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Abstract

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

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

Key index words: Bangiales; taxonomy; *COI*; *rbc*L; Chile; Intertidal distribution

Abbreviations: *COI*, cytochrome oxidase I; ML, Maximum Likelihood; PCA, Principal Component Analysis; PCR-RFLP, polymerase chain reaction restriction fragment length polymorphism analyses; *rbc*L, large subunit ribulose bis-phosphate carboxylase/oxygenase.

INTRODUCTION

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

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

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

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

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

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

MATERIALS AND METHODS

Sampling of foliose Bangiales *in Maitencillo beach*

Sampling was performed in the rocky intertidal shore of Maitencillo, Valparaíso (32° 39´S, 71° 26´ W, Fig. S1). This site is characterized by a rocky platform that gently slopes into the sea with small areas characterized by boulders and small, but steep rocky walls. Foliose Bangiales are prevalent in the upper intertidal zone while *Mazzaella laminaroides* (Rhodophyta) dominate the middle intertidal (Betancourtt et al. 2018). The lower intertidal zone is characterized by a sandy bottom. Two sampling schemes were used during the study. First, to characterize species diversity and small-scale distribution in Maitencillo we 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^2 placed on a steep wall adjacent to
149 the upper intertidal zone; and by 24 samples taken from four quadrants of 0.5 m^2 placed in
150 two boulder zones (two quadrats 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 rbc*L *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 *rbc*L was obtained
170 using the primers F-*rbc*L and R-*rbc*S (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 *rbc*L 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 *rbc*L, 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.

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

For each of the 90 specimens sequenced, affiliation to one of the 18 cryptic species observed in Chile (Guillemin et al. 2016) or to a newly encountered species was determined using the ML tree reconstruction. For the more problematic groups of sequences (i.e. corresponding to the group of closely related species *Pyropia* sp. CHJ, *Pyropia* sp. CHK and *Pyropia orbicularis*), we followed the grouping proposed by Guillemin et al. (2016) and a *COI* phylogeographic network was reconstructed using the median-joining algorithm implemented in HapView version Beta (Salzburger et al. 2011). For *Pyropia* sp. CHJ, *Pyropia* sp. CHK and *Pyropia orbicularis*, we calculated mean genetic distances within- and between-species for the *COI* in Mega6 (Tamura et al. 2013). Distances were calculated using the Maximum Composite Likelihood method and variation rate among sites was 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 *rbc*L available for the four more common species of foliose
218 Bangiales encountered in Maintecillo (this study, Ramírez et al. 2014, Guillemin 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 *rbc*L 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 *rbc*L-Pyr-F (5'-
227 AGGTGTTGACCCGATTGAAG-3'), producing a longer fragment of 1230 bp instead of
228 873 bp, was used instead of F-*rbc*L (Hommersand et al. 1994) in the PCR mix. The
229 digestion of 0.5-1 μg of *rbc*L 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 *rbc*L 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

Morphological measurements and statistical analyses

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

To describe in multivariate space and search for morphological differences between foliose Bangiales species present at Maitencillo, principal component analysis (PCA) was applied to microscopic and macroscopic measurements using the software PAST specifying a variance-covariance matrix (Hammer et al. 2001). All statistical analyses were done with normalized data, using the Box-Cox transformation option (Box and Cox 1964) available in the statistical software R (R core team, 2017). Statistical differences between species for all traits were assessed through multivariate nested, permutational ANOVA using the Adonis 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 *rbc*L
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 Guillemin et al. (2016). As shown by the Maximum Likelihood phylogenetic trees
274 reconstructed with *COI* sequences (Fig. 1 a and b) and *rbc*L 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 Guillemin 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 *rbc*L 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

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

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

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

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

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 µm 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 µm (Fig. 6 C-D). Sexual regions of the thalli are monoecious, 39.0-136.0 µm 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

(Chile: Valparaíso), 32° 39´S, 71° 26´ W, coll. Zapata, Contreras-Porcia, 10 Oct 2013. (Fig. 6A). GenBank accession numbers: *COI*, MH123945 and *rbc*L, MH124031.

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

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

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

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

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

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

399 monostromatic, and their thickness range from 38.0 to 122.0 µm (Figs. 7C-D). Sexual
400 regions of the thalli are monoecious, 36.0-94.0 µm 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-Porcia, 12 Aug 2014. (Fig.
413 7A). GenBank accession numbers: *COI*, MH123931 and *rbc*L, 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-Porcia, sp. nov.
419 corresponds to the genetic species *Porphyra* sp. CHC, originally identified by Guillemin et
420 al. (2016).

