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1 **Building-up knowledge on green macroalgae diversity in the Western Antarctic**
2 **Peninsula: data from two molecular markers reveals numerous species with amphipolar**
3 **distribution**

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21 Abstract:

22 Low levels of diversity and endemism, when compared to red or brown algae, have been
23 reported for Antarctic green macroalgae (Chlorophyta). However, recent studies including the
24 use of molecular markers have allowed to revisit the taxonomical status of species thought to
25 be well known, underlying the existence of unexpected Antarctic flora diversity at local and
26 regional scale. In the present study, samples of green macroalgae along the Western Antarctic
27 Peninsula (from the 62°S down to the 66°S) were sequenced for two genetic markers
28 regularly used for species determination and barcoding in Chlorophyta (i.e. the plastid genes
29 *tufA* and *rbcL*). From the 122 specimens of Chlorophyta sampled, 86 were sequenced for the
30 gene *tufA* and 15 for the gene *rbcL*. Using the NCBI Nucleotide Blast Tool and a threshold of
31 95% of similarity to compare our sequences to the ones available in public data depositories
32 allowed the identification of 12 species. Three new species were reported for the area:
33 *Rosenvingiella radicans*, *Urospora wormskioldii* and *Ulvella islandica*. Furthermore,
34 molecular identification revealed strong match (> 95%) between our Antarctic sequences and
35 the ones obtained for samples from the northern hemisphere for *Acrosiphonia arcta*, *Prasiola*
36 *crispa*, *Prasiola antarctica*, *R. radicans*, *Ulva sp. A-GW*, *U. islandica*, *Urospora*
37 *penicilliformis* and *U. wormskioldii* confirming the amphipolar distribution of various taxa of
38 Antarctic Trebouxiophyceae and Ulvophyceae. Amphipolar distribution seems more common
39 in green than red or brown Antarctic seaweeds, so here we hypothesize that recurrent
40 occurrence of long dispersal events could explain the low level of endemism observed for this
41 phylum along the Antarctic coasts.

42 Keywords : Antarctic - Chlorophyta - barcoding - *tufA* and *rbcL* - amphipolar distribution -
43 endemism.

44

45 **Résumé :**

46 Comparés aux algues rouges ou brunes, des niveaux faibles de diversité et d'endémisme ont
47 historiquement été reportés pour les macroalgues vertes de l'Antarctique (Chlorophyta).
48 Cependant, des études récentes incluant l'utilisation de marqueurs moléculaires ont permis de
49 revoir le statut taxonomique d'espèces que l'on croyait bien connues, révélant une diversité
50 inattendue de la flore antarctique à l'échelle locale et régionale. Dans cette étude, des
51 échantillons de macroalgues vertes prélevés le long de la péninsule Antarctique occidentale
52 (de 62°S à 66°S) ont été séquencés pour deux marqueurs génétiques régulièrement utilisés
53 pour l'identification des espèces de Chlorophytes (i.e gènes plastidiques *tufA* et *rbcL*). Sur les
54 122 spécimens de Chlorophyta échantillonnés, 86 ont été séquencés pour le gène *tufA* et 15
55 pour le gène *rbcL*. Grâce à l'utilisation de l'outil Nucleotide Blast de la plateforme NCBI et
56 d'un seuil de 95% de similarité pour comparer nos séquences à celles disponibles dans la base
57 de données publique, nous avons identifié 12 espèces dont trois nouvelles sont reportées pour
58 la première fois dans la région : *Rosenvingiella radicans*, *Urospora wormskioldii* et *Ulvella*
59 *islandica*. De plus, l'identification moléculaire a révélé une forte correspondance (> 95%)
60 entre nos séquences antarctiques et celles obtenues pour des espèces de l'hémisphère nord,
61 incluant *Acrosiphonia arcta*, *Prasiola crispa*, *Prasiola antarctica*, *R. radicans*, *Ulva* sp. A-
62 GW, *U. islandica*, *Urospora penicilliformis* et *U. wormskioldii* et confirmant la distribution
63 amphipolaire de divers taxons de Trébouxiophycées et Ulvophycées antarctiques. La
64 distribution amphipolaire semble plus fréquente chez les algues vertes antarctiques que chez
65 les algues rouges ou brunes. Nous émettons l'hypothèse que des évènements récurrents
66 d'épisodes de dispersion longues pourrait expliquer le faible niveau d'endémisme observé
67 pour ce phylum le long des côtes antarctiques.

68 Mots-clés : Antarctique - Chlorophyte - barcoding ADN - *tufA* et *rbcL* - distribution
69 amphipolaire - endémisme.

70 INTRODUCTION

71 In green algae, the so-called “core” Chlorophyta (including the classes Chlorophyceae,
72 Ulvophyceae, Trebouxiophyceae, Pedinophyceae and Chlorodendrophyceae, Fang et al.,
73 2018), groups taxa that have diverged early from the Prasinophyceae during the Paleozoic era
74 (Fučíková et al., 2014; Leliaert et al., 2012) and actually comprise 6,500 species (Guiry &
75 Guiry, 2019). These species are ecologically and morphologically very diverse and are found
76 in a wide variety of marine and freshwater environments. In the cold waters surrounding
77 Antarctica, fifteen to seventeen species belonging to Ulvophyceae, Trebouxiophyceae and
78 Chlorophyceae have historically been reported (Gallardo et al., 1999; Ramírez, 2010;
79 Wiencke & Clayton, 2002; Wiencke et al., 2014). However, the recent study of Pellizzari et
80 al. (2017) updated this number to 24 along the coasts of the South Shetland Islands (SSIs),
81 with five new records for the area (*Chaetomorpha (Lola) irregularis*, *Rhizoclonium*
82 *ambiguum*, *Monostroma grevillei*, *Spongomorpha (Acrosiphonia) arcta* and *Ulvella viridis*),
83 and two putative new species (*Callophyllis sp.* and *Prasiola sp.* distinct from *Prasiola crista*
84 already mentioned for the area). Some of these new records are supported by results obtained
85 with molecular markers (i.e. cytochrome c oxidase - COI-5P, UPA genes and Internal
86 Transcribed Spacer - ITS - region for *Callophyllis sp.*, *M. grevillei* *Protomonostroma sp.* and
87 *Prasiola sp.*).

88 Recent studies including molecular tools have allowed to revisit the taxonomical status
89 of species thought to be well known, improving knowledge on diversity and level of
90 endemism characterizing the Antarctic flora (red algae: Dubrasquet et al., 2018; Guillemain et
91 al., 2018; Hommersand et al., 2009; Ocaranza-Barrera et al., 2019; Pellizzari et al., 2017;
92 green algae: De Wever et al., 2009; Garrido-Benavent et al., 2017; Moniz et al., 2012;
93 Pellizzari et al., 2017; brown algae: Peters et al., 1997; 2000). In Chlorophyta, studies using
94 plastid sequences have underlined unexpected diversity at local and regional scale along the

95 Antarctic coasts for Chlorophyceae and Trebouxiophyceae (DeWever et al., 2009; Moniz et
96 al., 2012) and pointed out that the supposedly well-known Antarctic green macroalgae
97 diversity, with very few species reported in comparison with other marine realms (Griffiths,
98 2010), could be underestimated (DeWever et al., 2009; Moniz et al., 2012; Mystikou et al.,
99 2014). However, few molecular data are available in public data repository for Antarctic
100 green algae (i.e. 70 sequences of macroalgae obtained as result for a search for "Antarctic
101 marine Chlorophyta" in Genbank database considering all available molecular markers,
102 author's person. obs.).

