

1 Deep genetic divergence between austral populations of the red alga *Gigartina skottsbergii*  
2 reveals a cryptic species endemic to the Antarctic continent.

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20 Abstract

21 The almost complete isolation of Antarctica after the intensification the Antarctic Circumpolar  
22 Current (ACC) during the middle-Miocene has been challenged by recent molecular data  
23 showing the existence of allelic exchange across the ACC. For organisms present on both sides  
24 of the ACC, two hypotheses have then been discussed to explain the origin of the Antarctic  
25 populations: 1) they correspond to recent immigrants from adjacent continents or 2) they have  
26 evolved in situ and have survived the dramatic effects of the last Quaternary glaciations in this  
27 region. The red algae *Gigartina skottsbergii* presents a disjoint distribution and is reported in  
28 both Antarctica and southern South America, a distribution pattern that largely exceeds its  
29 dispersal capacity. Mitochondrial sequences of the intergenic region *cox2-3* (n=233) and partial  
30 chloroplastic *RuBisCo* large subunit gene (n=26) sequences were obtained for individuals from  
31 the Chilean sub-Antarctic ecoregion and Antarctic Peninsula localities. The results strongly  
32 support the persistence of populations on each side of the Drake Passage during glacial  
33 periods and the existence of dispersal barrier due to the ACC. On both sides of the ACC, the  
34 last Quaternary glaciations have induced strong bottlenecks that were followed by rapid  
35 colonization events.

36 Keywords: Phylogeography, glacial refugia, seaweed, Antarctica, *rbcl*, *cox2-3*,

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43 Introduction

44           The Southern Ocean is characterized by high levels of endemism of its fauna and flora  
45 (Clarke and Crame 1989; Brandt et al 1999; Clarke and Johnston 2003; Wulff et al. 2009) that  
46 has been related to the progressive isolation of the continent during the Mesozoic and the  
47 reinforcement of the Antarctic Circumpolar Current (ACC) during the mid-Miocene (Lawver  
48 and Gahagan 1998; Rogers et al. 2007; Poulin et al. 2014). Moreover, after the onset of  
49 icehouse conditions in Antarctic, both the radiation of groups that have adapted to this  
50 extreme environment and allopatric speciation driven by population fragmentation in  
51 Antarctic refugia during glacial period seem to have contributed to the high Antarctic diversity  
52 (Rogers et al. 2007; Thatje et al. 2008). Recent molecular data for several marine invertebrate  
53 taxa, especially those with strong dispersal capabilities, have shown that divergence between  
54 Antarctic and South American populations or sister species could be much more recent than  
55 the physical separation of the continental landmasses and may rather have been driven by  
56 more recent geographic and oceanographic changes like the evolution of the Scotia Arc and  
57 the deepening of the Drake Passage (González-Wevar et al. 2012a; Poulin et al. 2014). The ACC  
58 is generally considered to act as an impervious hydrographic barrier for most marine species  
59 (Clarke et al. 2005; Thatje et al. 2005). Indeed, many studies have shown the absence of gene  
60 flow between lineages across the ACC (Krabbe et al. 2010; Janosik et al. 2011; Stupnikova et al.  
61 2013; Poulin et al. 2014; Weis et al. 2014). However, the permeability of this barrier has been  
62 questioned by recent studies since low levels of exchanges across the ACC have been observed  
63 for spider crabs (Clarke et al. 2005), ribbon worms (Mahon et al. 2010) and the sea star  
64 *Odontaster meridionalis* (Janosik et al. 2011). These new evidences of the ability of species to  
65 permeate the Polar Front have raised questions about the importance of historical land mass  
66 connectivity versus more recent exchanges across the ACC in driving the distribution of the  
67 Southern Ocean benthic biota (Thatje and Fuentes 2003; Tavares and De Melo 2004; Clarke et  
68 al. 2005).

69           The persistence of high benthic marine diversity in the Antarctic continent is  
70 particularly puzzling when considering the major Quaternary climatic oscillations, which led to  
71 the formation of an ice-sheet reaching the limits of the continental plateau and likely  
72 eradicating life in shallow subtidal areas (Thatje et al. 2005). Many invertebrates are highly  
73 abundant and diverse along the Antarctic coasts (Clarke and Crame 1989; Clarke and Johnston  
74 2003; Linse et al. 2006; Aronson et al. 2007; Rogers et al. 2007), suggesting that major climatic  
75 and oceanographic changes in the region did not impede their evolutionary success (Clarke  
76 and Crame 1989; Aronson et al. 2007). Several hypotheses have been proposed to explain the  
77 occurrence of such diversity despite major changes in habitat availability. The “deep-sea  
78 refugia” model proposes that species of the Antarctic shelf shifted their bathymetric range  
79 toward the deep sea during events of maximum ice cover, and later recolonized shelf areas  
80 following the deglaciation process (Kussakin 1973; Thatje et al. 2005; Allcock and Strugnell  
81 2012). This hypothesis has been proposed for invertebrate species with wide eurybathic  
82 ranges, and has been confirmed by phylogeographic analyses (e.g. the crinoid *Promachocrinus*  
83 *kerguelensis*; Hemery et al. 2012). However, the model is not applicable to shallow benthic  
84 species, such as seaweeds and herbivores that feed on them, due to their dependence on light  
85 availability. Two alternative hypotheses, the “shelf in situ refugia” and the “island refugia”  
86 models, propose that some species might have survived in situ either because ice did not cover  
87 the entire shelf area at the same time, or alternatively because organisms sought refuge  
88 outside of the Antarctic continental shelf in more or less distantly surrounding islands (Thatje  
89 et al. 2005; Raupach et al 2010; Diaz et al. 2012; González-Wevar et al. 2013).

90           In parallel, sub-Antarctic species have also experienced important changes in their  
91 respective distribution ranges due to Quaternary glacial cycles. During the Last Glacial  
92 Maximum (LGM), the southern tip of South America was covered by the Patagonian ice-sheet  
93 that extended approximately from Chiloé Island (42°S) to the Fuegian low lands (56°S) (Hulton  
94 et al. 2002), and this had various effects on species of southern Chile and Argentina

95 (Valdovinos et al. 2003; Aguirre et al. 2013). However, coastal ice-sheets were absent in the  
96 Cape Horn region and along the Scotia Arc (Hulton et al. 1994, 2002; Fraser et al. 2012); this  
97 likely offered glacial refugia for marine species. Contrasting postglacial recolonization  
98 pathways have been inferred from the genetic evidence of several Patagonian species  
99 (González-Wevar et al. 2012a). Similarly, several terrestrial species including amphibians, river  
100 fish, mammals and plants were restricted to glacial refugia or became locally extinct, whereas  
101 others persisted *in situ* (i.e. in the areas putatively covered by ice-sheets; Jakob et al. 2009;  
102 Vianna et al. 2011; Zemlak et al. 2011; Fraser et al. 2012). To date few studies have focused on  
103 marine Patagonian species and the existing results indicated diverse scenarios. These scenarios  
104 include potential post-glacial recolonization from distant Sub-Antarctic sources (e.g. *Durvillaea*  
105 *antarctica*, Fraser et al. 2010), from northern, unglaciated regions (e.g. *Mazzaella*  
106 *laminarioides*, Montecinos et al. 2012), or from local refugia in the southern sub-Antarctic  
107 region (Valdovinos et al. 2003). The potential occurrence of glacial refugia between Cape Horn  
108 and the South Sandwich archipelagos raises questions about the origin of both sub-Antarctic  
109 and Antarctic diversity.

110         With its present distribution embracing the southern coast (up to 40°S) of Chile and  
111 Argentina, sub Antarctic islands (Falkland Islands), Antarctic Islands (South Shetland, South  
112 Orkney Islands and South Georgia) and the Antarctic Peninsula, the red alga *Gigartina*  
113 *skottsbergii* is a suitable model to investigate the impact of major climatic changes on the  
114 subtidal flora in high southern latitudes. This species is highly patchy, with populations  
115 generally less than a square kilometer in size (Ramirez and Santelices 1991). It belongs to the  
116 order Gigartinales, which appears to have originated on Antarctic coasts when this continent  
117 was still attached to Australasia and South America (Hommersand et al. 1994). The northern  
118 limit of *G. skottsbergii*'s distribution is set by contrasting topological and oceanic  
119 characteristics including changes in the seawater surface temperature (i.e. the transition  
120 between cold waters to more temperate ones) (Ramirez and Santelices 1991). This

121 carragenophyte alga is pseudo-perennial (Wiencke and Clayton 2002) and blades may reach  
122 up to 1–2m in diameter (Santelices 1988). *G. skottsbergii* is haploid-diploid and both phases of  
123 the isomorphic life cycle coexist in time and space (Piriz 1996; Avila et al. 1999; Westermeier et  
124 al. 1999). Propagation is achieved through sexual reproduction (Avila et al. 1999). Antarctic  
125 specimens are separated from South American plants by more than 2% *rbcL* base pair distance  
126 (Hommersand and Fredericq 2003), which is not uncommon among species within red algae  
127 (e.g. Gavio and Fredericq 2002). Furthermore, Antarctic and sub-Antarctic populations show  
128 physiological differences: while in Antarctica spores germinate at 0°C and juveniles grow only  
129 at temperatures below 5°C (Bischoff-Bäsmann and Wiencke 1996), spores from sub-Antarctic  
130 populations do not germinate at 0°C and can grow at up to 15°C (Buschmann et al. 1999)  
131 which might result from an adaptation to regional environmental conditions. These first  
132 genetic and physiological results suggest that there is some evolutionary divergence between  
133 *Gigartina* populations from Antarctica and South America. The objective of this study is to infer  
134 the evolutionary history of sub-Antarctic and Antarctic populations of the red alga *G.*  
135 *skottsbergii* (Setchell et Gardner) using mitochondrial and chloroplast markers *Cox2-3* and  
136 *rbcL*. Two main processes were investigated: the divergence between Antarctic and South  
137 American populations, and the genetic consequences of last glacial cycle on both continents.

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139 Materials and Methods.

140 *Sampling-* Samples were collected by autonomous diving in the shallow subtidal zones  
141 and includes a total of 233 individuals of *G. skottsbergii*. Samples were extracted from 18  
142 localities covering most of the distribution range of the species (Chilean and Antarctic coasts;  
143 Table 1). In order to avoid sampling genetically identical ramets we sampled fronds from  
144 distinct holdfasts. Individuals were sampled from the lower littoral down to the depth of 25m.

145 Each individual tissue sample was cut from a clean healthy frond and placed into a plastic bag  
146 filled with silica beds for rapid dehydration and preservation of DNA.

147 *DNA extraction, PCR amplification and sequencing-* Dried algal tissue was finely  
148 grounded using liquid nitrogen and DNA was extracted using the phenol–chloroform method  
149 described in Faugeron et al. (2001). The Cox2-3, an intergenic non-coding mitochondrial region  
150 located between the genes for cytochrome oxidase subunit 2 (COX2) and 3 (COX3) was  
151 amplified following Zuccarello et al. (1999). In total, 233 sequences of approximately 350 bp  
152 were obtained. Additionally, we amplified a 971 bp region of the chloroplastic gene *rbcl*,  
153 encoding the large subunit of the ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO)  
154 enzyme, using the primers F-*rbcl* and R-*rbcl* (Hommersand et al. 1994) and the PCR conditions  
155 described by Fredericq and Lopez-Bautista (2002). Sequences were obtained for a sub-sample  
156 of 26 individuals (Table 1) for the *rbcl* gene. All PCR reactions were performed in a Gene Amp  
157 PCR system 9700 (Applied Biosystems, Foster City, USA). The amplified samples from each  
158 individual were purified with the UltraClean™ kit (MO BIO Laboratories, Carlsbad, USA) and  
159 sequenced in both directions by Macrogen Inc. (Seoul, South Korea). Sequences were edited  
160 using Chromas v. 2.33 (McCarthy 1997) and alignments were obtained using the CLUSTAL  
161 function of Mega v 5 (Tamura et al. 2011). Sequences were deposited in Genbank with  
162 accession numbers KM261841 to KM261858 for the Cox2-3 region and accession numbers  
163 KM261859 to KM261862 for the *rbcl* region. Alignments of the sequences used for the Cox2-3  
164 region and the *rbcl* for phylogenetic reconstructions are available in Online Resources 1 and 2,  
165 respectively.

166 For the *rbcl* and the coding part of the Cox2-3 sequences obtained, the McDonald-  
167 Kreitman test (<http://mkt.uab.es>, Egea et al. 2008) was performed to detect selection. The  
168 neutrality index (NI) was calculated as follows:  $NI = (P_n/P_s)/(D_n/D_s)$ , where P is the  
169 polymorphism within the population, D is the divergence or fixed difference between  
170 populations, n is for non synonymous and s is for synonymous mutations.

