



**HAL**  
open science

## Genetic and morphological differentiation of *Porphyra* and *Pyropia* species (Bangiales, Rhodophyta) coexisting in a rocky intertidal in Central Chile

Andrés Meynard, Javier Zapata, Nicolás Salas, Claudia Betancourt, Gabriel Pérez-lara, Francisco Castañeda, María Eliana Ramírez, Cristian Bulboa Contador, Marie-laure Guillemin, Loretto Contreras-porcía

### ► To cite this version:

Andrés Meynard, Javier Zapata, Nicolás Salas, Claudia Betancourt, Gabriel Pérez-lara, et al.. Genetic and morphological differentiation of *Porphyra* and *Pyropia* species (Bangiales, Rhodophyta) coexisting in a rocky intertidal in Central Chile. *Journal of Phycology*, 2019, 55 (2), pp.297-313. 10.1111/jpy.12829 . hal-02147670

**HAL Id: hal-02147670**

**<https://hal.sorbonne-universite.fr/hal-02147670>**

Submitted on 4 Jun 2019

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



**Genetic and morphological differentiation of *Porphyra* and *Pyropia* species (Bangiales, Rhodophyta) coexisting in a rocky intertidal in Central Chile**

Journal:	<i>Journal of Phycology</i>
Manuscript ID	JPY-18-094-ART.R2
Manuscript Type:	Regular Article
Date Submitted by the Author:	30-Jul-2018
Complete List of Authors:	Meynard, Andres; Universidad Andres Bello Zapata, Javier; Universidad Andres Bello Salas, Nicolas; Universidad Andres Bello Betancourt, Claudia; Universidad Andres Bello Pérez-Lara, Gabriel; Universidad Andres Bello Castañeda, Francisco; Universidad Andres Bello Ramírez, Maria; Universidad Andres Bello Bulboa Contador, Cristian; Universidad Andres Bello Guillemin, Marie-Laure; Universidad Austral de Chile, Instituto de Ecología y Evolución, Facultad de Ciencias Contreras-Porcía, Loretto; Universidad Andrés Bello, Ecología y Biodiversidad
Keywords:	taxonomy, Macroalgae, Rhodophyta
Alternate Keywords:	Intertidal distribution, Bangiales, COI, rbcL, Chile
Note: The following files were submitted by the author for peer review, but cannot be converted to PDF. You must view these files (e.g. movies) online.	
Figure 2.tif Figure 6.tif Figure 7.tif Figure 8.tif Figure 9.tif	

1 **Genetic and morphological differentiation of *Porphyra* and *Pyropia* species**  
2 **(Bangiales, Rhodophyta) coexisting in a rocky intertidal in Central Chile**

3 *Andrés Meynard, Javier Zapata, Nicolás Salas, Claudia Betancourt,*

4 *Gabriel Pérez-Lara, Francisco Castañeda*

5 Departamento de Ecología y Biodiversidad, Facultad de Ciencias de la Vida, Universidad

6 Andrés Bello, República 440, Santiago, Chile

7 Centro de Investigación Marina Quintay (CIMARQ), Facultad de Ciencias de la Vida,

8 Universidad Andrés Bello, Quintay, Chile

9 *María Eliana Ramírez*

10 Departamento de Ecología y Biodiversidad, Facultad de Ciencias de la Vida, Universidad

11 Andrés Bello, República 440, Santiago, Chile

12 Museo Nacional de Historia Natural, Área Botánica, Casilla 787, Santiago, Chile

13 *Cristian Bulboa Contador*

14 Departamento de Ecología y Biodiversidad, Facultad de Ciencias de la Vida, Universidad

15 Andrés Bello, República 440, Santiago, Chile

16 *Marie-Laure Guillemin*

17 Instituto de Ciencias Ambientales y Evolutivas, Universidad Austral de Chile, Casilla 567

18 Valdivia, Chile

19 CNRS, Sorbonne Universités, UPMC University Paris VI, PUC, UACH, UMI 3614,

20 Evolutionary Biology and Ecology of Algae, Station Biologique de Roscoff, CS 90074,

21 Place G. Teissier, 29680 Roscoff, France

22 Centro FONDAP de Investigación en Dinámica de Ecosistemas Marinos de Altas Latitudes

23 (IDEAL)

24 *Loretto Contreras-Porcia*<sup>2</sup>  
25 Departamento de Ecología y Biodiversidad, Facultad de Ciencias de la Vida, Universidad  
26 Andrés Bello, República 440, Santiago, Chile  
27 Centro de Investigación Marina Quintay (CIMARQ), Facultad de Ciencias de la Vida,  
28 Universidad Andrés Bello, Quintay, Chile  
29 Center of Applied Ecology & Sustainability (CAPES-UC), Pontificia Universidad Católica  
30 de Chile, Av. Libertador Bernardo O'Higgins 340, Santiago, Chile

31

32 Running title: New *Porphyra* and *Pyropia* species from central Chile

33

34 <sup>1</sup>Date of submission and acceptance

35 <sup>2</sup>Author for correspondence: e-mail: [lorettocontreras@unab.cl](mailto:lorettocontreras@unab.cl)

36

### 37 **Abstract**

38 A recent molecular taxonomic study along the Chilean coast (18°S-53° S) described 18  
39 candidate species of bladed Bangiales of which only two were formally described. Few  
40 studies focused on local genetic and morphological diversity of bladed Bangiales and  
41 attempted to determine their intertidal distribution in contrasting habitats; and none were  
42 performed in Chile. To delimit intertidal distributions of genetic species, 66 samples of  
43 bladed Bangiales were collected at Maitencillo (32°S) in four zones: a rocky platform, a  
44 rocky wall, and two boulders zones surrounded by sandy and rocky bottoms, respectively.  
45 These samples were identified based on sequences of the mitochondrial *COI* and  
46 chloroplast *rbcL* markers. We also collected 87 specimens for morphological

47 characterization of the most common species, rapidly assessing their putative species  
48 identity using newly developed species-diagnostic (PCR-RFLP) markers. Eight  
49 microscopic and two macroscopic morphological traits were measured. We described and  
50 named three out of four species that predominate in Maitencillo (including *Pyropia*  
51 *orbicularis*): *Pyropia variabilis* Zapata, Meynard, Ramírez, Contreras-Porcia, sp. nov.,  
52 *Porphyra luchensis* Meynard, Ramírez, Contreras-Porcia sp. nov. and *Porphyra longissima*  
53 Meynard, Ramírez, Contreras-Porcia, sp. nov. With the exception of *Po. longissima*  
54 restricted to boulders **surrounded by sandy bottom**, and a morphotype of *Py. variabilis*  
55 restricted to rocky walls, the other species/morphotypes have overlapping intertidal  
56 **distributions**. Except for *Po. longissima* which is clearly differentiated morphologically  
57 (longest and thinnest blades), we conclude that morphology is not sufficient to differentiate  
58 bladed Bangiales. Our findings underscore the importance of refining our knowledge of  
59 intrinsic and environmental determinants on the distribution of bladed Bangiales.

60

61 *Key index words*: Bangiales; taxonomy; *COI*; *rbcL*; Chile; Intertidal distribution

62 *Abbreviations*: *COI*, cytochrome oxidase I; ML, Maximum Likelihood; **PCA, Principal**

63 **Component Analysis**; PCR-RFLP, polymerase chain reaction restriction fragment length

64 polymorphism analyses; *rbcL*, large subunit ribulose **bis-phosphate** carboxylase/oxygenase.

65

66

67

68

69

70

## INTRODUCTION

71 Prior to the last decade, all foliose Bangiales have been classified under the genus  
72 *Porphyra* sensu lato (Blouin et al. 2011, Sutherland et al. 2011). Nevertheless, classical  
73 taxonomic methods, based mainly on morphological and life history traits, were shown to  
74 be highly misleading in this order (Sutherland et al. 2011). Indeed, with the advent of  
75 molecular phylogenies, several cryptic genera of foliose Bangiales were discovered and  
76 various classical genera were redefined (Sutherland et al. 2011, Sánchez et al. 2014, 2015,  
77 Yang et al. 2018). The existence of nine genera of bladed Bangiales was then proposed  
78 based on these molecular studies: *Boreophyllum* S.C Lindstrom N. Kikuchi, M. Miyata et  
79 Neefus, *Clymene* WA Nelson, *Fuscifolium* S.C Lindstrom, *Miuraea* N. Kikuchi, S. Arai, G.  
80 Yoshida, J.A. Shin et M. Miyata, *Lysithea* WA Nelson, *Porphyra* C. Agardh, *Pyropia* J.  
81 Agardh, *Wildemania* De Toni and *Neothemis* A.Sánchez, A.Vergés, C.Peteiro, J.Sutherland  
82 & J.Brodie. Of particular note, most of the “nori” species cultivated in Asia **nowadays**  
83 **belong** to the genus *Pyropia* (Sutherland et al. 2011, Zuccarello 2011). At the genus level,  
84 the delimitation of species was undertaken recently using molecular tools, an **advent** that  
85 has redefined the frontiers of former taxa over the last ten years (e.g. Broom et al. 2002,  
86 Brodie et al. 2007, Neefus et al. 2008, Nelson et al. 2013, Guillemain et al. 2016, Dumilag et  
87 al. 2016, Reddy et al. 2018, Yang et al. 2018). A high level of cryptic genetic diversity was  
88 found within the two genera *Porphyra* and *Pyropia* (Brodie et al. 2007, Niwa et al. 2009,  
89 Broom et al. 2010, Lindstrom et al. 2015, Guillemain et al. 2016, Koh et al. 2018, Reddy et  
90 al. 2018, Yang et al. 2018). According to Zuccarello (2011), the discovery of these new  
91 taxa, that cannot be identified based on morphology, could drive field ecology studies to  
92 **misleading** conclusions and impair our understanding of the evolutionary processes that  
93 generated this diversity. Hence, to aid in the rapid, cheap and reliable identification of some

94 Asiatic bladed Bangiales, a number of studies carried by Niwa and collaborators (Teasdale  
95 et al. 2002, 2005, 2009, 2010a, b, and 2014, Niwa & Aruga 2006) developed molecular  
96 markers based on polymerase chain reaction restriction fragment length polymorphism  
97 (PCR-RFLP) analyses of nuclear and cytoplasmic genes.

98         Considering the economical, biotechnological, ecophysiological and evolutionary  
99 research potentials of species of bladed Bangiales, there is clearly a need to better define  
100 their taxonomic frontiers (Hurd et al. 2014). Moreover, this new appraisal of the taxonomic  
101 and biochemical diversity within the Bangiales could also unveil ecological patterns unseen  
102 under the old paradigm of identification based exclusively on morphology. Contradictory  
103 results **have been published** concerning intertidal zonation or habitat partitioning of  
104 *Porphyra* and *Pyropia* **species living in sympatry** (see, for example, West et al. 2005, for a  
105 positive answer, and Schweikert et al. 2012, for a negative one). Nevertheless, studies in  
106 other groups of red and brown algae showed that non-random small-scale distribution  
107 patterns occur locally between related algal species (e.g. Billard et al. 2010, Couceiro et al.  
108 2015, Muangmai et al. 2016, Montecinos et al. 2017).

109         In Chile, a recent study using molecular methods and samples spanning most of the  
110 coast (18°S-53°S), identified 18 species of bladed Bangiales belonging to the genus  
111 *Porphyra*, *Pyropia*, *Fuscifolium* and *Wildemanina* (Guillemin et al. 2016). Of the 18 species  
112 encountered in Chile only two are named and were characterized morphologically (Ramírez  
113 et al. 2014, Guillemin et al. 2016). A group of closely related species (namely *Pyropia* sp.  
114 CHJ, *Pyropia* sp. CHK and *Pyropia orbicularis* Ramírez, Contreras-Porcía & Guillemin  
115 (Ramírez et al. 2014, Guillemin et al. 2016) co-occur in central Chile. *Pyropia orbicularis*  
116 and *Pyropia* sp. CHK were even observed in sympatry in the locality of Maitencillo. It is  
117 well known that adaptation to different ecological micro-niches could facilitate the

118 coexistence of closely by related species in the same locality (Billard et al. 2010,  
119 Muangmai et al. 2016), but differences in intertidal zonation or habitat between *Pyropia* sp.  
120 CHJ, *Pyropia* sp. CHK and *Pyropia orbicularis* have not yet been studied.

121 The main aims of the present study was to (i) determine how many species of  
122 bladed Bangiales are present in the locality of Maitencillo when using a sampling method  
123 that takes into account the diversity of habitats present in the site, (ii) determine their  
124 distribution along the tidal gradient and in the different habitats sampled, (iii) develop  
125 simple and rapid species-diagnostic markers using polymerase chain reaction restriction  
126 fragment length polymorphism (PCR-RFLP) on *rbcL* gene amplicons for the  
127 discrimination of these bladed Bangiales species, and (iv) describe the unnamed species  
128 living at the study site using a combination of molecular tools and statistical analyses of  
129 morphological characters.

130

## 131 MATERIALS AND METHODS

132

### 133 *Sampling of foliose Bangiales in Maitencillo beach*

134 Sampling was performed in the rocky intertidal shore of Maitencillo, Valparaíso (32° 39' S,  
135 71° 26' W, Fig. S1). This site is characterized by a rocky platform that gently slopes into  
136 the sea with small areas characterized by boulders and small, but steep rocky walls. Foliose  
137 Bangiales are prevalent in the upper intertidal zone while *Mazzaella laminaroides*  
138 (Rhodophyta) dominate the middle intertidal (Betancourt et al. 2018). The lower intertidal  
139 zone is characterized by a sandy bottom. Two sampling schemes were used during the  
140 study. First, to characterize species diversity and small-scale distribution in Maitencillo we  
141 sampled 66 specimens of bladed Bangiales from July to December 2013 and 2014 (i.e.



142 winter-spring) in different intertidal zones and habitats. Sampling took place along three  
143 transects extending 20 m from the upper to lower intertidal zone of the rocky platform.  
144 Twenty-two specimens of *Bangiales* were sampled within three areas of 5-6 meters long  
145 per 0.5 meters wide along each transect. These three areas were defined as upper, middle  
146 and lower intertidal, respectively, according to animal and seaweed limits previously  
147 described in central Chile (Hoffmann & Santelices 1987). This sampling was completed by  
148 seventeen samples taken from two quadrants of 0.5 m<sup>2</sup> placed on a steep wall adjacent to  
149 the upper intertidal zone; and by 24 samples taken from four quadrants of 0.5 m<sup>2</sup> placed in  
150 two boulder zones (two quadrants in each zone, 12 samples per boulder zone). The boulder  
151 zones were located 150 m from the main transect. One was surrounded by rocky pools  
152 while the other was surrounded by pools of sandy bottoms. Some representative samples  
153 were chosen as voucher specimens that are housed in the herbarium of the National  
154 Museum of Natural History, Chile, under the SGO herbarium numbering system (see  
155 voucher numbers in Table S1; Museo Nacional de Historia Natural, herbarium code: SGO,  
156 <http://www.mnhn.cl>). To study the morphology of the four-dominant species encountered  
157 in Maitencillo (see below for more information about species determination), a second  
158 sampling was performed from August to December 2014 (i.e. winter-spring) where 87  
159 specimens of bladed *Bangiales* were sampled in different intertidal habitats.

160

161 *DNA extraction, amplification, sequencing and species delimitation by molecular tools*

162 *DNA extraction.* Total genomic DNA was extracted from dried algal tissue grounded in  
163 liquid nitrogen following the protocol originally described by Saunders (1993), with  
164 modifications by Faugeron et al. (2001).

165

166 *COI and rbcL amplification and sequencing.* A partial sequence of the *COI* was obtained  
167 using a newly defined forward primer COIF3 (5'-  
168 AATTAGRATGGAAYTAGCKCAACC-3') and the GazR1 primer, following the  
169 amplification protocols of Saunders (2005). A partial sequence of the *rbcL* was obtained  
170 using the primers F-*rbcL* and R-*rbcS* (Hommersand et al. 1994), following the previously  
171 published amplification protocols (Hommersand et al. 1994, Fredericq and López-Bautista  
172 2003). All PCR products were purified using UltraClean™ DNA Purification kits (MO  
173 BIO Laboratories, Carlsbad, CA, USA) and sequenced using the forward and reverse  
174 primers used for amplification by Macrogen Inc. (Seoul, South Korea). Sequences were  
175 aligned and edited using Bioedit (Hall 1999). Sixty-six specimens from the first sampling  
176 scheme and 24 from the second sampling scheme (for morphological analyses) were  
177 sequenced. During this study, 90 *COI* sequences (603 bp) and 46 *rbcL* sequences (873 bp)  
178 were obtained and deposited in GENBANK. Details about specimen collection information  
179 and GENBANK accession numbers are given in Table S1.

180

181 *Species delimitation and phylogenetic relationship reconstruction.* For the *rbcL*, in addition  
182 to the 46 sequences newly obtained from Maitencillo, 174 sequences of Chilean Bangiales  
183 specimens (including 20 sequences from Maitencillo, Guillemin et al. 2016) and 157  
184 sequences retrieved from GENBANK were included in our data set. For the *COI*, in  
185 addition to the 90 sequences newly obtained (i.e. first and second sampling scheme) from  
186 Maitencillo, 159 sequences of Chilean Bangiales (including 20 from sequences from  
187 Maitencillo, Guillemin et al. 2016) as well as 39 sequences retrieved from GENBANK  
188 were included in our data set. The complete list of specimens used in molecular analyses is  
189 available in Tables S1 and S2.

190 The monophyly of each *Pyropia* and *Porphyra* species, previously determined by  
191 Guillemin et al. (2016), was examined using a maximum likelihood (ML) phylogenetic  
192 relationship reconstruction performed using IQ-TREE online server (Trifinopoulos et al.  
193 2016). We selected the best-fit substitution model using the Akaike information criterion  
194 implemented in IQ-TREE (Nguyen et al. 2015, Trifinopoulos et al. 2016). The selected  
195 models were TIM3+F+G4 and TN+F+I+G4 for the *COI* (*Pyropia* and *Porphyra*,  
196 respectively) and TIM+I+G4 for the *rbcL*. Statistical support was estimated using 1,000  
197 ultrafast bootstrap replicates (Nguyen et al. 2014). *Minerva aenigmata* W.A. Nelson  
198 (EU570053) and *Dione arcuata* W.A. Nelson (EU570052) were used as outgroups in the  
199 phylogenetic analysis of *rbcL*. For the *COI* (Sutherland et al. 2011), tree reconstructions  
200 were done separately for *Pyropia* and *Porphyra* species, using as outgroups *Boreophylum*  
201 *birdiae* and *Bangia fuscopurpurea*, respectively, following Guillemin et al. (2016) and  
202 Sutherland et al. (2011).

203 For each of the 90 specimens sequenced, affiliation to one of the 18 cryptic species  
204 observed in Chile (Guillemin et al. 2016) or to a newly encountered species was determined  
205 using the ML tree reconstruction. For the more problematic groups of sequences (i.e.  
206 corresponding to the group of closely related species *Pyropia* sp. CHJ, *Pyropia* sp. CHK  
207 and *Pyropia orbicularis*), we followed the grouping proposed by Guillemin et al. (2016)  
208 and a *COI* phylogeographic network was reconstructed using the median-joining algorithm  
209 implemented in HapView version Beta (Salzburger et al. 2011). For *Pyropia* sp. CHJ,  
210 *Pyropia* sp. CHK and *Pyropia orbicularis*, we calculated mean genetic distances within-  
211 and between-species for the *COI* in Mega6 (Tamura et al. 2013). Distances were calculated  
212 using the Maximum Composite Likelihood method and variation rate among sites was  
213 modeled with a gamma distribution; codon positions included were 1st + 2nd + 3rd.

214

215 *Development of PCR-RFLP to rapidly assess putative species identity of Maitencillo foliose*  
216 *Bangiales*

217 Using the 44 sequences of *rbcL* available for the four more common species of foliose  
218 Bangiales encountered in Maitencillo (this study, Ramírez et al. 2014, Guillemín et al.  
219 2016, see results for more information), we developed a method of species identification  
220 using polymerase chain reaction restriction fragment length polymorphism analyses (PCR-  
221 RFLP) of plastid DNA.

