Phylogeny, connectivity and dispersal patterns of the giant kelp *Macrocystis* (Phaeophyceae)

by
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Photo: Kelp forest - Scorching Bay, Wellington, NZ.

"The number of living creatures of all orders, whose existence intimately depends on the kelp, is wonderful. A great volume might be written, describing the inhabitants of one of these beds of seaweed….

I can only compare these great aquatic forests of the southern hemisphere with the terrestrial ones in the tropical regions. Yet if in any country a forest was destroyed, I do not believe nearly so many species of animals would perish as would here, from the destruction of the kelp…"

Charles Darwin

Journal of Researches into the Geology and Natural History of the Various Countries Visited by the H.M.S. Beagle. (1839)

Abstract

Macrocystis represents the most widely distributed kelp genus, providing structure and energy for one of the most productive ecosystems on earth. Despite its ecological and economical importance, many aspects of its taxonomy, distribution and dispersal still remain unknown. Using different molecular markers I studied the taxonomy, phylogeography and dispersal patterns of *Macrocystis*. The analysis involves samples from different populations throughout the world. Using the DNA barcoding method I, confirmed previous suggestions that the genus must be considered as monospecific, *M. pyrifera* being the only species. The effects of historical and contemporary events on the haplotype distribution were determined by analyzing samples from the south-eastern Pacific (SEP) using the atp8-S mitochondrial marker. The last glacial maximum as well as oceanographic anomalies (El Niño phenomena) may be important factors driving the genetic pattern along the SEP. The genetic structure in southern Chile was also analyzed in more detail, especially in the Chilean Fjords. Samples from attached and floating kelp individuals revealed that dispersal via kelp rafts is possible. Finally, a global analysis using COI sequences showed shared haplotypes along vast distances in the Northern and Southern hemispheres, recent dispersal and high gene flow can explain such genetic homogeneity. Additionally, microsatellite analysis confirmed that gene flow along the Southern Ocean is occurring over ecological time scales, where rafting of detached reproductive kelps seems to be facilitated by the Antarctic Circumpolar Current connecting populations in the Southern Hemisphere. This study has provided valuable genetic evidence to understand factors shaping the genetic structure of this important ecologically and economically species. It also

contributes important knowledge for conservation and management strategies, especially in places where *M. pyrifera* has been harvested. In summary, the results of this study confirm previous suggestions of high gene flow among *M. pyrifera* populations at different scales. It also provides evidence suggesting that kelp rafts act as an important dispersal mechanism in this species, thus giving important information to understand the factors shaping the evolution of the largest seaweed on earth.

Dedicated to my dad Jose Erasmo Macaya

and grandparents Elias Horta and Natalia Perez.

Dedicada a my Padre Jose Erasmo Macaya,

abuelos Elias Horta y Natalia Perez

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Huamani, Andres Mansilla, Peter Martin, Jon Mee, Jean Sebastien Moore, Francisco Neira, Wendy Nelson, Lobo Orensanz, Anjali Pande, Alejandro Perez-Matus, Joost Pompert, Rafael Riosmena-Rodríguez, Claudio Sáez, Martin Thiel, Marcelo Valdebenito, Alonso Vega, Mario Villegas y Annelise Wiebkin.

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CHAPTER 1

Phylogeny, connectivity and dispersal patterns of the giant kelp *Macrocystis* (Phaeophyceae): Introduction & overview

Numerous factors known to be important in determining patterns of genetic diversity and structure include the spatial arrangement of the populations (patchy vs. continuous), distance among populations, the presence or effectiveness of vectors of dispersal, and physical barriers to dispersal (Palumbi 2003; Reed et al. 2006; Sotka and Palumbi 2006; Thiel and Haye 2006; Thiel et al. 2007). Determining the relative importance of these factors in structuring patterns of gene flow and genetic differentiation is critical to understanding how organisms and populations persist in nature (Coleman and Brawley 2005; Cowen and Sponaugle 2009). This valuable information is also essential for appropriate management especially of endangered or commercially valuable species, such as kelp species, which are harvested in several countries and possess an enormous ecological importance.

Macroalgae, especially the giant kelp *Macrocystis*, provide an ideal model for exploring patterns of genetic structure at different scales. This alga represents one of the largest benthic organisms on Earth, forming dense forests on temperate waters in Northern and Southern hemispheres (Graham et al. 2007). This kelp has received great attention, being one of the most studied macroalgal species; however most of the studies have an ecological bias. The genetic structure at local and global scales has received less attention. Thus genetic and

evolutionary consequences of recurrent phenomena, such as mass mortalities, extinction-recolonization processes, and variations in population connectivity, are presumably of critical significance for the maintenance of kelp populations but they are just beginning to be explored (e.g. Martinez et al. 2003). Understanding the genetic structure of *Macrocystis*, a keystone species of temperate ecosystems in Northern and Southern hemispheres, will provide relevant information for clarifying its evolutionary history as well as aiding in its management and conservation.

1.1 *Macrocystis* – Biology

Kelp are among the most ecologically and economically important seaweeds. They comprise brown algae from the order Laminariales and produce the largest biogenic structures found in benthic marine systems (Dayton 1985; Steneck et al. 2002). This diverse group of seaweeds of approximately 30 genera vary tremendously in size, morphology, life span, and habitat. They are particularly abundant where water temperature is generally lower than 20°C, often forming dense stands or forests, with individuals of some species reaching 30 m in length (Schiel and Foster 2006). Kelp communities are highly productive and sustain one of the most diverse, productive, and dynamic ecosystems on earth (Mann 1973; Graham et al. 2007). Their holdfasts and thalli constitute feeding areas, refuges against predation and bottom currents, spawning, settlement and nursery sites for a variety of organisms (Vásquez and Buschmann 1997; Vásquez et al. 1998; Vega et al. 2005).

Macrocystis represents the most easily recognized kelp genus, forming dense forests that span both hemispheres including the temperate west coasts of North and South America and also include Argentina, South Africa, Australia, New Zealand, and most of the subantarctic islands (Neushul 1971; North 1994; Graham et al. 2007). This genus is also an important economic resource used for alginate extraction (Hernandez-Carmona et al. 1998), as food for abalone and sea urchin aquaculture, organic fertilizer production and novel seafood products (Gutierrez et al. 2006). Recently the demand of alginate sources and food for cultivated abalone has dramatically increased harvesting *Macrocystis* in northern and southern Chile (Vásquez 2008).

This alga exhibits a complex life cycle with two morphologically different stages (Fig. 1.1): one conspicuous stage, the recognizable macroscopic sporophyte (the visible kelp) that produces zoospores, and a microscopic stage comprising of independent female and male plants (gametophytes) (Schiel and Foster 2006). Zoospores are released into the water column where they disperse via currents, until they reach a suitable substrate to settle and develop into microscopic gametophytes (Graham et al. 2007). Sporophytes are the product of gametic fusion, which is triggered by environmental factors (temperature, irradiance, photoperiod, and nutrient concentrations) (Schiel and Foster 2006).

The maximum age of *Macrocystis* sporophytes is unknown. Individual fronds generally senesce after 6–8 months (North 1994) although van Tüssenbroek (1989) observed maximum frond survival of 1 year, and *Macrocystis* sporophytes can produce new fronds from apical meristems (frond initials) retained above the holdfasts near the sporophylls (North 1994). As such, the sporophytes may survive as long as they remain attached to the substratum

and environmental conditions are adequate for growth. In any case, the life-span of *Macrocystis* sporophytes appears to be far less than that of other perennial kelp genera (Schiel and Foster 2006).

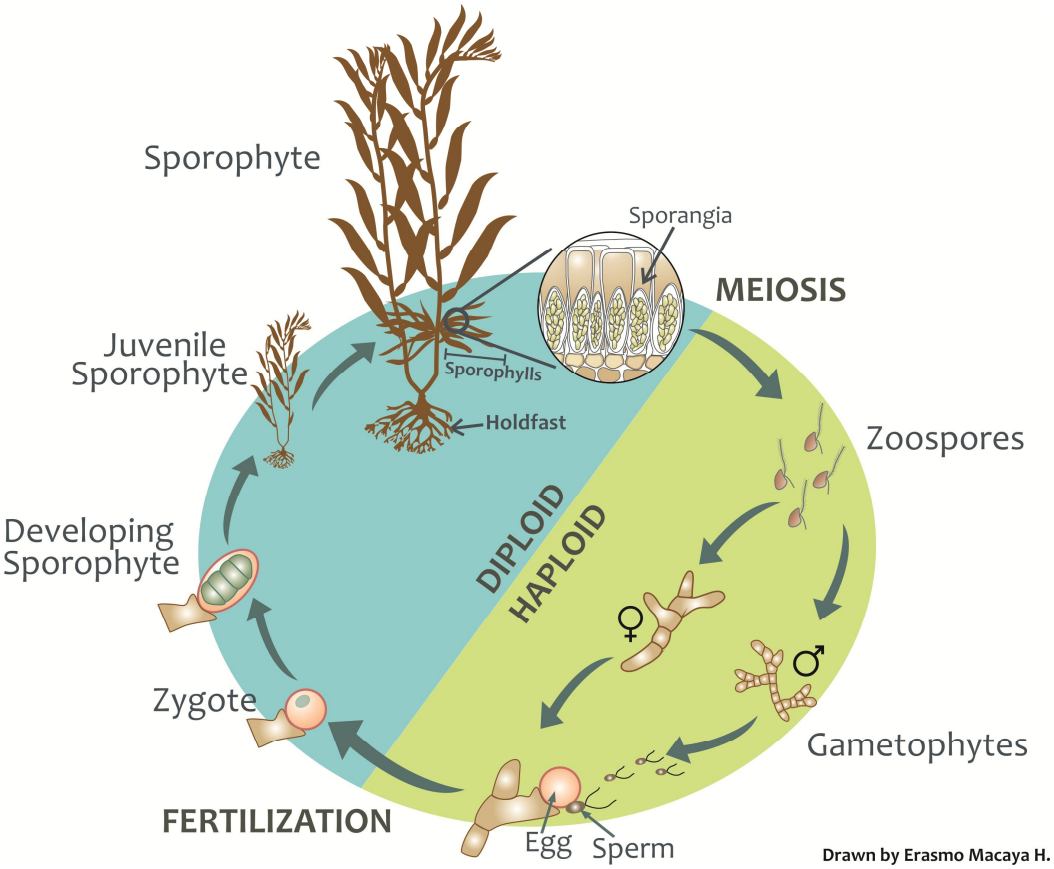

Figure 1.1 Life cycle of *Macrocystis pyrifera* and generally for all Laminariales.

1.2 *Macrocystis* – Dispersal

Dispersal is a process of fundamental biological importance in establishing connectivity between local populations and in facilitating the colonization of new habitats (Thiel et al. 2007). It can be quite important to recover populations after anthropogenic or natural environmental perturbations (Hutchings et al. 2007). In the marine environment, natural dispersal occurs primarily via planktonic larvae, propagules, transport by animals and rafting. Most benthic habitats have a predominance of species with complex life cycles, which include a free-swimming larval stage (Strathmann 1990), hence high dispersal capabilities are intuitively associated with most marine organisms. However, this is not a rule since there are always species with limited dispersal potential, such as most macroalgae and direct developers or brooding species (Reed et al. 1992; Shanks et al. 2003).

While larval dispersal has received substantial research attention during recent years (e.g. Grantham et al. 2003; Shanks et al. 2003; Gerber et al. 2005) little is known about other natural dispersal processes (e.g. dispersal on external surfaces of motile animals and rafting). Genetic studies show increasingly that populations of organisms without planktonic larval stages can also be widely dispersed, and rafting is most frequently invoked (Thiel and Haye 2006). This suggestion is supported by frequent reports of rafting organisms on the high seas (Kingsford and Choat 1985; Helmuth et al. 1994; Ingólfsson 1995; Hobday 2000; Smith 2000).

In *Macrocystis*, dispersal can be achieved in three ways: zoospores, sperm and kelp rafts (Fig. 1.2). Zoospore production is an important strategy of reproduction in seaweeds, but the dispersal capacity of spores from most benthic

algae is restricted, and they are rarely transported effectively over distances exceeding a few meters (Anderson and North 1966; Dayton 1973; Hoffmann 1987; Santelices 1990). Similarly sperm dispersal capacity is limited and reproduction requires a pheromone released from the egg cell, attracting sperm, over distances of about 1 mm (Maier and Muller, 1986) . In *Macrocystis pyrifera*, if the source of spores is an isolated plant, the measurable range of spore settlement does not exceed 5 m (Anderson and North 1966). However, spore dispersal can be enhanced by episodic periods of high zoospore production that coincide with storms (Reed et al. 1988; Reed et al. 1997) or large population sizes (and thus high source zoospore concentrations; Reed et al. 2004; Reed et al. 2006). In the absence of unidirectional currents, most giant-kelp zoospores are retained near the adults that released them (Graham 2003). Events with exceptionally far dispersal may occur via the transport of large sporophytes that become dislodged and set adrift (Macaya et al. 2005; Hernández-Carmona et al. 2006; Reed et al. 2006)

Table 1.1
Estimated dispersal distances of *Macrocystis pyrifera.*

Dispersal stage	Dispersal distance	Methods	Reference
zoospores	5-30 m	In situ spore dispersal (juveniles appearing around adult plants)	(Anderson and North 1966)
zoospores	< 10m	In situ spore dispersal (juveniles appearing around adult plants).	(Reed et al. 1988)
zoospores	1600m	Recruitment of juveniles on isolated reef.	(Ebeling et al. 1985)
zoospores	~4000m	In situ spore dispersal (juveniles appearing around adult plants after storms)	(Reed et al. 1988)
zoospores	3500m	In situ spore dispersal (juveniles appearing at varying distances from different sized groups of adults).	(Reed et al. 2004)
zoospores	~3000m	Mathematical model to estimation of dispersal.	(Gaylord et al. 2002)
zoospores	~1000m	Modelation by current speed, forest size, and the duration of viability of gamete-producing life stages derived from spores.	(Gaylord et al. 2006)
zoospores	>2000m	nonlinear regression analysis to estimate dispersal.	(Reed et al. 2006)
zoospores	150m	In situ spore dispersal (juveniles appearing at varying distances from different sized groups of adults).	(Reed et al. 2006)
floating algae	100 km	Inferred from kelp rafts along the Chilean coast	(Macaya et al. 2005)
floating algae	Over 900km	Inferred from kelp rafts in California, USA.	(Hernandez-Carmona et al. 2006)
floating algae	Over 1000 km	Inferred from kelp rafts, USA.	(Harrold and Lisin 1989; Hobday 2000)

Despite the apparent acceptance of floating sporophytes as the primary long distance dispersal vector for *Macrocystis* and other macroalgae (Macaya et al. 2005; Hérnandez-Carmona et al. 2006; Fraser et al. 2009), genetic data for *Macrocystis* is still needed it to confirm such suggestions and therefore support the connectivity of kelp populations through this method.

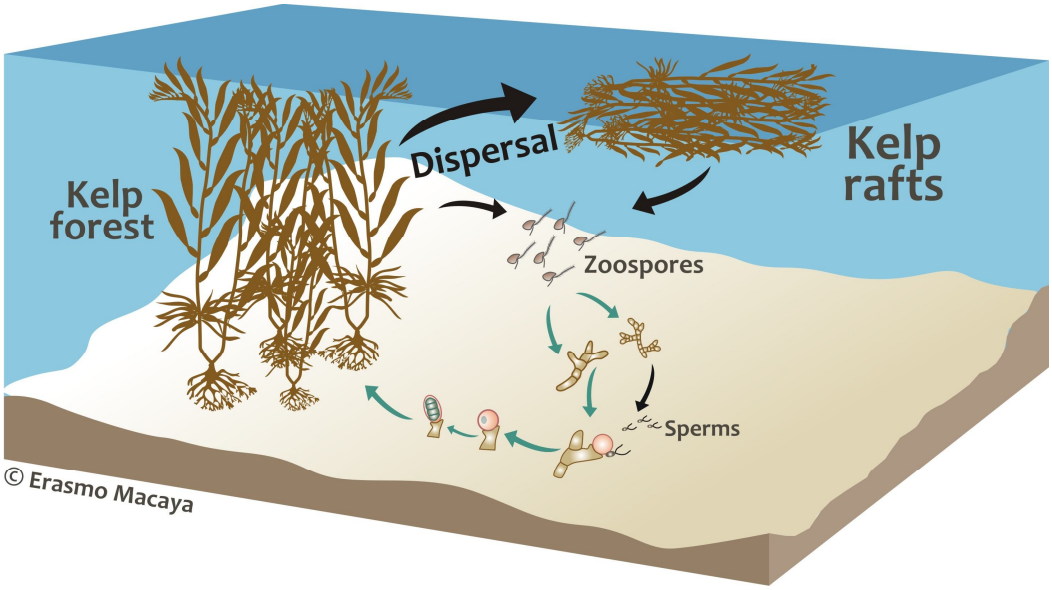

Figure 1.2 Dispersal mechanisms of *M. pyrifera.* Zoospores released from the sporophyte are likely to disperse relatively short distances (m-km) similarly to sperm, detached sporophytes form kelp rafts are transported by wind and currents reaching distant shores and might release zoospores for colonizing new habitats.

To date, only two studies have discussed whether *Macrocystis* sporophytes can remain reproductive while floating (Macaya et al. 2005; Hernández-Carmona et al. 2006). Both studies have produced evidence of floating reproductive *Macrocystis*. Macaya et al. (2005) determined that 26% of floating *Macrocystis* sporophytes along the Chilean coast had sporophylls (reproductive blades) and they estimated that fertility could be maintained for at least 21 days. Hernández-Carmona et al. (2006) found that floating *Macrocystis*

sporophytes remained fertile with high zoospore germination rates up to 125 days. They also measured the average displacement of radio-tagged floating algae at 7.12 km day^{-1}, suggesting that a sporophyte afloat for 125 days could disperse viable propagules (zoospores) over 1000 km. The key to long-distance colonization, therefore, is the arrival of a kelp propagule and the capacity of reproduction with local flora.

Figure 1.3 *Macrocystis* kelp raft, Breaker Bay, Wellington, New Zealand.

1.3 *Macrocystis* – Taxonomy, biogeography and phylogenetic aspects.

Despite their economic and ecological importance, *Macrocystis* phylogeny, taxonomy and dispersal patterns still remains unresolved. In a global molecular analysis of *Macrocystis* using ITS sequences, Coyer et al. (2001) suggested that the present taxonomy has no molecular systematic support. These authors and recognized *Macrocystis* as a single morphologically plastic species, with global populations linked by high gene flow. A similar suggestion was made by Mackenzie (1993) using molecular analysis with samples from Canada, California and Tristan da Cunha Island (S. Atlantic). Only recently the genus has been proposed to be merged in one species (Demes et al. 2009) based on evidence for morphological plasticity driven by environmental factors. Additionally, interfertility between species has been demonstrated (Lewis et al. 1986; Lewis and Neushul 1994; Lewis and Neushul 1995; Druehl et al. 2005; Westermeier et al. 2007). Intergeneric hybrids (e.g. between *Macrocystis* and *Pelagophycus*) also have been identified in the field (Coyer and Zaugg-Haglund 1982; Coyer et al. 1992) or produced artificially by crossing (Sanbonsuga and Neushul 1978). But still, genetic evidence is necessary to confirm the suggestion of a monospecific genus made by Demes et al. (2009).

The distribution of *Macrocystis* has been attributed to an origin in the Northern Hemisphere, followed by a migration across a diminished tropical belt during a cool period, and subsequent colonization of the Southern Hemisphere (Lindberg 1991). This suggestion is also supported by the study of the fossil record of kelp associated fauna, distribution of related Laminariales genera and paleoclimatic records (Nicholson 1978; Estes and Steinberg 1988; Lüning 1990). North (1971), on the other hand, proposed a Southern Hemisphere origin because

of the much more widespread distribution of the genus in this hemisphere. Alternatively, Chin et al. (1991) proposed a process of vicariant differentiation out of a Pacific Ocean/Southern Ocean ancestral complex.

To date, only one study has shown the global phylogeography of *Macrocystis*. Analyses of ITS1 and ITS2 sequences revealed little genetic differentiation across the Southern Hemisphere, but also suggests an origin in Northern Hemisphere due to high genetic divergence and paraphyletic clades of samples from California and Mexico in comparison with samples from the Southern Hemisphere (Coyer et al. 2001). Dispersal from the Northern Hemisphere involved populations in the Baja California Mexico and/or Santa Catalina Island (USA) as intermediate populations (Coyer et al. 2001). These authors also suggested that dispersal from the Northern to the Southern Hemisphere occurred approximately 0.01 to 3 Mya. Although the study involved a global analysis, few individuals per site were analyzed and further sampling populations from Canada, northern and Central Chile and sun-Antarctic islands is required in order to support such suggestions.

So far, limited data exist on the population genetics, dispersal pattern and connectivity of *Macrocystis*. The majority of molecular studies have reported on kelp evolution (Druehl and Saunders 1992; Saunders and Druehl 1992). Genetic structure of different kelp species has been reported, e.g. *Laminaria digitata* (Billot et al. 2003), *Postelsia palmaeformis* (Kusumo et al. 2004; Kusumo et al. 2006), *Lessonia nigrescens* (Martinez et al. 2003; Faugeron et al. 2005), *Ecklonia radiata* (Coleman et al. 2009) and *Durvillaea antarctica* (Fraser et al. 2009, 2010) . Using a hierarchical sampling and 7 microsatellite loci, Billot et al. (2003) demonstrated that forests of *L. digitata* were genetically differentiated at

distances greater than 10 km. A similar result has been reported in *P. palmaeformis* (Kusumo et al. 2006) where even populations separated by only 5 m were genetically different. In *Lessonia nigrescens*, Faugeron et al. (2005), using RAPD markers, reported a strong genetic structure in populations from northern Chile. All of these species lack floating structures (pneumatocysts), thus in contrast, Fraser et al. (2010) described low genetic differentiation among the buoyant *D. antarctica* samples collected along the sub-Antarctic region.

1.4 Thesis structure

In my research I studied the taxonomy, phylogeny, dispersal and connectivity of the giant kelp, *Macrocystis*. This alga provides a useful system for investigating connectivity since has a high dispersal potential due to floating kelp rafts. In Chapter 2, I analyze the taxonomy of *Macrocystis*, using DNA barcoding and samples collected worldwide. I also give a review of the taxonomic history of the genus highlighting the most important milestones since its creation (Appendix 1). In Chapter 3, I analyze the phylogeographic structure of this alga along the south-eastern Pacific coast. Since the distribution of *Macrocystis* spans well known biogeographic barriers, this study represents a good opportunity to examine the genetic structure of giant kelp along a vast coastal extension (5000 km) and to investigate the factors (historic and contemporary) involved in giant kelp phylogeography.

The role of kelp rafts in dispersal has been widely accepted, however little is known of dispersal routes and origin of kelp rafts floating offshore. Thus in Chapter 4, I analyze samples of floating and benthic *Macrocystis* in order to determine the possible dispersal routes of floating kelp rafts. The study was

carried out in the Patagonian fjords, since this is one area where the abundance of floating kelp rafts has been intensely investigated. In Chapter 5, using two molecular markers (mitochondrial DNA and microsatellites), I analyze the global phylogeography, with emphasis on connectivity and Nouthern Hemisphere dispersal.

Because this thesis has been written as a series of independent manuscripts, there is some repetition of general information in the individual chapters. Finally, Chapter 6 provides a summary and synthesis of the proceeding chapters and outlines directions for future research.

The different chapters have been submitted or will be submitted to journals as follows:

-Chapter 2:

Macaya EC, Zuccarello GC (2010) DNA barcoding and genetic divergence in the giant kelp *Macrocystis* (Laminariales). *Journal of Phycology* 46: 736-742

-Chapter 3:

Macaya E.C. and Zuccarello G. C. Genetic structure of the giant kelp *Macrocystis pyrifera* along the south-eastern Pacific. In press *Marine Ecology Progess Series.*

-Chapter 4:

Macaya E.C., Hinojosa, I., Thiel, M., and Zuccarello G. C. Genetic data reveal possible dispersal routes of floating kelp rafts in the Patagonian Fjords. To be submitted to *Marine Biology*.

-Chapter 5:

Global connectivity of giant kelp *Macrocystis pyrifera*, is currently in preparation for submission to *Molecular Ecology*.

1.5 References

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Billot C, Engel CR, Rousvoal S, Kloareg B, Valero M (2003) Current patterns, habitat discontinuities and population genetic structure: the case of the kelp *Laminaria digitata* in the English Channel. *Marine Ecology Progress Series* 253: 111-121

Chin NKM, Brown MT, Heads MJ (1991) The biogeography of Lessionaceae, with special reference to *Macrocystis C.* Agardh (Phaeophyta: Laminariales). *Hydrobiología* 215: 1-11

Coleman MA, Brawley SH (2005) Spatial and temporal variability in dispersal and population genetic structure of a rockpool alga. *Marine Ecology Progress Series* 300: 63-77

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CHAPTER 2

DNA barcoding and genetic divergence in the giant kelp *Macrocystis* (Laminariales)

2.1 Abstract

The brown alga *Macrocystis* C. Agardh is widely distributed throughout the cold temperate waters of the Northern and Southern Hemispheres, forming ecologically diverse and productive kelp forests. The taxonomy of this alga has been under constant discussion. Since first description, species have been mostly described by holdfast and blade morphology, however the importance of these taxonomic characters has been questioned. Based on a morphological study, the genus has recently been synonymized into a single species, *M. pyrifera*, but additional genetic evidence is still lacking. Using the "DNA barcoding" gene (COI), I examined the taxonomy of *Macrocystis* collected from 19 sites worldwide, covering the distribution of the four ecomorphs (*M. 'pyrifera', M. 'angustifolia', M 'integrifolia'* and *M. 'laevis'*). The molecular data strongly support the recognition of a single species, therefore the genus should contain only one species *M. pyrifera* (L.) C. Agardh, the oldest name. Results also reveal shared haplotypes in several distant sites around the Southern Hemisphere and very low variability among samples. Additionally, samples of the ecomorphs *M. 'integrifolia'* and *M. 'pyrifera'* from a sympatric population in California had the same haplotype. The revised taxonomy significantly changes the focus of questions of *Macrocystis* distribution from interspecific dispersal and

evolutionary questions to intraspecific ecological questions on the maintenance of *Macrocystis* in certain environments that produce particular morphologies.

2.2 Introduction

The genus *Macrocystis* was created by Agardh in 1820, although it was known long before as *Bulbus marinus crinitus* (Bahuine 1651) and described by Linnaeus as *Fucus pyriferus* (1771) from specimens collected by Koenig in "Oceano Aethiopico" during a voyage between Europe and India (Womersley 1954). The exact locality of these specimens is not clear and different localities have been mentioned by different authors: Cape of Good Hope by Hooker (1847); Kerguelen or Crozet Islands by Setchell (1932); Tristan da Cunha, Gough Island or Falkland Islands by Womersley (1954). As the locality cannot be designated, a neotype has been selected from the western Falkland Islands, King George's Sound (Spencer et al. 2009).

