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

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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 450 km. 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

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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

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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, the 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 Box 1 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 the 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, 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 10 km; 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 complete absence of genetic structure over 1600 km of coast sampled in the Antarctic region (Billard et al. 2015). The authors suggest that genetic homogeneity of *G. skottbergii* has resulted from a strong demographic bottleneck during the last Quaternary glaciations (i.e., range contraction) followed by sudden recolonization during postglacial expansion.

In this study, we sought to determine the impact of habitat reduction during glacial periods on genetic diversity in six species of red macroalgae commonly encountered along the Antarctic Peninsula and the South Shetland Islands. Depending on the distribution of refugia during the Last Glacial Maximum, two different outcomes could be expected for these organisms: (1) if a single, small-sized refugium has acted as the origin of a recent 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 isolated in various disjoint refugia, this should lead to the observation of highly differentiated genetic lineages, each mainly restricted to a single location or geographic area (see Fig. 1 in Allcock and Strugnell 2012).

Materials and methods

Study models

All our six study models correspond to fleshy red macroalgae with thallus size varying from some centimeters

(e.g., *Iridaea cordata* (Turner) Bory) to up to several dozen centimeters (e.g., *Gigartina skottsbergii* Setchell & N.L. Gardner) in length and/or diameter (see Fig. 1b). None possesses 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* 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*,
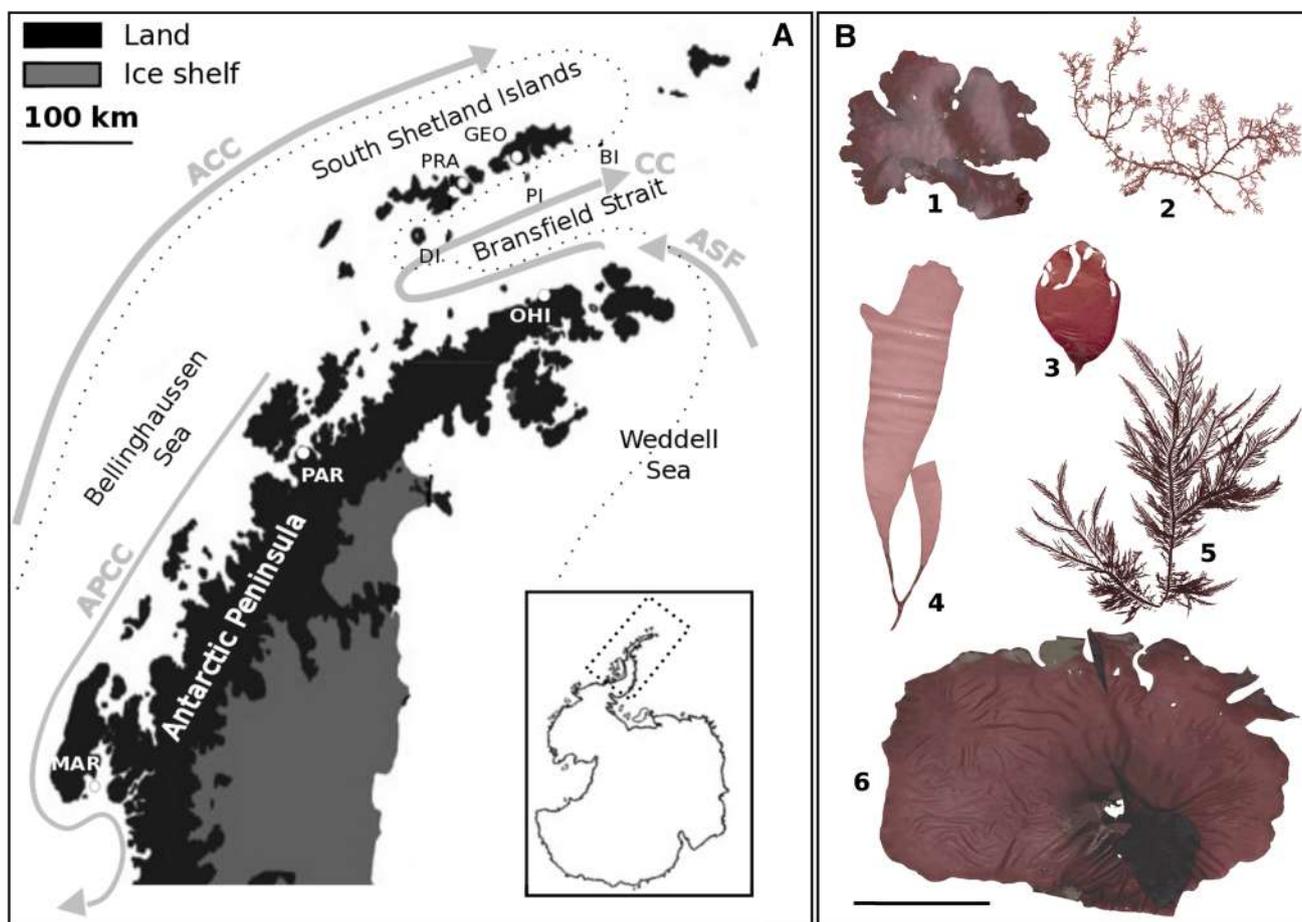
Fig. 1 Map of the study region and scans of herborized specimens of the six study models. **a** Map of the Antarctic Peninsula showing schematic paths of the main marine currents (synthesis from the data and maps presented in Mahon et al. 2008; Moffat et al. 2008; Savidge and Amft 2009). *ACC* Antarctic Circumpolar Current, *APCC* Antarctic Peninsula Coastal Current, *ASF* Antarctic Slope Front, and *CC* cyclonic circulation inside the Bransfield Strait. Lower arrow thickness represents currents only observed seasonally (i.e., APCC only during spring and summer; Moffat et al. 2008). The thin black dashed line corresponds to reconstructions of putative position of Antarctic Peninsula (Cofaigh et al. 2014) and South Shetland (Simms et al. 2011) ice caps during the Last Glacial Maximum. Volcanic areas active during the last 4 Myr include Deception Island (DI), Penguin Island (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