222 Differences in pattern of restriction digestion between species were examined in-  
223 silico using the program Webcutter 2.0 (<http://rna.lundberg.gu.se/cutter2/copyright> 1997  
224 Max Heiman). Amplification protocol of the *rbcL* used in the PCR-RFLP analyses was the  
225 same as described above. Nonetheless, PCRs were done using a different forward primer  
226 than previously mentioned. Our newly developed primer *rbcL*-Pyr-F (5'-  
227 AGGTGTTGACCCGATTGAAG-3'), producing a longer fragment of 1230 bp instead of  
228 873 bp, was used instead of F-*rbcL* (Hommersand et al. 1994) in the PCR mix. The  
229 digestion of 0.5-1 µg of *rbcL* PCR product with 1.5 U AfaI (Thermo Scientific, Pittsburgh,  
230 PA) was performed following manufacturer instructions, with slight modifications. After  
231 16 h at 37 °C, the reaction was stopped using a heat denaturation of 20 min at 65 °C. The  
232 fragments obtained after digestion with AfaI were separated on a 2 % TBE-agarose  
233 electrophoresis. Because the *rbcL* PCR-RFLP patterns obtained were extremely similar  
234 between the two *Pyropia* species (see results for more details), a partial sequence of the  
235 *COI* was obtained for 24 *Pyropia* samples using the same protocol as described above to  
236 confirm the species identification obtained by PCR-RFLP.

237

238 *Morphological measurements and statistical analyses*

239 For classical descriptions of foliose Bangiales species found at Maitencillo, the thallus  
240 shape, color, texture, and rhizoid position were described in 87 specimens previously  
241 identified at the species level using the newly developed PCR-RFLP method and *COI*  
242 sequences (see above). Microscopic observations of superficial and hand-cut transverse  
243 sections were used to establish tissue thickness and the number of cell layers, as well as to  
244 identify vegetative (generally at the thallus center) and reproductive structures (generally at  
245 the thallus border). More precisely, eight microscopic features of the gametophytic thallus  
246 were recorded, measuring three cells in three visual fields per photo, in a total of three  
247 photos per specimen. These morphological microscopic traits were the length and width of  
248 rhizoidal, vegetative, and zygotosporangial cells (surface views) and the thickness of  
249 vegetative and zygotosporangial lamina (hand-cut transverse sections). Moreover, the  
250 maximum length and maximum width of the thallus were also measured and considered in  
251 statistical analyses as macroscopic characteristics. Images were captured on an upright  
252 Leica ICC50 HD microscope (Wetzlar, Germany) using the Leica Application Suite  
253 Advanced Fluorescence EZ Imaging Software v.3.0 (Wetzlar, Germany).

254 To describe in multivariate space and search for morphological differences between  
255 foliose Bangiales species present at Maitencillo, principal component analysis (PCA) was  
256 applied to microscopic and macroscopic measurements using the software PAST specifying  
257 a variance-covariance matrix (Hammer et al. 2001). All statistical analyses were done with  
258 normalized data, using the Box-Cox transformation option (Box and Cox 1964) available in  
259 the statistical software R (R core team, 2017). Statistical differences between species for all  
260 traits were assessed through multivariate nested, permutational ANOVA using the Adonis  
261 function in R (Anderson 2001). The “plant” (i.e. individual) level was treated as nested

262 factor within the main factor “species”. Moreover, data for each morphological trait was  
263 subjected to univariate post-hoc Tukey multiple comparisons in R (Abdi and Williams  
264 2010) (after univariate ANOVA were done) to detect specific treatment differences  
265 between species.

266

267

268

## RESULTS

269

270 *Genetic species present at Maitencillo.* The Maximum Likelihood rooted tree for *rbcL*  
271 sequences of Bangiales reconstructed with 46 new sequences obtained in this study (Fig. S2  
272 a and b), reproduced the same general topology with almost the same support values as in  
273 Fig. 1 of Guillemain et al. (2016). As shown by the Maximum Likelihood phylogenetic trees  
274 reconstructed with *COI* sequences (Fig. 1 a and b) and *rbcL* sequences (Fig. S2 a and b),  
275 the 90 Bangiales samples from Maitencillo were assigned to 5 genetic species of bladed  
276 Bangiales (i.e. represented as black triangles in the tree reconstructions). All five-delimited  
277 species correspond to genetic entities previously identified by Guillemain et al. (2016).  
278 Among these five species, four were dominant at Maitencillo: *Pyropia orbicularis* (N =  
279 14), *Pyropia* sp. CHK (N = 47), *Porphyra* sp. CHE (N = 16) and *Porphyra* sp. CHC (N =  
280 12). Their habits are shown in Fig. 2. A fifth species, *Porphyra* sp. CHF, was also present  
281 but in low frequency (N = 2, Table 1 and Table S1). *Porphyra* sp. CHE and *Porphyra* sp.  
282 CHF were recovered as monophyletic sister groups both in the *rbcL* and *COI* phylogenetic  
283 trees (Fig. 1). *Porphyra* sp. CHC was also retrieved as a well-supported monophyletic  
284 group in the two phylogenetic trees (Fig. 1 and Fig. S2). On the other hand, phylogenetic  
285 trees were less clear concerning the number of distinct species that could be defined within

286 the *Pyropia orbicularis* / *Pyropia* sp. CHK / *Pyropia* sp. CHJ species complex. Mean  
287 sequence divergence between species pairs was higher than 6% between *Pyropia*  
288 *orbicularis* and the other two species, whereas the distance between *Pyropia* sp. CHK and  
289 *Pyropia* sp. CHJ was 3.7% (Table S3). All mean sequence divergences calculated within  
290 species were, at least, five times lower than the ones calculated between species (Table S3).  
291 Moreover, three groups of highly differentiated haplotypes (*Pyropia orbicularis*, *Pyropia*  
292 sp. CHK, and *Pyropia* sp. CHJ) were clearly shown by the reconstruction of the *COI*  
293 network for these recently diverged species (Supplementary material 4; Guillemin et al.  
294 2016), all our newly acquired sequences being part of the *Pyropia orbicularis* and *Pyropia*  
295 sp. CHK groups (Fig. S3).

296

297 *Microhabitats and intertidal distribution of genetic species.* Species distribution of foliose  
298 Bangiales reported here is based on the information obtained during our first sampling  
299 scheme (66 specimens sequenced for the *COI* and 46 for *rbcL*). Foliose Bangiales were  
300 never observed in medium or low intertidal zones of the rocky platform (Table 1). From  
301 twenty-five foliose Bangiales sampled at the high intertidal zone on the rocky platform, 13  
302 were assigned to *Pyropia* sp. CHK, nine to *Porphyra* sp. CHE, and three to *Pyropia*  
303 *orbicularis* (Table 1). On the rocky wall, 15 of the 17-sampled foliose Bangiales were  
304 identified as *Pyropia* sp. CHK, one as *Porphyra* sp. CHE, and one as *Pyropia orbicularis*  
305 (Table 1). In the boulder zone surrounded by rocky pools, six of the 12 samples were  
306 classified as *Porphyra* sp. CHE, four as *Pyropia* sp. CHK, and two as *Porphyra* sp. CHF  
307 (Table 1). In the boulder zone surrounded by pools of sandy bottom, all 12 samples were  
308 assigned to the genetic species *Porphyra* sp. CHC (Table 1). Fig. 2 shows the habits of the  
309 four dominant genetic species observed at Maitencillo beach.

310

311 *PCR-RFLP development and analysis for the rbcL.* Among the 1230 bp fragment amplified  
312 by PCR for the *rbcL*, 94 polymorphic sites can be observed between aligned sequences of  
313 the four Bangiales species dominating the intertidal of Maitencillo. The sequence positions  
314 (i.e. variable sites) corresponding to the restriction site of AFA I in the *rbcL*-amplified  
315 fragment are given in Table 2. As shown in Fig. 3, PCR-RFLP patterns of the *rbcL* allowed  
316 us to easily distinguish *Porphyra* sp. CHE from *Porphyra* sp. CHC and from the two  
317 species of *Pyropia*. One very intense fragment located at approximately 900 bp was  
318 observed in *Porphyra* sp. CHC, whereas two fragments of approximately 580 and 430 bp  
319 were the most visible and characteristic of *Porphyra* sp. CHE (Fig. 3). On the other hand,  
320 only the presence of two fragments of approximately 600 and 700 bp in *Pyropia*  
321 *orbicularis* and only one in *Pyropia* sp. CHK in the same size range, allowed  
322 differentiating the two closes by *Pyropia* species (Fig. 3). Restriction patterns were  
323 extremely similar between the two *Pyropia* species and generate doubts about species  
324 determination for most samples of *Pyropia*. Indeed, as visible in Fig. 3, **the two *Pyropia***  
325 ***orbicularis* bands of roughly similar size** are not easily separated and generate a very  
326 intense band/smear around 600-700 bp. This pattern can easily be confounded with *Pyropia*  
327 sp. CHK for which PCR-RFLP products are overloaded. In order to complement our PCR-  
328 RFLP analysis, 24 samples of *Pyropia* were then sequenced for the *COI* gene. Fifteen  
329 samples corresponded to *Pyropia* sp. CHK and 9 to *Pyropia orbicularis*. Only these 24  
330 sequenced individuals were used for morphological analyses when considering *Pyropia*  
331 samples.

332



333 *Variation in morphological characters.* Results of the PCA based on morphological  
334 characters are shown in Fig. 4. According to the eigenvalues, the cumulative proportion of  
335 the first two principal components explains 81% of the total variation in the data.  
336 Component 1 was represented mainly by thallus width and component 2 by thallus length.  
337 If we do not take into account the *Pyropia* sp. CHK data, specimens of *Porphyra* sp. CHE  
338 and *Pyropia orbicularis* would appear as discrete morphological clusters (data not shown).  
339 Nevertheless, both species show a high level of overlap with *Pyropia* sp. CHK in the PCA  
340 (Fig. 4). Conversely, *Porphyra* sp. CHC formed a discrete and distinctive morphological  
341 cluster in the PCA. Multivariate nested, permutational ANOVA (permanova) realized using  
342 the ten morphological traits measured, indicated that statistical differences exist between  
343 species and between plants (i.e. specimens) within species (Table 3). Indeed, post-hoc  
344 Tukey test for multiple comparisons identified significant differences ( $p < 0.05$ ) between all  
345 pairs of species for four out of ten traits (Fig. 5). The largest values in length and width of  
346 the thallus were observed in *Porphyra* sp. CHC and *Porphyra* sp. CHE, respectively, while  
347 the thinnest and thickest reproductive laminae characterized *Porphyra* sp. CHC and  
348 *Pyropia orbicularis*, respectively. Within *Porphyra*, *Porphyra* sp. CHE showed bigger  
349 vegetative and zygotosporangial cells than *Porphyra* sp. CHC. Within *Pyropia*, *Pyropia* sp.  
350 CHK reached a larger width of vegetative cells and a smaller length and width of  
351 zygotosporangial cells than *Pyropia orbicularis*. Even if statistical analyses indicate that the  
352 width of vegetative cells is different between all 4 species, the boxplots for this trait in Fig.  
353 5 show that the high variance observed in *Pyropia* sp. CHK overlap values measured in  
354 both *Porphyra* sp. CHC and *Porphyra* sp. CHE.

355

356 *Taxonomic treatment.* Below we describe in detail three previously unnamed species: one  
357 new species of *Pyropia* and two new species of *Porphyra* (See Fig. 2, Figs. 6-9, Table 4).  
358 For the *P. orbicularis* description, see Fig. S4 and Ramírez et al. (2014).

359 1.- *Porphyra luchensis* Meynard, Ramírez, Contreras-Porcia sp. nov.

360 Description: The gametangial blades are semi-translucent, 39.0-156.0  $\mu\text{m}$  thick in  
361 transverse section, reniform, rhomboid to spear-shaped and asymmetrical when fully  
362 extended, 5.0-22.0 cm long and 0.5-9.0 cm wide (Fig. 2A and Fig. 6A) with a loose,  
363 wrinkled surface. Blade margins are spiny to undulated, often folded. Color green on the  
364 center of the blade to reddish-brown on the margins. Blades are attached to rock substratum  
365 by a basal rhizoidal holdfast. Thalli are monostromatic and monoecious. In sectional view,  
366 blade vegetative portions are monostromatic and their thickness range from 68.0 to 156.0  
367  $\mu\text{m}$  (Fig. 6 C-D). Sexual regions of the thalli are monoecious, 39.0-136.0  $\mu\text{m}$  thick in  
368 sectional view (Fig. 6 E-H), with contiguous groups of cells forming either spermatangia or  
369 zygotosporangia and divided into separate male and female sectors. Reproductive cells are  
370 formed as continuous areas along the margins of the blade. The division formula of  
371 spermatangia is 128 (a/4, b/4, c/8) whereas the division formula of zygotosporangia is 64  
372 (a/2, b/4, c/8) or 128 (a/4, b/4, c/8) (Fig. 6 E-H). Table 4 summarizes the detailed  
373 morphological features of 32 blades of *Po. luchensis* identified through molecular assisted  
374 methods (i.e. **PCR-RFLP**).

375 Holotype: Voucher specimen = SGO168338, housed in the herbarium of the National  
376 Museum of Natural History, Chile; sample **code** = CHE0027; gametophytic blade  
377 (vegetative) collected from boulders **in the** upper intertidal zone from Maitencillo beach

378 (Chile: Valparaíso), 32° 39' S, 71° 26' W, coll. Zapata, Contreras-Porcía, 10 Oct 2013. (Fig.  
379 6A). GenBank accession numbers: *COI*, MH123945 and *rbcL*, MH124031.

380 Distribution: Chile, from Atacama, Chañaral de Aceituno (29°04' S, 71°29' W), to  
381 Valparaíso, Maitencillo beach, (32°39' S, 71°26' W).

382 Habitat: Thalli present year-round but more abundant in winter and spring, growing on  
383 boulders surrounded by stony ground pools at low tide. The species is less abundant in  
384 summer in the high and intermediate intertidal zones of rock platforms, where *Pyropia*  
385 *orbicularis* is dominant.

386 Etymology: The specific epithet *luchensis* refers to the word “luche”, a traditional name  
387 used by fishermen communities along the Chilean coast and referring to the bladed  
388 Bangiales harvested and sold for food consumption.

389 Comments: *Porphyra luchensis* Meynard, Ramírez, Contreras-Porcía sp. nov. corresponds  
390 to the genetic species *Porphyra* sp. CHE originally identified by Guillemín et al. (2016).

391

392 2.- *Porphyra longissima* Meynard, Ramírez, Contreras-Porcía sp. nov.

393 Description: The gametangial blades are semi-translucent, 36.0-122.0 µm thick in  
394 transverse section, linear to lanceolate and very long in comparison to their width, 16.0-  
395 50.0 cm long and 0.3-3.5 cm wide, and having a soft, relatively mucilaginous and flexible  
396 surface (Fig. 2B, and Fig. 7A). Blade margins are entire to sinuate. Color rosy pink to  
397 brown. Blades are attached to rock substratum by a basal rhizoidal holdfast. Thalli are  
398 monostromatic and monoecious. In sectional view, vegetative portions of blades are

399 monostromatic, and their thickness range from 38.0 to 122.0  $\mu\text{m}$  (Figs. 7C-D). Sexual  
400 regions of the thalli are monoecious, 36.0-94.0  $\mu\text{m}$  thick in transverse section and divided  
401 into separate male and female sectors by a vertical line. Spermatangia are light green while  
402 zygotosporangia are light reddish-brown (Figs. 7 E-H). Zygotosporangia are formed as  
403 continuous areas along most of the thallus, except the basal portion (vegetative and  
404 rhizoidal cells), whereas spermatangia develop along the margins of the blade. The division  
405 formula of spermatangia is 128 (a/4, b/4, c/8) whereas the division formula of  
406 zygotosporangia is 64 (a/4, b/4, c/4) (Figs. 7 E-H). Table 4 summarizes the detailed  
407 morphological features of 32 blades of *Po. longissima* identified through molecular assisted  
408 methods (i.e. **PCR-RFLP**).

409 Holotype: Voucher specimen = SGO168348, housed in the herbarium of the National  
410 Museum of Natural History, Chile; sample **code** = CHC0010; gametophytic blade  
411 (reproductive) collected from boulders of the upper intertidal zone of Maitencillo beach  
412 (Chile: Valparaíso, 32°39'S, 71°26'W), coll. Zapata, Contreras-Porcía, 12 Aug 2014. (Fig.  
413 7A). GenBank accession numbers: *COI*, MH123931 and *rbcL*, MH124021.

414 Distribution: Chile-from Atacama, Los Burros (28°55'S/71°31'W), to Los Ríos, Playa  
415 Rosada (39°48'S/73°24'W).

416 Habitat: Gametophytic thalli only registered in winter, growing on boulders surrounded by  
417 sandy **bottom or even** buried in sand (Fig. 2B).

418 Comments: *Porphyra longissima* Meynard, Ramírez, Contreras-Porcía, sp. nov.  
419 corresponds to the genetic species *Porphyra* sp. CHC, originally identified by Guillemín et  
420 al. (2016).

421

422 3.- *Pyropia variabilis* Zapata, Meynard, Ramírez, Contreras-Porcía sp. nov.

423 Description: The gametangial blades are monostromatic and monoecious. Blades are 63.0-

424 139.0  $\mu\text{m}$  thick in transverse section, oblong to lanceolate, sometimes cuneate or

425 acuminate, with wavy surface, 2.8-14.0 cm long and 1.1-7.0 cm wide, generally very

426 flexible and relatively resistant (Figs. 2D-E, Figs. 8A and 9A). Blade margins are entire to

427 undulate or lobate. Blades are attached to rock substratum by a basal or subcentral

428 rhizoidal holdfast. This species shows two different gametophytic morphotypes, occurring

429 in different intertidal microhabitats, and characterized principally by external morphology

430 (see Figs. 2D-E, Figs. 8A and 9A). The Green Morph (GM), consists of a single blade,

431 generally lanceolate and forest green to yellowish green in color (Figs. 2D and 8A). A

432 second morphotype, the Long Morph (LM), generally forestgreen to light-brown in color, is

433 characterized by a long central blade and one or two additional shorter blades arising from

434 a subcentral disc (Figs. 2E and 9A). LM blades are highly variable, being oblong to

435 lanceolate, cuneate or acuminate. In spite of their variable morphology, all fresh

436 gametophytic blades of *Py. variabilis* mostly display shades of green and present wavy

437 ruffled-margins (Figs. 2D-E). In sectional view, vegetative portions of blades are

438 monostromatic, and their thickness range from 63.0 to 137.0  $\mu\text{m}$  (Figs. 8C-D and 9C-D).

439 Sexual regions of the thalli are monoecious, 68.0-139.0  $\mu\text{m}$  thick (Figs. 8E-H and 9E-H),

440 with contiguous groups of cells forming either spermatangia or zygotosporangia and

441 divided into separate male and female sectors by a vertical line. Reproductive cells are

442 formed as continuous areas along the margins of the blade. The division formula of

443 spermatangia is 128 (a/4, b/4, c/8) whereas the division formula of zygotosporangia is 16

444 (a/2, b/2, c/4) or 32 (a/2, b/4, c/4) (Figs. 8E-H and 9E-H). Table 4 summarizes the detailed  
445 morphological features of 15 sequenced blades of *Py. variabilis* identified through  
446 molecular assisted methods (i.e. sequencing and PCR-RFLP).

447 Holotype: Voucher specimen = SGO168333, housed in the herbarium of the National  
448 Museum of Natural History, Chile; sample code = CHK0025; gametophytic blade  
449 (reproductive) collected from a rocky platform of the upper intertidal zone of Maitencillo  
450 beach (Chile: Valparaíso), 32° 39' S, 71° 26' W, coll. J. Zapata, L. Contreras-Porcía, 6  
451 September 2013 (Fig. 9A). GenBank accession numbers: *COI*, MH123975 and *rbcL*,  
452 MH124050.

453 Isotype: Voucher specimen = SGO168334, housed in the herbarium of the National  
454 Museum of Natural History, Chile; sample code = CHK0028; gametophytic blade  
455 (reproductive) collected from steep rock faces of the upper intertidal zone of Maitencillo  
456 beach (Chile: Valparaíso), 32° 39' S, 71° 26' W, coll. C. Fierro, F. Castañeda, 10 October  
457 2013 (Fig. 8A). GenBank accession numbers: *COI*, MH123978 and *rbcL*, MH124051.