Since the first description, several species of *Macrocystis* have been described (see Appendix 1). Most of these descriptions were based on drift specimens and pneumatocyst and blade morphology, factor considered the most important taxonomic features. Hooker (1847) combined all the species reported by the mid-nineteenth century into a single taxon, *M. pyrifera*, believing that variations among species were environmentally induced. This unification of *Macrocystis* species found general agreement (Harvey 1862, Skottsberg 1907). However, Areschoug (1883) and De Toni (1895) recognized the importance of the holdfast as a taxonomic character (Fig. 1). Howe (1914) reported *M. integrifolia* Bory de Saint-Vincent from Peru on the basis of holdfast morphology, while Setchell (1932) concluded that this species also occurred in the Northern Hemisphere (NE Pacific). Womersley (1954) continued reliance on

holdfast features reporting *M. angustifolia* Bory de Saint-Vincent in Southern Australia, Northern Tasmania and South Africa. The general agreement on the importance of the holdfast as a taxonomic feature was questioned when Hay (1986) described a fourth species, *M. laevis* C. H. Hay present at Marion Island (SW Indian Ocean), primarily based on the morphology of its smooth blades and vesiculate sporophylls, however it possessed a conical holdfast similar to *M. pyrifera*. Aguilar-Rosas et al. (2003) also collected *M. laevis* in southern Chile, but this record has been questioned (Gutierrez et al. 2006). North (1994) observed that if using blade features finds general acceptance by algal taxonomists, possibilities exist for the creation of additional species within the genus due to the high plasticity in blade and holdfast morphologies. The four species remained for more than 20 years, but recently Demes et al. (2009) proposed the conspecificity of *M. pyrifera, M. integrifolia, M. laevis* and *M. angustifolia*, with *M. pyrifera* as the only species. They demonstrated that environmental conditions influenced holdfast morphology of *M. pyrifera* and *M. integrifolia* in California and this character could therefore not be used to separate the species (Demes et al. 2009). For the purpose of this chapter and following Demes et al. (2009), I will call the former four species 'ecomorphs' (namely: *M. 'integrifolia, M. 'laevis', M. 'angustifolia'* and *M. 'pyrifera'*) and use *M. pyrifera* to refer to the taxonomic species.

Previous studies have shown inter-fertility among the three most widely distributed ecomorphs (*M. 'pyrifera', M. 'angustifolia'* and *M. 'integrifolia'*) (Lewis et al. 1986, Lewis and Neushul 1994, 1995, Druehl et al. 2005, Westermeier et al. 2007). In an early study, Coyer et al. (2001) addressed the taxonomy of *Macrocystis* using molecular methods. Using the internal

transcribed spacer (ITS1 and ITS2) they suggested that the genus is monospecific as their data showed the four species were not resolved in their phylogenetic analyses, but most of the 24 individuals analyzed corresponded to *M. 'pyrifera'* (70% of the samples) and their data did not include *M. 'integrifolia'* from the Southern Hemisphere.

Recently the use of a mitochondrial gene, cytochrome c oxidase subunit I, COI as a standardized marker has been suggested as useful for species identification in macroalgae (Saunders 2005, Robba et al. 2006, Lane et al. 2007, Kucera and Saunders 2008, McDevit and Saunders 2009). The COI gene is a relatively short piece of DNA that can be readily amplified and sequenced with one set of primers (Robba et al. 2006) and has the advantage of being an objective species identification tool in cases where identification is ambiguous (Kucera and Saunders 2008). According to McDevit and Saunders (2009) the ITS region is difficult to align above the genus level due to the large number of insertions and deletions, a problem not encountered in COI.

The origin and presence of *M. 'integrifolia'* in both hemispheres remains unresolved; on the basis of chloroplast DNA, Druehl and Saunders (1992) suggested that either *M. 'pyrifera'* and *M. 'integrifolia'* were separated prior to trans-hemispheric dispersal or diverged subsequent to dispersal through the equator during the Pleistocene glaciations. Druehl and Saunders (1992) revealed low sequence divergence between *M. 'pyrifera'* and *M. 'integrifolia'* from the Northern Hemisphere (0.08%), but samples of *M. 'integrifolia'* between hemispheres had higher divergence (0.3%).

Given the problematic morphology-based taxonomy, I investigated the species status among multiple *Macrocystis* samples collected worldwide using

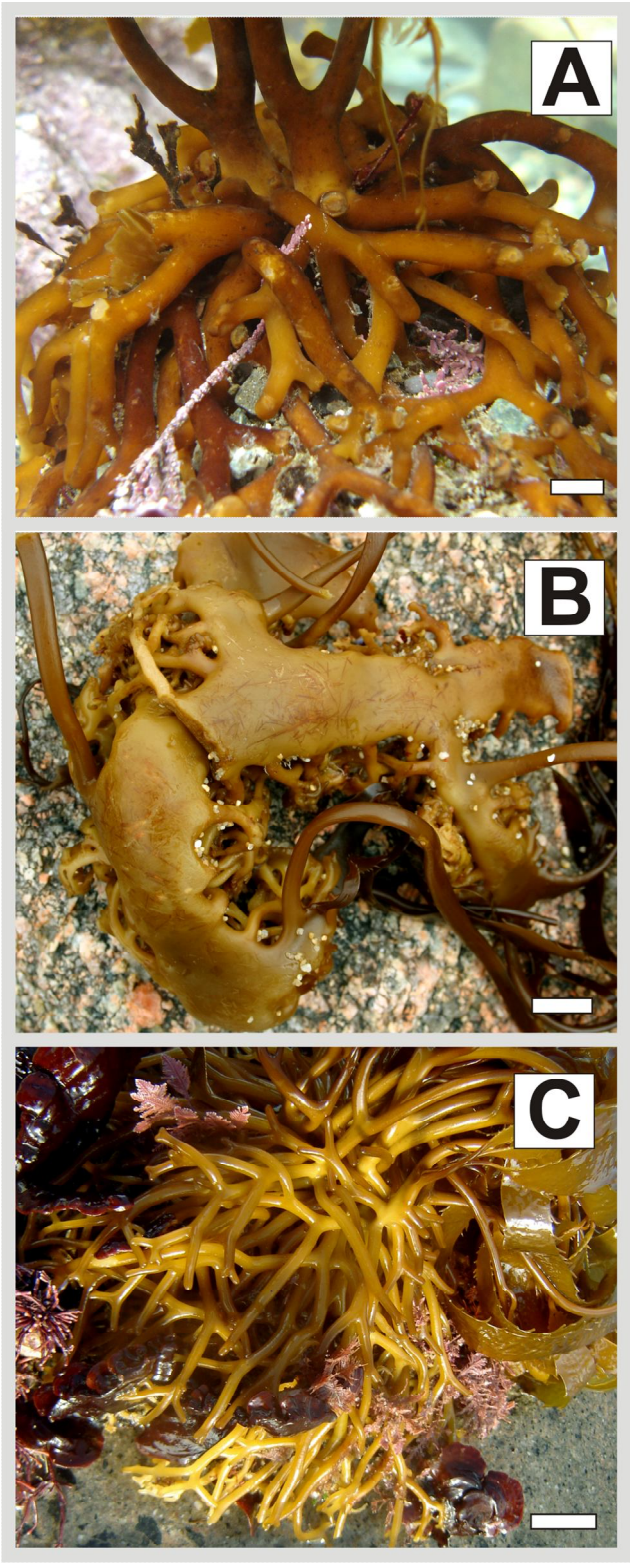

Figure 2.1 *Macrocystis* holdfasts, the main morphological feature used to distinguish the former four species. A) *Macrocystis 'pyrifera'* with a conical holdfast (individual from North Island, New Zealand), B) *Macrocystis 'integrifolia'* with a rhizomatous holdfast (individual from Northern Chile) and C) *Macrocystis 'angustifolia'* with a mounding rhizomatous holdfast (individual from Southern Australia). The holdfast of *Macrocystis 'laevis'* is the same as for *M. 'pyrifera'*. Scale bar = 2.5cm.

the DNA barcoding COI sequence. I analyzed sequences from 118 samples of the four *Macrocystis* ecomorphs enhancing sample size and geographic coverage from the previously published analysis (Coyer et al. 2001).

2.3 Methods

2.3.1 Sampling sites and collection

118 samples of *Macrocystis* were collected from 19 sites around the world, representing the distributional range of the four former described species (Fig. 2.2 and Table 2.1). At these sites, multiple individuals (5–7) were collected haphazardly in an area of ≥ 200 m^2. Healthy apical tips (2-3 cm^2) without epiphytes or epibionts were excised and preserved in sealed bags with silica gel until DNA extraction.

Samples were assigned to each ecomorph according to morphological features, predominantly based on holdfast morphology (Fig. 1). In most of the collecting sites one ecomorph was present, and no ambiguous morphologies were found. Samples with non-corrugated blades, conical holdfast and sporophylls having pneumatocysts were assigned to *M. 'laevis'* (Marion Island, type locality; Quihua and Curaco, southern Chile localities described by Aguilar-Rosas et al. 2003). Samples with a rhizomatous holdfast were assigned to *M. 'integrifolia'*, whilst samples with mounding rhizomatous holdfast were assigned to *M. 'angustifolia'*. Samples with a conical holdfast and corrugated blades were assigned to *M. 'pyrifera'*. Sympatric samples of *M. 'pyrifera'* and *M. 'integrifolia'* were collected at Stillwater Cove, Carmel Bay, California. Individuals of *M. 'integrifolia'* were collected intertidally whereas individuals of *M. 'pyrifera'* were collected subtidally, and the two populations were separated by 100 m.

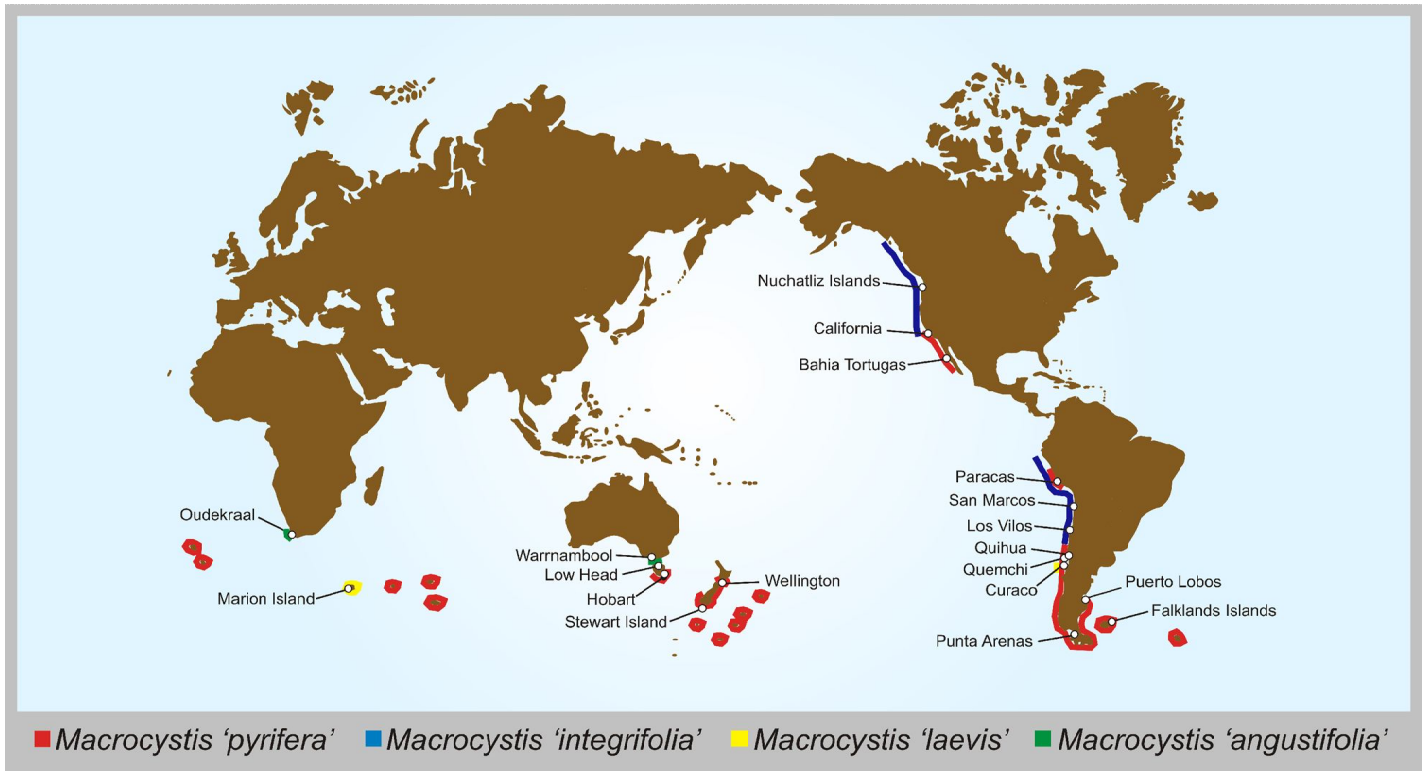

Figure 2.2 Map showing the world wide distribution of *Macrocystis* ecomorphs and sites where the individuals were collected.

2.3.2 DNA extraction and COI amplification.

DNA was extracted following a modified CTAB method (Zuccarello and Lokhorst 2005). The COI region was amplified using the primers GAZF2 and GAZR2 (Lane et al. 2007), which amplifies an approximately 610 bp fragment of the 5'-end of the COI gene.

PCR amplifications were performed in a 30 µL reaction volume consisting of 1X buffer (New England Biolabs, Massachusets, USA), 2.5 mM dNTPs, 2.5 mM $MgCl_2$, 0.025% BSA, 10 nM of each primer and 1 U *Taq* polymerase (New England Biolabs, Massachusets, USA), plus 1.5 µL of template DNA, previsouly diluted 100-fold. The PCR cycle had an initial denaturation step at 95°C for 5 min, followed by 5 cycles of 30 s at 95°C, 30 s at 60°C reduced by 1°C each cycle, and 45 s 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. PCR products were cleaned using ExoSAP-IT (USB, Cleveland, Ohio) and sequenced (Macrogen Inc, Seoul, South Korea).

Table 2.1 Sampling sites of *Macrocystis* and collection details.

Ecomorphs	Site	Coordinates	Collector	N
M. 'integrifolia'	N Chile - San Marcos	21° 00' S - 70° 09' W	E. Macaya	7
	N Chile - Los Vilos	31° 55' S - 71° 31' W	E. Macaya	7
	USA - California	36° 33' N - 121° 56' W	K. Demes & M Graham	6
	Canada - Nuchatliz Islands	49° 36' N - 126° 32' W	J. Mee & J. Moore	6
M. 'pyrifera'	Peru - Paracas	13°55' S - 76°17' W	A. Perez-Matus	5
	S Chile – Quemchi	42° 08' S - 73° 28' W	E. Macaya	7
	S Chile - Punta Arenas	53° 28' S - 70° 51' W	A. Mansilla	6
	Argentina – Puerto Lobos	42° 03' S - 65° 02' W	L. Orensanz	6
	New Zealand – Stewart Is.	46° 53' S - 168° 07' E	E. Macaya	6
	USA - California	49° 48' N - 126° 59' W	K. Demes & M Graham	5
	Falklands Islands	51° 37' S - 57° 45' W	J. Pompert	6
	Mexico - Bahia Tortugas	27° 40' N - 114° 53' W	R. Riosmena-Rodríguez	5
	New Zealand - Wellington	41° 17' S - 174° 49' E	E. Macaya	5
	S Tasmania - Hobart	43° 00' S - 147° 19' E	E. Macaya	5
M. 'laevis'	S Chile - Quihua	41° 45' S - 73° 09' E	I. Hinojosa, M. Thiel & O. Cerda	7
	S Chile - Curaco	42° 26' S - 73° 36' W	I. Hinojosa, M. Thiel & O. Cerda	6
	Marion Island	46° 50' S - 37° 50' E	C. Fraser	7
M. 'angustifolia'	S Africa - Oudekraal	33°59' S - 18° 21' E	R. Anderson	6
	S Australia - Warrnambool	38° 24' S - 142° 28' E	E. Macaya	5
	N Tasmania - Low Head	41° 03' S - 146° 47' E	E. Macaya	5

2.3.3 Data Analysis

COI sequences were aligned using ClustalW in the BIOEDIT program (Hall 1999). Haplotype frequencies were calculated using the software DnaSP Version 5.0 (Rozas and Rozas 1995). Estimates of haplotypic (He) and nucleotide (π) diversity were calculated for each species group and for the entire dataset using ARLEQUIN version 3.1 (Excoffier et al. 2005). Haplotype genealogies were reconstructed with a median-joining network by using NETWORK v 4.5 (Bandelt et al. 1999). Sequence divergences among species were calculated using MEGA 4.0 (Tamura et al. 2007).

2.4 Results

The COI sequences of the 118 analyzed individuals were: 56 of *M. 'pyrifera'*, 26 of *M. 'integrifolia'*, 16 of *M. 'angustifolia'* and 20 of *M. 'laevis'* (ecomorphs assigned according to morphological characteristics, see Methods section for more details). The 613 aligned bp showed 11 variable sites that yielded nine unique haplotypes (GenBank Accession Numbers HM153257 to HM153265) (Fig. 2.3). The ecomorph with the most haplotypes was *M. 'pyrifera'* with seven; this ecomorph also displayed the highest haplotype diversity, *He*= 0.71558 (Table 2.2). All samples of *M. 'laevis'* had the same haplotype (H1), despite the wide geographical range of collection for this ecomorph (southern Chile and Marion Island separated by approx. 8000 km). *Macrocystis 'integrifolia'* displayed the highest nucleotide diversity value π = 0.00320 (Table 2.2).

Table 2.2 Measure of COI haplotype and nucleotide diversity. N= sample size; H= numbers of haplotypes; *He*= haplotype diversity; π= nucleotide diversity.

Species	N	H	*He*	π
M. 'integrifolia'	26	3	0.66769	0.00320
M. 'pyrifera'	56	7	0.71558	0.00232
M. 'laevis'	16	1	0.00000	0.00000
M. 'angustifolia'	20	2	0.50000	0.00082

Table 2.3 Divergence in COI between *Macrocystis* ecomorphs based on uncorrected distances.

Species	*M. 'integrifolia'*	*M. 'pyrifera'*	*M. 'laevis'*	*M. 'angustifolia'*
M. 'integrifolia'	-			
M. 'pyrifera'	0.00348	-		
M. 'laevis'	0.00270	0.00126	-	
M. 'angustifolia'	0.00332	0.00187	0.00187	-

The hypothesized ancestral haplotype (H1) was found in all ecomorphs (Fig. 2.3 A) and had a wide distribution including samples from S. Australia, Northern, Central and Southern Chile, Marion Island and Argentina (Fig. 2.3 B). Some haplotypes were unique to a particular population (H4= Paracas, Peru, H3= San Marcos, Northern Chile, H5= Punta Arenas, Southern Chile, H6= Falklands Is., H7= Puerto Lobos, Argentina, H8= Oudekraal, South Africa, H9= Bahia Tortugas, Mexico) (Fig. 2.3 B). Sequence divergence between haplotypes is low (Table 2.3), ranging from 0.00126 to 0.00348, since six of the eight haplotypes vary with respect to H1 by only one substitution. Samples of *M. 'pyrifera'* and *M. 'integrifolia'* collected in California had the same haplotype (H2), which was also shared with samples of *M. 'integrifolia'* from Canada. Samples of *M. 'integrifolia'* from the Northern Hemisphere (H2) and Southern Hemisphere (H1) differ by 5 substitutions (0.82% divergence) and interestingly the divergence between *M. 'integrifolia'* and *M. 'pyrifera'* in both hemispheres was similar or lower.

2.5 Discussion

The molecular data confirm previous suggestions (Hooker 1847, Skottsberg 1907, Graham et al. 2007, Demes et al. 2009) that only one species of *Macrocystis*, *M. pyrifera*, should be recognized. The low genetic variation found in the worldwide collections and shared haplotypes between all ecomorphs suggests that all these ecomorphs share a very recent common ancestor. I also found no genetic distinction between two sympatric populations of *M. 'pyrifera'* and *M. 'integrifolia'* from California.

Previous taxonomy is not concordant with molecular evidence. The main morphological features applied to discriminate species, holdfast and blade morphology, have been reported as phenotypically plastic under different environmental conditions such as: temperature (North 1971), wave-exposure (Brandt 1923, Druehl 1978), currents (Wheeler 1980, Kain 1982, Hurd et al. 1996) and depth (Clendenning 1964, van Tüssenbroeck 1989a). Demes et al. (2009) based on observations in Chile and California suggests that polymorphism in *Macrocystis* holdfast morphology is determined by the depth at which the sporophytes grow (Fig. 1 in Demes et al. 2009). They also observed that the height of the basal stipe increases with deep. Furthermore, they carried out transplants of intertidal *M. 'integrifolia'* to the subtidal (2.5m), which resulted in a switch in holdfast morphology to that of *M. 'pyrifera'*.

One of the main morphological features used to describe *M. 'laevis'* is its non-corrugated blades. Smooth blades have also been described from the Falkland Islands (Skottsberg 1921, van Tüssenbroeck 1989b), and from French Farm, South Island, New Zealand (Fig. 4). The results show no genetic difference among samples of *M. 'laevis'* collected from Southern Chile and Marion Island and *M. 'pyrifera'* from different sites in the Southern Hemisphere.

Additional evidence to consider *Macrocystis* as monospecific comes from inter-fertility studies (Lewis et al. 1986, Lewis and Neushul, 1994, 1995, Druehl et al. 2005, Westermeier et al. 2007). Laboratory crosses between ecomorphs have been reported in the Northern (Lewis et al. 1986) and Southern Hemisphere (Westermeier et al. 2007), although many of these studies did not check for hybrid viability in the field or over multiple generations. These data would

strongly suggest that reproductive isolation and the biological species criterion do not apply to the previously named species of *Macrocystis*.

The divergence values in COI among species found in this study (0.00 – 1.80%) are under the threshold generally used for the characterization of species in barcoding studies of macroalgae (Lane et al. 2007, Kucera and Saunders 2008, McDevit and Saunders 2009, Saunders 2009). Using COI, Fraser et al. (2009a) found divergence values between 3.0 and 3.8% in two forms of *Durvillaea antarctica* (Chamisso) Hariot in New Zealand. Divergence among *Macrocystis* ecomorphs are much less than interspecific COI variation (2.2%–4.7%) detected within the brown algal genus *Alaria* (Lane et al. 2007). Previous molecular studies carried out in *Macrocystis* have also shown low genetic diversity but usually using restricted sampling (few samples and collection sites). Using chloroplast DNA, Yoon et al. (2001) reported low genetic divergence among three *Macrocystis* ecomorphs collected in California and Argentina, but they included few samples. Using the ITS1 and ITS2 regions, Coyer et al. (2001) were unable to differentiate *M. pyrifera* and *M. integrifolia* from the Northern Hemisphere, and samples from the Southern Hemisphere displayed very low sequence divergence. The results, obtained from a wider sampling area and more samples are consistent with these studies; these data also indicate more COI haplotypes in the Southern Hemisphere than in the Northern Hemisphere, which also reflects the global distribution with a major presence of the genus in the Southern Hemisphere.

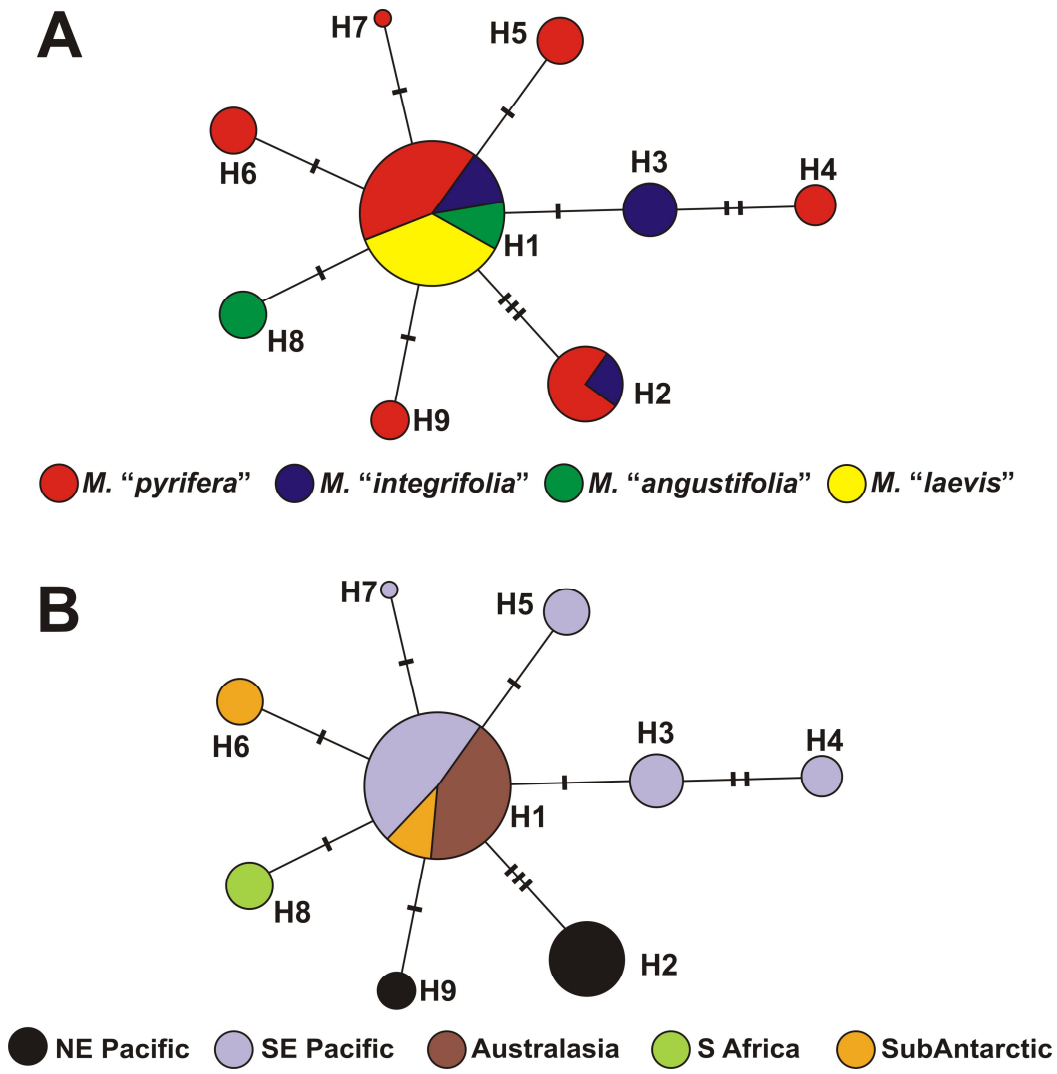

Figure 2.3 Haplotype network for COI of *Macrocystis* individuals, with circle size proportional to haplotype frequency. Lines connecting the haplotypes represent single bp mutations. Short cross lines represent undetected/hypothetical haplotypes. A) Haplotypes are shaded according to the respective four ecomorphs. B) Haplotypes are shaded according to the geographic origin (NE Pacific= Nuchatliz Islands, California and Bahia Tortugas; SE Pacific= Paracas, San Marcos, Los Vilos, Quihua, Quemchi, Curaco and Argentina; Australasia= Warnnambool, Low Head, Hobart, Wellington and Stewart Island; S Africa= Oudekraal; SubAntarctic= Marion Island and Falkland Islands).

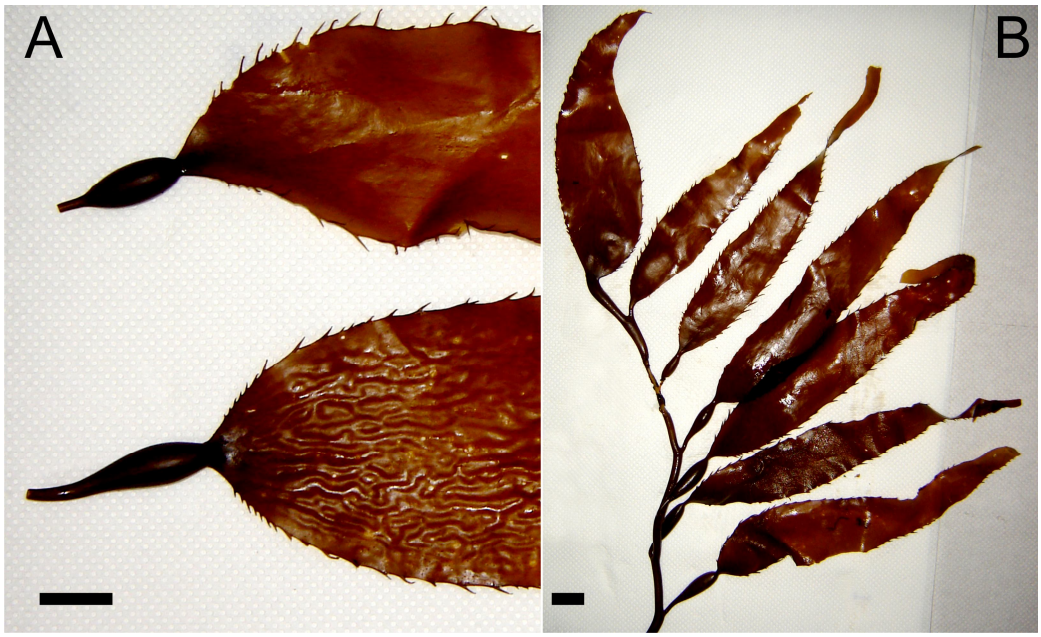

Figure 2.4 *Macrocystis pyrifera* samples collected at French Farm, South Island, New Zealand, A) Smooth and corrugated blades, and B) Apical section of a smooth *M. pyrifera* individual. Scale bar 2= cm.

