



Haplotype diversity of *Macrocystis pyrifera* (Phaeophyceae: Laminariales) in the central and southern coast of Peru

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Abstract. *Macrocystis pyrifera* is ecologically and commercially important seaweed; therefore, it is a well-studied species in terms of its morphology, geographical distribution and its economic uses in Perú. However, research on its genetic structure is scarce. A recent study conducted along the Southeastern coast of the Pacific, concluded that *Macrocystis* has low genetic variability. Nevertheless, this research unraveled a different genetic pattern in Perú, including unique haplotypes in two sites. The aim of this study was to analyze the genetic variability of *M. pyrifera*, using the mitochondrial markers atp8-S, in eight locations covering 2500 Km of coastline from Perú. With 57 sequences analyzed, the results showed low haplotype ($H_e = 0.562$) and nucleotide ($\pi = 0.00480$) diversity. Paired analysis distributions and the number of paired differences (k) were equal to 0.619. Four haplotypes were found: (1) The putative ancestral haplotype (Mpyr1), widely distributed in the study area, also reported in central Chile, (2) A shared haplotype between populations from southern Peru and northern Chile (Mpyr2) and (3) Two new haplotypes: (Mpyr8) from San Lorenzo Island, and (Mpyr9) from Los Bancos and San Nicolas Bay, Perú.

Key words: Genetic structure, mitochondrial DNA, Macroalgae, Eastern Pacific.

Resumen: Diversidad de haplotipos de *Macrocystis pyrifera* (Phaeophyceae: Laminariales) en las costas del centro y sur del Perú. *Macrocystis pyrifera* es una alga marina de importancia ecológica y comercial; por consiguiente, es una especie ampliamente estudiada en su morfología, distribución geográfica y su importancia económica en Perú. Sin embargo, las investigaciones sobre su estructura genética son escasas. Un estudio recientemente realizado a lo largo de la costa sudeste del Pacífico, concluyó que *Macrocystis* tiene baja variabilidad genética. No obstante, dicha investigación demostró un patrón genético diferente en Perú, incluyendo haplotipos únicos en dos sitios. El objetivo de este estudio fue analizar la variabilidad genética de *M. pyrifera*, usando el marcador mitocondrial atp8-S, en 8 localidades, cubriendo 2500 km de línea de costa del Perú. Con un total de 57 secuencias analizadas, los resultados mostraron baja diversidad haplotípica ($H_e = 0,562$) y nucleotídica ($\pi = 0,00480$). La distribución de análisis pareado y el número de diferencias pareadas (k) fue igual a 0,619. Cuatro haplotipos fueron encontrados: (1) El haplotipo ancestral putativo (Mpyr1), ampliamente distribuido en el área de estudio, también reportado en Chile central, (2) Un haplotipo compartido entre las poblaciones del sur del Perú y norte de Chile (Mpyr2) y (3) Dos nuevos haplotipos: (Mpyr8) de Isla San Lorenzo, (Mpyr9) de Los Bancos y Bahía San Nicolás.

Palabras clave: Estructura genética, ADN mitocondrial, Macroalga, Pacífico Oriental

Introduction

One of the main biogeographic regions of the Western Pacific is the Peruvian Province or Province of warm-temperate waters, located between 6°S and 30°S and characterized by the direct influence of the Humboldt Current (Thiel *et al.* 2007, Briggs & Bowen 2012). The biogeographic classification of the distribution of benthic marine algae along the coasts of the South American Pacific, reports for brown algae four well-distinguished groups (Meneses & Santelices 2000), one of which extends from 12°S (Callao) to 20°S (Iquique), represented by the presence of few sub-Antarctic taxonomic groups and widely distributed bipolar species.

