5
Forward Primer (10mM):	2.5
Reverse Primer (10mM):	2.5
10x PCR Buffer (unknown concentration)	2.5
Taq DNA Polymerase (5U/µl)	0.125
ddH$_2$O (nuclease-free water)	10.875
MgCl$_2$ (25 mM)	1
BSA (10 mM)	1
Template DNA (concentration variable)	1
Total	27

Primers were first tested using a gradient PCR, after which an optimal annealing temperature was chosen for each species. All PCR cycles used variations of the Thermal Cycler conditions listed in Table 8.1.2. Details of markers, primers, the number of PCR cycles and temperatures are given in each Chapter. PCR products were inspected using gel electrophoresis. Forward and reverse sequencing was performed by LGC Genomics (Berlin, Germany), using the same primers as used for PCR.

Table 8.1.2 Thermal Cycler Conditions used for PCR reactions.

Process	Time	Temperature
Initial denaturation	3 min	95 °C
3-step cycling (30-35 cycles*)		
Denaturation	30 sec	95 °C
Annealing	40 sec	50-60 °C *
Extension	2 min	72 °C
Final elongation	10 min	72 °C

*= Variable according to species, quality of DNA and marker

8.1.4 Gel electrophoresis

Agarose gels (1.5%) were made by adding 0.75 g agarose (Bioline, London, UK) to 47.5 ml of 1x TBE buffer (Tris Borate EDTA (TBE) buffer; made up of 10x solution; Severn Biotech Ltd., Kidderminster, UK) in an Erlenmeyer (with bigger gels were made according to the same ratio). The mixture was heated in a microwave and the whirled until completely mixed and dissolved. After the mixture was completely dissolved, it was cooled down slightly and ~5 ml of GelRed^{TM} (Biotium, Inc., Fremont, USA) was added and the mixture was mixed well by whirling. The mixture was then poored into a gel holder and cooled down for ~30 minutes until it was polymerized to a matrix. The gel was put in a gel tank, covered by 1x TBE buffer, after which DNA samples could be added. Between 2.5-5 µl of each DNA sample was loaded with a total concentration of ca 40-100 ng. The sample was mixed with a small drop (0.5-1 µl) of loading dye (CoralLoad PCR Buffer; part of the Taq PCR Core Kit; Qiagen GmbH, Hilden, Germany) before loaded into the gel. Before running the gel a ladder was added, typically HyperLadder^{TM} I and II (Bioline, London, UK) for genomic DNA and PCR products, respectively. The common running settings were 80V for 30-40 min.

8.1.5 Sequence editing and alignment

Forward and reverse sequences were manually examined and assembled using Codoncode Aligner v.5.0.2 (CodonCode Corp., Dedham, MA). Sequences were aligned using PRANK (Löytynoja & Goldman, 2008) or Geneious aligner within Geneious 9.0.4 (Biomatters, LTD, Auckland, NZ), using default settings, with obvious misaligned sequences re-aligned manually. Depending on the genetic marker, some sections of the alignment were excluded (see individual chapters for alignment treatments): i) short partially incomplete sections at the ends of each alignment, ii) homoplastic regions previously identified in the literature, or iii) identified hypervariable regions as identified using NOISY (Dress *et al.*, 2008) or GBLOCKS (Castresana, 2000), using default settings. The number of variable and parsimony informative sites was calculated using MEGA7 (Kumar *et al.*, 2016). Additionally, in Chapter 3 the coding gene *rps4* was tested for positive selection using a Z-test in MEGA7, and *ITS* was tested for recombination within the program RDP v4.71 (Martin *et al.*, 2015) (see Chapter 3 for settings for both tests).

8.1.6 Phylogenetic analyses

Suitable outgroups were chosen based on previous phylogenetic analyses from the literature (choosing taxa that were as closely related to the ingroup as possible), either by sequencing

new samples or by including sequences from Genbank. Models of sequence evolution were selected for each locus using jModeltest-2.1.7 (Darriba *et al.*, 2012) using the SPR tree topology search operation and the Akaike Information Criterion (AICc). Bayesian analyses were performed using MrBayes 3.2 (Ronquist *et al.*, 2012) and maximum likelihood (ML) analyses were performed using RAxML-GUI v1.3.1 (Silvestro & Michalak, 2012) (see individual chapters for run length and settings). In some chapters Bayesian analyses included indel information within the dataset. Here, indels were coded according to the simple indel coding method (SIC; Simmons & Ochoterena, 2000) in SeqState v1.0. Convergence of Bayesian runs was assessed by checking split frequencies had an average standard deviation of <0.01, and by using Tracer v.1.6 (Rambaut *et al.*, 2014) to check all parameters had effective sample sizes (ESS) >200. Maximum clade credibility trees were visualised using FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

8.1.7   Species delimitation

Possible species clusters were explored using the Automatic Barcode Gap Discovery (ABGD) web server (Puillandre *et al.*, 2012), using default settings. ABGD is a species delimitation method which uses a pairwise genetic distance-based clustering algorithm to distinguish non-overlapping values of intra- and interspecific genetic distances within a group of sequences to construct hypothetical candidate species. It uses a two-phased procedure; first, dividing the sequences based on a statistically inferred 'barcode gap' (where the intraspecific divergence is smaller than interspecific divergence), which, in the second step, is then applied to redefine a range of partitions (Puillandre *et al.*, 2012).

The ABGD method was chosen as it generally delimits fewer clusters than other methods (e.g. see Kekkonen *et al.* 2015 and references herein) and is therefore one of the more conservative delimitation methods, useful for grouping only those lineages that are genetically distinctively different. A range of 0.001-0.05 'maximum divergence of intraspecific diversity' (P_{max}) values was assessed in each case.

Previous examples of bryophyte studies to implement species delimitation methods are Aranda *et al.* (2014) and Lang *et al.* (2015).

8.1.8   Population diversity analyses

To examine the phylogeographic structure within species, TCS networks (Clement *et al.*, 2000) were produced within the program Popart (Leigh & Bryant, 2015), using default settings. To evaluate the demographic history of the different lineages genetic diversity

indices, pairwise Kimura-2P distances, demographic and spatial expansion models and neutrality tests Tajima's D (Tajima, 1989) and Fu's Fs (Fu, 1997) were calculated using Arlequin v3.5.1.2 (Excoffier & Lischer, 2010) (see individual chapters for population diversity analyses). In Chapter 3 and 5, genetic differentiation measures F_{ST} (differences in lineage frequency) and Φ_{ST} (genetic differentiation between lineages) were calculated in Arlequin v3.5.1.2, to assess genetic structure within and between regions. In Chapter 3 these measures were analysed using a hierarchical AMOVA analysis (within Arlequin v3.5.1.2), to assess the genetic structure within and between *a priori* defined worldwide geographic regions (see Chapter 3 for details).

8.1.9   Molecular dating

Divergence times of the different species and populations were assessed using BEAST v2.2.1 (Drummond *et al.*, 2012). For most dating analyses no suitable fossil material was present and a molecular rate analysis was performed (see individual chapters on rates and analysis settings). In Chapter 2 suitable fossil material was present and a two-step dating analysis was applied, in which, 1) first, using a larger dataset with more slowly evolving genes the age a particular node was calculated, after which 2) the resulting divergence times (and corresponding 95% quantiles) were applied as a secondary prior on the same node in a dating analyses with a more variable marker informative to calculate divergence on a population level (see Chapter 2 for analysis details).

In most studies several analyses were conducted with different tree priors, to investigate the impact of prior choice on divergence times. After all BEAST runs Tracer v1.6 (Rambaut *et al.*, 2014) was used to examine stationarity and effective sampling (ESS>100) from the posterior distribution. In some cases, log and tree files of several runs were combined using LogCombiner v2.3.1. Maximum clade credibility trees were constructed in TreeAnnotator v1.8.2 (Drummond & Rambaut, 2007), with 10% burn-in removed, and visualised using Figtree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

8.2 Supplementary tables and figures

8.2.1 Appendix Chapter 2

Table 8.2.1.1 Specimens of *Polytrichum juniperinum*, *P. strictum*, *P. piliferum*, and *Polytrichastrum alpinum* used in Chapter 2, including herbarium details, accession numbers, geographic locations and GenBank accession numbers. "Antarctic region" includes Antarctic, sub-Antarctic and southern South America. Brackets behind specific specimens represent multiple stems being taken from within ~5 cm, which could have represented the same individual and have been treated as one if the sequence was identical. Samples of *ITS* 2 only of *P. juniperinum* and *P. strictum* were not analysed in this study. - = not available.

Specimen name	Geographic origin (country)	Geographic region as used in this study	Herbarium + herbarium number (when applicable)	Collection + collection number (when applicable)	Latitude and longitude (decimal degr.)	*ITS* 1 + 2	*ITS* 2	*trnL-F*
Polytrichum juniperinum								
BR 282794391 Canada, Labrador	Canada	Holarctic	BR 5040282794391	G.R. Brassard	53.6, -64.32	MF180399	-	-
BR 282740824 Canada, British Columbia	Canada	Holarctic	BR 5040282740824	D.H. Vitt	52.11, -119.65 [1]	MF180400	-	MF180539
BR 282797422 Canada, Ontario	Canada	Holarctic	BR 5040282797422	B. Allen	48.77, -88.63 [1]	MF180401	-	MF180540
BR 069658120 Russia	Russia	Holarctic	BR 5040069658120	V. Vašák	43.4, 42.92 [1]	MF180402	-	-
BR 282698408 France	France	Holarctic	BR 5040282698408	J.L. De Sloover	44.89, -1.21 [1]	MF180403	-	-
BR 282721632 Russia	Russia	Holarctic	BR 5040282721632	I. Dabkowska	55.8, 37.68 [1]	MF180404	-	-
BR 282735776 France	France	Holarctic	BR 5040282735776	L. Leclercq	50.81, 1.61 [1]	MF180405	-	-
BR 341589524 Portugal	Portugal	Holarctic	BR 5040341589524	T. Arts	32.73, -16.89 [1]	MF180406	-	-
BR 341591541 Portugal	Portugal	Holarctic	BR 5040341591541	T. Arts	32.76, -17.08 [1]	MF180407	-	-
BR 355018959 Luxembourg	Luxembourg	Holarctic	BR 5040355018959	E. Jacques	49.91, 6.06	MF180408	-	-
BR 015357312 Bosnia and Herzegovina	Bosnia and Herzeg.	Holarctic	BR 5040015357312	A. Rusinska	43.57, 17.43 [2]	MF180409	-	MF180541

BR 089480466 Poland	Poland	Holarctic	BR 5040089480466	S. Lisowski, A. Rusinska & I. Melosik	50.83, 23.4 [1]	MF180410	-	MF180542
BR 182997557 Bulgaria	Bulgaria	Holarctic	BR 5040182997557	V. Vašák	41.77, 23.4 [1]	MF180411	-	MF180543
BR 246997356 Switzerland	Switzerland	Holarctic	BR 5040246997356	M. Onraedt	46.19, 7.63 [1]	MF180412	-	MF180544
BR 282736780 France	France	Holarctic	BR 5040282736780	J.L. De Sloover	44.04, -1.34 [1]	MF180413	-	MF180545
BR 282737794 Finland	Finland	Holarctic	BR 5040282737794	T. Lammes	60.45, 22.11 [1]	MF180414	-	MF180546
BR 357582403 Netherlands	Netherlands	Holarctic	BR 5040357582403	J.A.E. Slembrouck	51.42, 4.8 [1]	MF180415	-	MF180547
BR 113262631 Switzerland	Switzerland	Holarctic	BR 5040113262631	A. Lawalrée	46.14, 7.01	MF180416	-	MF180548
BR 104807476 Poland	Poland	Holarctic	BR 5040104807476	H. Bednarek-Ochyra & R. Ochyra	53.95, 22.3 [1]	-	-	MF180549
BR 022969775 Switzerland	Switzerland	Holarctic	BR 5040022969775	A. Lawalrée	46, 7.34 [1]	-	-	MF180550
BR 119152363 Georgia	Georgia	Holarctic	BR 5040119152363	V. Vašák	42.4, 43.94 [1]	MF180417	-	MF180551
BR 120321413 Russia	Russia	Holarctic	BR 5040120321413	V. Vašák	51.66, 103.7 [1]	MF180418	-	MF180552
BR 120324445 Russia	Russia	Holarctic	BR 5040120324445	V. Vašák	56.31, 101.66 [1]	MF180419	-	MF180553
BR 137958244 Papua New Guinea	Papua New Guinea	Holarctic	BR 5040137958244	J.L. De Sloover	-6.04, 143.89 [1]	MF180420	-	MF180554
BR 282694363 Papua New Guinea	Papua New Guinea	Holarctic	BR 5040282694363	J.L. De Sloover	-5.83, 143.4 [1]	MF180421	-	MF180555
BR 282738807 Mongolia	Mongolia	Holarctic	BR 5040282738807	A. Pacyna	47.5, 100 [1]	MF180422	-	MF180556
BR 282765100 Papua New Guinea	Papua New Guinea	Holarctic	BR 5040282765100	J.F. Veldkamp & M. Kuduk	-8.21, 146.78	MF180423	-	MF180557
BR 58446 South Africa	South Africa	S. African	BR 040058446523	E. Powrie	-34.06, 19.53 [1]	MF180424	-	-
AAS 1484 Crozet I.	Crozet I.	S. African	AAS	B.G. Bell 01484	-46.42, 51.83	-	-	MF180558
AAS 297 Prince Edward I.	Prince Edward I.	S. African	AAS	N.J.M. Gremmen 00297	-46.92, 37.75	-	-	MF180559
AAS 242500 New Zealand	New Zealand	Australasia	AAS	E.M. Chapman s.n.	-42.38, 172.4 [1]	MF180425	-	-
BR 282713552 New Zealand	New Zealand	Australasia	BR 5040282713552	s.n.	-45.54, 169.31 [1]	MF180426	-	MF180560
BR 282767128 Australia	Australia	Australasia	BR 5040282767128	H. Streimann	-37.07, 149.47	MF180427	-	MF180561
EMB New Zealand	New Zealand	Australasia	BAS dried samples	E.M. Biersma s.n.	-45.53, 167.86 [1]	MF180428	-	MF180562
Macquarie I. 3	Macquarie I.	Australasia	BAS frozen samples	D. Hodgson s.n.	-54.62, 158.86 [1]	MF180429	-	-
Macquarie I. 4	Macquarie I.	Australasia	BAS frozen samples	D. Hodgson s.n.	-54.62, 158.86 [1]	MF180430	-	-
AAS 1352 Campbell I.	Campbell I.	Australasia	AAS	D.H. Vitt	-52.55, 169.15	MF180431	-	-
AAS 433 Campbell I.	Campbell I.	Australasia	AAS	R.L. Oliver 00433	-52.55, 169.15	MF180432	-	-
BR 104558897 Brazil	Brazil	South America	BR 5040104558897	V. Nicolack & O.S. Ribas	-25.3, -49.06 [1]	MF180433	-	-
BR 282692345 Panama	Panama	South America	BR 5040282692345	M. Nee	8.87, -82.58 [1]	MF180434	-	-

