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Low genetic variation between South American and Antarctic populations of the bank-forming moss Chorisodontium aciphyllum (Dicranaceae)

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Title: Low genetic variation between South American and Antarctic populations of the bank-forming moss *Chorisodontium aciphyllum* (Dicranaceae)

Short running title: Genetic diversity in Antarctic peat moss

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Abstract

The Antarctic-South American bank-forming moss *Chorisodontium aciphyllum* is known for having the oldest sub-fossils of any extant plant in Antarctica as well as extreme survival abilities, making it a candidate species for possible long-term survival in Antarctica. Applying phylogeographic and population genetic methods using the plastid markers *trnL-F* and *rps4* and the nuclear Internal Transcribed Spacer (*ITS*) we investigated the genetic diversity within *C. aciphyllum* throughout its

range. Low genetic variation was found in all loci, both between and within Antarctic and southern South American populations, suggesting a relatively recent (likely within the last million years) colonization of this moss to the Antarctic, as well as a likely severe bottleneck during Pleistocene glaciations in southern South America. We also performed a simple atmospheric transfer modeling approach to study potential colonization rates of small (microscopic/microbial) or spore-dispersed organisms (such as many mosses and lichens). These suggested that the northern Antarctic Peninsula shows potentially regular connectivity from southern South America, with air masses transferring, particularly southbound, between the two regions. We found elevated genetic variation of *C. aciphyllum* in Elephant Island, also the location of the oldest known moss banks (>5500 years), suggesting this location to be a genetic hotspot for this species in the Antarctic.

Keywords: bryophyte – LGM – Last Glacial Maximum – peat moss – sub-Antarctic – wind

Introduction

The timing of origin of the contemporary 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 (e.g. Allegrucci et al. 2006, 2012; Convey et al. 2008, 2009b; 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. 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 thousands to multi-million year timescales (e.g Chong et al. 2015; Convey et al. 2008, 2009a; Convey and Stevens 2007; De Wever et al. 2009; Fraser et al. 2014; Iakovenko et al. 2015; McGaughran et al. 2010; Pisa et al. 2014; Stevens et al. 2006; Vyverman et al. 2010).

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, iii) perceived potentially high dispersal ability through spore and other propagule production, and iv) 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; see also Hills et al. 2010). 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. High genetic variation amongst Antarctic populations of *Polytrichum juniperinum* Hedw. (Biersma et

al. 2017) suggests this common Antarctic moss may also have had a long-term *in situ* persistence in the maritime Antarctic, although this requires further investigation.

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. *Chorisodontium aciphyllum* is a common moss in the sub- and maritime Antarctic (Antarctic Peninsula and Scotia Arc archipelagos). Its overall distribution includes southern South America (also including the Juan Fernandez Islands), the Falkland Islands, the Scotia Arc, the Antarctic Peninsula and associated islands, Tristan da Cunha, Amsterdam Island and the Kerguelen archipelago (Hyvönen, 1991; Ochyra et al. 2008, and references therein). New Zealand was previously also thought to be part of its range (Bartlett & Frahm, 1983), however a later consultation found the plant here to have been misidentified (Department of Conservation of New Zealand, 2013, see reference list for website link). The plant is thought to be sterile in the maritime Antarctic, but is known to locally produce sporophytes on sub-Antarctic South Georgia (Ochyra et al. 2008), and further north in southern South America (Hyvönen, 1991, Ochyra et al. 2008).

C. aciphyllum forms banks often up to 1-2 m in depth, with the deepest banks known reaching a depth of almost 3 m on Elephant Island in the South Shetland Islands (Björck et al. 1991; Collins 1976a, 1976b; Fenton 1980, 1982a; Fenton and Smith 1982; Smith 1972, 1979, 1996; Fig. 1). 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 (Björck et al. 1991; Fenton and Smith 1982), and deeper cores may potentially be older.

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 from a core obtained on Signy Island (South Orkney Islands) have revealed that old moss shoots deep within the peat banks are still viable and able to regrow after experimental 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, which are not produced by this moss in the maritime Antarctic) 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, for instance the Little Ice Age (1550–1850 BC), such as are inferred in various studies of glacial extent over time and through palaeoclimate proxies in the Antarctic (Guglielmin et al. 2015; Hodgson and Convey 2005). 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 thousands 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 transfer events between the regions. These analyses will increase our general understanding of the likely age of spore-dispersed organisms within Antarctica.

Materials and methods

Sampling and molecular methods

Material was sampled throughout the natural range of *C. aciphyllum* from 25 herbarium and 77 fresh (sub-)samples (the latter included spatially separated subsamples taken from eight different locations on four different islands, as described below; see Table 1 and Fig. 2). Most of the fresh (frozen) samples of *C. aciphyllum* included in this study were 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). 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 sample several sub-samples were taken at a finer-scale interval of approximately 5 cm (letters A-E). Several shoots were taken per sub-sample. 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.

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 and Quandt 2010): the nuclear Internal Transcribed Spacer (*ITS*) and the plastid markers *trnL-F* and *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.

