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1 Molecular-assisted revision of red macroalgal diversity and distribution along the Western Antarctic
2 Peninsula and South Shetland Islands

3

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15

16 RESUME

17 La flore marine de la zone Antarctique est suppos  e bien connue car relativement peu d'esp  ces y sont
18 observ  es par rapport    d'autres r  gions du monde. En utilisant la taxonomie traditionnelle combin  e
19 aux outils mol  culaires, nous avons   tudi   la diversit   et biog  ographie des communaut  s de
20 macroalgues rouges le long de la c  te Ouest de la P  ninsule Antarctique (WAP) et des   les Shetlands
21 du Sud (SShs) en consid  rant un gradient bathym  trique. Nous avons   galement compar   nos deux
22 m  thodes d'identification en terme de pr  cision et de nombre de taxa identifi  s. Nos r  sultats ont
23 d  montr   de faibles et homog  nes niveaux de diversit      l'  chelle de la zone d'  tude comme      chelle
24 locale, avec au total environ 50 taxa identifi  s. Nous avons   galement d  tect   trois complexes
25 d'esp  ces cryptiques chez les genres *Callophyllis*, *Curdiea* et *Georgiella*, et am  lior  e l'identification de

26 nos spécimens puisque 98% ont été identifiés jusqu'à l'espèce grâce aux outils moléculaires. Nos
27 résultats ont aussi permis de révéler des différences significatives entre les assemblages de macroalgues
28 rouges appartenant à trois sub-régions, correspondant à des latitudes différentes: SSHs et partie Nord de
29 la WAP ($\approx 63^\circ\text{S}$), la partie centrale de la WAP ($\approx 64^\circ\text{S}$) et la partie Centre-Sud de la WAP ($\approx 67^\circ\text{S}$). Nos
30 sub-régions ne correspondent pas à la séparation classique entre SSHs/WAP proposée dans les études
31 antérieures, et suggèrent d'une part, que le détroit de Bransfield n'est pas une barrière biogéographique
32 forte pour les macroalgues rouge et d'autre part, que la dynamique actuelle et passé de la couverture de
33 glace a pu jouer un rôle déterminant dans la distribution actuelle des assemblages de macroalgues
34 rouges. Finalement, notre travail a permis de construire une banque de données génétiques associée à
35 un herbier de collection de spécimens, et va permettre d'appuyer des travaux futurs pour mieux
36 comprendre la diversité et distribution des communautés d'algues rouges dans la zone d'étude.

37

38 Mots-clés: Barcoding ADN - Biogéographie - Espèces cryptiques - Inventaire de Biodiversité -
39 Rhodophyta - Taxonomie intégrative.

40

41 ABSTRACT:

42 The Antarctic marine flora is well known as a flora composed of relatively few species in comparison
43 with other marine realms. Using molecular taxonomy as complementary tool for traditional taxonomy,
44 we studied red macroalgal diversity and biogeography along the Western Antarctic Peninsula (WAP)
45 and South Shetlands Islands (SSHs) coasts and across a bathymetric gradient; in addition, we compared
46 both methods of identification in terms of accuracy and number of observed taxa. Our results show low
47 diversity levels at the regional and local scales, with around 50 taxa registered in total. Molecular tools
48 allowed us to detect putative cryptic species within the genera *Callophyllis*, *Curdiea* and *Georgiella*,
49 and to identify 98% of our specimens at species level. Our results also allowed us to identify significant

50 differences between red macroalgal assemblages of three distinct biogeographic sub-regions: SSHs and
51 Northern part of the WAP (at $\approx 63^{\circ}\text{S}$), Central part of the WAP (at $\approx 64^{\circ}\text{S}$) and Central-Southern part of
52 the WAP (at $\approx 67^{\circ}\text{S}$). Our sub-regions do not correspond to the classical separation of SSHs/WAP
53 reported in previous studies and suggest that the Bransfield Strait is not a strong biogeographic barrier
54 for red macroalgae. Since our three sub-regions correspond to three latitude levels, we propose that past
55 and present-day dynamics of sea-ice disturbance may have shaped the observed differences in red algal
56 communities. We believe that the construction of accessible genetic dataset associated with voucher
57 specimens in the present work will benefit future studies of macroalgal diversity and distribution in the
58 region.

59

60 Key-words: Biodiversity survey - Biogeography - Cryptic species - DNA barcoding - Integrative
61 taxonomy - Rhodophyta.

62

63 INTRODUCTION

64 With more than 7000 species described to the day (Guiry & Guiry, 2015), the phylum Rhodophyta
65 comprises the most diverse group of seaweeds, encompassing both macro- and microorganisms found
66 in a wide diversity of habitats. Antarctic macroalgal diversity is considered to be low compared to
67 temperate southern Pacific waters (one tenth of Australian algal diversity; Wiencke & Clayton, 2002),
68 but is at the same time characterized by high level of endemism ($\approx 35\%$, Wiencke & Clayton, 2002;
69 Ramírez, 2010; Wiencke et al., 2014). These characteristics have been linked to the history of
70 Antarctica. Indeed, the isolation of the Antarctic biota has begun with the sequential fragmentation of
71 the Gondwana super-continent (130 Mya BP) and was completed with the opening of the Drake
72 Passage and the separation of southern tip of South America and the Antarctic Peninsula about 40 Myr
73 ago (Scher & Martin, 2006). Moreover, the strong intensification of the Antarctic Circumpolar Current

74 (ACC) 25 Myr ago has also contributed to isolate Antarctic from sub-Antarctic waters, acting as an
75 oceanographic barrier to dispersion for most of marine species (Sanches et al., 2016). Since the
76 Mesozoic, Antarctica has become increasingly cooler and, for several million years, Southern Ocean
77 temperatures have been close to the freezing point for much of the year, and are considered as one of
78 the most constant thermal regimes on earth (Barnes & Conlan, 2007). This relatively stable Antarctic
79 environment and the strong isolation of Antarctica (but see Fraser et al., 2018) are presumed to be the
80 main reasons for the development of a unique biota, rich in endemic species well adapted to a cold-
81 water system (Rogers, 2007).

82 The description of the Antarctic flora has begun 200 years ago with the expedition of Gaudichaud and
83 Hooker (between 1817 and 1843, see Wiencke & Clayton, 2002) and new inventories have allowed to
84 increase the number of recorded species during the last decade in the region (from 96 species in 1964
85 to about 130 species listed in Wulff et al., 2009, and up to more 150 in Sanches et al., 2016). In
86 Antarctica, macroalgae are generally considered "well-known organisms" (Griffiths, 2010). Indeed, a
87 long list of studies have deepened our understanding about the structure and the function of macroalgal
88 communities, and have shown that significant differences exist between bioregions, habitats and ranges
89 of depth (Zacher et al., 2007; Oliveira et al., 2009; Quartino et al., 2013; Amsler et al., 2014; Valdivia
90 et al., 2014; Amsler et al., 2015; Griffiths & Waller, 2016; Sanches et al., 2016; Marcías et al., 2017).
91 However, when compared to marine animals, and especially invertebrates, very few occurrence records
92 are available along the coast of Antarctica for macroalgae (e.g. only 73 occurrence records in the
93 Register of Antarctic Marine Species - RAMS - in 2010 while 29,727 occurrence records exist for
94 molluscs alone, see De Broyer & Danis, 2011). It is clear that not all species have received the same
95 attention and that major drawbacks in understanding patterns of diversity can arise in group of
96 organisms characterized by simple morphology and for which errors in the taxonomic assignation of
97 specimens have been detected, like macroalgae. Because of their fairly simple anatomy, rampant
98 convergence, remarkable degrees of phenotypic plasticity in response to environmental factors, and

99 incompletely understood life histories with alternation of heteromorphic generations, marine
100 macroalgae are notoriously difficult to identify by means of traditional morphological characters
101 (Saunders, 2005; 2008).

