

## Comparative phylogeography of six red algae along the Antarctic Peninsula: extreme genetic depletion linked to historical bottlenecks and recent expansion

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1	Comparative phylogeography of six red algae along the Antarctic Peninsula: extreme
2	genetic depletion linked to historical bottlenecks and recent expansion
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14	ABSTRACT:
15	In the Southern Ocean, rapid climatic fluctuations during the Quaternary are thought to
16	have induced range contractions and bottlenecks, thereby instigating genetic divergence
17	and potentially even speciation of marine species. Specifically, ice scouring during glacial
18	events may have had drastic impacts on seaweed communities thus leading to genetic
19	diversification between algal populations that persisted on the Antarctic shelf in small
20	isolated refugia. Using the mitochondrial Cytochrome c Oxidase I (COI) gene and 279
21	individual macroalgal specimens collected from five geographic areas along the coasts of
22	the Antarctic Peninsula and the South Shetland Islands, we studied the genetic diversity of

23 six commonly encountered species of red algae. All six algae were characterized by very 24 low genetic diversity, and we found a significant signature of recent population expansion 25 of a single haplotype encountered over more than 450km. These results reflect the drastic 26 impact of historical perturbations on populations of Antarctic seaweeds. We propose that genetic drift during a glacial bottleneck had a strong effect and could have been amplified 27 28 by gene surfing effects during spatial expansion after ice sheet retreat. This led to the rapid spread of a single haplotype in the recolonized region. Unfortunately, the very low 29 level of genetic diversity encountered did not allow us to precisely pinpoint the putative 30 31 location of the glacial refugium inhabited by Antarctic seaweeds. Despite this, we propose 32 that future studies should test the role of active volcanic areas, such as Deception Island, 33 as long-term refugia in the region.

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KEYWORDS: Antarctic Peninsula, South Shetland, COI, Rhodophyta, glaciation, Pleistocene,
 refugia

38 INTRODUCTION

39 The 'species pump' or 'biodiversity pump' model (Haffer 1969) poses that past climatic cycles could have led to rapid divergence and speciation by promoting range 40 41 fragmentation and allopatric speciation. In particular, the importance of the periodic 42 changes in the earth's orbit, known as Milankovitch oscillations, that generated repeated 43 10–100 kyr glacial-interglacial shifts during the Pliocene and Pleistocene has been 44 acknowledged (Hewitt 2004; Allcock and Strugnell 2012). These rapid climatic fluctuations 45 produced changes in species ranges, population size, and connectivity. Furthermore, 46 during contractions of populations in refugia, altered selective regimes and increased drift 47 could have promoted incipient speciation (i.e. 'species pump' hypothesis, Haffer 1969; see 48 also Avise 2000). Glacial-interglacial shifts have been characterized not only by large and 49 rapid changes in the volume of global ice but also by variation in sea level, temperature, 50 and precipitation regimes (Haffer 1969; Hewitt 2004; Norris and Hull 2012). While the 51 amplitude of the effect of repeated glacial-interglacial shifts on divergence and allopatric 52 speciation is still debated (Knapp and Mallet 2003), the 'species pump' is believed to have played an important role in the diversification of terrestrial (alpine: Schoville et al. 2012; 53 54 boreal: Weir and Schluter 2004; Antarctic: Convey et al. 2009; and equatorial: Haffer 55 1969), freshwater (April et al. 2013), and marine (Clarke et al. 1992; Wilson et al. 2009) 56 taxa. During glacial periods in Antarctica, ice shelves have extended far from the 57 58 continent likely destroying most available habitat for benthic biota and forcing remnant

59 populations down the continental slope into deep-water refugia (Thatje et al. 2005).

60 However, for organisms restricted to shallow waters, such as photosynthetic macroalgae 61 or their associated fauna, survival in deep-water refugia is not possible; thus, the effect of glaciations could have led to complete eradication of some species from Antarctic coasts. 62 63 Indeed, at the present time, ice scouring has an extreme impact on shallow water 64 communities (Gutt 2001), and it has been postulated that ice caps had eliminated algal 65 populations in sub-Antarctic regions covered by ice during the Last Glacial Maximum 66 (Fraser et al. 2012). However, presence of polynyas on the continental shelf edge (Thatje et al. 2008) and diachrony of ice sheet extensions (i.e., regional variation in timing of ice-67 68 sheet formation; Anderson et al. 2002) suggest that ice-free regions persisted during 69 glacial periods along Antarctic coasts. These two phenomena could have provided small 70 refugia scattered around the Antarctic continental shelf edge, where shallow water 71 communities could have survived during glaciation (Allcock and Strugnell 2012). At least 38 glacial-interglacial cycles were suggested to have occurred over the last 5 million years 72 73 (Myr) (see Box1 in Allcock and Strugnell 2012), and repeated events of diversification 74 could have affected organisms persisting on the Antarctic shelf (Clarke et al. 1992). Recent phylogeographic studies have revealed the existence of numerous cryptic species or deep 75 76 genetic lineages in a wide array of marine animals (e.g., annelid polychaetes, nemerteans, 77 molluscs, arthropods, and echinoderms; see Janosik and Halanych 2010; Allcock and 78 Strugnell 2012; Riesgo et al. 2015 for review). The existence of these deep genetic lineages 79 has been reported even in organisms for which only a limited regional sampling was 80 performed (Janosik and Halanych 2010; Allcock and Strugnell 2012; Riesgo et al. 2015). 81 Speciation linked to isolation in small refugia along the Antarctic shelf has been