Twelve degree S (12°S), the latitude corresponds to the southern limit is the most important biogeographical breakpoints because warm surface waters extend up to this limit (Meneses & Santelices 2000, Santelices & Meneses 2000). The phylogeographic analyses have allowed registering the agreement between the genetic breaks and the biogeographic borders for the characterization of the distribution areas of the species. Among these, the studies carried out on the genetic structure of brown macroalgae and their geographical distribution (Tellier *et al.* 2009, Macaya & Zuccarello 2010, Montecinos *et al.* 2012, Billard *et al.* 2015, Guillemain *et al.* 2014, 2016, Hanyuda 2016.).

The 30°S latitude in the Peruvian Province shows important changes in the abundance and distribution in marine invertebrate species (Broitman *et al.* 2001, Rivadeneira *et al.* 2002), that is probably due to the changes in oceanographic conditions (Tapia *et al.* 2014). In some phylogeographic studies, the breakpoints have been explained by the climatic changes that occurred, such as Last Glacial Maximum (LGM) (Cárdenas *et al.* 2009) and events such as El Niño (ENSO) (Chávez *et al.* 2003). One of the great examples of the massive mortalities and subsequent reduced genetic diversity by such events is intertidal kelp *Lessonia berteroana* in northern Chile (Martínez *et al.* 2003, Tellier *et al.* 2009).

Till date several studies on the genetic structure of macroalgae have been performed on the southeast coast of the Pacific (Martínez *et al.* 2003, Faugeron *et al.* 2005, Vidal *et al.* 2008, Tellier *et al.* 2009, Macaya & Zuccarello 2010, Astorga *et al.* 2012) including Chile (Montecinos *et al.* 2012, Billard *et al.* 2015, Guillemain *et al.* 2014). However, reports on genetic diversity and haplotypic distribution of macroalgae in Peru are scarce. Few

studies on morphology, geographic distribution and uses of *Macrocystis* have been done in Peru (Acleto 1973, 1986,ACLEto & Zúñiga 1998); reporting two ecomorphs: pyriferia and integrifolia in the Peruvian coasts, however, reports on genetic studies are almost nil. Macaya & Zuccarello (2010) carried out studies on the genetic structure and haplotypic distribution in *M. pyriferia* covering a geographical area of more than 4000 km from Paracas to Punta Arenas (13 ° - 53 ° S). That included only two locations in Peru such as Paracas (13 ° 55'S, 73 ° 23'W) and Atico (15 ° 58'S, 74 ° 02'W).

The main aim of this study is to analyze the genetic variability of *M. pyriferia* in the coast of Peru using mitochondrial marker *atp8-S* and to compare the results with the genetic structure of reported *M. pyriferia* in the South American coast.

Materials and Methods

Sampling and collection sites: Fifty seven samples were collected from 8 locations of central and southern Peru (12°S to 16°S, covering a length of 2500 km) were analyzed. (Table I). The samples of *M. pyriferia* and its ecomorphs were identified on the basis of the morphology of the adhesive disc and fronds (Hoffman & Santelices 1997). The epical ends (2 cm²) of frond tissue samples of 10 individuals were collected from each sampling sites and preserved with silica gel, until DNA extraction.

DNA extraction and amplification: The DNA was extracted using the modified CTAB method as described by Zuccarello & Lokhorst (2005). The region of the interspace *atp8-S* was amplified using the primer pair (*atp8-trnS-F* 5'-TAGCAAACCAAGGCTTTCAAC-3' and *atp8-trnS-R* 5'TGTACGTTTCATATTACCTTCTTTAGC-3') (Voisin *et al.*, 2005). PCR amplifications were performed in 30 µl volume reaction consisting of 1X buffer (New England Biolabs, Beverly, MA, USA), 2.5 mM dNTPs, 2.5 mM MgCl₂, 0.025% BSA, 10 nM of each primer, 1 U of Taq polymerase (New England Biolabs) and 1.5 µl of DNA. The PCR cycle had a denaturation step at 95 ° C for 5 min, followed by 5 cycles of 30 sec at 95 ° C, 30 sec at 60 ° C decreasing by 1 ° C each cycle, and 45 sec at 72 ° C, followed by 30 cycles of 95 ° C for 30 s, 55 ° C for 30 s, and 72 ° C for 45 s with a final extension period of 10 min at 72 ° C. The PCR products were purified with ExoSAP-IT (USB, Cleveland, OH, USA) and subsequently sent for sequencing to the laboratories of MacroGen Inc., Seoul, South Korea.

