Molecular Data Reveal the Presence of Three Plocamium Lamouroux Species with Complex Patterns of Distribution in Southern Chile

Alejandro Montecinos, Oscar R Huanel, María Ramírez, Marie-Laure Guillemin

MOLECULAR DATA REVEAL THE PRESENCE OF THREE *PLOCAMIUM* SPECIES WITH COMPLEX PATTERNS OF DISTRIBUTION IN SOUTHERN CHILE.

Alejandro E. Montecinos[1], Oscar R. Huanel[2,3], María E. Ramírez[4], Marie-Laure Guillemin[1,3].

[1]Instituto de Ciencias Ambientales y Evolutivas, Facultad de Ciencias, Universidad Austral de Chile, Casilla 567, Valdivia, Chile.

[2]Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Casilla 114-D, Santiago, Chile.

[3]CNRS, Sorbonne Université, UMI 3614, Evolutionary Biology and Ecology of Algae, Station Biologique de Roscoff, CS 90074, Place G. Tessier, 296888, Roscoff, France.

[4]Museo Nacional de Historia Natural, Área Botánica, Casilla 787, Santiago, Chile.

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Corresponding author: marielaure.guillemin@gmail.com

ABSTRACT

Plocamium is a widespread genus for which forty-five species are currently recognized. However, classical taxonomy, based only on morphological characters, is problematic within this genus. The use of molecular tools has uncovered cryptic genetic species, mistakenly grouped under the name of morphological species that are common and widespread (including the generitype *Plocamium cartilagineum*). The aim of this work was to evaluate the species diversity of *Plocamium* in southern Chile. For this purpose, three independent molecular markers were sequenced in samples collected from seven populations located between 41°S and 54°S. The species diversity was evaluated using phylogenetic reconstructions and two independent methods for species delimitation (ABGD and GMYC). The outcomes of each method were congruent, suggesting the presence of three species in southern Chile. One species, named *Plocamium* sp. 1, is restricted to Punta Guabún, the only locality sampled north of the biogeographic barrier of the 42°S. The other two species, *Plocamium* sp. 2 and 3 are distributed in sympatry in Patagonia and Tierra del Fuego. The three Chilean species form a clade phylogenetically close to sequences obtained from New Zealand and Australia and a divergence along the coasts of Chile after past transoceanic dispersal is proposed. We propose that divergence in glacial microrefugia could have subsequently happen in the southern part of the coast, this hypothesis being supported by the strong impact of glacial maxima on population dynamics, especially in *Plocamium* sp. 3.

DES DONNÉES MOLÉCULAIRES RÉVÈLENT LA PRÉSENCE DE TROIS ESPÈCES DE *PLOCAMIUM* PRESENTANT UN PATRON COMPLEX DE DISTRIBUTION DANS LE SUD DU CHILI

RESUMÉ

Quarante-cinq espèces sont actuellement reconnues dans le genre *Plocamium*, un genre présentant une très ample distribution. Cependant, la taxonomie classique, basée uniquement sur les caractères morphologiques, est problématique au sein de ce genre. L'utilisation d'outils moléculaires a permis de révéler l'existence d'espèces génétiques cryptiques, groupées par erreur sous un même nom, celui d'espèces morphologiques courantes et répandues (y compris l'espèce type du genre *Plocamium*: *Plocamium cartilagineum*). Le but de notre travail était d'évaluer la diversité d'espèces de *Plocamium* dans le sud du Chili. A cet effet, trois marqueurs moléculaires indépendants ont été séquencés pour des échantillons prélevés dans sept populations situées entre 41°S et 54°S. La diversité en terme d'espèces a été évaluée à l'aide de reconstructions phylogénétiques et de deux méthodes indépendantes de délimitation d'espèces génétiques (ABGD et GMYC). Les résultats des différentes méthodes sont congruents, suggérant la présence de trois espèces dans le sud du Chili. Une espèce, nommée *Plocamium* sp. 1, est limité à Punta Guabún, la seule localité échantillonnée au nord de la barrière biogéographique du 42°S. Les deux autres espèces, *Plocamium* sp. 2 et 3 sont distribuées en sympatrie en Patagonie et en Terre de Feu. Les trois espèces chiliennes forment un clade phylogénétiquement proche de séquences obtenues en Nouvelle-Zélande et en Australie et une divergence le long des côtes du Chili après un évènement historique de dispersion transocéanique passée pourrait expliquer ce résultat. Nous proposons

des phénomènes postérieurs de divergence en micro-refuges glaciaires comme moteur de la spéciation en Patagonie et en Terre de Feu. Cette hypothèse est étayée par le fort impact des maxima glaciaires sur la dynamique des populations, en particulier dans le cas de *Plocamium* sp. 3.

Mots clés: Délimitation d'espèces, espèces génétiques, dispersion à longue distance, spéciation, Rhodophyta, algues rouges.

INTRODUCTION

Plocamium Lamouroux is a cosmopolitan genus of red seaweed for which 45 species have been recognized to the date (Guiry & Guiry 2020). These species have been recorded from the Arctic to the Antarctic, in intertidal and subtidal waters (Wynne 2002). This genus has recently received more attention due its relevance in the production of bioproducts (Calegario *et al.* 2019), including anti-cancer molecules (Antunes *et al.* 2011; Alves *et al.* 2018), herbicides (Gressler *et al.* 2011; Pereira & Vasconcelos 2014) and molecules with anti-herbivore properties (San-Martin *et al.* 1991; Pereira & Costa-Lotufo 2012). Thus, the development of molecular studies allowing clear species recognition and a better understanding of the evolutionary history of this genus, are critical for subsequent research on these biomolecules of potential importance.

Traditional taxonomy of *Plocamium* species is based on the number of ramuli (i.e., small protrusions occurring along the thalli main or secondary axes) in alternating series, the width, colour, length, consistency of the thallus, the morphology of the lower ramulus, the arrangement of tetrasporangial structures and cystocarps (Simons 1964; Womersley 1971; South & Adams 1979; Gabrielson & Scagel 1989; Cremades *et al.* 2011). However, these morphological characters have been recognized as insufficient to describe and distinguish between some species (Yano *et al.* 2004; Saunders & Lehmkuhl 2005; Cremades *et al.* 2011). For example, the generitype species, *Plocamium cartilagineum* (Linnaeus) P.S.Dixon is purportedly very widespread, being recorded in the North Atlantic, eastern and western North Pacific, northern Arabian Sea, Australia, New Zealand, Antarctica and Chile (Bischoff-Basmann & Wiencke 1996; Wynne 2002). However, molecular studies have demonstrated that specimens named as

105 *P. cartilagineum* based on morphological characters actually represented various
106 cryptic species that could be easily distinguished genetically. For example, in a
107 study using molecular nuclear marker LSU sequences for numerous
108 morphological specimens of *P. cartilagineum* from northern Europe, four genetic
109 cryptic species were revealed (Saunders & Lehmkuhl 2005). Moreover, another
110 case of cryptic genetic species has been reported using the molecular marker
111 5P-COI, in individuals of *P. angustum* (J.Agardh) J.D.Hooker & Harvey from
112 Australia and New Zealand (Cremades *et al.* 2011).

113 Contrasting with *P. cartilagineum* and *P. angustum*, some taxonomically
114 recognized species, as for example *P. nanum* G.W.Saunders & Lehmkuhl, show
115 restricted distributions (Saunders & Lehmkuhl 2005) probably linked to the
116 presence of biogeographical barriers. Biogeographical barriers are zones defined
117 by rapid changes in biota that can act as barriers to migration (Dawson 2001). In
118 the marine realm, biogeographical barriers have often been associated with
119 landscape features, such as the presence of strong currents or topographical
120 features (e.g., sandy beaches, river mouth, sea mount) limiting gene flow
121 between populations and have been reported as important drivers of speciation,
122 especially in taxa presenting low dispersal capacity (Avise 2000; Kuo & Avise
123 2005). Deep phylogeographic discontinuities, congruent with biogeographic
124 barriers, have commonly been encountered in widespread species with
125 distributions encompassing various biogeographic areas (Dawson 2001; Hurt *et*
126 *al.* 2009). For example, numerous studies have uncovered deep genetic
127 divergence in coastal marine taxa that coincide with recognized transition zones,
128 such as the California transition zone (Dawson 2001; Kelly & Palumbi 2010) and
129 the 30°S-33°S area located along the Chilean coast (Tellier *et al.* 2009;

Montecinos *et al.* 2012; Haye *et al.* 2014). These transition zones have been related to the effect of historical processes, mostly linked to eustatic or climatic changes associated with Pleistocene glacial cycles (Avise 2000). In the case of *Plocamium*, various widespread morphological species, encompassing more than one biogeographic area, have been reported. In these taxa, the use of molecular markers and genetic species delimitation approaches can help in reevaluating species diversity and distribution.

The Chilean coast is subdivided in three major biogeographical regions (Camus 2001): the Peruvian Province (PP), located from Peru to a southern limit around 30-33°S on the northern coast of Chile; the Magellanic Province (MP) extending from Cape Horn (56°S) north to 41-42°S (Chiloé Island) and an Intermediate Area (IA) limited by the PP and MP provinces. These biogeographical regions are characterized by distinct biota from warm-temperate in PP to cold-water and sub-Antarctic species in MP. The IA is characterized by a gradual overlap of biota characteristic of the other two provinces (Camus 2001). Phylogeographic breaks concordant with the biogeographic limit at 41°S - 42°S have been reported for various marine or coastal species, such as *Acanthina monodon* Pallas, a brooding gastropod, (Sánchez *et al*. 2011) and the Patagonian otter *Lontra provocax* Thomas (Vianna *et al.* 2011). The coastline north of 42°S is continuous, linear and dominated by rocky shores only intersected by a few small rivers and sandy beaches, while a high density of islands, fjords and channels, influenced by sub-Antarctic oceanographic and climatic conditions, characterizes the shoreline south of 42°S. These distinct coastal morphologies are the results of major topographic transformations due to interglacial/glacial cycles during the Pliocene and Pleistocene in southern Chile (Mercer 1976; McCulloch *et al.* 2000).