Possible explanations for the observed low genetic diversity are either a recent common ancestor and rapid dispersal and/or high levels of gene flow between populations. The Laminariales are thought to have arisen in the Northern Hemisphere (Estes and Steinberg 1988, Lüning and tom Dieck 1990, Vermeij 1992, 2001, Coyer et al. 2001) and specifically *Macrocystis* is thought to have spread southward along the West Pacific coast (see Lindberg 1991). Trans-tropical dispersal probably occurred during the cooler Pleistocene (approx. 20,000 years ago) when the tropics were compressed (Estes and Steinberg 1988), suggesting that this event was recent, although the data were not able to locate the putative ancestral haplotype in Northern Hemisphere populations.

Alternatively, high levels of gene flow are related to the high dispersal potential in some brown algae. Fraser et al. (2009b) revealed colonization of a single haplotype of the floating kelp *Durvillaea antarctica* into recently deglaciated areas around the Southern Ocean. Other studies have also produced evidence of reproductive viability of detached *Macrocystis* (Macaya et al. 2005, Hernández-Carmona et al. 2006). Muhlin et al. (2008) demonstrated the importance of detached thalli on genetic differentiation in *Fucus vesiculosus* L. Connectivity through floating kelp along the Antarctic Circumpolar Current might explain the low divergence among samples from Chile, South Africa, Australia, Tasmania and New Zealand in *Macrocystis* (Coyer et al. 2001). Further analysis including an intensive sampling along the Southern Ocean and more variable markers might corroborate this hypothesis.

The revised taxonomy changes the focus of questions of *Macrocystis* distribution from interspecific dispersal and speciation, to intraspecific ecological questions about the causes that trigger variation in *Macrocystis* morphologies. In addition, management and fisheries statistics as well as governmental policies have to be changed to *M. pyrifera* in countries were two or more ecomorphs are present (e.g. Chile, Australia, Peru).

DNA barcoding has been used to resolve taxonomic ambiguity in a variety of different organisms but has also stimulated intense debate about its reliability at the species level (Plaisance et al. 2009). It should not be used as the only taxonomic tool; more traditional approaches are also necessary. To date, the International Code of Botanical Nomenclature (ICBN) does not include indication about the use of DNA barcoding, however I believe that this study

together with previous evidence supports (e.g Demes et al. 2009) the recognition of a single *Macrocystis* species.

In conclusion, shared haplotypes between several distinct ecomorphs of *Macrocystis* (indicating a recent ancestor or high gene flow between species), low genetic variation among samples collected worldwide, the known plasticity of holdfast morphology (a main criterion for former species designation) and reproductive compatibility between all ecomorphs corroborates previous suggestions that the four ecomorphs must be considered as a single species. Henceforth the only valid name should be *M. pyrifera*.

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CHAPTER 3

Genetic structure of the giant kelp *Macrocystis pyrifera* along the south-eastern Pacific.

3.1 Abstract

I assessed the phylogenetic relationships and genetic diversity of the giant kelp *Macrocystis pyrifera* along the south-eastern Pacific coast (SEP). Specifically, I analyzed the concordance of biogeographic breaks with genetic discontinuities and the effect of historical and contemporaneous events on the genetic patterns of this important seaweed. A total of 723 samples were analyzed using mitochondrial DNA sequences of the intergenic spacer atp8-S. A haplotype network, the genetic diversity and phylogenetic relationships were calculated. Only five haplotypes were found among all individuals collected along 4800 kilometres of coastline, with very low haplotype diversity and a short genealogy compared with other macroalgal species. The distribution of haplotypes showed few disjunctions that are partially congruent with the breaks in established biogeographic regions. On the southern coast a genetic break was found 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 it does reflect a break associated with other marine taxa. The low genetic diversity in northern Chile might be related to contemporary events (i.e. El Niño Southern Oscillation, areas subjected to intensive harvesting), whilst in southern Chile the haplotype distribution might reflects the effect of an historical event (Last

Glacial Maximum). Present and historic events are responsible for the genetic pattern of *Macrocystis pyrifera* observed along the SEP. The high dispersal potential by kelp rafts, recent contemporary colonization and local extinctions might explain the low genetic diversity in the SEP.

3.2 Introduction

Biogeographic regions are often described based on the overlapping ranges of many species and boundaries between these regions may derive from historical and contemporary processes (Riginos and Nachman 2001), such as glaciations and oceanographic features. Along the south-eastern Pacific coast (SEP) the presence of biogeographic barriers has been long recognized and discussed (e.g. Brattström and Johanssen 1983; Lancellotti and Vásquez 1999; Santelices and Meneses 2000; Camus 2001; Vidal et al. 2008). Most biogeographic studies have proposed two main biogeographic regions: the Peruvian or warm-temperate province (between 6ºS and 30ºS) and the Magellan or cold-temperate province (between 40-42ºS and 56ºS) and several authors have recognized a transitional area between 30-33ºS and 40-42ºS made of mixed components from the two neighboring regions (e.g. Brattström and Johanssen 1983) (Fig. 1). The distribution of algal species along the SEP has also shown geographic breaks in species composition (Santelices 1980; Meneses and Santelices 2000; Santelices and Meneses 2000). Two main break points have been reported: one at 30ºS is explained by the prevailing oceanographic conditions, resultant of upwelling events (Meneses and Santelices 2000; Santelices and Meneses 2000) and shows marked discontinuity especially in brown algae (Meneses and Santelices 2000). The second major breaking point is located at 40-42º S which has also been

reported for several marine organisms (Brattström and Johanssen 1983; Fernandez et al. 2000; Camus 2001; Thiel et al. 2007) and has been explained by both changed water conditions (lower salinity, less wave exposure) (Meneses and Santelices 2000) and topographical breakup of the coastline caused by the increased number of fjords from which large amounts of fresh water enter the sea (Lancellotti and Vásquez 1999).

Species with high dispersal potential may not show evidence of a genetic break associated with these biogeographic breaks (Thiel et al. 2007). High levels of gene flow have been reported in marine animals along the SEP. 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*: this result was attributed to its long-lived planktonic larvae (60 days). Similar lack of genetic structure along major parts of the Chilean coast has been reported in: the Chilean gastropod *Concholepas concholepas* (Gallardo and Carrasco 1996 - allozyme analysis; Cardenas et al. 2009 – COI sequences), the blue mussel *Mytilus chilensis* (Toro et al. 2006 - RAPDs) and the pelagic fish *Merluccius gayi* (Galleguillos et al. 2000 - allozyme analysis)

A less explored group of marine organisms are macroalgae. To date only one study has addressed concordance between phylogeographic patterns and biogeographic transition at the SEP. A major phylogeographic break at 30º S in the intertidal kelp *Lessonia nigrescens* was found by Tellier et al. (2009) using mitochondrial, nuclear and chloroplast DNA, this brown alga has a reduced gene flow (Martinez et al. 2003; Faugeron et al. 2005). It might be expected that a different result is observed 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 and North 1966; Dayton 1973). Long distance dispersal has been suggested for zoospores by Reed et al. (2004; 2006), however recent evidence suggests that floating kelp is 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 26% of floating *Macrocystis* rafts observed possessed functional reproductive blades (i.e. viable spores released) and it was estimated that fertility could be maintained for at least 21 days. In addition, spore dispersal from kelp rafts might play a valuable role in occasional long-distance dispersal events that are important for biogeographical expansion and genetic exchange (Reed et al. 2006). This would suggest substantial inter-populational genetic homogeneity in *Macrocystis*. 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 in southern Chile: Punta Pucatrihue (N=1) and Metri Bay (N=4) (40º- 41ºS respectively) separated only by 200 km.

During the Last Glacial Maximum (LGM, 18,000-20,000 years ago), ice sheets covered broad areas of southern Chile from 35ºS to 54ºS (McCulloch et al. 2000; Hulton et al. 2002) including the whole area of the Chilean fjords (Fig. 2). The impact of glaciations on the distribution and genetic variation of species in this area has been well documented, but most of the studies have been carried out

on terrestrial (Muellner et al. 2005; Marchelli and Gallo 2006; Himes et al. 2008; Rodriguez-Serrano et al. 2008; Victoriano et al. 2008) and freshwater biota (Ruzzante et al. 2008; Zemlak et al. 2008; Xu et al. 2009). A recent colonization, after the LGM, of *Durvillaea antarctica* in the sub-antarctic region by a series of long-distance rafting events has been suggested by Fraser et al. (2009a), however fine-scale studies along the SEP are still lacking.

Events such as the El Niño Southern Oscillation (ENSO) produce massive mortality of kelp species in the Peruvian province and the northern part of the Intermediate province (Vega et al. 2005; Vásquez et al. 2006; Thiel et al. 2007). Such sharp population declines, or bottlenecks, such as those seen during recent ENSO events, might translate into losses of genetic variation of marine organisms (Steinfartz et al. 2007). A study carried out in *Lessonia nigrescens* along the northern and central Chilean coast by Martinez et al. (2003), using RAPDs determined that after the 1982-83 ENSO, the genetic diversity of individuals from sites impacted by ENSO was lower than individuals from non-impacted sites.

Macrocystis pyrifera is one of the the largest known seaweed on earth (up to 30-40 m long) and the most widely distributed kelp species, forming extensive submarine forests that harbour a rich diversity of marine life (Neushul 1971; North 1994). *Macrocystis* also provide a valuable economic resource used for alginates extraction - 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). The distribution of *M. pyrifera* encompasses the three different biogeographic provinces along the SEP, areas affected by the ENSO phenomena and it also represents an important economic resource in Peru

and northern-central Chile, which recently, showed evidence of overexploitation (Vásquez 2008). Therefore, knowledge of genetic diversity and phylogeographic patterns might aid in the application of management and conservation policies and also provide insights of the ecological and evolutionary processes driving the actual *M. pyrifera* distribution along the SEP. The aim of this study was to: 1) analyze the genetic diversity of *M. pyrifera* over a wide latitudinal range (13˚S - 53˚S) along the SEP coast using mitochondrial gene sequences; 2) evaluate the possible coincidence of phylogeographic breaks in this genus with known biogeographic breaks, and 3) evaluate whether the genetic diversity of this alga could be related with historical and contemporaneous events affecting the SEP (e.g. LGM and ENSO).

3.3 Methods

3.3.1 Sampling sites and collection

A total of 723 samples of *Macrocystis pyrifera* were collected between 2006 and 2009 from 38 sites between 13°S and 53°S, covering the entire geographical range of the species along the SEP (Fig. 3.1 and Table 3.1). At these sites, multiple individuals (7–20) were collected haphazardly in an area of at least 200 m^2. Healthy apical tips (2-3 cm^2) without epiphytes or epibionts from individuals were excised and preserved in zip lock bags with silica gel until DNA extraction.

3.3.2 DNA extraction and atp8-S amplification

DNA was extracted following the CTAB method described by Zuccarello and 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 and Saitoh 2005; Voisin et al. 2005; Uwai et al. 2006; Tellier et al. 2009) and recently 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). PCR product (3 μL) was mixed with 9 μL 95% formamide, 0.1% aqueous bromophenol blue/xylene cyanol and 10 mM NaOH, subsequently denatured at 95^{o}C for 5 min, then snap cooled on ice before loading. Gels contained 20% 37.5:1 acrylamide/bis-acrylamide (Sigma Aldrich), 0.5X TBE buffer, 0.5% ammonium persulphate, 0.05% tetramethylethylenediamine (TEMED). Electrophoresis was carried out for 14-16 hr at 4W in 0.5X TBE buffer at 4^{o}C on 225 mm long and 0.75 mm thick gels (BioRad, Hercules, CA, USA). After electrophoresis, gels were silver stained following Bassam *et al.* (1991) and band identities were assigned by eye. To check the accuracy of SSCP typing, 3-4 individuals for each location were sequenced in both directions (Macrogen Inc., Seoul Korea). No ambiguous SSCP profiles were found and each one has the same unique sequence.

3.3.3 Data Analysis

Haplotype frequencies were calculated using the software DnaSP version 5.10 (Rozas and Rozas 1995). Sequences were aligned by 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). Haplotype genealogies were reconstructed with a median-joining network using NETWORK v 4.5 (Bandelt et al. 1999). Because of the lack of variation within populations, and low numbers of haplotypes, further analysis of genetic variation was not undertaken.

Table 3.1 Sampling sites of *Macrocystis* along the South Eastern Pacific coast, collection details and haplotype frequency.

Site	Coordinates	Collector	n	Haplotype Frequency
Paracas	13° 55' S - 73° 23' W	Alejandro Perez-Matus	20	H1=1.0
Atico	15° 58' S - 74° 02' W	Alex Gamarra	20	H2=0.26; H3=0.74
San Marcos	21° 00' S - 70° 09' W	Erasmo Macaya	20	H4=1.0
Rio Seco	21° 06' S - 70° 07' W	Erasmo Macaya	20	H4=1.0
Chipana	21° 20' S - 70° 05' W	Erasmo Macaya	20	H4=1.0
Caleta Constitucion	23° 25' S - 70° 35' W	Erasmo Macaya	20	H4=1.0
Playa Blanca	28° 11' S - 71° 09' W	Ivan Hinojosa	20	H4=1.0
Punta Choros	29° 15' S - 71° 28' W	Luciano Hiriart	20	H4=1.0
Totoral	30° 21' S - 71° 40' W	Marcelo Valdebenito	20	H4=1.0
Los Vilos	31° 55' S - 71° 31' W	Erasmo Macaya	20	H4=1.0
Totoralillo	32° 01' S - 71° 30' W	Erasmo Macaya	20	H4=1.0
Pichicuy	32° 20' S - 71° 27' W	Alonso Vega	20	H4=1.0
Algarrobo	33° 21' S - 71° 40' W	Erasmo Macaya	20	H4=1.0
La Castilla	33° 27' S - 71° 40' W	Erasmo Macaya	20	H4=1.0
Matanzas	33° 56' S - 71° 52' W	Erasmo Macaya	20	H1=1.0
Pichilemu	34° 22' S - 72° 01' W	Erasmo Macaya	20	H1=1.0
Infiernillo	34° 23' S - 72° 01' W	Erasmo Macaya	20	H1=1.0
Duao	34° 53' S - 72° 09' W	Ivan Hinojosa	20	H1=1.0
Tumbes	36° 37' S - 73° 05' W	Erasmo Macaya	20	H1=1.0
Cuidico	37° 22' S - 73° 39' W	Ivan Hinojosa	9	H1=1.0
Loncoyen	39° 49' S - 73° 24' W	Ivan Hinojosa	20	H1=1.0
Los Molinos	39° 50' S - 73° 24' W	Erasmo Macaya	20	H1=1.0
Pucatrihue	40° 32' S - 73° 43' W	Alejandro Buschmann	20	H1=1.0
Bahia Mansa	40° 34' S - 73° 44' W	Erasmo Macaya	20	H1=1.0
Ancud	41° 51' S - 73° 49' W	Erasmo Macaya	20	H1=1.0
Metri	41° 36' S - 72° 42' W	Alejandro Buschmann	20	H5=1.0
Quihua	41° 36' S - 72° 42' W	Ivan Hinojosa	20	H5=1.0
Pumillahue	41° 52' S - 74° 00' W	Ivan Hinojosa	20	H1=1.0
Quemchi	42° 08' S - 73° 28' W	Erasmo Macaya	20	H5=1.0
Dalcahue	42° 22' S - 73° 39' W	Erasmo Macaya	7	H5=1.0
Huinay	42° 22' S - 72° 24' W	Ivan Hinojosa	7	H5=1.0
Curaco	41° 26' S - 73° 36' W	Ivan Hinojosa	20	H5=1.0
Achao	42° 28' S - 73° 29' W	Erasmo Macaya	20	H5=1.0
Cucao	42° 40' S - 74° 07' W	Ivan Hinojosa	20	H1=1.0
Quellon	43° 08' S - 73° 36' W	Erasmo Macaya	20	H5=1.0
Isla Merino	47° 26' S - 74° 10' W	Ivan Hinojosa	20	H5=1.0
Isla Madre Dios	50° 08' S - 74° 40' W	Ivan Hinojosa	20	H5=1.0
Punta Arenas	53° 28' S - 70° 51' W	Andres Mansilla	20	H5=1.0

3.4 Results

A region of 133 bp was compared among 723 samples of *Macrocystis pyrifera*, collected from 38 sampling locations along the SEP. Despite the large geographic area analyzed only five haplotypes were found, distinguished by only seven variable sites (Fig. 1). Haplotype H1 was the most common, present in 37% of the samples, follow by the haplotypes H4 and H5, present in 33% and 27% of the samples respectively, and haplotypes H2 and H3 with the minor proportion in only 3% of the samples. In general, all the haplotypes were restricted to a specific geographic area, H4 ranging from San Marcos (21°S) to Algarrobo (33°S), H1 from Matanzas (33°S) to Cucao (42°S), H5 from Metri (41°S) to Punta Arenas (53°S), whereas the haplotypes H2 and H3 were restricted to Atico, Peru (15°S). Interestingly the most northern sampling site, Paracas (13°S), displayed the same haplotype as central Chile (H1). Each sampling site displayed a unique haplotype and no shared haplotypes were found among populations, with the only exception of Atico where haplotypes H2 and H3 were both found (Fig.1, Table 1).

The haplotype diversity for all the samples was 0.7561 (±0.013 SD) and nucleotide diversity was 0.01563 (±0.00056 SD). The haplotype network (Fig. 1, II) indicates haplotype H1 as a central haplotype, separated only by one substitution from haplotypes H2 and H3, and by three substitutions from haplotype H4.

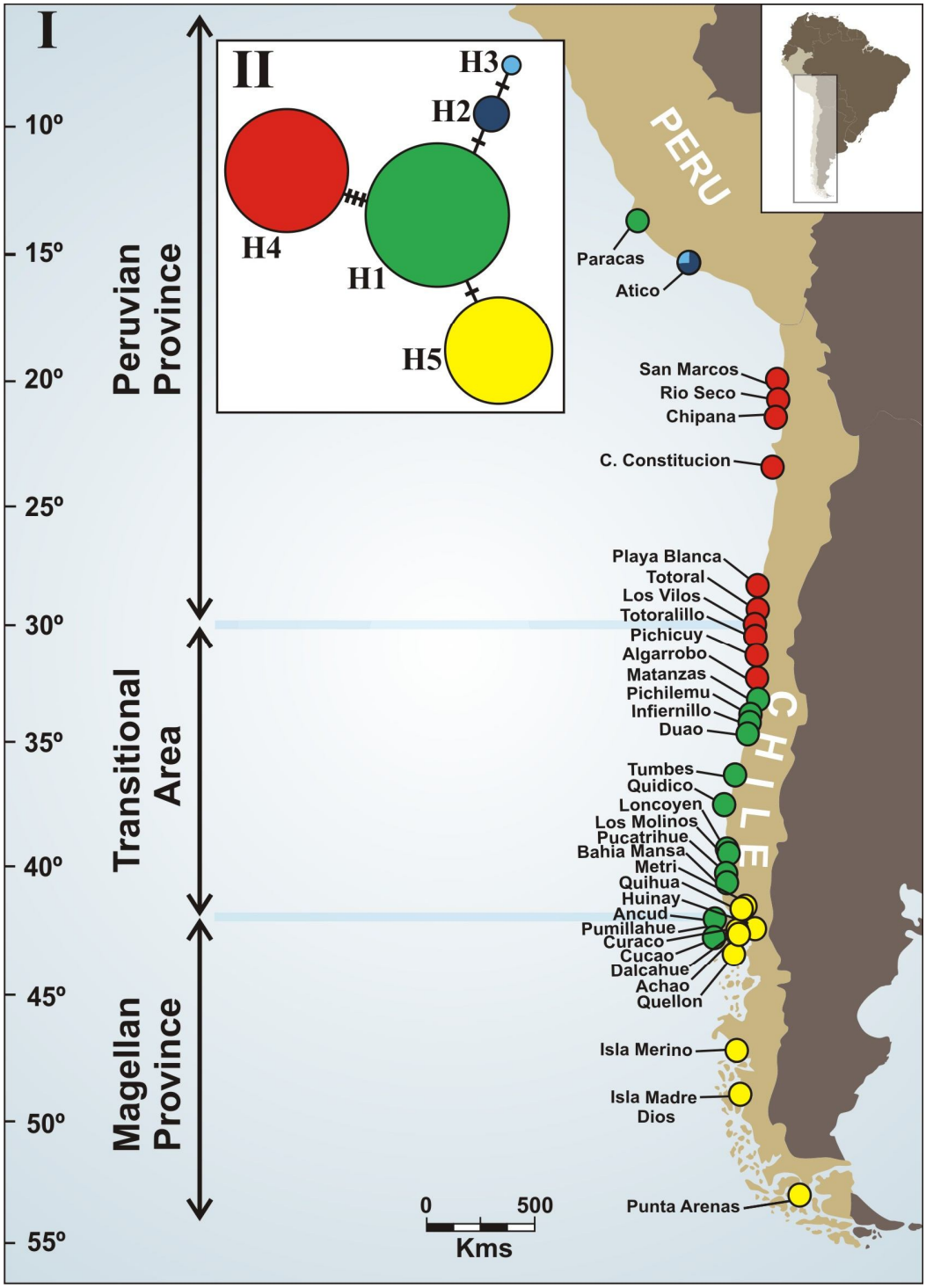

Figure 3.1 I) Map of South Eastern Pacific showing the location of collecting sites, detailed descriptions of sites are presented in Table 1. Different colours represent different haplotypes. Map also shows the three different biogeographic provinces reported to SE Pacific coast. Light blue lines represent the biogeographic breaks at 30 ºS and 42 ºS. II) Statistical parsimony network of all sampled populations, the size of each circle is proportional to the haplotype frequency, connecting lines shows mutational

pathways among haplotypes and small lines represent single site substitutions, circle size proportional to haplotype frequency.

Another interesting result was found in Chiloé Island were two different haplotypes were present: haplotype H1 was restricted to the west side of the island, whereas haplotype H5 was found only on the east side (Fig. 3.2).

At 30° no sign of a phylogeographic break was observed. At 42°S I found an overlap of two haplotypes: H1 ends its distribution whilst the haplotype H2 begins its distribution that ends at 53°S.

3.5 Discussion

This study showed a low amount of genetic differentiation among atp8-S sequences of *Macrocystis pyrifera* along the SEP. The phylogeographic structure does reveal an interesting pattern related with contemporary and historical events. Two clear genetic breaks were found and their relations with biogeographic barriers is discussed below.

3.5.1 Genetic diversity and dispersal potential

The data showed very low genetic diversity in the SEP. Along nearly 5000 km only five haplotypes were found: three common haplotypes (H1, H4 and H5) were present in 97% of the sampling sites and two haplotypes (H2 and H3) restricted to one population (Atico, Peru). The difference between haplotypes was also minimal with a maximum of seven substitutions. Haplotypes were distributed over very large geographic areas: haplotype H4 in northern Chile between 21°S to 33°S, (approx. 1500 km); haplotype H1 in central Chile between 33°S to 42°S (approx. 1000 km); and haplotype H5 in southern Chile between 41°S to 53°S (approx.1400 km). A similar low genetic variation has

been shown in several marine species along the Chilean coast, both invertebrates (Gallardo and Carrasco 1996 – *Concholepas concholepas,* allozymes; Toro and Aguila 1996 – *Ostrea chilensis,* allozymes; Gallardo et al. 2003 – *Eurhomalea,* allozymes; Gomez-Uchida et al. 2003, *Cancer setosus,* AFLP; Toro et al. 2006 - *Mytilus chilensis,* allozymes) and fish (Galleguillos et al. 2000 *Merluccius gayi gayi,* allozymes), which has been associated with long lived larvae that have considerable dispersal ability. Along the SEP, a few studies have analyzed the genetic structure of macroalgae (e.g. Martinez et al. 2003; Faugeron et al. 2005; Vidal et al. 2008; Tellier et al. 2009) and have found high genetic differentiation between populations. In contrast, I provide evidence for low genetic variation in a macroalga over a wide areas of the SEP.

The low genetic variation might have different explanations. First, a recent colonization in this area, *M. pyrifera* is thought to have originated in the Northern Hemisphere and spread towards the Southern Hemisphere reaching western South America recently (0.01 to 3 Ma) (Coyer et al. 2001). This is supported by genetic evidence from ITS (Coyer et al. 2001) and mitochondrial COI sequences (Chapter 2), where little divergence was found among samples collected on a wide geographic scale in Southern Hemisphere. Alternatively high levels of gene flow between populations, might have a direct relationship with the high dispersal potential of *Macrocystis,* 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 *Macrocystis* rafts along the Chilean coast has been reported (Macaya et al. 2005). Rafting of detached *Fucus spiralis* adults has been proposed to lead to increased gene flow (Coleman and Brawley 2005). Furthermore dispersal by floating thalli has been suggested for

many macroalgal species (Dayton 1973; van den Hoeck 1987; Hernández-Carmona et al. 2006; McKenzie and Bellgrove 2008; Fraser et al. 2009a). Floating kelp rafts of *M. pyrifera* can survive for long periods at the sea surface in cooler conditions (<15°C), where degradation is lower (Rothausler et al. 2009). This would suggest that gene flow might be favoured by kelp rafting in high latitudes on the SEP. Finally, recent colonization of unoccupied habitats along the coast can be also pointed out, a suggestion made be Fraser et al. (2010) for rafter of *D. antarctica*.

3.5.2 Concordance of biogeographic and phylogeographic breaks

Along the SEP, *M. pyrifera* displayed five haplotypes. Three of them were more frequent and their distribution is partially correlated with previously described biogeographic provinces and biogeographic breaks (30°S and 42°S). Haplotypes H4, H1 and H5 are partially restricted to the Peruvian, Intermediate and Magellan provinces described respectively for the SEP.

No phylogeographic break was found for *Macrocystis pyrifera* at 30°S, given that haplotype H4 has a continuous distribution from 18°S to 33°S. However for the intertidal kelp *Lessonia nigrescens* a clear genetic break has been recently established at 30^{o}S (Tellier et al. 2009) suggesting the limited dispersal might have contributed to the maintenance of this genetic pattern, a brown alge with similar life history but withouth floating structures. The presence of upwelling in this area is thought to be responsible for a biogeographic break in brown macroalgae (Santelices 1980; Meneses and Santelices 2000). However there is a clear phylogeographic break at 33°S for *M. pyrife*ra, which might be related to some environmental adaptation of

Macrocystis 'ecomorphs' (Graham et al. 2007; Demes et al. 2009). In northern-central Chile two different 'ecomorphs' are present (Chapter 2), haplotype H4 whose southern distribution limit is at 33°S represents samples of the '*M. integrifolia*' ecomorph, while the ecomorph '*M. pyrifera*' have their northern distributional limit at 33°S. No overlap of haplotype distributions 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). Both environments differ in factors known to regulate *Macrocystis* growth, productivity and reproduction (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). On the other hand, biogeographic breaks for different taxa do exhibit some latitudinal scattering throughout northern Chile, but they are significantly concentrated around 30°S and 33°S (Thiel et al. 2007). Thus, the phylogeographic break in *Macrocystis* is still within the range of biogeographic breaks described for other marine taxa.