171 *Phylogenetic reconstructions and estimation of divergence time* – Phylogenetic  
172 reconstructions for each marker dataset were performed with the Maximum Likelihood (ML)  
173 method using TreeFinder v March 2011 (Jobb et al. 2004) and a Bayesian inference (BI) using  
174 MrBayes v 3.1.2 (Huelsenbeck and Ronquist 2001). Outgroup species belonging to the genus  
175 *Sarcothalia* and *Iridaea* were chosen since they represent the closest known sister-taxa of  
176 *Gigartina skottsbergii* in the phylogenetic systematics of the Gigartinaceae (Hommersand et al.  
177 1999). Outgroup sequences considered for the *rbcl* consisted of four species of *Sarcothalia* (*S.*  
178 *crispata*, SCU03085; *S. stiriata*, SSU03089; *S. livida*, SLU03087 and *S. circumcincta*, AF146219)  
179 and two sequences of *Iridaea cordata* from Antarctica (U02989 and GQ323780). For the *cox2-*  
180 *3*, two sequences of *S. crispata* (KM275591 and KM275592, both from Punta Estaquilla) and  
181 one sequence of *I. cordata* (KM275593 from Punta Hanna, South Shetland Islands) were used  
182 as outgroup. We also included in the analyses available *rbcl* sequences for *G. skottsbergii*  
183 (U03432, Ancud, Chile and AF146206, King George Island, South Shetland Islands;  
184 Hommersand et al. 1999). For both phylogenetic reconstruction methods, large indels within  
185 the non-coding intergenic region of the *Cox2-3* were treated as single mutation events.

186 ML analyses were performed using a mixed model taking into account the position of  
187 codons for the *rbcl* gene while only one model was used for the *cox2-3* intergenic region  
188 analysis. TreeFinder v March 2011 (Jobb et al. 2004) allows to choose between 32 substitution  
189 models for each partition of the data set. The best-fitted substitution models were selected  
190 using the Akaike Information Criterion implemented in the ModelTest package of the  
191 TreeFinder program (Posada and Crandall 1998; Jobb et al. 2004). The selected models for the  
192 *rbcl* data were TN+G for the first codon position, HKY+G for the second codon position and  
193 J3+G for the third codon position. For *cox2-3* the selected model was HKY+G. Using TreeFinder  
194 v March 2011, we performed a heuristic search in order to reconstruct the trees and node  
195 supports were assessed by non-parametric bootstrapping (1000 pseudo-replicates, Felsenstein  
196 1985).



197 Bayesian inference was performed using the general criteria of the best fit model  
198 parameters defined for each dataset. Four independent analyses were run with four chains  
199 each (3 heated chains and one “cold” chain) for ten million generations. The settings were a  
200 heating parameter value of 0.2 and sampling every 1000 generations with randomly generated  
201 starting trees. The first 25% of sampled trees were discarded as “burn-in” to ensure  
202 convergence. The remaining trees were used to compute a consensus topology and posterior  
203 probability values. The split frequency (variance between the four independent runs) was  
204 below 0.0005, confirming that the posterior probability distribution was accurately sampled.  
205 Because the posterior probability bootstrap values were essentially identical in the  
206 independent runs starting from different, random topologies, we considered that the chains  
207 had converged.

208 Even though the lack of fossils impedes a precise calibration of molecular clocks in red  
209 algae, we used divergence rates already published for this group to estimate the historical  
210 divergence event between South American and Antarctic populations. A divergence rate of  
211 0.109-0.127% per site per million years (Myr) has been proposed for *rbcL* (Kamiya et al. 2004).  
212 For *cox2-3*, a site mutation rate of 0.756-0.426% per Myr, based on the divergence of the red  
213 algae *Asparagopsis* spp. associated to the Panama isthmus closure, was proposed by Andreakis  
214 et al. (2007). Divergence time was estimated in BEAST v1.8 (Drummond et al. 2012) using the  
215 Yule model of tree prior, a gamma site heterogeneity model to allow variation among sites of  
216 the mutation rate, and a Log-normal relaxed clock with a uniform sampling within the range of  
217 published mutation rates. Four runs of ten millions MCMC iterations each were performed and  
218 the combined results were analyzed with Tracer v1.8 (Drummond et al. 2012). Effective sample  
219 size of the posterior distribution, the parameter of accuracy of the parameter estimation, was  
220 always superior to 300 in each individual run and in the combined analyses, indicating the  
221 MCMC appropriately converged to estimated values.

222           *Genetic diversity* - For *cox2-3*, we calculated five diversity indices for each sampled  
223 location and for the two phylogenetic lineages (i.e. *G. skottsbergii* from Chile and the Falkland  
224 Islands and *G. skottsbergii* from sub-Antarctic and Antarctic) using Arlequin v 3.5 (Excoffier and  
225 Lisher 2010): the number of haplotypes (nH); the number of private haplotypes (i.e.  
226 haplotypes found in a single population, nHpriv); the number of polymorphic sites (S); gene  
227 diversity (Hd, Nei 1987) and nucleotide diversity ( $\pi$ , Nei and Li 1979). For *rbcl*, only nH was  
228 calculated.

229           *Network reconstruction and historical demography* - Haplotype networks were  
230 reconstructed for *cox2-3* using the median-joining algorithm implemented in NETWORK v  
231 4.510 (Bandelt et al. 1999). Moreover, for this molecular marker, three complementary  
232 approaches were used to infer the historical demography of *G. skottsbergii* from Chile and the  
233 Falkland Islands.

234           First, Tajima's D (Tajima 1989) and Fu's Fs (Fu 1997) statistics were calculated to detect  
235 significant past changes in population size. Significant departure from mutation-drift  
236 equilibrium was tested by 1000 bootstrap replicates in Arlequin (Excoffier and Lisher 2010).  
237 Under the assumption of neutrality, negative values characterize populations in expansion  
238 while positive values, associated with the loss of rare haplotypes, are considered as a signature  
239 of recent bottlenecks (Tajima 1989, Fu 1997).

240           Second, the observed distributions of pairwise differences were compared to  
241 estimated values under a model of sudden pure demographic expansion (Roger and  
242 Harpending 1992) using Arlequin (Excoffier and Lisher 2010). The model fit between the  
243 observed and estimated mismatch distributions was calculated through a generalized least  
244 squares approach, which was then tested with 1000 permutations. The date of growth/decline  
245 ( $\tau=2\mu t$ ), measured in units of  $1/2 \mu$  generations where  $t$ =time in years and  $\mu$ =mutation rate per  
246 sequence per generation, was estimated using the demographic expansion parameters as

247 determined in the nonlinear least squares approach implemented in Arlequin (Excoffier and  
248 Lisher 2010).

249 Third, population growth rate and timing was estimated from coalescent simulations  
250 implemented in LAMARC 2.1.9 (Kuhner 2006). The maximum likelihood approach was applied  
251 using the Metropolis-coupled Markov chain Monte Carlo (MCMC) method with replication of  
252 chains and adaptive heating to achieve optimal sampling of the parameter space. The MCMC  
253 runs were performed three times with random seeds; each run used 10 initial chains with 500  
254 samples and two long final chains with 10 000 samples. All initial chains and final chains were  
255 simulated using a sampling interval of 20 and a burn-in of 1000 samples. A tenfold  
256 evolutionary rate (4.26-7.56% per million years) was considered at population level, following  
257 the correction for time dependence of molecular rate proposed by Ho et al. (2011).

258

259 Results.

260 Four chlorotypes were detected for the chloroplast marker *rbcL*, with 23 polymorphic  
261 sites along the 971 base pair fragments sequenced (11 sequenced individuals, Table 1). For the  
262 *cox2-3* mitochondrial marker, 18 mitotypes were observed (233 sequenced individuals, Table  
263 1). For this marker, 48 polymorphic sites were observed including two indels: one indel of 1bp  
264 characteristic of the mitotype C13 and one indel of 12bp for mitotypes C8 and C17. Sequence  
265 length of *cox2-3* sequences varied from 337 to 350bp.

266 Figure 1 shows the Maximum Likelihood (ML) phylogenetic trees constructed using the  
267 two molecular markers. For both markers, tree topologies based on Bayesian and ML analyses  
268 were largely congruent and shared comparable support values for major nodes (Figure 1).  
269 Regardless of the marker used, tree topologies were broadly similar among phylogenetic  
270 reconstruction methods and clearly showed that all *G. skottsbergii* sequences obtained in this  
271 study form a monophyletic group, clearly split into two well supported lineages (Figure 1,

272 support values >89%) that are strongly divergent from the outgroup species. The first lineage is  
273 composed of all individuals from Chile and the Falkland Islands while the second is composed  
274 of individuals from the Antarctic Peninsula, the South Shetland Islands and the South Orkney  
275 Islands (Figure 1). The available *rbcL* sequence AF146206 in GenBank for *G. skottsbergii*  
276 previously sampled in King George Island in the South Shetland Islands (Hommersand et al.  
277 1999) corresponds exactly to the chlorotype R4 present in our Antarctic lineage. The  
278 uncorrected p-distance, measured using only 665bp, between the *rbcL* sequence U03432 and  
279 the closest sequence in our data set (i.e. chlorotype R1, present only in Ancud, Chile, see Table  
280 1) is of 0.3%. This is congruent with the location where the specimen corresponding to the  
281 U03432 sequence was collected, on Chiloé Island within the bay of Ancud (Hommersand et al.  
282 1999). P distances between sequences from the Antarctic and South American *G. skottsbergii*  
283 lineages were of  $9.2 \pm 1.5$  % for *cox2-3* and of  $2.1 \pm 0.5$  % for *rbcL*. When performed between  
284 the Antarctic and South American *G. skottsbergii* lineages, no significant departure from  
285 neutrality was detected using the McDonald-Kreitman test for the *rbcL* and the coding part of  
286 the *Cox2-3* sequences analyzed (NI= 0.969, p = 0,980; NI= 2.191, p= 0.536, for the *rbcL* and the  
287 *Cox2-3*, respectively).

288         Divergence between the Antarctic and South American *G. skottsbergii* lineages was  
289 estimated at 9.4 Myr (95% CI: 3.2-16.4Myr) based on *cox2-3* data and at 14.9 Myr (95% CI: 2.6-  
290 35.9Myr) based on *rbcL* data.

291         For both markers, no genetic diversity was observed in the Antarctic lineage of *G.*  
292 *skottsbergii*. Only one haplotype was detected for the *cox2-3* region and for the *rbcL* gene, the  
293 mitotype C18 and the chlorotype R4, respectively (Table 1). The Antarctic lineage is spread  
294 over more than 1600 km of coast, from Marguerite Bay (67°S) to the South Orkney Islands  
295 (60°S) (Table 1, Figure 2C). Within the South American lineage of *G. skottsbergii* the genetic  
296 diversity in *cox2-3* was generally low with the number of mitotypes per sampling site (nH)

297 being lower than 3 and the gene diversity ( $H_d$ ) as lower than 0.3 in 6 of the 11 studied  
298 populations (Table 1). The highest genetic diversity was observed in the population of PAG,  
299 BCH and FAL ( $n_H = 5$  and  $H_d > 0.5$  in all three populations, Table 1). These three populations  
300 were also the ones with the highest number of private haplotypes, with three private  
301 haplotypes in PAG and FAL and two in BCH (Table 1). For the *rbcl* gene, only three chlorotypes  
302 were observed within the South American lineage of *G. skottsbergii*. Only one chlorotype was  
303 found in each single population and the only private chlorotype (R1) was observed in the ANC  
304 population (Table 1). The chlorotype R3 was shared between BOR in Tierra del Fuego and FAL  
305 located in the Falkland Islands (Table 1).

306         The *cox2-3* mitotype network revealed the presence of two main haplogroups, one for  
307 South America and one for Antarctica, which are separated by 31 bp (Figure 2A), a result fully  
308 congruent with the phylogenetic reconstructions (Figure 1). Within the South American  
309 haplogroup, characterized by a typical star-like topology, pairs of mitotypes were separated by  
310 1 to 5 bp, except for the mitotypes C17 and C8 that were differentiated by a unique 12bp  
311 indel. This haplogroup is also characterized by one frequent and widespread mitotype (C1, 81%  
312 of the samples, Table 1) and several less frequent mitotypes (Figure 2A). Low frequency  
313 mitotypes were predominantly restricted to a single or few nearby local populations. For  
314 example, the mitotype C3 was observed in BLO and PAG, these being two populations located  
315 in the Moraleda Channel. Additionally, mitotype C7 was observed in BCH, BOR and TOR which  
316 are located in the southern part of the Magellanic region and Tierra del Fuego (Figure 2B). One  
317 mitotype, C13, was also shared between BCH in the southern part of the Magellanic region and  
318 FAL located in the Falkland Islands (Figure 2B). As expected, the star-like topology is coupled  
319 with a unimodal mismatch distribution (Figure 3A), and the values of Tajima's  $D$  and Fu's  $F_s$   
320 statistics were both negative and significant ( $D = -2.01$ ,  $p = 0.001$ ;  $F_s = -1.26$ ,  $p = 0.0001$ ) a  
321 result congruent with a sudden demographic population expansion model. Population size  
322 changes depicted from the mismatch distribution was  $\tau = 0.73$  (90% confidence interval of 0.00

323 to 2.23). Assuming the start of exponential demographic expansion when population size was  
324 1% of present-day's estimate, it was estimated to initiate 20 000 – 36000 years before present.  
325 A large positive exponential growth rate,  $g= 3004$  (2458-4666 95% confidence interval), was  
326 also detected. It is possible that demographic expansion led to a large increase in  $N_e$   
327 (approximately 170 000 to 300 000-fold increase, Fig. 3B), which may have started during or  
328 just before the LGM, depending on the combination of growth and mutation rates considered  
329 within their respective confidence limits.

