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2

3 **Seaweed biodiversity in the south-western Antarctic Peninsula: Surveying**  
4 **macroalgal community composition in the Adelaide Island / Marguerite Bay**  
5 **region over a 35-year time span**

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21

22 **Abstract**

23 The diversity of seaweed species of the south-western Antarctic Peninsula region is poorly  
24 studied, contrasting with the substantial knowledge available for the northern parts of the  
25 Peninsula. However, this is a key region affected by contemporary climate change. Significant  
26 consequences of this change include sea ice recession, increased iceberg scouring, and increased  
27 inputs of glacial melt water, all of which can have major impacts on benthic communities. We  
28 present a baseline seaweed species checklist for the southern Adelaide Island and northern  
29 Marguerite Bay region, combining data obtained during a small number of surveys completed in  
30 1973-5 and a six week intensive diving-based field campaign in 2010-2011. Overall, with a total  
31 of 41 macro-algal species recorded (7 brown, 27 red, 6 green, 1 chrysophyte), the region is  
32 species-poor compared to the north of the Antarctic Peninsula, and even more so in comparison  
33 with the sub-Antarctic. The key canopy-forming species is *Desmarestia menziesii*, which is  
34 abundant in Antarctic Peninsula waters, but lacking in the sub-Antarctic. *Himantothallus*  
35 *grandifolius*, which is a common species further north in the Antarctic phytobenthos, was absent  
36 in our recent collections. This paper also reports the first record of *Aplanochytrium* sp.  
37 (Labyrinthulomycetes) from this part of Antarctica and in association with *Elachista* sp..

38

39 **Keywords**

40 *Aplanochytrium* sp., climate change, *Desmarestia menziesii*, marine macroalgae,  
41 maritime Antarctic, ice recession

## 42           **Introduction**

43           Seaweeds, in particular brown algae, are the major primary producers in temperate and  
44 polar rocky inshore environments. They are important contributors to global biogeochemical  
45 cycles, for instance through the transfer of iodine from the marine environment to the  
46 atmosphere and the land (Küpper et al. 2011). Compared to the sub-Antarctic region, the  
47 Antarctic is generally considered depauperate in terms of seaweed species diversity (Wiencke  
48 and Clayton 2002). Pioneering studies of Antarctic seaweed biodiversity, taxonomy and  
49 biogeography were conducted over a century ago by Skottsberg (1907), with a recent synopsis  
50 provided by Wiencke and Clayton (2002). Polar seaweeds show adaptations enabling survival in  
51 temperatures around freezing, and of months of winter darkness (Wiencke et al. 2009). In clear  
52 contrast to temperate and tropical bioregions, polar regions are characterized by an intertidal  
53 almost devoid of seaweeds. This is due to the extreme environmental conditions in the intertidal  
54 zone – with temperature extremes ranging from -50 to +5°C (Peck et al. 2006; Waller et al. 2006)  
55 and strong impacts of abrasion by sea ice (Barnes and Souster 2011; Barnes et al. 2014).  
56 Remarkably, the Antarctic phytobenthos has no representatives of the Laminariales, which are  
57 present in Arctic and all other cold and cold-temperate bioregions of the world. Instead, their  
58 ecological niche and role, as canopy providers, is largely fulfilled by members of the  
59 Desmarestiales (Moe and Silva 1977).

60           Climate change is altering parts of the Antarctic and Arctic faster than any other region  
61 on Earth. In the Antarctic, this applies particularly to the Antarctic Peninsula, where major  
62 changes have been observed in only the last 20-50 years (Meredith and King 2005; Turner et al.  
63 2009, 2013; Convey et al. 2009). Changes in the physical environment are characterized by  
64 increasing temperatures, receding sea ice cover and increased iceberg scouring of the inshore  
65 seabed caused by the combination of increased calving of shelf ice and glaciers coinciding with  
66 resulting icebergs being less restrained by sea ice (Barnes and Souster 2011; Barnes et al. 2014).  
67 Population expansions of alien microbes, fungi, plants and animals have been recorded in sub-  
68 Antarctic and Antarctic areas, although most documented examples are from the terrestrial  
69 environment (Frenot et al. 2005; Greenslade et al. 2012; Molina-Montenegro et al. 2012).  
70 Southward range expansion into previously inaccessible or uninhabitable areas of the Antarctic  
71 has been documented for some penguins (Lynch et al. 2012) and has been highlighted as a likely

72 scenario for toxic cyanobacteria (Kleinteich et al. 2012). So far it is not clear whether, or to what  
73 extent, this also applies to sub-Antarctic and Antarctic seaweeds, but it is reasonable to  
74 hypothesize that such changes in distribution will occur in the foreseeable future.

75 In this study, we have revisited the south-eastern Adelaide Island area, which has been  
76 much less studied in terms of seaweed diversity than the more northern regions of the Antarctic  
77 Peninsula. While numerous phycological investigators (DeLaca and Lipps 1976; Moe and De  
78 Laca 1976; Quartino et al. 2001; Wiencke and Clayton 2002; Oliveira et al. 2009) have worked  
79 in particular around King George Island and Anvers Island since the 1960s, and the region of  
80 Adelaide Island is well studied for other marine biota (Barnes and Brockington 2003; Smale et  
81 al. 2007), little consideration has been given to the seaweeds of the latter. In this respect, the  
82 work of Moe and DeLaca (1976) stands out in its extensive coverage of the western Antarctic  
83 Peninsula over a wide latitudinal gradient, including an unsurpassed number of study sites, and  
84 its relatively long duration. However, even though this remains the most comprehensive survey  
85 of the phytobenthos of the western Antarctic Peninsula to date, this study includes 24 recorded  
86 taxa from only three dives in the Adelaide Island / Marguerite Bay area.