421

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

423 Description: The gametangial blades are monostromatic and monoecious. Blades are 63.0-
424 139.0 µm 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 µm (Figs. 8C-D and 9C-D).
439 Sexual regions of the thalli are monoecious, 68.0-139.0 µm 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-Porcia, 6
451 September 2013 (Fig. 9A). GenBank accession numbers: *COI*, MH123975 and *rbc*L,
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 *rbc*L, 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).

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

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

DISCUSSION

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

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

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

Our study suggests that only one out of the four-dominant foliose Bangiales species 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

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

However, in our study, we observed what seems to be a variable or specialized 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, Betancourtt 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 *rbc*L 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 As pointed out by Sutherland et al. 2011 (following Matsuyama-Serisawa et al.
582 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

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

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

852 Table 2. AFA I cut sites (base position) among the variable bases of the aligned *rbc*L
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 *rbc*L 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
Pyropia sp. CHK	T	C	T	T
Pyropia orbicularis	T	C	T	A
Porphyra sp. CHE	C	T	A	A
Porphyra 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^2	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	***Pyropia orbicularis***	***Pyropia variabilis***	***Porphyra luchensis***	***Porphyra longissima***
Size blade (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
Shape	Orbicular (Several laminae interwined)	Oblong to lanceolate (one or several laminae)	Reniform to spear-shaped asymmetrical (One lamina)	Linear to lanceolate, very long (one lamina)
Color	Green-brown	Forestgreen to light-brown	Redish-brown to green	Rosy pinky to brown
Habitat	Upper-mid intertidal zone	Upper-mid intertidal zone and steep rock faces	Boulders surrounded by stony ground and rocky platforms	Boulders surrounded by sand
Seasonality	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
Sexuality	Monoecious	Monoecious	Monoecious	Monoecious
Vegetative thickness (µm)	68–128	63–137	68-156	38-122
Reproductive thickness (µm)	72–130	68–139	39-136	36-94
Vegetative cells (µm, length × width)	16–30 x 10–22	12–35 x 8–27	17–43 x 12–29	9–42 x 6–30
Rhizoidal cells (µm, length × width)	17–46 x 15–41	16–55 x 14–48	24–59 x 21–48	13–49 x 4–40
Spermatangium	a4/b4/c4	a4/b4/c8	a4/b4/c8	a4/b4/c8
Zygotosporangium	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 *Boreophylum*
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 *rbc*L
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.

Figure 4. 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.*

Figure 5. Boxplot of the sizes of two out of eight microscopic traits (all in Log µm) 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*.

Figure 6. Images of macro and micromorphology of *Porphyra luchensis* sp. nov., SGO168338, holotype, Museo Nacional de Historia Natural, Santiago, Chile (Table S1). 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 μm.

Figure 7. Images of macro and micromorphology of *Porphyra longissima* sp. nov., SGO168348, holotype, Museo Nacional de Historia Natural, Santiago, Chile (Table S1). 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 μm.

Figure 8. Images of macro and micromorphology of *Pyropia variabilis* sp. nov. (CHK) Green Morph (GM), SGO168334, isotype, Museo Nacional de Historia Natural, Santiago, Chile (Table S1). 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 μm.

Figure 9. Images of macro and micromorphology of *Pyropia variabilis* sp. nov. (CHK) Long Morph (LM), SGO168333, holotype, Museo Nacional de Historia Natural, Santiago, Chile (Table S1). 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 μm.

