Logan Couraud

Giant kelp forests of the Falkland Islands - a metapopulation structured by past historical colonization events and by present habitat continuity and oceanographic transportation

UNIVERSIDADE DO ALGARVE

Faculdade de Ciências e Tecnologia

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Mestrado em Biologia Marinha

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Declaração de autoria de trabalho

Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

_____ Logan Couraud

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Abstract

The genetic structure of *Macrocystis pyrifera* around the Falkland Islands and the Magallean region was determined. In fact, genetic discontinuities related to biogeographic breaks and the role of predictors such as habitat continuity, dispersal, oceanographic currents, and bathymetry were assessed to understand the metapopulation's structure around the Falklands. In total, 9 microsatellites and one mitochondrial marker were used to genotyped 433 individuals from 22 different populations. Nuclear DNA and Mitochondrial DNA analysis were carried out to comprehend the effects of historical and contemporary effects on species distribution and range shifts. At a large scale, the analysis shows that *Macrocystis pyrifera* is subdivided into four main genetic clusters, one in the Magallean region, two in the Falkland Islands, and one in South Africa. Furthermore, populations in the Magallean region have displayed a low genetic diversity that can be linked to recent colonization events while around the Falklands a higher genetic diversity have been found that may reflect historical events. The role played by the Falkland Islands as a refugia for this species is well supported by the phylogeographic structure. Genetic analysis revealed that the distribution around the Falklands has been shaped by contemporary and historical events. Multiple genetic breaks have been observed and are concordant with biogeographic and oceanographic breaks. The present genetic structure can be best explained by transportation through ocean currents and habitat continuity for stepping-stone migration rather than by geographical distance. Kelps are under increasing anthropogenic pressure and environmental changes and unlike most other marine species they can be easily monitored. Thus, further studies need to be carried out to highlight more biogeographic and phylogeographic breaks at a global scale to understand the ecological and evolutionary processes that shape the distribution of kelps. In consequence, it will provide more information for management and conservation policies to be taken.

Keywords: *Macrocystis pyrifera*, connectivity, genetic structure, phylogeography, LGM

Resumo

Foi determinada a estrutura genética de *Macrocystis pyrifera* em torno das Ilhas Malvinas e da região de Magalhães. *M. pyrifera*, comummente chamada de alga gigante, forma extensas florestas submarinas e como a alga mais amplamente distribuída na terra. Os indivíduos podem crescer até 40 a 50 m de comprimento e é descrito como o organismo bentónico mais alto. De facto, a *M.pyrifera* é agora definida como uma espécie de fundação, na medida em que desempenha um forte papel na estruturação da comunidade, criando uma estrutura espacial complexa em 3D, influenciando as condições físicas e químicas, e a complexidade dos processos dos ecossistemas. *M. pyrifera* é também considerada como um engenheiro de ecossistemas na medida em que fornece alimento, habitat e substrato de desova a numerosas espécies, tais como mamíferos, peixes, caranguejos, ouriços do mar, moluscos, epífitos e comunidades bacterianas. Através deste complexo ecossistema estruturado, as florestas de algas não só ampliam a produção secundária e primária, como também apoiam uma diversificada teia alimentar costeira. *Macrocystis pyrifera* está distribuída ao longo de uma gama latitudinal e longitudinal muito ampla, do Alasca ao México no hemisfério norte, e ao longo da costa sudeste da América do Sul, do Peru à Argentina, mas também, em locais dispersos como a África do Sul, Tasmânia, e várias regiões sub-Antárcticas. Esta ampla gama distributiva mostra a eficiência da aclimatação de *Macrocystis* para lidar com tal painel de factores ambientais como, temperatura, salinidade, profundidade, luz, nutrientes, e exposição a ondas. Os atributos biológicos, morfologia interna e externa, e traços de história de vida são particularmente relevantes para a ecologia das algas, a fim de compreender como os *Macrocystis* interagem e lidam com o seu ambiente. A está entre as algas marinhas castanhas mais desenvolvidas, mostrando uma morfologia interna e externa muito complexa na qual cada parte da planta tem de ser capaz de funcionar e lidar com o ambiente que pode ser extremamente variável ao longo do tempo e da profundidade. Os factores bióticos limitam a capacidade de dispersão desta espécie. As literaturas sugerem que a capacidade de dispersão a longa distância é restrita e raramente eficiente. Contudo, estudos recentes têm demonstrado o valioso papel das balsas de algas e dos adultos flutuantes em termos de dispersão a longa distância. Além disso, as jangadas flutuantes demonstraram não só uma elevada capacidade de sobrevivência, aproximadamente 100 dias para alguns indivíduos, mas também o potencial de percorrer centenas de quilómetros enquanto continuam a ser férteis. A alga gigante habita

manchas rochosas e tem uma capacidade de dispersão limitada, mas um elevado potencial de dispersão. Parece que a dispersão de longas distâncias por jangadas de algas permite uma fixação eficiente em novo habitat. Assim, pensa-se que a conectividade entre as populações é principalmente impulsionada pelas correntes oceânicas. As Ilhas Malvinas, também chamadas Islas Malvinas, estão localizadas no hemisfério sul, na faixa de vento ocidental na zona temperada a frio sul e situam-se a norte da frente polar, 450 km a nordeste da Terra do Fogo entre a latitude (51-53°S) e longitude (57-61°W) no Atlântico Sul. As Malvinas fornecem um habitat de reprodução essencial para muitas aves marinhas, mamíferos marinhos e espécies de aves costeiras, podendo assim ser caracterizadas como um importante ponto de atracção da biodiversidade. Alguns estudos identificaram 57 espécies de aves marinhas, incluindo 17 espécies de pinguins e 17 espécies de mamíferos marinhos, contudo, para a maioria das espécies foi observada uma variação interanual. A plataforma patagónica de águas pouco profundas produz com as duas correntes uma região de afloramento, o que significa uma água muito rica em nutrientes na origem de um copioso local de alimentação. Foram avaliadas descontinuidades genéticas relacionadas com quebras biogeográficas e o papel de preditores como a continuidade do habitat, dispersão, correntes oceanográficas e batimetria, para compreender a estrutura da metapopulação em redor das Malvinas. No total, 9 microsatélites e um marcador mitocondrial foram utilizados para genotipular 433 indivíduos de 22 populações diferentes. As populações de *Macrocystis pyrifera* foram amostradas de 2018 a 2019 a partir de cinco sítios na região de Magallean, localizados na Patagónia chilena, de um sítio na África do Sul e de 16 sítios em redor das Ilhas Malvinas (latitude -51,563412 e longitude -59,820557). Foram realizadas análises de ADN nuclear e de ADN mitocondrial para compreender os efeitos dos efeitos históricos e contemporâneos na distribuição das espécies e nas mudanças de distribuição. Em grande escala, a análise mostra que *Macrocystis pyrifera* está subdividida em quatro grupos genéticos principais, um na região de Magalhães, dois nas Ilhas Malvinas, e um na África do Sul. Além disso, as populações na região de Magallean demonstraram uma baixa diversidade genética que pode ser ligada aos recentes eventos de colonização, enquanto que em redor das Malvinas foi encontrada uma maior diversidade genética que pode reflectir eventos históricos. O papel desempenhado pelas Ilhas Malvinas como refúgio para esta espécie é bem suportado pela estrutura filogeográfica. A análise genética revelou que a distribuição em torno das Malvinas foi moldada por acontecimentos contemporâneos e históricos. Múltiplas quebras genéticas foram observadas e são concordantes com quebras biogeográficas e

oceanográficas. A estrutura genética actual pode ser melhor explicada pelo transporte através das correntes oceânicas e pela continuidade do habitat para a migração das estepes, e não pela distância geográfica. As algas estão sob pressão antropogénica crescente e alterações ambientais e, ao contrário da maioria das outras espécies marinhas, podem ser facilmente monitorizadas. A alga gigante é um bom modelo para explorar os efeitos da distribuição espacial no padrão de conectividade devido à sua vasta gama de tamanhos de manchas, distribuição e distâncias que as separam. Os padrões espaciais de diversidade genética são essenciais para determinar os mecanismos evolutivos que conduzem a esta diferenciação genética, tanto quanto para compreender as consequências ecológicas da perda da diversidade genética. A *Macrocystis* é uma das algas marinhas mais importantes ecológica e economicamente do hemisfério sul. As políticas de gestão e conservação devem ser aplicadas para conservar as florestas de *M. pyrifera* e espécies ameaçadas, na medida em que aumentará a biodiversidade. Assim, é necessário realizar mais estudos para destacar mais quebras biogeográficas e filogeográficas à escala global, a fim de compreender os processos ecológicos e evolutivos que moldam a distribuição das algas. Em consequência, fornecerá mais informação para as políticas de gestão e conservação a serem tomadas

Palavras-chave: *Macrocystis pyrifera*, conectividade, estrutura genética, filogeografia, LGM

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Abbreviations

DNA	Deoxyribonucleic acid
POM	Particulate Organic Matter
POC	Particulate Organic Carbon
LGM	Last Glacial Maximum
PCR	Polymorphism Chain Reaction
Tm	Melting Temperature
ENSO	El Nino Southern Oscillation

I. Introduction

Circum-Antarctic islands of the Southern Ocean and adjacent continental coastlines form a unique oceanographic system in the world, interconnected by strong oceanographic currents, but with varying degrees of exposure to past climatic variations in sea ice and sea level. Among these, one of the most striking examples are the Falkland Islands, which during the last glacial maximum were not covered by permanent ice and were connected to South America due to low sea level. Thus, these are expected to have long-term persistent populations with longer evolutionary time, possibly reflected in high genetic diversity for marine populations such as the important marine forests of giant kelp, *Macrocystis pyrifera*. The role of predictors such as habitat continuity, dispersal, and spatial distance will be evaluated to assess the genetic structure of giant kelp populations along its broad distribution range, from the southern coast of Chile to New Zealand, passing by Australia, sub-Antarctic regions and more specifically around the Falkland Islands. These populations are mainly distributed along non-continuous habitats, such as islands, which are expected to display variable levels of connectivity between sites. Recent literature suggests that marine forests connectivity might be explained by oceanographic currents and habitat continuity and/or by past events of one or several periodical colonization histories. This thesis will examine the genetic pattern of the Falkland Island and the Chilean Patagonia to highlight genetic breaks associated with habitat discontinuity but also to understand the effect of contemporary and historical events that have shaped the genetic patrimony of the giant kelp *Macrocystis*. Contemporary climatic events may be at the origin of the low genetic diversity observed in northern Chile according to the literature, whereas populations in the southern regions may have been affected by past historical events such as the last glacial maximum (LGM) which can explain the high genetic structure (**Macaya & Zuccarello, 2010**).

The use of microsatellites as a tool to understand the population genetics of a species has revolutionized the field of conservation biology (**Ellegren, 2004; DeSalle and Amato, 2004**).

These repetitive sequences undergo higher mutation rates than other part in the DNA in which repeat units are added or subtracted, meaning they are highly polymorphic among individuals. They provide excellent resolution for assessing intraspecific genetic variability and differentiation.

Moreover, mitochondrial marker has been suggested to be a good marker for phylogeographic analysis on brown seaweed (**Engel et al., 2008**).

Kelps have a huge economic and ecological importance for coastal ecosystems in which it provides essential resources for the food web but also for coastal people that rely on. In effect, genetic structure and diversity will help understand ecological and evolutionary processes that have shaped the distribution of *Macrocystis pyrifera* around the Falkland Islands.
The results of this study will provide information on the genetic diversity pattern of *M. pyrifera* around the Falkland Island to highlight phylogeographic breaks and understand the role played by contemporary and historical events in structuring metapopulations.

1.1 The study species

Brown algae *Ochrophyta*, is a group of photosynthetic heterokonts characterized by their accessory photosynthetic pigments called fucoxanthin (**Schiel et Foster., 2015**), class *Phaeophyceae*, order *Laminariales*, family *Laminariaceae,* and monospecific genus *Macrocystis* (**Agardh., 1820**). Macrocystis species have undergone multiple taxonomic rearrangement since its first description by Linnaeus in 1771. At first, four distinct species of Macrocystis were initially described: *M. pyrifera*, *M. integrifolia*, *M. angustifolia*, and *M. laevis* based on the holdfast and blade morphologies (**Guiry & Guiry., 2009**). However, due to the high polymorphic nature of this genus, four distinct species of *Macrocystis* are yet unjustifiable. Then, new molecular taxonomic work on rDNA internal transcribed spacer region carried out by **Coyer et al. (2001),** breeding, and DNA barcoding researches have brought the evidence that the three species are ecotypes of *M. pyrifera* (**Graham et al., 2007**), and indicate that *Macrocystis* is a monospecific genus **(Astorga et al., 2012**).

Multiple hypothesis was set concerning the origin and spread of Macrocystis based on the number of species in the genus, its distribution, fossil evidence, and morphological traits. Early literature has suggested a southern hemisphere origin followed by northward expansion (**Parker and Dawson., 1965**) whereas **Nicholson (1979)** has favored a northern origin with a southward expansion based on kelp genera diversity and fossil evidence. Recent studies, using genetic analysis have not only highlighted high genetic similarity in the southern hemisphere populations, potentially due to gene flow or recent dispersal, but also a greater diversity of sequences in individuals inhabiting the northern hemisphere (**Coyer et al., 2001**; **Macaya and**

Zuccarello., 2010b). These two evidences combined have strengthened the idea of a northern origin.

1.2 General background

M. pyrifera, commonly called the giant kelp, form extensive submarine forests and as the most widely distributed kelp on earth. Individuals can grow up to 40 to 50 m long (**Macaya & Zuccarello., 2010**) and is described as the tallest benthic organism (**Steneck et al., 2002**). They grow on rocky substrate between the low intertidal zone and up to 50 m depth.

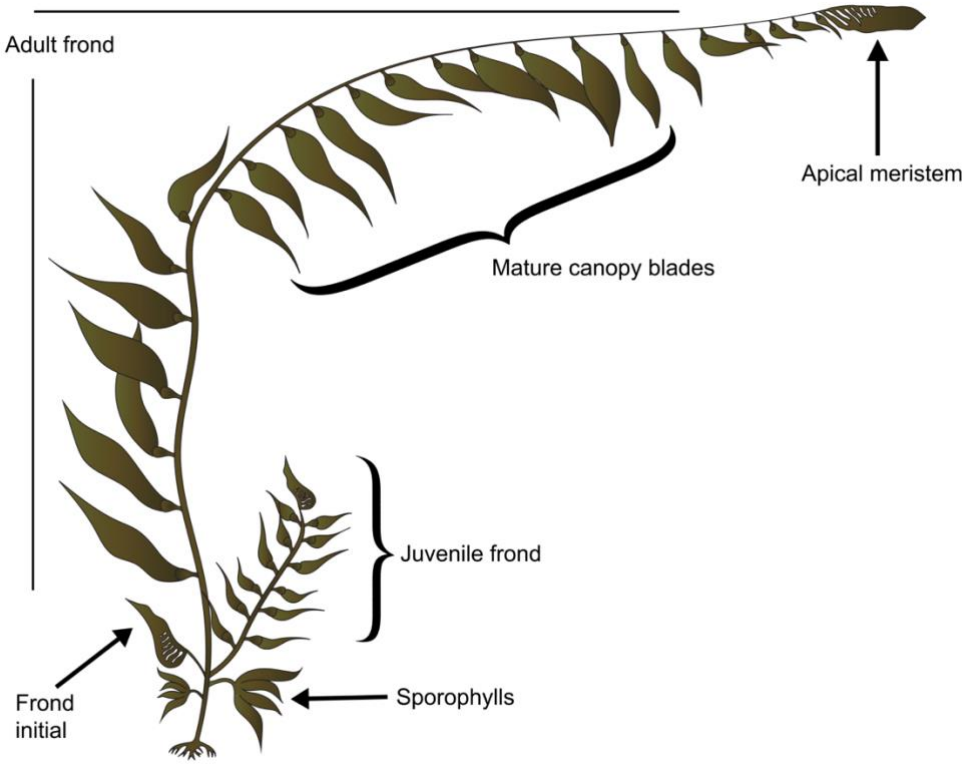

Figure1. 1 Morphological features of Macrocystis pyrifera

Macrocystis pyrifera, sustains one of the most productive, dynamic, and diverse ecosystems on earth (**Macaya and Erasmo., 2010**). Kelp ecosystems are energy rich and play an important role in influencing and controlling coastal processes, sedimentation, recruitment, erosion, water flow, primary and secondary production (**Steneck et al., 2002**). *M. pyrifera* is considered as an ecosystem engineer (**Coleman and Williams., 2002**) in the extent it provides food,

habitat, and spawning substrate for numerous species such as mammals, fishes, crabs, sea urchins, molluscs, epiphytes, and bacterial communities. Through this complex structured ecosystem, kelp forests not only magnify secondary and primary production but also support a diversified coastal food web (**Mann 2000**).

