

01-May-2017

Dear Dr Campos De Paula:

Ref: NZJB-2017-0015.R2 Molecular evidence of the presence of *Dictyota dichotoma* in Argentina.

Thank you for your revision which has been accepted for publication in the New Zealand Journal of Botany. If there are any formatting or production issues with the revised text or figures you have sent me, the editorial office will be in contact to request further revision.

The formal date of acceptance is 01-May-2017, or the date on which all electronic files are received in a production-ready state by the editorial office.

Final editing and production of manuscripts is handled by Taylor & Francis, Melbourne who will contact you regarding proofs etc. However, please feel free to send any enquiries on these matters to the main editorial office of the Royal Society journals at: publish@royalsociety.org.nz.

Please also let us know if you intend to be away for extended periods of time in the next few months. If this is the case, we would like you to nominate a co-author or colleague to deal with urgent enquiries and checking of page proofs and to provide us with their contact details (email, fax, telephone).

The journal cover will feature a colour image representing a paper published in the issue. I am inviting all authors to send a portrait-oriented colour photo or file (300 dpi cmyk tif. or > 1 MB jpg) of an image relating to their paper suitable for the 297mm High x 210mm Wide image area on the front cover. Please also supply a brief caption and details of the photographer (name, affiliation). Please note that candidate photos must not have any copyright restrictions on their use. Potential cover images should be sent directly to: publish@royalsociety.org.nz, clearly referencing the paper to which they relate.

Please note that author names are published as listed in the final accepted version of the manuscript. If you or a co-author requires this to be changed (e.g. from full name to initials-surname) please contact the Royal Society editorial office by return email (publish@royalsociety.org.nz). As the submitting author, it is your responsibility to check all names are accurate on the final file at page-proof stage.

Yours

Dr Roberta D'Archino

Associate Editor, New Zealand Journal of Botany

roberta.darchino@niwa.co.nz

1 Molecular evidence of the presence of *Dictyota dichotoma* in Argentina based on sequences
2 from mtDNA and cpDNA and a discussion of its possible origin

3
4 Erick Alves Pereira Lopes-Filho^a, Fabiano Salgueiro^a, Silvia Mattos Nascimento^a, M. Cecilia
5 Gauna^b, Elisa R. Parodi^b, Joel Campos De Paula^{a,*}

6
7 ^aPrograma de Pós-Graduação em Biodiversidade Neotropical, Universidade Federal do Estado
8 do Rio de Janeiro, CEP 22290-255, Rio de Janeiro, Brazil

9 ^bCONICET- Instituto Argentino de Oceanografía. Universidad Nacional del Sur, Camino La
10 Carrindanga Km 7, CONICET-Bahía Blanca Edificio E1, B8000FWB Bahía Blanca, Argentina

11 *Corresponding author: depaula.joelc@gmail.com

12
13 ABSTRACT

14 *Dictyota* is a brown alga genus inhabiting tropical to warm temperate environments where it
15 performs an important ecological role, such as a food source, shelter and substrate to several
16 species of invertebrates and other algae. The taxonomy of this genus is troublesome, with poor
17 species delimitation and doubtful records in the literature. *Dictyota dichotoma*, the type species
18 of the genus, was commonly accepted as a cosmopolitan species because of the inaccurate
19 geographical distribution range as a consequence of its misidentification in several parts of the
20 world. Recent studies with molecular data revealed new species and a new understanding of the
21 evolution and biogeography of the genus *Dictyota*. These studies confined populations of *D.*
22 *dichotoma* to Europe (Atlantic and Mediterranean coasts) and the Macaronesian Islands (Azores,
23 Madeira and Canary archipelagos). Also, they confirmed its presence in South Africa, but,
24 whether the species was native or introduced in South Africa could not be verified. In the present
25 study two regions of cpDNA (*psbA*, *rbcL*) and one region of mtDNA (*nad1*) from Argentine
26 samples, identified morphologically as *D. dichotoma*, were analyzed and compared with other
27 *Dictyota* species. The identity of these samples as *D. dichotoma* was confirmed. A haplotype
28 network analysis using all available *psbA* sequences distinguished seven haplotypes divided into
29 two geographic groups: Atlantic-Mediterranean and Canarian. In Argentina and South Africa, only
30 the most common haplotype of the Atlantic–Mediterranean group was observed. According to the
31 paleoceanographic currents and the presence of European haplotype, the introduced nature of *D.*
32 *dichotoma* is indicated.

33
34 Keywords: Dictyotaceae; South Atlantic Ocean; Exotic Species; *psbA*; *rbcL*; *nad1*

35
36 INTRODUCTION

37 *Dictyota* J.V. Lamouroux is an important component of the marine flora in tropical and
38 subtropical environments and is commonly found from the lower range of the intertidal zone to
39 deeper areas (~80m) of the subtidal zone (Littler & Littler 2000; De Clerck et al. 2006; Bittner et
40 al. 2008). The genus has attracted the attention of researchers for several reasons, including the

41 production of bioactive metabolites (Vallim et al. 2005; De Paula et al. 2011) and its ecological
42 role, such as the high biomass that may be used as a food source, shelter and that serves as a
43 substrate to several species of invertebrates and other algae (Genzano & Rodríguez 1998;
44 Stachowicz & Hay 2000; Gauna et al. 2014; Moore & Eastman 2015), as well as the high number
45 of species that make this genus very diverse (Tronholm et al. 2010b). The identification of *Dictyota*
46 species may be challenging due to phenotypic plasticity, simple morphology and poor delineation
47 between species, which is the reason for the description of hundreds of names for new taxa and
48 doubtful geographical distributional ranges (Schnetter et al. 1987; De Clerck 2003; Tronholm et
49 al. 2010a, 2010b; Gauna et al. 2013). In the southwestern Atlantic, the genus *Dictyota* remains
50 understudied and the species recorded have never been the subject of a formal review, although
51 there have been some nomenclatural corrections (Oliveira-Filho 1977; Széchy & De-Paula 2016).