87 Here we present the results of a six week diving-based field campaign in the vicinity of  
88 Rothera Point (south-eastern Adelaide Island) in 2010-2011, integrating our data with that of  
89 Moe and DeLaca (1976). The main objective of this work was to establish an inventory for this  
90 region, where currently little knowledge about seaweed biodiversity exists. This will provide  
91 important baseline data for future biogeographical and comparative studies. Given that  
92 eukaryotic pathogens have been documented for most marine bioregions outside Antarctica (e.g.  
93 Strittmatter et al. 2009) and considering their potentially significant impact on seaweed ecology  
94 (Küpper and Müller 1999; Gachon et al. 2010), the seaweed survey presented here is  
95 complemented by the first ever such survey of filamentous brown algae for such pathogens in  
96 Antarctica.

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99 **Material and Methods**

100 Nine sites were surveyed in the vicinity of the British Antarctic Survey's Rothera  
101 Research Station (Adelaide Island): Anchorage Island, Biscoe Wharf, Cheshire Island, Hangar  
102 Cove, Honeybucket, Lagoon Island, Léonie Island, Shack's Crack and South Cove (Fig. 1). A  
103 total of 17 scuba dives (duration 10-52 min, maximum depth 35.6 m) were conducted at all of  
104 these sites. Destructive purposive sampling took place along the full depth profile (0 – 35m). For  
105 safety reasons due to the presence of leopard seals, snorkeling was not permitted.

106 Immediately following each day of diving herbarium specimens were prepared by  
107 mounting seaweed thalli on Bristol paper (Online Resource 1), or samples were fixed as  
108 permanent mounts on microscope slides, using acetocarmine (to preferentially stain for  
109 pathogens) and 50% Karo Syrup™ and subsequently sealed with nail polish once dried (Küpper  
110 and Müller 1999). They were deposited in the herbarium of the British Antarctic Survey (BAS,  
111 Cambridge, UK). Fragments of all specimens were kept in silica gel or CTAB buffer (Phillips et  
112 al. 2001), both of which conserve DNA for further molecular studies. Filamentous brown algae  
113 were surveyed for eukaryotic pathogens as described previously (Küpper and Müller 1999;  
114 Strittmatter et al. 2013).

115 Given the limited time and logistic constraints at these remote locations, inevitably  
116 leading to a limited coverage of the smaller representatives of the flora, collections of seaweed  
117 specimens were supplemented by collections of substratum samples in sterile tubes. Following  
118 return to Europe and based upon a protocol developed for a similar study in the Juan Fernandez  
119 Islands (Müller and Ramirez 1994), these samples were incubated in Provasoli-enriched sea  
120 water (Starr and Zeikus 1993) under light and temperature regimes corresponding to their region  
121 of origin. Over approximately 3 months, they were monitored for algal outgrowth, from which  
122 unialgal isolates were made. Isolates were characterized and identified, both morphologically  
123 using a Zeiss PrimoVert™ inverted microscope and a Zeiss Axio Imager.D2™ compound  
124 microscope (Online Resource 2), and by DNA sequencing and comparison with published data.  
125 The isolates have been deposited in the Culture Collection of Algae and Protozoa (CCAP, Oban).

126 DNA extractions were performed using CTAB buffer as described previously (Gachon et  
127 al. 2009). Polymerase chain reactions (PCR) were performed to amplify a fragment of nuclear  
128 ribosomal DNA containing 3'-SSU, ITS1, 5.8S, ITS2 and 5'-LSU, using the primer pair ITS-

129 ITSPI/KIRI, ITSP1 (5' GGAAGGAGAAGTCGTAACAAGG 3'; Tai et al. 2001) and KIR1 (5'  
130 TTCAAAGTTTTGATGATT 3'; Lane et al. 2006), was used. PCR was carried out with an  
131 initial denaturation at 94°C for 5 min, followed by 40 cycles of amplification consisting of  
132 denaturation at 94°C for 30 sec, annealing at 45°C for 30 sec, and elongation at 72°C for between  
133 1 min. The 40 cycles were followed by a final extension at 72°C for 5 min. PCR amplification  
134 was performed in a total volume of 25 µL, containing 1.25 units of Taq DNA Polymerase  
135 (Promega), 1x GoTaq Buffer, 5mM MgCl<sub>2</sub>, 0.5mM dNTPs, 0.3mM of each primer and 1µL of  
136 template DNA. The alignment of each DNA sequence was conducted with the BioEdit Sequence  
137 Alignment Editor™ (Hall 1999). For identifying taxa, sequences were compared to published  
138 data by means of NCBI BLAST searches (Altschul et al. 1997).

139 Identification of herbarium specimens and live cultures was conducted (Online Resource  
140 3) using available keys, in particular that of Wiencke and Clayton (2002). For present-day  
141 taxonomic and nomenclatural aspects AlgaeBase (Guiry & Guiry 2013) was consulted.  
142 Taxonomic details of species recorded by Moe and DeLaca (1976) have been updated (Table 1,  
143 see also Moe and Silva 1981; Moe 1986; Hommersand et al. 2009; Lin et al. 2012).

144 Our study also used diversity data obtained in 1975 at three sites in the region of the  
145 2010-2011 sampling points, also sampled by scuba diving (maximum depth 33 m) (Moe and  
146 DeLaca 1976; Online Resource 4 ). These were Henkes Island (off the southern tip of Adelaide  
147 Island), Horseshoe Island and Square Bay (Fig. 1).

148 Affinities of seaweed species composition in the three sites that were sampled by Moe  
149 and DeLaca in 1975 (Henkes Island, Horseshoe Island and Square Bay) and the seven sites of the  
150 current study (Anchorage Island, Biscoe Wharf, Cheshire Island, Hangar Cove, Honey-bucket,  
151 Shack's Crack and South Cove) were compared using the Sørensen similarity index (Sørensen  
152 1948).