BR 282726682 Brazil	Brazil	South America	BR 5040282726682	H.S. Irwin, E. Onishi, S.F. Da Fonseca, R. Souza, R. Reis Dos Santos & J. Ramos	-18.08, -43.74 [1]	MF180435	-	-
BR 307706224 Bolivia	Bolivia	South America	BR 5040307706224	I.G. Vargas	-18.72, -64.02	MF180436	-	-
BR 307711273 Bolivia	Bolivia	South America	BR 5040307711273	I.G. Vargas	-17.82, -64.62	MF180437	-	-
BR 314920583 Ecuador	Ecuador	South America	BR 5040314920583	T. Arts	-0.46, -78.45 [1]	MF180438	-	-
BR 320589040 Costa Rica	Costa Rica	South America	BR 5040320589040	T. Arts	9.56, -83.8 [1]	MF180439	-	-
AAS 35759 Brazil	Brazil	South America	AAS	W.R. Anderson, M. Stieber & J.H. Kirkbride Jr. s.n.	-18.4, -43.35 [2]	MF180440	-	-
AAS Colombia	Colombia	South America	AAS	C.E. Chardón Palacios s.n.	5.3, -75.25 [1]	MF180441	-	-
BR 271959688 Bolivia	Bolivia	South America	BR 5040271959688	M. Nee	-18.07, -63.92	MF180442	-	MF180563
BR 282695377 Ecuador	Ecuador	South America	BR 5040282695377	L.J. Dorr & L.C. Barnett	-2.88, -78.77	MF180443	-	MF180564
BR 314921597 Ecuador	Ecuador	South America	BR 5040314921597	T. Arts	-0.68, -78.44	MF180444	-	MF180565
BR 240328590 Ecuador	Ecuador	South America	BR 5040240328590	M. Lewis	0.37, -78.33	-	-	MF180566
BR 282739811 Brazil	Brazil	South America	BR 5040282739811	J.-P. Frahm	-23.86, -46.21 [1]	-	-	MF180567
AAS 1115 S. Orkney Is.	S. Orkney Is.	Antarctic region	AAS	R.E. Longton	-60.7, -45.67	MF180445	-	-
AAS 898 S. Georgia	South Georgia	Antarctic region	AAS	B.G. Bell 00898	-54.5, -36.17	MF180446	-	-
AAS 98 South Georgia	South Georgia	Antarctic region	AAS	S.W. Greene 00098	-54.23, -36.52	MF180447	-	-
AAS 194 S. Sandwich Is.	S. Sandwich Is.	Antarctic region	AAS	P. Convey 00194	-57.07, -26.7	MF180448	-	MF180568
AAS 379 S. Orkney Is.	S. Orkney Is.	Antarctic region	AAS	R.I.L. Smith 00379	-60.7, -45.67	MF180449	-	MF180569
AAS 1714 S. Sandwich Is.	S. Sandwich Is.	Antarctic region	AAS	R. Ochyra 01714	-62.08, -58.25	-	-	MF180570
AAS 202 Chile	Chile	Antarctic region	AAS	S.W. Greene	-50.68, -72.37	MF180450	-	-
AAS 2832 Chile	Chile	Antarctic region	AAS	C.M. Matteri	-51.4, -73.07	MF180451	-	-
AAS 4231 Antarctic Peninsula	Antarctic Peninsula	Antarctic region	AAS	R.I.L. Smith 04231	-64.73, -62.55	MF180452	-	-
AAS 68 S. Shetland Is.	S. Shetland Is.	Antarctic region	AAS	R.I.L. Smith 00068	-62.98, -60.58	MF180453	-	-
AAS 7645 Antarctic Peninsula	Antarctic Peninsula	Antarctic region	AAS	R.I.L. Smith 07645	-63.93, -57.82	MF180454	-	-
AAS 1865 S. Shetland Is.	S. Shetland Is.	Antarctic region	AAS	R. Ochyra 01865	-62.17, -58.48	MF180455	-	MF180571
AAS 3171 S. Orkney Is.	S. Orkney Is.	Antarctic region	AAS	R.I.L. Smith 03171	-60.7, -45.67	MF180456	-	MF180572
AAS 3331 Antarctic Peninsula	Antarctic Peninsula	Antarctic region	AAS	R.I.L. Smith 03331	-65.42, -64.23	MF180457	-	MF180573
AAS 4125 Antarctic Peninsula	Antarctic Peninsula	Antarctic region	AAS	R.I.L. Smith 04125	-64.33, -62.93	MF180458	-	MF180574
AAS 66A S. Shetland Is.	S. Shetland Is.	Antarctic region	AAS	D. Mason 00066A	-62.98, -60.58	MF180459	-	MF180575

AAS 4640 Antarctic Peninsula	Antarctic Peninsula	Antarctic region	AAS	R.I.L. Smith 4640	-67.97, -67.32	-	-	MF180576

Polytrichum strictum

BR 282761065 Canada, Quebec	Canada	Holarctic	BR 5040282761065	J.L. De Sloover	47.42, -61.76 [1]	MF180460	-	-
BR 282746888 Canada, Quebec	Canada	Holarctic	BR 5040282746888	J.L. De Sloover	47.84, -69.53 [1]	MF180461	-	MF180577
BR 282759048 Canada, Quebec	Canada	Holarctic	BR 5040282759048	J.L. De Sloover	47.38, -61.87 [1]	-	MF180301	-
BR 282760051 Canada, Quebec	Canada	Holarctic	BR 5040282760051	J.L. De Sloover	47.25, -61.93 [1]	MF180462	-	MF180578
BR 282926747, Canada, Newfoundland	Canada	Holarctic	BR 5040282926747	G.R. Brassard	55.26, -59.65 [1]	MF180463	-	MF180579
BR 282932809 Canada, Yukon	Canada	Holarctic	BR 5040282932809	D.H. Vitt	64.28, -140.45	MF180464	-	MF180580
BR 282756016 Canada, British Columbia	Canada	Holarctic	BR 5040282756016	D.H. Norris	52.55, -125.72	MF180465	-	MF180581
BR 089717890 U.S.A., Alaska	U.S.A.	Holarctic	BR 5040089717890	P. Alpert	66.36, -147.39 [1]	-	MF180302	-
BR 282916649 U.S.A., Michigan	U.S.A.	Holarctic	BR 5040282916649	H. Crum & N.G. Miller	45.61, -84.73 [1]	MF180466	-	-
BR 282910586 U.S.A., Michigan	U.S.A.	Holarctic	BR 5040282910586	J. Jaworski	42.4, -84.22 [1]	MF180467	-	MF180582
BR 017303379 U.S.A., Minnesota	U.S.A.	Holarctic	BR 5040017303379	J.A. Janssens	47.23, -94.95	MF180468	-	MF180583
BR 017038639 U.S.A., Minnesota	U.S.A.	Holarctic	BR 5040017038639	J.A. Janssens	48.34, -94.54	-	-	MF180584
BR 027965291 United Kingdom	United Kingdom	Holarctic	BR 5040027965291	S.L. Jury & F.J. Rumsey	51.88, -3.7	MF180469	-	-
BR 036605362 Norway	Norway	Holarctic	BR 5040036605362	R.E. Longton	71.05, 28.02 [1]	MF180470	-	-
BR 311557904 Norway	Norway	Holarctic	BR 5040311557904	T. Arts	69.27, 20.01 [1]	MF180471	-	-
TROM B 320005 Norway	Norway	Holarctic	TROM B 320005	A. Elvebakk & A.A. Frisvoll s.n.	69.27, 20.52 [1]	MF180472	-	-
TROM B 320007 Norway	Norway	Holarctic	TROM B 320007	A. Sortland s.n.	69.1, 18.05 [1]	MF180473	-	MF180585
BR 282924729 Norway	Norway	Holarctic	BR 5040282924729	R. Alava	69.58, 20.16 [1]	-	-	MF180586
BR 217573022 Slovakia	Slovakia	Holarctic	BR 5040217573022	J. Bouharmont	49.17, 20.08 [1]	MF180474	-	MF180587
BR 117682213 France	France	Holarctic	BR 5040117682213	J.-P. Frahm	47.94, 6.59 [1]	MF180475	-	MF180588
BR 130336650 Svalbard	Svalbard	Holarctic	BR 5040130336650	B. Godzik & K. Grodzinska	77.26, 16.34 [1]	MF180476	-	MF180589
BR 026484038 Iceland	Iceland	Holarctic	BR 5040026484038	C. Van den Berghen	65.69, -18.12 [1]	-	MF180303	-
BR 138015816 Russia, Chukotka	Russia	Holarctic	BR 5040138015816	O. Dopokuka	69.76, 162.14 [1]	MF180477	-	-
BR 282936845 Japan, Hokkaido	Japan	Holarctic	BR 5040282936845	s.n.	43.11, 144.4 [1]	MF180478	-	-
BR 120329495 Russia	Russia	Holarctic	BR 5040120329495	V. Vašák	55.29, 100.95 [1]	-	-	MF180590
AAS 3335A Falklands	Falkland Is.	Antarctic region	AAS	s.n.	69.27, 20.01 [1]	MF180479	-	-
EMB Chile	Chile	Antarctic region	BAS dried samples	E.M. Biersma s.n.	-54.94, -67.63 [1]	MF180480	-	-

AAS 5457 Falkland Is.	Falkland Is.	Antarctic region	AAS	R.E. Longton 05457	-51.68, -57.92	-	MF180304	-
AAS 249B S. Sandwich Is.	S. Sandwich Is.	Antarctic region	AAS	P. Convey 00249B	-56.67, -28.13	MF180481	-	MF180591
AAS 24B S. Sandwich Is.	S. Sandwich Is.	Antarctic region	AAS	P. Convey 00024B	-59.43, -27.08	MF180482	-	MF180592
AAS 163A S. Sandwich Is.	S. Sandwich Is.	Antarctic region	AAS	P. Convey 00163A	-57.07, -26.7	MF180483	-	MF180593
AAS 126A S. Sandwich Is.	S. Sandwich Is.	Antarctic region	AAS	P. Convey 00126A	-57.07, -26.7	-	MF180305	MF180594
AAS 5058 South Georgia	South Georgia	Antarctic region	AAS	R.E. Longton 05058	-54.22, -36.67	-	MF180306	-
AAS 5066 South Georgia	South Georgia	Antarctic region	AAS	R.E. Longton 05066	-54.17, -36.72	-	MF180307	-
AAS 5047 South Georgia	South Georgia	Antarctic region	AAS	R.E. Longton 05047	-54.23, -36.63	MF180484	-	-
S. Shetland Is. Ardley I. 2C	S. Shetland Is.	Antarctic region	BAS / Cambridge U.	J. Royles s.n.	-62.22, -58.93 [1]	MF180486	-	MF180595
S. Shetland Is. Ardley I. 2D	S. Shetland Is.	Antarctic region	BAS / Cambridge U.	J. Royles s.n.	-62.22, -58.93 [1]	MF180485	-	MF180596
S. Shetland Is. Ardley I. 1J	S. Shetland Is.	Antarctic region	BAS / Cambridge U.	J. Royles s.n.	-61.11, -55.14 [1]	MF180487	-	MF180597
S. Shetland Is. Elephant I. 1A (1)	S. Shetland Is.	Antarctic region	BAS / Cambridge U.	J. Royles s.n.	-61.11, -55.14 [1]	-	MF180308	-
S. Shetland Is. Elephant I. 1A (2)	S. Shetland Is.	Antarctic region	BAS / Cambridge U.	J. Royles s.n.	-61.11, -55.14 [1]	-	MF180309	-
S. Shetland Is. Elephant I. 1A (4)	S. Shetland Is.	Antarctic region	BAS / Cambridge U.	J. Royles s.n.	-61.11, -55.14 [1]	-	MF180310	-
S. Shetland Is. Elephant I. 1B (1,2)	S. Shetland Is.	Antarctic region	BAS / Cambridge U.	J. Royles s.n.	-61.11, -55.14 [1]	MF180488	-	MF180598
S. Shetland Is. Elephant I. 1B (3)	S. Shetland Is.	Antarctic region	BAS / Cambridge U.	J. Royles s.n.	-61.11, -55.14 [1]	MF180489	-	-
S. Shetland Is. Elephant I. 1B (4,5)	S. Shetland Is.	Antarctic region	BAS / Cambridge U.	J. Royles s.n.	-61.11, -55.14 [1]	MF180490	-	-
S. Shetland Is. Elephant I. 1E	S. Shetland Is.	Antarctic region	BAS / Cambridge U.	J. Royles s.n.	-61.11, -55.14 [1]	MF180491	-	MF180599
S. Shetland Is. Elephant I. 1J	S. Shetland Is.	Antarctic region	BAS / Cambridge U.	J. Royles s.n.	-61.11, -55.14 [1]	MF180492	-	MF180600
S. Shetland Is. Elephant I. 2B	S. Shetland Is.	Antarctic region	BAS / Cambridge U.	J. Royles s.n.	-61.11, -55.14 [1]	MF180493	-	MF180601
Antarctic Peninsula, Norsel Point 1H	Antarctic Peninsula	Antarctic region	BAS / Cambridge U.	J. Royles s.n.	-64.45, -64.05 [1]	MF180494	-	-
Antarctic Peninsula, Norsel Point 1E	Antarctic Peninsula	Antarctic region	BAS / Cambridge U.	J. Royles s.n.	-64.45, -64.05 [1]	MF180495	-	-
AAS 1690 Ant. Pen., Palmer Coast	Antarctic Peninsula	Antarctic region	AAS	D.C. Lindsay 01690	-63.55, -59.85	MF180496	-	-
AAS 824 Ant. Pen., Axander I.	Antarctic Peninsula	Antarctic region	AAS	P. Convey 00824	-69.37, -71.85	MF180497	-	MF180602
AAS 832 Ant. Pen., Axander I.	Antarctic Peninsula	Antarctic region	AAS	P. Convey 00832	-69.37, -71.85	MF180498	-	MF180603
AAS 1318 Ant. Pen., Danco Coast	Antarctic Peninsula	Antarctic region	AAS	R.E. Longton 01318	-64.82, -64.03	-	MF180311	-
AAS 2 Ant Pen., Trinity Coast	Antarctic Peninsula	Antarctic region	AAS	C.G. Brading 00002	-63.4, -57	-	MF180312	-
AAS 4269 Antarctic Peninsula	Antarctic Peninsula	Antarctic region	AAS	R.I.L. Smith 04269	-65.23, -64.23	MF180499	-	MF180604
AAS 4897 Antarctic Peninsula	Antarctic Peninsula	Antarctic region	AAS	R.I.L. Smith 04897	-65.27, -64.13	MF180500	-	MF180605
AAS 27 Ant. Pen., Trinity Coast	Antarctic Peninsula	Antarctic region	AAS	C.G. Brading 00027	-63.4, -57	MF180501	-	MF180606
AAS 3368a Ant. Pen., Graham Coast	Antarctic Peninsula	Antarctic region	AAS	R.I.L. Smith 03368A	-65.32, -64.17	MF180502	-	MF180607

AAS 700a Ant. Pen., Palmer Coast	Antarctic Peninsula	Antarctic region	AAS	R.I.L. Smith 00700A	-63.75, -60.67	MF180503	-	MF180608

Polytrichum piliferum

H 3120679 Canada, Yukon	Canada	Holarctic	H 3120679	D. Vitt	64.37, -136.46	MF180504	-	-
H 3120727 Canada, Quebec	Canada	Holarctic	H 3120727	D. Weber	57.06, -65.23	MF180505	-	-
BR 225742236 Canada, British Columbia	Canada	Holarctic	BR 5040225742236	F. Demaret	50.12, -123.03 [1]	MF180506	-	-
BR 282869174 Canada, Quebec	Canada	Holarctic	BR 5040282869174	J.L. De Sloover	45.8, -74.64 [1]	-	MF180313	-
H WS70685 Canada	Canada	Holarctic	H WS70685	W.D. Schofield	62.34, -128.32	-	MF180314	-
H 3120695 Canada, Newfoundland	Canada	Holarctic	H 3120695	G. Brassard	48.8, -54.21 [1]	-	MF180315	-
H 3120704 Canada, Ontario	Canada	Holarctic	H 3120704	R.R. Ireland	45, -81.15	-	MF180316	-
BR 282809545 Canada, Newfoundland	Canada	Holarctic	BR 5040282809545	J. Bridgland	53.7, -57.02	-	-	MF180609
H 3120547 U.S.A., Missouri	U.S.A.	Holarctic	H 3120547	B. Allen	37.65, -90.68 [1]	-	MF180317	-
H 3120769 U.S.A., Hawaii	U.S.A.	Holarctic	H 3120769	W.J. Hoe	20.71, -156.15 [1]	-	MF180318	-
BR 016853721 U.S.A., Alaska	U.S.A.	Holarctic	BR 5040016853721	M. Lewis	66.92, -156.92	-	MF180319	-
BR 282846915 U.S.A., Hawaii	U.S.A.	Holarctic	BR 5040282846915	W.J. Hoe	20.64, -156.12 [1]	-	MF180320	-
H 3120771 U.S.A., Hawaii	U.S.A.	Holarctic	H 3120771	W.J. Hoe	20.71, -156.14 [1]	-	MF180321	-
BR 225791722 U.S.A., Washington	U.S.A.	Holarctic	BR 5040225791722	F.J. Hermann	46.83, -121.76 [1]	MF180507	-	MF180610
BR 017183148 U.S.A., Minnesota	U.S.A.	Holarctic	BR 5040017183148	J.A. Janssens	45.4, -92.66	-	MF180322	MF180611
BR 019183740 U.S.A., Washington	U.S.A.	Holarctic	BR 5040019183740	M.P. Harthill	47.97, -123.5 [1]	-	MF180323	MF180612
BR 282801464 U.S.A., Missouri	U.S.A.	Holarctic	BR 5040282801464	B. Allen	37.92, -93.75 [1]	-	MF180324	MF180613
BR 282833786 U.S.A., Washington	U.S.A.	Holarctic	BR 5040282833786	M.P. Harthill	47.97, -123.5 [1]	-	MF180325	MF180614
H 3120730 Greenland	Greenland	Holarctic	H 3120730	K. Holmen	66.06, -37.13	MF180508	-	-
TUR WW1152 Poland	Poland	Holarctic	TUR WW1152	W. Wilczynska	51.03, 17.15 [1]	MF180509	-	-
BR 016108067 Portugal	Portugal	Holarctic	BR 5040016108067	Stud. Biol. Rheno-trai in Itinere	41.68, -8.43 [1]	MF180510	-	-
BR 189174241 Georgia	Georgia	Holarctic	BR 5040189174241	V. Vašák	41.71, 44.91 [1]	MF180511	-	-
BR 225821054 United Kingdom	United Kingdom	Holarctic	BR 5040225821054	G. Raeymaekers	50.59, -3.95 [1]	MF180512	-	-
BR 341607709 Norway	Norway	Holarctic	BR 5040341607709	T. Arts	61.33, 8.15 [1]	MF180513	-	-
BR 117747868 France	France	Holarctic	BR 5040117747868	J.-P. Frahm	47.85, 6.59 [1]	MF180514	-	MF180615
BR 217565911 Slovakia	Slovakia	Holarctic	BR 5040217565911	J. Bouharmont	48.93, 19.75 [1]	MF180515	-	MF180616
BR 282836817 France	France	Holarctic	BR 5040282836817	J.L. De Sloover	45.01, 6.12 [1]	MF180516	-	MF180617
AY396439 Finland	Finland	Holarctic	H 6205	J. Hyvönen	60.3, 24.28 [1]	-	AY396439	-

