‘Sinister Southerners’: a trans-continental study of the gall-forming protist genus *Maullinia* on southern bull-kelp (*Durvillaea*).

‘Swiss cheese’: *Durvillaea* sp. nov infected with *Maullinia ectocarpii* at Mallacoota, NSW.

by

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Candidate's Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of the author's knowledge, it contains no material previously published or written by another person, except where due reference is made in the text.

Date: 26/5/16

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Abstract

Species within the brown algal genus *Durvillaea* are major ecosystem engineers throughout the sub-antarctic and cold-temperature Southern Hemisphere. Many provide habitat and sustenance for a range of organisms, and food and fibre for human communities. Any large-scale pathogenic outbreaks could prove devastating on this keystone genus, particularly as species are placed under increased stress in a changing climate. *Maullinia* is a genus of protistan parasites recently found infecting *Durvillaea antarctica* in Chile, however little is known about interactions between these parasites and their hosts. This study examined the biogeography and infection dynamics of the *Durvillaea-Maullinia* (DM) pathosystem, through sampling of intertidal populations of *Durvillaea* sp. nov and *D. potatorum* in Australia (-36'S to -38'S), and *D. antarctica* in Chile (-32'S to -42'S) during austral Spring/ Summer 2015-2016. As previous research suggested *Maullinia* is able to infect a range of algal hosts, it was hypothesised that the parasite would be found infecting *Durvillaea* species throughout the sampling range. It was expected that infection incidence would be highest at northern range limits due to increased physiological stress, and infected hosts would show reduced length due to detrimental pathogen effects. *Durvillaea antarctica* is known to disperse through rafting across the Pacific, and so it was hypothesised that connectivity between distant parasite populations would be reflected in genetic similarity of *Maullinia* samples.

Using genetic, ecological, and morphological approaches, I confirmed for the first time that this parasite genus infects Australian *Durvillaea* species. Phylogenetic analyses based on 18S rRNA data indicated the parasite may have undergone a host shift in Australia and may contain host-specific lineages. No evidence for long-distance dispersal with *Durvillaea* hosts was found, although short-distance dispersal was inferred based on limited phylogeographic structure along Australian and Chilean coasts. Generalised linear modelling showed a consistent trend of increasing infection prevalence towards higher latitudes, which did not support my inference that range-limit stress would increase infection intensity towards hosts' northern range limits. Alternative variables such as host density, temperature, or ecotypes were instead proposed as factors likely to be shaping trends. While infection was not found to relate to host length, seasonal differences in infection incidence and qualitative observations of gall morphology indicate that this parasite has important impacts on *Durvillaea* populations, increasing frond breakage and dispersal into the marine environment. The findings of this research have broad implications, as *Durvillaea* populations will likely be forced southwards in a warming climate and be less able to resist infection. Future research should use a combination of morphological and molecular approaches to determine the temporal dynamics and patterns of contemporary connectivity for the DM pathosystem.

Table of Contents

1.1 *List of Figures*

1.2 *List of Tables*

1.3 *Glossary, Acronyms and Terms*

- **Biogeography**: the study of factors shaping the distribution of an organism over spatial and temporal scales.
- **Dispersal**: the movement of an organism from a place of origin to a new environment, through either passive or active abilities.
- **Genetic marker**: a length of DNA (usually a gene) analysed to identify and describe whether differences between organisms suggest population or species level relationships.
- **Haplotype**: set of genetic characteristics passed through successive generations. Individuals with the same haplotype are closely related. This thesis uses 'haplotype' to refer to unique genetic lineages for the 18S rRNA marker.
- **Infection dynamics:** the study of factors shaping infections (i.e. environmental variables, biotic characteristics).
- **Intertidal:** denotes the coastal area between high and low tides, where organisms are periodically exposed to a range of environmental conditions (i.e. desiccation at low tide).
- **Keystone species:** an organism with disproportionately significant importance for the maintenance of ecosystem structure and function.

- **Parasite:** an organism that lives on a host and requires the host for some aspect food or shelter. A non-mutualistic symbiotic relationship where one organism benefits to the detriment of the other.
- **Pathogen:** an organism or agent that causes damage or illness.
- **Pathosystem:** refers to multiple organisms within their environment. DM pathosystem refers to *Durvillaea-Maullinia* interactions within the host species' ranges.
- **PCR**: polymerase chain reaction, a standard method used to copy segments of DNA for sequencing.
- **Phylogenetic:** analyses of the evolutionary dynamics of organisms using molecular (genetic) information.
- **Phylogeography (phylogeographical):** the analysis of spatial patterning of genetic information
- **Protist(an):** a range of largely unicellular eukaryotic organisms from kingdom Protista.
- **Raft(ing):** long-distance dispersal of floating individuals on ocean surfaces.
- **Sp**.: used when the species-level classification of an organism is unknown.
- **Sp. nov**: new species. Used in this thesis as one species of *Durvillaea* has been described in recent research, but its taxonomy has not yet been published.
- **Spp**.: refers to multiple species within a genus.

Chapter 1: Introduction

In the face of a changing climate, ecologists predict significant global shifts in the abundances and distributions of species. Parasitic organisms are anticipated to increase their infection intensities and geographic ranges with changing environmental conditions (Eggert *et al.*, 2010; Gleason *et al.*, 2013). These increases may prove devastating where infections affect species in lower trophic levels, on whom disease can, in extreme cases, lead to ecosystem collapse (see Mouritsen *et al.*, 2005; Collinge *et al.*, 2008). Despite this, the spatial extent of many parasitic species, and the environmental and biological elements shaping their patterns of infection, remain largely unexplored.

Parasitic protists represent one of the least understood groups of infective organisms, despite their potential for large-scale outbreaks and detrimental impacts on organisms of economic, ecological, and social significance (Foissner, 2006). Such parasites have the ability to disturb entire ecosystems by infecting keystone species (Collinge *et al.*, 2008). This is of serious concern as recent increases in rates of global marine disease are disproportionately affecting kelps (Buschmann *et al.*, 2014; Wernberg *et al.*, 2010), which are central to the structure and nutrient cycling of coastal ecosystems, and the survival of a broad range of species (Pereira *et al.*, 2015).

In this research I used molecular, morphological, and ecological data to analyse the biogeography and infection dynamics of the protistan parasite genus *Maullinia* on its kelp host genus *Durvillaea*, with the complex referred to as the *Durvillaea-Maullinia* (DM) pathosystem. The term pathosystem is used as this study seeks to understand interactions between a parasite, host, and their environment (see Robinson, 1977), rather than describing simple morphological characters such as infection pathways, which have dominated historical parasite research (Eggert *et al.*, 2010).

1.1 *Model system*

Host

Species within the genus *Durvillaea* provide significant ecological and economic benefits through the provision of habitat and food for a variety of organisms, as well as food and fibre for human communities (Abbott, 1996). They are the key determinants of intertidal community structure in the sub-antarctic and cold-temperate Southern Hemisphere (Smith, 2002) (Figure 1a). *Durvillaea antarctica* has cultural and economic significance for the indigenous peoples of New Zealand and Chile (locally referred to as ‘rimu’ and ‘cochayuyo’ respectively) (Figure 1b), and is a component of Chile’s substantial contributions to the world’s brown seaweed products industry (Saavedra, 2011). Until recently, *Durvillaea potatorum* was considered the sole member of the genus found in Australia, being described as a key determinant of the structure, composition and patchiness of population assemblages of rocky intertidal zones on the continent’s south-

eastern coast (Underwood and Kennelly, 1990). The closely related and newly described *Durvillaea* sp. nov, while morphologically similar to *D. potatorum*, occurs in slightly deeper water in south-eastern Australia. It is thought to have diverged from its sister species through separation during geological and oceanic changes in the Pliocene and Pleistocene (Weber, 2015). The genus is being placed under increasing stress with a changing environment (Millar, 2007), and increases in rates of infection for pathogens such as *Maullinia* could prove devastating under future climate scenarios. Impacts may be heightened as parasites are known to disperse long distances on *Durvillaea* hosts (see Fraser and Waters, 2013), suggesting increases in infections in one location could spread to others across vast distances.

