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

Marie-Laure Guillemin, Hélène Dubrasquet, Janette Reyes, Myriam Valero

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

Marie-Laure Guillemin[1,2,3*], Hélène Dubrasquet[1,2], Janette Reyes[1], Myriam Valero[2]

[1]Instituto de Ciencias Ambientales y Evolutivas, Facultad de Ciencias, Universidad Austral de Chile, Casilla 567 Valdivia, Chile

[2]CNRS, Sorbonne Universités, UPMC University Paris VI, PUC, UACH, UMI 3614, Evolutionary Biology and Ecology of Algae, Station Biologique de Roscoff, CS 90074, Place G. Teissier, 29680 Roscoff, France

[3]Centro FONDAP de Investigación en Dinámica de Ecosistemas Marinos de Altas Latitudes (IDEAL)

*Author for correspondence: marielaure.guillemin@gmail.com

ABSTRACT:

In the Southern Ocean, rapid climatic fluctuations during the Quaternary are thought to have induced range contractions and bottlenecks, thereby instigating genetic divergence and potentially even speciation of marine species. Specifically, ice scouring during glacial events may have had drastic impacts on seaweed communities thus leading to genetic diversification between algal populations that persisted on the Antarctic shelf in small isolated refugia. Using the mitochondrial Cytochrome c Oxidase I (COI) gene and 279 individual macroalgal specimens collected from five geographic areas along the coasts of the Antarctic Peninsula and the South Shetland Islands, we studied the genetic diversity of

six commonly encountered species of red algae. All six algae were characterized by very low genetic diversity, and we found a significant signature of recent population expansion of a single haplotype encountered over more than 450km. These results reflect the drastic 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 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 level of genetic diversity encountered did not allow us to precisely pinpoint the putative location of the glacial refugium inhabited by Antarctic seaweeds. Despite this, we propose that future studies should test the role of active volcanic areas, such as Deception Island, as long-term refugia in the region.

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

INTRODUCTION

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 fragmentation and allopatric speciation. In particular, the importance of the periodic changes in the earth’s orbit, known as Milankovitch oscillations, that generated repeated 10–100 kyr glacial-interglacial shifts during the Pliocene and Pleistocene has been acknowledged (Hewitt 2004; Allcock and Strugnell 2012). These rapid climatic fluctuations produced changes in species ranges, population size, and connectivity. Furthermore, during contractions of populations in refugia, altered selective regimes and increased drift could have promoted incipient speciation (i.e. ‘species pump’ hypothesis, Haffer 1969; see also Avise 2000). Glacial-interglacial shifts have been characterized not only by large and rapid changes in the volume of global ice but also by variation in sea level, temperature, and precipitation regimes (Haffer 1969; Hewitt 2004; Norris and Hull 2012). While the amplitude of the effect of repeated glacial-interglacial shifts on divergence and allopatric 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; boreal: Weir and Schluter 2004; Antarctic: Convey et al. 2009; and equatorial: Haffer 1969), freshwater (April et al. 2013), and marine (Clarke et al. 1992; Wilson et al. 2009) taxa.

During glacial periods in Antarctica, ice shelves have extended far from the continent likely destroying most available habitat for benthic biota and forcing remnant populations down the continental slope into deep-water refugia (Thatje et al. 2005).

However, for organisms restricted to shallow waters, such as photosynthetic macroalgae 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. Indeed, at the present time, ice scouring has an extreme impact on shallow water communities (Gutt 2001), and it has been postulated that ice caps had eliminated algal populations in sub-Antarctic regions covered by ice during the Last Glacial Maximum (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-sheet formation; Anderson et al. 2002) suggest that ice-free regions persisted during glacial periods along Antarctic coasts. These two phenomena could have provided small refugia scattered around the Antarctic continental shelf edge, where shallow water 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 (Myr) (see Box1 in Allcock and Strugnell 2012), and repeated events of diversification 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 genetic lineages in a wide array of marine animals (e.g., annelid polychaetes, nemerteans, molluscs, arthropods, and echinoderms; see Janosik and Halanych 2010; Allcock and Strugnell 2012; Riesgo et al. 2015 for review). The existence of these deep genetic lineages has been reported even in organisms for which only a limited regional sampling was performed (Janosik and Halanych 2010; Allcock and Strugnell 2012; Riesgo et al. 2015). Speciation linked to isolation in small refugia along the Antarctic shelf has been

hypothesized to be source of the unexpectedly high species diversity in many taxa, 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 existence of divergent lineages and cryptic species have focused on fishes and marine invertebrates (Allcock and Strugnell 2012). Surprisingly, until now, genetic structure of macroalgae has been largely neglected (but see Fraser et al. 2012; Fraser et al. 2013; Billard et al. 2015; Fraser 2016) despite the fact that they represent an important part of benthic communities both as resources (i.e., primary producers) and as community structural components (Amsler et al. 2014). The diversity pump has been proposed to be particularly relevant in Antarctic organisms with a limited capacity for dispersal since populations located in refugia could easily be thoroughly isolated, leading to allopatric 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 poor dispersers compared to other marine organisms (Kinlan and Gaines 2003). Indeed, strong spatial structure of genetic diversity can be observed at small scales (i.e., less than 10km; Valero et al. 2011 and Durrant et al. 2014). Thus, the small population sizes and restrictive dispersal (Montecinos et al. 2012 and references therein) of macroalgae could lead to an increased susceptibility to neutral processes of genetic differentiation, and genetic divergence could arise rapidly even between neighboring populations (Neiva et al. 2012). However, contrary to our expectations, a recent study focusing on the red macroalga, *Gigartina skottbergii,* and utilizing two genetic markers (the mitochondrial 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
107 (i.e. range contraction) followed by sudden recolonization during post-glacial expansion.
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
114 and low genetic diversity over the whole sampled area or 2) if macroalgae have been
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
120 Study models- All our six study models correspond to fleshy red macroalgae with thallus-
121 size varying from some centimeters (e.g. *Iridaea cordata* (Turner) Bory) to up to several
122 dozen of centimeters (e.g. *Gigartina skottsbergii* Setchell & N.L. Gardner) in length and/or
123 diameter (see Figure 1B). None possess floating structures. *Georgiella confluens* (Reinsch)
124 Kylin, *Curdiea racovitzae* Hariot, and *Palmaria decipiens* (Reinsch) Ricker are reported as
125 mostly Antarctic but have also been observed in some sub-Antarctic Islands (*C. racovitzae*