330

### 331 Discussion

332 The patterns of genetic structure for *G. skottsbergii* seem to confirm the absence of gene flow  
333 between Antarctic and South American populations. Regardless of the marker analyzed,  
334 phylogenetic reconstructions using mtDNA and cpDNA sequences showed strong congruence  
335 and clear support for two distinct lineages consisting of populations from South America and  
336 the Falklands on the one hand and populations from the Antarctic Peninsula, Shetlands and  
337 Orkney Islands on the other hand. The absence of shared haplotypes between the two regions  
338 may reflect the isolation created by the Antarctic Circumpolar Current between the Antarctic  
339 Peninsula and the South American continent. Divergences among lineages of *G. skottsbergii* for  
340 the Cox2-3 ( $9.2 \pm 1.5$  %) and for the *rbcl* ( $2.1 \pm 0.5$  %) are within the range reported for  
341 interspecific distances between sister species in Rhodophyta (ranging from 2.55% to 4.70% and  
342 from 0.77% to 5.08% for the Cox2-3 and the *rbcl*, respectively; McIvor et al. 2001; Destombe  
343 et al. 2010; Hernández-Kantún et al. 2014). The large range estimated, of 2.6 to 35.9 My  
344 depending on the genetic marker and the mutation rate considered (with central tendency  
345 around 9 to 15My), for divergence time between the two lineages seems to predate  
346 Pleistocene glaciations. This time of divergence is however more recent than the separation of  
347 the Antarctic continent from the South American continent (approximately 24-40 Myr ago),

348 and seems to include the period of intensification of the ACC circulation 11-12 Myr ago (Dalziel  
349 et al. 2013). These climatic and oceanographic changes have been shown to be major drivers in  
350 the isolation of marine Antarctic fauna. For example, González-Wevar et al. (2010) have shown  
351 that the diversification within a genus of mollusk (*Nacella*) took place long after the separation  
352 of the continents. For these marine mollusks, the appearance of the most genetically distant  
353 clades (Kerguelen, Antarctic, and South America) took place between 9 and 5 Myr. These  
354 results were further supported by a recent study of comparative phylogeography of different  
355 invertebrate taxa showing a shared Antarctic and South American distribution (González-  
356 Wevar et al. 2012a; Poulin et al. 2014). In these studies, divergence times between Antarctic  
357 and South American lineages ranged from 1.0 to 13.6 Myr, largely overlapping the estimates  
358 for *G. skottsbergii*. Interestingly, divergence estimates in the mitochondrial markers for *G.*  
359 *skottsbergii* (9.2%) fall within the 7-11% range for shallow subtidal and intertidal invertebrates  
360 (González-Wevar et al. 2010, 2012a; Poulin et al. 2014), corroborating the occurrence of a  
361 shared evolutionary history despite imprecisions in the respective mutation rates. These  
362 authors proposed that the connection between Antarctic and South American populations  
363 could have been maintained by a stepping stone process along the archipelago of the Scotia  
364 Arc. Indeed, geological evidence has recently been reported of a now-submerged volcanic arc  
365 in the Central Scotia Sea that existed during the early Miocene (Dalziel et al. 2013). This  
366 archipelago was located closer to the Antarctic Peninsula and the Cape Horn region than the  
367 South Sandwich Islands are today, and may thus have provided a corridor for genetic  
368 connectivity across the Drake Passage until approximately 10-11 Myr ago (Poulin et al. 2014).  
369 Our study further shows that *G. skottsbergii*, a seaweed characterized by a very restricted  
370 dispersal capacity of its spores, may also have maintained a certain level of connectivity (even  
371 after the mid-Miocene) between the continents of Antarctica and South America through the  
372 volcanic arc of islands linking both sides of the Scotia Sea. This connection could have been

373 maintained until the beginning of Pliocene's glaciations, as in the case of brooding  
374 invertebrates, through rafting of adults (Nikula et al. 2010; Haye et al. 2012; Poulin et al. 2014).

375 The Antarctic and South American lineages of *G. skottsbergii* exhibit different patterns of  
376 genetic diversity. While a strong demographic expansion was inferred in the sub-Antarctic  
377 region, a total absence of genetic diversity was observed in the Antarctic lineage. The presence  
378 of a single haplotype over more than 1600 km from Marguerite Bay to the South Orkney  
379 Islands is intriguing. Even if this lack of diversity limits our capacity to test for different  
380 demographic scenarios in Antarctica, it suggests that a very strong demographic bottleneck  
381 occurred during glacial contraction, followed by a sudden and recent recolonization process  
382 that did not allow for new mutations. Similarly, the overall low genetic diversity and the  
383 presence of the same common haplotype C1 in every population from the Chilean coast to the  
384 Falkland Islands seems to support the hypothesis of persistence in a single glacial refugium  
385 followed by a massive demographic expansion over 2500 km. Such a pattern of genetic  
386 homogeneity over a broad geographical range has usually been related to high dispersal  
387 potential (Bortolotto et al. 2011; González-Wevar et al. 2012b). The scenario of rapid  
388 recolonization leading to the presence of only one haplotype over thousands of kilometers of  
389 coast is, however, difficult to envision for *G. skottsbergii*. Indeed, this alga is a non-buoyant  
390 species, and spore dispersal is considered to be very limited (Ramirez and Santelices 1991).

391 Nevertheless, signatures of long distance dispersal have been observed in other apparently  
392 non-dispersive algal species like *Adenocystis utricularis* or *Bostrychia intricata* and have been  
393 explained by the organisms' potential ability to raft on floating substrates (Fraser et al. 2010,  
394 2013). In contrast with our results, in a previous study using RAPDs nuclear markers (Faugeron  
395 et al. 2004), significant genetic differentiation among South American populations was  
396 observed and has been related to the poor dispersal capacity of for *G. skottsbergii*. Differences  
397 in mutation rates and/or level of drift effects (effective population size of uniparentally  
398 inherited loci is only one-fourth that of nuclear loci) may account for the differences between



399 level of genetic structure obtained with RAPDs (Faugeron et al. 2004) and cytoplasmic  
400 sequences (our study). Also, during spatial expansion, gene surfing effects may contribute to  
401 the reduction of diversity in cytoplasmic markers in the recolonized region (Excoffier and Ray  
402 2008). Indeed, large-scale spread of mitochondrial genetic variants has been observed during  
403 recolonization process in seaweeds (Fraser et al. 2010). The ACC is a strong west to east  
404 current that connects the Antarctic Peninsula and the South Shetland Islands to the Antarctic  
405 Islands located in the South Scotia Ridge. Particle movement modeling and particle tracking  
406 has shown that passive drifters travel northeastwards across the Scotia Sea, connecting the  
407 Antarctic Peninsula and the South Shetland Islands to South Orkney Islands and South Georgia  
408 (Thorpe et al. 2004). Movement of drifting seaweed along the ACC could be connected to  
409 west-east spatial expansion pattern. On the other hand, the postglacial colonization of the  
410 Peninsula could have been promoted by stepwise spatial expansion through spore  
411 propagation. Such spatial expansion could have been facilitated by strong coastal currents, as  
412 the Antarctic Peninsula Coastal Current (APCC; Moffat et al. 2008), a southward current that  
413 forms during the ice-free seasons and extends to Alexander Island. However, we lack  
414 knowledge on the actual mechanisms of dispersal in *G. skottbergii* to appropriately determine  
415 the role of dispersal during post-glacial population dynamics on the present day genetic  
416 diversity.

417 The location of both Antarctic and South American glacial refugia is difficult to infer from the  
418 results presented here. The extreme genetic homogeneity within the *G. skottsbergii* Antarctic  
419 clade is in disagreement with the hypothesis that genetic structure and global genetic diversity  
420 in Antarctica may have been promoted by the fragmentation and isolation of micro-refugia  
421 where marine species were able to survive during repeated ice advances and retreats (Thatje  
422 et al. 2005; Allcock and Strugnell 2012). The possible presence of micro-refugia has been  
423 particularly highlighted in the archipelagoes located near the West Antarctic Peninsula and the  
424 South Shetland Island (See Fraser et al. 2012 for review). Indeed, new studies using molecular

425 data have shown that the South Shetland Islands is the most speciose region for endemic  
426 Southern Ocean octopuses of the genus *Pareledone* (Allcock et al. 2011). Even though the data  
427 herein lack the explicit molecular diversity to test adequately for the number and location of  
428 glacial refugees in this region, the survival of *G. skottsbergii* during the LGM seems more likely  
429 in the northernmost latitude (South Shetland and South Orkney Islands). Indeed, whereas *G.*  
430 *skottsbergii* is found in dense beds within these northern archipelagos, less than ten specimens  
431 could be collected in Paradise Bay and only one specimen was sampled in Marguerite Bay  
432 (Note that the same sampling effort was applied in Paradise Bay, Marguerite Bay, O'Higgings  
433 and Punta Prat; Table 1; M-L Guillemin, personal communication). It is understood that an  
434 increased sampling effort could better elucidate the southern limits of *G. skottsbergii*, but it  
435 seems that in Marguerite Bay the alga has reached a biotic or abiotic limit to its distribution.

436 The absence of genetic structure for the mitochondrial and chloroplast markers in southern  
437 South America also limits our ability to infer the number and location of glacial refugia.  
438 Because of their longstanding demographic stability, populations from glacial refugia are  
439 expected to present higher levels of genetic diversity than those populations that have formed  
440 following postglacial expansions (Provan and Bennett 2008). In addition, long-term isolation in  
441 distant refugia often leads to genetic differentiation due to mutation accumulation and genetic  
442 drift (Hewitt 2000). Along the Chilean coast, recolonization pathways have differed between  
443 organisms in terms of the number and origin of sources. A recent recolonization from a single  
444 distant/exterior source has been proposed to explain the high genetic homogeneity of  
445 populations of the red alga *Mazaella laminarioides* (Montecinos et al. 2012) and the brown  
446 alga *Durvillaea antarctica* that exist south of 42°S (Fraser et al. 2010). In *M. laminarioides*, a  
447 single refugium has been proposed, located in the North of the Island of Chiloe and the  
448 mainland coast north of the 41°S (Montecinos et al. 2012). In *D. antarctica*, broad sampling  
449 across the southern hemisphere has shown that all individuals present off the Patagonian  
450 coast of Chile share the same haplotype (Fraser et al. 2010) and the authors have indicated

451 that this species likely recolonized the region from a refugium in New Zealand sub-Antarctic  
452 archipelagoes. Yet others have highlighted the role that the Southern Fjords and Channels  
453 Region played as a refuge for several animal species, like marine mollusks (Valdovinos et al.  
454 2003; González-Wevar et al. 2010) and the river otter (Vianna et al. 2011). The existence of  
455 multiple refugia, scattered among the Southern Fjords and Channels, has been proposed to  
456 explain the high levels of endemism and the high diversity of mollusks in this region  
457 (Valdovinos et al. 2003). In *G. skottsbergii*, the overall low genetic diversity and the presence of  
458 the same common haplotype, C1, in every population from the Chilean coast to the Drake  
459 Passage and the Falkland Islands seem to support the hypothesis of a single glacial refugium.  
460 Strong currents (i.e. the Cape Horn Current and the Malvinas-Falkland Current) connect the  
461 southern coast of Chile from 42°S to Cape Horn and the Falkland Islands. These currents have  
462 been shown to provide high connectivity among all inhabiting regions for the genus *Nacella*  
463 (González-Wevar et al. 2012b). In this genus, the existence of asymmetric gene flow, from  
464 West to East, was shown to be related to the prevailing circulation patterns in this region  
465 (González-Wevar et al. 2012b). Due to these strong directional currents, the hypothesis of a  
466 postglacial recolonization of the Chilean coast by *G. skottsbergii* from a refuge located in the  
467 Falkland Islands is most unlikely. Again, more polymorphic markers should help identifying  
468 regions with higher / lower genetic diversity as putative locations of glacial refugia /  
469 recolonized areas, respectively, for *G. skottsbergii*.

470

#### 471 Conclusions

472 In accordance with previous results of Hommersand et al. (2009) the data presented here  
473 clearly show two divergent and respectively monophyletic clades of *G. skottsbergii* that may  
474 correspond to two cryptic species. *G. skottsbergii* (Type locality: Slogget Bay, Fuegia - Silva  
475 1996; <http://www.algaebase.org>) is distributed in the Southern and sub-Antarctic coast of

476 Chile and the Falkland Islands. The Antarctic species, still to be formally described and named,  
477 occurs in the Antarctic Peninsula, the South Shetland Islands and the South Orkney Islands.  
478 Despite drastic reductions in population size, as revealed by strong signals of bottlenecks, both  
479 species persisted and maintained their separation on either side of the ACC even during the  
480 glacial period. The divergence time between the two cryptic species, estimated as 9.4 to  
481 14.9 Myr (comparable with other invertebrate species, but with a large confidence interval of  
482 2.6-35.9Myr), indicates that algae with limited dispersal capabilities were able to cross the  
483 Scotia Sea after separation of the continents, potentially via a stepping stone process through  
484 the volcanic arc of islands. Our work also sheds light on the possible importance of dispersal by  
485 rafting in the recolonization process of a species for which natural propagation is mainly  
486 achieved via sexual reproduction (Avila et al. 1999; Westermeier et al. 2012). Future prospects  
487 on glacial refugia for Antarctic and sub-Antarctic seaweeds shall also contribute to better  
488 understand the dispersal mechanisms during demographic expansion in these regions  
489 characterized by complex topography of coastal shelf and coastal currents.

490

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507

508 Figure 1: Maximum likelihood rooted trees for the *cox2-3* and the *rbcl* haplotypes of *Gigartina*  
509 *skottsbergii*. Maximum likelihood bootstrap values are indicated above each node and  
510 Bayesian posterior probabilities are noted between brackets. Only high support values are  
511 shown (>75 for bootstraps and >0.95 for posterior values, respectively; - correspond to  
512 branches not observed in the Bayesian inference reconstruction).

