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# Molecular divergence between *Iridaea cordata* (Turner) Bory de Saint-Vincent 1826 populations from Antarctic Peninsula and the Magellan Region

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Paula Ocaranza-Barrera<sup>1,4</sup>, Claudio A. González-Wevar<sup>1,2,3</sup>, Marie-Laure Guillemin<sup>4,5,6</sup>, Sebastián Rosenfeld<sup>7</sup>,
 Andrés Mansilla<sup>1,6</sup>

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7 <sup>1</sup> Laboratorio de Ecología Molecular Antártica y Subantártica, Facultad de Ciencias, Universidad de Magallanes.<sup>2</sup> Instituto Milenio de Ecología y Biodiversidad (IEB).<sup>3</sup> Instituto de Ciencias Marinas y 8 9 Limnológicas (ICML), Facultad de Ciencias, Universidad Austral de Chile, Casolla 567, Valdivia, Chile.<sup>4</sup> 10 Instituto de Ciencias Ambientales y Evolutivas, Universidad Austral de Chile, Valdivia, Chile, <sup>5</sup> Centro 11 FONDAP de Investigación en Dinámica de Ecosistemas Marinos de Altas Latitudes (IDEAL). 6 CNRS, 12 Sorbonne Universités, UPMC University Paris VI, PUC, UACH, UMI 3614, Evolutionary Biology and 13 Ecology of Algae, Station Biologique de Roscoff, Roscoff, France <sup>7</sup> Laboratorio de Ecosistemas Marinos 14 Antárticos y Subantárticos (LEMAS).

15

#### 16 Abstract

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18 The distribution of the Southern Ocean (SO) biota is the result of major geologic, oceanographic and climate 19 changes during the last 50 million years (Ma). Several groups of marine benthic organisms exhibit marked 20 taxonomic similarities between Antarctic Peninsula and southern South America where families, genera and 21 even species are currently co-distributed in these continents. Several species of macroalgae such as Gigartina 22 skottsbergii, Plocamium cartilagineum, Iridaea cordata, are currently found at both sides of the Drake 23 Passage. Advances in molecular techniques have allowed to determine their phylogenetic relationships and 24 level of divergence between populations from these continents in order to determine whether they constitute 25 separate evolutionary units. In this study we determine whether Iridaea cordata represents the same 26 evolutionary unit in southern South America and the Antarctic Peninsula or if populations at both sides of the 27 Drake Passage, separated by the Antarctic Polar Front (APF), represent putative cryptic species. According to 28 our results, I. cordata populations from Antarctic Peninsula and South America are clearly distinguishable 29 evolutionary units with a 8.31% and a 3.17% mtDNA and cpDNA molecular divergence, respectively. The 30 separation between Antarctic and South American populations of I. cordata occurred at the end of the 31 Miocene between 5 Ma (rbcL) and 9 Ma (COI-5P). These results are similar to those recorded in G. 32 skottsbergii on both sides of the Drake Passage. Accordingly, I. cordata populations at both sides of the 33 Drake Passage include two cryptic species. In this context, cryptic speciation plays an important role in the 34 evolution of the Southern Ocean and accordingly, the systematic, biogeography and biodiversity of the region 35 require major revisions.

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37 Keywords: Southern Ocean, Rhodophyte, biogeography, Antarctic Circumpolar Current, cryptic speciation,

38 vicariance.