Molecular analyses

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 all three regions derived from the same original specimens as outgroups in all alignments: *Dicranoloma cylindrothecium* (Mitt.) Sakurai. and *D. robustum* (Hook.f. & Wils.) Paris. (see Table 1). Additionally, as the above mentioned *rps4* outgroup sequences were only partial, we included several other *Dicranoloma* sequences in the *rps4* alignment (*D. billardieri* (Brid.) Paris., *D. blumii* (Nees) Paris.. and *D. eucamptodontoides* (Broth. & Geh.) Paris.), as well as extra *Chorisodontium* sequences (*C. mittenii*, and *C. setaceum*). In the *trnL-F* alignment, we added additional outgroup sequences (*D. cylindrothecium* and *D. robustum*, respectively) and two *Chorisodontium* sequences (*C. mittenii* and *C. setaceum*, respectively). Loci were aligned per 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 numbers of variable and parsimony informative sites were calculated per locus in MEGA7 (Kumar et al. 2016) using ingroup sequences with *Chorisodontium* species only.

165 Bayesian analyses using MrBayes 3.2 (Ronquist et al. 2012) were performed on each locus separately.
166 Nucleotide substitution models were selected according to the SPR tree topology search operation and
167 AICc calculations as implemented by jModeltest-2.1.7 (Darriba et al. 2012) for each individual marker,
168 resulting in the TIM2, TPM1uf and TPM3uf (n=6, rates=equal for all) for *rps4*, *trnL-F* and *ITS*,
169 respectively. For the MrBayes analysis indels in *ITS* were coded in SeqState v1.0. (Simmons and
170 Ochoterena 2000) using the simple indel coding. MrBayes runs of all markers were continued for
171 1000000 generations, sampling every 1000, ensuring all parameters exceeded effective sample sizes
172 (ESS) >200 and split frequencies reached values >0.01 using Tracer v.1.6 (Rambaut et al. 2014), and
173 discarding the first 25% as burn-in. Maximum clade credibility trees with mean node heights were
174 visualised using Figtree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).
175 We examined phylogeographic structure within ingroup specimens with TCS networks produced for
176 each locus using the program Popart (Leigh and Bryant 2015), using default settings.
177
178 *Aerial modeling*
179 The potential relative frequency of atmospheric dispersal events between different locations was
180 evaluated using a method of following trajectories of air-mass movements from reconstructions of past
181 atmospheric winds. Simplifying assumptions were made that (i) particles are blown by the wind
182 without any independent movement (e.g. fall-out) and that (ii) there are no thresholds on survival in
183 terms of environmental conditions such as temperature or humidity. For a given location of interest
184 three-dimensional forward trajectories were calculated at daily intervals over a 10 y period from 1979.
185 In other words, for every day, starting at a specified location, a calculation was conducted which
186 estimates the path that a particle released at that location at midnight would follow if it were blown by
187 the wind over the following two days. For the purpose of this study we used two different starting
188 locations in the area of interest: one from southern South America (55°S, 67.5°W) and one in the South
189 Shetland Islands (62.5°S, 57.5°W) in the maritime Antarctic.
190 The atmospheric winds were taken from a reconstruction of past winds available from the European
191 Centre for Medium-Range Weather Forecasts (ECMWF). The specific version used was ERA‐40
192 (Uppala et al. 2005) and the post-1979 period was chosen, which is known to be more reliable due to
193 the introduction of widespread data from satellites in late 1978 (Marshall 2003). The three-dimensional
194 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.

Results

Molecular analyses

Sequence lengths within *rps4*, *trnL-F* and *ITS* alignments ranged between 649-650 bp, 454-462 bp and 744-777 bp (including outgroups), respectively. Variation between *Chorisodontium* species was low in all markers (including only *Chorisodontium* sequences: 2, 3 and 9 variable sites, and 2, 2 and 3 parsimony informative sites in *rps4*, *trnL-F* and *ITS*, respectively). 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. 3a-c). Haplotype networks of each locus are shown next to each phylogenetic tree in Fig. 3.

Both phylogenetic and haplotype analyses revealed that in the loci *trnL-F* and *ITS* (Figs 3b 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 the specimens were initially misidentified. In the *trnL-F* phylogenic 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. 3b and c, respectively) some specimens identified as *C. aciphyllum* (Chile 00504, 11472A, 02015) were resolved as sister-species or together with other *Chorisodontium* species, again 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 (*rps4* and *ITS*; Fig. 3a and c, respectively) or no (*trnL-F*; Figs. 3b) 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.

The *ITS* marker (Fig. 3c) 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. 3a-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). The two added nucleotides were only found in Elephant Island samples, and were not present in any other locations of *C. aciphyllum*.

Aerial modeling studies

Two 95%-probability distribution figures were produced that show the relative connectivity between southern South America and the northern maritime Antarctic (Figs. 5a, 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. 5a) is more likely than *vice versa* (Fig. 5b). 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.