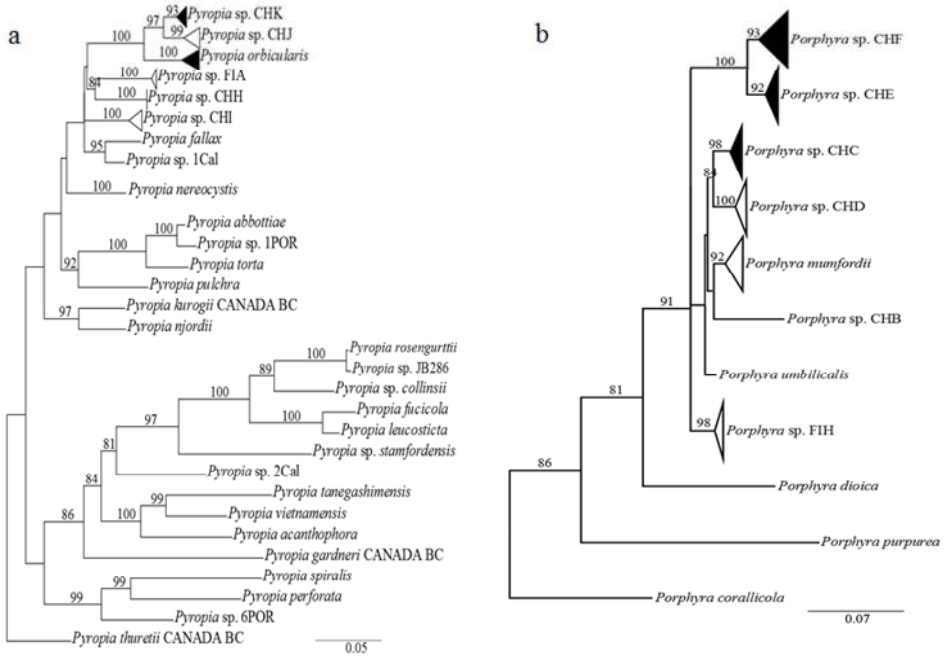

Figure 1. Maximum likelihood (ML) trees of Pyropia and Porphyra using Boreophylum birdiae and Bangia fuscopurpurea as outgroups, respectively, and based on DNA sequences of the cytochrome oxidase I (COI) gene. a) Details of the Pyropia COI ML tree (outgroup not shown). b) Details of the Porphyra COI 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.

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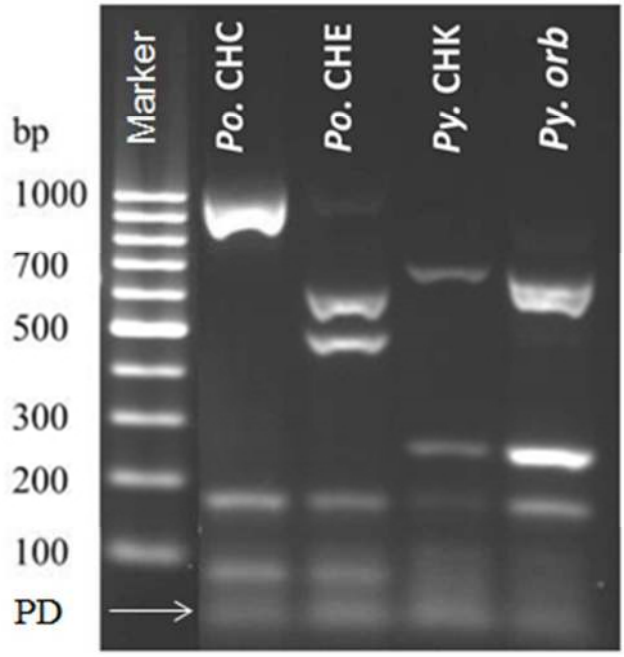
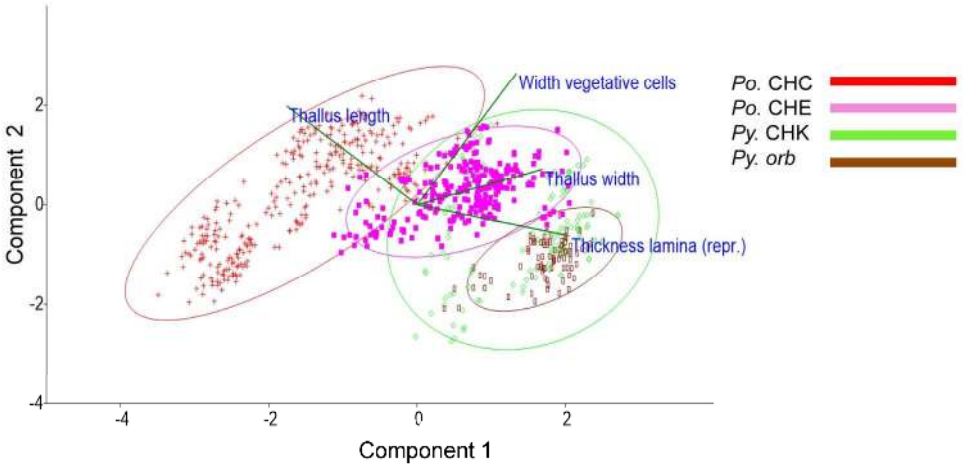