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., the growth of *G. confluens*, *P. cartilagineum*, and *I. cordata* occurs between 0 °C and 5 °C, with an upper survival temperature of 11–16 °C; Wiencke et al. 2014).

Sampling

For *G. confluens*, *G. skottsbergii*, and *P. cartilagineum*, samples were collected by scuba diving from shallow subtidal zones down to 30 m. Samples of *C. racovitzae*, *I. cordata*, and *P. decipiens* were obtained from both intertidal rock pools and the shallow subtidal (i.e., mostly at depth of 0 to 15 m). In order to sample individuals corresponding to distinct genotypes and coming from sexual reproduction and through spore settlement, only one thallus sample was taken from each macroalgal holdfast, and all holdfasts were sampled well separated on the rocky subtract. Samples were collected in five distinct areas (see Fig. 1a), two located in the South Shetland Islands (GEO: King George Island, 62°12′S/58°57′W and PRA: Greenwich Island, 62°28′S/59°40′W) and three located along 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 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 de Chile and are available from the contact author on request. In total, 42 specimens were sampled for *C. racovitzae*, 20 for *G. confluens*, 28 for *G. skottsbergii*, 90 for *I. cordata*, 35 for *P. decipiens,* and 64 for *P. cartilagineum* (Table 1; Fig. 2).

DNA extraction, PCR amplification, and sequencing

DNA extraction was undertaken according to the methods described in Faugeron et al. (2001). DNA amplification of the 5′ part of the mitochondrial Cytochrome c Oxidase I gene (COI) was done following the amplification protocols of Saunders (2005) using the primer pair GazF1 (5′-TCA ACA AAT CAT AAA GAT ATT GG-3′) and GazR1 (5′-ACT TCT GGA TGT CCA AAA AAY CA-3′). Purified PCR products (UltraCleanTM kit, MO BIO Laboratories, Carlsbad, USA) were sequenced (Macrogen Inc., Seoul, South Korea). The 279 generated COI sequences (632 bp) have been archived with the GENBANK accession numbers KY559671-KY559712, KY559727-KY559746, KY559753-KY559780, KY559817-KY559905, KY559935-KY559947, KY559949-KY559970, KY560012-KY560065, and KY560067-KY560076. For each accession, information about the region, sampling locality, and voucher code (i.e., given under the "organism" category) has been provided. Sequences were aligned using the CLUSTAL function of MEGA v.5 (Tamura et al. 2011).

Data analysis

For each species, levels of polymorphism over the whole sampled area were calculated using the following standard genetic diversity indices: number of sampled haplotypes (k), number of polymorphic sites (S), haplotype diversity (H), average number of pairwise differences (Π), and nucleotide diversity (π). These indices were calculated using DnaSP v.4 (Rozas et al. 2003). Haplotype networks were reconstructed using the median joining algorithm implemented in NET-WORK v.4 (Bandelt et al. 1999). Haplotype frequencies, for each species in each area, were calculated using DnaSP v.4 (Rozas et al. 2003).