Table I. Geographic location of the 8 localities along the central/southern coast of Peru and frequency of haplotypes

Locations	Latitude - Longitude	N	N hap	Haplotype frequency
Isla San Lorenzo	12°04' 13.1"S - 77°13' 12.9"O	8	3	Mpyr1= 0,8; Mpyr2= 0,1; Mpyr8= 0,1
Isla La Vieja	14° 17' 15,23"S - 76° 10' 38,90"O	11	1	Mpyr1= 1,0
Isla Santa Rosita	14° 18,9' 80"S - 76° 10' 30,80"O	5	2	Mpyr1= 0,40; Mpyr2= 0,60
Pan de Azúcar	14° 18' 52,80"S - 76° 10' 1,96"O	8	2	Mpyr1= 0,25; Mpyr2= 0,75
Bahía San Nicolás	15° 13' 58.1"S - 75° 13' 18.5"O	7	2	Mpyr2= 0,86; Mpyr9= 0,14
Los Bancos	15° 14' 59.0"S - 75° 13' 18.4"O	5	2	Mpyr1= 0,67; Mpyr9= 0,33
Punta San Juanito	15° 14' 43.2"S - 75° 15' 31.9"O	8	2	Mpyr1= 0,75; Mpyr2= 0,25
Caleta del Inca	16° 30' 51.61"S - 72° 58' 40.33"O	4	1	Mpyr2= 1,0

N: number of individuals sequenced; N hap: number of haplotypes.

Bioinformatics data analysis: To compare the overall population of *M. pyrifera* belongs to the coast of the Southeast Pacific 3 previously reported *M. Pyrifera* atp8 sequences from Peru, 2 previously reported sequences from northern and southern Chile and 2 from New Zealand and Subantarctic Islands (Macaya & Zuccarello 2010) were added to the current group of 57 Peruvian individuals. Subsequently, we also compared individuals of *M. integrifolia* the ecomorph of *M. Pyrifera* to find any genetic differences between them. For comparative data analysis purpose the same nomenclature (haplotypes: "Mpyr") was used in this study as previously used by Macaya & Zuccarello (2010).

The consensus were generated from the sequencing data using BIOEDIT (Hall 1999) and CAP3 program (Huang & Madan 1999). For haplotype identification the number of polymorphic sites (S) and the average of the nucleotide differences (k) were estimated using the DnaSP program (Rozas & Rozas 1995) and subsequently neutrality tests (Tajima 1989, Fu 1997) was performed using the Arlequin v 3.1 program (Excoffier *et al.* 2005). Paired distributions (mismatch distributions) were also calculated using the DnaSP v. program. 5.1 to evaluate any sudden

demographic expansion in the populations (Rogers & Harpending 1992). Genetic distances between and within populations were estimated using the MEGA v.4.0 program (Tamura *et al.* 2007) using the proportion of variable sites between sequences (p-distance) (Nei & Kumar 2000) and finally NETWORK program (Bandelt *et al.* 1999) was used to generate haplotype networks.

Results

The mitochondrial marker atp8-S based analysis revealed that there are no genetic differences between the two ecomorphs of *Macrocystis (pyrifera* and *integrifolia*). In both the ecomorphs polymorphic sites showed 4 haplotypes (h) with a haplotypic diversity of 0.562 (He), the nucleotide diversity (π) of 0.00480 and the average number of nucleotide differences (k) of 0.619 (Table I).

The 60 sequences from the Peruvian populations showed 4 polymorphic sites (S) which revealed 5 haplotypes (h) with a haplotype diversity, nucleotide diversity (π) and average number of nucleotide differences(k) of 0.575 (He), 0.00490 and 0.65 respectively. When the sequences of Peruvian population compared with the sequences of the

populations of the Southeast of the Pacific (64 sequences in total) 132 aligned sites, 10 polymorphic sites (S) and 9 haplotypes were generated (GenBank accessions nos MH000472 to MH000528). The haplotype diversity (H_e), nucleotide diversity (π) and the average of the nucleotide differences (k) were found to be 0.627, 0.00619, and 0.817 respectively. (Table II).