H 3120607 Germany	Germany	Holarctic	H 3120607	H. Hämäläinen	59.27, 13.02	-	MF180326	-
H 3120660 Canary Is.	Spain	Holarctic	H 3120660	R. Rajalin	28.36, -17.5	-	MF180327	-
BR 031803848 Switzerland	Switzerland	Holarctic	BR 5040031803848	A. Lawalrée	46.15, 7.06 [1]	-	MF180328	-
H B3992 Faroe Is.	Faroe Is.	Holarctic	H B3992	s.n.	62.06, -6.83 [1]	-	MF180329	-
H 6205 Finland	Finland	Holarctic	H 6205	J. Hyvönen	60.3, 24.28 [1]	-	MF180330	-
H 3120658 Madeira	Portugal	Holarctic	H 3120658	R. Ruotsalo-Aario & L. Aario	32.73, -17.05 [1]	-	MF180331	-
TUR 88-130 Finland	Finland	Holarctic	TUR 88-130	Y. Mäkinen	60.63, 21.29 [1]	-	MF180332	-
BR 018305693 Poland	Poland	Holarctic	BR 5040018305693	S. Lisowski	49.2, 19.75 [1]	-	MF180333	-
BR 022118029 Sweden	Sweden	Holarctic	BR 5040022118029	H. Möller	58.57, 11.37 [1]	-	MF180334	-
BR 022119033 Sweden	Sweden	Holarctic	BR 5040022119033	H. Möller	61.29, 13.91 [1]	-	MF180335	-
BR 026466829 Sweden	Sweden	Holarctic	BR 5040026466829	L. Cooreman	68.35, 18.83 [1]	-	MF180336	-
AAS 157 Falkland Is.	Falkland Is.	Holarctic	AAS	J.J. Engel 00157	-51.75, -59.5	-	MF180337	-
BR 225749303 Portugal	Portugal	Holarctic	BR 5040225749303	Stud. Biol. Rheno-trai in Itinere	40.45, -7.64 [1]	-	MF180338	-
BR 282891397 Norway	Norway	Holarctic	BR 5040282891397	Stud. Biol. Rheno-Trai in Itinere	62.15, 9.17 [1]	-	MF180339	-
BR 314397200 Finland	Finland	Holarctic	BR 5040314397200	T. Arts	69.05, 20.82 [1]	-	MF180340	-
BR 341596591 Ireland	Ireland	Holarctic	BR 5040341596591	T. Arts	53.4, -9.96 [1]	-	MF180341	-
BR 103358535 France	France	Holarctic	BR 5040103358535	J.L. De Sloover	43.42, 2.46 [1]	-	MF180342	MF180618
BR 207875044 Switzerland	Switzerland	Holarctic	BR 5040207875044	J.A.E. Slembrouck	47.1, 9.61 [1]	-	MF180343	MF180619
BR 225751320 Poland	Poland	Holarctic	BR 5040225751320	J. Mickiewicz	52.33, 20.44 [1]	-	MF180344	MF180620
BR 251012730 Italy	Italy	Holarctic	BR 5040251012730	M. Onraedt	45.68, 6.88 [1]	-	MF180345	MF180621
BR 282803482 Faroe Is.	Faroe Is.	Holarctic	BR 5040282803482	J. Lewinsky	62.3, -6.72	-	MF180346	MF180622
BR 089481470 Poland	Poland	Holarctic	BR 5040089481470	S. Lisowski, A. Rusinska, & I. Melosik	50.83, 23.4	-	-	MF180623
H 3120647 Russia	Russia	Holarctic	H 3120647	P. Alanko	51.34, 139.51	MF180517	-	-
AAS 287 South Georgia	South Georgia	Holarctic	AAS	G.C.S. Clarke 00287	-54.28, -36.5	MF180518	-	MF180624
H 3120653 China, Jilin	China	Holarctic	H 3120653	T. Koponen	41.3, 128.1	-	MF180347	-
BR 189173237 Georgia	Georgia	Holarctic	BR 5040189173237	V. Vašák	42.93, 41.11 [1]	-	MF180348	-
BR 119154381 Georgia	Georgia	Holarctic	BR 5040119154381	V. Vašák	42.93, 41.11 [1]	-	MF180349	MF180625
BR 120327477 Georgia	Georgia	Holarctic	BR 5040120327477	V. Vašák	43, 41.02 [1]	-	MF180350	MF180626
BOL 109447 South Africa	South Africa	Holarctic	BOL 109447	T.A. Hedderson	-32.36, 19.12	-	MF180351	-

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BOL 109448 South Africa	South Africa	Holarctic	BOL 109448	T.A. Hedderson	-32.23, 19.1	-	MF180352	-
H JH5997 Chile	Chile	Antarctic region	H JH5997	J. Hyvönen	-38.42, -71.41	MF180519	-	-
H 3120779 Argentina	Argentina	Antarctic region	H 3120779	A. Kalela	-40.66, -71.42 [1]	-	MF180353	-
H BR23-2-66 Chile	Chile	Antarctic region	H BR23-2-66	B. Ruthsatz	-39.93, -72.03 [1]	-	MF180354	-
H 2168 Argentina	Argentina	Antarctic region	H 2168	J. Hyvönen	-53.24, -68.5	-	MF180355	-
H 2282 Argentina	Argentina	Antarctic region	H 2282	J. Hyvönen	-53.46, -69.47	-	MF180356	-
H 4970 Argentina	Argentina	Antarctic region	H 4970	J. Hyvönen	-50.1, -72.47	-	MF180357	-
H 3120781 Falkland Is.	Falkland Is.	Antarctic region	H 3120781	J. Engel	-51.21, -60.42	-	MF180358	-
H RLS73 South Georgia	South Georgia	Antarctic region	H RLS73	R.I.L. Smith	-54.23, -36.58 [3]	MF180520	-	-
H RLS75 South Georgia	South Georgia	Antarctic region	H RLS75	R.I.L. Smith	-54.1, -36.42	MF180521	-	-
H 3120767 South Georgia	South Georgia	Antarctic region	H 3120767	R. Longton	-54.28, -36.5 [1]	-	MF180359	-
AAS 67 S. Shetland Is.	S. Shetland Is.	Antarctic region	AAS	R.I.L. Smith 00067	-62.98, -60.58	-	MF180360	-
S. Shetland Is. Barrientos I. 3C	S. Shetland Is.	Antarctic region	BAS / Cambridge U.	J. Royles s.n.	-62.41, -59.75 [1]	MF180522	-	-
H 3120778 S. Shetland Is.	S. Shetland Is.	Antarctic region	H 3120778	R. Ochyra	-62.04, -58.25	-	MF180361	-
AAS 3332 Antarctic Peninsula	Antarctic Peninsula	Antarctic region	AAS	R.I.L. Smith 3332	-65.42, -64.23	-	MF180362	-
AAS 2770A Antarctic Peninsula	Antarctic Peninsula	Antarctic region	AAS	M. Sharp 02770A	-67.62, -64.75	MF180523	-	-

Polytrichastrum alpinum

TUR 63-18-11, U.S.A., Colorado	U.S.A.	Holarctic	TUR 63-18-11	A. Vaarama	39.6, -105.64	MF180524	-	-
BR 282719615 U.S.A., Montana	U.S.A.	Holarctic	BR 5040282719615	L. E. Anderson	48.17, -115.83 [1]	MF180525	-	MF180627
H 3118718 Canada, Ontario	Canada	Holarctic	H 3118718	R.R. Ireland	45.04, -79.45	-	MF180363	-
H 3118657 USA, Minnesota	USA	Holarctic	H 3118657	B. Allen	47.79, -90.19	-	MF180364	-
H 3118596 Canada, Newfoundland	Canada	Holarctic	H 3118596	T.A. Hedderson	48.35, -53.55	-	MF180365	-
L SN20 Svalbard	Svalbard	Holarctic	L SN20	E.M. Biersma & S. Lubbe	78.08, 18.55	MF180526	-	-
BR 282697395 Greenland	Greenland	Holarctic	BR 5040282697395	J. Lewinsky	63.93, -50.92	MF180527	-	-
BR 138177491 Switzerland	Switzerland	Holarctic	BR 5040138177491	J. Bouharmont	46.52, 9.9 [1]	MF180528	-	-
H 10.7.85 Czech Republic	Czech Republic	Holarctic	H 10.7.85	J. Vána	50.68, 15.65	-	MF180366	-
H 3118412 Faroe Is.	Faroe Is.	Holarctic	H 3118412	J. Lewinsky	62.02, -7.13	-	MF180367	-
H JP10813 Finland	Finland	Holarctic	H JP10813	J. Pykälä	60.13, 23.49	-	MF180368	-
H 3118511 Russia, Perm Prov.	Russia	Holarctic	H 3118511	M. Ignatov & A. Bezgodov	58.48, 58.28	-	MF180369	-
H SH102 Slovakia	Slovakia	Holarctic	H SH102	S. Huttunen & H. Jalkanen	49.11, 19.59	-	MF180370	-
H 3118486 Poland	Poland	Holarctic	H 3118486	H. Bednarek-Ochyra & R.	49.24, 19.92 [1]	-	MF180371	-

Ochyra

H SH1083 Russia	Russia	Holarctic	H SH1083	S. Huttunen	67.09, 31.51	-	MF180372	-
H SH928 Russia	Russia	Holarctic	H SH928	S. Huttunen & H. Wahlberg	61.45, 30.47	-	MF180373	-
TROM B 320018 Norway	Norway	Holarctic	TROM B 320018	I. Hagen	63.42, 10.45 [1]	-	MF180374	-
BR 138106750 Austria	Austria	Holarctic	BR 5040138106750	J. Bouharmont	47.19, 13.3 [1]	-	-	MF180628
BR 120322427 Georgia	Georgia	Holarctic	BR 5040120322427	V. Vašák	42.4, 43.94 [1]	MF180529	-	-
H 3118618 China, Xinjiang	China	Holarctic	H 3118618	B. Tan	43.32, 89.44	-	MF180375	-
H JH3524 Taiwan	Taiwan	Holarctic	H JH3524	J. Hyvönen	24.08, 121.17	-	MF180376	-
H JH3538 Taiwan	Taiwan	Holarctic	H JH3538	J. Hyvönen	24.08, 121.17	-	MF180377	-
H JH3709 Taiwan	Taiwan	Holarctic	H JH3709	J. Hyvönen	23.27, 120.57	-	MF180378	-
H MI97543 Russia	Russia	Holarctic	H MI97543	M. Ignatov	51.32, 133.54	-	MF180379	-
H 3118602 China	China	Holarctic	H 3118602	T. Koponen	41.3, 128.1	-	MF180380	-
H 3118745 Australia	Australia	Australasia	H 3118745	H. Streimann	-36.53, 147.18	-	MF180381	-
Macquarie I.	Macquarie I.	Australasia	BAS frozen samples	D. Hodgson s.n.	-54.62, 158.86 [1]	MF180530	-	-
H JH6872 Argentina	Argentina	Antarctic region	H JH6872	J. Hyvönen	-54.49, -68.33	-	MF180382	-
AAS 5456 Falkland Is.	Falkland Is.	Antarctic region	AAS	R.E. Longton 05456	-51.67, -57.92	-	MF180383	-
H RLS67 S. Orkney Isl.	S. Orkney Is.	Antarctic region	H RLS67	R.I.L. Smith	-60.72, -45.6 [3]	-	MF180384	-
H RLS71 South Georgia	South Georgia	Antarctic region	H RLS71	R.I.L. Smith	-54.16, -36.3	-	MF180385	-
H RLS76 South Georgia	South Georgia	Antarctic region	H RLS76	R.I.L. Smith	-54.1, -36.42	-	MF180386	-
S. Shetland Is., Deception I.	S. Shetland Is.	Antarctic region	BAS frozen samples	E.M. Biersma s.n.	-62.98, -60.71 [1]	MF180531	-	-
S. Shetland Is., Arctowski	S. Shetland Is.	Antarctic region	BAS frozen samples	E.M. Biersma s.n.	-62.16, -58.47 [1]	MF180532	-	-
S. Shetland Is. Hannes Point	S. Shetland Is.	Antarctic region	BAS frozen samples	E.M. Biersma s.n.	-62.64, -60.6 [1]	MF180533	-	-
S. Shetland Is. Barrientos I. 1A	S. Shetland Is.	Antarctic region	BAS / Cambridge U.	J. Royles s.n.	-62.41, -59.75 [1]	MF180534	-	-
S. Shetland Is. Barrientos I. 1B	S. Shetland Is.	Antarctic region	BAS / Cambridge U.	J. Royles s.n.	-62.41, -59.75 [1]	MF180535	-	MF180629
S. Shetland Is. Barrientos I. 1C (1,2,4)	S. Shetland Is.	Antarctic region	BAS / Cambridge U.	J. Royles s.n.	-62.41, -59.75 [1]	-	MF180387	MF180630
S. Shetland Is. Barrientos I. 1C (3)	S. Shetland Is.	Antarctic region	BAS / Cambridge U.	J. Royles s.n.	-62.41, -59.75 [1]	MF180536	-	MF180631
S. Shetland Is. Barrientos I. 1C (5)	S. Shetland Is.	Antarctic region	BAS / Cambridge U.	J. Royles s.n.	-62.41, -59.75 [1]	-	MF180388	-
S. Shetland Is. Barrientos I. 1D	S. Shetland Is.	Antarctic region	BAS / Cambridge U.	J. Royles s.n.	-62.41, -59.75 [1]	-	MF180389	MF180632
S. Shetland Is. Barrientos I. 1D (2)	S. Shetland Is.	Antarctic region	BAS / Cambridge U.	J. Royles s.n.	-62.41, -59.75 [1]	-	MF180390	-
S. Shetland Is. Barrientos I. 1D (5)	S. Shetland Is.	Antarctic region	BAS / Cambridge U.	J. Royles s.n.	-62.41, -59.75 [1]	-	MF180391	-
S. Shetland Is. Barrientos I. 1E	S. Shetland Is.	Antarctic region	BAS / Cambridge U.	J. Royles s.n.	-62.41, -59.75 [1]	MF180537	-	-

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S. Shetland Is. Barrientos I. 3A	S. Shetland Is.	Antarctic region	BAS / Cambridge U.	J. Royles s.n.	-62.41, -59.75 [1]	MF180538	-	-
H 12.II.80 S. Shetland Is.	S. Shetland Is.	Antarctic region	H 12.II.80	Y. Petrov	-62, -58 [1]	-	MF180392	-
AAS 115 Ant. Pen., Fallieres Coast	Antarctic Peninsula	Antarctic region	AAS	J. Killingbeck 00115	-67.73, -68.42	-	MF180393	-
H RLS83 Ant. Pen., Lagoon I.	Antarctic Peninsula	Antarctic region	H RLS83	R.I.L. Smith	-67.58, -68.25 [3]	-	MF180394	-
AAS 2304 Ant. Pen., Danco Coast	Antarctic Peninsula	Antarctic region	AAS	R.E. Longton 02304	-64.58, -63.5	-	MF180395	-
AAS 25 Ant. Pen., Trinity Coast	Antarctic Peninsula	Antarctic region	AAS	C.G. Brading 00025	-63.42, -57.02	-	MF180396	-
AAS 427 Ant. Pen., Fallieres Coast	Antarctic Peninsula	Antarctic region	AAS	B.J. Taylor 00427	-67.73, -68.42	-	MF180397	-
AAS 686B Ant. Pen., Graham Coast	Antarctic Peninsula	Antarctic region	AAS	R.W.M. Corner 00686B	-65.27, -64.13	-	MF180398	-