Additionally the presence of unique haplotypes in Atico and the putative ancestral haplotype H1 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. But there is concordance with the presence of Haplotype H1 representing ecomorph '*M. pyrifera*' and the description of the same ecomorph on the central-northern Peruvian coast (Dawson et al. 1964; Acleto 1986). However to date there have been no genetic or phylogeographic studies

on macroalgae from Peru that facilitate a comparison and furthermore *Macrocystis* distribution in Peru remains unclear (Lleellish et al. 2001)

At 42°S the phylogeographic breaks coincide with a main biogeographic break described for the SEP (Camus 2001). The haplotype distribution at the contact area overlaps with haplotype H1 distributed up to Cucao at 42°S and haplotype H5 which begins its distribution in Metri at 41°S. The biogeographic break at 40-42º S has been hypothesized to be due to topographical breakup of the coastline by fjords where large volumes of freshwater enter the sea (Lancellotti and Vásquez 1999) and the divergence of main oceanic currents (Humboldt and Cape Horn, Fig. 3.2) (Cardenas et al. 2009). Interestingly both haplotypes were locally separate, haplotypes H1 and H5 were found on the west and east coast of Chiloé Island respectively (Fig. 3.2). This particular distribution of haplotypes might be related to the Last Glacial Maximum LGM (see below). To my knowledge no studies have investigated concordance between biogeographic and phylogeographic breaks in macroalgae at this latitude. Additional studies on other macroalgal species might also revealed a similar pattern.

3.5.3 Contemporaneous and historical events

Along the area of the SEP affected by ENSO (6-30°S) four haplotypes were found, 3 haplotypes in Peru and only 1 in northern central Chile up to 33°S. Local extinction of kelp populations during ENSO events is common in the area between 10°S and 21°S (Camus 1990; Vásquez et al. 2006; Thiel et al. 2007). Reduced genetic variation in the kelp *Lessonia nigrescens* in two sites at northern Chile, Iquique (20°S) and Antofagasta (23°S) together with a slow recolonization

(less than 60 km in 20 years) has been reported by Martinez et al. (2003). These authors associated results with ENSO which produced massive mortalities of kelp along 600 km in northern Chile. It is likely that *M. pyrifera* was similarly effected. 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 might colonize following a northwards direction from the Humboldt Current, much more quickly than do non-floating algae such as *Lessonia nigrescens*. Further research with more variable molecular markers (microsatellites) might reveal a detailed genetic structure in populations affected by ENSO and might also display the location of donor populations after extinctions.

Alternatively the distribution of haplotype H5 in central-southern Chile might be related to historical processes such as the extension of the ice sheet during the LGM. This event had a major impact on the distribution of species (Hewitt et al. 2003). In brown algae it has been suggested that entire populations were completely eliminated (Fraser et al. 2009a). The data suggests an effect from the LGM on the haplotype distribution of *Macrocystis pyrifera* from 42˚S southwards because there is a perfect match between hypothesized ice sheet extent and haplotype H5 distribution (Fig. 2). The effect of the LGM on algal distribution and genetic structure has been studied 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 and Brawley 2009). Studies in the Southern Hemisphere are restricted to the genus *Durvillaea* (Fraser et al. 2009a; Fraser et al. 2009b). A recent recolonization after the LGM via kelp rafts in the sub-Antarctic region has been suggested by Fraser et al. (2009a). A similar scenario

might be possible for *Macrocystis pyrifera* along the SEP, with kelp rafts colonizing areas after the LGM. However, the origin of the source populations remains unknown as very low variation among haplotypes is found. A wider sampling in the area and samples from the Atlantic (Falklands, Argentina) might help tease apart distribution and colonization process. This is the first study to show evidence of an effect of the LGM on the genetic structure of a marine organism in the SEP.

3.6 Conclusions

Present and historic climate change might be responsible for genetic patterns of *Macrocystis pyrifera* in the SEP. Although sampling was extensive (over 4800 km) only five haplotypes were found with few mutations separating them. High dispersal potential and a recent colonization history might lead to this genetic homogeneity. Despite its dispersal potential, phylogeographic breaks were evident, associated with known biogeographic breaks. The main biogeographic break described at 42˚S corresponds with a phylogeographic break in the same area. The low genetic diversity in lower latitudes might also be related with local extinctions of kelp bed populations due to ENSO events, whilst historic changes due principally to the LGM have shaped the genetic features of *Macrocystis* in southern Chile. More recent events related with anthropogenic disturbances (e.g. intense harvesting of kelp beds) might also produce an effect the genetic structure of this important kelp, but the markers used here might not reflect such recent events. This results contribute to the management of this important ecologically and economically 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. For example, the effect (genetically or ecologically) of kelp transplanting from southern to northern Chile, which is a common practice, is unknown. Finally, further research using more variable molecular markers will be useful to detect and understand colonization routes, connectivity and the effect of anthropogenic disturbances on *Macrocystis*.

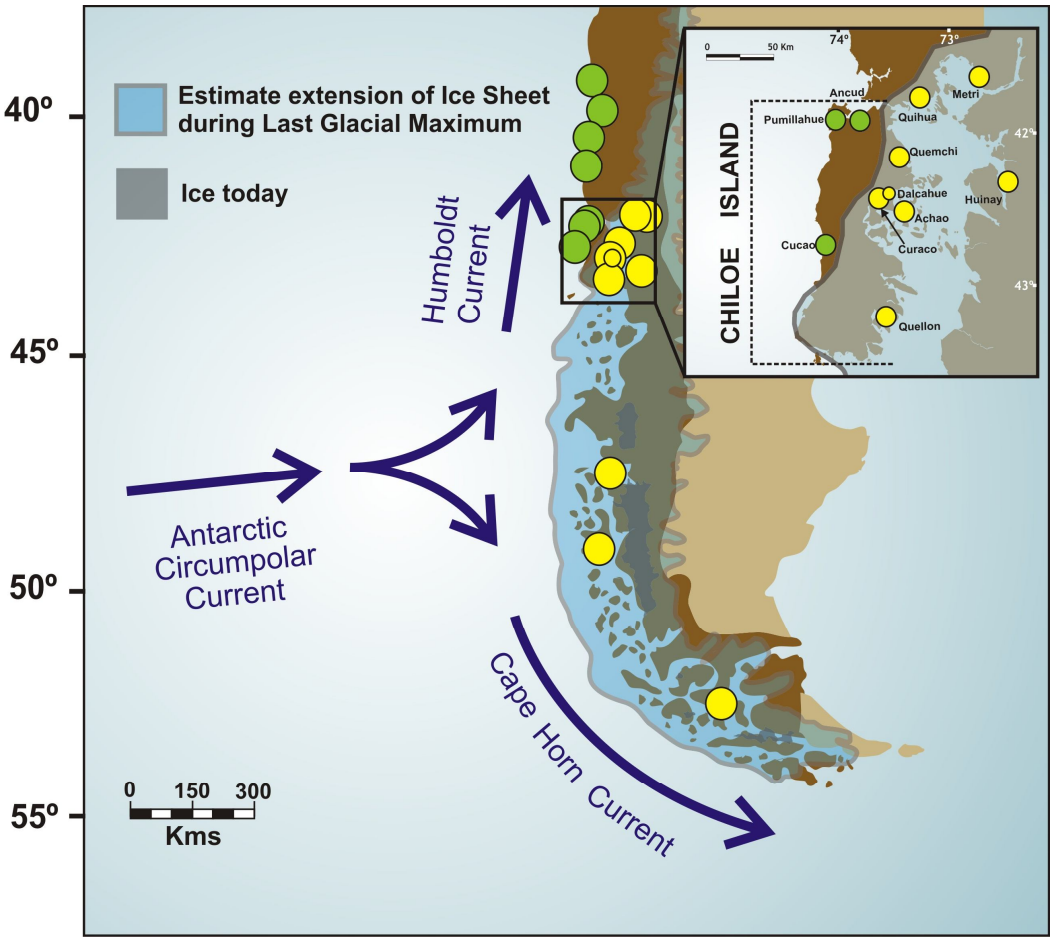

Figure 3.2 Distribution of haplotypes in southern Chile showing the extent of the Last Glacial Maximum ice sheet and the distribution of existing icefields. Circle sizes are proportional to the number of individuals from each population (green circle represent hapltype H1 whereas yellow cuircles represent hapltype H5). Insert display detailed haplotype distribution at Chiloé Island and neighbour sampling sites. Arrows show directions of major currents in Southern Chile.

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CHAPTER 4

Genetic data reveal possible dispersal routes of floating kelp rafts in the Patagonian Fjords

4.1 Abstract

Buoyant macroalgae can persist at the sea surface for weeks or months after detachment, which may represent an important mechanism of long distance dispersal. The brown alga *Macrocystis pyrifera* (L.) C. Agardh represents a good model for studying dispersal, because (i) it forms kelp rafts that might travel long distances (up to 900 km), and (ii) kelp rafts are capable of reproduction after detachment. Identifying the possible origin of a kelp raft is one of the first steps to establish the importance of rafting as a dispersal mechanism. Using two mitochondrial markers (atp8-S and COI), I examined the genetic structure of floating and benthic samples of *M. pyrifera* in the Patagonian fjords and then try to infer the possible origin of these kelp rafts. The results revealed shared haplotypes between floating and specific benthic sampling sites (200 km apart), which provides indication of possible travelling distances and dispersal routes. Although low genetic variation was found and one haplotype was dominant throughout most of the sampling sites, both molecular markers revealed that kelp rafts were mixtures of sporophytes from different sources. Comparison with populations north and south of the study area revealed possible southwards

dispersal by rafting. This study represents the first step to identify the relevance of rafting in the connectivity among kelp populations in the Patagonian Fjords.

4.2 Introduction

Rafting has been proposed as an important alternative dispersal mechanism for marine and terrestrial organisms (see Queiroz 2005; Thiel and Haye 2006 and references therein), and several studies have provided evidence that long-distance rafting is possible (Waters and Roy 2004; Donald et al. 2005; Fraser et al. 2009, Fraser et al. in press). Some of the most common floating objects from coastal environments are macroalgae (Thiel and Gutow 2005). The dominant floating macroalgal species in the southern hemisphere are those with air-filled cells (e.g. *Durvillaea antarctica* (Chamisso) Hariot) or pneumatocysts (e.g. *Macrocystis pyrifera*). These structures keep the algae afloat after detachment, and algal rafts can potentially travel over long distances. Floating times of kelp rafts may be up to 3 months (Hobday 2000b; Hernández-Carmona et al. 2006), and *M. pyrifera* rafts remain reproductively viable while afloat (Macaya et al. 2005; Hernández-Carmona et al. 2006). Thus, rafting could facilitate connectivity among kelp populations, and actual measurements of the influence of kelp rafts on the genetic structure of *M. pyrifera* populations are lacking. Using mitochondrial and chloroplast markers (COI; *rbc*L) Fraser et al. (2009) found genetic homogeneity in the bull kelp *Durvillaea antarctica* throughout the subantartic region. They suggested that populations had been wiped out during the Last Glacial Maximum (LGM) and that subantarctic sites were rapidly recolonized via long-distance rafting after ice retraction. Results from a more local study show that rafting can also influence present-day connectivity among local populations (Muhlin et al.

2008). Recently Fraser et al. (in press) analyzing genetically washed ashore bull kelp *D. antarctica* specimens, have demonstrated rafting of several invertebrate species as well the alga itself, these organisms travelled approximated 600 km.

Usually, two main questions arise when a kelp raft is found floating offshore: (1) How long has it been afloat? and (2) Where does it come from? The answers to these questions are crucial to determine the importance of rafting as a dispersal mechanism. Estimates of kelp floating time have been obtained using morphological measurements (change in length of blades following detachment) of benthic and floating plants (Hobday 2000a, E. C. Macaya et al. unpublished data), size of associated fauna (e.g. stalked barnacles) (Macaya et al. 2005; Hernández-Carmona et al. 2006; Fraser et al. in press), and distance from potential source regions (Helmuth et al. 1994). However, it is very difficult to determine the origin of a kelp raft. The trajectory of algal rafts can be followed by buoys and drifters (e.g. Harrold and Lisin 1989; Komatsu et al. 2008), but this only allows tracking rafts from determined release sites. Molecular markers provide a useful tool to infer the origin of kelp rafts, when examining benthic and floating samples. Thus, DNA can be turned into a mapping device for determining dispersal (Palumbi et al. 2003) and sources of rafted communities (Fraser et al. in press).

The Patagonian fjords (41°S–55°S) are one of the largest estuarine ecosystems in the world, over 1,600 km long and around 84,000 km of coastline when considering all the contours of its multiple islands and peninsulas (Palma and Silva 2004). Extensive kelp forests of *M. pyrifera* are present in this area (Dayton 1985). The study of this area is particulary interesting since its oceanographic features are well documented , furthermore the abundance and

density of floating macroalge have been intensively studied (Hinojosa et al. 2007a, 2007b, 2010, in press) indicating a suitable area of study due to high densities of *M. pyrifera* rafts reporteded (Hinojosa et al. 2010). In this study I used two mitochondrial markers to infer the possible origin and dispersal routes of *M. pyrifera* kelp rafts in the Patagonian fjords by analyzing floating and benthic individuals (as potential sources).

4.3 Methods

Benthic and floating samples of *Macrocystis pyrifera* were collected at 13 sites from the Patagonian fjords between 46°S and 50°S (Fig. 1, Table 1), during an oceanographic cruise in late austral spring (November 2008) as part of the research program 'CIMAR-Fiordo' (Marine Research Cruises in Remote Areas). Benthic samples were collected from attached adult thalli (n = 4-6) at random locations within an area of at least 200 m^2 per site. Healthy apical tips (2-3 cm^2) without epiphytes or epibionts were cut off and preserved in zip lock bags with silica gel. Floating samples were collected where kelp rafts were encountered. A small auxiliary boat was lowered and samples were taken using a special dip-net (ring diameter 60 cm, mesh size 3 mm in the upper part and 0.5 mm in the cod end) or by a diver who carefully separated individual samples from large algal patches. Four to six different individuals were sampled per raft, each algal piece came from an stipe attached to a different holdfast.

DNA was extracted following the CTAB method described by Zuccarello and Lokhorst (2005). Two mitochondrial sequences were amplified: Cytochrome c oxidase subunit I (COI) and the intergenic spacer region between genes atp8 and trnS (atp8-S). The COI region was amplified using the primers GAZF2 and

GAZR2 (Lane *et al.*, 2007), whereas the atp8-S region was amplified using the primers atp8-trnS-F and atp8-trnS-R (Voisin et al. 2005).

The PCR amplifications followed (Methods from Chapter 2) for COI and Voisin et al. (2005) for atp8-S. PCR products were cleaned using ExoSAP-IT (USB, Cleveland, Ohio) and commercially sequenced (Macrogen Inc, Seoul, South Korea). DNA sequences were aligned using ClustalW in the BIOEDIT program (Hall 1999). Haplotype frequencies were calculated using the software DnaSP Version 5.0 (Rozas and Rozas 1995). Estimates of haplotypic (*h*) and nucleotide (π) diversity were calculated for each marker dataset using ARLEQUIN version 3.1 (Excoffier et al. 2005). Additionally, *M. pyrifera* DNA sequences (Macaya and Zuccarello 2010 - Chapter 2) from localities north and south of the study area were included for comparison as potential sources of rafts (North: Chiloé Island, Bahía Mansa, Quihua and South: Punta Arenas, Puerto Lobos-Argentina) (Fig. 4.1).

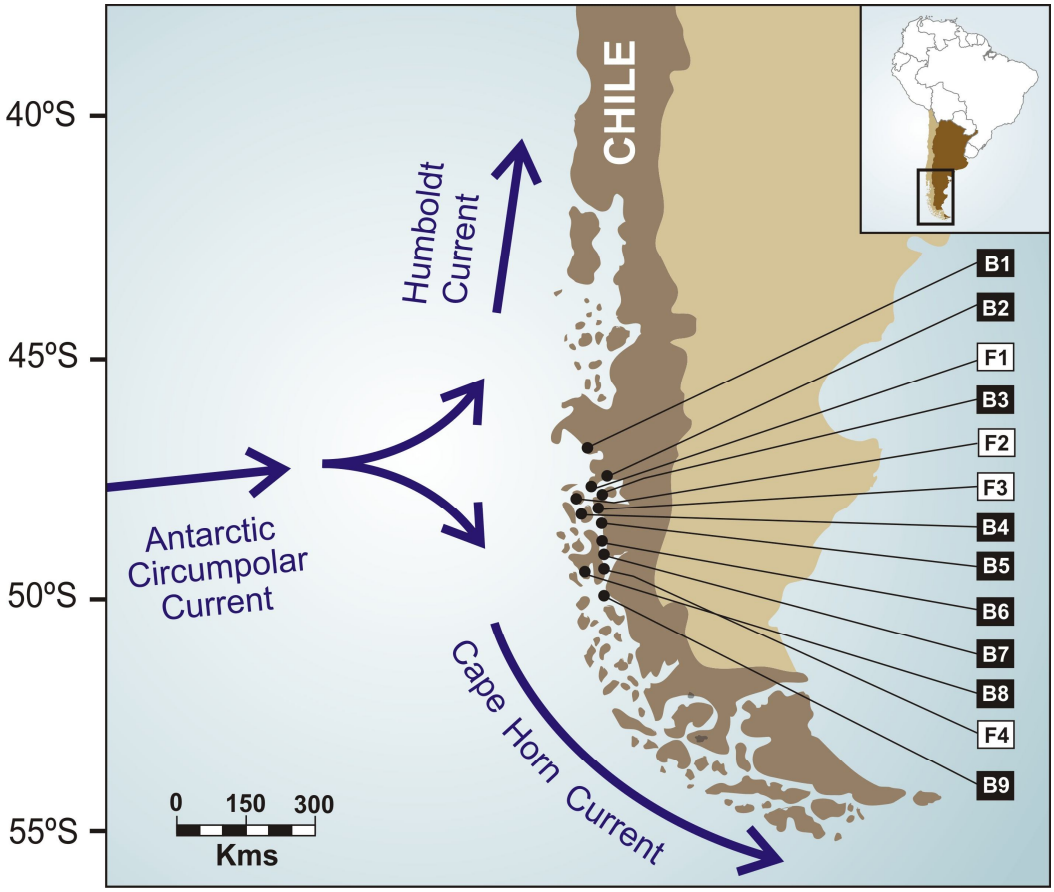

Figure 4.1 Sampling sites in the Patagonian fjords. Floating samples indicated by white boxes whereas benthic samples indicated by black boxes. Blue arrows shows directions of major currents in Southern Chile.

Table 1. Sampling sites of *Macrocystis pyrifera* showing number of samples and haplotypes found. Bold letters indicate samples from floating kelp rafts.

		N° of samples sequenced		Haplotypes	
Site Label	Coordinates	atp8-S	COI	atp8-S	COI
B1	46° 49.6' S - 74° 20.9' W	4	4	A I	H I; H II
B2	47° 46.3' S - 74° 10.4' W	4	4	A II	H III
B3	47° 56.3' S - 74° 27.1' W	4	4	A II	H III
B4	48° 28.6' S - 74° 33.2' W	4	4	A II	H III
B5	48° 18.6' S - 75° 04.9' W	4	4	A II	H III
B6	49° 03.0' S - 74° 25.0' W	4	4	A II	H III
B7	49° 09.5' S - 75° 17.5' W	4	4	A II	H III
B8	49° 40.7' S - 75°16.4' W	3	3	A II	H III
B9	50° 08.9' S - 74°39.9' W	4	4	A II	H III
F1	**47° 47.2' S - 74° 30.7' W**	**6**	**4**	**A II**	**H III**
F2	**48° 19.3' S - 75° 05.4' W**	**6**	**4**	**A I; A II**	**H I; H IV**
F3	**48° 20.7' S - 74° 29.8' W**	**4**	**4**	**A I; A II**	**H II**
F4	**49° 11.7' S - 74° 22.7' W**	**6**	**4**	**A II**	**H III**
TOTAL		57	51		

4.4 Results

A total of 55 and 51 *Macrocystis pyrifera* specimens were sequenced for atp8-S and COI, respectively (Table 1). For atp8-S one variable site was found over the 132 bp fragment amplified; the analysis yielded two haplotypes (AI-AII), and the most common haplotype (AI) was present in 78% of the samples and in 12 of the 13 sampling sites. For COI three variable sites were found on the 613 bp fragment amplified. The analysis of sequences yielded 4 haplotypes (HI-HIV),

and the most common haplotype (HIII) was present in 80% of the samples and 10 sampling sites. Most of the sampling sites had single haplotypes.

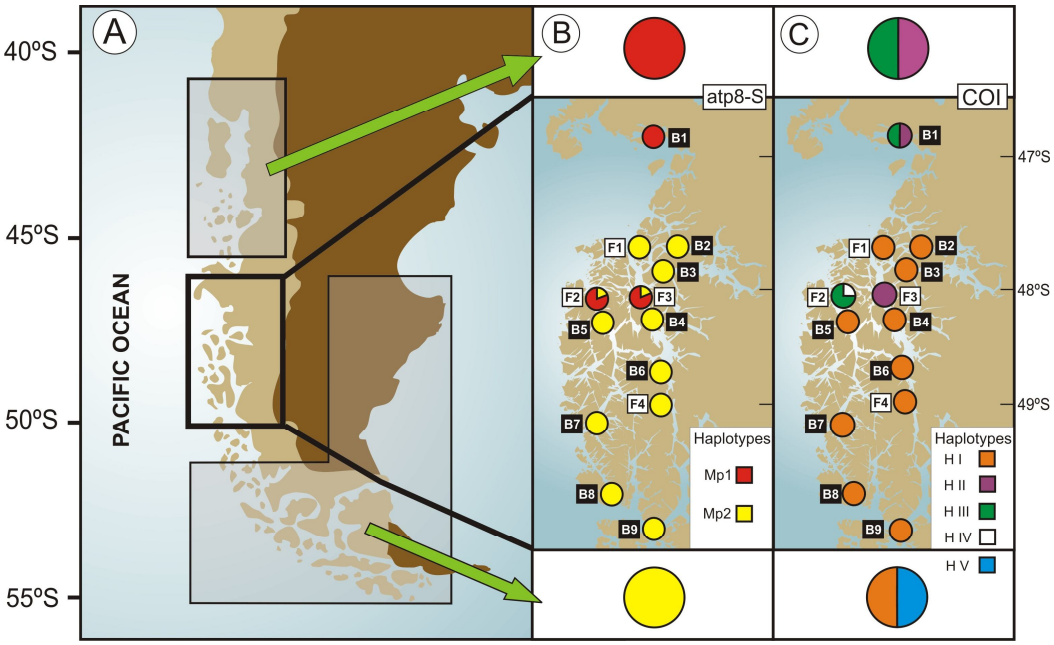

Figure 4.2 A) Map showing the sampling area in the Patagonian Fjords (middle box) and additional sampling areas north and south of the study area (upper and lower boxes). B) and C) Distribution of atp8-S and COI haplotypes respectively; pie chart on the map indicate the relative proportion of haplotypes at each site. Sampling sites in white boxes represent floating samples, black boxes represent benthic samples. Pie charts above and below map represent additional data from areas north and south of the sampling site (Macaya and Zuccarello 2010 - Chapter 2, Macaya et al. unpublished data).

Samples from floating kelp rafts at two sites (F2 and F3) shared haplotypes with a northern site (B1), approximately 200 km away. For atp8-S, 75% of the samples from these two floating raft collections shared the haplotype AI with B1 (Fig. 4.2 B). A similar result was found for COI: floating samples from sites F2 and F3 shared two haplotypes (HI and HII) with site B1, whereas haplotype HIV was found only in floating kelp samples from F2 (Fig. 4.2 C). Comparison with published sequences revealed that kelp rafts F2 and F3 also shared haplotypes with the northern area (Fig. 4.2 B-C). The mtDNA diversity was low, haplotype diversity (*h*) was 0.3470 and 0.6390 for atp8-S and COI respectively, whereas the nucleotide diversity (π) was 0.0026 and 0.0008 for atp8-S and COI respectively.

4.5 Discussion

Floating reproductive thalli have been widely suggested to play an important role as a dispersal mechanism in macroalgae (e.g. van den Hoeck 1987; Macaya et al. 2005; Hernández-Carmona et al. 2006). The data showed that floating kelp rafts in the Patagonian fjords were composed of individuals from distant populations (shared haplotypes between benthic samples B1 and floating samples F2- F3, 200 km apart) representing the first study to demonstrate putative source populations in floating kelp samples through molecular techniques. I have also found that kelp rafts are composed of sporophytes from different sources, since the different haplotypes found in some rafts came from both distant and nearby populations. Providing evidence of possible dispersal routes in the Patagonian fjords is the first step to understand the role that kelp rafts might play in connectivity of populations.

Rafting will only be a significant ecological and evolutionary process if rafters are capable of establishing new populations after reaching new coastal habitats (Thiel and Gutow 2005). Successful dispersal therefore requires a suitable new habitat and the capacity of an arriving organism to reproduce or proliferate in the new habitat (Thiel and Haye 2006). To my knowledge, little information is available on the reproductive success of arriving floating kelp rafts (but see, McKenzie and Bellgrove 2008, for *Hormosira banksii*), although Fraser et al. (2009) suggested that the putative success of floating *Durvillaea antarctica* on subantarctic islands could be due to the fact that most suitable habitats were available for colonists after local kelp extinction during the LGM. The fact that floating samples do not share haplotypes with southern localities indicate that rafts come from northern areas. At least one benthic population composed of these northern haplotypes has already established south of Peninsula Taitao, further suggesting that this peninsula is not a rigorous biogeographic barrier for subtidal biota (e.g. Häussermann 2006). The low resolution of molecular makers utilized in this study (i.e. low levels of variation, which is common in this species; Macaya and Zuccarello (2010, Chapter 2)) does not allow us to evaluate the present role of rafting in the connectivity of kelp populations. Competition with local flora or substratum availability might be an important factor determining the population dynamics of *M. pyrifera* in southern Chile (Santelices and Ojeda 1984). In the future, more extensive sampling and more variable molecular markers may reveal whether establishment of new populations following dispersal by kelp rafts in the area is currently taking place.

In the Patagonian fjords a high biomass of *M. pyrifera* kelp rafts has been found, ranging from ~100 kg km^2 to 1500 kg km^2 (Hinojosa et al. 2010). The

strong westerly winds in combination with estuarine seaward surface outflow leads to an accumulation of many of these kelp rafts in the openings of the channels (I. Hinojosa personal observation). This also coincides with the major proportion of kelp forest in the north-eastern area of the sampling site (Hinojosa et al. unpublished data) (Fig. 4.3). Occasionally some of the larger rafts might be transported out of the channels and carried in a southward direction by the Cape Horn Current (Fig. 4.3), which could explain the shared haplotypes between northern site B1 and floating samples from F2 and F3. Dispersal might occur in a stepping stone fashion, with kelp rafts travelling short distances southwards, but this suggestion must necessarily be verified using more variable markers.

In summary, this study has shown the presence of individuals from distant populations in kelp rafts thus providing the first step to understand the role of kelp in explaining the connectivity among populations. Furthermore, results suggest southward dispersal is common in kelp rafts, which should be examined in the future with more variable molecular markers (microsatellites or nuclear DNA). Rafting dispersal might also be relevant for the population dynamics of associated fauna capable of surviving on kelp rafts.