52 *Dictyota dichotoma* (Hudson) J.V. Lamouroux was described as *Ulva dichotoma* in
53 England in 1762 and later it was transferred to *Dictyota* in 1809 (Lamouroux 1809). During the
54 19th and 20th centuries, *D. dichotoma* was considered to be widely distributed from tropical to
55 warm temperate areas of the world (Hwang et al. 2005; Tronholm et al. 2008, 2010b) and it
56 was placed in an eurythermic group of species (Van den Hoek 1982). Tronholm et al. (2010b)
57 reviewed the genus *Dictyota* for Europe using molecular data and a large dataset with sequences
58 from several places of the world and they restricted the occurrence of *D. dichotoma* to the
59 northeastern Atlantic (Europe and Africa). The specimens from other oceans, previously identified
60 as *D. dichotoma*, were assigned to other species and so this taxon should not be considered as
61 a cosmopolitan species. So, the native range of *Dictyota dichotoma* on the European Atlantic
62 coasts was established, from Scotland and southern Norway to Portugal, the Mediterranean Sea
63 and most of the Macaronesia islands, except for the tropical Cape Verde where it was not found
64 (Van den Hoek 1982; Schnetter et al. 1987; Tronholm et al. 2010b). *D. dichotoma* could not be
65 attributed to natural or introduced origin in South Africa despite that its occurrence there had been
66 questioned by De Clerck (2003), the intriguing record of this species by Tronholm et al. (2010b)
67 using a *psbA* marker, and its disjunct distribution.

68 On the Atlantic coast of South America, there are many records of misidentification of
69 species of *Dictyota* as *D. dichotoma* in earlier phycological studies (Saint-Hilaire 1833; Dunal
70 1833; Martius et al. 1833; Montagne 1839). Recently, *D. dichotoma* has been considered as a
71 cryptogenic species in Argentina (Gauna et al. 2014; Raffo et al. 2014; Schwindt et al. 2014).

72 Exotic species are one of the major threats to biodiversity and one of the major concerns
73 throughout the oceans (Schwindt et al. 2014; Abreu et al. 2016). Invasion of marine macroalgae
74 have been reported around the world in recent decades, such as *Undaria pinnatifida* (Harvey)
75 Suringar, *Sargassum muticum* (Yendo) Fensholt, *Codium fragile* ssp. *fragile* (Suringar) Hariot,
76 *Caulerpa taxifolia* (M. Vahl) C. Agardh and *Schizymenia dubyi* (Chauvin ex Duby) J. Agardh
77 (Ramírez et al. 2002; Casas et al. 2004, 2008; Raffo et al. 2009; Irigoyen et al. 2011). Recently,
78 Schwindt et al. (2014) sampled six of the ten main marine ports in Argentina and found the
79 presence of 32 non-indigenous taxa, including exotic and cryptogenic taxa.

80 This study aims to (i) confirm the molecular identity of specimens previously identified as
81 *Dictyota dichotoma* from Argentina and (ii) discuss the possible origin of the South Atlantic
82 populations.

83

84 MATERIALS AND METHODS

85 *Dictyota* specimens were collected from Las Grutas, Argentina (San Matías Gulf, 40° 48'
86 S, 64° 48' W) in 2014 from a population previously studied by Gauna et al. (2013, 2014) with
87 respect to the morphology of the specimens, phenology and ecology (epiphytic communities).
88 The algae were collected by SCUBA diving from the subtidal zone and then screened in the field
89 to remove possible epiphytes. Each individual was separated into two sub-samples, one of which
90 was preserved in silica gel for molecular studies, and the other preserved in 4% formalin solution,
91 and deposited at the BBB - Herbarium of Universidad Nacional del Sur (Bahía Blanca, Argentina).

92 DNA was extracted using HiPura™ Plant Genomic DNA Miniprep Purification Kit
93 (MolBio™ HIMEDIA). The Plastid-encoded PSII reaction center D1 (*psbA*), NADH
94 dehydrogenase subunit 1 (*nad1*) and RUBISCO large Subunit (*rbcL*) were amplified
95 via Polymerase Chain Reaction (PCR) utilizing the primers from Tronholm et al. (2010b) and
96 Bittner et al. (2008) and then sequenced by MacroGen Inc. Korea.

97 The sequences were edited on Mega 6.0 (Tamura et al. 2013) and then analyzed with
98 others retrieved from Genbank (www.ncbi.nlm.nih.gov/genbank) (Table S1). Three datasets were
99 generated with alignments of 695bp for *nad1*, 775bp for *psbA* and 1149bp for *rbcL*. The
100 phylogenetic reconstructions were performed for each gene separately. The concatenation would
101 not be reliable in this case because sequences available on Genbank are from distinct vouchers
102 and from different locations.

103 The evolution model of GTR+I+G was obtained by jModelTest 2.14 for each marker
104 (Darriba et al. 2012) and then used in the Bayesian Inference (BI) analysis on MrBayes 3.1.2
105 (Ronquist et al. 2012). For *nad1* and *psbA*, BI was carried out with 1 million generations in 2 runs
106 and 4 chains, sampling every 1000th generation, discarding the first 30 and 50 trees for *nad1* and
107 *psbA*, respectively. For *rbcL*, BI was carried out with 2 million generations in 2 runs and 4 chains,
108 sampling every 1000th generation, discarding the first 50 trees. The Maximum Likelihood (ML)
109 analysis was performed on Mega 6.0, using the GTR+I+G model with bootstrap of 1000
110 replications.

111 The gene with a higher number of *Dictyota dichotoma* sequences available from Genbank
112 was *psbA* and therefore it was used to build a haplotype network with Dnasp v.5 (Librado & Rozas
113 2009) and Network v.5 by Median Joining. The initial alignment used the 220 available sequences
114 (Table S2). Then, the shorter sequences and those with many missing data were removed from
115 the analysis, leaving 149 sequences. To account for the disparity in the number of sequences
116 available from different geographic regions/localities, up to 10 sequences/haplotypes per
117 population were used in the analysis, considering that sequences retrieved from samples
118 collected from up to 100 km apart were considered as part of the same population. The final
119 dataset used in the haplotype network analysis included 68 sequences with 638bp (Table S2).