Longitudes and latitudes not provided with sample. Approximate location found via:

[1] = from http://mynasadata.larc.nasa.gov/latitudelongitude-finder/, [2] = from http://tools.wmflabs.org/geohack/, [3] = coordinates from similar location from AAS herbarium website
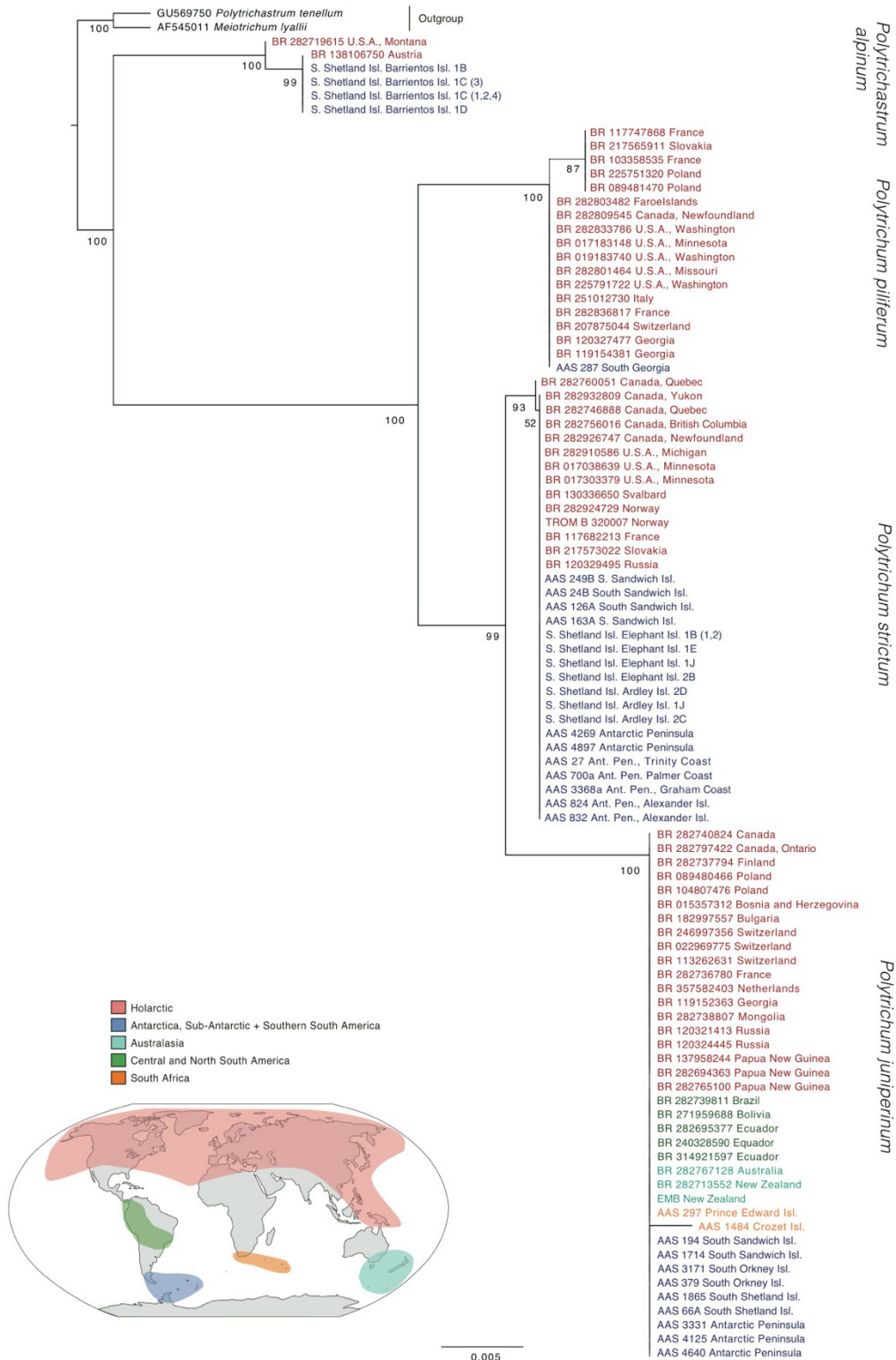
Fig. 8.2.1.2 Maximum Likelihood tree of *trnL-F* for *Polytrichastrum alpinum, Polytrichum. piliferum*, *P. strictum* and *P. juniperinum*. Bootstrap support is provided below or next to the relevant node. Taxon colours refer to the different continents or regions (see map). Outgroups are indicated in black. The scale bar represents the mean number of nucleotide substitutions per site.
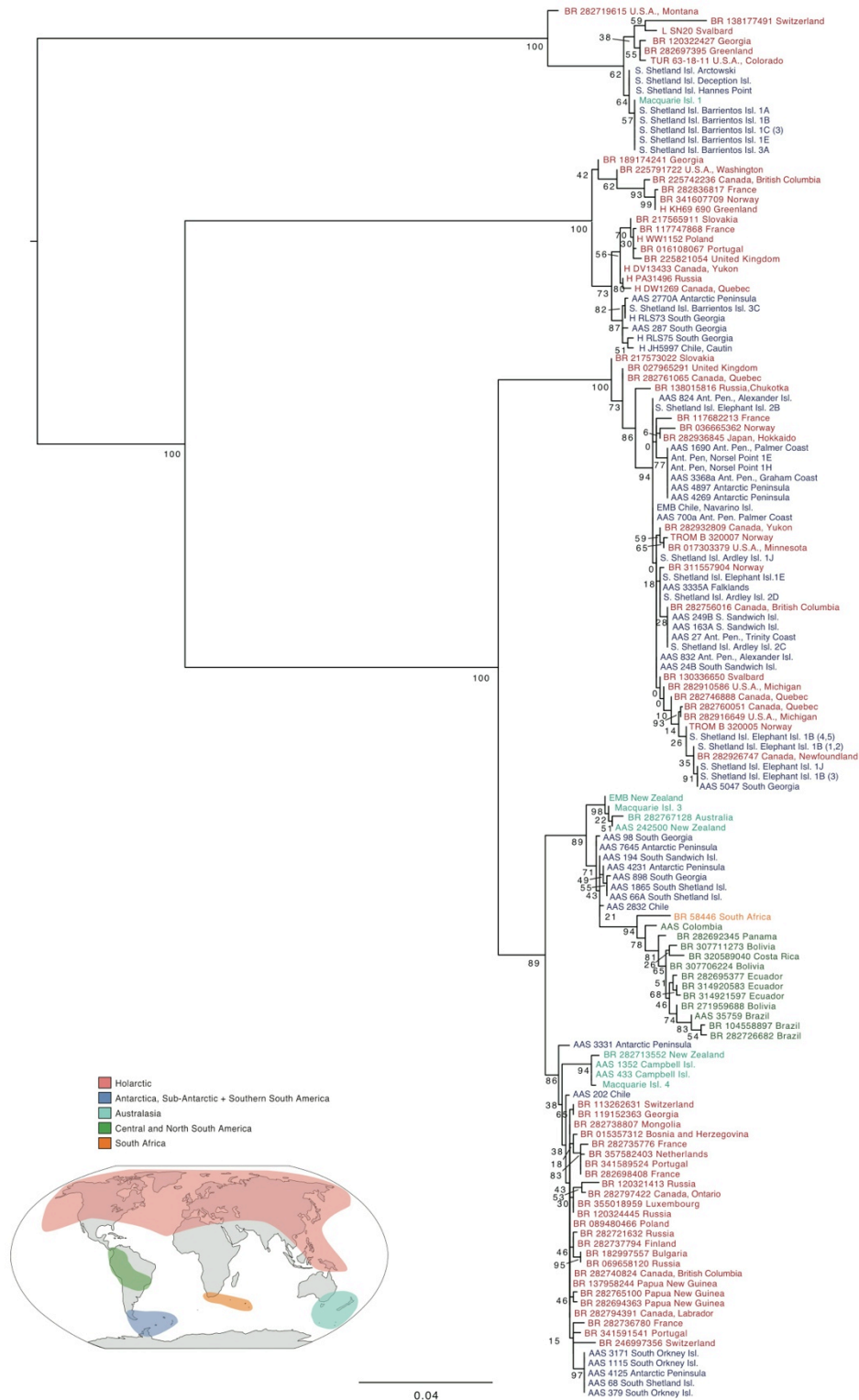
Fig. 8.2.1.3 Maximum Likelihood tree of *ITS* for *Polytrichastrum alpinum, Polytrichum. piliferum, P. strictum* and *P. juniperinum*. Bootstrap support is provided below or next to the relevant node. Taxon colours refer to the different continents or regions (see map). The scale bar represents the mean number of nucleotide substitutions per site.
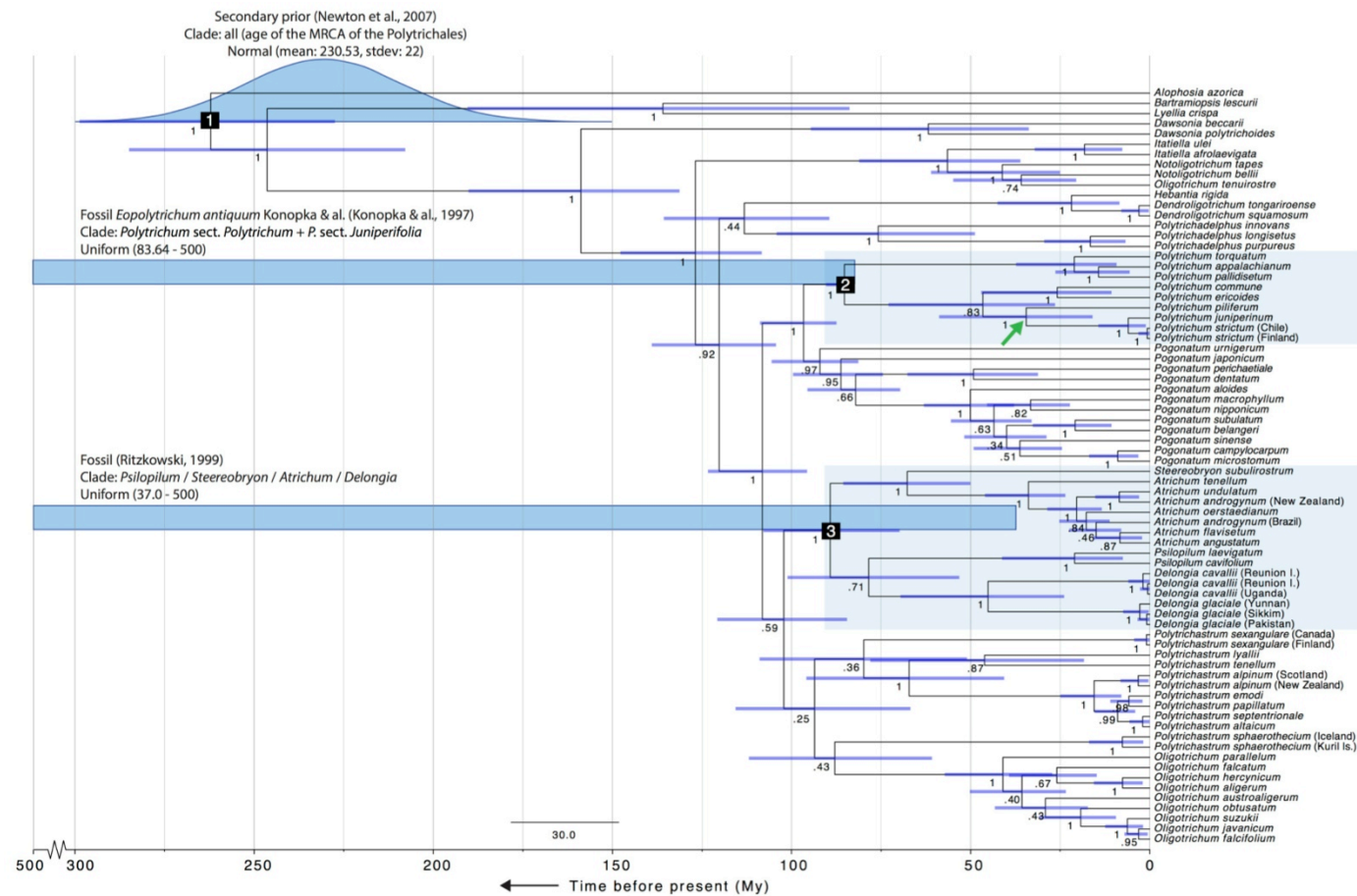
Fig. 8.2.1.4 Analysis I1a (including *Eopolytrichum antiquum*), with a graphic representation of all prior information and the key node of interest (*P. piliferum*/(*P. juniperinum* + *P. strictum*); green arrow). The priors include 1) a normal (230.53, stdev 22) prior on the age of the MRCA of the Polytrichales (Newton *et al.*, 2007); 2) a uniform prior (83.64, 500) on the stem lineage of *Polytrichum* sect. *Polytrichum* + *P.* sect. *Juniperifolia*; and 3) a uniform prior (37.0, 500) on the *Psilopilum*/*Steereobryon*/*Atrichum*/*Delongia* clade (Ritzkowski, 1999). See text on two-step dating analyses for more information.

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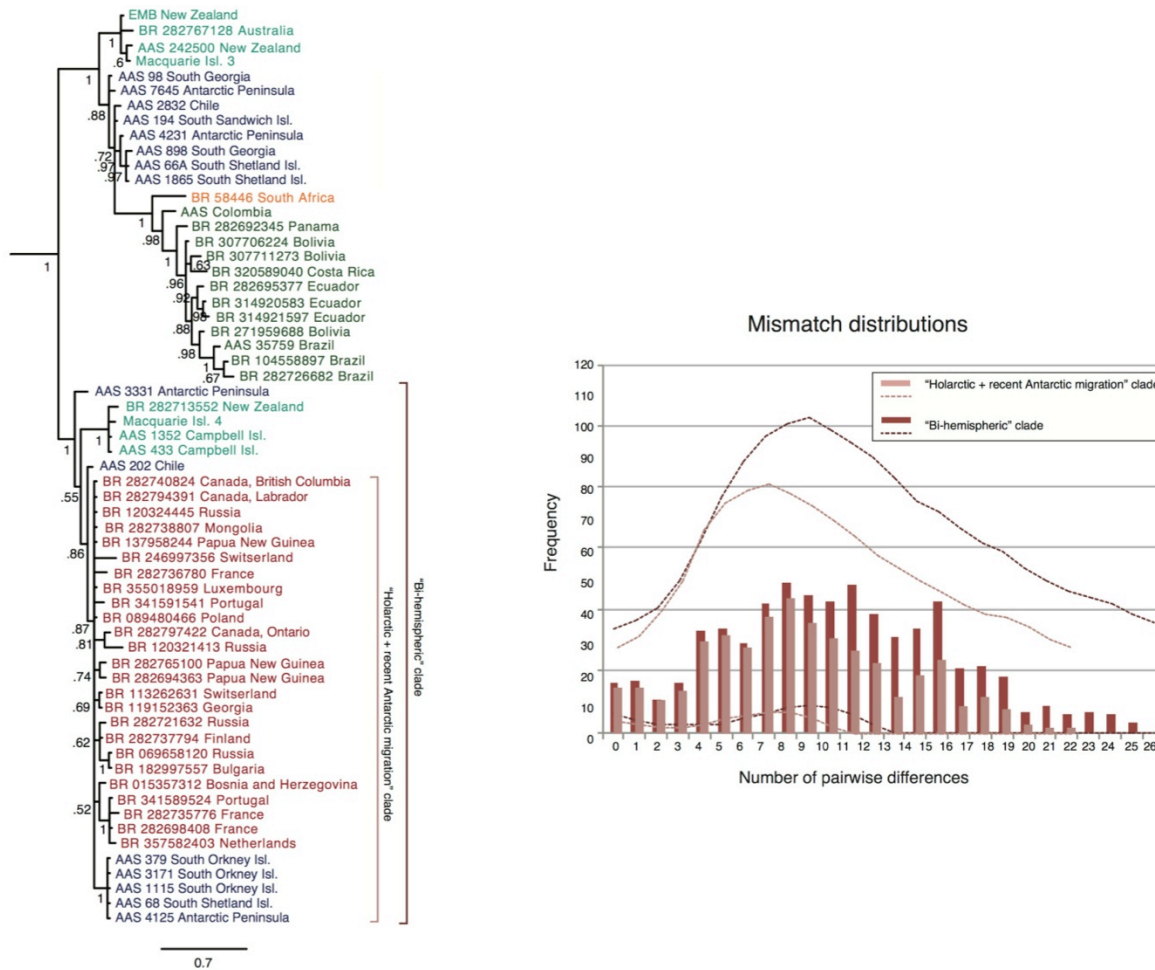
Fig. 8.2.1.5 Observed mismatch distributions (bars) of sub-clades within *Polytrichum juniperinum* compared to the lower and upper bound of the expected distribution (dashed lines). See Fig. 2.4 for details regarding tree; see Table 2.1 for demographic analyses.