513 Figure 2: Haplotype networks of *Gigartina skottsbergii* and their geographic distribution based  
514 on *cox2-3*. The haplotype networks are presented in (A) while the pie charts showing the  
515 geographical distribution of haplotypes of the South American haplogroup are shown in (B)  
516 and the pie charts showing the geographical distribution of haplotypes of the Antarctic  
517 haplogroup are shown in (C). In the networks, each circle represents a haplotype and its size is  
518 proportional to the frequency in which the haplotype was encountered (correspondence  
519 between circle sizes and numbers of individuals is indicated in the box A). The black square in-  
520 between C7, C9 and C14 represents a hypothetical un-sampled haplotype. For haplotypes  
521 separated by more than one mutational step, the number of steps is indicated in bp. Pie  
522 charts' color-code corresponds to the one used in haplotype networks. Abbreviations for  
523 population codes are as in Table 1. Numbers of sequenced individuals are given between  
524 brackets.

525 Figure 3: Mismatch distributions (A) and population growth rate estimates (B) of the South  
526 American haplogroup of *Gigartina skottsbergii* for the *cox2-3* marker. Growth rate and timing  
527 of population dynamic changes were estimated from coalescent simulations implemented in  
528 LAMARC 2.1.9 (Kuhner 2006). The observed distribution of the number of pair base differences  
529 between sequences is indicated by the grey bars while the expected distribution under a  
530 model of sudden demographic expansion is represented by a black line. Effective population  
531 size (i.e.  $N_e$ ) fluctuations throughout time are represented in the LAMARC graph. The dotted  
532 lines represent the 95% confidence intervals of the estimated growth rate, whereas the grey

533 shaded area corresponds to the average growth rate combined with the range of mutation  
534 rates proposed by Andreakis et al (2007) corrected by a tenfold evolutionary rate as suggested  
535 at population level for time dependence of molecular rate by Ho et al. (2011).

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1 Deep genetic divergence between austral populations of the red alga *Gigartina skottsbergii*  
2 reveals a cryptic species endemic to the Antarctic continent.

3

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20 Abstract

21 The almost complete isolation of Antarctica after the intensification the Antarctic Circumpolar  
22 Current (ACC) during the middle-Miocene has been challenged by recent molecular data  
23 showing the existence of [allelic](#) exchange across the ACC. For organisms present on both sides  
24 of the ACC, two hypotheses have then been discussed to explain the origin of the Antarctic  
25 populations: 1) they correspond to recent [immigrants](#) from [adjacent](#) continents or 2) they have  
26 evolved in situ and have survived the dramatic effects of the last Quaternary glaciations in this  
27 region. The red algae *Gigartina skottsbergii* presents a disjoint distribution and is reported in  
28 both Antarctica and southern South America, a distribution pattern that largely exceeds its  
29 dispersal capacity. Mitochondrial sequences of the intergenic region cox2-3 (n=233) and partial  
30 chloroplastic RuBisCo large subunit gene (n=26) sequences were obtained for individuals from  
31 the Chilean sub-Antarctic ecoregion and Antarctic Peninsula localities. The results strongly  
32 support the persistence of populations on each side of the Drake Passage during glacial  
33 periods and the existence of dispersal barrier due to the ACC. On both sides of the ACC, the  
34 last Quaternary glaciations have induced strong bottlenecks that were followed by rapid  
35 colonization events.

36 Keywords: Phylogeography, glacial refugia, seaweed, Antarctica, *rbcl*, *cox2-3*,

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43 Introduction

44           The Southern Ocean is characterized by high levels of endemism of its fauna and flora  
45 (Clarke and Crame 1989; Brandt et al 1999; Clarke and Johnston 2003; Wulff et al. 2009) that  
46 has been related to the progressive isolation of the continent during the Mesozoic and the  
47 reinforcement of the Antarctic Circumpolar Current (ACC) during the mid-Miocene (Lawver  
48 and Gahagan 1998; Rogers et al. 2007; Poulin et al. 2014). Moreover, after the onset of  
49 icehouse conditions in Antarctic, both the radiation of groups that have adapted to this  
50 extreme environment and allopatric speciation driven by population fragmentation in  
51 Antarctic refugia during glacial period seem to have contributed to the high Antarctic diversity  
52 (Rogers et al. 2007; Thatje et al. 2008). Recent molecular data for several marine invertebrate  
53 taxa, especially those with [strong dispersal](#) capabilities, have shown that divergence between  
54 Antarctic and South American populations or sister species could be much more recent than  
55 the physical separation of the continental landmasses and may rather have been driven by  
56 more recent geographic and oceanographic changes like the evolution of the Scotia Arc and  
57 the deepening of the Drake Passage (González-Wevar et al. 2012a; Poulin et al. 2014). The ACC  
58 is generally considered to act as an impervious hydrographic barrier for most marine species  
59 (Clarke et al. 2005; Thatje et al. 2005). Indeed, many studies have shown the absence of gene  
60 flow between lineages across the ACC (Krabbe et al. 2010; Janosik et al. 2011; Stupnikova et al.  
61 2013; Poulin et al. 2014; Weis et al. 2014). However, the permeability of this barrier has been  
62 questioned by recent studies since low levels of exchanges across the ACC have been observed  
63 for spider crabs (Clarke et al. 2005), ribbon worms (Mahon et al. 2010) and the sea star  
64 *Odontaster meridionalis* (Janosik et al. 2011). These new evidences of the ability of species to  
65 permeate the Polar Front have raised questions about the importance of historical land mass  
66 connectivity versus more recent exchanges across the ACC in driving the distribution of the  
67 Southern Ocean benthic biota (Thatje and Fuentes 2003; Tavares and De Melo 2004; Clarke et  
68 al. 2005).

69           The persistence of high benthic marine diversity in the Antarctic continent is  
70 particularly puzzling when considering the major Quaternary climatic oscillations, which led to  
71 the formation of an ice-sheet reaching the limits of the continental plateau and likely  
72 eradicating life in shallow subtidal areas (Thatje et al. 2005). Many invertebrates are highly  
73 abundant and diverse along the Antarctic coasts (Clarke and Crame 1989; Clarke and Johnston  
74 2003; Linse et al. 2006; Aronson et al. 2007; Rogers et al. 2007), suggesting that major climatic  
75 and oceanographic changes in the region did not impede their evolutionary success (Clarke  
76 and Crame 1989; Aronson et al. 2007). Several hypotheses have been proposed to explain the  
77 occurrence of such diversity despite major changes in habitat availability. The “deep-sea  
78 refugia” model proposes that species of the Antarctic shelf shifted their bathymetric range  
79 toward the deep sea during events of maximum ice cover, and later recolonized shelf areas  
80 following the deglaciation process (Kussakin 1973; Thatje et al. 2005; Allcock and Strugnell  
81 2012). This hypothesis has been proposed for invertebrate species with wide eurybathic  
82 ranges, and has been confirmed by phylogeographic analyses (e.g. the crinoid *Promachocrinus*  
83 *kerquelenensis*; Hemery et al. 2012). However, the model is not applicable to shallow benthic  
84 species, such as seaweeds and herbivores that feed on them, due to their dependence on light  
85 availability. Two alternative hypotheses, the “shelf in situ refugia” and the “island refugia”  
86 models, propose that some species might have survived in situ either because ice did not cover  
87 the entire shelf area at the same time, or alternatively because organisms sought refuge  
88 outside of the Antarctic continental shelf in more or less distantly surrounding islands (Thatje  
89 et al. 2005; Raupach et al 2010; Diaz et al. 2012; González-Wevar et al. 2013).

90           In parallel, sub-Antarctic species have also experienced important changes in their  
91 respective distribution ranges due to Quaternary glacial cycles. During the Last Glacial  
92 Maximum (LGM), the southern tip of South America was covered by the Patagonian ice-sheet  
93 that extended approximately from Chiloé Island (42°S) to the Fuegian low lands (56°S) (Hulton  
94 et al. 2002), and this had various effects on species of southern Chile and Argentina

95 (Valdovinos et al. 2003; Aguirre et al. 2013). However, coastal ice-sheets were absent in the  
96 Cape Horn region and along the Scotia Arc (Hulton et al. 1994, 2002; Fraser et al. 2012); this  
97 likely offered glacial refugia for marine species. Contrasting postglacial recolonization  
98 pathways have been inferred from the genetic evidence of several Patagonian species  
99 (González-Wevar et al. 2012a). Similarly, several terrestrial species including amphibians, river  
100 fish, mammals and plants were restricted to glacial refugia or became locally extinct, whereas  
101 others persisted *in situ* (i.e. in the areas putatively covered by ice-sheets; Jakob et al. 2009;  
102 Vianna et al. 2011; Zemlak et al. 2011; Fraser et al. 2012). To date few studies have focused on  
103 marine Patagonian species and the existing results indicated diverse scenarios. These scenarios  
104 include potential post-glacial recolonization from distant Sub-Antarctic sources (e.g. *Durvillaea*  
105 *antarctica*, Fraser et al. 2010), from northern, unglaciated regions (e.g. *Mazzaella*  
106 *laminarioides*, Montecinos et al. 2012), or from local refugia in the southern sub-Antarctic  
107 region (Valdovinos et al. 2003). The potential occurrence of glacial refugia between Cape Horn  
108 and the South Sandwich archipelagos raises questions about the origin of both sub-Antarctic  
109 and Antarctic diversity.

110           With its present distribution embracing the southern coast (up to 40°S) of Chile and  
111 Argentina, sub Antarctic islands (Falkland Islands), Antarctic Islands (South Shetland, South  
112 Orkney Islands and South Georgia) and the Antarctic Peninsula, the red alga *Gigartina*  
113 *skottsbergii* is a suitable model to investigate the impact of major climatic changes on the  
114 subtidal flora in high southern latitudes. This species is highly patchy, with populations  
115 generally less than a square kilometer in size (Ramirez and Santelices 1991). It belongs to the  
116 order Gigartinales, which appears to have originated on Antarctic coasts when this continent  
117 was still attached to Australasia and South America (Hommersand et al. 1994). The northern  
118 limit of *G. skottsbergii*'s distribution is set [by contrasting topological and oceanic](#)  
119 [characteristics including changes in the seawater surface temperature \(i.e. the transition](#)  
120 [between cold waters to more temperate ones\)](#) (Ramirez and Santelices 1991). This

121 carragenophyte alga is pseudo-perennial (Wiencke and Clayton 2002) and blades may reach  
122 up to 1–2m in diameter (Santelices 1988). *G. skottsbergii* is haploid-diploid and both phases of  
123 the isomorphic life cycle coexist in time and space (Piriz 1996; Avila et al. 1999; Westermeier et  
124 al. 1999). Propagation is achieved through sexual reproduction (Avila et al. 1999). Antarctic  
125 specimens are separated from South American plants by more than 2% *rbcL* base pair distance  
126 (Hommersand and Fredericq 2003), which is not uncommon among species within red algae  
127 (e.g. Gavio and Fredericq 2002). Furthermore, Antarctic and sub-Antarctic populations show  
128 physiological differences: while in Antarctica spores germinate at 0°C and juveniles grow only  
129 at temperatures below 5°C (Bischoff-Bäsmann and Wiencke 1996), spores from sub-Antarctic  
130 populations do not germinate at 0°C and can grow at up to 15°C (Buschmann et al. 1999)  
131 which might result from an adaptation to regional environmental conditions. These first  
132 genetic and physiological results suggest that there is some evolutionary divergence between  
133 *Gigartina* populations from Antarctica and South America. The objective of this study is to infer  
134 the evolutionary history of sub-Antarctic and Antarctic populations of the red alga *G.*  
135 *skottsbergii* (Setchell et Gardner) using mitochondrial and chloroplast markers *Cox2-3* and  
136 *rbcL*. Two main processes were investigated: the divergence between Antarctic and South  
137 American populations, and the genetic consequences of last glacial cycle on both continents.

138

139 Materials and Methods.

140 *Sampling-* Samples were collected by autonomous diving in the shallow subtidal zones  
141 and includes a total of 233 individuals of *G. skottsbergii*. Samples were extracted from 18  
142 localities covering most of the distribution range of the species (Chilean and Antarctic coasts;  
143 Table 1). [In order to avoid sampling genetically identical ramets we sampled fronds from](#)  
144 [distinct holdfasts. Individuals were sampled from the lower littoral down to the depth of 25m.](#)

145 Each individual tissue sample was cut from a clean healthy frond and placed into a plastic bag  
146 filled with silica beds for rapid dehydration and preservation of DNA.