During glacial maxima, in particular during the Last Glacial Maximum (LGM, 20,000 years ago), ice sheets covered a broad region of southern Chile from the Chiloé Island at 41°S to Cape Horn at 56°S (Hulton *et al.* 2002; Saillard *et al.* 2009). Glacial periods were characterized by the retraction of the temperate biota in glacial refugia while interglacial periods were characterized by range expansion of these species in areas previously covered by ice.

Along the Chilean coast, three species of *Plocamium* identified using morphology have a distribution range that span more than one Chilean bioregion: *P. cartilagineum* (in the Peruvian Province, the Intermediate Area and the Magellanic Province), *P. secundatum* (Kützing) Kützing (in the Peruvian and Magellanic Province), and *P. pacificum* Kylin (in the Peruvian Province and Intermediate Area) (Etcheverry 1986; Ramírez & Santelices 1991; Ramírez 2010). In addition to the extensive distribution of these morphological species, cryptic species have already been reported in *P. cartilagineum* (Saunders & Lehmkuhl 2005; Cremades *et al.* 2011), casting doubts about *Plocamium* diversity and distribution in Chile. The present work aims to explore *Plocamium* species diversity and evolutionary history in southern Chile (41°S-54°S) using genetic markers encompassing the three cell compartments (i.e., mitochondria, chloroplast and nucleus). Using these molecular data sets, we applied different methods to delimit species (as conceptualized by the phylogenetic species concept; de Queiroz 1998) and improve knowledge on *Plocamium* diversity. We hypothesized that genetic species, if existent, will show distributions limited by the biogeographic barrier at 42°S and that species encountered in Patagonia and Tierra del Fuego will present historical population dynamics (i.e., contraction

during the LGM and recent expansion) strongly affected by the glacial/interglacial cycles.

MATERIALS AND METHODS

Sampling

A total of 107 individuals of *Plocamium* spp. were collected from 7 localities (Table 1). One locality was located north of the 42°S (i.e., Punta Guabún) and six localities were located south of the 42°S (i.e., San Gregorio, Parque Chabunco, Faro Porvenir, Los Canelos, Faro San Isidro and Fiordo Yendegaia). Intertidal samplings were conducted during diurnal low tide in Los Canelos and Fiordo Yendegaia while subtidal samplings were done by means of SCUBA diving in the rest of the localities studied. Each sample corresponds to a single frond cut from an isolated holdfast.

In the field, all the samples were first named '*P. cartilagineum*'. However, more precise observations in the lab show that some individuals from the southern part of the country presented a ramification pattern not fully congruent with the one expected for *P. cartilagineum* (slender ramuli, not all ramification unilateral, very bushy in appearance). In the same way, samples from Punta Guabún did not present the typical *P. cartilagineum* morphology. These small plants were characterized by an intense red color with sympodial branching characterized by profuse ramifications almost from the base and third and fourth order branches arranged in a scalloped manner. Because of these slightly distinct morphologies and since only a few mature tetrasporophytes were sampled we choose to refer to all individuals studied here as *Plocamium* spp. For most individuals (i.e., 96)

only small tissue fragments were conserved in silica and no observation in the lab could be made. The 11 samples guarded as voucher specimens are housed in the herbarium of the National Museum of Natural History, Chile (SGO, see voucher numbers in Supplementary Table 1)

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DNA extraction, PCR amplification, sequencing and sequence alignment

Dry tissues were ground by hand in liquid nitrogen and DNA was extracted using extraction kit E.Z.N.A.® Poly-Gel DNA Extraction (Omega Bio-Tek Inc., Norcross, USA). Three independent genetic markers were used to provide complementary species identification based on molecular criterion: a partial sequence of the mitochondrial Cytochrome c Oxidase I gene (5P-COI); a partial sequence of the plastid gene *rbc*L, encoding the large subunit of the ribulose-1,5-bisphosphate (*rbc*L) and the partial large subunit ribosomal RNA gene (LSU). The amplification of 5P-COI was performed, for all specimens sampled, using the primers developed by Saunders (2005). The amplification of the *rbc*L marker was performed for a subset of samples (n = 18) using the primers developed by Hommersand *et al.* (1994). The LSU was amplified as three overlapping fragments with previously published primer combinations (Harper & Saunders 2001), in a subset of five samples. For the 5P-COI, PCR were performed using conditions described in Dubrasquet *et al.* (2018) while *rbc*L and LSU markers were amplified using conditions described in Hommersand *et al.* (1994) and Harper & Saunders (2001), respectively. For both *rbc*L and LSU genes, sub samples included individuals from the three genetic groups recovered by the 5P-COI (more details in the results section below). PCR products were purified using commercial kit E.Z.N.A.® DNAProbe Purification (Omega Bio-Tek Inc., Norcross,

USA) and sequenced with primers used for amplification at the AUSTRAL-omics Core-Facilities (Valdivia, Chile). Sequences were aligned manually using Mega X (Kumar *et al.* 2018) and checked by eye; only traces with high quality values and no ambiguities were retained for further analyses.

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Phylogenetic analyses

To explore phylogenetic relationships between *Plocamium* species, 250 5P-COI sequences from the genus available in GenBank were added to our data set. In the same way, 24 and 20 sequences from GenBank were added to the *rbc*L and LSU data sets, respectively. Phylogenetic analyses were conducted independently for each gene, using both Maximum Likelihood (ML) and Bayesian Inference (BI) methods. ML analyses were performed using W-IQ-Tree (Trifinopoulos *et al.* 2016). The best-fit substitution model was selected using the Bayesian Information Criterion (Kalyaanamoorthy *et al.* 2017) implemented in W-IQ-Tree. The selected model was K3Pu+F+I+G4 for the 5P-COI, TN+F+I+G4 for the *rbc*L and GTR+F+I+G4 for the LSU. BI analyses were conducted using MrBayes v3.2.7 (Ronquist *et al.* 2012). Two independent analyses were run using, for each one, four chains and 20 million generations. Trees and parameters were sampled every 1,000 generations and the default parameters for temperature and branch swapping were used. The first 20% of the sampled trees were discarded as “burn-in” to ensure stabilization. The remaining trees were used to compute a consensus topology and posterior probability values. The split frequency (variance among the four independent runs) was below 0.005, confirming that the posterior probability distribution was accurately sampled.

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253 Delimitation of genetic species

254 To evaluate the existence of genetic species, two independent analyses were
255 conducted using the 5P-COI dataset. First, the Automatic Barcode Gap
256 Discovery (ABGD) was remotely run at
257 https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html. ABGD identifies a value
258 separating the frequency distribution of intraspecific pairwise genetic distances
259 from the ones of interspecific pairwise genetic distances, even when they overlap,
260 and use it as a threshold to delimit species (Puillandre *et al.* 2012). We computed
261 Kimura two-parameter (K2P) genetic distances and used default ABGD settings.
262 Second, a General Mixed Yule Coalescent (GMYC) analysis was run. GMYC
263 identifies a threshold value for the shift in branching rate from coalescent lineage
264 branching to interspecific diversification on an ultrametric tree and explicitly
265 delimits "independently evolving" clusters (i.e., putative species; Pons *et al.* 2006;
266 Monaghan *et al.* 2009). Before the analysis, duplicated haplotypes were removed
267 from the data set using DnaSP v6.12.03 (Rozas *et al.* 2017). Branch lengths were
268 estimated under a relaxed log-normal clock using the Bayesian analysis
269 implemented in BEAST v1.10.4 (Suchard *et al.* 2018). A coalescent (constant
270 size) prior was used and Markov Chains Monte Carlo (MCMC) were run for 20
271 million generations. Trees were sampled each 1,000 generations with a 10%
272 burn-in. A visual inspection of MCMC progression using Tracer v1.7.1 (Rambaut
273 *et al.* 2018) was performed to corroborate stabilization. An ultrametric tree was
274 constructed using Tree Annotator v1.10.4 (Rambaut & Drummond 2018). Since
275 the multiple-thresholds approach tends to overestimate the number of delineated
276 species (Fujisawa & Barraclough 2013) only the single-threshold (Pons *et al.*

2006) versions of GMYC was fitted on the ultrametric tree using the SPLITS v1.0-19 package for R (https://r-forge.r-project.org/projects/splits/).

Genetic diversity, network reconstruction and historical demography

Within each genetic species, defined using ABGD and GMYC, four diversity indices were calculated for the 5P-COI gene using DnaSP v6.12.03 (Rozas *et al.* 2017): the number of haplotypes (nH), the number of polymorphic sites (S), gene diversity (H, Nei 1987) and nucleotide diversity (π, Nei & Li 1979). Moreover, within each genetic species, haplotype networks were reconstructed for the 5P-COI using the median-joining algorithm implemented in NETWORK v10.1.0.0 (Bandelt *et al.* 1999).

Finally, to evaluate changes in the demographic history of the genetic species, two complementary approaches were used to infer the historical demography using the 5P-COI dataset. First, Tajima's D (Tajima 1989) and Fu's Fs (Fu 1997) statistics were calculated to detect significant past changes in population size. Significant departure from selection-drift equilibrium was tested using 1,000 bootstrap replicates in Arlequin v3.5.2.2 (Excoffier & Lischer 2010). Under the presumption of neutrality, negative values distinguish populations in expansion while positive values, associated to the loss of rare alleles, are considered as signature of recent bottleneck (Tajima 1989; Fu 1997). Second, the observed mismatch distributions of the number of differences between pairs of 5P-COI sequences were compared to estimated values under a model of demographic expansion (Roger & Harpending 1992) using Arlequin v3.5.2.2 (Excoffier & Lischer 2010). Multimodal distributions generally characterize populations in

demographic equilibrium while unimodal distributions are associated with recent expansion.

RESULTS

A total of 107 sequences of *Plocamium* spp. collected from 7 localities were obtained for the 5P-COI (575bp; Table 1). Moreover, a sub sample of 18 and 5 individuals were sequenced for the *rbc*L (641bp) and the LSU (2960bp), respectively (Table 1). GenBank accession numbers for the three molecular markers sequenced are available in the Supplementary Table 1.