82 hypothesized to be source of the unexpectedly high species diversity in many taxa, 83 particularly in sea slugs (Wilson et al. 2009) and sea cucumbers (O'Loughlin et al. 2011). In the Southern Ocean, most studies using molecular approaches to determine the 84 85 existence of divergent lineages and cryptic species have focused on fishes and marine 86 invertebrates (Allcock and Strugnell 2012). Surprisingly, until now, genetic structure of 87 macroalgae has been largely neglected (but see Fraser et al. 2012; Fraser et al. 2013; 88 Billard et al. 2015; Fraser 2016) despite the fact that they represent an important part of 89 benthic communities both as resources (i.e., primary producers) and as community 90 structural components (Amsler et al. 2014). The diversity pump has been proposed to be 91 particularly relevant in Antarctic organisms with a limited capacity for dispersal since 92 populations located in refugia could easily be thoroughly isolated, leading to allopatric 93 divergence (Clarke et al. 1992; Wilson et al. 2009; Allcock and Strugnell 2012; Verheye et al. 2016). Macroalgae lacking specialized floating structures are generally considered very 94 95 poor dispersers compared to other marine organisms (Kinlan and Gaines 2003). Indeed, 96 strong spatial structure of genetic diversity can be observed at small scales (i.e., less than 97 10km; Valero et al. 2011 and Durrant et al. 2014). Thus, the small population sizes and 98 restrictive dispersal (Montecinos et al. 2012 and references therein) of macroalgae could 99 lead to an increased susceptibility to neutral processes of genetic differentiation, and 100 genetic divergence could arise rapidly even between neighboring populations (Neiva et al. 101 2012). However, contrary to our expectations, a recent study focusing on the red 102 macroalga, Gigartina skottbergii, and utilizing two genetic markers (the mitochondrial 103 intergenic region Cox2-3 and the chloroplastic RuBisCo large subunit gene) showed a

104 complete absence of genetic structure over 1600 km of coast sampled in the Antarctic 105 region (Billard et al. 2015). The authors suggest that genetic homogeneity of G. skottbergii 106 has resulted from a strong demographic bottleneck during the last Quaternary glaciations (i.e. range contraction) followed by sudden recolonization during post-glacial expansion. 107 108 In this study, we sought to determine the impact of habitat reduction during glacial 109 periods on genetic diversity in six species of red macroalgae commonly encountered along 110 the Antarctic Peninsula and the South Shetland Islands. Depending on the distribution of 111 refugia during the Last Glacial Maximum, two different outcomes could be expected for 112 these organisms: 1) if a single, small-sized refugium has acted as the origin of a recent 113 expansion wave, this should be reflected in our genetic data by high genetic homogeneity and low genetic diversity over the whole sampled area or 2) if macroalgae have been 114 115 isolated in various disjoint refugia this should lead to the observation of highly 116 differentiated genetic lineages, each mainly restricted to a single location or geographic 117 area (see Figure 1 in Allcock and Strugnell 2012). 118 119 MATERIALS AND METHODS

Study models- All our six study models correspond to fleshy red macroalgae with thallussize varying from some centimeters (e.g. *Iridaea cordata* (Turner) Bory) to up to several dozen of centimeters (e.g. *Gigartina skottsbergii* Setchell & N.L. Gardner) in length and/or diameter (see Figure 1B). None possess floating structures. *Georgiella confluens* (Reinsch) Kylin, *Curdiea racovitzae* Hariot, and *Palmaria decipiens* (Reinsch) Ricker are reported as mostly Antarctic but have also been observed in some sub-Antarctic Islands (*C. racovitzae* 

