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Genetic structure of the giant kelp *Macrocystis pyrifera* along the southeastern Pacific

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ABSTRACT: We assessed the genetic structure of the giant kelp *Macrocystis pyrifera* across a broad latitudinal range along the southeastern Pacific coast (SEP). Specifically we analyzed the concordance of putative biogeographic breaks with genetic discontinuities and the effect of historical and contemporary events on the genetic pattern of this important seaweed. Mitochondrial DNA and single-strand DNA conformation polymorphism (SSCP) analysis for a total of 730 samples were carried out. Only 5 haplotypes were found among individuals collected along 4800 km of coastline, with very low haplotype diversity and a shallow genealogy compared with other macroalgal species. Some phylogeographic disjunctions in *M. pyrifera* were found to correspond roughly to established biogeographic breaks. On the southern coast we found a genetic break at 42°S (Chiloé Island) coincident with a well-known biogeographic boundary, while the genetic break found between samples in central/northern Chile (33° S) does not correspond to any known biogeographic breaks in other brown algae, but does reflect a break associated with other marine taxa. The low genetic diversity in northern Chile may be related to contemporary events (e.g. El Niño Southern Oscillation) while in southern Chile the haplotype distribution may reflect the effect of historical events (Last Glacial Maximum; LGM). Additionally, we compared the SEP data with samples from some of the subantarctic islands and New Zealand. The results showed shared haplotypes among some of the subantarctic islands and southern-central Chile, suggesting a recent colonization of the subantarctic region. The high dispersal potential of kelp rafts may also help to explain the low genetic diversity observed. We conclude that both present and historic events are responsible for the genetic structure of *M. pyrifera* along the SEP.

KEY WORDS: Dispersal · Biogeography · ENSO · Kelp · *Macrocystis pyrifera* · Mitochondrial DNA Phylogeography · Southeastern Pacific · Subantarctic

INTRODUCTION

Biogeographic regions are generally delimited by discontinuities between biotic assemblages, and may be derived from both historical (e.g. glaciations) and/or contemporary (e.g. oceanographic) processes (Riginos & Nachman 2001). Along the southeastern Pacific coast of Peru and Chile (hereafter SEP) the boundaries of biogeographic regions have long been discussed (e.g. Brattström & Johanssen 1983, Lancellotti & Vásquez 1999, Santelices & Meneses 2000, Camus 2001, Vidal et al. 2008). Most biogeographic studies have proposed 2 main biogeographic regions: the Peruvian or warm-temperate province (between 6 and 30° S) and the Magellan or cold-temperate province (between 40 or 42° S and 56° S); additionally, several authors have recognized an intermediate area between 30 and 33° S and 40 and 42° S made of mixed components from the 2 neighboring regions (e.g. Brattström & Johanssen 1983) (Fig. 1). The distribution of algal species along the SEP has also shown geographic breaks in species composition (Santelices 1980, Meneses & Santelices 2000, Santelices & Meneses 2000). Two main breaks have been reported: one at