103 Accurate and exhaustive understanding of the native algal flora biodiversity and
104 distribution is a key factor for monitoring Antarctic seaweeds (Wiencke et al., 2014). In the
105 Western Antarctic Peninsula (WAP), recent transformations of the physical environment
106 linked to global climate change (e.g. increasing sea temperatures and sea ice melting:
107 Etourneau et al., 2019; Holland et al., 2019; Meelh et al., 2019; Valdivia et al., 2020) may for
108 example favor the arrival and settlement of non-native species, affecting the whole benthic
109 communities functions (Hughes et al., 2020; Hughes & Ashton, 2016; McCarthy et al., 2019;
110 Wiencke et al., 2014). The development of molecular tools associated to comprehensive
111 sampling have allowed for rapid and efficient detection of marine non-native species (Bott et
112 al., 2010). In Antarctica, the Patagonian mussel *Mytilus cf. platensis* (Cárdenas et al., 2020)
113 and the bryozoan *Membranipora membranacea* (Avila et al., 2020) were reported for the first
114 time in the WAP in 2020. Both species have been categorized as "invasive non-native species
115 likely to threaten biodiversity and ecosystems" in Antarctica (Hughes et al., 2020). Regarding
116 green algae, some species of Chlorophyta are recorded among the most invasive marine
117 organisms (Williams & Smith, 2007) and have demonstrated to drastically affect coastal
118 ecosystems functions (e.g., *Caulerpa taxifolia* introduction in Mediterranean Sea;
119 BellanSantini et al., 1996; Jousson et al., 1998). The only non-native photosynthetic marine

120 organism reported to be recently established in Antarctica is the green alga *Ulva intestinalis*
121 (Clayton et al., 1997). The species was observed in highly touristic sites and closed to human
122 settlements (e.g., scientific bases) around the SSHs and the WAP, and its arrival was related to
123 maritime transport (i.e. specimens found as biofouling on ship hull; Clayton et al., 1997;
124 Chown et al., 2012; Chown et al., 2015; Hughes & Ashton, 2016). As increasing shipping
125 traffic augment propagule pressure of potential new colonizer (Cárdenas et al., 2020; Hughes
126 et al., 2020; Hughes & Ashton, 2016; Lee and Chown, 2009), being able to detect early arrival
127 of non-native species and to monitor their possible settlement and distribution range extension
128 will rely on a comprehensive sampling design associated with long-term monitoring and
129 available molecular data (McCarthy et al., 2019; Wiencke et al., 2014). However, apart from
130 Wiencke et al. (2014) effort to resume the current state of knowledge about Antarctic seaweed
131 diversity and distribution, long-term data monitoring is still lacking for these taxa at regional
132 scale (De Broyer & Danis, 2011; Grant & Linse, 2009).

133 We propose to revisit Antarctic green macroalgae diversity using two molecular
134 markers regularly used for species determination and barcoding in Chlorophyta (i.e., the
135 plastid genes *tufA* and *rbcL*; Leliaert et al., 2012; Lewis & Lewis 2005; Pröschold & Leliaert
136 2007). Our survey encompasses a wide area (i.e., more than 450 km) from the SSHs to the
137 center part of the WAP. The present work is part of an ongoing effort to monitor the benthic
138 Antarctic flora, a group of taxa under increasing threat in the region due to the acceleration of
139 climate change and intensification of anthropogenic activities.

140

141 **MATERIALS AND METHODS**

142 Sampling - Sampling was realized during austral summers between 2011 and 2014 within the
143 framework of four campaigns organized by the Chilean Antarctic Institute (INACH). Five

144 areas were sampled (Figure 1), two located in the SSHs (near the Chilean Capitán Arturo Prat
145 base in Greenwich Island and at Bahía Fildes in King George Island, hereafter referred as
146 PRAT and KGI, respectively) and three areas along the Northern and Central part of the WAP
147 (near the Chilean O'Higgins Antarctic base, noted OHI; in Paradise Bay, near the Chilean
148 Presidente Gabriel González Videla Antarctic base, noted GGV and in Marguerite Bay, noted
149 MAR). In all areas, intertidal samplings were conducted during diurnal low tide hours while
150 subtidal samples were collected by SCUBA diving. Specimens showing different
151 morphotypes (e.g., presenting noticeable variations in thallus shape, color or thickness and
152 elasticity) were collected. All specimens were pressed as vouchers after removing a small
153 portion of the thallus that was stored in silica gel for subsequent DNA analysis. Voucher
154 specimens are housed in the herbarium of the Universidad Austral de Chile and available on
155 request. All voucher specimens were identified, to the lowest possible taxonomic level, on the
156 basis of morphological criteria using floristic keys and species list available for the region
157 (Ramirez, 2010; Pellizarri et al., 2017; Wiencke & Clayton, 2002).

158 DNA extraction, PCR amplification and sequencing - For each specimen stored in the
159 herbarium, a fraction corresponding to some 30 mm² of dried tissue was milled in a Mini-
160 BeadBeater 24 (BioSpec Products, Inc. Bortlesville, USA) and DNA extraction was
161 performed with an E.Z.N.A tissue DNA kit (Omega Bio-tek, Inc. Georgia, USA) following
162 the manufacturer instructions.

163 A fragment of the plastid gene *tufA*, encoding for protein synthesis elongation factor
164 Tu (EF-Tu), was amplified in all samples. This gene, well conserved in a wide variety of
165 photosynthetic species, allows for reliable plant and green algae species determination (Fama
166 et al., 2002; Saunders & Kucera, 2010) and has been largely used to infer green macroalgae
167 phylogeny (Leliaert et al., 2012). Amplification of *tufA* was realized using the primers
168 (TufAgf4: 5TGAAACAGAAMAWAWCGTCATTATGC-3 and TufAR:

169 5CCTTCNCGAATMGCRAAWCGC-3) developed by Fama et al. (2002) following the
170 published protocol.

171 For a subsampling of green algae specimens (i.e. one or a few specimens per distinct
172 genetic entities detected with the gene *tufA*), the plastid gene *rbcL* coding for the large
173 subunit of ribulose 1,5 bisphosphate carboxylase/oxygenase was amplified. The primers
174 GrbcLnF (5' GCTGGWGTAAAAGATTAYCG 3') and GrbcLR (5'
175 TCACGCCAACGCATRAASGG 3') developed by Saunders & Kucera (2010) were used and
176 PCR reaction mix and program follow the protocol of Pirian et al. (2016).

177 All PCR reactions were performed in a Perkin Elmer Gene Amp PCR system 9700
178 thermal cycler (Applied Biosystems, Foster City, USA). PCR products were purified using
179 the commercial kit UltraClean™ (MO BIO Laboratories, Carlsbad, USA). Quality and
180 concentration of purified PCR products were verified by electrophoresis on 2% agarose gel
181 dyed with GelRed™ (Biotium Inc, Hayward, USA). Sequencing was performed in
182 AUSTRAL-omics Core-Facility (Universidad Austral de Chile, Chile) using a ABI
183 PRISM®310 Genetic Analyzer (Applied Biosystems, Foster City, USA).

184

185 Data analyses - Sequences were edited using Chromas v.2.33 (McCarthy, 1997), and aligned
186 using MEGA v.5 (Tamura et al., 2011). Molecular species identification was performed using
187 the basic local alignment search tool (BLAST) from NCBI (Altschul et al., 1990) and
188 comparing the sequences obtained in this study with those available in GenBank. Given the
189 scarcity of available sequences, a threshold of 95% identity was used as a cut-off value to
190 record a match (Manghisi et al., 2019).

191

192 **RESULTS**

193 A total of 122 specimens of Chlorophyta were sampled between the high intertidal down to a
194 depth of 30 m in the sampling area: 34 in PRAT, 22 in KGI, 15 in OHI, 34 in GGV and 17 in
195 MAR. Because of low quality and/or quantity of DNA extracted for some specimens, *tufA*
196 sequences were obtained only for 93 specimens, representing 76 % of the samples. Among
197 these 93 sequences, four were contaminated by the bacteria *Granulosicoccus antarcticus* (Lee
198 et al., 2008) and three by the diatom *Seminavis robusta* D.B.Danielidis & D.G.Mann and
199 removed from the dataset. The remaining 86 sequences obtained for green macroalgae
200 belonged to Ulvophyceae (69 specimens of Ulotrichales and 12 specimens of Ulvales) and
201 Trebouxiophyceae (5 specimens belonging to Prasiolales). Twelve distinct putative species
202 were detected in the present study using *tufA* molecular dataset: nine are part of the
203 Ulvophyceae class and three of the Trebouxiophyceae class (Suppl Mat 1). In order to
204 confirm the species assignment based on the *tufA* gene, the *rbcL* gene was amplified in a sub-
205 sample of randomly selected specimens (N=31 in total) belonging to each putative species.
206 The *rbcL* sequences were obtained for only half of tested specimen (N=15) belonging to
207 seven putative species out of the twelve detected with the *tufA* gene (Suppl Mat 2). No *rbcL*
208 PCR products were obtained for *Monostroma hariotii*, even after testing amplification using
209 the DNA of the 37 specimens available. Among the 15 *rbcL* obtained sequences, 11 were
210 congruent with *tufA* sequences identification (Suppl Mat 1). The remaining four sequences
211 were identified as *Ulva sp.* A-GW and congruent with morphological identification but *tufA*
212 gene sequences were lacking for these specimens. All sequences were deposited in the public
213 depository (GenBank NCBI Public Database; see Suppl Mat 1 and 2). Following the
214 classification of Guiry & Guiry (2019), taxonomic status regarding species-specific results
215 provided by *tufA* and *rbcL* datasets are registered below. Reported distribution also follows
216 Guiry & Guiry (2019).