102 During the past two decades, molecular tools have been increasingly used to rapidly and accurately
103 identify macroalgal specimens (Le Gall and Saunders, 2010) and the emerging field of molecular-
104 assisted alpha taxonomy has allowed to revisit the phylogeny and species diversity in various orders
105 and families (e.g. for red algae: Hommersand et al., 2009; Sutherland et al., 2011; Billard et al., 2015;
106 Guillemin et al., 2016). Along the tip of South America and the Antarctic Peninsula, recent genetic
107 studies have warranted the description of new genera and species of macroalgae (Hommersand et al.,
108 2009; Moniz et al., 2012) and have pointed out the existence of various cryptic species in brown, red
109 and green algae yet to be described (Young et al., 2013; Mystikou et al., 2014; Billard et al., 2015;
110 Guillemin et al., 2016; Garrido-Benavent et al., 2017; Pellizzari et al., 2017). The DNA barcoding
111 approach (Hebert et al., 2003), based on the use of a standard genetic marker (i.e. generally the COI
112 mitochondrial gene coding for subunit 1 of cytochrome oxidase, see Leliaert et al., 2012 and reference
113 therein), has been proposed as a simple tool to decipher species diversity and distribution in highly
114 speciose groups. Even if the method presents well-known limitations (DeSalle et al., 2005), it has been
115 proved useful to detect cryptic diversity of algae (Saunders, 2005; LeGall & Saunders, 2010) and has
116 been used to revise spatial patterns of biodiversity in Atlantic kelp forests (Robuchon et al., 2015).

117 Despite the problems linked to their species delimitation and determination, Antarctic algae have not
118 been cited as organisms where possible gaps in knowledge exist (see De Broyer & Danis, 2011). It is
119 clear, however, that Antarctic benthic diversity has been seriously underestimated and that much fewer
120 taxa have large circumpolar distributions or cross the Antarctic Circumpolar Current (ACC) than was
121 previously postulated (see review in Clarke et al., 2007 and Convey et al., 2014) — macroalgae being
122 no exception (Hommersand et al., 2009; Billard et al., 2015).

123 Within the Antarctic waters, the existence of various sub-regions / eco-regions was postulated and the
124 authors generally have proposed a separation of the South Shetland Islands (SShs) from the Western
125 Antarctic Peninsula (WAP; Linse et al., 2006; Spalding et al., 2007; Terauds et al., 2012) and even the
126 existence of fine scale differences between the Northern, Central and Southern part of the WAP (Linse
127 et al., 2006; Terauds et al., 2012). These regions are characterized by different histories (e.g. different
128 deglaciation ages: Ó Cofaigh et al., 2014), marine current systems and environmental conditions such
129 as temperature, salinity, light and substrate availability and period of sea ice coverage. At large
130 geographic scales, recent studies have demonstrated that macroalgal species tend to form characteristic
131 groups broadly following the delimitation of the proposed biogeographic regions (Griffiths & Waller,
132 2016; Sanches et al., 2016; Pellizzari et al., 2017). Of all the studies focused on macroalgae, only the
133 one made by Pellizzari et al. (2017) has taken into account the existence of unnamed cryptic genetic
134 lineages in some of the taxa under study. Species identification being an essential first step in
135 ecological studies, it has been demonstrated that incorrect taxonomic classification of specimens can
136 significantly bias any survey of biodiversity, producing an underestimation of both local and regional
137 biodiversity (Bortolus, 2008). Indeed, in their recent survey of algal communities associated to distinct
138 kelp forests in French Brittany, Robuchon et al. (2015) have shown that DNA barcoding significantly
139 increases the alpha-diversity and generally allows to improve the taxonomic level of identification (i.e.
140 higher number of samples resolved to the species level when using genetic data).

141 In the present study our aim was to contrast results of classical and molecular assisted taxonomy to
142 investigate the variation of red macroalgal assemblages among Antarctic sub-regions. For the first time,
143 a barcoding approach was consistently used to classify the whole red algal community in five areas
144 located in the SSIs and along the Northern and Central part of the WAP at the lowest taxonomic level
145 achievable. We expected the integrative classification method to increase species diversity when
146 compared to the morphological classification (Robuchon et al., 2015) since cryptic diversity could be
147 taken into account (Saunders, 2005; LeGall & Saunders, 2010; Young et al., 2013; Guillemin et al.,

148 2016; Garrido-Benavent et al., 2017). Finally we tested for differences in species assemblage between
149 biogeographic sub-regions in the area under study.

150

151 MATERIALS AND METHODS

152 Sampling- Red macroalgae were sampled during the 2013 and 2014 Antarctic summer campaigns in
153 five areas located in the SSHs (near the Chilean Capitán Arturo Prat base in Greenwich Island, and at
154 Bahia Fildes in King George Island, hereafter referred to as PRAT and KGI, respectively) and along
155 the Northern (near the Chilean O'Higgins Antarctic base, noted OHI) and Central part of the WAP (in
156 Paradise Bay, near the Chilean Presidente Gabriel González Videla Antarctic base, noted GGV and in
157 Marguerite Bay, noted MAR) (Figure 1, Annex 1). The whole sampling region covered two distinct
158 biogeographic sub-regions proposed by Linse et al (2006): the SSHs and the WAP (see Figure 1).
159 Along the Central part of the WAP, GGV is located in the Gerlache Strait while MAR is located within
160 the Bellingshausen Sea.

161 In each area, 4 to 7 sites separated by at most 80 km were sampled during our study (Annex 1). In
162 PRAT, OHI, GGV and MAR, the sampling was done along transects (perpendicular to the shore, each
163 site correspond to one transect), with three bathymetric zones defined for each transect (i.e. intertidal
164 zone, subtidal zone from 0 to 15 m and subtidal zone from 15 to 30 m). An exhaustive research of
165 different morphotypes was completed during a 30 min sampling effort in each bathymetric zone for
166 each site. Specimens for which we were able to observe noticeable variations in thallus shape, color or
167 thickness and elasticity were considered as distinct morphotypes and at least one specimen of each
168 morphotype was sampled. In KGI, a non-exhaustive research of morphotypes, without controlling the
169 sampling time effort, was conducted in seven sites along a bathymetric gradient going from the
170 intertidal zone to a 40 m depth. Intertidal samplings were conducted during diurnal low tide hours;
171 subtidal samplings were done by means of SCUBA diving.

172 All specimens were pressed as vouchers after removing a small portion of the thallus that was stored in
173 silica gel for subsequent DNA analysis (see Annex 1). Voucher specimens are housed in the herbarium
174 of the Universidad Austral de Chile and available from the corresponding author on request (i.e.
175 voucher code correspond to "Specimen ID" in Annex 1). In this study, the specimens of the
176 Corallinaceae were not included since the sampling was highly biased to collecting in the intertidal
177 zone only in most of our sites.

178 Identification process of red algal specimens- A total of 882 specimens of red macroalgae were
179 collected over the sampling region (Annex 1). Two methods were used during the process of
180 identification and taxonomic assignation of specimens. First, we used floristic keys, species list and
181 field guides available for the region (Wiencke & Clayton 2002, Hommersand et al. 2009, Ramírez
182 2010) to identify red macroalgae to the lowest possible taxonomic level on the basis of morphological
183 criteria alone [see Annex 1, "Species (Identification based on morphological characters)". Second, two
184 genetic markers were used to provide a complementary species identification based on molecular
185 criteria: a partial sequence of the mitochondrial Cytochrome c Oxidase I gene (5P-COI) and a partial
186 sequence of the plastid gene *rbcL*, encoding the large subunit of the ribulose-1,5-bisphosphate. Dry
187 tissues were grinded by hand in liquid nitrogen. All samples belonging to the genus *Georgiella*,
188 *Gigartina*, *Iridaea*, *Picconiella* and *Trematocarpus* were extracted using extraction protocol from
189 Saunders (1993) modified by Faugeron et al. (2001). For all the other samples, DNA extraction was
190 performed using extraction kit E.Z.N.A.® Poly-Gel DNA Extraction (Omega Bio-Tek Inc., Norcross,
191 USA). For all specimens, the amplification of 5P-COI was performed using the primers developed by
192 Saunders (2005) (GaZF1: TCAACAAATCATAAAGATATTGG and GaZR1:
193 ACTTCTGGATGTCCAAAAAYCA). *rbcL* amplification was performed only for a subsample of the
194 red algae including: 1) all specimens that did not amplify for the 5P-COI and 2) up to 23 specimens for
195 each group of samples assigned to the same species with the 5P-COI (more details available below).
196 *rbcL* amplification was performed using the primers developed by Hommersand et al. (1994) (F-*rbcL*:

197 TTGCATAYGATATTGATY TATTTGAA and R-*rbcL*: RAGCTGTTTKTAAAGGWCCACAA). For
198 both gene fragments the same PCR mix was used. Amplifications were performed in a final volume of
199 30 μL that included: 0.12 μL of Kapa Taq (5 U/ μl), 3 μL of Kappa Taq buffer B (10x) (Kapa
200 Biosystems); 0.6 μL of MgCl_2 (25 mM); 0.6 μL dNTPs (2.5 μM) and 1.5 μL of each primer (10 μM).
201 Depending on the species and marker under study, 0.2 to 0.6 $\mu\text{g}/\mu\text{L}$ BSA (100X) (New England
202 Biolabs, MA, USA) was added in each PCR mix. For both markers, cycling conditions consisted of an
203 initial denaturing step of 5 min at 94°C, followed by 5 cycles (94°C for 30 s, T_{m1} °C for 45 s, and 72°C
204 for 45 s; T_{m1} = 40°C for 5P-COI and 45°C for *rbcL*), 25 additional cycles (94°C for 30 s, T_{m2} °C for
205 30 s, and 72°C for 30 s; T_{m2} = 54°C for 5P-COI and 56°C for *rbcL*) and a final elongation step of 7
206 min at 72°C. PCR products were purified using commercial kit E.Z.N.A.® DNAProbe Purification
207 (Omega Bio-Tek Inc., Norcross, USA) and sequenced with primers used for amplification at the
208 AUSTRAL-omics Core-Facilities (Valdivia, Chile). Please note that both the forward and reverse
209 primers were used for the 5P-COI sequencing while only the F-*rbcL* primer was used for the *rbcL*
210 sequencing.