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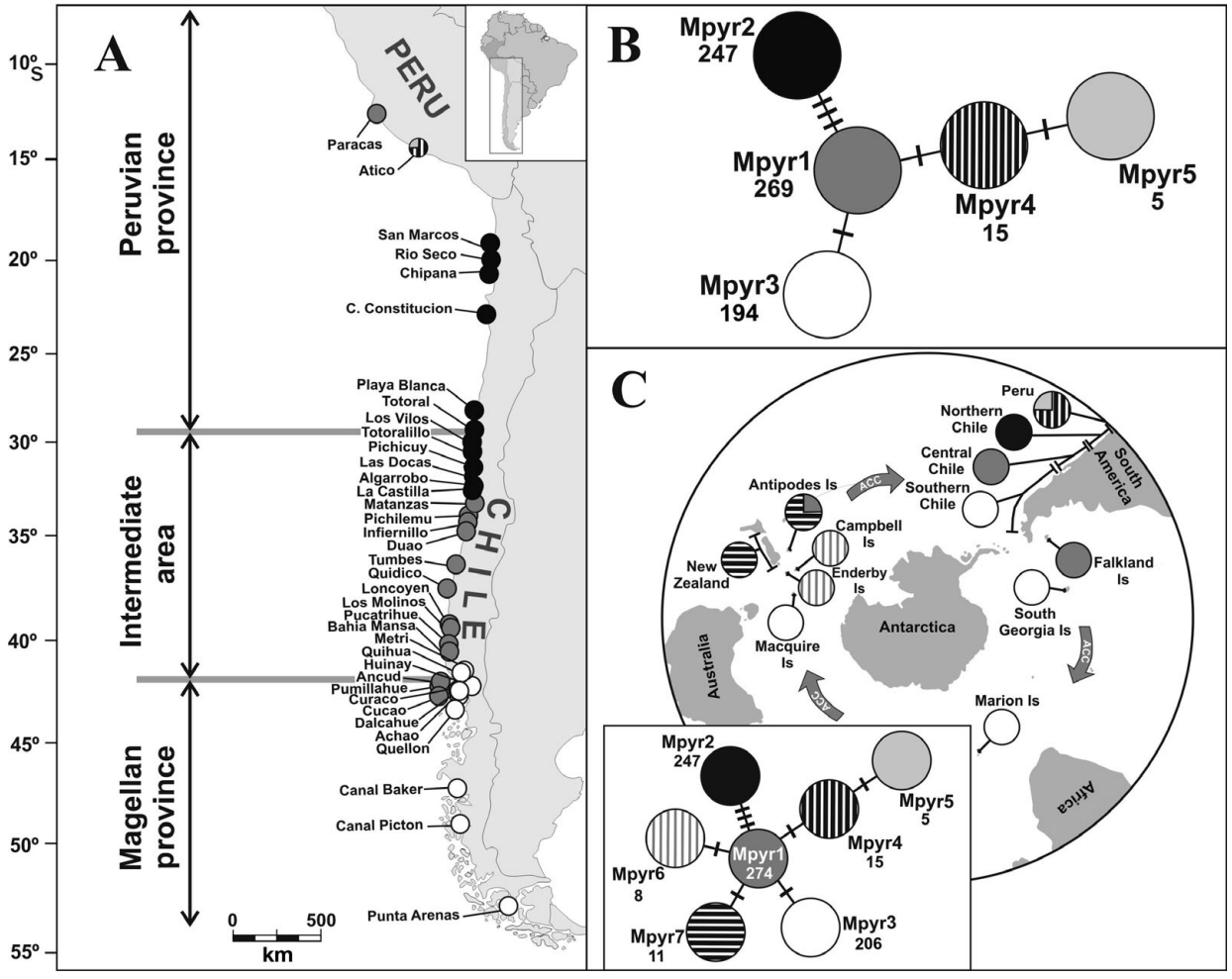

Fig. 1. (A) Southeastern Pacific coast (SEP) showing the location of collecting sites. Different shading represents different haplotypes. For detailed descriptions of sites see Table 1. Map also shows the 3 biogeographic provinces reported for SEP: Peruvian province, Intermediate area, and Magellan province. Grey lines represent the biogeographic breaks at 30 and 42° S. (B) Statistical parsimony network of all sampled populations from SEP, connecting lines shows mutational pathways among haplotypes, and small lines represent single-site substitutions. (C) Haplotype distribution including samples from subantarctic islands and New Zealand. The frequency of each haplotype is shown below each haplotype for (B) and (C). ACC: Antarctic Circumpolar Current

30° S is explained by the prevailing oceanographic conditions, resultant of upwelling events (Meneses & Santelices 2000, Santelices & Meneses 2000) and shows marked discontinuity especially in brown algae (Meneses & Santelices 2000). The second major break is located at 40 to 42° S, which has also been reported for several marine organisms (Brattström & Johanssen 1983, Fernández et al. 2000, Camus 2001, Thiel et al. 2007) and has been explained by changed water conditions (lower salinity, less wave exposure) (Meneses & Santelices 2000), topographical breakup of the coastline caused by the increased number of fjords from which large amounts of freshwater enter the sea (Lancellotti & Vásquez 1999), and divergence of the main oceanographic currents (Cárdenas et al. 2009).

Species with high dispersal potential would theoretically be expected to show limited genetic disjunctions associated with biogeographic breaks (Thiel et al. 2007), and, indeed, high levels of gene flow along the SEP have been reported for several marine taxa. Using allozymes and amplified fragment length polymorphisms, Gomez-Uchida et al. (2003) reported genetic homogeneity over 2500 km of the Chilean coast for the hairy edible crab *Cancer setosus*, which the authors attributed to its long-lived planktonic larval stage (60 d). Similar lack of genetic structure along major parts of the Chilean coast has been reported for the Chilean abalone *Concholepas concholepas* (Gallardo & Carrasco 1996, Cárdenas et al. 2009), the blue mussel *Mytilus chilensis* (Toro et al.

2006), and the pelagic fish *Merluccius gayi* (Galleguillos et al. 2000).