and *G. confluens* in South Georgia, and *P. decipiens* in South Georgia, Macquarie Island, 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, Tierra del Fuego, and the Southern part of the Chilean and Argentinian Patagonia in studies using classical taxonomy (Wiencke et al. 2014; Pellizzari et al. 2017). However, molecular studies support that specimens sampled on both sides of the Antarctic Circumpolar Current correspond to different cryptic species (Hommersand et al. 2009; Billard et al. 2015). *Plocamium cartilagineum* (Linnaeus) Dixon has been reported on most Antarctic and Sub-Antarctic coasts although these observations are based on taxonomical 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* 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. skottsbergii*, *C. racovitzae,* and *P. decipiens;* or deeper, as understory of large brown macroalgae, for *G. confluens* and *P. cartilagineum*. Most Antarctic macroalgae studied have also proven to be highly stenothermic. Thus, their spore production, settlement, and survival are highly limited by temperature; and characteristics such as growth and reproduction often follow a strong seasonal pattern, mirroring changes in abiotic conditions (e.g. *G. confluens*, *P. cartilagineum* and *I. cordata* growth occur between 0°C and 5°C; with an upper survival temperature of 11°C-16°C°; Wiencke et al. 2014).

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.*
149 *cordata* and *P. decipiens* were obtained from both intertidal rock pools and the shallow
150 subtidal (i.e., mostly at depth of 0 to 15m). In order to sample individuals corresponding to
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,
157 64°50'S/62°52'W and MAR: Marguerite Bay, 67°45'S/68°52'W). Specimens were pressed
158 as vouchers after removing a small portion of the thallus stored in silica gel for subsequent
159 DNA analysis. Voucher specimens are housed in the herbarium of the Universidad Austral
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 (Π), and nucleotide diversity (π). These indices were calculated
180 using DnaSP v.4 (Rozas et al. 2003). Haplotype networks were reconstructed using the
181 median-joining algorithm implemented in NETWORK v.4 (Bandelt et al. 1999). Haplotype
182 frequencies, for each species in each area, were calculated using DnaSP v.4 (Rozas et al.
183 2003).
184 Three tests were performed to assess whether each species is at mutation-drift
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 (τ) and 90% percentile
203 values of τ 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

213 (Muangmai et al. 2014) before estimating the timing since the beginning of population
214 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 *C. racovitzae* and *G. skottsbergii* to
221 seven in *I. cordata* and *P. decipiens*. The highest values of mtDNA diversity were
222 encountered in *I. cordata* (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. cordata* to 98% of the samples in *C. racovitzae*) 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.*
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.*
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. cordata* (Table 2).

245

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
251 than 450km. Some limitations of our study, such as the low number of samples available
252 for some species (mostly for *G. confluens*, *G. skottsbergii,* and *P. decipiens,* for which less
253 than 40 samples were studied), the relatively limited area studied compared to the
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 reflection of historical glacial perturbations during the late Quaternary.

There are reports of distinct glacial refugia in the South Shetland Islands and 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. 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 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 Peninsula area was encountered.

The existence of a single glacial refugium (Billard et al. 2015) associated with massive population size reduction during the late Quaternary and a recent recolonization 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 studied in the Southern Ocean (Allcock and Strugnell 2012), and this includes animals with high dispersal capacities and large population sizes (e.g., the Antarctic limpet *Nacella concinna*: González-Wevar et al. 2011; the krill *Euphausia superba*: Goodall-Copestake et al. 2010; Bortolotto et al. 2011; and the shrimp *Chorismus antarcticus*: Raupach et al. 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),

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

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

323 organisms collected along those regions of the Antarctic coasts that could have been less
324 affected by ice scour during the Last Glacial Maximum (e.g., Deception Island, Penguin
325 Island, or Bridgeman Island among others; Simms et al. 2011) and from the more northern
326 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
335 very limited. The western Antarctic Peninsula represents a highly fragmented and isolated
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

345 development: *Doris kerguelenensis*, Wilson et al. 2009). For these species as well as for
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
357 dispersal ability can quickly colonize newly available habitats stripped bare by ice during
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

established, locally adapted communities (Waters et al. 2013). It is interesting to note that *G. skottsbergii*, *I. cordata*, *P. decipiens,* and *P. cartilagineum* are among the first macroalgae colonizing new areas available after glacial retreat (Quartino et al. 2013). West of the Antarctic Peninsula, complex oceanic circulation patterns have been described (Moffat et al. 2008; Savidge and Amft 2009; see also Figure 1A) that likely played a role in the postglacial expansion of regional marine flora and fauna. Spatial expansion from refugia could have been facilitated by fronds drifting on one of the strong 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 Bellingshausen Sea through the South Shetland Islands and follows the western shelf-edge limb, and by the Antarctic Slope Front, which flows westward along the whole Antarctic 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 Coastal Current (APCC) that connect the Bransfield Strait to Margerite Bay during spring and summer could easily lead to recurring connectivity in our study region (Moffat et al. 2008; Savidge and Amft 2009; Figure 1A). The strength of the APCC is tightly linked to the amount of melt-water fluxes from the coast, and since global warming affects these fluxes (i.e., affecting glacier melting rates and snowfall rates among others), one can only wonder how connectivity of marine organisms along the Antarctic Peninsula will be altered in the near future (Moffat et al. 2008).