211 Sequences were edited using Chromas v.2.33 (McCarthy, 1997), and aligned using MEGA v.5
212 (Tamura et al., 2011). For the 5P-COI, uncorrected p-distances were calculated in Mega v 5 (Tamura et
213 al., 2011) and sequences separated by p-distances higher than 1% were considered as potentially
214 belonging to distinct species (Robuchon et al., 2015). Molecular species identification was performed
215 using the basic local alignment search tool (BLAST) from NCBI (Altschul et al., 1990) and comparing
216 the sequences obtained in this study with those already deposited in GenBank. Before phylogenetic tree
217 reconstructions, GenBank sequences presenting more than 95% of similitude from the ones obtained in
218 our study were added to the 5P-COI and the *rbcL* datasets (i.e. 19 and 42 sequences for the 5P-COI and
219 the *rbcL*, respectively). For both markers, and in order to limit the complexity of the trees for some
220 highly diverse genera, only the first six most similar species present in GenBank were added to our

221 data sets. Tree reconstructions were conducted separately for the COI-5P and the *rbcL* markers using a
222 neighbour-joining (NJ) algorithm and the Juke-Cantor model in MEGA v.5 (Tamura et al., 2011).

223 Previous to analyses of species diversity and composition, two datasets were generated for Antarctic
224 red algae: a "morphological" dataset based only on species identification using the morphological
225 criteria and an "integrative" dataset that combines information given by the morphological and
226 molecular criteria. In order to generate the integrative dataset, morphological identification was
227 contrasted for each specimen for which genetic data were available, with the results of a species
228 identification based on molecular criteria. When a complete concordance was found between both
229 methodologies of species identification, the species name given in the morphological identification was
230 kept in the "integrative" dataset. When a discordance was observed between the two methods and
231 sequences were available in NCBI that allow to name our specimens, the name given by molecular
232 species identification tools (i.e. 5P-COI and/or *rbcL*) were kept in the "integrative" dataset. For
233 specimens for which a discordance was observed between the two methods but the information present
234 in NCBI was not sufficient to name our samples, the name of the genus followed by sp.1 (2, 3, etc.)
235 was given to the genetic entities. In this last case, for all except four specimens, the name of the genus
236 obtained after comparing our two molecular markers and the NCBI database were concordant between
237 the 5P-COI and the *rbcL* sequences. Four specimens were identified as *Cryptonemia* in the
238 morphological dataset, as *Grateloupia* when using the 5P-COI marker and as *Cryptonemia* when using
239 the *rbcL* marker. *Grateloupia* and *Cryptonemia* are closely related genera and we chose to classify
240 these specimens as species of *Cryptonemia* (Annex 1).

241 Specimens for which no sequences were available were named in the "integrative" dataset depending
242 on the situation: 1) if a complete congruence between morphological and molecular identification was
243 observed for all specimens of a morphological species for which sequences were obtained, species
244 name given by the morphological identification were kept in the "integrative" dataset for all specimens

245 (i.e. with and without molecular data); 2) if we observed a discordance between morphological and
246 molecular identification for at least one specimen sequenced within a morphological species, non
247 sequenced specimens were given the name corresponding to the ones identified by molecular criteria
248 and sampled at the same site and at the same depth; 3) if, for a species defined using the morphological
249 criteria no sequences were obtained at all, all specimens kept the name given by the morphological
250 identification in the "integrative" dataset.

251 Data analyses- To characterize pattern of red seaweed diversity, presence / absence of species was
252 recorded for each site within the five areas under study (i.e. KGI, PRAT, OHI, GGV and MAR). For
253 each site, information available for the three bathymetric zones was pooled together and analyzed
254 jointly. Within each sampled area, absolute values of species richness were determined using both the
255 "morphological" and the "integrative" datasets. Moreover, for the whole sampled region, we
256 constructed species accumulation curves for both the "morphological" and the "integrative" datasets.
257 Accumulation curves and standard errors were calculated using the statistical software R version 3.01
258 (R Core Team, 2014) via the specaccum function from vegan package (Oksanen et al., 2014) and
259 combining data from increasing numbers of sites in random order repeated 1000 times. On the basis of
260 the presence / absence data, Jaccard index was used to construct a matrix of similarity between our
261 areas, for both the "morphological" and the "integrative" datasets, using PRIMER 6 (Clarke &
262 Warwick, 2001).

263 Compositional changes in species assemblages across sampled areas were analyzed by nonmetric
264 multidimensional scaling (nMDS) ordination of distance matrices calculated using Jaccard's index. All
265 sites were used for the analysis with a total sampling effort over the whole sampling region of 15, 38
266 and 41 in the intertidal, shallow subtidal (0-15 m) and deep subtidal (15-30 m) zone, respectively. A
267 stress value was also calculated for the ordination in order to judge the quality of the plot. Stress values
268 <0.1 generally indicate good representation of the similarity matrix and a low potential of

269 misinterpretation whereas caution should be applied when interpreting the nMDS results when stress
270 values >0.2. Finally an analysis of similarities (ANOSIM) was used to identify statistically significant
271 differences between species assemblages among sampling areas, considering data from all bathymetric
272 zones pooled together, and to test for biogeographic delimitations based on the results of the nMDS
273 analysis (see Results for more details).

274

275 RESULTS

276 A total of 882 specimens of the phylum Rhodophyta, 511 sequences of 5P-COI (632 bp) and 98
277 sequences of *rbcL* (958 bp) were obtained (Annex 1). For 362 of the 882 specimens (i.e. 41%) no
278 sequences are available. All GenBank numbers are listed in Annex 1.

279 Comparison between the two methods of species identification- Fifty-one taxa in total, ranging from
280 order to species taxonomic ranks, were detected using classical taxonomy, i.e. based on morphological
281 characters only (Annex 1). The use of the integrative method allowed us to identify forty-nine taxa
282 (Annex 1). These 49 taxa were classified as part of 11 orders and 17 families (Figure 2, Figure 3,
283 Annex 1). We were unable to define the family level for a group of 8 samples for which no sequences
284 were obtained, keeping the order level identification [i.e. Gigartinales (Kallymeniaceae or
285 Palmariaceae); Annex 1]. Among the 868 algae that were determined at least at the genus level using
286 classical taxonomy, the genus name was changed for only 16% (i.e. 143) of them in our integrative
287 dataset (Annex 1). More than half (i.e. 76, 53%) of these 143 specimens were Bangiaceae (namely,
288 specimens determined morphologically as *Porphyra* changed to *Pyropia* and *Wildemanina* after using
289 molecular tools, Annex 1), a family in which genera are notoriously difficult to recognize using
290 classical taxonomy (see for reference Sutherland et al. 2011).

