



Molecular divergence between *Iridaea cordata* (Turner) Bory de Saint-Vincent from the Antarctic Peninsula and the Magellan Region

Paula Ocaranza-Barrera¹ · Claudio A. González-Wevar^{2,3,4} · Marie-Laure Guillemin^{4,5,6} · Sebastián Rosenfeld¹ · Andrés Mansilla¹

Received: 28 March 2018 / Revised and accepted: 3 October 2018 / Published online: 5 November 2018
© Springer Nature B.V. 2018

Abstract

The distribution of the Southern Ocean (SO) biota is the result of major geological, oceanographic, and climate changes during the last 50 million years (Ma). Several groups of marine benthic organisms exhibit marked taxonomic similarities between the Antarctic Peninsula and southern South America, where families, genera, and even species are currently co-distributed in these continents. Several species of macroalgae including *Gigartina skottsbergii*, *Plocamium cartilagineum*, and *Iridaea cordata* are currently found on both sides of the Drake Passage. Advances in molecular techniques have allowed estimating phylogenetic relationships, levels of differentiation and divergence time estimates between populations from these continents in order to determine whether they constitute separate evolutionary units. In this study, we determine whether *Iridaea cordata* represents the same evolutionary unit in southern South America and the Antarctic Peninsula or if populations on the two sides of the Drake Passage represent different genetic lineages. According to our results, *I. cordata* populations from the Antarctic Peninsula and South America are clearly distinguishable evolutionary units with 8.31% and 3.17% mtDNA and cpDNA molecular divergence, respectively. The separation between Antarctic and South American populations of *I. cordata* occurred at the end of the Miocene, between 5 Ma (rbcL) and 9 Ma (COI-5P). These results are similar to those reported in *G. skottsbergii* on both sides of the Drake Passage. Thus, *I. cordata* populations on the two sides of the Drake Passage should be considered two sister species. Cryptic speciation plays an important role in the evolution of the Southern Ocean; thus, the systematics, biogeography, and biodiversity of the region require major revisions.

Keywords Southern Ocean · Rhodophyta · Biogeography · Antarctic Circumpolar Current · Cryptic speciation · Vicariance

✉ Paula Ocaranza-Barrera
pau.ocaranza.barrera@gmail.com

- ¹ Laboratorio de Ecosistemas Marinos Antárticos y Subantárticos, Universidad de Magallanes (LEMAS), Casilla 113-D, Punta Arenas, Chile
- ² Instituto de Ciencias Marinas y Limnológicas (ICML), Facultad de Ciencias, Universidad Austral de Chile, Casilla 567, Valdivia, Chile
- ³ Instituto Milenio de Ecología y Biodiversidad (IEB), Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425, Ñuñoa, Santiago, Chile
- ⁴ Centro FONDAF de Investigación en Dinámica de Ecosistemas Marinos de Altas Latitudes (IDEAL), Valdivia, Chile
- ⁵ Instituto de Ciencias Ambientales y Evolutivas, Facultad de Ciencias, Universidad Austral de Chile, Casilla 567, Valdivia, Chile
- ⁶ CNRS, Sorbonne Universités, UPMC University Paris VI, PUC, UACH, UMI 3614, Evolutionary Biology and Ecology of Algae, Station Biologique de Roscoff, Roscoff, France

Introduction

The Southern Ocean (SO) covers an area of around 35×10^6 km² encircling the Antarctic continent. It represents 8% of the world's ocean surface and is considered to be a major driver of global oceanic circulation (Rintoul et al. 2001). This vast region harbors ~5% of the global marine biodiversity (Barnes et al. 2009); the distribution of its biota reflects the dynamic geological, oceanographic, and climate history (Crame 1999; Aronson et al. 2007; Clarke 2008). In spite of the complex oceanographic and tectonic settings, there are strong links between the Antarctic Peninsula and the Magellan Province biota (Arntz 1999; Arntz et al. 2005); families, genera, and even species of marine near-shore benthic organisms are co-distributed in these continents (Crame 1999). Several studies have demonstrated that larvae and rafted organisms may travel across the Antarctic Polar Front

(APF) using eddies or intrusions of sub-Antarctic water masses through the Antarctic Circumpolar Current (ACC) (Clarke et al. 2005). New evidence from observations of floating kelps indicates that the APF can be actually crossed by some marine organisms, suggesting that the absence of many sub-Antarctic taxa in the Antarctic might be largely due to environmental differences on the two sides of the APF (Fraser et al. 2017). Recent genome-based analyses revealed that rafting keystone macroalgae traveled for more than 20,000 km across oceanographic barriers to reach the Antarctic Peninsula from mid-latitude source populations located in sub-Antarctic islands (South Georgia and Kerguelen Islands) (Fraser et al. 2018). These results highlight that Antarctica is not as biologically isolated as previously proposed and that rafting is a frequent process across the ACC.

Integrative biogeographic studies in SO marine benthic organisms have helped to understand the relative role of vicariance versus dispersal in the biogeography of the region and have aided taxonomic revisions showing the presence of hidden cryptic diversity (Billard et al. 2015; Dornburg et al. 2016). Several groups of marine organisms including invertebrates (Patarnello et al. 1996), vertebrates (Clarke and Johnston 1996), and macroalgae (Hommersand et al. 1994; Silberfeld et al. 2010) exhibit major levels of genetic divergence between SO provinces, supporting the role of vicariance. However, new molecular studies are showing that the origin and diversification of several marine groups occurred long after the physical separation of the continental landmasses (Near et al. 2012; Poulin et al. 2014; Billard et al. 2015; González-Wevar et al. 2017).

This study aims to estimate patterns of genetic divergence between co-distributed Antarctic and South American populations of the Rhodophyta *Iridaea cordata*, a cold-adapted species widely distributed in different provinces of the SO (Wiencke 1990). This species is a member of the Gigartinales and common in shallow sub-tidal communities but can also be found down to a depth of 30 m (Wiencke and Amsler 2012). The species produces large quantities of carrageenans (Craigie 1990), which have economic importance; it is harvested in southern Chile (Mansilla et al. 2012; Astorga-España et al. 2017) jointly with other carragenophytes (*G. skottsbergii*, *Mazzaella laminarioides*, and *Sarcothalia crispata*). Economic income from carrageenan extraction is particularly important in Patagonia and Tierra del Fuego and has been affected by the steady decline of *G. skottsbergii* natural beds due to overexploitation (Buschmann et al. 2001). A shift to complementary exploitation of other carragenophyte genera including *Iridaea* has been proposed as a mechanism to allow the recovery of the *G. skottsbergii* stands by diversification of exploited resources (Buschmann et al. 2001). Except for *Gigartina*, higher latitude carragenophyte genera have been poorly studied and key information concerning taxonomy, distribution of genetic diversity, and potential ecological

adaptation are required. Preliminary phylogenetic analyses in *I. cordata* recorded 3.4% cpDNA divergence between Antarctic and South American populations (Hommersand et al. 1999; Hommersand and Fredericq 2003). We performed molecular comparisons using cpDNA (*rbcL*) and mtDNA (COI-5P) sequences to understand better the biogeography of *Iridaea* at higher Antarctic and sub-Antarctic latitudes.

Materials and methods

Sampling

Individuals of *Iridaea cordata* (Rhodophyta; Fig. 1) were collected between 2011 and 2016 from five localities across the Antarctic Peninsula and the Magellan Province. Antarctic Peninsula sampling localities were (1) Avian Island, Marguerite Bay (67° 45' S; 68° 52' W) $n=2$; (2) Hannah Point, Livingstone Island, South Shetland Islands (62° 41' S; 60° 52' W) $n=4$; and (3) Fildes Bay, King George Island, South Shetland Islands (62° 11' S; 58° 56' W) $n=4$. South American sampling localities included: (1) Diego Ramírez Island, Cape Horn (56° 27' S; 68° 43' W) $n=5$; and (2) Otway Sound, Strait of Magellan (52° 58' S; 71° 13' W) $n=5$. Fronds were sampled from different holdfasts to avoid analyzing genetically identical ramets (Billard et al. 2015). Sampling was conducted in the intertidal during low tides; healthy fronds were cut and placed into a plastic bag filled with silica beads for rapid desiccation of the tissue and DNA preservation.