In summary, even if the effects of population contraction on genetic diversity were 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
401 genetic drift) compared to nuclear DNA (Dowling et al. 2008; Durand et al. 2013). In order
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
410 ACKNOWLEDGMENTS

411 This research was supported by the Instituto Antártico Chileno (INACH) T_16-11 and
412 RG_15-16 projects, and sampling in King George Island was funded by the Project Anillo
413 ART1101 from the Comisión Nacional de Investigación Cientıfíca y Tecnológica. Additional
414 support came from the International Research Network "Diversity, Evolution, and
415 Biotechnology of Marine Algae" (GDRI N° 0803) and from the Centro FONDAP IDEAL no.
416 15150003. The authors thank P. Brunning, J. L. Kappes, T. Heran, Y. Henriquez, and L.
417 Vallejos for their help in the field. The authors would also like to thank the Chilean Navy
418 (especially the captain and crew of the ships, Almirante Oscar Viel and Lautaro), the staff
419 from the Chilean Army in the O'Higgins base and the Air Force of Chile (FACh) for the
420 logistic support of our fieldwork in sub-Antarctica and Antarctica.

421

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
426 Peninsula Coastal Current, ASF: Antarctic Slope Front, and CC: cyclonic circulation inside
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

433 Island (GEO), Greenwich Island (PRA), O'Higgins (OHI), Paradise Bay (PAR), and Marguerite
434 Bay (MAR). B) Study models: *Curdiea racovitzae* (1), *Plocamium cartilagineum* (2), *Iridaea*
435 *cordata* (3), *Palmaria decipiens* (4), *Georgiella confluens* (5), and *Gigartina skottsbergii* (6).
436 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.
445 The five localities correspond, from north to south, to King George Island (GEO),
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.
449
450
451 REFERENCES
452 Allcock AL, Barratt I, Eleaume M, Linse K, Norman MD, Smith PJ, Steinke D, Stevens DW,
453 Strugnell JM (2011) Cryptic speciation and the circumpolarity debate: A case study

454 on endemic Southern Ocean octopuses using the COI barcode of life. Deep-Sea Res
455 II 58:242–249
456 Allcock AL, Strugnell JM (2012) Southern Ocean diversity: new paradigms from molecular
457 ecology. Trends Ecol Evol 27:520–528
458 Amsler CD, McClintock JB, Baker BJ (2014) Chemical mediation of mutualistic interactions
459 between macroalgae and mesograzers structure unique coastal communities along
460 the western Antarctic Peninsula. J Phycol 50:1–10
461 Anderson JB, Shipp SS, Lowe AL, Wellner JS, Mosola AB (2002) The Antarctic ice sheet
462 during the last glacial maximum and its subsequent retreat history: a review. Quat
463 Sci Rev 21:49–70
464 April J, Hanner RH, Dion-Côté AM, Bernatchez L (2013) Glacial cycles as an allopatric
465 speciation pump in north-eastern American freshwater fishes. Mol Ecol 22:409–
466 422
467 Aris-Brosou S, Excoffier L (1996) The impact of population expansion and mutation rate
468 heterogeneity on DNA sequence polymorphism. Mol Biol Evol 13:494–504
469 Arntz WE (2005) The Magellan-Antarctic connection: links and frontiers at high latitudes.
470 Sci Mar 69:359–365
471 Avise JC (2000) Phylogeography: The History and Formation of Species. Harvard University
472 Press, Cambridge, USA
473 Bandelt HJ, Forster P, Röhl A (1999) Median-Joining networks for inferring intraspecific
474 phylogenies. Mol Biol Evol 16:37–48

475 Billard E, Reyes J, Mansilla A, Faugeron S, Guillemin M-L (2015) Deep genetic divergence
476 between austral populations of the red alga *Gigartina skottsbergii* reveals a cryptic
477 species endemic to the Antarctic continent. Polar Biol 38:2021–2034
478 Bortolotto E, Bucklin A, Mezzavilla M, Zane L, Patarnello T (2011) Gone with the currents:
479 lack of genetic differentiation at the circum-continental scale in the Antarctic krill
480 *Euphausia superba*. BMC Genetics 12:32
481 Clarke A, Crame JA, Stromberg JO, Barker PF (1992) The Southern Ocean benthic fauna
482 and climate change: a historical perspective [and discussion]. Philos Trans R Soc
483 Lond Ser B Biol Sci 338:299–309
484 Clarke A, Crame JA (2010) Evolutionary dynamics at high latitudes: speciation and
485 extinction in polar marine faunas. Philos Trans R Soc Lond Ser B Biol Sci 365:3655–
486 3666
487 Cofaigh CÓ, Davies BJ, Livingstone SJ, Smith JA, Johnson et al. (2014) Reconstruction of ice-
488 sheet changes in the Antarctic Peninsula since the Last Glacial Maximum. Quat Sci
489 Rev 100:87–110
490 Convey P, Stevens M, Hodgson D, Smellie J, Hillenbrand C, Barnes D, Clarke A, Pugh P,
491 Linse K, Cary S (2009) Exploring biological constraints on the glacial history of
492 Antarctica. Quat Sci Rev 28:3035–3048
493 Dornburg A, Federman S, Eytan RI, Near TJ (2016) Cryptic species diversity in sub-Antarctic
494 islands: A case study of *Lepidonotothen*. Mol Phyl Evol 104:32–43
495 Dowling DK, Friberg U, Lindell J (2008) Evolutionary implications of non-neutral
496 mitochondrial genetic variation. Trends Ecol Evol 23:546–554