291 No incongruence was observed between the results obtained for the COI and the *rbcL* for the 89
292 samples sequenced for both markers (Figure 2, Figure 3, Annex 1). In various cases, the molecular
293 dataset led to lumping entities recognized as different taxa using morphological criteria. For
294 example, all specimens identified in morphological dataset as *Plocamium aff. hookeri*, *Plocamium aff.*
295 *secundatum*, *Plocamium sp.* and *Plocamium cartilagineum* (Linnaeus) P.S.Dixon corresponded to a
296 unique genetic entity determined both by COI and *rbcL* gene (Figure 2, Figure 3, Annex 1). Thus, all
297 specimens were identified as *Plocamium cartilagineum* in the integrative dataset. In the same way, the
298 COI sequences of two specimens recognized morphologically as *Gracilaria pulvinata* Skottsberg and
299 *Rhodymenia subantarctica* R.W.Ricker were lumped within *Gymnogongrus* specimens under the name
300 *Gymnogongrus antarcticus* Skottsberg (Annex 1). The only specimen recognized morphologically as
301 *Trematocarpus dichotomus* Kützing was also lumped with the rest of the *Trematocarpus antarcticus*
302 (Harriot) Fredericq & R.L.Moe specimens after sequencing the COI gene (Annex 1). On the other hand,
303 we detected putative cryptic species in three genera: *Curdiea*, *Georgiella* and *Callophyllis*. In *Curdiea*
304 (between *Curdiea racovitzae* Harriot and *Curdiea sp.1*) and *Callophyllis* (between *Callophyllis sp.1*, *sp.*
305 *2* and between *Callophyllis sp. 3* and *sp. 4*) the divergence between putative cryptic species were low,
306 just above our 1% threshold. Five specimens of *Georgiella* showed a COI sequence divergence of 6%
307 when compared to the rest of the specimens of *Georgiella confluens* (Reinsch) Kylin studied and were
308 renamed *Georgiella sp.1*. Interestingly, specimens of *Curdiea sp.1* were only found in the SSHs and the
309 northern part of the WAP (i.e. KGI and OHI), whereas specimens of *Curdiea racovitzae* were found
310 over the whole sampling region (Figure 1, Annex 1).

311 Geographic distribution- Both accumulation curves for sampling effort including all sites at all depths
312 for the “Morphological dataset” and the “Integrative dataset” were highly similar (Figure 4). They both
313 reached an asymptote point around 50 taxa in our study region. Species richness expressed in absolute
314 values of the number of taxa displayed the same pattern between the “Morphological dataset” and the
315 “Integrative dataset” (Figure 5). The species-specific richness varied between 22 taxa in GGv to 28

316 taxa in PRAT (estimation computed using the “Integrative dataset”, Figure 5). For the nMDS and
317 ANOSIM analyses, both the “Morphological dataset” and the “Integrative dataset” gave very similar
318 results and only the “Integrative dataset” results are presented here. The existence of three distinct
319 groups was suggested by a separation of the three northern areas of sampling (i.e. KGI and PRAT in
320 the SShs and OHI in the northern part of the WAP), the Gerlache Strait area (GGV) and the
321 Bellingshausen Sea (MAR) (Figure 6).

322 The results of the ANOSIM analysis support this finding, with significant differences encountered
323 between the three northern areas, and the two southern areas while no significant differences were
324 detected between KGI and PRAT, KGI and OHI and OHI and PRAT (Table 1). The results of the
325 ANOSIM analysis also detected a significant difference between red algal assemblages of GGV and
326 MAR (i.e. the two southern sites located along the WAP, Table 1, Figure 6).

327

328 DISCUSSION

329 Our work is the first study where integrative taxonomy, coupling information from morphological
330 characters and molecular tools, has been consistently used to study red macroalgal diversity along the
331 coasts of the South Shetland Islands and the Northern and Central part of the Western Antarctic
332 Peninsula. Our qualitative sampling allowed us to register around 50 taxa over the whole sampled
333 region, being consistent with previously registered number of species in these areas (Wiencke &
334 Clayton, 2002; Pellizarri et al., 2017). Our complementary identification method (i.e. "Integrative
335 Dataset"), based on both morphology and molecular markers, confirmed that the region is characterized
336 by a low specific diversity level; nevertheless, it also allowed us to detect putative cryptic species
337 within three genera: *Curdiea*, *Georgiella* and *Callophyllis*. *Georgiella* had been reported previously as
338 a monotypic genus restricted to Antarctic waters (including some islands of the Scotia Arc, Wiencke et
339 al., 2014). Finally, our results allowed us to identify significant differences between red algal
340 assemblages of three distinct geographic sub-regions around the South Shetland Islands and the

341 Western part of the Antarctic Peninsula, following a latitudinal gradient: SSHs and Northern part of the
342 WAP (i.e. all areas located on both side of the Bransfield Strait: KGI, PRAT and OHI, 63°S), Central
343 part of the WAP (GGV, 64°S) and Center-Southern part of the WAP (MAR, 67°S).

344

345 *Three major biogeographic sub-regions across South Shetland Islands and West Antarctic Peninsula*

346 In our study, significant differences between red macroalgal assemblages were detected between three
347 geographic sub-regions: the South Shetland Islands and the Northern West coast of the Antarctic
348 Peninsula (i.e. KGI, PRAT and OHI); the Gerlache Strait (i.e. GGV) and Marguerite Bay located
349 within the Bellingshausen Sea (i.e. MAR). Some widespread species, as *Curdiea racovitzae*,
350 *Georgiella confluens*, *Gigartina skottsbergii* Setchell & N.L.Gardner, *Iridaea cordata* (Turner) Bory de
351 Saint-Vincent, *Myriogramme manginii* (Gain) Skottsberg, *Palmaria decipiens* (Reinsch) R.W.Ricker,
352 *Plocamium cartilagineum* and *Trematocarpus antarcticus* were common to the three identified sub-
353 regions. Nevertheless, some species were found to be characteristic of each of the different geographic
354 sub-regions: bladed Bangiales (both *Porphyra* sp.1 and *Pyropia* sp.1), *Cystoclonium* sp.1 and
355 *Phycodrys antarctica* Skottsberg were recorded only in the South Shetland Islands and the Northern
356 West coast of the Antarctic Peninsula; *Delisea pulchra* (Greville) Montagne and *Kallymenia* sp.1 were
357 recorded only in the Gerlache Strait and *Hymenocladopsis crustigena* R.L.Moe and *Notophycus*
358 *fimbriatus* R.L.Moe were recorded only in Marguerite Bay. *Callophyllis* sp.4, *Cryptonemia* sp.1 and
359 *Myriogramme smithii* (J.D.Hooker & Harvey) Kylin were limited to the two most southern areas of
360 GGV and MAR. Species distribution uncovered in our study remains subject to sampling limitations
361 and some species could have been overlooked during our 2013 and 2014 field surveys. This could
362 especially be the case within the two bio-regions of the Gerlache Strait and the Bellingshausen Sea,
363 each of them only being represented by one sampling area and a reduced sampling effort (~15 days in
364 2014). For example, both *Hymenocladopsis crustigena* and *Notophycus fimbriatus*, recorded only in

365 the Bellingshausen Sea in our study (i.e., MAR) where first described along the coastlines of Anvers
366 Island and Argentine Island, respectively (Hommersand et al. 2009). Anvers and Argentine Islands are
367 located within the Gerlache Strait at less than 100 km of GGV. The absence of these two species from
368 our GGV samples could be more related to local oceanic and climatic conditions in our sampling area
369 (GGV is a much more sheltered area than the coasts of Anvers and Argentine Islands) than to a clear
370 separation of the Central part of the WAP and Center-Southern part of the WAP in distinct geographic
371 sub-regions. Even if significant, separations between bioregions have then to be taken with caution and
372 new sampling localities in the Gerlache Strait and the Bellingshausen Sea will be needed to confirm
373 our results.

374 Our three detected biogeographic sub-regions do not correspond to the ones proposed by Linse et al
375 (2006) or to the marine ecoregions proposed by Spalding et al (2007). In these works the South
376 Shetlands Islands were distinguished from the Center/North of the Western Antarctic Peninsula,
377 considered as a single region. Moreover, the environmental domains and expert-defined bioregions
378 described by Terauds et al. (2012) separate the Center/North of the Western Antarctic Peninsula in
379 three distinct latitudinal zones: North-east Antarctic Peninsula (where OHI is located, 63°S), North
380 Antarctic Peninsula (where GGV is located, 64°S) and South Antarctic Peninsula (where MAR is
381 located, 67°S). For red macroalgae we did not detect the proposed separation between the North-east
382 Antarctic Peninsula (OHI) and the South Shetlands Islands (KGI and PRAT) (Terauds et al., 2012),
383 suggesting that the Bransfield Strait is not a strong biogeographic barrier for these organisms. In the
384 same way, the previous work of (Griffiths & Waller, 2016) reported a high overlap in terms of
385 intertidal species composition (27% of species) between King George Island and the Antarctic
386 Peninsula (considering both invertebrates and macroalgae).