(a) **(b)**

Figure 1 (a) <u>Durvillaea antarctica</u> growing in southern Chile; (b) 'Cochayuyo' for sale in a supermarket in Coquimbo, Chile.

Parasite

Maullinia is a genus of protistan parasites (class Phytomyxea) with an obligate intracellular lifestyle, meaning aside from dispersive zoosporic stages they can only survive and reproduce within host cells (Figure 2a). Described on the seaweed *Ectocarpus siliculosus* by Maier *et al.* (2000), *Maullinia ectocarpii* is the only recognised species within the genus, and thus most data are representative of interactions within the *Ectocarpus-Maullinia* pathosystem. The parasite is assumed to have a broad distribution (Neuhauser *et al.*, 2011b), however few data have been collected to confirm this. Previous research has suggested that infections on *Durvillaea antarctica* in southern Chile may be another species (*Maullinia* sp.) (Goecke *et al.*, 2012), presenting as warty, gall-like growths (Figure 2b). Such growths have been implicated in alterations to the metabolic processes, reproductive structures, and mechanical strength and flexibility of a range

of algal hosts (Neuhauser *et al.*, 2011b), suggesting possible detrimental impacts for *Durvillaea* populations.

Previous research by Jahnke (1978) and James et al (1987) (in Aguilera, 1988) suggested the presence of an unnamed gall-forming parasite similar to *Maullinia* sp. on *Durvillaea potatorum* in Victoria, Australia. As *M. ectocarpii* has been confirmed on *E. siliculosus* in Australian waters (Saavedra, 2011), it may be infecting a range of other kelp species in local coastal environments.

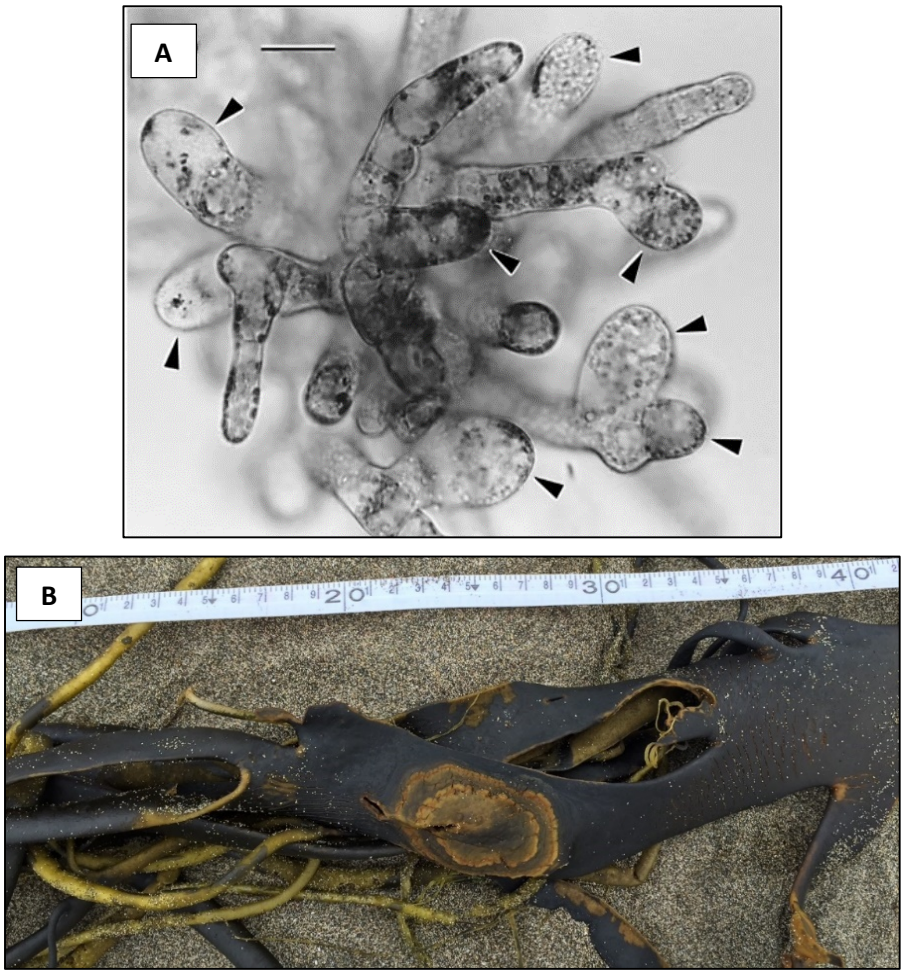


Figure 2: A- Infection of M. ectocarpii on gametophytes of Macrocystis pyrifera under laboratory conditions, showing a range of developmental stages (arrows). Scale bar= 25µm (Maier et al., 2000); B- Gall formed by Maullinia sp. infection on beach-cast Durvillaea antarctica in central Chile.

1.2 *Research direction*

Despite the potential for significant impacts, no research has, to date, sought to analyse the distribution and interactions between *Maullinia* species and *Durvillaea* hosts. This study set out to address the questions:

- **What is the geographical distribution of *Maullinia* spp. on *Durvillaea* hosts?**

- Do infection trends suggest a relationship to environment or host characteristics?

The aim was to examine the infection dynamics, distribution and dispersal characteristics of these two genera, as a model for understanding host-parasite-environmental interactions. A pressing need exists to analyse marine algal-protist interactions, moving away from the historical bias of characterising simple morphological aspects to more broad-scale research (Eggert *et al.*, 2010). As such, the objectives were:

a) To ascertain whether *Maullinia* sp. occurs across the distributional range of its host,

b) To assess whether *Maullinia* sp. is dispersing long-distance on kelp rafts based on population genetic characteristics,

c) To assess whether physiological stress in host populations determines infection incidence along two latitudinal gradients, and

d) To determine whether *Maullinia* infections can be reliably identified in the field based on infection morphology for future monitoring programs.

Objectives were addressed through a trans-continental study of kelp populations across approximately 2000 km of coastline in central and southern Chile, and approximately 500 km of coastline in south-eastern continental Australia. Samples and data originated from two sources:

1. **Intertidal and beach fieldwork**: field sampling occurred at sites accessible for shore-based intertidal work in southern Australia, representative of the continental populations of *Durvillaea potatorum* and *Durvillaea* sp. nov. Sites visited in Chile represented populations of *Durvillaea antarctica* from the 'central' region described by Fraser *et al.* (2010a).

2. **FSES *Durvillaea* spp. tissue collection**: gall-like samples from sub-antarctic locations in the Molecular Ecology laboratory of the Fenner School of Environment and Society (FSES) at the Australian National University (ANU) were used to test the large-scale distribution of *Maullinia* spp. They were hoped to be supplemented through collections made by researchers in other sub-antarctic locales. Samples consisted of infected tissue from *Durvillaea* species that did not amplify as the algal parasite *Herpodiscus durvillaea* from the work of Fraser and Waters (2013), and might thus have been from *Maullinia.*

1.3 *Hypotheses*

Data were used to address the hypotheses proposed and justified in the following sections.

1.3.1 Based on the buoyant nature of the host *Durvillaea antarctica* and its ability to raft long-distance, *Maullinia* sp. will show a broad distribution across the Southern Ocean.