39

#### 40 Introduction

41 The Southern Ocean (SO) covers an area of around 35 x  $10^6$  km<sup>2</sup> encircling the Antarctic continent. It 42 represents 8% of the world's ocean surface and is considered as a major driver of global oceanic circulation 43 (Rintoul et al. 2001). This vast region harbors  $\sim 5\%$  of the marine biodiversity (Barnes et al. 2009) and the 44 distribution of its biota mainly reflects the dynamic geologic, oceanographic, and climate history (Crame 45 1999; Aronson et al. 2007; Clarke 2008; Saucède et al. 2014). Marine benthic communities in the SO are 46 unique and exhibit particular characteristics that are probably associated to ecological and evolutionary 47 responses to low temperatures, strong seasonal variations in light conditions, and to short periods of primary 48 production making them particularly vulnerable to climate change (Barnes & Peck 2008; Griffiths et al. 2017). 49 Biogeographers recognize three periods that dramatically affected the distribution of the biota in the region 50 (Mackensen 2004; Poulin et al. 2014). First, the Eocene/Oligocene boundary (~34 Ma) related to major 51 oceanic gateway openings, the onset of the Antarctic isolation, and the initiation of the Antarctic Circumpolar 52 Current (ACC) (Zachos et al. 2001). The middle Miocene (~14 Ma) represents a second period of major 53 oceanographic and climatic changes that affected the intensity of the ACC and thermal latitudinal gradients 54 (Zachos et al. 2001). Finally, the Quaternary characterized by the alternation between glacial and interglacial 55 periods strongly influenced the seasonality and the intensity of sea-ice formation (Gersonde et al. 2005; Kemp 56 et al. 2010).

57 In spite of the complex oceanographic and tectonic settings there are strong biogeographic links between the 58 biota from Antarctic Peninsula and the Magellan Province (Arntz 1999; Arntz et al. 2005). Families, genera, 59 and even species of marine near-shore benthic organisms are shared between these areas and the general 60 explanation of this "Antarctic-Magellanic Connection" is that these provinces were once contiguous and 61 progressively drifted apart through the opening of the Drake Passage (Crame 1999). Currently, the ACC is 62 delimited by two main fronts: i) the Antarctic Polar Front (APF) and the sub-Antarctic Front (SAF) and their 63 respective positions have major influence over the biogeography of the region. However, several studies have 64 demonstrated that larvae and rafted organisms may travel across the APF using eddies or intrusions of sub-65 Antarctic waters masses through the ACC (Clarke et al. 2005). New evidence from observations of floating 66 kelps at sea indicate that the APF can actually be crossed by some marine organisms suggesting that the 67 absence of many sub-Antarctic taxa in the Antarctic might be largely due to environmental differences at both 68 sides of the front more than a complete APF impermeability (Fraser et al. 2017).

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 helped for taxonomic revisions showing the presence of hidden cryptic diversity (Krabbe et al. 2008; Dornburg et al. 2016; Billard et al. 2015). 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. Nevertheless, new molecular studies are showing that the origin and diversification of several marine groups occurred long after

76 the physical separation of the continental landmasses (Near et al. 2012; Poulin et al. 2014; Billard et al. 2015). 77 This study aims at estimating patterns of genetic divergence between co-distributed Antarctic and Magellanic 78 populations of the rhodophyte Iridaea cordata, a cold adapted species broadly distributed in different 79 provinces of the SO (Wiencke 1990; Wiencke and Clayton 2002). This species is a member of the 80 Gigartinaceae and common in shallow sub-tidal communities but can also be found down to a depth of 30 m 81 (Wiencke and Amsler 2012). The species produces large quantities of carrageenans (Craigie 1990) have 82 economic importance and is harvested in southern Chile jointly with other carragenophytes (i.e. G. 83 skottsbergii, Mazzaella laminarioides and Sarcothalia crispata). Economic incomes from carrageenan 84 extraction is particularly important in Patagonia and Tierra del Fuego and have been affected by the steady 85 decline of G. skottsbergii natural stands due to over exploitation (Buschmann et al. 2001). A shift through 86 complementary exploitation of other carragenophytes genera, including Iridea has been propose as a 87 mechanism to allow the recovery of the G. skottsbergii stands by diversification of exploited resources. 88 Nevertheless, apart from Gigartina, higher latitude carragenophytes genera have not been intensely studied 89 and key information concerning taxonomy, distribution of genetic diversity, and potential ecological 90 adaptation are strongly required. Preliminary phylogenetic analyses in I. cordata recorded a 3.4% of cpDNA 91 divergence between Antarctic and Magellanic populations (Hommersand et al. 1999; Hommersand and 92 Fredericg 2003). In order to corroborate these results, we performed molecular comparisons using cpDNA 93 (rbcL) and mtDNA (COI-5P) sequences to determine the actual number of evolutionary units and to 94 determine the divergence time between populations of the species from both sides of the Drake Passage.