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8.2.2 Appendix Chapter 3

Table 8.2.2.1 Sample list including herbarium and location details. Literature references of samples published before are given below.

Label in study	Geographic Origin	Ref.	Herbarium or	Collection	Coordinates	*rps4*	*atpB-rbcL*	*trnL-F*	*ITS*
AAS 03988 Ant. Peninsula	Recess Cove, Charlotte Bay, Ant. Peninsula		AAS	Smith R.I.L. 03988	-64.58, -61.63	GenBank n.	GenBank n.	GenBank n.	GenBank n.
AAS 07625 Ant. Peninsula	James Ross I., Ant. Peninsula		AAS	Smith R.I.L. 07625	-63.87, -57.90	GenBank n.	GenBank n.	GenBank n.	
CP24 S. Orkney Is.	Moe I, off Signy Is., South Orkney Is.	1,2	AAS	Smith R.I.L. 5237	-60.44, -45.41	GenBank n.			GenBank n.
BR 12045376 Papoua New Guinea	Mt. Kaindi, Papua New Guinea		BR 5040120453763	Hoffmann I. 90-414	-7.33, 146.68 [1]	GenBank n.	GenBank n.	GenBank n.	GenBank n.
BR 32032632 Costa Rica	Serra de la Muerte, Costa Rica		BR 5040320326324	Arts. T. CR 14/27	9.56, -83.80 [1]	GenBank n.	GenBank n.	GenBank n.	
BR 27280945 Bolivia	NW of Quime, Bolivia		BR 5040272809456	Lewis M. 87585	-16.95, -67.28	GenBank n.	GenBank n.	GenBank n.	
AY881045 Reunion I.	Piton de la Fournaise, Reunion I.	3	DUKE	Arts REU 44/15	-21.24, 55.71 [1]		AY881045		
BM 20823 Mexico	W of Popocatépetl, Sierra Nevada, Mexico		BM	Herman F.J. 20823	19.02, -98.63 [1]	GenBank n.		GenBank n.	
CP17 Nepal	Sindure, Nepal	1,2	RBGE	Long D.G. 16417	28.17, 84.30	GenBank n.		GenBank n.	
BR 27272962 Greece	Chania, Omalos, Crete, Greece		BR 5040272729624	Gradstein S.R. & Smittenberg J.H. 1214	35.34, 23.90 [1]	GenBank n.	GenBank n.	GenBank n.	GenBank n.
AY881059 Greece	Argalasti, Greece	3	DUKE	Cano & Ros 23.3.1999	39.23, 23.22 [1]		AY881059		
BM 27 Canary Is.	Above Aguamansa, Canary Is.		BM	Champion C.L. 27	28.37, -16.50 [1]	GenBank n.		GenBank n.	
BM R08277a Reunion I.	Le Maido, W of Cirque de Mafate, Reunion I.		BM	Ellis L. & Wilbraham J. R08-277a	-21.40, 55.23	GenBank n.	GenBank n.	GenBank n.	GenBank n.
CP13 Malawi	Lichenya, Malawi, Africa	1,2	RBGE	Longton R.E. M8593A	-15.55, 35.83 [1]	GenBank n.	GenBank n.	GenBank n.	
AY881052 Reunion I.	Piton de la Fournaise, Reunion I.	3	DUKE	Arts REU 44/15	-21.24, 55.71 [1]		AY881052		
AY881057 South Africa	Table Mt, W Cape Prov, Sount	3	DUKE	Hedderson 13371	-33.96, 18.41 [1]		AY881057		
AB980065 Uganda	Rwenzori Mountains, Stanlay Plateau, Uganda		Uetake et al., unpubl.	Uetake J. ("taxon:3225")	0.38, 29.88	AB848717	AB980065		
AY881031 Indonesia	Amlapura, Bali, Indonesia	3	DUKE	Schäfer-Verwimp 21099	-8.46, 115.61 [1]		AY881031		
AY881034 Chile	PN Alerces Osorno, Chile	3	DUKE	Cox 602/00	-41.11, -72.36 [1]		AY881034		
AY881046 Wilkes sector, Antarctica	Casey Base, Antarctica	3	DUKE	Robinson & Wasley 99/00	-66.28, 110.53 [1]		AY881046		

ID	Locality	Ref	Herbarium	Collector	Coordinates	GenBank	GenBank	GenBank	GenBank
AY881048 Chile	Navarino I., Magellanes, Chile	3	DUKE	Goffinet 6990	-55.11, -67.71 [1]			AY881048	
AY881053 Australia	Kangaroo I., S. Australia	3	NY	Streimann 54883	-35.77, 137.21 [1]			AY881053	
AY881051 Tasmania, Australia	Hobart, Tasmania, Australia	3	DUKE	Newton & Bell 5780	-42.88, 147.32 [1]			AY881051	
AY881049 Australia	Oberon, New South Wales,	3	NY	Gilmore 65	-33.70, 149.85 [1]			AY881049	
AY881043 Oregon, USA	Columbia, R. Gorge, Oregon, USA	3	DUKE	Sargent 6.VIII.00	45.71, -121.52 [1]			AY881043	
AY881037 California, USA	Pt. Reyes, NS California, USA	3	DUKE	Shaw 10 090	38.04, -122.80 [1]			AY881037	
AY881040 Australia	Stanthorpe, Queensland, Australia	3	NY	Streimann 52925	-28.65, 151.94 [1]			AY881040	
AAS 03708 Ant. Peninsula	Joinville I., Ant. Peninsula		AAS	Smith R.I.L. 03708	-63.25, -55.75	GenBank n.	GenBank n.	GenBank n.	GenBank n.
AAS 04324 Ant. Peninsula	W of Lahille I., Ant. Peninsula		AAS	Smith R.I.L. 04324	-65.53, -64.37	GenBank n.	GenBank n.	GenBank n.	GenBank n.
BR 01907765 Idaho, USA	Boundary County, Copeland, Idaho, USA		BR 5040019077650	Anderson L.E. 22618	48.90, -116.38 [1]	GenBank n.	GenBank n.	GenBank n.	GenBank n.
BM BM000977576 Montana, USA	Targhee Pass, W Yellowstone, Montana, USA		BM	Stern R.C. BM000977576	44.67, -111.28 [1]	GenBank n.	GenBank n.	GenBank n.	
BM 21661 Alaska, USA	Valdez Quadrangle, Old Valdez, Alaska, USA		BM	Herman F.J. 21661	61.11, -146.26 [1]	GenBank n.	GenBank n.	GenBank n.	GenBank n.
CP25 S. Shetland Is.	West Baily Head, Deception I., S. Shetland Is.	1,2	AAS	Mason D. 00001	-62.58, -60.30	GenBank n.	GenBank n.	GenBank n.	
CP32 Ant Peninsula	Charcot Is, Ant. Peninsula	1,2	AAS	Convey P. 00368	-69.45, -75.15	GenBank n.	GenBank n.	GenBank n.	
CP28 Ant. Peninsula	Danco Coast, Cuverville I, Ant. Peninsula	1,2	AAS	Leeuw C. de 00020	-64.41, -62.38	GenBank n.	GenBank n.	GenBank n.	GenBank n.
CP34 Ant. Peninsula	W Anchorage I., Ant. Peninsula	1,2	AAS	Smith R.I.L. 09212	-67.36, -68.14	GenBank n.	GenBank n.	GenBank n.	GenBank n.
CP30 S. Sandwich Is.	Irving Point, Visokoi I., S.	1,2	AAS	Convey P. 00242	-56.43, -27.04	GenBank n.	GenBank n.	GenBank n.	GenBank n.
AY881058 India	Lam Pokhari, Sikkim, India	3	DUKE	Long 22687	27.50, 88.20 [1]			AY881058	
AY881047 Illinois, USA	Schuyler, Illinois, USA	3	NY	Hill 30219	40.18, -90.63 [1]			AY881047	
AY881036 Alaska, USA	Denali N. P., Alaska, USA	3	DUKE	Vanderpoorten 4998	63.06, -151.00 [1]			AY881036	
BR 02840177 S. Shetland Is.	King George I., Admiralty Bay, S. Shetland Is.		BR 5040028401774	Ochyra R. 4887/79	-62.17, -58.44	GenBank n.		GenBank n.	
AAS 00064 S. Shetland Is.	Deception I., S. Shetland Is.		AAS	Smith R.I.L. 00064	-63.00, -60.52	GenBank n.			
CP23 S. Shetland Is.	Pendulum Cove, Deception I, S. Shetland Is.	1,2	AAS	Smith R.I.L. 11191	-62.56, -60.36	GenBank n.		GenBank n.	GenBank n.
CS30 S. Shetland Is.	Carlini Station, King George I., S. Shetland Is.		WOLL	Casanova-Katny A. CS30	-62.23, -58.67	GenBank n.			
AAS 725 Argentina	Argentina		AAS ACHE	Matteri-Schiavone 725	-54.50, -68.00	GenBank n.	GenBank n.	GenBank n.	

AAS 4545909 Wilkes sector, Antarctica	Clark Pen, Wilkes Sector, Antarctica		AAS ACHE	Seppelt R.D. 5909 ACHE 454	-66.15, 110.36	GenBank n.	GenBank n.	GenBank n.	
AAS 04120 Ant. Peninsula	Omega I., Melchior I., Ant.		AAS	Smith, R.I.L. 04120	-64.33, -62.93	GenBank n.	GenBank n.	GenBank n.	
AAS 09644A Ross sector, Antarctica	Harrow Peaks, Victoria Land.		AAS	Smith, R.I.L. 09644A	-74.07, 164.75	GenBank n.	GenBank n.	GenBank n.	GenBank n.
AAS 00165 S. Orkney Is.	W of Matthews I., S. Orkney Is.		AAS	Smith, R.I.L. 00165	-60.75, -45.17	GenBank n.	GenBank n.	GenBank n.	GenBank n.
Biersma 18 Chile	Navarino I., Magellanes, Chile		BAS collections	Biersma, E.M. 18	-54.93, -67.28 [1]	GenBank n.	GenBank n.	GenBank n.	GenBank n.
BM 33207 Chile	5 km E of Quidico, Prov. Arauco, Chile		BM	Ireland, R.R. & Bellolio, G. 33207	-38.15, -73.27	GenBank n.	GenBank n.	GenBank n.	
CP5 Australia	Mt Beauty, Victoria, Australia	1,2	WOLL ARC	Clarke, L.J. CP5	-36.74, 147.17	GenBank n.	GenBank n.	GenBank n.	GenBank
CP44 Heard I.	Dovers Moraine, Heard I.	1,2	WOLL ARC	Turnbull, J.D. CP44	-53.12, 73.70	GenBank n.	GenBank n.	GenBank n.	GenBank n.
CP21 Maud Land, Antarctica	Ice Axe Peak, Maud Land,	1,2	AAS	Watkins, B.P. 00020A	-71.47, -3.25	GenBank n.	GenBank n.	GenBank n.	GenBank n.
CP39 Heard I.	Paddock Valley, Heard I.	1,2	WOLL ARC	Turnbull, J.D. CP39	-53.08, 73.50	GenBank n.	GenBank n.	GenBank n.	
BR 02155925 Switzerland	Valais, Bagnes, Versegers,		BR 5040021559250	Lawalrée, A. 23282	46.06, 7.23 [1]	GenBank n.	GenBank n.	GenBank n.	GenBank n.
BR 02316781 Switzerland	Valais, Bagnes, Lourtier,		BR 5040023167811	Lawalrée, A. 24147	46.04, 7.26 [1]	GenBank n.	GenBank n.	GenBank n.	GenBank n.
BR 08930464 Poland	Plaine de Walcz, Ilowiec, Poland		BR 5040089304649	Lisowski, S., Rusinska, A. & Melosik, I. 27	50.83, 23.40 [1]	GenBank n.	GenBank n.	GenBank n.	
BR 12018401 Siberia, Russia	Baikal lake, Listvianka, Siberia,		BR 5040120184018	Vašák, V. s.n.	51.85, 104.83 [1]	GenBank n.	GenBank n.	GenBank n.	
BR 13051247 Svalbard	Isbjörnkamna, Honsund, Svalbard		BR 5040130512474	Godzik, B. & Grodzinska, K. s.n.	77.02, 15.57	GenBank n.	GenBank n.	GenBank n.	GenBank n.
BR 13622234 France	Foret de Fontainebleau, France		BR 5040136222346	Bamps, P. 4685	48.41, 2.63 [1]	GenBank n.	GenBank n.	GenBank n.	
BR 30658767 Japan	Campus Hiroshima University, Japan		BR 5040306587671	Yamaguchi, T. & Iwatsuki, Z. 29	36.07, 138.08 [1]	GenBank n.	GenBank n.	GenBank n.	
BR 31255218 Hawaii, USA	Near Pu'u hulu-hulu, Big Island, Hawaii, USA		BR 5040312552182	Arts, T. HAW 11/04	19.37, -155.20 [1]	GenBank n.	GenBank n.	GenBank n.	
BR 31460231 Ecuador	Imbabura, Lago Cuicocha, Equador		BR 5040314602311	Arts, T. 13/007A	0.38, -78.11 [1]	GenBank n.	GenBank n.	GenBank n.	
BR 36261428 Vermont, USA	Chittenden, Vermont, USA		BR 5040362614281	White, H. & White, C. 1158	43.73, -72.94 [1]	GenBank n.	GenBank n.	GenBank n.	GenBank n.
BR 27271447 Hawaii, USA	Oahu, Kaala, Hawaii, USA		BR 5040272714477	Hoe W.J. 3048.0	21.44, -158.00 [1]	GenBank n.	GenBank n.	GenBank n.	GenBank n.
BR 27272356 Quebec, Canada	Iles-de-la-Madeleine, Quebec,		BR 5040272723561	De Sloover J.L. 7786	47.38, -61.88 [1]	GenBank n.	GenBank n.	GenBank n.	GenBank n.
BR 27270639 Colorado, USA	Clear Creek County, Colorado,		BR 5040272706397	Miller J.S. 6887	39.82, 105.75	GenBank n.	GenBank n.	GenBank n.	
CP8 Hawaii, USA	Mauna Kea, Hawaii, USA	1,2	RBGE	Degener O. & Degener I. 34420	19.82, -155.47	GenBank n.	GenBank n.	GenBank n.	GenBank n.(x2)
CP11 New York, USA	New York (Botanical Gardens),	1,2	NY	S.N.	40.86, -73.88	GenBank n.	GenBank n.	GenBank n.	GenBank n.