In contrast to these zoological species, marine macroalgae are less explored. To date, only 1 study has addressed concordance between phylogeographic patterns and the 2 main biogeographic transitions along the SEP. Tellier et al. (2009) found a major phylogeographic break at 30° S in the intertidal kelp *Lessonia nigrescens*, a brown alga with reduced gene flow (Martínez et al. 2003, Faugeron et al. 2005) and no floating structures that could facilitate dispersal of adult thalli. We may expect a different result in kelps with high dispersal potential, such as *Macrocystis pyrifera*. The dispersal of this alga can be achieved by microscopic spores (zoospores) or by transport of large sporophytes that become dislodged and set adrift (Reed et al. 2006). The dispersal capacity of kelp spores is restricted, and they are rarely transported effectively over distances exceeding a few meters (Anderson & North 1966, Dayton 1973). Long-distance dispersal has been suggested for *M. pyrifera* zoospores by Reed et al. (2004, 2006); however, recent evidence suggests that floating adult kelp are more likely to be important in long-distance dispersal (Macaya et al. 2005, Hernández-Carmona et al. 2006). Along the Chilean coast, Macaya et al. (2005) determined that 27% of floating *M. pyrifera* rafts possessed functional reproductive blades (i.e. viable spores released), and it was estimated that fertility could be maintained for at least 21 d. Spore dispersal from kelp rafts may play a valuable role in long-distance dispersal events that are important for biogeographic expansion and genetic exchange (Reed et al. 2006). This would suggest substantial inter-population genetic homogeneity in *M. pyrifera*. Using ITS sequences, Coyer et al. (2001) found little genetic differentiation in samples collected across a wide geographic range (Chile, South Africa, Marion Island, Tasmania, Australia, and New Zealand). Specifically, along the SEP these authors looked at only 5 samples collected from southern Chile: Punta Pucatrihue (n = 1) and Metri Bay (n = 4) (40 to 41° S, respectively) separated only by 200 km. Similarly, a recent study by Macaya & Zuccarello (2010), has analyzed mitochondrial cytochrome c oxidase subunit I (COI) sequences globally including few (7) sites along the SEP. Although their goal was focused on taxonomic issues, they did note low genetic structure and shared haplotypes among distant areas from the southern hemisphere (Macaya & Zuccarello 2010). An extensive sampling along the SEP is required to have a better understanding of the genetic pattern in this area.

During the Last Glacial Maximum (LGM; 18000 to 20000 yr ago), ice sheets covered broad areas of southern Chile from 35 to 54° S (McCulloch et al. 2000, Hulton et al. 2002) including the whole of the Chilean fjords (Fig. 2). The impact of glaciations on the distribution and genetic variation of species in southern Chile has been documented, but mostly for terrestrial (Muellner et al. 2004, Marchelli & Gallo 2006, Himes et al. 2008, Rodriguez-Serrano et al. 2008, Victoriano et al. 2008) or freshwater biota (Ruzzante et al. 2008, Zemlak et al. 2008, 2010, Xu et al. 2009). A recent colonization, after the LGM and the retreat of sea ice, of *Durvillaea antarctica* in the subantarctic region by a series of long-distance rafting events has been suggested (Fraser et al. 2009). A recent article described the effect of the most recent ice age on the genetic imprints patterns of *D. antarctica* at the Chilean Patagonia (Fraser et al. 2010); COI and rbc sequences revealed genetic homogeneity on this area and suggested a possible recolonization from transoceanic sources for this intertidal kelp. Studies on additional species will determine whether the effect of the LGM is a common feature in structuring genetic diversity among marine algae in this area.

Events such as the El Niño Southern Oscillation (ENSO) produce massive mortality of kelp in the Peruvian province and the northern part of the Intermediate area (Vega et al. 2005, Vasquez et al. 2006, Thiel et al. 2007). Such sharp population declines, or bottlenecks, such as those seen during recent ENSO events, may translate into losses of genetic variation of marine organisms (Steinfartz et al. 2007). Martínez et al. (2003) found that, in sites in northern and central Chile impacted by the 1982–83 ENSO, genetic diversity of *Lessonia nigrescens* was lower than in non-impacted sites.

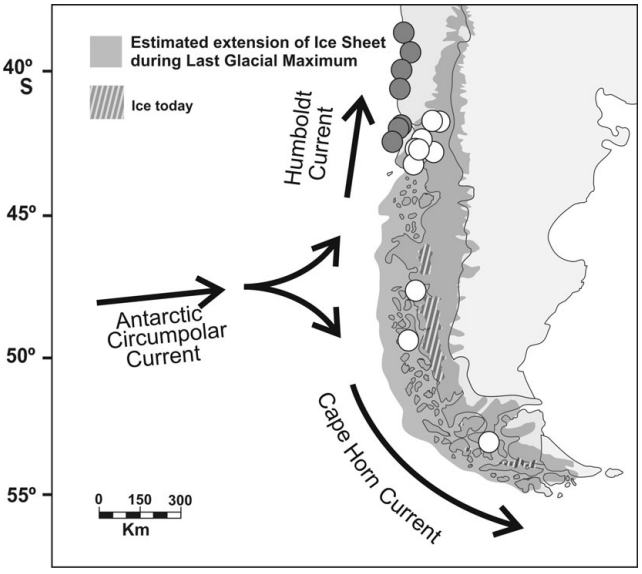

Fig. 2. Distribution of haplotypes in southern Chile showing the extent of the Last Glacial Maximum (LGM) ice sheet over the Chilean fjords and the distribution of existing icefields. Arrows show directions of major currents in southern Chile. For sampling locations details, see Fig. 1 and Table 1