497 Durand JD, Blel H, Shen KN, Koutrakis ET, Guinand B (2013) Population genetic structure of
498 *Mugil cephalus* in the Mediterranean and Black Seas: a single mitochondrial clade
499 and many nuclear barriers. Mar Ecol Prog Ser 474:243–261
500 Durrant H, Burridge CP, Kelaher BP, Barrett NS, Edgar GJ, Coleman MA (2014) Implications
501 of macroalgal isolation by distance for networks of marine protected areas.
502 Conserv Biol 28:438–445
503 Excoffier E, Ray N (2008) Surfing during population expansions promotes genetic
504 revolutions and structuration. Trends Ecol Evol 23:347–351
505 Excoffier L, Lisher H (2010) Arlequin suite ver 3.5: A new series of programs to perform
506 population genetics analyses under Linux and Windows. Mol Ecol Resour 10:56
507 Fahrbach E (2006) The Expedition ANTARKTIS-XXII/3 of the Research Vessel "*Polarstern*" in
508 2005 (Ed). Ber Polar Meeresforsch (Rep Polar Mar Res) 533:1–246
509 Faugeron S, Valero M, Destombe C, Martínez EA, Correa JA (2001) Hierarchical spatial
510 structure and discriminant analysis of genetic diversity in the red alga *Mazzaella*
511 *laminarioides* (Gigartinales, Rhodophyta). J Phycol 37:705–716
512 Fraser CI, Spencer HG, Waters JM (2009) Glacial oceanographic contrasts explain
513 phylogeography of Australian bull kelp. Mol Ecol 18:2287–2296
514 Fraser CI, Nikula R, Ruzzante DE, Waters JM (2012) Poleward bound: biological impacts of
515 Southern Hemisphere glaciation. Trends Ecol Evol 27:462–471
516 Fraser CI, Zuccarello GC, Spencer HG, Salvatore LC, Garcia GR, Waters JM (2013) Genetic
517 affinities between trans-oceanic populations of non-buoyant macroalgae in the
518 high latitudes of the Southern Hemisphere. PLoS One 8:e69138

519 Fraser CI, Terauds A, Smellie J, Convey P, Chown SL (2014) Geothermal activity helps life
520 survive glacial cycles. Proc R Soc B 111:5634–5639
521 Fraser CI (2016) Change in Southern Hemisphere intertidal communities through climate
522 cycles: the role of dispersing algae. In Hu Z-M and Fraser C (eds) Seaweed
523 Phylogeography. Springer, Netherlands
524 Fu YX (1997) Statistical tests of neutrality of mutations against population growth,
525 hitchhiking and background selection. Genetics 147:915–925
526 González-Wevar CA, David B, Poulin E (2011) Phylogeography and demographic inference
527 in *Nacella* (Patinigera) *concinna* (Strebel, 1908) in the western Antarctic Peninsula.
528 Deep-Sea Res II 58:220–229
529 González-Wevar CA, Chown SL, Morley S, Coria N, Saucéde T, Poulin E (2016) Out of
530 Antarctica: quaternary colonization of sub-Antarctic Marion Island by the limpet
531 genus *Nacella* (Patellogastropoda: Nacellidae). Polar Biol 39:77–89
532 Goodall-Copestake WP, Perez-Espona S, Clark MS, Murphy EJ, Seear PJ, Tarling GA (2010)
533 Swarms of diversity at the gene cox1 in Antarctic krill. Heredity 104:513–518
534 Gutt J (2001) On the direct impact of ice on marine benthic communities, a review. Polar
535 Biol 24:553–564
536 Haffer J (1969) Speciation in Amazonian forest birds. Science 165:131–137
537 Harpending HC (1994) Signature of ancient population growth in a low-resolution
538 mitochondrial DNA mismatch distribution. Hum Biol 66:591–600
539 Hewitt GM (2004) Genetic consequences of climatic oscillations in the Quaternary. Philos
540 Trans R Soc Lond Ser B Biol Sci 359:183–195

541 Ho S, Lanfear R, Bromham, Phillips LMJ, Soubrier J, Rodrigo AG, Cooper A (2011) Time-
542 dependent rates of molecular evolution. Mol Ecol 20:3087–3101
543 Hoffman JI, Clarke A, Linse K, Peck LS (2011) Effects of brooding and broadcasting
544 reproductive modes on the population genetic structure of two Antarctic
545 gastropod molluscs. Mar Biol 158:287–296
546 Hommersand MH, Moe RL, Amsler CD, Fredericq S (2009) Notes on the systematics and
547 biogeographical relationships of Antarctic and sub-Antarctic Rhodophyta with
548 descriptions of four new genera and five new species. Bot Mar 52: 509–534
549 Hunter RL, Halanych KM (2008) Evaluating connectivity in the brooding brittle star
550 *Astrotoma agassizii* across the drake passage in the Southern Ocean. J Hered
551 99:137–148
552 Janosik AM, Halanych KM (2010) Unrecognized Antarctic biodiversity: a case study of the
553 genus *Odontaster* (Odontasteridae; Asteroidea). Integr Comp Biol 50:981–992
554 Janosik AM, Mahon AR, Halanych KM (2011) Evolutionary history of Southern Ocean
555 *Odontaster* sea star species (Odontasteridae; Asteroidea). Polar Biol 34:575–586
556 Kinlan BP, Gaines SD (2003) Propagule dispersal in marine and terrestrial environments: a
557 community perspective. Ecology 84:2007–2020
558 Knapp S, Mallet J (2003) Refuting refugia? Science 300:71–72
559 Macaya EC, Zuccarello GC (2010) Genetic structure of the giant kelp *Macrocystis pyrifera*
560 along the southeastern Pacific. Mar Ecol Prog Ser 420:103–112
561 Macaya EC, López B, Tala F, Tellier F, Thiel M (2016) Float and raft: role of buoyant
562 seaweeds in the phylogeography and genetic structure of non-buoyant associated

563 flora. In Hu Z-M and Fraser C (eds) Seaweed Phylogeography. Springer,
564 Netherlands
565 Mahon AR, Arango CP, Halanych KM (2008) Genetic diversity of *Nymphon* (Arthropoda:
566 Pycnogonida: Nymphonidae) along the Antarctic Peninsula with a focus on
567 *Nymphon australe* Hodgson 1902. Mar Biol 155:315–323
568 Moffat C, Beardsley RC, Owen B, van Lipzig N (2008) A first description of the Antarctic
569 Peninsula Coastal Current. Deep-Sea Res II 55:277–293
570 Montecinos A, Broitman BR, Faugeron S, Haye PA, Tellier F, Guillemin ML (2012) Species
571 replacement along a linear coastal habitat: phylogeography and speciation in the
572 red alga *Mazzaella laminarioides* along the south east pacific. BMC Evol Biol 12:17
573 Muangmai N, West JA, Zuccarello GC (2014) Evolution of four Southern Hemisphere
574 *Bostrychia* (Rhodomelaceae, Rhodophyta) species: phylogeny, species delimitation
575 and divergence times. Phycologia 53:593–601
576 Neiva J, Pearson G, Valero M, Serrao E (2010) Surfing the wave on a borrowed board:
577 range expansion and spread of introgressed organellar genomes in the seaweed
578 *Fucus ceranoides* L. Mol Ecol 19:4812–4822
579 Neiva J, Pearson GA, Valero M, Serrao EA (2012) Fine-scale genetic breaks driven by
580 historical range dynamics and ongoing density-barrier effects in the estuarine
581 seaweed *Fucus ceranoides* L. BMC Evol Biol 12:78
582 Norris RD, Hull PM (2012) The temporal dimension of marine speciation. Evol Ecol 26:393–
583 415
584 O'Loughlin MP, Paulay G, Davey N, Michonneau F (2011) The Antarctic region as a marine

biodiversity hotspot for echinoderms: diversity and diversification of sea cucumbers. Deep Sea Res Part II 58:264–275