Discussion

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 ky, the age of the oldest fossil evidence of *C. aciphyllum* in the Antarctic (Björck et al. 1991; Fenton and Smith 1982). 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. site^{-1} my^{-1},

255 originally derived from angiosperms (Les et al. 2003, and references therein) we would expect one
256 substitution to have happened every 982,415 years in a 754 bp long *ITS* sequence (the *ITS* sequence
257 length of *C. aciphyllum* haplotype IV, Fig 3c; 0.00135 subst. site^{-1} my^{-1} results in 1.0179 subst. 754
258 sites^{-1} my^{-1}, which is one mutation every 982,414.78 years). This simplistically suggests populations in
259 South America and the Antarctic have likely been separated no longer than one million years, and a
260 minimum of ~5.5 ky, the age of the oldest dated *C. aciphyllum* peat core in the Antarctic (see above).
261 However, we acknowledge the rate used in this rough estimation does not take into account a rate
262 standard deviation (which is not available), and that this rate might be different in bryophytes
263 compared to angiosperms, and may also vary within bryophytes. From the genetic variation in this
264 study it is not possible to assess the direction of spread, but it is perhaps more plausible that the species
265 has spread from South America to the maritime Antarctic and/or sub-Antarctic, as the extant
266 distributions of sister-species of *C. aciphyllum* only include South America. The 95%-probability
267 distribution figures from the aerial modeling studies (Fig. 5) also suggest local wind patterns are more
268 likely to transfer particles from southern South America to the northern maritime Antarctic than *vice*
269 *versa*. Long-distance migration of moss particles *via* migratory birds may also have been a possibility
270 for dispersal (and in either direction) (Lewis et al. 2014; Viana et al. 2016), although further research
271 efforts are still needed to validate this mode of transfer in mosses.
272 Even though using three markers that are often variable at species and population level (particularly
273 *ITS*; Stech and Quandt 2010), there was no genetic variation within South American populations of *C.*
274 *aciphyllum*, whereas the opposite would be expected of an 'ancestral' population. Further sampling
275 might provide clarification on the genetic variation of *C. aciphyllum* in South American populations
276 (many of the Chilean specimens used in this study identified as *C. aciphyllum* in herbarium records
277 turned out to be misidentified and represent *C. sphagneticola*; see below). It is likely that these
278 southern South American populations experienced a strong bottleneck throughout the LGM and
279 possibly other Pleistocene glacial maxima, when the region was extensively glaciated (Hulton et al.
280 2002). Molecular studies on a wide range of terrestrial biota strongly suggest the existence of local
281 refugia in Patagonia throughout the LGM and previous glaciations, rather than recolonisation from
282 northern regions (Sersic et al. 2011, and references therein). This scenario matches the still restricted
283 distribution of *C. aciphyllum*, essentially limited to the far southern latitudes within South America.
284 Despite the potential in *C. aciphyllum* for regeneration from viable shoots preserved in permafrost

285 (Roads et al. 2014), and therefore a possible survival strategy for long-term persistence in the Antarctic
286 *in situ*, this study reveals very little genetic variation exists between South American and Antarctic
287 populations. This suggests the species has not been present in the Antarctic on a multi-million year
288 timescale, unlike for example the suggested Antarctic presence of *Bryum argenteum* (Pisa et al. 2014;
289 Hills et al. 2010). If the oldest known bank of *C. aciphyllum* in the Antarctic (~5500 yrs old, on
290 Elephant I., South Shetland Is.; Björck et al. 1991) represents the approximate arrival date of this
291 species in the Antarctic, such a recent arrival would likely not have generated a strong detectable
292 genetic differentiation, a finding consistent with the genetic signals in our study. The moss banks on
293 Signy Island on the South Orkney Islands are also estimated to have begun to accumulate
294 approximately 5.59-5.49 kya (Fenton 1982b; Smith 1990), suggesting this was one of the earliest
295 periods with suitable conditions for post-glacial colonization. A similar implication of recent (post-
296 LGM) arrival of an Antarctic moss was reported by Kato et al. (2013), studying the moss *Leptobryum*
297 *wilsonii* (Mitt.) Broth., a species found growing uniquely in lakes of the Sôya Coast region in East
298 Antarctica. Using the same makers as applied here (*rps4*, *trnL-F* and *ITS*) very low genetic variation
299 (one base substitution and three to four indels) was detected between samples of *L. wilsonii* from East
300 Antarctica and Chile, locations separated by a considerably greater distance than those separating
301 *Chorisodontium* populations in the current study. Both Kato et al. (2013) and the current study provide
302 examples of species whose genetic diversity is consistent with the widespread but generally untested
303 assumption that Antarctic moss species may be post-LGM arrivals (e.g. Convey et al. 2008; Ochyra et
304 al. 2008; Peat et al. 2007). However, other features of the biology of both *C. aciphyllum* and *L.*
305 *wilsonii*, in particular that neither produce sporophytes in the Antarctic and/or sub-Antarctic (Ochyra et
306 al. 2008) where both rely solely on asexual reproduction, might (due to a lack of genetic variation
307 associated with asexual reproduction) considerably slow their rates of evolution and hence
308 underestimate the timing of their arrival in the continent. It should be noted, however, that we also
309 observe little genetic variation within southern South American populations of *C. aciphyllum* (see Fig.
310 3), as well as southern South American versus maritime Antarctic populations, despite the occurrence
311 of sexual reproduction in the former population.
312 We found evidence of local genetic variation in *C. aciphyllum* within several locations on Elephant
313 Island (Figs. 3 and 4). Although this genetic variation was only small (two nucleotide additions in *ITS*),
314 it revealed more variation in *ITS* between samples from 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 single shoots alone.