Macrocystis pyrifera is the largest seaweed on earth (up to 40 to 50 m long) and the most widely distributed kelp species, forming extensive submarine forests that harbor a rich diversity of marine life (Neushul 1971, North 1994). *M. pyrifera* also provides a valuable economic resource used for alginates, as food for abalone aquaculture, organic fertilizer, and recently as novel seafood (Hernández-Carmona et al. 1998, Gutierrez et al. 2006, Graham et al. 2007, Vásquez 2008). Along the SEP *M. pyrifera* distribution encompasses all 3 biogeographic provinces. It is present in areas both affected or unaffected by the ENSO phenomenon, and represents an important economic resource in Peru and Chile, though recent evidence suggests that it is over exploited (Vásquez 2008). Knowledge of genetic diversity and phylogeographic patterns will aid in the application of management and conservation policies, and can also provide insights into the ecological and evolutionary processes driving the distribution of marine macroalgae along the SEP. The aim of this study was to: (1) analyze the genetic diversity of *M. pyrifera* over a wide latitudinal range (13 to 53°S) along the SEP using mitochondrial DNA sequences; (2) evaluate the possible coincidence of phylogeographic breaks in this species with known biogeographic breaks; (3) evaluate whether the genetic diversity of this alga could be related to historical or contemporary events affecting the SEP (e.g. LGM and ENSO), and (4) compare the results with additional samples collected from New Zealand and some subantarctic islands.

MATERIALS AND METHODS

Sampling sites and collection. A total of 730 samples of *Macrocystis pyrifera* was collected between 2006 and 2009 from 39 sites between 13 and 53° S, covering almost the entire geographical range of the species along the SEP (Fig. 1A–C, Table 1). At each site, multiple individuals (7 to 20) were collected haphazardly in an area of at least 200 m^2. Healthy apical tips (2 to 3 cm^2) without obvious epiphytes or epibionts were excised and preserved in Ziplock bags with silica gel until DNA extraction. Additionally, 40 samples from the subantarctic region and New Zealand were included in the analysis (Table 1).

DNA extraction and atp8-S amplification. DNA was extracted following the modified 1% N-cetyl N,N,N-trimethylammonium bromide (CTAB) method described by Zuccarello & Lokhorst (2005). The mitochondrial intergenic spacer region between genes atp8 and trnS (atp8-S) was amplified using the primers atp8-trnS-F and atp8-trnS-R (Voisin et al. 2005). This region has been used for phylogeographic studies in several kelp species (e.g. Muraoka & Saitoh 2005, Voisin et al. 2005, Uwai et al. 2006, Tellier et al. 2009), and it has been suggested that this marker is a useful tool for phylogeographic studies in brown seaweeds (Engel et al. 2008).

PCR amplifications were performed following Voisin et al. (2005). Due to the large number of samples, single-stranded DNA conformation polymorphism (SSCP) analysis was carried out. SSCP allows discrimination between DNA fragments of the same size that are different in their nucleotide sequence (Sunnucks et al. 2000). Three µl of PCR product was mixed with 9 µl 95% formamide, 0.1% aqueous bromophenol blue/xylene cyanol, and 10mM NaOH, subsequently denatured at 95°C for 5 min, then snap cooled on ice before loading. Gels contained 20% 37.5:1 acrylamide/bis-acrylamide (Sigma Aldrich), 0.5× TBE buffer, 0.5% ammonium persulphate, 0.05% tetramethylethylenediamine (TEMED). Electrophoresis was carried out for 14 to 16 h at 4 W in 0.5× TBE buffer at 4°C on 225 mm long and 0.75 mm thick gels (BioRad). After electrophoresis, gels were silver stained following Bassam et al. (1991), and banding patterns were assigned by eye. To check the accuracy of SSCP typing, 3 to 4 individuals for each location were sequenced in both directions (Macrogen). One or more of each haplotype indicated by SSCP were sequenced from each sampling site. No ambiguous SSCP profiles were found, and each one has the same unique sequence.

Data analysis. Haplotype frequencies were calculated using DnaSP version 5.10 (Rozas & Rozas 1995). Sequences were aligned using ClustalW in the BIOEDIT program (Hall 1999). Estimates of haplotype (He) and nucleotide (π) diversity were calculated for each population and for the entire dataset using ARLEQUIN V. 3.1 (Excoffier et al. 2005).

An unrooted statistical parsimony network was reconstructed using TCS 1.21 (Clement et al. 2000). Because of the lack of variation within populations and the low numbers of haplotypes, further analysis of genetic variation was not undertaken.