DNA preparation, PCR amplification, and sequence editing

Dried algal tissue was ground using a bead beater and DNA was prepared following two protocols: (i) a phenol-chloroform methodology (Faugeron et al. 2001) and (b) a cetyltrimethylammonium bromide (CTAB) methodology (Martínez et al. 2003). A partial fragment of the mitochondrial gene cytochrome c oxidase subunit I (COI-5P) was amplified using the specific primers GazF1 and GazR1 (Saunders 2005). At the same time, a partial fragment of the chloroplast gene ribulose-1,5-biphosphate carboxylase/oxygenase (*rbcL*) was amplified using specific primers F-*rbcL* and R-*rbcL* (Guillemin et al. 2016a). PCR amplification was done in a 25- μ L reaction containing 2.5 μ L 10 \times Buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.0), 0.7 μ L 50 mM MgCl₂, 200 mM dNTPs, 1.25 μ L each primer (10 pg μ L⁻¹), 1 μ L DMSO, 1 U Taq polymerase (Invitrogen), 15.6 μ L double-distilled water, and 50 ng DNA. Thermal cycling parameters for COI-5P included an initial denaturation step at 94 °C for 5 min, 5 cycles of 94 °C (30 s), 40 °C (30 s), and 72 °C (30 s), followed by 30 cycles of 94 °C (30 s), 56 °C (30 s), and 72 °C (30 s), and a final extension at 72 °C for 7 min. Similarly, thermal cycling

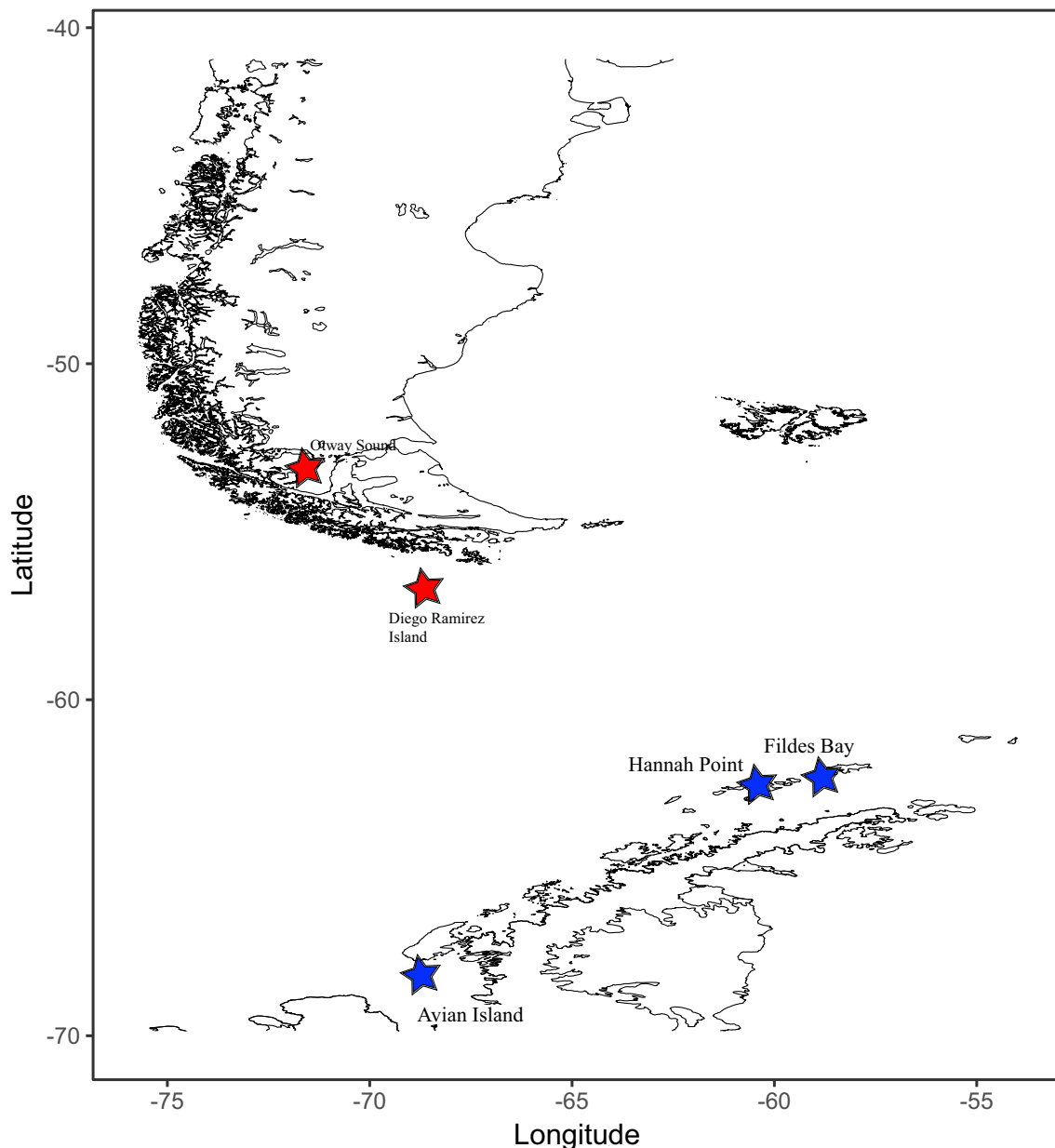


Fig. 1 Sampling localities of *Iridaea cordata* in Antarctic Peninsula and South America. Shapefiles of the coastlines available in the database GEODas (NOAA) were filtered using GEODas Coastline Extractor v.1.1.3.1 (<https://www.ngdc.noaa.gov/mgg/geodas/geodas.html>)

parameters for *rbcL* included an initial denaturation step at 96 °C for 4 min, 5 cycles of 94 °C (30 s), 45 °C (30 s), and 72 °C (30 s), followed by 30 cycles of 94 °C (30 s), 56 °C (30 s), and 72 °C (30 s), and a final extension at 72 °C for 7 min. PCR amplicons were purified and sequenced in both directions with an ABI3730 Automatic Sequencer at MacroGen Inc. (Seoul, South Korea). Chromatograms were inspected and edited using Proseq v.3.5 (Filatov 2009), and multiple alignments were obtained independently for each molecular marker using Muscle (Edgar 2004). Base composition of nucleotide sequences was analyzed separately for each marker using MEGA 7.0 (Kumar et al. 2016). DNA saturation analyses following Xia and Xie (2001) were performed using DAMBE and

codon usage was determined using the Effective Number of Codons (ENC) value (Wright 1990) in DnaSP 5.0 (Librado and Rozas 2009). New COI-5P and *rbcL* sequences in *I. cordata* are available in GenBank under accession numbers MH892484–MH892491 and MH892492–MH892496, respectively.