387

388 *Potential processes underpinning the biogeography of Antarctic macroalgae*

389 At large scale, macroalgal community structure has been deemed to be primarily determined by both
390 historical factors and the present dynamics of currents and sea-ice disturbance (Spalding et al., 2007;
391 Pellizzari et al., 2017). Historical effects of Antarctic species range contraction in refugia during glacial
392 periods and subsequent allopatric divergence and speciation could have led to regional differences in
393 species community structure. Indeed, cryptic species and/or genetic lineages have been detected in a
394 wide array of marine animals (see Allcock & Strugnell, 2012 for review) and, for most species
395 complex, allopatric speciation while in isolation in small refugia along the Antarctic shelf has been
396 hypothesized to be at the origin of this unsuspected diversity. It is possible that the slight, but
397 significant, differences in red algal community structure detected in our study between the three
398 distinct latitudinal zones could be linked to the historical glacial legacy of the region. However, within
399 the red algal samples under study, we detected only a low amount of cryptic species and the pattern
400 encountered for community structure was not mirrored by specific richness. Indeed, no clear gradient
401 of taxa diversity was observed, with 28 taxa recovered in KGI, our northernmost sampling point, and
402 27 taxa recovered in MAR, our southernmost sampling point (considering species richness calculated
403 with our “Integrative dataset”). Moreover, no genetic structure was observed among our three detected
404 biogeographic sub-regions for six common species of red algae sampled from KGI to MAR (i.e.
405 *Curdiea racovitzae*, *Iridaea cordata*, *Georgiella confluens*, *Gigartina skottsbergii*, *Palmaria decipiens*
406 and *Plocamium cartilagineum*; Guillemain et al., 2018). This result has been related with the drastic
407 effect of genetic bottleneck during glaciation and subsequent expansion while rafting on the strong
408 currents flowing along the coast of the South Shetlands Islands and the Western Antarctic Peninsula
409 (Guillemain et al., 2018). Indeed, Griffiths & Waller (2016) already reported the importance of rafting in
410 explaining the existence of wide-ranging macroalgae shaping in great extent the biogeography of
411 Antarctic and Sub-Antarctic intertidal communities in the Southern Ocean. In the same way, fronds
412 drifting on the cyclonic circulation inside the Bransfield Strait (Savidge et al., 2009) could easily lead
413 to recurring connectivity between the North-east Antarctic Peninsula (OHI) and the South Shetlands

414 Islands (KGI and PRAT). At last, since our three sub-regions correspond to three latitude levels, the
415 dynamic of sea-ice disturbance in the present day could also partly shape the observed differences in
416 red algal communities (Clarke et al. 2007, Terauds et al., 2012). Indeed, the distribution of some
417 species seems limited to the northern most areas of our sampling region, as for example *Porphyra* sp.1
418 and *Pyropia* sp.1 encountered only in the high intertidal zone of the three milder areas sampled.

419 Species richness has been described to decline with increasing latitude along the Antarctic Peninsula
420 (Moe & Delaca, 1976), a pattern commonly explained by an increasing effect of ice scour and
421 encasement at high latitudes (Waller, 2008). Indeed, these results seem well supported by the study of
422 diversity of macroalgal communities following a glacial retreat in King George Island (Quartino et al.,
423 2013), where species diversity correlated positively with time elapsed since substrate availability for
424 recolonization. In our study no clear gradient of taxa diversity was observed, even if slightly less taxa
425 can be observed in the southern part of our study region (i.e. GGV and MAR) than in the SSHs (i.e.
426 PRAT and KGI). The low species richness in OHI may be explained by specific and local limitations
427 (e.g. substrate availability, oceanic conditions increasing time of ice encasement or probability of ice
428 scouring) blurring possible observation of an existing latitudinal gradient. Nevertheless, variation in
429 level of ice disturbance could still explain the strong difference between the intertidal and the subtidal
430 communities in our study (Mystikou et al., 2014; Valdivia et al., 2014; Marcías et al., 2017). Indeed, at
431 least 2.5 more taxa were encountered in the subtidal zone than in the intertidal zone, whatever the area
432 under study, and this ratio was the most extreme in MAR (Bahia Margarita) where the subtidal zone
433 was eight times more diverse than the intertidal zone (Annex 1). Only *Palmaria decipiens* and *Iridaea*
434 *cordata* were commonly encountered in the intertidal zone of the five areas under study, two species
435 that were reported to be able to survive to extreme environmental conditions and were even recorded in
436 the Ross Sea (Wiencke & Clayton, 2002) and the continental coasts of East Antarctica (Wiencke et al.
437 2014).

438

439 *Integrative identification as a tool to study Antarctic macroalgal diversity and biogeography*

440 Morphological delimitation of macroalgal species commonly faces problems linked with a lack or
441 plasticity of characters and morphological stasis (Leliaert et al., 2014). This has led to taxon
442 misidentification, incorrect taxon delineation and a global underestimation of floral diversity, a
443 phenomenon also affecting our understanding of Antarctic macroalgal community structure (Moniz et
444 al., 2012; Young et al., 2013; Pellizzari et al., 2017). In our study, some molecular data contrasted with
445 morphological data, leading to 1) the detection of uncovered cryptic diversity and 2) of the lumping
446 under the same integrative name of various morphologically different (polymorphic) taxa, showing
447 very little to no genetic divergence. In previous studies, acquisition of molecular data sets has led to
448 both lumping (González-Wever et al., 2010) and splitting (see review in Kaiser et al., 2013)
449 morphologically recognized Antarctic taxa. However, simple approximation based on unique
450 divergence threshold adopted to delimit taxa, as in our study (i.e., sequences considered as
451 characteristic of distinct species when separated by more than 1% of divergence for the 5P-COI), only
452 represents a first step in the estimation of red macroalgal diversity in Antarctica. This relatively rapid
453 and low-cost method is usually used for large-scale barcode-based identification whereas combined
454 species delineation approaches, based on sequences from multiple independent genes, are
455 recommended for reliably identifying species (e.g. Montecinos et al., 2017). Complementary studies
456 will be needed to confirm the existence of cryptic species in the genera *Curdiea*, *Georgiella* and
457 *Callophyllis*.

458 Most studies where cryptic species were detected show that cosmopolitan species are much less
459 common than previously reported within Antarctic communities (Kaiser et al., 2013; see also De
460 Wever et al., 2009 for an example of microchlorophyte cryptic species). These observations have raised
461 questions about our capacity to clearly measure species diversity and detect fine-scale biogeographic
462 divisions in taxa where classical taxonomy is problematic (Kaiser et al., 2013). However, contrasting
463 with Robuchon et al. (2015), the use of integrative taxonomy in our study did not lead to an increase in

464 alpha-diversity in Antarctic red algae. Molecular data mostly led to lumping entities previously
465 recognized as different taxa using morphological criteria, ultimately reducing the number of taxa in the
466 "Integrative dataset" when compared to the "Morphological dataset" (51 and 49 taxa, respectively).
467 Interestingly, in the case of the genus *Plocamium*, all samples were lumped under the name *Plocamium*
468 *cartilagineum*, supporting the doubts raised by Hommersand and collaborators (2009) about the
469 validity of the species *Plocamium hookeri* and *Plocamium secundatum*. Even if the complementary use
470 of molecular data and classical taxonomy in our work had had little impact on results obtained about
471 diversity and structure of communities it had, nonetheless, improved level of species identification
472 (98% of our specimens in the "Integrative dataset" were determined at species level, while only 89% of
473 them had been determined at species level in the "Morphological dataset").

474 The relative low availability of sequences allowing comparisons of Antarctic macroalgae within
475 databanks, still prevented us going further than the genus on specimen name attribution in some cases.
476 We believe that the construction of accessible genetic dataset associated with voucher specimens in the
477 present work will benefit future studies of macroalgal diversity and distribution in the region. The 49
478 taxa of red macroalgae detected in the "Integrative dataset", with more than 20 taxa per area, are
479 consistent with the number of species previously listed for the region. A total of 75 species of red
480 macroalgae has been listed in Wiencke & Clayton (2002) for the whole Antarctic waters while only 21
481 has been reported on King George Island (Oliveira et al., 2009), 27 for the Marguerite Bay region
482 (Mystikou et al., 2014) and 46 along the coasts of the South Shetland archipelago (i.e. excluding
483 Corallinaceae, Pellizzari et al., 2017). Nevertheless, it is worth to note that our work represents only an
484 incomplete view of the Antarctic red macroalgal diversity since molecular data has been obtained for
485 only 59% of our collected specimens and some families have been particularly difficult to sequence
486 (i.e. Delesseriaceae, Kallymeniaceae; less than 55% of specimens with molecular data; Annex 1). For
487 example, we did not detect the divergent genetic group named "*P. cartilagineum* isolate A" reported in

488 the study of Young et al. (2013) even with molecular data obtained for 65 of our 88 samples of
489 *Plocamium*.