Phylogenetic relationships

Phylogenetic relationships based on 5P-COI, for both the ML and BI analyses, recovered all specimens sequenced in the present study as a single, well-supported monophyletic group, strongly divergent from all the other sequences of *Plocamium* available in GenBank (Fig. 1). This Chilean clade appear as nested within a poorly resolved group including most specimens sampled in Australia and New Zealand, and as sister to a well-supported monophyletic group composed by specimens of *P. cartilagineum* from New Zealand, *P. patagiatum* J.Agardh from Australia and *P. angustum* from Australia and New Zealand (Fig. 1). It is interesting to note that specimens sampled within the same ocean tend to be genetically related (Fig. 1). The Chilean clade was also recovered as sister of South Hemisphere *Plocamium* species for the *rbc*L (i.e., as sister to KC174809,

U21703, U26821 and HQ224543 from New Zealand, Fig. S1) and for the LSU (as sister to AY881712 and AY881714 from Australia, Fig. S2). Finally, Chilean sequences from the present study form three monophyletic lineages observed for the three genetic markers used (Fig. 1, Fig. S1, Fig. S2). Whatever the gene under study, these lineages were generally well supported for both the ML and BI analyses (Fig. 1, Fig. S1, Fig. S2).

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Species delimitation

Genetic pairwise K2P distances for the 5P-COI ranged from 0 to 0.062 and the ABGD located the barcode gap within the 0.010–0.030 distance range (Fig. S3b). Primary partitions using this threshold suggested the existence of three genetic groups (Fig. 2, Fig. S3a). The likelihood of the GMYC model for the single threshold model was (LGMYCsingle = 44.04); a value significantly higher than the one obtained for the null model (L0 = 40.86). The number of partitions obtained for the GMYC was three, with confidence limits of three to five (Fig. 2, Fig. S4). The three monophyletic groups recovered using phylogenetic reconstructions (Fig. 1, S1 and S2) were supported as putative species by both the ABGD and the GMYC single-threshold results (Fig. 2). Moreover, values of Kimura 2-parameter (K2P) were more than ten times higher when measured between genetic species (*Plocamium* sp. 1 - *Plocamium* sp. 2 = 0.05361 ± 0.00936; *Plocamium* sp. 1 - *Plocamium* sp. 3 = 0.03063 ± 0.00684; *Plocamium* sp. 2 - *Plocamium* sp. 3 = 0.04652 ± 0.00865) than between haplotypes sequenced within a single genetic species (within *Plocamium* sp. 1 = 0.00019 ± 0.00019; within *Plocamium* sp. 2 = 0.00040 ± 0.00014; within *Plocamium* sp. 3 = 0.00144 ± 0.00079). The three putative genetic species of *Plocamium* from

southern Chile were then named *Plocamium* sp. 1, *Plocamium* sp. 2 and *Plocamium* sp. 3. *Plocamium* sp. 1 was restricted to Punta Guabún, the only locality sampled north of the 42ºS in the present study, while the two other species were distributed in sympatry in southern Chile (Fig. 3). The two species *Plocamium* sp. 2 and *Plocamium* sp. 3 were collected at the same sites in both intertidal (i.e., Los Canelos and Fiordo Yendegaia) and subtidal (i.e., San Gregorio, Faro Porvenir and Faro San Isidro) (Fig. 3). The 19 samples from Parque Chabunco were identified as *Plocamium* sp. 3.

Genetic diversity, haplotype network and demographic history.

For the 5P-COI data set the number of haplotypes (nH) and number of polymorphic sites (S) were the lowest in *Plocamium* sp. 1 (nH = 2; S = 1) and the highest in *Plocamium* sp. 3 (nH = 6; S = 6; Table 2). The highest values of genetic and nucleotide diversity were encountered in *Plocamium* sp. 2 ($H = 0.684 \pm 0.099$ and $\pi = 0.00144 \pm 0.00111$), while the lowest values were encountered in *Plocamium* sp. 1 ($H = 0.118 \pm 0.101$ and $\pi = 0.00019 \pm 0.00048$) (Table 2). *Plocamium* sp. 2 presented a haplotype network slightly more reticulated than the two other species, for which star-like type of networks were observed (Fig. 3). In the haplotype network *Plocamium* sp. 2 was connected to *Plocamium* sp. 3 by 25 mutational steps, while *Plocamium* sp. 1 was connected to *Plocamium* sp. 2 by 18 mutational steps. Whatever the species under study, all values of Tajima's D and Fu's Fs statistics were negative, but these were significant only for *Plocamium* sp. 3 (Table 3). Mismatch distributions were unimodal with the most commonly calculated number of differences between pairs of sequences equal to 0 in *Plocamium* sp. 1 and *Plocamium* sp. 3 and to 1 in *Plocamium* sp. 2 (Fig.

4). Tests for goodness-of-fit based on the sum of square deviations (SSD) for the demographic expansion model give values ranking from 0.00012 (p-value = 0.38400) for *Plocamium* sp. 1 and 0.00028 (p-value = 0.34400) for *Plocamium* sp. 3 up to 0.01945 (p-value = 0.23400) for *Plocamium* sp. 2 (Table 3). These results did not reject the null hypothesis of a population expansion in any of the three genetic species studied.

DISCUSSION

Our study revealed the existence of three genetic species of *Plocamium* in southern Chile, with *Plocamium* sp. 2 and *Plocamium* sp. 3 located in sympatry in Patagonia and Tierra del Fuego while *Plocamium* sp. 1 was only encountered north of the biogeographical limit at 42°S. Interestingly, phylogenetic analyses recovered the Chilean clade as sister to a well-supported monophyletic group composed by specimens from New Zealand and Australia, suggesting the occurrence of transoceanic dispersal in the past. On the other hand, various paraphyletic taxa were observed within *Plocamium* phylogenetic trees (e.g., *P. cartilagineum, P. patagiatum, P. angustum, P. fimbriatum* M.J.Wynne*, P. violaceum and P. pacificum*; Fig. 1), clearly pointing out the difficulty of species identification based on morphological characters in this genus (Cremades *et al.* 2011). Concordance across results obtained with different methods (here GMYC and ABGD) and monophyly recovered in trees reconstructed with unlinked markers are now widely acknowledged as suitable for genetic species delimitation (Carstens *et al.* 2013; Modica *et al.* 2014). Our results confirm the relevance of information obtained from molecular markers encompassing the three cell compartments (i.e., mitochondria, chloroplast and nucleus) to delimit

399 species in the genus *Plocamium* and better estimate species diversity,
400 distribution and understand the evolutionary history in these highly
401 morphologically variable red algae.

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403 Transoceanic dispersal as potential origin of Chilean *Plocamium* species
404 ancestral clade

405 In the phylogenetic trees, the three Chilean *Plocamium* species formed a clade
406 embedded in sequences from the Southern Hemisphere and genetically close to
407 clades from New Zealand and Australia. We propose that the Chilean *Plocamium*
408 clade has diverged from Australian or New Zealand colonists, after their arrival
409 by rafting in Chile. Transoceanic dispersal has commonly been reported,
410 especially in taxa with high capacity of dispersal, for example fish (Blower *et al.*
411 2012) or crustacea (Page *et al.* 2005). Recurrent dispersal, more than vicariance,
412 has indeed been postulated to be the mechanism leading to the biogeographic
413 patterns and disjunct species distributions observed nowadays in the Southern
414 Ocean (Waters 2008; Fraser *et al.* 2013). The importance of transoceanic rafting
415 is less recognized in marine species with limited dispersal capacity (i.e., lack of
416 larvae or short lived propagules) even if recent studies have demonstrated that
417 this mechanism, depending on the species physiological/reproductive tolerance,
418 could be highly efficient, allowing rapid expansion of their distribution ranges
419 (Thiel & Gutow 2005a; 2005b; Fraser *et al.* 2011; Fraser *et al.* 2013; Waters *et*
420 *al.* 2013; Guillemin *et al.* 2014; Guillemin *et al.* 2016; Tala *et al.* 2019). In the
421 Southern Hemisphere, currents are dominated by the Antarctic Circumpolar
422 Current (ACC) and the West Wind Drift (WWD) (Waters 2008). Recurrent
423 dispersal from Australia and/or New Zealand to Chile have been registered using

molecular data in various macroalgae including the buoyant seaweed *Durvillaea antarctica* (Chamisso) Hariot (Fraser *et al.* 2009), but also the non-buoyant species *Bostrychia intricata* (Bory) Montagne*, Adenocystis utricularis* (Bory) Skottsberg (Fraser *et al.* 2013), *Capreolia implexa* Guiry & Womersley (Boo *et al.* 2014) and *Agarophyton chilense* (C.J.Bird, McLachlan & E.C.Oliveira) Gurgel, J.N.Norris et Fredericq (as *Gracilaria chilensis* C.J.Bird, McLachlan & E.C.Oliveira; Guillemin *et al.* 2014). All these species show genetic signatures of recent west to east dispersal across vast oceanic distances. In the case of the genus *Durvillaea* Bory there is evidence of a long-distance dispersal event from New Zealand to temperate Chile that was followed by genetic divergence leading to the speciation of *D. incurvata* (Suhr) Macaya (a species restricted to Chilean temperate waters) some 3 – 10 Million years ago (Fraser *et al.* 2013; Fraser *et al.* 2019). Studies in other organisms, as in the coastal sac spiders of the genus *Amaurobioides* O. Pickard-Cambridge, show repeated events of long-distance dispersal along the WWD followed by divergence, revealing a remarkable pattern of "stepping-stone" speciation all around the Southern Ocean (Ceccarelli *et al.* 2016).