BM A1003 Alaska, USA	Chandler Lake, Endicott Mts, Alaska, USA		BM	Smith, G.L. A1003	68.12, -152.47	GenBank n.	GenBank n.	GenBank n.	GenBank n.
BM 1228 Japan	Honshu, Tsukuba-shi, Amakubo,		BM	Higuchi, M. 1228	36.06, 140.06	GenBank n.	GenBank n.	GenBank n.	
BR 02873422 UK	Bedfordshire, UK		BR 5040028734223	Jury, S.L. J2359	51.92, 0.66	GenBank n.	GenBank n.	GenBank n.	
AY881062 Morocco	Bab Bou Idir, Morocco	3	DUKE	Cano and Ros 21.4. 1997	34.07, -4.12 [1]		AY881062		
AY881061 Arizona, USA	Madira Canyon, Arizona, USA	3	NY	Schofield 105061	34.05, -111.09 [1]		AY881061		
AY881044 Ecuador	Otavalo, Ecuador	3	DUKE	McDaniel E112	0.23, -78.26 [1]		AY881044		
AY881038 Colorado, USA	Boulder, Colorado, USA	3	DUKE	Weber 19426	40.01, -105.27 [1]		AY881038		
AY881060 New York, USA	Adirondack Mts, New York, USA	3	NY	Miller 11911	43.95, -73.73 [1]		AY881060		
AY881055 Germany	Baden-Württemberg, Germany	3	DUKE	Schäfer-Verwimp 25550	48.66, 9.35 [1]		AY881055		
AY881054 Alberta, Canada	Edson, Alberta, Canada	3	NY	Schofield 103437	53.58, -116.44 [1]		AY881054		
AY881050 Siberia, Russia	Kurgan, Siberia, Russia	3	DUKE	Kurbatova 21 V 2000	55.46, 65.30 [1]		AY881050		
AY881042 New York, USA	Ithaca, New York, USA	3	DUKE	Shaw 12.VI. 2000	42.44, -76.50 [1]		AY881042		
AY881041 Sweden	Hamra, Halsingland, Sweden	3	S	Hedenäs B38313	61.66, 15.01 [1]		AY881041		
AY881039 Liaoning, China	Baishilaji Reserve, Liaoning, China	3	NY	Buck 23783	41.83, 123.43 [1]		AY881039		
AY881035 Nova Scotia,	Digby, Nova Scotia, Canada	3	NY	Schofield 95576	44.62, -65.76 [1]		AY881035		
AY881033 France	Soultzeren, Alsace, France	3	DUKE	Untereiner 12.05.00	48.06, 7.10 [1]		AY881033		
AY881032 Germany	Baden-Württemberg, Germany	3	DUKE	Schäfer-Verwimp 25550	48.66, 9.35 [1]		AY881032		
AY881056 Pennsylvania, USA	Palmerton, Pennsylvania, USA	3	DUKE	McDaniel B135	40.80, -75.61 [1]		AY881056		
Biersma 21 Chile	Navarino I., Magellanes, Chile		BAS collections	Biersma, E.M. 21	-54.95, -67.26 [1]	GenBank n.		GenBank n.	GenBank n.
BM 31149 Chile	S of Contulmo, Prov. Arauco, Chile		BM	Ireland, R.R. & Bellolio, G. 31149	-38.00, -73.13	GenBank n.		GenBank n.	GenBank n.
BM 33951 Chile	Waterfalls near Santa Juana, S of Curali, Chile		BM	Ireland, R.R. & Bellolio, G. 33951	-37.15, -72.57	GenBank n.		GenBank n.	
CP16 Australia	Australian Nat. Uni., Canberra, Australia	2	WOLL ARC	Clarke, L.J. CP16	-35.28, 149.12	GenBank n.		GenBank n.	
219 Wilkes sector, Antarctica	Clark Peninsula, Antarctica	2,4	WOLL ARC	Robinson, S.A. 219	-66.25, 110.57	GenBank n.		GenBank n.	GenBank n.
Biersma 1 Chile	Navarino I., Magellanes, Chile		BAS collections	Biersma, E.M. 1	-54.93, -67.60 [1]	GenBank n.			GenBank n.
Biersma 4 Chile	Navarino I., Magellanes, Chile		BAS collections	Biersma, E.M. 4	-54.93, -67.61 [1]	GenBank n.			GenBank n.
Biersma 20 Chile	Navarino I., Magellanes, Chile		BAS collections	Biersma, E.M. 20	-54.95, -67.21 [1]	GenBank n.			
BR 27264979 Australia	Leura Gap, Bimberi Range, Canberra, Australia		BR 5040272649793	Streimann H. 34959	-35.63, 148.77	GenBank n.			
CP6 Wilkes sector, Antarctica	Melt Lake, Casey, Windmill Is., Antarctica	2,4	WOLL ARC	Robinson, S.A. CP6	-66.33, 110.47 [1]	GenBank n.		GenBank n.	

Name	Location		Collection	Collector	Coordinates	GenBank	GenBank	GenBank
CP7 Australia	Macquarie University, Ryde, Sydney, Australia	2	WOLL ARC	Downing, A. CP7	-33.77, 151.11	GenBank n.		
76 Wilkes sector, Antarctica	Red Shed, Casey, Windmill Is., Antarctica	2,4	WOLL ARC	Robinson, S.A. 76	-66.28, 110.53	GenBank n.		
CPMP Wilkes sector, Antarctica	Melt puddle, Casey, Windmill Is., Antarctica	2,4	WOLL ARC	Robinson, S.A. CPMP	-66.28, 110.54	GenBank n.		
WPD8 Wilkes sector, Antarctica	Clark Peninsulla, Antarctica	2,4	WOLL ARC	Robinson, S.A. WPD8	-66.25, 110.56	GenBank n.		
B10 Australia	Wollongong, Australia	2	WOLL ARC	Wyber, R. B10	-34.40, 150.86	GenBank n.		
B11 Australia	Wollongong, Australia	2	WOLL ARC	Wyber, R. B11	-34.40, 150.86	GenBank n.		
B12 Australia	Wollongong, Australia	2	WOLL ARC	Wyber, R. B12	-34.40, 150.86	GenBank n.		
B20 Wilkes sector, Antarctica	Casey Station, Windmill Is.,	2,4	WOLL ARC	Robinson, S.A. B20	-66.28, 110.69	GenBank n.		GenBank n.
WPD5 Wilkes sector, Antarctica	Clark Peninsulla, Antarctica	2,4	WOLL ARC	Robinson, S.A. WPD5	-66.25, 110.56	GenBank n.		GenBank n.
166CP Wilkes sector, Antarctica	Melt Lake, ASPA135, Windmill Is., Antarctica	2,4	WOLL ARC	Robinson, S.A. 166CP	-66.28, 110.54	GenBank n.		
Biersma 2 Chile	Navarino I., Magellanes, Chile		BAS collections	Biersma, E.M. 2	-54.93, -67.60 [1]	GenBank n.		
CP20 Victoria Land, Antarctica	Edmonson Pnt, Victoria Land, Antarctica	1,2	AAS	Smith R.I.L. S.N.	-74.20, 165.08	GenBank n.	GenBank n.	
AJ554004 UK	Berkshire, Reading, UK	5	RNG	S.N.	51.45, -0.98 [1]	AJ554004		
BR 01726701 Wisconsin, USA	St Croix Falls, Wisconsin, USA		BR 5040017267015	Janssens, J.A. 8222	45.41, -92.64 [1]	GenBank n.	GenBank n.	
BR 31381116 Spain	Erjos, near the Casa Forestal,		BR 5040313811165	Arts, T. 24078	28.29, -16.62 [1]	GenBank n.	GenBank n.	GenBank n.
BR 33783783 Luxemburg	Consdorf, Kalkesbaach, Luxemburg		BR 5040337837837	Arts, T. 15180	49.82, 6.35 [1]	GenBank n.	GenBank n.	
BM 14349 British Colombia,	Howe Sound, British Columbia,		BM	Schofield, W.B. 14349	49.15, 123.15	GenBank n.	GenBank n.	GenBank n.
BR 01997691 Poland	Sejny Lake District, Giby-Podkaczan, Poland		BR 5040019976915	Ochyra R. 220	54.11, 23.35 [1]	GenBank n.		GenBank n.
BR 33783581 Switzerland	Luzern, Gütschenwald, Switzerland		BR 5040337835819	Arts, T. 1218	47.05, 8.28 [1]	GenBank n.		GenBank n.
BR 11900583 Siberia, Russia	Pikhtovaia gora, Bratsk, Siberia,		BR 5040119005836	Vašák, V. s.n.	56.17, 101.60 [1]	GenBank n.		GenBank n.
BR 01997893 Cuacasus,	Itkol, Baksan, Mnt. Elbrus,		BR 5040019978933	Vašák, V. s.n.	43.68, 43.54 [1]	GenBank n.		GenBank n.
BR 16775643 British Columbia, Canada	SE of Chilliwack Lake, British Columbia, Canada		BR 5040167756438	Schofield, W.B. 57917	49.13, 121.23	GenBank n.		
BR 31460433 Equador	Volcan Cotopaxi, near Sindipamba, Equador		BR 5040314604339	Arts, T. 23/026A	-0.68, -78.43 [1]	GenBank n.		

AF435271 British Columbia, Canada	Bennett Bay, Mayne I., British Columbia, Canada	6	DUKE	Belland & Schofield 17530	48.84, -123.24	AF435271		
FJ572605 NE China	Changbai, NE China	7	SHNU CB158	S.N.	42.00, 128.00	FJ572605		
FJ572589 NE China	Changbai, NE China	7	SHNU CB43	S.N.	42.00, 128.00	FJ572589		
AY908122 UK	St. Abbs, Scotland, UK	8	DUKE	Long, D.L. 21805	55.89, -2.14[1]	AY908122		
AY908121 W Virginia, USA	Larenim County Park, W Virginia, USA	8	DUKE	Bachmann 538	38.59, -80.45[1]	AY908121		
AAS 06209B Wilkes sector, Antarctica	E of Wilkes Station, Clark Peninsula, Antarctica		AAS	Smith, R.I.L. 06209B	-66.25, 110.55		GenBank n.	GenBank n.
AAS 06213A Wilkes sector, Antarctica	E of Wilkes Station, Clark Peninsula, Antarctica		AAS	Smith, R.I.L. 06213A	-66.25, 110.55		GenBank n.	GenBank n.
BR 33783278 Madeira	Madeira, Boca de Encumeade		BR 5040337832788	Arts, T. 15982	32.76, -16.96[1]		GenBank n.	
AAS 363835 New Zealand	Doubtful Sound, Fjordland, New Zealand		AAS CHR	Allan, H.H. 363835	-45.43, 167.72[1]		GenBank n.	
AAS 02192 Enderby sector Antarctica	West Ongul Island.		AAS	Shimizu, H. 02192	-69.02, 39.53		GenBank n.	
AAS 05301A Ant. Peninsula	Dundee I., Trinity Peninsula, Ant. Peninsula		AAS	Booth, R.G. 05301A	-63.48, -56.25		GenBank n.	
AAS BA 02827 Chile	Puerto Toro, Prov. Magallanes, Chile		AAS BA	Matteri, C.M. 02827	-51.40, -73.07		GenBank n.	
CP02 Australia	Lucas Heights, Australia		WOLL ARC	Clarke, L.J. CP02	-34.05, 150.98		GenBank n.	
AAS 03231 S. Orkney Is.	Coronation I., S. Orkney Is.		AAS	Smith, R.I.L. 03231	-60.67, -45.60		GenBank n.	
BR 10334136 France	Aude, Pic de Nore, France		BR 5040103341360	De Sloover J.L. 45210	43.42, 2.46[1]		GenBank n.	GenBank n.
AAS 00139 Chile	S shore Lago Porteno, Prov. Magallanes, Chile		BA AAS	Greene, S.W. 00139	-51.37, -72.75		GenBank n.	
AAS 09876A Victoria Land, Antarctica	Harrow Peaks, Victoria Land, Antarctica		AAS	Smith, R.I.L. 09876A	-74.07, 164.75		GenBank n.	GenBank n.
AY156591 Victoria Land,	Edmonson Point, N. Victoria Land	9	-	S.N.	-74.03, 165.00			AY156591
AY613336 Victoria Land,	Crater Cirque, N. Victoria Land	9	-	S.N.	-72.62, 169.37			AY613336
AY156589 Macquarie I.	Macquarie I.	9	-	S.N.	-54.62, 158.85			AY156589
AY156590 Victoria Land,	Botany Bay, Victoria Land,	9	-	S.N.	-76.88, 162.73			AY156590
AY156587 Australia	Sydney, Australia	9	-	S.N.	-33.87, 151.20			AY156587
AY156586 Australia	Canberra, Australia	9	-	S.N.	-35.30, 149.13			AY156586
AAS 00070A S. Shetland Is.	Deception I., S. Shetland Is.		AAS	Smith, R.I.L. 00070A	-63.00, -60.57			GenBank n.
BR 30659878 Italy	Sardegna, Broncu Spina, Gennargentu, Italy		BR 5040306598783	Pedrotti, C.C. s.n.	40.12, 9.01[1]			GenBank n.

| AY156588 Heard I. | Heard I. | 9 | - | S.N. | -53.10, 73.52 | AY156588 |

[1] = Longitudes and latitudes not provided with sample. Approximate location found via http://mynasadata.larc.nasa.gov/latitudelongitude-finder/

References: 1: (Clarke *et al.*, 2008), 2: (Wyber, 2013), 3: (McDaniel & Shaw, 2005), 4: (Clarke *et al.*, 2009), 5: (Hedderson *et al.*, 2004), 6: (La Farge *et al.*, 2002), 7: (Liu *et al.*, 2010), 8: (Shaw *et al.*, 2005), 9: (Skotnicki *et al.*, 2004)
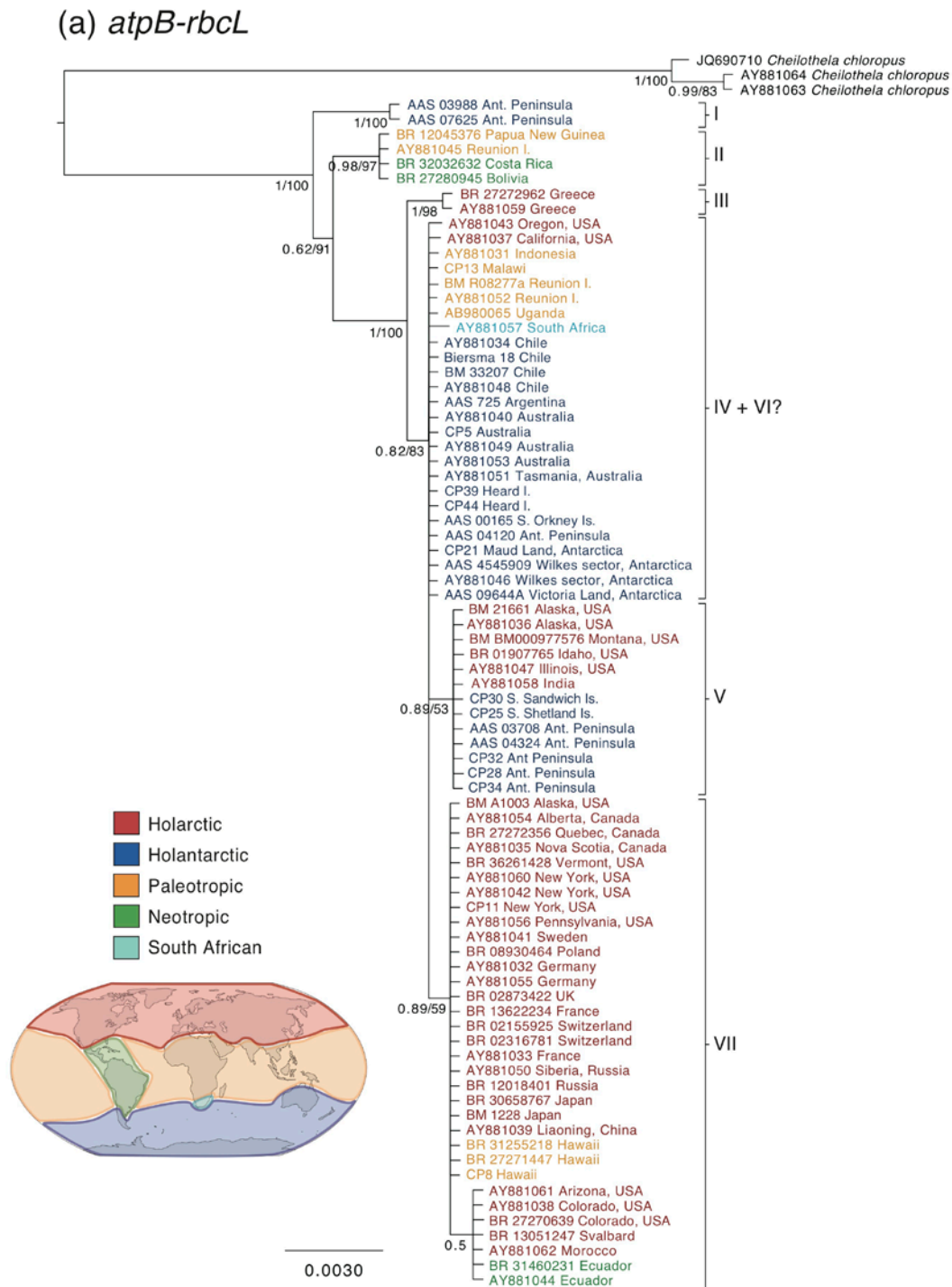
Fig. 8.2.2.1 Bayesian phylogenies for *Ceratodon purpureus* constructed with cpDNA markers (a) *atpB-rbcL*, (b) *rps4*, (c) *trnL-F* and (d) a concatenated dataset of *atpB-rbcL* and *rps4* (for b-d see continuation in the next three pages). Taxon colours refer to the bryofloristic kingdoms of the world (see map; redrawn from Schofield, 1992) and do not follow the same colour scheme as Fig. 3.3 and 3.4 in Chapter 3, as singe gene trees do not show evidence of all clades. Outgroups are shown in black. The scale bar represents the mean number of nucleotide substitutions per site.