In both *trnL-F* and *ITS* phylogenies (see Figs. 3b, 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. 3c) of *C. dicranellatum* was genetically identical to *C. aciphyllum*. Similarly, the *trnL-F* spacer (Fig. 3b) 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 of the specific material examined. This is exemplified by the *rps4* sequences of *C. setaceum* (i.e. *C. wallisii*) and *C. mittenii*, which do differ from *C. aciphyllum* (Fig. 3a), while *rps4* is often less divergent between species than *ITS* and *trnL-F* (Stech and 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. 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.

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Figure legends

Fig. 1 Extensive *Chorisodontium aciphyllum* moss bank growing on Signy Island, South Orkney Islands. For scale, the yellow post on the left is one meter long. Photographs: James Fenton

Fig. 2 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 1)

Fig. 3 Bayesian phylogenetic trees and haplotype networks constructed with (a) plastid loci *rps4* and (b) *trnL-F*, and (c) nuclear marker *ITS* 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 network 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)

Fig. 4 Partial alignment of *ITS* showing the within-population variation in *Chorisodontium aciphyllum* populations on 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

Fig. 5 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, re-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

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Table 1. *Chorisodontium* specimens used in this study 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. UC = University of Cambridge

Specimen (Species, Geographic origin, herbarium no.)	**Herbarium/ Collection + Coll. number**	**Collection**	**Latitude + Longitude**	***ITS***	***rps4***	***trnL-F***
Chile 11472A	AAS 11472A	Smith, R.I.L.	-55.98,-67.27	MG076984	MG077055	MG077031
C. magellanicum, Chile 00522	AAS 00522	Roivainen, H.	-54.56,-69.80 [a]	MG076982		
Chile 00507	AAS 00507	Roivainen, H.	-54.45,-70.67	MG076991		
Chile 00504	AAS 00504	Roivainen, H.	-54.45,-70.67			MG077030
C. lanigerum, Chile 00512	AAS 00512	Roivainen, H.	-54.45,-70.67	MG076986		
C. spegazzini, Chile 00523	AAS 00523	Roivainen, H.	-54.08,-71.03	MG076987		MG077029
Argentina 00173	AAS 00173	Castellanos	-54.78,-64.25	MG076992		
Argentina 00712	AAS 00712	Matteri, C.M.	-54.30,-68.00	MG076993		
C. dicranellatum, Argentina 00509	AAS 00509	Roivainen, H.	-53.60,-69.55 [b]	MG076988		
C. dicranellatum, Argentina 00511	AAS 00511	Roivainen, H.	-53.64,-69.65 [b]	MG076989		
C. sphagneticola, Chile 00525	AAS 00525	Roivainen, H.	-53.64,-69.65 [b]	MG076983		MG077028
Chile 02015	AAS 02015	Matteri, C.M.	-51.47,-73.27	MG076985		MG077027
C. sp, Chile 00355	AAS 00355	Pisano, E.	-52.08,-71.92	MG076990		MG077042