Phylogenetic reconstructions, maximum parsimony networks, and divergence time estimations

Phylogenetic reconstructions included *I. cordata* populations from the Antarctic Peninsula and the Magellan province. For comparative purposes, we also included in the analyses

available COI-5P and *rbcL* sequences of the Rhodophyta groups *Chondrus*, *Chondracanthus*, *Gigartina*, *Mazzaella*, and *Sarcothalia* (Table 1). For *rbcL* and COI-5P reconstructions, we included the same outgroups (*Dumontia contorta*,

Table 1 Species name, collection location or reference, and GenBank numbers for rhodophyte sequences used in this study

Species	<i>rbcL</i>	Reference	COI-5P	Reference
<i>Dumontia contorta</i>	N/A	N/A	AY970583	Saunders 2005
<i>Mastocarpus stellatus</i>	U02992	Hommersand et al. 1994	KY572683	Bringloe and Saunders 2018
<i>Phyllophora crispa</i>	U02990	Hommersand et al. 1994	KJ960955	Robuchon et al. 2015
<i>Chondracanthus acicularis</i>	N/A	N/A	KR909521	Saunders 2005
<i>Chondracanthus canaliculatus</i>	U02939	Hommersand et al. 1994	N/A	N/A
<i>Chondracanthus chamissoi</i>	N/A	N/A	KP059072	Saunders 2005
<i>Chondracanthus corymbiferus</i>	U02941	Hommersand et al. 1994	GQ398090	Le Gall and Saunders 2010
<i>Chondracanthus intermedius</i>	U02942	Hommersand et al. 1994	KR909534	Saunders 2005
<i>Chondracanthus sp.</i>	N/A	N/A	KR909524	Saunders 2005
<i>Chondracanthus spinosus</i>	U02943	Hommersand et al. 1994	N/A	N/A
<i>Chondrus crispus</i>	U02984	Hommersand et al. 1994	AY970567	Saunders 2005
<i>Chondrus giganteus</i>	U02986	Hommersand et al. 1994	N/A	N/A
<i>Chondrus ocellatus</i>	U02987	Hommersand et al. 1994	N/A	N/A
<i>Chondrus verrucosus</i>	U02988	Hommersand et al. 1994	N/A	N/A
<i>Gigartina clavifera</i>	U03424	Hommersand et al. 1994	N/A	N/A
<i>Gigartina muelleriana</i>	U03427	Hommersand et al. 1994	N/A	N/A
<i>Gigartina pistillata</i>	U03429	Hommersand et al. 1994	KJ960715	Robuchon et al. 2015
<i>Gigartina skottsbergii</i> (FAK)	AF146206	Billard et al. 2015	N/A	N/A
<i>Gigartina skottsbergii</i> (ANT)	U03432	Billard et al. 2015	N/A	N/A
<i>Mazzaella affinis</i>	N/A	N/A	AY970577	Saunders 2005
<i>Mazzaella californica</i>	U03082	Hommersand et al. 1994	N/A	N/A
<i>Mazzaella cornucopiae</i>	U03377	Hommersand et al. 1994	N/A	N/A
<i>Mazzaella flaccida</i>	U03378	Hommersand et al. 1994	AY970574	Saunders 2005
<i>Mazzaella lilacina</i>	U03382	Hommersand et al. 1994	N/A	N/A
<i>Mazzaella linearis</i>	U03383	Hommersand et al. 1994	AY970584	Saunders 2005
<i>Mazzaella rosea</i>	N/A	N/A	AY970600	Saunders 2005
<i>Mazzaella sanguinea</i>	N/A	N/A	AY970599	Saunders 2005
<i>Mazzaella splendens</i>	U03385	Hommersand et al. 1994	AY970594	Saunders 2005
<i>Mazzaella volans</i>	U03386	Hommersand et al. 1994	N/A	N/A
<i>Sarcothalia crispata</i>	U03085	Hommersand et al. 1994	N/A	N/A
<i>Sarcothalia livida</i>	U03087	Hommersand et al. 1994	N/A	N/A
<i>Sarcothalia stiriata</i>	U03089	Hommersand et al. 1994	N/A	N/A
<i>Iridaea tuberculosa</i>	AF146208	Hommersand et al. 1999	N/A	N/A
<i>Iridaea cordata</i> (ICPH01)	N/A	N/A	MH892484	Our sequence
<i>Iridaea cordata</i> (ICPH05)	MH892495	Our sequence	N/A	N/A
<i>Iridaea cordata</i> (ICPH07)	MH892496	Our sequence	N/A	N/A
<i>Iridaea cordata</i> (ICBF19)	N/A	N/A	MH892485	Our sequence
<i>Iridaea cordata</i> (ICDR01)	N/A	N/A	MH892486	Our sequence
<i>Iridaea cordata</i> (ICDR11)	N/A	N/A	MH892487	Our sequence
<i>Iridaea cordata</i> (ICSO01)	MH892492	Our sequence	MH892488	Our sequence
<i>Iridaea cordata</i> (ICSO03)	N/A	N/A	MH892489	Our sequence
<i>Iridaea cordata</i> (ICSO04)	MH892493	Our sequence	N/A	N/A
<i>Iridaea cordata</i> (ICSO09)	N/A	N/A	MH892491	Our sequence
<i>Iridaea cordata</i> (ICSO15)	MH892494	Our sequence	N/A	N/A
<i>Iridaea cordata</i> (ICSO16)	N/A	N/A	MH892490	Our sequence

Mastocarpus stellatus, and *Phyllophora crispa*), following Hommersand et al. (1994).

Phylogenetic relationships in Rhodophyta were estimated using maximum parsimony (MP), maximum likelihood (ML), and Bayesian analysis (BA). MP and ML reconstructions were performed using MEGA. For MP reconstructions, characters were treated as equally weighted using a tree bisection and reconnection (TBR) heuristic search with the branch swapping option. Non-parametric bootstrap (bs) values were estimated to infer nodal support for both MP and ML, with 1000 pseudo-replicates (Felsenstein 1981). Nucleotide substitution models for ML and BA were determined with jModelTest (Darriba et al. 2012) using the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC), respectively. The TN93 + G (*rbcL*) and the GTR + G (COI-5P) substitution models were used for ML and BA reconstructions using MEGA and MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001), respectively. Bayesian inference posterior probabilities were estimated using the Metropolis-coupled Markov chain Monte Carlo (MCMCMC) algorithm, running four chains for 50×10^6 generations with trees sampled every 1000 generations. Stationarity of the analyses was inferred when the average standard deviation of split frequencies was less than 0.01 following Huelsenbeck and Ronquist (2001). The first 10% of the trees were discarded (burn-in) and posterior probabilities were estimated as the fraction of trees showing a particular nodal association. Posterior probability densities were summarized as a Maximum Clade Credibility Tree using Treeannotator v.1.6.1. (<http://beast.bio.ed.ac.uk/TreeAnnotator>) and visualized with Figtree v.1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree>).

For comparative purposes, we also reconstructed mtDNA and cpDNA genealogical relationships within Antarctic and South American *I. cordata* populations using maximum parsimony networks in Hapview (Salzburger et al. 2011). This method allows the simple reconstruction of phylogenetic relationships based on intra- and inter-specific molecular datasets.

Divergence time estimations were performed in BEAST v.1.6.2 (Drummond et al. 2012). The best-fit model for the mtDNA COI-5P and *rbcL* datasets was a relaxed molecular clock analysis with an uncorrelated lognormal model of evolutionary rate heterogeneity using the GTR + G and the TN93 + G substitution models, respectively. A birth-death speciation prior was used for branching rates in the phylogeny; four chains were run twice for 100×10^6 generations and trees were sampled every 1000 generations. Because of the absence of clear fossil records in Rhodophyta, we used conservative mutation rates (0.5% for COI-5P and 0.2% for *rbcL*), following previous studies in the group (Kamiya et al. 2004; Billard et al. 2015). Convergence of model parameters was estimated by plotting the marginal posterior probabilities versus

generations in TRACER. Effective sample size values were estimated for each parameter to ensure adequate mixing of the MCMCMC (ESSs > 1000).