217 **CHLOROPHYTA, Ulotrichales, Ulotrichaceae**

218 *Acrosiphonia arcta*, (Dillwyn) Gain (SShs: PRAT, WAP: GGV). Reported distribution:
219 Arctic - North Europe (Sweden - Denmark - Britain - Faroe Islands) - North America
220 (Alaska - Oregon - Canada - British Columbia) - South America (Chile - Argentina -
221 Falkland Islands) - Asia (East Russia and Kamchatka - Bering Sea) - Antarctic and
222 subAntarctic islands (South Georgia - SShs - Kerguelen Islands - Auckland Islands -
223 Campbell Island) - New Caledonia. Closest match (98,31%, GenBank Access Number
224 HQ610211, Suppl Mat 1) with Antarctic *tufA* sequences was a Canadian specimen of
225 *Acrosiphonia arcta* from British Columbia. No *rbcL* sequences were obtained for this species.
226 *Acrosiphonia arcta* has previously been reported in Antarctic waters (Ramirez, 2010), mainly
227 under the name *Spongomorpha arcta* (Papenfuss, 1964; Pellizzari et al., 2017) considered as
228 synonymous for this species (Guiry&Guiry, 2019). We provide here the first genetic data for
229 *A. arcta* in the southern part of its area of distribution (i.e., GGV in the WAP), and confirm its
230 amphipolar distribution (Saunders & Kucera, 2010; VanOppen et al., 1993, Figure 2).

231 *Capsosiphon* sp. (WAP: GGV). Reported distribution: *Capsosiphon groelandicus* has
232 been reported in Arctic (Svalbard) - North Asia (China - Japan-East Russia -
233 Kamchatka - Commander Islands) - Antarctic and subAntarctic islands (Adelaide
234 Island). *Capsosiphon fulvescens* has been reported in Europe (UK - Belgium - Crimea -
235 Denmark - Faroe Islands - Greenland - Iceland - France - Germany - Ireland - Italy -
236 Netherlands - Norway - Sweden - Spain - Ukraine) - USA (Alaska - California -
237 Connecticut - Maine - New Hampshire - New Jersey) - Canada (British Columbia - New
238 Brunswick) - North Asia (China - Japan - Korea) - Argentina - SubAntarctic Islands
239 (Saint Paul). No close match was found in GenBank for the *tufA* sequences obtained in the
240 present work. Close match was found for *rbcL* sequences of specimens sampled in the
241 Gerlache Strait (formerly identified as *Urospora penicilliformis* using morphological
242 characters in the present study) with the *Capsosiphon fulvescens* plastid complete genome

243 (98.53%, GenBank Access Number NC_039920, Suppl Mat 2). *Capsosiphon fulvescens* has
244 never been reported in the Antarctic. However, using morphological identification combined
245 to the information from various nuclear markers (18S rRNA gene, ITS1, 5.8S rRNA gene,
246 ITS2 and 50-28S rRNA gene), Mystikou et al. (2014) reported the presence of *Capsosiphon*
247 *groelandicus* in the WAP (i.e Adelaide Island in Marguerite Bay). Since species identification
248 could not be clarified with the help of our two molecular markers, we choose to keep the
249 name *Capsosiphon* sp. for the specimens sampled in the present study until further taxonomic
250 work.

251 ***Protomonostroma* sp. A-GW (SShs: PRAT). Reported distribution: *P. undulatum* has**
252 **been reported in Europe (UK - Norway - Germany - Faroe Islands - Iceland - Greenland**
253 **- Denmark) - USA (Maine - Alaska) - Canada (British Columbia - New Brunswick) -**
254 **North Asia (Japan - China - Korea - Kamchatka - East Russia) - Argentina. *P.***
255 ***rosulatum* Vinogradova, has only been reported in the South Shetland Islands.** Closest
256 match for *tufA* gene (99.87%, GenBank Access Number MG646367, Suppl Mat 1) was found
257 with *Protomonostroma* sp. A-GW from King George Island. Exact match with *rbcL*
258 sequences of *Protomonostroma* sp. A-GW from King George Island was observed (100%,
259 GenBank Access Number MG711514, Suppl Mat 2). Based on previous reports (Medeiros,
260 2013) and complementing morphological characters with molecular markers, Pellizzari et al
261 (2017) reported the presence of *Protomonostroma rosulatum* in the SShs instead of *P.*
262 *undulatum*, as described in the early study of Vinogradova (1984). However, since closest
263 matches obtained for both genes for our sequences were with *Protomonostroma* sp. A-GW we
264 decided to use this last name for specimens sampled in the present study until further
265 taxonomic work.

266 ***Urospora* sp. 1 *penicilliformis* (SShs: PRAT, WAP: GGV, MAR) Reported distribution**
267 **of *Urospora penicilliformis*: Worldwide except tropical waters.** Exact match was found

268 with *Urospora sp. 1 penicilliformis tufA* sequence from Nome, Alaska (GenBank Access
269 Number MH571163, Suppl Mat 1). Another close match was found (99.48%, GenBank
270 Access Number HQ610440, Suppl Mat 1) with *Urospora sp. 1 penicilliformis tufA* sequence
271 from Canada. Exact match was found for the *rbcL* gene with *Urospora sp. 1 penicilliformis*
272 sequence from USA, Maine (GenBank Access Number HQ603674, Suppl Mat 2). Type
273 locality for *Urospora penicilliformis* is located in the northern hemisphere, probably in
274 Germany (Guiry & Guiry, 2019), but the species has previously been reported (based on
275 morphological character) in the southern hemisphere along the Chilean and Argentinean
276 coasts (Boraso de Zaixso, 2004,2013; Ramirez y Santelices, 1991), Antarctica and Sub-
277 Antarctic Islands (Papenfuss, 1964; Mystikou et al, 2014; Wiencke & Clayton, 2002),
278 Australia and New Zealand (Broady et al, 2012; Womersley, 1984). Amphipolar distribution
279 of *Urospora sp 1 penicilliformis* is supported by molecular data (Alaska: Bringloe et al. 2019;
280 British Columbia: Saunders & Kucera, 2010; Antarctic and subAntarctic Islands: the present
281 study, Figure 2).

282 ***Urospora wormskioldii* (Mertens) Rosenvinge (WAP: OHI). Reported distribution:**
283 **Arctic (Canada - Svalbard - Greenland - Iceland & Faroe Islands) - North Europe**
284 **(Germany - Denmark - Brittany - Baltic Sea - Norway - Spitzberg) - North America**
285 **(both Pacific and Atlantic coasts down to Mexico) - North Asia (China - East Russia -**
286 **Kamchatka).** Closest matches for three specimens formerly identified as *U. penicilliformis*
287 based on morphological characters were observed with sequences of *Urospora wormskioldii*
288 from British Columbia, Canada for both *tufA* and *rbcL* genes (99.87%, GenBank Access
289 Number HQ610441 and HQ603676 for *tufA* and *rbcL*, respectively, Suppl Mat 1 and 2). The
290 present molecular data represent the first report of a second *Urospora* species in Antarctic
291 waters, underlying the unknown amphipolar distribution pattern of *U. wormskioldii*
292 (Lindstrom & Hanic, 2005, Figure 2).