This hypothesis explores the biogeography of the DM pathosystem. Biogeographical methodologies are appropriate for pathosystem analyses as they use genetic, morphological, and ecological data to study interactions between organisms (Gleason *et al.*, 2013) and explain patterns of biodiversity over spatial and temporal scales. Applications of biogeographic principles in pathosystem research remain limited however, as many have argued that the distribution of a parasite is by definition limited to the distribution of its host (Hoberg and Klassen, 2002; Poulin *et al.*, 2011b). This fails to recognise the role of the environment, which interacts with the distribution of species through two primary mechanisms: dispersal and vicariance.

Dispersal biogeography concerns itself with how organisms move from a point of origin to new environments through intrinsic active abilities or the passive use of vectors (Gillespie *et al.*, 2012). Vicariance analyses look at how species' biogeographies are shaped by separation due to physical environmental factors (e.g. mountains) (Stigall, 2012). With time, both mechanisms can alter the genetic composition of populations and communities, such that speciation may occur if the flow of genetic material is significantly reduced or blocked (Figure 3) (Stigall, 2012).

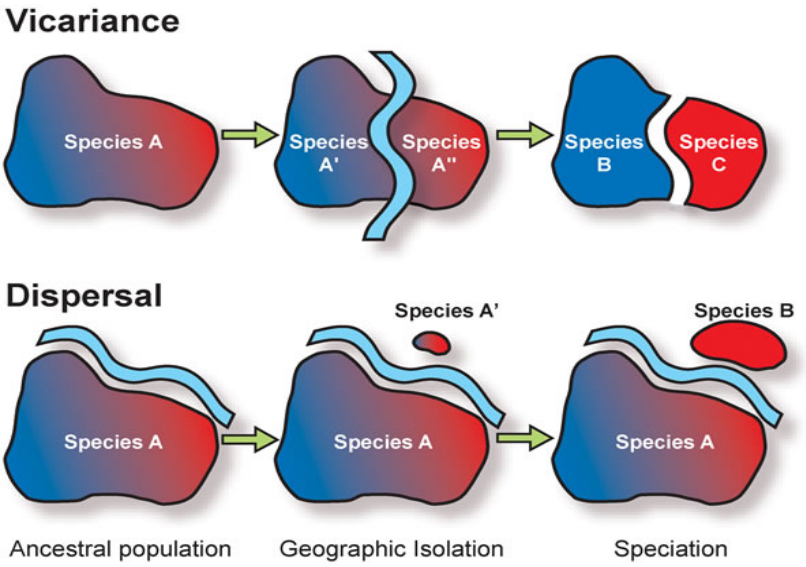


Figure 3 Illustration of vicariance and dispersal effects leading to speciation (Stigall, 2012)

Organisms with poor intrinsic dispersal capacities may passively disperse on highly-mobile organisms ('vectors') (Thiel and Gutow, 2005; Smith, 2002; Johannesson, 1988; Nikula *et al.*, 2011). This suggests a need to consider how factors external to the life-history of a species shape its biogeography. While historical marine research characterised organisms' distributions

as depending on dispersal in larval stages, increasing evidence suggests alternative forces may be at work (Hedgecock, 1986; Nikula *et al.*, 2011; Bradbury *et al.*, 2008; Cowen and Sponaugle, 2009; Johannesson, 1988). Vectors for trans-oceanic dispersal include wind, currents, birds (Gillespie *et al.*, 2012), human activity (e.g. shipping vessels) (Padilla *et al.*, 1996), and floating algae including *Durvillaea antarctica* (Thiel and Gutow, 2005). As such, epibiota found on this kelp species may be transported to new environments through passive dispersal events.

1.3.2 Due to the high dispersal capacity of *D. antarctica*, populations of *Maullinia* spp. will be connected and show genetic similarity over large distances.

Dispersal events may be 'read' through genetic analyses in order to understand biogeographical processes (Avise, 2009), as information collected from existing populations can be interpreted to infer past phenomena. For instance, genetic dissimilarity between populations may suggest long-term separation of organisms across a range of distances (Figure 3). While few morphological characters are comparable between all living organisms, a number of genes can be found across all species and sequenced for comparison (Hillis and Dixon, 1991).

DNA barcoding through Polymerase Chain Reaction (PCR), a widely used method in biogeographical research, compares short genetic sequences from the same genomic region and can be used to identify organisms based on the idea that genetic variation between species exceeds that within species (Hajibabaei *et al.*, 2007). Sequencing a known gene and comparing it to a library of similar genetic information allows researchers to positively identify an organism, detect new species, and occasionally reveal co-speciation, colonisation or invasion events (Hajibabaei *et al.*, 2007; Criscione *et al.*, 2005; Emerson and Hewitt, 2005; Blakeslee *et al.*, 2008). While the approach has caused tension between ecologists and taxonomists (Moritz and Cicero, 2004; Weisse, 2008), it has generated a wealth of information regarding species delineations, distributions, and phylogenetic processes. This is particularly important for the study of protist organisms, whose genetic variability, size and morphology often make it difficult to distinguish between species (Sogin and Silberman, 1998; Foissner, 2006; Cumming *et al.*, 2014).

A lack of distributional data for microorganisms such as *Maullinia* has led to the use of two descriptive terms in protist biogeography. 'Cosmopolitan' is used to describe microbes that should be found everywhere one would find suitable habitat, due to their small sizes, high numbers, and high dispersal ability (Foissner, 2006). Moderate endemicity, in contrast, conceptualises protist species as having limited ranges like other organisms. Of the 300,000 extant species of protists, it is believed up to a third have restricted distributions (Foissner, 2008), and genetic data have begun to support this idea (Lara *et al.*, 2011). These data have, however, remained largely constrained to terrestrial species, despite recognition that distributions and

infection dynamics on land may not accurately represent patterns seen in the world's oceans (Eggert *et al.*, 2010).

Maullinia is widely described as a cosmopolite, however few data have been collected to support this proposed distribution. DNA barcoding of samples of diseased host tissue may assist in understanding the genus' biogeography. 'Cosmopolitan' fails to recognise that variability is often seen in species abundance within their distribution, which is largely shaped by environmental factors.

1.3.3 Infection incidence will increase towards host range limits due to increased host physiological stress.

Several factors have been implicated in shaping patterns of infection for pathosystems, most notably related to spatio-temporal variations in climatic features (Eggert *et al.*, 2010). This hypothesis seeks to analyse the infection dynamics of the DM pathosystem, examining the role of the environment in shaping species distributions and intensities. While the seasonality of infectious disease has received significant attention, particularly in light of fluctuations in temperature (Andrews, 1976), the impact of non-seasonal climate variation throughout species' ranges remains less well understood. Within the range of an organism, variations in environmental conditions affect rates of survival and reproduction, with changes in latitude associated with alterations in climatic factors (Tuya *et al.*, 2012), species taxonomy, and even rates of parasitism (Poulin *et al.*, 2011b). At the extremes of a species' latitudinal range, physiological stress is often highest, making it more susceptible to infection by pathogens (Hopper *et al.*, 2014).

Range effects are well-recognised for algal organisms (Peters and Breeman, 1993), and variation in environmental conditions is known to affect the survival of *Durvillaea* species (Tala *et al.*, 2013; Cruces *et al.*, 2012). As such, the DM pathosystem represents an ideal model for understanding latitudinal infection dynamics and their impacts on component species.

1.3.4 Infected and uninfected hosts will show differences in size due to stress from pathogen interaction.

Morphological impacts often result from pathogenic interactions. Parasites can affect the metabolism of their hosts (Neuhauser *et al.*, 2011b), leading to changes in growth rates, reproductive potential, and dispersal. *Maullinia* causes hypertrophies of host tissue ('galls') which may lead to a reduction in growth potential as nutrients are preferentially distributed to infected tissue.