Results

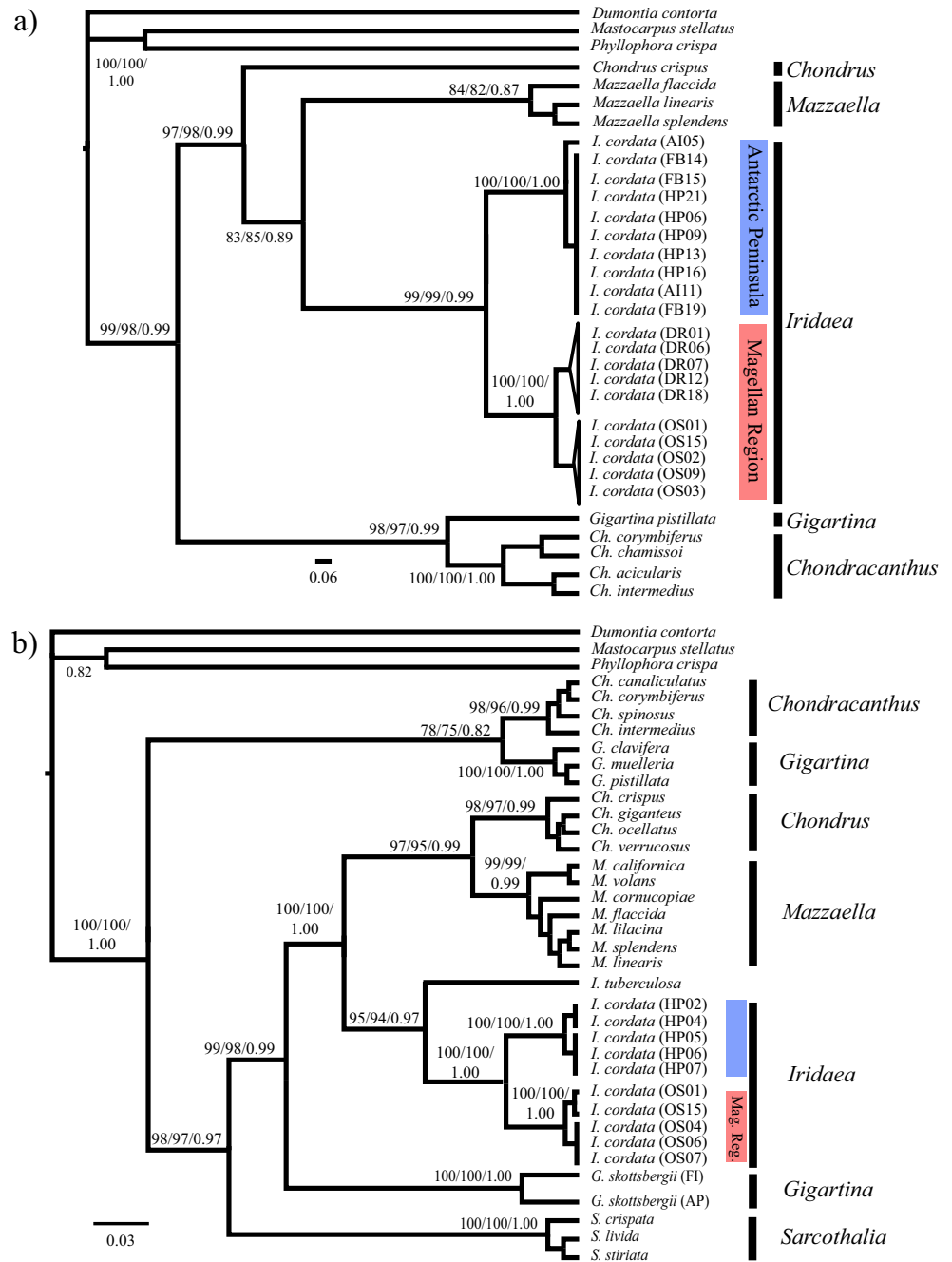
Mitochondrial DNA (COI-5P) alignments in *Iridaea cordata* populations (609 bp) coded for 203 amino acids and included 50 variable positions (8.21%) of which 49 (98%) were parsimoniously informative. Chloroplast DNA (*rbcL*) sequences in the species (869 bp) coding for 289 amino acids were more conserved, with only 24 variable positions (2.76%), and all were (100%) parsimoniously informative. Mitochondrial and chloroplast sequences were A-T rich. Mitochondrial and nuclear sequences were not saturated at any position and no evidence for codon bias was detected for COI-5P (ENC = 43.23) or *rbcL* (ENC = 39.47). No indels or stop codons were identified within the *I. cordata* populations analyzed. For COI-5P, we found five amino acid changes (positions 1, 48, 143, 149, and 164) between Antarctic and South American populations. In contrast, only a single amino acid change was recorded for *rbcL* between Antarctic and South American populations.

All the methods (MP, ML, and BA) and markers showed that *I. cordata* populations from the two sides of the Drake Passage represent different evolutionary units and sister species (Fig. 2a, b). Uncorrected *p* values between these units were 8.31% and 3.17% for COI-5P and *rbcL* (Fig. 3a, b), respectively. Divergence time estimates based on COI-5P sequences under 95% Bayesian confidence credible intervals for BCI for relative divergence times (Fig. 4) suggest that the separation between *I. cordata* populations from the Antarctic Peninsula and the Magellan province occurred during the Miocene around 9.0 Ma (13–7 Ma). Divergence time estimates based on *rbcL* sequences suggest that the separation between Antarctic Peninsula and South American populations of *I. cordata* occurred approximately 5 Ma (4–6 Ma).

Discussion

Advances in molecular techniques have allowed better understanding of the evolution and biogeography of the SO biota and resolving major taxonomic questions (Aronson et al. 2007; Poulin et al. 2014; Billard et al. 2015). Phylogenetic and phylogeographic studies have demonstrated how life-history characteristics are associated with the molecular signals found, providing insight into the main biogeographic patterns across the SO, the origin and diversification of several marine benthic groups, and how species survived the last glacial maximum at higher latitudes (Allcock and Strugnell 2012; Near et al. 2012; González-Wevar et al. 2017). Studies on SO

Fig. 2 Bayesian maximum credibility tree of rhodophyte relationships, with special emphasis on *Iridaea cordata* populations from the Antarctic Peninsula and South America. **a** Phylogenetic reconstructions based on mtDNA COI-5P sequences. **b** Phylogenetic reconstructions based on cpDNA *rbcL* sequences. Bootstrap support values (MP and ML) and BPP are shown above the nodes (in that order). AI Avian Island, FB Fildes Bay, HP Hannah Point, OS Otway Sound, DR Diego Ramírez Island, FI Falkland Islands, AP Antarctic Peninsula