Falkland Is. 5440	AAS 5440	Smith, R.I.L.	-51.68,-58.83 [a]	MG077015		MG077032
Falkland Is. 00131A	AAS 00131A	Engel, J.J.	-51.75,-59.50	MG076998		
South Georgia 05031	AAS 05031	Smith, R.I.L.	-54.00,-38.08	MG077022	MG077058	MG077038
South Georgia 00295	AAS 00295	Briggs, M.	-54.30,-36.52	MG076994	MG077057	MG077036
South Georgia 00291	AAS 00291	Cable, S.	-54.18,-36.72	MG076995	MG077056	MG077035
South Georgia 01154	AAS 01154	Smith, R.I.L.	-54.28,-36.50	MG076996		
S. Orkney Is. 04965	AAS 04965	Walton, D.W.H.	-60.63,-45.58	MG077023		MG077037
S. Orkney Is. 05251	AAS 05251	Smith, R.I.L.	-60.73,-45.68	MG077024	MG077059	MG077039
S. Orkney Is. 08007	AAS 08007	Smith, R.I.L.	-60.60,-46.05	MG077025	MG077060	MG077040
SSI, Ardley I. 1A (4)	UC 1A (1-4)	Royles, J.	-62.21,-58.93	MG076999		MG077044
SSI, Ardley I. 1B (5)	UC 1B (1-5)	Royles, J.	-62.21,-58.93	MG077000	MG077063	MG077045
SSI, Ardley I. 1D (5)	UC 1D (1-5)	Royles, J.	-62.21,-58.93	MG077001		MG077046
SSI, Ardley I. 2A (5)	UC 2A (1-5)	Royles, J.	-62.21,-58.94	MG077002	MG077064	
SSI, Ardley I. 2E (5)	UC 2E (1-5)	Royles, J.	-62.21,-58.94	MG077003		
SSI, Elephant I. 1A b (1)	UC 1A (1)	Royles, J.	-61.14,-54.70	MG077004	MG077065	
SSI, Elephant I. 1C a (2)	UC 1C (2)	Royles, J.	-61.14,-54.70	MG077009		
SSI, Elephant I. 1C b (1)	UC 1C (1)	Royles, J.	-61.14,-54.70	MG077005		
SSI, Elephant I. 1D a (2)	UC 1D (2)	Royles, J.	-61.14,-54.70	MG077010		
SSI, Elephant I. 1D b (3)	UC 1D (2)	Royles, J.	-61.14,-54.70	MG077006		
SSI, Elephant I. 2A a (4)	UC 2A (4)	Royles, J.	-61.14,-54.70	MG077011		
SSI, Elephant I. 2A b (1)	UC 2A (1)	Royles, J.	-61.14,-54.70	MG077007		
SSI, Elephant I. 3A a (4)	UC 3A (4)	Royles, J.	-61.14,-54.71	MG077012	MG077066	
SSI, Elephant I. 3B a (1)	UC 3B (1)	Royles, J.	-61.14,-54.71	MG077013		
SSI, Elephant I. 3B b (4)	UC 3B (4)	Royles, J.	-61.14,-54.71	MG077008	MG077067	MG077047
SSI, Robert I.	BAS s.n.	Biersma, E.M.	-62.38,-59.66	MG077014	MG077062	MG077043
AP, Norsel Point 1A (5)	UC 1A (1-5)	Royles, J.	-64.76,-64.08	MG077016	MG077068	MG077048
AP, Norsel Point 1B (5)	UC 1B (1-5)	Royles, J.	-64.76,-64.08	MG077017	MG077069	MG077049
AP, Norsel Point 1C (5)	UC 1C (1-5)	Royles, J.	-64.76,-64.08	MG077018		MG077050
AP, Norsel Point 2A (5)	UC 2A (1-5)	Royles, J.	-64.76,-64.08	MG077019	MG077070	MG077051
AP, Norsel Point 2B (5)	UC 2B (1-5)	Royles, J.	-64.76,-64.08	MG077020		MG077052
AP, Norsel Point 2C (5)	UC 2C (1-5)	Royles, J.	-64.76,-64.08	MG077021		MG077053
AP, Danco Coast 11938A	AAS 11938A	Smith, R.I.L.	-64.68,-62.63	MG076997		MG077034
AP, Danco Coast 08801	AAS 08801	Weinstein, R.	-64.68,-62.63	MG077026	MG077061	MG077041
AP, Graham Coast 10661	AAS 10661	Fowbert, J.A.	-65.28,-64.13		MG077054	MG077033
C. mittenii Bolivia AY908107	MO 19750	Churchill et al	-16.27,-67.83		AY908107	
C. mittenii AF435272/AF435311	DUKE PV 1515	Griffin & Lopez	-		AF435272	AF435311
C. setaceum AF435273/AF435312	DUKE 9168	Allen	-		AF435273	AF435312