147 *DNA extraction, PCR amplification and sequencing-* Dried algal tissue was finely  
148 grounded using liquid nitrogen and DNA was extracted using the phenol–chloroform method  
149 described in Faugeron et al. (2001). The Cox2-3, an intergenic non-coding mitochondrial region  
150 located between the genes for cytochrome oxidase subunit 2 (COX2) and 3 (COX3) was  
151 amplified following Zuccarello et al. (1999). In total, 233 sequences of approximately 350 bp  
152 were obtained. Additionally, we amplified a 971 bp region of the chloroplastic gene *rbcl*,  
153 encoding the large subunit of the ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO)  
154 enzyme, using the primers F-*rbcl* and R-*rbcl* (Hommersand et al. 1994) and the PCR conditions  
155 described by Fredericq and Lopez-Bautista (2002). Sequences were obtained for a sub-sample  
156 of 26 individuals (Table 1) for the *rbcl* gene. All PCR reactions were performed in a Gene Amp  
157 PCR system 9700 (Applied Biosystems, Foster City, USA). The amplified samples from each  
158 individual were purified with the UltraClean™ kit (MO BIO Laboratories, Carlsbad, USA) and  
159 sequenced in both directions by Macrogen Inc. (Seoul, South Korea). Sequences were edited  
160 using Chromas v. 2.33 (McCarthy 1997) and alignments were obtained using the CLUSTAL  
161 function of Mega v 5 (Tamura et al. 2011). Sequences were deposited in Genbank with  
162 accession numbers KM261841 to KM261858 for the Cox2-3 region and accession numbers  
163 KM261859 to KM261862 for the *rbcl* region. Alignments of the sequences used for the Cox2-3  
164 region and the *rbcl* for phylogenetic reconstructions are available in Online Resources 1 and 2,  
165 respectively.

166 For the *rbcl* and the coding part of the Cox2-3 sequences obtained, the McDonald-  
167 Kreitman test (<http://mkt.uab.es>, Egea et al. 2008) was performed to detect selection. The  
168 neutrality index (NI) was calculated as follows:  $NI = (P_n/P_s)/(D_n/D_s)$ , where P is the  
169 polymorphism within the population, D is the divergence or fixed difference between  
170 populations, n is for non synonymous and s is for synonymous mutations.

171 *Phylogenetic reconstructions and estimation of divergence time* – Phylogenetic  
172 reconstructions for each marker dataset were performed with the Maximum Likelihood (ML)  
173 method using TreeFinder v March 2011 (Jobb et al. 2004) and a Bayesian inference (BI) using  
174 MrBayes v 3.1.2 (Huelsenbeck and Ronquist 2001). Outgroup species belonging to the genus  
175 *Sarcothalia* and *Iridaea* were chosen since they represent the closest known sister-taxa of  
176 *Gigartina skottsbergii* in the phylogenetic systematics of the Gigartinaceae (Hommersand et al.  
177 1999). Outgroup sequences considered for the *rbcl* consisted of four species of *Sarcothalia* (*S.*  
178 *crispata*, SCU03085; *S. stiriata*, SSU03089; *S. livida*, SLU03087 and *S. circumcincta*, AF146219)  
179 and two sequences of *Iridaea cordata* from Antarctica (U02989 and GQ323780). For the *cox2-*  
180 *3*, two sequences of *S. crispata* (KM275591 and KM275592, both from Punta Estaquilla) and  
181 one sequence of *I. cordata* (KM275593 from Punta Hanna, South Shetland Islands) were used  
182 as outgroup. We also included in the analyses available *rbcl* sequences for *G. skottsbergii*  
183 (U03432, Ancud, Chile and AF146206, King George Island, South Shetland Islands;  
184 Hommersand et al. 1999). For both phylogenetic reconstruction methods, large indels within  
185 the non-coding intergenic region of the *Cox2-3* were treated as single mutation events.

186 ML analyses were performed using a mixed model taking into account the position of  
187 codons for the *rbcl* gene while only one model was used for the *cox2-3* intergenic region  
188 analysis. TreeFinder v March 2011 (Jobb et al. 2004) allows to choose between 32 substitution  
189 models for each partition of the data set. The best-fitted substitution models were selected  
190 using the Akaike Information Criterion implemented in the ModelTest package of the  
191 TreeFinder program (Posada and Crandall 1998; Jobb et al. 2004). The selected models for the  
192 *rbcl* data were TN+G for the first codon position, HKY+G for the second codon position and  
193 J3+G for the third codon position. For *cox2-3* the selected model was HKY+G. Using TreeFinder  
194 v March 2011, we performed a heuristic search in order to reconstruct the trees and node  
195 supports were assessed by non-parametric bootstrapping (1000 pseudo-replicates, Felsenstein  
196 1985).



197 Bayesian inference was performed using the general criteria of the best fit model  
198 parameters defined for each dataset. Four independent analyses were run with four chains  
199 each (3 heated chains and one “cold” chain) for ten million generations. The settings were a  
200 heating parameter value of 0.2 and sampling every 1000 generations with randomly generated  
201 starting trees. The first 25% of sampled trees were discarded as “burn-in” to ensure  
202 convergence. The remaining trees were used to compute a consensus topology and posterior  
203 probability values. The split frequency (variance between the four independent runs) was  
204 below 0.0005, confirming that the posterior probability distribution was accurately sampled.  
205 Because the posterior probability bootstrap values were essentially identical in the  
206 independent runs starting from different, random topologies, we considered that the chains  
207 had converged.

208 Even though the lack of fossils impedes a precise calibration of molecular clocks in red  
209 algae, we used divergence rates already published for this group to estimate the historical  
210 divergence event between South American and Antarctic populations. A divergence rate of  
211 0.109-0.127% per [site per](#) million years (Myr) has been proposed for *rbcL* (Kamiya et al. 2004).  
212 For *cox2-3*, a site mutation rate of 0.756-0.426% per Myr, based on the divergence of the red  
213 algae *Asparagopsis* spp. associated to the Panama isthmus closure, was proposed by Andreakis  
214 et al. (2007). Divergence time was estimated in BEAST v1.8 (Drummond et al. 2012) using the  
215 Yule model of tree prior, a gamma site heterogeneity model to allow variation among sites of  
216 the mutation rate, and a Log-normal relaxed clock with a uniform sampling within the range of  
217 published mutation rates. Four runs of ten millions MCMC iterations each were performed and  
218 the combined results were analyzed with Tracer v1.8 (Drummond et al. 2012). Effective sample  
219 size of the posterior distribution, the parameter of accuracy of the parameter estimation, was  
220 always superior to 300 in each individual run and in the combined analyses, indicating the  
221 MCMC appropriately converged to estimated values.

222           *Genetic diversity* - For *cox2-3*, we calculated five diversity indices for each sampled  
223 location and for the two phylogenetic lineages (i.e. *G. skottsbergii* from Chile and the Falkland  
224 Islands and *G. skottsbergii* from sub-Antarctic and Antarctic) using Arlequin v 3.5 (Excoffier and  
225 Lisher 2010): the number of haplotypes (nH); the number of private haplotypes (i.e.  
226 haplotypes found in a single population, nHpriv); the number of polymorphic sites (S); gene  
227 diversity (Hd, Nei 1987) and nucleotide diversity ( $\pi$ , Nei and Li 1979). For *rbcl*, only nH was  
228 calculated.

229           *Network reconstruction and historical demography* - Haplotype networks were  
230 reconstructed for *cox2-3* using the median-joining algorithm implemented in NETWORK v  
231 4.510 (Bandelt et al. 1999). Moreover, for this molecular marker, three complementary  
232 approaches were used to infer the historical demography of *G. skottsbergii* from Chile and the  
233 Falkland Islands.

234           First, Tajima's D (Tajima 1989) and Fu's Fs (Fu 1997) statistics were calculated to detect  
235 significant past changes in population size. Significant departure from mutation-drift  
236 equilibrium was tested by 1000 bootstrap replicates in Arlequin (Excoffier and Lisher 2010).  
237 Under the assumption of neutrality, negative values characterize populations in expansion  
238 while positive values, associated with the loss of rare haplotypes, are considered as a signature  
239 of recent bottlenecks (Tajima 1989, Fu 1997).

240           Second, the observed distributions of pairwise differences were compared to  
241 estimated values under a model of sudden pure demographic expansion (Roger and  
242 Harpending 1992) using Arlequin (Excoffier and Lisher 2010). The model fit between the  
243 observed and estimated mismatch distributions was calculated through a generalized least  
244 squares approach, which was then tested with 1000 permutations. The date of growth/decline  
245 ( $\tau=2\mu t$ ), measured in units of  $1/2 \mu$  generations where  $t$ =time in years and  $\mu$ =mutation rate per  
246 sequence per generation, was estimated using the demographic expansion parameters as

247 determined in the nonlinear least squares approach implemented in Arlequin (Excoffier and  
248 Lisher 2010).

249 Third, population growth rate and timing was estimated from coalescent simulations  
250 implemented in LAMARC 2.1.9 (Kuhner 2006). The maximum likelihood approach was applied  
251 using the Metropolis-coupled Markov chain Monte Carlo (MCMC) method with replication of  
252 chains and adaptive heating to achieve optimal sampling of the parameter space. The MCMC  
253 runs were performed three times with random seeds; each run used 10 initial chains with 500  
254 samples and two long final chains with 10 000 samples. All initial chains and final chains were  
255 simulated using a sampling interval of 20 and a burn-in of 1000 samples. A tenfold  
256 evolutionary rate (4.26-7.56% per million years) was considered at population level, following  
257 the correction for time dependence of molecular rate proposed by Ho et al. (2011).

258

259 Results.

260 Four chlorotypes were detected for the chloroplast marker *rbcL*, with 23 polymorphic  
261 sites along the 971 base pair fragments sequenced (11 sequenced individuals, Table 1). For the  
262 *cox2-3* mitochondrial marker, 18 mitotypes were observed (233 sequenced individuals, Table  
263 1). For this marker, 48 polymorphic sites were observed including two indels: one indel of 1bp  
264 characteristic of the mitotype C13 and one indel of 12bp for mitotypes C8 and C17. Sequence  
265 length of *cox2-3* sequences varied from 337 to 350bp.

266 Figure 1 shows the Maximum Likelihood (ML) phylogenetic trees constructed using the  
267 two molecular markers. For both markers, tree topologies based on Bayesian and ML analyses  
268 were largely congruent and shared comparable support values for major nodes (Figure 1).  
269 Regardless of the marker used, tree topologies were broadly similar among phylogenetic  
270 reconstruction methods and clearly showed that all *G. skottsbergii* sequences obtained in this  
271 study form a monophyletic group, clearly split into two well supported lineages (Figure 1,

272 support values >89%) that are strongly divergent from the outgroup species. The first lineage is  
273 composed of all individuals from Chile and the Falkland Islands while the second is composed  
274 of individuals from the Antarctic Peninsula, the South Shetland Islands and the South Orkney  
275 Islands (Figure 1). The available *rbcL* sequence AF146206 in GenBank for *G. skottsbergii*  
276 previously sampled in King George Island in the South Shetland Islands (Hommersand et al.  
277 1999) corresponds exactly to the chlorotype R4 present in our Antarctic lineage. The  
278 uncorrected p-distance, measured using only 665bp, between the *rbcL* sequence U03432 and  
279 the closest sequence in our data set (i.e. chlorotype R1, present only in Ancud, Chile, see Table  
280 1) is of 0.3%. This is congruent with the location where the specimen corresponding to the  
281 U03432 sequence was collected, on Chiloé Island within the bay of Ancud (Hommersand et al.  
282 1999). P distances between sequences from the Antarctic and South American *G. skottsbergii*  
283 lineages were of  $9.2 \pm 1.5$  % for *cox2-3* and of  $2.1 \pm 0.5$  % for *rbcL*. When performed between  
284 the Antarctic and South American *G. skottsbergii* lineages, no significant departure from  
285 neutrality was detected using the McDonald-Kreitman test for the *rbcL* and the coding part of  
286 the *Cox2-3* sequences analyzed (NI= 0.969, p = 0,980; NI= 2.191, p= 0.536, for the *rbcL* and the  
287 *Cox2-3*, respectively).

288         Divergence between the Antarctic and South American *G. skottsbergii* lineages was  
289 estimated at 9.4 Myr (95% CI: 3.2-16.4Myr) based on *cox2-3* data and at 14.9 Myr (95% CI: 2.6-  
290 35.9Myr) based on *rbcL* data.

291         For both markers, no genetic diversity was observed in the Antarctic lineage of *G.*  
292 *skottsbergii*. Only one haplotype was detected for the *cox2-3* region and for the *rbcL* gene, the  
293 mitotype C18 and the chlorotype R4, respectively (Table 1). The Antarctic lineage is spread  
294 over more than 1600 km of coast, from Marguerite Bay (67°S) to the South Orkney Islands  
295 (60°S) (Table 1, Figure 2C). Within the South American lineage of *G. skottsbergii* the genetic  
296 diversity in *cox2-3* was generally low with the number of mitotypes per sampling site (nH)

297 being lower than 3 and the gene diversity ( $H_d$ ) as lower than 0.3 in 6 of the 11 studied  
298 populations (Table 1). The highest genetic diversity was observed in the population of PAG,  
299 BCH and FAL ( $n_H = 5$  and  $H_d > 0.5$  in all three populations, Table 1). These three populations  
300 were also the ones with the highest number of private haplotypes, with three private  
301 haplotypes in PAG and FAL and two in BCH (Table 1). For the *rbcl* gene, only three chlorotypes  
302 were observed within the South American lineage of *G. skottsbergii*. Only one chlorotype was  
303 found in each single population and the only private chlorotype (R1) was observed in the ANC  
304 population (Table 1). The chlorotype R3 was shared between BOR in Tierra del Fuego and FAL  
305 located in the Falkland Islands (Table 1).