1.4 *Thesis structure*

Acknowledging the aims and hypotheses of this research, Chapter 2 reviews literature relevant to the distribution and infection dynamics of parasites including protists in order to develop a theoretical understanding of the biogeography of the DM pathosystem. Range stress literature is analysed to explore the relationships between pathogens, hosts, and their environment; and previous research related to the DM pathosystem is summarised to contextualise this thesis' contribution to marine pathosystem research. Chapter 3 describes the methods used in field, laboratory, and statistical work, with results presented in Chapter 4. The findings of this thesis are discussed in Chapter 5, with conclusions drawn and suggestions for future research summarised in Chapter 6.

Chapter 2: Literature Review

This chapter will review how biotic and abiotic factors interact to shape the intensity and distribution of parasitic infections, and how understanding these factors may assist in predicting the biogeography of a pathosystem. How these elements change within species ranges will be explored, with particular emphasis placed on the relationship between range stress and latitude. Finally, current findings related to components of the DM pathosystem will be described to illustrate how species-specific characteristics interact with factors of interest in this research.

2.1 *The development and spread of pathogens: host biology and environmental parameters*

Patterns of infection within a given pathosystem depend on two elements: characteristics of the host and pathogen, and the characteristics of the environment in which they operate. Anderson and Gordon (1982) describe these elements as 'demographic stochasticity', where chance factors act to shape population growth, and 'environmental stochasticity', where parameters such as climate alter rate processes (deaths/ births/ infections) over temporal or spatial scales. The interactions of a wide range of biotic and abiotic ecological factors influence parasite distributions (Kennedy and Bush, 1994) (Figure 4), as outlined in the following sections.

2.1.1 Biotic factors

Interactions within pathosystems are complex, with hosts and parasites affecting, and being affected by, each another (Figure 4). The factors shaping these systems are dynamic and represent opposing forces over time (Anderson and Gordon, 1982). The 'biological arms race' between parasites and hosts constitutes a co-evolving system where each organism's prevalence is dependent on the other. Biotic control of pathosystem distribution is defined by two factors: parasite virulence and host resistance.

Parasite virulence

A parasite's virulence (i.e. infection potential) can be understood through its host range and specificity, with heterogeneity in virulence considered a key factor contributing to the spectrum of disease severity seen across the globe (Gupta *et al.*, 1994).

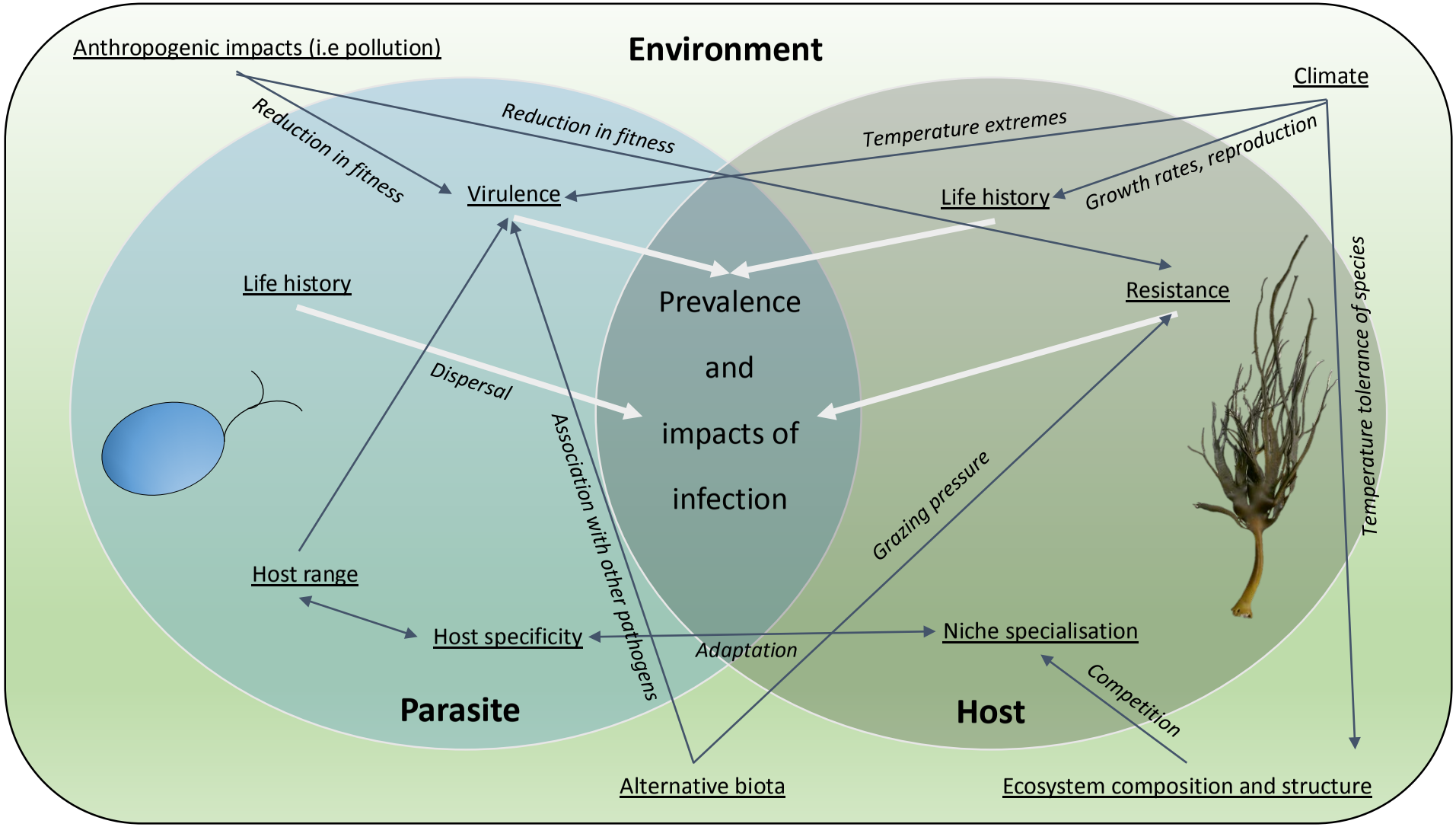


Figure 4 Illustration of some of the complex host-parasite-environment interactions affecting the prevalence and impacts of infection in an algal pathosystem. Example interactions are written in italics.

Host specificity

Host specificity is the prevalence or intensity of infection of a parasite for a particular host type (Rohde, 2002) or the genetic lineages able to infect specific hosts (Poulin *et al.*, 2011a) (i.e. whether a parasite species can infect only one, or several host species). The concept has received significant academic attention, particularly with regard to how it affects species' geographical ranges. As a general trend, parasites with lower host specificity show broader ranges (Krasnov *et al.*, 2008), however this trend is complicated by various characteristics of parasite and host.

While several studies have examined marine algal pathosystem dynamics, a substantial body of literature has analysed the host specificity of *Eurychasma dicksonii*, a 'cosmopolitan' oomycete affecting a range of seaweeds and kelp including species of *Ectocarpus*. The parasite exhibited differential rates of development between hosts from differing geographical locations (Müller *et al.*, 1999), with the strong possibility of host genetic factors affecting rates of infection (Gachon *et al.*, 2009). Variability in infection trends on differing hosts supported the notion that a parasite's host specificity is based on a range of biological and environmental factors (Rohde, 2002), which proved of particular interest considering its broad distribution and large host range.

Host range

The host range of a parasite refers to the variety of organisms it is able to infect (Rohde, 2002). The broad host range of *E. dicksonii* (Müller *et al.*, 1999; Kupper *et al.*, 2006) is often proposed as a reason for its broad geographical distribution, based on a widely accepted paradigm that organisms infecting a range of hosts show greater geographical spread. Simply analysing a parasite's host specificity may not accurately predict its distribution, however, as 'rare' infections in a particular host may act as a bridge to species more suited to the pathogen's proliferation.