95

#### 96 Material and Methods

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#### 98 Samplings

99 Individuals of Iridea cordata (Rhodophyta; Fig. 1) were collected between 2011-2016 from five localities 100 across Antarctic Peninsula and the Magellanic Province. Sampling localities are as follows: 1) Avian Island, 101 Marguerite Bay (67°45'S; 68°52'W, n = 2); 2) Hannah Point, Livingstone Island, South Shetland Islands 102 (62°41'S; 60°52'W, n = 4); 3) Fildes Bay, King George Island, South Shetland Islands (62°11'S; 58°56'W, n 103 = 4); 4) Diego Ramírez Island, Cape Horn ( $56^{\circ}27^{\circ}S$ ;  $68^{\circ}43^{\circ}W$ , n = 5); and 5) Otway Sound, Strait of 104 Magellan ( $52^{\circ}58^{\circ}S$ ;  $71^{\circ}13^{\circ}W$ , n = 5). Fronds were sampled from distinct holdfasts to avoid the analyses of 105 genetically identical ramets (Billard et al. 2015). Samplings were conducted in the intertidal during low tides 106 and healthy fronds were cut and placed into a plastic bag filled with silica beads for rapid desiccation of the 107 tissue and DNA preservation.

108

#### 109 DNA preparation, PCR amplification and sequence edition

110 Dried algal tissue was grounded using a Bead beater and DNA was prepared following two protocols: i) a

phenol-clorophorm methodology (Faugeron et al. 2001) and b) a cetyl trimethylammonium bromide (CTAB)

112 methodology (Martinez 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

114 time, a partial fragment of the chloroplastic gene Ribulose-1,5-biphosphate carboxylase/oxygenase (rbcL) 115 was amplified using specific primers F-rbcL and R-rbcL (Guillemin et al. 2015). PCR amplifications were 116 done in a 25 ul reaction containing 2.5 ul 10X Buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.0). 0.7 ul of 50 117 mM MgCl<sub>2</sub>, 200 mM dNTPs, 1.25 ul of each primer (10 pg/ul), 1 ul DMSO, 1 U Tag polymerase 118 (Invitrogen), 15.6 ul of double-distilled water and 50 ng of DNA. Thermal cycling parameters for COI-5P 119 included an initial denaturation step at 94°C for 5 min, 5 cycles at 94°C (30 sec), 40°C (30 sec), and 72°C (30 120 sec), followed by 30 cycles of 94°C (30 sec), 56°C (30 sec) and 72° (30 sec), and a final extension at 72°C for 121 7 min. Thermal cycling parameters for *rbcL* included an initial denaturation step at 96°C for 4 min, 5 cycles 122 of 94°C (30 sec), 45°C (30 sec), and 72°C (30 sec), followed by 30 cycles of 94°C (30 sec), 56°C (30 sec), and 123 72° (30 sec), and a final extension at 72°C for 7 min. PCR amplicons were purified and sequenced in both 124 directions with an ABI3730 Automatic Sequencer at Macrogen Inc. (Seoul, South Korea). Chromatograms 125 were inspected and edited using Proseq v.3.5 (Filatov 2009), and multiple alignments were obtained 126 independently for each molecular marker using Muscle (Edgar 2004). Base composition of nucleotide 127 sequences was analyzed independently for each marker using MEGA 7.0 (Kumar et al. 2016) and codon 128 usage was determined using the effective number of codon value (ENC; Wright 1990) with DnaSP 5.0 129 (Librado and Rozas 2009). New COI-5P and rbcL sequences in I. cordata have been deposited in Genbank 130 under the Accession Numbers XXXXX-XXXXX and XXXXX-XXXXX, respectively.

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#### 132 Phylogenetic reconstructions, maximum parsimony networks and divergence time estimations

Phylogenetic reconstructions included *I. cordata* populations from Antarctic Peninsula and the Magellan region. For comparative purposes we also included in the analyses available COI-5P and *rbcL* sequences of other rhodophytes such as *Chondrus*, *Chondracanthus*, *Gigartina*, *Mazzaella*, and *Sarcothalia* (Table 1). For *rbcL* reconstructions we used two outgroups (*Mastocarpus stellatus* and *Phyllophora crispa*) following Hommersand et al. (1994). Similarly, in the case of COI-5P we used two outgroups *Dumontia contorta* and *Asteromenia bermudensis* following Saunders (2005).