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/

531

Figure

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Figure

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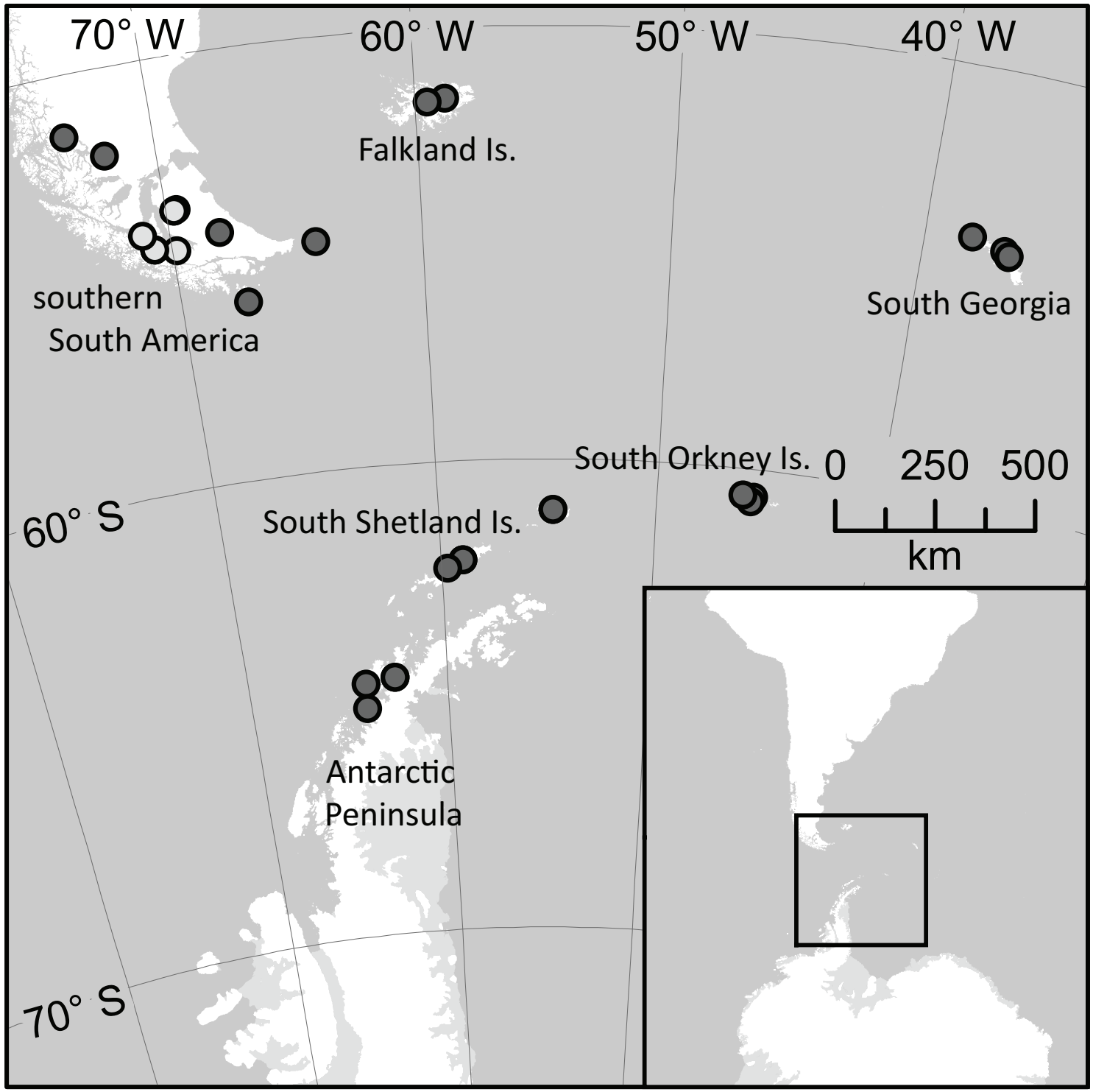
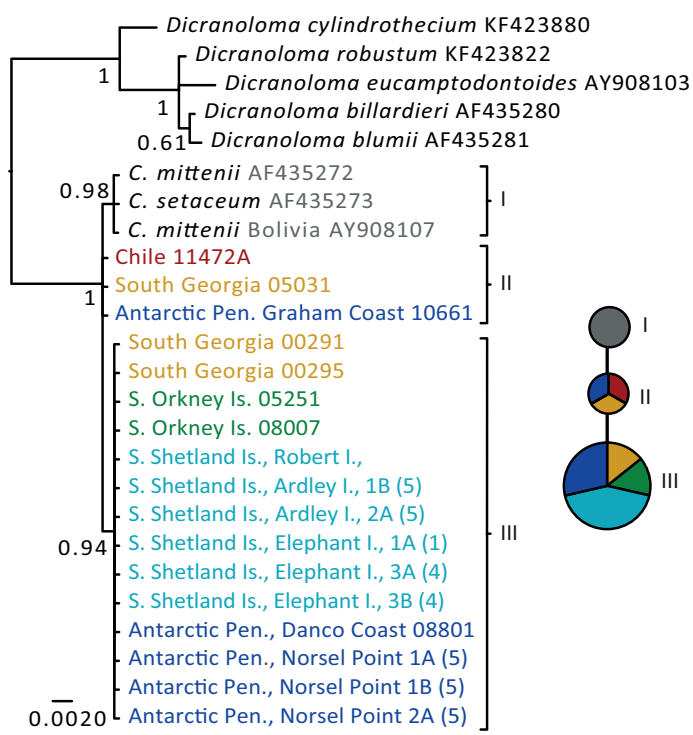