Payo DA, Leliaert F, Verbruggen H, D'hondt S, Calumpong HP, De Clerck O (2013) Extensive cryptic species diversity and fine-scale endemism in the marine red alga *Portieri*a in the Philippines. Proc R Soc B 280:20122660

Pellizzari F, Silva MC, Silva EM, Medeiros A, Oliveira MC, Yokoya NS, Pupo D, Rosa LH, Colepicolo P (2017) Diversity and spatial distribution of seaweeds in the South Shetland Islands, Antarctica: an updated database for environmental monitoring under climate change scenarios. Polar Biol doi:10.1007/s00300-017-2092-5

Quartino ML, Deregibus D, Campana GL, Latorre GEJ, Momo FR (2013) Evidence of macroalgal colonization on newly ice-free areas following glacial retreat in Potter Cove (South Shetland Islands), Antarctica. PLoS One 8:e58223

Raupach MJ, Thatje S, Dambach J, Rehm P, Misof B, Leese F (2010) Genetic homogeneity and circum-Antarctic distribution of two benthic shrimp species of the Southern Ocean, *Chorismus antarcticus* and *Nematocarcinus lanceopes*. Mar Biol 157:1783–1797

Riesgo A, Taboada S, Avila C (2015) Evolutionary patterns in Antarctic marine invertebrates: an update on molecular studies. Mar Genom 23:1–13

Roger AR, Harpending H (1992) Population growth makes waves in the distribution of pairwise genetic differences. Mol Biol Evol 9:552–569

Rozas J, Sánchez-DeI Barrio JC, Messeguer X, Rozas R (2003) DnaSP DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496–2497

607 Saunders GW (2005) Applying DNA barcoding to red macroalgae: a preliminary appraisal
608 holds promise for future applications. Philos Trans R Soc Lond Ser B Biol Sci
609 360:1879–1888
610 Savidge DK, Amft JA (2009) Circulation on the West Antarctic Peninsula derived from 6
611 years of shipboard ADCP transects. Deep-Sea Res I 56:1633–1655
612 Schoville SD, Roderick GK, Kavanaugh DH (2012) Testing the 'Pleistocene species pump' in
613 alpine habitats: lineage diversification of flightless ground beetles (Coleoptera:
614 Carabidae: *Nebria*) in relation to altitudinal zonation. Biol J Linn Soc 107:95–111
615 Simms AR, Milliken KT, Anderson JB, Wellner JS (2011) The marine record of deglaciation
616 of the South Shetland Islands, Antarctica since the Last Glacial Maximum. Quat Sci
617 Rev 30:1583-1601
618 Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA
619 polymorphism. Genetics 123:585–595
620 Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular
621 Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance,
622 and Maximum Parsimony Methods. Mol Biol Evol 28: 2731–2739
623 Thatje S, Hillenbrand C-D, Larter R (2005) On the origin of Antarctic marine benthic
624 community structure. Trends Ecol Evol 20:534–540
625 Thatje S, Hillenbrand CD, Mackensen A, Larter R (2008) Life hung by a thread: endurance
626 of Antarctic fauna in glacial periods. Ecology 89:682–692

627 Thornhill DJ, Mahon AR, Norenburg JL, Halanych KM (2008) Open-ocean barriers to
628 dispersal: a test case with the Antarctic Polar Front and the ribbon worm
629 *Parborlasia corrugatus* (Nemertea: Lineidae). Mol Ecol 17:5104–5117
630 Valero M, Destombe C, Mauger S, Ribout C, Engel CR, Daguin-Thiebaut C, Tellier F (2011)
631 Using genetic tools for sustainable management of kelps: a literature review and
632 the example of *Laminaria digitata*. Cah Biol Mar 52:467
633 Verheye ML, Backeljau T, d'Acoz CDU (2016) Looking beneath the tip of the iceberg:
634 diversification of the genus *Epimeria* on the Antarctic shelf (Crustacea,
635 Amphipoda). Polar Biol 39:925–945
636 Waters JM, Fraser CI, Hewitt GM (2013) Founder takes all: density-dependent processes
637 structure biodiversity. Trends Ecol Evol 28:78-85
638 Weir JT, Schluter D (2004) Ice sheets promote speciation in boreal birds. Proc R Soc B
639 271:1881–1887
640 Wiencke C, Clayton M (2002) Antarctic seaweeds. In: Wägele JW (ed) Synopsis of the
641 Antarctic Benthos. ARG Gantner, Ruggell
642 Wiencke C, Amsler CD, Clayton MN (2014) Macroalgae. In: De Broyer C, Koubbi P, Griffiths
643 HJ, Raymond B, d'Udekem d'Acoz C, Van de Putte AP, Danis B, David B, Grant S,
644 Gutt J, Held C, Hosie G, Huettmann F, Post A, Ropert-Coudert Y (eds) Biogeographic
645 Atlas of the Southern Ocean. SCAR, Cambridge
646 Wilson NG, Hunter RL, Lockhart SJ, Halanych KM (2007) Multiple lineages and absence of
647 panmixia in the ''circumpolar'' crinoid *Promachocrinus kerguelensis* from the
648 Atlantic sector of Antarctica. Mar Biol 152:895–904