293 **CHLOROPHYTA, Ulotrichales, Monostromaceae**

294 *Monostroma hariatii* Gain (SShs: PRAT, KGI; WAP: OHI, GGV and MAR). Reported
295 **distribution: Antarctic and SubAntarctic Islands (Kerguelen Islands - Macquarie Island**
296 **- South Georgia - South Orkney Islands - SShs - Antarctic Peninsula - Wilkes Land) -**
297 **South America (Argentina - Falkland Islands).** Exact match for *tufA* gene was observed
298 with sequence of a specimen named *Monostroma angicava* from King Georges Island (100%,
299 GenBank Access Number MG646366, Suppl Mat 1). Comparison with other sequences of
300 specimens of *Monostroma* available in public repositories showed a lower percentage of
301 similarity (e.g. 92.60% of similarity with *Monostroma grevillei* sp. 1 from Canada, GenBank
302 Access Number HQ610257). The species *Monostroma grevillei* sp. 1 has, however, been
303 reported, identified by molecular approaches, in the South Shetland Islands (Pellizzarri et al.
304 2017). No *rbcL* sequences were obtained for this specie in the present study. Since our
305 samples were first determined as *M. hariatii* based on morphological characters and due to the
306 fact that *M. hariatii* has been reported as an emblematic specie of the Antarctic and
307 SubAntartic waters (Wiencke & Clayton, 2002) while *M. angicava* has only been reported in
308 the northern hemisphere, we decided to retain the name *M. hariatii* for specimens sequenced
309 in the present study. *Monostroma hariatii* has been reported as common in the Falklands,
310 Kerguelen and Macquarie Islands (Wiencke & Clayton, 2002), South Orkney Islands
311 (Wiencke & Clayton, 2002), South Shetlands Islands including King George Island (Al-
312 Handal & Wulff 2008; Pellizzarri et al., 2017; Quartino et al., 2005; Wiencke & Clayton,
313 2002), Wilkes Land (Runcie & Riddle, 2006) and the Antarctic Peninsula (Amsler et al..
314 2005; Lamb & Zimmermann, 1977; Mystikou et al.. 2014; Peters et al., 2005).

315

316 **CHLOROPHYTA, Ulvales, Ulvaceae**

317 *Ulva* sp. A-GW, (WAP: GGV, MAR). **Reported distribution: Unknown.** Both genes *tufA*
318 and *rbcL* confirmed the assignation of our samples to the species *Ulva* sp. A-GW with exact
319 matches with sequences from King George Island, SSHs (100%, GenBank Access Number
320 MG646368 and MG711515 for *tufA* and *rbcL* genes, respectively, Suppl. Mat. 1 and 2).
321 Close matches were also observed with *tufA* sequences from British Columbia, Canada
322 (98.83%, GenBank Access Number KM254999) and Nome, Alaska (98.61%; GenBank
323 Access Number MF124264; Suppl Mat 1) suggesting that *Ulva* sp. A-GW displays an
324 amphipolar distribution (Figure 2). Our specimens were sampled in the southern part of the
325 WAP (e.g. Gerlache Strait and Marguerite Bay), about 250 km from King George Island,
326 suggesting an extensive distribution of *Ulva* sp. A-GW, at least in the Western Antarctic.

327 **Unknown specimen of Ulvales (WAP: GGV). Reported distribution: Unknown.** One
328 sample formerly identified as *Urospora penicilliformis* show a close match with a specimen
329 of Ulvales isolated from *Mastocarpus stellatus* in England for the *tufA* gene (95.98%, *Ulvales*
330 sp. 6 BER, GenBank Access Number EF595318, Suppl Mat 1). Since the *rbcL* gene failed to
331 amplify, no accurate identification is available for this sample. The sequence obtained in the
332 present study could correspond to an epiphytic Ulvales specimen living on *U. penicilliformis*.

333

334 **CHLOROPHYTA, Ulvales, Ulvellaceae**

335 *Ulvella islandica* R. Nielsen & K. Gunnarsson (SSHs: PRAT). **Reported distribution:**
336 **North Europe (Iceland).** After sequencing one specimen formerly identified as *Monostroma*
337 *hariotii* using morphological characters, molecular data provided unexpected close match
338 with *Ulvella islandica tufA* sequence from Iceland (98.12%, GenBank Access Number
339 KF444924, Suppl Mat 1). It is probable that the sequence obtained in the present study
340 corresponds to an epiphytic *Ulvella* stage living on *M. hariotii* thallus. No *rbcL* sequence was

341 obtained for this specimen. *Ulvella islandica* has been recently described in Icelandic waters
342 (Nielsen et al. 2014) but has never been reported in Antarctic waters. The *tufA* sequence
343 obtained in the present study was less related to other *Ulvella* sequences deposited in
344 GenBank (*Ulvella reticulata*, 96.54%, GenBank Access Number JQ303009; *Ulvella viridis*,
345 95.61%, GenBank Access Number EF595286; *Ulvella leptochaete*, 95,07%, GenBank Access
346 Number JQ303013, Suppl Mat 1), all species reported in Antarctica (Mystikou et al., 2014;
347 Pellizzari et al., 2017). This could represent the first evidence of an amphipolar distribution
348 for *Ulvella islandica*, but caution should be taken as our study relies upon one single
349 specimen sampled in PRAT.

350

351 **CHLOROPHYTA, Trebouxiophyceae, Prasiolales**

352 ***Prasiola crispa* (Lightfoot) Kützing (WAP: OHI, GGV). Reported distribution:**
353 **Worldwide.** Two of the three specimens sequenced for *tufA* showed exact matches with
354 GenBank sequence of *Prasiola crispa* strain n°43 from King George Island (100%, GenBank
355 Access Number KF993450, Suppl Mat 1) while the other one exactly matched a sequence of
356 *P. crispa* from Svalbard (100%, GenBank Access Number LN877821, Suppl Mat 1). *rbcL*
357 gene confirms *Prasiola crispa* identification and an exact match was encountered with a
358 Canadian specimen from British Columbia (100%, GenBank Access Number KR017748,
359 Suppl Mat 2). Our findings are congruent with previous works reporting the presence of the
360 species in Antarctica and its amphipolar distribution pattern (Garrido-Benavant et al., 2017;
361 Moniz et al., 2012).

362 ***Prasiola crispa* subsp. antarctica (Kützing) Knebel (WAP: GGV). Reported distribution:**
363 **Antarctic and the subAntarctic islands (Macquarie Island - South Georgia - SShs -**
364 **Antarctic Peninsula) - South America (Chile - Argentina).** Close match was found for a

365 single specimen formerly identified as *Prasiola sp.* with *P. antarctica* strain P31 from the
366 SSHs for the *tufA* gene (99.31%, GenBank Access Number KF993447, Suppl Mat 1). Exact
367 match for *rbcL* sequence was found with the same specimen of *P. antarctica* strain P31 from
368 the SSHs (100%, GenBank Access Number JQ669712, Suppl Mat 2). Moniz et al. (2012)
369 proposed the resurrection of *P. antarctica* as a true species. However this decision has not yet
370 been approved and *P. antarctica* is still considered as a synonym of *Prasiola crispa* in
371 AlgaeBase. Our sequence was thus named *Prasiola crispa* subsp. antarctica, after AlgaeBase
372 nomenclature. Our work expands the distribution of *Prasiola crispa* subsp. antarctica from the
373 SSHs (Moniz et al., 2012) down to the Gerlache Strait.

374 ***Rosenvingiella radicans* (Kützing) Rindi, L.McIvor & Guiry (SSHs: PRAT). Reported**
375 **distribution: North Europe (Britain - Ireland - Baltic Sea - France - Faroe Island -**
376 **Spain) - North America (California - Washington) - Arctic (White Sea) - Australia &**
377 **New Zealand - Argentina.** One specimen, formerly identified as *Blidingia minima* using
378 morphological characters, showed close match with *Rosenvingiella radicans* from Norway for
379 the *tufA* gene (98.02%, GenBank Access Number LN877834, Suppl Mat 1). *Rosenvingiella*
380 *radicans* has only been described in the Northern hemisphere. Another species of
381 *Rosenvingiella*, *R. simplex*, has been described along the coasts of King George Island
382 (Vinogradova, 1984). To the extent of our knowledge, no *tufA* sequence representing this
383 species has been deposited in public repositories, while 8 *rbcL* sequences are available from
384 Norway (GenBank Access Number LN877833 - LN877833 - AY694199:AY694204, Heesch
385 et al., 2016). Unfortunately, *rbcL* gene failed to amplify in the present study limiting further
386 identification.