The special architecture formed by the holdfast and the blades support a complex food web in which 43 to 114 taxa are associated to the Giant kelp (**Rios et al., 2007**). Most of them are detrital feeders and feed on detritus in the form of sedimented particulate organic matter as the primary source of food (**Schaal et al., 2012**). Environmental factors plus natural senescence can trigger the release of an enormous quantity of kelp detritus through the water column (**Hobday 2000b**). **KrumHansl and Scheibling (2012)** have shown that 80% of the productivity finishes as detached detritus. However, due to its size and complex morphology, productivity is very difficult to estimate. Kelp are extremely productive but also highly vulnerable to detachment from winds and currents creating rafts of giant kelp. They are colonized by a multitude of species going from pelagic invertebrates to larval, juvenile and adult fishes and thus contribute to the transport to nearshore habitat. *Macrocystis* forests increase recruitment efficiency of juvenile fishes by providing shelters and nursery areas (**Macaya and Erasmo., 2010**). Moreover, associated species with drifting rafts are a source of food for higher trophic level predators (**Schiel and Foster., 2015**). Decomposition and senescence produce particulate organic matter (POM) that is essential for suspension feeders inhabiting the kelp forest. **Miller and Page (2012)**, thanks to carbon isotope composition analysis pointed out that nearly 70% of POC eaten by suspension feeders are directly from kelp. In addition to its ecological value, *M. pyrifera* has an economical interest for the use of alginates, to feed abalone, seafood consumption, and organic fertilizer so the kelp demand continues to increase rapidly (**Vasquez 2008; Graham et al., 2007**).

Macrocystis is one of the most ecologically and economically important seaweeds of the southern hemisphere.

In fact, *M.pyrifera* is now defined as a foundation species, in the extent it plays a strong role in structuring the community, by creating a complex 3D spatial structure, influencing physical and chemical conditions, and ecosystem processes complexity (**Dayton 1972; van der Zee et al., 2016**) such as sand beach, hydrodynamics, and biochemistry (**Miller et al., 2018**). Kelp forests enhance species richness and food-web (**Baiser et al., 2013**)

Thus, *M. pyrifera* is ecologically and economically valuable for the entire food web, so management strategies for conservation should be designed.

Commercially kelp harvesting, plant competition due to habitat shrinking, grazing by fish, sea urchins, storms, El Niño event, the pacific decadal oscillation, and pollution strongly influenced the productivity and abundance of this species. For instance, natural and periodic event such as El Niño Southern Oscillation can cause a decline or a bottleneck and therefore trigger the loss of genetic variation for the next generations. The main threat of concern is the overfishing of coastal ecosystem by which apex predators are removed (**Jackson et al., 2001**) and triggered the increase of kelp-grazing herbivore that promotes kelp forests decline (**Steneck et al., 2002**). Industrial uses and the increase demand of kelp biomass in the form of alginate intensify the exploitation pressure of *Macrocystis* and therefore jeopardise the sustainable management of natural populations (**Camus et al., 2018**).
Management and conservation policies to restore biodiversity and conserve threatened species, should take into account connectivity of these essential habitat-forming populations.

Figure1. 2 Picture taken in the Beagle channel (Patagonia) showing the complex structure formed by the canopy of *Macrocystis pyrifera*

1.3 Distribution

Macrocystis pyrifera is distributed along a very broad latitudinal and longitudinal range, from Alaska to Mexico in the northern hemisphere, and along the southeast coast of south America from Peru to Argentina but also, in scattered locations such as South Africa, Tasmania, and several sub-Antarctic regions.

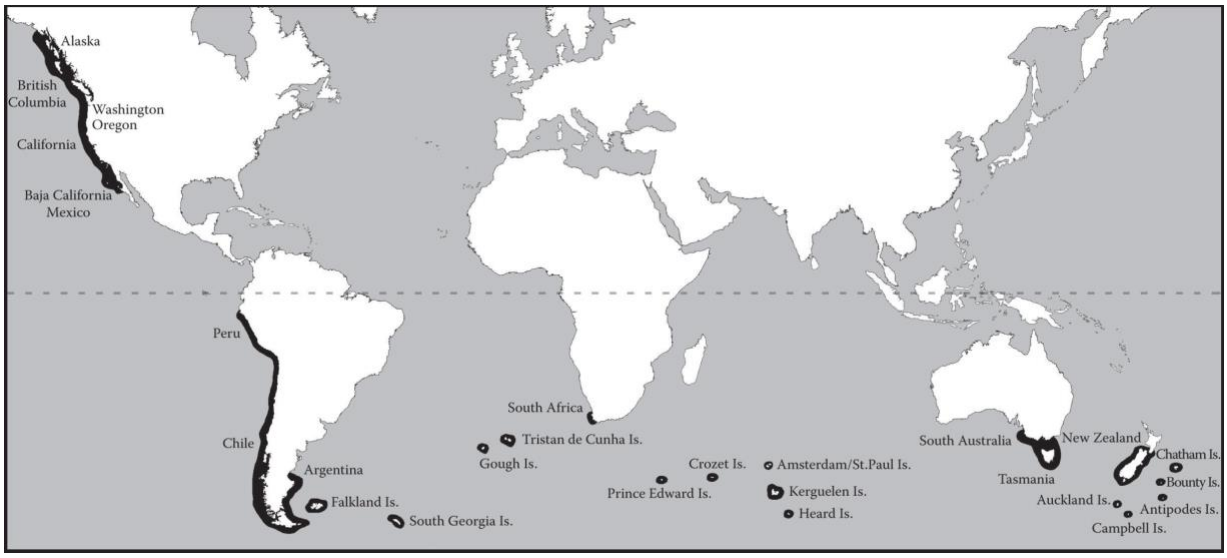

Figure1. 3 Worldwide distribution of *Macrocystis pyrifera* (**Graham et al., 2007**)

This wide distributional range shows the acclimatization efficiency of *Macrocystis* to cope with such panel of environmental factors like, temperature, salinity, depth, light, nutrient, and wave exposure (**Buschmann et al., 2014**). *Macrocystis* species are very sensitive to biotic and abiotic factors for their growth, dispersal, and reproduction. In fact, climate change, for instance recent glaciation and deglaciation period have produced major range shifts in term of distribution and abundance but also have shaped the phylogeographic structure of the species (**Assis et al., 2018**; **Assis et al., 2016; Neiva et al., 2018**). Moreover, footprints on the genetic patrimony and evolutionary traits of populations arose through climate driven range shift. Thus, differentiation occurs by the accumulation of mutations in the different populations, creating a unique gene pool (**Assis et *al*., 2014**)

Range contraction might have reduced genetic diversity due to bottleneck and/or genetic drift (**Leimu et al., 2006**) as well as range expansion can erode genetic diversity due to "Founder effect" at the leading edge (**Assis et al., 2014**)

Biotic factors limit the dispersal capacity of this species (**Assis et al., 2016**) and it directly affect the distribution of kelp forests. Moreover, they are physiologically strained and controlled by light (increased desiccation, high ultraviolet and photosynthetically active radiation), and nutrient at high latitude and temperature at low latitude. Macrocystis pyrifera have shown evidence of high specialization for different environmental gradients which may have driven divergent selection among populations.

1.4 Life cycle

Biological attributes, inner and extern morphology, and life history traits are particularly relevant for the ecology of kelp in order to comprehend how *Macrocystis* interact and cope with its environment. Macrocystis is among the most highly developed of large brown seaweeds, showing a very complex internal and external morphology in which each plant parts have to be able to function and cope in the environment that can be extremely variable over time and depth.

The giant kelp exhibits a complex biphasic life cycle (**Figure 4**) (**Graham et al., 2007**) during which microscopic zoospores and macroscopic sporophytes are produced (**Macaya and Erasmo., 2010; Schiel and Foster., 2006**) and disperse through the water column by ocean currents. From the release of zoospores (1N) by the sporophylls, through the development into gametophytes (1N) to the fertilization into sporophytes (2N), the giant kelp undergoes an alternation of generations that occur over several weeks. Thus, it requires an understanding of both of these life stages to bring out its ecological relationship because they behave like two different individuals in term of structure, development and requirements **(Schiel and Foster., 2015).** Moreover, fertilization in M. pyrifera occurs after dispersal **(Alberto et al., 2010)**

Biotic factors limit the dispersal capacity of this species (**Assis et al., 2016**). Dayton (1972) suggests that long-distance dispersal capacity is restricted and rarely efficient. However, recent studies have shown the valuable role of kelp rafts and floating adults in term of long-distance dispersal. Moreover, drifting raft have not only shown a high survival capacity, approximately 100 days for some individuals (**Hobday 2000a**), but also the potential to travel hundreds of kilometers while continuing to be fertile **(Macaya et al., 2005; Hernandez-Carmona et al., 2006.**

In effect, this allows to understand biogeographic expansion and genetic exchange among populations (**Reed et al., 2006**)

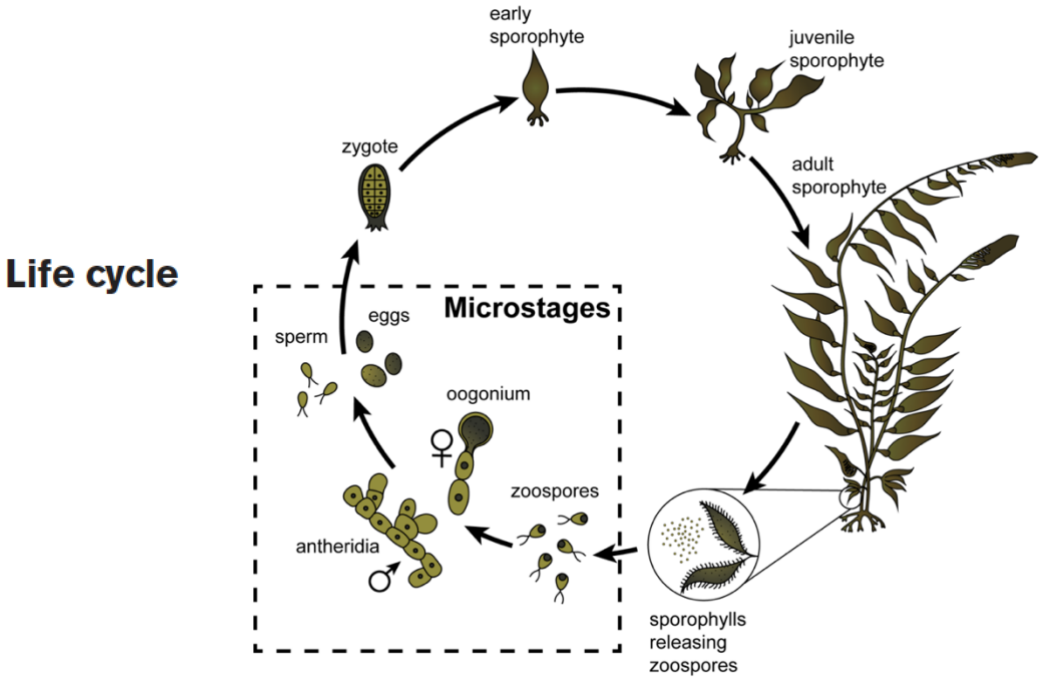

Figure1. 4. Biphasic life cycle of *Macrocystis pyrifera*. Blue part is the diploid phase following the gametic fusion triggered by environmental factors. Whereas the pale green correspond to the haploid phase during which zoospores are released and develop into gametophytes (**Macaya 2010**)

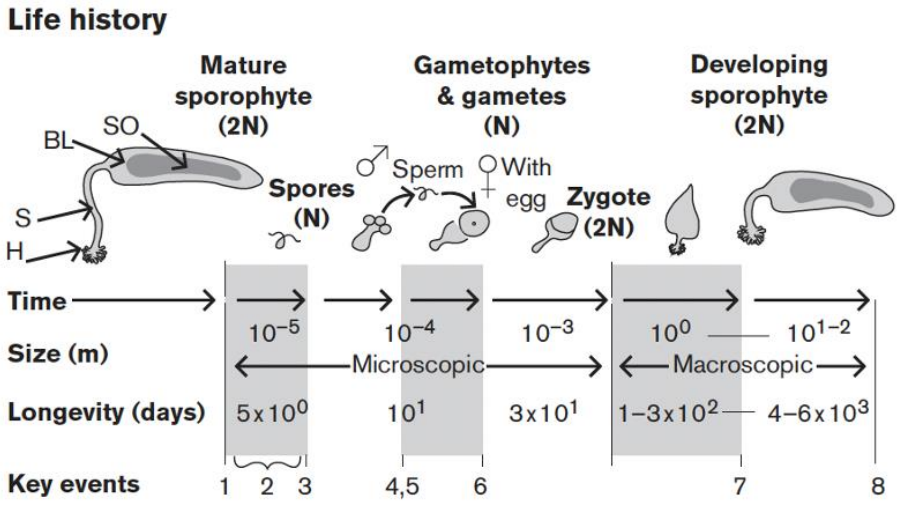

Figure1. 5 Life history representing the main stages of development, Time, Size (m), Longevity (Days), and Key events. 1: spore production; 2: spore dispersal; 3: spore settlement; 4: gamete production; 5: sperm dispersal; 6: fertilization; 7: sporangia development; 8: sporophyte / adult plant mortality; 9: sperm and egg dispersal; 10: egg settlement; 11: zygote settlement; 12: zygote dispersal; 13: development of gametangia (**Schiel and Foster., 2006**)

1.5 Population genetic connectivity of *Macrocystis*

Although the giant kelp is probably one of the most studied macroalgae in the world and much is known about its ecology, there is still limited understanding of the patterns and levels of connectivity along its distribution. Many demographic characteristics such as settlement, growth, mortality, and reproduction are key components to understand dispersal and thus connectivity among metapopulations. The giant kelp essentially inhabits rocky substrata that form patches varying in term of size and spatial distributions (**Schiel and Foster., 2015**). Thus, affecting the connectivity and dispersal efficiency through time and space. The concept of metapopulation suggests an occasional connectivity in between patches of different populations through outbreeding, inbreeding, and for re-establishment if major storm events have led population to extinction (**Schiel and Foster., 2015**).