120

121 RESULTS

122 The topology of the trees for *psbA*, *rbcl* and *nad1* obtained in the present study agreed
123 with previous studies (Tronholm et al. 2010a, 2010b). The samples from Argentina formed a clade
124 with genuine European sequences of *D. dichotoma*. For *psbA* the clade with sequences from
125 Argentina and from the neotype elected by Tronholm et al. (2010b) obtained bootstrap values of
126 100 to ML and 1.00 of posterior probability in the BI analysis (Figure 1). For *rbcl* (Figure S1) high
127 values of bootstrap and posterior probability were obtained for the clade with sequences from
128 Argentina and Ireland. High values were also observed for *nad1* (Figure S2) for the clade with
129 sequences from Argentina and France.

130 The haplotype network (Figure 2) revealed seven haplotypes in the northeast Atlantic
131 ocean that were geographically divided into two groups: Atlantic-Mediterranean and Canarian.
132 The Atlantic-Mediterranean group consisted of two haplotypes, with H1 being the most
133 widespread and present on the Atlantic coasts of the Iberian Peninsula, France, the British Islands
134 and the North Sea, the entire Mediterranean Sea and part of the Macaronesia Islands (Azores
135 and Madeira Archipelagos). The second haplotype (H2) seems to be less frequent in general and
136 was only detected in the Gulf of Lion (France), in the Mediterranean Sea. Samples from Argentina
137 and South Africa corresponded to the H1 haplotype. The Canarian group consisted of five
138 haplotypes (H3, H4, H5, H6 and H7) that are almost restricted to Macaronesia (Canary and
139 Madeira Islands), whereas H5 is the most common one, widely found throughout these islands.
140 The H3 haplotype from the Canarian group is the only one that was detected away from these
141 islands in the Gulf of Lion.

142

143 DISCUSSION

144 The higher haplotype diversity observed for *D. dichotoma* in the northeastern Atlantic
145 (Figure 2) is consistent with the hypothesis presented by Tronholm et al. (2010b) explaining the
146 low diversity of the genus *Dictyota* in Europe. This hypothesis states that following the desiccation
147 of the Mediterranean Sea, which caused the extinction of most of the marine biota during the
148 Messinian salinity crisis (6.8 - 5.3 Ma), the Mediterranean Sea was recolonized by Atlantic species
149 from adjacent areas, such the Macaronesian Islands and the northwestern African coast when it
150 was re-flooded (Coll et al. 2010; Tronholm et al. 2010b). The results from the present study
151 demonstrated that European Atlantic and Macaronesian populations of *D. dichotoma* were
152 probably separated during this vicariant event and, later, northern populations (haplotype H1)
153 were successful in recolonizing the Mediterranean Sea and subsequently reached Macaronesia
154 (Azores and Madeira Archipelagos). Despite having a greater diversity, the Canarian group
155 remained restricted to Macaronesia with the only exception of the haplotype H3, in contrast to
156 what seems to have happened with other *Dictyota* species from Macaronesia that were able to
157 recolonize the Mediterranean Sea (Tronholm et al. 2010b). However, the presence of the H1
158 haplotype in the South Atlantic would contradict this scenario.

159 The results of Tronholm et al. (2012), using the relaxed molecular clock for the genus,
160 revealed *D. dichotoma* as the only extant species of one of the oldest lineages in the genus, that
161 split early from the others circa 44.37 Ma (53-34 Ma) and being one of the first clades to disperse
162 through the Tethys Seaway to the westernmost part of the Tethys realm, where it most likely
163 evolved and, later dispersed to the Atlantic coast of Europe and Macaronesia. Since the opening
164 of the South Atlantic (100 – 80 Ma) the northward paleocurrents along the African coast allowed
165 the water transport of the Tethys sea to the Atlantic via Southern Africa while most of the transport
166 continued on account of the circum-equatorial circulation directly to the North Atlantic until 12-18
167 myr (Stille 1992; Stille et al. 1996; Cowman 2013), when the circulation in the North Atlantic was
168 governed by the Pacific-North Atlantic current (Iturralde-Vinent 2006). Therefore, the colonization
169 of the South Atlantic coast of Africa by *D. dichotoma* would have been prevented because it would
170 have been against the direction of the ocean currents.

171 Furthermore, there was no connection between the westernmost part of Tethys realm
172 and South America, thereby a warm paleocurrent (probably originating in the Caribbean) crossed
173 the Atlantic coast of South America to Patagonia and Tierra del Fuego, where the marine
174 communities were tropical until middle-late Miocene (Del Río 2004a, 2004b; Le Roux 2012).
175 The hypotheses of trans-oceanic dispersal in this scenario fail mainly due to biology of *D.*
176 *dichotoma* because culture experiments demonstrated that this species does not possess
177 affinities to tropical conditions (Biebl 1959; Bogaert et al. 2016) which would be required for it to
178 be successfully dispersed along the South American coast until the late Miocene. The adequate
179 temperate marine conditions for *D. dichotoma* in the southwestern Atlantic were only established
180 after the complete development of the Circumpolar Antarctic Current which lead to the full
181 operation of the Malvinas/Falklands Current and the establishment of the Benguela Upwelling
182 System in the Middle-Late Miocene (10 – 9 Ma). As a consequence, there was a decrease in the
183 seawater temperature in Patagonia and along the southwestern African coast (Heinrich et al.
184 2011; Rommerskirchen et al. 2011; Le Roux 2012), the extinction of the Patagonian tropical
185 marine communities and the retraction of the warm Brazilian current to the north of
186 Argentina/Uruguay (Del Río 2004a, 2004b). Therefore, it is difficult to explain how *D. dichotoma*
187 would have reached the coast of Argentina through natural dispersion.