387

388 **DISCUSSION AND CONCLUSION**

389 **New records of Chlorophyta in Antarctic waters**

390 Molecular data obtained for 86 specimens of our 122 Chlorophyta samples allowed detecting
391 twelve species including three new reports (*Rosenvingiella radicans*, *Urospora wormskioldii*
392 and *Ulvella islandica*) in the SSHs and WAP area. *Urospora wormskioldii* was previously
393 reported in the northern hemisphere close to the polar circle, along the coasts of Greenland,
394 Canada, Europe and East Russia (Guiry & Guiry, 2019; Figure 2), *Ulvella islandica* in Iceland
395 and *Rosenvingiella radicans* at mid-high latitudes in both hemispheres (Guiry & Guiry,
396 2019). A cosmopolitan distribution (except in the tropics, Guiry & Guiry, 2019) has been
397 reported for *Urospora penicilliformis*, a specie considered as common in the intertidal zone
398 and reported in the SSHs and WAP since first register in the middle of the 20th century (Lamb
399 & Zimmermann, 1977; Mystikou et al., 2014; Papenfuss 1964; Roleda et al., 2009; Wiencke
400 & Clayton, 2002). We reported here, for the first time, the presence of a second specie of
401 *Urospora*, *U. wormskioldii*, in Antarctica.

402 The present work improves the Chlorophyta genetic database in a region within which only a
403 few green macroalgae have been sequenced (Ulvophyceae: *Monostroma grevillei*, Pellizzari
404 et al. 2017; *Ulva sp.*: Khan et al., 2017; Trebouxiophyceae: *Prasiola crispa* and *Prasiola*
405 *antarctica*: Garrido-Benavant et al. 2017; Moniz et al. 2012). Lack of molecular data,
406 especially of sequences available in public repositories for comparison in barcoding studies,
407 has been identified as a clear limitation for studies focused on Antarctic algae (Dubrasquet et
408 al., 2018). Even if only a few species of green algae are reported in Antarctica, the use of
409 morphological characters without confirmation by molecular data could lead to confusion and
410 inaccuracy in assessing marine flora diversity. In the present study, even if only a few
411 sequences of Antarctic green algae were available in public repositories, identification match
412 with GenBank reference sequences were obtained with at least one of the two genetic markers
413 (i.e. *tufA* and *rbcL* genes) for each putative species, most of them with sequences from

414 specimens sampled in the northern hemisphere. These new *tufA* and *rbcL* data, including
415 sequences of common intertidal species such as *Acrosiphonia arcta*, *Monostroma hariotii*,
416 *Ulva* sp. A-GW and *Urospora penicilliformis* could help in Antarctic algae diversity long-
417 term monitoring. However, as in other polar areas (i.e. Alaska, Bringloe et al. 2019), assessing
418 the current state of marine flora diversity in Antarctica will imply important sampling effort in
419 order to include specimens from other non-glaciated coasts, such as East Antarctic coasts
420 located between 45°E and 160°E (Wiencke et al., 2014), to complete the information already
421 obtained for the SSHs and the WAP (Papenfuss, 1964; Ramirez, 2010; Wiencke & Clayton,
422 2002; Wiencke et al., 2014; present study). Our sampling strategy was limited by logistics of
423 the Antarctic campaigns and sampling restrictions: sampling effort happening only during
424 summer season, one sampling event in each region and scuba diving down to 30m (e.g. the
425 emblematic species *Lambia antarctica*, that generally live at greater depth as reported by
426 Wiencke et al., 2014, was not sampled in any of our five sites). However, our effort still
427 allowed us to sample and sequenced half of the reported species in the SSHs and WAP (12
428 over 24, Pellizzarri et al., 2017).

429 **Various Antarctic green algae are amphipolar species**

430 The use of molecular data allows a better understanding of marine flora diversity but also to
431 better define species biogeographic limits and study their evolutionary history. Recent studies
432 have focused on current diversity and distribution pattern of red (Billard et al., 2015;
433 Dubrasquet et al., 2018; Guillemain et al., 2018; Ocaranza-Barrera et al., 2019) and brown
434 (Peters et al., 1997; 2000) Antarctic algae. Cryptic species have been found in several well-
435 known and widely distributed red algae (Billard et al., 2015; Dubrasquet et al., 2018;
436 Guillemain et al., 2018) and in terrestrial green algae (DeWever et al., 2009), underlying the
437 limitation of taxonomic knowledge for these taxa.

438 For Antarctic species, as for the canopy forming brown algae *Desmarestia* spp. and the
439 common red algae *Gigartina skottsbergii*, divergence from species living outside of the
440 Antarctic waters has been estimated to date back some 10 Million years (Mya) (Billard et al.,
441 2015; Peters et al., 1997). As a result of long-time isolation from the rest of the marine realms,
442 red and brown Antarctic algae display a high percentage of endemic species nowadays (36%
443 and 44% respectively) and clear adaptations to Antarctic marine environment. However, some
444 cold-water species of *Desmarestia* (i.e *Desmarestia aculeata*, *D. viridis/confervoides*, Peters
445 et al., 1997; *D. viridis/willii*, VanOppen et al., 1993) have been reported in both cold Arctic
446 and Antarctic waters (note that *D. confervoides* is considered as synonymous of *D. willii*,
447 Guiry & Guiry. 2019). For Antarctic green algae, a much lower percentage of endemic
448 species has been recorded (18%, Wiencke and Clayton. 2002) and amphipolar distribution has
449 been observed for the common intertidal species *Acrosiphonia arcta* (VanOppen et al., 1993).
450 For both *D. viridis/willii* and *A. arcta*, amphipolar distribution has been related to recurrent
451 equator-barrier crossing during the cooling temperatures events of the Pleistocene (VanOppen
452 et al., 1993). The ability of early life stages to survive extreme temperatures is crucial when
453 considering a possible connection from pole to pole and phytogeographical patterns and
454 endemism levels are shaped by this physiological requirement (Bartsch et al., 2012 and
455 references therein). Gametophyte stages of *D. viridis/willii* and *A. arcta* present great
456 tolerance to warm temperatures (i.e. survival up to 26-27°C for *D. viridis/willii* and at least up
457 to 25°C for *A. arcta*; Peters and Breeman 1992; VanOppen et al, 1993). These two species
458 could have survived the passage of the tropics through deep-water dispersion of gametophyte
459 stages. Early life stages of several Antarctic marine green algae as *A. arcta*, *Ulva* sp. and *U.*
460 *penicilliformis* have been shown to present better tolerance to high temperatures (upper
461 survival temperature above 20°C) than endemic red or brown algae (upper survival
462 temperature between 11°C and 19°C, Wiencke & Dieck, 1990). In general, green algae

463 propagules have been shown to support long dark periods (e.g., *Ulva flexulosa*; Imchen, 2012)
464 and to be able to travel over very long distances on oceanic currents (more than hundreds of
465 kilometers; Watanabe et al., 2009) attached to rafting algae (Arroyo & Bonsdorff, 2016;
466 Macaya et al., 2016; Saunders, 2014). This could explain in part their success as invasive
467 species (e.g *Caulerpa taxifolia*: Arnaud-Haond et al., 2017; Fama et al., 2002; BellanSantini
468 et al., 1996; Smith & Walters. 1999; *Codium fragile* sp *fragile*: Watanabe et al., 2009) or the
469 high number of species with a reported amphipolar distribution. A recent study on
470 evolutionary history of the lichen-associated green algae *Prasiola crispa* complex species
471 proposed a combined theory of vicariance events associated with long distance deep-water
472 dispersal across the tropics during the Pleistocene for explaining their disjoint distribution in
473 both polar areas (Garrido-Benavant et al., 2017). Our findings confirm the existence of an
474 amphipolar distribution for *A. arcta*, *P. crispa*, *U. penicilliformis*, *U. wormskioldii*, *Ulva* sp.
475 *A-GW* and *Ulvella islandica* and show that amphipolar distribution seems to be much more
476 common in Antarctic green than red or brown algae. It could explain in part the low level of
477 endemism found in Antarctica for these studied taxa (Wiencke & Clayton, 2002).

478 **Conclusion**

479 Studies of species diversity including genetic data provide key information for correct
480 assessment of flora and fauna diversity in Antarctica, an area still difficult to access
481 (Dubrasquet et al., 2018; De Broyer & Danis, 2011; Grant & Linse, 2009; Leliaert et al.,
482 2014). Fast environmental changes have been reported in Antarctica, especially in the SShs
483 and the WAP, leading to increasing pressures and threats over the Antarctic biota (Chown et
484 al., 2015). Among them, introduction of non – native species associated to human activities
485 such as scientific research and tourism have been reported as an important threat (Broady et
486 al., 1994; McCarthy et al., 2019; Olech, 1996; Radulovici et al., 2010, Cardenas et al. 2020).
487 However, basic information is still lacking that could allow to properly monitor the timing

488 and magnitude of these arrivals. Quick detection of alien species settlement in Antarctic
489 waters could help building environmental recommendation for shipping, including tourism
490 and fishing activities. As these organisms present great dispersal potential and includes
491 several potential invaders, availability of genetic sequences in public depository for species
492 commonly found in the Western Antarctic Peninsula and the South Shetland Islands will help
493 to monitor the state of green Antarctic flora.