Furthermore, living in a very dynamic environment with harsh water motion make the giant kelp more exposed to complete or partial removal by storm for instance, but in fact these environmental conditions have morphologically and genetically shaped and reinforced its ability to persist and replenish populations. A multitude of factors are influencing the effectiveness of recruitment, settlement, and renewal by regulating spore dispersal, gametogenesis, fertilization, and sporophyte growth.

Coyer et al (2001) have used noncoding rDNA internal transcribed spacer regions (ITS1 and ITS2) to provide insight of the evolution and relatedness of *Macrocystis* population in both hemispheres. Genetic results have shown more diversity in the Northern hemisphere with paraphyletic clades than in the Southern hemisphere with less diversity and monophyletic clades. This indicate that dispersal and colonization started from North to South. In addition, **Macaya and Zuccarello (2010b**) have explored the genetic structure of the giant kelp across the south Pacific region and they observed shared genotypes among southern Chile and other regions in the SE Pacific. In this study, metapopulation of the Falklands were genetically examined and the data suggest an admixture of multiple genetic types from distinct spatial sources and/or from distinct events of colonization taking place at different times. The study of **Fraser et al (2009**) on *Durvillaea antarctica* suggests that the Patagonian ice sheet present during the Last Glacial Maximum (LGM) and the Antarctic Circumpolar Current have facilitated several colonization.

1.6 The region under study: Falkland Islands

The Falkland Islands, also called Islas Malvinas, are located in the Southern hemisphere westerly wind belt in the southern cold-temperate zone and lie north of the polar front (**Scaife et al., 2019**), 450 km north east of Tierra Del Fuego between latitude (51-53°S) and longitude (57-61°W) in the South Atlantic (**Greenway 1972; Rosenfield et al., 2014**). The archipelagos are composed of 780 islands aggregated around two principal islands, the West Falkland of 3.500 km^2 and the East Falkland of 5.000 km^2 (**Greenway 1972**). Two branches of the Antarctic Circumpolar Current surround the Falkland Islands: the Patagonian Current to the west and the Falkland (Malvinas) Current to the east (**White et al., 2002)**

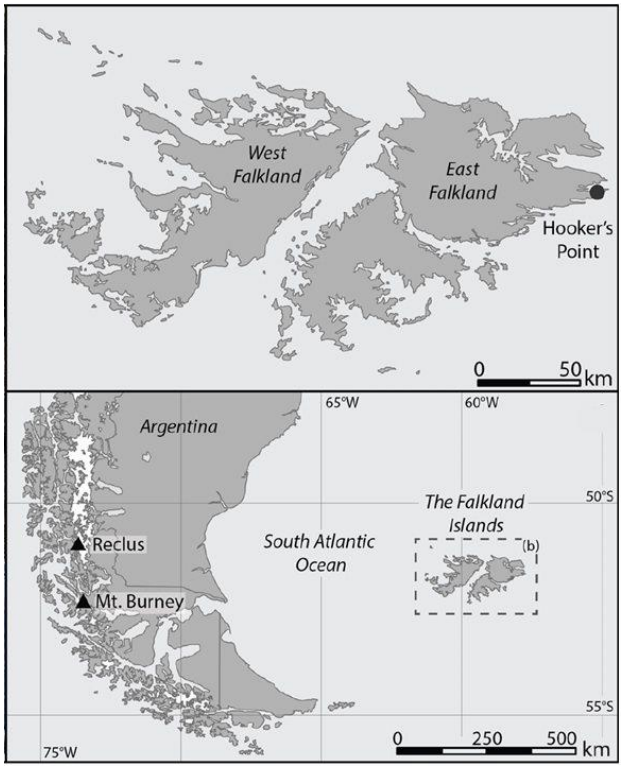

Figure1. 6. (Top) Picture of the Falkland Islands showing the two main Islands (West and East Falkland). (Down) Latitudinal and longitudinal position of the Falkland Islands in relation to the South America (**Scaife et al., 2019**)

The Falklands provide an essential breeding habitat for many seabirds, marine mammals and shorebirds species and thus can be characterized as an important biodiversity hotspot **(Groff 2018)**. **White et al in 2002** have identified 57 species of seabirds including 17 species of penguins and 17 species of marine mammals, however for most species an inter-annual variation has been observed. The shallow water Patagonian shelf produces with the two currents an upwelling region, meaning a very nutrient-rich water at the origin of a copious feeding ground (**White et al., 2002**).

In the Falkland Islands, *M. pyrifera* forests play a key role as habitat or spawning substrate for numerous taxa but more specifically as nursery for the Patagonian squid *Doryteuthis gahi* which is, according to **Rosenfield, 2014** the only squid species in the Falkland Island that use

kelp to attach their egg masses. Moreover, due to its structural complexity, kelp will positively affect local biodiversity by affecting water motion and light intensity through the water column. The Patagonian squid is the main marine food resource that sustains not only valuable fisheries activities but also high trophic level predators, including endangered seabirds and marine mammals that feed mainly on this species. *Macrocystis* is one of the most ecologically and economically important seaweeds of the southern hemisphere. Management and conservation policies should be applied to conserve *M. pyrifera* forests and threatened species in the extent it will increase biodiversity in the region.

Figure1. 7. Egg masses of Doryteuthis gahi attached to Macrocystis pyrifera stipes (photo by Mathias Hüne), (**Rosenfeld et al., 2014**)

1.7 Phylogeographical background

1.7.1 Marine Forests

The giant kelp is a good model to explore the effects of spatial distribution on connectivity pattern due to its wide range of patches sizes, distribution, and distances separating them.

Spatial patterns of genetic diversity are essential to determine the evolutionary mechanisms leading to this genetic differentiation as much as understanding the ecological consequences of the loss of genetic diversity. From the first isolation by distance model built by **Wright (1943)** to the development of new software such as GIS allowing to incorporate habitat features that relate to population connectivity (**Epps et al., 2007; Michels et al., 2001**).

As mentioned by **Reed et al (2006)** *Macrocystis pyrifera* is one of the few marine species globally geo-referenced thanks to its very dense canopy detectable from the air.

Reed et al (2006) have studied the role played by the size of kelp patches and their isolation by distance related to extinction. They find out that more the patches were small and isolated the more probabilities they have to go extinct.

DNA fingerprints development have led to a greater understanding of the population genetic structure of *Macrocystis* (**Macaya and Zuccarello., 2010a**) and allowed to evaluate the cost of inbreeding, self-fertilization, and crossbreeding among discrete kelp patches. Multiple studies have corroborated empirical and theoretical models to understand the effect of geographic distance and habitat continuity on the genetic distance. **Alberto et al. (2009)** have shown that genetic distance increases when geographic distance increased, and habitat continuity decreased. Therefore, habitat fragmentation will lead to severe consequences on metapopulation structure (**Alberto et al., 2010**). However, environmental factors such as ocean circulation, nature and direction of flows, and transport time between patches need to be added as predictors to measure gene flow and how accountable are these factors in the genetic differentiation (**Alberto et al., 2011**). The giant kelp inhabits rocky patches and have a limited dispersal capacity but a high dispersal potential. It appears that long-distance dispersal by kelp raft allows efficient settlement in new habitat. Thus, connectivity among populations thought to be mainly driven by ocean currents (**Watson et al., 2010**).

According to these studies, the present genetic structure is best explained by predicted transportation by ocean currents and habitat continuity for stepping-stone migration, rather than by geographical distance.

1.7.2 Marine species of the region Falklands, Patagonia and Sub-Antarctic region in general

The broad latitudinal and longitudinal range occupied by Macrocystis plus its limited dispersal make it a very good model to not only investigate processes affecting species range, speciation, vicariance,

and "founder effect" but also to understand the role of climate change in shaping intraspecific diversity through several shift of the abundance and distribution **(Assis et al., 2018)**

Historical and contemporary events are known to leave footprint on the genetic but also on evolutionary trait. Furthermore, literature has highlighted the fact that long term population persistence displays a high genetic diversity and usually a unique gene pool **(Assis et al., 2014)**

The climate driven range shift caused by glacial and interglacial cycles, for instance, is a critical question in evolutionary ecology and conservation management. However, many more environmental factors such as El Niño Southern Oscillations (ENSO) can lead to species range shift but in this study, we will go over the effects of glacial periods.

Glacial and Interglacial cycles played an important role in shaping the distribution and abundance of marine species. During these periods, populations might have loose or accumulate genetic diversity depending on their effective size, genetic drift, bottleneck, and local extinction **(Assis et al., 2018)**. Throughout these periods, variation in sea level and sea surface temperature have modified coastal topology gave rise to barriers limiting dispersal.

During glacial maxima (e.g., Last Glacial Maximum, LGM: ~ 21 ka) range contraction pushed populations to settle in unfavorable niche, retreating from the poles into lower latitude refugia, thus genetic diversity might be reduced due to genetic drift, gene flow, and bottleneck (**Leimu et al., 2006**). In contrast, during interglacial period (post LGM), populations recolonized previously glaciated regions in which founder effect at the leading edge of colonization might have caused genetic erosion through range expansion **(Hewitt, 1996; Assis et al., 2016)**.

Sea ice influences solar radiation, ocean circulation and thus rate of climate change. **Fraser et al (2009)** have been investigated the effects of sea ice during the Last Glacial Maximum on *Durvillaea Antarctica* and they have noticed a latitudinal contrast in genetic diversity suggesting a southward colonization from multiple northern refugia.

The Falkland Islands were not covered by permanent ice during the Last Glacial Maximum and had a continuous habitat with Argentina due to low sea level. In this archipelago the giant kelp exhibits a high genetic diversity maybe due to long term persistence with longer evolutionary time. Moreover, the bathymetry analyses highlighted a southern seamount that was a suitable habitat in the past when the sea level was 136 m below present

1.8 Objectives

This thesis asks the following research questions for populations of giant kelp forests along the Falkland Islands. The first research question concerns the pattern of population connectivity along the archipelago which can be inferred by genetic structure and/or by biophysical oceanographic dispersal models? Thus, we hypothesize that the populations will be highly connected along some regions, but there will be sharp genetic breaks across certain areas, forming a system with well-defined geographical locations of genetic transitions rather than gradual variation. The genetic structure of the archipelago is explored by using microsatellites to highlight genetic signatures of more recent events. Moreover, biophysical oceanographic dispersal models based on ocean current allows to obtain theoretical map showing the genetic structure according to the geographical location.

The second research question asks if there is evidence for effects of the past climate history on present population genetic structure? and is the origin of the Falkland populations comes from vicariance or colonization or both, with multiple origin, vicariance from the West and colonization from the East? Glacial and interglacial cycle have drastically changed soft and physical barriers in the extent that the habitats that were unsuitable in the recent past glacial period have lower genetic diversity and lower differentiation. Then, during the Last Glacial Maximum, period of low sea level, the bridge with South America has facilitated colonization or at the opposite has acted as a physical barrier and promotes vicariance. Moreover, the effects of multiple colonization to explain the admixed composition found in the Falkland Islands, such as from South Georgia or from the Southern Island which is now a seamount. The last hypothesis made about the second research question concerns the evolutionary pump, with multiple glacial cycles, expanding fronts southwards and eastwards interspersed with local extinctions in the South, resulting in the observed pattern.

Finally, the last research question will explore which present factors best explain the present population genetic structure? Several researches such as the one done by **Fraser et al (2008**) on *Durvillaea antarctica*, **Assis et al (2018**) on the golden kelp *Laminaria ochroleuca*, and **Neiva et al (2018**) on *Saccharina latissima*,, reinforce the idea that transportation by ocean currents and habitat continuity are more accountable for stepping-stone migration rather than geographical distance.

II. Materials and methods

2.1 Study area and focal species

Populations of *Macrocystis pyrifera* were sampled from 2018 to 2019 from five sites in the Magallean region, located in the Chilean Patagonia, from one site in South Africa and from16 sites around the Falkland Islands (latitude -51.563412 and longitude -59.820557).

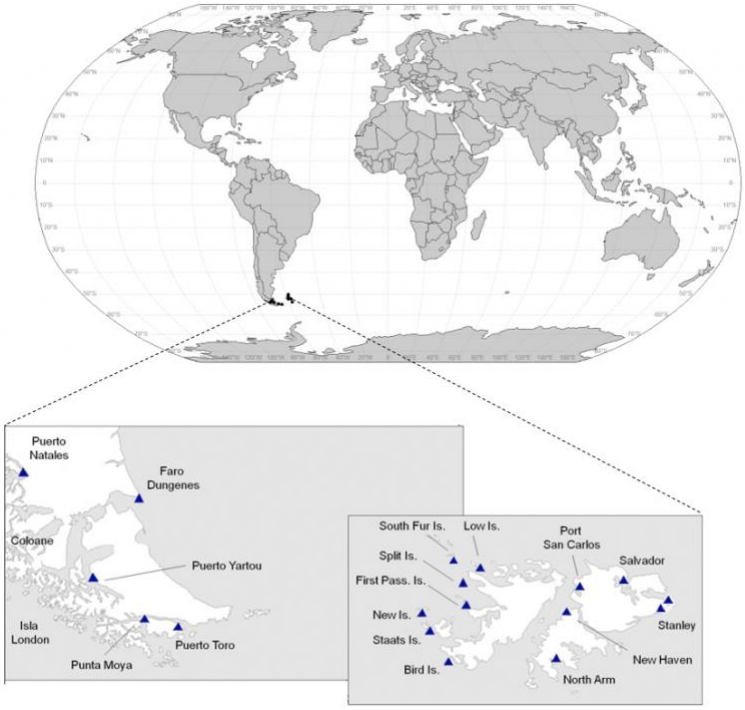

Figure 2. 1. Map showing sample region in the southern hemisphere

In total, 412 individuals of *Macrocystis pyrifera* were collected from 16 locations in the Falkland Islands and 5 in Patagonia. At each site, 200 meters transect was deployed and every 50 meters, around 15 individuals were collected, in a 10 m^2 area, haphazardly by the diver. Healthy blades, with no epiphytes or epibionts were selected, collected and sorted. A 2 to 5 cm^2 section was cut off from each blade and cleaned out with fresh water to remove the mucus and to ease dehydration before being organized in ziplock bags containing silica gel until DNA extraction.