153 Permanent mounts of filamentous algae, prepared at Rothera were surveyed after the  
154 expedition using a ZEISS Axio imager D2™ compound microscope at magnifications of 40-  
155 1000x, in search of novel pathogens and saprotrophs and imaged using Zeiss Zen 2011™ image  
156 processing software. Upon identification of organisms of interest, cultures were subjected to  
157 morphological examination, using a Zeiss Primo Vert™ inverted microscope initially to inspect

158 cultures and then by creating wet slides for investigation using the aforementioned compound  
159 microscope to try to reveal the affinities of these organisms.

160 Cultures which revealed pathogenic / saprotrophic organisms were also investigated  
161 molecularly with the SSU rRNA of existing DNA extractions being amplified using the primer  
162 pair ALG1 & ALG8 (Moro et al. 2003). The resulting amplicon was then ligated into the pJet™  
163 cloning vector following the protocol of the CloneJet™ PCR cloning kit (ThermoScientific) and  
164 transformed into competent *Escherichia coli* cells (ActivMotif™) using the supplied protocol,  
165 through heat shock utilizing a water bath. These cells were then plated onto LB media<sup>+Ampicillin</sup>  
166 and left at 37°C overnight according to the manufacturer's instructions. Single colonies were  
167 picked and placed into a colony PCR using the pJet Forward™ and pJet Reverse™ sequencing  
168 primers. The PCR reaction was made up of 1x PCR buffer, 1.5 mM MgCl<sub>2</sub>, 8 mM dNTPs, 0.2  
169 mM primers and half a unit of GoTaq™ (Promega) in a 20 µl reaction, ran for 30 cycles (95°C-  
170 30s, 60°C-30s, 72°C-60s) with an initial 95°C denaturation step for 3 minutes. No final  
171 extension step was employed. A 5 µl aliquot was then run on a 1% (w/v) agarose gel and a single  
172 reaction was purified using the GeneJet™ PCR purification kit and sent for sequencing using the  
173 Eurofins Value Read sequencing service, with primers ALG1 and ALG8, to obtain the brown  
174 algal SSU rRNA sequence. Following the tentative identification of *Aplanochytrium* sp., this  
175 sequence was placed in an alignment with Labyrinthulomycete sequences and restriction enzyme  
176 sites were located and assessed for conservations with the members of the labyrinthulomycetes.  
177 *PleI* (New England Biolabs) was then used to digest 5µl of the colony PCR product following  
178 the manufacturers guidelines (37°C 1hr) and representatives of each restriction pattern were sent  
179 for sequencing using the primers ALG1, ALG8 and internal sequencing primers F706 (5'-  
180 TGTTGTCTCCAGCCATCC -3') and R796 (5'- ATTTTGGTCTCCAACGAGG -3').  
181 Acquired ABI files were checked for quality, trimmed and aligned with one another using  
182 Bioedit (Hall 1999). A consensus sequence was then produced and the sequence was imported  
183 into an alignment, in MEGA 6.0, containing several members of the Labyrinthulomyceteye class,  
184 specifically *Aplanochytrium* sp., *Oblongichytrium* sp. and *Thraustochytrium* sp., the accession  
185 numbers of sequences contained within the alignment can be found on the resulting cladogram  
186 (Fig. 2). *Aplanochytrium minuta* is listed in the NCBI database *Labyrinthuloides minuta*  
187 (L27634; Leander et al. 2004), the species name label was therefore changed in the alignment.

188 The cladogram was produced by firstly using the ClustalW alignment tool available in MEGA  
189 6.0 (Tamura et al. 2013) and manually checking the alignment to ensure parsimony. The  
190 alignment was then tested with a Tamura-Nei Maximum Likelihood model, with a Nearest  
191 Neighbour Interchange heuristic model. Gaps/missing data with a site coverage above 95% were  
192 treated as partial deletions and 1000 bootstraps were used as a test of phylogeny.

193

## 194 **Results**

195 All data on species encountered are provided in Table 1. A total of 110 macroalgal  
196 samples were collected, augmented by 3 live isolates from substratum samples. Among the 24  
197 species recorded in the vicinity of Rothera Point during the 2010-2011 field season (Table 1), six  
198 were Phaeophyceae (brown algae), 12 Rhodophyta (red algae), five Chlorophyta (green algae)  
199 and one Chrysophyceae (golden algae). Two taxa of Chlorophyta were only identified among the  
200 three live isolates obtained from substratum samples (confirmed by both morphological and  
201 molecular approaches), and constitute new records for this region.

202 Sørensen's Similarity Index (Table 3) showed very low overlap in species composition of  
203 the communities sampled in the current study and those sampled in 1975. The highest similarity  
204 that was recorded between the two campaigns was at Henkes Island (1975) and South Cove  
205 (2010-2011) with 3 shared species, *Desmarestia menziesii*, *Plocamium cartilagineum* and  
206 *Trematocarpus antarcticus*, and a similarity index value of 0.18. In contrast, the highest  
207 similarity between the sampled areas in this study (2010-2011) was observed between  
208 Honeybucket and South Cove with 9 shared species and a similarity index value of 0.69, but also  
209 between Cheshire Island and South Cove with 9 shared species and a similarity index of 0.6.