458 Distribution: Chile-from Antofagasta, Pan de Azúcar (26°10'S/70°38'W) to Coquimbo,  
459 Horcón (32°42'S/71°29'W).

460 Habitat: Thalli of the GM morphotype grow on shady and humid steep rock faces (Fig.  
461 2D). This morphotype is present mainly during the winter and spring seasons and is almost  
462 absent in summer. Thalli of the LM morphotype grow on the sunnier and drier platforms of  
463 the high intertidal (Fig. 2E). It is present mainly during the winter and spring seasons  
464 (Zapata 2016).

465 Etymology: *variabilis*-variable in shape. The name *variabilis* aims to describe the very  
466 variable morphology of the gametophytic habit.

467 Comments: *Pyropia variabilis* Zapata, Meynard, Ramírez, Contreras-Porcía, sp. nov.  
468 corresponds to the genetic species *Pyropia* sp. CHK, originally identified by Guillemin et  
469 al. (2016).

470

## 471 DISCUSSION

472 Few molecular studies have characterized genetic and morphological local diversity  
473 of bladed Bangiales and considered different types of habitats of the intertidal to describe  
474 their distribution within this environment. We have confirmed the presence of five species  
475 of bladed Bangiales in the rocky intertidal of Maitencillo beach, four of them being  
476 dominant, during winter-spring 2013-2014. All correspond to species previously detected  
477 by Guillemin et al. (2016) along the coasts of Chile, but our study report for the first time  
478 the presence of *Porphyra* sp. CHF, *Porphyra*. sp. CHE and *Porphyra*. sp. CHC in  
479 Maitencillo. *Porphyra*. sp. CHE was reported by Guillemin et al. (2016) in only one site  
480 located at 29°S of latitude in Chile. It is clear that the species is probably more common  
481 and broadly distributed than previously described, extending at least to Central Chile (32°S  
482 of latitude). Phylogenetic analyses of *rbcL* and *COI* gene sequences (Figs. 1 and S2) were  
483 not fully concordant, only the *COI* allowing to clearly separate *Pyropia* sp. CHJ, *Pyropia*  
484 *orbicularis* and *Pyropia* sp. CHK as three highly supported monophyletic clades (Figs. 1  
485 and S3). **Incongruence between *rbcL* and *COI* results could be the result of the lower base-**  
486 **substitution mutation rate of the *rbcL* gene for which incomplete lineage sorting and lack of**

487 monophyly has been observed in macroalgae species complex (Tellier et al. 2009,  
488 Montecinos et al. 2012). *COI* sequences, in contrast, allowed to determine genetic species  
489 in these recently diverged species complex. In red macroalgae, hybridization and  
490 introgression have also been linked to the existence of incongruence between mitochondrial  
491 and chloroplast markers (Destombe et al. 2010). We cannot discard the possibility of past  
492 and/or ongoing gene flow between the three *Pyropia* sp. CHJ, *Pyropia orbicularis* and  
493 *Pyropia* sp. CHK, especially in central Chile where they co-occur (see Niwa et al. 2009 for  
494 an example of introgression in Bangiales, between *Py. Yezoensis* and *Py. tenera*).

495 In spite of significant differences between all pairs of species for four  
496 morphological characters, the huge variance in traits observed for *Pyropia* sp. CHK only  
497 allow to clearly separate the species *Porphyra* sp. CHC from the other three dominant  
498 bladed *Bangiales* of Maitencillo Beach using morphology. Indeed, *Porphyra* sp. CHC  
499 consistently shows extreme values of length and width of the thallus and thickness of the  
500 reproductive lamina (see Fig. 5). *Porphyra* sp. CHC is also the only species showing a  
501 specific habitat preference in the intertidal and was encountered only on boulders  
502 surrounded by pools of sandy bottom. This suggests that, unlike the other three dominant  
503 species present at the study site, *Porphyra* sp. CHC morphology and/or other associated  
504 characteristics (e.g. metabolites or physiological traits) could be central in explaining its  
505 local distribution. Interestingly, a recent study reported the presence of a morphologically  
506 very similar species of *Porphyra* with long thin blades, *Po. mumfordii*, on boulders  
507 surrounded by sand in central Chile (see Fig. 2 of Muñoz-Muga et al. 2018), supporting the  
508 possibility of morphs adapted to particular habitats.

509 Our study suggests that only one out of the four-dominant foliose *Bangiales* species  
510 show specific distributional ranges in the intertidal at Maitencillo since the distribution of



511 *Pyropia orbicularis*, *Porphyra* sp. CHE and *Pyropia* sp. CHK overlap at the high intertidal  
512 zone on the rocky platform and at the boulder zone surrounded by rocky pools.  
513 Nevertheless, even if the three species were generally observed sympatrically, some  
514 habitats seem mostly dominated by one species (e.g. *Pyropia* sp. CHK along the rocky  
515 wall). As previously reported in distributional studies supported by molecular tools for  
516 intertidal sites in New England, USA (West et al. 2005) and in the southern West Cape,  
517 South Africa (Griffin et al. 1999), we expected that the abundance and occurrence of  
518 specific intertidal zones by bladed Bangiales would differ. Indeed, in a highly  
519 heterogeneous environment, in terms of landscape complexity, physical and chemical  
520 variables and biotic interactions, differences in microhabitats could be expected among  
521 related species (Billard et al. 2010, Couceiro et al. 2015, Muangmai et al. 2016, Montecinos  
522 et al. 2017). Nonetheless, in our study, most specimens occurred in the high intertidal,  
523 probably experiencing relatively homogeneous abiotic or biotic conditions during late  
524 winter and early spring (i.e. study period). Accordingly, Scweikert et al. (2012) found no  
525 distinct intertidal zonation patterns for bladed *Porphyra* sp. at Brighton Beach, southeast  
526 New Zealand, with two dominant species showing a similar distributional pattern across  
527 intertidal zones and seasons and seven other species being present only sporadically.

528         In the present study we have focused only on differences in zonation, but we did  
529 not, however, test if *Pyropia orbicularis*, *Porphyra* sp. CHE and *Pyropia* sp. CHK  
530 presented any differences in micro-ecological niches. Supporting this possibility, Scweikert  
531 et al. (2012) hypothesized that the differing distributional patterns within and between  
532 seasons observed in the generally overlapping *Porphyra* sp. in Brighton Beach could still  
533 be explained by their differing degrees of physiological adaptation to abiotic factors.  
534 Previous studies integrating finer sampling scale than in our study, or the one of Scweikert

535 et al. (2012), and records of physical environmental factors in distinct micro-habitats, have  
536 revealed differences in micro-niche partitioning between related species of macroalgae. The  
537 study of Muangmai et al. (2016) describing the small-scale distribution of three sympatric  
538 cryptic species of the red alga *Bostrychia intricata* (Bory) Montagne (Ceramiales,  
539 Rhodophyta) along the shore of Moa Point, Wellington, New Zealand, selected sampling  
540 patches according to three a priori contrasting factors: tidal position, wave exposure, and  
541 sun exposure. Muangmai et al. (2016) demonstrated that the distribution of three cryptic  
542 species of *Bostrychia* in the intertidal was non-random, and highly influenced by tidal  
543 height and wave exposure. In the case of the brown algae *Ectocarpus crouaniorum* Thuret  
544 in Le Jolis and *E. siliculosus* (Dillwyn) Lyngbye, Coucerio et al. (2015) revealed that  
545 differences in micro-niches existed between species and phases (i.e. gametophytes and  
546 sporophytes). Along the European coast, the *Fucus* species complex (Fucales, Ochrophyta)  
547 was observed as the succession of different ecological species along a tidal gradient of  
548 contrasting habitats with a segregation between species in less than 50 cm on the vertical  
549 shore gradient (Billard et al. 2010). As in the marine gastropod *Littorina saxatilis* (Olivi),  
550 where ecotypes are segregated in different habitats along the shore (Butlin et al. 2008),  
551 divergence and speciation has been deemed to be driven by slight differences in ecological  
552 selective pressures as desiccation stress in *Fucus* (Billard et al. 2010). It is possible that our  
553 sampling was too coarse to detect microhabitat differences between *Pyropia orbicularis*,  
554 *Porphyra* sp. CHE and *Pyropia* sp. CHK in Maitencillo, and new field observation along a  
555 fine vertical shore gradient linked with the acquisition of data on physical environmental  
556 factors and biotic local interaction should now be performed.

557           However, in our study, we observed what seems to be a variable or specialized  
558 ecotype of *Pyropia* sp. CHK (see Figs. 2E-D, and Figs. 8A and 9A). Indeed, gametophytes

559 of the GM morphotype of *Pyropia* sp. CHK were dominant on rocky walls, with very low  
560 presence of gametophytes of the LM morphotype of *Pyropia* sp. CHK or other bladed  
561 Bangiales species therein (Zapata 2016, Betancourt et al. 2018, this study). Interestingly, in  
562 the study of Coucerio et al. (2015), two ecotypes were observed among the *E. siliculosus*  
563 sporophytes sampled in North West France with one ecotype encountered as epiphyte on  
564 several different algal hosts and the other attached to abiotic substrates. The authors  
565 proposed that these two ecotypes probably arose due to phenotypic plasticity in the case of  
566 *E. siliculosus*. Our molecular data sets include only sequences of the *COI* and *rbcL* genes  
567 and these were not sufficiently variable to detect genetic difference between our two  
568 *Pyropia* sp. CHK ecotypes. However, these two molecular markers are characterized by  
569 low mutational rates in comparison to microsatellites (Jarne and Lagoda 1996), and we  
570 cannot discard the hypothesis of possible genetic adaptation of the GM morphotype in  
571 *Pyropia* sp. CHK to the conditions encountered on the step walls of Maitencillo. To  
572 determine if the two ecotypes within *Pyropia* sp. CHK are due to phenotypic plasticity or  
573 represent traits related to intraspecific genetic differentiation and adaptation, samples  
574 should be genotyped using more variables markers (i.e. microsatellites or SNP's) that allow  
575 testing for signatures of selection (e.g. Schlotterer 2000, Haasl et al. 2014). We also  
576 propose that transplant experiments and/or common garden experiments should be  
577 considered to complement the population genetic analyses and to test for adaptation (e.g. de  
578 Villemereuil et al. 2016).

579

580

## CONCLUSIONS

581

582

As pointed out by Sutherland et al. 2011 (following Matsuyama-Serisawa et al. 2004) the very simple and at the same time highly variable morphology of foliose

583 Bangiales make taxonomic identification using key morphological traits an unresolved  
584 problem in this group. On the other hand, phylogenetic reconstructions using molecular  
585 markers have revealed cryptic diversity at the genus and species level in *Porphyra* and  
586 *Pyropia* (e.g. Guillemin et al. 2016 in Chile), and allowed the accurate reassignment of  
587 these organisms (e.g. Nelson and Broom 2010 in New Zealand). In our study, in spite of  
588 substantial sample sizes and significant differences between all pairs of species for various  
589 morphological characters, the overlap observed in morphological trait values between  
590 *Porphyra* and *Pyropia* species suggests that morphology is not sufficient to delineate  
591 genetic species in this group. In spite of this, some clear morphological differences were  
592 observed among the four species of foliose Bangiales analyzed, such as the differential  
593 thickness of the blade. This trait was associated with a differential degree of palatability  
594 (Niwa et al. 2008) and would have a potential utility in the selection of strains (each  
595 belonging to one or more species) for the food industry. Scweikert et al. (2012) proposed  
596 that the very similar morphology between some foliose Bangiales implies that they  
597 developed the most suited thallus shape for their gametophytes in their habitat. In this case,  
598 an optimal (and common) structure in phenotypic morphology in this group of algae would  
599 have been shaped by the particular genetic, developmental and environmental constraints  
600 operating on these algal taxa (Rosen 1967). The rapid and accurate identification of local  
601 genetic diversity in foliose Bangiales recently achieved using molecular techniques  
602 (Scweikert et al. 2012, Nelson et al. 2013, Dumilag et al. 2016, Guillemin et al. 2016,  
603 Reddy et al. 2018, Yang et al. 2018) will allow researchers to refine the knowledge about  
604 the intrinsic and environmental determinants of their distribution across tidal gradients of  
605 contrasting habitats and climates.

606

607

## ACKNOWLEDGEMENTS

608 This work was supported by FONDECYT 1120117, 1170881, and DI-501-14/R & DI-  
609 1245-16/R (Universidad Andrés Bello) awarded to LC-P. We would like to thank two  
610 anonymous reviewers for their insightful and constructive comments.

611

612

## REFERENCES

- 613 Abdi, H. & Williams, L. J. 2010. Honestly significant difference (HSD) test. In Salkind,  
614 N.J., Dougherty, D.M. & Frey, B. [Ed.] *Encyclopedia of Research Design*, Sage,  
615 Thousand Oaks, CA, USA (2010), pp. 583-585.
- 616 Aguilar-Rosas, R. & Aguilar-Rosas, L. E. 2003. El género *Porphyra* (Bangiaceae,  
617 Rhodophyta) en la costa Pacífico de México. I. *Porphyra suborbiculata* Kjellman.  
618 *Hidrobiológica*, 13:51–56.
- 619 Anderson, M.J. 2001. A new method for non-parametric multivariate analysis of variance.  
620 *Austral Ecol.*, 26:32–46.
- 621 Bandelt, H-J., Forster, P. & Röhl, A. 1999. Median-Joining Networks for Inferring  
622 Intraspecific Phylogenies. *Mol. Biol. Evol.* 16:37–48.
- 623 Betancourt, C., Zapata, J., Latorre, N., Anguita, C., Castañeda, F., Meynard, A., Fierro, C.,  
624 Espinoza, C., Guajardo, E., Núñez, A., Salas, N., González, C., Ramírez, M.L.,  
625 Bulboa-Contador, C. & Contreras-Porcía, L. 2018. Spatio-temporal variation in the  
626 composition of the macroalgae assemblage of the intertidal rocky zone from  
627 Maitencillo, Valparaíso, central coast of Chile. *Rev. Biol. Mar. Oceanogr.* 53(1):105–  
628 117.
- 629 Billard, E., Serrao, E., Pearson, G; Destombe, C. & Valero, M. 2010. *Fucus vesiculosus*  
630 and *spiralis* species complex: a nested model of local adaptation at the shore level.  
631 *Mar. Ecol. Prog. Ser.* 405:163–174.
- 632 Blouin, N. A., Brodie, J. A., Grossman, A. C., Xu, P. & Brawley, S. H. 2011. *Porphyra*: a  
633 marine crop shaped by stress. *Trends Plant Sci.* 16:29–37.
- 634 Box, G.E. P. & Cox, D. R. 1964. An analysis of transformations (with discussion). *J. R.*  
635 *Stat. Soc. Series B. Stat. Methodol.* 26:211–252.
- 636 Brodie, J., Bartsch, I., Neefus, C., Orfanidis, S., Bray, T. & Mathieson, A. C. 2007. New  
637 insights into the cryptic diversity of the North Atlantic–Mediterranean *Porphyra*  
638 *leucosticta* complex: *P. olivii* sp. nov. and *P. rosenfurtii* (Bangiales, Rhodophyta).  
639 *Eur. J. Phycol.* 42:3–28.
- 640 Broom, J. E., Nelson, W. A., Yarish, C., Jones, W. A., Aguilar-Rosas, R. & Aguilar-Rosas,  
641 L. E. 2002. A reassessment of the taxonomic status of *Porphyra suborbiculata*,  
642 *Porphyra carolinensis* and *Porphyra lilliputiana* (Bangiales, Rhodophyta) based on  
643 molecular and morphological data. *Eur. J. Phycol.* 37:227–35.
- 644 Butlin, R. K., Galindo, J. & Grahame, J. W. 2008. Sympatric, parapatric or allopatric: the  
645 most important way to classify speciation? *Transt. R. Soc. B.* 363: 2997–3007. Collén

- 646 J & Davison IR. 1999a. Reactive oxygen production and damage in intertidal *Fucus*  
647 spp.(Phaeophyceae). *J. Phycol.* 35: 54–61.
- 648 Collén, J., & Davison, I. R. 1999b. Stress tolerance and reactive oxygen metabolism in the  
649 intertidal red seaweeds *Mastocarpus stellatus* and *Chondrus crispus*. *Plant, Cell &*  
650 *Environment*. 22(9): 1143–1151.
- 651 Couceiro, L., Le Gac, M., Hunsperger, H.M., Mauger, S., Destombes, C., Cock, J.M.,  
652 Ahmed, S., Coelho, S.M., Valero, M. & Peters, A.F. 2015. Evolution and  
653 maintenance of haploid-diploid life cycles in natural populations: The case of the  
654 marine brown alga *Ectocarpus*. *Evolution* 69-7:1808–1822.
- 655 Destombe, C., Valero, M., & Guillemin, M-L. 2010. Diversity and natural hybridization in  
656 two related red algae species: *Gracilaria gracilis* and *Gracilaria dura* using multi  
657 DNA markers: resurrection of the species *G. dura* previously described in the  
658 Northern Atlantic 200 years ago. *J. Phycol.* 46:720–727.
- 659 de Villemereuil, Gaggiotti, O.E., Mouterde, M. & Till-Bottraud, I. 2016. Common garden  
660 experiments in the genomic era: new perspectives and opportunities. *Heredity*  
661 116:249–254.
- 662 Dillehay, T.D. 2008. Monte Verde: Seaweed, Food, Medicine, and the Peopling of South  
663 America. *Science* 320:784–6.
- 664 Dumilag, R.V., Aguinaldo, Z-Z.A., Mintu, C.B., Quinto, M.P., Ame, E.C., Andres, R.C.,  
665 Monotilla, W.D. & Yap, S.L. 2016. Morphological and molecular confirmation of the  
666 occurrence of *Pyropia tanegashimensis* (Bangiales, Rhodophyta) from Palaui Is., Sta.  
667 Ana, Cagayan, Philippines. *Phytotaxa* 255:83–90.
- 668 Faugeron, S., Valero, M., Destombe, C., Martínez, E. A. & Correa J. A. 2001. Hierarchical  
669 spatial structure and discriminant analysis of genetic diversity in the red alga  
670 *Mazzaella laminarioides* (Gigartinales, Rhodophyta). *J. Phycol.* 37:705–16.
- 671 Flores-Molina, M.R., Thomas, D., Lovazzano, C., Núñez, A., Zapata, J., Kumar, M.,  
672 Correa, J.A. & Contreras-Porcia, L. 2014. Desiccation stress in intertidal seaweeds:  
673 effects on morphology, antioxidant responses and photosynthetic performance.  
674 *Aquat. Bot.* 113:90–99.
- 675 Fredericq, S., Anderson, R. J. & Lopez-Bautista, J. M. 2003. Circumscription of some  
676 Phylloporaceae (Gigartinales, Rhodophyta) from the Cape region, South Africa,  
677 based on molecular evidence. *Proc. Int. Seaweed Symp.* 17:263–73.
- 678 Guillemin, M. L., Contreras-Porcia, L., Ramírez, M. E., Macaya, E. C., Bulboa-Contador,  
679 C., Woods, H., Wyatt, C. & Brodie, J. 2016. The bladed Bangiales (Rhodophyta) of

- 680 the South Eastern Pacific: Molecular species delimitation reveals extensive diversity.  
681 *Mol. Phylogenet. Evol.* 94:814–26.
- 682 Griffin, N. J., Bolton, J. J. & Anderson, R. J. 1999. *Porphyra aeodis* sp. nov. (Bangiales,  
683 Rhodophyta), an epiphyte of *Aeodes orbitosa* from South Africa. *Eur. J. Phycol.*  
684 34:505–12.
- 685 Haasl, R.J., Jonson, R.C. & Payseur, B.A. 2014. The effects of microsatellite selection on  
686 linked sequences diversity. *Genome Biol. Evol.* 6(7):1843–1861.
- 687 Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis  
688 program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.* 41:95–8.
- 689 Hammer, Ø., Harper, D.A.T., and P. D. Ryan, 2001. PAST: Paleontological Statistics  
690 Software Package for Education and Data Analysis. *Palaeontologia Electronica* 4(1):  
691 9pp.
- 692 Hilton, Z., Wellenreuther, M., & Clements, K. D. 2008. Physiology underpins habitat  
693 partitioning in a sympatric sister species pair of intertidal fishes. *Funct. Ecol.* 22:  
694 1108–1117.
- 695 Hoffmann, A., Santelices, B., 1997. Flora marina de Chile central (Marine Flora of Central  
696 Chile). Universidad Católica de Chile, Santiago, Chile (in Spanish).
- 697 Hommersand, M. H., Fredericq, S. & Freshwater, D. W. 1994. Phylogenetic systematics  
698 and biogeography of the Gigartinae (Gigartinales, Rhodophyta) based on the  
699 sequence analysis of *rbcL*. *Bot. Mar.* 37:193–203.
- 700 Hurd, C.L., Harrison, P.J., Bischof, K. & Lobban, C.H. 2014. *Seaweed Ecology and*  
701 *Physiology*. 2nd ed. Cambridge University Press, Cambridge, 551 pp.
- 702 Jarne, P. & Lagoda, P.J.L. 1996. Microsatellites, from molecules to populations and back.  
703 *TREE*. 11 (10):424–429.
- 704 Koh, Y.H. & Kim, M.S. 2018. DNA barcoding reveals cryptic diversity of economic red  
705 algae, *Pyropia* (Bangiales, Rhodophyta): description of novel species from Korea. *J.*  
706 *Appl. Phycol.* <https://doi.org/10.1007/s10811-018-1529-8>.
- 707 Krueger-Hadfield, S.A., Collén, J., Daguin-Thiébaud, C. & Valero, M. 2011. Genetic  
708 population structure and mating system in *Chondrus crispus* (Rhodophyta). *J Phycol.*  
709 47:440–450.
- 710 Krueger-Hadfield, S.A., Roze, D., Mauer, S. & Valero, M. 2013 Intergametophytic selfing  
711 and microgeographic genetic structure shape populations of the intertidal red seaweed  
712 *Chondrus crispus*. *Mol. Ecol.* 22:3242–60.