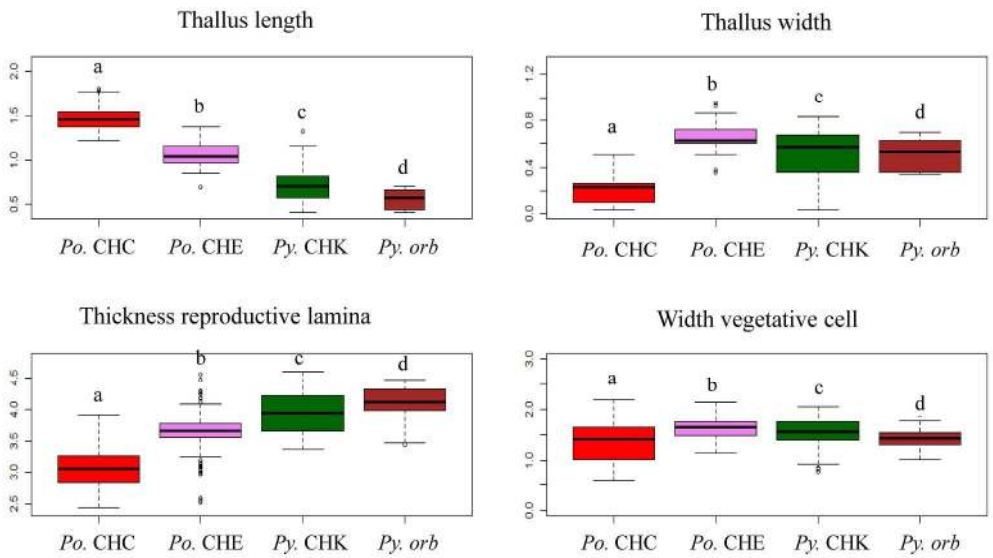
Figure 3. PCR-RFLP profiles resulting from the digestion of the 1230 bp rbcL amplification fragment by the enzyme AFA I. Restriction pattern observed for the four dominant genetic species found at the intertidal in Maitencillo Beach are shown. Legend for each lane corresponds to: Marker, molecular weight 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 µm) 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.

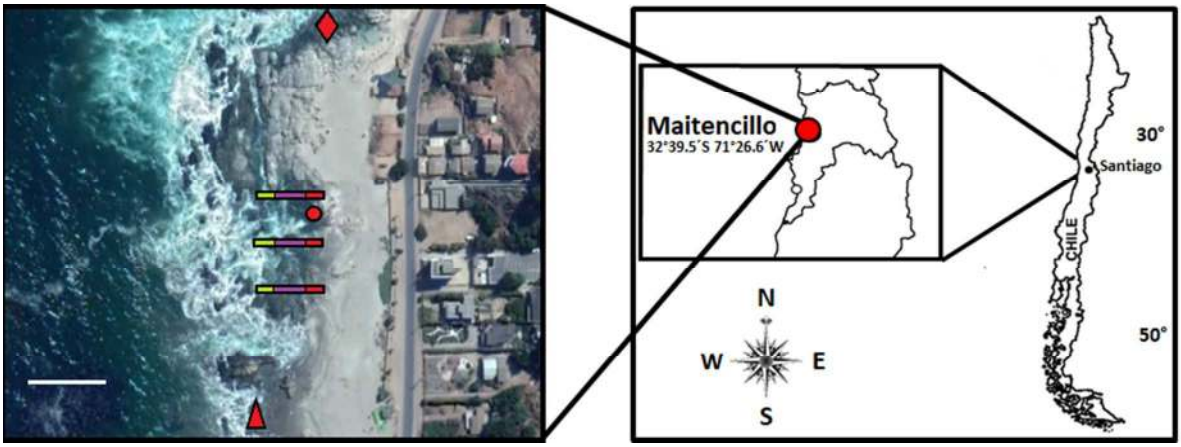
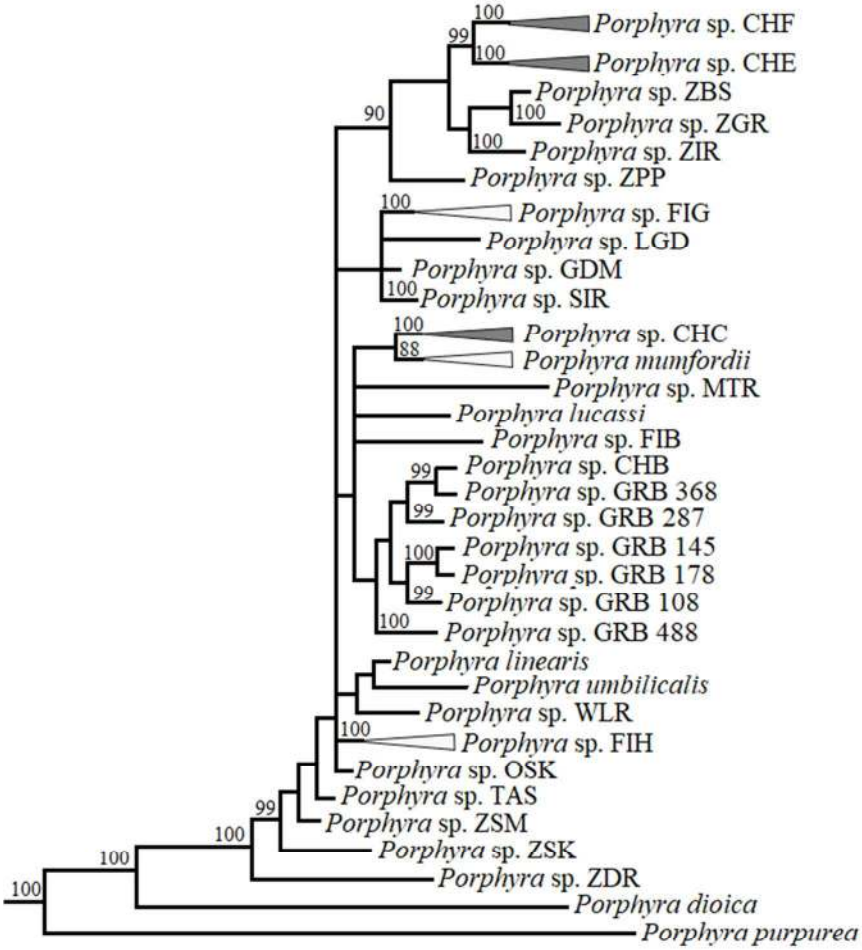