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506

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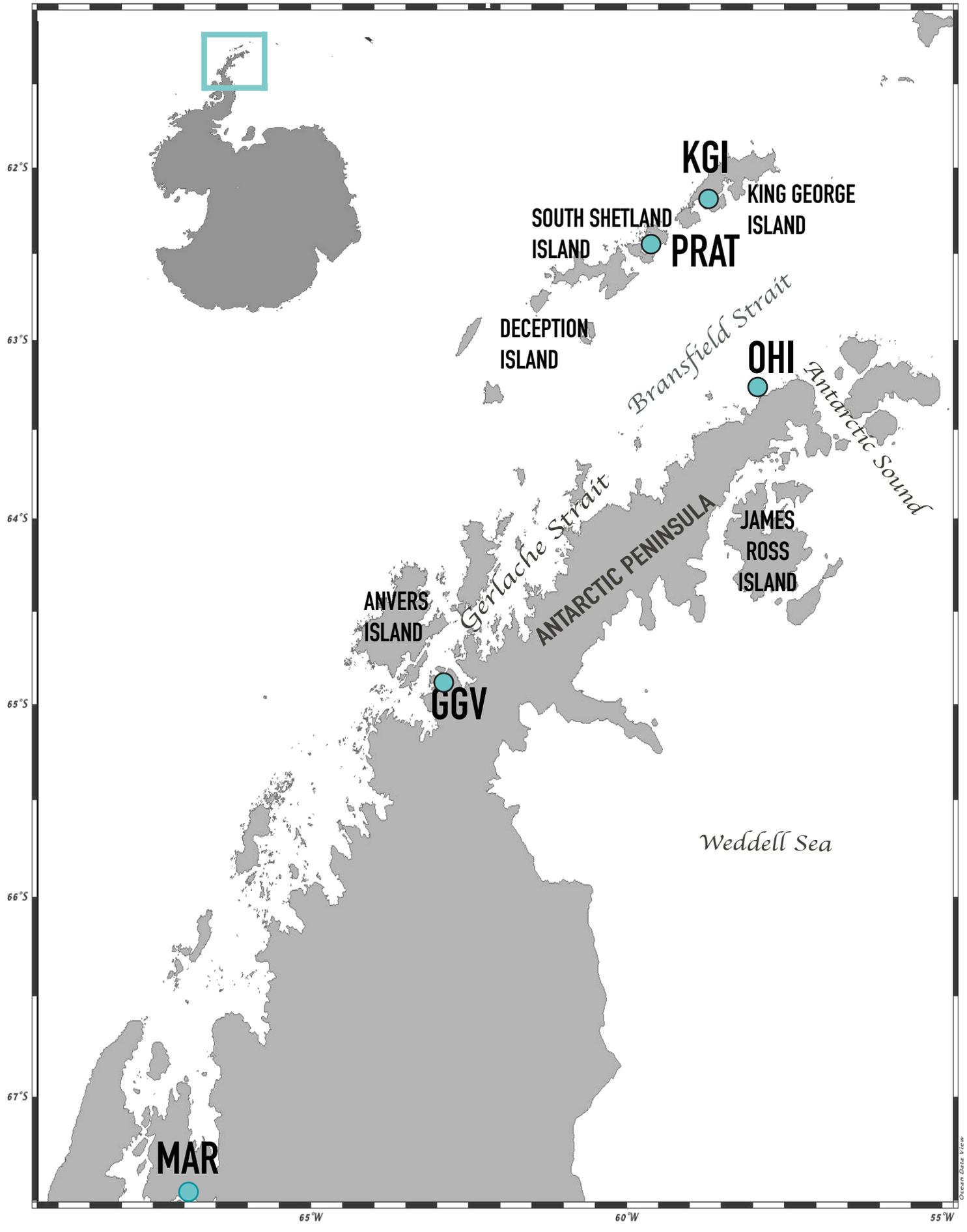
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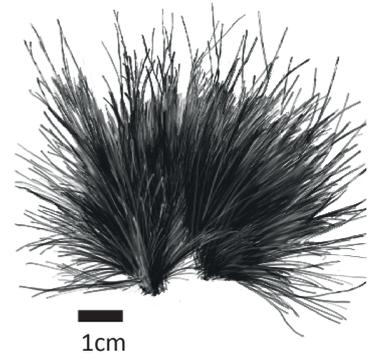
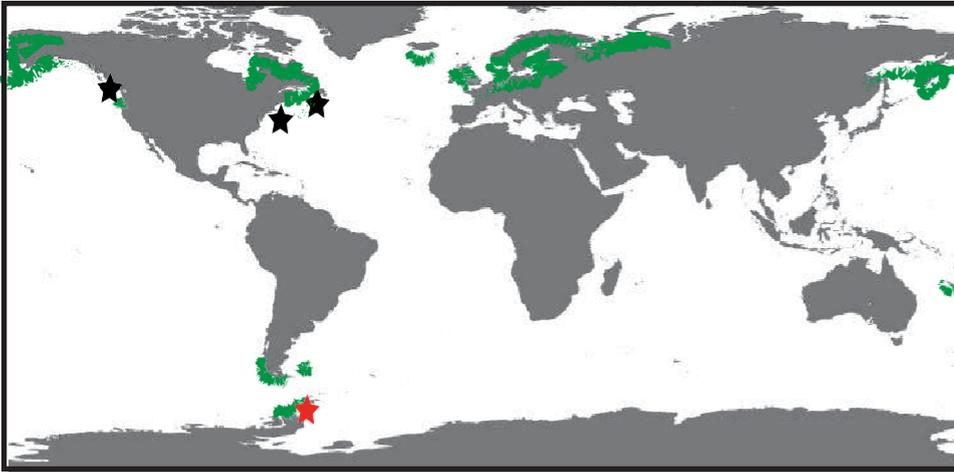
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774 FIGURES LEGEND

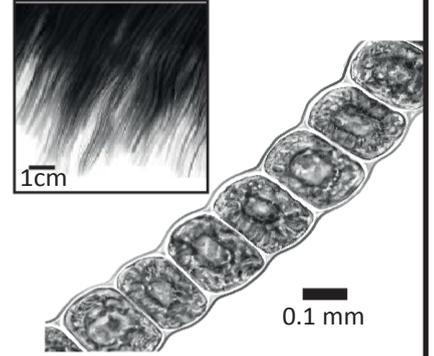
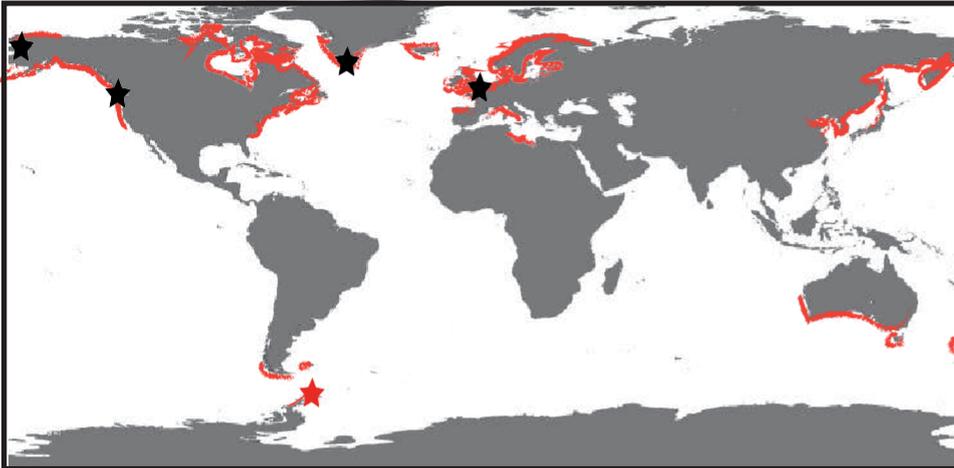
775 Figure 1: Map of the study area. Sampling Sites are shown as blue diamonds. Two sites are
776 located in the South Shetland Islands (KGI, King George Island; PRAT, Greenwich Island)
777 and three sites along the Western Antarctic Peninsula (OHI, Base O'Higgins; GGV, Paradise
778 Bay; MAR, Margarita Bay).