Regarding the distribution of haplotypes by sampling localities, 2 frequent haplotypes were observed: Mpyr1 with 31 individuals (54.39%) and Mpyr2 with 22 individuals (38.60%) present in 6 localities, followed by Mpyr9 with 3 individuals (5.26%) in 2 localities and Mpyr8 with 1 individual (1.75%) in a single locality. Comparison of the geographic distribution of Peruvian haplotypes with the Chilean haplotypes (Macaya & Zuccarello 2010. Fig. 1) showed that Mpyr1 haplotype from the northern zone (12° S) and the central zone (between 14° S and 15° S) was also reported from central and southern Chile (between 33°S and 42°S); while the Mpyr2 haplotype from the central zone (between 14°S and 16°S) is also present in northern Chile (between 21° and 33° S). The Mpyr8 and Mpyr9 haplotypes are found to be unique, each in the north and center of Peru, respectively.

The haplotype network of the populations of the Peruvian central and southern coast showed a star type topology with the putative ancestral haplotype Mpyr1 at the center of the network. The mutational connection between haplotypes was from one to three mutational steps: The ancestral haplotype Mpyr1 was presented separately from the haplotypes Mpyr8 and Mpyr9 by a substitution, and from the Mpyr2 by three substitutions. The haplotype network for the populations of Peru evidenced the ancestral haplotype Mpyr1, the shared haplotype Mpyr2, the haplotypes Mpyr4 and Mpyr5 from Atico locality, the Mpyr8 haplotype from San Lorenzo Island (12° 05' 09" S 77° 13' 19" W) and the Mpyr9 haplotype from Los Bancos (15° 14' 59.0" S 75° 13' 18.4" W) and San Nicolás Bay (15° 11' 41" S 75° 16' 27" W) (Fig. 1A, 1B). When comparing the samples from the southeast coast of the Pacific with the populations of Peru, the haplotype network maintains the top topology. Although a greater number of sequences was incorporated ($N = 64$), Mpyr3 limited to southern Chile and the Sub Antarctic Islands. The network does not show greater variation compared to the results obtained in the populations of Peru (Fig. 1C).

Discussion

The results obtained in this study show the low genetic diversity of *M. pyrifera*. However, identification of new haplotypes evidenced the moderate genetic diversity for the Chilean species. Peru represents a greater number of haplotypes, which suggests a recent dispersion of *Macrocystis* to the southern hemisphere by an adaptation to the warm waters that allowed migrations through a tropical barrier from 3.1 million to 10,000 years ago (Coyer *et al.* 2001). Our results suggest that current ecomorphs came from a common ancestor Mpyr1 (ancestral haplotype) while Mpyr2 haplotype shared with the coastal zone of northern Chile, made up of both *pyrifera* and *integrifolia* ecomorphs.

Some macroalgae, when they are detached from the primary substrate, remain drifting, and are washed away by marine currents (Nikula *et al.* 2010). This could increase the dispersal capacity for some species, especially when they have the property to keep their reproductive structures viable for long periods of time, eg. *M. pyrifera* (Macaya *et al.* 2005). These structures are effective and functional at medium or high latitudes where sea temperature lower, creating favorable conditions for the dispersion of *M. pyrifera* (Rothausler *et al.* 2011); this could explain the presence of Mpyr8 and Mpyr9 haplotypes in Peru where, on the contrary, temperatures are higher, restricting the dispersion capacity of *Macrocystis*. The genetic distance calculated among the populations of *Macrocystis* merely showed any differences, revealing high similarity between the populations studied and indicating the balance between the processes of gene drift and migration. The results of the analysis of paired distributions present a unimodal distribution for the *M. pyrifera* and *M. integrifolia* ecomorphs, adjusting to the hypothesis of population expansion.