139 Phylogenetic relationships in rhodophytes were estimated using different reconstruction methods including 140 maximum parsimony (MP), maximum likelihood (ML), and bayesian analysis (BA). MP and ML 141 reconstructions were performed using MEGA. For maximum parsimony reconstructions we used the 142 following assumptions: characters were treated as equally weighted using a tree bisection and reconnection 143 (TBR) heuristic search with the branch swapping option. Non-parametric bootstrap (bs) values were estimated 144 to infer nodal support for both the MP and the ML with 1000 pseudo-replicates (Felsenstein 1981). 145 Nucleotide substitution models for ML and BA were determined with iModelTest (Darriba et al. 2012) 146 through the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC), respectively. 147 The TN93 + G (rbcL) and the GTR + G (COI-5P) substitution models were used for ML and BA 148 reconstructions using MEGA and MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001), respectively. Bayesian 149 inference posterior probabilities were estimated using the Metropolis coupled Markov chain Monte Carlo 150 (MCMC) algorithm running four chains for 50 x  $10^6$  generations with tree sampled every 1000 generations.

151 Stationarity of the analyses was inferred when the average standard deviation of split frequencies was less

than 0.01 following Huelesenbeck 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.

154 Posterior probability densities were summarized as a Maximum Clade Credibility Tree (MCCT) using

155 Treeannotator v.1.6.1. (http://beast.bio.ed.ac.uk/TreeAnnotator) and visualized with Figtree v.1.4.3

156 (http://tree.bio.ed.ac.uk/software/figtree).

157 For comparative purposes, we also reconstructed mtDNA and cpDNA genealogical relationships within

158 Antarctic and South American *I. cordata* populations using maximum parsimony networks in Hapview 159 (Salzburger et al. 2011). This method allows the simple reconstruction of phylogenetic relationships based on

- 160 intra- and inter-specific molecular data sets.
- 161 Divergence time estimations were performed in BEAST v.1.6.2 (Drummond et al. 2012). The best-fit model 162 for the mtDNA COI-5P and rbcL data sets was a relaxed molecular-clock analyses with an uncorrelated 163 lognormal (ucln) model of evolutionary rate heterogeneity using the GTR + G and the XXX substitution 164 models, respectively. A birth-death speciation prior was used for branching rates in the phylogeny, four 165 chains were run twice for  $100 \times 10^6$  generations, and trees were sampled every 1000 generations. Because of 166 the absence of clear fossil records in rhodophytes we used conservative mutation rates (0.5% for COI-5P and 167 0.2% for *rbcL*), following previous studies in the group (Kamiya et al. 2004; Billard et al. 2015). 168 Convergence of model parameters was estimated by plotting the marginal posterior probabilities versus the 169 generations in TRACER. Effective sample-size values were estimated for each parameter to ensure adequate 170 mixing of the MCMC (ESSs > 1000).
- 171

#### 172 Results

173 Mitochondrial DNA (COI-5P) alignments in Iridaea cordata populations (609 bp) coded for 203 amino acids 174 and included 50 variable positions (8.21%) of which 49 (98%) were parsimoniously informative. Chloroplast 175 DNA (*rbcL*) sequences in the species (869 bp) coding for 289 amino acids, were more conserved with only 24 176 variable positions (2.76%), and all of them (100%) parsimoniously informative. Mitochondrial and 177 chloroplast sequences were A-T rich. Mitochondrial and nuclear sequences were not saturated at any position 178 and no evidence for codon bias was detected for COI-5P (ENC = 43.23) and *rbc*L (ENC = 39.47). No indels 179 or stop codons were identified within the analyzed *I. cordata* populations. In the case of COI-5P we found 180 five amino acid changes (positions 1, 48, 143, 149, and 164) between Antarctic and Magellan populations. In 181 contrast, just a single amino acid change was recorded for the *rbc*L between Antarctic and Magellan 182 populations.