Three tests were performed to assess whether each species is at mutation–drift equilibrium or if there was a signature of post-glacial recent expansion. Tajima's D (Tajima 1989) and Fu's FS (Fu 1997) neutrality tests were conducted using DnaSP v.4 (Rozas et al. 2003). Significance for these

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	$\pi \cdot 10^{-2}$	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* nonsignificant. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$
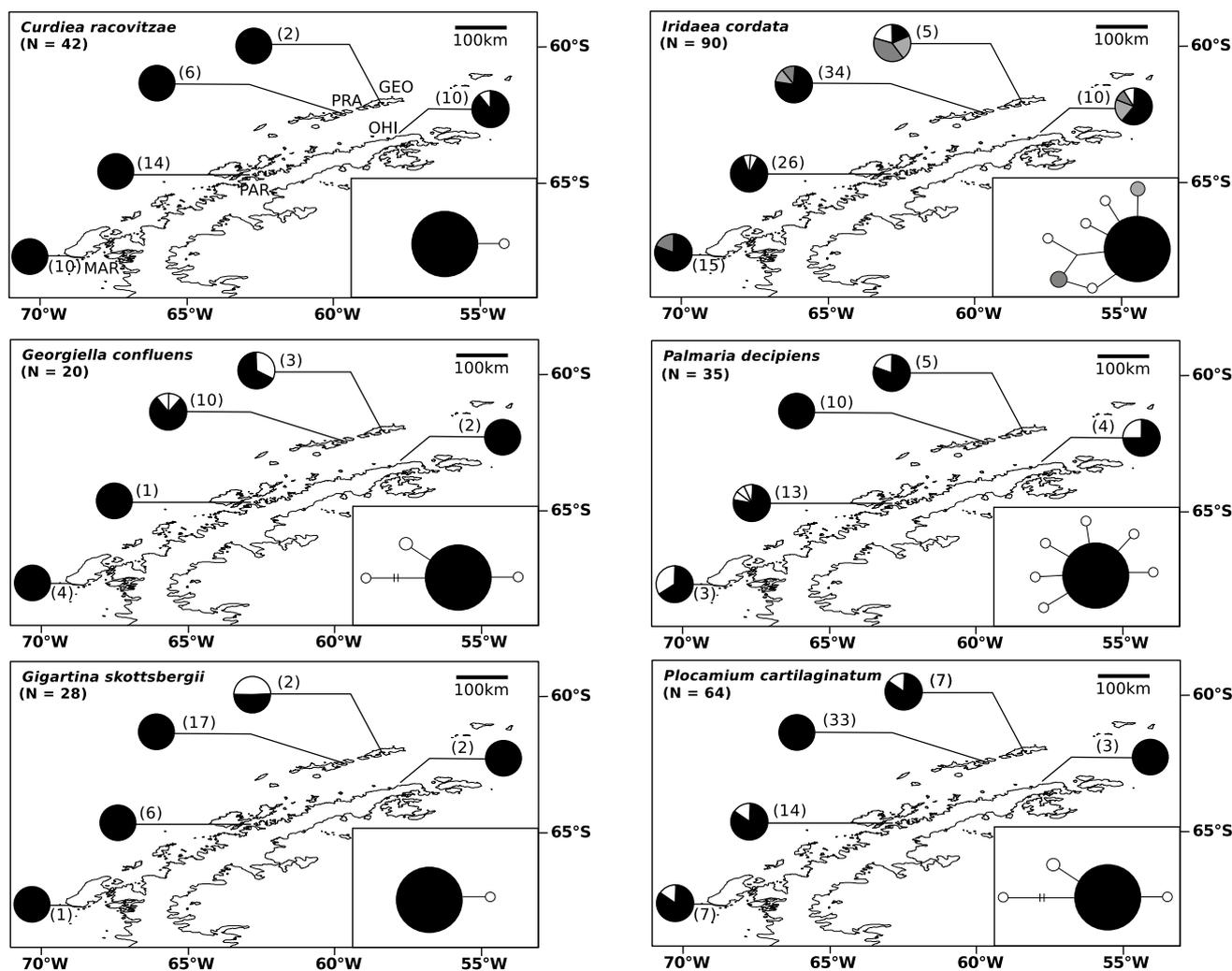
Fig. 2 Haplotype networks and pie charts showing the geographical distribution of haplotypes for the genetic marker COI in six macroalgae in the western Antarctic Peninsula area. Haplotype networks are given within boxes in the lower right corner of each graph. In the networks, each circle represents a haplotype and its size is proportional to the frequency in which the haplotype was encountered. For haplotypes separated by more than one mutational step, the number of steps is indicated by small lines. Black and gray haplotypes are shared between localities, while white ones are private haplotypes. The five localities correspond, from north to south, to King George Island (GEO), Greenwich Island (PRA), O'Higgins (OHI), Paradise Bay (PAR), and Marguerite Bay (MAR) (localization given in the *Curdiea racovitzae* map on the upper left; see also Fig. 1a). For each species, the number of sequenced individuals is given between brackets

two neutrality tests was obtained by simulating 1000 samples using the coalescent approach developed in DnaSP v.4 (Rozas et al. 2003). Negative and significant values for Tajima's D and Fu's FS neutrality tests reflect an excess of rare polymorphisms in a population, which indicates either positive selection or a recent increase in population size (Aris-Brosou and Excoffier 1996). Moreover, we tested the frequency distribution of pairwise differences between haplotypes (i.e., mismatch analysis) against a model of sudden expansion (Roger and Harpending 1992). This was done using 1000 replicates in the program ARLEQUIN v.3.5 (Excoffier and Lisher 2010). The goodness-of-fit between the observed and estimated distributions was assessed by calculating the sum of squared differences (SSD) between the observed and expected distributions and the Harpending's raggedness index (Rag) (Harpending 1994). Calculations were performed in ARLEQUIN v.3.5 (Excoffier and Lisher 2010), and significance was assessed by bootstrapping (1000 replicates).