126 and G. confluens in South Georgia, and P. decipiens in South Georgia, Macquarie Island, 127 and Kerguelen Island; Wiencke and Clayton 2002; Wiencke et al. 2014). Iridaea cordata and G. skottsbergii have been registered in Antarctica, South Georgia, Falkland Islands, 128 Tierra del Fuego, and the Southern part of the Chilean and Argentinian Patagonia in 129 130 studies using classical taxonomy (Wiencke et al. 2014; Pellizzari et al. 2017). However, 131 molecular studies support that specimens sampled on both sides of the Antarctic 132 Circumpolar Current correspond to different cryptic species (Hommersand et al. 2009; 133 Billard et al. 2015). Plocamium cartilagineum (Linnaeus) Dixon has been reported on most 134 Antarctic and Sub-Antarctic coasts although these observations are based on taxonomical 135 characteristics only, and the existence of cryptic species could be suspected in this highly variable morphospecies (Wiencke et al. 2014; specimens reported as *P. aff. cartilagineum* 136 137 in Pellizzari et al. 2017). The six study species are fairly common and can be encountered forming mats mostly in the intertidal down to the shallow subtidal for *I. cordata*, *G.* 138 139 skottsbergii, C. racovitzae, and P. decipiens; or deeper, as understory of large brown 140 macroalgae, for G. confluens and P. cartilagineum. Most Antarctic macroalgae studied have also proven to be highly stenothermic. Thus, their spore production, settlement, and 141 142 survival are highly limited by temperature; and characteristics such as growth and 143 reproduction often follow a strong seasonal pattern, mirroring changes in abiotic 144 conditions (e.g. G. confluens, P. cartilagineum and I. cordata growth occur between 0°C 145 and 5°C; with an upper survival temperature of 11°C-16°C°; Wiencke et al. 2014).

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147 Sampling- For G. confluens, G. skottsbergii and P. cartilagineum, samples were collected 148 by scuba diving from shallow subtidal zones down to 30m. Samples of C. racovitzae, I. cordata and P. decipiens were obtained from both intertidal rock pools and the shallow 149 subtidal (i.e., mostly at depth of 0 to 15m). In order to sample individuals corresponding to 150 151 distinct genotypes and coming from sexual reproduction and through spore settlement, 152 only one thallus sample was taken from each macroalgal holdfast, and all holdfasts were 153 sampled well separated on the rocky subtract. Samples were collected in five distinct 154 areas (see Figure 1A), two located in the South Shetland Islands (GEO: King George Island, 155 62°12'S/58°57'W and PRA: Greenwich Island, 62°28'S/59°40'W) and three located along 156 the Antarctic Peninsula (OHI: O'Higgins, 63°18'S/57°53'W; PAR: Paradise Bay, 64°50'S/62°52'W and MAR: Marguerite Bay, 67°45'S/68°52'W). Specimens were pressed 157 158 as vouchers after removing a small portion of the thallus stored in silica gel for subsequent DNA analysis. Voucher specimens are housed in the herbarium of the Universidad Austral 159 160 de Chile and are available from the contact author on request. In total, 42 specimens were 161 sampled for C. racovitzae, 20 for G. confluens, 28 for G. skottsbergii, 90 for I. cordata, 35 162 for P. decipiens, and 64 for P. cartilagineum (Table 1, Figure 2). 163 DNA extraction, PCR amplification and sequencing- DNA extraction was undertaken 164 according to the methods described in Faugeron et al. (2001). DNA amplification of the 5' 165 part of the mitochondrial Cytochrome c Oxidase I gene (COI) was done following the 166 amplification protocols of Saunders (2005) using the primer pair GazF1 (5'-TCA ACA AAT 167 CAT AAA GAT ATT GG -3') and GazR1 (5'-ACT TCT GGA TGT CCA AAA AAY CA -3'). Purified 168 PCR products (UltraCleanTM kit, MO BIO Laboratories, Carlsbad, USA) were sequenced

169 (Macrogen Inc., Seoul, South Korea). The 279 generated COI sequences (632 bp) have 170 been archived with the GENBANK accession numbers KY559671-KY559712, KY559727-171 KY559746, KY559753-KY559780, KY559817-KY559905, KY559935-KY559947, KY559949-172 KY559970, KY560012-KY560065 and KY560067-KY560076. For each accession, information 173 about the region, sampling locality, and voucher code (i.e., given under the "organism" 174 category) has been provided. Sequences were aligned using the CLUSTAL function of 175 MEGA v.5 (Tamura et al. 2011). 176 Data analysis- For each species, levels of polymorphism over the whole sampled area were 177 calculated using the following standard genetic diversity indices: number of sampled 178 haplotypes (k), number of polymorphic sites (S), haplotype diversity (H), average number 179 of pairwise differences ( $\Pi$ ), and nucleotide diversity ( $\pi$ ). These indices were calculated 180 using DnaSP v.4 (Rozas et al. 2003). Haplotype networks were reconstructed using the median-joining algorithm implemented in NETWORK v.4 (Bandelt et al. 1999). Haplotype 181 182 frequencies, for each species in each area, were calculated using DnaSP v.4 (Rozas et al. 183 2003). Three tests were performed to assess whether each species is at mutation-drift 184 185 equilibrium or if there was a signature of post-glacial recent expansion. Tajima's D (Tajima 186 1989) and Fu's FS (Fu 1997) neutrality tests were conducted using DnaSP v.4 (Rozas et al. 187 2003). Significance for these two neutrality tests was obtained by simulating 1,000 188 samples using the coalescent approach developed in DnaSP v.4 (Rozas et al. 2003). 189 Negative and significant values for Tajima's D and Fu's FS neutrality tests reflect an excess 190 of rare polymorphisms in a population, which indicates either positive selection or a