Reconstructions using different sources of DNA (mtDNA and cpDNA) corroborated previous molecular analyses in rhodophytes and discriminated major taxonomic groupings (Hommersand et al. 1999; Saunders 2005) with high bootstrap values and posterior probabilities (Fig. 2A and 2B). All the methods (MP, ML, and BA) and markers showed that *I. cordata* populations from both sides of the Drake Passage represent different evolutionary units and sister species (Fig. 2A and 2B). Uncorrected p-values between these units are 8.31% and 3.17% for COI-5P and *rbcL* (Fig. 3A and 3B), respectively. Divergence time estimates based on COI-5P sequences, under a 95% Bayesian confidence credible interval for BCI for relative divergence times (Fig. 4),

- 190 suggest that the separation between *I. cordata* populations from Antarctic Peninsula and the Magellan region
- 191 occurred during the Miocene around 9.0 Ma (16 7 Ma). Similarly, divergence time estimates based on *rbc*L
- 192 sequences suggest that the separation between Antarctic Peninsula and South American populations of *I*. 193 *cordata* occurred approximately 5 Ma (4 - 6 Ma).
- 195 *cordata* occurred ap
- 194

#### 195 Discussion

196 Recent advances in molecular technologies have allowed to further understand the evolution and 197 biogeography of the SO biota (Allcock and Strugnell 2012; Poulin et al. 2014; Billard et al. 2015). During the 198 last decade, SO macroalgae have been subjected to several studies that generated key advances in the 199 comprehension of phylogenetic relationships, systematics, biogeographies and actual patterns of diversities 200 (Hommersand et al. 2009; Wiencke et al. 2014; Guillemin et al. 2018). Macroalgae exhibit a remarkably wide 201 range of life cycles and several species represent important resources with a wide range of uses in the food, 202 cosmetic, and fertilizer industry (Craigie 1990). Accordingly, they represent interesting models for 203 evolutionary biology, physiological, and induced environmental adaptation studies (Coelho et al. 2010). At 204 the same time, macroalgae represent suitable sentinels for global warming-induced processes such as ocean 205 acidification, atmospheric CO<sub>2</sub> rise, increased ultraviolet B (UVB) irradiance, and temperatures (Ji et al. 206 2016).

207 In this study, multi-locus phylogenetic reconstructions support previous molecular studies (Hommersand et al. 208 1999) in *Iridaea cordata* and recovered the presence of two sister species at each side of the Drake Passage. 209 Patterns of molecular differentiation and divergence time estimates between Antarctic and South American 210 lineages of Iridaea suggest that they have been separated for several million of years. Indeed, divergence time 211 estimates suggest that the effective separation between South American and Antarctic populations of I. 212 cordata occurred between 5 (cpDNA) and 9 (mtDNA) Ma, long after the physical separation of these 213 continental landmasses estimated between 41 Ma (Livermore et al. 2005) and 23.9 Ma (Pfuhl and McCave 214 2005) and the initiation of the ACC (Zachos et al. 2001). Divergence time estimations in I. cordata are 215 consistent with recent molecular studies in notothenioid fishes (Near et al. 2012), invertebrates (Page and 216 Linse 2002; Wilson et al. 2009), and macroalgae (Billard et al. 2015) showing that the effective separation 217 between Antarctic and sub-Antarctic populations of several marine benthic organisms occurred no more than 218 10 Ma ago (Poulin et al. 2014). Accordingly, several studies have demonstrated that the separation between 219 Antarctic and South American populations of near-shore marine benthic organisms occurred near the Mio-220 Pliocene boundary and the onset of these divergences does not seem to be a direct consequence of continental 221 drift processes (Poulin et al. 2014 and references therein).