649 Wilson NG, Schrodl M, Halanych KM (2009) Ocean barriers and glaciation: evidence for
650 explosive radiation of mitocondrial lineages in the Antarctic sea slug *Doris*
651 *kerguelenensis* (Mollusca, Nudibranchia). Mol Ecol 18:965–984
652
653

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

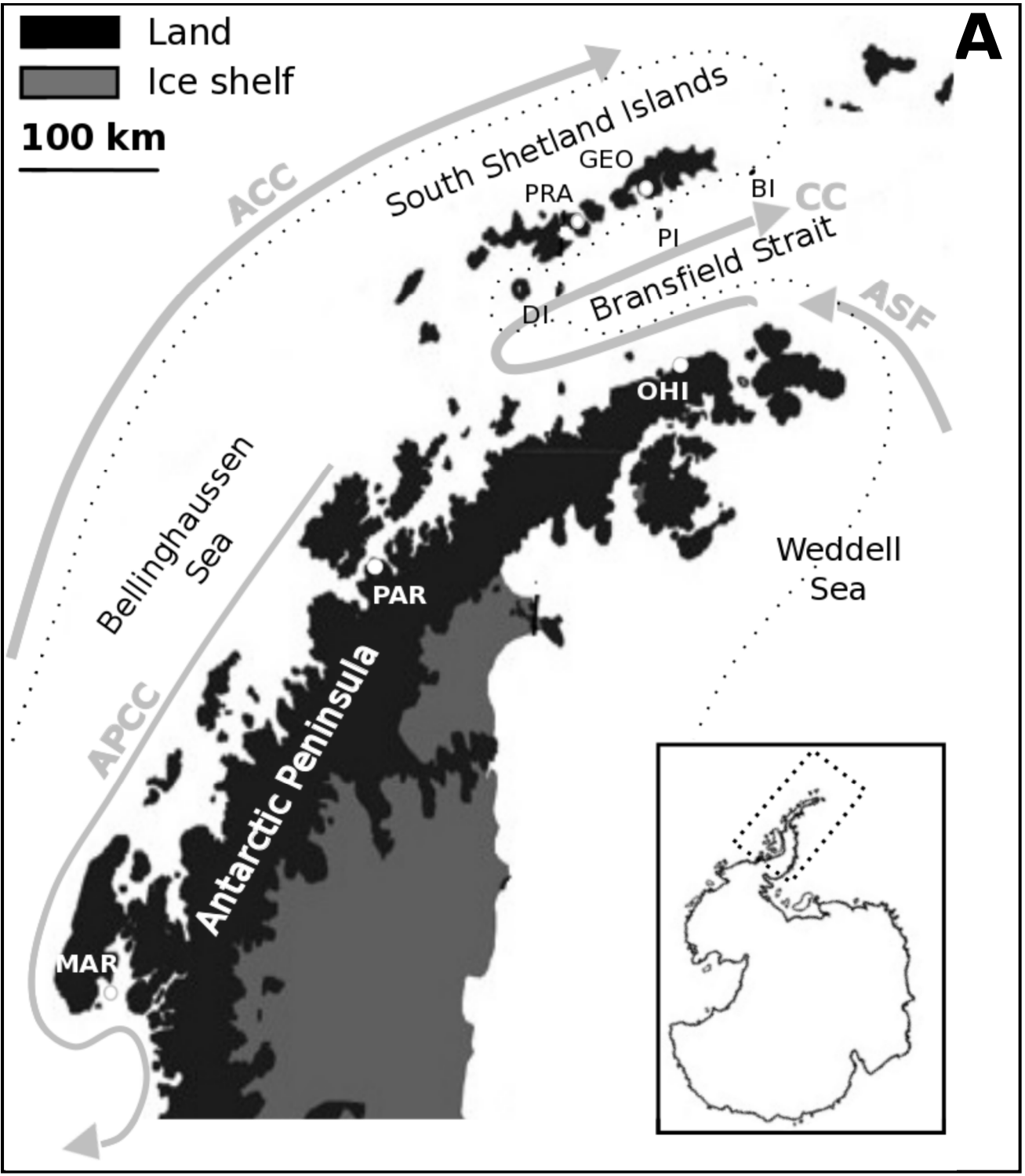
Species	**N**	**k**	**S**	**H**	**Π**	**π .10^{-2}**	**Tajima's D**	**Fu's FS**
Curdiea racovitzae	42	2	1	0.048	0.048	0.008	-1.120*	-1.491^{ns}
Georgiella confluens	20	4	4	0.363	0.489	0.077	-1.638*	-1.613^{ns}
Gigartina skottsbergii	28	2	1	0.071	0.071	0.011	-1.151^{ns}	-1.155^{ns}
Iridaea cordata	90	7	5	0.398	0.623	0.102	-0.797^{ns}	-2.882^{ns}
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**