191	recent increase in population size (Aris-Brosou and Excoffier 1996). Moreover, we tested
192	the frequency distribution of pairwise differences between haplotypes (i.e. mismatch
193	analysis) against a model of sudden expansion (Rogers and Harpending 1992). This was
194	done using 1,000 replicates in the program ARLEQUIN v.3.5 (Excoffier and Lisher 2010).
195	The goodness-of-fit between observed and estimated distributions was assessed by
196	calculating the sum of squared differences (SSD) between observed and expected
197	distributions and the Harpending's raggedness index (Rag) (Harpending 1994).
198	Calculations were performed in ARLEQUIN v.3.5 (Excoffier and Lisher 2010), and
199	significance was assessed by bootstrapping (1,000 replicates).
200	We approximated the timing since the beginning of the most recent population
201	expansion using the equation $\tau$ =2ut, where t is the number of years since expansion and u
202	is the per-sequence-per-year mutation rate. The parameter Tau ( $ au$ ) and 90% percentile
203	values of $\tau$ were inferred directly from the model of sudden expansion in ARLEQUIN v.3.5
204	(Excoffier and Lisher 2010). Since no precise molecular clock exists for the COI in red algae,
205	we used as an approximation the divergence rate of 0.14 % per Myr published by
206	Muangmai et al. (2014). These authors have computed this rough estimate for COI of the
207	genus Bostrychia using two samples of Bostrychia calliptera collected from both sides of
208	the Isthmus of Panama, considering 2.5–3.0 Myr ago as the final closure date of the
209	Isthmus. Since substitution rates are usually much lower than mutation rates (i.e., because
210	natural selection tends to remove deleterious mutations), we applied the tenfold
211	evolutionary rate correction for intra-species time dependence of molecular rates as
212	proposed by Ho et al. (2011); this was applied to the divergence rate proposed for COI

- (Muangmai et al. 2014) before estimating the timing since the beginning of population
  expansion (mutation rate, u = 1.4 % per Myr for the COI).
- 215

216 RESULTS

217	Over the whole sampled area (i.e. more than 450 km of sampled coast, Figure 1A), all six
218	red macroalgae showed very low levels of mtDNA genetic diversity (Figure 2). Depending
219	on the species under study, one to six polymorphic sites were detected (Table 1). The
220	number of observed haplotypes varied from two in <i>C. racovitzae</i> and <i>G. skottsbergii</i> to
221	seven in <i>I. cordata</i> and <i>P. decipiens</i> . The highest values of mtDNA diversity were
222	encountered in <i>I. cordata</i> (haplotype diversity = 0.398, average number of pairwise
223	differences = 0.623, and nucleotide diversity = 0.001), the species with the largest sample
224	size (N = 90; Table 1). Simple, star-like haplotype network topologies were observed for
225	each species (Figure 2). For all species, the central haplotype was the most frequent (from
226	77% of the samples in <i>I. cordata</i> to 98% of the samples in <i>C. racovitzae</i> ) and was present in
227	all localities (Figure 2), while the remaining haplotypes were rare and private (i.e.,
228	haplotype confined to a single geographic locality). One exception to this was the <i>I</i> .
229	cordata network (Figure 2). For this species two haplotypes (i.e. shaded in grey in Figure 2)
230	showed intermediate frequency (11 and 8 %, respectively) and were also widely
231	distributed in most sampled areas. These haplotypes were related to the most common <i>I</i> .
232	cordata haplotype through branch length of at most two mutational steps (Figure 2).
233	In all studied species, both Tajima's D and Fu's Fs tests gave negative values (Table
234	1). Associated probabilities were significant in C. racovitzae, G. confluens, P. decipiens, and

235	P. cartilagineum for the Tajima's D test, and in P. decipiens and P. cartilagineum for the
236	Fu's Fs test (Table 1). Congruent with these findings and the fact that the majority of the
237	individuals within each species shared the same haplotype, distributions of pairwise
238	differences between sequence pairs were L-shaped for all six species (Online Resource 1).
239	From the results of the goodness-of-fit tests, the null hypothesis of sudden expansion
240	could not be rejected for the six species tested (Table 2; all $p$ values for Expected SSD >
241	Observed SSD and Expected Rag > Observed Rag were much higher than 0.05). The
242	population expansion was estimated to have begun during the late Quaternary. Times of
243	expansion were estimated to range from 0 - 36,000 years in C. racovitzae to 0 - 240,000
244	years in <i>I. cordata</i> (Table 2).