We approximated the timing since the beginning of the most recent population expansion using the equation $\tau = 2 ut$, where t is the number of years since expansion and u is the per-sequence-per-year mutation rate. The parameter Tau (τ) and 90% percentile values of τ were inferred directly

from the model of sudden expansion in ARLEQUIN v.3.5 (Excoffier and Lisher 2010). Since no precise molecular clock exists for the COI in red algae, we used as an approximation of the divergence rate of 0.14% per Myr published by Muangmai et al. (2014). These authors have computed this rough estimate for COI of the genus *Bostrychia* using two samples of *Bostrychia calliptera* collected from both sides of the Isthmus of Panama, considering 2.5–3.0 Myr ago as the final closure date of the Isthmus. Since substitution rates are usually much lower than mutation rates (i.e., because natural selection tends to remove deleterious mutations), we applied the tenfold evolutionary rate correction for intraspecies time dependence of molecular rates as 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).

Results

Over the whole sampled area (i.e., more than 450 km of sampled coast, Fig. 1a), all six red macroalgae showed very low levels of mtDNA genetic diversity (Fig. 2). Depending on the species under study, one to six polymorphic sites were detected (Table 1). The number of observed haplotypes varied from two in *C. racovitzae* and *G. skottsbergii* to seven in *I. cordata* and *P. decipiens*. The highest values of mtDNA diversity were encountered in *I. cordata* (haplotype diversity = 0.398, average number of pairwise differences = 0.623, and nucleotide diversity = 0.001), the species with the largest sample size (N = 90; Table 1). Simple, starlike haplotype network topologies were observed for each species (Fig. 2). For all species, the central haplotype was the most frequent (from 77% of the samples in *I. cordata* to 98% of the samples in *C. racovitzae*) and was present in all localities (Fig. 2), while the remaining haplotypes were rare and private (i.e., haplotype confined to a single geographic locality). One exception to this was the *I. cordata* network (Fig. 2). For this species, two haplotypes (i.e., shaded in gray in Fig. 2) showed intermediate frequency (11 and 8%, respectively) and were also widely distributed in most sampled areas. These haplotypes were related to the most common *I. cordata* haplotype through branch length of at most two mutational steps (Fig. 2).

In all studied species, both Tajima's D and Fu's Fs tests gave negative values (Table 1). Associated probabilities were significant in *C. racovitzae*, *G. confluens*, *P. decipiens,* and *P. cartilagineum* for the Tajima's D test, and in *P. decipiens* and *P. cartilagineum* for the Fu's Fs test (Table 1). Congruent with these findings and the fact that the majority of the individuals within each species shared the same haplotype, distributions of pairwise differences between sequence pairs were L-shaped for all six species (Online Resource 1). From the results of the goodness-of-fit tests, the null hypothesis of sudden expansion could not be rejected for the six species tested (Table 2; all *p* values for Expected SSD > Observed SSD and Expected Rag > Observed Rag were much higher than 0.05). The population expansion was estimated to have begun during the late Quaternary. Times of expansion were estimated to range from 0–36,000 years in *C. racovitzae* to 0–240,000 years in *I. cordata* (Table 2).

Discussion

Throughout the South Shetland Islands and the Antarctic Peninsula, no differentiated genetic groups were observed within the six species studied here. On the contrary, all six red macroalgae were characterized by very low genetic diversity and a significant signature of recent population expansion of a single haplotype encountered over more than 450 km. Some limitations of our study, such as the low number of samples available 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 entirety of the Antarctic coast, or the fact that only one gene was sequenced (but see Billard et al. 2015, the same results observed using two different markers, more samples, and more sample locations for *G.*

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

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), and they are organisms that typically have restricted belt-like distributions along the coast. Differing then from pelagic or broad depth-ranging benthic animals threatened by ice scour in Antarctica (Allcock and Strugnell 2012), macroalgae cannot use refugia located in the deepest parts of the continental shelf. Indeed, a complete or nearly complete eradication of macroalgal populations from the sub-Antarctic and Antarctic coasts during the Last Glacial Maximum has been inferred based on molecular data in the Southern hemisphere (Macaya and Zuccarello 2010; Fraser et al. 2012; Montecinos et al. 2012; Billard et al. 2015), and the impact of ice scouring has been deemed to be particularly important in Antarctica during this period (Thatje et al. 2005). Due to their ecological and life cycle characteristics, the dramatic impact of ice scour on Antarctic macroalgal genetic diversity is not surprising. In our study, the very low level of genetic diversity and the large-scale spread of common genetic variants prohibit us from precisely pinpointing the location of the glacial refugium of Antarctic macroalgae.