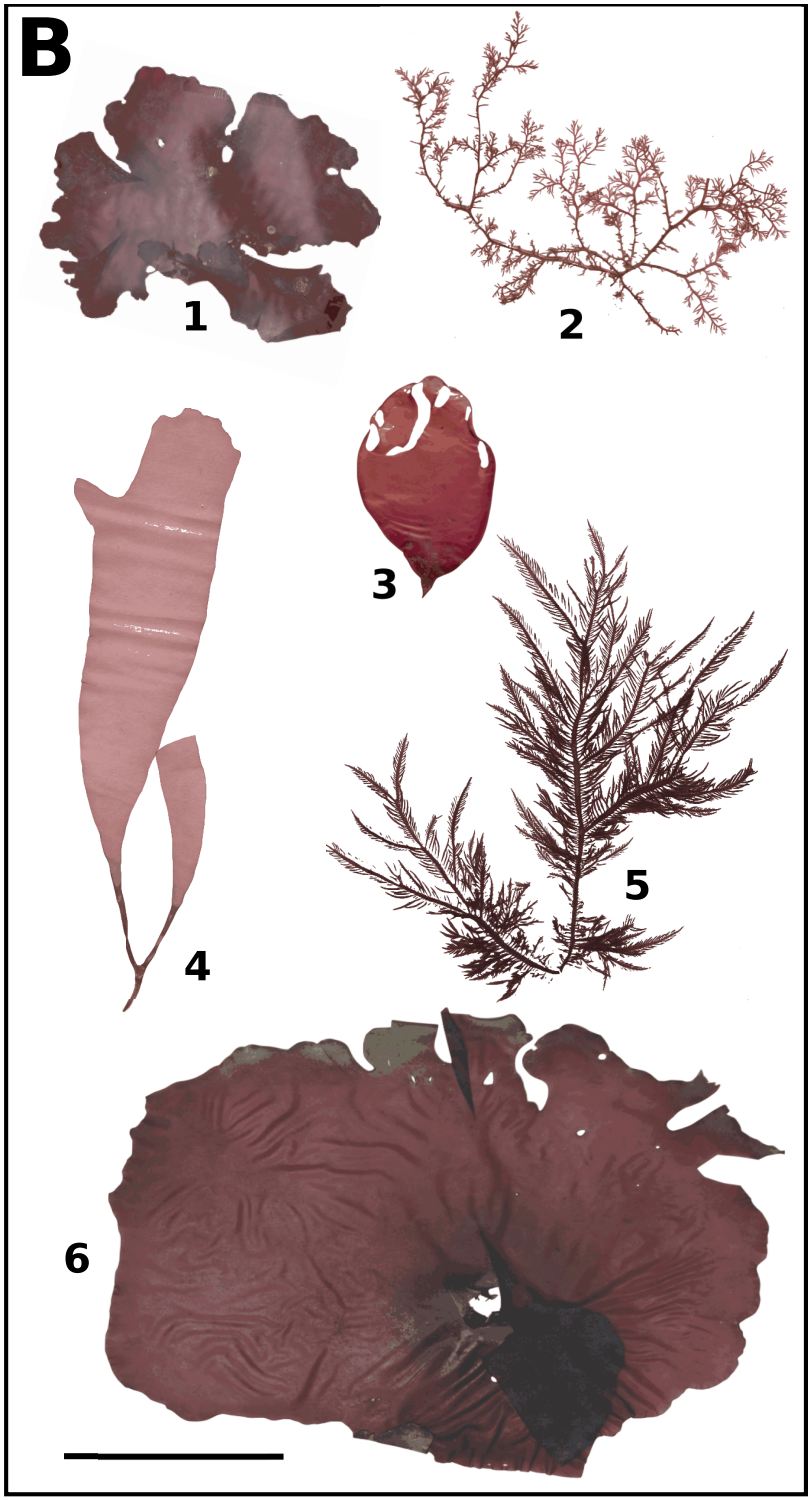
N: number of sampled specimens; k: number of haplotypes; S: polymorphic sites; H: haplotype diversity; Π: average number of nucleotide differences; π: nucleotide diversity. ns: non-significant; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

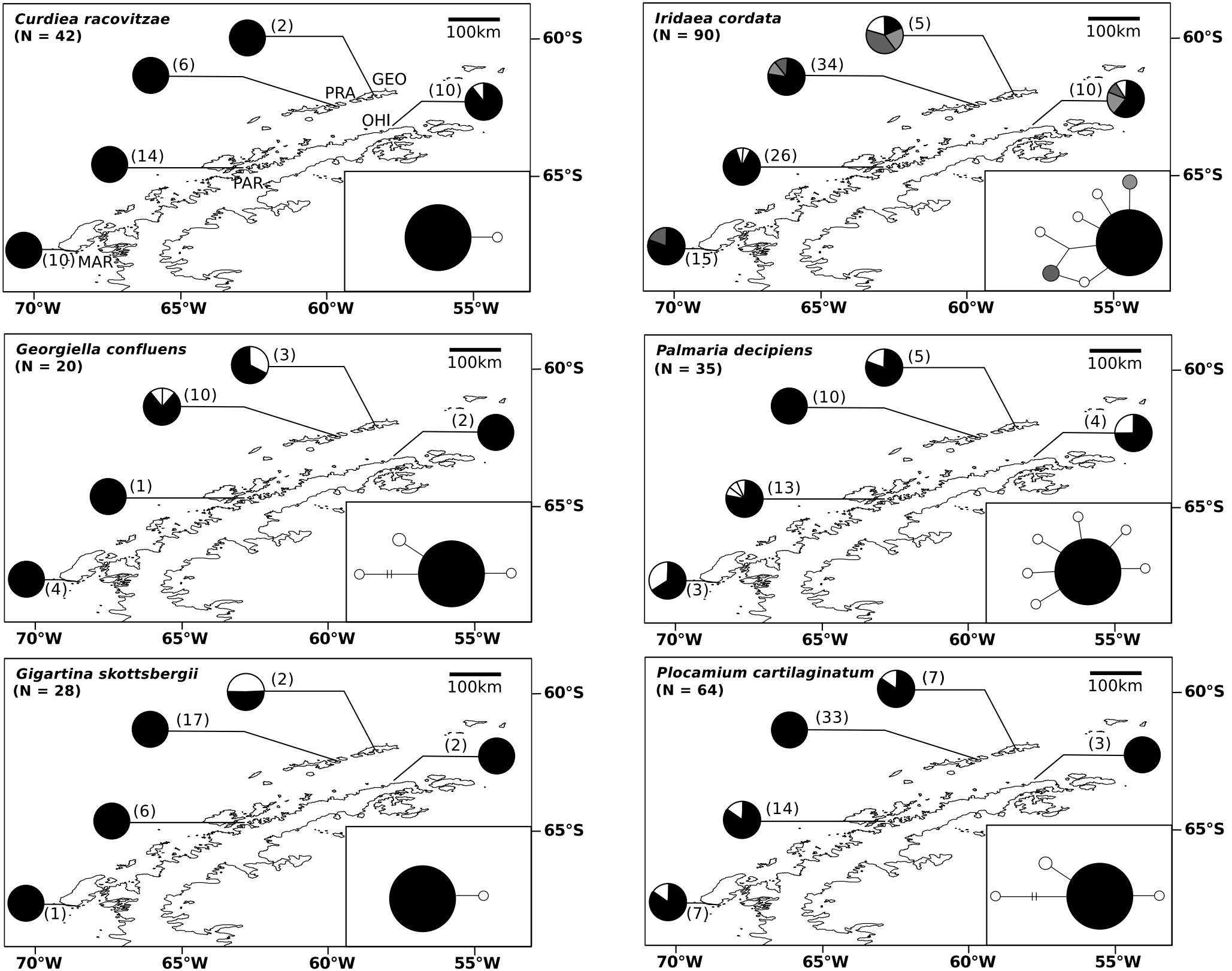
Table 2: Goodness of fit tests for a model of sudden expansion calculated for the sum of squared deviation (SSD) and the Harpending's raggedness index (Rag) and approximated time since the beginning of the most recent population expansion.