779 Figure 2: Amphipolar distribution of four species of Chlorophyta detected in the SSHs and/or
780 WAP during our study. General distribution (color lines) follow information given in
781 AlgaeBase repository (Guiry & Guiry, 2019). Genetic data (i.e., *tufA*) available for the
782 species are indicated with stars: red stars, present study; black stars, already published in
783 GenBankNCBI Database. All illustrations were kindly realized by Enzo Mardones, based on
784 photographs available in www.algaebase.com (for *Acrosiphonia arcta*),
785 www.seaweedssofaraska.com (for *Acrosiphonia arcta*, *Urospora wormskioldii*, *Urospora* sp.1
786 *penicilliformis*, *Ulva* sp A-GW). Cells illustrations for *Urospora wormskioldii* and *Urospora*
787 sp.1 *penicilliformis* were based on www.algaebase.com photographs available for *Urospora*
788 *penicilliformis* and *Urospora wormskioldii*. *Ulva* sp. A-GW illustration was based on *Ulva*
789 *linza* photography available at www.seaweedssofaraska.com/

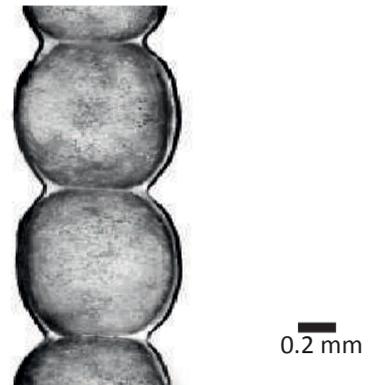




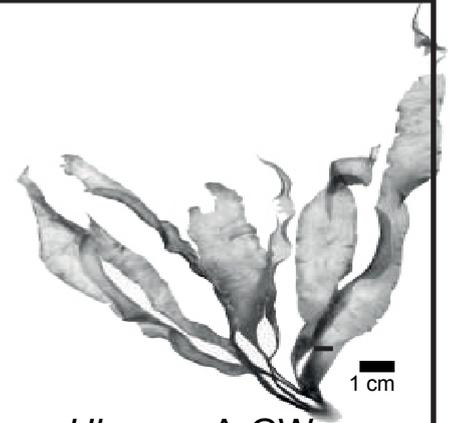
Acrosiphonia arcta



Urospora sp. 1 penicilliformis



Urospora wormskioldii



Ulva sp. A-GW

★ Existing genetic data

★ New genetic data

Supplementary table 1: Genbank (GB) Access Number for the *tufA* gene for green macroalgae specimens from the South Shetlands Islands and Western Antarctic Peninsula. Specie names are given following molecular assignation. Closest match with existing *tufA* sequence in Genbank data repository are given (percentage of similarity). References are given for closest match sequences. Specimens ID marked with * or ** correspond to subtidal samples (*sampling depth between 0-15m and ** sampling depth between 15-30m). Specimens ID written in bold correspond to samples for which *rbcl* sequences are available.

Specie name (AlgaeBase Current Accepted name)	Specimen ID	Sampling Area - Sampling Site	GB Access Number for <i>tufA</i> gene	Closest match for <i>tufA</i> in GB repository	References
Ulvophyceae					
<i>Acrosiphonia</i> (Dillwyn) Gain	<i>arcta</i> MLG-0234B	South Shetland Islands - Greenwich Island	MN145911	HQ610211 (98,31%) with <i>Acrosiphonia arcta</i> from British Columbia, Canada.	Saunders & Kucera. 2010
	MLG-0234C	South Shetland Islands - Greenwich Island	MN145912		
	MLG-0582A	Antarctic Peninsula - Paradise Bay	MN145913		
	MLG-0582B	Antarctic Peninsula - Paradise Bay	MN145914		
	MLG-0582C	Antarctic Peninsula - Paradise Bay	MN145915		
	MLG-0610A	Antarctic Peninsula - Paradise Bay	MN145916		
	MLG-0610B	Antarctic Peninsula - Paradise Bay	MN145917		
	MLG-0610C	Antarctic Peninsula - Paradise Bay	MN145918		
<i>Capsosiphon</i> sp.	MLG-0585	Antarctic Peninsula - Paradise Bay	MN145920	No close match was found for these specimens.	-
	MLG-0609	Antarctic Peninsula - Paradise Bay	MN145921		
	MLG-0612	Antarctic Peninsula - Paradise Bay	MN145922		
	MLG-0523	Antarctic Peninsula - Paradise Bay	MN145919		

<i>Monostroma hariotii</i> Gain	ANT-2004-2	South Shetland Islands - King George Island	MK507414	MG646366 (100%) with <i>Monostroma angicava</i> from King George Island, South Shetland Islands.	Khan et al. Unpublished.
	ANT-2004-3	South Shetland Islands - King George Island	MK507415		
	ANT-2005-1	South Shetland Islands - King George Island	MK507416		
	ANT-2005-2	South Shetland Islands - King George Island	MK507417		
	ANT-2005-3	South Shetland Islands - King George Island	MK507418		
	ANT-2005-4	South Shetland Islands - King George Island	MK507419		
	ANT-2009-2	South Shetland Islands - King George Island	MK507420		
	ANT-2134-1*	South Shetland Islands - King George Island	MK507421		
	ANT-2134-3*	South Shetland Islands - King George Island	MK507422		
	ANT-2134-4*	South Shetland Islands - King George Island	MK507423		
	ANT-2164*	South Shetland Islands - King George Island	MK507424		
	MLG-0032*	Antarctic Peninsula - O'Higgins	MK507425		
	MLG-0109*	Antarctic Peninsula - O'Higgins	MK507426		
	MLG-0124**	Antarctic Peninsula - O'Higgins	MK507427		
	MLG-0142	Antarctic Peninsula - O'Higgins	MK507428		
	MLG-0153	Antarctic Peninsula - O'Higgins	MK507429		
	MLG-0185	Antarctic Peninsula - O'Higgins	MK507430		
	MLG-0223*	South Shetland Islands - Greenwich Island	MK507431		
	MLG-0233	South Shetland Islands - Greenwich Island	MK507432		
	MLG-0241	South Shetland Islands - Greenwich Island	MK507433		
	MLG-0259*	South Shetland Islands - Greenwich Island	MK507434		
	MLG-0329B	South Shetland Islands - Greenwich Island	MK507435		
	MLG-0506A*	Antarctic Peninsula - Paradise Bay	MK507443		
	MLG-0506B*	Antarctic Peninsula - Paradise Bay	MK507444		
	MLG-0526C	Antarctic Peninsula - Paradise Bay	MK507445		
	MLG-0530*	Antarctic Peninsula - Paradise Bay	MK507436		
	MLG-0541	Antarctic Peninsula - Paradise Bay	MK507446		
	MLG-0550**	Antarctic Peninsula - Paradise Bay	MK507447		
	MLG-0564*	Antarctic Peninsula - Paradise Bay	MK507437		
	MLG-0575	Antarctic Peninsula - Paradise Bay	MK507438		
	MLG-0607	Antarctic Peninsula - Paradise Bay	MK507439		
	MLG-0617*	Antarctic Peninsula - Paradise Bay	MK507440		