Speciation in the genus *Plocamium* along the coast of southern Chile

After transpacific colonization, distinct processes of divergence seem to have taken place during the radiation of the genetic Chilean *Plocamium* species ancestral clade. The species *Plocamium* sp. 1 is found isolated north of the biogeographical limit of 42°S and we propose that parapatric or allopatric speciation could have taken place in this case. A strong biogeographic discontinuity has been described at 41-42°S (Camus 2001), generally related to

449 the latitudinal migration of the southern Westerlies during the Miocene-
450 Pleistocene. The split of the WWD into the northward Humboldt Current and the
451 southward Cape Horn Current, located at these latitudes, has been demonstrated
452 to represent a major oceanic barrier that has contributed to the origin of the
453 biogeographic break. Nowadays, contrasted ecologic, climatic and topographic
454 features characterize both sides of the 41-42°S biogeographical limit (Camus
455 2001). In the past, major currents restricting gene flow could have led to the
456 divergence of *Plocamium* sp. 1 from *Plocamium* sp. 2 and 3; while more subtle
457 differences in term of coastal topography or salinity could also help in maintaining
458 these species genetic integrity nowadays. Our sampling does not allow
459 separation of patterns of allopatric from parapatric speciation nor to precisely
460 pinpoint the phylogeographic break in *Plocamium* (i.e., the gap between IA and
461 MP sampling sites span more than 1,200 kilometers). However, other genetic
462 studies described a phylogeographic break at the 41°S-42°S zone or nearby (the
463 buoyant kelps *Durvillaea spp.*, Fraser *et al.* 2010; in the brooding gastropod,
464 *Acanthina monodon*, Sánchez *et al.* 2011; the Patagonian otter *Lontra provocax,*
465 Vianna *et al.* 2011). Theoretical studies emphasize the possible quick genetic
466 divergence that could be observed in scenarios of parapatric speciation in
467 organisms with low dispersal capacity, as is the case for *Plocamium,* (Gravilets
468 *et al.* 2000; Kuo & Avise 2005). Moreover, parapatric speciation has been
469 suggested as a common mechanism of speciation in macroalgae along the
470 Chilean coast (brown algae: *Lessonia trabeculata* Villouta & Santelices and *L.*
471 *spicata* (Suhr) Santelices, as *L. nigrescens* Bory in Tellier *et al.* 2009; red alga:
472 *Mazzaella laminarioides* (Bory) Fredericq, Montecinos *et al.* 2012).

473 Contrasting with *Plocamium* sp. 1, the species *Plocamium* sp. 2 and 3 were in
474 sympatry in most localities sampled in Patagonia and Tierra del Fuego. Existence
475 of cryptic genetic species or diverged haplotypic groups distributed in sympatry
476 in southern Chile have already been reported in three macroalgae: *Adenocystis*
477 *utricularis*, *Bostrychia intricata* (Fraser *et al.* 2013) and *Iridaea cordata* (Turner)
478 Bory (Ocaranza *et al.* 2019). In these cases, divergence in sympatry or micro-
479 allopatry could be hypothesized. Exhaustive surveys have revealed common
480 patterns of genetic divergence, consistent with isolation in refugia during glacial
481 periods, in various organisms (Hewitt 2004; Sérsic *et al.* 2011). During the
482 Pleistocene glacial/interglacial cycles populations of temperate species could
483 have survived in isolated microrefugia in Patagonia and Tierra del Fuego (for
484 example, terrestrial organisms: Sérsic *et al.* 2011; freshwater fish: Zemlak *et*
485 *al.* 2010). In the region, during the glacial maxima, *Plocamium* populations could
486 have survived in small pockets of suitable habitat located at the edge of, or even
487 within, glaciated areas (Rull 2009; Mosblech *et al.* 2011). During isolation periods
488 (i.e., glacial maxima), divergence between microrefugia could be favored by drift
489 and/or selection. After interglacial expansion from refugia, differentiated genetic
490 groups or species (e.g., *Plocamium* sp. 2 and 3) could then be observed in
491 sympatry in localities where secondary contact takes place (Zemlak *et al.* 2008;
492 Zhang *et al.* 2008; Durand *et al.* 2009). Supporting the impact of
493 glacial/interglacial cycles on marine Chilean species living at high latitude, our
494 results suggest that both *Plocamium* sp. 2 and 3 have been affected by these
495 cycles but that the bottleneck and demographic expansion is more recent in
496 *Plocamium* sp. 3 (i.e., bottleneck probably linked to the LGM) than in *Plocamium*
497 sp. 2. Strong ice impact during the LGM and recent demographic expansion has

also been observed in other Patagonian macroalgae as *Mazzaella laminarioides* (Montecinos *et al.* 2012), *Gigartina skottsbergii* Setchell & N.L.Gardner (Billard *et al.* 2015) and *Durvillaea antarctica* (Fraser *et al.* 2009).

Various cryptic species, incorrectly named *P. cartilagineum* using morphological characters, are present in distinct parts of the Southern Hemisphere. One interesting example is the presence of *P. cartilagineum* sequences from individuals collected in Antarctica forming a genetic group fairly distinct from *Plocamium* sp. 1, 2 and 3 from Chile (see Fig. 1). Similar results were obtained for *Iridaea cordata* (Ocaranza *et al.* 2019) and *Gigartina skottsbergii* (Billard *et al.* 2015), where cryptic sister species were encountered on both side of the Drake Passage. The main difference between *Plocamium* and *Iridaea cordata* and *Gigartina skottsbergii* is that Antarctic and Chilean species are not sister species in the case of *Plocamium*. Complex patterns of long distance colonization followed by speciation seem to characterize Southern Hemisphere macroalgae; with some colonization routes following the main currents (ACC and WWD; *Durvillaea*: Fraser *et al.* 2019; *Trematocarpus* Kützing and *Mazzaella* G.De Toni f.: Hommersand & Fredericq 2003; *Plocamium*: present study) and some crossing them (ACC; *Iridaea cordata* and *Gigartina skottsbergii*: Hommersand & Fredericq 2003; Billard *et al.* 2015; Ocaranza *et al.* 2019). More efforts are needed to understand the evolutionary history of *Plocamium* in the Southern Hemisphere including the sub-Antarctic Islands and the coasts of the Antarctic Peninsula. Moreover, further work increasing the number of sites sampled (especially in the PP and the IA) could help to better understand *Plocamium* species diversity in Chile and to study speciation processes in this ecologically important group of red algae.

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DATA SHARING AND DATA ACCESSIBILITY

The authors confirm that all data underlying the findings are fully available without restriction. All sequences are available in GenBank (accession numbers in Supplementary Table 1).

COMPETING INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTION

MLG conceived the study. AEM and ORH generated molecular data sets. AEM performed molecular and statistical analyses. AEM and MLG drafted the manuscript. MER deposited the individuals into the museum (SGO) and obtained the vouchers code. All authors contributed to discussions resulting in the final manuscript.

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859 TABLES

860 Table 1 – Sampling localities and number of individuals sequenced for the three
861 molecular markers used in the present study. Abbreviation (CODE) and
862 geographic coordinates are indicated.

Locality	CODE	Coordinates	5P-COI	*rbc*L	LSU
Punta Guabún	GUA	41°38'S/74°02'W	17	6	2
San Gregorio	GRE	52°33'S/70°02'W	2	-	-
Parque Chabunco	CHA	52°59'S/70°48'W	19	-	-
Faro Porvenir	POR	53°18'S/70°27'W	18	-	-
Los Canelos	CAN	53°28'S/70°11'W	9	1	1
Faro San Isidro	FSI	53°46'S/70°58'W	24	2	1
Fiordo Yendegaia	YEN	54°54'S/68°42'W	18	9	1
		TOTAL	107	18	5

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864

865 Table 2 – Genetic diversity estimates for the molecular marker 5P-COI. Each
866 *Plocamium* genetic species, as delimited by ABGD and GMYC, was treated
867 separately.

Species	N	nH	H	SD	π	SD	S
Plocamium sp. 1	17	2	0.118	0.101	0.00019	0.00048	1
Plocamium sp. 2	17	5	0.684	0.099	0.00144	0.00111	4
Plocamium sp. 3	73	6	0.133	0.054	0.00026	0.00093	6
TOTAL	107						

868 N: number of sequences; nH: number of haplotypes; H: gene diversity; π:
869 nucleotide diversity; S: number of polymorphic sites; SD: Standard deviation.

870870

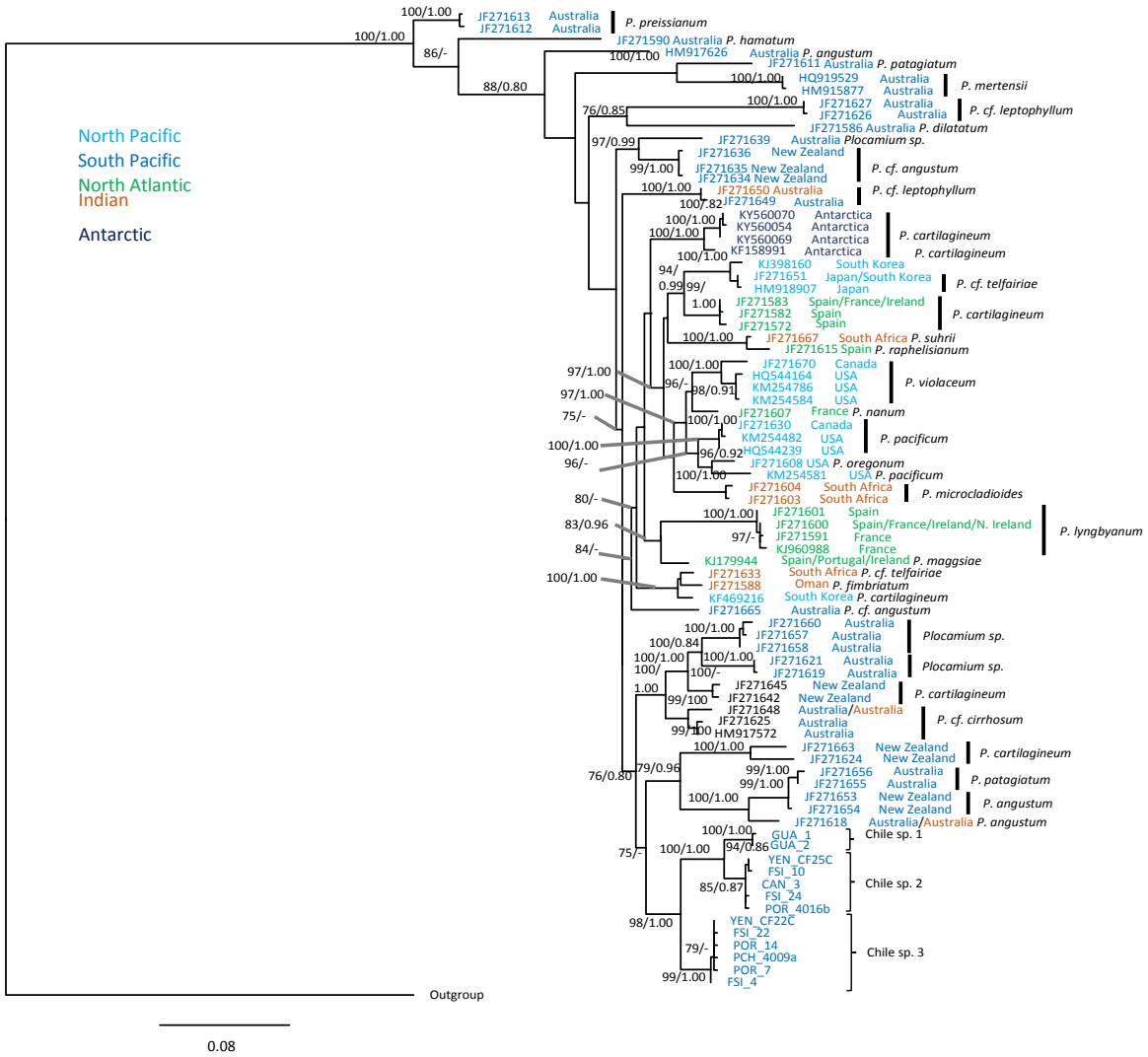
871 Table 3 – Neutrality tests calculated using the 5P-COI marker data set. Results
872 are given separately for each *Plocamium* genetic species (as delimited by ABGD
873 and GMYC).