- 713 Lindstrom, S. C., Hughey, J. R. & Aguilar-Rosas, L. E. 2015. Four new species of *Pyropia*  
714 (Bangiales, Rhodophyta) from the west coast of North America: the *Pyropia*  
715 *lanceolata* species complex updated. *PhytoKeys* 52:1–22.
- 716 Martin Henry H. Stevens, adapted to vegan by Jari Oksanen: Community Ecology Package.  
717 2009. R package vegan version 1.16-32. <http://cran.r-project.org/>, [http://vegan.r-](http://vegan.r-forge.r-project.org/)  
718 [forge.r-project.org/](http://vegan.r-forge.r-project.org/)
- 719 Matsuyama-Serisawa, K., Couceiro, L., Yamazaki, S., Kitade, Y., Serisawa, Y., Kuwano,  
720 K. & Saga, N. 2004. Tracing of systematic characters of Bangiales (Rhodophyta)  
721 based on molecular phylogeny inferred from sequences of nuclear small subunit  
722 rRNA genes. *Suisanzoshoku* 52:185–198.
- 723 Montecinos, A., Broitman, B.R., Faugeron, S., Haye, P.A., Tellier, F. & Guillemin, M.L.  
724 2012. Species replacement along a linear coastal habitat: phylogeography and  
725 speciation in the red alga *Mazzaella laminaroides* along the south east pacific. *BMC.*  
726 *Evol. Biol.* 12(1):97.
- 727 Montecinos, A.E., Couceiro, L., Peters, A.F., Desrut, A., Valero, M., & Guillemin, M.L.  
728 2017. Species delimitation and phylogeographic analyses in the *Ectocarpus* subgroup  
729 *siliculosi* (Ectocarpales, Phaeophyceae). *J. Phycol.* 53:17–31.
- 730 Muangmai, N., von Ammon, U. & Zuccarello, G.C. 2016. Cryptic species in sympatry:  
731 nonrandom small-scale distribution patterns in *Bostrychia intricata* (Ceramiales,  
732 Rhodophyta). *Phycologia* 55:424–30.
- 733 Muñoz-Muga, P., Romo, H., Calderón, C., Evrard, O. & Díaz, H. 2018. Amplificación del  
734 gen *rbcL* revela primer registro de *Porphyra mumfordii* (Bangiales, Rhodophyta) en  
735 la Bahía de Valparaíso, Chile central. *Rev. Biol. Mar. Oceanogr.* 53(1):131–140.
- 736 Neefus, C. D., Mathieson, A. C., Bray, T. L. & Yarish, C. 2008. The distribution,  
737 morphology, and ecology of three introduced asiatic species of *Porphyra* (Bangiales,  
738 Rhodophyta) in the Northwestern Atlantic. *J. Phycol.* 44:1399–414.
- 739 Nelson, W. A. & Broom, J.E.S. 2010. *Porphyra columbina* (Bangiales, Rhodophyta):  
740 originally described from the New Zealand subantarctic islands. *Austral. Syst.Bot.*  
741 23:16–26.
- 742 Nelson, W. A. 2013. *Pyropia plicata* sp. nov. (Bangiales, Rhodophyta): naming a common  
743 intertidal alga from New Zealand. *PhytoKeys* 21:17–28.
- 744 Nguyen, L.T., Schmidt, H.A., von Haeseler, A. & Minh, B.Q. 2015. IQ-TREE: a fast and  
745 effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol.*  
746 *Biol. Evol.* 32: 268–274.

- 747 Niwa, K., Kobiyama, A. & Aruga, Y. 2005. Confirmation of cultivated *Porphyra tenera*  
748 (Bangiales, Rhodophyta) by polymerase chain reaction restriction fragment length  
749 polymorphism analyses of the plastid and nuclear DNA. *Phycol. Res.* 53: 296–302.
- 750 Niwa, K. & Aruga, Y. 2006. Identification of currently cultivated *Porphyra* species by  
751 PCR-RFLP analysis. *Fisheries Science.* 72:143–48.
- 752 Niwa, K., Furuita, H. & Yamamoto, T. 2008. Changes of growth characteristics and free  
753 amino acid content of cultivated *Porphyra yezoensis* Ueda (Bangiales, Rhodophyta)  
754 blades with the progression of the number of harvest in a nori farm. *Fisheries*  
755 *Science.* 72:143–48.
- 756 Niwa, K., Iida, S., Kato, A., Kawai, H., Kikuchi, N., Kobiyama, A. & Aruga, Y. 2009.  
757 Genetic diversity and introgression in two cultivated species (*Porphyra yezoensis* and  
758 *Porphyra tenera*) and closely related wild species of *Porphyra* (Bangiales,  
759 Rhodophyta). *J. Phycol.* 45:493–502.
- 760 Niwa, K., Kobiyama, A. & Sakamoto, T. 2010a. Interspecific hybridization in the haploid  
761 blade-forming marine crop *Porphyra* (Bangiales, Rhodophyta): occurrence of  
762 allodiploidy in surviving F1 gametophytic blades. *J. Phycol.* 46:693–702.
- 763 Niwa, K. & Sakamoto, T. 2010b. Allopolyploidy in natural and cultivated populations of  
764 *Porphyra* (Bangiales, Rhodophyta). *J. Phycol.* 46:1097–1105.
- 765 Niwa, K. & Kobiyama, A. 2014. Speciation in the marine crop *Pyropia yezoensis*  
766 (Bangiales, Rhodophyta). *J. Phycol.* 50:897–900.
- 767 Niwa, K., Kikuchi, N., Hwang, M.S., Choi, H.G., Aruga, Y., 2014. Cryptic species in the  
768 *Pyropia yezoensis* complex (Bangiales, Rhodophyta): sympatric occurrence of two  
769 cryptic species even on same rocks. *Phycol. Res.* 62: 36–43.
- 770 Pons, J., Barraclough, T. G., Gomez-Zurita, J., Cardoso, A., Duran, D. P., Hazell, S.,  
771 Kamoun, S., Sumlin, W. D. & Vogler, A. P. 2006. Sequence-Based species  
772 delimitation for the DNA taxonomy of undescribed insects. *Syst. Biol.* 55:595–609.
- 773 R Development Core Team. 2017. R: A language and environment for statistical  
774 computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-  
775 900051-07-0, URL <http://www.R-project.org>.
- 776 Ramírez, M.E., Contreras-Porcía, L., Guillemin, M.L., Brodie, J., Valdivia, C., Flores-  
777 Molina, M.R., Núñez, A, Bulboa Contador, C. & Lovazzano, C. 2014. *Pyropia*  
778 *orbicularis* sp. nov. (Rhodophyta, Bangiaceae) based on a population previously  
779 known as *Porphyra columbina* from the central coast of Chile. *Phytotaxa* 158:133–  
780 153.

- 781 Reddy, M.M., A, De Clerck, O., Leliaert, F., Anderson, R.J. & Bolton, J.J. 2018. A rosette  
782 by any other name: species diversity in the Bangiales (Rhodophyta) along the South  
783 African coast. *Eur. J. Phycol.* 53:267–82.
- 784 Rosen, R. 1967. Optimality Principles in Biology. In: The Vascular System, Butterworths,  
785 London, Chapter 3, 41-60.
- 786 Sánchez, N., Vergés, A., Peteiro, C., Sutherland, J.E. & Brodie, J. 2014. Diversity of bladed  
787 Bangiales (Rhodophyta) in western Mediterranean: recognition of the genus *Themis*  
788 and descriptions of *T. iberica* sp. nov., and *Pyropia parva* sp. nov. *J. Phycol.* 50:908–  
789 29.
- 790 Salzburger, W., Ewing, G.B. & Von Haeseler, A. 2011. The performance of phylogenetic  
791 algorithms in estimating haplotype genealogies with migration. *Mol. Ecol.* 20:1952–  
792 1963.
- 793 Saunders, G.W. 1993. Gel purification of red algal genomic DNA: an inexpensive and  
794 rapid method for the isolation of polymerase-chain reaction-friendly DNA. *J. Phycol.*  
795 29:251–54.
- 796 Saunders, G.W. 2005. Applying DNA barcoding to red macroalgae: a preliminary appraisal  
797 holds promise for future applications. *Phil. Trans. R. Soc. B.* 360:1879–1888.
- 798 Schlotterer, C. 2002. A microsatellite-based multilocus screen for the identification of local  
799 selective sweeps. *Genetics* 160:753–763.
- 800 Schweikert, K., Sutherland, J.E., Burritt, D.J. & Hurd, C.L. 2012. Analysis of spatial and  
801 temporal diversity and distribution of *Porphyra* (Rhodophyta) in southeastern New  
802 Zealand supported by the use of molecular tools. *J. Phycol.* 48:530–38.
- 803 Sutherland, J.E., Lindstrom, S., Nelson, W., Brodie, J., Lynch, M., Hwang, M.S., Choi, H.  
804 G., Miyata, M., Kikuchi, N., Oliveira, M., Farr, T., Neefus, C., Mortensen, A.,  
805 Milstein, D. & Müller, K. 2011. A new look at an ancient order: generic revision of  
806 the Bangiales. *J. Phycol.* 47:1131–1151.
- 807 Tamura, K., Stecher, G., Peterson, D., FilipSKI, A. & Kumar, S. 2013. Molecular  
808 evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 30:2725–2729.
- 809 Teasdale, B., West, A., Taylor, H.E. & Klein, A.S. 2002. A simple Restriction Fragment  
810 Length Polymorphism (RFLP) Assay to discriminate common *Porphyra*  
811 (Bangioophyceae, Rhodophyta) taxa from the Northwest Atlantic. *J. Appl. Phycol.*  
812 38:293–298.
- 813 Tellier, F., Meynard, A.P., Correa, J.A., Faugeron, S. & Valero, M. 2009. Phylogeographic  
814 analysis of the 30°S south-east Pacific biogeographic transition zone establish the

- 815 occurrence of a sharp genetic discontinuity in the kelp *Lessonia nigrescens*:  
816 Vicariance or parapatry? *Mol. Phyl. Evol.* 53(3): 679-693.
- 817 Trifinopoulos, J., Nguyen, L.T., von Haeseler, A. & Minh, B.Q. 2016. W-IQ-TREE: a fast-  
818 online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Res.*  
819 44(W1): W232-5.
- 820 Vanellander, B., Creach, V., Vanormelingen, P., Ernst, A., Chepurinov, V. A., Sahan, E., ...  
821 & Sabbe, K. 2009. Ecological differentiation between sympatric pseudocryptic  
822 species in the estuarine benthic diatom *Navicula phyllepta* (Bacillariophyceae). *J.*  
823 *Phycol.* 45: 1278–1289.
- 824 West, A.L., Mathieson, A.C., Klein, A.S., Neefus, C.D. & Bray, T.L. 2005. Molecular  
825 ecological studies of New England species of *Porphyra* (Rhodophyta, Bangiales).  
826 *Nova Hedwigia* 80:1–24.
- 827 Yang, L.E., Zhou, W., Hu, C.M., Deng, Y.Y., Xu, G.P., Zhang, T., Russell, S., Zhu,  
828 J.Y., Lu, Q.Q. & Brodie, J. 2018. A molecular phylogeny of the bladed Bangiales  
829 (Rhodophyta) in China provides insights into biodiversity and biogeography of the  
830 genus *Pyropia*. *Mol. Phyl. Evol.* 120:94–102.
- 831 Zapata, J. 2016. Diferenciación ecológica entre ecotipos de dos especies hermanas de  
832 *Pyropia* (Bangiales, Rhodophyta) mediado por estrés ambiental. Tesis de grado en  
833 Magister en Biología Marina, Facultad de Ecología y Recursos Naturales,  
834 Universidad Andrés Bello.
- 835 Zuccarello, J. 2011. What are you eating? It may be nori, but it is probably not *Porphyra*  
836 anymore. *J. Phycol.* 47:967–8.
- 837
- 838
- 839
- 840
- 841
- 842
- 843
- 844
- 845

846 Table 1. Distribution of the five taxa of foliose Bangiales sampled in the site of Maitencillo  
 847 along different intertidal habitats: *Pyropia* sp. CHK, *Pyropia orbicularis*, *Porphyra* sp.  
 848 CHE, *Porphyra* sp. CHC and *Porphyra* sp. CHF. Species delimitation was based on  
 849 molecular criteria using sequences of *COI* (66 sequences) and *rbcL* (46 sequences).

INTERTIDAL HABITATS						
Species	Rocky Wall	High	Medium	Low	Boulders	Boulders
					(rocky pools)	(sandy pools)
<i>Pyropia</i> sp. CHK	15	13	0	0	4	0
<i>Pyropia orbicularis</i>	1	3	0	0	0	0
<i>Porphyra</i> sp. CHE	1	9	0	0	6	0
<i>Porphyra</i> sp. CHC	0	0	0	0	0	12
<i>Porphyra</i> sp. CHF	0	0	0	0	2	0

850

851

852 Table 2. AFA I cut sites (base position) among the variable bases of the aligned *rbcL*  
 853 sequences of *Pyropia* sp. CHK, *Pyropia orbicularis*, *Porphyra* sp. CHE and *Porphyra* sp.  
 854 CHC. A total of 873 sites were analyzed using the program Webcutter 2.0. We show base  
 855 positions going from the forward to the reverse primers and indicate as reference for base  
 856 position the *rbcL* sequence AB818919.1 of *Pyropia yezoensis* retrieved from Genbank. It is  
 857 worth mentioning that amplified fragments used for PCR-RFLP were larger (1230 bp) than  
 858 our obtained sequences (873 bp). See Materials and Methods for more details.

Base Position	582	765	996	1134
<i>Pyropia</i> sp. CHK	T	C	T	T
<i>Pyropia orbicularis</i>	T	C	T	A
<i>Porphyra</i> sp. CHE	C	T	A	A
<i>Porphyra</i> sp. CHC	T	T	A	A

Table 3. Nested Permanova carried out on the eight microscopic and two macroscopic morphological characters measured for four dominant sympatric *Pyropia* and *Porphyra* species. Null hypothesis: no morphological differences between species.

Source of Variation	df	Sums of Sqs	Mean Sqs	F. Model	R <sup>2</sup>	Pr(>F)
Species	3	1.10743	0.36914	1583.01	0.44084	0.001***
Species/Plant	88	1.23304	0.01401	60.09	0.49084	0.001***
Residuals	736	0.17163	0.00023		0.06832	
Total	827	2.51210			1.00000	

- 1 Table 4. Habits and morphological features of *Pyropia variabilis* sp. nov. (CHK), *Porphyra*  
 2 *luchensis* sp. nov. (CHE), *Porphyra longissima* sp. nov. (CHC), and *Pyropia orbicularis*  
 3 from Maitencillo beach, Valparaíso, Chile.

4

Feature	<i>Pyropia orbicularis</i>	<i>Pyropia variabilis</i>	<i>Porphyra luchensis</i>	<i>Porphyra longissima</i>
<b>Size blade</b> (cm, length × width)	2.8–5.5 x 2.4–5.3	2.8–14.0 x 1.1–7.0	5.4–22.0 x 0.5–9.0	16.0–50.0 x 0.3–3.5
<b>Shape</b>	Orbicular (Several laminae intertwined)	Oblong to lanceolate (one or several laminae)	Reniform to spear-shaped asymmetrical (One lamina)	Linear to lanceolate, very long (one lamina)
<b>Color</b>	Green-brown	Forestgreen to light-brown	Redish-brown to green	Rosy pinky to brown
<b>Habitat</b>	Upper-mid intertidal zone	Upper-mid intertidal zone and steep rock faces	Boulders surrounded by stony ground and rocky platforms	Boulders surrounded by sand
<b>Seasonality</b>	Year-round: More abundant in Spring and Summer	Year-round: More abundant in Winter and Spring	Year-round: More abundant in Winter and Spring	Present only in Winter
<b>Sexuality</b>	Monoecious	Monoecious	Monoecious	Monoecious
<b>Vegetative thickness</b> (µm)	68–128	63–137	68–156	38–122
<b>Reproductive thickness</b> (µm)	72–130	68–139	39–136	36–94
<b>Vegetative cells</b> (µm, length × width)	16–30 x 10–22	12–35 x 8–27	17–43 x 12–29	9–42 x 6–30
<b>Rhizoidal cells</b> (µm, length × width)	17–46 x 15–41	16–55 x 14–48	24–59 x 21–48	13–49 x 4–40
<b>Spermatangium</b>	a4/b4/c4	a4/b4/c8	a4/b4/c8	a4/b4/c8
<b>Zygotosporangium</b>	a2/b2/c4	a2/b2/c4 or a2/b4/c4	a2/b4/c8 or a4/b4/c8	a4/b4/c4

5

6



7

8

## FIGURE LEGENDS

9

10 Figure 1. Maximum likelihood (ML) trees of *Pyropia* and *Porphyra* using *Boreophyllum*  
11 *birdiae* and *Bangia fuscopurpurea* as outgroups, respectively, and based on DNA  
12 sequences of the cytochrome oxidase I (*COI*) gene. a) Details of the *Pyropia COI* ML tree  
13 (outgroup not shown). b) Details of the *Porphyra COI* ML tree (outgroup not shown). For  
14 each node, ML bootstrap values are indicated. Only high support values (>80) are shown.  
15 For species for which two or more sequences were considered in the analyses branches  
16 have been collapsed for easier reading and are represented as triangles. Black triangles  
17 indicate species present at the study site, Maitencillo Beach.

18

19 Figure 2. Habit of the four dominant genetic species of foliose Bangiales observed at  
20 Maitencillo beach. Scale bar = 10 cm. For taxonomical characteristics see Table 4 and  
21 Results section. A) *Porphyra* sp. CHE, B) *Porphyra* sp. CHC, C) *Pyropia orbicularis*, D)  
22 *Pyropia* sp. CHK LM (Long Morph), E) *Pyropia* sp. CHK GM (Green Morph).