Recent studies have estimated that the Last Glacial Maximum ice cap was thick and extended as far as the shelf edge around the South Shetland Islands and the Antarctic Peninsula (Simms et al. 2011; Cofaigh et al. 2014). The ice sheet retreat began earlier in the Northern part of the region, and it is very likely that Marguerite Bay, where grounded ice was hypothesized to have existed up to 14,000 years ago, is a recently colonized area (Cofaigh et al. 2014). Interestingly, the Last Glacial Maximum ice coverage proposed by Simms et al. (2011) does not include Deception Island, the largest and most active volcano in the area (see Fig. 1a). It is possible that volcanoes, like Deception Island, Penguin Island, and Bridgeman Island, have been active during the last 4 Myr and remained free of ice during glacial periods (Simms et al. 2011; Fig. 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 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).

In this study, the geographic spread of haplotypes of six species was determined for populations inhabiting more than 450 km of coast. Haplotypes distributed over hundreds or even thousands of kilometers has been reported in various pelagic and benthic species with long-lived pelagic larvae inhabiting the Southern Ocean (Thornhill et al. 2008; Raupach et al. 2010; Bortolotto et al. 2011; González-Wevar et al. 2011; Janosik et al. 2011). The wide-ranging distribution of haplotypes was attributed to the impact of strong oceanic currents and to a high degree of connectivity. However, none of the six macroalgae under study has floating structures, and their dispersal capacity should be very limited. The western Antarctic Peninsula represents a highly fragmented and isolated habitat for coastal marine species since ice-free rocky shores are rare and separated from each other by extensive areas covered by thick ice sheets, strong currents, and steep variation in shelf topography (e.g., Bransfield Strait). These physical properties likely limit gene flow between coastal populations, especially for organisms with low dispersal potential, such as macroalgae that lack buoyant structures (Hoffman et al. 2011). Nevertheless, extreme mtDNA monomorphism along the South Shetland Islands and the coasts of the Antarctic Peninsula has been observed in other species with limited dispersal ability (brooding invertebrates: the brittle star *Astrotoma agassizii*, Hunter and Halanych 2008; the sea spider *Nymphon australe*, Mahon et al. 2008; sea slug with direct development: *Doris kerguelenensis*, Wilson et al. 2009). For these species as well as for red macroalgae, the strong effect of genetic drift during population contraction in glacial refugia could have been amplified by gene surfing effects during spatial expansion, after ice sheet retreat; this, in turn, would lead to the rapid spread of a dominant haplotype in the recolonized region. Indeed, during interglacial recolonization, random sampling of haplotypes through successive founder events can result in the drastic reduction of genetic diversity; thus, a single haplotype can easily spread over vast geographic areas at expanding range margins (Excoffier and Ray 2008). An emblematic example of gene surfing in macroalgae is the case of the post-glacial, European northwards range expansion of introgressed organelle lineages of *Fucus vesiculosus* with the *F. ceranoides* nuclear gene pool (Neiva et al. 2010). Moreover, passive transport of detached fronds by ocean currents can explain how even macroalgae characterized by low autonomous dispersal ability can quickly colonize newly available habitats stripped bare by ice during the Last Glacial Maximum (Macaya et al. 2016). Rare events of long-distance colonization by rafting have been documented even among macroalgae lacking floating structures (Fraser et al. 2013; Fraser 2016; Macaya et al. 2016). Antarctic macroalgae fronds enclosed in drift ice have been observed at sea (Guillemin M-L pers. obs.), and these fronds can also be recovered far away from the coasts (e.g., frond of a *Desmarestia* sp. sampled at a 2500 m depth in the Weddell Sea more than 250 km away from the closest coast; Fahrbach 2006). In the particular case of recolonization of newly available substrates (e.g., recently deglaciated substrates), the installation of few migrants arriving 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 Fig. 1a) that likely played a role in the post-glacial 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; Fig. 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 (Fig. 1a). Moreover, the cyclonic circulation inside the Bransfield Strait and the Antarctic Peninsula Coastal Current (APCC) that connect the Bransfield Strait to Marguerite Bay during spring and summer could easily lead to recurring connectivity in our study region (Moffat et al. 2008; Savidge and Amft 2009; Fig. 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 Pliocene and Pleistocene glacial and interglacial cycles (Hommersand et al. 2009; Convey et al. 2009; Clarke and Crame 2010; Allcock and Strugnell 2012; Fraser et al. 2014; Billard et al. 2015). The strong water currents in the region likely played a prominent role in the rapid recolonization that took place at the end of the Quaternary. However, in the six macroalgae under study, the presence of one dominant haplotype over the whole studied area could simply be linked to the past glacial demographic history and might not necessarily imply the existence of actual homogenizing gene flow between the Antarctic Peninsula and the South Shetland Islands. Indeed, recent studies have reported strong dissimilarities between genetic diversity and structure encountered in marine species depending on the genetic markers used (e.g., in the flathead mullet; Durand et al. 2013). Differences in patterns of