246 DISCUSSION

247 Throughout the South Shetland Islands and the Antarctic Peninsula, no differentiated 248 genetic groups were observed within the six species studied here. On the contrary, all six 249 red macroalgae were characterized by very low genetic diversity and a significant 250 signature of recent population expansion of a single haplotype encountered over more than 450km. Some limitations of our study, such as the low number of samples available 251 252 for some species (mostly for G. confluens, G. skottsbergii, and P. decipiens, for which less than 40 samples were studied), the relatively limited area studied compared to the 253 254 entirety of the Antarctic coast, or the fact that only one gene was sequenced (but see 255 Billard et al. 2015, same results observed using two different markers, more samples, and 256 more sample locations for G. skottsbergii) could explain the results found here. However,

the fact that the same pattern was encountered in all six species allows us to assume that

the genetic structure and diversity of Antarctic macroalgal populations is actually a

259 reflection of historical glacial perturbations during the late Quaternary.

260 There are reports of distinct glacial refugia in the South Shetland Islands and

261 Antarctic Peninsula harboring cryptic species that have diverged recently in micro-

allopatry (marine animals: Wilson et al. 2007, 2009; Allcock et al. 2011; Verheye et al.

263 2016). In other marine bioregions, like the southeastern tip of Australia (Fraser et al. 2009)

or the Philippine archipelago (Payo et al. 2013), processes of divergence and speciation in

265 marine macroalgae have also been connected to fluctuations in sea level and water

temperature during glacial periods. For the six species of Antarctic red macroalgae studied

here, however, no trace of an effect of a diversity pump in the western Antarctic Peninsulaarea was encountered.

269 The existence of a single glacial refugium (Billard et al. 2015) associated with 270 massive population size reduction during the late Quaternary and a recent recolonization 271 of the Antarctic Peninsula and the South Shetland Islands could explain our results. Severe bottlenecks during glacial periods have been inferred for many taxa that have been 272 273 studied in the Southern Ocean (Allcock and Strugnell 2012), and this includes animals with 274 high dispersal capacities and large population sizes (e.g., the Antarctic limpet Nacella 275 concinna: González-Wevar et al. 2011; the krill Euphausia superba: Goodall-Copestake et 276 al. 2010; Bortolotto et al. 2011; and the shrimp *Chorismus antarcticus*: Raupach et al. 277 2010). Macroalgae are fairly limited in terms of the depth that they inhabit (as these are

photosynthetic organisms that need to live close enough to the surface to get sunlight),

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279 and they are organisms that typically have restricted belt-like distributions along the 280 coast. Differing then from pelagic or broad depth-ranging benthic animals threatened by 281 ice scour in Antarctica (Allcock and Strugnell 2012), macroalgae cannot use refugia located 282 in the deepest parts of the continental shelf. Indeed, a complete or nearly complete 283 eradication of macroalgal populations from the sub-Antarctic and Antarctic coasts during 284 the Last Glacial Maximum has been inferred based on molecular data in the Southern 285 hemisphere (Macaya and Zuccarello 2010; Fraser et al. 2012; Montecinos et al. 2012; 286 Billard et al. 2015), and the impact of ice scouring has been deemed to be particularly 287 important in Antarctica during this period (Thatje et al. 2005). Due to their ecological and 288 life cycle characteristics, the dramatic impact of ice scour on Antarctic macroalgal genetic 289 diversity is not surprising. In our study, the very low level of genetic diversity and the 290 large-scale spread of common genetic variants prohibit us from precisely pinpointing the 291 location of the glacial refugium of Antarctic macroalgae. Recent studies have estimated 292 that the Last Glacial Maximum ice cap was thick and extended as far as the shelf edge 293 around the South Shetland Islands and the Antarctic Peninsula (Simms et al. 2011; Cofaigh 294 et al. 2014). The ice sheet retreat began earlier in the Northern part of the region, and it is 295 very likely that Marguerite Bay, where grounded ice was hypothesized to have existed up 296 to 14,000 years ago, is a recently colonized area (Cofaigh et al. 2014). Interestingly, the 297 Last Glacial Maximum ice coverage proposed by Simms et al. (2011) does not include 298 Deception Island, the largest and most active volcano in the area (see Figure 1A). It is 299 possible that volcanoes, like Deception Island, Penguin Island, and Bridgeman Island, have 300 been active during the last 4 Myr and remained free of ice during glacial periods (Simms et