Figure S2. Details of the Maximum Likelihood (ML) rooted tree for *rbc*L sequences (873 bp) of Bangiales. The complete tree is not shown but is similar to the one presented in Fig. 1 in Guillemin et al. (2016), save for some difference in branch support. a) Details of the *Porphyra* clade of the Bangiales *rbc*L ML rooted tree. b) Details of the *Pyropia* clade of the Bangiales *rbc*L 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

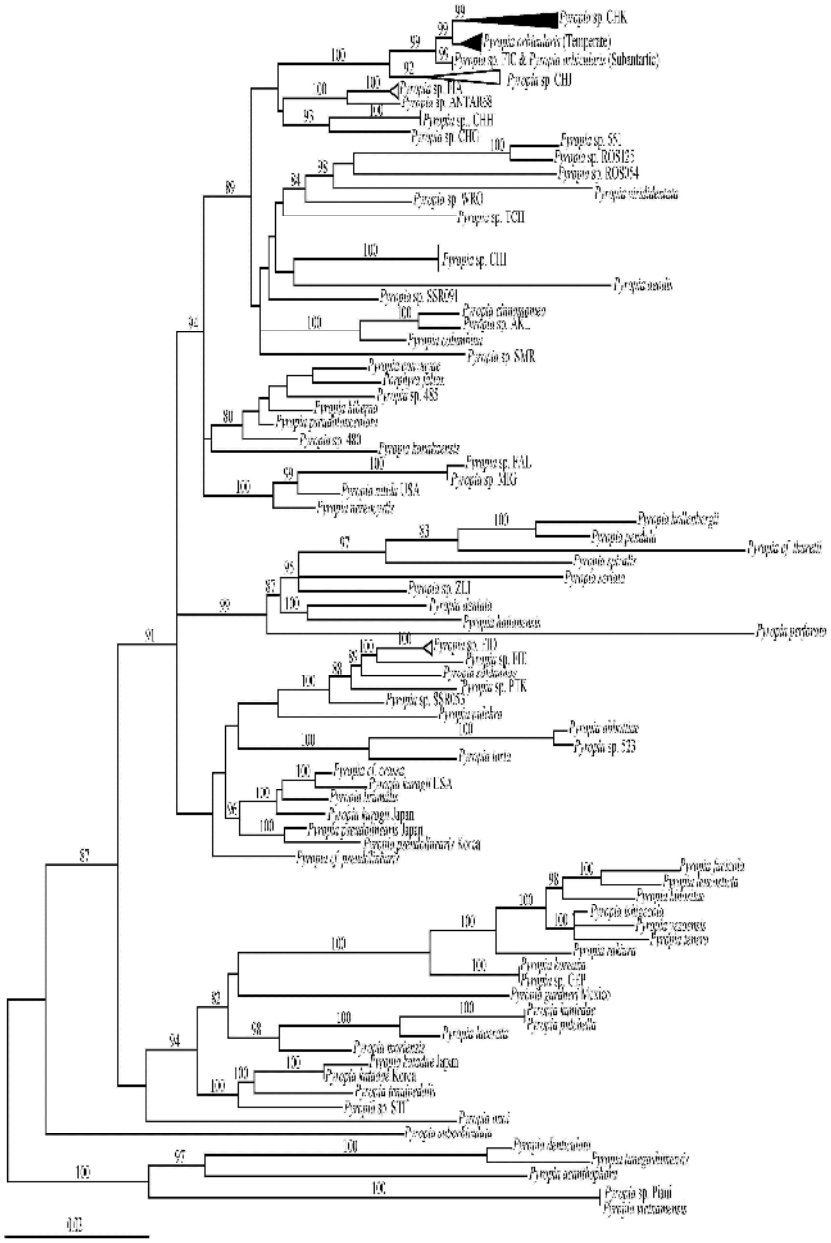

Pyropia sp. CHK
Pyropia yezoensis (Temperate)
Pyropia sp. TTC & Pyropia yezoensis (Subantartic)
Pyropia sp. CHD
Pyropia sp. PTA
Pyropia sp. ANTAR88
Pyropia sp. CHH
Pyropia sp. CHC
Pyropia sp. SS2
Pyropia sp. ROS125