306         The *cox2-3* mitotype network revealed the presence of two main haplogroups, one for  
307 South America and one for Antarctica, which are separated by 31 bp (Figure 2A), a result fully  
308 congruent with the phylogenetic reconstructions (Figure 1). Within the South American  
309 haplogroup, characterized by a typical star-like topology, pairs of mitotypes were separated by  
310 1 to 5 bp, except for the mitotypes C17 and C8 that were differentiated by a unique 12bp  
311 indel. This haplogroup is also characterized by one frequent and widespread mitotype (C1, 81%  
312 of the samples, Table 1) and several less frequent mitotypes (Figure 2A). Low frequency  
313 mitotypes were predominantly restricted to a single or few nearby local populations. For  
314 example, the mitotype C3 was observed in BLO and PAG, these being two populations located  
315 in the Moraleda Channel. Additionally, mitotype C7 was observed in BCH, BOR and TOR which  
316 are located in the southern part of the Magellanic region and Tierra del Fuego (Figure 2B). One  
317 mitotype, C13, was also shared between BCH in the southern part of the Magellanic region and  
318 FAL located in the Falkland Islands (Figure 2B). As expected, the star-like topology is coupled  
319 with a unimodal mismatch distribution (Figure 3A), and the values of Tajima's  $D$  and Fu's  $F_s$   
320 statistics were both negative and significant ( $D = -2.01$ ,  $p = 0.001$ ;  $F_s = -1.26$ ,  $p = 0.0001$ ) a  
321 result congruent with a sudden demographic population expansion model. Population size  
322 changes depicted from the mismatch distribution was  $\tau = 0.73$  (90% confidence interval of 0.00

323 to 2.23). Assuming the start of exponential demographic expansion when population size was  
324 1% of present-day's estimate, it was estimated to initiate 20 000 – 36000 years before present.  
325 A large positive exponential growth rate,  $g= 3004$  (2458-4666 95% confidence interval), was  
326 also detected. It is possible that demographic expansion led to a large increase in  $N_e$   
327 (approximately 170 000 to 300 000-fold increase, Fig. 3B), which may have started during or  
328 just before the LGM, depending on the combination of growth and mutation rates considered  
329 within their respective confidence limits.

330

### 331 Discussion

332 The patterns of genetic structure for *G. skottsbergii* [seem to](#) confirm the absence of gene flow  
333 between Antarctic and South American populations. Regardless of the marker analyzed,  
334 phylogenetic reconstructions using mtDNA and cpDNA sequences showed strong congruence  
335 and clear support for two distinct lineages consisting of populations from South America and  
336 the Falklands on the one hand and populations from the Antarctic Peninsula, Shetlands and  
337 Orkney Islands on the other hand. The absence of shared haplotypes between the two regions  
338 may reflect the isolation created by the Antarctic Circumpolar Current between the Antarctic  
339 Peninsula and the South American continent. Divergences among lineages of *G. skottsbergii* for  
340 the Cox2-3 ( $9.2 \pm 1.5$  %) and for the *rbcl* ( $2.1 \pm 0.5$  %) are within the range reported for  
341 interspecific distances between sister species in Rhodophyta (ranging from 2.55% to 4.70% and  
342 from 0.77% to 5.08% for the Cox2-3 and the *rbcl*, respectively; McIvor et al. 2001; Destombe  
343 et al. 2010; Hernández-Kantún et al. 2014). The large range estimated, of 2.6 to 35.9 My  
344 depending on the genetic marker and the mutation rate considered (with central tendency  
345 around 9 to 15My), for divergence time between the two lineages seems to predate  
346 Pleistocene glaciations. This time of divergence is however more recent than the separation of  
347 the Antarctic continent from the South American continent (approximately 24-40 Myr ago),

348 and seems to include the period of intensification of the ACC circulation 11-12 Myr ago (Dalziel  
349 et al. 2013). These climatic and oceanographic changes have been shown to be major drivers in  
350 the isolation of marine Antarctic fauna. For example, González-Wevar et al. (2010) have shown  
351 that the diversification within a genus of mollusk (*Nacella*) took place long after the separation  
352 of the continents. For these marine mollusks, the appearance of the most genetically distant  
353 clades (Kerguelen, Antarctic, and South America) took place between 9 and 5 Myr. These  
354 results were further supported by a recent study of comparative phylogeography of different  
355 invertebrate taxa showing a shared Antarctic and South American distribution (González-  
356 Wevar et al. 2012a; Poulin et al. 2014). In these studies, divergence times between Antarctic  
357 and South American lineages ranged from 1.0 to 13.6 Myr, largely overlapping the estimates  
358 for *G. skottsbergii*. Interestingly, divergence estimates in the mitochondrial markers for *G.*  
359 *skottsbergii* (9.2%) fall within the 7-11% range for shallow subtidal and intertidal invertebrates  
360 (González-Wevar et al. 2010, 2012a; Poulin et al. 2014), corroborating the occurrence of a  
361 shared evolutionary history despite imprecisions in the respective mutation rates. These  
362 authors proposed that the connection between Antarctic and South American populations  
363 could have been maintained by a stepping stone process along the archipelago of the Scotia  
364 Arc. Indeed, geological evidence has recently been reported of a now-submerged volcanic arc  
365 in the Central Scotia Sea that existed during the early Miocene (Dalziel et al. 2013). This  
366 archipelago was located closer to the Antarctic Peninsula and the Cape Horn region than the  
367 South Sandwich Islands are today, and may thus have provided a corridor for genetic  
368 connectivity across the Drake Passage until approximately 10-11 Myr ago (Poulin et al. 2014).  
369 Our study further shows that *G. skottsbergii*, a seaweed characterized by a very restricted  
370 dispersal capacity of its spores, may also have maintained a certain level of connectivity (even  
371 after the mid-Miocene) between the continents of Antarctica and South America through the  
372 volcanic arc of islands linking both sides of the Scotia Sea. This connection could have been

373 maintained until the beginning of Pliocene's glaciations, as in the case of brooding  
374 invertebrates, through rafting of adults (Nikula et al. 2010; Haye et al. 2012; Poulin et al. 2014).

375 The Antarctic and South American lineages of *G. skottsbergii* exhibit different patterns of  
376 genetic diversity. While a strong demographic expansion was inferred in the sub-Antarctic  
377 region, a total absence of genetic diversity was observed in the Antarctic lineage. The presence  
378 of a single haplotype over more than 1600 km from Marguerite Bay to the South Orkney  
379 Islands is intriguing. Even if this lack of diversity limits our capacity to test for different  
380 demographic scenarios in Antarctica, it suggests that a very strong demographic bottleneck  
381 occurred during glacial contraction, followed by a sudden and recent recolonization process  
382 that did not allow for new mutations. Similarly, the overall low genetic diversity and the  
383 presence of the same common haplotype C1 in every population from the Chilean coast to the  
384 Falkland Islands seems to support the hypothesis of persistence in a single glacial refugium  
385 followed by a massive demographic expansion over 2500 km. Such a pattern of genetic  
386 homogeneity over a broad geographical range has usually been related to high dispersal  
387 potential (Bortolotto et al. 2011; González-Wevar et al. 2012b). The scenario of rapid  
388 recolonization leading to the presence of only one haplotype over thousands of kilometers of  
389 coast is, however, difficult to envision for *G. skottsbergii*. Indeed, this alga is a non-buoyant  
390 species, and spore dispersal is considered to be very limited (Ramirez and Santelices 1991).

391 Nevertheless, signatures of long distance dispersal have been observed in other apparently  
392 non-dispersive algal species like *Adenocystis utricularis* or *Bostrychia intricata* and have been  
393 explained by the organisms' potential ability to raft on floating substrates (Fraser et al. 2010,  
394 2013). In contrast with our results, in a previous study using RAPDs nuclear markers (Faugeron  
395 et al. 2004), significant genetic differentiation among South American populations was  
396 observed and has been related to the poor dispersal capacity of for *G. skottsbergii*. Differences  
397 in mutation rates and/or level of drift effects (effective population size of uniparentally  
398 inherited loci is only one-fourth that of nuclear loci) may account for the differences between



399 level of genetic structure obtained with RAPDs (Faugeron et al. 2004) and cytoplasmic  
400 sequences (our study). Also, during spatial expansion, gene surfing effects may contribute to  
401 the reduction of diversity in cytoplasmic markers in the recolonized region (Excoffier and Ray  
402 2008). Indeed, large-scale spread of mitochondrial genetic variants has been observed during  
403 recolonization process in seaweeds (Fraser et al. 2010). The ACC is a strong west to east  
404 current that connects the Antarctic Peninsula and the South Shetland Islands to the Antarctic  
405 Islands located in the South Scotia Ridge. Particle movement modeling and particle tracking  
406 has shown that passive drifters travel northeastwards across the Scotia Sea, connecting the  
407 Antarctic Peninsula and the South Shetland Islands to South Orkney Islands and South Georgia  
408 (Thorpe et al. 2004). Movement of drifting seaweed along the ACC could be connected to  
409 west-east spatial expansion pattern. On the other hand, the postglacial colonization of the  
410 Peninsula could have been promoted by stepwise spatial expansion through spore  
411 propagation. Such spatial expansion could have been facilitated by strong coastal currents, as  
412 the Antarctic Peninsula Coastal Current (APCC; Moffat et al. 2008), a southward current that  
413 forms during the ice-free seasons and extends to Alexander Island. However, we lack  
414 knowledge on the actual mechanisms of dispersal in *G. skottbergii* to appropriately determine  
415 the role of dispersal during post-glacial population dynamics on the present day genetic  
416 diversity.

417 The location of both Antarctic and South American glacial refugia is difficult to infer from the  
418 results presented here. The extreme genetic homogeneity within the *G. skottsbergii* Antarctic  
419 clade is in disagreement with the hypothesis that genetic structure and global genetic diversity  
420 in Antarctica may have been promoted by the fragmentation and isolation of micro-refugia  
421 where marine species were able to survive during repeated ice advances and retreats (Thatje  
422 et al. 2005; Allcock and Strugnell 2012). The possible presence of micro-refugia has been  
423 particularly highlighted in the archipelagoes located near the West Antarctic Peninsula and the  
424 South Shetland Island (See Fraser et al. 2012 for review). Indeed, new studies using molecular

425 data have shown that the South Shetland Islands is the most speciose region for endemic  
426 Southern Ocean octopuses of the genus *Pareledone* (Allcock et al. 2011). Even though the data  
427 herein lack the explicit molecular diversity to test adequately for the number and location of  
428 glacial refugees in this region, the survival of *G. skottsbergii* during the LGM seems more likely  
429 in the northernmost latitude (South Shetland and South Orkney Islands). Indeed, whereas *G.*  
430 *skottsbergii* is found in dense beds within these northern archipelagos, less than ten specimens  
431 could be collected in Paradise Bay and only one specimen was sampled in Marguerite Bay  
432 (Note that the same sampling effort was applied in Paradise Bay, Marguerite Bay, O'Higgings  
433 and Punta Prat; Table 1; M-L Guillemin, personal communication). It is understood that an  
434 increased sampling effort could better elucidate the southern limits of *G. skottsbergii*, but it  
435 seems that in Marguerite Bay the alga has reached a biotic or abiotic limit to its distribution.

436 The absence of genetic structure for the mitochondrial and chloroplast markers in southern  
437 South America also limits our ability to infer the number and location of glacial refugia.  
438 Because of their longstanding demographic stability, populations from glacial refugia are  
439 expected to present higher levels of genetic diversity than those populations that have formed  
440 following postglacial expansions (Provan and Bennett 2008). In addition, long-term isolation in  
441 distant refugia often leads to genetic differentiation due to mutation accumulation and genetic  
442 drift (Hewitt 2000). Along the Chilean coast, recolonization pathways have differed between  
443 organisms in terms of the number and origin of sources. A recent recolonization from a single  
444 distant/exterior source has been proposed to explain the high genetic homogeneity of  
445 populations of the red alga *Mazaella laminarioides* (Montecinos et al. 2012) and the brown  
446 alga *Durvillaea antarctica* that exist south of 42°S (Fraser et al. 2010). In *M. laminarioides*, a  
447 single refugium has been proposed, located in the North of the Island of Chiloe and the  
448 mainland coast north of the 41°S (Montecinos et al. 2012). In *D. antarctica*, broad sampling  
449 across the southern hemisphere has shown that all individuals present off the Patagonian  
450 coast of Chile share the same haplotype (Fraser et al. 2010) and the authors have indicated

451 that this species likely recolonized the region from a refugium in New Zealand sub-Antarctic  
452 archipelagoes. Yet others have highlighted the role that the Southern Fjords and Channels  
453 Region played as a refuge for several animal species, like marine mollusks (Valdovinos et al.  
454 2003; González-Wevar et al. 2010) and the river otter (Vianna et al. 2011). The existence of  
455 multiple refugia, scattered among the Southern Fjords and Channels, has been proposed to  
456 explain the high levels of endemism and the high diversity of mollusks in this region  
457 (Valdovinos et al. 2003). In *G. skottsbergii*, the overall low genetic diversity and the presence of  
458 the same common haplotype, C1, in every population from the Chilean coast to the Drake  
459 Passage and the Falkland Islands seem to support the hypothesis of a single glacial refugium.  
460 Strong currents (i.e. the Cape Horn Current and the Malvinas-Falkland Current) connect the  
461 southern coast of Chile from 42°S to Cape Horn and the Falkland Islands. These currents have  
462 been shown to provide high connectivity among all inhabiting regions for the genus *Nacella*  
463 (González-Wevar et al. 2012b). In this genus, the existence of asymmetric gene flow, from  
464 West to East, was shown to be related to the prevailing circulation patterns in this region  
465 (González-Wevar et al. 2012b). Due to these strong directional currents, the hypothesis of a  
466 postglacial recolonization of the Chilean coast by *G. skottsbergii* from a refuge located in the  
467 Falkland Islands is most unlikely. Again, more polymorphic markers should help identifying  
468 regions with higher / lower genetic diversity as putative locations of glacial refugia /  
469 recolonized areas, respectively, for *G. skottsbergii*.