188 Those facts agree with the current data that do not show any exclusive haplotypes in
189 the South Atlantic populations (Argentina and South Africa) of *D. dichotoma*, which would be
190 expected in the case of an old dispersal from the northeastern Atlantic (over 10 myr), or any
191 haplotype shared with the Canarian group, which would be expected in the case of a recent
192 dispersal (less than 6 myr) and so, against the direction of the ocean currents. The occurrence of
193 the H1 haplotype in the South Atlantic ocean is inconsistent with the hypothesis of natural
194 dispersal with subsequent genetic differentiation and supports the hypothesis of human-mediated
195 introduction, where propagules of *D. dichotoma* settled successfully in Argentina and in South
196 Africa because both areas belong to temperate provinces, similar to its native area in the
197 northeastern Atlantic (Spalding et al. 2007).

198 The first evidence of the presence of *D. dichotoma* in Argentina based on
199 chemosystematics, rather than just morphology, is present by Palermo et al. (1994) who studied
200 a population from Nuevo Gulf and identified three prenylated diterpenes expected to occur (and
201 its precursors) in this species (Amico et al. 1976; Fattorusso et al. 1976; Faulkner et al. 1977;
202 Siamopoulou et al. 2004; Vallim et al. 2005). The locations where *D. dichotoma* was collected by
203 Palermo et al. (1994) for this study are near to two of the six main marine ports studied
204 by Schwindt et al. (2014). These authors demonstrate that the collection areas are in natural bays
205 with anthropic influences (but with abiotic conditions adequate for *Dictyota dichotoma*) and have
206 high maritime activities, which enable the high percentage of non-indigenous marine taxa found
207 in them. Port areas provide artificial structures that favor the introduction of exotic fouling/benthic
208 species (recruitment, survival and dispersal) which are mainly transported by ballast water
209 (Schwindt et al. 2014; Abreu et al. 2016; Lin & Zhan 2016). In South Africa most of the introduced
210 species reported in both the cool and warm temperate provinces are from the Northern
211 hemisphere (65%). At the Agulhas ecoregion, where the presence of *D. dichotoma* was confirmed
212 (Tronholm et al., 2010), 73 non-indigenous (alien, invasive or cryptogenic) taxa have been
213 reported (Mead et al. 2011; Robinson 2015).

214 Introduction of exotic species may be overlooked for decades (Abreu et al. 2016),
215 especially when a group (such as *Dictyota*) has not been formally revised. It is not clear at present
216 how *D. dichotoma* has affected the marine species in Argentina, especially on the northern parts
217 of the Patagonian coast where the genus *Dictyota* (reported as *D. dichotoma*) is abundant (Casas
218 et al. 2004; Gauna et al. 2014). For example, in the Gulf of San José and nearby areas (e.g.
219 Nuevo Gulf and San Matias Gulf) *D. dichotoma* may cover up to 30% of the entire area at depths
220 up to 10 meters (Boraso de Zaixso & Zaixso 2007; A. Boraso pers. com.).

221 In the same way, the impact on native *Dictyota* species could not be estimated. The
222 known distribution of the genus *Dictyota* in Argentina (Figure S3) covers the final part of the Warm
223 Temperate Southwestern Atlantic province and the Atlantic part of the Magallanic province
224 (Spalding et al. 2007). The persistent misidentification for almost two centuries (since Montagne
225 1839) resulted in *D. dichotoma* being considered as the only species in Argentina and the reports
226 of other species, such as *Canistrocarpus cervicornis* (as *Dictyota cervicornis*), *D. divaricata* and
227 *D. dichotoma* var. *intricata* (Taylor 1939; Asensi 1966; Van den Hoek 1982; Boraso de Zaixso
228 1995; Mendoza & Nizovoy 2000; Piriz et al. 2003; Boraso & Zaixo 2012; Croce et al. 2015), were
229 considered as representing a wide morphological variation of it (Boraso 2012). Although the
230 genus has also been found on the Beagle Channel (A. Boraso, pers. com.), Mystikou et al. (2016)
231 published the southernmost records of *Dictyota* in the southwestern Atlantic. Their molecular data
232 confirmed that the species are distinct from *D. dichotoma* (as also verified in Figure 1), which is
233 the first step towards uncovering the diversity of the genus in Argentina.

234 The spread of *D. dichotoma* northward along the southwestern Atlantic from the
235 Argentine populations is unexpected because of (i) the Confluence Zone of the southward warm
236 Brazilian current and northward cold Malvinas/Falklands current - that spans from about 25°S to
237 45°S-, where the water masses are reflected eastward as a South Atlantic current (approximate

238 average axis at 39°S, Bisbal 1995), and (ii) the La Plata river (at 35°S), which results in brackish
239 and turbid water, as well as the lack of a suitable substrate that prevents the occurrence of
240 seagrass and marine benthic macroalgae in the La Plata estuary region, which is the reason for
241 the depauperate flora on the Argentine, Uruguayan and Brazilian coasts under its influence (Coll
242 & Oliveira 1999; Calliari et al. 2003; Acha et al. 2008; Braga et al. 2008; Campos et al. 2008).
243 Also, the only Uruguayan record of *Dictyota* (as *D. dichotoma*) was questioned by Coll & Oliveira
244 (1999) because no specimens were found in any of the mentioned herbaria in the original study
245 and no other specimens have been collected subsequently. Therefore, *Dictyota* species are
246 separated by more than 1200 km between Mar del Plata (Argentina) and Torres (Brazil) due to
247 the lack of any hard substrate (Baptista 1977; Oliveira Filho 1977).