210 A microscopic survey of filamentous brown algae (226 x *Pylaiella* sp., 58 x  
211 *Geminocarpus* sp., 1 x *Elachista antarctica*) did not reveal any unambiguous symptoms of  
212 eukaryotic pathogens, even though in several instances structures reminiscent of early-stage  
213 infections of *Eurychasma dicksonii* or *Anisolpidium* sp. were observed. Observations of  
214 permanent mounts of *E. antarctica* revealed single cells, not of algal origin, attached to the  
215 surface of algal filaments. Dimensions of the cells are approximately 35 µm in diameter. This,  
216 together with other morphological features comparable to previous reports of the  
217 labyrinthulomycete class (Moro et al. 2003; Damare and Raghukumar 2006) such as the presence



218 of an ectoplasmic net (Fig. 2 B, arrowed), which does not enrobe the cell (i.e. *Labyrinthula* sp.;  
219 Leander et al. 2004), led to the tentative identification of the organism as an *Aplanochytrium*. and  
220 is seen to attach the cells to the brown algal filament. Evidence for the association of this cell  
221 with the brown algal filament includes the observation that the cell was not washed away during  
222 the creation of permanent mounts, something that occurs to small organic matter that is not  
223 attached to the main body of the filament during permanent mount preparation. Due to the nature  
224 of the observations (i.e. within a permanent mount) the investigation of cellular movement along  
225 the ectoplasmic net and spore generation was not possible. Whether the processing of this  
226 material to permanent mounts has any effect upon the dimensions of the *Aplanochytrium*  
227 cell/ectoplasmic net is unknown. A 1635 base pair SSU rRNA sequences was successfully  
228 obtained from the organism under study here, which is shown to branch within the  
229 *Aplanochytrium* clade (94/100). The specimen appears to be a basal species of this genus, sitting  
230 on a long branch at an equal distance from all other *Aplanochytrium* sp. (97/100) (Fig. 3). The  
231 cladogram has been coded to allow easy interpretation of linkage between species. From this it  
232 can be noted that the substrate of the *Aplanochytrium* specimen can be a good indicator of its  
233 relations with other species, yet this new specimen, does not appear to have any close affinities  
234 to *Aplanochytrium stocchinoi* previously isolated from Antarctica or *Aplanochytrium* sp. PR1-  
235 1 (*A. minuta*) previously isolated from brown algae.

236

## 237 **Discussion**

238 ***Seaweed biodiversity.*** The Antarctic is generally known for its low diversity of marine  
239 algae, attributed to the presence of sea ice and icebergs for much of the year. Comparison of the  
240 records from 2010-2011 with the previous 1975 study (Moe and DeLaca 1976) reveals a number  
241 of new records for this part of the Antarctic Peninsula, both at species and genus level (18  
242 species and 14 genera; Table 1). The new records include four brown, eight red, five green and  
243 one golden algae. Seven species were observed in both sampling campaigns, separated by 35  
244 years, while 18 species were only observed in 1975 and 18 species were only observed in 2010-  
245 2011.

246 Only three species in total grew in the incubated substratum samples in which common  
247 Antarctic species, particularly gametophytes of *Desmarestia*, were missing. It is possible that the

248 latter and other particularly temperature-sensitive Antarctic endemics (Wiencke and Tom Dieck  
249 1989, 1990; Wiencke et al. 1994) did not survive the conditions during transport to the European  
250 laboratory. The fact that two Chlorophyte taxa were not seen macroscopically *in situ* but  
251 emerged from incubated substratum samples underlines the value of isolation / culturing work to  
252 underpin macroalgal biodiversity surveys especially in remote regions and demonstrates that the  
253 taxa were present at least as propagules if not as full-grown thalli. For one of these isolates, the  
254 most similar available ITS1 sequence (*Ulvella leptochaete*) had only 82% similarity, and future  
255 studies on the variability of ITS1 in these microscopic taxa may show whether it rather belongs  
256 to a related species. The second green alga and the brown alga were clearly identified to species  
257 level, as their ITS1 sequences were highly similar to previously sequenced specimens (Table 2).  
258 Confidence in molecular identification of these samples is high since all taxa had been collected  
259 and sequenced before from localities outside Antarctica. These sequences identities were then  
260 strongly correlated with morphological characters, ensuring that no doubt remains over the  
261 identities subscribed here.

262         The datasets available at the current time are clearly not sufficiently robust to support  
263 speculation on whether the largely non-overlapping data obtained in the two surveys are  
264 representative of genuine differences in diversity between the sampled areas or of any response  
265 to environmental changes in the general region. It has to be highlighted that due to logistical  
266 reasons, the sampling sites in 2010-2011 were not the same as those surveyed in the region in  
267 1975, and there is also a lack of detailed information on habitat conditions at any of these  
268 locations. As potential explanations we propose the following hypotheses: (1) limited range and  
269 number of surveys (especially in 1975, when only 3 dives were conducted in this region); (2)  
270 large variation between sites; (3) local loss of species observed in 1975, and replacement by the  
271 species found in the current study. Lack of both baseline and repeat survey data are increasingly  
272 recognized as a fundamental impediment to Antarctic biodiversity and biogeographical research  
273 (Convey 2011; Convey et al. 2012). In this context, the combined records of both campaigns  
274 presented here represent a useful dataset and checklist for future comparative studies aimed at  
275 assessing the impact of climate or other changes on benthic communities. For most regions of the  
276 world, there are few historic datasets of seaweed biodiversity (e.g. Asensi and Küpper 2012). In

277 this context, the value of records such as those of Lamb and Zimmerman (1977) and Moe and  
278 DeLaca (1976) for the Antarctic Peninsula cannot be overestimated.

279 ***Pathogenic and saprotrophic organisms on Antarctic seaweeds.*** The question as to  
280 whether eukaryotic pathogens occur in Antarctica in epidemic outbreaks similar to those reported  
281 from temperate latitudes (Küpper and Müller 1999; Strittmatter et al. 2013) cannot be  
282 conclusively answered as no pathogens were observed – however, it is well known that such  
283 outbreaks are sporadic (Küpper and Müller 1999) and the period of the survey may have been  
284 too short. Instead further sampling at other sites and during other seasons should be seen as an  
285 important step to unveiling the potential role that algal pathogens play in Antarctic seaweed  
286 ecology.