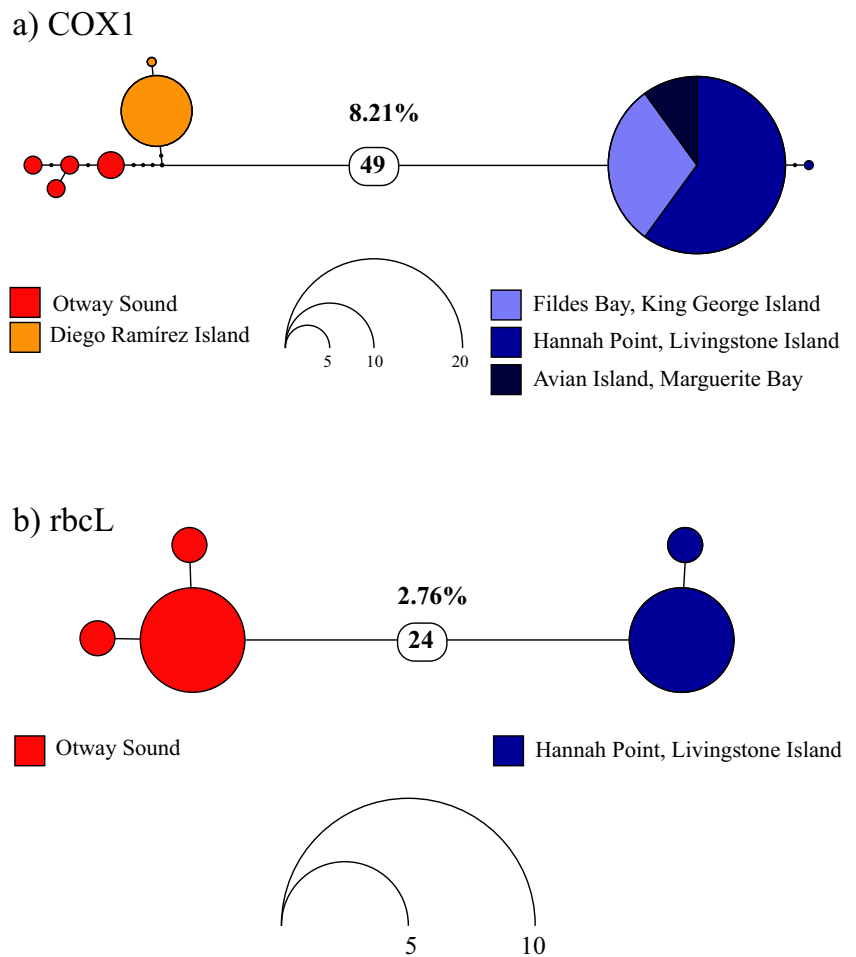


macroalgae during the last decade generated major advances in the comprehension of phylogenetic relationships, systematics, biogeography, and current patterns of diversity across the region (Hommersand et al. 2009; Wiencke et al. 2014; Billard et al. 2015; Guillemin et al. 2018). Macroalgae represent suitable sentinels for global warming-induced processes including ocean acidification, atmospheric CO₂ rise, increased ultraviolet B (UVB) irradiance, and temperature (Ji et al. 2016).

Multi-locus phylogenetic reconstructions in this study corroborated previous mtDNA molecular analyses in Rhodophyta and discriminated major taxonomic groups

(Hommersand et al. 1999; Saunders 2005), as well as the presence of two genetic lineages within the nominal species *I. cordata* on each side of the Drake Passage (Fig. 2a, b). Patterns of molecular differentiation and divergence time estimates between Antarctic and South American lineages of *Iridaea* suggest that they have been separated for several million years. Indeed, divergence time estimates suggest that the effective separation between South American and Antarctic populations of *I. cordata* occurred between 5 (cpDNA) and 9 Ma (mtDNA), long after the physical separation of these continental landmasses, estimated to be between 41 Ma

Fig. 3 Maximum parsimony haplotype Networks in Antarctic and South American populations of *Iridaea cordata*. **a** Mitochondrial COI-5P sequences. **b** Chloroplast DNA *rbcL* sequences. Each haplotype is represented by a circle, and the colors indicate collection sites. The size of the circles is proportional to the frequencies in the entire dataset



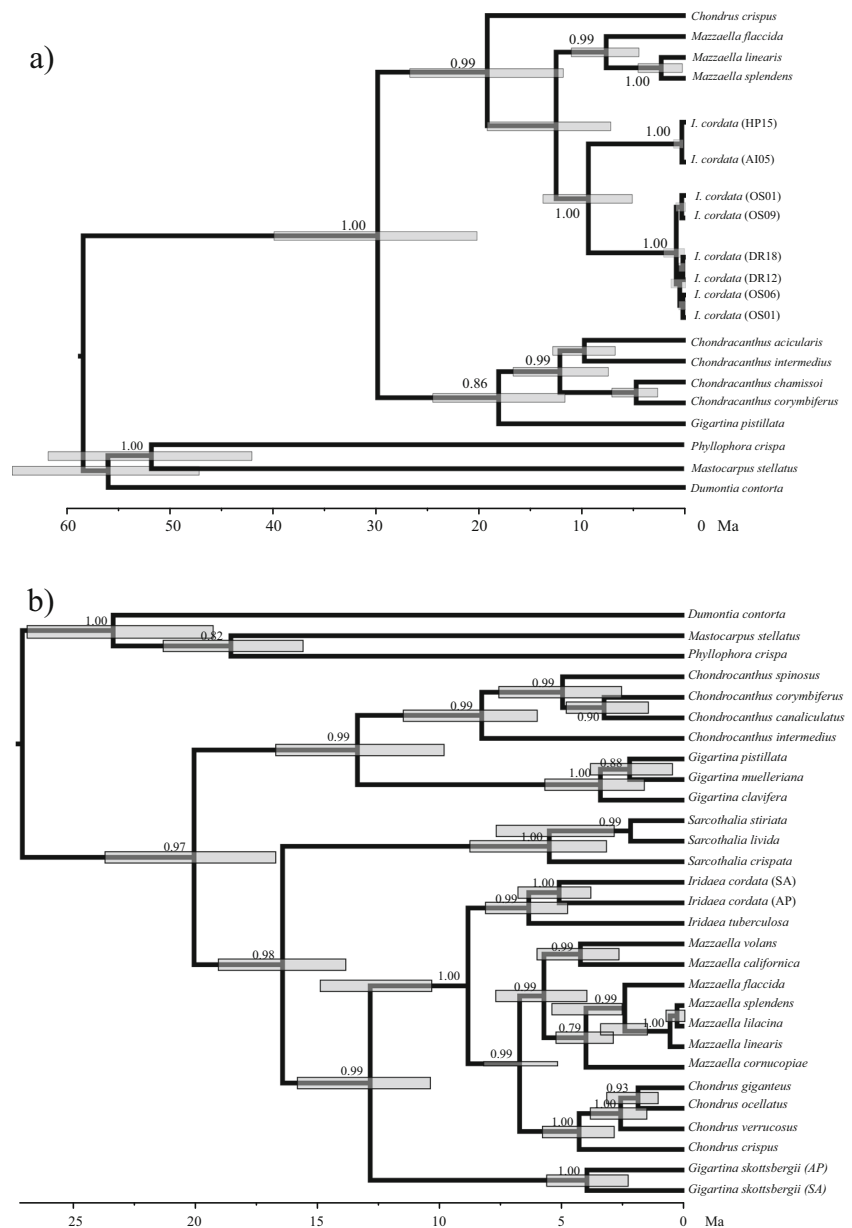
(Livermore et al. 2005) and 23.9 Ma (Pfuh and McCave 2005) and the initiation of the ACC (Zachos et al. 2001).

Divergence time estimations in *I. cordata* are consistent with recent molecular studies in notothenioid fishes (Near et al. 2012), invertebrates (Page and Linse 2002; Wilson et al. 2009; González-Wevar et al. 2017), and macroalgae (Billard et al. 2015), showing that the effective separation between Antarctic and sub-Antarctic populations of several marine benthic organisms occurred no more than 10 Ma (Poulin et al. 2014). Accordingly, several studies have demonstrated that the separation between Antarctic and South American populations of near-shore marine benthic organisms occurred near the Mio-Pliocene boundary and the onset of these divergences does not seem to be a direct consequence of continental drift processes (Poulin et al. 2014 and references therein).