Pyropia sp. ROS064
Pyropia sp. WKO
Pyropia sp. TCH
Pyropia sp. CHJ
Pyropia sp. SSR094
Pyropia sp. AK
Pyropia sp. SMR
Pyropia sp. 488
Pyropia sp. BAL
Pyropia sp. MIG
Pyropia sp. ZLI
Pyropia sp. PTK
Pyropia sp. 323
Pyropia sp. Pini
0.02

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.

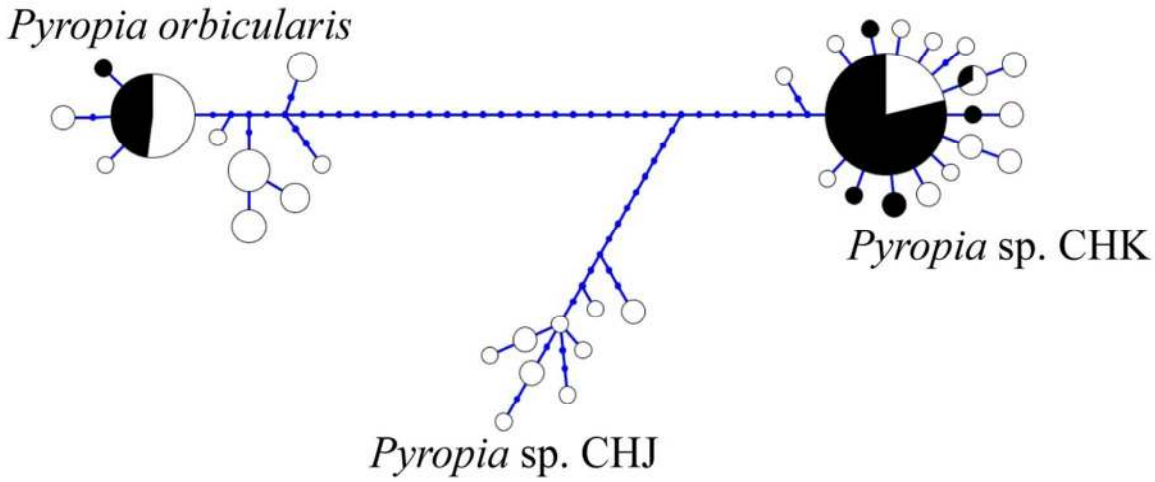


Figure S4. Images of macro and micromorphology of *Pyropia orbicularis* Ramírez, Contreras-Porcia & Guillemin. A) Habit of the foliose gametophyte sampled from the intertidal zone in Maitencillo beach, Valparaíso, Chile (scale bar= 10 cm). B) Surface view of basal, rhizoidal cells. C) Surface view of 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 µm.