Parasites with large host ranges may create linkages between populations of common hosts through other host species. Research on *Kudoa thyrsites*, a myxozoan parasite of marine fishes (Whipps and Kent, 2006), provides insight into how a pathogen's host range and specificity can influence its biogeography. While most species within this genus are highly host specific, infecting fishes of a particular family or even species, *K. thyrsites* has been identified on 37 different hosts from 18 different families in largely similar intensities. Findings suggest that this broad host range and low host specificity promoted the species' broad geographical distribution, homogenising populations within, but not between, oceanic regions (Whipps and Kent, 2006). It remains possible for species exhibiting high host specificity to increase their geographical range, as primary hosts are supplemented by low-intensity infections in persistent alternatives (Neuhauser et al., 2014). As such, predicting the potential impacts and distribution of parasitic

organisms based on their host range and specificity is often inadequate as organismal interconnectivity has impacts on virulence in pathosystems.

For instance, hyperparasites, or organisms that parasitise parasites, have been implicated in controlling pathogen virulence, population size and diversity (Gleason *et al.*, 2014), altering pathosystem trends. Debate continues as to whether organisms associated with parasites decrease virulence or increase rates of infection (Neuhauser *et al.*, 2014; Maier *et al.*, 2000; Parodi *et al.*, 2010), particularly for organisms such as the Phytomyxea, which are known to transmit viruses that may weaken hosts and facilitate further infection (Parodi *et al.*, 2010). The multiple scales on which parasitism occurs may complicate patterns of infection, particularly when hosts present some capacity to resist pathogenic attack.

Host resistance

The ability of an organism to resist infection depends on a range of morphological and genetic characteristics, and these characteristics shape pathosystem distribution. Several factors are considered of particular importance to host resistance in marine algal pathology, including damage to tissue (wounds), population density (crowding), and life stage or genetic lineage (Andrews, 1976).

For systems in which primary producers such as kelp act as hosts for a pathogen, resistance is often decreased due to other biota existing within the system. Faunal grazing of algae has been described as a key factor determining primary producers' abilities to survive in high energy wave environments, as algal species break at sites of grazing damage (Koehl, 1979). It has long been assumed that the structure, health and composition of Australian kelp assemblages are primarily shaped by grazing pressure (Underwood and Kennelly, 1990), with fully developed, non-damaged algal individuals believed to be most resistant to infection in populations where crowding does not promote the transmission of disease. Despite this, data have indicated that pathogens can infect hosts with a range of morphological characteristics, suggesting genetic factors may act in concert with morphological and ecological factors to drive patterns of infection for a range of pathosystems (Müller *et al.*, 1999).

Analyses of kelp pathosystems have revealed strong evidence for a molecular basis to host resistance, supporting the idea that particular species or lineages of marine algae may have an ability to resist infection based on genetic composition (Gachon *et al.*, 2009; Gachon *et al.*, 2010; Carius *et al.*, 2001). The possibility of *Eurychasma dicksonii* presenting differential rates of infection on brown algal hosts based on their lineage (Gachon *et al.*, 2009), has been inferred to indicate that host-pathogen coevolution may have shaped patterns of infection. As such, knowledge regarding the spatial distribution of genetic information between host populations is

crucial to conserving kelp biodiversity, as genetic determinism is increasingly seen as a key driver of pathology patterns (Hoberg and Klassen, 2002; Alberto *et al.*, 2010).

When analysing infection trends in a given pathosystem, it must be recognised that morphological differences between system components may interact with underlying genetic factors. All of these factors are in turn shaped by the environment in which they operate, affecting the distribution and intensity of infection on a range of scales.

2.1.2 Abiotic factors: the role of the environment

Recognition of the critical role of the environment in shaping pathosystem trends has led many to assume that a pathogen found in a particular environment will be found in all areas with similar conditions, resulting in inaccurate descriptions of species distributions (Hoberg and Klassen, 2002; Weisse, 2008). Abiotic factors operate on a number of scales, with localised factors such as pollution or mechanical damage interacting with broader conditions, such as climate, to shape marine algal pathosystem trends.

A number of population-scale environmental parameters affect host susceptibility and pathogen virulence, including pollutants, mechanical habitat alterations, and fishery practices (Lafferty and Kuris, 1999; Crowe *et al.*, 2000). Environmental factors are a 'balancing act', in that the same parameter may improve conditions for a parasite or host, or lead to its decline. For instance, habitat alterations from the removal of biomass may alter ecosystem structure in such a way as to reduce or increase the prevalence of hosts implicated in a particular pathosystem, with flow-on effects on the prevalence of parasites and organisms in other trophic levels. While marine pollution has been implicated in a reduction in fitness for a range of algae (Li *et al.*, 2010) and is expected to increase in importance into the future, further research is needed to understand the implications of pollution for the prevalence of disease. Temperature remains the most studied abiotic factor implicated in marine parasite dynamics.

Temperature effects

Temperature plays an important role in determining how infections develop on local and regional scales. Both host and parasite have 'optimal' temperatures in which they can exist, with the overlap of these representing the ideal temperature range for a pathosystem. While it is widely proposed that the requirements for obligate, endobiotic organisms follow those of their host, this concept lacks empirical support and requires further research (Neuhauser *et al.*, 2011b).

Temperature effects have been found for patterns of larval development and dispersal in parasites and hosts, suggesting broad implications for long distance population connectivity in a range of taxonomic groups (Bradbury *et al.*, 2008). Thermal effects on the long-term survival of planktonic larvae suggest the survival of organisms with cysts disseminated into the marine environment is highly dependent on the temperatures they experience (Cowen and Sponaugle, 2009). A large

body of literature has analysed kelp susceptibility to heat stress, suggesting that responses can vary both between and within species, having direct impacts on organismal distributions and rates of colonisation (Pereira *et al.*, 2015). Responses also vary over different temporal scales, as seasonal changes alter distributions of infection over periods of months, while broader climatic changes may impact over years (Andrews, 1976). For instance, seasonal fluctuations measured in densities of diseased thalli for the alga *Iridaea laminarioides* infected with an endophytic bacterium were linked to temperature effects and the dislodgement of kelp during winter storms (Correa *et al.*, 1993), suggesting climate and weather may both interact to shape pathosystem trends.

On broader temporal scales, a changing climate has received significant attention regarding possible impacts on pathosystem development. While numerous predictions have been made about how terrestrial pathogen systems will respond to a changing climate, their marine counterparts remain less well understood (Eggert *et al.*, 2010). Predictions based on existing data suggest more frequent and intense disease outbreaks as pathogens' growth rates accelerate, more survive winter seasonal changes, and hosts become stressed due to shifts in their 'optimum' ranges (Eggert *et al.*, 2010). It has been suggested that currently under-studied parasites, including the Phytomyxea, may develop into 'devastating' future diseases with a warming marine environment (Gleason *et al.*, 2013; Neuhauser *et al.*, 2014), suggesting a need to understand what factors shape infection trends to control outbreaks.

Environmental variables may interact with host and pathogen biotic factors to shape patterns of infection. Research has shown anthropogenic and natural elements act in concert to determine the intensity of pathogen infection, with broader environmental factors implicated in where pathosystems are found. Understanding how the variability of such factors alters infection trends is key to determining the relative contribution of biotic and abiotic elements in pathosystem research.

2.2 *Patterns within patterns: variability in biogeography*

Population-level patterns of infection reveal important characteristics that shape the broad-scale distribution of a pathosystem. Rohde (2002), in reviewing the biogeography of marine parasites, identified that infections may be shaped along gradients and indicate dispersal routes, biogeographical regions, and barriers. Understanding variability of infection intensities within the distribution of the DM pathosystem may help us to discover how environmental effects structure the biogeography of both host and parasite.