287 Significant to this study is the finding of a presumed saprotrophic *Aplanochytrium*  
288 species upon *E. antarctica*. This genus diagnosis is completed by the morphological  
289 characteristics presented here, with the presence of an ectoplasmic net (Fig. 2), not encasing the  
290 spore, being the defining feature of this genus from other members of the labyrinthulomycte  
291 class (Leander and Porter 2001, Leander et al. 2004). Though members of the genus  
292 *Aplanochytrium* have been previously recorded from Antarctica (Moro et al. 2003) and upon a  
293 brown alga (Leander et al. 2004), respectively, this finding is still of significant interest because  
294 the specimen under investigation here appears to fall on a long branch an equal distance away  
295 from the previously surveyed species (Fig.2). Given that all 8 previously described species have  
296 yet to be molecularly characterized, it is conceivable that it does fall within one of these,  
297 however as only the previously surveyed *A. minuta* has been described in association with brown  
298 algae (Leander et al. 2004), it does seem possible that the organism observed in this study may  
299 constitute a new species. Unfortunately isolation attempts of this organism were not successful  
300 so far and only a single permanent mount is currently available for morphological  
301 characterization, it is not suitable here to attempt to attribute a species name. The specimen here  
302 is presumed saprotrophic, as the majority of previously reported interactions between  
303 *Aplanochytrium* and algae/seagrasses are (Tsui et al. 2009), however given the  
304 pathogenic/predator-prey/commensalist relationship *Aplanochytrium* species have with  
305 zooplankton (Damare and Ragkhumar 2010, Damare et al. 2013), it is possible that the specimen  
306 investigated here has other affinities with the algal substrate. Indeed this would be a suitable line

307 of enquiry, should this species, or a similar species of the same lineage, be successfully isolated  
308 in the future.

309

310 ***Climate change.*** Antarctic seaweeds display plasticity and adaptability in response to  
311 extreme environmental conditions such as low temperatures and limited light availability  
312 (Wiencke and Amsler 2012). It is important to examine how environmental alterations, such as  
313 those caused by climate change, are going to affect algal seasonality, depth zonation and  
314 biogeography. As sea ice extent reduces along the Antarctic Peninsula (Turner et al. 2013), sub-  
315 Antarctic seaweeds can be expected to migrate to more southerly regions. When assessing the  
316 further consequences of these developments, the role of algal communities in structuring food  
317 webs - especially of the zoobenthos – must be considered (Wiencke 1996). In the Antarctic,  
318 shallow water benthic macroalgal communities are strongly affected by the grazing pressure of  
319 amphipods. Filamentous algae can therefore be found mostly in the intertidal zone where  
320 amphipods are rare (Amsler et al. 2011). The disappearance of sea ice, leading to increased light  
321 availability but also to increased habitat instability and damage through ice scouring, is therefore  
322 likely to alter the distribution and depth zonation of filamentous macroalgae, with knock-on or  
323 reciprocal effects on amphipod population density.

324 It should also be highlighted that species numbers from limited collections alone cannot  
325 be considered as a reliable proxy to estimate changes in algal communities impacted by climate  
326 change over a time span of several decades. In this context, local processes such as retreating  
327 glaciers with subsequent changes in bottom and water column characteristics (e.g. turbidity) can  
328 cause changes in local biodiversity (Quartino et al. 2013). Further analyses of present-day  
329 patterns of composition and distribution along environmental gradients (e.g. depth) or spatial  
330 scales could enable detection of differences with previous surveys.

331 The decline in sea ice cover off the Western Antarctic Peninsula, along with increasing  
332 atmospheric temperatures, has consequences for populations of marine biota, including several  
333 keystone species (Meredith and King 2005). The large brown algae *Himantothallus grandifolius*  
334 and *Ascoseira mirabilis* are major structuring elements of seaweed communities in the northern  
335 part of the Antarctic Peninsula. They are not widely established in the Adelaide Island area (there  
336 is only a single record of *H. grandifolius* from Henkes Islands in 1975, and none from the area in

337 2010-2011) but, as canopy-forming species, their arrival and more widespread occurrence would  
338 mark a major change in the phytobenthos. At present, the only dominant, large canopy-forming  
339 species around Adelaide Island is *Desmarestia menziesii*. Even though reported by Moe and  
340 DeLaca (1976), this species is thought anecdotally to have increased in abundance in the last 10  
341 years (unpublished observations by divers of the British Antarctic Survey at Rothera).

342

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**Table 1. Taxa recorded in 1975 and 2010-2011 around the Southwest Antarctic Peninsula (Adelaide Island / Margaret Bay).**

Taxa in bold: New records of seaweed taxa for the Adelaide Island / Marguerite Bay region in 2010-2011. Third column (ML/now/both): This indicates whether a taxon was only recorded by Moe & De Laca 1975 ("ML"), only by the investigators of this study ("now") or by both surveys ("both").