248 In the southeastern Brazil ecoregion, which includes two important Brazilian harbors, there
249 are seasonal coastal upwelling events and the cold (< 18 °C) water masses may reside throughout
250 the year in a deep benthic system (Coelho-Souza et al. 2012). These waters could sustain
251 organisms with cold waters affinities, such as the brown alga *Laminaria abyssalis* Joly & Oliveira,
252 which occurs below 50 meters (Guimarães et al. 1986). Moreover, the intertidal *Jolyana*
253 *laminarioides* Guimarães and *Elachistiella leptoneumatoides* Cassano, Yoneshigue-Valentin &
254 Wynne (Valentin 2001; Cassano et al. 2004) only occur during the upwelling period. This can
255 promote a temporary niche for the introduction of species with cold water affinities. There is no
256 evidence, so far, of the presence of *D. dichotoma* and most of the morphologically similar
257 specimens are *D. menstrualis* (unpublished data). Recent reports of *D. dichotoma* and *D.*
258 *dichotoma* var. *intricata* by Villaça et al. (2010) and Crespo et al. (2014) are due to the citation of
259 old studies

260 In conclusion, the natural dispersal of the species from the northeast Atlantic to Argentina
261 and South Africa would be unlikely, as this temperate species would have to cross the whole
262 equatorial and tropical Atlantic ocean against the direction of the current. The occurrence of a
263 single, and the most common, haplotype of the Atlantic–Mediterranean group in the South Atlantic
264 populations suggests that *D. dichotoma* was introduced. The human-assisted introduction of other
265 taxa of Dictyotaceae has already been reported elsewhere, such as the cases of *Rugulopteryx*
266 *okamurae* (E.Y. Dawson) I.K. Hwang, W.J. Lee & H.S. Kim (Verlaque et al. 2009), *Dictyota*
267 *cyanoloma* Tronholm, De Clerck, A. Gómez-Garreta & Rull Lluç (Tronholm et al. 2010b; García
268 et al. 2016) and *Dictyota furcellata* (C.Agardh) Greville (Nelson & Wilcox 2010). Future studies
269 including a more comprehensive sampling are necessary and may reveal whether the introduction
270 of *D. dichotoma* has occurred once or multiple times, its actual geographical range in Argentina
271 and how it has affected the benthic marine community

272

273 SUPPLEMENTARY MATERIAL

274 Figure S1: Phylogenetic tree based on *rbcl* sequences, presenting a consensus topology
275 estimated by Maximum likelihood (ML) and Bayesian Inference (BI) analyses.

276 Figure S2: Phylogenetic tree based on *nad1* sequences, presenting a consensus topology
277 estimated by Maximum likelihood (ML) and Bayesian Inference (BI) analyses.

278 Figure S3: Localities where *Dictyota* has been reported from Argentina, including the populations
279 of *D. dichotoma* discussed in this paper

280 Table S1: Sequences used on the phylogenetic analyses

281 Table S2: Sequences used on haplotype network of *psbA* from *Dictyota dichotoma*

282

283 ACKNOWLEDGEMENTS

284 We would like to thank Dr. Alicia Boraso for her valuable information and Clariana Ferraz
285 Sampaio for proof reading the English version. Also, to Dr. Roberta D'Archino and the two
286 anonymous referees for the suggestions that improved this paper. This work was supported by
287 Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ E-26/111/397/2012)
288 and by the scholarships received by EAPFL during his final year in college (UNIRIO) and during
289 his Master Program (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES).
290

291 REFERENCES

- 292 Abreu NMN, Marçal I., Duarte AB, Pitombo FB, Vilasboa A, Gusmao J. 2016. Microsatellite
293 markers for barnacle studies: Isolation and characterization of polymorphic
294 microsatellite loci from the invasive barnacle *Megabalanus coccopoma* (Darwin, 1854)
295 and its cross-amplification in the Southern Atlantic endemic species *Megabalanus*
296 *vesiculosus* (Darwin, 1854). *Biochem Syst Ecol*, 66:224-228
- 297 Acha EM, Mianzan H, Guerrero R, Carreto J, Giberto D, Montoya N, Carignan M. 2008. An
298 overview of physical and ecological processes in the Rio de La Plata Estuary. *Cont*
299 *Shelf Res*, 28:1579–1588
- 300 Amico V, Oriente G, Piattelli M, Tringa R. 1976. Dilophol, a new ten-membered -ring diterpene
301 alcohol from the brown alga *Dilophus ligulatus*. *JCS Chem Comm*. 24:1024-1025
- 302 Asensi AO 1966. Guía para reconocer los géneros de algas pardas de la Argentina. *Contrib Inst*
303 *Antárt Argent*. 103:1-51.
- 304 Baptista LRM. 1977. Flora ilustrada do Rio Grande do Sul. Flora marinha de Torres. *Bolm. Inst.*
305 *Biociênc. (Bot.)*. 37(7):1-246.
- 306 Biebl R. 1959. Temperatur-und osmotische Resistenz von Meeresalgen der bretonischen Küste.
307 *Protoplasma* 50: 217-242
- 308 Bisbal GA. 1995. The Southeast South American shelf large marine ecosystem. Evolution and
309 components. *Mar Policy*. 19(1):21-38
- 310 Bittner L, Payri CE, Couloux A, De Reviere B, De Rousseau F. 2008. Molecular phylogeny of the
311 Dictyotales and their position within the Phaeophyceae, based on nuclear, plastid and
312 mitochondrial DNA sequence data. *Mol Phylog and Evol*. 49:211-226
- 313 Bogaert K, Beeckman T, De Clerck O. 2016. Abiotic regulation of growth and fertility in the
314 sporophyte of *Dictyota dichotoma* (Hudson) J.V. Lamouroux (Dictyotales,
315 Phaeophyceae). *J Appl Phycol*. 28(5):2915–2924
- 316 Boraso AL. 2012. Elementos para el estudio de las macroalgas marinas de Argentina. Instituto
317 de Desarrollo Costero (IDC). Comodoro Rivadavia: Editorial Universitaria de la
318 Patagonia.
- 319 Boraso AL, Rico A, Perales S, Perez L, Salazar I. 2004. Algas marinas de la Patagonia, una guía
320 ilustrada. Buenos Aires: Editorial Vazquez Mazini.
- 321 Boraso de Zaixso A. 1995. Algas bentónicas de Puerto Deseado (Santa Cruz). Composición de
322 la flora luego de la erupción del volcan Hudson. *Nat Patagon Ser Cienc Biol*. 3:129–
323 152.
- 324 Boraso de Zaixso AL, Zaixso J M. 2007. Algas marinas bentónicas. In: Atlas de Sensibilidad
325 ambiental del mar y de la costa. Boltovskoy D, editor. Secretaria de Ambiente y
326 Desarrollo Sustentable, República Argentina.
- 327 Braga ES, Chiozzini VC, Berbel GBB, Maluf JCC, Aguiar VMC, Charo M, Molina D, Romero SI,
328 Eichler BB. 2008. Nutrient distributions over the Southwestern South Atlantic continental
329 shelf from Mar del Plata (Argentina) to Itajaí (Brazil): Winter– summer aspects. *Cont*
330 *Shelf Res*. 28:1649–1661