genetic structure have been linked to the fact that organelle 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 to test for the existence of current gene flow between localities within the region, it is imperative to develop new nuclear markers (e.g., microsatellites or SNPs) in macroalgae species of interest. Since the common species of macroalgae in Antarctica grow in widely heterogeneous habitats in terms of salinity and turbidity (for example, see Savidge and Amft 2009; Quartino et al. 2013), it could also be of interest to test for the existence of local adaptation using recently developed genomics and transcriptomics tools (see Riesgo et al. 2015 for a review).

References

Allcock AL, Strugnell JM (2012) Southern Ocean diversity: new paradigms from molecular ecology. Trends Ecol Evol 27:520–528

Allcock AL, Barratt I, Eleaume M, Linse K, Norman MD, Smith PJ, Steinke D, Stevens DW, Strugnell JM (2011) Cryptic speciation and the circumpolarity debate: a case study on endemic Southern Ocean octopuses using the COI barcode of life. Deep-Sea Res II 58:242–249

Amsler CD, McClintock JB, Baker BJ (2014) Chemical mediation of mutualistic interactions between macroalgae and mesograzers structure unique coastal communities along the western Antarctic Peninsula. J Phycol 50:1–10

Anderson JB, Shipp SS, Lowe AL, Wellner JS, Mosola AB (2002) The Antarctic ice sheet during the last glacial maximum and its subsequent retreat history: a review. Quat Sci Rev 21:49–70

April J, Hanner RH, Dion-Côté AM, Bernatchez L (2013) Glacial cycles as an allopatric speciation pump in north-eastern American freshwater fishes. Mol Ecol 22:409–422

Aris-Brosou S, Excoffier L (1996) The impact of population expansion and mutation rate heterogeneity on DNA sequence polymorphism. Mol Biol Evol 13:494–504

Arntz WE (2005) The Magellan-Antarctic connection: links and frontiers at high latitudes. Sci Mar 69:359–365

Avise JC (2000) Phylogeography: The History and Formation of Species. Harvard University Press, Cambridge

Bandelt HJ, Forster P, Röhl A (1999) Median-Joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16:37–48

Billard E, Reyes J, Mansilla A, Faugeron S, Guillemin M-L (2015) Deep genetic divergence between austral populations of the red alga *Gigartina skottsbergii* reveals a cryptic species endemic to the Antarctic continent. Polar Biol 38:2021–2034

Bortolotto E, Bucklin A, Mezzavilla M, Zane L, Patarnello T (2011) Gone with the currents: lack of genetic differentiation at the circum-continental scale in the Antarctic krill *Euphausia superba*. BMC Genet 12:32

Clarke A, Crame JA (2010) Evolutionary dynamics at high latitudes: speciation and extinction in polar marine faunas. Philos Trans R Soc Lond Ser B Biol Sci 365:3655–3666

Clarke A, Crame JA, Stromberg JO, Barker PF (1992) The Southern Ocean benthic fauna and climate change: a historical perspective [and discussion]. Philos Trans R Soc Lond Ser B Biol Sci 338:299–309

Cofaigh CÓ, Davies BJ, Livingstone SJ, Smith JA, Johnson JS et al (2014) Reconstruction of ice-sheet changes in the Antarctic Peninsula since the Last Glacial Maximum. Quat Sci Rev 100:87–110

Convey P, Stevens M, Hodgson D, Smellie J, Hillenbrand C, Barnes D, Clarke A, Pugh P, Linse K, Cary S (2009) Exploring biological constraints on the glacial history of Antarctica. Quat Sci Rev 28:3035–3048

Dornburg A, Federman S, Eytan RI, Near TJ (2016) Cryptic species diversity in sub-Antarctic islands: a case study of *Lepidonotothen*. Mol Phyl Evol 104:32–43

Dowling DK, Friberg U, Lindell J (2008) Evolutionary implications of non-neutral mitochondrial genetic variation. Trends Ecol Evol 23:546–554

Durand JD, Blel H, Shen KN, Koutrakis ET, Guinand B (2013) Population genetic structure of *Mugil cephalus* in the Mediterranean and Black Seas: a single mitochondrial clade and many nuclear barriers. Mar Ecol Prog Ser 474:243–261