874874

Species	Tajima's D	p-value	Fu's Fs	p-value	SSD	p-value
Plocamium sp. 1	-1.16387	0.14500	-0.74844	0.07800	0.00012	0.38400
Plocamium sp. 2	-0.74003	0.26300	-1.61645	0.08000	0.01945	0.23400
Plocamium sp. 3	-2.16969	0.00000	-7.46656	0.00000	0.00028	0.34400

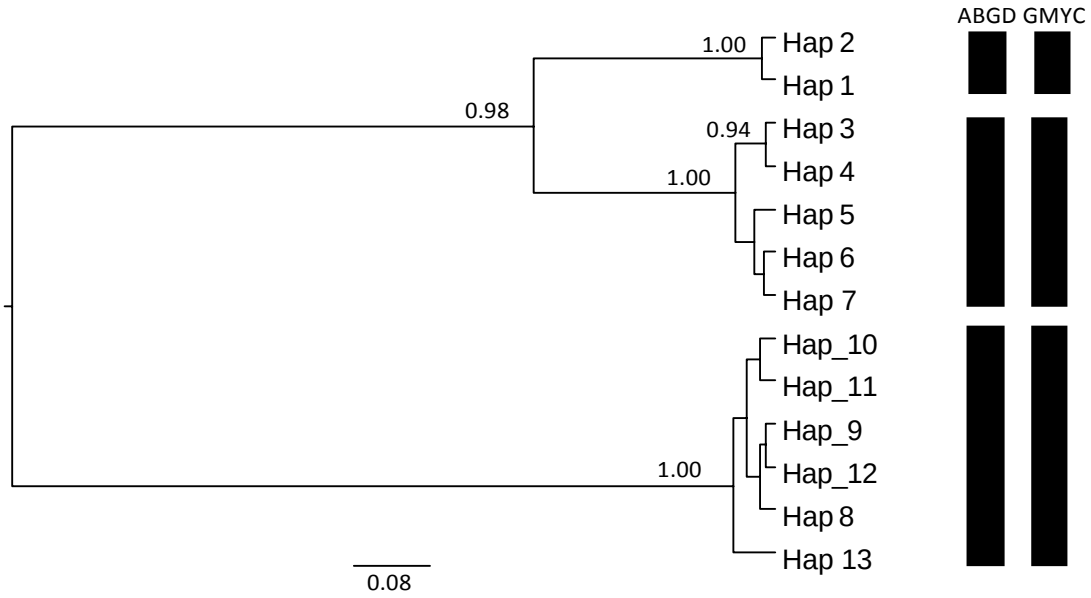
875 SSD: Sum of square deviations.

876 FIGURES



8778
7
7

878 Figure 1 - Maximum likelihood (ML) phylogram of the genus *Plocamium* based
879 on 5P-COI sequences. ML bootstrap (BS)/Bayesian posterior probability (PP)
880 values are shown above each branch and only values superior to 75 and 0.75,
881 respectively, are given. Colors correspond to oceans where individuals
882 sequenced where sampled. Species names, as reported in GenBank, are given
883 on the right. Outgroup corresponds to *Asparagopsis armata* (GenBank
884 accession: KJ960344).



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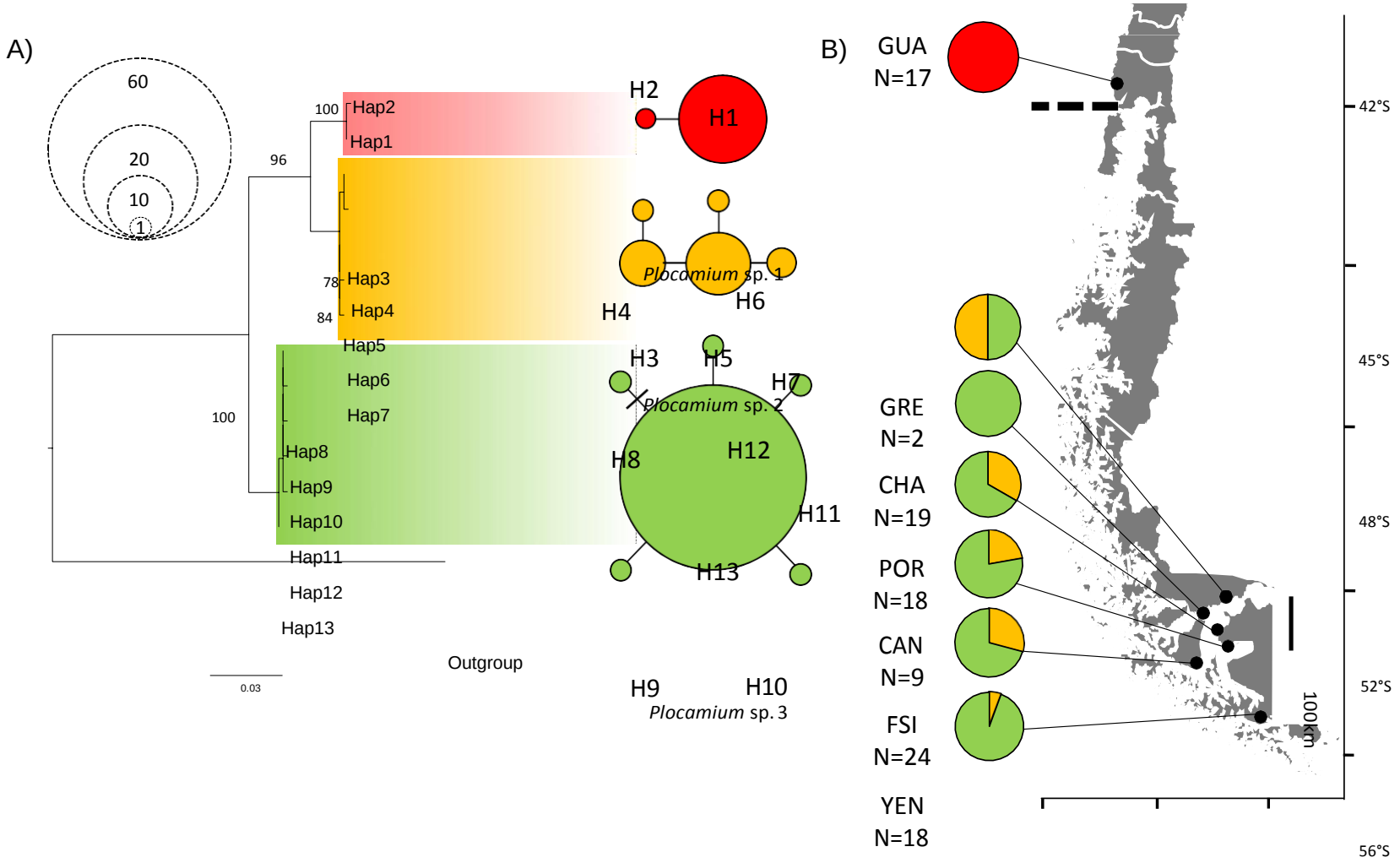
8
5

886 Figure 2 - Bayesian inference ultrametric gene tree (5P-COI). Species
887 delimitation results from ABGD and GMYC are given on the right. Only distinct
888 haplotypes sequenced during the present study are represented. Haplotype code
889 as in Supplementary Table 1.