2.2.1 Range stress

Environmental parameters change along gradients, having direct effects on the distribution of a species. Range stress, the physiological stress increasingly experienced by an

organism towards its distributional limit, operates on a number of scales, and is known to play a role in shaping organismal biogeography. For instance, range stress is known to operate in the zonation of rocky shorelines, with the community composition of shallow intertidal areas being vastly different to those submerged for most of the day due to differential ability to survive desiccation (Pereira *et al.*, 2015). Similarly, changing distributional characteristics within species ranges reflect different abilities to cope with environmental conditions. Central Abundance Theory, which states that species are most abundant at the centre of their geographic range and rarer towards their range limits, is empirically well-supported for a large number of organisms (Samis and Eckert, 2007) although exceptions do exist (Hochberg and Ives, 1999). This concept aligns with Range Limit Theory, which conceptualises an organisms range limit as the expression of its ecological niche in space, beyond which no living individuals occur due to increasing ecological stress (Sexton *et al.*, 2009). Its effect on a host's resistance to infection is of particular interest, as hosts at their latitudinal range limit may be more susceptible to infection (Gleason *et al.*, 2014).

Many studies have supported the notion that species inhabiting areas near their climatic pessimal may be more susceptible to pathogen infection, drawing direct links between range limits and bioclimatic suitability (Rödder *et al.*, 2008). For instance, increasing sea temperature towards an algal species' range limit in Western Australia was directly linked to the resilience of kelp beds, with impacts on the species' responsiveness to perturbation, canopy recovery, and juvenile recruitment (Wernberg *et al.*, 2010). Similarly, research exploring how habitat structure transitions along a latitudinal gradient on the Portuguese coast showed abrupt changes in kelp composition related to sea surface temperature (Tuya *et al.*, 2012), suggesting susceptibility to local conditions. This susceptibility to conditions within species ranges would suggest parasite infection incidence would increase in areas under highest stress.

Despite this, Levins (1968) (in Sexton et al., 2009) postulated that organisms at their range limit may have an increased capacity to resist infection due to the natural selection of individuals with greater adaptive plasticity. 'Ecotype' effects, whereby organisms existing in adverse conditions show behavioural, morphological, or genetic adaptations to survive (Gao *et al.*, 2012), have been shown for a range of organisms (e.g. Mitchell *et al.*, 2005). This further supports the concept of 'parasite escape', whereby organisms at their range limits may show reduced pathogen loads due to intrinsic abilities to resist infection or conditions adverse to the survival of the parasite (Hopper *et al.*, 2014). In fact, as species' ranges change with a changing climate, research has suggested that certain hosts may be able to 'escape' their pathogens as the movement of parasites may occur at a different speed (Hopper *et al.*, 2014). Factors shaping pathogen infections within species ranges are a 'balancing act' (Lafferty and Kuris, 1999), and research must recognise that variables including range stress may act to promote or suppress the spread of a pathosystem by acting on both host and parasite. A growing body of research has also shown numbers and diversity of

marine parasites fluctuate based on gradients of depth, longitude, and latitude. As such, there exists a need for further large-scale research into geographical variation in pathosystem infection trends (Rohde, 2002).

2.2.2 Latitude and parasitism

While much research has analysed the role of latitudinal gradients in shaping parasite biodiversity, showing species richness increasing towards lower latitudes (Lindenfors et al., 2007), less has considered how the same gradient may influence the distribution and intensity of infections. Rapaport's rule, which relates niche breadth with latitude, proposes species have broader geographic ranges at higher latitudes, and has been supported in some parasitic systems (Krasnov *et al.*, 2008). Its application to parasitic species has suggested those at higher latitudes may show less host specificity, and as such broader ranges and greater infection intensity, than their equatorial counterparts. However, variability is still often seen in the incidence of parasite infections across species ranges, suggesting interactions with local conditions (Poulin *et al.*, 2012). Parasites with little variation in abundance along environmental gradients may have lower rates of local extinction (Poulin *et al.*, 2012), and as such identifying and seeking to explain variability in infection trends is highly important.

It has been suggested that parasitism may directly shape the distribution of hosts (Correa, 1996), however data support competition and abiotic factors being of greater importance in promoting latitudinal trends (Sexton *et al.*, 2009). Parasites and predators have also been proposed as factors implicated in expanding the range of their prey by pushing them to colonise new areas to survive (Holt and Barfield, 2009), suggesting the dispersal of host and parasite are particularly important in determining their survival as range limits shift in a changing climate (Eggert *et al.*, 2010). With the distribution of some species shrinking and others expanding, further data are needed to understand how dispersal events shape pathosystem biogeography.

2.2.3 Dispersal capacity

Dispersal is central to variability in the emergence of disease (Suzuki *et al.*, 2015), with the dispersal ability of both host and parasite interacting to shape the broad-scale distribution of infections. High dispersal capacities may lead to the homogenisation of parasite communities along host ranges (Poulin *et al.*, 2011b). It remains unclear whether hosts with large dispersal abilities are able to escape parasites from within their range or rather transport pathogens to new populations, increasing the geographical spread of the pathosystem (e.g. Fraser and Waters, 2013). It has been argued that dispersal can both increase the persistence of pathogenic species through supplementing infections in areas of low abundance, or lead to a parasitic species' decline as homogenised populations are more likely to be uniformly adversely affected by stochastic processes (Abbott, 2011). As such, model systems are needed to analyse the factors involved in shaping parasite dynamics.

2.3 *Bull-kelp and their pathogens: understanding the DM pathosystem*

The DM pathosystem represents an ideal model for understanding biogeographic and parasitological processes. The host genus *Durvillaea* has a large distribution across the southern hemisphere, and its ecological and economic significance has prompted much research into factors such as the role of rafting in dispersal. *Maullinia* remains a largely understudied genus of protists, with previous research concentrated on understanding infection pathways rather than broader geographical trends.

2.3.1 The genus Durvillaea

The genus *Durvillaea*, commonly referred to as 'southern bull-kelp', has been the focus of a number of research projects seeking to understand how kelp systems allow complex ecological communities to persist (Kennelly, 1987).

Species ranges

Fraser *et al.* (2010b) described nine distinct evolutionary lineages within the genus, with several clades existing within the five species recognised by Hay (1994) (in Fraser *et al.*, 2010b). Several species, such as *Durvillaea potatorum* and *Durvillaea* sp. nov, have restricted continental distributions within Australasia, with the latter species found along the south-eastern coast of New South Wales, Victoria, and Tasmania (Weber, 2015), and the former western Victoria and Tasmania (Figures 5 and 6). The ranges of these species are believed to be shrinking due to warming ocean temperatures, with the current northernmost limit of *Durvillaea* sp. nov occurring at Tathra in southern New South Wales (Weber, 2015). Conversely, *Durvillaea antarctica* has a broad, circum-polar distribution containing five distinct clades (Fraser *et al.*, 2010b). For further detail regarding the phylogenetic placement and geographical range of species and clades within the genus, see Fraser *et al.* (2010b).

Based on the broad geographical range of the genus *Durvillaea,* a Southern-Hemispherical, cold-temperate distribution may be expected for the DM pathosystem if *Maullinia* species are able to reach disjunct populations and infect a range of species. Connectivity may be promoted through some species in the host genus being able to raft long-distance (Fraser *et al.*, 2010b), allowing the dispersal of epibiota (including parasites) able to survive rafting events.