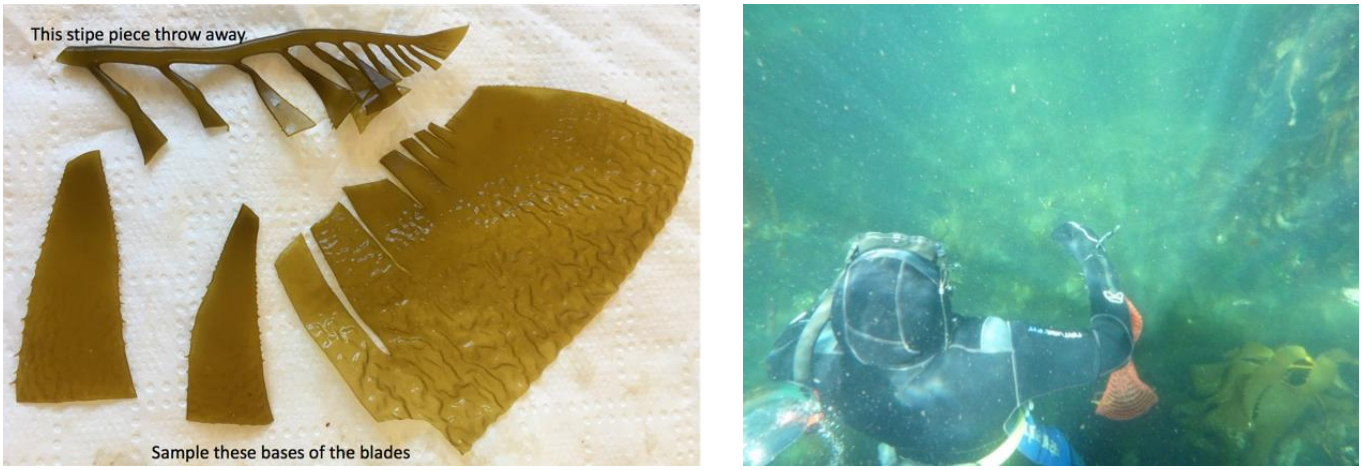

Figure 2. 2. *(Left*) Basal parts of apical (young) blades of *Macrocystis pyrifera* separated from the stipe. The distal part which represent the older part was thrown away. (*Right*) Sampling in the Beagle Channel

2.2 Genetic analysis

The DNA of each individuals was chemically isolated from the membrane, proteins and other cellular materials. The isolation and purification of DNA is a key starting point for other experimental protocols such as polymorphism chain reaction (PCR). The extraction requires careful handling, efficient pipetting and good organization in order to prevent risks of contamination and crossover. Indeed, the extraction follows multiple steps, depending on the protocol used. In this thesis, DNA is extracted by the nucleospin 96 plant II extraction kit (Macherey-Nagel, Düren, Germany), following the manufacturer's protocol. A total of nine microsatellites developed by **Alberto et al., 2009** and one mitochondrial marker were selected (**Table 2.1**). The extraction was done in a DNA extraction plate composed of 96 wells (**Appendix A, Figure A1).**

Table 2. 1 Characterization of nineteen microsatellites loci for Macrocystis pyrifera

Locus name	Primer sequences	Repeat motif	Tm	Range
Mp-BC-18	F-TTGCTCCTCCTGCTGCTAC	(CT)9	65	150 - 190
	R-GACCAGATGCAGAGATGACAG			
Mp-BC-4	F-AACCCACTCCACTCCT	(CT)11	65	210 - 250
	R-CTTCATAGTGCCCTTGTAT			
Mp-BC-25	F-CGGAAGGAGAGAGGGCAAG	(AG)11	65	150 - 190

	R-CTGCGTCCATTTGAGCCAC			
Mpy-8	F-CAACAACTAGCGTACCTTGAG	(CT)19	60-55	90 - 150
	R-TTCGGTTCATCTACATACTCG			
Mp-BC-19	F-TGACGCGTTCATCGTGTTG	(CT)10	65	130 - 170
	R-CGGAGAACAGGGAGAGCAG			
Mpy-14	F-ACTCGCTCAAGGTAAGCC	(CT)32	62-60	150 - 270
	R-AAAAGGGTGTGGCATCTT			
Mpy-11	F-GTTCCAGCTTGGTATTCAAA	(GA)10…(GA)3…(GA)8	65-60	210 - 250
	R-ACCGTGTAGCATGAGTCTATG			
Mpy-7	F-CGCATTCATTTTTCGCAC	(GA)16	60-55	130 - 190
	R-CAGGCTTGGTGTTGTTGC			
Mpy-17	F-GGAAATGCGGCACTAAAG	(GA)15..(GA)7	60-55	170 - 270
	R-GGCAGGTCTCGTCTTCTG			
Atp-8	F- TAGCAAACCAAGGCTTTCAAC	(CT)22	50-55	177 - 200
	R-TGTACGTTTCATATTACCTTCTTTAGC			

One mitochondrial region atp-8 (170bp) was amplified using the primer pair (atp-8-R: 5'-TGTACGTTTCATATTACCTTCTTTAGC-3'; atp-8-F:5'-TAGCAAACCAAGGCTTTCAAC -3') (**Voisin et al, 2005**). PCR amplification of the mitochondrial region was performed using a 20 µl PCR mix containing: 5x goTaq flexi buffer, 2mM $MgCl_2$ (25mM), 0.125mM each dNTP's (2mM), 0.5 µM each primer, 1U GoTaq (5U/ µL), sterile H2O, and 5 microlitre of isolated and purified DNA extract (1:50). A negative control was included in every set of PCRs plus a technical positive control. The negative control is used to detect any contamination, so we expect no amplification and a flat spectrum whereas the positive control is a technical control in which the DNA sequence of interest is known and thus, amplification and spectrum will appear. PCRs were performed on an ABI GeneAmp 9700 thermocycler (Applied Biosystems, Foster City, California, USA). Then PCR cycle conditions are set and are composed of a denaturation step at 95° for 5 mins, followed by 5 cycles at 95°C during 30 sec, 60°C during 30sec with a decrease of 1°C each cycle, and 1 min at 72°C, followed-up by 30 cycles at 95°C during 30 sec, then an annealing step at 55°C for 30 sec, 72°C for 45 sec with a final extension at 72°C during 10 mins.

2.3 Microsatellite amplification and scoring

Microsatellites PCRs were carried out following **Alberto et al (2009)** protocol for all the primers. A total of seven PCRs were carried out per plate including two multiplex and five

simplexes. Multiplex PCR allows for simultaneous amplification of multiple target sequences using specific primer sets in combination with probes labelled with spectrally distinct fluorophores whereas simplex PCR allows the amplification of a single target. PCRs mix and cycle conditions are adjusted and specific to each primer (**Appendix A, Figure A2**)

Then, the quality of PCR products was visualized under UV light on a 1% agarose gel stained by Gelred. In the extent there is amplifications, a genotyping plate is prepared by mixing a constant volume of 9.75 ul of hi-di formamide with 0.25 ul of LIZ 500 plus a volume from 1.0 – 2.0 ul of the PCR mix depending on the intensity of the amplification.

PCRs amplification are then sequenced on an ABI PRISM 3130 (Applied Biosystems) genetic analyser.

Table 2. 2 Genotyping mix according to the different markers

MpM8	Mpy1114	MpM1
Genotying mix: 9.25 ul hi-di formamide 0.25 ul Liz 500 1.8-2.0 ul PCR Mix	**Genotying mix:** 9.25 ul hi-di formamide 0.25 ul Liz 500 1.0-1.5 ul PCR Mix	**Genotying mix:** 9.25 ul hi-di formamide 0.25 ul Liz 500 1.5-2.0 ul PCR Mix

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Table 2. 3 Population genetics statistics summary by population of *Macrocystis pyrifera*

n, sample size; He, expected heterozygosity (corrected for sampling bias); Ho, observed heterozygosity; F_{is}, inbreeding coefficient (* significant Fis values at 0.05); Ar, allelic richness (averaged per population); Private alleles

Region	Population	Code	Year	Lat	Lon	n	He	Ho	Fis	Ar	Private alleles
Magallanes	Faro Dungenes	DU	2019	-52.362	-68.443	25	0.344	0.271	0.22*	2.16	-
	Puerto Natales	PN	2019	-51.716	-72.505	25	0.361	0.289	0.20*	2.34	1
	Puerto Yartou	PY	2019	-53.890	-70.144	25	0.366	0.280	0.24*	2.35	1
	Punta Moya	PM	2019	-54.917	-68.233	25	0.385	0.289	0.25*	2.08	-
	Puerto Toro	PT	2019	-55.082	-67.061	25	0.374	0.209	0.45*	2.19	4
Falkland Island	Bird Island 1	BI	2018	-52.167	-60.919	20	0.366	0.303	0.17*	2.28	1
	Bird Island 2	BIN	2019	-52.167	-60.919	13	0.353	0.316	0.11	2.21	-
	Staats Island	SI	2018	-51.899	-61.191	20	0.379	0.267	0.30*	2.20	1
	Gultches	GU	2018	-51.738	-61.312	3	0.333	0.250	0.28	2.11	-
	Settlement Whaling Station	SWS	2018	-51.732	-61.296	20	0.361	0.294	0.19*	2.08	-
	Whaling Station	WS	2018	-51.739	-61.304	13	0.369	0.299	0.20*	2.12	2
	First Passage Island	FPI	2018	-51.666	-60.666	20	0.410	0.361	0.12*	2.49	-
	Split Island	SPI	2018	-51.466	-60.701	20	0.374	0.367	0.02	2.21	-
	South Fur Island	SFI	2018	-51.388	-61.055	20	0.391	0.350	0.11*	2.48	3
	Low Island	LI	2018	-51.333	-60.588	20	0.434	0.400	0.08	2.90	2
	North Arm	NA	2018	-52.143	-59.370	20	0.466	0.350	0.25*	2.46	3
	New Haven	NH	2018	-51.723	-59.210	20	0.394	0.350	0.11*	2.39	2
	Port San Carlos	PSC	2018	-51.497	-59.022	20	0.416	0.328	0.22*	2.40	1
	Salvador	SAL	2018	-51.442	-58.393	20	0.372	0.322	0.14*	2.20	1
	Kidney Island	KI	2018	-51.624	-57.757	20	0.450	0.339	0.25*	2.43	1
	Stanley	ST	2018	-51.692	-57.857	20	0.333	0.317	0.05	1.96	-
South Africa	Kommetjie	CP	2018	-34.140	18.329	20	0.159	0.122	0.23*	1.47	3

2.4 Genetic diversity

Before estimating the genetic diversity, raw microsatellite alleles are scored with STRand (Veterinary Genetics Laboratory, University of California, Davis; http://www.vgl.ucdavis.edu/STRand) software and tidy into allele classes with the MsatAllele package in R software (**Toonen et Hughes., 2001; Alberto et al., 2009).**

Summary statistics of the microsatellite genetic diversity was performed with GENETIX software 4.05 (**Belkhir et al., 2004)** and by using the R package PopGenKit. This summary includes the observed heterozygosity (Ho), expected heterozygosity (He), mean allelic richness per population (Ar), and the number of private alleles per population. The inbreeding

coefficient (Fis) and departures from Hardy-Weinberg equilibrium were estimated with GENODIVE 3.04 (**Meirmans 2020**) per population.

The number of genetic clusters among the 22 sampling sites was inferred by using the software STRUCTURE without a population assignment and allowing admixture. The analysis was performed by running the correlated allele frequency model with a burning time of 10^5 and 10^6 iterations for a K ranging from 1 to 20. The number of genetic groups inside the study region was determined by delta K criterion: $DeltaK = mean(|L''(K)|) / sd(L(K))$

(Evanno, Regnaut, & Goudet, 2005).

Structure software allows preliminary examination of population structure by detecting allele frequency differences and thus bin individuals into sub-populations based on likelihood analysis (**Porras-Hurtado et al., 2013**). This step permit to establish genetic delimitation present in the study regions.

The patterns of genetic differentiation were computed on GENETIX with the help of a Factorial Correspondence Analysis (FCA) (**Belkhir et al., 2004; Assis et al., 2013**). Pairwise differentiation of populations, Fst and Jost's D were calculated with GENODIVE to assess genetic differentiation between sites within regions (Meirmans & Van Tienderen, 2004).

The molecular variance based on the allele frequency was computed using GENODIVE, the significance was tested using 999 permutations and 95% confidence intervals. The AMOVA analysis was performed at two hierarchical STRUCTURE level to assess the proportion of genetic variability within individuals (F-it), among individuals nested in population (F-is), among population nested in the first hierarchal STRUCTURE level (F-sc), and among regions (F-ct).

Analysis of the mitochondrial data were assessed by constructing a median-joining network using PopART (http://popart.otago.ac.nz) to determine the genealogical relationship at the intra-specific level with the biogeography and history of populations at the different sampling site.

III. Results

3.1 Population genetics summary statistics

The haplotype network reveals two main widespread haplotypes (A and B) and three localized haplotypes (**Figure 3.1**). The A haplotype was the most common among populations within regions, follow by the B haplotype. The haplotypes are usually restricted to a specific geographic area. The B is the most common haplotype found among populations within the Magallean region. Interestingly, one site in the eastern Falkland island shares the same haplotype than the populations in the Magallean region. The A haplotype is the main widespread haplotype found in the west Falkland island that is also shared by two populations located up north of the east Falkland (PSC and SAL). The western Falkland displays a unique genetic diversity. Two other sites (KI and ST) display two distinct unique haplotypes. One centered site (NH) exhibit one shared haplotype with the two most common

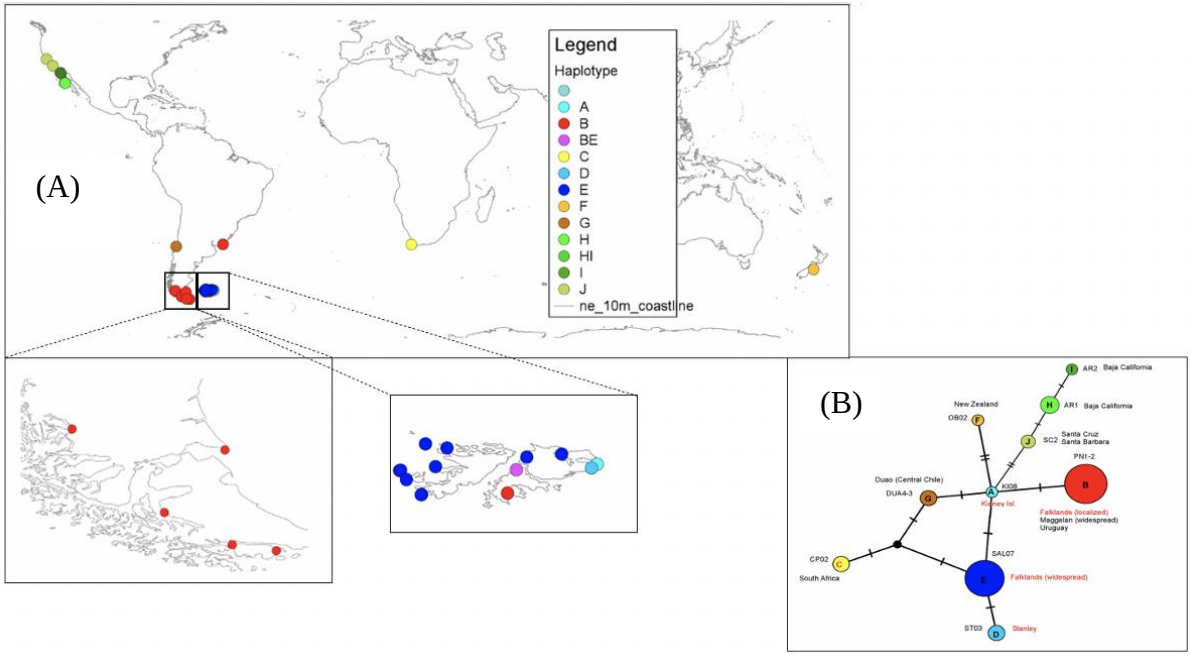


Figure 3. 1. (A) Map and (B) Haplotype network with concordant colors**.**

A total number of 105 alleles among 9 loci were found for 432 individuals genotyped. Gene diversity like standardized allelic richness varies from 1.47 (Kommetjie, South Africa) to 2.90 (Low Island, Falkland Island) (**Table 2.3)**. Endemism represented by private alleles ranged

from 4 to no private allele in the Magallean region, from 3 to no private alleles in the Falkland Island (**Figure 3.2**)

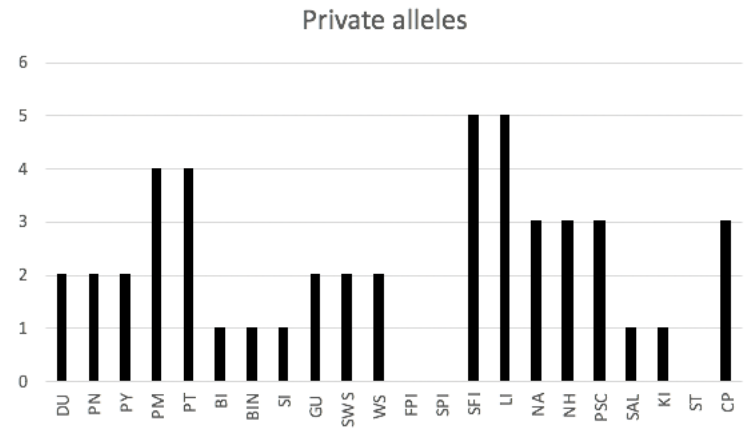


Figure 3. 2. Private alleles associated to genetic group

Observed heterozygosity ranged from 0.20 to 0.2889 in the Magallean region, from 0.25 to 0.4 in the Falkland Islands while expected heterozygosity ranged from 0.3439 to 0.3846, and from 0.3333 to 0.4662, respectively. Gene diversity (He) among populations within regions shows a gradual increase from west to east (**Figure 3.3**). The Magallean region displays the lowest genetic diversity with 0.366, follows by the western Falkland island with 0.377, and finally the eastern Falkland island with the highest 0.405. In 17 out of 22 sites, significant F_{is} values were obtained. This trend is fully supported by the genetic differentiation indices. Pairwise Fst ranged between 0.125 (Magallean region), 0.25 (western Falkland), and 0.238 (eastern Falkland). Josts's D absolute population differentiation ranged between 0.112 (Magallean), 0.189 (western Falkland), and 0.206 (eastern Falkland).