- 331 Calliari D, Defeo O, Cervetto G, Gómez M, Giménez L, Scarabino F, Brazeiro A, Norbis W. 2003.
332 Marine life of Uruguay: critical update and priorities for future research. *Gayana*.
333 67(2):341-370
- 334 Campos EJD, Piola AR, Matano RP, Miller JL. 2008. PLATA: A synoptic characterization of the
335 southwest Atlantic shelf under influence of the Plata River and Patos Lagoon outflows.
336 *Cont Shelf Res.* 28:1551-1555
- 337 Casas GN, Piriz ML, Parodi ER. 2008. Population features of the invasive kelp *Undaria pinnatifida*
338 (Phaeophyceae: Laminariales) in Nuevo Gulf (Patagonia, Argentina). *J Mar Biol Assoc*
339 *UK.* 88(1):21–28
- 340 Casas G, Scrosati R, Piriz ML. 2004. The invasive kelp *Undaria pinnatifida* (Phaeophyceae,
341 Laminariales) reduces native seaweed diversity in Nuevo Gulf (Patagonia, Argentina).
342 *Biol Invasions.* 6:411-416
- 343 Cassano V, Yoneshigue-Valentin Y, Wynne MJ. 2004. *Elachistiella leptoneumatoides* gen. et sp.
344 nov. (Elachistaceae, Phaeophyceae) from Brazil. *Phycologia.* 43:329-340.
- 345 Coelho-Souza SA, López MS, Guimarães JRD, Coutinho R, Candella RN. 2012. Biophysical
346 interactions in the Cabo Frio upwelling system, southeastern Brazil. *Braz J Oceanogr.*
347 60(3):353-365.
- 348 Coll J, Oliveira EC. 1999. The benthic marine algae of Uruguay. *Bot Mar.* 42: 129-135
- 349 Coll M, Piroddi C, Steenbeek J, Kaschner K, Ben Rais Lasram F, Aguzzi J, Ballesteros E, Bianchi
350 CN, Corbera J, Dailianis T, et al. 2010 The Biodiversity of the Mediterranean Sea:
351 Estimates, Patterns, and Threats. *PLoS ONE* 5(8): e11842.
- 352 Crespo TM, Bahia RG, Maneveldt GW, Filho GMA. 2014. Floristic composition of crustose
353 coralline algae from the St. Peter and St. Paul Archipelago, a summit of the Mid-Atlantic
354 Ridge. *Phytotaxa.* 190(1):17–37
- 355 Croce ME, Gauna MC, Fernández C, Parodi ER. 2015. Intertidal seaweeds from North Atlantic
356 Patagonian coasts, Argentina. *Check List.* 11(5):1739
- 357 Darriba D, Taboada GI, Doallo R, Posada D. 2012. Jmodeltest 2: More Models, New Heuristics
358 and Parallel Computing". *Nat Methods.* 9(8):772.
- 359 De Clerck O. 2003. The genus *Dictyota* in the Indian Ocean. *Opera Botánica Belgica* 13. Meise:
360 National botanic Garden of Belgium
- 361 De Paula JC, Vallim MA, Teixeira VL. 2011. What are and where are the bioactive terpenoids
362 metabolites from Dictyotaceae (Phaeophyceae). *Braz J Pharmacog.* 21(2):216-228
- 363 Dunal F. 1833. *Bulletin Bibliographique*. "Voyage dans le District des Diamans et sur le litoral du
364 Brésil" par M. Auguste de Saint-Hillaire, membre de l'Académie des sciences, etc. 2 vol.
365 In-8° - Paris, Gide, 1833. *Arch Bot* 2:444-456
- 366 Fattorusso E, Magno S, Mayol L, Santacrose C, Sica D, Amico V, Oriente G, Piattelli M, Tringali
367 C. 1976. Dictyol A and B, two novel diterpene alcohols from the brown alga *Dictyota*
368 *dichotoma*. *J Chem Soc, Chem Commun.* 14:575-576
- 369 Faulkner DJ, Ravi BN, Finer J, Clardy J. 1977. Diterpenes from *Dictyota Dichotoma*.
370 *Phytochemistry.* 16:991-993
- 371 García M, Weitzmann B, Pinedo S, Cebrian E, Ballesteros E. 2016. First Report on the Distribution
372 and Impact of Marine Alien Species in Coastal Benthic Assemblages Along the Catalan
373 Coast. In: Munné A, Ginebreda A, Prat N., editors. *Experiences from Ground, Coastal*
374 *and Transitional Water Quality Monitoring. The EU Water Framework Directive*
375 *Implementation in the Catalan River Basin District (Part II). The Handbook of*
376 *Environmental Chemistry* 43. Cham (CH): Springer; p. 249-270
- 377 Gauna MC, Cáceres EJ, Parodi ER. 2013. Temporal variations of vegetative features, sex ratios
378 and reproductive phenology in a *Dictyota dichotoma* (Dictyotales, Phaeophyceae)
379 population of Argentina. *Helgoland Mar Res.* 67: 721-732.
- 380 Gauna MC, Cáceres EJ, Parodi ER. 2014. Spatial and temporal variability in algal epiphytes on
381 Patagonian *Dictyota dichotoma* (Dictyotales, Phaeophyceae). *Aquat Bot.* 120:338–345
- 382 Genzano GN, Rodriguez GM. 1998. Associations between hydroid species and their substrates
383 from the intertidal zone of Mar del Plata (Argentina). *Misc Zool.* 21(1):21-29
- 384 Guimarães SMPB, Braga MRA, Cordeiro-Marino M, Pedrini AG. 1986. Morphology and taxonomy
385 of *Jolyra laminarioides*, a new member of the Scytosiphonales (Phaeophyceae) from
386 Brazil. *Phycologia.* 25:99-108
- 387 Hwang IK, Kim HS, Lee WJ. 2005. Polymorphism in the brown alga *Dictyota dichotoma*
388 (Dictyotales, Phaeophyceae) from Korea. *Mar Biol.* 147:999–1015
- 389 Irigoyen AJ, Trobbiani G, Sgarlatta MP, Raffo MP. 2011. Effects of the alien algae *Undaria*
390 *pinnatifida* (Phaeophyceae, Laminariales) on the diversity and abundance of benthic