| Taxa  | Phylum / Class | ML/now/both | Locations 1975 |                  |            | Locations 2010-2011 |              |                 |             |              |               |            |  |   |  |  |   |   |
|---|----------------|-------------|----------------|------------------|------------|---------------------|--------------|-----------------|-------------|--------------|---------------|------------|--|---|--|--|---|---|
|   |                |             | Henkes Island  | Horseshoe Island | Square Bay | Anchorage Island    | Biscoe Wharf | Cheshire Island | Hangar Cove | Honey-bucket | Shack's Crack | South Cove |  |   |  |  |   |   |
| <i>Adenocystis urticularis</i> (Bory de Saint-Vincent) Skottsberg             | Phaeophyceae   | now         |                |                  |            | X                   |              |                 |             | X            |               |            |  |   |  |  |   |   |
| <i>Anartocaccion oplanatum</i> (Gaim) Delépine                                | Chrysophyceae  | now         |                |                  |            | X                   |              |                 |             |              |               |            |  |   |  |  |   |   |
| <i>Balita callitricha</i> (C. Agardh) Kützing                                 | Rhodophyta     | ML          | X              |                  |            |                     |              |                 |             |              |               |            |  |   |  |  |   |   |
| <i>Callophyllis</i> sp. Kützing   | Rhodophyta     | ML          | X              |                  |            |                     |              |                 |             |              |               |            |  |   |  |  |   |   |
| <i>Capsosiphon groenlandicus</i> (J. Agardh) K.L.Vinogradova #                | Chlorophyta    | now         |                |                  | X          |                     |              |                 |             |              |               |            |  |   |  |  |   |   |
| <i>Clathromorphum</i> sp. Fostle  | Rhodophyta     | ML          | X              |                  |            |                     |              |                 |             |              |               |            |  |   |  |  |   |   |
| <i>Codium</i> sp. A. Braun  | Chlorophyta    | ML          | X              |                  |            |                     |              |                 |             |              |               |            |  |   |  |  |   |   |
| <i>Cordia racovitzae</i> Hariot   | Rhodophyta     | ML          | X              |                  |            |                     |              |                 |             |              |               |            |  |   |  |  |   |   |
| <i>Desmarestia menziesii</i> J. Agardh  | Phaeophyceae   | both        | X              |                  |            |                     | X            |                 |             |              |               |            |  |   |  |  |   |   |
| <i>Elaeisia antarctica</i> Skottsberg #                                       | Phaeophyceae   | now         |                |                  |            |                     |              |                 |             |              | X             |            |  |   |  |  |   |   |
| <b><i>Geminoacarpus ausorgeorgiae</i></b> Skottsberg S                        | Phaeophyceae   | now         |                |                  |            |                     | X            |                 |             |              |               |            |  | X |  |  |   | X |
| <i>Geminoacarpus geminatus</i> (J.D.Hooker & Harvey) Skottsberg               | Phaeophyceae   | ML          | X              |                  |            |                     |              |                 |             |              |               |            |  |   |  |  |   |   |
| <i>Himantolabus grandifolius</i> (A. Gepp & E.S. Gepp) Zinova*                | Phaeophyceae   | ML          | X              |                  |            |                     |              |                 |             |              |               |            |  |   |  |  |   |   |
| <i>Hymenochorda</i> sp. J. Agardh   | Rhodophyta     | ML          | X              |                  |            |                     |              |                 |             |              |               |            |  |   |  |  |   |   |
| <b><i>Hymenodliopsis cruxifera</i></b> R.L.Moe                                | Rhodophyta     | now         |                |                  |            |                     | X            |                 |             |              |               |            |  |   |  |  |   | X |
| <i>Iridaea cordata</i> (Turner) Bory de Saint-Vincent                         | Rhodophyta     | now         |                |                  |            |                     | X            |                 |             |              |               |            |  | X |  |  |   | X |
| <i>Lithoderma antarcticum</i> Skottsberg                                      | Phaeophyceae   | both        |                | X                |            |                     | X            |                 |             |              |               |            |  | X |  |  |   | X |
| <i>Lithophyllum antarcticum</i> (J.D.Hooker & Harvey) Rosano†                 | Rhodophyta     | ML          | X              |                  |            |                     |              |                 |             |              |               |            |  |   |  |  |   |   |
| <i>Mesophyllum</i> sp. M.L. Lemoine   | Rhodophyta     | ML          | X              |                  |            |                     |              |                 |             |              |               |            |  |   |  |  |   |   |
| <b><i>Monostroma harloti</i></b> Gaim   | Chlorophyta    | now         |                |                  |            |                     |              |                 |             |              |               | X          |  |   |  |  |   |   |
| <i>Myriogramme manginii</i> (Gaim) Skottsberg                                 | Rhodophyta     | ML          | X              |                  |            |                     |              |                 |             |              |               |            |  |   |  |  |   |   |
| <i>Myriogramme smithii</i> (J.D.Hooker & Harvey) Kytlin                       | Rhodophyta     | ML          | X              |                  |            |                     |              |                 |             |              |               |            |  |   |  |  |   |   |
| <i>Notophycus fimbriatus</i> R.L.Moe**  | Rhodophyta     | ML          | X              |                  |            |                     |              |                 |             |              |               |            |  |   |  |  |   |   |
| <b><i>Palmaria decipiens</i></b> (Reinsch) R.W.Rieker                         | Rhodophyta     | now         |                |                  |            |                     | X            |                 |             |              |               |            |  | X |  |  |   | X |
| <b><i>Panoneura placamioides</i></b> Kytlin                                   | Rhodophyta     | now         |                |                  |            |                     |              |                 |             |              |               |            |  | X |  |  |   | X |
| <i>Paraglossum salicifolium</i> (Reinsch) S.-M.Lin, Fredericq & Hommersand*** | Rhodophyta     | both        | X              |                  |            |                     |              |                 |             |              |               |            |  | X |  |  |   |   |
| <i>Phycodrys antarctica</i> (Skottsberg) Skottsberg                           | Rhodophyta     | ML          |                |                  | X          |                     |              |                 |             |              |               |            |  |   |  |  |   |   |
| <b><i>Phycodrys austrorgeorgica</i></b> Skottsberg                            | Rhodophyta     | now         |                |                  |            |                     |              |                 |             |              |               |            |  |   |  |  |   |   |
| <i>Phyllophora abyssalis</i> Skottsberg                                       | Rhodophyta     | ML          |                |                  | X          |                     |              |                 |             |              |               |            |  |   |  |  |   | X |
| <i>Phyllophora antarctica</i> A. Gepp & E.S. Gepp                             | Rhodophyta     | ML          |                | X                |            |                     |              |                 |             |              |               |            |  |   |  |  |   |   |
| <i>Placomium cartilagineum</i> (Linnaeus) P.S.Dixon                           | Rhodophyta     | both        | X              |                  |            |                     |              |                 |             |              |               |            |  |   |  |  |   | X |
| <i>Placomium hookeri</i> Harvey in J.D. Hooker & Harvey                       | Rhodophyta     | both        | X              |                  |            |                     |              |                 |             |              |               |            |  |   |  |  |   | X |
| <b><i>Placomium secundatum</i></b> (Kützing) Kützing                          | Rhodophyta     | now         |                |                  |            |                     |              |                 |             |              |               |            |  |   |  |  |   | X |
| <b><i>Polyphra placamiestrus</i></b> R.W.Rieker                               | Rhodophyta     | now         |                |                  |            |                     |              |                 |             |              |               |            |  |   |  |  | X | X |