470

#### 471 Conclusions

472 In accordance with previous results of Hommersand et al. (2009) the data presented here  
473 clearly show two divergent and respectively monophyletic clades of *G. skottsbergii* that may  
474 correspond to two cryptic species. *G. skottsbergii* (Type locality: Slogget Bay, Fuegia - Silva  
475 1996; <http://www.algaebase.org>) is distributed in the Southern and sub-Antarctic coast of

476 Chile and the Falkland Islands. The Antarctic species, still to be formally described and named,  
477 occurs in the Antarctic Peninsula, the South Shetland Islands and the South Orkney Islands.  
478 Despite drastic reductions in population size, as revealed by strong signals of bottlenecks, both  
479 species persisted and maintained their separation on either side of the ACC even during the  
480 glacial period. The divergence time between the two cryptic species, estimated as 9.4 to  
481 14.9 Myr (comparable with other invertebrate species, but with a large confidence interval of  
482 2.6-35.9Myr), indicates that algae with limited dispersal capabilities were able to cross the  
483 Scotia Sea after separation of the continents, potentially via a stepping stone process through  
484 the volcanic arc of islands. Our work also sheds light on the possible importance of dispersal by  
485 rafting in the recolonization process of a species for which natural propagation is mainly  
486 achieved via sexual reproduction (Avila et al. 1999; Westermeier et al. 2012). Future prospects  
487 on glacial refugia for Antarctic and sub-Antarctic seaweeds shall also contribute to better  
488 understand the dispersal mechanisms during demographic expansion in these regions  
489 characterized by complex topography of coastal shelf and coastal currents.

490

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507

508 Figure 1: Maximum likelihood rooted trees for the *cox2-3* and the *rbcl* haplotypes of *Gigartina*  
509 *skottsbergii*. Maximum likelihood bootstrap values are indicated above each node and  
510 Bayesian posterior probabilities are noted between brackets. Only high support values are  
511 shown (>75 for bootstraps and >0.95 for posterior values, respectively; - correspond to  
512 branches not observed in the Bayesian inference reconstruction).

513 Figure 2: Haplotype networks of *Gigartina skottsbergii* and their geographic distribution based  
514 on *cox2-3*. The haplotype networks are presented in (A) while the pie charts showing the  
515 geographical distribution of haplotypes of the South American haplogroup are shown in (B)  
516 and the pie charts showing the geographical distribution of haplotypes of the Antarctic  
517 haplogroup are shown in (C). In the networks, each circle represents a haplotype and its size is  
518 proportional to the frequency in which the haplotype was encountered (correspondence  
519 between circle sizes and numbers of individuals is indicated in the box A). The black square in-  
520 between C7, C9 and C14 represents a hypothetical un-sampled haplotype. For haplotypes  
521 separated by more than one mutational step, the number of steps is indicated in bp. Pie  
522 charts' color-code corresponds to the one used in haplotype networks. Abbreviations for  
523 population codes are as in Table 1. Numbers of sequenced individuals are given between  
524 brackets.

525 Figure 3: Mismatch distributions (A) and population growth rate estimates (B) of the South  
526 American haplogroup of *Gigartina skottsbergii* for the *cox2-3* marker. Growth rate and timing  
527 of population dynamic changes were estimated from coalescent simulations implemented in  
528 LAMARC 2.1.9 (Kuhner 2006). The observed distribution of the number of pair base differences  
529 between sequences is indicated by the grey bars while the expected distribution under a  
530 model of sudden demographic expansion is represented by a black line. Effective population  
531 size (i.e.  $N_e$ ) fluctuations throughout time are represented in the LAMARC graph. The dotted  
532 lines represent the 95% confidence intervals of the estimated growth rate, whereas the grey

533 shaded area corresponds to the average growth rate combined with the range of mutation  
534 rates proposed by Andreakis et al (2007) corrected by a tenfold evolutionary rate as suggested  
535 at population level for time dependence of molecular rate by Ho et al. (2011).

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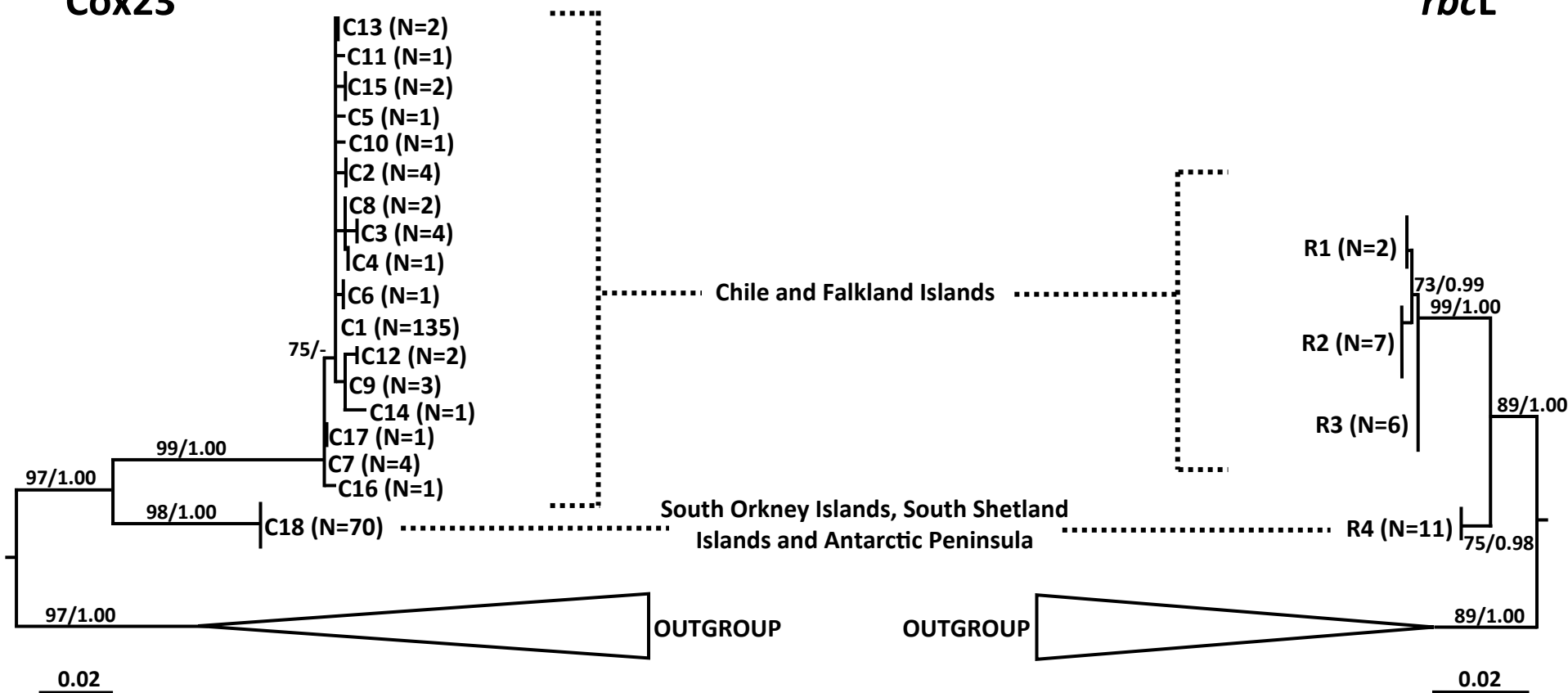
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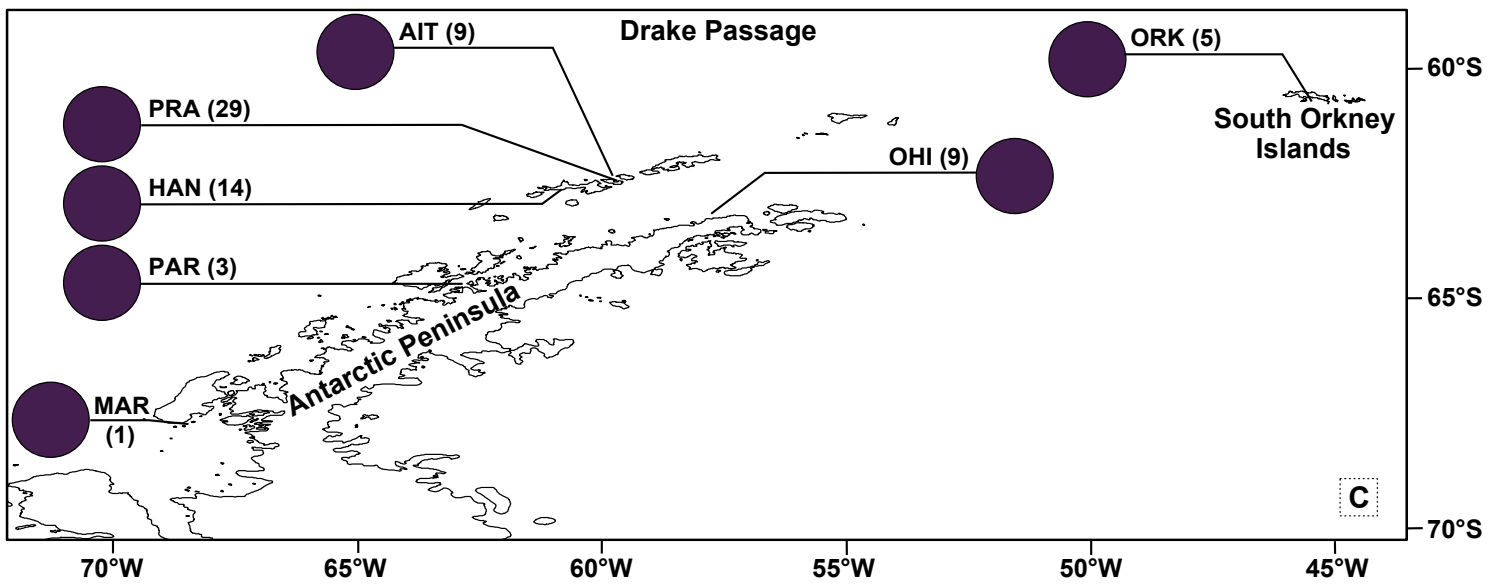
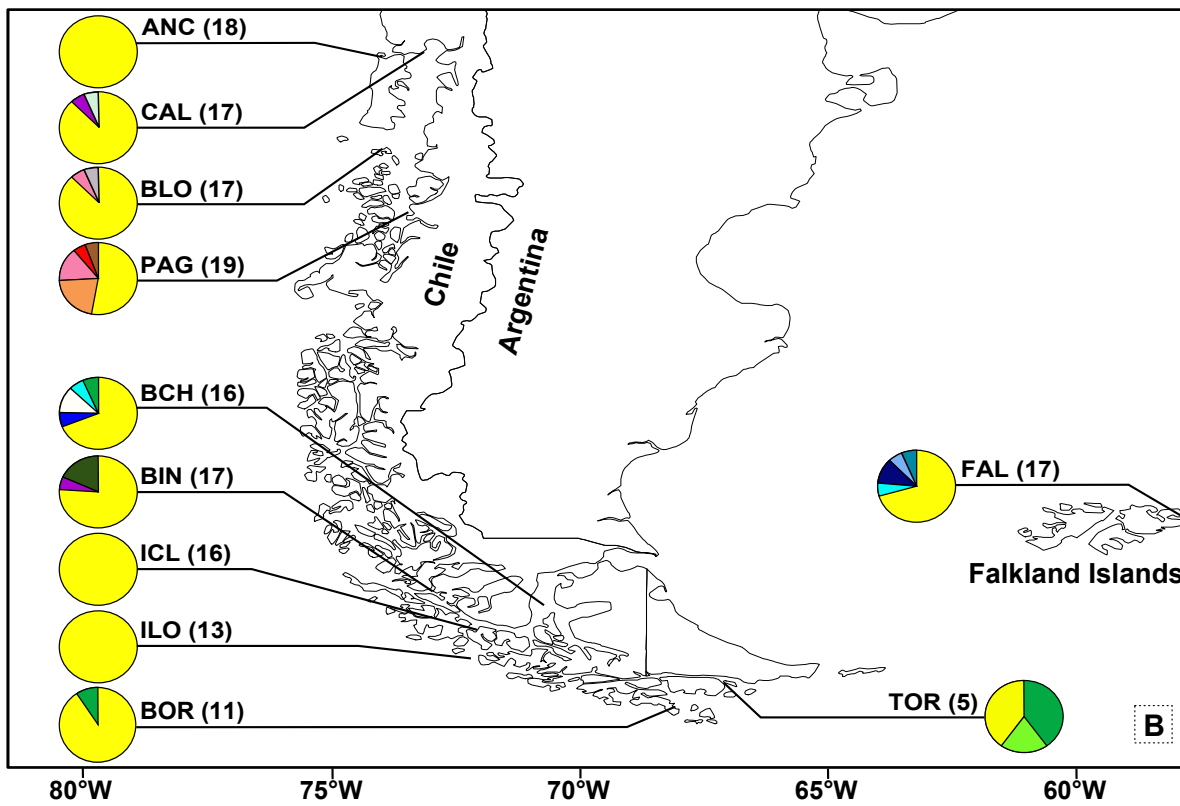
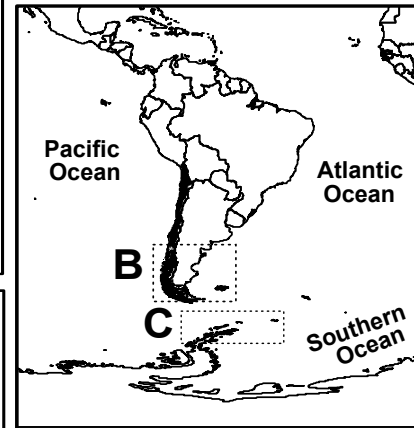
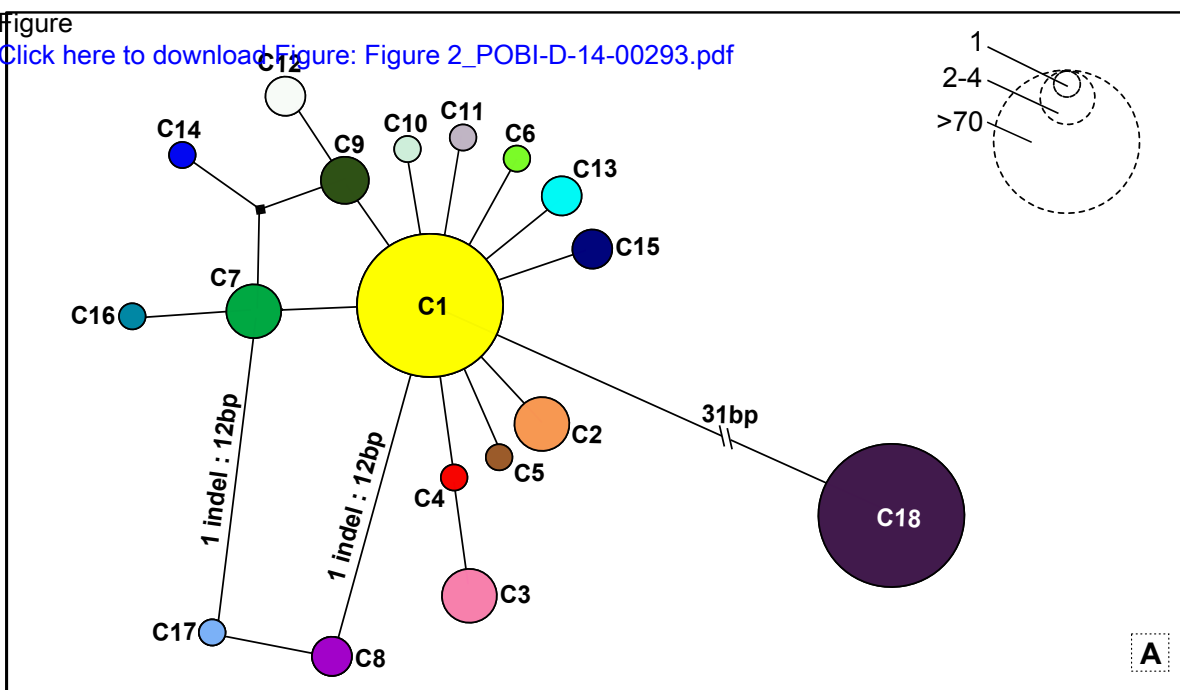
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### Cox23