23

24 Figure 3. PCR-RFLP profiles resulting from the digestion of the 1230 bp *rbcL*  
25 amplification fragment by the enzyme AFA I. Restriction pattern observed for the four  
26 dominant genetic species found at the intertidal in Maitencillo Beach are shown. Legend for  
27 each lane corresponds to: Marker, molecular weight marker (bp: base pairs); *Po.* CHC =  
28 *Porphyra* sp. CHC; *Po.* CHE = *Porphyra* sp. CHE, *Py.* CHK = *Pyropia* sp. CHK; and *Py.*  
29 *orb* = *Pyropia orbicularis*. PD: primer dimer products.

30 Figure 4. PCA plot carried out for two microscopic and two macroscopic traits (i.e.  
31 normalized data after Box-Cox transformation) measured in specimens of the four-  
32 dominant foliose Bangiales observed at Maitencillo beach. Points correspond to scores for  
33 each specimen (gametophyte) in the coordinates of the principal components PC1 and PC2,  
34 and ellipses indicate the 95% confidence intervals of the scores for each species. Green  
35 vectors are projections of the original variables into the new axes of the principal  
36 components. The greatest contribution to PC1 is from the thallus length and thallus width  
37 and allows separating *Porphyra* sp. CHC (with the longest and thinnest blades) from the  
38 other three species. Red - *Porphyra* sp. CHC; pink - *Porphyra* sp. CHE; green - *Pyropia* sp.  
39 CHK and brown - *Pyropia orbicularis*.

40

41 Figure 5. Boxplot of the sizes of two out of eight microscopic traits (all in Log  $\mu\text{m}$ ) and two  
42 out of two macroscopic traits (in Log cm) measured in the four-dominant foliose Bangiales  
43 species observed at Maitencillo beach. Different letters indicate statistically significant  
44 differences between species in Tukey multiple comparisons test. *Po.* CHC = *Porphyra* sp.  
45 CHC; *Po.* CHE = *Porphyra* sp. CHE; *Py.* CHK = *Pyropia* sp. CHK; and *Py. orb.* = *Pyropia*  
46 *orbicularis*.

47

48 Figure 6. Images of macro and micromorphology of *Porphyra luchensis* sp. nov.,  
49 SGO168338, holotype, Museo Nacional de Historia Natural, Santiago, Chile (Table S1). A)  
50 Habit of the foliose gametophyte sampled from the intertidal zone in Maitencillo beach,  
51 Valparaíso, Chile (scale bar = 10 cm). B) Surface view of basal, rhizoidal cells. C) Surface  
52 view of vegetative region of the thallus. D) Cross-section of vegetative region of thallus. E)

53 Surface view of zygotosporangia. F) Cross-section of zygotosporangial region of thallus.  
54 G) Surface view of spermatangia (smaller and colourless). H) Cross-section of  
55 spermatangial region of thallus. Scale bar B-H = 20  $\mu$ m.

56

57

58 Figure 7. Images of macro and micromorphology of *Porphyra longissima* sp. nov.,  
59 SGO168348, holotype, Museo Nacional de Historia Natural, Santiago, Chile (Table S1). A)  
60 Habit of the foliose gametophyte sampled from the intertidal zone in Maitencillo beach,  
61 Valparaíso, Chile (scale bar = 10 cm). B) Surface view of basal, rhizoidal cells. C) Surface  
62 view of vegetative region of the thallus. D) Cross-section of vegetative region of thallus. E)  
63 Surface view of zygotosporangia. F) Cross-section of zygotosporangial region of thallus.  
64 G) Surface view of spermatangia (smaller and colourless). H) Cross-section of  
65 spermatangial region of thallus. Scale bar B-H = 20  $\mu$ m.

66

67

68 Figure 8. Images of macro and micromorphology of *Pyropia variabilis* sp. nov. (CHK)  
69 Green Morph (GM), SGO168334, isotype, Museo Nacional de Historia Natural, Santiago,  
70 Chile (Table S1). A) Habit of the foliose gametophyte sampled from the intertidal zone in  
71 Maitencillo beach, Valparaíso, Chile (scale bar = 10 cm). B) Surface view of basal,  
72 rhizoidal cells. C) Surface view of vegetative region of the thallus. D) Cross-section of  
73 vegetative region of thallus. E) Surface view of zygotosporangia. F) Cross-section of  
74 zygotosporangial region of thallus. G) Surface view of spermatangia (smaller and  
75 colourless). H) Cross-section of spermatangial region of thallus. Scale bar B-H = 20  $\mu$ m.

76

77 Figure 9. Images of macro and micromorphology of *Pyropia variabilis* sp. nov. (CHK)  
78 Long Morph (LM), SGO168333, holotype, Museo Nacional de Historia Natural, Santiago,  
79 Chile (Table S1). A) Habit of the foliose gametophyte sampled from the intertidal zone in  
80 Maitencillo beach, Valparaíso, Chile (scale bar = 10 cm). B) Surface view of basal,  
81 rhizoidal cells. C) Surface view of vegetative region of the thallus. D) Cross-section of  
82 vegetative region of thallus. E) Surface view of zygotosporangia. F) Cross-section of  
83 zygotosporangial region of thallus. G) Surface view of spermatangia (smaller and  
84 colourless). H) Cross-section of spermatangial region of thallus. Scale bar B-H = 20  $\mu\text{m}$ .

85

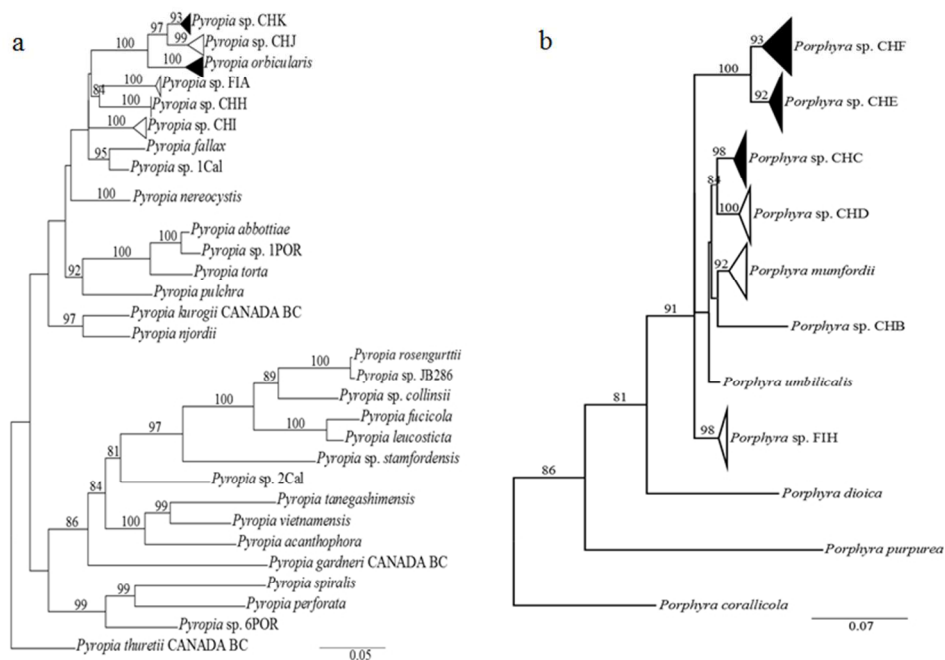


Figure 1. Maximum likelihood (ML) trees of *Pyropia* and *Porphyra* using *Boreophyllum birdiae* and *Bangia fuscopurpurea* as outgroups, respectively, and based on DNA sequences of the cytochrome oxidase I (COI) gene. a) Details of the *Pyropia* COI ML tree (outgroup not shown). b) Details of the *Porphyra* COI ML tree (outgroup not shown). For each node, ML bootstrap values are indicated. Only high support values (>80) are shown. For species for which two or more sequences were considered in the analyses branches have been collapsed for easier reading and are represented as triangles. Black triangles indicate species present at the study site, Maitencillo Beach.

231x163mm (96 x 96 DPI)

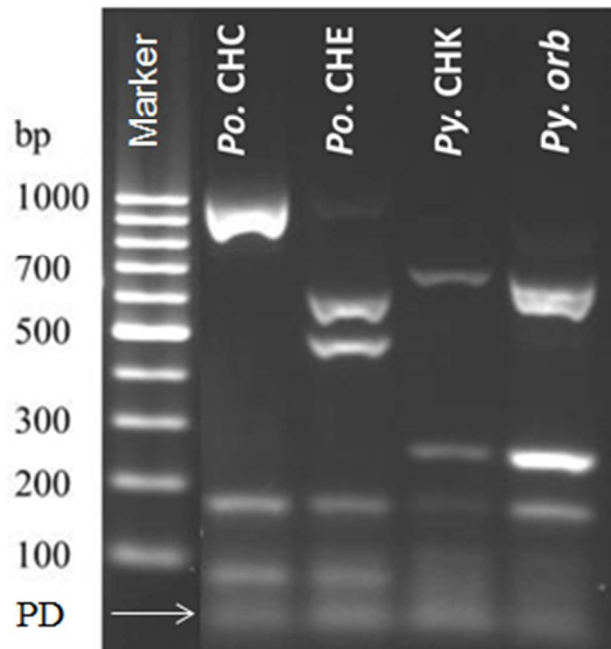
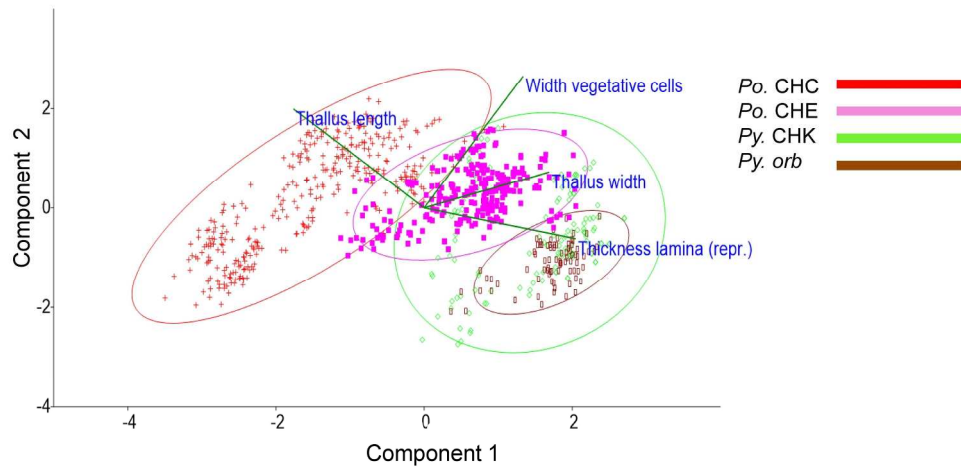


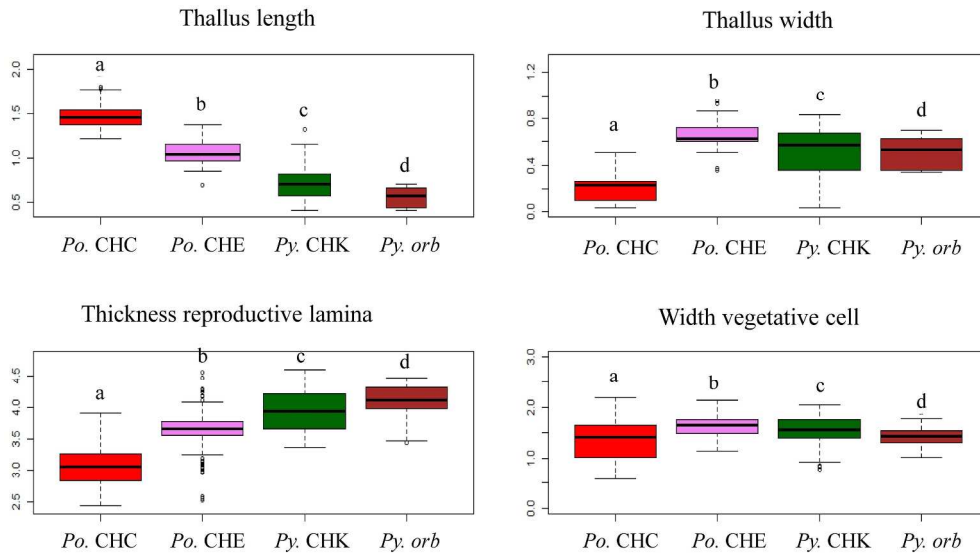
Figure 3. PCR-RFLP profiles resulting from the digestion of the 1230 bp *rbcl* amplification fragment by the enzyme AFA I. Restriction pattern observed for the four dominant genetic species found at the intertidal in Maitencillo Beach are shown. Legend for each lane corresponds to: Marker, molecular weight marker (bp: base pairs); Po. CHC = *Porphyra* sp. CHC; Po. CHE = *Porphyra* sp. CHE, Py. CHK = *Pyropia* sp. CHK; and Py. orb = *Pyropia orbicularis*. PD: primer dimer products.

84x90mm (96 x 96 DPI)



PCA plot carried out for two microscopic and two macroscopic traits (i.e. normalized data after Box-Cox transformation) measured in specimens of the four-dominant foliose Bangiales observed at Maitencillo beach. Points correspond to scores for each specimen (gametophyte) in the coordinates of the principal components PC1 and PC2, and ellipses indicate the 95% confidence intervals of the scores for each species. Green vectors are projections of the original variables into the new axes of the principal components. The greatest contribution to PC1 is from the thallus length and thallus width and allows separating *Porphyra* sp. CHC (with the longest and thinnest blades) from the other three species. Red - *Porphyra* sp. CHC; pink - *Porphyra* sp. CHE; green - *Pyropia* sp. CHK and brown - *Pyropia orbicularis*.

108x53mm (600 x 600 DPI)



Boxplot of the sizes of two out of eight microscopic traits (all in Log  $\mu\text{m}$ ) and two out of two macroscopic traits (in Log cm) measured in the four-dominant foliose Bangiales species observed at Maitencillo beach. Different letters indicate statistically significant differences between species in Tukey multiple comparisons test. *Po. CHC* = *Porphyra* sp. CHC; *Po. CHE* = *Porphyra* sp. CHE; *Py. CHK* = *Pyropia* sp. CHK; and *Py. orb.* = *Pyropia orbicularis*.

134x75mm (600 x 600 DPI)



Figure S1. Study site (map is given on the right) and google earth image of the Maitencillo beach including the locations of the intertidal habitats sampled during this study (given on the left). The three central lines, perpendicular to the beach, represent three sampling transects on the rocky platform. Colors correspond to the three intertidal zones sampled (for more details on zone delimitation see Materials and Methods): lower intertidal (green), middle intertidal (purple) and upper intertidal (red). The circle indicates the steep wall, the rhombus indicates the boulder zone surrounded by rocky pools, and the triangle indicates the boulder zone surrounded by pools of sandy bottoms (all are in colored in red since they were located in the upper intertidal zone). Scale bar, in white, 100 m.

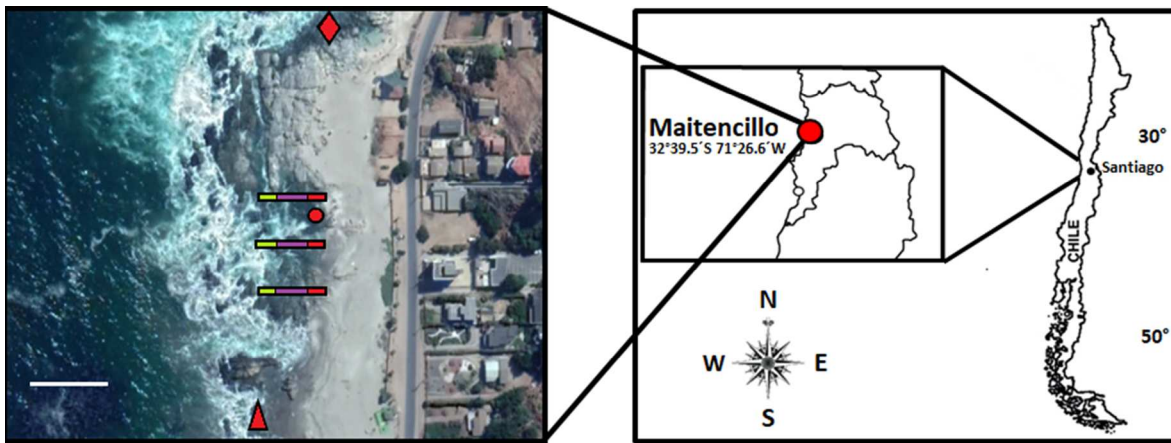
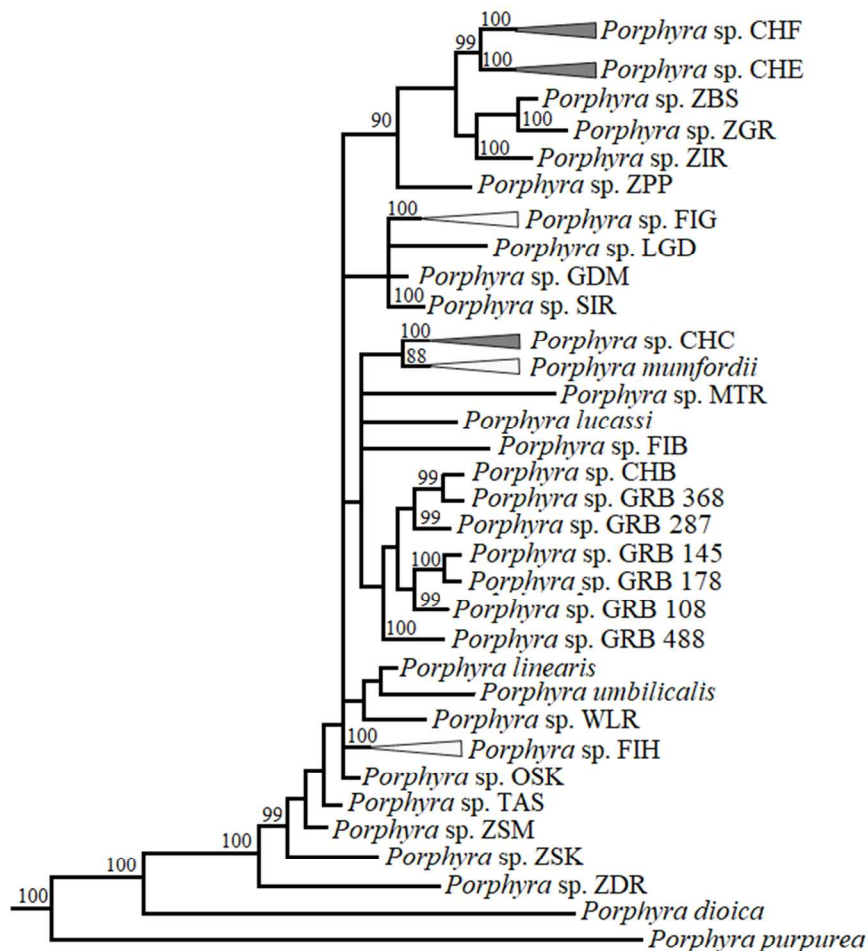


Figure S2. Details of the Maximum Likelihood (ML) rooted tree for *rbcL* sequences (873 bp) of Bangiales. The complete tree is not shown but is similar to the one presented in Fig. 1 in Guillemain et al. (2016), save for some difference in branch support. a) Details of the *Porphyra* clade of the Bangiales *rbcL* ML rooted tree. b) Details of the *Pyropia* clade of the Bangiales *rbcL* ML rooted tree. For each node, ML bootstrap values are indicated. Only high support values (>80) are shown. For species for which two or more sequences were considered in the analyses branches have been collapsed for easier reading and are represented as triangles. Grey triangles indicate species present at the study site, Maitencillo Beach.