The analysis of phylogeography in the populations of the *M. pyrifera* and *M. integrifolia* ecomorphs of the southeastern Pacific coast, including the populations of Peru revealed low values of diversity statistics due to the low number of polymorphic sites; suggesting an event of sudden population expansion prior to a bottleneck event, the haplotype network with star topology showed a population expansion. Events such as the LGM have had effects on the distribution and genetic structure of algae (Provan *et al.* 2001, Gabrielsen *et al.* 2002, Coyer *et al.* 2003, Fraser *et al.* 2009, 2010). The high gene flow between populations of *M. pyrifera* probably occurred due to the transport of sporophylls (that release viable spores) (Macaya *et al.* 2005, Hernández-Carmona *et al.* 2006) during

Table II. Direct relationship between the estimators of the populations grouped by geographic regions analyzed with the mitochondrial marker *atp8-S*.

Regions	N	N hap	S	He	π	k	Tajima's D	Fu Fs
*Central and southern coast of Perú	57	4	3	0,562	0,0048	0,619	-0,09717	-0,24
**Perú	60	5	4	0,575	0,0049	0,651	-0,52519	-1,121
***Southeastern coast of Pacific SEP	64	9	10	0,627	0,00619	0,817	-1,68123	-4,695

* Sequences analyzed for this study. ** Sequences reported for Peru; *** Sequences analyzed for Peru and those reported for the PSE. N: number of individuals sequenced; N hap: Number of haplotypes; S: number of polymorphic sites; He: Haplotypic diversity; K: Average number of pairs difference; π : Nucleotide diversity. Value of Tajima's D with: $0.10 > P\text{-value} > 0.0$