890890


A)
60
20
10
1
100
Hap2
Hap1
96
78
Hap3
84
Hap4
Hap5
Hap6
100
Hap7
Hap8
Hap9
Hap10
Hap11
Hap12
Hap13
0.03
Outgroup
H2
H1
Plocamium sp. 1
H6
H4
H3
H5
H7
Plocamium sp. 2
H8
H12
H11
H13
H9
H10
Plocamium sp. 3
B)
GUA
N=17
GRE
N=2
CHA
N=19
POR
N=18
CAN
N=9
FSI
N=24
YEN
N=18
42°S
45°S
48°S
52°S
56°S
100km

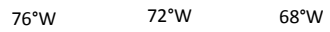
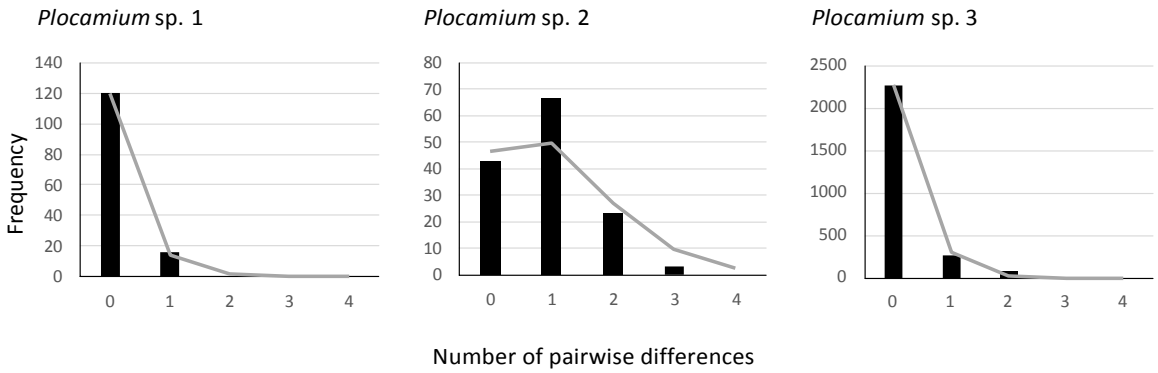

76°W
72°W
68°W

Figure 3 – A) ML tree (left) and Neighbour joining (NJ) network (right) inferred from 5P-COI sequences dataset of *Plocamium* specimens from the present study. In the tree, numbers above the branches are support values as inferred from ML analysis, only values superior to 75 are given. In the NJ networks, haplotypes are represented by open circles with size proportional to frequency within each genetic species (see upper left corner for correspondence between number of sequences and circle size). For haplotypes separated by more than one mutational step, black bars indicate the additional number of steps. B) *Plocamium* species distribution; the number (N) of individuals sequenced is indicated for each sampling locality. Code for each locality as in Table 1; haplotype code as in Supplementary Table 1. Dashed line represents the biogeographic transition zone located at 42°S.

912912



9139
1
3

9149
1
4

915 Figure 4 - Mismatch distributions calculated for the 5P-COI data set; each
916 *Plocamium* genetic species (as delimited by ABGD and GMYC) was treated
917 separately. Observed distributions (black histograms) and expected distributions
918 under a model of demographic expansion (grey lines) of the number of pair base
919 differences between sequences of 5P-COI.

920920

SUPPLEMENTARY MATERIAL

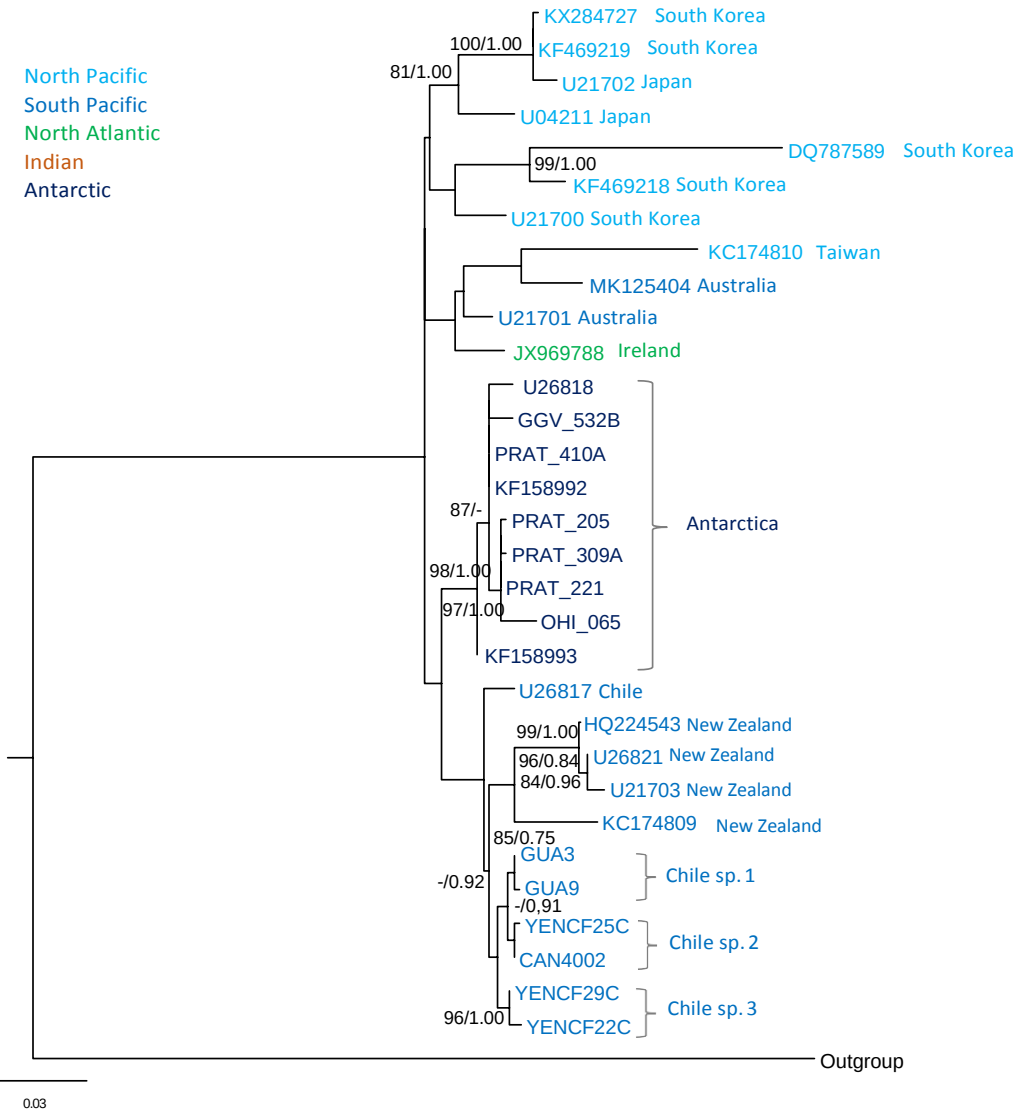


Figure S1 - Maximum likelihood (ML) phylogram of the genus *Plocamium* based on *rbc*L sequences. ML bootstrap (BS)/Bayesian posterior probability (PP) values are shown above or close to each branch and only values superior to 75 and 0.75, respectively, are given. Colors correspond to oceans where individuals sequenced where sampled. Outgroup corresponds to *Sarcodia ciliata* (GenBank accession: KM360040).

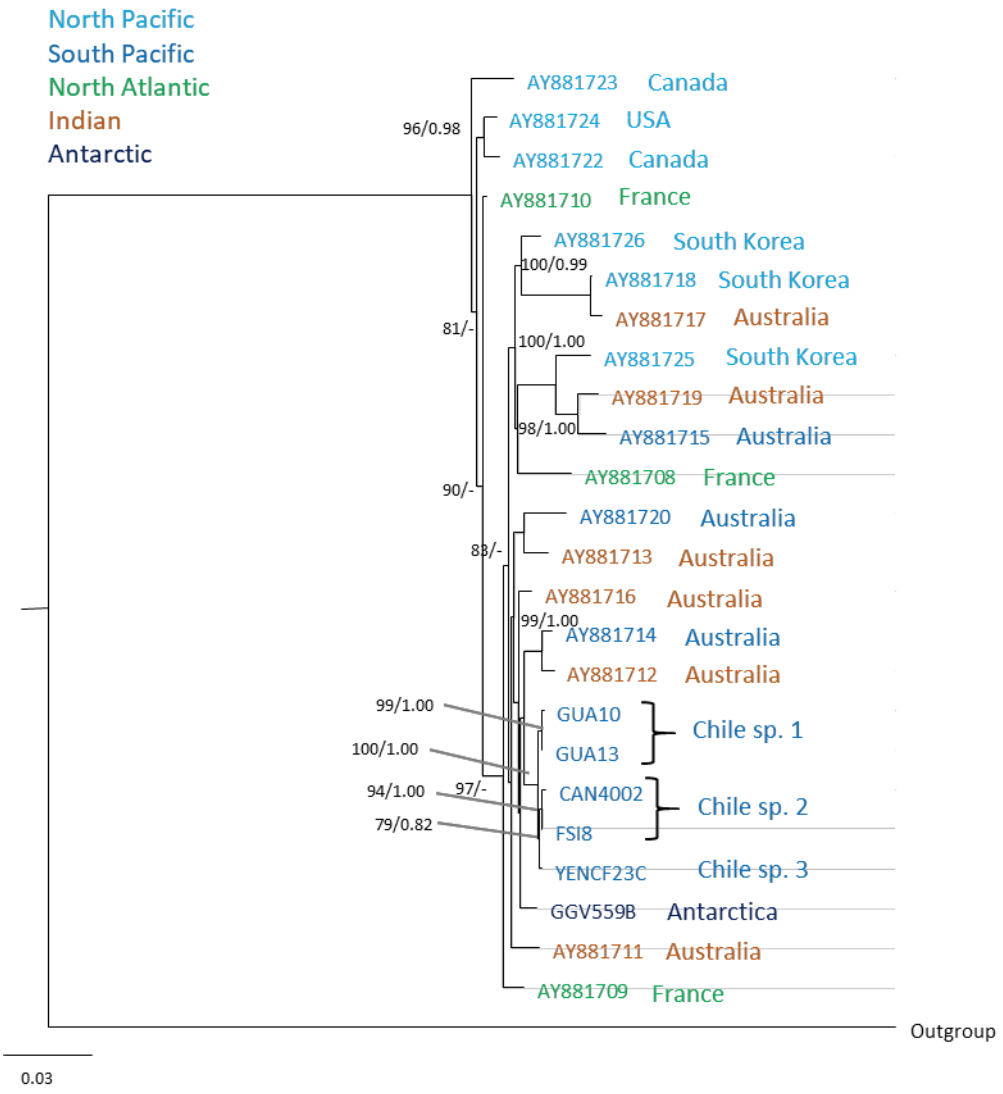


Figure S2 - Maximum likelihood (ML) phylogram of the genus *Plocamium* based on LSU sequences. ML bootstrap (BS)/Bayesian posterior probability (PP) values are shown above or close to each branch and only values superior to 75 and 0.75, respectively, are given. Colors correspond to oceans where individuals sequenced where sampled. Outgroup corresponds to *Sarcodia ciliata* (GenBank accession: DQ343708).