301 al. 2011; Figure 1A), thus, representing potential refugia for Antarctic flora and fauna 302 (Convey et al. 2009; Fraser et al. 2014). Except for C. racovitzae, all the macroalgae studied 303 here have been recorded in both Deception and Penguin Islands (Pellizzari et al. 2017). 304 Unfortunately, no sites located along these volcanic coasts were included in our work. 305 Other glacial contraction-expansion scenarios have also proposed recolonization occurring 306 from peri-Antarctic refugia, in sub-Antarctic Islands, to the Antarctic coasts in marine 307 organisms (Fraser et al. 2012; González-Wevar et al. 2016). In particular, South Georgia 308 has been reported to be located at an intermediate position between the Magellan and 309 Antarctic biogeographic regions (i.e., representing the southernmost limit for exclusive 310 Magellan species and the northernmost limit for exclusive Antarctic species; Arntz 2005), 311 providing a potential glacial refugium for Antarctic marine species (González-Wevar et al. 312 2016). However, contrarily to what was reported by González-Wevar and collaborators (2016), a recent molecular study on Lepidonotothen nudifrons, a notothenioid fish 313 314 formerly believed to be distributed from the Antarctic Peninsula up to South Georgia, has 315 described the existence of two genetic cryptic species, one located in the northern part of 316 the Scotia Arc (South Georgia and Sandwich Islands) and the other one restricted to more 317 Antarctic waters, which reveals the long term isolation of these two regions (Dornburg et 318 al. 2016). When only taxonomical tools are taken into account, the distribution of the six 319 macroalgae under study includes South Georgia (Wiencke and Clayton 2002; Wiencke et 320 al. 2014), however, no sampling has been undertaken to date to resolve the genetic 321 identity of these populations. In order to better locate glacial refugia in the region, future 322 studies should consider identifying the genetic diversity present in populations of marine

organisms collected along those regions of the Antarctic coasts that could have been less
affected by ice scour during the Last Glacial Maximum (e.g., Deception Island, Penguin
Island, or Bridgeman Island among others; Simms et al. 2011) and from the more northern
islands of the Scotia Arc (e.g., South Georgia or the South Sandwich Islands).

327 In this study, the geographic spread of haplotypes of six species was determined 328 for populations inhabiting more than 450km of coast. Haplotypes distributed over 329 hundreds or even thousands of kilometers has been reported in various pelagic and 330 benthic species with long-lived pelagic larvae inhabiting the Southern Ocean (Thornhill et 331 al. 2008; Raupach et al. 2010; Bortolotto et al. 2011; González-Wevar et al. 2011; Janosik 332 et al. 2011). The wide ranging distribution of haplotypes was attributed to the impact of 333 strong oceanic currents and to a high degree of connectivity. However, none of the six 334 macroalgae under study has floating structures, and their dispersal capacity should be very limited. The western Antarctic Peninsula represents a highly fragmented and isolated 335 336 habitat for coastal marine species since ice-free rocky shores are rare and separated from 337 each other by extensive areas covered by thick ice sheets, strong currents, and steep 338 variation in shelf topography (e.g. Bransfield Strait). These physical properties likely limit 339 gene flow between coastal populations, especially for organisms with low dispersal 340 potential, such as macroalgae that lack buoyant structures (Hoffman et al. 2011). 341 Nevertheless, extreme mtDNA monomorphism along the South Shetland Islands and the 342 coasts of the Antarctic Peninsula has been observed in other species with limited dispersal 343 ability (brooding invertebrates: the brittle star Astrotoma agassizii, Hunter and Halanych 344 2008; the sea spider Nymphon austral, Mahon et al. 2008; sea slug with direct