As proposed by Poulin et al. (2014)<sub>a</sub> an explanation for the incongruence in the molecular divergence between Antarctic and sub-Antarctic benthic organisms and the expected separation based on geologic evidence may be a consequence <u>of</u> populations from Antarctica and South America maintaining connectivity after the physical separation of these continents through passive dispersal of pelagic larval stages or rafting. <u>Connectivity between geographically separated populations plays a key role in the biogeography of the</u> Southern Ocean, particularly between sub-Antarctic provinces (Fraser et al. 2009, 2011; Macaya & 228 Zuccarello 2010; Nikula et al. 2010; Cumming et al. 2014; Moon et al. 2017; González-Wevar et al. 2018). 229 The disruption of connectivity between Antarctic and sub-Antarctic populations seems to be related to more 230 recent oceanographic and climatic changes that have occurred in the Southern Ocean after the Middle 231 Miocene Climatic Transition (MMCT). Sea Surface Temperatures (SST), salinity and ice-volume 232 accumulation after the MMCT support the occurrence of major changes in ocean circulation that triggered 233 global cooling, the initiation of sub-zero conditions in the Southern Ocean and the intensification of the ACC 234 (Shevenell et al. 2004; Lewis et al. 2008). The intensification of the ACC after the MMCT is supported by 235 different sources of data including the strengthening of the westerly winds, atmospheric circulation shifts, 236  $\delta_{18}$ O signatures (Heinrich et al. 2011) and by faunal isotopic changes (Lewis et al. 2008; Verducci et al. 2009). 237 Recent studies in the Central Scotia Sea indicate that a remnant, currently-submerged volcanic arc may have 238 formed a barrier to deep eastward oceanic circulation until the mid-Miocene, 11.2 Ma (Dalziel et al. 2013) 239 when the full establishment of a deep ACC was achieved, ultimately separating Antarctic and South 240 American benthos. In this context, I. cordata as other higher latitude rhodophytes, exhibit low dispersive 241 potential with free-living stages (i.e. spores) lasting only for a couple of hours (Hansen 1977). However, 242 oceanographic conditions could have favoured connectivity between Antarctic and South America through 243 rafting of adult fronds until the mid-Miocene, even for this non-buoyant species (Fraser et al. 2013; Billard et 244 al. 2015). In summary, as proposed for other Southern Ocean marine organisms (González-Wevar et al. 2017), 245 changes in the latitudinal position of the ACC and the development of a strengthened current system may 246 constitute key mechanisms explaining the recent Mio-Pliocene separation of Antarctic and sub-Antarctic 247 lineages of red algae. As recorded in other macroalgae groups, after the separation, the resulting evolutionary 248 lineages at both sides of the Drake Passage, followed different evolutionary pathways (Lin et al. 2001; Billard 249 et al. 2015).

250 Even though I. cordata populations from Antarctic Peninsula and southern South America are recognized as 251 the same nominal species, the levels of molecular divergence recorded between populations located on these 252 two continents raises questions about its taxonomy (pairwise percent of divergence: 8.31% for COI-5P and 253 3.17% for *rbcL*). These values are well above the threshold generally used to discriminate between species in 254 red algae, including rhodophytes of the order Gigartinales (Le Gall and Saunders 2010, Calderon and Boo 255 2016). In fact, several studies in Southern Ocean marine organisms have demonstrated the presence of 256 genetically distinct species that have not been previously distinguished through morphological criteria (Held 257 and Wägele, 2005; Allcock et al. 2011; Billard et al. 2015). Cryptic speciation seems to constitute a common 258 evolutionary process in the Southern Ocean and a plausible explanation for the high degree of divergence 259 recorded between Antarctic and South American population of marine organisms (see for example Poulin et 260 al. 2014). In this context, islands of the Scotia Sea seems to have played a major role in the evolution of the 261 Southern Ocean biota (Linse et al. 2007). These islands constitute hotspots of biodiversity and currently 262 represent the northernmost limit of many Antarctic species, as well as the southernmost distribution of several 263 South American ones. Future studies should attempt to elucidate the phylogenetic and phylogeographic 264 affinities of Scotia Arc islands populations of *I. cordata* in order to better understand the role of the geologic and oceanographic processes separating Antarctic and sub-Antarctic populations of this rhodophyte.