490

491 CONCLUSION

492 Our work is the first attempt to document red seaweeds diversity along the Antarctic Peninsula and the
493 South Shetlands Islands coasts using integrative taxonomy. It highlighted the importance of using
494 complementary tools to lead biodiversity surveys even in areas considered as well known, as cryptic
495 diversity and morphology-based assignation errors can be underlined. On the contrary to our
496 expectations, molecular tools did not increase alpha-diversity, but allowed us to be more accurate when
497 identifying species. Errors in naming species can have strong implications when studying
498 biogeographic patterns or species role in ecological processes (Bortolus, 2008) and integrative
499 taxonomy can clearly offers better possibilities to understand structure and dynamics of marine benthic
500 ecosystems. We believe that the collection of COI and *rbcL* sequences associated to voucher specimens
501 of red macroalgae sampled in our study region (i.e from the SSHs down to the center-southern of the
502 WAP) can be used as a platform to generate new phylogenetic, ecological and biogeographical studies
503 in Antarctica and the Southern Ocean. Indeed, Antarctic macroalgae are extremely stenothermic
504 organisms for which rapid climatic changes in the WAP could affect resilience and distribution and
505 more work will be needed to clarify their actual diversity and distribution and test for potential changes
506 depending on future scenarios (Wiencke et al., 2014). Human activities could also add to threats linked
507 to global climatic changes. Economic interests in sub-Antarctic and Antarctic marine communities
508 have grew recently and, as red macroalgae offers great economic prospects for molecules extraction,
509 they could be particularly at risk.

510

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522

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716

717 FIGURE LEGENDS

718 Figure 1: Map of the study region including the five sampling areas: PRAT = Greenwich Island, KGI =
719 King George Island, OHI = O'Higgins Antarctic base, GGV = Paradise Bay and MAR = Marguerite
720 Bay. The study region covered the two distinct biogeographic regions proposed by Linse et al (2006);
721 the South Shetlands Islands (SShs) and the Antarctic Peninsula (AP).

722 Figure 2: Unrooted neighbour-joining (NJ) phylogenetic tree based on the COI sequences (632 bp) of
723 Antarctic red algae. Sequences downloaded from public database are indicated by their GenBank
724 number while specimens collected during our surveys are noted in bold. Species names, as given in the
725 “Integrative dataset”, are noted next to collapsed branches.

726 Figure 3: Unrooted neighbour-joining (NJ) phylogenetic tree based on the *rbcL* sequences (958 bp) of
727 Antarctic red algae. Sequences downloaded from public database are indicated by their GenBank
728 number while specimens collected during our surveys are noted in bold. Species names, as given in the
729 “Integrative dataset”, are noted next to collapsed branches.

730 Figure 4: Taxa accumulation curves obtained from identification based only on morphological
731 characters (“Morphological dataset”) and from integrative identification including molecular markers
732 (“Integrative dataset”) for red macroalgae communities sampled in our study region.

733 Figure 5: Absolute values of specific richness calculated for each sampling area using the
734 "Morphological dataset" and the "Integrative dataset". For each site, information available for the three
735 bathymetric zones was pooled together and analysed jointly.

736 Figure 6: Non-metric multidimensional scaling (nMDS) of taxa detected within our “Integrative
737 dataset” and based on distance matrix calculated with Jaccard’s. Geographic areas: PRAT = Greenwich
738 Island, KGI = King George Island, OHI = O'Higgins Antarctic base, GGV = Paradise Bay and MAR =
739 Marguerite Bay.

740

741 TABLE

742 Table 1: Results of the pairwise ANOSIM test. Matrix was calculated using Jaccard’s index and the
 743 “Integrative dataset”. Significant values are indicated with a star. Geographic areas: PRAT =
 744 Greenwich Island, KGI = King George Island, OHI = O’Higgins Antarctic base, GGV = Paradise Bay
 745 and MAR = Marguerite Bay.

Results of pairwise tests between geographic areas, all bathymetric zones pooled together

Global R Statistic = 0.253

Groups	R Statistic	<i>p</i> -value
KGI, MAR	0.390	0.001*
KGI, GGV	0.273	0.011*
KGI, OHI	0.007	0.467
KGI, PRAT	0.067	0.212
MAR, GGV	0.514	0.001*
MAR, OHI	0.405	0.005*
MAR, PRAT	0.443	0.001*
GGV, OHI	0.256	0.01*
GGV, PRAT	0.172	0.025
OHI, PRAT	0.151	0.096