Fig. 8.2.2.1 (continued)

(b) *rps4*

AY908124 *Cheilothela chloropus*
AJ554005 *Cheilothela chloropus*
1/100

AAS 03988 Ant. Peninsula
AAS 07625 Ant. Peninsula
CP24 S Orkney Is.
1/100 — I

BR 27272962 Greece
BM 27 Canary Is.
1/99 — III

CP17 Nepal
BR 12045376 Papua New Guinea
BM 20823 Mexico
BR 32032632 Costa Rica
BR 27280945 Bolivia
1/95 / 1/98 — II

BM A1003 Alaska, USA
AF435271 British Columbia, Canada
BM 14349 British Colombia, Canada
BR 16775643 British Columbia, Canada
BR 27272356 Quebec, Canada
BR 36261428 Vermont, USA
BR 01726701 Wisconsin, USA
BR 27270639 Colorado, USA
CP11 New York, USA
AY908121 W Virginia, USA
BR 11900583 Siberia, Russia
BR 01997893 Cuacasus, Russia
BR 12018401 Russia
BM 1228 Japan
BR 30658767 Japan
FJ572605 NE China
FJ572589 NE China
BR 13051247 Svalbard
BR 08930464 Poland
BR 01997691 Poland
BR 02873422 UK
AJ554004 UK
AY908122 UK
BR 33783783 Luxemburg
BR 02155925 Switzerland
BR 02316781 Switzerland
BR 33783581 Switzerland
BR 13622234 France
BR 31381116 Spain
CP8 Hawaii
BR 27271447 Hawaii
BR 31255218 Hawaii
CP13 Malawi
BM R08277a Reunion I.
AB848717 Uganda
BR 31460433 Equador
BR 31460231 Ecuador
0.96/92 — IV + VII?

BM 21661 Alaska, USA
BM BM000977576 Montana, USA
BR 01907765 Idaho, USA
CP30 S. Sandwich Is.
AAS 00064 S. Shetland Is.
BR 02840177 S. Shetland Is.
CP23 S. Shetland Is.
CS30 S. Shetland Is.
CP25 S. Shetland Is.
AAS 03708 Ant. Peninsula
AAS 04324 Ant. Peninsula
CP32 Ant. Peninsula
CP28 Ant. Peninsula
CP34 Ant. Peninsula
0.98/95 — V

AAS 725 Argentina
BM 33207 Chile
BM 31149 Chile
BM 33951 Chile
Biersma 1 Chile
Biersma 4 Chile
Biersma 20 Chile
Biersma 21 Chile
BR 27264979 Australia
CP5 Australia
CP7 Australia
CP16 Australia
B10 Australia
B11 Australia
B12 Australia
CP44 Heard I.
AAS 00165 S. Orkney Is.
AAS 04120 Ant. Peninsula
CP6 Wilkes sector, Antarctica
76 Wilkes sector, Antarctica
CPMP Wilkes sector, Antarctica
WPD8 Wilkes sector, Antarctica
219 Wilkes sector, Antarctica
WPD5 Wilkes sector, Antarctica
B20 Wilkes sector, Antarctica
166CP Wilkes sector, Antarctica
AAS 4545909 Wilkes sector, Antarctica
0.98/94 — VI

Biersma 2 Chile
Biersma 18 Chile
CP39 Heard I.
CP21 Maud Land, Antarctica
CP20 Victoria Land, Antarctica
AAS 09644A Victoria Land, Antarctica
1/96

Holarctic
Holantarctic
Paleotropic
Neotropic
South African

0.0020

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Fig. 8.2.2.1 (continued)

(c) *trnL-F*

Legend:
- Holarctic
- Holantarctic
- Paleotropic
- Neotropic
- South African

Scale: 0.0020

Fig. 8.2.2.1 (continued)

(d) *rps4 + atpB-rbcL*

Fig. 8.2.2.2 Haplotype networks of cpDNA markers (a) *atpB-rbcL* (b) *rps4*, (c) *trnL-F*, and (d) all cpDNA markers combined within the species *Ceratodon purpureus* with geographical information. Haplotype circle sizes correspond to the number of specimens. Colours refer to the bryofloristic kingdoms of the world (see map; redrawn from Schofield, 1992) and do not follow the same colour scheme as Fig. 3.3 in Chapter 3, as singe loci do not show evidence of all clades. Branches represent mutations between haplotypes, with mutations shown as 1-step edges.
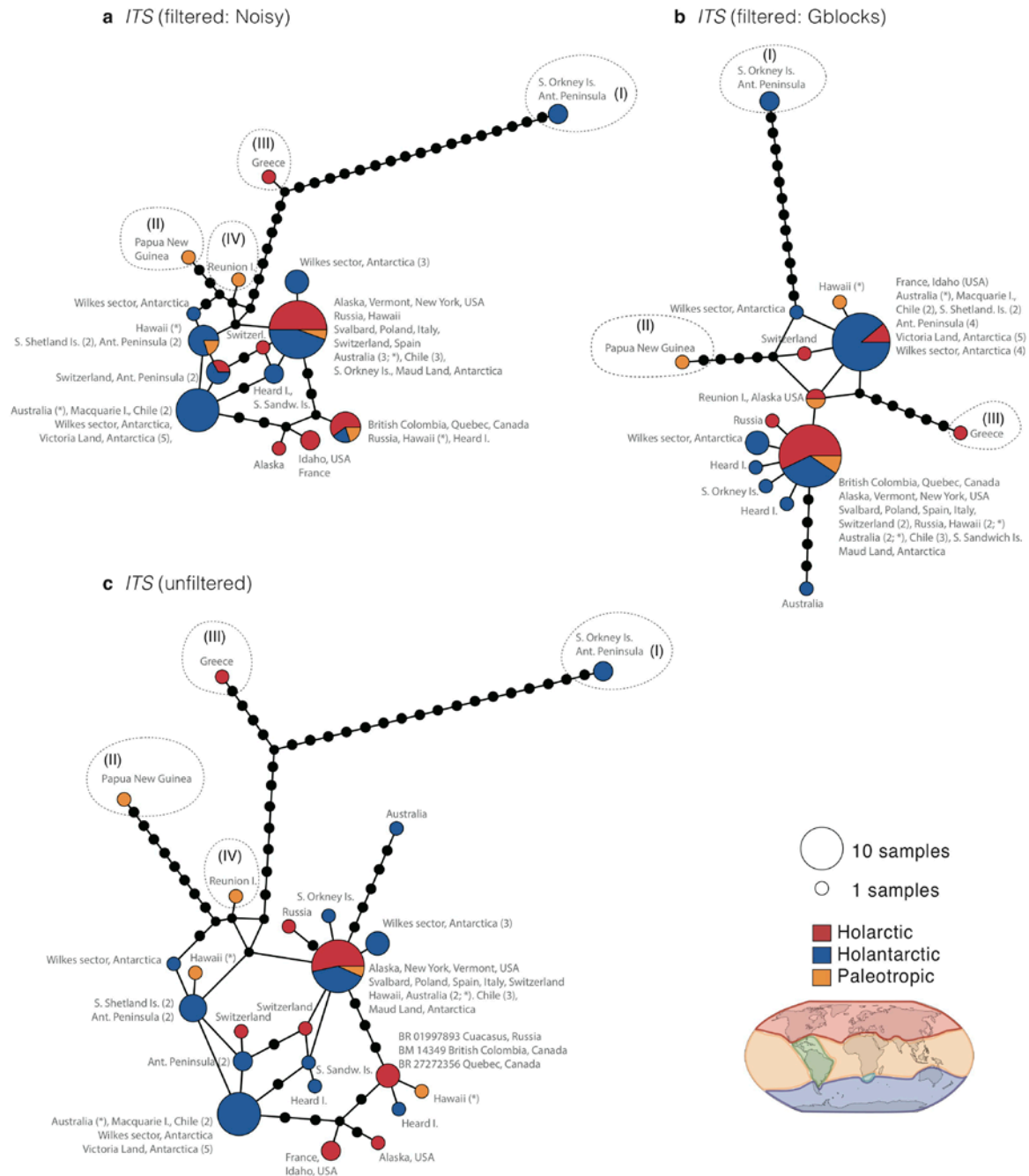
Fig. 8.2.2.3 Haplotype networks of *ITS* within the species *Ceratodon purpureus*, after treatment with NOISY (a) Gblocks (b) or as original data (c). Haplotype circle sizes and colours correspond to the number of specimens and globally recognised bryofloristic kingdoms (see legend; Schofield, 1992), respectively. Branches represent mutations between haplotypes, with mutations shown as 1-step edges.

8.2.3 Appendix Chapter 4

Table 8.2.3.1 *Chorisodontium* specimens used in Chapter 4 including herbarium details, collection coordinates (in decimal degrees) and accession numbers. Specimens include *C. aciphyllum* as well as several specimens from other *Chorisodontium* species (if species name is not mentioned the specimen is identified as *C. aciphyllum)*. SSI= South Shetland Islands, AP= Antarctic Peninsula. Numbers in brackets behind some taxa from the South Shetland Islands and the Antarctic Peninsula represent the number of replicates of a particular location (within ~5 cm) with identical haplotypes. In case of identical sequences in all replicates of one location (e.g. SSI, Ardley I. 1A (4)) only one sequence is uploaded to GenBank. U. Cambr. = University of Cambridge

| Specimen (Species, Geographic origin, herbarium no.)	Herbarium/ Collection + Coll.	Collection	Latitude + Longitude	*ITS*	*rps4*	*trnL-F*
Chile 11472A	AAS 11472A	Smith, R.I.L.	-55.98,-67.27	GenBank n.	GenBank n.	GenBank n.
C. magellanicum, Chile 00522	AAS 00522	Roivainen, H.	-54.56,-69.80 a	GenBank n.		
Chile 00507	AAS 00507	Roivainen, H.	-54.45,-70.67	GenBank n.		
Chile 00504	AAS 00504	Roivainen, H.	-54.45,-70.67			GenBank n.
C. lanigerum, Chile 00512	AAS 00512	Roivainen, H.	-54.45,-70.67	GenBank n.		
C. spegazzini, Chile 00523	AAS 00523	Roivainen, H.	-54.08,-71.03	GenBank n.		GenBank n.
Argentina 00173	AAS 00173	Castellanos	-54.78,-64.25	GenBank n.		
Argentina 00712	AAS 00712	Matteri, C.M.	-54.30,-68.00	GenBank n.		
C. dicranellatum, Argentina 00509	AAS 00509	Roivainen, H.	-53.60,-69.55 b	GenBank n.		
C. dicranellatum, Argentina 00511	AAS 00511	Roivainen, H.	-53.64,-69.65 b	GenBank n.		
C. sphagneticola, Chile 00525	AAS 00525	Roivainen, H.	-53.64,-69.65 b	GenBank n.		GenBank n.
Chile 02015	AAS 02015	Matteri, C.M.	-51.47,-73.27	GenBank n.		GenBank n.
C. sp, Chile 00355	AAS 00355	Pisano, E.	-52.08,-71.92	GenBank n.		GenBank n.

Falkland Is. 5440	AAS 5440	Smith, R.I.L.	-51.68,-58.83 a	GenBank n.		GenBank n.
Falkland Is. 00131A	AAS 00131A	Engel, J.J.	-51.75,-59.50	GenBank n.		
South Georgia 05031	AAS 05031	Smith, R.I.L.	-54.00,-38.08	GenBank n.	GenBank n.	GenBank n.
South Georgia 00295	AAS 00295	Briggs, M.	-54.30,-36.52	GenBank n.	GenBank n.	GenBank n.
South Georgia 00291	AAS 00291	Cable, S.	-54.18,-36.72	GenBank n.	GenBank n.	GenBank n.
South Georgia 01154	AAS 01154	Smith, R.I.L.	-54.28,-36.50	GenBank n.		
S. Orkney Is. 04965	AAS 04965	Walton,	-60.63,-45.58	GenBank n.		GenBank n.
S. Orkney Is. 05251	AAS 05251	Smith, R.I.L.	-60.73,-45.68	GenBank n.	GenBank n.	GenBank n.
S. Orkney Is. 08007	AAS 08007	Smith, R.I.L.	-60.60,-46.05	GenBank n.	GenBank n.	GenBank n.
SSI, Ardley I. 1A (4)	U. Cambr. 1A (1-4)	Royles, J.	-62.21,-58.93	GenBank n.		GenBank n.
SSI, Ardley I. 1B (5)	U. Cambr. 1B (1-5)	Royles, J.	-62.21,-58.93	GenBank n.	GenBank n.	GenBank n.
SSI, Ardley I. 1D (5)	U. Cambr. 1D (1-5)	Royles, J.	-62.21,-58.93	GenBank n.		GenBank n.
SSI, Ardley I. 2A (5)	U. Cambr. 2A (1-5)	Royles, J.	-62.21,-58.94	GenBank n.	GenBank n.	
SSI, Ardley I. 2E (5)	U. Cambr. 2E (1-5)	Royles, J.	-62.21,-58.94	GenBank n.		
SSI, Elephant I. 1A b (1)	U. Cambr. 1A (1)	Royles, J.	-61.14,-54.70	GenBank n.	GenBank n.	
SSI, Elephant I. 1C a (2)	U. Cambr. 1C (2)	Royles, J.	-61.14,-54.70	GenBank n.		
SSI, Elephant I. 1C b (1)	U. Cambr. 1C (1)	Royles, J.	-61.14,-54.70	GenBank n.		
SSI, Elephant I. 1D a (2)	U. Cambr. 1D (2)	Royles, J.	-61.14,-54.70	GenBank n.		
SSI, Elephant I. 1D b (3)	U. Cambr. 1D (2)	Royles, J.	-61.14,-54.70	GenBank n.		
SSI, Elephant I. 2A a (4)	U. Cambr. 2A (4)	Royles, J.	-61.14,-54.70	GenBank n.		
SSI, Elephant I. 2A b (1)	U. Cambr. 2A (1)	Royles, J.	-61.14,-54.70	GenBank n.		
SSI, Elephant I. 3A a (4)	U. Cambr. 3A (4)	Royles, J.	-61.14,-54.71	GenBank n.	GenBank n.	