Poulin et al. (2014) proposed an explanation for the incongruence in the molecular divergence between Antarctic and sub-Antarctic benthic organisms and the expected separation based on geological evidence. According to them, inconsistencies between geological and molecular dating may be a consequence of populations from Antarctica and South America maintained connectivity even when these continents

were physically separated through passive dispersal of pelagic larval stages or rafting. Connectivity between geographically separated populations plays a key role in the biogeography of the Southern Ocean, particularly between sub-Antarctic provinces (Fraser et al. 2009, 2011; Macaya and Zuccarello 2010; Nikula et al. 2010; Cumming et al. 2014; Moon et al. 2017; González-Wevar et al. 2018). The disruption of connectivity between Antarctic and sub-Antarctic populations seems to be related to more recent oceanographic and climatic changes that have occurred in the Southern Ocean after the Middle Miocene Climatic Transition (MMCT). Sea surface temperatures (SSTs), salinity, and ice-volume accumulation after the MMCT support the occurrence of major changes in ocean circulation that triggered global cooling, the initiation of sub-zero conditions in the Southern Ocean, and the intensification of the ACC (Shevenell et al. 2004; Lewis et al. 2008). The intensification of the ACC after the MMCT is supported by different sources of data including strengthening of the westerly winds, atmospheric circulation shifts, $\delta_{18}\text{O}$ signatures (Heinrich et al. 2011), and isotope changes in fauna (Lewis et al. 2008; Verducci et al. 2009). Recent studies in the Central Scotia Sea indicate that a remnant, currently submerged volcanic arc may have formed a barrier to deep

Fig. 4 Bayesian maximum clade credibility tree based on mtDNA (COI-5P) showing divergence time estimates among rhodophytes and particularly between *Iridaea cordata* populations from the Antarctic Peninsula and South America. Posterior probabilities are shown above the clades. Gray-boxed regions depict 95% Bayesian credibility intervals (BCIs) for relative divergence times. HP Hannah Point, Livingstone Island, South Shetland Islands; AI Avian Island, Marguerite Bay, Antarctic Peninsula; OS Otway Sound, Strait of Magellan; DR Diego Ramírez Island, Cape Horn; AP Antarctic Peninsula; SA South America



eastward oceanic circulation until the mid-Miocene, 11.2 Ma (Dalziel et al. 2013) when the full establishment of a deep ACC was achieved, ultimately separating Antarctic and South American benthos. Considering the low dispersive potential exhibited by higher latitude Rhodophyta including *Iridaea* (Hansen 1977), we propose that oceanographic conditions could have favored connectivity between Antarctic and South America through rafting of adult fronds until the mid-Miocene, even for this non-buoyant species (Fraser et al. 2013; Billard et al. 2015). In summary, as proposed for other Southern Ocean marine organisms (González-Wevar et al. 2017), changes in the latitudinal position of the ACC and the development of a strengthened current system may constitute key mechanisms explaining the recent Mio-Pliocene separation of Antarctic and sub-Antarctic lineages of red algae.

As recorded in other macroalga groups, the evolutionary lineages resulting after the separation on the two sides of the Drake Passage followed different evolutionary pathways (Lin et al. 2001; Billard et al. 2015).

Levels of genetic divergence found between *Iridaea* populations from the Antarctic Peninsula and southern South America raise questions about the taxonomy of the genus across the SO (pairwise percent divergence, 8.31% for COI-5P and 3.17% for *rbcL*). These values are well above the threshold generally used to discriminate between species Rhodophyta of the order Gigartinales (Le Gall and Saunders 2010; Calderon and Boo 2016). In fact, several studies in Southern Ocean marine organisms have demonstrated the presence of genetically distinct species that had not been previously distinguished using morphological criteria (Held and Wägele

2005; Billard et al. 2015). Cryptic speciation seems to constitute a common evolutionary process in the Southern Ocean and a plausible explanation for the high degree of divergence recorded between Antarctic and South American populations of marine organisms (see for example Poulin et al. 2014). The islands of the Scotia Sea seem to have played a major role in the evolution of the Southern Ocean biota (Linse et al. 2006). These islands constitute hotspots of biodiversity and currently represent the northernmost limit of many Antarctic species, as well as the southernmost limit of several South American ones. Future studies should attempt to elucidate the phylogenetic and phylogeographic affinities of Scotia Arc islands populations of *I. cordata* in order to understand better the role of the geologic and oceanographic processes separating the Antarctic and sub-Antarctic populations of *Iridaea*.

Preliminary mtDNA phylogeographic results in *Iridaea* populations (Fig. 3a) suggest the presence of a single genetic unit in the Antarctic Peninsula across more than 700 km, from Fildes Bay, South Shetland Islands, to Avian Island in Marguerite Bay. These results corroborate a recent phylogeographic study in the species including more individuals ($n = 90$) from five study sites evidencing a complete absence of genetic structure across the Antarctic Peninsula and the South Shetland Islands (Guillemin et al. 2018). Low levels of genetic diversity and absence of population structure have also been recorded in other Antarctic macroalgae including *G. skottsbergii* (Billard et al. 2015) and other rhodophytes (Guillemin et al. 2018). These results could reflect the drastic effect of historical perturbations on Antarctic macroalgae, where genetic drift during glacial bottlenecks was followed by rapid recolonization after the deglaciation process (Billard et al. 2015). In contrast, the two analyzed South American populations of *Iridaea* showed marked levels of genetic differentiation. Fragmentation of populations and subsequent divergence in southern South America may have been promoted by isolation in various micro-refugia during glacial maxima, a trend reported in different Patagonian freshwater (Zemlak et al. 2010), terrestrial (Sérsic et al. 2011), and marine organisms (Vianna et al. 2011). Nevertheless, the high levels of genetic structure recorded between South American populations of *Iridaea* analyzed contrast with previous studies in other macroalgae across this province where several species exhibit absence of genetic structure across their distributions in this continent (reviewed in Guillemin et al. 2016b). Low levels of genetic diversity and absence of phylogeographic signals have been related to the biological characteristics of the macroalgae studied, for which perturbations by glacial cycles and associated ice-scouring seem to have completely wiped out the southernmost populations along the coast of Chile (Fraser et al. 2009). Fragmentation of populations into refugia during glacial maxima and subsequent expansions during interglacial periods may have enhanced the differentiation processes in southern South America. The low potential

dispersal of *Iridaea*, the complex geography of the region, and the main oceanographic regimes may explain the pattern of genetic structure found in South American populations of *Iridaea*. Otway Sound is located inside the Strait of Magellan system and represents an isolated gulf, while the Diego Ramírez Islands are located more than 400 km south in the southernmost extreme of South America. The Diego Ramírez Islands are also washed by the Cape Horn Current, a southern branch of the West Wind Drift that flows south across the Pacific margin of South America. In contrast, Otway Sound within the Strait of Magellan is considered an inlet or fjord with a basic estuarine circulation model (Valdenegro and Silva 2003). Thus, the patterns of genetic structure recorded in *Iridaea* could be a consequence of adaptive divergence associated with life-history traits of the species, as well as main differences in geographic position and oceanographic circulation. However, these results must be considered with precaution considering the low number of individuals analyzed here. Future studies in the species should include more expanded phylogeographic analyses in South American populations of *Iridaea*, including fjord areas where high endemism has been reported for macroalgae (Guillemin et al. 2016a) and possible periglacial refugia as in islands of the Scotia Sea (Linse et al. 2006), in order to understand better the legacy of the Quaternary glacial cycles over patterns of genetic diversity and structure.