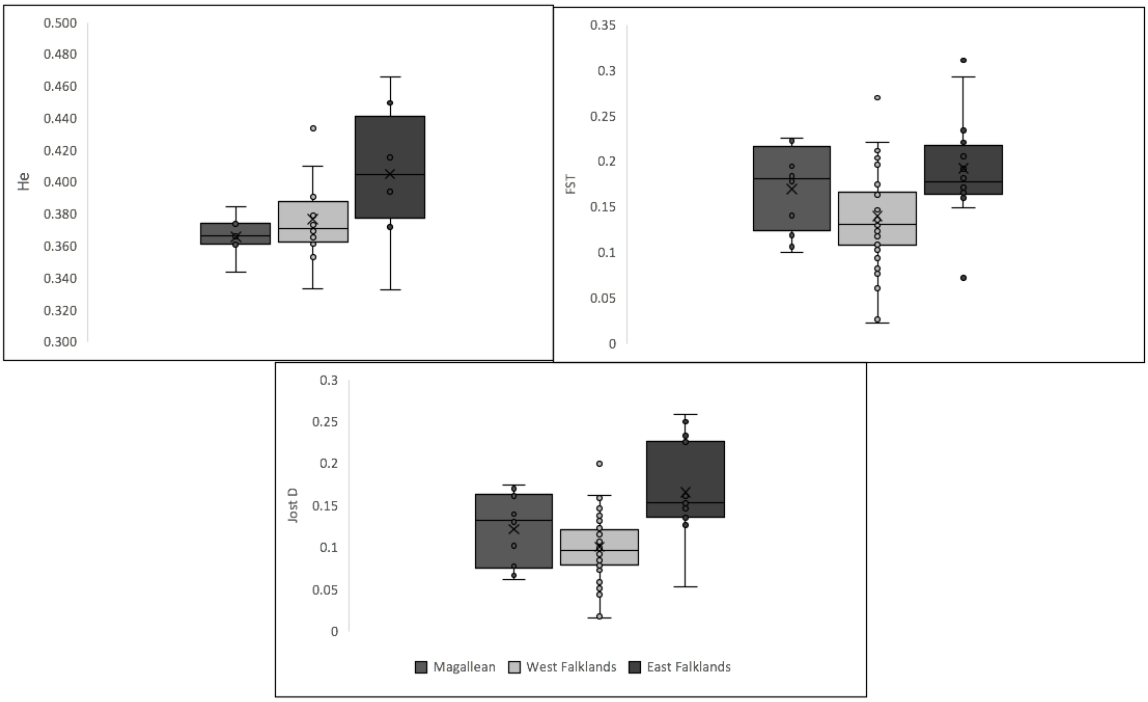


Figure 3. 3. Genetic diversity and differentiation of populations of *Macrocystis pyrifera* within selected regions. Top left expected heterozygosity (He) at population (boxplot). Top right fixation index (Fst) and Bottom differentiation of populations (Jost's D) within regions. Box plots depict the median (horizontal line), the 25thand 75th percentiles (bottom and top of the box) and the minimum/maximum values (horizontal lines).

At a first STRUCTURE level, F-statistics using AMOVA shows 60% of genetic variations within individuals which explain the low percentage of variation among populations within genetic cluster (14 %) and among genetic clusters (11%) (**Table 3.1**). At a subsequent STRUCTURE level, F-statistics support the same percentages of variation within individuals (61%) and among genetic clusters (15%), however a very low percentage of 1% among populations within genetic clusters.

Table 3. 1 F-statistics using AMOVA to assess proportion of genetic variability within individuals (F-it), among individuals nested in population (F-is), among population nested in the first hierarchal STRUCTURE level (F-sc), and among regions (F-ct)

Source of Variation	Nested in	%var	F-stat	F-value
Within Individual	--	0.599	F_it	0.401
Among Individual	Population	0.142	F_is	0.192
Among Population	Clusters_level_1	0.145	F_sc	0.164
Among Clusters_level_1	--	0.114	F_ct	0.114

Source of Variation	Nested in	%var	F-stat	F-value
Within Individual	--	0.616	F_it	0.384
Among Individual	Population	0.146	F_is	0.192
Among Population	Cluster_level_2	0.093	F_sc	0.108
Among Cluster_level_2	--	0.145	F_ct	0.145

At a large scale, *Macrocystis pyrifera* was subdivided into four main genetic groups to Magallean, West Falklands, East Falkland, and South Africa. Structure clustering analysis performed mainly expose 4 distinct clusters showing a geographical break between the Magallean, among Falkland Islands, and South Africa sites (**Figure 3.4 (A))**. At the first hierarchical STRUCTURE level K=3) it appears that Magallean and South Africa are stable genetic clusters with no admixture whereas the Falkland appeared as an admixed zone where we found genetic footprint of both the Magallean and South African region. In order to have extra level of information and more resolution, we tried to map with the second-best delta K=8 (not mapped), however we didn't manage to extract any relevant information and it was even very confusing. The next level of genetic structure mapped for K=5 instead (**Figure 3.4**) was used and it allowed to highlight more smaller subsequent genetic structuring in the Falkland island.

At a large scale the 3D FCA analysis performed with GENETIX software almost support similar results showed by the Structure analysis in which four distinct clusters were defined: Magallean, western Falkland and eastern Falklands, and South Africa (**Figure 3.4 (C)**). This analysis shows only three principal clusters and not the one within the Falklands. Moreover, the genetic distance of the South African population looks biased due to the fact that only one population has been sampled. In addition, three individuals from New Zealand were added which form another distinct group. The Factorial correspondence analysis also shows that inside the Falkland and Patagonia there is lower genetic differentiation between sites than between these two regions.

(A)

(B)

(C)

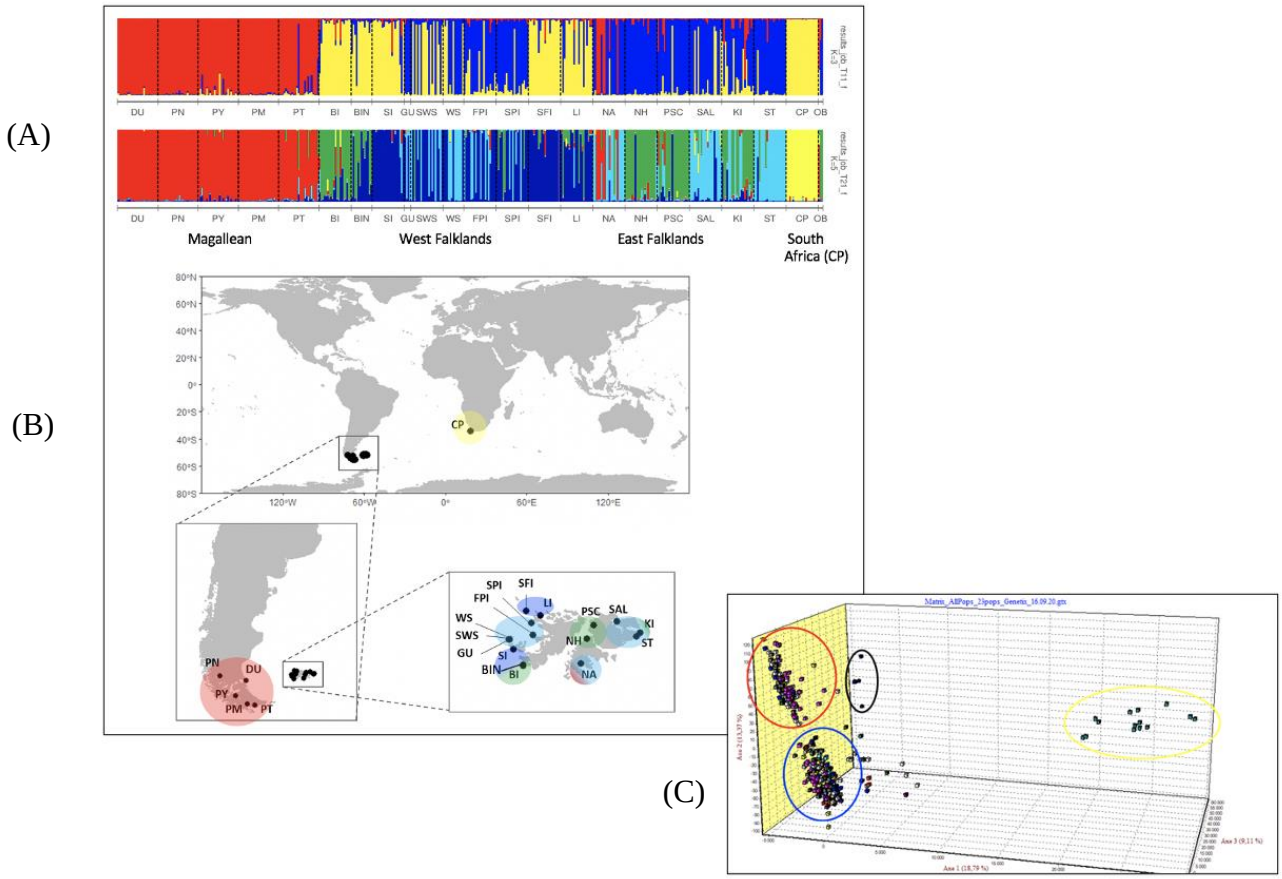


Figure 3. 4. Analysis of the 22 populations. (A) Hierarchical structure plots assuming K=3 and K=5. (B) Sampling of *Macrocystis pyrifera* and genetic structure inferred from multi-locus microsatellite genotypes. Sampling locations coloured according to genetic structure. Different colours represent percentage ancestry of each genotyped individual (vertical bars). (C) 3D Factorial Correspondence Analysis (FCA).

3.2 Magallean genetic structure and diversity

On the large-scale STRUCTURE analysis gathering all the population sampled, when K=3 is mapped the Magallean region appears as a homogenous region with no or very few admixtures. As well, for k=5 this region appears as one distinct cluster. However, considering the five populations present in the Magallean region, the STRUCTURE analysis revealed two distinct clusters for the best delta K=2 One north with the sites of Puerto Natales (PN), Dungenes (DU), and Puerto Yartou (PY) and one south separated by the Beagle Channel with the sites of Punta Moya (PM), and Puerto Toro (PT) (**Figure 3.5**)

Inside the Magallean region, the 3D FCA analysis supports the STRUCTURE results, confirming the presence of two clusters. Moreover, it appears that inside the southern

cluster, Punta Moya and Puerto Toro seems to be slightly differentiated. Allelic richness per sites was highest in the northern cluster, Puerto Yartou and Puerto natales, followed by Puerto Toro and Faro Dungenes (**Table 2.3**). Despite a higher allelic richness in the north, more private alleles were found in the south in Puerto Toro, decreasing northward. Gene diversity (He) at the opposite shows similar pattern than the private alleles, higher in the south.

(A)

(B)

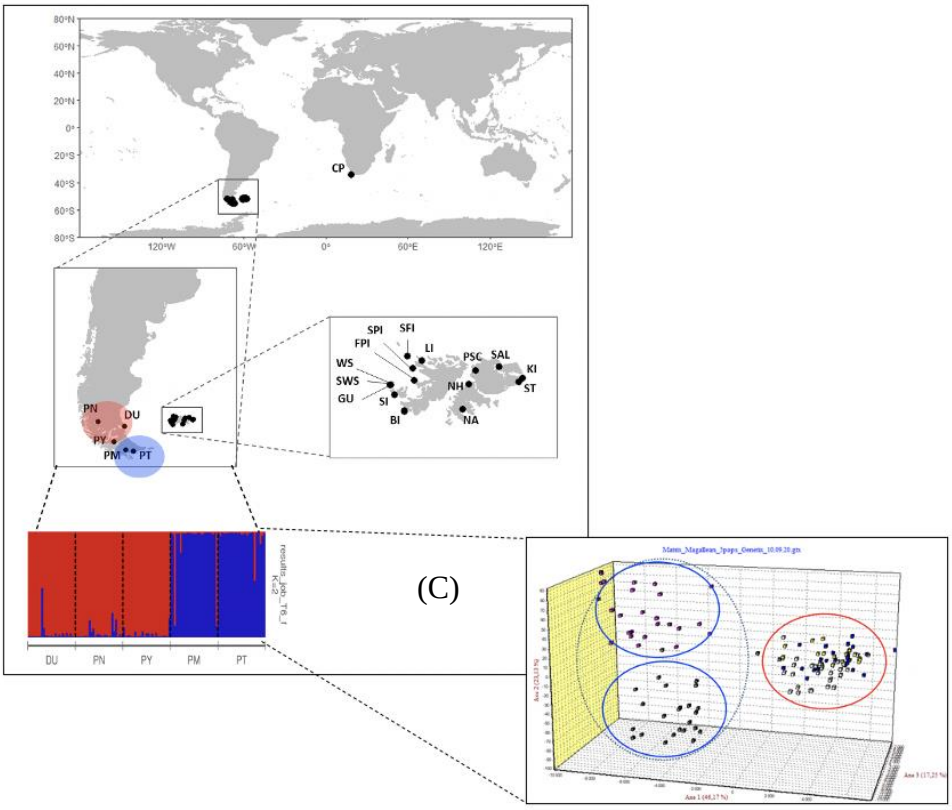


Figure 3. 5. Analysis of the five populations in the Magallean region. (A) Sampling of *Macrocystis pyrifera* and genetic structure inferred from multi-locus microsatellite genotypes. Sampling locations coloured according to genetic structure. Different colours represent percentage ancestry of each genotyped individual (vertical bars). (B) Hierarchical structure plots assuming K=2. (C) 3D Factorial Correspondence Analysis (FCA).