Figure 3 *rps4*



b *trnL-F*

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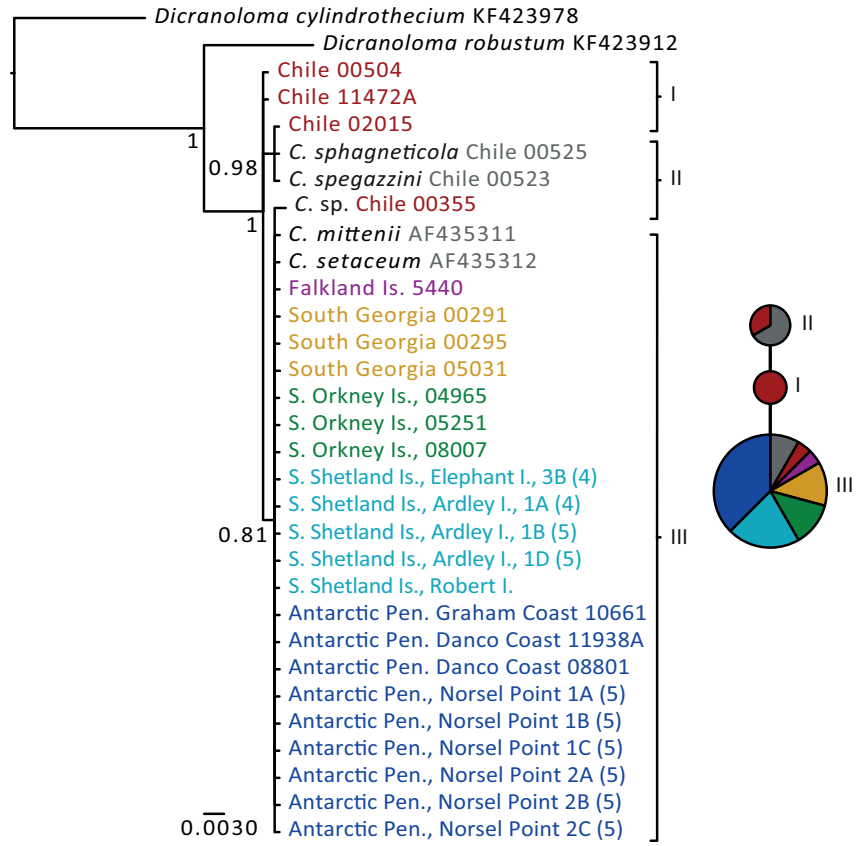
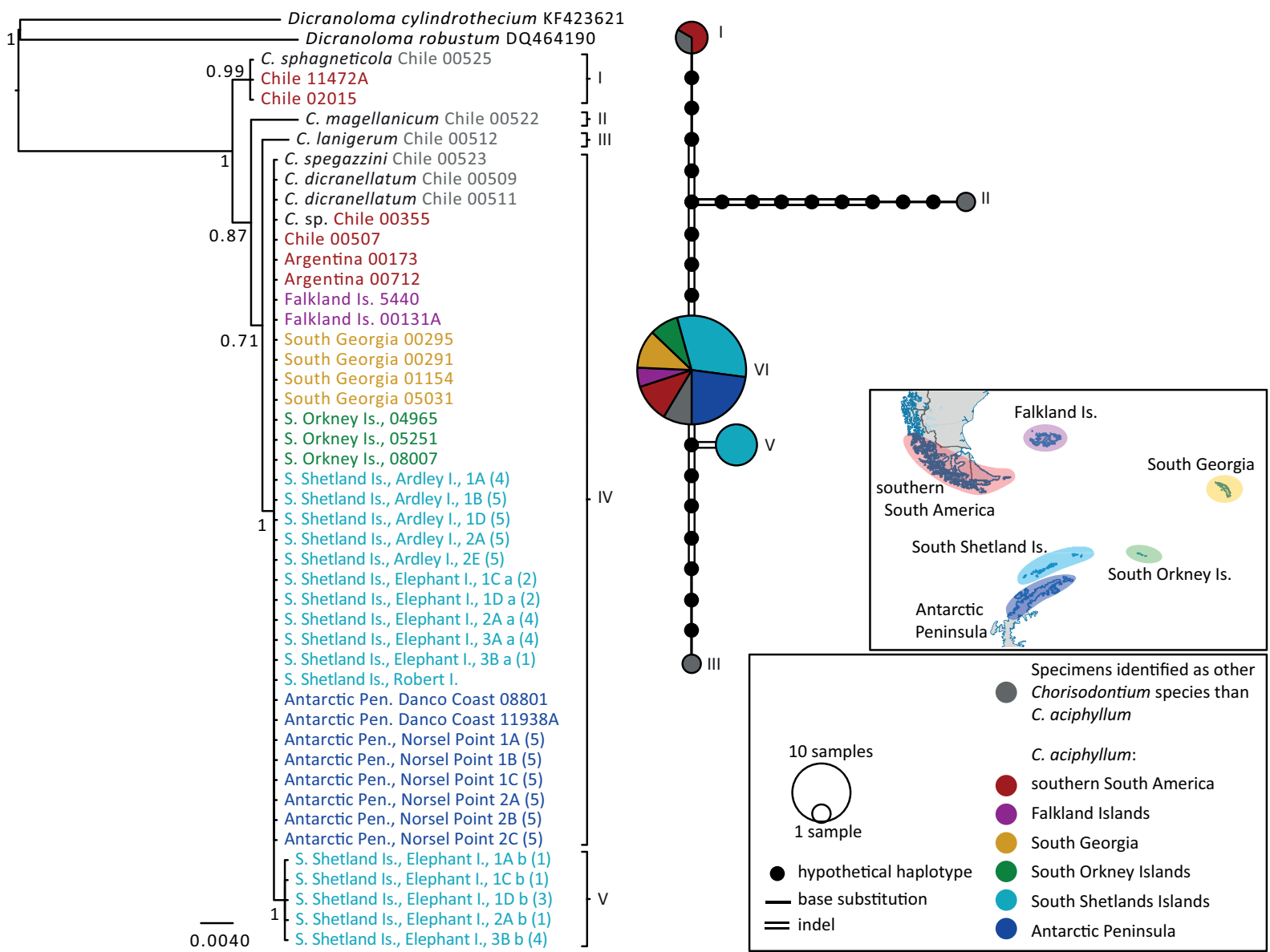