- 266 Preliminary mtDNA phylogeographic results in *Iridaea* populations (Figure 3a) suggest the presence of a 267 single genetic unit in Antarctic Peninsula across more than 700 km, from Fildes Bay, South Shetland Island to 268 Avian Island, Marguerite Bay, These results corroborate a recent phylogeographic study in the species 269 including more individuals (n = 90) from five study sites that showed a complete absence of genetic structure 270 across its distribution in Antarctic Peninsula and the South Shetland Island (Guillemin et al. 2018). Moreover, 271 low levels of genetic diversity and absence of population structure have also been recorded in other Antarctic 272 macroalgae including G. skottsbergii (Billard et al. 2015), and other rhodophytes (Guillemin et al. 2018). 273 These results could reflect the drastic effect of historical perturbations on Antarctic macroalgae where genetic 274 drift during glacial bottlenecks where followed by rapid recolonization following the deglaciation process 275 (Billard et al. 2015). In contrast, the two analyzed South American populations of Iridaea showed marked 276 levels of genetic differentiation. In southern South America, fragmentation of populations and subsequent 277 divergence may have been promoted by isolation in various in-situ microrefugia during glacial maxima, a 278 trend reported in different Magellanic freshwater (Zemlak et al. 2010), terrestrial (Sérsic et al. 2011) and 279 marine organisms (Vianna et al. 2011). Nevertheless, our results in Iridaea contrast with previous studies on 280 macroalgae phylogeography; where recent recolonization from a single source has been proposed to explain 281 the high genetic homogeneity of populations in southern South America (review in Guillemin et al. 2016b). 282 These results has been related to the biological characteristics of the intertidal, low subtidal macroalgae 283 studied to the date for which perturbations by glacial cycles and associated ice-scouring seem to have 284 completely whipped out southernmost populations along the coast of Chile (Fraser et al. 2009). Nevertheless, 285 highly isolated Gulfs as the Otway Sound, which basically exhibits an estuarine circulation pattern 286 (Valdenegro and Silva 2003), have not been included in previous macroalgae studies (Guillemin et al. 2016b). 287 Future studies should perform more expanded phylogeographic analyses in South American populations of 288 Iridaea, including fjord areas where high endemism's has been reported for macroalgae (Guillemin et al. 289 2016a) and possible periglacial refugia as islands of the Scotia Sea (Linse et al. 2006), in order to better 290 understand the legacy of the Quaternary glacial cycles over its patterns of genetic diversity and structure.
- 291

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- 503
- 504 Conflict of interest
- 505 The authors declare no conflict of interest.
- 506

#### 507 Figure Captions

- Fig. 1 Sampling localities of *Iridaea cordata* in Antarctic Peninsula and the Magellan region. Shapefiles of
   the coastlines available in the database GEOdas (NOAA) were filtered using GEOdas Coastline Extractor
- 510 v.1.1.3.1 (https://www.ngdc.noaa.gov/mgg/geodas/geodas.html).
- 511
- 512 **Fig. 2** Bayesian maximum credibility tree of rhodophytes relationships with special emphasis in *Iridaea*
- 513 *cordata* populations form the Antarctic Peninsula and the Magellan region. a) phylogenetic reconstructions
- 514 based on mtDNA COI-5P sequences. b) phylogenetic reconstructions based on cpDNA *rbc*L sequences.
- 515 Bootstrap support values (MP and ML) and BPP are shown above the nodes (in that order).
- 516
- 517 Fig. 3 Maximum parsimony haplotype Networks in Antarctic and Magellanic populations of *Iridaea cordata*.
- 518 a) Mitochondrial COI-5P sequences. b) Chloroplast DNA *rbc*L sequences. Each haplotype is represented by a
- 519 <u>circle, color</u> indicated the collection site. The size of the circles is proportional to its frequency in the whole
- 520 sampling effort.
- 521

- 522 Fig. 4. Bayesian maximum clade credibility tree based on mtDNA (COI-5P) showing divergence time
- 523 estimates within rhodophytes and particularly between *Iridaea cordata* populations from <u>the</u> Antarctic
- 524 Peninsula and the Magellanic region. Posterior probabilities are shown above the clades. Grey boxed regions
- 525 depict 95% Bayesian credibility intervals (BCIs) for relative divergence times.
- 526

### 527 Table Captions

- 528 Table 1. Species name, collection location or reference, and GenBank numbers for <u>rhodophyte sequences</u>
- 529 used in this study.
- 530
- 531

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

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





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)

Fig. 2 Bayesian maximum credibility tree of rhodophyte relationships, with special emphasis on Iridaea cordata populations form 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



Fig. 3 Maximum parsimony haplotype Networks in Antarctic and South American populations of *Iridaea cordata*. **a** Mitochondrial COI-5P sequences. **b** Chloroplast DNA *rbc*L 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



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