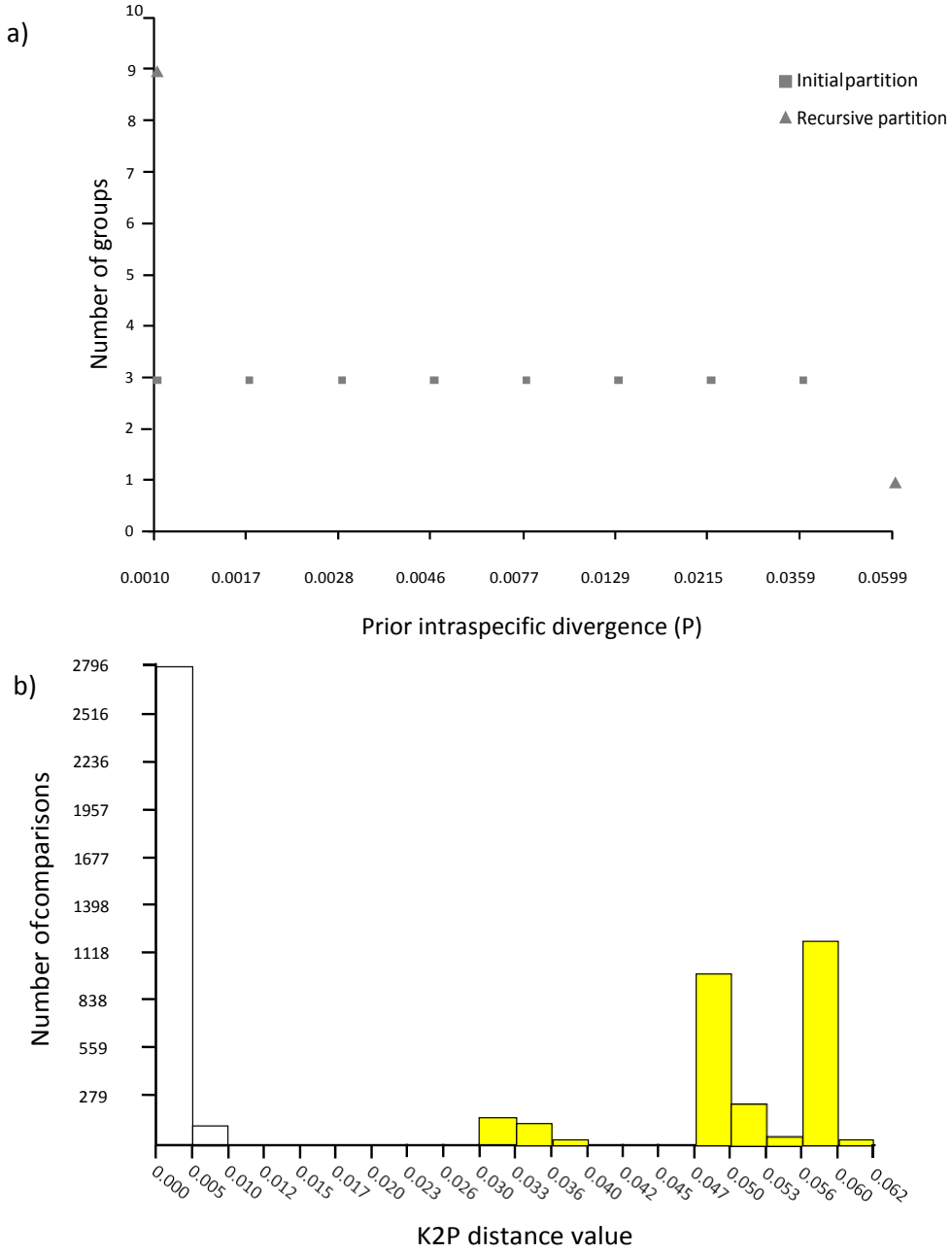


Figure S3 - Automatic Barcode Gap Discovery (ABGD) results and distribution of pairwise distances for the marker 5P-COI. (a) ABGD results showing the number of groups (primary partitions) obtained for a range of prior maximum divergence of intraspecific diversity. (b) Bar chart showing the proportion of pairwise comparisons of 5P-COI gene at each range of sequence divergence (K2P distance). Intraspecific divergences are represented in white bars and divergences belonging to different species are represented in yellow bars.

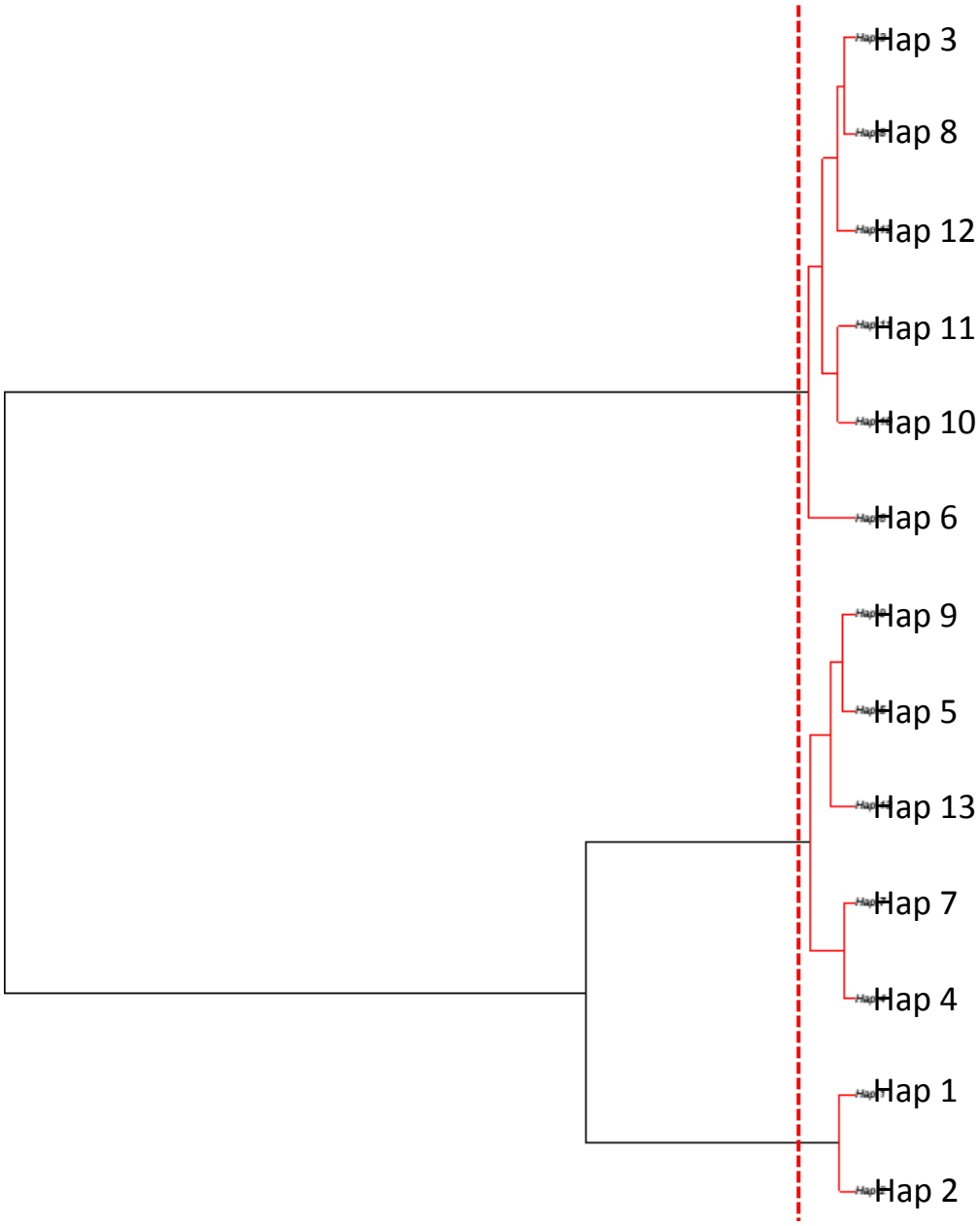


Figure S4 - Ultrametric Bayesian tree reconstructed with the 5P-COI marker. The dotted vertical red line indicates the maximum likelihood transition point of the switch in branching rates, as estimated by a General Mixed Yule-Coalescent (GMYC) model. The GMYC analysis was performed using a single threshold.

Table S1 – GenBank accession numbers of 5P-COI, *rbc*L and LSU sequences obtained for Chilean *Plocamium* spp. Haplotype code for COI-5P, as in Figure 2 and 3, is given for each individual. The voucher code and phase or sex observed for deposited individuals is indicated.

N°	Sample Code	Locality	Coordinates	GenBank Accession COI	Haplotype code COI-5P	GenBank Accession *rbc*L	GenBank Accession LSU	Voucher code (phase/sex)
1	GUA_1	Punta Guabún	41°38'S/74°02'W	MN967353	Hap1			SGO170286 (tetrasporophyte)
2	GUA_2	Punta Guabún	41°38'S/74°02'W	MN967354	Hap2			
3	GUA_3	Punta Guabún	41°38'S/74°02'W	MN967355	Hap2	MT151849		
4	GUA_4	Punta Guabún	41°38'S/74°02'W	MN967356	Hap2			
5	GUA_6	Punta Guabún	41°38'S/74°02'W	MN967357	Hap2			
6	GUA_7	Punta Guabún	41°38'S/74°02'W	MN967358	Hap2			
7	GUA_9	Punta Guabún	41°38'S/74°02'W	MN967359	Hap2	MT151850		
8	GUA_10	Punta Guabún	41°38'S/74°02'W	MN967360	Hap2	MT151851	MT151869	
9	GUA_11	Punta Guabún	41°38'S/74°02'W	MN967361	Hap2			
10	GUA_13	Punta Guabún	41°38'S/74°02'W	MN967362	Hap2	MT151852	MT151870	
11	GUA_14	Punta Guabún	41°38'S/74°02'W	MN967363	Hap2			
12	GUA_15	Punta Guabún	41°38'S/74°02'W	MN967364	Hap2			
13	GUA_16	Punta Guabún	41°38'S/74°02'W	MN967365	Hap2	MT151853		
14	GUA_17	Punta Guabún	41°38'S/74°02'W	MN967366	Hap2	MT151854		
15	GUA_19	Punta Guabún	41°38'S/74°02'W	MN967367	Hap2			
16	GUA_22	Punta Guabún	41°38'S/74°02'W	MN967368	Hap2			
17	GUA_23	Punta Guabún	41°38'S/74°02'W	MN967369	Hap2			
18	GRE4001A	San Gregorio	52°33'S/70°02'W	MN967421	Hap5			SGO170291 (tetrasporophyte)