RESULTS

A region of 133 bp was compared among 730 samples of *Macrocystis pyrifera*, collected from 38 sampling locations along the SEP. Each SSCP profile was found to have the same unique sequence, while some differed by only 1 base pair. Despite the large geographic area analyzed only 5 haplotypes were found, distinguished by only 6 variable sites (Fig. 1) (GenBank Accession no. HQ336480 to HQ336486). Haplotype Mpyr1 was the most common, present in 36.85% of the samples, followed by the haplotypes Mpyr2 and

Table 1. *Macrocystis pyrifera.* Sampling sites along the southeastern Pacific coast (SEP), collection details and haplotype frequency. Mpyr indicates different haplotype number (see Fig. 1 for details)

Region – sampling site	Coordinates	Collector	Sample size	Haplotype frequency
SEP				
Paracas	13° 55′S, 73°23′W	Alejandro Perez-Matus	20	Mpyr1 = 1.0
Atico	15°58′S, 74°02′W	Alex Gamarra	20	Mpyr4 = 0.74, Mpyr5 = 0.26
San Marcos	21°00′S, 70°09′W	Erasmo Macaya	20	Mpyr2 = 1.0
Rio Seco	21°06′S, 70°07′W	Erasmo Macaya	20	Mpyr2 = 1.0
Chipana	21°20′S, 70°05′W	Erasmo Macaya	20	Mpyr2 = 1.0
Caleta Constitucion	23°25′S, 70°35′W	Erasmo Macaya	20	Mpyr2 = 1.0
Playa Blanca	28°11′S, 71°09′W	Ivan Hinojosa, Martin Thiel	20	Mpyr2 = 1.0
Punta Choros	29°15′S, 71°28′W	Luciano Hiriart	20	Mpyr2 = 1.0
Totoral	30°21′S, 71°40′W	Marcelo Valdebenito	20	Mpyr2 = 1.0
Los Vilos	31°55′S, 71°31′W	Erasmo Macaya	20	Mpyr2 = 1.0
Totoralillo	32°01′S, 71°30′W	Erasmo Macaya	20	Mpyr2 = 1.0
Pichicuy	32°20′ S, 71°27′W	Alonso Vega	20	Mpyr2 = 1.0
Las Docas	32°08′S, 71°42′W	Claudio Saez	7	Mpyr2 = 1.0
Algarrobo	33°21′S, 71°40′W	Erasmo Macaya	20	Mpyr2 = 1.0
La Castilla	33°27′S, 71°40′W	Erasmo Macaya	20	Mpyr2 = 1.0
Matanzas	33°56′S, 71°52′W	Erasmo Macaya	20	Mpyr1 = 1.0
Pichilemu	34°22′S, 72°01′W	Erasmo Macaya	20	Mpyr1 = 1.0
Infiernillo	34°23′S, 72°01′W	Erasmo Macaya	20	Mpyr1 = 1.0
Duao	34°53′S, 72°09′W	Ivan Hinojosa	20	Mpyr1 = 1.0
Tumbes	36°37′S, 73°05′W	Erasmo Macaya	20	Mpyr1 = 1.0
Quidico	37°22′S, 73°39′W	Ivan Hinojosa, Martin Thiel	9	Mpyr1 = 1.0
Loncoyen	39°49′S, 73°24′W	Ivan Hinojosa, Martin Thiel	20	Mpyr1 = 1.0
Los Molinos	39°50′S, 73°24′W	Erasmo Macaya	20	Mpyr1 = 1.0
Pucatrihue	40°32′S, 73°43′W	Alejandro Buschmann	20	Mpyr1 = 1.0
Bahia Mansa	40°34′S, 73°44′W	Erasmo Macaya	20	Mpyr3 = 1.0
Metri	41°36′S, 72°42′W	Alejandro Buschmann	20	Mpyr2 = 1.0
Quihua	41°36′S, 72°42′W	Ivan Hinojosa, Martin Thiel	20	Mpyr2 = 1.0
Ancud	41°51′S, 73°49′W	Erasmo Macaya	20	Mpyr3 = 1.0
Pumillahue	41°52′S, 74°00′W	Ivan Hinojosa, Martin Thiel	20	Mpyr3 = 1.0
Quemchi	42°08′S, 73°28′W	Erasmo Macaya	20	Mpyr3 = 1.0
Dalcahue	42°22′S, 73°39′W	Erasmo Macaya	7	Mpyr3 = 1.0
Huinay	42°22′S, 72°24′W	Ivan Hinojosa, Martin Thiel	7	Mpyr3 = 1.0
Curaco	41°26′S, 73°36′W	Ivan Hinojosa	20	Mpyr3 = 1.0
Achao	42°28′S, 73°29′W	Erasmo Macaya	20	Mpyr3 = 1.0
Cucao	42°40′S, 74°07′W	Ivan Hinojosa, Martin Thiel	20	Mpyr1 = 1.0
Quellon	43°08′S, 73°36′W	Erasmo Macaya	20	Mpyr3 = 1.0
Canal Baker	47°26′S, 74°10′W	Ivan Hinojosa	20	Mpyr3 = 1.0
Canal Picton	50°08′S, 74°40′W	Ivan Hinojosa	20	Mpyr3 = 1.0
Punta Arenas	53°28′S, 70°51′W	Andres Mansilla	20	Mpyr3 = 1.0
Subantarctic region				
Marion Island	46°50′S, 37°50′E	Ceridwen Fraser	4	Mpyr3 = 1.0
Antipodes Island	49°40′S, 178°48′E	Ceridwen Fraser	4	Mpyr7 = 0.75, Mpyr1 = 0.25
Campbell Island	52°32′S, 169°11′E	Ceridwen Fraser	4	Mpyr6 = 1.0
Enderby Island	50°37′S, 166°15′E	Ceridwen Fraser	4	Mpyr6 = 1.0
Falkland Islands	51°37′S, 57°45′W	Joost Pompert	4	Mpyr1 = 1.0
South Georgia Island	54°17′S, 36°29′W	Anjali Pade	4	Mpyr3 = 1.0
Macquarie Island	54°38′S, 158°48′E	Annelise Wiebkin	4	Mpyr3 = 1.0
New Zealand				
Kau Bay	41°17′S, 174°49′E	Erasmo Macaya	4	Mpyr7 = 1.0
Fiordland	45°16′S, 166°50′E	Wendy Nelson	4	Mpyr7 = 1.0
Stewart Island	46°53′S, 168°07′E	Erasmo Macaya	4	Mpyr7 = 1.0