Durrant H, Burridge CP, Kelaher BP, Barrett NS, Edgar GJ, Coleman MA (2014) Implications of macroalgal isolation by distance for networks of marine protected areas. Conserv Biol 28:438–445

Excoffier L, Lisher H (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10:56

Excoffier E, Ray N (2008) Surfing during population expansions promotes genetic revolutions and structuration. Trends Ecol Evol 23:347–351

Fahrbach E (2006) The expedition ANTARKTIS-XXII/3 of the research vessel "*Polarstern*" in 2005 (Ed). Ber Polar Meeresforsch (Rep Polar Mar Res) 533:1–246

Faugeron S, Valero M, Destombe C, Martínez EA, Correa JA (2001) Hierarchical spatial structure and discriminant analysis of genetic diversity in the red alga *Mazzaella laminarioides* (Gigartinales, Rhodophyta). J Phycol 37:705–716

Fraser CI (2016) Change in Southern Hemisphere intertidal communities through climate cycles: the role of dispersing algae. In: Hu Z-M, Fraser C (eds) Seaweed Phylogeography. Springer, Netherlands

Fraser CI, Spencer HG, Waters JM (2009) Glacial oceanographic contrasts explain phylogeography of Australian bull kelp. Mol Ecol 18:2287–2296

Fraser CI, Nikula R, Ruzzante DE, Waters JM (2012) Poleward bound: biological impacts of Southern Hemisphere glaciation. Trends Ecol Evol 27:462–471

Fraser CI, Zuccarello GC, Spencer HG, Salvatore LC, Garcia GR, Waters JM (2013) Genetic affinities between trans-oceanic populations of non-buoyant macroalgae in the high latitudes of the Southern Hemisphere. PLoS ONE 8:e69138

Fraser CI, Terauds A, Smellie J, Convey P, Chown SL (2014) Geothermal activity helps life survive glacial cycles. Proc R Soc B 111:5634–5639

Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915–925

González-Wevar CA, David B, Poulin E (2011) Phylogeography and demographic inference in *Nacella* (Patinigera) *concinna* (Strebel, 1908) in the western Antarctic Peninsula. Deep-Sea Res II 58:220–229

González-Wevar CA, Chown SL, Morley S, Coria N, Saucéde T, Poulin E (2016) Out of Antarctica: quaternary colonization of sub-Antarctic Marion Island by the limpet genus *Nacella* (Patellogastropoda: Nacellidae). Polar Biol 39:77–89

Goodall-Copestake WP, Perez-Espona S, Clark MS, Murphy EJ, Seear PJ, Tarling GA (2010) Swarms of diversity at the gene cox1 in Antarctic krill. Heredity 104:513–518

Gutt J (2001) On the direct impact of ice on marine benthic communities, a review. Polar Biol 24:553–564

Haffer J (1969) Speciation in Amazonian forest birds. Science 165:131–137

Harpending HC (1994) Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. Hum Biol 66:591–600

Hewitt GM (2004) Genetic consequences of climatic oscillations in the Quaternary. Philos Trans R Soc Lond Ser B Biol Sci 359:183–195

Ho S, Lanfear R, Bromham Phillips LMJ, Soubrier J, Rodrigo AG, Cooper A (2011) Time-dependent rates of molecular evolution. Mol Ecol 20:3087–3101

Hoffman JI, Clarke A, Linse K, Peck LS (2011) Effects of brooding and broadcasting reproductive modes on the population genetic structure of two Antarctic gastropod molluscs. Mar Biol 158:287–296

Hommersand MH, Moe RL, Amsler CD, Fredericq S (2009) Notes on the systematics and biogeographical relationships of Antarctic and sub-Antarctic Rhodophyta with descriptions of four new genera and five new species. Bot Mar 52: 509–534

Hunter RL, Halanych KM (2008) Evaluating connectivity in the brooding brittle star *Astrotoma agassizii* across the drake passage in the Southern Ocean. J Hered 99:137–148

Janosik AM, Halanych KM (2010) Unrecognized Antarctic biodiversity: a case study of the genus *Odontaster* (Odontasteridae; Asteroidea). Integr Comp Biol 50:981–992

Janosik AM, Mahon AR, Halanych KM (2011) Evolutionary history of Southern Ocean *Odontaster* sea star species (Odontasteridae; Asteroidea). Polar Biol 34:575–586

Kinlan BP, Gaines SD (2003) Propagule dispersal in marine and terrestrial environments: a community perspective. Ecology 84:2007–2020

Knapp S, Mallet J (2003) Refuting refugia? Science 300:71–72

Macaya EC, Zuccarello GC (2010) Genetic structure of the giant kelp *Macrocystis pyrifera* along the southeastern Pacific. Mar Ecol Prog Ser 420:103–112