19	GRE4002B	San Gregorio	52°33'S/70°02'W	MN967422	Hap3			SGO170292 (tetrasporophyte)
20	PCH_4008B	Parque Chabunco	52°59'S/70°48'W	MN967423	Hap3			SGO170290 (female)
21	PCH_4008C	Parque Chabunco	52°59'S/70°48'W	MN967424	Hap3			
22	PCH_4009A	Parque Chabunco	52°59'S/70°48'W	MN967425	Hap10			SGO170293 (female)
23	PCH_4009B	Parque Chabunco	52°59'S/70°48'W	MN967426	Hap3			SGO170294 (tetrasporophyte)
24	PCH_4009C	Parque Chabunco	52°59'S/70°48'W	MN967427	Hap3			
25	PCH_4010	Parque Chabunco	52°59'S/70°48'W	MN967428	Hap3			
26	PCH_4011	Parque Chabunco	52°59'S/70°48'W	MN967429	Hap3			
27	PCH_4012	Parque Chabunco	52°59'S/70°48'W	MN967430	Hap3			
28	PCH_4013	Parque Chabunco	52°59'S/70°48'W	MN967431	Hap3			
29	PCH_4014	Parque Chabunco	52°59'S/70°48'W	MN967432	Hap3			
30	PCH_4015	Parque Chabunco	52°59'S/70°48'W	MN967433	Hap3			
31	PCH_4016	Parque Chabunco	52°59'S/70°48'W	MN967434	Hap3			
32	PCH_4018	Parque Chabunco	52°59'S/70°48'W	MN967435	Hap3			
33	PCH_4019	Parque Chabunco	52°59'S/70°48'W	MN967436	Hap3			

34	PCH_4020	Parque Chabunco	52°59'S/70°48'W	MN967437	Hap3			
35	PCH_4022	Parque Chabunco	52°59'S/70°48'W	MN967438	Hap3			
36	PCH_4023	Parque Chabunco	52°59'S/70°48'W	MN967439	Hap3			
37	PCH_4024	Parque Chabunco	52°59'S/70°48'W	MN967440	Hap3			
38	PCH_4025	Parque Chabunco	52°59'S/70°48'W	MN967441	Hap3			
39	POR_1	Faro Porvenir	53°18'S/70°27'W	MN967442	Hap3			
40	POR_2	Faro Porvenir	53°18'S/70°27'W	MN967443	Hap3			
41	POR_3	Faro Porvenir	53°18'S/70°27'W	MN967444	Hap3			
42	POR_4	Faro Porvenir	53°18'S/70°27'W	MN967445	Hap3			
43	POR_5	Faro Porvenir	53°18'S/70°27'W	MN967446	Hap3			
44	POR_6	Faro Porvenir	53°18'S/70°27'W	MN967447	Hap5			
45	POR_7	Faro Porvenir	53°18'S/70°27'W	MN967448	Hap11			
46	POR_8	Faro Porvenir	53°18'S/70°27'W	MN967449	Hap4			
47	POR_9	Faro Porvenir	53°18'S/70°27'W	MN967450	Hap5			
48	POR_10	Faro Porvenir	53°18'S/70°27'W	MN967451	Hap3			
49	POR_12	Faro Porvenir	53°18'S/70°27'W	MN967452	Hap3			
50	POR_13	Faro Porvenir	53°18'S/70°27'W	MN967453	Hap3			
51	POR_14	Faro Porvenir	53°18'S/70°27'W	MN967454	Hap12			
52	POR_16	Faro Porvenir	53°18'S/70°27'W	MN967455	Hap3			
53	POR_17	Faro Porvenir	53°18'S/70°27'W	MN967456	Hap3			
54	POR_18	Faro Porvenir	53°18'S/70°27'W	MN967457	Hap4			
55	POR_4016A	Faro Porvenir	53°18'S/70°27'W	MN967458	Hap5			SGO170287 (not mature)

56	POR_4016B	Faro Porvenir	53°18'S/70°27'W	MN967459	Hap13			SGO170288 (tetrasporophyte)
57	CAN_1	Los Canelos	53°28'S/70°11'W	MN967388	Hap3			
58	CAN_2	Los Canelos	53°28'S/70°11'W	MN967389	Hap3			
59	CAN_3	Los Canelos	53°28'S/70°11'W	MN967390	Hap5			
60	CAN_4	Los Canelos	53°28'S/70°11'W	MN967391	Hap3			
61	CAN_5	Los Canelos	53°28'S/70°11'W	MN967392	Hap3			
62	CAN_6	Los Canelos	53°28'S/70°11'W	MN967393	Hap3			
63	CAN_7	Los Canelos	53°28'S/70°11'W	MN967394	Hap3			
64	CAN_8	Los Canelos	53°28'S/70°11'W	MN967395	Hap3			
65	CAN_4002	Los Canelos	53°28'S/70°11'W	MN967396	Hap5	MT151864	MT151867	
66	FSI_1	Faro San Isidro	53°46'S/70°58'W	MN967397	Hap3			
67	FSI_3	Faro San Isidro	53°46'S/70°58'W	MN967398	Hap3			
68	FSI_4	Faro San Isidro	53°46'S/70°58'W	MN967399	Hap6			
69	FSI_5	Faro San Isidro	53°46'S/70°58'W	MN967400	Hap3			
70	FSI_6	Faro San Isidro	53°46'S/70°58'W	MN967401	Hap3			
71	FSI_7	Faro San Isidro	53°46'S/70°58'W	MN967402	Hap3			
72	FSI_8	Faro San Isidro	53°46'S/70°58'W	MN967403	Hap4	MT151865	MT151868	
73	FSI_9	Faro San Isidro	53°46'S/70°58'W	MN967404	Hap3			
74	FSI_10	Faro San Isidro	53°46'S/70°58'W	MN967405	Hap7	MT151866		
75	FSI_11	Faro San Isidro	53°46'S/70°58'W	MN967406	Hap3			
76	FSI_12	Faro San Isidro	53°46'S/70°58'W	MN967407	Hap3			
77	FSI_13	Faro San Isidro	53°46'S/70°58'W	MN967408	Hap3			
78	FSI_14	Faro San Isidro	53°46'S/70°58'W	MN967409	Hap3			
79	FSI_15	Faro San Isidro	53°46'S/70°58'W	MN967410	Hap5			
80	FSI_16	Faro San Isidro	53°46'S/70°58'W	MN967411	Hap5			
81	FSI_17	Faro San Isidro	53°46'S/70°58'W	MN967412	Hap3			

82	FSI_18	Faro San Isidro	53°46'S/70°58'W	MN967413	Hap3			
83	FSI_20	Faro San Isidro	53°46'S/70°58'W	MN967414	Hap3			
84	FSI_21	Faro San Isidro	53°46'S/70°58'W	MN967415	Hap3			
85	FSI_22	Faro San Isidro	53°46'S/70°58'W	MN967416	Hap8			
86	FSI_23	Faro San Isidro	53°46'S/70°58'W	MN967417	Hap3			
87	FSI_24	Faro San Isidro	53°46'S/70°58'W	MN967418	Hap9			
88	FSI_26	Faro San Isidro	53°46'S/70°58'W	MN967419	Hap5			
89	FSI_27	Faro San Isidro	53°46'S/70°58'W	MN967420	Hap9			
90	YEN_CF22C	Fiordo Yendegaia	54°54'S/68°42'W	MN967370	Hap3	MT151855		
91	YEN_CF23C	Fiordo Yendegaia	54°54'S/68°42'W	MN967371	Hap3	MT151856	MT151871	
92	YEN_CF25C	Fiordo Yendegaia	54°54'S/68°42'W	MN967372	Hap4	MT151863		
93	YEN_CF26C	Fiordo Yendegaia	54°54'S/68°42'W	MN967373	Hap3	MT151857		
94	YEN_CF27C	Fiordo Yendegaia	54°54'S/68°42'W	MN967374	Hap3	MT151858		
95	YEN_CF28C	Fiordo Yendegaia	54°54'S/68°42'W	MN967375	Hap3	MT151859		SGO170296 (tetrasporophyte)
96	YEN_CF29C	Fiordo Yendegaia	54°54'S/68°42'W	MN967376	Hap3	MT151860		SGO170297 (tetrasporophyte)
97	YEN_CF30C	Fiordo Yendegaia	54°54'S/68°42'W	MN967377	Hap3	MT151861		
98	YEN_CF31C	Fiordo Yendegaia	54°54'S/68°42'W	MN967378	Hap3			
99	YEN_CF32C	Fiordo Yendegaia	54°54'S/68°42'W	MN967379	Hap3			
100	YEN_CF33C	Fiordo Yendegaia	54°54'S/68°42'W	MN967380	Hap3			

101	YEN_CF34C	Fiordo Yendegaia	54°54'S/68°42'W	MN967381	Hap3			
102	YEN_CF35C	Fiordo Yendegaia	54°54'S/68°42'W	MN967382	Hap3			
103	YEN_CF36C	Fiordo Yendegaia	54°54'S/68°42'W	MN967383	Hap3	MT151862		SGO170298 (tetrasporophyte)
104	YEN_CF38C	Fiordo Yendegaia	54°54'S/68°42'W	MN967384	Hap3			
105	YEN_CF39C	Fiordo Yendegaia	54°54'S/68°42'W	MN967385	Hap3			
106	YEN_CF40C	Fiordo Yendegaia	54°54'S/68°42'W	MN967386	Hap3			
107	YEN_CF42BC	Fiordo Yendegaia	54°54'S/68°42'W	MN967387	Hap3			

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