**Table 2.** Live isolates of three algal taxa included in the present study.

| <b>Isolate number</b> | <b>Species name</b>   | <b>Date of collection</b> | <b>Locality</b>  | <b>% identity to closest relative with publicly available sequences</b> | <b>Query cover</b> | <b>e value</b> | <b>EBI accession numbers for new sequences</b><br>(each containing 3'-18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 5'-28S rRNA gene) |
|-----------------------|---|---------------------------|------------------|---|--------------------|----------------|---|
| CCAP 6000/1 (ANT6)    | <i>Ulveila leptochaete</i> (Huber) R.Nielsen                | 20/01/2011                | Anchorage Island | 82%   | 81%                | 2.00E-52       | HG931702  |
| CCAP 6004/1 (ANT10.1) | <i>Capsosiphon groenlandicus</i> (J.Agardh) K.L.Vinogradova | 15/01/2011                | South Cove       | 98%   | 99%                | 3.00E-156      | HG931701  |
| CCAP 1308/1 (ANT10.3) | <i>Elachista antarctica</i> Skottsberg                      | 15/01/2011                | South Cove       | 99%   | 91%                | 0              | HG931703  |

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**Table 3.** Similarity (measured by Sørensen Similarity Index) between the assemblages at each pair of sites.

|   |    | Sørensen Similarity Index |      |      |      |      |      |    |      |      |      |
|---|----|---------------------------|------|------|------|------|------|----|------|------|------|
|   |    | He                        | Ho   | Sb   | Ai   | Bw   | Ci   | Hc | Hb   | Sc   | Co   |
| Shared species                                  | He |                           | 0.09 | 0.10 | 0    | 0.09 | 0.2  | 0  | 0.08 | 0    | 0.18 |
|   | Ho | 1                         |      | 0.2  | 0.13 | 0    | 0.21 | 0  | 0.13 | 0.18 | 0.09 |
|   | Sb | 1                         | 1    |      | 0    | 0    | 0    | 0  | 0    | 0    | 0.10 |
|   | Ai | 0                         | 1    | 0    |      | 0.38 | 0.52 | 0  | 0.53 | 0.67 | 0.44 |
|   | Bw | 1                         | 0    | 0    | 3    |      | 0.53 | 0  | 0.53 | 0.55 | 0.52 |
|   | Ci | 3                         | 2    | 0    | 6    | 5    |      | 0  | 0.46 | 0.44 | 0.6  |
|   | Hc | 0                         | 0    | 0    | 0    | 0    | 0    |    | 0    | 0    | 0    |
|   | Hb | 1                         | 1    | 0    | 5    | 4    | 5    | 0  |      | 0.43 | 0.69 |
|   | Sc | 0                         | 1    | 0    | 5    | 3    | 4    | 0  | 3    |      | 0.36 |
|   | Co | 3                         | 1    | 0    | 6    | 6    | 9    | 0  | 9    | 4    |      |
| He: Henkes Island - 1975 (Moe & DeLaca 1976)    |    |                           |      |      |      |      |      |    |      |      |      |
| Ho: Horseshoe Island - 1975 (Moe & DeLaca 1976) |    |                           |      |      |      |      |      |    |      |      |      |
| Sb: Square Bay - 1975 (Moe & DeLaca 1976)       |    |                           |      |      |      |      |      |    |      |      |      |
| Ai: Anchorage Island - 2010-2011                |    |                           |      |      |      |      |      |    |      |      |      |
| Bw: Biscoe Wharf - 2010-2011                    |    |                           |      |      |      |      |      |    |      |      |      |
| Ci: Cheshire Island - 2010-2011                 |    |                           |      |      |      |      |      |    |      |      |      |
| Hc: Hangar Cove - 2010-2011                     |    |                           |      |      |      |      |      |    |      |      |      |
| Hb: Honey-bucket - 2010-2011                    |    |                           |      |      |      |      |      |    |      |      |      |
| Sc: Shack's Crack - 2010-2011                   |    |                           |      |      |      |      |      |    |      |      |      |
| Co: South Cove - 2010-2011                      |    |                           |      |      |      |      |      |    |      |      |      |

543 **Figure 1.** Study sites around Rothera Point, Adelaide Island, Antarctica.

544

545 **Figure 2.** *Aplanochytrium* cell associated with *Elachista antarctica* (ANT10.3) at 100x  
546 magnification (scale bars = 20µm). The *Aplanochytrium* cell can be seen to be rounded, around  
547 35 µm in diameter. Internally no zoospores can be seen, thus it is assumed that this is a somatic  
548 cell. The ectoplasmic net (arrowed) is seen to attach the cell to the brown algal filament and  
549 measures approximately 34-35 µm in length and 1-3 µm in width. The ectoplasmic net does not  
550 encase the cell and migration of the cell along the ectoplasmic net was not observable due to the  
551 nature of the mount.