746

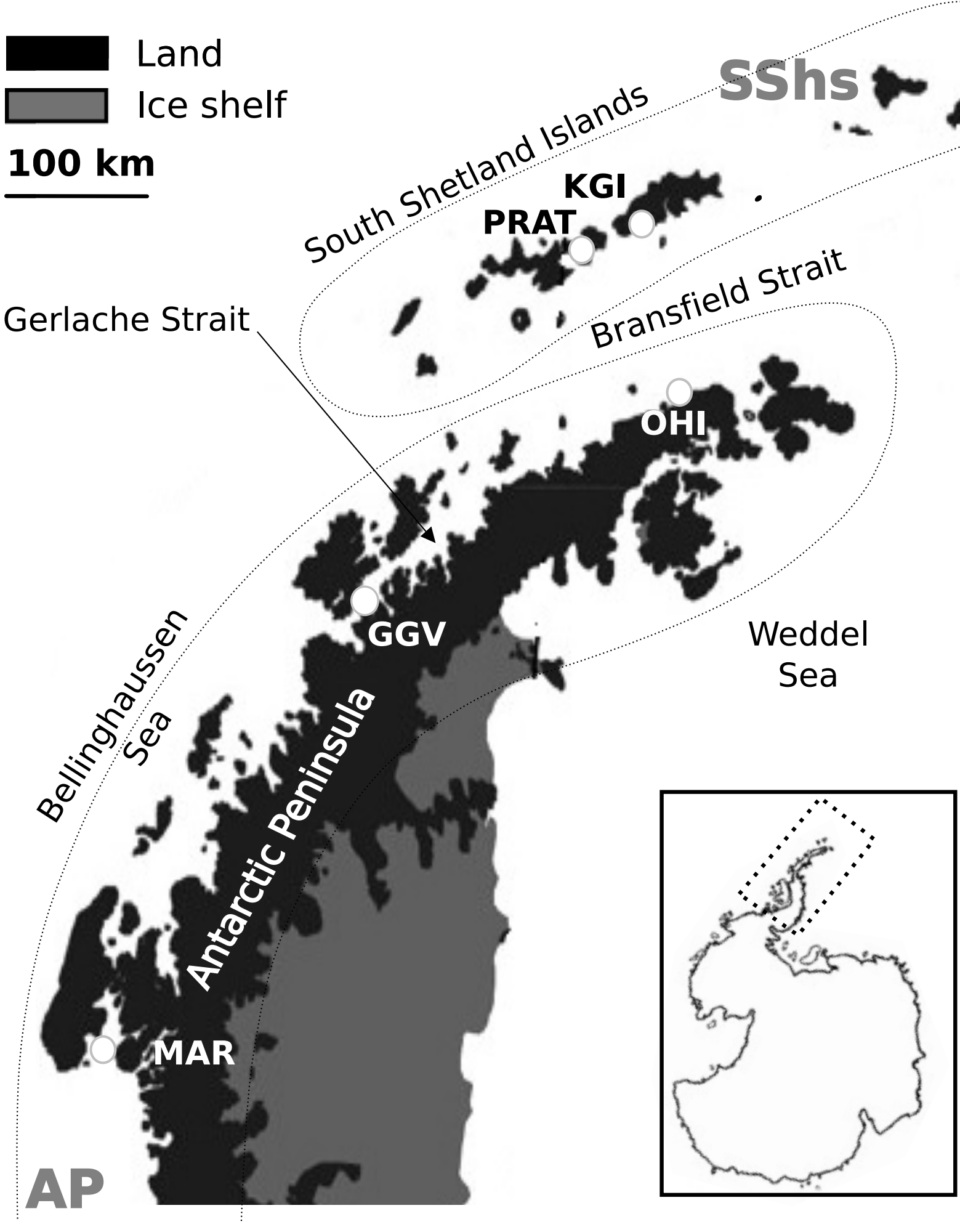
747 ANNEX

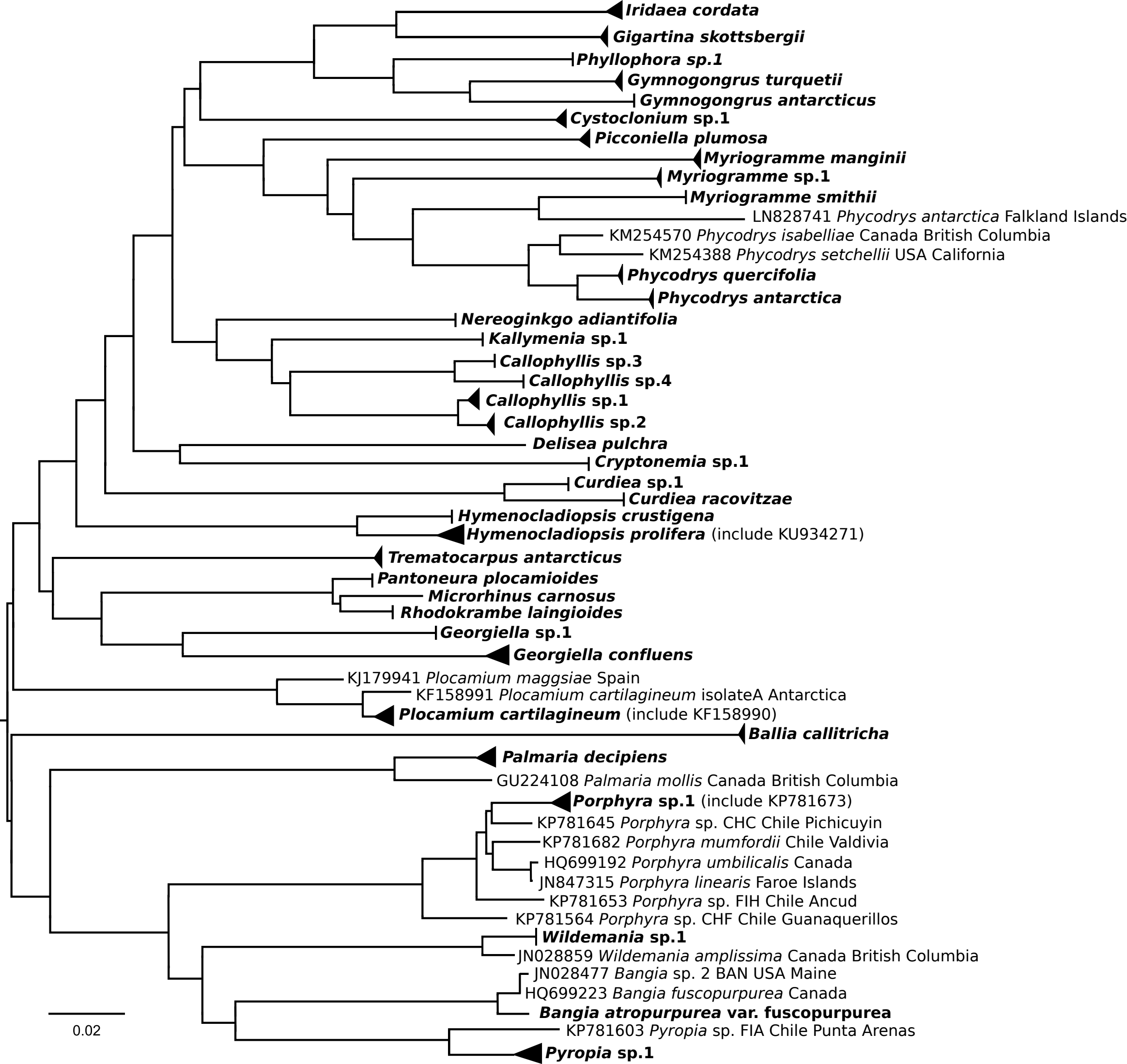
748 Annex 1: Specimen collection information including specimen ID code; taxa identification based only
 749 on morphological characters (i.e. corresponding to the “Morphological dataset”); taxa identification
 750 from integrative identification including molecular markers (i.e. corresponding to the “Integrative

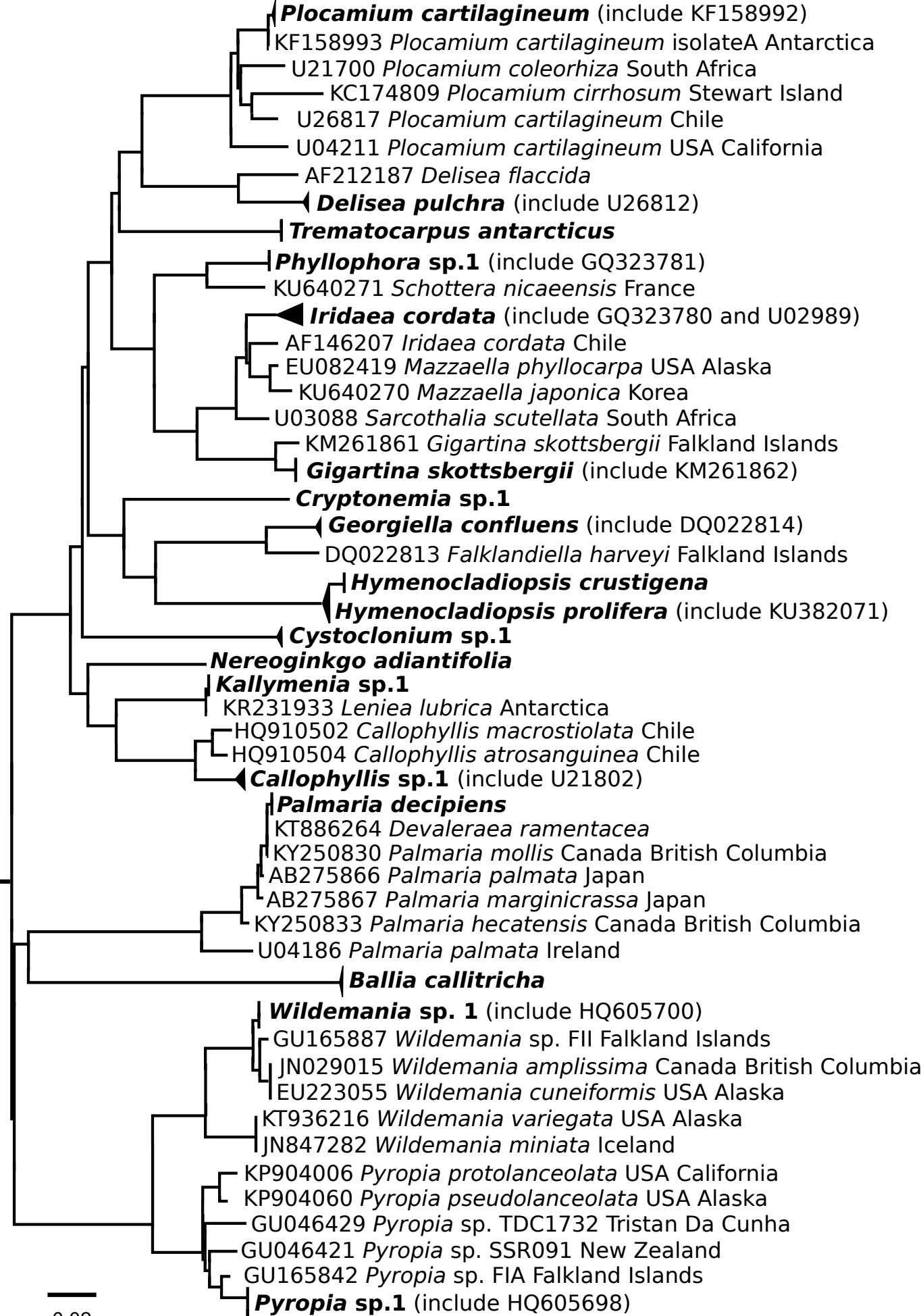
751 dataset”); GenBank accession numbers for the COI and the *rbcL*, when available (i.e. “_” no sequence
752 available); Sampling region and area; Sampling sites and their GPS coordinates; sampling year;
753 bathymetric zone; collectors; researchers in charge of the taxa identification based on morphological
754 characters; orders and families for each specimen, classified when taking into account the “Integrative
755 dataset”. Specimens are classified in increasing specimen ID code order.