development: Doris kerguelenensis, Wilson et al. 2009). For these species as well as for 345 346 red macroalgae, the strong effect of genetic drift during population contraction in glacial 347 refugia could have been amplified by gene surfing effects during spatial expansion, after 348 ice sheet retreat; this, in turn, would lead to the rapid spread of a dominant haplotype in 349 the recolonized region. Indeed, during interglacial recolonization, random sampling of 350 haplotypes through successive founder events can result in the drastic reduction of 351 genetic diversity; thus, a single haplotype can easily spread over vast geographic areas at 352 expanding range margins (Excoffier and Ray 2008). An emblematic example of gene 353 surfing in macroalgae is the case of the post-glacial, European northwards range 354 expansion of introgressed organelle lineages of Fucus vesiculosus with the F. ceranoides 355 nuclear gene pool (Neiva et al. 2010). Moreover, passive transport of detached fronds by 356 ocean currents can explain how even macroalgae characterized by low autonomous dispersal ability can quickly colonize newly available habitats stripped bare by ice during 357 358 the Last Glacial Maximum (Macaya et al. 2016). Rare events of long distance colonization 359 by rafting have been documented even among macroalgae lacking floating structures 360 (Fraser et al. 2013; Fraser 2016; Macaya et al. 2016). Antarctic macroalgae fronds 361 enclosed in drift ice have been observed at sea (Guillemin M-L pers. obs.), and these 362 fronds can also be recovered far away from the coasts (e.g., frond of a Desmarestia sp. 363 sampled at a 2500m depth in the Weddell Sea more than 250km away from the closest 364 coast; Fahrbach 2006). In the particular case of recolonization of newly available 365 substrates (e.g., recently deglaciated substrates), the installation of few migrants arriving 366 to the coast can be enhanced since these recruits do not have to compete with

367 established, locally adapted communities (Waters et al. 2013). It is interesting to note that 368 G. skottsbergii, I. cordata, P. decipiens, and P. cartilagineum are among the first 369 macroalgae colonizing new areas available after glacial retreat (Quartino et al. 2013). 370 West of the Antarctic Peninsula, complex oceanic circulation patterns have been 371 described (Moffat et al. 2008; Savidge and Amft 2009; see also Figure 1A) that likely 372 played a role in the postglacial expansion of regional marine flora and fauna. Spatial 373 expansion from refugia could have been facilitated by fronds drifting on one of the strong 374 currents present in the region (Moffat et al. 2008; Savidge and Amft 2009; Figure 1A); specifically, by the Antarctic Circumpolar Current, which flows northeastward from the 375 376 Bellingshausen Sea through the South Shetland Islands and follows the western shelf-edge 377 limb, and by the Antarctic Slope Front, which flows westward along the whole Antarctic 378 continental shelf entering the Bransfield Strait near the South Scotia Ridge (Figure 1A). Moreover, the cyclonic circulation inside the Bransfield Strait and the Antarctic Peninsula 379 380 Coastal Current (APCC) that connect the Bransfield Strait to Margerite Bay during spring 381 and summer could easily lead to recurring connectivity in our study region (Moffat et al. 382 2008; Savidge and Amft 2009; Figure 1A). The strength of the APCC is tightly linked to the 383 amount of melt-water fluxes from the coast, and since global warming affects these fluxes 384 (i.e., affecting glacier melting rates and snowfall rates among others), one can only 385 wonder how connectivity of marine organisms along the Antarctic Peninsula will be 386 altered in the near future (Moffat et al. 2008). 387 In summary, even if the effects of population contraction on genetic diversity were

388 particularly drastic, a rich flora and fauna assemblage has clearly survived through the

389 Pliocene and Pleistocene glacial and interglacial cycles (Hommersand et al. 2009; Convey 390 et al 2009; Clarke and Crame 2010; Allcock and Strugnell 2012; Fraser et al. 2014; Billard et 391 al. 2015). The strong water currents in the region likely played a prominent role in the 392 rapid recolonization that took place at the end of the Quaternary. However, in the six 393 macroalgae under study, the presence of one dominant haplotype over the whole studied 394 area could simply be linked to the past glacial demographic history and might not 395 necessarily imply the existence of actual homogenizing gene flow between the Antarctic 396 Peninsula and the South Shetland Islands. Indeed, recent studies have reported strong 397 dissimilarities between genetic diversity and structure encountered in marine species 398 depending on the genetic markers used (e.g. in the flathead mullet; Durand et al. 2013). 399 Differences in patterns of genetic structure have been linked to the fact that organelle 400 DNA is more sensitive to introgression and/or rapid sweeps (due to selection or strong genetic drift) compared to nuclear DNA (Dowling et al. 2008; Durand et al. 2013). In order 401 402 to test for the existence of current gene flow between localities within the region, it is 403 imperative to develop new nuclear markers (e.g. microsatellites or SNPs) in macroalgae 404 species of interest. Since the common species of macroalgae in Antarctica grow in widely 405 heterogeneous habitats in terms of salinity and turbidity (for examples see Savidge and 406 Amft 2009; Quartino et al. 2013), it could also be of interest to test for the existence of 407 local adaptation using recently developed genomics and transcriptomics tools (see Riesgo 408 et al. 2015 for a review).