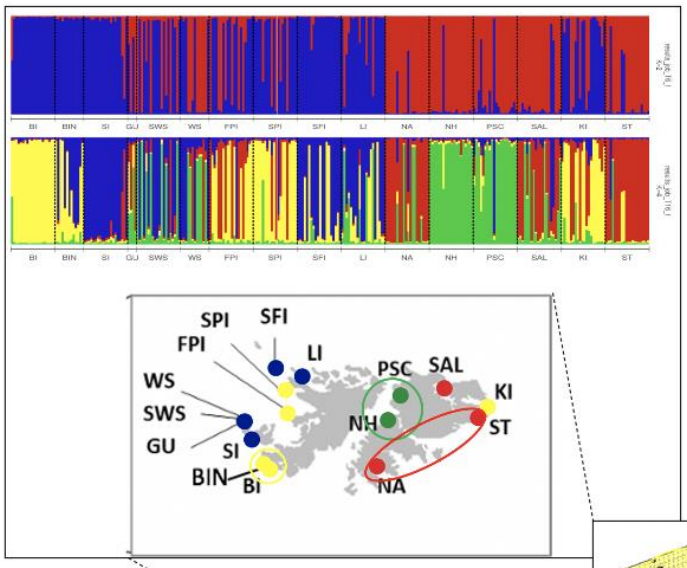
3.3 Falkland genetic diversity and structure

The main focus of this thesis was about the Falklands. Considering the 16 populations sampled in this region, the first hierarchal level of STRUCTURE analysis exposes two main clusters for K=2. It appears than one cluster corresponds to the populations located on the eastern island from Bird island (South) to Low Island (South) whereas the populations

on the western island form a different cluster. Despite having two clusters on different island we observe admixture in both (**Figure 3.6**). The second level from K=2 to K=4, additional breaks appear within the two islands. On the eastern island, inner population such as New Haven (NH) and Port San Carlos (PSC) form one sub-cluster; Stanley (ST); North Arm (NA) another one; Kidney island (KI) a distinct one; Salvador (SAL) appears to be an admixture site near transition zones. On the western island, the STRUCTURE analysis revealed additional clusters that are geographically coherent. Bird island (BI, sampled in 2018), Bird island (BIN, sampled in 2019) are two sites separated by a peninsula. They show similar genetic pattern in the first hierarchal STRUCTURE analysis whereas in the second level BIN appears as an admixture zone between Bi and Staat island (SI). Populations of Settlement whaling station (SWS) and Whaling station (WS) share similarities as well as First passage island (FPI) and Split island (SPI). However, although Gultches (GU) have too few individuals sampled, this site shows interesting pattern. Finally, South fur island (SFI) and Low island (Li) that are the two far north sites are mixed and share similarities with few populations located in the south such as Staat island (SI) and Bird island (BI).

(A)

(B)



(C)

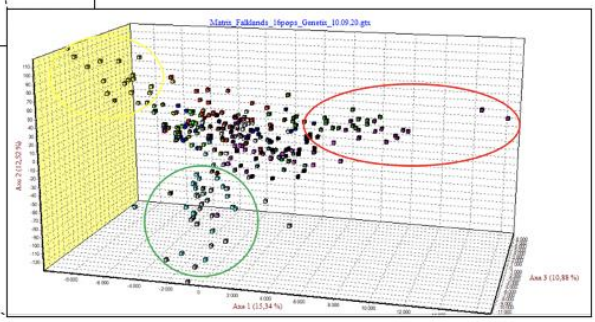


Figure 3. 6. Analysis of the 15 populations around the Falkland Islands. (A) Hierarchical structure plots assuming K=3 and K=5. (B) Sampling of *Macrocystis pyrifera* and genetic structure inferred from multi-locus microsatellite genotypes. Sampling locations coloured according to genetic structure. Different colours represent percentage ancestry of each genotyped individual (vertical bars). (C) 3D Factorial Correspondence Analysis (FCA).

IV. Discussion

The results show interesting genetic structuring and genetic variation among populations of *Macrocystis pyrifera* within the regions of interest. The role played by the Falkland Islands as a refugia for this species is well supported by the phylogeographic structure. Genetic analysis revealed that the distribution around the Falklands has been shaped by contemporary and historical events. At a large scale, this study shows that *Macrocystis pyrifera* is subdivided into four main genetic clusters, one in the Magallean region, two in the Falkland Islands, and one in South Africa. Multiple genetic breaks have been observed that are concordant with biogeographic and oceanographic breaks. Samples comparisons between Magallean and Falklands reveals that these regions have distinct and common haplotypes. However, interesting pattern emerged, showing that one common haplotype in Magallean is also found in one site on the eastern Falkland. These results are concordant with oceanographic currents and suggest recent colonization events after the LGM.

4.1 Genetic diversity and dispersal potential

The first expectations were that populations will be highly connected along some regions, but there will be sharp genetic breaks across certain areas, forming a system with well-defined geographical locations of genetic transitions rather than gradual variation. The results of this study show low level of genetic diversity for the populations along the Magallean region whereas, as expected, populations around the Falkland Islands show higher genetic diversity. The high genetic diversity and high number of private alleles compared to the Magallean region suggest that these populations have been stable, and persistent throughout climatic changes to

accumulate mutations and fixed alleles. In fact, Falklands and specifically the east Falkland island, considering its higher genetic differentiation and structure might have been a glacial refugia. Similar patterns, low genetic diversity and differentiation have been suggested in the literature concerning the Chilean region. Whereas studies on Macroalgae have found high genetic differentiation along the Chilean Coast (**Faugeron et al., 2005**). The giant kelp, *Macrocystis pyrifera* have shown high survival capacity (100 days) **(Hobday 2000a**) but also long-distance dispersal while continuing to be fertile. Long distance dispersal has been suggested to be a powerful mechanism for biogeographic expansion and genetic exchange, however, is considered as poor to maintain gene flow among populations (**Collins et al., 2010**; **Reed et al., 2006**). The low genetic variation among population in Magallean could be the results of a recent colonization event(s) and knowing the ability of *Macrocystis* to float over long-distance, it can explain the high level of gene flow (**Macaya et al., 2005**). Kelp rafts can play an important role to promote connectivity among populations (**Batista et al., 2018**). This has been suggested for other species such as *Durvillae antarctica* (**Collins et al., 2010**; **Buschmann et al., 2006**). Moreover, short distance genetic breaks have been observed in the Magallean region and in the Falklands without any strong oceanographic barriers. That can be explain by the density which create a barrier to dispersal (**Assis et al., 2016**).

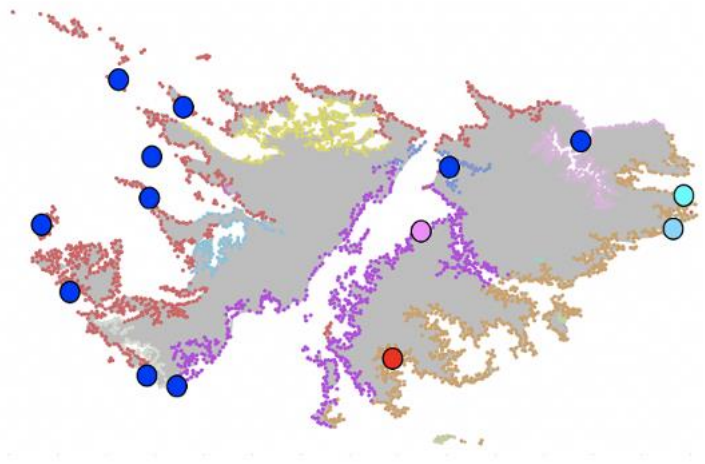


Figure 4. 1. Hypothesis for connectivity patterns. The colours indicate coastlines with high probability of dispersal by oceanographic currents.

The biophysical oceanographic dispersal model hypothetically estimates connectivity pattern around Falklands based on oceanographic currents **(Figure 4.1).** This model is consistent with microsatellite structure results and suggests asymmetrical dispersion pattern around this region.

Both regions display same habitat so it can be explained by differences in exposure level, population density or by kelp-detachment rate (**Collins et al., 2010**).

With its particular morphology and more specifically thanks to aerocysts, *Macrocystis* has a high buoyancy allowing a long-distance dispersal.

Major oceanographic currents surrounding the Magallean region and the Falklands are unidirectional **(Figure 4.2).** The directionality of transport is directly link to the direction of gene flow in between populations (**Alberto et al., 2011**). Thus, the results can suggest a greater gene flow from west to east than from east to west. However, caution is taken because the oceanographic transport time is an important predictor that has not been taken into consideration in those results. **Johansson et al (2015**) suggested that minimum transport time for the population in California is the best predictor of genetic differentiation. The Antarctic circumpolar current going from west to east divides itself into two current that go up north when passing under the Falkland Islands. The Patagonian current passing west to the Falklands and the Malvinas current passing east. These two currents that encounter the shallow Patagonian shelf bring cold rich water around the Falklands, and thus create an upwelling system which favour *Macrocystis* populations. The high genetic diversity and private alleles around the Falklands can be explained by this rich nutrient environment created by the upwelling. The private and unique diversity found in this region support the idea of a long population's persistence. Whereas, Magallean populations tend to have lower genetic diversity and fewer private alleles that is concordant with post glacial colonization events from founder effects at the leading edge (**Hewitt, 1996**)

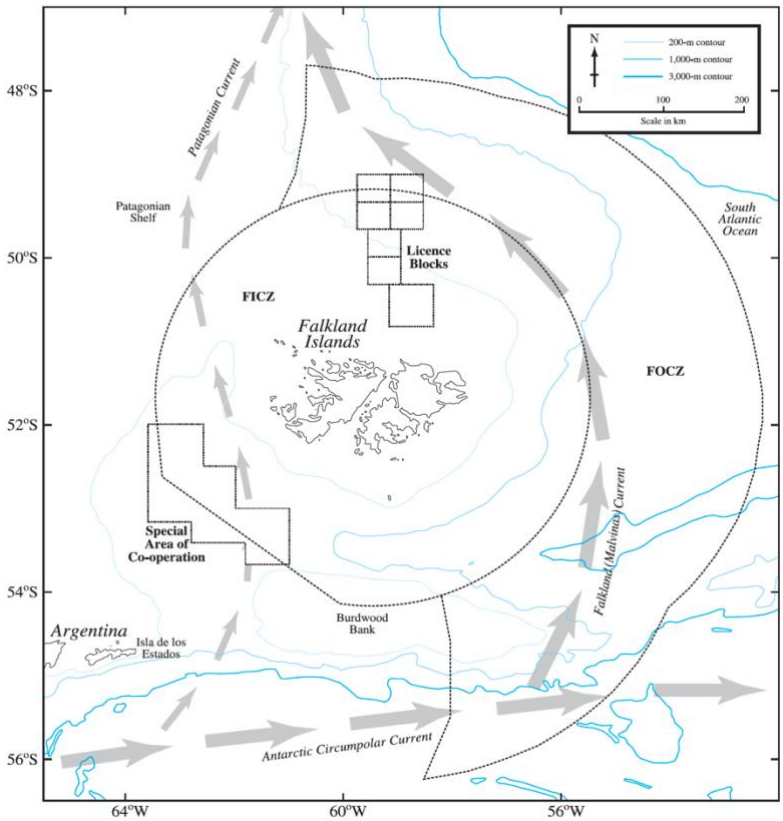


Figure 4. 2. Position and heading of the three currents surrounding the Falkland Islands: At the bottom, the Antarctic Circumpolar Current; to the West, the Patagonian current; and to the East, the Falkland Malvinas Current (White et al., 2002)

It seems that environmental factors such as oceanographic currents and wind surface are key components for kelp dispersion and settlement. Moreover, physical factors such as light penetration, temperature (**Batista et al, 2018**) and nutrient concentration tend to limit immigrant spore survival. The present genetic structure can be best explained by transportation through ocean currents and habitat continuity for stepping-stone migration rather than by geographical distance.

4.2 Contemporary and historical events

The main interest is the Falkland Islands because they were expected to be a refuge during the Last Glacial Maximum as they were not covered by ice during this period. In contrast, ice sheets were covering most of the Southern fjord in Chile, including the Magallean region (**Hulton et al., 2002**) (**Figure 4.3**). Several studies have mentioned the role played by glaciation and deglaciation events on genetic variations and distribution (**Fraser et al., 2009**). In his study on *Durvillae antarctica*, he mentioned a set of colonization events towards antarctica through long distance drifting, following the LGM and the retreat of ice.

During this period, the habitats in southern Chile were unsuitable for *Macrocystis pyrifera*. In effect, according to the results, Magallean populations show lower genetic diversity and lower genetic differentiation in the extent extinction or bottleneck might have led to losses of genetic variation among marine organisms. At the opposite Falkland populations have persisted and display higher genetic diversity and differentiation which has led to more diverse and stable populations. The Haplotypes breaks between these two regions is mainly explained by topographical breaks and oceanic currents. Moreover, this haplotype distribution is also a consequence of the Last glacial Maximum.

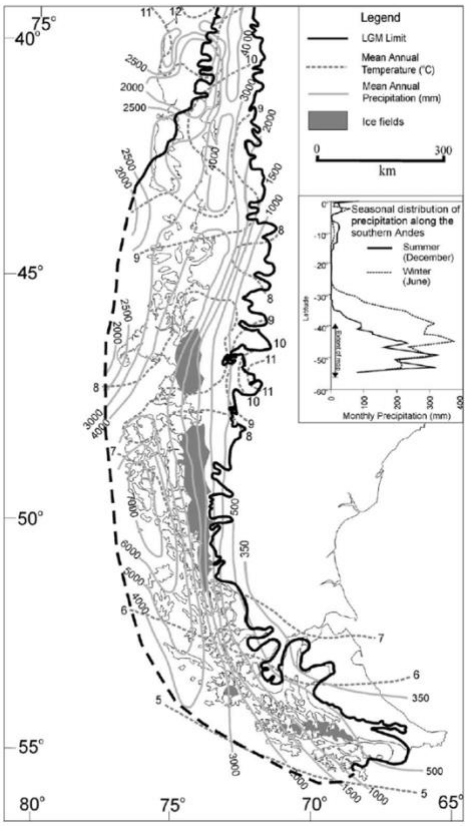


Figure 4. 3. The limits of the Last Glacial Maximum and the distribution of existing icefields (**Hulton et al., 2002**)

The high allelic diversity and high number of private alleles found among the populations of the Falklands compared to the Magallean region lead to the possibility that it's an ancient climate refugia during the Last Glacial Maximum (LGM). At the opposite, Magallean region was covered by ice and recently underwent El Nino Southern Oscillation (ENSO). These environmental events might have led to increase genetic differentiation and loss of allelic diversity by creating local extinctions and local bottlenecks. Thus, kelp rafts might be a crucial dispersal mechanism for populations with recurrent extinctions and recolonization, specifically for species that have slow recolonization speed (**Martinez et al., 2003**). Furthermore, by its unique combination of life history and life cycles, M. pyrifera has a huge reproductive output that allows a quick response to environmental disturbances to ensure local replenishment. However, the results do not fully support these expectations and thus explained that Macrocystis pyrifera is capable of maintaining high genetic diversity during those periods (**Johansson et al., 2015**).