**a**



b

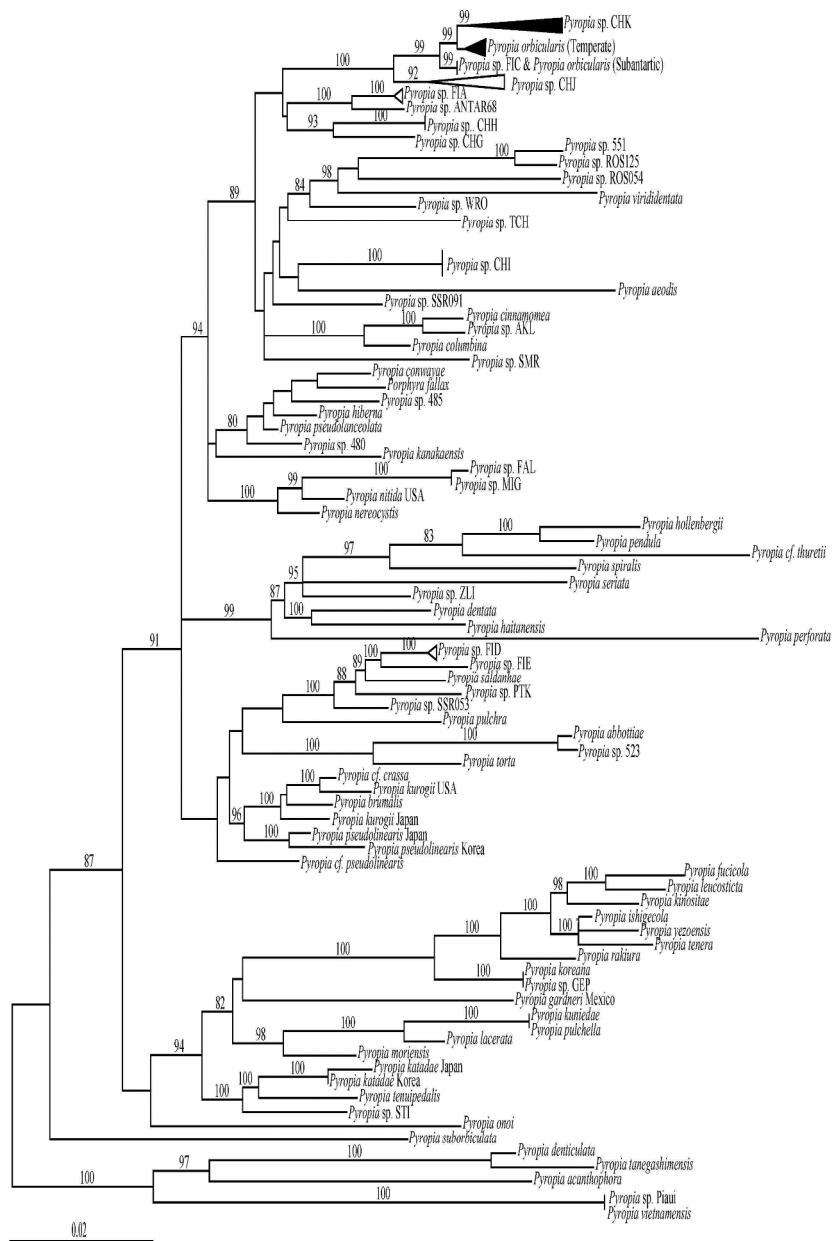


Figure S3: Haplotype network reconstructed using the *COI* data set for the *Pyropia orbicularis*, *Pyropia* sp. CHJ and *Pyropia* sp. CHK species complex. Each circle represents one haplotype and its size is proportional to the frequency in which the haplotype was encountered. White represents sequences from previous studies already deposited in GenBank and black the sequences acquired during this study. Non-interrupted blue lines represent one mutation step. When more than one mutational step separated two haplotypes, the number of segments (i.e. blue line disrupted by blue dots) indicated the number of steps.

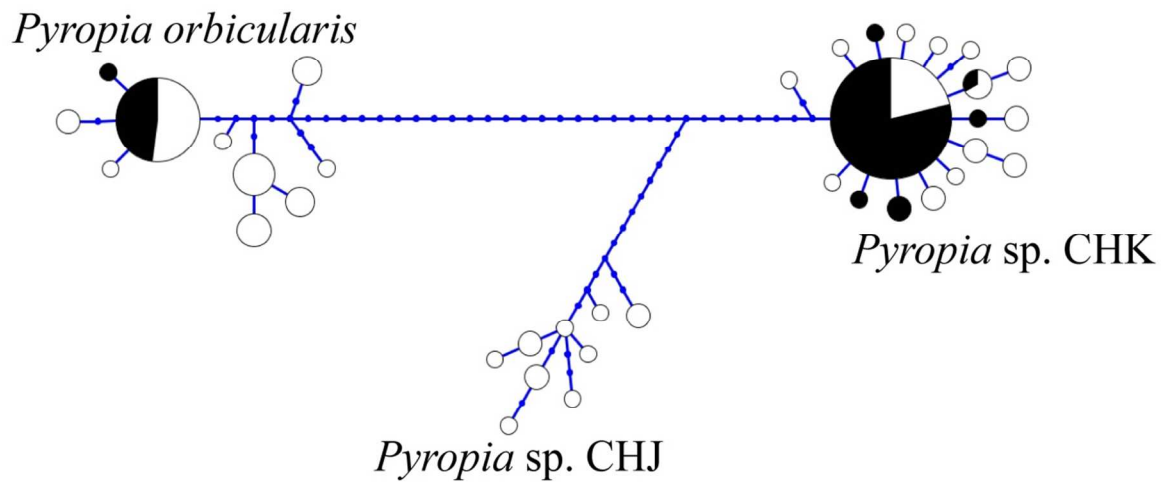




Figure S4. Images of macro and micromorphology of *Pyropia orbicularis* Ramírez, Contreras-Porcía & Guillemin. A) Habit of the foliose gametophyte sampled from the intertidal zone in Maitencillo beach, Valparaíso, Chile (scale bar= 10 cm). B) Surface view of basal, rhizoidal cells. C) Surface view of vegetative region of the thallus. D) Cross-section of vegetative region of thallus. E) Surface view of zygotosporangia. F) Cross-section of zygotosporangial region of thallus. G) Surface view of spermatangia (smaller and colourless). H) Cross-section of spermatangial region of thallus. Scale bar B-H = 20  $\mu$ m.

*Pyropia orbicularis*

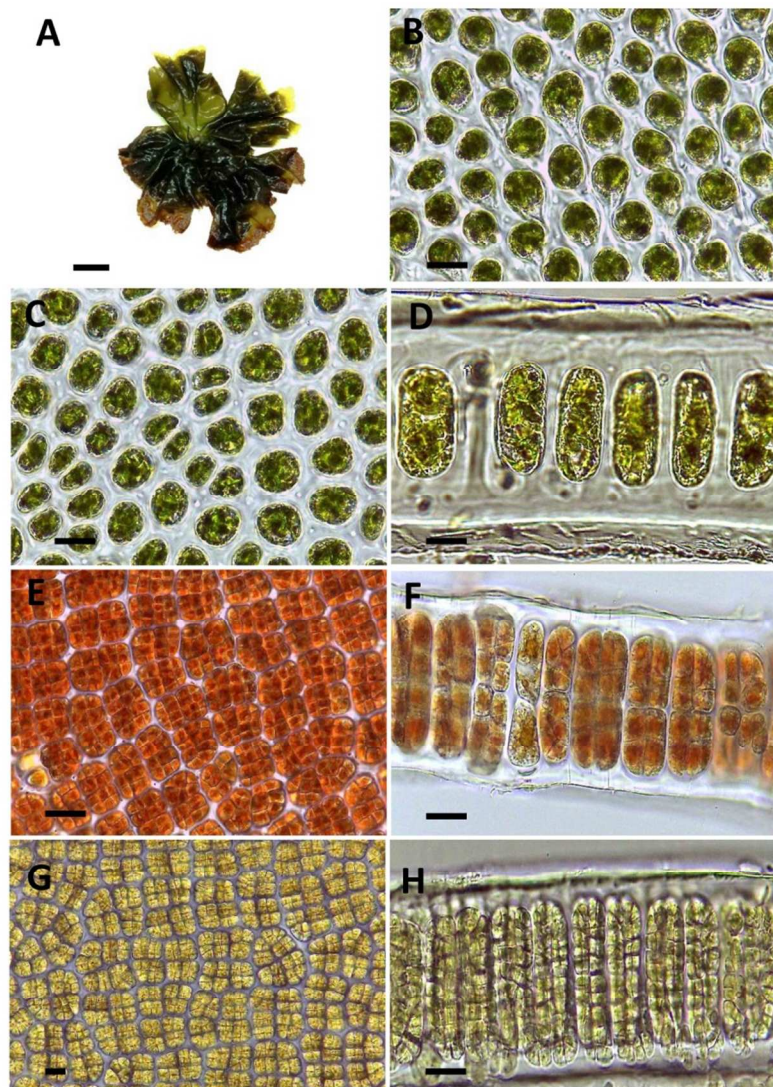


Table S1. Specimen collection information, voucher numbers and GENBANK accession numbers of *Pyropia* sp. CHK, *Porphyra* sp. CHC, *Porphyra* sp. CHE and *Pyropia orbicularis* from Maitencillo beach, Valparaíso, Chile, sequenced during this work.

Collection data	Sample code/ voucher number	GENBANK accession number	
		<i>COI</i>	<i>rbcL</i>
12/08/2014. Collector: J. Zapata, L.Contreras-Porcia	CHC0001/ SGO168341	MH123922	MH124012
12/08/2014. Collector: J. Zapata, L.Contreras-Porcia	CHC0002/ SGO168342	MH123923	MH124013
12/08/2014. Collector: J. Zapata, L.Contreras-Porcia	CHC0003/ SGO168343	MH123924	MH124014
12/08/2014. Collector: J. Zapata, L.Contreras-Porcia	CHC0004/ SGO168344	MH123925	MH124015
12/08/2014. Collector: J. Zapata, L.Contreras-Porcia	CHC0005/ SGO168345	MH123926	MH124016
12/08/2014. Collector: J. Zapata, L.Contreras-Porcia	CHC0006	MH123927	MH124017
12/08/2014. Collector: J. Zapata, L.Contreras-Porcia	CHC0007/ SGO168346	MH123928	MH124018
12/08/2014. Collector: J. Zapata, L.Contreras-Porcia	CHC0008/ SGO168347	MH123929	MH124019
12/08/2014. Collector: J. Zapata, L.Contreras-Porcia	CHC0009	MH123930	MH124020
12/08/2014. Collector: J. Zapata, L.Contreras-Porcia	CHC0010/ SGO168348	MH123931	MH124021
12/08/2014. Collector: J. Zapata, L.Contreras-Porcia	CHC0011/SGO168349	MH123932	MH124022

12/08/2014. Collector: J. Zapata, L.Contreras-Porcia	CHC0012/ SGO168350	MH123933	MH124023
08/09/2014. Collector: J. Zapata, L.Contreras-Porcia	CHE0001	MH123934	MH124024
08/09/2014. Collector: J. Zapata, L.Contreras-Porcia	CHE0002	MH123935	MH124025
08/09/2014. Collector: J. Zapata, L.Contreras-Porcia	CHE0004	MH123936	MH124026
08/09/2014. Collector: J. Zapata, L.Contreras-Porcia	CHE0005	MH123937	MH124027
08/09/2014. Collector: J. Zapata, L.Contreras-Porcia	CHE0007	MH123938	-
08/09/2014. Collector: J. Zapata, L.Contreras-Porcia	CHE0008	MH123939	MH124028
08/09/2014. Collector: J. Zapata, L.Contreras-Porcia	CHE0012	MH123940	MH124029
08/09/2014. Collector: J. Zapata, L.Contreras-Porcia	CHE0019	MH123941	-
08/09/2014. Collector: J. Zapata, L.Contreras-Porcia	CHE0031	MH123942	-
08/09/2014. Collector: J. Zapata, Francisco Castañeda	CHE0020	MH123943	-
08/09/2014. Collector: J. Zapata, Francisco Castañeda	CHE0015/SGO168337	MH123944	MH124030
10/10/2013. Collector: J. Zapata, L.Contreras-Porcia	CHE0027/SGO168338	MH123945	MH124031
08/09/2014. Collector: C.Fierro,	CHE0029/SGO168339	MH123946	MH124032



F.Castañeda			
08/09/2014. Collector: C.Fierro,	CHE0030/SGO168340	MH123947	-
F.Castañeda			
08/09/2014. Collector: J. Zapata,	CHE0036	MH123948	-
L.Contreras-Porcía			
08/09/2014. Collector: J. Zapata,	CHE0035	MH123949	MH124033
L.Contreras-Porcía			
08/09/2014. Collector: J. Zapata,	CHF0003	MH123950	MH124034
L.Contreras-Porcía			
08/09/2014. Collector: J. Zapata,	CHF0010	MH123951	MH124035
L.Contreras-Porcía			
08/09/2014Collector: J. Zapata,	CHK006p	MH123953	MH124037
L.Contreras-Porcía			
08/09/2014Collector: J. Zapata,	CHK009p	MH123954	MH124038
L.Contreras-Porcía			
08/09/2014Collector: J. Zapata,	CHK0011p	MH123952	MH124040
L.Contreras-Porcía			
28/07/2014. Collector: J. Zapata,	CHK0001/SGO168324	MH123955	-
L.Contreras-Porcía			
28/07/2014. Collector: J. Zapata,	CHK0002/SGO168325	MH123956	MH124041
L.Contreras-Porcía			
28/07/2014. Collector: J. Zapata,	CHK0003/SGO168326	MH123957	-
L.Contreras-Porcía			
28/07/2014. Collector: J. Zapata,	CHK0005	MH123958	-
L.Contreras-Porcía			
28/07/2014. Collector: J. Zapata,	CHK005b	MH123959	-
L.Contreras-Porcía			

28/07/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK0006	MH123960	-
28/07/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK006b	MH123961	MH124043
28/07/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK007b	MH123962	-
28/07/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK0008	MH123963	-
28/07/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK0009/SGO168327	MH123964	MH124042
28/07/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK0010	MH123965	MH124044
28/07/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK0012	MH123966	MH124055
28/07/2014. Collector: C.Lovazzano, F.Castañeda	CHK0013/SGO168328	MH123967	MH124045
28/07/2014. Collector: C.Lovazzano, F.Castañeda	CHK0014/SGO168329	MH123968	MH124046
28/07/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK0017/SGO168330	MH123969	MH124047
28/07/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK0018/SGO168331	MH123970	MH124057
28/07/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK0021	MH123971	MH124036
28/07/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK0022	MH123972	MH124039
28/07/2014.. Collector: J. Zapata,	CHK0023	MH123973	-

L.Contreras-Porcia			
28/07/2014. Collector: J. Zapata,	CHK0024/SGO168332	MH123974	MH124049
L.Contreras-Porcia			
06/09/2013. Collector: J. Zapata,	CHK0025/SGO168333	MH123975	MH124050
F.Castañeda			
28/07/2014. Collector: J. Zapata,	CHK0026	MH123976	-
L.Contreras-Porcia			
28/07/2014. Collector: J. Zapata,	CHK026b	MH123977	-
L.Contreras-Porcia			
10/10/2013. Collector: C.Fierro-	CHK0028/SGO168334	MH123978	MH124051
F.Castañeda			
28/07/2014. Collector: J. Zapata,	CHK0032	MH123979	MH124056
L.Contreras-Porcia			
28/07/2014. Collector: J. Zapata,	CHK0033	MH123980	MH124052
L.Contreras-Porcia			
28/07/2014. Collector: J. Zapata,	CHK0034	MH123981	MH124053
L.Contreras-Porcia			
28/07/2014. Collector: J. Zapata,	CHK0037/SGO168335	MH123982	MH124054
L.Contreras-Porcia			
28/07/2014. Collector:	CHK0040/SGO168336	MH123983	MH124048
C.Lovazzano- F.Castañeda			
28/07/2014. Collector: J. Zapata,	CHK036o	MH123984	-
L.Contreras-Porcia			
28/07/2014. Collector: J. Zapata,	CHK038o	MH123985	-
L.Contreras-Porcia			
28/07/2014. Collector: J. Zapata,	CHK044o	MH123986	-
L.Contreras-Porcia			

28/07/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK050o	MH123987	-
28/07/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK055o	MH123988	-
28/07/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK015v	MH123989	-
28/07/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK030L	MH123990	-
28/09/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK035o	MH123991	-
28/09/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK039o	MH123992	-
28/09/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK071o	MH123993	-
28/09/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK106o	MH123994	-
28/09/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK024v	MH123995	-
28/09/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK033v	MH123996	-
28/09/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK037L	MH123997	-
28/09/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK105o	MH123998	-
02/01/2014. Collector: J. Zapata, L.Contreras-Porcia	ORB0004	MH123999	MH124058
02/12/2013. Collector: J. Zapata,	ORB0011/SGO168323	MH124000	-

L.Contreras-Porcia			
02/01/2014. Collector: J. Zapata,	ORB0015	MH124001	-
L.Contreras-Porcia			
02/01/2014. Collector: J. Zapata,	ORB0016	MH124002	-
L.Contreras-Porcia			
28/09/2014. Collector: J. Zapata,	ORB0046	MH124003	-
L.Contreras-Porcia			
28/09/2014. Collector: J. Zapata,	ORB0061	MH124004	-
L.Contreras-Porcia			
28/09/2014. Collector: J. Zapata,	ORB0062	MH124005	-
L.Contreras-Porcia			
28/09/2014. Collector: J. Zapata,	ORB0064	MH124006	-
L.Contreras-Porcia			
28/09/2014. Collector: J. Zapata,	ORB0066	MH124007	-
L.Contreras-Porcia			
28/09/2014. Collector: J. Zapata,	ORB0063	MH124008	-
L.Contreras-Porcia			
28/09/2014. Collector: J. Zapata,	ORB0052	MH124009	-
L.Contreras-Porcia			
28/09/2014. Collector: J. Zapata,	ORB0075	MH124010	-
L.Contreras-Porcia			
28/09/2014. Collector: J. Zapata,	ORB0072	MH124011	-
L.Contreras-Porcia			

Table S2. Specimen collection information and GENBANK accession numbers of sequences of COI and *rbcl* used in Maximum Likelihood tree reconstruction to complement our data set (see Table S1). Specimens are in alphabetical order.