Mpyr3, present in 33.84 and 26.58% of the samples, respectively, while haplotypes Mpyr4 and Mpyr5 were detected in only 2.73% of samples. Haplotypes corresponded to particular geographic areas, with Mpyr2 ranging from San Marcos (21° S) to Algarrobo (33° S), Mpyr1 from Matanzas (33° S) to Cucao (42° S), Mpyr3 from Metri (41° S) to Punta Arenas (53° S), and haplotypes Mpyr4 and Mpyr5 restricted to Atico, Peru (15° S) (Fig. 1). Interestingly, the most northern sampling site, Paracas, Peru (13° S), displayed the same haplotype as central Chile (Mpyr1). At each sampling site only 1 haplotype was detected, with the exception of Atico,

where haplotypes Mpyr4 and Mpyr5 were both found (Fig.1, Table 1).

Haplotype diversity for all SEP samples was 0.7561 (±0.013 SD), and nucleotide diversity was 0.01563 (±0.00056 SD). The statistical parsimony network, which indicates the relationships among haplotypes with a 95% connection limit (Fig. 1B) resulted with Mpyr1 as a central haplotype, separated only by 1 substitution from haplotypes Mpyr3 and Mpyr4, and by 3 substitutions from haplotype Mpyr2.

At 30° S no sign of a phylogeographic break was observed. At 42° S we found an overlap of 2 haplotypes, Mpyr1 and Mpyr3. Both of these Chiloe Island haplotypes had different distributions, with haplotype Mpyr1 restricted to the west side of the island, whereas haplotype Mpyr3 was found only on the east side (Fig. 2).

The comparison of the SEP *Macrocystis pyrifera* data with data from some of the subantarctic islands and New Zealand (Fig. 1C) revealed a shared Mpyr3 haplotype between southern Chile and 3 subantarctic islands: South Georgia, Marion, and Macquarie. Haplotype Mpyr1 was shared among Central Chile, the Falklands, and the Antipodes. Additionally, 2 new haplotypes were found: Mp6 from the islands Campbell and Enderby, and Mpyr7 from New Zealand and the Antipodes. The haplotype network showed a star-like shape with haplotypes Mpyr6 and Mpyr7 separated by only 1 base pair from the central haplotype Mpyr1.

DISCUSSION

Our study showed fairly low genetic variation among atp8-S sequences of *Macrocystis pyrifera* along the SEP. The limited phylogeographic structure detected does, nonetheless, reveal an intriguing pattern that is likely the result of both contemporary and historical events.

Two distinct genetic breaks were observed along the SEP; their relation to putative biogeographic barriers is discussed in 'Concordance of biogeographic and phylogeographic breaks'. Comparison with samples from subantarctic islands and New Zealand revealed that these localities shared haplotypes with central and southern Chile, suggesting a recent colonization after the LGM in some locations of the subantarctic region probably from central Chile.