391 macrofauna in Golfo Nuevo (Patagonia, Argentina): potential implications for local food
392 webs. *Biol Invasions*. 13:1521–1532

393 Lamouroux JVF. 1809. Observations sur la physiologie des algues marines, et description de cinq
394 nouveaux genres de cette famille. *Nouv Bull Sci Soc Philom*, Paris. 1:330–333

395 Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA
396 polymorphism data. *Bioinformatics*. 25(11):1451-1452

397 Lin Y, Zhan A. 2016. Population genetic structure and identification of loci under selection in the
398 invasive tunicate, *Botryllus schlosseri*, using newly developed EST-SSRs. *Biochem*
399 *Syst Ecol*. 66:331-336

400 Littler DS, Littler MM. 2000. Caribbean reef plants: an identification guide to the reef plants of the
401 Caribbean, Bahamas, Florida and Gulf of Mexico. Washington, DC : OffShore Graphics,
402 Inc..

403 Martius KFP, Eschweiller GG, Esenbeck CGDN. 1833. Flora Brasiliensis; seu Enumeratio
404 plantarum in Brasilia tam sua sponte quam accedente cultura provenientium, quas in
405 itinere auspiciis Maximiliani Josephi I. Bavariae regis annis 1817-1820 peracto collegit,
406 partim descripsit; alias a Maximiliano seren. Principe Widensi, sellovio aliisque advectas
407 addidit. 1. 1: IV + 390 (Algae 1-50), 8 vol. Stuttgartiae et Tubingen: Sumptibus J.G.
408 Cottae, p. 1-50

409 Mendoza ML, Nizovoy A. 2000. Géneros de macroalgas marinas de la Argentina
410 fundamentalmente de Tierra del Fuego: Secretaria de Desarrollo y Planeamiento de la
411 Provincia de Tierra del Fuego. Ushuaia, Tierra del Fuego.

412 Montagne C. 1839. Cryptogames de la Patagonie. In: Voyage Dans L'amérique Méridionale (Le
413 Brésil, La République Orientale De L'uruguay, La République Argentine, La Patagonie,
414 La République Du Chili, La République de Bolivie, La République Du Pérou), Exécité
415 Pendant Les Années 1826, 1827, 1828, 1829, 1830, 1831, 1832 Et 1833. Vol. 7:
416 Botanique. Part I. Sertum Patagonium. (D'orbigny, A. Eds), p. 1-19. Paris:

417 Moore PG, Eastman LB. 2015. The tube-dwelling lifestyle in crustaceans and its relation to
418 feeding. In: Thiel M, Watling L, editors. Lifestyles and feeding biology. The natural
419 history of the crustacea.V.2. New York: Oxford University Press. ; p.35-77

420 Mystikou A, Asensi AO, De Clerck O, Müller DG, Peters AF, Tsiamis K, Fletcher KI, Westermeier
421 R, Brickle P, West P, Küpper FC. 2016. New records and observations of macroalgae
422 and associated pathogens from the Falkland Islands, Patagonia and Tierra del Fuego.
423 *Bot Mar* 59(2-3):105–121

424 Nelson WA, Wilcox MD. 2010. *Rosenvingea* (Ectocarpales, Scytosiphonaceae) – a new brown
425 macroalgal record for New Zealand. *New Zeal J Bot*. 48(3-4):193-196

426 Oliveira Filho EC. 1977. Algas marinhas bentônicas do Brasil [Thesis]. São Paulo (SP):
427 Universidade de São Paulo.

428 Palermo JA, Bernardo JJ, Seldes AM. 1994. Dictyol-D-2b-acetate and other diterpenoids from
429 the brown alga *Dictyota dichotoma*. *Ann Assoc Quím Argent*. 82:355–358

430 Piriz ML, Eyra MC, Rotagno CM. 2003. Changes in biomass and botanical composition of
431 beachcoast seaweeds in a disturbed coastal area from Argentine Patagonia. *J Appl*
432 *Phycol*. 15:67-74

433 Raffo MP, Eyra MC, Iribarne OO. 2009. The invasion of *Undaria pinnatifida* to a *Macrocystis*
434 *pyrifera* kelp in Patagonia (Argentina, south-west Atlantic). *J Mar Biol Assoc UK*.
435 89(8):1571–1580

436 Raffo MP, Russo VL, Schwindt E. 2014. Introduced and native species on rocky shore macroalgal
437 assemblages: Zonation patterns, composition and diversity. *Aquat Bot*. 112:57–65

438 Ramírez ME, Nuñez JD, Ocampo EH, Matula CV, Suzuki M, Hashimoto T, Cledón M. 2012.
439 *Schizymenia dubyi* (Rhodophyta, Schizymeniaceae), a new introduced species in
440 Argentina, *New Zeal J Bot*. 50(1):51-58

441 Ronquist F, Teslenko M, Der Mark PV, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard
442 MA, Huelsenbeck AP. 2012. MrBayes3.2: Efficient bayesian phylogenetic inference
443 and model choice across a large model space. *Syst Biol*. 61(3):539–542

444 Saint-Hillaire A. Voyage dans le District des Diamans et sur le litoral du Brésil. Vol.1, i-xx + 402pp.
445 Paris

446 Schnetter R, Hörnig I, Weber-Peukert G. 1987. Taxonomy of some North Atlantic *Dictyota* species
447 (Phaeophyta). *Hydrobiologia*. 151/152:193-197.