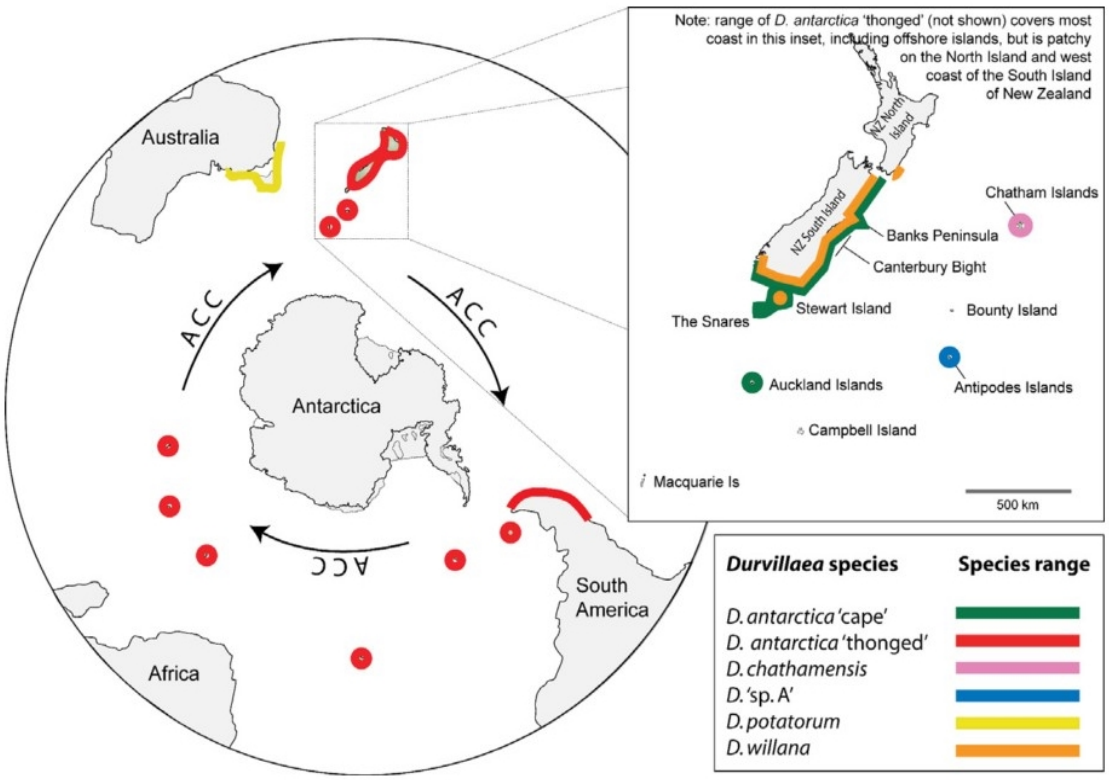


Figure 5 Geographic ranges of Durvillaea species (Fraser et al., 2010)

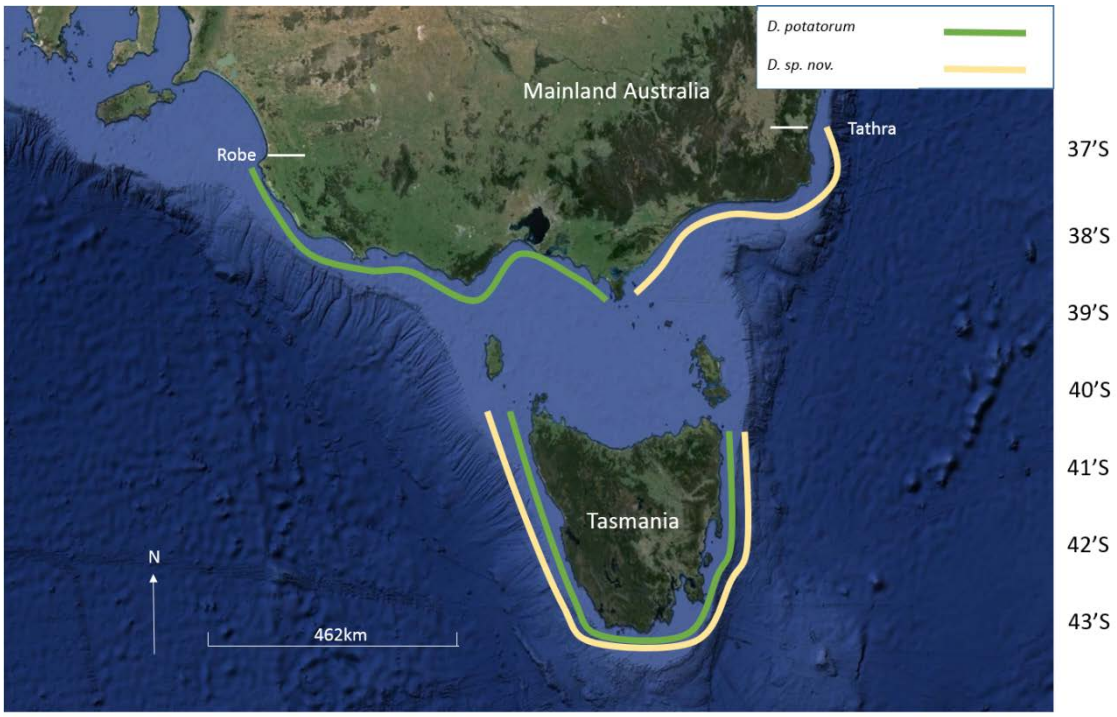


Figure 6 Ranges of Durvillaea potatorum and Durvillaea sp. nov in Southern Australia, based off Weber (2015) (Google Earth, 2015b)

Rafting ability

Molecular and ecological data have suggested that *Durvillaea antarctica* is capable of rafting long distances over extended periods of time due to the 'honeycombed' structure found in the tissues of certain clades (Smith, 2002; Fraser *et al.*, 2009b).

The wide geographic distributions of a range of organisms with limited ability for autonomous dispersal have supported the importance of marine algal rafts in shaping species distributions (Thiel and Gutow, 2005). Cumming *et al.* (2014) describe long-distance genetic similarity between populations of kelp-associated biota as evidence of the role of rafting in the marine environment. They implicate rafting on macroalgae such as *Durvillaea* in the connectivity and gene-flow of populations of kelp-associated organisms such as chitons, adding to existing knowledge regarding the role of rafting in the re-colonisation of sub-antarctic areas by *Durvillaea* species post glaciation (Fraser and Waters, 2013). Rafts of *Durvillaea antarctica* have, on rare occasions, also been found on the Australian coast where the species does not grow (Moore and Cribb, 1952), suggesting long-distance population connectivity.

Pathology

This is particularly interesting considering several factors are known to affect the structure and health of *Durvillaea* populations. As the primary producer in a number of marine ecosystems, the genus experiences significant grazing pressure which may damage tissue and weaken individuals, altering the structure of communities (Underwood and Kennelly, 1990) and producing a greater number of rafts. The high energy wave environment in which the genus exists both suppresses competition through whiplash effects (Taylor and Schiel, 2005) and removes individuals weakened by damage (Kennelly, 1987). Like other macroalgae, the growth and reproductive status of *Durvillaea* species are linked to seasonal conditions such as temperature and solar radiation, both when attached to the shore and when rafting (Tala *et al.*, 2013).

Few parasites are allied with the genus, with most pathological investigations to date concentrating on highly conspicuous infections of the algal parasite *Herpodiscus durvillaeae* (Saavedra, 2011). Molecular research on this parasite supports the notion of *D. antarctica* transporting epibionts long-distance in the pacific (Fraser and Waters, 2013). As such, the possibility exists for other parasites, such as the recently identified *Maullinia* sp., to disperse long-distance on *D. antarctica* rafts.

2.3.2 The genus *Maullinia*

Infection dynamics

Host range and specificity

The broad host ranges of *Maullinia* and other Phytomyxids on marine algae have been proposed to promote increased infection severity under future climate scenarios (Neuhauser et al.,

2014). A large range of filamentous brown algae are susceptible to infections of *Maullinia* (Maier *et al.*, 2000; Goecke *et al.*, 2012), leading to proposals of a broad geographical distribution on southern-hemispherical cold-temperate coasts. The organism's two types of zoospores and complex life cycle have the potential to infect different host tissues at different times (Parodi *et al.*, 2010), suggesting host specificity may change over time. The relative severity of infection within and between hosts, however, remains to be quantified.