### *rbcl*





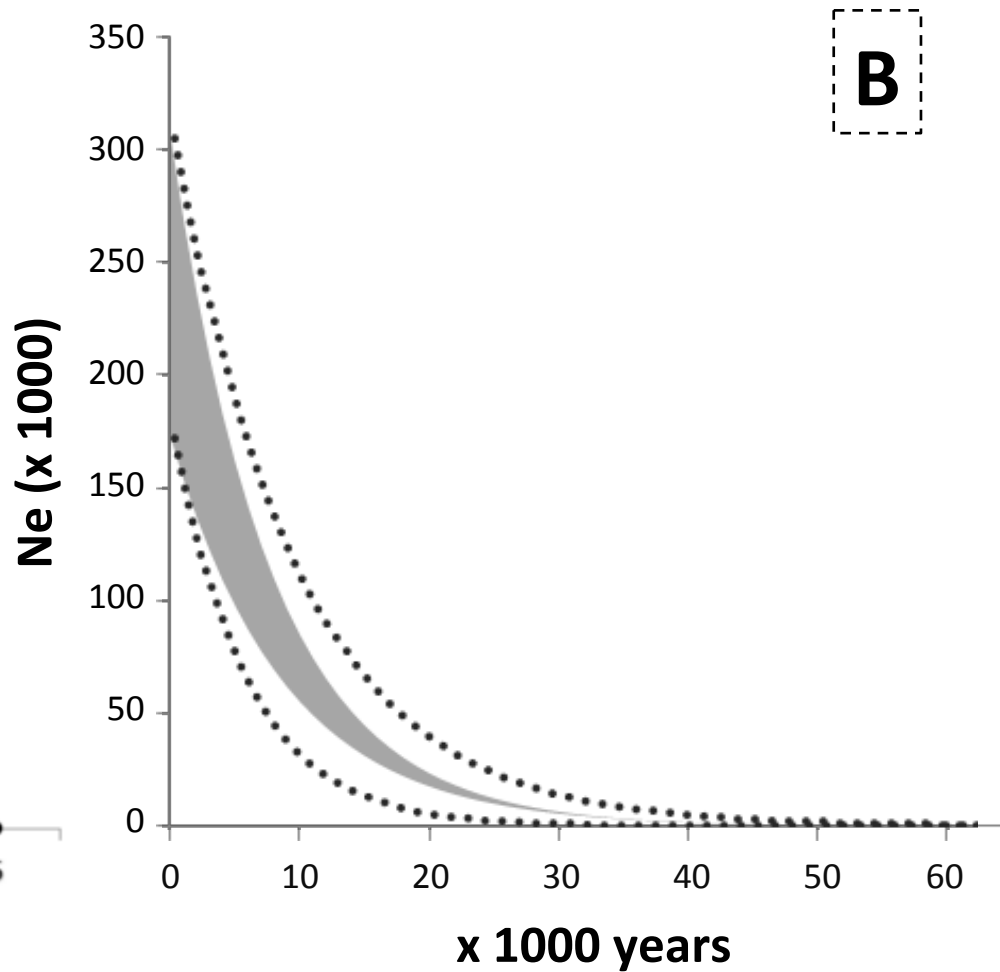
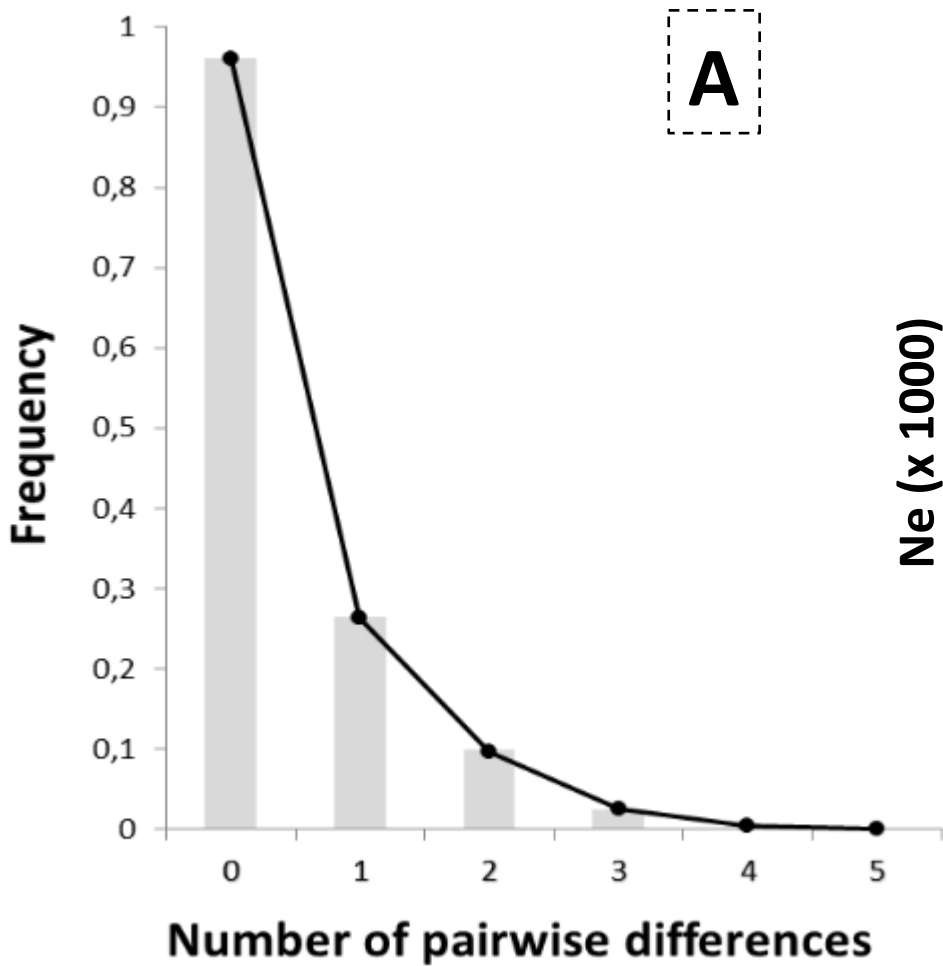


Table1: Sampling sites of *G. skottsbergii* and associated genetic diversity for two molecular markers (cox23: mitochondrial; rbcL: chloroplast). For each site, the abbreviation (code) and the geographic coordinates are indicated. For more information on molecular diversity indices see foot note references.

Sampling site	Code	Coordinates	Cox23							<i>rbcL</i>			
			N	nH	Hd	$\pi$ (.10-2)	S	nHpriv	Hap.	N	nH	Hap.	
<b>Chile</b>													
Ancud	ANC	41°51'S/73°47'W	18	1	0	0	0	0	0	C1(18)	2	1	<b>R1(2)</b>
Calbuco	CAL	41°48'S/73°13'W	17	3	0.18	0.44	13	1	1	C1(15), C8(1), <b>C10(1)</b>			
Bahía Low	BLO	43°47'S/73°58'W	17	3	0.23	0.10	3	1	1	C1(15), C3(1), <b>C11(1)</b>	1	1	R2(1)
Puerto Aguirre	PAG	45°10'S/73°32'W	19	5	0.68	0.31	4	3	3	C1(10), <b>C2(4)</b> , C3(3), <b>C4(1)</b> , <b>C5(1)</b>			
Bahía Chilota	BCH	53°20'S/70°43'W	16	5	0.53	0.30	5	2	2	C1(11), C7(1), <b>C12(2)</b> , C13(1), <b>C14(1)</b>	2	1	R2(2)
Bahía Inútil	BIN	53°10'S/72°55'W	17	3	1.72	0.49	13	1	1	C1(13), C8(1), <b>C9(3)</b>	2	1	R2(2)
Isla clarence	ICL	54°03'S/71°58'W	16	1	0	0	0	0	0	C1(16)	1	1	R2(1)
Isla London	ILO	54°57'S/72°20'W	13	1	0	0	0	0	0	C1(13)	1	1	R2(1)
Bahía Orange	BOR	55°31'S/68°08'W	11	2	0.18	0.05	1	0	0	C1(10), C7(1)	1	1	R3(1)
Puerto Toro	TOR	55°06'S/67°06'W	5	3	0.80	0.29	2	1	1	C1(2), <b>C6(1)</b> , C7(2)			
<b>Falkland Islands</b>													
Falkland	FAL	51°37'S/57°45'W	17	5	0.51	0.59	15	3	3	C1(12), C13(1), <b>C15(2)</b> , <b>C16(1)</b> , <b>C17(1)</b>	5	1	R3(5)
<b>South Orkney Islands</b>													
Orkney	ORK	60°44'S/45°37'W	5	1	0	0	0	0	0	C18(5)			
<b>South Shetland Islands</b>													
Punta Hanna	HAN	62°39'S/60°38'W	14	1	0	0	0	0	0	C18(14)	1	1	R4(1)
Punta Prat	PRA	62°28'S/59°40'W	29	1	0	0	0	0	0	C18(29)	2	1	R4(2)
Isla Aitcho	AIT	62°25'S/59°44'W	9	1	0	0	0	0	0	C18(9)	1	1	R4(1)
<b>Antarctic Peninsula</b>													
O'Higgins	OHI	63°18'S/57°53'W	9	1	0	0	0	0	0	C18(9)	4	1	R4(4)
Paradise Bay	PAR	64°50'S/62°52'W	3	1	0	0	0	0	0	C18(3)	2	1	R4(2)
Marguerite Bay	MAR	67°45'S/68°52'W	1	1	0	0	0	0	0	C18(1)	1	1	R4(1)
<b>Chile and Falkland Antarctic Peninsula and Islands</b>			166	17	0.34	1.05	24				15	3	
<b>Chile and Falkland Antarctic Peninsula and Islands</b>			70	1	0	0	0				11	1	

Molecular diversity indices are as: N: number of sequences; nH: number of haplotypes; Hd: gene diversity;  $\pi$ : nucleotide diversity; H<sub>priv</sub>: number of private haplotypes; S: number of polymorphic sites; Hap.: list of haplotypes present in each population. In the haplotype list the name of the haplotype is directly followed by the number of sampled individuals presenting the haplotype between parenthesis and private haplotype are noted in bold characters.

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