Land
Ice shelf

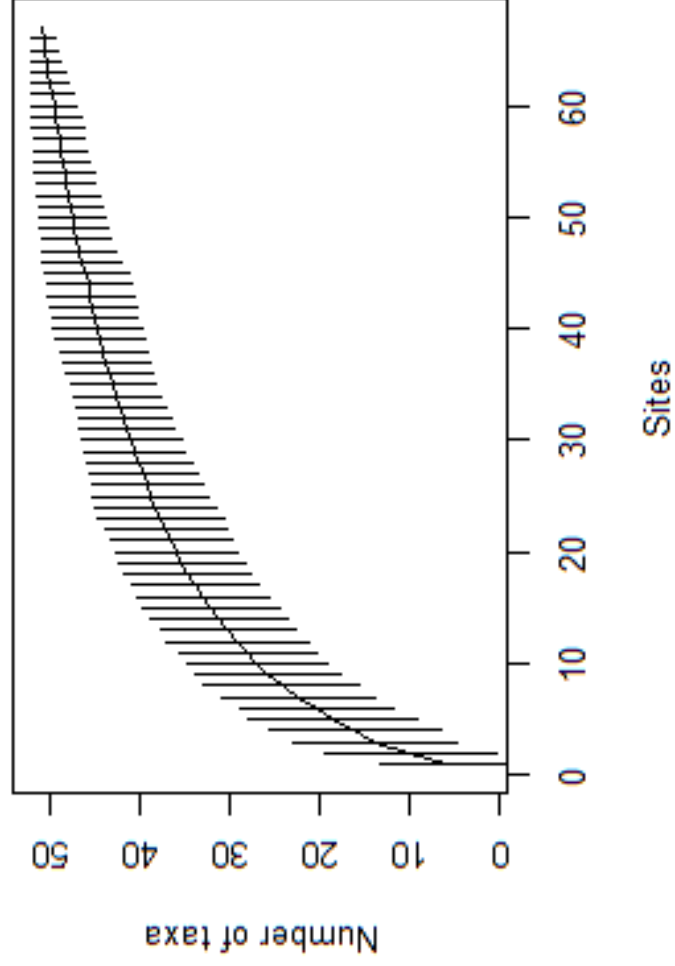
100 km



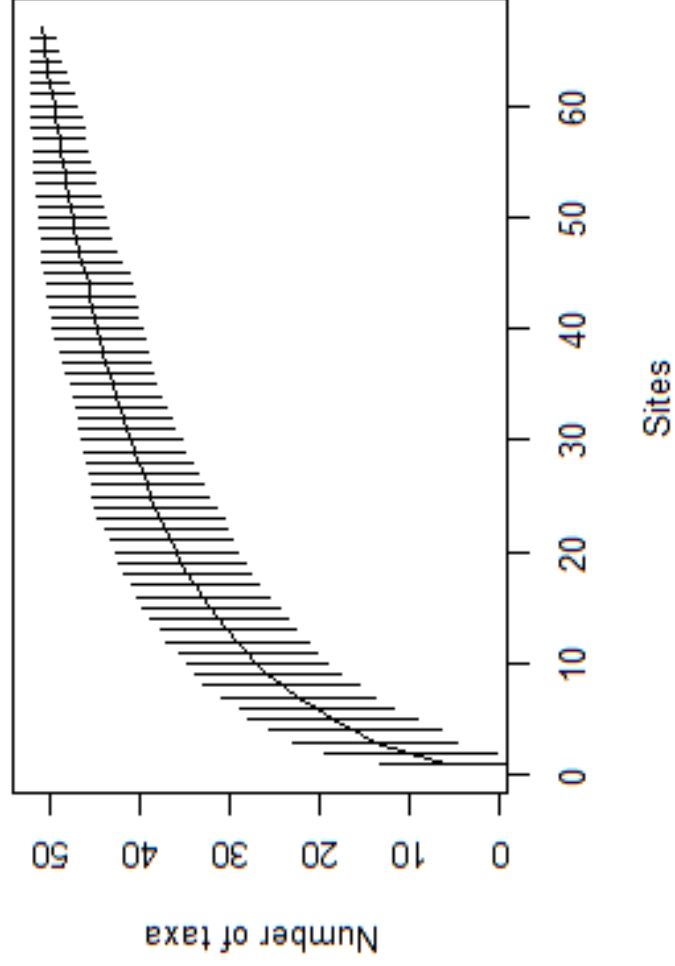


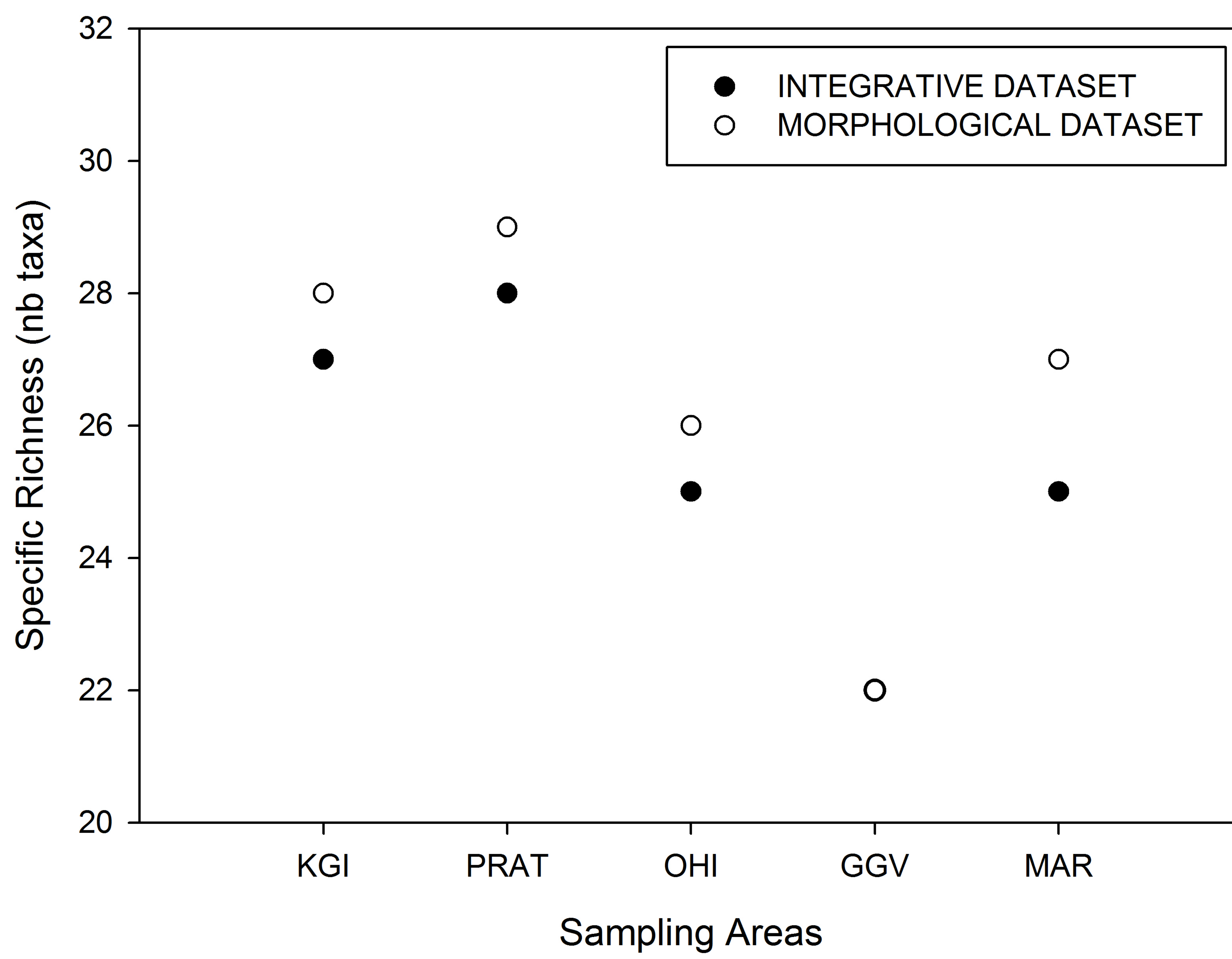


Morphological Dataset



Integrative Dataset





Stress value : 0.11

 KGI

 OHI

MAR 

PRAT 

GGV