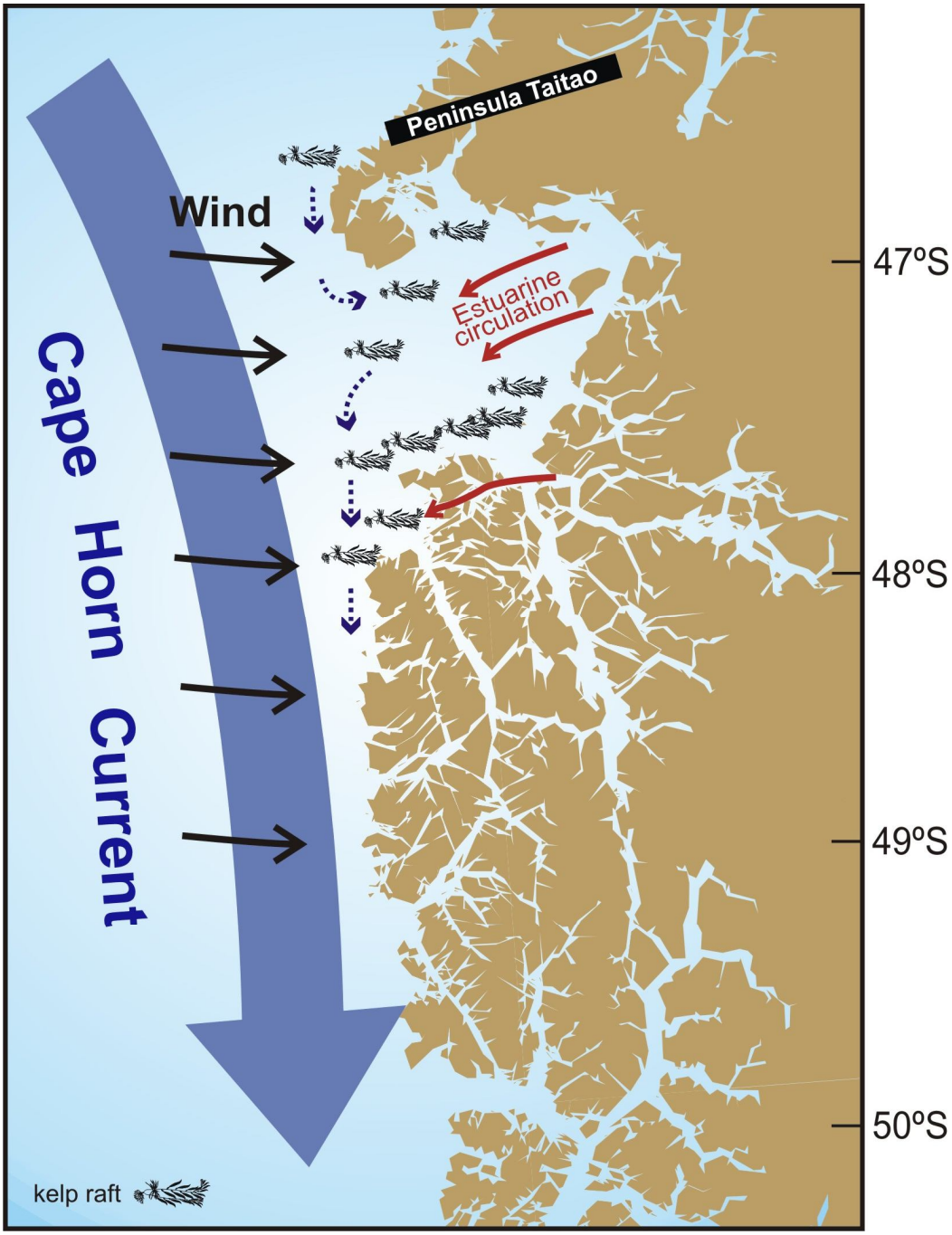

Figure 4.3 Conceptual model of the oceanographic features of the sampling area, showing the suggested dispersal routes of *M. pyrifera* kelp rafts in the central Patagonian region (dotted blue line).

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CHAPTER 5

Global genetic structure of the giant kelp *Macrocystis pyrifera* and the influence of the Antarctic Circumpolar Current on population connectivity

5.1 Abstract

Macrocystis pyrifera represents the most widely distributed kelp taxon in the world. The anti-tropical distribution of this large brown alga has been explained as a result of vicariance or origin in one hemisphere with a subsequent trans-tropical migration. The present distribution is also believed to be the as the result of high gene flow principally by floating reproductive kelp rafts because of the restricted dispersal distances of zoospores and sperms. However few studies have confirmed this idea by analyzing the global genetic structure of this kelp species. In this chapter I analyzed the genetic structure of *M. pyrifera* collected from more than 45 sites globally using COI - mtDNA sequences. Additionally, more than 700 samples from the Southern Hemisphere were analyzed using 6 microsatellites, in order to understand the connectivity along the subantarctic region, most directly impacted by the Antarctic Circumpolar Current, and to determine the possible role of kelp rafts as an important dispersal mechanism in this area. Results from COI sequences revealed a pattern of vast areas dominated by a single haplotype in both the Northern and Southern hemispheres. The haplotype network suggests a Southern Hemisphere origin, contrary to previous research using ITS - DNA sequences, the fossil record and the phylogeny of the

Laminariales which all suggest an origin in the Northern Hemisphere. Analysis of microsatellites confirms the previous suggestion of high gene flow in the Southern Ocean, with no significant genetic structure and no isolation by distance, indicating ongoing gene flow. The Antarctic Circumpolar Current might be responsible for such connectivity, where kelp rafts are transported along the subantarctic region among populations.

5.2 Introduction

Gene flow is a fundamental process in the evolution of populations, and an important influence on population genetic structure (Wagner and McCune 2009). Extensive gene flow may lead to genetic homogenization of populations; in contrast, reduced gene flow may permit the evolution of reproductive barriers between populations and can compromise stability and recovery potential (Rundle and Nosil 2005; Hellberg 2009), as well as reduce or increase their ability to adapt to changing environmental conditions (Worm et al. 2006). Several factors are important in determining patterns of genetic diversity and structure, including the spatial arrangement of populations (patchy vs. continuous), distance among populations, the presence or effectiveness of vectors of dispersal, climatic effects associated with glaciations and physical barriers to dispersal (Palumbi 2003; Reed et al. 2006; Sotka and Palumbi 2006; Thiel and Haye 2006). It is necessary to determine the importance of these factors affecting genetic differentiation in organisms and populations, to understand how they persist in nature (Coleman and Brawley 2005; Cowen and Sponaugle 2009).

The degree of genetic connectivity between populations is determined principally by the dispersal process (Palumbi 2003; Thiel and Haye 2006). Many

marine organisms are capable of dispersing remarkably far, along lengths of entire coasts and even across oceans (Grosberg and Cunningham 2001). Terrestrial and freshwater organisms have also been shown to undergo long distance dispersal (LDD) (Sanmartin and Ronquist 2004; Knapp et al. 2005; Sanmartin et al. 2007). Wind and ocean currents are probably the two major abiotic processes that lead to LDD in several taxa (Raven 1973; Wardle 1978; Gaines et al. 2003; McDowall 2004; Munoz et al. 2004). For example, the strong, eastwards flowing Antarctic Circumpolar Current (ACC) has been considered as promoting the dispersal of marine organisms throughout the waters of the Southern Ocean (Bargelloni et al. 2000; Waters and Roy 2004; Donald et al. 2005; Fraser et al. 2009; Nikula et al. 2010). The ACC is thought to be mainly responsible for dispersal and recolonization of the brown alga *Durvillaea antarctica* after the last glacial maximum in the subantartic region (Fraser et al. 2009). Floating kelp rafts of *D. antarctica* might travel along the ACC in a stepping stone fashion, colonizing ice-free localities. The findings from Fraser et al. (2009) have been recently confirmed by Nikula et al. (2010), analyzing connectivity in the subantarctic region of *D. antarctica* holdfast-associated fauna (two peracarids). They found broad genetic similarity of populations in the region, suggesting that theACC is an important dispersal mechanism for organisms capable of rafting.

The giant kelp *Macrocystis pyrifera* provides a good model for exploring patterns of genetic structure at different scales. Discontinuities in hard substrate in the near-shore cause *M. pyrifera* to be distributed in discrete patches of varying size that expand and contract in response to biotic and abiotic changes in the environment (Vásquez and Buschmann 1997; Vega et al. 2005; Reed et al.

2006). It also represents the most widely distributed kelp species on the planet, being the only large brown alga with an antitropical distribution. *Macrocystis* kelp forests are present in the northeast Pacific (from Alaska to Mexico) and are extensively distributed throughout the Southern Hemisphere (SH) (Peru, Chile, Argentina, South Africa, Australia, New Zealand and most of the subantarctic islands). The taxonomy and distribution of *Macrocystis* has long been the subject of debate, but recent, morphological and genetic studies have demonstrated the monospecific status of the genus (Demes et al. 2009; Macaya and Zuccarello 2010 - Chapter 2). The wide distribution of this alga has been attributed to several causes, such as vicariance (Chin et al. 1991), origin in northern (Coyer et al. 2001) and SH (Nicholson 1978) followed by migration either southwards or northwards. Chin et al. (1991) proposed a process of vicariant differentiation out of a Pacific Ocean/Southern Ocean ancestral complex. However such a scenario cannot explain the antitropical distribution in the eastern Pacific Ocean (Coyer et al. 2001). A Northern Hemisphere (NH) center of origin has been hypothesized based on biogeography of extant kelps in the north Pacific, paleoclimatic record, and fossil records of certain obligate facultative kelp-associated molluscs (Nicholson 1978; Estes and Steinberg 1988; Lüning 1990). Furthermore, analysis of the ITS regions of *Macrocystis* supports a NH origin because of the greater genetic diversity of samples from that area in comparison with samples from the SH (Coyer et al. 2001). North (1971) proposed a southern hemisphere origin because of the much more widespread distribution of the genus in this hemisphere; however abundance and distribution alone are not proof of an area of origin (Nicholson 1978).

Long distance dispersal of reproductively viable sporophytes has been suggested as being an important dispersal mechanism for this species, connecting populations in the SH (Nicholson 1978; Estes and Steinberg 1988; Coyer et al. 2001). Despite the apparent acceptance of floating sporophytes as the primary LDD vector for *M. pyrifera* (Lüning 1990; Macaya et al. 2005), little genetic data exist that support the ability of reproductively viable *Macrocystis* sporophytes to connect isolated populations. Thus, in this study I investigated the genetic structure of the giant kelp *M. pyrifera* in a world-wide analysis using DNA COI sequences in order to understand global phylogeography and determine the possible origin of the species. Given that dispersal potential of kelp zoospores and gametes are limited to just a few meters or kilometres (Anderson and North, 1966, Dayton 1973, Reed 1990, Graham 2003), gene flow in the SH might be facilitated by reproductive floating kelp rafts transport by the ACC. Thus, here I also analyzed samples from the SH using microsatellites in order to determine whether dispersal occurs over ecological time scales and is facilitated by the ACC.

5.3 Methods

5.3.1 Sampling sites and collection

Samples of *Macrocystis pyrifera* were collected between 2006 and 2009 from 50 locations globally (Tables 5.1 and 5.2). At these sites, multiple individuals (7–40) were collected haphazardly in an area of 200 m^2 or more. Samples consisted of small pieces (2-3 cm^2) of tissue from attached *M. pyrifera*. Apical tips without epiphytes or epibionts were preserved in silica gel until DNA extraction. A total

of 215 *M.pyrifera* individuals were analyzed using COI sequences and 708 individuals using microsatellites.

5.3.2 DNA extraction and COI amplification.

DNA was extracted following the CTAB method described by Zuccarello and Lokhorst (2005). A 613 bp region of mitochondrial cytochrome c oxidase I (COI) was amplified using primers GazF2 and GazR2 (Lane et al. 2007). PCR amplifications were performed following Macaya and Zuccarello (2010 - Chapter 2).

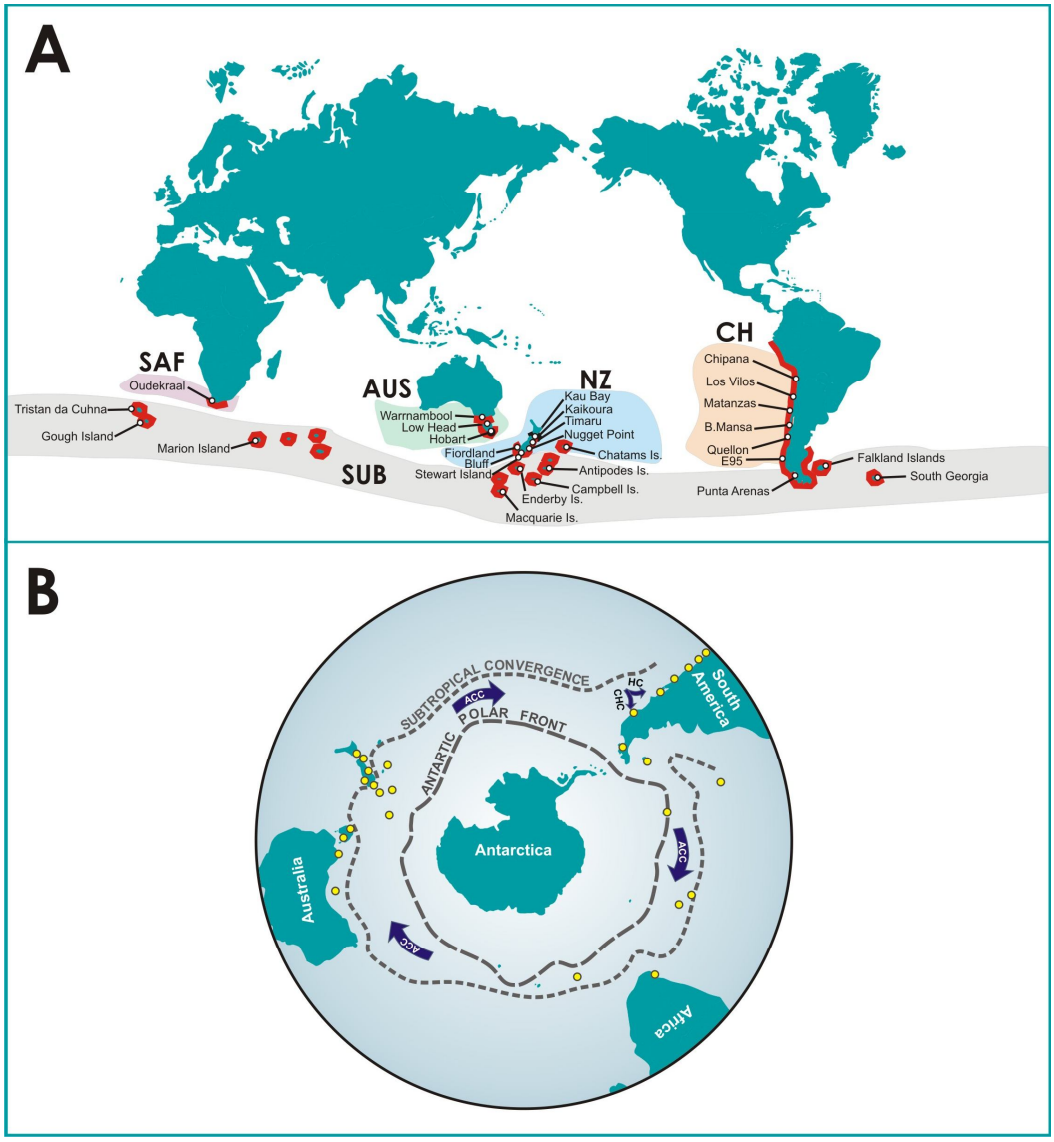

Figure 5.1 A) Sampling sites for microsatellites study. In order to facilitate the analysis, data was grouped into different areas as shown on the image: CH=Chile, SUB=Subantarctic region, SAF=South Africa, AUS=Australia, NZ=New Zealand. Red line indicates *Macrocystis* distribution. B) Polar view of the subantarctic region, showing sampling locations. The Antarctic Circumpolar Current (ACC) runs between the Subtropical Convergence and the Antarctic Polar Front (dashed lines). HC= Humboldt Current and CHC= Cape Horn Current.

Table 5.1 Sampling sites of *Macrocystis pyrifera* and number of samples sequenced for COI.

Location - Country	Latitude	Longitude	n
Northern Hemisphere			
Nuchatliz Islands - Canada	49° 36' N	126° 32' W	7
Pebble Beach, California - USA	36° 33' S	121° 56' E	5
Bulito - USA	34° 27' N	120° 20' W	4
Cojo - USA	34° 24' N	120° 26' W	4
Carpinteria - USA	34° 23' N	119° 31' W	4
Emma Wood - USA	34° 16' N	118° 18' W	4
Leo Carrillo - USA	34° 02' N	118° 53' W	4
Santa Catalina - USA	33° 24' N	118° 28' W	4
Point Loma - USA	32° 41' N	117° 14' W	5
Punta Banda - Mexico	31° 42' N	116° 38' W	4
Bahia Tortugas - Mexico	27° 40' N	114° 53' W	5
Southern Hemisphere			
Paracas - Peru	13° 55' S	76° 17' W	5
Atico - Peru	15° 57' S	74° 02' W	4
Chipana - Chile	21° 20' S	70° 05' W	4
Caleta Constitucion - Chile	23° 25' S	70° 35' W	4
Playa Blanca - Chile	28° 11' S	71° 09' W	4
Los Vilos - Chile	31° 55' S	71° 31' W	5
La Castilla - Chile	33° 27' S	71° 40' W	4
Matanzas - Chile	33° 56' "S	71° 52' W	4
Tumbes - Chile	36° 37' S	73° 05' W	4
Bahia Mansa - Chile	40° 34' S	73° 44' W	4
Quihua - Chile	41° 45' S	73° 09' E	5
Ancud - Chile	41° 51' S	73° 49' W	4
Curaco - Chile	42° 26' S	73° 36' W	4
Quellon - Chile	43° 08' S	73° 36' W	4
E95 Fjords - Chile	46° 49' S	74° 27' W	4
E40 Fjords - Chile	48° 24' S	74° 28' W	4
Punta Arenas - Chile	53° 28' S	70° 51' W	4
Oudekraal – South Africa	33° 59' S	18° 21' E	7
Tristan da Cunha - UK	37° 03' S	12° 18' W	5
Gough Island - UK	40° 20' S	09° 57' W	4
Marion Island - South Africa	46° 50' S	37° 50' E	7
Stewart Island - New Zealand	46° 53' S	168° 07' E	6
Antipodes Island - New Zealand	49° 40' S	178° 48' E	6
Campbell Island - New Zealand	52° 32' S	169° 11' E	7
Enderby Island - New Zealand	50° 37' S	166° 15' E	5
Falkland Islands - UK	51° 37' S	57° 45' W	6
South Georgia - UK	54° 17' S	36° 29' W	4
Macquarie Island - Australia	54° 38' S	158° 48' E	4
Low Head, Tasmania - Australia	41° 03' S	146° 47' E	5
Hobart, Tasmania - Australia	43° 00' S	147° 19' E	5
Warrnambool - Australia	38° 24' S	142° 28' E	4
Picton - New Zealand	41° 16' S	174° 05' W	4
Kau Bay - New Zealand	41° 17' S	174° 49' E	5
Kaikoura - New Zealand	42° 25' S	173° 42' W	4
Timaru - New Zealand	44° 22' S	171° 15' W	4
		TOTAL	**215**

Table 5.2 Sampling sites of *Macrocystis pyrifera* and number of samples sequenced for microsatellites. Additional information regarding grouping for analysis purposes is given.

Location - Country	Latitude	Longitude	n
Chile – CH			
Chipana - Chile	21° 20' S	70° 05' W	40
Los Vilos - Chile	31° 55' S	71° 31' W	29
Matanzas - Chile	33° 56' S	71° 52' W	41
Bahia Mansa - Chile	40° 34' S	73° 44' W	38
Quellon - Chile	43° 08' S	73° 36' W	39
E95 Fjords - Chile	46° 49' S	74° 27' W	36
Punta Arenas - Chile	53° 28' S	70° 51' W	22
South Africa - SAF			
Oudekraal – South Africa	33° 59' S	18° 21' E	38
Subantarctic - SUB			
Tristan da Cunha - UK	37° 03' S	12° 18' W	9
Gough Island - UK	40° 20' S	09° 57' W	8
Marion Island - South Africa	46° 50' S	37° 50' E	8
Stewart Island - New Zealand	46° 53' S	168° 07' E	24
Antipodes Island - New Zealand	49° 40' S	178° 48' E	6
Campbell Island - New Zealand	52° 32' S	169° 11' E	9
Enderby Island - New Zealand	50° 37' S	166° 15' E	10
Falkland Islands - UK	51° 37' S	57° 45' W	39
South Georgia - UK	54° 17' S	36° 29' W	8
Macquarie Island - Australia	54° 38' S	158° 48' E	13
Australia - AUS			
Low Head, Tasmania - Australia	41° 03' S	146° 47' E	40
Hobart, Tasmania - Australia	43° 00' S	147° 19' E	28
Warrnambool - Australia	38° 24' S	142° 28' E	40
New Zealand - NZ			
Kau Bay - New Zealand	41° 17' S	174° 49' E	39
Kaikoura - New Zealand	42° 25' S	173° 42' W	25
Timaru - New Zealand	44° 22' S	171° 15' W	19
Fiordland - New Zealand	45° 16' S	166° 50' E	22
Nugget Point - New Zealand	46° 26' S	169° 47' E	39
Bluff - New Zealand	46° 36' S	168° 21' W	39
		TOTAL	**708**

5.3.3 Microsatellites amplification.

All individuals were genotyped for 6 microsatellite loci (Table 5.3). PCR reactions were carried out using forward 5´fluorochrome labelled primers following the methods by Alberto et al. (2009). Primers were developed by Filipe Alberto and all PCR reactions and sequencing were carried out in CIMAR Laboratory University of Algarve, Campus Gambelas, Faro, Portugal, between January-February 2009. Fragment length was visualized on an ABI PRISM 3130 DNA analyzer (Applied Biosystems) using the GeneScan-500 LIZ standard. Raw allele sizes were scored with STRAND

(http://www.vgl.ucdavis.edu/informatics/STRand), binned using the R package msatAllele (Alberto 2009) and manually checked for ambiguities.

Table 5.3 Microsatellites loci used in this study (From Alberto et al. 2009).

Locus name	Primer sequences	Repeat motif	Size range (bp)
Mp-BC-4	F-AACCCACTCCACTCCT R-CTTCATAGTGCCCTTGTAT	(CT)11	222–247
Mp-BC-18	F-TTGCTCCTCCTGCTGCTAC R-GACCAGATGCAGAGATGACAG	(CT)9	173–181
Mp-BC-19	F-TGACGCGTTCATCGTGTTG R-CGGAGAACAGGGAGAGCAG	(CT)10	156–164
Mp-BC-25	F-CGGAAGGAGAGAGGGCAAG R-CTGCGTCCATTTGAGCCAC	(AG)11	164–172
Mpy-8	F-CAACAACTAGCGTACCTTGAG R-TTCGGTTCATCTACATACTCG	(CT)19	116–132
Mpy-11	F-GTTCCAGCTTGGTATTCAAA R-ACCGTGTAGCATGAGTCTATG	(GA)10…(GA)3 …(GA)8	224–237

5.3.4 Data Analysis

COI sequences were aligned using ClustalW in the BIOEDIT program (Hall 1999). Haplotype frequencies were calculated using the software DnaSP Version 4.0 (Rozas and Rozas 1995). Estimates of haplotypic (H_e) and nucleotide (π) diversity were calculated for Northern and Southern hemispheres groups, for subantarctic islands and for the entire dataset using ARLEQUIN version 3.1 (Excoffier et al. 2005). Haplotype genealogies were estimated with the statistical parsimony method, implemented by TCS 1.21 (Clement et al. 2000).

For microsatellites, allele frequencies were estimated for each locus at selected sites in the SH (Fig. 5.1). Standard genetic diversity indices were calculated at each site and at all six loci; the number of alleles at each locus, gene diversity (H_e), observed heterozygosity (H_O) and fixation indices (F_{IS}) were computed using Fstat 2.9.3.2 (Goudet 2001). Samples were also grouped into 5 regions to facilitate analysis and to compare the subantarctic region (mostly

subantarctic islands) with the rest of the sampling sites (See Fig. 5.1 and Table 5.2 for details). Allelic richness was calculated for each region by standardizing to the smallest sample size (n = 35) using the rarefaction method in Fstat. In addition, heterozygote deficiencies and excesses were tested using 1000 randomizations of alleles among individuals. Genetic structure was estimated by testing Weir and Cockerham's F_{ST} (Wright's fixation index) and F_{IS} estimates (Weir and Cockerham 1984) using permutation test (1000 permutations). Pairwise F_{ST} valueswere estimated between regions. Genepop 3.4 (Raymond & Rousset 1995) was used to perform exact tests (Guo & Thompson 1992) for deviations from Hardy-Weinberg genotypic proportions at each locus. The data were tested for linkage equilibrium using an exact test based on a Markov chain method implemented in Genepop 3.4. The false discovery rate (FDR) technique was used to eliminate false assignment of significance by chance (Verhoeven et al. 2005). Genetic structure was also visualized using Factorial Correspondence Analysis (FCA) of GENETIX (Belkhir et al. 2004)

5.3.5 Isolation by Distance

A linear regression method was used to test for isolation by distance (IBD) among samples from the subantarctic region for microsatellite data. A matrix of pairwise values of F_{ST} /(1 – F_{ST}) was regressed on ocean distance between sites, following Rousset (1997). Significance of the regression was assessed by the Mantel test with 10,000 permutations using Isolation By Distance Web Service IBDWS (Turner et al. 2009). A positive relationship over a range of distances indicates isolation by distance.

5.4 Results

5.4.1 COI Sequences

A 613 bp fragment of the mtDNA COI was sequenced for a total of 217 individuals of *M. pyrifera* revealing 17 different haplotypes among 47 populations worldwide (Fig. 5. 2). Samples from the NH had four haplotypes whereas the SH had 13 haplotypes. The total haplotype diversity was $H_e = 0.719$ (st dev = 0.027) with low nucleotide diversity $\pi = 0.003$, whereas haplotype diversity for Northern and SH was $H_e = 0.444$ and 0.612 respectively. The most frequent haplotype was H7 present at 22 sampling sites and in 99 individuals (46%). The Haplotype H1 was common in the NH with 36 samples in 9 sampling sites. Punta Loma represents the only site where 3 haplotypes were present, the remaining sampling sites only had one haplotype.

Along the South American coast 6 haplotypes were present. Haplotype H7 was the most common along the subantarctic region, present in most of the subantarctic islands and Australia and New Zealand (Fig. 5.2 and 5.3). Haplotype diversity for the subantarctic region was 0.5687 with a very low nucleotide diversity $\pi = 0.0013$. Haplotype H8 was also present in a wide geographic area including South America, South Georgia, Gough Island, Tristan da Cunha and South Africa.

The statistical parsimony network revealed a central haplotype (H7) from which most of the haplotypes derived (Fig. 5.2). Most of the haplotypes were separated by only one mutational step from the central haplotype. Similar results were found for the statistical parsimony network with samples only from the subantarctic region (Fig. 5.3). Most of the islands possessed the Haplotype H7,

but Gough Island had two different haplotypes separated by two bp from the putative ancestral haplotype H7.

Along the whole sampling area only a few sites displayed unique haplotypes (Point Loma, Bahia Tortugas Paracas, Punta Arenas, Falkand Is., Gough Island Is., Antipodes Is. and Enderby Is.).