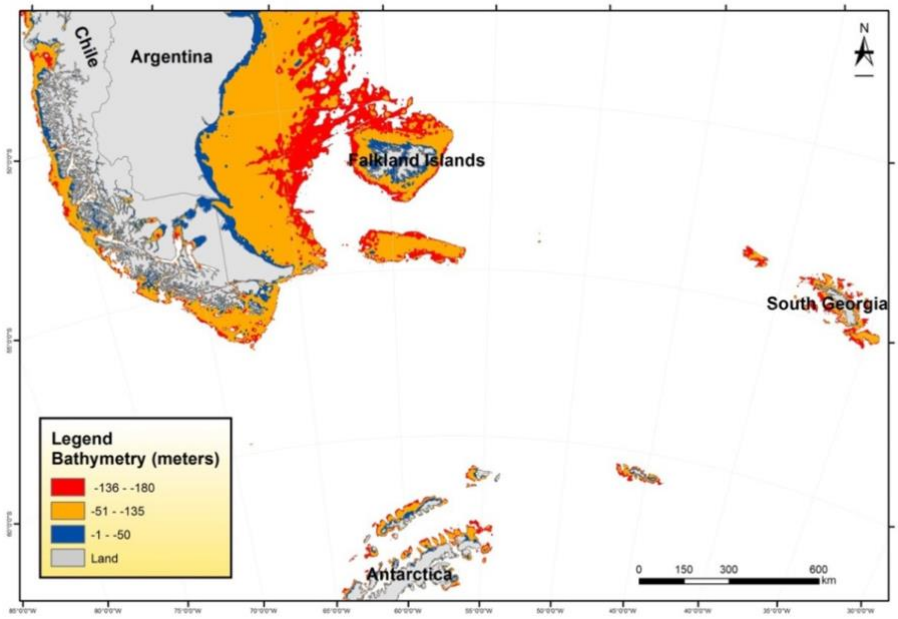


Figure 4. 4. Map showing the bathymetry during period of lower sea level

The bathymetry analyses reveal that the giant kelp of the Falklands had a continuous habitat with Argentina during lower sea level period (-120m) (**Figure 4.4**). It is possible that Falkland populations have originated from vicariance or multitude colonisation events. As shown by the genetic diversity and structure observed in the results, the admixed composition in the Falklands can be the effects of multiple colonization sources expanding southwards and eastwards interspersed by local extinctions in the south.

V. Conclusion

The literature about kelps reinforced the fact that they play a key role, economically and ecologically for the entire food web, in the extent it provides food, habitat, and spawning substrate for numerous species such as mammals, fishes, crabs, sea urchins, molluscs, epiphytes, and bacterial communities. Through this structured ecosystem, kelp forests not only magnify secondary and primary production but also support a diversified coastal food web. By its complex 3D structure, *M. pyrifera* control and influence ecosystem processes such as coastal currents, sedimentation, sand beach, hydrodynamics and biochemistry. The wide distributional range shows how efficient is this species to cope with such panel of environmental factors and how the morphology and physiology is highly variable in response to these environmental

conditions. The giant kelp has limited mobility and thus limited dispersal capacity but high dispersal potential through its ability to float over long-distance, over long time while being fertile. Its prodigious capacity of recruitment and replenishment allow this species to surpass cyclical environmental disturbances leading to local extinctions and bottleneck by maintaining a high genetic diversity. The results of this study bring evidence on evolutionary mechanisms leading to this present distribution. In the Magallean region, lower genetic diversity is found due to a recent colonization while the Falkland is a glacial refugia where populations have been able to persist and increase genetic diversity by accumulating mutations and private alleles. The present genetic structure can be best explained by transportation through ocean currents and habitat continuity for stepping-stone migration rather than by geographical distance. Despite the increasing anthropogenic pressure on *M. pyrifera*, the literature continues to show how amazing this species cope with disturbances and is able to constantly adapt to new environment. Thus, Management and conservation policies should be applied to conserve *M. pyrifera* forests.

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Appendix A: Supplementary Information

		1	2	3	4	5	6	7	8	9	10	11	12
Plate 1	A	MPGU01	MPWS01	MPWS09	MPSWS04	MPSWS12	MPSWS20	MPBI08	MPBI16	MPSI04	MPSI12	MPSI20	MPKI08
	B	MPGU02	MPWS02	MPWS10	MPSWS05	MPSWS13	MPBI01	MPBI09	MPBI17	MPSI05	MPSI13	MPKI01	MPKI09
	C	MPGU03	MPWS03	MPWS11	MPSWS06	MPSWS14	MPBI02	MPBI10	MPBI18	MPSI06	MPSI14	MPKI02	MPKI10
	D	MPGU04	MPWS04	MPWS12	MPSWS07	MPSWS15	MPBI03	MPBI11	MPBI19	MPSI07	MPSI15	MPKI03	MPKI11
	E	MPGU05	MPWS05	MPWS13	MPSWS08	MPSWS16	MPBI04	MPBI12	MPBI20	MPSI08	MPSI16	MPKI04	MPKI12
	F	MPGU06	MPWS06	MPSWS01	MPSWS09	MPSWS17	MPBI05	MPBI13	MPSI01	MPSI09	MPSI17	MPKI05	MPKI13
	G	MPGU07	MPWS07	MPSWS02	MPSWS10	MPSWS18	MPBI06	MPBI14	MPSI02	MPSI10	MPSI18	MPKI06	Control +
	H	MPGU08	MPWS08	MPSWS03	MPSWS11	MPSWS19	MPBI07	MPBI15	MPSI03	MPSI11	MPSI19	MPKI07	Control -

Figure A1. DNA extraction and PCR plates

		1	2	3	4	5	6	7	8	9	10	11	12
Plate 2	A	MPKI14	MPST02	MPST10	MPST18	MPSAL06	MPSAL14	MPPSC02	MPPSC10	MPPSC18	MPNH06	MPNH14	MPNA02
	B	MPKI15	MPST03	MPST11	MPST19	MPSAL07	MPSAL15	MPPSC03	MPPSC11	MPPSC19	MPNH07	MPNH15	MPNA03
	C	MPKI16	MPST04	MPST12	MPST20	MPSAL08	MPSAL16	MPPSC04	MPPSC12	MPPSC20	MPNH08	MPNH16	MPNA04
	D	MPKI17	MPST05	MPST13	MPSAL01	MPSAL09	MPSAL17	MPPSC05	MPPSC13	MPNH01	MPNH09	MPNH17	MPNA05
	E	MPKI18	MPST06	MPST14	MPSAL02	MPSAL10	MPSAL18	MPPSC06	MPPSC14	MPNH02	MPNH10	MPNH18	MPNA06
	F	MPKI19	MPST07	MPST15	MPSAL03	MPSAL11	MPSAL19	MPPSC07	MPPSC15	MPNH03	MPNH11	MPNH19	MPNA07
	G	MPKI20	MPST08	MPST16	MPSAL04	MPSAL12	MPSAL20	MPPSC08	MPPSC16	MPNH04	MPNH12	MPNH20	Control +
	H	MPST01	MPST09	MPST17	MPSAL05	MPSAL13	MPPSC01	MPPSC09	MPPSC17	MPNH05	MPNH13	MPNA01	Control -

		1	2	3	4	5	6	7	8	9	10	11	12
Plate 3	A	MPPM 1-1	MPPM 2-4	MPPM 4-3	MPPM 5-5	MPPT 2-3	MPPT 4-1	MPPT 5-4	MPDU 2-2	MPDU 3-5	MPDU 5-3	MPPNC5-14	
	B	MPPM 1-2	MPPM 2-5	MPPM 4-4	MPPT 1-1	MPPT 2-4	MPPT 4-2	MPPT 5-5	MPDU 2-3	MPDU 4-1	MPDU 5-4	MPALG2-11	
	C	MPPM 1-3	MPPM 3-1	MPPM 4-5	MPPT 1-2	MPPT 2-5	MPPT 4-3	MPDU 1-1	MPDU 2-4	MPDU 4-2	MPDU 5-6	MPDUA4-3	
	D	MPPM 1-4	MPPM 3-2	MPPM 4-6	MPPT 1-3	MPPT 3-1	MPPT 4-4	MPDU 1-2	MPDU 2-5	MPDU 4-3	MPOB01	MPDUA5-13	
	E	MPPM 1-5	MPPM 3-3	MPPM 5-1	MPPT 1-4	MPPT 3-2	MPPT 4-5	MPDU 1-3	MPDU 3-1	MPDU 4-4	MPOB02		
	F	MPPM 2-1	MPPM 3-4	MPPM 5-2	MPPT 1-5	MPPT 3-3	MPPT 5-1	MPDU 1-4	MPDU 3-2	MPDU 4-5	MPOB03		
	G	MPPM 2-2	MPPM 3-5	MPPM 5-3	MPPT 2-1	MPPT 3-4	MPPT 5-2	MPDU 1-5	MPDU 3-3	MPDU 5-1		Control +	
	H	MPPM 2-3	MPPM 4-2	MPPM 5-4	MPPT 2-2	MPPT 3-5	MPPT 5-3	MPDU 2-1	MPDU3-4	MPDU 5-2		Control -	

		1	2	3	4	5	6	7	8	9	10	11	12
Plate 4	A	MPPN 1-1	MPPN 2-4	MPPN 4-2	MPPN 5-12	MPPY 2-3	MPPY 4-1	MPPY 5-4	MPNA14	MPCP02	MPCP10	MPCP18	MPPDD06
	B	MPPN 1-2	MPPN 2-5	MPPN 4-3	MPPY 1-1	MPPY 2-4	MPPY 4-2	MPPY 5-5	MPNA15	MPCP03	MPCP11	MPCP19	MPPDD07
	C	MPPN 1-3	MPPN 3-1	MPPN 4-4	MPPY 1-2	MPPY 2-5	MPPY 4-3	MPNA08	MPNA16	MPCP04	MPCP12	MPCP20	MPPDD08
	D	MPPN 1-4	MPPN 3-2	MPPN 4-8	MPPY 1-3	MPPY 3-1	MPPY 4-4	MPNA09	MPNA17	MPCP05	MPCP13	MPPDD01	
	E	MPPN 1-5	MPPN 3-5	MPPN 5-1	MPPY 1-4	MPPY 3-2	MPPY 4-5	MPNA10	MPNA18	MPCP06	MPCP14	MPPDD02	
	F	MPPN 2-1	MPPN 3-11	MPPN 5-3	MPPY 1-5	MPPY 3-3	MPPY 5-1	MPNA11	MPNA19	MPCP07	MPCP15	MPPDD03	
	G	MPPN 2-2	MPPN 3-15	MPPN 5-4	MPPY 2-1	MPPY 3-4	MPPY 5-2	MPNA12	MPNA20	MPCP08	MPCP16	MPPDD04	Control +
	H	MPPN 2-3	MPPN 4-1	MPPN 5-5	MPPY 2-2	MPPY 3-5	MPPY 5-3	MPNA13	MPCP01	MPCP09	MPCP17	MPPDD05	Control -

		1	2	3	4	5	6	7	8	9	10	11	12
Plate 5	A	MPSI1	MPSI9	MPSI17	MPFPI5	MPFPI13	MPLI1	MPLI9	MPLI17	MPSFI5	MPSFI13	MPBI1	MPBI9
	B	MPSI2	MPSI10	MPSI18	MPFPI6	MPFPI14	MPLI2	MPLI10	MPLI18	MPSFI6	MPSFI14	MPBI2	MPBI10
	C	MPSI3	MPSI11	MPSI19	MPFPI7	MPFPI15	MPLI3	MPLI11	MPLI19	MPSFI7	MPSFI15	MPBI3	MPBI11
	D	MPSI4	MPSI12	MPSI20	MPFPI8	MPFPI16	MPLI4	MPLI12	MPLI20	MPSFI8	MPSFI16	MPBI4	MPBI12
	E	MPSI5	MPSI13	MPFPI1	MPFPI9	MPFPI17	MPLI5	MPLI13	MPSFI1	MPSFI9	MPSFI17	MPBI5	MPBI13
	F	MPSI6	MPSI14	MPFPI2	MPFPI10	MPFPI18	MPLI6	MPLI14	MPSFI2	MPSFI10	MPSFI18	MPBI6	
	G	MPSI7	MPSI15	MPFPI3	MPFPI11	MPFPI19	MPLI7	MPLI15	MPSFI3	MPSFI11	MPSFI19	MPBI7	Control +
	H	MPSI8	MPSI16	MPFPI4	MPFPI12	MPFPI20	MPLI8	MPLI16	MPSFI4	MPSFI12	MPSFI20	MPBI8	Control -

Figure A2. PCR Protocols

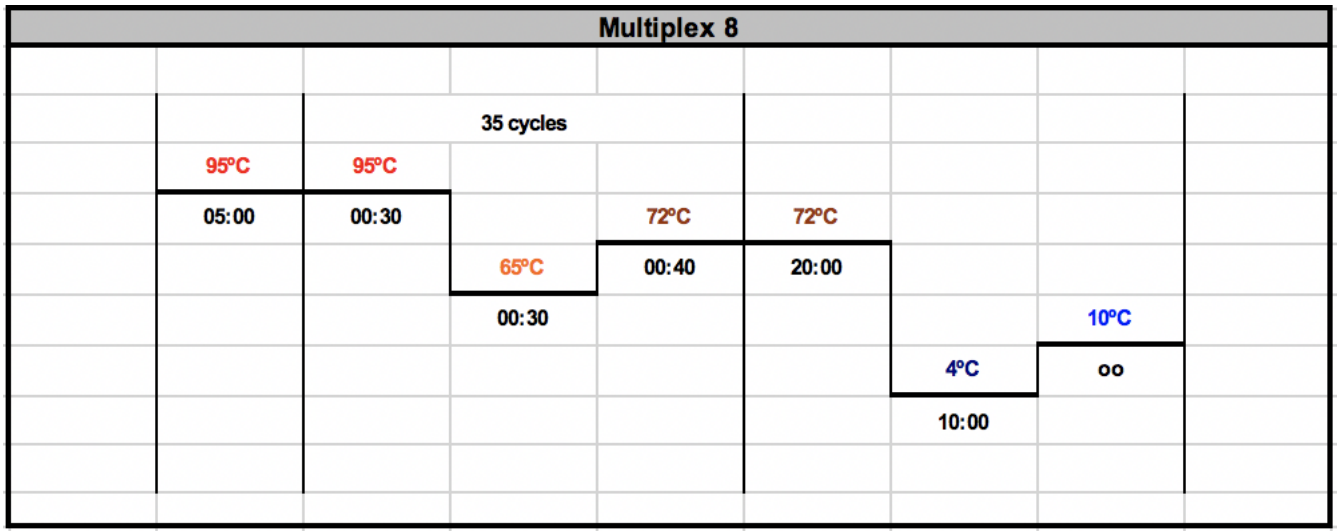
MpM8

Multiplex 8 (MpBC-18, BC-19, BC-20)