Funding information This study was supported by different projects and institutions: INACH project MG_07-17 to P.O-B; Fondecyt Initiation project 11140087, INACH RG_18-17, and GAB (ACT172065) to C.A.G-W; projects P05-002 ICM and PFB023 (Instituto de Ecología y Biodiversidad IEB) to C.A.G-W. and A.M.; Fondecyt Regular project 1140940 to A.M. and P.O-B.; INACH project RG_15-16 to M-L. G.; and FONDAF program project no. 15150003 to M-L.G. and C.A.G-W.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Allcock AL, Strugnell JM (2012) Southern Ocean diversity: new paradigms from molecular ecology. *Trends Ecol Evol* 27:520–528
- Arntz WE (1999) Magellan—Antarctic: ecosystems that drifted apart. Summary Review. *Sci Mar* 63:503–511
- Arntz WE, Thatje S, Gerdes D, Gili J-M, Gutt J, Jacob U, Montiel A, Orejas C, Teixidó N (2005) The Antarctic-Magellan connection: macrobenthos ecology on the shelf and upper slope, a progress report. *Sci Mar* 69:237–269
- Aronson RB, Thatje S, Clarke A, Peck LS, Blake DB, Wilga CD, Seibel BA (2007) Climate change and invasibility of the Antarctic benthos. *Annu Rev Ecol Syst* 38:129–154
- Astorga-España MS, Mansilla A, Ojeda J, Marambio J, Rosenfeld S, Mendez F, Rodríguez JP, Ocaranza P (2017) Nutritional properties

- of dishes prepared with sub-Antarctic macroalgae—an opportunity for healthy eating. *J Appl Phycol* 29:2399–2406
- Barnes DKA, Griffiths HJ, Kaiser S (2009) Geographic range shift responses to climate change by Antarctic benthos: where we should look. *Mar Ecol Prog Ser* 393:13–26
- 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
- Bringloe TT, Saunders GW (2018) Mitochondrial DNA sequence data reveal the origins of postglacial marine macroalgal flora in the Northwest Atlantic. *Mar Ecol Prog Ser* 589:45–58
- Buschmann A, Correa J, Westermeier R, Hernandez M, Norambuena R (2001) Red algal farming in Chile: a review. *Aquaculture* 194:203–220
- Calderon MS, Boo SM (2016) Phylogeny of Phylloporaceae (Rhodophyta, Gigartinales) reveals *Asterfilopsis* gen. nov. from the southern hemisphere. *Phycologia* 55:543–554
- Clarke A (2008) Antarctic marine benthic diversity: patterns and processes. *J Exp Mar Biol Ecol* 366:48–55
- Clarke A, Johnston IA (1996) Evolution and adaptive radiation of Antarctic fishes. *Trends Ecol Evol* 11:212–218
- Clarke A, Barnes DKA, Hodgson DA (2005) How isolated is Antarctica? *Trends Ecol Evol* 20:004
- Craigie JS (1990) Cell walls. In: Cole KM, Sheath RG (eds) *Biology of the red algae*. Cambridge University Press, Cambridge, pp 221–257
- Crame JA (1999) An evolutionary perspective on marine faunal connections between southernmost South America and Antarctica. *Sci Mar* 63:1–14
- Cumming RA, Nikula R, Spencer HG, Waters JM (2014) Transoceanic genetic similarities of kelp-associated sea slug populations: long-distance dispersal via rafting? *J Biogeogr* 41:2357–2370
- Dalziel IWD, Lawver LA, Pearce JA, Barker PF, Hastie AR, Barford DN, Schenke HW, Davis MB (2013) A potential barrier to deep Antarctic circumpolar flow until the late Miocene? *Geology* 41:947–950
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* 9:772–772
- Dornburg A, Federman S, Eytan RI, Near TJ (2016) Cryptic species diversity in sub-Antarctic islands: a case study of *Lepidonotothen*. *Mol Phylogenet Evol* 104:32–43
- Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol Biol Evol* 29:1969–1973
- Edgar RC (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5:1–19
- 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
- Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 17:368–376
- Filatov DA (2009) Processing and population genetic analysis of multi-genic datasets with ProSeq3 software. *Bioinformatics* 25:3189–3190
- Fraser CI, Nikula R, Spencer HG, Waters JM (2009) Kelp genes reveal effects of subAntarctic sea ice during the Last Glacial Maximum. *Proc Natl Acad Sci U S A* 106:3249–3253
- Fraser CI, Nikula R, Waters JM (2011) Oceanic rafting by a coastal community. *Proc R Soc Lond B* 278:649–655
- 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, Kay GM, du Plessis M, Ryan PG (2017) Breaking down the barrier: dispersal across the Antarctic polar front. *Ecography* 40:235–237
- Fraser CI, Morrison AK, Hogg AMC, Macaya EC, van Sebille E, Ryan PG, Padovan A, Jack C, Valdivia N, Waters JM (2018) Antarctica's ecological isolation will be broken by storm-driven dispersal warming. *Nat Clim Chang* 8:704–708
- González-Wevar CA, Hüne M, Segovia NI, Nakano T, Spencer HG, Chown SL, Saucède T, Johnstone G, Mansilla A, Poulin E (2017) Following the Antarctic Circumpolar Current: patterns and processes in the biogeography of the limpet *Nacella* (Mollusca: Patellogastropoda) across the Southern Ocean. *J Biogeogr* 44:861–874
- González-Wevar CA, Segovia NI, Rosenfeld S, Ojeda J, Hüne M, Naretto J, Saudède T, Brickle P, Morley S, Féral JP et al (2018) Unexpected absence of island endemics: long-distance dispersal in higher latitude sub-Antarctic *Siphonaria* (Gastropoda: Euthyneura) species. *J Biogeogr* 45:874–884
- Guillemin ML, Contreras-Porcía L, Ramírez ME, Macaya EC, Bulboa Contador C, Woods H, Wyatt C, Brodie J (2016a) The bladed Bangiales (Rhodophyta) of the South Eastern Pacific: molecular species delimitation reveals extensive diversity. *Mol Phylogenet Evol* 94:814–826
- Guillemin ML, Valero M, Tellier F, Macaya EC, Destombe C, Faugeron S (2016b) Phylogeography of seaweeds in the South East Pacific: complex evolutionary processes along a latitudinal gradient. In: Hu ZM, Fraser C (eds) *Seaweed Phylogeography*. Springer, Dordrecht, pp 251–277
- Guillemin ML, Dubrasquet H, Reyes J, Valero M (2018) Comparative phylogeography of six red algae along the Antarctic peninsula: extreme genetic depletion linked to historical bottlenecks and recent expansion. *Polar Biol* 41:827–837
- Hansen JE (1977) Ecology and natural history of *Iridaea cordata* (Gigartinales, Rhodophyta) growth. *J Phycol* 13:395–402
- Heinrich S, Zonneveld KAF, Bickert T, Willems H (2011) The Benguela upwelling related to the Miocene cooling events and the development of the Antarctic Circumpolar Current: evidence from calcareous dinoflagellate cysts. *Paleoceanography* 26:PA3209
- Held C, Wägele J-W (2005) Cryptic speciation within the widespread Antarctic crustacean *Ceratoserolis trilobitoides* (Crustacea, Isopoda). In: Huiskes AHL, Gieskes WWC, Rozema RML, Schomo SM, van der Vies SM, Wolff WJ (eds) *Antarctic biology in a global context*. Backhuys, Leiden, pp 305–309
- Hommersand MH, Fredericq S, Freshwater DW (1994) Phylogenetic systematics and biogeography of the Gigartineae (Gigartinales, Rhodophyta) based on sequence analysis of *rbcL*. *Bot Mar* 37:193–203
- Hommersand MH, Fredericq S, Freshwater DW, Hughey J (1999) Recent developments in the systematics of the Gigartineae (Gigartinales, Rhodophyta) based on *rbcL* sequence analysis and morphological evidence. *Phycol Res* 47:139–151
- Hommersand MH, Fredericq S (2003) Biogeography of the marine red algae of the South African West Coast: a molecular approach. *Proceedings XVIIth International Seaweed Symposium*, Oxford University Press, pp. 325–336
- 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
- Huelsbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17:754–755
- Ji Y, Xu Z, Zou D, Gao K (2016) Ecophysiological responses of marine macroalgae to climate change factors. *J Appl Phycol* 28:2953–2967
- Kamiya M, Zuccarello GC, West JA (2004) Phylogeography of *Caloglossa leprieurii* and related species (Delesseriaceae,