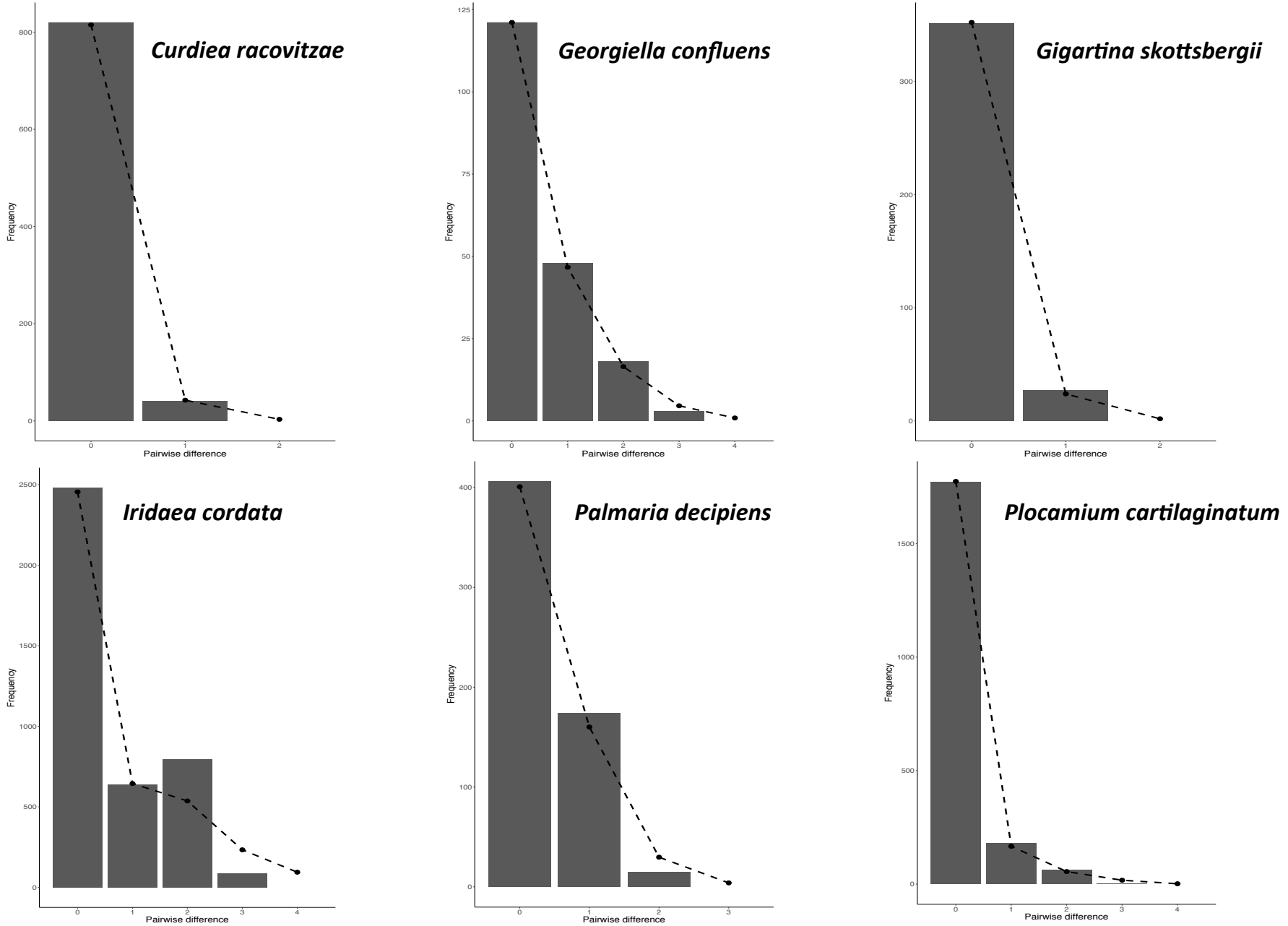
Species	**SSD**	***p* value**	**Rag**	***p* value**	**τ**	**(90% values)**	**Range estimation of times of expansion (years)**
Curdiea racovitzae	0.000	0.19	0.821	0.83	0.16	(0-0.636)	0-36,000
Georgiella confluens	0.000	0.74	0.179	0.77	0.48	(0-2.263)	0-128,000
Gigartina skottsbergii	0.000	0.22	0.740	0.80	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 (τ) and time of expansion see details in the text.


A
Land
Ice shelf
100 km
ACC
South Shetland Islands
GEO
PRA
BI
CC
PI
Bransfield Strait
DI
ASF
OHI
Bellinghaussen Sea
PAR
Weddell Sea
APCC
Antarctic Peninsula
MAR


B
1
2
3
4
5
6


Curdiea racovitzae
(N = 42)
100km
(2)
(6)
GEO
PRA
(10)
OHI
(14)
PAR
(10)
MAR
Iridaea cordata
(N = 90)
100km
(5)
(34)
(10)
(26)
(15)
Georgiella confluens
(N = 20)
100km
(3)
(10)
(2)
(1)
(4)
Palmaria decipiens
(N = 35)
100km
(5)
(10)
(4)
(13)
(3)
Gigartina skottsbergii
(N = 28)
100km
(2)
(17)
(2)
(6)
(1)
Plocamium cartilaginatum
(N = 64)
100km
(7)
(33)
(3)
(14)
(7)
60°S
65°S
70°W
65°W
60°W
55°W



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.