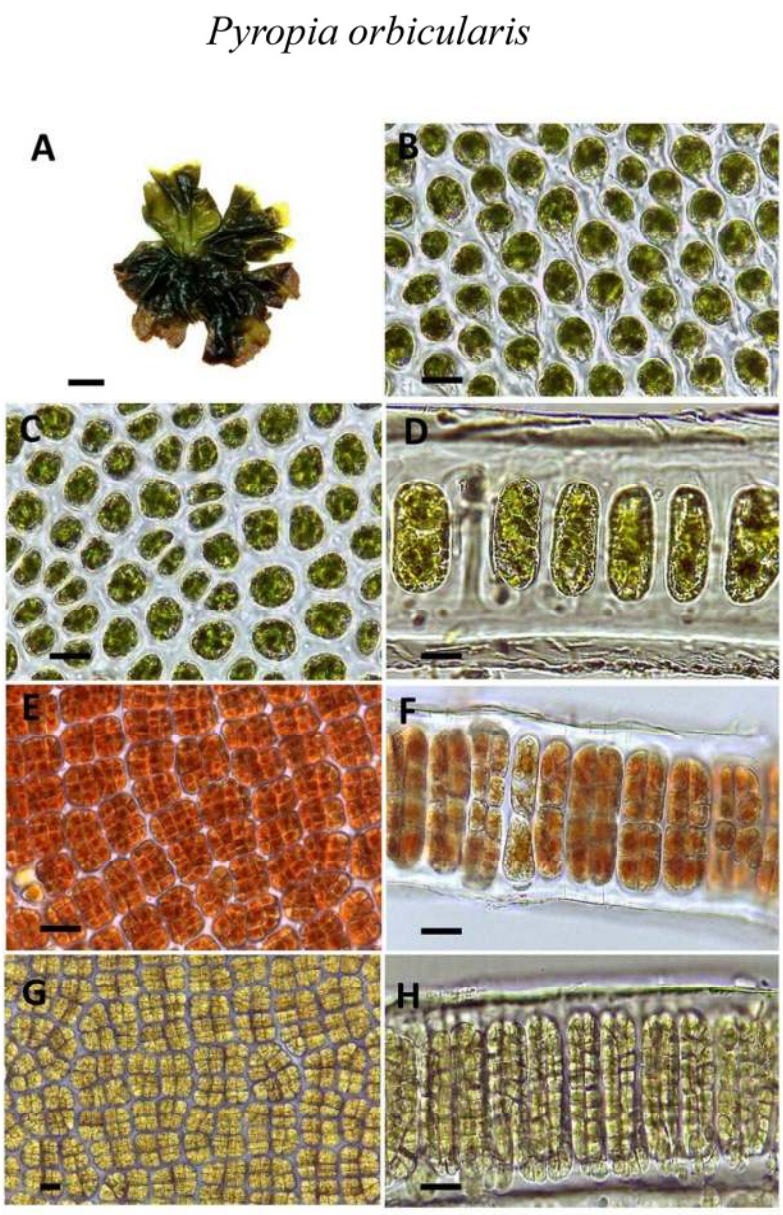


Table S1. Specimen collection information, voucher numbers and GENBANK accession numbers of *Pyropia* sp. CHK, *Porphyra* sp. CHC, *Porphyra* sp. CHE and *Pyropia orbicularis* from Maitencillo beach, Valparaíso, Chile, sequenced during this work.

Collection data	Sample code/ voucher number	GENBANK accession number	
		COI	*rbc*L
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, F.Castañeda	CHE0030/SGO168340	MH123947	-
08/09/2014. Collector: J. Zapata, L.Contreras-Porcia	CHE0036	MH123948	-
08/09/2014. Collector: J. Zapata, L.Contreras-Porcia	CHE0035	MH123949	MH124033
08/09/2014. Collector: J. Zapata, L.Contreras-Porcia	CHF0003	MH123950	MH124034
08/09/2014. Collector: J. Zapata, L.Contreras-Porcia	CHF0010	MH123951	MH124035
08/09/2014Collector: J. Zapata, L.Contreras-Porcia	CHK006p	MH123953	MH124037
08/09/2014Collector: J. Zapata, L.Contreras-Porcia	CHK009p	MH123954	MH124038
08/09/2014Collector: J. Zapata, L.Contreras-Porcia	CHK0011p	MH123952	MH124040
28/07/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK0001/SGO168324	MH123955	-
28/07/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK0002/SGO168325	MH123956	MH124041
28/07/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK0003/SGO168326	MH123957	-
28/07/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK0005	MH123958	-
28/07/2014. Collector: J. Zapata, L.Contreras-Porcia	CHK005b	MH123959	-