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SSI, Elephant I. 3B a (1)	U. Cambr. 3B (1)	Royles, J.	-61.14,-54.71	GenBank n.	GenBank n.	
SSI, Elephant I. 3B b (4)	U. Cambr. 3B (4)	Royles, J.	-61.14,-54.71	GenBank n.	GenBank n.	GenBank n.
SSI, Robert I.	BAS s.n.	Biersma, E.M.	-62.38,-59.66	GenBank n.	GenBank n.	GenBank n.
AP, Norsel Point 1A (5)	U. Cambr. 1A (1-5)	Royles, J.	-64.76,-64.08	GenBank n.	GenBank n.	GenBank n.
AP, Norsel Point 1B (5)	U. Cambr. 1B (1-5)	Royles, J.	-64.76,-64.08	GenBank n.	GenBank n.	GenBank n.
AP, Norsel Point 1C (5)	U. Cambr. 1C (1-5)	Royles, J.	-64.76,-64.08	GenBank n.		GenBank n.
AP, Norsel Point 2A (5)	U. Cambr. 2A (1-5)	Royles, J.	-64.76,-64.08	GenBank n.	GenBank n.	GenBank n.
AP, Norsel Point 2B (5)	U. Cambr. 2B (1-5)	Royles, J.	-64.76,-64.08	GenBank n.		GenBank n.
AP, Norsel Point 2C (5)	U. Cambr. 2C (1-5)	Royles, J.	-64.76,-64.08	GenBank n.		GenBank n.
AP, Danco Coast 11938A	AAS 11938A	Smith, R.I.L.	-64.68,-62.63	GenBank n.		GenBank n.
AP, Danco Coast 08801	AAS 08801	Weinstein, R.	-64.68,-62.63	GenBank n.	GenBank n.	GenBank n.
AP, Graham Coast 10661	AAS 10661	Fowbert, J.A.	-65.28,-64.13		GenBank n.	GenBank n.
C. mittenii Bolivia AY908107	MO 19750	Churchill et al	-16.27,-67.83		AY908107	
C. mittenii AF435272/AF435311	DUKE PV 1515	Griffin &	-		AF435272	AF435311
C. setaceum AF435273/AF435312	DUKE 9168	Allen	-		AF435273	AF435312

a/b = Longitudes and latitudes not provided with sample. Approximate location found via:

a = http://mynasadata.larc.nasa.gov/latitudelongitude-finder/

b= Global Plants database; http://plants.jstor.org/

8.2.4　Appendix Chapter 5

Table 8.2.4.1 *Schistidium* specimens used in Chapter 5 including herbarium details, collection coordinates (in decimal degrees) and accession numbers. Species clades as found in the study are given (left column), as well as the original identification (as listed on the herbarium voucher). Numbers in brackets behind specimens represent replicates (additional extraction of the same herbarium sample) of particular specimens. U. Cambr. = University of Cambridge

Clade, collection no. + geographical area	Original identification	Herbarium + no. (or accession no. if published before)	Collector	Latitude + Longitude		Location details	*ITS*
Schistidium sp.							
9346 Ant. Pen., Alexander I.	*S. antarctici*	AAS 09346	Smith, R.I.L.	-71.33	-68.28	Fossil Bluff, Alexander I.	GenBank n.
9322 Ant. Pen., Alexander I.	*S. antarctici*	AAS 09322	Smith, R.I.L.	-71.33	-68.28	Fossil Bluff, Alexander I.	GenBank n.
508 Ant. Pen., Alexander I.	*S. antarctici*	AAS 00508	Taylor, B.J.	-71.33	-68.28	Fossil Bluff, Alexander I.	GenBank n.
S. leptoneurum							
11296A S. Shetland I.	*S. leptoneurum*	AAS 11296A	Smith, R.I.L.	-62.97	-60.70	Fumarole Bay, Deception I.	GenBank n.
9123 Ant. Pen., near Adelaide I.	*S. antarctici*	AAS 09123	Smith, R.I.L.	-67.6	-68.35	N side of Leonie I.	GenBank n.
S. amblyophyllum/S.cupulare							
11171 S. Shetland I.	*S. amblyophyllum*	AAS 11171	Smith, R.I.L.	-62.95	-60.55	N of Mt. Pond, Deception I.	GenBank n.
11288 S. Shetland I.	*S. amblyophyllum*	AAS 11288	Smith, R.I.L.	-62.97	-60.70	Fumarole Bay, Deception I.	GenBank n.
3788 S. Shetland I.	*S. cupulare*	AAS 03788	Smith, R.I.L.	-62.63	-61.08	Byers Peninsula, Livingston I.	GenBank n.
S. lewis-smithii							
3787 S. Shetland I.	*S. lewis-smithii*	AAS 03787	Smith, R.I.L.	-62.63	-61.08	Byers Peninsula, Livingston I.	GenBank n.
S. falcatum							
2818 South Georgia	*S. falcatum*	AAS 02818	Smith, R.I.L.	-54.17	-37.62	N of Samuel Is., South Georgia	GenBank n.
S. andinum/S. apocarpum							
123A South America, Chile	*S. apocarpum*	AAS 00123A	Greene, S.W.	-51.30	-72.72	SE of La Peninsula, Lago del Toro	GenBank n.
3299 South Georgia	*S. apocarpum*	AAS 03299	Bell, B.G.	-54.70	-35.93	Iris Bay, South Georgia	GenBank n.

S. andinum

494 Macquarie I.	_S. apocarpum_	AAS 00494	Seppelt, R.D.	-54.50	158.95	Macquarie I.	GenBank n.
7468B NE Ant. Pen., James Ross I.	_S. antarctici_	AAS 07468B	Smith, R.I.L.	-63.93	-57.82	James Ross I.	GenBank n.
11534 S. Shetland Is.	_S. andinum_	AAS 11534	Smith, R.I.L.	-62.97	-60.70	Fumarole Bay, Deception I.	GenBank n.
11168 S. Shetland Is.	_S. andinum_	AAS 11168	Smith, R.I.L.	-62.95	-60.55	N of Mt. Pond, Deception I.	GenBank n.

S. rivulare

HM053934 Altai, Russia	_S. rivulare_	HM053934	Ignatova E.A.	50.62	86.22 *	Altai, Russia	HM053934
HM053935 Caucasus, Russia	_S. rivulare_	HM053935	Ignatova E.A.	43.88	41.73 *	Caucasus, Russia	HM053935
HM053936 Kuril Is., Russia	_S. rivulare_	HM053936	s.n.	45.54	149.01 *	Russia, Kuril Is., Bakalin	HM053936
HM053937 Vologda, Russia	_S. rivulare_	HM053937	Ignatova E.A	59.22	39.90 *	Vologda, Russia, Vologda Province	HM053937
5040295385203 Belgium	_S. rivulare_	BR 5040295385203	Rubers, W. V.	50.16	5.67 *	Le Herou, S. of Nadrin, Belgium	GenBank n.
5040295380154 Germany	_S. rivulare_	BR 5040295380154	De Sloover, J.L.	50.51	10.07 *	Rhön, Eisgraben, Germany	GenBank n.
5040036490180 France	_S. rivulare_	BR 5040036490180	De Sloover, J.L.	45.00	5.93 *	Les Deux Alpes, Isère, France	GenBank n.
5040295620618 Faroe Is.	_S. rivulare_	BR 5040295620618	Lewinsky, J.	62.27	-6.92 *	Eysturoy, Faroe Is.	GenBank n.
2901B South Georgia	_S. rivulare_	AAS 02901B	Greene, S.W.	-54.27	-36.52	Cumberland West Bay	GenBank n.
10796 (1) S. Orkney Is.	_S. rivulare_	AAS 10796	Smith, R.I.L.	-60.70	-45.62	Changing Lake, Signy I.	GenBank n.
10796 (2) S. Orkney Is.	_S. rivulare_	AAS 10796	Smith, R.I.L.	-60.70	-45.62	Changing Lake, Signy I.	GenBank n.
10906 S. Orkney Is.	_S. rivulare_	AAS 10906	Smith, R.I.L.	-60.68	-45.65	Signy I.	GenBank n.

S. antarctici

151 South Georgia	_S. antarctici_	AAS 00151	Tallowin, J.	-54.50	-36.60	Undine South Harbour, South Georgia	GenBank n.
207 S. Orkney Is.	_S. antarctici_	AAS 00207	Smith, R.I.L.	-60.68	-45.05	SE coast of Powell I.	GenBank n.
273 S. Orkney Is.	_S. antarctici_	AAS 00273	Smith, R.I.L.	-60.73	-45.05	W of Falkland Harbour, Powell I.	GenBank n.
236 S. Orkney Is.	_S. antarctici_	AAS 00236	Smith, R.I.L.	-60.68	-45.05	NW coast of Powell I.	GenBank n.
171 S. Orkney Is.	_S. antarctici_	AAS 00171	Webb, R.	-60.63	-45.50	Coronation I.	GenBank n.

8125 S. Orkney Is.	*S. antarctici*	AAS 08125	Smith, R.I.L.	-60.73	-45.65	Cummings Cove, Signy I.	GenBank n.
10823 S. Orkney Is.	*S. antarctici*	AAS 10823	Smith, R.I.L.	-60.72	-45.62	Orwell Glacier moraine, Signy I.	GenBank n.
5981A NE Ant. Pen., James Ross I.	*S. antarctici*	AAS 05981A	Cain, A. & Poole, P.	-64.12	-57.82	James Ross I.	GenBank n.
5385D NE Ant. Pen., James Ross I.	*S. antarctici*	AAS 05385D	Burkilt, D.	-63.83	-57.97	Brandy Bay, James Ross I.	GenBank n.
7854 NE Ant. Pen., Vega I.	*S. antarctici*	AAS 07854	Smith, R.I.L.	-63.90	-57.30	W of Pastorizo Bay, Vega I.	GenBank n.
11136B NE Ant. Pen., James Ross I.	*S. antarctici*	AAS 11136B	Smith, R.I.L.	-63.93	-57.82	James Ross I.	GenBank n.
5381A NE Ant. Pen., James Ross I.	*S. antarctici*	AAS 05381A	Burkilt, D.	-64.37	-57.28	Hamilton Point, James Ross I.	GenBank n.
7624 NE Ant. Pen., James Ross I.	*S. antarctici*	AAS 07624	Smith, R.I.L.	-63.87	-57.90	James Ross I.	GenBank n.
1771 S. Shetland Is.	*S. antarctici*	AAS 01771	Ochyra, R.	-62.10	-58.30	King George I., S. Shetland Is.	GenBank n.
141 S. Shetland. Is.	*S. antarctici*	AAS 00141	Allison, J.S.	-61.12	-55.47	S of Emma Cove, Elephant I.	GenBank n.
21 S. Shetland Is.	*S. antarctici*	U. Cambr. 21	Biersma, E.M.	-62.38	-59.66	Robert I., S. Shetland Is.	GenBank n.
128 S. Shetland Is.	*S. antarctici*	AAS 00128	Mason, D.	-62.90	-60.53	Macaroni Point, Deception I.	GenBank n.
11178 S. Shetland Is.	*S. antarctici*	AAS 11178	Smith, R.I.L.	-62.95	-60.55	Mt. Pond, Deception I.	GenBank n.
31 S. Shetland Is.	*S. antarctici*	AAS 00031	Taylor, B.J.	-62.95	-60.57	NW of Ronald Hill, Deception I.	GenBank n.
11297A S. Shetland Is.	*S. antarctici*	AAS 11297A	Smith, R.I.L.	-62.97	-60.70	Fumarole Bay, Deception I.	GenBank n.
5735A S. Shetland Is.	*S. antarctici*	AAS 05735A	Morton, A.	-62.97	-60.73	Deception I.	
3600 S. Shetland Is.	*S. antarctici*	AAS 03600	Smith, R.I.L.	-63.00	-60.58	Collins Point, Deception I.	GenBank n.
139 S. Shetland Is.	*S. antarctici*	AAS 139	Mason, D.	-63.02	-60.62	Deception I.	GenBank n.
1946 Ant. Pen., Danco Coast	*S. urnulaceum*	AAS 01946	Smith, R.I.L.	-64.77	-64.12	Norsel Point, Anvers I.	GenBank n.
5633 Ant. Pen., Graham Coast	*S. antarctici*	AAS 05633	Longton, R.E.	-65.25	-64.27	Skua I., Argentine Is.	GenBank n.
649 Ant. Pen., Graham Coast	*S. antarctici*	AAS 00649	Corner, R.W.M.	-65.23	-64.10	Edge Hill, Graham Coast	GenBank n.
847 Ant. Pen., Loubet Coast	*S. antarctici*	AAS 00847	Smith, R.I.L.	-67.55	-67.00	Blaiklock I., Loubet Coast	GenBank n.
1880 (1) Ant. Pen., Loubet Coast	*S. antarctici*	AAS 01880	Smith, R.I.L.	-67.57	-68.13	Adelaide I., Loubet Coast	GenBank n.
1880 (2) Ant. Pen., Loubet Coast	*S. antarctici*	AAS 01880	Smith, R.I.L.	-67.57	-68.13	N of Rothera Point, Adelaide I.	GenBank n.

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4772 Ant. Pen., Fallieres Coast	*S. antarctici*	AAS 04772	Smith, R.I.L.	-67.68	-67.50	Pourquoi Pas I.	GenBank n.
4754 Ant. Pen., near Adelaide I.	*S. antarctici*	AAS 04754	Smith, R.I.L.	-67.73	-68.38	N side, Jenny I.	GenBank n.
4673 Ant. Pen., Fallieres Coast	*S. antarctici*	AAS 04673	Smith, R.I.L.	-67.97	-67.32	Marguerite Bay	GenBank n.
850 Ant. Pen., Alexander I.	*S. antarctici*	AAS 00850	Convey, P.	-69.37	-71.85	Lazarev Bay, NW Alexander I.	GenBank n.
850 Ant. Pen., Alexander I.	*S. antarctici*	AAS 00850	Convey, P.	-69.37	-71.85	Lazarev Bay, NW Alexander I.	GenBank n.
9289 Ant. Pen., Alexander I.	*S. antarctici*	AAS 09289	Smith, R.I.L.	-70.82	-68.42	Ablation Valley, Alexander I.	GenBank n.
9255A Ant. Pen., Alexander I.	*S. antarctici*	AAS 09255A	Smith, R.I.L.	-70.82	-68.43	Ablation Valley, Alexander I.	GenBank n.
9278 Ant. Pen., Alexander I.	*S. antarctici*	AAS 09278	Smith, R.I.L.	-70.82	-68.43	Ablation Valley, Alexander I.	GenBank n.
11874A Ant. Pen., Alexander I.	*S. antarctici*	AAS 11874A	Lewis, N.	-71.67	-68.87	Astarte Horn, Alexander I.	GenBank n.
5415 Ant., Byrd Sector	*S. antarctici*	AAS 05415	Cleary, P.	-72.45	-98.07	Mt Dowling, Thurston I., Eights Coast	GenBank n.
9640 Ant., Ross Sector	*S. antarctici*	AAS 09640	Smith, R.I.L.	-74.07	164.75	Harrow Peaks, Victoria Land	GenBank n.
9642 Ant., Ross Sector	*S. antarctici*	AAS 09642	Smith, R.I.L.	-74.07	164.75	Harrow Peaks, Victoria Land	GenBank n.
6219 Ant., Wilkes Sector	*S. antarctici*	AAS 06219	Smith, R.I.L.	-66.25	110.62	Clark Peninsula, Budd Coast	GenBank n.
439 Ant., Wilkes Sector	*S. antarctici*	AAS ADT 00439	Seppelt, R.D.	-66.25	110.60	Clark Peninsula	GenBank n.
SCSA1 Ant., Wilkes Sector	*S. antarctici*	WOLL SCSA1	Robinson, S.A.	-66.25	110.59	Stevenson Cove (Clarke 2)[1], ASPA 136, Clark Peninsula, Windmill Is.	GenBank n.
SCSA2 Ant., Wilkes Sector	*S. antarctici*	WOLL SCSA2	Robinson, S.A.	-66.25	110.59	Stevenson Cove (Clarke 2)[1], ASPA 136, Clark Peninsula, Windmill Is.	GenBank n.
221 Ant., Wilkes Sector	*S. antarctici*	WOLL 221	Clarke L.J.	-66.25	110.57	Clarke 1[2], ASPA 136, Clark Peninsula, Windmill Is.	GenBank n.
199 Ant., Wilkes Sector	*S. antarctici*	WOLL 199	Clarke L.J.	-66.28	110.54	ASPA 2[3], ASPA135, Bailey Peninsula, Windmill Is.	GenBank n.
RSSA1 Ant., Wilkes Sector	*S. antarctici*	WOLL RSSA1	Robinson, S.A.	-66.28	110.53	Red Shed[4], Casey Station, Bailey Peninsula, Windmill Is.	GenBank n.
RRSA6 Ant., Wilkes Sector	*S. antarctici*	WOLL RRSA6	Robinson, S.A.	-66.36	110.59	Robinson Ridge[4], Windmill Is.	GenBank n.
RRSA5 Ant., Wilkes Sector	*S. antarctici*	WOLL RRSA5	Robinson, S.A.	-66.37	110.59	Robinson Ridge[4], Windmill Is.	GenBank n.

RRSA3 Ant., Wilkes Sector	*S. antarctici*	WOLL RRSA3	Robinson, S.A.	-66.37	110.59	Robinson Ridge[4], Windmill Is.	GenBank n.
941 Ant., Wilkes Sector	*S. antarctici*	AAS 00941	Markov, K.K.	-66.55	93.02	Mirny	
6325 Ant., Wilkes Sector	*S. antarctici*	AAS 06325	Smith, R.I.L.	-66.58	110.68	Haupt Nunataks, Budd Coast	GenBank n.

* = Longitudes and latitudes not provided with sample. Approximate location found via http://mynasadata.larc.nasa.gov/latitudelongitude-finder/

[1] = Stevenson Cove (SCSA1 & 2) samples, collected in 2012, is described in Clarke *et al.* (2012);

[2] = Clark 1 (Clarke Ridge) site collected in 2005, described in Clarke *et al.* (2009),

[3] = ASPA 2 collected in 2005, described in Dunn and Robinson (2006);

[4] = Robinson Ridge and Red Shed sites samples collected in 2012, described in Lovelock and Robinson (2002)