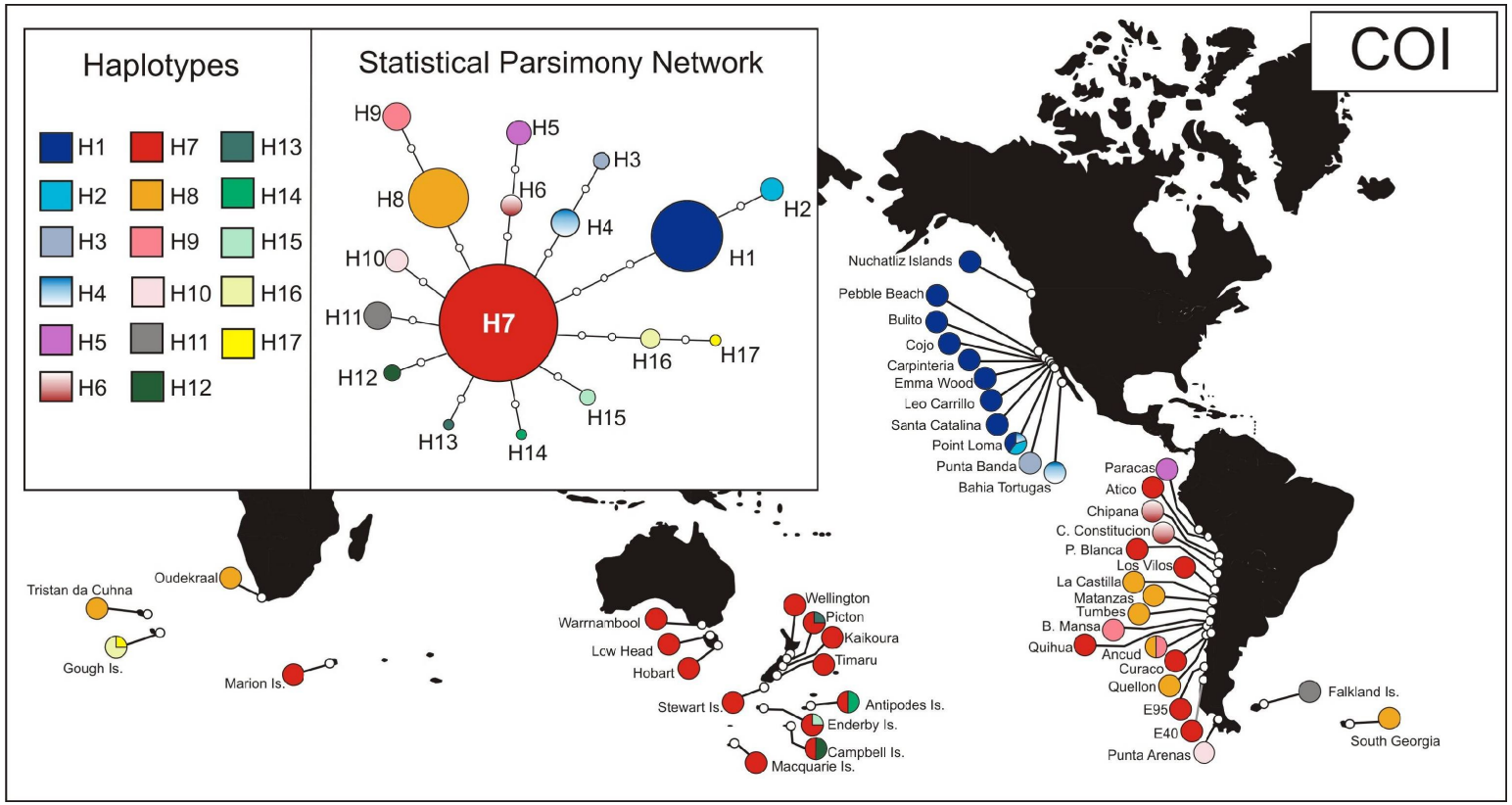

Figure 5.3 Distribution of the 17 COI haplotypes detected in *M. pyrifera* populations sampled around the world. Color-motif pie charts display relative frequencies of each haplotype in each population, haplotype H7 represent most probable ancestral haplotype. Small white circles represent undetected haplotypes.

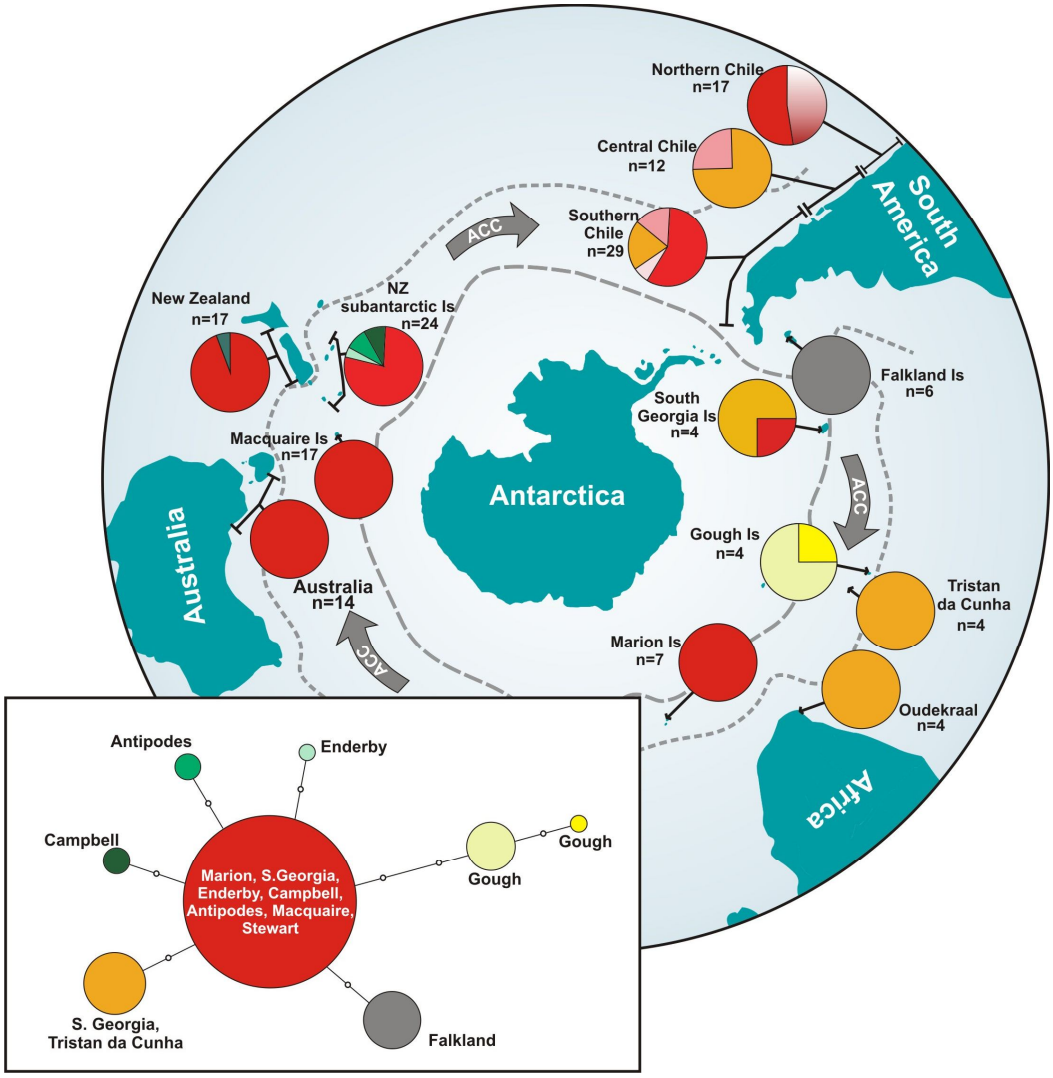

Figure 5.3 Haplotype distribution and proportion at each sampling site. Colour circles display relative frequencies of each haplotype in each population. Haplotype network constructed only for subantarctic islands (inset), small white circles represent undetected haplotypes..

5.4.2 Microsatellites

All selected loci showed high polymorphism: 77 alleles were characterized across six microsatellites and 708 individuals, with an average of 12.83 alleles per locus. The locus Mpy8 displayed the greatest number of alleles (23) whilst BC18 the lowest number of alleles (10).

Gene diversity or unbiased expected heterozygosity (Nei, 1987) varied between 0.042 and 0.460. Sites from the subantarctic region had lower levels of gene diversity than sites from New Zealand, Australia, South Africa and Chile (average He = 0.248). However no statistical difference was found among groups (Kruskal Wallis test H = 3.866, p = 0.4245).

Allelic richness (AR) was lower in South Africa (AR=2.99), but there was no significant AR difference between regions (ANOVA F = 0.715, p = 0.640) (Fig. 5.4) despite the wide geographic area among samples. In most populations F_{IS} was greater than zero, and only in the subantarctic region three sites had values that were below zero. The highest F_{IS} value was found in South Africa (0.783).

The global F_{ST} value was 0.464 and the pairwise F_{ST} estimates revealed the greatest differentiation between South Africa and Chile (F_{ST} =0.3128) (Table 5.4). The lowest genetic differentiation was found between the subantarctic region and Chile (F_{ST}=0.1126). Interestingly two regions as distant as Chile and Australia, also displayed one of the lowest F_{ST} values (F_{ST} = 0.1337).

The microsatellite genotypic data for *M. pyrifera* for the FCA were plotted on three principle axes, which cumulatively explained 85% of the genetic variation. The FCA plot showed a partial separation of the South African samples, however this region considered only one site. The subantarctic region

overlaps with Australia and part of the Chilean and New Zealand samples, indicating that there is a not clear genetic structure among the southern hemisphere sites (Fig. 5.5).

The IBD analysis from subantarctic populations did not yield a significant slope (Mantel test: Z = 778643.3326, P = 0.8033 and r^2 = 0.0825 over all populations), thus very little of the variation in genetic distance was explained by geographical distance even though a vast geographic area was sampled. (Fig. 5.6).

Table 5.4. Pairwise F_{ST} estimates among regions in the Southern Hemisphere. F_{ST} estimates are based on Weir and Cockerham (1984). All values in italics refers to significant values after false discovery rate (FDR).

	CHI	SAF	SUB	AUS	NZ
CHI	-				
SAF	*0.3128*	-			
SUB	*0.1126*	*0.2659*	-		
AUS	*0.1337*	*0.2906*	*0.1712*	-	
NZ	*0.1996*	*0.2916*	*0.1902*	*0.1809*	-

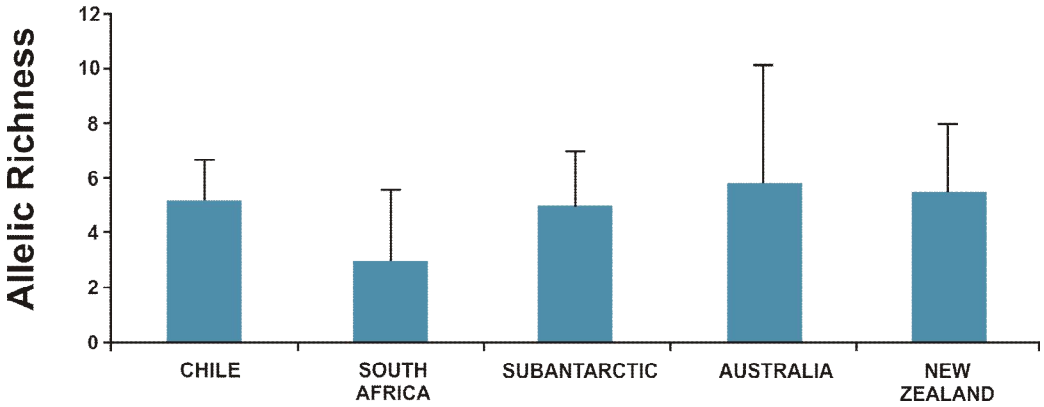

Figure 5.4 Average Allelic Richness of microsatellites loci, in different regions of the southern hemisphere. Bar represenst one st. dev.

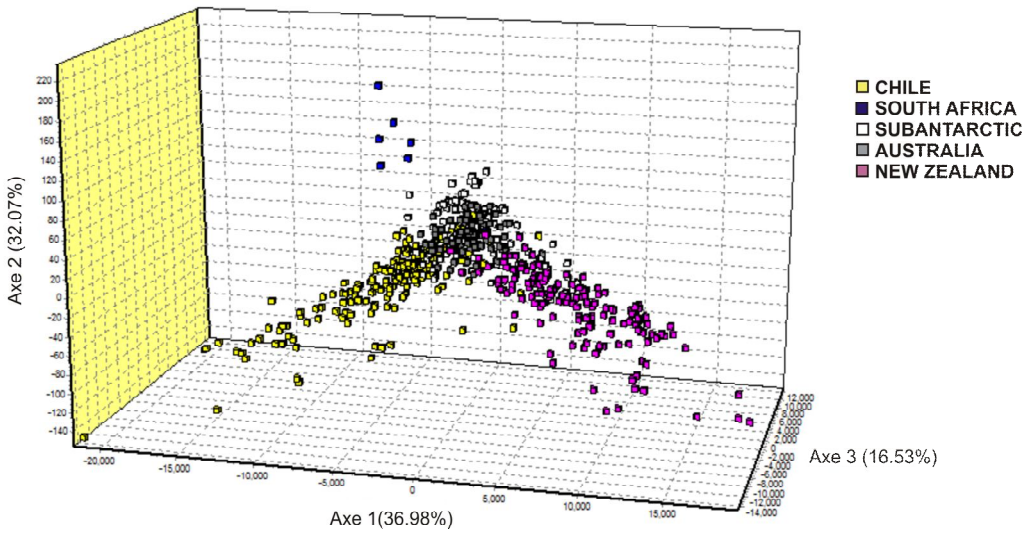

Figure 5.5 Factorial Correspondence Analysis (FCA) scores of *M. pyrifera* microsatellite genotypes plotted using GENETIX.

Table 5.3 Number of individuals sampled (n), Number of alleles (Na), expected and observed heterozygosity (H_e and H_O) and F_{IS} (a measure of inbreeding within populations) for each site in Southern Hemisphere.

Region	Site	n	Na	H_e	H_O	F_{IS}
Chile	Chipana	40	16	0.364	0.357	0.031
	Los Vilos	29	13	0.232	0.160	0.362
	Matanzas	41	14	0.206	0.166	0.206
	Bahia Mansa	38	26	0.265	0.267	0.011
	Quellon	39	22	0.372	0.293	0.226
	E95 Fjords	36	21	0.343	0.288	0.177
	Punta Arenas	22	15	0.249	0.141	0.459
South Africa	Oudekraal	38	18	0.294	0.065	0.783
Subantarctic	Falkland Islands	39	21	0.368	0.329	0.119
	South Georgia	8	14	0.254	0.238	0.155
	Tristan da Cunha	9	11	0.264	0.274	0.033
	Gough Island	8	8	0.131	0.179	-0.154
	Marion Island	8	8	0.042	0.045	0.000
	Stewart Island	24	19	0.318	0.246	0.250
	Antipodes Island	6	14	0.377	0.333	0.211
	Campbell Island	9	14	0.275	0.304	-0.038
	Enderby Island	10	17	0.387	0.304	0.267
	Macquarie Island	13	8	0.072	0.088	-0.156
Australia	Warnambool	40	28	0.405	0.399	0.029
	Low Head	28	18	0.226	0.090	0.613
	Hobart	40	16	0.280	0.196	0.318
New Zealand	Kau Bay	39	17	0.194	0.063	0.685
	Kaikoura	25	16	0.314	0.112	0.669
	Timaru	19	19	0.460	0.353	0.280
	Nugget Point	22	16	0.255	0.256	0.013
	Bluff	39	17	0.371	0.228	0.399
	Fiordland	39	19	0.315	0.277	0.146

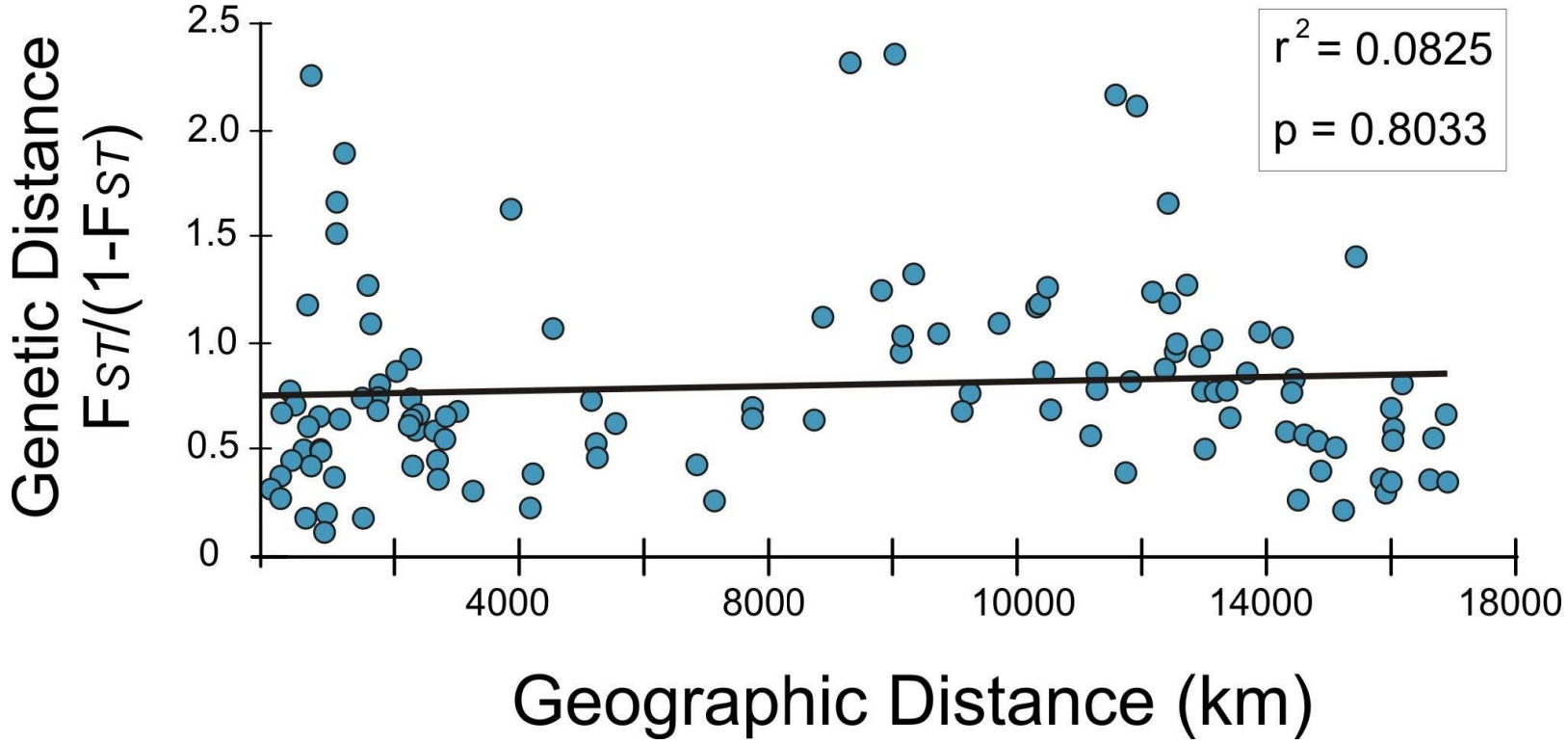

Figure 5.6 Isolation-by-distance analysis of *Macrocystis pyrifera* samples from the subantarctic region. Regression of $F_{ST}/(1 - F_{ST})$ on linear distance between population pairs.

5.5 Discusion

This study reprersents the most comprehensive global analysis of *Macrocystis pyrifera* using molecular markers. COI sequences and microsatellites revealed an interesting pattern of genetic homogeneity along wider geographic areas in Northern and Sourthern Hemisphere. The haplotype network analysis using mt DNA places the possible ancestral haplotype in the SH. Microsatellites analysis confirms the COI results in the SH, with no isolation by distance and no genetic structure in the subantarctic region despite the wide geographic area analyzed. The results confirm previous suggestion of high gene flow in this kelp and also suggest the ACC as an important dispersal mechanism along the Southern Ocean (Fraser et al. 2009).

5.5.1 Global analysis – COI sequences.

Previous global analyses carried out on *Macrocystis pyrifera* (Coyer et al. 2001) used ITS sequences and suggested a NH origin based on a higher genetic diversity in samples from the NH. Recently, Macaya and Zuccarello (2010-Chapter 2) used COI sequences with global sampling but with a taxonomic purpose. The present analysis incorporated more sampling sites than Coyer et al. (2001), including most of the subantarctic islands. Despite the wide geographic area sampled in the northern hemisphere (~ 2800 km) only one haplotype was dominant at most of the sites (H1). Interestingly, Point Loma had three different haplotypes :this site represents one of the largest *Macrocystis* kelp forests in the world (Dayton et al. 1999) and has been previously described as a genetic break for fish species (Bernardi 2005). During recent years, El Niño events have resulted in widespread mortality of *M. pyrifera* in this area (southwest California,

USA and Baja California, Mexico) (Dayton et al. 1992; Ladah et al. 1999; Edwards 2004; Edwards and Estes 2006). The populations from Nuchatliz Islands (Canada) to Santa Catalina (California, USA) might reflect more stable environmental conditions permitting high gene flow, whereas samples from Point Loma and Mexico (Punta Banda and Bahia Tortugas) have been under a severe bottleneck. Colonization in this area might be recent and come from northern and southern populations as reflected in the haplotype diversity found in Point Loma sharing haplotypes with northern (haplotype H1, Canada to Santa Catalina) and southern populations (haplotype H4 from Bahia Tortugas, Mexico), this has been also suggested by Coyer et al. (2001) using ITS analysis. Thus the dynamics of mortality and recovery of kelp forests might account for the genetic pattern observed in the area.

Most previous evidence suggested on *M. pyrifera* an origin in the NH because: a) the distribution of related genera is in this Hemisphere (Nicholson 1978), b) the sister genus based on genetic analysis corresponds to *Pelagophycus porra* (Boo et al. 1999; Coyer et al. 2001; Yoon et al. 2001) which is only found in California and Baja California (Parker and Bleck 1966; Coyer and Zaugg-Haglund 1982), and c) the presence of a fossil record and the biogeography of kelp-associated animal species (Estes and Steinberg 1988; Vermeij 2001). In contrast my results, the greater haplotype diversity found in the SH with COI sequences, as well as the haplotype network both suggest a SH origin. Samples from both hemispheres are separated by only 1 or 2 bp, which suggests a recent expansion. In a global study of the bryozoan *Membranipora membranacea*, Schwaninger (1999), suggested a northern hemisphere origin for the organisms based on COI sequences, he also suggested southwards dispersal through the

tropics during the last glacial maximum. The variation found in the SH may also indicate the presence of glacial refugia that have been suggested for populations of the bull kelp *Durvillaea antarctica* from New Zealand and northern central Chile (Fraser et al. 2009).

Genetic homogeneity was observed along the subantarctic region. The haplotype H7 was present at most of the sampling sites are similar results were found by Coyer et al. (2001) using ITS sequences and COI sequences by Macaya and Zuccarello (2010 - Chapter 2). Furthermore, a similar lack of variation was found in the bull kelp *Durvillaea antarctica* using COI and *rbc*L sequences (Fraser et al. 2009). A lack of variation has been attributed to high dispersal potential but also a probable recent colonization after the LGM (Coyer et al. 2001, Fraser et al. 2009, Chapter 2 and 3). Lack of genetic variation and high dispersal in the Southern Hemisphere has also been reported for marine animals (e.g. Waters and Roy 2004; Donald et al. 2005; Nikula et al. 2010). Most genetic homogeneity has been attributed to dispersal by rafting on floating kelps. Smith (2002) estimated 20 million floating *Durvillaea antarctica* at one time in the Southern Ocean. Similarly, Macaya et al. (unpublished data) reported high abundance of *M. pyrifera* rafts along the Chilean coast with values estimated at 170 million kelp rafts. Such high abundances of floating kelp rafts might contribute to dispersal along the southern hemisphere, especially in high latitudes where low temperatures facilitate longevity of kelp rafts on the sea surface (Rothausler et al. 2009).

5.5.2 Connectivity along the ACC

Analysis of COI and microsatellites showed high levels of connectivity among populations in the southern hemisphere. No genetic structure and no evidence of isolation by distance were found. These findings are in concordance with previous suggestions of high gene flow of marine organisms in the area (Coyer et al. 2001; Waters and Roy 2004; Donald et al. 2005; Nikula et al. 2010). Furthermore, due to the restricted dispersal distances of zoospores (Anderson and North 1966, Graham 2003; Raimondi et al. 2004; Reed et al. 2004), rafting of floating kelp can be suggested as the main mechanism driving such connectivity as zoospores from fertile algal kelp rafts can be viable for long periods following detachment (Macaya et al. 2005; Hernández-Carmona et al. 2006, Hawes 2008). Dispersal of floating algal rafts has been suggested to be an important dispersal mechanism in other brown algal species with floating structures such as *Ascophyllum nodosum* (John, 1974; Olsen et al. 2010), *Phyllosphora comosa* (Coleman and Kelaher 2009), *Fucus vesiculosus* (Muhlin et al. 2008), *Hormosira banksii* (McKenzie and Bellgrove 2008; McKenzie and Bellgrove 2009), *Halydris dioca* (Lu and Williams 1994) and *Sargassum polyceratium* (Engelen et al. 2001). In contrast, kelp species without floating structures displayed limited dispersal distances: for example, *Lessonia nigrescens* (Martinez et al. 2003; Faugeron et al. 2005), *Laminaria digitata* (Billot et al. 2003), *Postelsia palmaeformis* (Kusumo et al. 2006) and *Ecklonia radiata* (Coleman et al. 2009).

Several studies have shown that long distance dispersal via the ACC is possible (Queiroz 2005; Sanmartin et al. 2007; Waters 2008; Fraser et al. 2009). Shared haplotypes among 2 peracarid species in the SH have been reported using mitochondrial DNA by Nikula et al. (2010). These organisms live on *Durvillaea*

antarctica holdfasts and display similar patterns of genetic structuring as the host. These peracarid species are also found on *Macrocystis pyrifera* holdfasts (Edgar 1987). Thus LDD is also possible using *Macrocystis* as a floating sustrata vector for these species.

Interestingly no genetic structure and no pattern of isolation by distance was found for *Macrocystis pyrifera* using microsatellite data, despite the large geographic area (17,000 km). Long distance colonization via kelp rafts might facilitate natural recolonization events, especially after disturbance (Thiel and Haye 2006, Coleman and Kelaher 2009). The microsatellite data confirm that significant dispersal over ecological time-scales is occurring in the southern hemisphere and that the ACC promotes such dispersal. For *Fucus vesiculosus*, nearshore currents have been suggested as the most likely mechanism driving gene flow around and between coastal points via detached rafts (Muhlin et al. 2008).

F_{ST} pairwise comparison aids to the understanding the genetic differentiation among regions in the SH. F_{ST} values between the subantarctic region and adjacent areas were low and similar to values found in other kelp species but at smaller scales (Billot et al. 2003; Coleman et al. 2009). Despite the high connectivity found in *M. pyrifera*, some particular sites had low genetic diversity (H_e) (Gough Island, Marion Island and Macquarie Island). These sites represent isolated volcanic (Gough and Marion) and tectonic (Macquarie) islands and the low genetic diversity might represent recent colonizations, as these islands were affected by LGM ice scours (Fraser et al. 2009). Additionally, in a biogeographic study, van den Hoek (1987) proposed that Gough Island received its marine flora by dispersal from Argentina or the Falkland Islands due to the

similar composition of these sites. Other subantarctic islands did not have low genetic diversity, a pattern also reflected by COI sequences. However due to the small number of samples analyzed from the subantartic islands, the interpretation of these analyses must be viewed with caution.

In conclusion, the global analysis using COI showed shared haplotypes over vast areas of the northern and southern hemisphere. Higher haplotype diversity in samples from the SH and the ancestral haplotype derived from the haplotype network analysis, suggest an origin in this hemisphere, contrary to previous evidence that supports a NH origin. Since global analysis with nuclear and mitochondrial DNA have show contrasting results, the question still remains open and future work with more molecular markers (e.g. SNPs) and sampling sites might help to explain the anti-tropical distribution of this kelp species. Analysis of microsatellites support the hypothesis of dispersal mediated by the ACC in the Southern Ocean, due to a lack of significant population differentiation or isolation-by-distance confirming previous suggestions of floating reproductive kelp rafts as an important mechanism shaping the connectivity in the Southern Hemisphere.

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CHAPTER 6

Summary, final considerations and future research

Before this research many aspects of the taxonomy, connectivity, and dispersal of *Macrocystis pyrifera* were unknown. Using different molecular markers I have addressed some of these gaps in the knowledge. The study of DNA sequences (mitochondrial and nuclear) has given important insights into the different factors shaping the genetic structure, distribution and dispersal of *Macrocystis*. Recent dispersal and high levels of gene flow can explain the genetic homogeneity observed at different spatial scales, as well as historical and recent events which might explain the distribution of haplotypes in this large brown macroalga. This study also presents genetic evidence supporting the importance of detached kelp individuals in the dispersal of *Macrocystis*, although additional analysis are needed to confirm this suggestion. Thus this research provides substantial information for future studies in kelp connectivity and dispersal, and it provides a model that can be used in other algal species with similar life cycles and dispersal mechanisms.