Macaya EC, López B, Tala F, Tellier F, Thiel M (2016) Float and raft: role of buoyant seaweeds in the phylogeography and genetic structure of non-buoyant associated flora. In: Hu Z-M, Fraser C (eds) Seaweed Phylogeography. Springer, Netherlands

Mahon AR, Arango CP, Halanych KM (2008) Genetic diversity of *Nymphon* (Arthropoda: Pycnogonida: Nymphonidae) along the Antarctic Peninsula with a focus on *Nymphon australe* Hodgson 1902. Mar Biol 155:315–323

Moffat C, Beardsley RC, Owen B, van Lipzig N (2008) A first description of the Antarctic Peninsula Coastal Current. Deep-Sea Res II 55:277–293

Montecinos A, Broitman BR, Faugeron S, Haye PA, Tellier F, Guillemin ML (2012) Species replacement along a linear coastal habitat: phylogeography and speciation in the red alga *Mazzaella laminarioides* along the south east Pacific. BMC Evol Biol 12:17

Muangmai N, West JA, Zuccarello GC (2014) Evolution of four Southern Hemisphere *Bostrychia* (Rhodomelaceae, Rhodophyta) species: phylogeny, species delimitation and divergence times. Phycologia 53:593–601

Neiva J, Pearson G, Valero M, Serrao E (2010) Surfing the wave on a borrowed board: range expansion and spread of introgressed organellar genomes in the seaweed *Fucus ceranoides* L. Mol Ecol 19:4812–4822

Neiva J, Pearson GA, Valero M, Serrao EA (2012) Fine-scale genetic breaks driven by historical range dynamics and ongoing density-barrier effects in the estuarine seaweed *Fucus ceranoides* L. BMC Evol Biol 12:78

Norris RD, Hull PM (2012) The temporal dimension of marine speciation. Evol Ecol 26:393–415

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. https://doi.org/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

Saunders GW (2005) Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. Philos Trans R Soc Lond Ser B Biol Sci 360:1879–1888

Savidge DK, Amft JA (2009) Circulation on the West Antarctic Peninsula derived from 6 years of shipboard ADCP transects. Deep-Sea Res I 56:1633–1655

Schoville SD, Roderick GK, Kavanaugh DH (2012) Testing the 'Pleistocene species pump' in alpine habitats: lineage diversification of flightless ground beetles (Coleoptera: Carabidae: *Nebria*) in relation to altitudinal zonation. Biol J Linn Soc 107:95–111

Simms AR, Milliken KT, Anderson JB, Wellner JS (2011) The marine record of deglaciation of the South Shetland Islands, Antarctica since the Last Glacial Maximum. Quat Sci Rev 30:1583–1601

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739

Thatje S, Hillenbrand C-D, Larter R (2005) On the origin of Antarctic marine benthic community structure. Trends Ecol Evol 20:534–540

Thatje S, Hillenbrand CD, Mackensen A, Larter R (2008) Life hung by a thread: endurance of Antarctic fauna in glacial periods. Ecology 89:682–692

Thornhill DJ, Mahon AR, Norenburg JL, Halanych KM (2008) Open-ocean barriers to dispersal: a test case with the Antarctic Polar Front and the ribbon worm *Parborlasia corrugatus* (Nemertea: Lineidae). Mol Ecol 17:5104–5117

Valero M, Destombe C, Mauger S, Ribout C, Engel CR, Daguin-Thiebaut C, Tellier F (2011) Using genetic tools for sustainable management of kelps: a literature review and the example of *Laminaria digitata*. Cah Biol Mar 52:467

Verheye ML, Backeljau T, d'Acoz CDU (2016) Looking beneath the tip of the iceberg: diversification of the genus *Epimeria* on the Antarctic shelf (Crustacea, Amphipoda). Polar Biol 39:925–945

Waters JM, Fraser CI, Hewitt GM (2013) Founder takes all: density-dependent processes structure biodiversity. Trends Ecol Evol 28:78–85

Weir JT, Schluter D (2004) Ice sheets promote speciation in boreal birds. Proc R Soc B 271:1881–1887

Wiencke C, Clayton M (2002) Antarctic seaweeds. In: Wägele JW (ed) Synopsis of the Antarctic Benthos. ARG Gantner, Ruggell

Wiencke C, Amsler CD, Clayton MN (2014) Macroalgae. In: De Broyer C, Koubbi P, Griffiths HJ, Raymond B, d'Udekem d'Acoz C, Van de Putte AP, Danis B, David B, Grant S, Gutt J, Held C, Hosie G, Huettmann F, Post A, Ropert-Coudert Y (eds) Biogeographic Atlas of the Southern Ocean. SCAR, Cambridge

Wilson NG, Hunter RL, Lockhart SJ, Halanych KM (2007) Multiple lineages and absence of panmixia in the ''circumpolar'' crinoid *Promachocrinus kerguelensis* from the Atlantic sector of Antarctica. Mar Biol 152:895–904

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