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

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

Table S2. Specimen collection information and GENBANK accession numbers of sequences of COI and *rbc*L 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.
Bangia atropurpurea	Devon, Sidmouth, UK	DQ442886
Bangia atropurpurea	Dorset, Kimmeridge, UK	DQ442887
Bangia fuscopurpurea	Rhode Island, Brenton Point., Newport, USA	JN028460
Bangia sp. 1BAN	British Columbia, Whiffen Spit, Sooke Harbour, Vancouver Island, Canada	JN028465
Bangia sp. 2BAN	Quebec, Escoumins (Rue des Pilotes), Canada	JN028476
Bangiales sp. HK-2011a	British Columbia, Ramsey Island (beach on NW coast), Gwaii Haanas, Canada	JN028481
Bangiales sp. HK-2011c	British Columbia, Ridley Island, Prince Rupert, Canada	JN028495
Boreophyllum birdiae	Newfoundland and Labrador, from Bonne Bay Station, Canada	JN028485
Fuscifolium sp. CHA	Región de Coquimbo, Choapa, Puerto Oscuro	KP781631
Porphyra corallicola	New Brunswick, Maces Bay, Bay of Fundy, Canada	JN028496
Porphyra dioica	Talmine, Sutherland, Scotland, UK	DQ191339
Porphyra sp. JB286	Sussex, Bracklesham Bay, England, UK	DQ191335
Porphyra mumfordii	British Columbia, Bamfield, Bradys Beach, Canada	JN028502
Porphyra mumfordi	Región de Los Lagos Hueihue, Chiloé, Chile	KP781651
Porphyra mumfordi	Región de Los Ríos, Los Molinos,Valdivia, Chile	KP781682
Porphyra purpurea	New Brunswick, Richebucto Cape Breakwater, Canada	JN028518
Porphyra rosengurttii	Faroe Islands	AM943399
Porphyra sp. 1FIH	Chacao, Chiloe, Chile	JN028551.1
Porphyra sp. CHB	Región de Los Lagos, Cucao, Chiloé	KP781663
Porphyra sp. CHC	Región de Los Ríos, Playa Rosada, Valdivia, Chile	KP781684
Porphyra sp. CHC	Región de Valparaíso, Petorca, Salinas de Pullay	KP781638/ KP781639
Porphyra sp. CHC	Región de la Araucanía, Cautin, Cheuque	KP781643
Porphyra sp. CHC	Región de Los Ríos, Pichicuyin, Valdivia, Chile	KP781644/ KP781645
Porphyra sp. CHD	Región de Magallanes, Magallanes, Buque Quemado	KP781673
Porphyra sp. CHF	Región de Antofagasta, Antofagasta, Playa El Lenguado	KP781562/ KP781563
Porphyra sp. CHF	Región de Coquimbo, Elqui, Guanaquerillos	KP781559/ KP781564/ KP781565
Porphyra sp. CHF	Región de Coquimbo, Caleta las Conchas, Choapa, Chile	KP781560
Porphyra sp. CHF	Región de Valparaíso, Quintay-Playa Chica, Valparaíso, Chile	KP781567
Porphyra sp. CHF	Región de Valparaíso, Playa Amarilla, Valparaíso, Chile	KP781689/ KP781647
Porphyra sp. CHF	Región de Valparaíso, Valparaíso, Curaumilla, Chile	KP781674/ KP781675/ KP781691/ KP781649
Porphyra sp. CHF	Región de Valparaíso, Valparaíso, Playa El Encanto, Chile	KP781690/ KP781692/ KP781693
Porphyra sp. CHF	Región de Valparaíso, San Antonio, Punta de Tralca, Chile	KP781568/ KP781569/ KP781570/ KP781571/ KP781572
Porphyra sp. CHF	Región de Valparaíso, San Antonio, Las Cruces, Chile	KP781573/

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

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

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

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

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

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

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

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

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

Pyropia sp. TCH	Torias Corner, Stewart I, NZ	GU046418
Pyropia sp. WRO	Punakaiki, Westland, South I, NZ	HQ687541
Pyropia sp. ZLI	Paternoster, South Africa	GU165839
Pyropia spiralis	Vila Velha, Espírito Santo, Brazil	HQ605696
Pyropia suborbiculata	Wonsando, Chungchungnamdo, Korea	HQ728201
Pyropia tanegashimensis	Iseki, Tanegashima, Kagoshima, Japan	HQ687542
Pyropia tenera	Kawaura, Kumamoto, Japan	HQ687543
Pyropia tenuipedalis	Urayasu, Chiba, Japan	EU521649
Pyropia torta	Vista Mar, San Juan Island, WA, USA	EU223236
Pyropia vietnamensis	Thangeseri (Kerala) India	HQ687544
Pyropia viridiidentata	Lyall Bay, Wellington, North I, NZ	EU521650
Pyropia yezoensis	Galmok, Tongyoung, Gyeongsangnamdo, Korea	HQ728197
Wildemania amplissima	Nosappu, Nemuro, Hokkaido, Japan	HQ687560
Wildemania norrisii	Harling Point, Victoria, BC, Canada	EU223212
Wildemania occidentalis	Cape Palmerston, Vancouver Island, BC, Canada	EU223118
Wildemania schizophylla	Van Damme State Park, Mendocino County, CA, USA	GU319871
Wildemania sp. Antar23	Admiralty Bay, King George Island, South Shetlands Archipelago, Antarctica	HQ605700
Wildemania sp. FII	Región de Magallanes, Magallanes, Buque Quemado	KP781817/ KP781818/ KP781820
Wildemania sp. FII	Hill cove kelp forest west of jetty, West Falkland, Falkland Is.	GU165883
Wildemania sp. HM080	Songjiho, Gangwondo, Korea	HQ728202
Wildemania variegata AK	Kagamil Island, AK, USA	EU223237
Wildemania variegata 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 Guillemin et al. (2016).

	Pyropia sp. CHJ	*Pyropia orbicularis*	*Pyropia* sp. CHK
Pyropia sp. CHJ	**0.008**		
Pyropia orbicularis	0.073	**0.006**	
Pyropia sp. CHK	0.038	0.063	**0.001**