Genetic diversity and dispersal potential

Our data showed low levels of genetic diversity in *Macrocystis pyrifera* populations along the SEP. Haplotypes were distributed over very large geographic areas: haplotype Mpyr2 in northern Chile between 21 and 33° S, (~1500 km); haplotype Mpyr1 in central Chile between 33 and 42° S (~1000 km); and haplotype Mpyr3 in southern Chile between 41 and 53° S (~1400 km). A similar low genetic variation has been shown in several marine species along the Chilean coast, both invertebrates (Gallardo & Carrasco 1996, Toro & Aguila 1996, Gallardo et al. 2003, Gomez-Uchida et al. 2003, Toro et al. 2006) and fish (Galleguillos et al. 2000), presumably associated with the high dispersal potential of these taxa, which have long-lived larvae. Along the SEP, few studies have analyzed the genetic structure of macroalgae (Martínez et al. 2003, Faugeron et al. 2005, Vidal et al. 2008, Tellier et al. 2009), and most of them have found high genetic differentiation among populations. In contrast, we provide evidence for low genetic variation in a macroalga over a wide area of the SEP and throughout the subantarctic region.

The low genetic variation observed along the SEP could be the result of several factors. First, a recent colonization event or events; *Macrocystis pyrifera* is thought to have originated in the northern hemisphere and spread to the southern hemisphere reaching western South America recently (10^4 to 3×10^6 yr ago) (Coyer et al. 2001). This is supported by genetic evidence from ITS (Coyer et al. 2001) and mitochondrial COI sequences (Macaya & Zuccarello 2010), where little divergence was found among samples collected across a wide geographic area of the southern hemisphere. Alternatively high levels of gene flow among populations may have a direct relationship with the high dispersal potential of *M. pyrifera*, especially given its ability to float once detached (Macaya et al. 2005, Hernández-Carmona et al. 2006). Continuous growth and production of viable zoospores from *M. pyrifera* rafts along the Chilean coast has been reported (Macaya et al. 2005). Similarly, rafting of storm-detached thalli of *Fucus vesiculosus*, which release gametes when deposited at a new site, has been proposed to lead to connectivity between populations (Muhlin et al. 2008), and dispersal by floating thalli has been suggested for other macroalgal species (e.g., Dayton 1973, van den Hoek 1987, Buschmann et al. 2006, McKenzie & Bellgrove 2008, Fraser et al. 2009, Buchanan & Zuccarello in press, and see review by Thiel & Gutow 2005).

Concordance of biogeographic and phylogeographic breaks

Along the SEP, genetic disjunctions in *Macrocystis pyrifera* corresponded roughly to previously described biogeographic breaks (30 and 42° S). Haplotypes Mpyr2, Mpyr1, and Mpyr3 were largely restricted to the Peruvian province, Intermediate, and Magellan

provinces described for the SEP, respectively (Fig. 1). However, the genetic disjunction between haplotypes Mpyr2 and Mpyr1 was somewhat further south than breaks observed in other taxa in this region, occurring in *M. pyrifera* at around 33° S rather than 30° S. For the intertidal kelp *Lessonia nigrescens*, for example, a clear genetic break has been recently established at 30° S (Tellier et al. 2009), and the authors suggest the limited dispersal may have contributed to the maintenance of this genetic pattern. The presence of upwelling in this area is thought to be responsible for a biogeographic break in brown macroalgae (Santelices 1980, Meneses & Santelices 2000). Although there is a clear phylogeographic break at 33° S for *M. pyrife*ra, this break may be related to some environmental adaptation of *Macrocystis* 'ecomorphs' (Graham et al. 2007, Demes et al. 2009). In northern-central Chile, 2 different ecomorphs are present (Macaya & Zuccarello 2010), haplotype Mpyr2 whose southern distribution limit is at 33° S represents samples of the *M. integrifolia* ecomorph, while the ecomorph *M. pyrifera* has its northern distributional limit at 33° S. No overlap of haplotypes and ecomorphs was found. These ecomorphs are generally adapted to specific environments, *M. integrifolia* is generally found in shallow waters, whereas *M. pyrifera* is generally found in intermediate to deep waters (Graham et al. 2007). Studies on kelp physiology in different environments and on the role of the mitochondrial genome in potential adaptations to specific environments are needed (Tellier et al. 2009). Additionally the presence of unique haplotypes in Atico and the putative ancestral haplotype Mpyr1 in Paracas is intriguing, additional sampling along the Peruvian coast, and comparison with other areas (e.g. the northern hemisphere), plus analysis with more molecular markers are needed to understand variation from this low latitude.

At 42° S the phylogeographic break coincides with a primary biogeographic break previously suggested for the SEP (Camus 2001). The haplotype distribution at the contact area overlaps, with haplotype Mpyr1 distributed up to Cucao at 42° S and haplotype Mpyr3 beginning its distribution in Metri at 41° S. The biogeographic break at 40 to 42° S has been explained mainly because of the topographical breakup of the coastline by fjords where large amount of fresh water enter the sea (Lancellotti & Vásquez 1999) and the divergence of the main oceanic currents (Humboldt and Cape Horn; Fig. 2) (Cárdenas et al. 2009). Interestingly the haplotypes were locally separate on Chiloé Island, with haplotype Mp1 on the west coast and Mpyr3 on the east coast. This particular distribution of haplotypes may be related with the LGM (see 'Contemporary and historical events'). Similarly Fraser et al. (2010) suggested extirpation of *Durvillaea antarctica* populations during the last glacial period and described a postglacial recolonization in southern Chile (see 'Contemporary and historical events' below). Additional studies on other species may also show a similar pattern.