448 Schwindt E, Gappa JL, Raffo MP, Tatian M, Bortolus A, Orensanz JM, Alonso G, Diez ME, Doti
449 B, Genzano G, Lagger C, et al. 2014. Marine fouling invasions in ports of Patagonia

- 450 (Argentina) with implications for legislation and monitoring programs. *Mar Environ Res.*
 451 99:60-68
- 452 Siamopoulou P, Bimplakis A, Iliopoulou D, Vagias C, Cos P, Berghe DV, Roussis V. 2004.
 453 Diterpenes from the brown algae *Dictyota dichotoma* and *Dictyota linearis*.
 454 *Phytochemistry.* 65:2025–2030
- 455 Spalding MD, Fox HE, Allen GR, Davidson N, Ferdaña ZA, Finlayson M, Halpern BS, Jorge MA,
 456 Lombana A, Lourie SA, Martin KD, et al. 2007. Marine Ecoregions of the World: A
 457 Bioregionalization of Coastal and Shelf Areas. *BioScience.* 57(7):573-583
- 458 Stachowicz JJ, Hay ME. 2000. Geographic variation in camouflage specialization by a decorator
 459 crab. *Am Nat.* 156(1):59-71
- 460 Széchy MTM, De Paula JC. Phaeophyceae Lista de Espécies da Flora do Brasil [Internet]. Rio
 461 de Janeiro: Jardim Botânico do Rio de Janeiro. [cited 2016 Sept 25]. Available from:
 462 <http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB99411>
- 463 Tamura K, Stecher G, Peterson D, Filipowski A, Kumar S. 2013. Mega6: Molecular Evolutionary
 464 Genetics Analysis Version 6.0. *Mol Phylogenet Evol.* 30:2725-2729.
- 465 Taylor WR. 1939. Algae collected by the "Hassler", "Albatross" and Schmitt expeditions. II. Marine
 466 algae from Uruguay, Argentina, the Falkland Islands, and the Strait of Magellan. *Papers*
 467 *Mich Ac Sc Arts Let.* 24:127-164.
- 468 Tronholm A, Sansón M, Afonso-Carrillo J, De Clerck O. 2008. Distinctive morphological features,
 469 life-cycle phases and seasonal variations in subtropical populations of *Dictyota*
 470 *dichotoma* (Dictyotales, Phaeophyceae). *Bot Mar.* 51:132–144
- 471 Tronholm A, Sansón M, Afonso-Carrillo J, Verbruggen H, De Clerck O. 2010a. Niche partitioning
 472 and the coexistence of two cryptic *Dictyota* (Dictyotales, Phaeophyceae) species from
 473 the Canary Islands. *J Phycol.* 46:1075-1087
- 474 Tronholm A, Steen F, Tyberghein L, Leliaert F, Verbruggen H, Siguan MAR, De Clerck O. 2010b.
 475 Species delimitation, taxonomy and biogeography of *Dictyota* in Europe (Dictyotales,
 476 Phaeophyceae). *J Phycol.* 46:1301-1321
- 477 Tronholm A, Leliaert F, Sansón M, Afonso-Carrillo J, Tyberghein L, Verbruggen H, De Clerck O.
 478 2012. Contrasting geographical distributions as a result of thermal tolerance and long-
 479 distance dispersal in two allegedly widespread tropical brown algae. *Plos One*7:E30813
- 480 Valentin JL. 2001. The Cabo Frio Upwelling System, Brazil. In: Seeliger U, Kjerfve B. Coastal
 481 Marine Ecosystems of Latin America.. *Ecol Stud.* 144. Berlin: Springerp.97-105.
- 482 Vallim MA, De Paula JC, Pereira RC, Teixeira VL. 2005. The diterpenes from Dictyotacean marine
 483 brown algae in the Tropical Atlantic American region. *Biochem Syst Ecol.* 33:1–16
- 484 Van den Hoek C. 1982. Phytogeographic distribution groups of benthic marine algae in the north
 485 Atlantic Ocean. A review of experimental evidence from life history studies. *Helgol*
 486 *Meeresunters.* 35:153-214
- 487 Verlaque M, Steen F, De Clerck O. 2009. *Rugulopteryx* (Dictyotales, Phaeophyceae), a genus
 488 recently introduced to the Mediterranean. *Phycologia.* 48(6):536–542
- 489 Villaça R, Fonseca AC, Jensen VK, Knoppers B. 2010. Species composition and distribution of
 490 macroalgae on Atol das Rocas, Brazil, SW Atlantic. *Bot Mar.* 53:113–122

491
 492 Captions:

493 **Figure 1:** Phylogenetic tree based on *psbA* sequences, presenting a consensus topology
 494 estimated by Maximum Likelihood (MP) and Bayesian Inference (BI) analyses. The numbers
 495 associated with each branch represent the statistical support values (only values above 95% are
 496 shown), where the first is the bootstrap values from ML and the second is the posterior probability
 497 from BI.

498 **Figure 2:** Haplotype diversity of *psbA* from *Dictyota dichotoma*. The red dots are the locations
 499 where the sequences of *D. dichotoma* on GenBank come from. A) Haplotype network of *psbA*
 500 showing the two groups: Atlantic-Mediterranean and Canarian. B) Distribution of the seven
 501 haplotypes in the North Atlantic population C) South Atlantic populations with the single haplotype
 502 H1.