Taxon	Collection location	COI - GENBANK Accession no.
<i>Bangia atropurpurea</i>	Devon, Sidmouth, UK	DQ442886
<i>Bangia atropurpurea</i>	Dorset, Kimmeridge, UK	DQ442887
<i>Bangia fuscopurpurea</i>	Rhode Island, Brenton Point., Newport, USA	JN028460
<i>Bangia</i> sp. 1BAN	British Columbia, Whiffen Spit, Sooke Harbour, Vancouver Island, Canada	JN028465
<i>Bangia</i> sp. 2BAN	Quebec, Escoumins (Rue des Pilotes), Canada	JN028476
<i>Bangiales</i> sp. HK-2011a	British Columbia, Ramsey Island (beach on NW coast), Gwaii Haanas, Canada	JN028481
<i>Bangiales</i> sp. HK-2011c	British Columbia, Ridley Island, Prince Rupert, Canada	JN028495
<i>Boreophyllum birdiae</i>	Newfoundland and Labrador, from Bonne Bay Station, Canada	JN028485
<i>Fuscofolium</i> sp. CHA	Región de Coquimbo, Choapa, Puerto Oscuro	KP781631
<i>Porphyra corallicola</i>	New Brunswick, Maces Bay, Bay of Fundy, Canada	JN028496
<i>Porphyra dioica</i>	Talmine, Sutherland, Scotland, UK	DQ191339
<i>Porphyra</i> sp. JB286	Sussex, Bracklesham Bay, England, UK	DQ191335
<i>Porphyra mumfordii</i>	British Columbia, Bamfield, Bradys Beach, Canada	JN028502
<i>Porphyra mumfordi</i>	Región de Los Lagos Hueihue, Chiloé, Chile	KP781651
<i>Porphyra mumfordi</i>	Región de Los Ríos, Los Molinos, Valdivia, Chile	KP781682
<i>Porphyra purpurea</i>	New Brunswick, Richebucto Cape Breakwater, Canada	JN028518
<i>Porphyra rosenгурttii</i>	Faroe Islands	AM943399
<i>Porphyra</i> sp. 1FIH	Chacao, Chiloe, Chile	JN028551.1
<i>Porphyra</i> sp. CHB	Región de Los Lagos, Cucao, Chiloé	KP781663
<i>Porphyra</i> sp. CHC	Región de Los Ríos, Playa Rosada, Valdivia, Chile	KP781684
<i>Porphyra</i> sp. CHC	Región de Valparaíso, Petorca, Salinas de Pullay	KP781638/ KP781639
<i>Porphyra</i> sp. CHC	Región de la Araucanía, Cautin, Cheuque	KP781643
<i>Porphyra</i> sp. CHC	Región de Los Ríos, Pichicuyin, Valdivia, Chile	KP781644/ KP781645
<i>Porphyra</i> sp. CHD	Región de Magallanes, Magallanes, Buque Quemado	KP781673
<i>Porphyra</i> sp. CHF	Región de Antofagasta, Antofagasta, Playa El Lenguado	KP781562/ KP781563
<i>Porphyra</i> sp. CHF	Región de Coquimbo, Elqui, Guanaquerillos	KP781559/ KP781564/ KP781565
<i>Porphyra</i> sp. CHF	Región de Coquimbo, Caleta las Conchas, Choapa, Chile	KP781560
<i>Porphyra</i> sp. CHF	Región de Valparaíso, Quintay-Playa Chica, Valparaíso, Chile	KP781567
<i>Porphyra</i> sp. CHF	Región de Valparaíso, Playa Amarilla, Valparaíso, Chile	KP781689/ KP781647
<i>Porphyra</i> sp. CHF	Región de Valparaíso, Valparaíso, Curaumilla, Chile	KP781674/ KP781675/ KP781691/ KP781649
<i>Porphyra</i> sp. CHF	Región de Valparaíso, Valparaíso, Playa El Encanto, Chile	KP781690/ KP781692/ KP781693
<i>Porphyra</i> sp. CHF	Región de Valparaíso, San Antonio, Punta de Tralca, Chile	KP781568/ KP781569/ KP781570/ KP781571/ KP781572
<i>Porphyra</i> sp. CHF	Región de Valparaíso, San Antonio, Las Cruces, Chile	KP781573/

<i>Porphyra</i> sp. CHF	Región del Biobío, Concepción, Coliumo, Chile	KP781574 KP781584/ KP781585
<i>Porphyra</i> sp. CHF	Región del Libertador General Bernardo O'Higgins, Cardenal Caro, Pichilemu, Chile	KP781575
<i>Porphyra</i> sp. CHF	Región del Libertador General Bernardo O'Higgins, Cardenal Caro, La Boca, Chile	KP781677/ KP781694/ KP781678/ KP781695/ KP781680
<i>Porphyra</i> sp. CHF	Región de Valparaíso, Valparaíso, Playa El Encanto; Chile	KP781693
<i>Porphyra</i> sp. FIH	Región de Los Lagos, Llanquihue, Metri, Chile	KP781592/ KP781593/ KP781594/ KP781595/ KP781596
<i>Porphyra</i> sp. FIH	Región de Los Lagos, Llanquihue, Carelmapu, Chile	KP781597
<i>Porphyra</i> sp. FIH	Puerto Montt, Pargua, Punta Corona, Chile	KP781650/ KP781646
<i>Porphyra</i> sp. FIH	Región de Los Lagos, Chiloé, Ancud, Fátima, Chile	KP781653
<i>Porphyra</i> sp. FIH	Región de Los Lagos, Chiloé, Ancud-Arena Gruesa, Chile	KP781654
<i>Porphyra</i> sp. FIH	Región de Los Lagos, Chiloé, Puñihuil, Chile	KP781655/ KP781656
<i>Porphyra</i> sp. FIH	Región de Los Lagos, Chiloé, Quemchi, Chile	KP781657/ KP781658/ KP781659/ KP781660/ KP781661
<i>Porphyra</i> sp. FIH	Región de Los Ríos, Valdivia, Playa Rosada, Chile	KP781686/ KP781688
<i>Porphyra</i> sp. FIH	Región de Magallanes, Magallanes, Seno Otway-Punta Canelo, Chile	KP781670/ KP781671
<i>Porphyra</i> sp. FIH	Región de Magallanes, Magallanes, Seno Skyring, Rio Verde, Chile	KP781672
<i>Porphyra</i> sp. FIH	Región de Los Ríos, Valdivia, Isla Mancera, Chile	KP781696/ KP7816797 KP781681
<i>Porphyra</i> sp. FIH	Región de la Araucanía, Cautin, Cheuque, Chile	KP781641/ KP781642
<i>Porphyra</i> sp. HK-2011c	Rhode island, Jamestown, USA	HM917381.1
<i>Porphyra</i> sp. HK-2011d	Rhode island, Narragansett, USA	JN028798.1
<i>Porphyra umbilicalis</i>	Newfoundland and Labrador, St. Brides, Canada	JN028569
<i>Pyropia abbottiae</i>	British Columbia, Ridley Island, Canada	JN028597
<i>Pyropia acanthophora</i>	Praia da Lagoinha, Ubatuba, Sao Paulo, Brazil	JN222750
<i>Pyropia fallax</i>	British Columbia, Murchison Island Lagoon, Gwaii Haanas, Canada	HQ969862
<i>Pyropia fucicola</i>	British Columbia, Island #40 on Esperanza Inlet Chart, Tahsis, Canada	JN028614
<i>Pyropia gardneri</i>	British Columbia, Chaatl Island across from Newton Point, Haida Gwaii, Canada	HM915300
<i>Pyropia kurogii</i>	British Columbia, Ridley Island, Prince Rupert, Canada	JN028655
<i>Pyropia leucosticta</i>	New Brunswick, Lepreau exposed biodiversity site, Bay of Fundy, Canada	JN028662
<i>Pyropia nereocystis</i>	British Columbia, Cape Beale, exposed front, Bamfield, Canada	JN028685
<i>Pyropia nereocystis</i>	British Columbia, Graham Island, Canada	HQ919419.1
<i>Pyropia njordii</i>	Nova Scotia, Peggys Cove, Canada	JN028693
<i>Pyropia njordii</i>	Quebec, Les Escoumins, Canada	JN028690.1
<i>Pyropia orbicularis</i>	Región de Valparaíso, Petorca, Salinas de Pullay, Chile	KP781637/

<i>Pyropia orbicularis</i>	Región de Valparaíso, Maitencillo, Chile	KP781640 KF479515/ KF479507/ KF479516/ KF479502/ KF479503/ KF479504/ KF479505/ KF479506/ KF479508/ KF479509/ KF479512/ KF479513/ KF479514
<i>Pyropia orbicularis</i>	Región del Maule, Duao, Curico, Chile	KP781576/ KP781577/ KP781578/ KP781579
<i>Pyropia orbicularis</i>	Región del Maule, Constitución, Talca, Chile	KP781580/ KP781581/ KP781582/ KP781583
<i>Pyropia orbicularis</i>	Región del Biobío, Lota, Concepción, Chile	KP781587
<i>Pyropia orbicularis</i>	Región de Los Ríos, Calfuco, Valdivia, Chile	KP781589
<i>Pyropia orbicularis</i>	Región de Los Ríos, Niebla, Valdivia, Chile	KP781591
<i>Pyropia orbicularis</i>	Región de Los Lagos, Chonchi, Chiloé, Chile	KP781602
<i>Pyropia orbicularis</i>	Región de Los Lagos, Llanquihue, Puerto Montt-Estaquilla	KP781652
<i>Pyropia orbicularis</i>	Región de Los Ríos, Valdivia, Playa Rosada	KP781685/ KP781697
<i>Pyropia orbicularis</i>	Región de Magallanes, Magallanes, Fuerte Bulnes	KP781665/ KP781666/ KP781667
<i>Pyropia perforata</i>	California, Montara Beach, USA	HQ919270.1
<i>Pyropia</i> sp. 1Cal	California, Jade Cove, USA	JN028786
<i>Pyropia</i> sp. 1POR	British Columbia, Whiffen Spit, SookeHarbour, Vancouver Island, Canada	JN028790
<i>Pyropia</i> sp. 2Cal	California, McAbee Beach, Monterey, USA	JN028791
<i>Pyropia</i> sp. 6POR	Texas, South Jetty, Port Arkansas, USA	JN028792
<i>Pyropia</i> sp. CHH	Región de Los Ríos, Valdivia, Niebla (Playa Grande)	KP781676
<i>Pyropia</i> sp. CHI	Región de Arica y Parinacota, Arica, Playa Corazón, Chile	KP781561
<i>Pyropia</i> sp. CHI	Región de Coquimbo, Elqui, Abalonera Chica, Chile	KP781620/ KP781621/ KP781622
<i>Pyropia</i> sp. CHJ	Región del Biobío, Coliumo, Concepción, Chile	KP781586
<i>Pyropia</i> sp. CHJ	Concepción, Lebu, Chile	KP781588
<i>Pyropia</i> sp. CHJ	Región de Los Ríos, Valdivia, Niebla, Chile	KP781590
<i>Pyropia</i> sp. CHJ	Región de Los Ríos, Valdivia, Niebla (Playa Grande), Chile	KP781687
<i>Pyropia</i> sp. CHJ	Región de Los Lagos, Chiloé, Playa Mar Brava, Chile	KP781600
<i>Pyropia</i> sp. CHJ	Región de Los Lagos, Chiloé, Cucao, Chile	KP781662/ KP781648
<i>Pyropia</i> sp. CHJ	Región de Los Ríos, Valdivia, Los Molinos, Chile	KP781683
<i>Pyropia</i> sp. CHJ	Región del Biobío, Concepción, Coliumo, Chile	KP781586
<i>Pyropia</i> sp. CHJ	Región de Los Lagos, Llanquihue, Carelmapu, Chile	KP781598
<i>Pyropia</i> sp. CHJ	Región de Los Lagos, Chiloé, Cucao, Chile	KP781601
<i>Pyropia</i> sp. CHJ	Región de Los Lagos, Chiloé, Playa Mar Brava, Chile	KP781599
<i>Pyropia</i> sp. CHK	Región de Valparaíso, Horcón, Valparaíso, Chile	KP781566
<i>Pyropia</i> sp. CHK	Región de Atacama, Los Burros, Huasco, Chile	KP781611/



<i>Pyropia</i> sp. CHK	Región de Atacama, Chañaral de Aceituno, Huasco, Chile	KP781612/ KP781613/ KP781614/ KP781615/ KP781616/ KP781617/ KP781618
<i>Pyropia</i> sp. CHK	Región de Coquimbo, Elqui, Abalonera Chica, Chile	KP781619/ KP781623/ KP781624
<i>Pyropia</i> sp. CHK	Región de Coquimbo, Puerto Aldea, Elqui, Chile	KP781625
<i>Pyropia</i> sp. CHK	Región de Coquimbo, Mina Talca, Limarí, Chile	KP781626/ KP781627/ KP781628
<i>Pyropia</i> sp. CHK	Región de Coquimbo, Choapa, Puerto Oscuro, Chile	KP781629/ KP781630/ KP781632
<i>Pyropia</i> sp. CHK	Región de Valparaíso, Petorca, Los Molles, Chile	KP781633/ KP781634/ KP781635
<i>Pyropia</i> sp. CHK	Región de Valparaíso, Valparaíso, Maitencillo, Chile	KP781604/ KP781605/ KP781606/ KP781607/ KP781608/ KP781609/ KP781610
<i>Pyropia</i> sp. CHK	Región de Valparaíso, Petorca, Salinas de Pullay, Chile	KP781636
<i>Pyropia</i> sp. FIA	Región de Magallanes, Punta Arenas, Chile	KP781603
<i>Pyropia</i> sp. FIA	Región de Magallanes, Fuerte Bulnes, Chile	KP781668
<i>Pyropia</i> sp. FIA	Región de Magallanes, Agua Fresca, Chile	KP781669
<i>Pyropia</i> sp. HK-2011d	Rhode Island, Governor Sprague Bridge 17, Narragansett, USA	JN028798
<i>Pyropia spiralis</i>	Espírito Santo, Vila Velha, Brazil	JN222754
<i>Pyropia tanegashimensis</i>	Sao Paulo, Praia Dura, Ubatuba, Brazil	JN222752
<i>Pyropia thuretii</i>	British Columbia, Island south of Clotchman I., Spanish Pilot Group, Tahsis, Canada	JN028801
<i>Pyropia torta</i>	British Columbia, Alder Island, Gwaii Haanas, Canada	JN028802
<i>Pyropia vietnamensis</i>	Parnaíba, Pedra do Sal, Piauí, Brazil	JN222751
<i>Wildemanía amplissima</i>	British Columbia, Murchison Island Lagoon, Gwaii Haanas, Canada	HQ969863
<i>Wildemanía miniata</i>	Quebec, Escoumins, Canada	JN028874
<i>Wildemanía occidentalis</i>	British Columbia, Tahsis Nuchatliz Island, Canada	JN028923
<i>Wildemanía</i> sp. FII	Región de Magallanes, Buque Quemado, San Gregorio Chile	KP781664

Taxon	Collection location	RBCL - GENBANK Accession no.
<i>Bangia atropurpurea</i>	Ysselmeer, Enschede, Netherlands	AF169330
' <i>Bangia fuscopurpurea</i> ' BB Bf 1	Bolinas Bay, CA, USA	EU289018
' <i>Bangia fuscopurpurea</i> '	Banda, Tateyama, Chiba, Japan	HQ687502
' <i>Bangia fuscopurpurea</i> ' France	Nice, France	AF168659
' <i>Bangia fuscopurpurea</i> ' Taiwan	Taiwan	AF168654
' <i>Bangia fuscopurpurea</i> ' WA	Fisherman's Bay, WA, USA	AF169329
' <i>Bangia</i> ' <i>gloiopeltidicola</i>	Shinori, Hakodate, Hokkaido, Japan	HQ687503
' <i>Bangia</i> ' <i>maxima</i>	Bolinas Bay, CA, USA	EU289020
' <i>Bangia</i> ' sp. BC Can	Ogden Point, Victoria, BC, Canada	AF043376
' <i>Bangia</i> ' sp. BCH	Taylor's Mistake, Christchurch, South I, NZ	HQ687504
' <i>Bangia</i> ' sp. BFK	Frank Kitts Lagoon, Wellington, North I, NZ	HQ687505
' <i>Bangia</i> ' sp. BGA	Gentle Annie, Westland, South I, NZ	HQ687506
' <i>Bangia</i> ' sp. BHH	14 Mile Bluff, Westland, South I, NZ	GU046404
' <i>Bangia</i> ' sp. BJB	Causet Cove, Doubtful Sound, Fiordland, NZ	HQ687507
' <i>Bangia</i> ' sp. BMW	Makawhio, North I, NZ	HQ687508
' <i>Bangia</i> ' sp. BNS	Bawley Point, N. of Bateman's Bay, NSW, Australia	HQ687509
' <i>Bangia</i> ' sp. BPL	Maketu, Bay of Plenty, North I, NZ	HQ687510
' <i>Bangia</i> ' sp. BRM	Kaka Point, Otago, South I, NZ	HQ687511
' <i>Bangia</i> ' sp. BWP	Woodpecker Bay, Paparoa, Westland, South I, NZ	EU570051
' <i>Bangia</i> ' sp. CH620	Supseom, Jejudo, Korea	HQ728203
<i>Bangia</i> sp. Ireland	Shannon River, Ireland	AF043371
' <i>Bangia</i> ' sp. MA	Woods Hole, MA, USA	AF043369
' <i>Bangia</i> ' sp. NthBC Can	Triple Island, BC, Canada	AF043372
' <i>Bangia</i> ' sp. NWT	Rankin Inlet, NWT, Canada	AF043366
' <i>Bangia</i> ' sp. OR	Lincoln City, OR, USA	AF043367
' <i>Bangia</i> ' sp. SB Bf 1	Solana Beach, CA, USA	EU289019
' <i>Bangia</i> ' sp. TX	Port Aransas, TX, USA	AF043377
' <i>Bangia</i> ' <i>vermicularis</i>	Golden Gate, San Francisco Bay, CA, USA	EU289022
<i>Boreophyllum aestivale</i>	Captains Bay, Amaknak Island, AK, USA	EU223033
<i>Boreophyllum birdiae</i>	Herring Cove, NS, Canada	AY180909
<i>Boreophyllum</i> <i>pseudocrassum</i>	Erimo, Hokkaido, Japan	HQ687512
<i>Boreophyllum</i> sp. 148	Village of Akutan, AK, USA	EU223240
<i>Clymene coleana</i>	Leigh, North I, NZ	FJ263672
<i>Clymene</i> sp. OTA	Brighton, Otago, South I, NZ	GU214023
<i>Clymene</i> sp. TTS	Trial Harbour, Tasmania, Australia	HQ687514
<i>Dione arcuata</i>	Ohau Stream, Kaikoura, South I, New Zealand	EU570052
<i>Fusciifolium papenfussii</i>	Seldovia Point, AK, USA	EU223120
<i>Fusciifolium</i> sp. CHA	Región de Coquimbo, Choapa, Puerto Oscuro	KP781730
<i>Fusciifolium tasa</i>	Spray Cape, Unalaska Island, AK, USA	EU223226
<i>Miuraea migitae</i>	Osaka Bay, Osaka, Japan	EU521643
<i>Porphyra dioica</i>	Sidmouth, UK	HQ687546
<i>Porphyra linearis</i>	Aberystwyth, UK	HQ687547
<i>Porphyra lucasii</i>	Trigg Beach, WA, Australia	AY139687
<i>Porphyra mumfordii</i>	Región de Los Ríos, Valdivia, Los Molinos, Chile	KP781809
<i>Porphyra mumfordii</i>	Región de la Araucanía, Cautin, Cheuque, Chile	KP781768
<i>Porphyra purpurea</i>	Litstock, Somerset, UK	HQ687516
<i>Porphyra</i> sp. CHB	Región de Los Lagos, Chiloé, Cuaao, Chile	KP781808
<i>Porphyra</i> sp. CHC	Región de Valparaíso, Petorca, Salinas de Pullay, Chile	KP781737/ KP781738
<i>Porphyra</i> sp. CHC	Región de Los Ríos, Valdivia, Playa Rosada, Chile	KP781811
<i>Porphyra</i> sp. CHC	Región de la Araucanía, Cautin, Cheuque, Chile	KP781769

<i>Porphyra</i> sp. CHC	Región de Los Ríos, Valdivia, Pichicuyin, Chile	KP781770/ KP781771
<i>Porphyra</i> sp. CHC	Región de Atacama, Huasco, Los Burros, Chile	KP781705
<i>Porphyra</i> sp. CHF <i>Porphyra</i> sp. CHF	Región de Antofagasta, Antofagasta, Playa El Lenguado, Chile Región de Coquimbo, Elqui, Guanaquerillos, Chile	KP781699 KP781719/ KP781720
<i>Porphyra</i> sp. CHF	Región de Coquimbo, Elqui, Puerto Aldea, Chile	KP781723/ KP781724
<i>Porphyra</i> sp. CHF	Región de Atacama, Huasco, Chañaral de Aceituno, Chile	KP781707/ KP781708
<i>Porphyra</i> sp. CHF <i>Porphyra</i> sp. CHF	Región de Coquimbo, Choapa, Caleta las Conchas, Chile Región de Valparaíso, San Antonio, Punta de Tralca, Chile	KP781731 KP781749/ KP781750
<i>Porphyra</i> sp. CHF	Región del Libertador General Bernardo O'Higgins, Cardenal Caro, Pichilemu, Chile	KP781751
<i>Porphyra</i> sp. CHF	Región del Libertador General Bernardo O'Higgins, Cardenal Caro, La Boca, Chile	KP781847/ KP781848/ HQ687552/ KP781849/ KP781850/ KP781854
<i>Porphyra</i> sp. CHF <i>Porphyra</i> sp. CHF <i>Porphyra</i> sp. CHF	Región del Biobío, Concepción, Coliumo, Chile Región de Valparaíso, Valparaíso, Playa Amarilla, Chile Región de Valparaíso, Valparaíso, Playa El Encanto, Chile	KP781764 KP781838 KP781839/ KP781844/ KP781846
<i>Porphyra</i> sp. CHF	Región de Valparaíso, Valparaíso, Curaumilla, Chile	KP781840/ KP781841/ KP781842/ KP781843
<i>Porphyra</i> sp. FIB <i>Porphyra</i> sp. FIG <i>Porphyra</i> sp. FIG <i>Porphyra</i> sp. FIG	Falkland Is Weddell Inn, Weddell Is, Falkland Is Región de Magallanes, Magallanes, Buque Quemado, Chile Región de Magallanes, Magallanes, Fuerte Bulnes, Chile	GU165840 GU165885 KP781819 KP781828/ KP781832
<i>Porphyra</i> sp. FIH <i>Porphyra</i> sp. FIH	Región de la Araucanía, Cautin, Cheuque, Chile Región de Los Lagos, Llanquihue, Metri, Chile	KP781767 KP781774/ KP781775/ KP781776/ KP781777/ KP781778/ KP781779/ KP781780/
<i>Porphyra</i> sp. FIH <i>Porphyra</i> sp. FIH <i>Porphyra</i> sp. FIH	Región de Magallanes, Magallanes, Punta Arenas, Chile Región de Los Lagos, Chiloé, Playa Mar Brava, Chile Región de Los Lagos, Llanquihue, Carelmapu, Chile	KP781781 KP781787 KP781782 KP781792
<i>Porphyra</i> sp. FIH	Puerto Montt, Pargua, Punta Corona, Chile	KP781794/ KP781795
<i>Porphyra</i> sp. FIH <i>Porphyra</i> sp. FIH <i>Porphyra</i> sp. FIH <i>Porphyra</i> sp. FIH	Región de Los Lagos, Chiloé, Ancud, Fátima, Chile Región de Los Lagos, Chiloé, Ancud-Arena Gruesa, Chile Región de Los Lagos, Chiloé, Puñihuil, Chile Región de Los Lagos, Chiloé, Quemchi, Chile	KP781798 KP781799 KP781800 KP781801/ KP781802/ KP781803/ KP781804/