The taxonomy of *Macrocystis* has changed constantly over years but since 1986 four species have been recognized. However, it is recognized that morphological characters (the main feature to separate species) could be plastic environmental adaptations rather than genetic differences. Today, DNA barcoding is a powerful tool to discriminate among species of brown algae (Lane et al. 2007; Kucera and Saunders 2008; McDevit and Saunders 2009). Thus the

use of COI sequences allows confirmation of previous suggestions. Results showed low genetic variation in a global analysis and shared haplotypes among former "species" (Chapter 2). Consequently, the genus *Macrocystis* should now be considered as monospecific, *M. pyrifera* being the only species, containing four ecomorphs: *M. 'integrifolia', M. 'pyrifera', M. 'angustifolia'* and *M. 'laevis'*. Therefore, this changes questions of *Macrocystis* distribution from interspecific dispersal and evolutionary questions to intraspecific ecological questions and the maintenance of *Macrocystis* in certain environments that produce particular morphologies. For example, the distinct ecomorphs that can dominate are different habitats often adjacent to each other (Graham et al. 2007).

My study shows for the first time an effect of the last glacial maximum (LGM) on the haplotype distribution of a marine organism in southern Chile (Chapter 3). Recolonization after the LGM in high-latitude habitats can occur rapidly, especially in highly dispersive taxa (Hewitt 1996; Fraser et al. 2009). The high dispersal potential of detached reproductive kelp rafts might be an important factor shaping the genetic pattern of *Macrocystis* populations, as the high abundance of floating kelp rafts has been described in southern Chile (Hinojosa et al. 2010). Furthermore, the longevity of floating kelp rafts can be increased at temperatures characterisctic of high latitudes (Rothäusler et al. 2009). I also described the phylogeography of this species along approximately 5000 km iof the SEP, which was characterized by a small number of haplotypes and a poor phylogeographic structure, with haplotypes separated by only one base pair. Additionally the presence of genetic breaks might be related partially to the presence of biogeographics barriers. The SEP upwelling (30º S) does not seem to restrict gene flow in northern Chile, contrary to results described for other marine

organisms elsewhere and in this region (e.g. Apte and Gardner 2002; e.g. Ayers and Waters 2005; Tellier et al. 2009). A major biogeographic break in southern Chile (42º S) is partially in concordance with the haplotype disjunction found in the area, although the presence of different haplotypes might be related to the LGM (Chapter 3).

Dispersal via reproductively detached kelp individuals has been long suggested as an important dispersal mechanism maintaining connectivity among populations (van den Hoeck 1987; Norton 1992; Coleman and Brawley 2005; Macaya et al. 2005; Hernández-Carmona et al. 2006; Muhlin et al. 2008). In Chapter 4, using two different mitochondrial markers, I infer the possible origin of detached *Macrocystis* individuals from the Chilean fjords. These results contrasted floating individuals with adjacent attached populations. This study represents the first step to determine routes for floating kelp rafts, and results suggest a southward dispersal in this area. Recently, Nikula et al. (2010) demonstrated that macroalgal rafting may explain similarities in the genetic feature of two peracarid species associated with *Durvillaea antarctica* holdfasts across the subantarctic. Similar approaches as used by Nikula et al. (2010) and this thesis (Chapter 4) can be used to analyse samples from floating and detached kelps in a more complete community analysis. Additionally, the use of more variable molecular markers from floating kelp rafts might help to determine the exact origin of donor populations (although intense sampling of attached populations must be carrying out).

In Chapter 5 the analysis of COI sequences from world wide sampling confirms high gene flow of this species, since vast areas of the Northern and Southern hemisphere shared haplotypes. Along approximately 2800 km of

sampling in the Northern Hemisphere, only one haplotype was found. A similar result was found for the subantarctic region. The haplotype network suggests a Southern Hemisphere origin, but most previous research indicates a Northern Hemisphere origin,.Further analysis is required to answer this question. But, what is clear is a probable recent dispersal either from south-to-north or north-to-south, with the species crossing the tropics during the LGM (Coyer et al. 2001). Microsatellites analysis revealed no genetic structure and no isolation by distance in the Southern Hemisphere These findings highlight the presence of high gene flow over ecological time scales along vast areas of the Southern Ocean.

The data presented in this thesis contributes towards an understanding of connectivity and dispersal patterns of the largest seaweed on the planet, and are useful to understand the dynamics and distribution of this species. Results can also be extrapolated to species with similar dispersal potential and to associated fauna that travel on kelp rafts as a method of reach long distance dispersal.

My study is different from previous research on *Macrocystis* (taxonomy, dispersal, connectivity) because it has incorporated analysis on a global scale as well as on a fine (local) scale, thus the understanding of kelp dynamics can be explained considering dispersal variation in both levels. Genetics studies (like this one) also can be combined with ecological, morphological and physiological research, in order to understand and explain the evolution and biogeography of globally distributed species like the giant kelp. Thus, the information in this thesis can be incorporated on collaborative research to uncover the factors that for example regulated the different phenotypic expression under distinct environmental conditions.

The information here is also quite useful to be incorporated in models that try to explain the effect of climate change in distribution and resilience of macroalgal populations, especially in kelp communities, which are highly susceptible to anthropogenic and natural perturbations such el Niño effect and over-harvesting.

6.1 Future research directions

This thesis has investigated the taxonomy, phylogeography and dispersal patterns of *Macrocystis pyrifera*. There are still additional questions that remain and others have arisen during this study and might extend the findings of this thesis.

6.1.1 Factors regulating the expression of different ecomorphs in *Macrocystis*

There is still no clarity behind the factors regulating the development of specific morphologies in *Macrocystis*. Future research might consider the genetic source of the phenotypic variation under different environments. Thus, gene expression can be explored in order to elucidate mechanisms underlying plastic response. Physiological, biomechanic and biochemical studies can be also used in this task.

6.1.2 Effect of the Last Glacial Maximum on genetic pattern

With more intensive sampling and incorporating more molecular markers (ITS, *rbc*L, microsatellites). Future research might address the effect of the LGM on the genetic structure of populations from southern Chile. In addition, recolonization processes can be addressed by trying to determine the source of populations either from central Chile, New Zealand or elsewhere. The recolonization of Patagonia from populations from NZ has been suggested for

the bull kelp *Durvillaea antartica* (Fraser 2009). This could be tested with the markers available for *M. pyrifera*.

6.1.3 Rafting as an important dispersal factor

The analysis of two mitochondrial markers from attached and floating *Macrocystis* individuals from the Chilean Fjords has revealed possible dispersal routes; however further analyses are required to confirm such findings. Analysis with more variable molecular markers (e.g. microsatellites) from floating and attached individuals including more individuals and more sampling sites can be used to compare allelic frequencies in order to determine dispersal routes and donor populations. Molecular analysis can also include samples of associated fauna, especially from organisms without larval stages (e.g peracarid fauna), as rafting is the most likely dispersal method used to travel long distances.

6.1.4 Further analysis of Peruvian samples

The analysis of a few individuals from Peru (Paracas and Atico) revealed the presence of exclusive haplotypes (Chapter 3 and 4) but also the only site (Atico) along the south-eastern Pacific coast with more than one haplotype using the atp8-S marker. Further sampling including more populations might give insights into the cause behind such variation and genetic structure. In the case of a Northern Hemisphere origin of the species and subsequent colonization of South America, Peru must have been the first colonized area; thus the consequences of such interesting processes can be analyzed.

6.1.5 Effect of anthropogenic and environmental disturbances on kelp genetic structure

The demand for alginate sources and food for cultivated abalone has dramatically increased the harvesting pressure of *Macrocystis* in northern and southern Chile. Additionally, the use of individuals from different regions has been suggested to develop aquaculture farms. The genetic consequences of these disturbances need to be addressed in order to aid the application of conservation and management plans. Furthermore, the effect of 'El Niño' phenomena should be analyzed with more details, increasing the number of sampling sites from northern Chile and Peru, so that samples can be contrasted with populations from non-affected areas (central Chile) to determine the effect of this important environmental disturbance on the evolution and genetic structure of *Macrocystis* populations. These analyses can be also contrasted with samples from Northern Hemisphere where El Niño also affects populations (e.g. California and Baja California).

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APPENDIX 1 Taxonomic review of *Macrocystis*

Table 1. Taxonomic review of *Macrocystis*. Important milestones are highlighted in bold. N.I. = No Information, D.L. = Specimens described from samples collected at different locations.

Author	**Year**	**Species**	**Geographic Location**	**Remark**	**Reference**
Bauhin, J.	1651	*Bulbus marinus crinitus*	N.I.	First known description	Bahuine 1651
Banks, J.and Solander, D.	1769	*Fucus giganteus*	SE Pacific (Patagonia)	Specimen seen on the first voyage of James Cook, described as 126 feet long	Banks 1771
Linnaeus, C.	**1771**	***Fucus pyriferus***	**Oceano Aethiopico**	**Described from specimens collected by Emmanuel Koenig**	**Linnaeus 1771**
Menzies, A.	1792	*Fucus pyriferus*	NE Pacific	The first known record of *Fucus pyriferus* in California on a expedition led by G. Vancouver 1791-1795	Menzies 1923
Turner, D.	1809	*Fucus pyriferus*	SE Pacific (Patagonia), S Africa (Cape Good Hope)	The first detailed description of *Fucus pyriferus* with information on its distribution, natural history and diagrams	Turner 1809
Humboldt, A. and Bonpland, A.	1809	*Fucus humboldtii* and *Fucus hirtus*	SE Pacific (Guanchaco and Callao - Peru)	Described apparently from drifting material	Kunth 1822
Lamouroux, J. V. F.S.	1812	*Laminaria pyrifera* and *Laminaria pomifera*	D.L.	Assigned the group to *Laminaria* genus	Lamourou x 1813

Agardh, C. A.	**1820**	***M. pyrifera (var. minor) M. comosa (var. integrifolia)* and *M. mentziensii*.**	**D.L.**	**The genus *Macrocystis* is created**	**Agardh 1820**
Bory de Saint Vincent, J. B. G. M.	**1826**	***M. integrifolious, M. latifoliuos, M. angustifolius, M. menziesii, M. pomiferus* and *M. communis***	**SE Pacific (Valparaiso and Concepción - Chile), (Peru), SW Pacific (Nouvelle Hollande)**	**Described several species on the 'Dictionnaire classique D'Historie Naturelle'**	**Bory de Saint-Vincent 1826**
Greville, R.	1830	*M. pyrifera*	N.I.	Described M*acrocystis* as the longest of all known algae (500 to 1500 feet in length)	Greville 1830
Agardh, C. A.	1839	*M. latifolia,; M. pyrifera, M. planicaulis, M. angustifolia, M. zosteraefolia* and *M. humboldtii*	D.L.	First monograph of the genus, recognized 6 species	Agardh 1839
Montagne, J. F. C.	1839	*M. orbignyana*	SE Pacific (Patagonia)	Described *M. orbignyana* based on blade morphology	Montagne 1839
Postels, A. and Ruprecht, F.	1840	*M. pyrifea*, *M. tenuifolia* and *M. angustifolia*	NE Pacific	Described *M. tenuifolia* based on blade morphology	Postels and Ruprecht 1840

Hooker, J. D.	**1847**	***M. pyrifera (var. integrifrons, angustifrons, zosterofolia, luxurians, membranacea, humboldtii)***	**Throughout the Antarctic seas, between 40° and 64°**	**Referred some previously described species as forms of one species, *M. pyrifera*. He also called the Antarctic Ocean the *"Macrocystis Ocean"***	**Hooker 1847**
Harvey, W.H.	1862	*M. pyrifera (var dubenii)*	D.L.	Adhere to the opinion that the various forms of *Macrocystis*, are merely varieties, dependent on local circumstances or on age, etc.	Harvey 1862
Areschoug, J. E.	1883	*M. angustifolia*	N.I.	Recognized the importance of holdfast morphology	Areschoug 1883
De Toni, G. B.	1895	*M.pyrifera, M. angustifolia* and *M. obtusa*	SE Pacific (Chile), NE Pacific (California)	Described 3 species using holdfast morphology	De Toni 1895
Skottberg, C.	**1907**	***M. pyrifera***	**N.I.**	**Recommended the genus be collapsed back to the single species *M. pyrifera***	**Skottsberg 1907**
Howe, M. A.	1914	*M. integrifolia* and *M. pyrifera var. humboldtii*	SE Pacific (Peru)	Description of *Macrocystis* from Peru based on holdfast morphology	Howe 1914
Setchell, W.A and Gadner, N. L.	1925	*M. integrifolia* and *M. pyrifera*	NE Pacific	Described *M integrifolia* and *M. pyrifera* based on holdfast morphology (rhizome form and hapteres)	Setchell and Gardner 1925
Laing, R. M.	1927	*M. pyrifera* and *M. humboldtii*	SW Pacific (New Zealand)	Described *M.pyrifera* and *M. humboldtii* from New Zealand	Laing 1927

Setchell, W. A.	1932	*M. integrifolia* and *M. pyrifera*	NE Pacific	Reported that *M.integrifolia* also occurs on the west coast of N America	Setchell 1932
Papenfuss, G. F.	1942	*M. pyrifera*	S Africa	Described the first stages of *M. pyrifera* in S. Africa, gametophytes and juvenile sporophytes	Papenfuss 1942
Smith, G.M.	1942	*M. integrifolia* and *M. pyrifera*	NE Pacific (USA)	Discussed the presence of *Macrocystis* species in Monterrey, California	Smith 1942
Moore, L. B.	1943	*M. pyrifera*	SW Pacific (New Zealand)	Described a free-living form of *Macrocystis* (without holdfast) in Paterson Inlet and Berkely Sound, NZ	Moore 1943
Scagel, R. F.	1947	*M. integrifolia*	NE Pacific	Described *M. integrifolia* from Vancouver Island based on holdfast morphology	Scagel 1947
Cribb, A. B.	1954	*M. pyrifera*	SW Pacific, Tasmania	Described *M. pyrifera* from Tasmania; also described a different morphology from plants colleted in N Tasmania (smaller plants)	Cribb 1954
Womersley, H. B. S.	**1954**	***M. integrifolia, M. pyrifera* and *M. angustifolia***	**SW Pacific (S. Australia)**	**Described a new species *M. angustifolia* present in S. Africa and S. Australia based on holdfast morphology**	**Womersley 1954**
Neushul, M.	1971	*M integrifolia, M. pyrifera* and *M. angustifolia*	D.L.	Suggested a need for further reconsideration of taxonomy and species distribution. Described *M. angustifolia* in southern California	Neushul 1971

Hay, C.	**1986**	***M. laevis***	**S Indian Ocean (Marion Island)**	**Described a new species, *M. laevis* based on blade morphology**	**Hay 1986**
Brostoff, W. N.	1988	*M. integrifolia* and *M. pyrifera*	NE Pacific	Pointed out that *Macrocystis* in southern California must be recognized as a single species, *M. pyrifera*	Brostoff 1988
van Tussenbroeck, B. I.	1989	*M. pyrifera*	SE Pacific (Falklands Islands)	Mentioned that morphological differences (blades) do not define a new species. Also questioned the classification made by C. Hay in 1986	van Tussenbroeck 1989
Coyer, J. et al.	2001	*M. pyrifera*	World sampling	Using ITS sequences suggested *Macrocystis* as a monotypic genus based on the low variability among samples	Coyer et al. 2001
Aguilar-Rosas, L. E. et al.	2003	*M. laevis*	SE Pacific (S Chile)	Reported *M. laevis* from southern Chile	Aguilar-Rosas et al. 2003
Graham, M. H. et al.	2007	*M. pyrifera*	N.I.	Suggest a taxonomy revision and refer to the different species as 'forms'	Graham et al. 2007
Demes, K. et al.	**2009**	***M. pyrifera***	**NE Pacific (USA)**	**Morphological variability driven by environmental factors, genus collapsed back to *M. pyrifera***	**Demes et al. 2009**
Macaya, E. C.	**2010**	***M. pyrifera***	**World sampling**	**Using COI DNA barcoding confirm that *Macrocystis i*s a monospecific genus**	**This Study**

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APPENDIX 2 Additional research

During the course of this PhD research, I have also made a significant contribution to a number of additional studies. I was actively involved in design, analysis and/or writing of different articles:

-Gutow, L., Hinojosa, I. & **Macaya E.** 2009. Epifauna on benthic macroalgae. *Global Marine Environment*. 9:27.

-**Macaya E.** & Thiel M. 2008. In situ tests on inducible defenses in *Dictyota kunthii* and *Macrocystis integrifolia* (Phaeophyceae) from the Chilean coast. *Journal of Experimental Marine Biology and Ecology.* 354: 28–38.

-Thiel M., **Macaya E.,** Acuña E., Arntz W., Bastias H., Brokordt K., Camus P, Castilla JC, Castro L., Cortés M., Dumont C., Escribano R., Fernandez M., Gajardo J., Gaymer C., Gomez I., González A., Gonzalez H., Haye P., Illanes JE, Iriarte JL., Lancellotti D., Luna-Jorquera G., Luxoro C., Manriquez P., Marín V., Muñoz P., Navarrete SA., Perez E., Poulin E., Sellanes J., Sepúlveda H., Stotz W., Tala F., Thomas A., Vargas C, Vásquez J. & Vega A. 2007. The Humboldt current system of northern-central Chile: Oceanographic processes, ecological interactions and socio-economic feedback. *Oceanography and Marine Biology: an Annual Review.* 45:195-345.

-Bulboa C.R., Macchiavello J., Veliz K., **Macaya E**. & Oliveira E.C. 2007. In vitro recruitment of *Ulva* sp. *and Enteromorpha* sp. on gametophytic and tetrasporophytic thalli of four populations of *Chondracanthus chamissoi* from Chile. *Journal of Applied Phycology*. 19: 247-254.

I’m extremely honoured by colleagues from NIWA and UCN-Chile who named a new amphipod species (found on the Wellington coast) after me: *Bircenna macayai*

Journal of the Marine Biological Association of the United Kingdom, page 1 of 9.
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Algal-dwelling Eophliantidae (Amphipoda): description of a new species and key to the world species, with notes on their biogeography

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Eophliantidae are poorly studied marine algal-dwelling amphipods with a wide distribution. A new species was found to excavate burrows across the main stem of Carpophyllum maschalocarpum *(Turner) Grev. in the New Zealand subtidal, and a detailed morphological description of this amphipod is given.* Bircenna macayai *sp. nov. can be distinguished from other* Bircenna *species by a combination of the following characters: bilobed coxa 1, merus and carpus of pereopods 5–7 strongly extended posteriorly, crenulate basis of pereopod 7 and smooth posterior margin of epimeron 3, pereopod 7 basis longer than wide. A key to the fourteen world species of Eophliantidae is provided. Taxonomy, evolutionary sequences, functional morphology and biogeography of the Eophliantidae are briefly discussed. New Zealand and Australian shallow waters show the highest species diversity of Eophliantidae, containing both species bearing plesiomorphic and highly derived characters, suggesting that Australasia is an evolutionary centre for this amphipod family.*

Keywords: Amphipoda, Eophliantidae, *Bircenna macayai*, new species, *Carpophyllum maschalocarpum*, functional morphology, algal-dwelling, biogeography, New Zealand, Australasia, evolutionary centre

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INTRODUCTION

According to Barnard (1972) the New Zealand intertidal zone can be considered to be a distinct gammeridean biogeographical province, since more than 50 per cent of its species are endemic. More than 100 species of gammaridean amphipods are known to live amongst algae in New Zealand, but few are considered to be obligate algal dwellers or tunnellers. The family Eophliantidae, however, are one such group.

The Eophliantidae contain 14 species belonging to six genera. The main distribution is in the southern hemisphere, with the exception of *Ceinina japonica* Stephensen, 1933 found in Japan and *Wandelia orghidani* Ortiz & Lalana, 1997 from Indonesia, just north of the equator. *Lignophliantis pyrifera* J.L. Barnard, 1969 is described from California, but the placement of this species in the Eophliantidae is uncertain. Its taxonomic position is briefly discussed below. Information regarding species of the Eophliantidae is scattered throughout the literature, and to facilitate identification we have also included a key to the world species. Finally, a new species was found burrowing in the algae *Carpophyllum maschalocarpum* (Turner) Grev. off New Zealand and is described in detail below.

All eophliantids are assumed to be algal dwellers, although no record of algae association is found for *Cylindryllioides kaikoura* Barnard, 1972 or for *Wandelia orghidani* Ortiz & Lalana, 1997. Several species from the family Eophliantidae live in positively buoyant macroalgae, such as *Macrocystis pyrifera* (L.) C. Agardh, *Durvillaea antarctica* (Cham.) Har. or *C. maschalocarpum*. These algae are commonly found floating in coastal waters of New Zealand (Kingsford, 1992) and in the Southern Ocean (Helmuth *et al.*, 1994). Several recent studies indicate that the biota (including Eophliantidae) associated with these floating macroalgae might be dispersed via algal rafting (e.g. Donald *et al.*, 2005). This will also influence the phylogeography of these organisms. We propose that algal rafting might have contributed to the evolutionary radiation of the Eophliantidae, originating in Australasia.

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MATERIALS AND METHODS

Field collecting

The alga *Carpophyllum maschalocarpum* was collected by snorkelling in about 2 m depth at Kau Bay, Wellington Peninsula, New Zealand, in May 2009. The brown alga *Carpophyllum maschalocarpum* is common in coastal regions of New Zealand (including the Wellington area), where it grows in shallow subtidal waters. *Carpophyllum* is a common habitat for several groups of peracarids (e.g. Taylor & Cole, 1994; Taylor, 1998) and a food source for herbivorous labroid fish (Choat & Clements, 1993). It has a distinct main stem with alternating lateral blades, and its texture is

characterized as leathery and tough (Adams, 1997). Several pieces were kept in seawater before examination under a stereomicroscope. Photographs were taken of live amphipods in their holes in the algae stem. Amphipods were removed from small holes in the algae by forceps and fixed in either buffered formalin or in 90% ethanol.

Morphological description

Specimens were examined and dissected under a Leica MZ9.5 stereomicroscope and drawn using a camera lucida. One of the selected paratypes was completely dissected and mounted on one slide in Faure's solution. The holotype was temporarily mounted in glycerol. These specimens were examined and drawn using a Nikon compound microscope fitted with a camera lucida. The body lengths of specimens examined were measured by tracing individual's mid-trunk lengths (tip of the rostrum to end of telson) using a camera lucida. All illustrations were digitally 'inked' following Coleman (2003). Setal terminology follows Watling (1989).

Type material is held in the National Institute of Water and Atmospheric Research Invertebrate Collection, Wellington, New Zealand (NIWA). Comparative material of *Wandelia dronga* (Myers, 1985) was borrowed from the Australian Museum, Sydney.

Functional morphology

An extensive literature search was conducted for all species of Eophliantidae. Morphometric measurements were taken from published descriptions (e.g. length of coxae related to length of pereonites).

RESULTS

SYSTEMATICS
Order AMPHIPODA Latreille, 1816
Suborder GAMMARIDEA Latreille, 1803
Superfamily TALITROIDEA Rafinesque, 1815
Family EOPHLIANTIDAE Sheard, 1936

DIAGNOSIS (AFTER BARNARD & KARAMAN, 1991)
Body cylindroid (vermiform), coxae small, often discontiguous. Head spheroid. Cuticle smooth. Eyes bilateral. Antennae short, sparsely articulate, accessory flagellum absent. Mandibular palp vestigial or absent; molar nontriturative, often absent or spinose, rakers sparse to absent. Palp of maxilla 1 vestigial or absent. Gnathopods thin, feeble, parachelate, or minutely subchelate. Pereopods short, article 2 of pereopods 5–7 expanded. Uropod 3 vestigial, ramus absent. Telson usually deeply cleft or fully bilobate, with exception of *Lignophliantis* bearing an entire telson. The telson lobes usually forming tent and slightly fleshy. Urosomites 2–3 occasionally coalesced.

Genus *Bircenna* Chilton, 1884

DIAGNOSIS (AFTER BARNARD & KARAMAN, 1991)
Pereonite 1 with ventral cradle for support of head.

Flagella of antennae 1–2 with four or more articles. Coxae 2–4 or 4–5 discontiguous. Posterior lobe on articles 4–5 of pereopods 5–7 with 2–3 medium size setae. Pleopods bira mous, peduncles expanded. Telson almost fully cleft.

REMARKS
Examination of the type material of *Bircenna dronga* Myer 1985 revealed that this species lacks a cradle on pereonite and its coxae are contiguous. Based on this we herewit remove this species from *Bircenna* to the genus *Wandeli* Chevreux, 1906.

Bircenna macayai sp. nov.
(Figures 1–4)

TYPE MATERIAL
Holotype: adult male 1.52 mm, in ethanol; New Zealand, Ka Bay, Wellington Peninsula, 174°49′48″E–41°17′16″S, subtid water depth 2 m, NIWA 49241. Collected by snorkelling May 2009.

Paratype: adult female 2.6 mm, (bearing 4 spherical egg 0.2 mm in diameter each) NIWA 49242 collection details a for holotype, in ethanol.

Specimen of undetermined sex, 1.4 mm, NIWA 4924 fully dissected on one slide.

COMPARATIVE MATERIAL EXAMINED
Bircenna dronga Myers, 1985, holotype AMP35194 and para type AMP35195 from the Australian Museum, Sydney. Fij Makuluva Isaland, Viti Levu, collected 13 August 1979 fro mixed red algae, by A. Myers.

Bircenna fulva Chilton, 1884 from the NIWA Invertebrat Collection, NIWA7188, NIWA 7181. New Zealan 174°49′60″E–41°20′05″S, collected November 1968 by Barnard, 0 m depth.

ETYMOLOGY
The species is named for Erasmo Macaya Horta, in acknowl edgement of his hospitality, participation in collection algae, and taking photographs of specimens during the vis of M. Thiel to Wellington.

DESCRIPTION OF *BIRCENNA MACAYAI* SP. NOV.
Body shape cylindrical, head rounded with hemispheric incision anteroventrally. Eyes round. Coxae 1–5 small an discontiguous. Pereopods 5–7 increasing in length, pereopo 7 twice the length of pereonite 7. Urosomites two and three ar fused (Figure 1A).