Contemporary and historical events

Along the area affected by ENSO (~6 to 30° S) 4 haplotypes were found: 3 haplotypes in Peru and only 1 in northern central Chile. Local extinction of kelp populations during ENSO events is common in the area between 10 and 23° S (Camus 1990, Vasquez et al. 2006, Thiel et al. 2007). Reduced genetic variation in the kelp *Lessonia nigrescens* in 2 sites in northern Chile, Iquique (20° S) and Antofagasta (23° S), together with slow recolonization (<60 km in 20 yr) has been reported by Martínez et al. (2003). It is likely that *Macrocystis pyrifera* was similarly affected but more sampling from 10 to 20° S are needed to confirm this. On the other hand, rafting may be a very important dispersal mechanism for populations that suffer recurrent extinctions and recolonizations (Thiel et al. 2007). Along the SEP, kelp rafts may colonize following the northwards direction of the Humboldt Current much more quickly than in non-floating algae such as *Lessonia nigrescens*. Further research with more variable molecular markers (microsatellites) may reveal a more detailed genetic structure in populations affected by ENSO and may also determine the location of source populations.

The distribution of haplotype Mpyr3 in central-southern Chile corresponds precisely to the extent of the Patagonian ice sheet at the LGM (Fig. 2). This event had a major effect on the global distributions of species, with many taxa forced out of areas covered by encroaching ice sheets (Hewitt et al. 2003). The glaciations may have had massive effects on distribution, abundance, and productivity of *Macrocystis pyrifera* (Graham et al. 2007). The effect of the LGM on algal distribution and genetic structure has been studied mainly in the northern hemisphere (e.g. van Oppen et al. 1995, Provan et al. 2001, Gabrielsen et al. 2002, Coyer et al. 2003, Hoarau et al. 2007, Muhlin & Brawley 2009), but recent research on phylogeographic structure in the southern hemisphere bull-kelp *Durvillaea antarctica* has shown that the species likely recolonised much of the subantarctic region following elimination by ice scour at the LGM (Fraser et al. 2009). Recent work has also confirmed this result (Fraser et al. 2010), with genetic homogeneity in *D. antarctica* collected south of 44° S, they suggested recolonization from a transoceanic source because of the close genetic relation with samples from subantarctic islands and New Zealand. The comparison with *M. pyrifera*

from the subantarctic region revealed a similar result, a shared haplotype (Mpyr3) along the area affected by LGM (see Fraser et al. 2009 for details). Furthermore, areas not affected by ice at the LGM (such as New Zealand, Antipodes Island, Campbell Island, and Enderby Island) displayed unique haplotypes closely related with the putative ancestral haplotype Mpyr1 (Fig. 1C). Antipodes Island and Falkland Island share haplotype with central Chile (Mpyr1) suggesting that Mpyr1 was probably widely present in the subantarctic region before the LGM. Similar to *D. antarctica* (Fraser et al. 2009, 2010) and fauna inhabiting their holdfasts (Nikula et al. 2010), the Antarctic Circumpolar Current may facilitate the recolonization after the LGM of *M. pyrifera* via detached kelp rafts. The recolonization of *M. pyrifera* in southern Chile therefore is similar to *D. antarctica*, although in *M. pyrifera* case only subantarctic islands shared the same haplotype.

CONCLUSIONS

Both contemporary and historic environmental factors are likely responsible for the genetic pattern of *Macrocystis pyrifera* in the SEP. Although sampling was extensive (over 4800 km), only 5 haplotypes were found with few mutations separating them. The high dispersal potential and a recent colonization history may explain the genetic homogeneity in this area. Despite its dispersal potential, however, distinct phylogeographic breaks were evident and correspond roughly to known biogeographic breaks in other marine taxa. The low genetic diversity in low latitudes may be due to local extinctions of kelp bed populations due to ENSO effect. The presence of the ice sheet at the LGM has shaped the genetic features of *M. pyrifera* in southern Chile. Shared haplotypes among vast areas of the subantarctic region suggests recolonization via detached kelp rafts facilitated by the Antarctic Circumpolar Current. Our results contribute to the management of this ecologically and economically important kelp species. Kelp harvesting and aquaculture regulations must take into account the low genetic variation and the presence of exclusive haplotypes in vast areas of the SEP coast. Finally, further research using more variable molecular markers will be useful to detect and understand colonization routes, connectivity and the effect of anthropogenic disturbances.

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