409

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422 Figure 1: Map of the study region and scans of herborized specimens of the six study 423 models. A) Map of the Antarctic Peninsula showing schematic paths of the main marine 424 currents (synthesis from the data and maps presented in Mahon et al. 2008; Moffat et al. 425 2008; and Savidge and Amft 2009). ACC: Antarctic Circumpolar Current, APCC: Antarctic Peninsula Coastal Current, ASF: Antarctic Slope Front, and CC: cyclonic circulation inside 426 427 the Bransfield Strait. Lower arrow thickness represents currents only observed seasonally 428 (i.e. APCC only during spring and summer; Moffat et al. 2008). The thin black dashed line 429 corresponds to reconstructions of putative position of Antarctic Peninsula (Cofaigh et al. 430 2014) and South Shetland (Simms et al. 2011) ice caps during the Last Glacial Maximum. 431 Volcanic areas active during the last 4 Myr include Deception Island (DI), Penguin Island 432 (PI), and Bridgeman Island (BI). White circles represent sampling localities: King George

Island (GEO), Greenwich Island (PRA), O'Higgins (OHI), Paradise Bay (PAR), and Marguerite
Bay (MAR). B) Study models: *Curdiea racovitzae* (1), *Plocamium cartilagineum* (2), *Iridaea cordata* (3), *Palmaria decipiens* (4), *Georgiella confluens* (5), and *Gigartina skottsbergii* (6).
Black line represents a 10 cm scale.

437

438 Figure 2: Haplotype networks and pie charts showing the geographical distribution of 439 haplotypes for the genetic marker COI in six macroalgae in the western Antarctic 440 Peninsula area. Haplotype networks are given within boxes in the low right corner of each 441 graph. In the networks, each circle represents a haplotype, and its size is proportional to 442 the frequency in which the haplotype was encountered. For haplotypes separated by 443 more than one mutational step, the number of steps is indicated by small lines. Black and 444 grey haplotypes are shared between localities while white ones are private haplotypes. The five localities correspond, from north to south, to King George Island (GEO), 445 446 Greenwich Island (PRA), O'Higgins (OHI), Paradise Bay (PAR), and Marguerite Bay (MAR) 447 (localization given in the *Curdiea racovitzae* map on the upper left; see also Figure 1A). For 448 each species, number of sequenced individuals is given between brackets.

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Table 1: Genetic diversity indices and neutrality test in six common red algae sampled along the Antarctic Peninsula and South Shetland Islands.

Species	Ν	k	S	Н	П	π.10 <sup>-2</sup>	Tajima's D	Fu's FS
Curdiea racovitzae	42	2	1	0.048	0.048	0.008	-1.120*	-1.491 <sup>ns</sup>
Georgiella confluens	20	4	4	0.363	0.489	0.077	-1.638*	-1.613 <sup>ns</sup>
Gigartina skottsbergii	28	2	1	0.071	0.071	0.011	-1.151 <sup>ns</sup>	-1.155 <sup>ns</sup>
Iridaea cordata	90	7	5	0.398	0.623	0.102	-0.797 <sup>ns</sup>	-2.882 <sup>ns</sup>
Palmaria decipiens	35	7	6	0.318	0.343	0.054	-2.103***	-7.041***
Plocamium cartilagineum	64	4	4	0.122	0.155	0.025	-1.759***	-3.466**

N: number of sampled specimens; k: number of haplotypes; S: polymorphic sites; H: haplotype diversity;  $\Pi$ : average number of nucleotide differences;  $\pi$ : nucleotide diversity. ns: non-significant; \*: p<0.05; \*\*: p<0.01; \*\*\*: p<0.001.

Table 2: Goodness of fit tests for a model of sudden expansion calculated for the sum of squared deviation (SSD) and the Harpending's raggedness index (Rag) and approximated time since the beginning of the most recent population expansion.

Species	SSD	p value	Rag	p value	τ	(90% values)	Range estimation of times of
							expansion (years)
Curdiea racovitzae	0.000	0.19	0.821	0.83	0.16	(0-0.636)	0-36,000
Georgiella confluens	0.000	0.74	0.179	0.77	0.48	(0-2.263)	0-128,000
Gigartina skottsbergii	0.000	0.22	0.740	0.80	0.18	(0-0.715)	0-40,000
Iridaea cordata	0.007	0.56	0.244	0.63	1.47	(0-4.241)	0-240,000
Palmaria decipiens	0.001	0.32	0.224	0.56	0.39	(0.081-0.987)	5,000-56,000
Plocamium cartilagineum	0.000	0.38	0.627	0.74	0.64	(0-2.199)	0-124,000

For details about calculations of the parameter Tau ( $\tau$ ) and time of expansion see details in the text.









Online Resource 1: Observed (grey bar) and sudden expansion simulated (black line) mismatch distributions of pairwise COI haplotype differences in six red algae sampled in the western Antarctic Peninsula area.