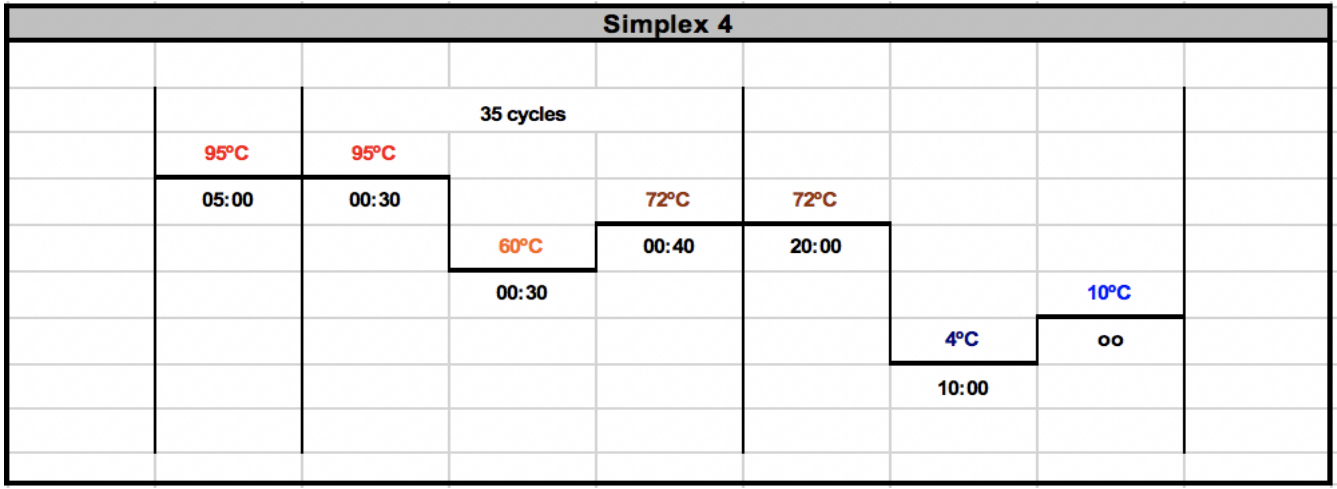
Simplex 4 (MpBC-04)

Simplex 8 (Mpy-8)

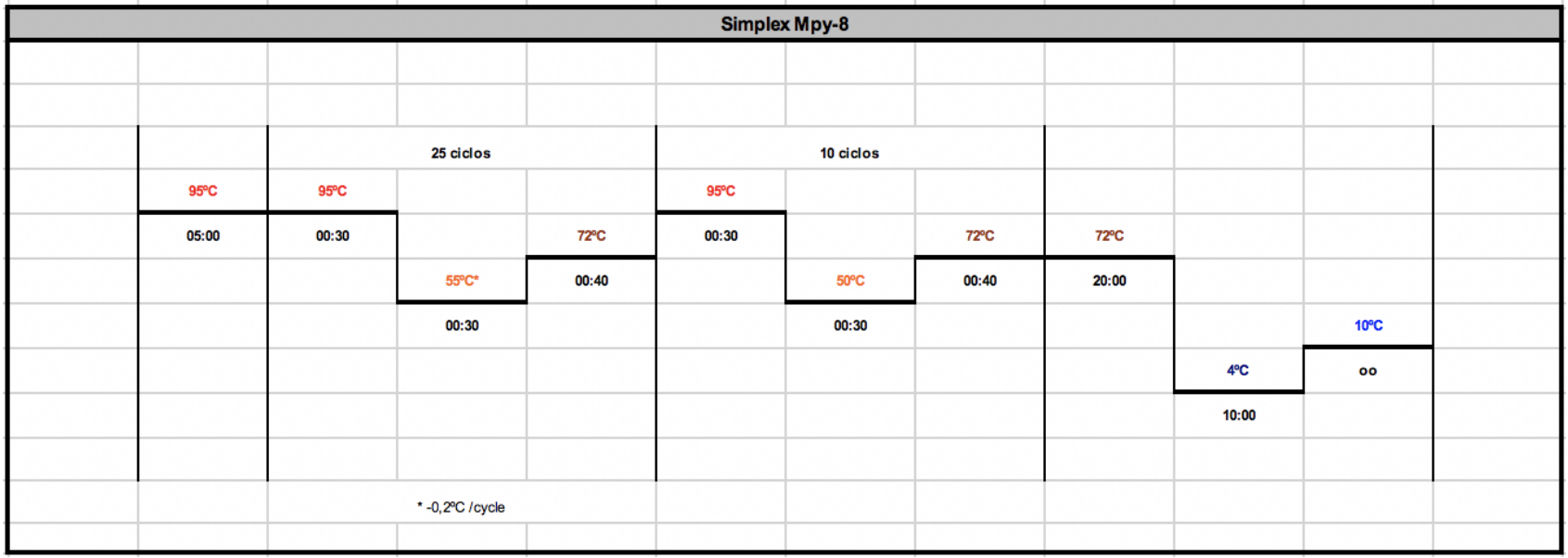
Multiplex 8	1x ul
Buffer 5x	3
MgCl2 (25mM)	1.2
dNTPs (1mM)	0.5
Mp-BC-18F (5 uM)	0.4
Mp-BC-18R (10 uM)	0.4
Mp-BC-25F (5 uM)	0.4
Mp-BC-25R (10 uM)	0.4
Mp-BC-19F (5 uM)	0.5
Mp-BC-19R (10uM)	0.5
Taq	0.15
H2O	2.55
DNA (1:100)	5
Total	10



Simplex 4	1x (ul)
Buffer 5x	3
MgCl2 (25mM)	1.2
dNTPs (1mM)	0.5
Mp-BC-4F (5 uM)	0.3
Mp-BC-4R (10 uM)	0.3
Taq	0.15
H2O	4.55
DNA (1:100)	5
Total	10



Simplex Mpy-8	1x (ul)
Buffer 5x	3
MgCl2 (25mM)	1.2
dNTPs (1mM)	0.5
Mpy-8F (5 uM)	0.3
Mpy-8R (10 uM)	0.3
Taq	0.1
H2O	4.6
DNA (1:100)	5
Total	10

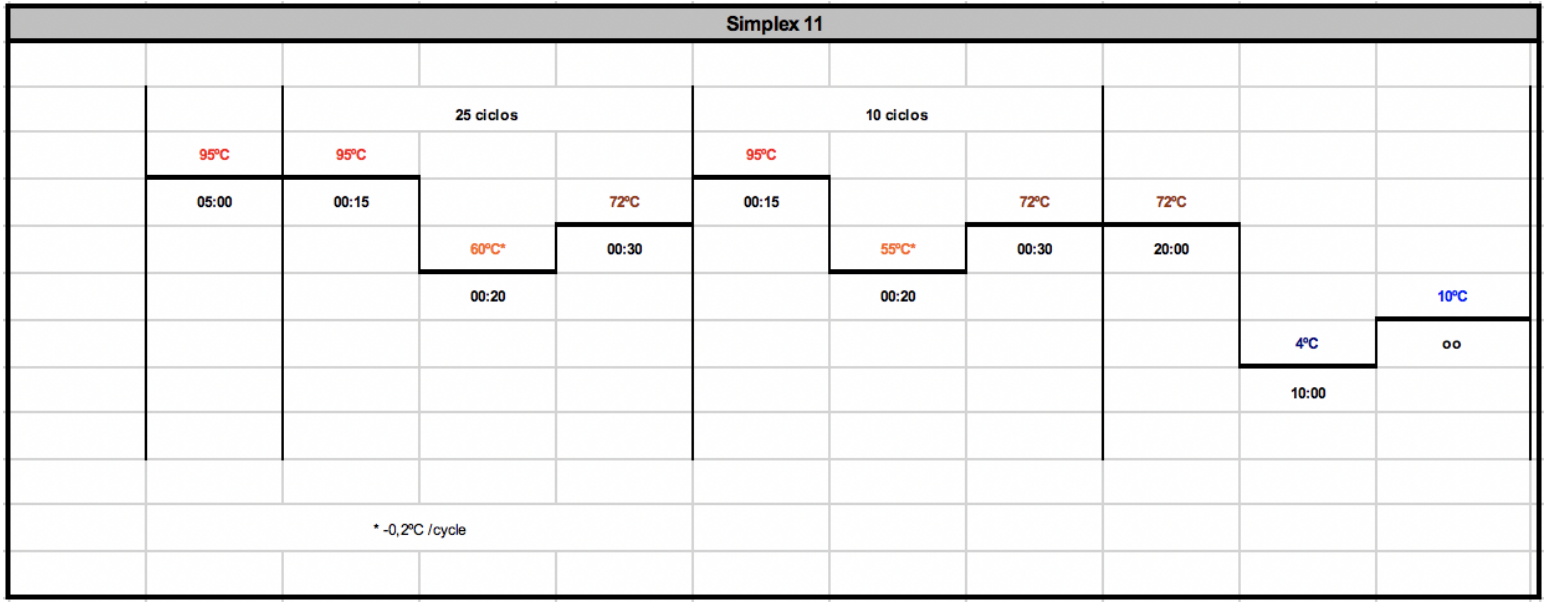


Mpy1114

Simplex 11 (Mpy-11)

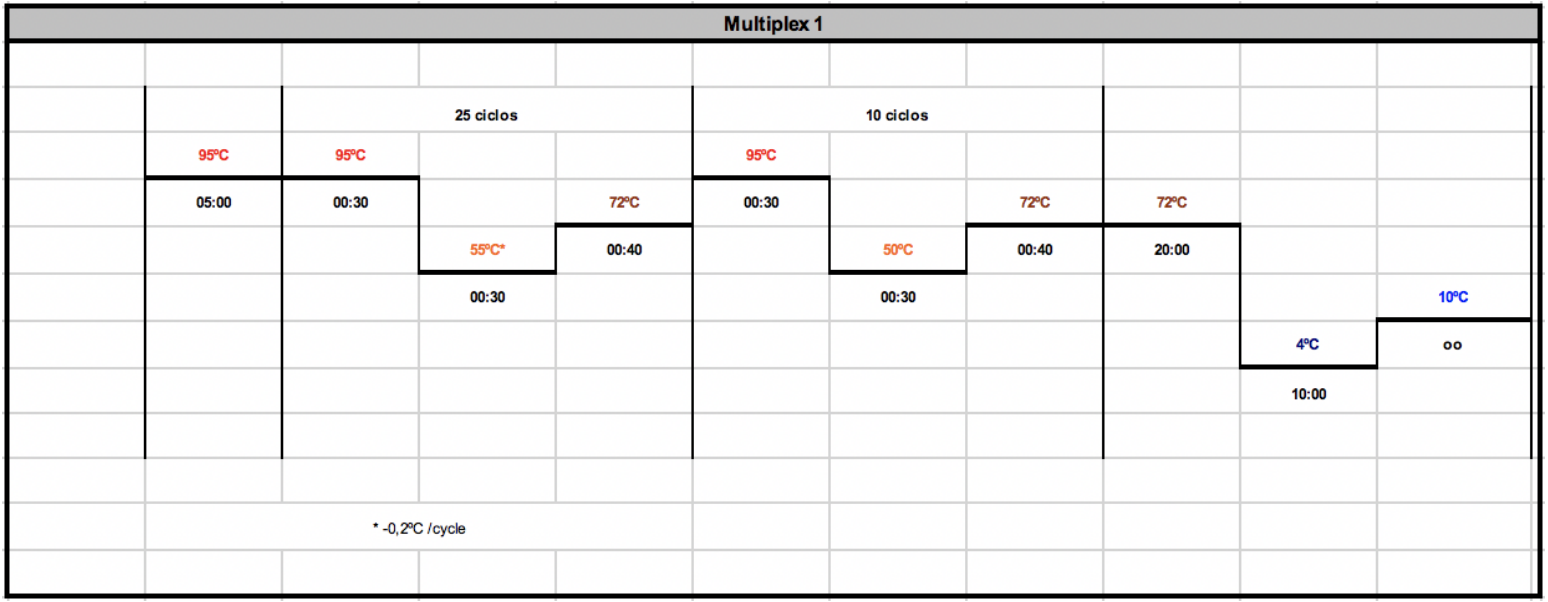
Simplex 14 (Mpy-14)

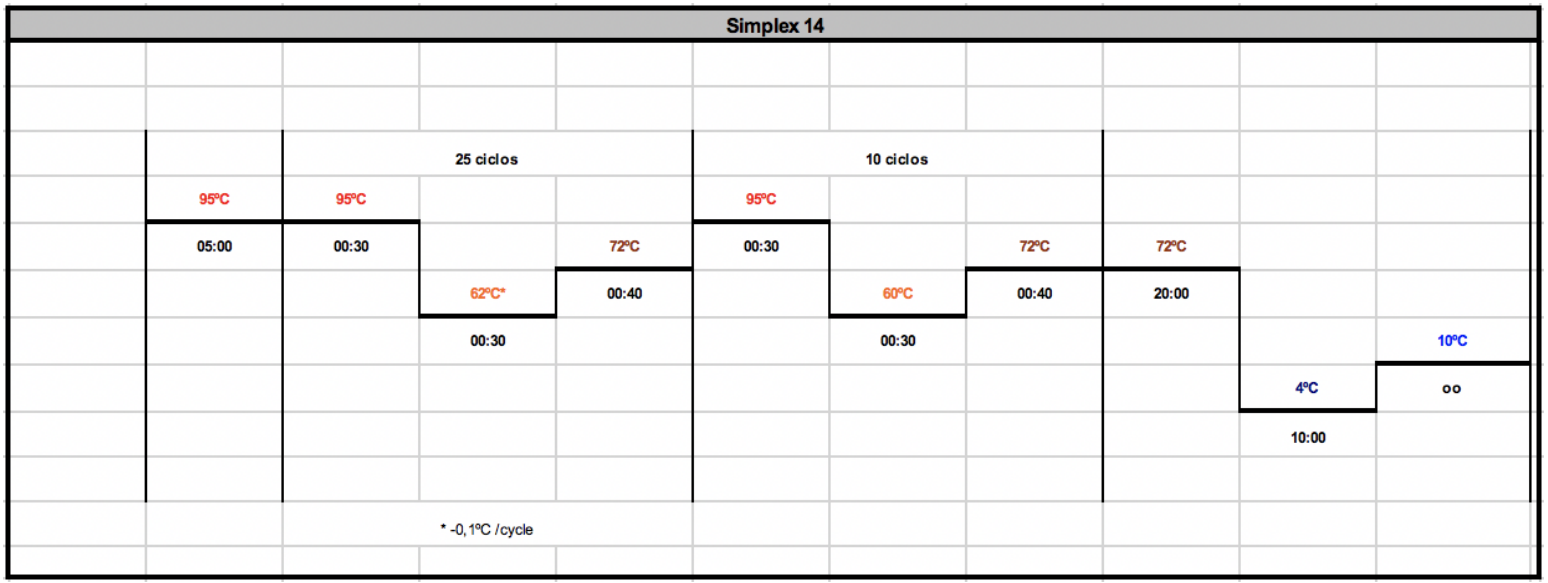
Simplex 11	1x (ul)
Buffer 5x	3
MgCl2 (25mM)	1.2
dNTPs (1mM)	0.5
Mpy-11F (5 uM)	0.5
Mpy-11R (10 uM)	0.5
Taq (5U /ul)	0.15
H2O	4.15
DNA (1:100)	5
Total	10



Simplex 14	1x (ul)
Buffer 5x	3
MgCl2 (25mM)	1.2
dNTPs (1mM)	0.5
Mpy-14F (5 uM)	0.3
Mpy-14R (10 uM)	0.3
Taq (5U /ul)	0.15
H2O	4.55
DNA (1:100)	5
Total	10

Multiplex 1	1x ul
Buffer 5x	3.00
MgCl2 (25mM)	1.20
dNTPs (1mM)	0.50
Mpy-17F (5 uM)	0.70
Mpy-17R (10uM)	0.70
Mpy-19F (5 uM)	0.30
Mpy-19R (10 uM)	0.30
Taq	1.05
H2O	2.25
DNA (1:100)	5.00
Total	10.0





Simplex 7	1x (ul)
Buffer 5x	3.00
MgCl2 (25mM)	1.2
dNTPs (1mM)	0.5
Mpy-7F (5 uM)	0.30
Mpy-7R (10 uM)	0.30
Taq (5U /ul)	0.15
H2O	4.55
DNA (1:100)	5.00
Total	10.00