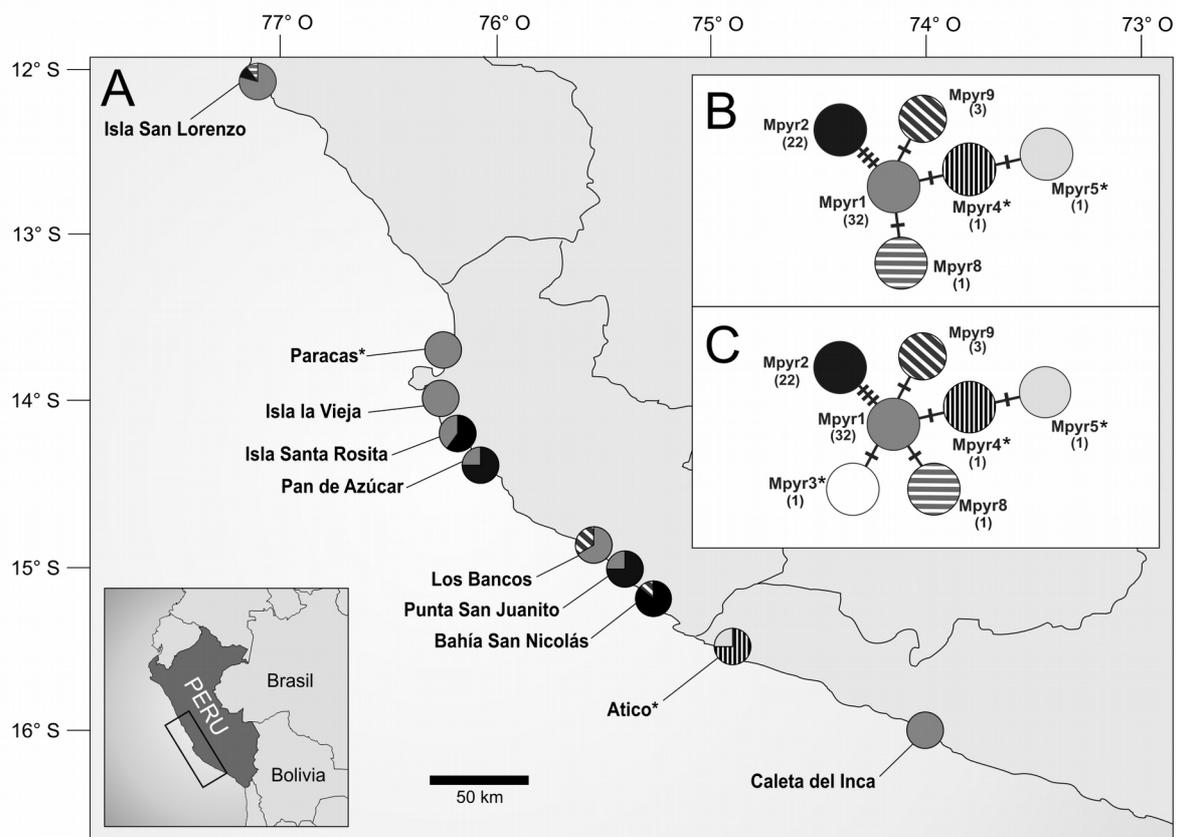


Figure 1. A) Map of Peru indicating the sampling locations from 12°S to 16°S in the Peruvian Province and the different haplotypes found in this study. B) A network of haplotypes with mitochondrial marker *atp8-S* for 57 individuals from the 8 sampled localities also includes sequences previously reported (Macaya & Zuccarello, 2010a) for Atico and Paracas indicated with *. C) A network of haplotypes for 64 individuals from the localities of Peru and the Southeast Pacific coasts PSE. The lines connecting the haplotypes represent mutational pathways and short lines represent the number of substitutions between haplotypes. The number of individuals from which each haplotype was obtained is shown under each haplotype.

the historical events such as the LGM, resulting in geographic isolation of the species. The global bipolar distribution of the *M. Pырifera* population and the absence in latitudes where ocean

temperatures exceed 20°C giving rise to migratory events in macroalgae.

Two new and unique haplotypes were obtained corresponding to populations in the towns of San Lorenzo Island in Callao, Los Bancos and

Bahía San Nicolás in San Juan de Marcona indicating a different genetic structure. Although areas not affected by events such as El Niño maintained the reduced genetic variation, but these events strongly affect *Macrocystis* populations in latitudes 10°S to 23°S (Vásquez *et al.* 2006, Thiel *et al.* 2007). Favoring the presence of species with greater affinity for higher temperatures and reducing the abundance of some native marine species (Castilla & Camus 1992). The incidence of El Niño on brown macroalgae populations results in reduced genetic variation (Martínez *et al.* 2003, Macaya & Zuccarello 2010, Montecinos *et al.* 2012). Corroborating the data of *Lessonia berteriana* reported from northern Chile (Martínez *et al.* 2003, Tellier *et al.* 2009, 2011) our results also showed reduced genetic variation in *M. pyrifera* from latitudes 12°S to 16°S.

Changes in oceanographic conditions, such as the presence of nutrient-rich outcrops, affect the distribution patterns and ecological processes of marine communities on the Southeast Pacific coast (Hormazabal *et al.* 2004, Navarrete *et al.* 2008). These changes are more evident in the different biogeographical provinces and can intervene in the flow of genes between populations through migratory processes by the movement of genes from one population to another and allow to determine if populations evolve at random (genetic drift) or by adaptive processes (natural selection), such as, *L. berteriana* in 30°S (Martínez *et al.* 2003, Tellier *et al.* 2009, 2011, Oppliger *et al.* 2012). In the Peruvian Province, the Peruvian Coastal Surge System or Humboldt Current has a direct impact on the structure and population dynamics of the communities sublittoral zone on the coasts of Peru (Zuta & Guillen 1970). The localities of central and southern Peru analyzed in this study was found to be the most important coastal upwelling centers in front of Peru, e.g. 12°S Callao, 15°S San Juan de Marcona (Graco *et al.* 2007).

The presence of unique haplotypes reported in this study constitute the starting point for the development of new investigations to confirm these new haplotypes and in future this genetic information can be used for the proper management of these populations.

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