<i>Porphyra</i> sp. FIH	Región de Los Ríos, Valdivia, Playa Rosada, Chile	KP781805 KP781814/ KP781816
<i>Porphyra</i> sp. FIH	Región de Magallanes, Magallanes, Fuerte Bulnes, Chile	KP781829/ KP781830/ KP781834/ KP781835
<i>Porphyra</i> sp. FIH	Región de Magallanes, Magallanes, Seno Otway-Punta Canelo, Chile	KP781836
<i>Porphyra</i> sp. FIH	Región de Magallanes, Magallanes, Seno Skyring, Rio Verde, Chile	KP781851/ KP781852/ KP781853
<i>Porphyra</i> sp. FIH	Región de Los Ríos, Valdivia, Isla Mancera, Chile	GU046415
<i>Porphyra</i> sp. GDM	Dead Man Beach, Stewart I, NZ	GU214021
<i>Porphyra</i> sp. GRB108	Cape Wanbrow, Otago, South I, NZ	HQ687548
<i>Porphyra</i> sp. GRB145	East Cape, Te Wharenaonao Point, North I, NZ	HQ687549
<i>Porphyra</i> sp. GRB178	Wharariki Beach, Nelson, NZ	HQ687550
<i>Porphyra</i> sp. GRB287	Paparoa, Westland, South I, NZ (2 collections: Seal Island & opposite coast)	HQ687551
<i>Porphyra</i> sp. GRB368	Kaka Point, Otago, NZ	GU046405
<i>Porphyra</i> sp. GRB488	Chatham Is, NZ	HQ687552
<i>Porphyra</i> sp. JBCH26A	Playa Amarillo, nr Valparaiso, Chile	GU046409
<i>Porphyra</i> sp. LGD	Lyll Bay, Wellington, NZ	HQ687553
<i>Porphyra</i> sp. MTR	Maunganui Bluff, Northland, North I, NZ	HQ687554
<i>Porphyra</i> sp. OSK	Ohau Stream, Kaikoura, South I, NZ	GU046414
<i>Porphyra</i> sp. SBA	Derry Castle, Auckland Island, NZ	GU046417
<i>Porphyra</i> sp. SIR	Ringaringa, Stewart I (nrSSU); Torias Corner, Stewart I ( <i>rbcl</i> ) NZ	GU046427
<i>Porphyra</i> sp. TAS	Blinkers Billy Pt, Derwent Estuary, (nrSSU) & Taroona Point ( <i>rbcl</i> ) Tasmania, Australia	
<i>Porphyra</i> sp. WLR	Brighton, Otago, South I, NZ	GU165837
<i>Porphyra</i> sp. ZBS	St Helena Bay, South Africa	HQ687555
<i>Porphyra</i> sp. ZCE	The Boulders, False Bay, South Africa	GU046424
<i>Porphyra</i> sp. ZDR	The Boulders, South Africa	GU046425
<i>Porphyra</i> sp. ZGR	St Helena Bay, South Africa	HQ687556
<i>Porphyra</i> sp. ZIR	St Helena Bay, South Africa	GU214022
<i>Porphyra</i> sp. ZPP	Port Alfred breakwater, South Africa	HQ687557
<i>Porphyra</i> sp. ZSM	Tietiesbaai, South Africa	HQ687558
<i>Porphyra umbilicalis</i>	Sidmouth, UK	HQ687559
<i>Pyropia abbottiae</i>	Harling Point, Victoria, BC, Canada	EU223024
<i>Pyropia acanthophora</i>	Ubatuba, São Paulo, Brazil	HQ605695
<i>Pyropia aeodis</i>	Paternoster, South Africa	GU165843
<i>Pyropia brumalis</i>	Stanley Park, Vancouver, BC, Canada	EU223038
<i>Pyropia cf crassa</i>	Nosappu, Nemuro, Hokkaido, Japan	HQ687518
<i>Pyropia cf pseudolinearis</i>	Bridget Cove, AK, USA	EU223172
<i>Pyropia cf thuretii</i>	Olas Altas, Mazatlan, Sinaloa, Mexico	HQ687519
<i>Pyropia cinnamomea</i>	Bruce's Rock, Otago, South I, NZ	EU521637
<i>Pyropia columbina</i>	Top Island, Port Stanley, East Falkland, Falkland Is	GU046423
<i>Pyropia conwayae</i>	French Beach, BC, Canada	EU223045
<i>Pyropia dentata</i>	Shirahama, Chiba, Japan	HQ687520
<i>Pyropia denticulata</i>	Mooloolaba, Queensland, Australia	HQ687521
<i>Pyropia fallax</i>	Avatanak Island, AK, USA	GU319865
<i>Pyropia fucicola</i>	SW side, 29 end of runway, Sitka airport, Japonski Island, AK, USA	EU223088

<i>Pyropia gardneri</i> AK	Northeast Ushagat Island, AK, USA	EU223096
<i>Pyropia gardneri</i>	Caminitos (Cabo Punta Banda), Baja California, Mexico	HQ687522
<i>Pyropia haitanensis</i>	Yuge, Ehime, Japan	AB118585
<i>Pyropia hiberna</i>	foot of 15th Street, Pacific Grove, Monterey County, CA, USA	GU319866
<i>Pyropia hollenbergii</i>	Agua Verde, Baja California Sur, Mexico	HQ687523
<i>Pyropia ishigecola</i>	Yoshio, Katsuura, Chiba, Japan	HQ687524
<i>Pyropia kanakaensis</i>	between Makah Bay and Sekui, Olympic Peninsula, WA, USA	EU223099
<i>Pyropia katadae</i> Japan	Ise, Mie, Japan	HQ687525
<i>Pyropia katadae</i> Korea	Pohang, Gyeongsangbukdo, Korea	HQ728199
<i>Pyropia kinositae</i>	Arito, Suttu, Hokkaido, Japan	EU521641
<i>Pyropia koreana</i>	Ohuri, Gangwondo, Korea	HQ728198
<i>Pyropia kuniedae</i>	Sachon, Namhae, Gyeongsangnamdo, Korea	HQ728200
<i>Pyropia kurogii</i> AK	Sandy Beach, Sitka, AK, USA	EU223105
<i>Pyropia kurogii</i> Japan	Utoro, Hokkaido, Japan	HQ687526
<i>Pyropia lacerata</i>	Shirahama, Chiba, Japan	HQ687527
<i>Pyropia leucosticta</i>	Sidmouth, UK	HQ687528
<i>Pyropia moriensis</i>	Otaru, Hokkaido, Japan	EU521645
<i>Pyropia nereocystis</i>	Northeast Ushagat Island, AK, USA	EU223117
<i>Pyropia onoi</i>	Mori, Hokkaido, Japan	HQ687529
<i>Pyropia orbicularis</i>	Región de Valparaíso, Petorca, Salinas de Pullay	KP781735/ KP781736/ KP781739
<i>Pyropia orbicularis</i>	Región de Valparaíso, Valparaíso, Maitencillo	KF479481/ KF479482/ KF479484/ KF479485/ KF479486/ KF479488/ KF479489/ KF479490/ KF479491/ KF479492/ KF479497/ KF479498/ KF479499
<i>Pyropia orbicularis</i>	Región del Maule, Curico, Duao	KP781752/ KP781753/ KP781754/ KP781755/ KP781756/ KP781757
<i>Pyropia orbicularis</i>	Región del Maule, Talca, Constitución	KP781758/ KP781759/ KP781761/ KP781762/ KP781763
<i>Pyropia orbicularis</i>	Región del Biobío, Concepción, Cocholgue	KP781765
<i>Pyropia orbicularis</i>	Región del Biobío, Concepción, Lota	KP781766
<i>Pyropia orbicularis</i>	Región de Los Ríos, Valdivia, Calfuco	KP781772
<i>Pyropia orbicularis</i>	Región de Los Lagos, Chiloé, Playa Mar Brava	KP781788

<i>Pyropia orbicularis</i>	Región de Los Lagos, Chiloé, Chonchi	KP781791
<i>Pyropia orbicularis</i>	Región de Los Lagos, Llanquihue, Punta Corona	KP781796
<i>Pyropia orbicularis</i>	Región de Los Lagos, Llanquihue, Puerto Montt-Estaquilla	KP781797
<i>Pyropia orbicularis</i>	Región de Los Lagos, Chiloé, Achao, Punta Palqui	KP781806
<i>Pyropia orbicularis</i>	Región de Los Ríos, Valdivia, Playa Rosada	KP781812
<i>Pyropia orbicularis</i>	Región de Los Ríos, Valdivia, Playa Rosada	KP781813
<i>Pyropia orbicularis</i>	Región de Magallanes, Magallanes, Fuerte Bulnes	KP781825/ KP781827
<i>Pyropia pendula</i>	Calerita, Baja California Sur, Mexico	HQ687530
<i>Pyropia perforata</i>	Punta Poptla, Baja California, Mexico (nrSSU); Crescent City Lighthouse, Del Norte County, CA, USA (rbcl)	EU223127
<i>Pyropia pseudolanceolata</i>	Harling Point, Victoria, BC, Canada	EU223145
<i>Pyropia pseudolinearis</i> Japan	Choshi, Chiba, Japan	HQ687531
<i>Pyropia pseudolinearis</i> Korea	Dokdo, Gyeongsangbukdo, Korea	HQ728196
<i>Pyropia pulchella</i>	Waihau Bay East, North I, NZ	HQ687532
<i>Pyropia rakiura</i>	Ocean View, Kaikoura, South I, NZ	EU521646
<i>Pyropia saldanhae</i>	Kommetjie, South Africa	GU165838
<i>Pyropia seriata</i>	Hondo, Kumamoto, Japan	HQ687533
<i>Pyropia smithii</i>	Nudibranch Point, Barkley Sound, Vancouver Island, BC, Canada	EU223224
<i>Pyropia</i> sp. 480	Spanish Bay, Monterey County, CA, USA	GU319867
<i>Pyropia</i> sp. 485	Mussel Rock, Cape Mendocino, CA, USA	GU319868
<i>Pyropia</i> sp. 523	outside Chichagof Harbor, Attu Island, AK, USA	GU319869
<i>Pyropia</i> sp. 551	Gerringong boat harbor, NSW, Australia	GU319870
<i>Pyropia</i> sp. AKL	Southeast Sandy Bay, Enderby I, Auckland Is, NZ	GU046403
<i>Pyropia</i> sp. Antar68	Admiralty Bay, King George Island, South Shetlands Archipelago, Antarctica	HQ605698
<i>Pyropia</i> sp. CHG	Región de Magallanes, Magallanes, Punta Carrera	KP781837
<i>Pyropia</i> sp. CHH	Región de Los Ríos, Valdivia, Niebla (Playa Grande)	KP781845
<i>Pyropia</i> sp. CHH	Región de Los Lagos, Chiloé, Playa Mar Brava	KP781784
<i>Pyropia</i> sp. CHI	Región de Arica y Parinacota, Arica, Playa Corazón, Chile	KP781698
<i>Pyropia</i> sp. CHI	Región de Coquimbo, Elqui, Abalonera chica	KP781713/ KP781714/ KP781715
<i>Pyropia</i> sp. CHJ	Región del Maule, Talca, Constitución	KP781760
<i>Pyropia</i> sp. CHJ	Región de Los Ríos, Valdivia, Niebla	KP781773
<i>Pyropia</i> sp. CHJ	Región de Los Lagos, Llanquihue, Carelmapu	KP781783
<i>Pyropia</i> sp. CHJ	Región de Los Lagos, Chiloé, Playa Mar Brava	KP781785/ KP781786
<i>Pyropia</i> sp. CHJ	Región de Los Lagos, Chiloé, Cucao	KP781789/ KP781790/ KP781807
<i>Pyropia</i> sp. CHJ	Región de Los Ríos, Valdivia, Los Molinos	KP781810
<i>Pyropia</i> sp. CHJ	Región de Los Ríos, Valdivia, Niebla (Playa Grande)	KP781815
<i>Pyropia</i> sp. CHK	Región de Atacama, Huasco, Los Burros, Chile	KP781701/ KP781702/ KP781703/ KP781704

<i>Pyropia</i> sp. CHK	Región de Atacama, Huasco, Chañaral de Aceituno, Chile	KP781706/ KP781709/ KP781710/ KP781711
<i>Pyropia</i> sp. CHK	Región de Coquimbo, Elqui, Abalonera Chica, Chile	KP781712/ KP781716/ KP781717/ KP781718
<i>Pyropia</i> sp. CHK	Región de Coquimbo, Elqui, Puerto Aldea, Chile	KP781721/ KP781722
<i>Pyropia</i> sp. CHK	Región de Coquimbo, Limarí, Mina Talca, Chile	KP781725/ KP781726/ KP781727
<i>Pyropia</i> sp. CHK	Región de Coquimbo, Choapa, Puerto Oscuro, Chile	KP781728/ KP781729
<i>Pyropia</i> sp. CHK	Región de Valparaíso, Petorca, Los Molles, Chile	KP781732/ KP781733
<i>Pyropia</i> sp. CHK	Región de Valparaíso, Petorca, Salinas de Pullay, Chile	KP781734
<i>Pyropia</i> sp. CHK	Región de Valparaíso, Valparaíso, Maitencillo, Chile	KP781740/ KP781741/ KP781742/ KP781743/ KP781744/ KP781745/ KP781746/ KP781747
<i>Pyropia</i> sp. CHK	Región de Valparaíso, Valparaíso, Playa Caucao	KP781748
<i>Pyropia</i> sp. DRB	Sumner, Christchurch, South I, NZ	HQ687534
<i>Pyropia</i> sp. FAL	Saldamando, Baja California, Mexico	HQ687535
<i>Pyropia</i> sp. FIA	Top Island, Port Stanley, East Falkland, Falkland Is.	GU165842
<i>Pyropia</i> sp. FIA	Región de Magallanes, Magallanes, Gregorio	KP781821/ KP781822
<i>Pyropia</i> sp. FIA	Región de Magallanes, Magallanes, Fuerte Bulnes	KP781831
<i>Pyropia</i> sp. FIA	Región de Magallanes, Magallanes, Agua Fresca	KP781833
<i>Pyropia</i> sp. FIC	Top Island, Port Stanley, East Falkland, Falkland Is.	GU046422
<i>Pyropia</i> sp. FID	East Loafers, Sea Lion I, Falkland Is.	GU046406
<i>Pyropia</i> sp. FID	Región de Magallanes, Magallanes, Fuerte Bulnes	KP781793/ KP781823/ KP781826
<i>Pyropia</i> sp. FIE	Top Island, Port Stanley, East Falkland, Falkland Is.	GU046408
<i>Pyropia</i> sp. GEP	Avon/Heathcote Estuary, Christchurch, South I, NZ	GU165841
<i>Pyropia</i> sp. MIG	Faru de San Miguel, Baja California, Mexico	HQ687536
<i>Pyropia</i> sp. Piaui	Parnaiba, Piaui, Brazil	HQ605697
<i>Pyropia</i> sp. PTK	Northwest Bay, Manawa Tawhi, Three Kings Islands, NZ	HQ687537
<i>Pyropia</i> sp. ROS054	Ocean View, Kaikoura, South I, NZ	GU046410
<i>Pyropia</i> sp. ROS125	Henderson Point, North I, NZ	HQ687538
<i>Pyropia</i> sp. SMR	Curio Bay, Southland, South I, NZ	HQ687539
<i>Pyropia</i> sp. SSR053	Ocean View, Kaikoura, South I, NZ	GU046411
<i>Pyropia</i> sp. SSR091	Brighton, Otago, South I, NZ ( 2 collections, 11 Feb 1997 nrSSU & 21 May 2001 rbCL)	GU046421
<i>Pyropia</i> sp. STI	Campbell Point, Otago, South I, NZ	HQ687540

<i>Pyropia</i> sp. TCH	Torias Corner, Stewart I, NZ	GU046418
<i>Pyropia</i> sp. WRO	Punakaiki, Westland, South I, NZ	HQ687541
<i>Pyropia</i> sp. ZLI	Paternoster, South Africa	GU165839
<i>Pyropia spiralis</i>	Vila Velha, Espírito Santo, Brazil	HQ605696
<i>Pyropia suborbiculata</i>	Wonsando, Chungchungnamdo, Korea	HQ728201
<i>Pyropia tanegashimensis</i>	Iseki, Tanegashima, Kagoshima, Japan	HQ687542
<i>Pyropia tenera</i>	Kawaura, Kumamoto, Japan	HQ687543
<i>Pyropia tenuipedalis</i>	Urayasu, Chiba, Japan	EU521649
<i>Pyropia torta</i>	Vista Mar, San Juan Island, WA, USA	EU223236
<i>Pyropia vietnamensis</i>	Thangeseri (Kerala) India	HQ687544
<i>Pyropia virididentata</i>	Lyll Bay, Wellington, North I, NZ	EU521650
<i>Pyropia yezoensis</i>	Galmok, Tongyoung, Gyeongsangnamdo, Korea	HQ728197
<i>Wildemanina amplissima</i>	Nosappu, Nemuro, Hokkaido, Japan	HQ687560
<i>Wildemanina norrisii</i>	Harling Point, Victoria, BC, Canada	EU223212
<i>Wildemanina occidentalis</i>	Cape Palmerston, Vancouver Island, BC, Canada	EU223118
<i>Wildemanina schizophylla</i>	Van Damme State Park, Mendocino County, CA, USA	GU319871
<i>Wildemanina</i> sp. Antar23	Admiralty Bay, King George Island, South Shetlands Archipelago, Antarctica	HQ605700
<i>Wildemanina</i> sp. FII	Región de Magallanes, Magallanes, Buque Quemado	KP781817/ KP781818/ KP781820
<i>Wildemanina</i> sp. FII	Hill cove kelp forest west of jetty, West Falkland, Falkland Is.	GU165883
<i>Wildemanina</i> sp. HM080	Songjiho, Gangwondo, Korea	HQ728202
<i>Wildemanina variegata</i> AK	Kagamil Island, AK, USA	EU223237
<i>Wildemanina variegata</i> Japan	Mori, Hokkaido, Japan	GU046430

---



Table S3. Mean genetic distances calculated within- and between species for *Pyropia* sp. CHJ, *Pyropia* sp. CHK and *Pyropia orbicularis* for the *COI*. Genetic distances calculated within species are noted in bold along the diagonal. Genetic distances calculated between species pairs are noted below the diagonal. Sequences obtained in Maitencillo during this work were completed by the ones already available in Guillemín et al. (2016).

	<i>Pyropia</i> sp. CHJ	<i>Pyropia orbicularis</i>	<i>Pyropia</i> sp. CHK
<i>Pyropia</i> sp. CHJ	<b>0.008</b>		
<i>Pyropia orbicularis</i>	0.073	<b>0.006</b>	
<i>Pyropia</i> sp. CHK	0.038	0.063	<b>0.001</b>