c *ITS*



Figure

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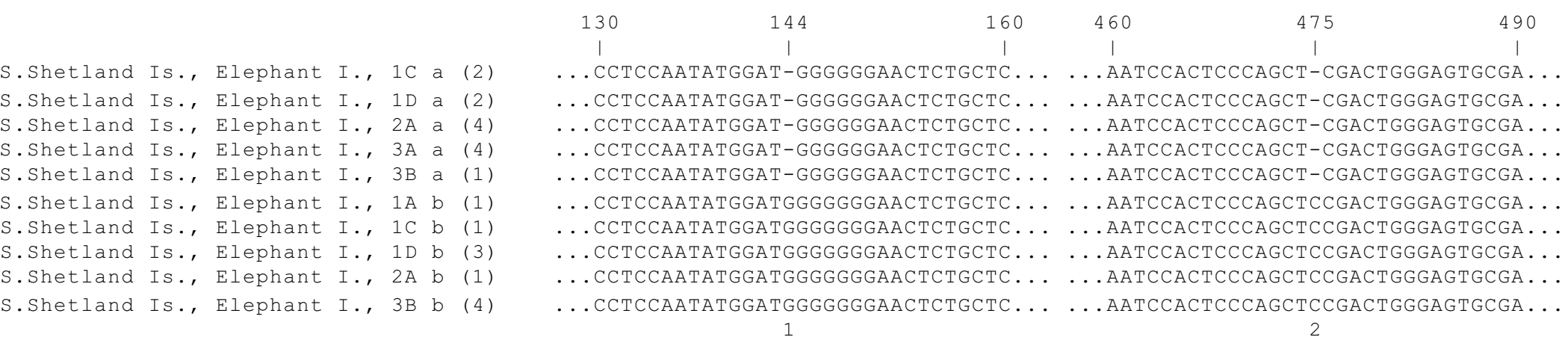
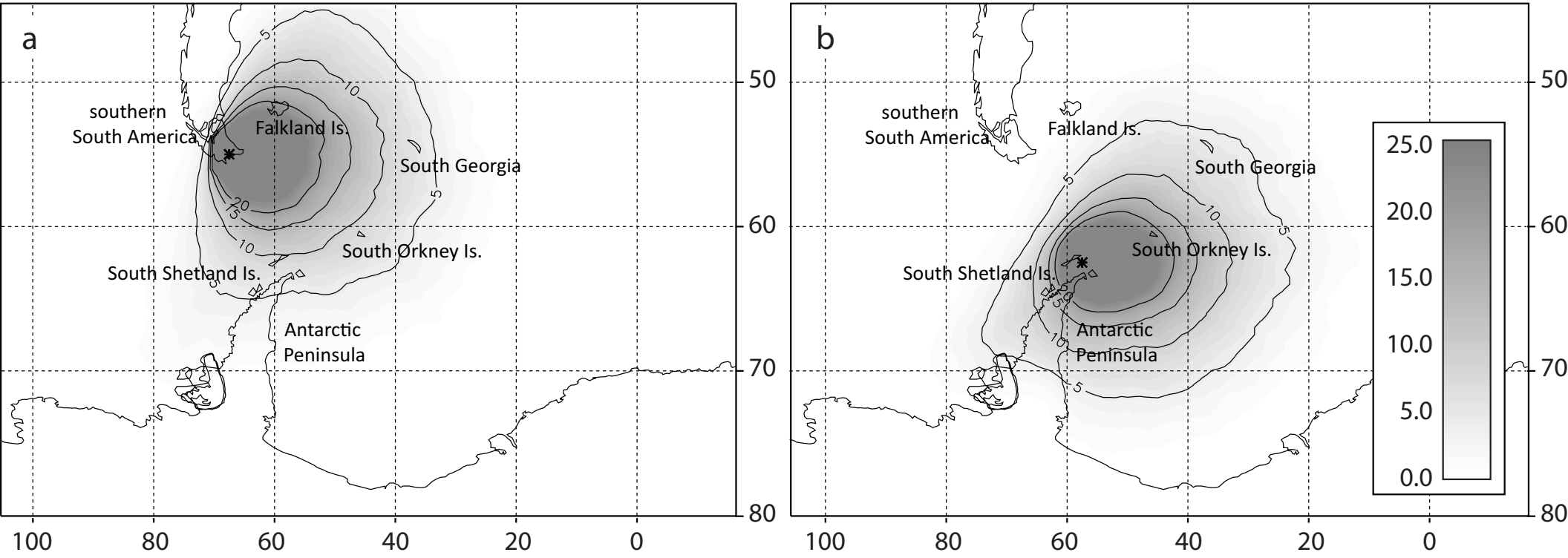


Figure

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