Antennae 1 and 2 subequal in length (Figure 1B, C Antenna 1 flagellum with four articles. Upper lip rounde and slightly setose apically (Figure 1D). Mandible lackin palp, incisor dentate, bearing 4 to 5 teeth (Figure 2A, B lacinia mobilis not apparent, potentially modified to resembl spine in spine row. Maxilla 1 lacking palp; inner plate slende bearing 1 stout seta; outer plate with 7 setal teeth (Figure 1F Lower lip slender lobe, apically setose (Figure 1E). Maxillipe palp four articulate; article four blunt; inner plate long, reach ing fourth article of palp; inner plate bearing three apic robust setae; outer plate shorter than inner plate (Figure 2C

Gnathopod 1 coxa bilobed, twice as wide as long; ischiur two-thirds of basis length; carpus and merus subequal i length; propodus posterodistally produced into triangula parachela; dactylus unguiform (Figure 2D). Gnathopod coxa small, rectangular; basis to dactylus similar to gnathopo 1 (Figure 2E). Pereopods 3 and 4 very similar; meru

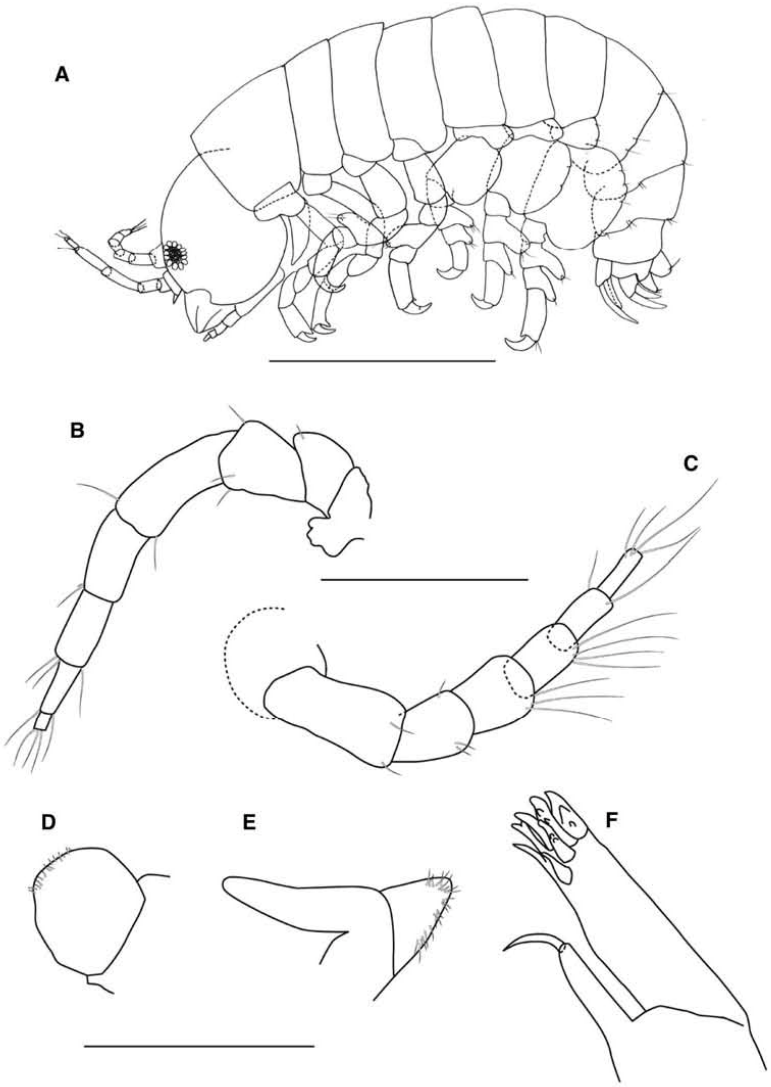

Fig. 1. *Bircenna macayai* sp. nov. (A) Holotype: adult male 1.52 mm, NIWA 49241; (B–F) paratype undetermined sex, 1.4 mm, NIWA 49243. (A) Habitus; (B) antenna 1; (C) antenna 2; (D) labrum; (E) hypopharynx (lower lip); (F) maxilla 1. Scale bars; A: 0.4 mm, B–F: 0.1 mm.

expanded anterodistally (Figure 2F). Pereopod 5 basis subrectangular, merus and carpus anterodistally produced; rounded protrusion of merus bearing five long setae, protrusion of carpus bearing one seta; dactylus unguiform (Figure 3A). Pereopod 6 basis as wide as long; merus and carpus anterodistally produced; rounded protrusion of merus bearing eight long setae, protrusion of carpus bearing three seta; dactylus unguiform (Figure 3B). Pereopod 7 basis rounded posteriorly and crenulated; merus and carpus anterodistally produced; protrusion of merus bearing two long setae, protrusion of carpus bearing one seta; dactylus unguiform (Figure 3C).

Pleopods 1–3 peduncle broad; pleopods 1–3 biramous (not drawn). Urosomite 1 more than double the length of fused urosomites 2 and 3; uropod 1 peduncle shorter than outer ramus; outer ramus about two-thirds the length of inner ramus (Figure 3D). Uropod 2 peduncle shorter than outer ramus; outer ramus about 75% of inner ramus (Figure 3D). Uropod 3 very small, uniarticulate, two apical slender setae. Telson fleshy, bilobed, deeply cleft; each lobe with 1 apical slender seta (Figure 3D).

DISTRIBUTION

On *Carpophyllum maschalocarpum* in Kau Bay and Breaker's Bay, Wellington, New Zealand, subtidal.

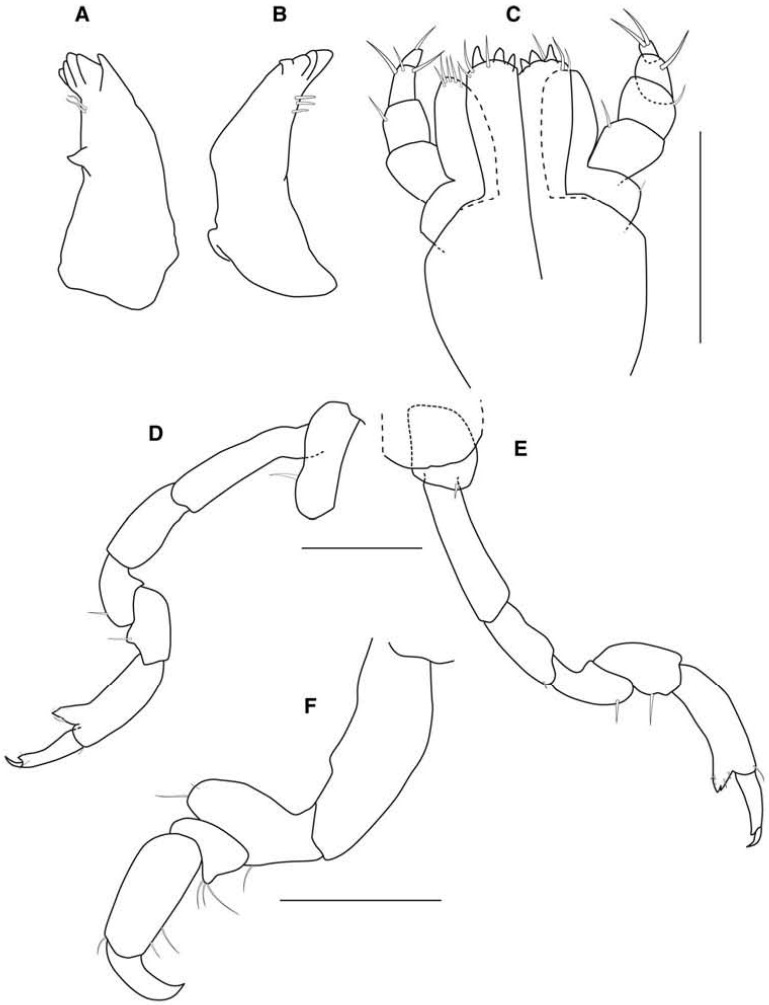

Fig. 2. *Bircenna macayai* sp. nov. (A–E) Paratype undetermined sex, 1.4 mm, NIWA 49243; (F) holotype: adult male 1.52 mm, NIWA 49241 (A, B) mandible; (C) maxilliped; (D) gnathopod 1; (E) gnathopod 2; (F) pereopod 4. Scale bars: 0.1 mm.

NOTES ON THE BIOLOGY OF *BIRCENNA MACAYAI* SP. NOV.

Bircenna macayai sp. nov. excavate burrows across the main stem of *Carpophyllum maschalocarpum* (Figure 4A–C). Burrows of large individuals have two openings on either side of the stem, but burrows of smaller individuals may have only a single opening. Around the openings of the burrows, circular holes of brownish-yellowish colour indicate the presence of active amphipod burrows (Figure 4A, B). These holes are produced by the grazing activity of the amphipods, which removes the dark brownish meristoderm layer on the blade-like stems of the thallus. Living amphipods can frequently be seen consuming algal tissues within their burrows (Figure 4C). Occasionally they ventilate their burrows with repeated pleopod beats. They can easily turn around in their burrows. Specimens of *Bircenna macayai* sp. nov. appear quite reluctant to abandon their burrows: only cutting away of surrounding tissues and slight squeezing of the remaining burrow walls induces amphipods to leave. They can then be seen walking around on the blade stems. Eophliantids are also relatively agile swimmers.

Several burrows are often found together on individual thallus stems of *C. maschalocarpum*. Other grazers (e.g. snails) appear to be attracted to burrows of *Bircenna macayai* sp. nov. where they can be seen feeding in the burrow holes. In combination with a high prevalence of amphipod burrows, the combined grazing activity of different grazer species causes weakening and subsequent breakage of the stems.

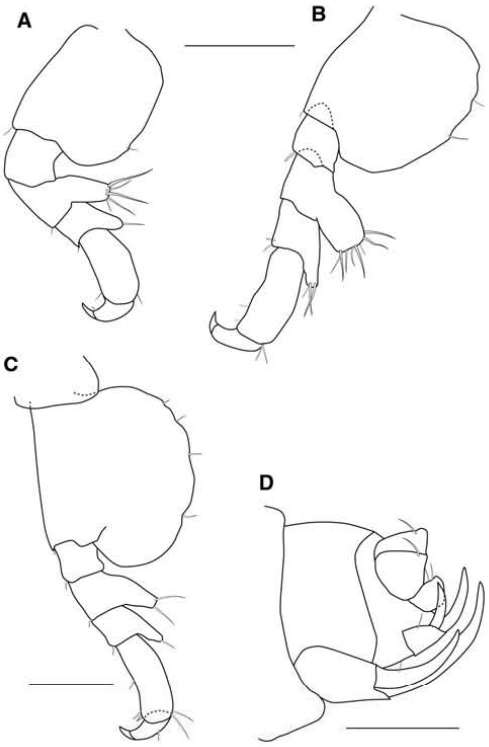

Fig. 3. *Bircenna macayai* sp. nov. (A–C) Holotype, adult male 1.52 mm, NIWA 49241; (D) paratype undetermined sex, 1.4 mm, NIWA 49243. (A) pereopod 5; (B) pereopod 6; (C) pereopod 7; (D) urosome including uropods and telson. Scale bars 0.1 mm.

KEY TO THE WORLD SPECIES OF EOPHLIANTIDAE, 14 SPECIES IN TOTAL

1. Telson uncleft and fused to urosomites 2–3, flagella of antennae 1–2 with one article only ... (*Lignophliantis*, 1 species) ... *Lignophliantis pyrifera* J.L. Barnard, 1969
   — Telson cleft, distinct from urosome, flagella of antennae 1–2 with more than 1 article 2
2. Pleopods with 1 ramus ..... 3 (*Cylindryllioides*, 2 species)
   — Pleopods with 2 rami 4
3. Uropod 3 with heavy apical jewel spine; telson rounded, bilobed; upper lip evenly rounded. ..... *Cylindryllioides kaikoura* Barnard, 1972
   — Uropod 3 lacking apical jewel spine; telson pointed, deeply cleft, upper lip bilobed. ..... *Cylindryllioides mawsoni* Nicholls, 1938
4. All coxae contiguous ..... 5 (*Wandelia*, 4 species)
   — Some or all coxa discontiguous 8
5. Slightly bilobed coxa 1, posterior lobe longer than anterior lobe, not overlapping coxa 2; upper lip rounded and setulose; telson lobes triangular shaped ..... *Wandelia crassipes* Chevreux, 1906
   — Coxa 1 not bilobed, telson lobes rectangular shaped 6
6. Broad coxa 1, overlapping coxa 2; upper lip quadrate and asetulose; distinct incision of head for reception of antenna 2; antenna 1 flagellum 2 articulate. ... *Wandelia wairarapa* Barnard, 1972
   — Head not incised; flagellum of antenna 1 more than 2 articles 7
7. Uropod 1 outer ramus distinctly shorter than inner ramus; epimeral plate 3 posterior margin smooth, ... *Wandelia orghidani* Ortiz & Lalana, 1997
   — Uropod 1 outer ramus about subequal to inner ramus; crenulate posterior margin of epimeral plate 3 ..... *Wandelia dronga* (Myers, 1985)
8. Pereonite 1 with ventral cradle ..... 9 (*Bircenna*, 4 species)
   — Pereonite 1 lacking ventral cradle. 12

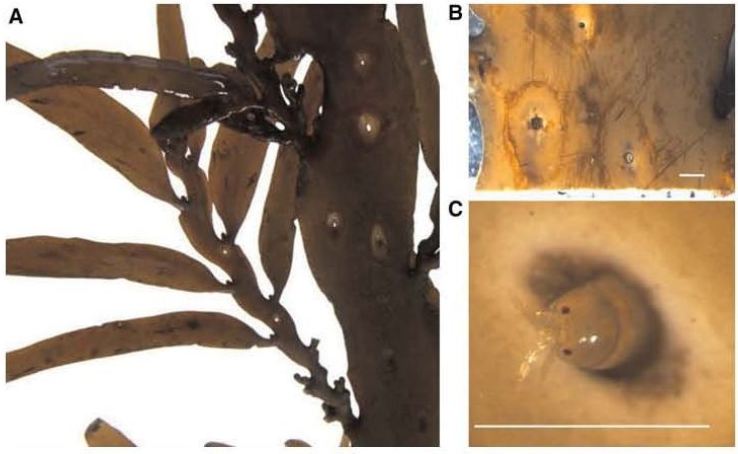

Fig. 4. (A) *Carpophylum maschalocarpum*; (B) thallus detail of *Carpophylum maschalocarpum* with holes burrowed by *Bircenna macayai* sp. nov. (C) *Bircenna macayai* sp. nov. within its hole. Scale bars: 1 mm.

9. Outer rami of uropods 1–2 reaching less than 75 per cent along inner rami, locking spine on pereopods 1–5 very small, parachela of gnathopods strong. . . . *Bircenna fulva* Chilton, 1884
   — Outer rami of uropods 1–2 reaching more than 75 per cent along inner rami, locking spine on pereopods 1–5 large, parachela of gnathopods weak. 10
10. Parchela of gnathopods quadrate, pereopod 7 length about equal to height of pereonite 7, antenna slender. . . . . *Bircenna nichollsi* Sheard, 1936
    — Parachela of gnathopods triangular, pereopod 7 length about twice height of pereonite 7, bilobed coxa 1. 11
11. Posterior margin of epimeron 3 and of pereopod 7 basis smooth, merus and carpus of pereopods 5–7 weakly extended posteriorly, pereopod 7 basis subequal in length and width . . . . . *Bircenna ignea* Nicholls, 1939
    — Merus and carpus of pereopods 5–7 strongly extended posteriorly, crenulate basis of pereopod 7 and smooth posterior margin of epimeron 3; pereopod 7 basis longer than wide. . . . . *Bircenna macayai* sp. nov.
12. Posterior lobe on articles 4–5 of pereopods 5–7 with 0-1 vestigal seta . . . . . 13 (*Ceinina*, 2 species)
    — Posterior lobe on articles 4–5 of pereopods 5–7 densely setose, setae elongate . . . . . (*Eophliantis*, 1 species) *Eophliantis tindalei* Sheard, 1936
13. Basis of pereopods 6 and 7 posteroventral corner enlarged, carpus of pereopods 6 and 7 posteriorly produced . . . . . *Ceinina japonica* Stephensen, 1933
    — Basis of pereopods 6 and 7 inflated, carpus of pereopods 6 and 7 not produced. . . . . *Ceinina latipes* Ledoyer, 1978

DISCUSSION

Taxonomy

We assume the Eophliantidae (excluding *Lignophliantis*) are a monophyletic line. Discussing evolutionary patterns in gammaridean amphipods, Barnard (1974) noted that the cylindroid Eophliantidae may be a monophyletic line based on a neotenous phliantid. Hatched juveniles of phliantids resemble eophliantids, but phliantids have special cuticular craters found also in ceinids and rudimentarily in hyalids, whereas eophliantids have apparently lost these structures. The eophliantid uropod 3 is slightly more complex than that of phliantids, the juveniles recapitulating their phylogeny. Two genera have been removed from the Eophliantidae by Barnard (1972): *Amphitholina* Ruffo 1953 (to the Ampithoidae) based on its biramous ampithoid uropod 3 and *Biancolina* Della Valle 1893 (to the Biancolinidae) based on the shape of the uropod 3 and the maxillipedal plates

the position of this genus. We have thus excluded the genus *Lignophliantis* in the following discussion regarding evolutionary trends in the Eophliantidae.

Barnard (1972) noted a general trend within eophliantid genera towards reduction of coxae in the sequence of *Wandelia* – *Bircenna* – *Eophliantis* – *Ceinina* – *Cylindryllioides*. Based on the body shape, after measuring the coxa height in relation to height of the corresponding pereonite we suggest that *Wandelia wairarapa* and *Wandelia dronga* are primitive species, wheras *Cylindryllioides kaikoura* and *Bircenna macayai* sp. nov. are highly developed species. While we therefore in general agree with Barnard's (1972) observations regarding evolutionary trends, based on the description of new species and additional data since 1972, we regard our new species of *Bircenna* as highly derived. Barnard (1972) also noted an evolutionary trend within eophliantid genera towards a loss or reduction of lacinia mobilis in the sequence of *Wandelia* to *Ceinina*, incision of head from *Bircenna* to *Ceinina*, loss of inner lobes on lower lip from *Wandelia* to the others.

Functional morphology

We agree with Barnard's (1972) suggestion that the evolutionary trend of the eophliantids is characterized by morphological adaptations for life in an algal habitat. The species benefit from three main morphological characters: (a) narrow, cylindrical body shape; (b) mouthparts combined with a strongly rotating head suitable for shaving algal tissue; and (c) reduced pleopods.

A cylindrical body shape confers distinct advantages to algae-boring arthropods, streamlining the body to enable the animal to fit neatly into and move easily within its tunnel. As such, this trait has also evolved convergently in other algal-dwelling taxa such as the amphipod *Biancolina* (previously considered to be an eophliantid until removed to its own family by Barnard, 1972), and in the wood- and kelp-boring limnoriid isopods (Kreibhom de Paternoster & Escofet, 1976).

The loss or reduction of the lacinia mobilis would seem to be a disadvantage to a burrowing organism, as the lacinia mobilis should double the rasping ability of the mandible; however, the strongly rotating head coupled with the flattening of the incisor may together be better for boring soft living tissues of algae (Barnard, 1972). Barnard (1972) noted that living plant tissues may actually have to be shaved rather than rasped and the buccal mass of *Wandelia wairarapa* (from New Zealand) appears well suited to that task; the mandibles project in such a way as to suggest a blade in a razor. Our preliminary examination of the mouthparts of the Eophliantidae revealed this to be true for all fourteen species.

The reduction of pleopodal peduncles from *Wandelia* to *Bircenna* and *Ceinina* with an aberrancy in *Cylindryllioides* suggests that the prototypical eophliantid may have had expanded peduncles and that narrowing those is the evolutionary trend (Barnard, 1972). Our initial morphological examination of the eophliantids agrees with Barn

from its congeners. However, since *C. mawsoni* is found on *Macrocystis* sp. (Table 1) and *B. fulva* is also found on *Macrocystis* sp., we assume that the genus *Cylindryllioides* does not have an algal habitat different from other members of Eophliantidae.

Ceinina is reported to penetrate the stem of the brown alga *Undaria pinnatifida* (Harv.) Suringar and the pleopodal paddles seem very solid, although the peduncle is not as expanded as in other eophliantids. *Ceinina* does retain a strong, sharply serrate lacinia mobili, has the thinnest body of eophlinatids, and generally the shortest legs and antennae, and is therefore well adapted as a tunneller. Perhaps the other species bore into more tender species of algae (Barnard, 1972). Unfortunately, most authors describing species of eophliantids do not name the species of the associated algae (Table 1). Nevertheless two species bearing very differently shaped pleopods (*Lignophliantis pyrifera* and *Bircenna fulva*) are reported from the same species of algae: *Macrocystis pyrifera*. *Cylindryllioides mawsoni*, bearing very narrow pleopodal peduncles, also lives on *M. pyrifera* (in the original publication this alga was reported as *M. laevis*).

Biogeography

Currently our knowledge about the biogeography of the eophliantids is so speculative, that it is only briefly discussed in the following. A more substantial biogeography of the Eophliantidae will follow a detailed phylogenetic analysis which is presently in preparation.

All species of Eophliantidae are found in shallow waters, from 0–4 m (see Table 1), except *Bircenna fulva* (0–25 m) and *Wandelia crassipes* (1–126 m). Since the reported depth below 10 m often refers to stations sampled by dredges (e.g. Chevreux, 1906; De Broyer *et al.*, 2007) and this gear has no closing mechanism, thus also sampling the water column, it is very likely that the eophliantids were taken on pieces of algae close to the surface. All eophliantids are algal dwellers,

Table 1. Record of distribution and host algae of the Eophliantidae.

Species	Author	Type locality	Other known areas	Host algae	Water depth
Lignophliantis pyrifera	J.L. Barnard, 1969	Goleta, California		Wash of rhizomes of *Macrocystis pyrifera*	3 m
Cylindryllioides kaikoura	Barnard, 1972	Morgan's Pool, Kaikoura, New Zealand		Not given	0–2 m
Cylindryllioides mawsoni	Nicholls, 1938	Macquarie Island	Kerguelen, Îles Crozet, Marion Island	*Macrocystis* sp., *Durvillia antarctica, Desmarestia rossi, Durvillaea* sp.	0–2 m
Wandelia crassipes	Chevreux, 1906	Wilhelm Archipelago, Antarctic peninsula	Palmer Archipelago, South Orkney Islands, South Shetland Islands, Tristan de Cunha	'avec des eponges, dragage'	1–126 m
Wandelia wairarapa	Barnard, 1972	Wellington, New Zealand		'intertidal wash of algae'	0–4 m
Wandelia orghidani	Ortiz & Lalana, 1997	Bunaken Island, Indonesia		Not given	2–2.5 m
Wandelia dronga	(Myers, 1985)	Makuluva Island, Fiji		'mixed red algae from leeward side of reef flat'	0–3 m
Bircenna fulva	Chilton, 1884	Lyttelton Harbour, New Zealand	Australia, Argentina (Chubut and the Magellan area, Southern Tierra del Fuego), Chile	*Macrocystis pyrifera*	0–25 m
Bircenna nichollsi	Sheard, 1936	Gulf St Vincent, South Australia		'among algae growing on the film of sand covering rocks below low tide mark'	~2 m
Bircenna ignea	Nicholls, 1939	Shelly Beach, south-western Australia		'among fine seaweed and sand'	0 m?
Bircenna macayai sp. nov.	Lörz, Kilgallen & Thiel (this publication)	Kau Bay, Wellington, New Zealand		*Carpophyllum maschalocarpum*	2 m
Eophliantis tindalei	Sheard, 1936	Port Wynyard, Tasmania		'in fine sand and algae, on tidal rocks, periodically flooded with river water'	0 m
Ceinina japonica	Stephensen, 1933	Yoichi, Hokkaido, Japan		Stem of *Undaria pinnatifida*	0 m?
Ceinina latipes	Ledoyer, 1978	Recif de Balaclava, Mauritius		Zone of *Melobesia* (Corallines)	0–5 m

even though not all authors mention the associated algae (Table 1).

There are currently two main mechanisms hypothesized for long-range biotic distributions in the southern hemisphere; vicariance due to continental drift and rafting dispersal via the West Wind Drift (Waters, 2008). Numerous studies have remarked on biogeographical connections among biota from regions linked by the West Wind Drift (Thiel & Haye, 2006). For example, recent molecular studies have identified biogeographical links between South Africa, Tasmania and New Zealand (e.g. Waters & Roy, 2004). An extensive literature search has revealed five algae identified to species level that are associated with eophliantids (Table 1). *Ceinina japonica* is described from the stem of *Undaria pinnatifida* in Yoichi, Hokkaido (Stephensen, 1933). *Cylindryllioides mawsoni* has been found on Macquarie Island, Kerguelen, Crozet Island and Marion Island on *Macrocystis pyrifera* (reported as *M. laevis*) and *Durvillaea antarctica* (e.g. Beckley & Branch, 1992). *Macrocystis pyrifera* is also the habitat of *Lignophliantis pyrifera* from California (Barnard, 1972), while *Bircenna fulva* is known from *M. pyrifera* off Argentina (Kreibohm de Paternoster & Escofet, 1976; Alonso, 1980), off Chile (Martin Thiel, personal observation), and off New Zealand (Chilton, 1884). Thus, due to the algal-dwelling habitat of these animals, we assume that they are ideal candidates for dispersal via rafting.

Biotic connections between New Zealand and South America (e.g. Donald *et al.*, 2005; Fraser *et al.*, 2009) are often discussed controversially (e.g. IIeads, 2005). Studies on the alpha-diversity of peracarids from New Zealand and Chile have reported several peracarid species that are apparently common to both regions (e.g. Barnard, 1972; Thiel, 2002; Gonzalez *et al.*, 2008). However, there is a high possibility that these 'bi-regional' species such as *Bircenna fulva* are mis-identifications and/or represent (several) cryptic species but at the generic level the connections may be significant. If algal rafting was the main explanation for the biogeography of the Eophliantidae family, the same species should be found in, e.g. Chile, Australia and New Zealand. The current generic level connectivity may support a vicariant biogeography because similarities at generic level indicate an archaic relationship.

The highest eophliantid species diversity, four of the 14 known species, occurs in New Zealand. These represent three of the six genera. Another species is found on Macquarie Island, south of New Zealand, and a further three species in southern Australia (including Tasmania) (Figure 5). At this point, we therefore speculate that Australasia is the evolutionary origin of this amphipod family, and species have radiated to Antarctica, Chile, Mauritius and Japan from here. Again, without a detailed phylogenetic analysis of the family, this biogeographical hypothesis cannot be confirmed.

Summary and outlook

Very little is known about the systematics and evolution of obligate algae dwelling amphipods. This initial study, focusing on the taxonomy, has highlighted that the family

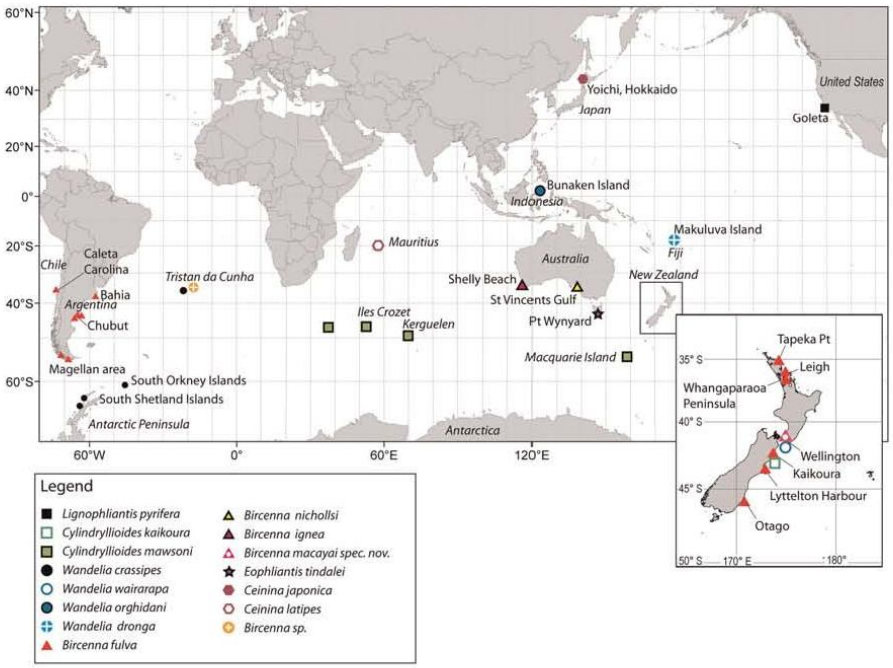

Fig. 5. Worldwide distribution of the species of Eophliantidae (Amphipoda).

Eophliantidae is a suitable model group to study the phylogeography of obligate algal dwellers.

We currently postulate Australasia as the evolutionary centre of the Eophliantidae, having four of the six currently known genera. Both species with pututatively plesiomorphic and derived characters are found in Australasia. Based on the ecology of the algae dwelling Eophliantidae, their biogeographical distribution and that of their host algae, we believe rafting on macroalgae, e.g. on *Macrocystis* sp., is a viable distributional mechanism for this amphipod family. At present, the observed distributions could be consistent with vicariant or dispersalist mechanisms. A detailed phylogenetic analysis of the Eophliantidae will be conducted in a following paper to help clarify this issue.

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