552

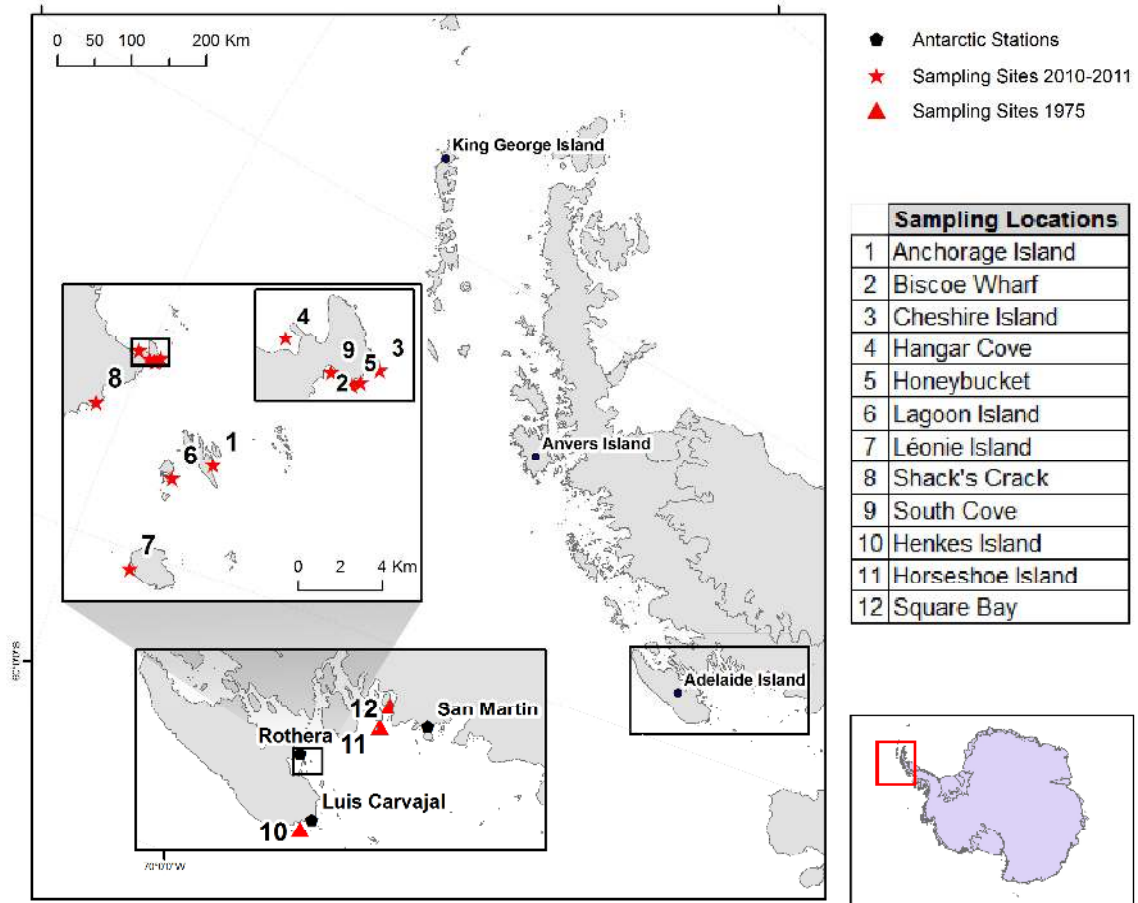
553 **Figure 3.** Maximum likelihood test of phylogeny of the 1635bp SSU rRNA sequence obtained  
554 from the *Aplanochytrium* sp. under investigation in this study. The sequence obtained shows  
555 strong support that this specimen falls within the *Aplanochytrium* clade (94/100) and that it is at  
556 an equal distance from all other *Aplanochytrium* sequences surveyed here (97/100). The key to  
557 the right indicates firstly the geographic location and secondly the substrate association of each  
558 sequenced tested. A trend can clearly be seen that substrate is a good predictor of branching  
559 affiliations within the genus. All sequences obtained associated with zooplankton, from three  
560 separate studies, form a monophyletic clade, while those obtained from sea grasses/algae, from  
561 six separate studies, with the exception of this novel basal sequence, form a paraphyletic clade.  
562 Within this second clade are two sequences labelled as being associated to unknown/unrecorded  
563 substrates: The first of these (*Aplanochytrium* sp. S1a) was found in salt marshes in Taiwan, the  
564 second (*Aplanochytrium kerguelense*) was taken from a culture collection and was originally  
565 described from sub-Antarctic waters.

566

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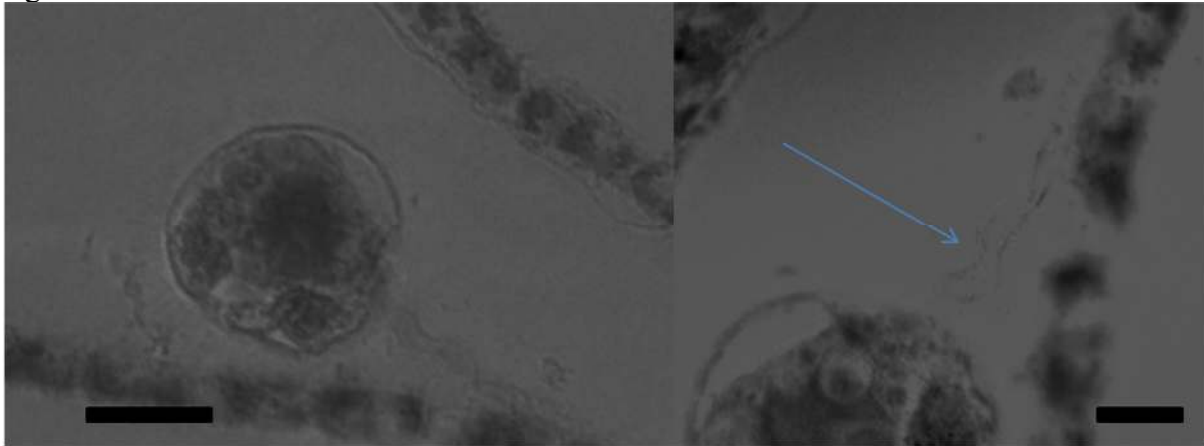


568 Fig. 1



569

570 Fig. 2



571  
572  
573