- Rhodophyta) based on the *rbcL* gene sequences. *Jpn J Phycol* 52: 147–151
- Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33: 1870–1874
- Le Gall L, Saunders GW (2010) DNA barcoding is a powerful tool to uncover algal diversity: a case study of the Phylloporaceae (Gigartinales, Rhodophyta) in the Canadian flora. *J Phycol* 46: 374–389
- Lewis AR, Marchant DR, Ashworth AC, Hedenäs L, Hemming SR, Johnson JV, Leng MJ, Machlus ML, Newton AE, Raine JI et al (2008) Mid-Miocene cooling and the extinction of tundra in continental Antarctica. *Proc Natl Acad Sci U S A* 105:10676–10680
- Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25:1451–1452
- Lin S, Fredericq S, Hommersand MH (2001) Systematics of the Delesseriaceae (Ceramiales, Rhodophyta) based on large subunit rDNA and *rbcL* sequences, including the Phycodryoidae, subfam, *Nov. J Geophys Res* 37:881–899
- Linse K, Griffiths HJ, Barnes DKA, Clarke A (2006) Biodiversity and biogeography of Antarctic and sub-Antarctic mollusca. *Deep-Sea Res* 53:985–1008
- Livemore R, Nankivell A, Eagles G, Morris P (2005) Paleogene opening of Drake Passage. *Earth Planet Sci Lett* 236:459–470
- Macaya EC, Zuccarello GC (2010) DNA barcoding and genetic divergence in the giant kelp *Macrocystis* (Laminariales). *J Phycol* 46: 736–742
- Mansilla A, Ávila M, Yokoya NS (2012) Current knowledge on biotechnological interesting seaweeds from the Magallanes region, Chile. *Rev Bras* 22:760–767
- Martínez EA, Cárdenas L, Pinto R (2003) Recovery and genetic diversity of the intertidal kelp *Lessonia nigrescens* (Phaeophyceae) 20 years after El Niño 1982/83. *J Phycol* 39:504–508
- Moon KL, Chown SL, Fraser CI (2017) Reconsidering connectivity in the sub-Antarctic. *Biol Rev* 92:2164–2181
- Near TJ, Dornburg A, Kuhn KL, Eastman JT, Pennington JN, Patemello T, Zane L, Fernández DA, Jones CD (2012) Ancient climate change, antifreeze, and the evolutionary diversification of Antarctic fishes. *Proc Natl Acad Sci U S A* 109:3434–3439
- Nikula R, Fraser CI, Spencer HG, Waters JM (2010) Circumpolar dispersal by rafting in two sub-Antarctic kelp-dwelling crustaceans. *Mar Ecol Prog Ser* 405:221–230
- Page TJ, Linse K (2002) More evidence of speciation and dispersal across Antarctic Polar Front through molecular systematics of Southern Ocean *Limatula* (Bivalvia: Limidae). *Polar Biol* 25:818–826
- Patarnello T, Bargelloni L, Varotto V, Battaglia B (1996) Krill evolution and the Antarctic Ocean currents: evidence of vicariant speciation as inferred by molecular data. *Mar Biol* 126:603–608
- Pfuh HA, McCave IN (2005) Evidence for late Oligocene establishment of the Antarctic Circumpolar Current. *Earth Planet Sci Lett* 235: 715–728
- Poulin E, González-Wevar C, Díaz A, Gerard K, Hüne M (2014) Divergence between Antarctic and South American marine invertebrates: what molecular biology tells us about Scotia Arc geodynamics and the intensification of the Antarctic Circumpolar Current. *Glob Planet Chang* 123:392–399
- Rintoul SR, Hughes C, Olbers D (2001) The Antarctic Circumpolar Current system. In: Siedler G, Church J, Gould J (eds) *Ocean circulation and climate*. New York Academic Press, New York, pp 271–302
- Robuchon M, Valero M, Gey D, Le Gall L (2015) How does molecular-assisted identification affect our estimation of α , β , and γ biodiversity? An example from understory red seaweeds (Rhodophyta) of *Laminaria* kelp forests in Brittany, France. *Genetica* 143:207–223. <https://doi.org/10.1007/s10709-014-9796-z>
- Salzburger W, Ewing GB, Von Haeseler A (2011) The performance of phylogenetic algorithms in estimating haplotype genealogies with migration. *Mol Ecol* 20:1952–1963
- Saunders GW (2005) Applying DNA barcoding to red macroalgae: a preliminary appraisal holds promise for future applications. *Philos Trans R Soc B* 360:1879–1888
- Sérsic AN, Cosacov A, Cocucci AA, Johnson LA, Pozner R, Avila LJ, Sites JW Jr, Morando M (2011) Emerging phylogeographical patterns of plants and terrestrial vertebrates from Patagonia. *Biol J Linn Soc* 103:475–494
- Shevenell AE, Kennett JP, Lea DW (2004) Middle Miocene Southern Ocean cooling and Antarctic cryosphere expansion. *Science* 305: 1766–1770
- Silberfeld T, Leigh JW, Verbruggen H, Cruaud C, de Reviers B, Rousseau F (2010) A multi-locus time-calibrated phylogeny of the brown algae (Heterokonta, Ochrophyta, Phaeophyceae): investigating the evolutionary nature of the “brown algal crown radiation”. *Mol Phylogenet Evol* 56:659–674
- Valdenegro A, Silva N (2003) Caracterización oceanográfica física y química de la zona de canales y fiordos australes de Chile entre el Estrecho de Magallanes y Cabo de Hornos (CIMAR 3 Fiordos). *Revista Ciencia y Tecnología del Mar* 26:19–60
- Verducci M, Foresi LM, Scott GH, Sprovieri M, Lirer F, Pelosi N (2009) The Middle Miocene climatic transition in the Southern Ocean: evidence of paleoclimatic and hydrographic changes at Kerguelen plateau from planktonic foraminifers and stable isotopes. *Palaeogeogr Palaeoclimatol Palaeoecol* 280:371–386
- Vianna JA, Medina-Vogel G, Chehébar C, Sielfeld W, Olavarría C, Faugeton S (2011) Phylogeography of the Patagonian otter *Lontra provocax*: adaptive divergence to marine habitat or signature of southern glacial refugia? *BMC Evol Biol* 11:53
- Wiencke C (1990) Seasonality of red and green macroalgae from Antarctica—a long-term culture study under fluctuating Antarctic daylengths. *Polar Biol* 10:601–607
- Wiencke C, Amsler CD (2012) Seaweeds and their communities. In: Bischof K, Wiencke C (eds) *Polar Regions, Seaweed biology: novel insights into ecophysiology, ecology and utilization*. Springer, Berlin, pp 265–291
- Wiencke C, Amsler CD, Clayton MN (2014) Macroalgae. In: De Broyer C, Koubbi P, Griffiths HJ, Raymond B, Udekem d’Acoz C et al (eds) *Biogeographic atlas of the Southern Ocean*. Scientific Committee on Antarctic Research, Cambridge, pp 66–73
- Wilson NG, Schrödl M, Halaných 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
- Wright F (1990) The 'effective number of codons' used in a gene. *Gene* 87:23–29. [https://doi.org/10.1016/0378-1119\(90\)90491-9](https://doi.org/10.1016/0378-1119(90)90491-9)
- Xia X, Xie Z (2001) DAMBE: software package for data analysis in molecular biology and evolution. *J Hered* 92:371–373
- Zachos J, Pagani M, Sloan L, Thomas E, Billups K (2001) Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* 292:686–693
- Zemlak TS, Habit EM, Walde SJ, Carrea C, Ruzzante DE (2010) Surviving historical Patagonian landscapes and climate: molecular insights from *Galaxias maculatus*. *BMC Evol Biol* 10:67