<i>Monostroma hariatii</i> Gain	MLG-0645	Antarctic Peninsula - Marguerite Bay	MK507441	MG646366 (100%) with <i>Monostroma angicava</i> from King George Island, South Shetland Islands.	Khan et al. Unpublished.
	MLG-655**	Antarctic Peninsula - Marguerite Bay	MK507442		
	MLG-0666A*	Antarctic Peninsula - Marguerite Bay	MK507448		
	MLG-0666B*	Antarctic Peninsula - Marguerite Bay	MK507449		
	MLG-0680*	Antarctic Peninsula - Marguerite Bay	MK507450		
<i>Protomonostroma</i> sp. A- GW	MLG-0236A	South Shetland Islands - Greenwich Island	MN145890	MG646367 (99.87%) with <i>Protomonostroma</i> sp. AGW from King George Island, South Shetland Islands.	Khan et al. Unpublished
	MLG-0236B	South Shetland Islands - Greenwich Island	MN145891		
	MLG-0234A	South Shetland Islands - Greenwich Island	MN145889		
<i>Ulva</i> sp. A-GW	MLG-0524	Antarctic Peninsula - Paradise Bay	MN145923	MG646368 (100%) with <i>Ulva</i> <i>sp. A-GW</i> from King George Island, South Shetlands Islands.	Khan et al. Unpublished
	MLG-0543A	Antarctic Peninsula - Paradise Bay	MN145924		
	MLG-0543B	Antarctic Peninsula - Paradise Bay	MN145925		
	MLG-0581B	Antarctic Peninsula - Paradise Bay	MN145926	KM254999 (98.83%) with <i>Ulva</i> sp. A-GW from British Columbia, Canada.	Saunders. 2014
	MLG-0583B	Antarctic Peninsula - Paradise Bay	MN145901		
	MLG-0608B	Antarctic Peninsula - Paradise Bay	MN145927		
	MLG-0608C	Antarctic Peninsula - Paradise Bay	MN145928		
	MLG-0729A	Antarctic Peninsula - Marguerite Bay	MN145930		
	MLG-0647	Antarctic Peninsula - Marguerite Bay	MN145929		
<i>Ulvella islandica</i> R.Nielsen & K.Gunnarsson 2014	MLG-0249	South Shetland Islands - Greenwich Island	MN145931	KF444924 (98,12%) with <i>Ulvella islandica</i> from Iceland	Nielsen et al. 2014
				JQ303009 (96,54%) with <i>Ulvella reticulata</i> from cultured specimen.	Nielsen et al. 2013

<i>Ulvella islandica</i> R.Nielsen & K.Gunnarsson 2014	MLG-0249	South Shetland Islands - Greenwich Island	MN145931	JQ303013 (95.07%) with <i>Ulvella leptochaete</i> from culture specimen (see References).	Nielsen et al. 2013
				EF595286 (95,61%) with <i>Ulvella viridis</i> from U.K.	Rinkel et al. 2012
<i>Urospora</i> sp. 1 <i>penicilliformis</i>	MLG-0226	South Shetland Islands - Greenwich Island	MN145895	MH571163 (100%) with	Bringloe et al. 2019
	MLG-0238	South Shetland Islands - Greenwich Island	MN145896	<i>Urospora</i> sp. 1	
	MLG-0291	South Shetland Islands - Greenwich Island	MN145897	<i>penicilliformis</i> from Nome, Alaska.	
	MLG-0314	South Shetland Islands - Greenwich Island	MN145898		
	MLG-0316	South Shetland Islands - Greenwich Island	MN145899		
	MLG-0412	Antarctic Peninsula - Paradise Bay	MN145900	HQ610440 (99,48%) with	Saunders & Kucera. 2010
	MLG-0584	Antarctic Peninsula - Marguerite Bay	MN145902	<i>Urospora</i> sp. 1 <i>penicilliformis</i>	
	MLG-0644	Antarctic Peninsula - Marguerite Bay	MN145903	from Canada.	
	MLG-0676	Antarctic Peninsula - Marguerite Bay	MN145904		
	MLG-0677	Antarctic Peninsula - Marguerite Bay	MN145905		
	MLG-0709	Antarctic Peninsula - Marguerite Bay	MN145906		
	MLG-0724	Antarctic Peninsula - Marguerite Bay	MN145907		
	MLG-0726	Antarctic Peninsula - Marguerite Bay	MN145908		
	MLG-0746	Antarctic Peninsula - Marguerite Bay	MN145909		
<i>Urospora wormskioldii</i> (Mertens) Rosenvinge	MLG-0145	Antarctic Peninsula - O'Higgins	MN145892	HQ610441 (99.87%) with <i>U.</i>	Saunders & Kucera. 2010
	MLG-0148	Antarctic Peninsula - O'Higgins	MN145893	<i>wormskioldii</i> from British	
	MLG-0181	Antarctic Peninsula - O'Higgins	MN145894	Colombia, Canada.	
Unknown specimen of <i>Ulvaes</i>	MLG-0577	Antarctic Peninsula - Paradise Bay	MN145910	EF595318 (96,10%) with <i>Ulvaes</i> sp. 6 BER from U.K.	Rinkel et al. 2012

Trebouxiophyceae

<i>Prasiola crisa</i> (Lightfoot) Kützing	MLG-0150 MLG-0538	Antarctic Peninsula - O'Higgins Antarctic Peninsula - Paradise Bay	MN145932 MN145934	KF993450 (100%) with <i>Prasiola crisa</i> strain P43 from King George Island, South Shetland Islands.	Moniz et al.2012
	MLG-0189	Antarctic Peninsula - O'Higgins	MN145933	LN877821 (100%) with <i>Prasiola crisa</i> from Billefjorden, Svalbard.	Heesh et al. 2016
<i>Prasiola crisa</i> subsp. <i>antarctica</i> (Kützing) Knebel	MLG-0576	Antarctic Peninsula - Paradise Bay	MN145935	KF993447 (99,31%)with <i>Prasiola antarctica</i> strain P31 from King George Island, South Shetland Islands.	Moniz et al. 2012
<i>Rosenvingiella radicans</i> (Kützing) Rindi, L.Mclvor & Guiry.	MLG-0313	South Shetland Islands - Greenwich Island	MN145936	LN877834 (98.02%) with <i>Rosenvingiella radicans</i> from Nordland, Norway.	Heesh et al. 2016

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Supplementary table 2: Genbank (GB) Access Number for the *rbcL* gene for green macroalgae specimens from the South Shetlands Islands and Western Antarctic Peninsula. Specie names are given following molecular assignation. All specimens were collected in the intertidal zone. Closest match with existing *rbcL* sequence in Genbank data repository are given (percentage of similarity). References are given for closest match sequences deposited in Genbank. Specimens ID written in bold correspond to samples for which *tufA* sequences are available.

Specie Name	Specimen ID	Sampling Area - Sampling Site	GB Access Number for <i>rbcL</i> gene	Closest match with <i>rbcL</i> in GB repository	References
Ulvophyceae					
<i>Capsosiphon</i> sp.	MLG-0523	Antarctic Peninsula - Paradise Bay	MN164670	NC_039920 (98.53%) with <i>Capsosiphon fulvescens</i> plastid complete genome from South Korea.	Kim et al., 2018. In Press.
	MLG-0609	Antarctic Peninsula - Paradise Bay	MN164671		
	MLG-0612	Antarctic Peninsula - Paradise Bay	MN164672		
<i>Protomonostroma</i> sp. A-GW	MLG-0236A	South Shetland Islands - Greenwich Island	MN164665	MG711514 (100%) with <i>Protomonostroma</i> sp. A-GW from King George Island, South Shetland Islands.	Khan et al., Unpublished.
<i>Ulva</i> sp. A-GW	MLG-0317	South Shetland Islands - Greenwich Island	MN164676	MG711515 (100%) with <i>Ulva</i> sp. A-GW from King George Island, South Shetland Islands.	Khan et al., Unpublished.
	MLG-0390D	South Shetland Islands - Greenwich Island	MN164674		
	MLG-0413A	South Shetland Islands - Greenwich Island	MN164677		
	MLG-0729A	Antarctic Peninsula - Marguerite Bay	MN164678		
	MLG-0608A	Antarctic Peninsula - Paradise Bay	MN164675		

<i>Urospora</i> sp.1 <i>penicilliformis</i>	MLG-0226 MLG-0677	South Shetland Islands - Greenwich Island Antarctic Peninsula - Marguerite Bay	MN164666 MN164667	HQ603674 (100%) with <i>Urospora</i> sp. 1 <i>penicilliformis</i> from British Columbia, Canada.	Saunders & Kucera., 2010
<i>Urospora</i> <i>wormskioldii</i> (Mertens) Rosenvinge	MLG-0148 MLG-0181	Antarctic Peninsula - O'Higgins Base Antarctic Peninsula - O'Higgins Base	MN164668 MN164669	HQ603676 (99.85%) with <i>Urospora wormskioldii</i> from British Columbia, Canada.	Saunders & Kucera., 2010
Trebouxiophyceae					
<i>Prasiola crispa</i> (Lightfoot) Kützing	MLG-0189	Antarctic Peninsula - O'Higgins	MN164679	KR017748 (99.85%) with <i>Prasiola crispa</i> from Antarctica (unknown location).	Carvalho et al., 2015.
<i>Prasiola crispa</i> subsp. <i>antarctica</i> (Kützing) Knebel	MLG-0576	Antarctic Peninsula - Paradise Bay	MN164680	JQ669712 (100%) with <i>Prasiola antarctica</i> from Amsler Island, Antarctic Peninsula.	Moniz et al., 2012.

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