Infection pathway

The genus *Maullinia* presents similar infection characteristics to other phytomyxids, with a complex life cycle and association with viruses (see Maier *et al.*, 2000; Parodi *et al.*, 2010). It produces motile zoospores which disperse and infect new hosts (Neuhauser *et al.*, 2011b) (Figure 7), where infections cause gall-like hypertrophies in host tissue. The production of resting spores suggests the parasite may be able to passively disperse long-distance and survive adverse conditions (Neuhauser *et al.*, 2011b). While some research has described Phytomyxids- including *Maullinia*- as parasites that infect hosts without directly leading to mortality (Neuhauser *et al.*, 2014), on flexible species such as *Durvillaea antarctica* the formation of galls has been hypothesised to reduce the host's ability to survive in high-energy wave environments (Eggert *et al.*, 2010). *M. ectocarpii* has been shown to heavily infect the gametophytes of algal species such as *Macrocystis pyrifera* (Maier *et al.*, 2000), which may have implications for the reproductive potential of its host. The virulence of *M. ectocarpii* may also relate to its association with a range of viruses, much like other Phytomyxids (Neuhauser *et al.*, 2014), however previous infections on filamentous algae have been shown to be independent of viral infection, suggesting viral association to be of limited importance (Maier *et al.*, 2000).

While data regarding the host range, life cycle, and infection pathway of *M. ectocarpii* are well characterised, little is known about the factors that shape its virulence in natural populations. Aguilera (1988) found differences in infection characteristics for an unclassified organism likely to be *Maullinia* sp. between adult and juvenile kelp in Chile, suggesting adult plants are infected to a greater severity than their younger counterparts. The author further suggested rates of infection increase towards southern latitudes, calling for more research to determine how the parasite acts on broad scales.

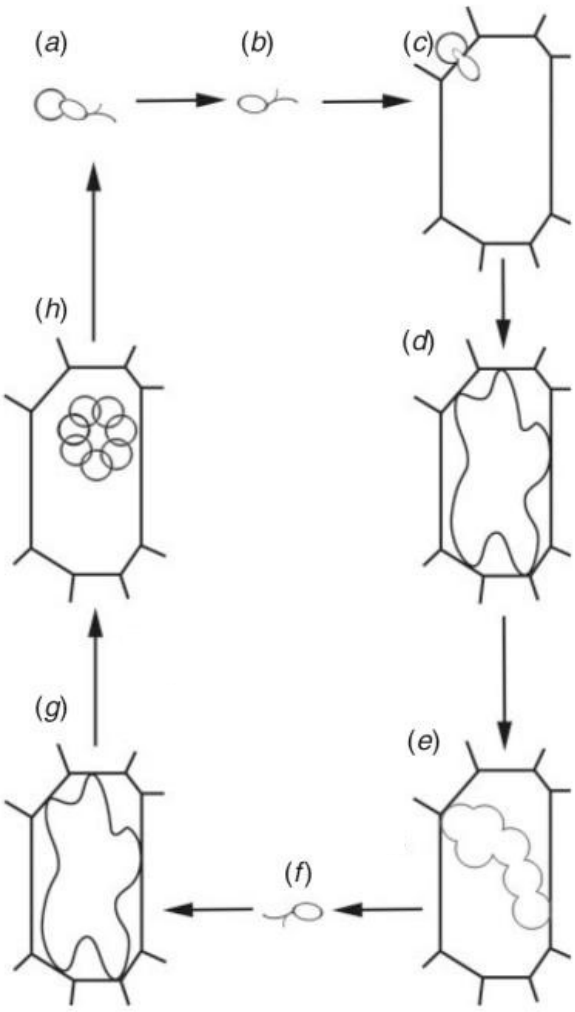


Figure 7 Diagrammatic representation of the life cycle of phytomyxids such as Maullinia ectocarpii, showing dispersive zoosporic stages: (a) release of primary zoospore from resting spore, (b) dispersal of zoospore, and (f) secondary zoospore (Neuhauser et al., 2011b)

Distribution

While several studies have analysed the phylogenetic placement and lifecycle of *Maullinia ectocarpii*, the samples have originated from few geographical locations. The parasite found on *Durvillaea antarctica* hosts has received less attention than that associated with *Ectocarpus siliculosus*, which has been confirmed in single areas in Australia and Chile (Figure 8). Academic literature discussing areas in which *Maullinia* organisms have been confirmed are presented alongside highly likely sightings in Table 1, providing a baseline description of the organism's distribution. Although *Durvillaea* species occur from 36'S southwards in Australia, and 30'S southwards in Chile, to date infections on this genus have only been recorded at 38'S (Australia) and around 41'S (Chile).

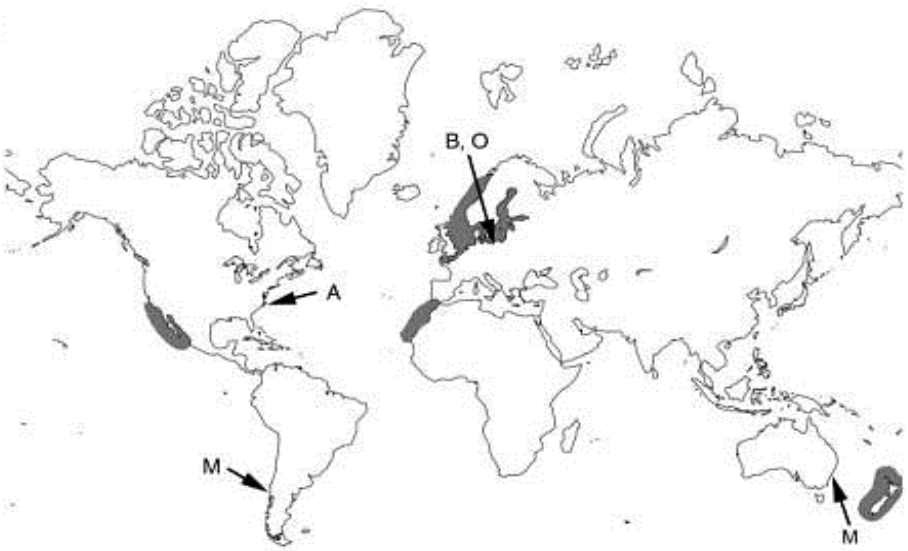


Figure 8 Map of known distribution of Phytomyxean parasites (M=Maullinia ectocarpii; B= Phagomyxa bellerochae; A= Phagomyxa algarum; O= Phagomyxa odontellae) (Neuhauser et al., 2011b)

Table 1 Summary of literature related to Maullinia spp. infections

Reference	Discipline/ approach	Sample origin	Host	Confirmed / highly likely
Jahncke (1978)	Microscopy	Sorrento, Victoria, Australia	*Durvillaea potatorum*	Highly likely
James *et al.* (1987) (unpublished- in Aguilera et al, 1988)	n/a	Australia	*Durvillaea potatorum*	Highly likely
Aguilera *et al.* (1988)	Ecology/ microbiology	Valdivia, Chile Concepcion, Chile Mehuin, Chile	*Durvillaea antarctica*	Highly likely
Maier *et al.* (2000)	Microscopy and description of life cycle	La Pasada, Chile Flinders, Victoria, Australia	*Ectocarpus siliculosus*	Confirmed
Parodi *et al.* (2010)	Microscopy, analysis of ultrastructure	Maullin city/ river, Chile	*Ectocarpus siliculosus*	Confirmed.
Goecke *et al.* (2012)	Phylogenetics/ ecology	Coliumo bay, Chile	*Durvillaea antarctica*	Confirmed

Genetic diversity

Goecke *et al.* (2012), who first described *Maullinia* sp. infections on *D. antarctica* in Chile, suggested the possibility of the organism representing a novel species. Such a claim is difficult to confirm, however, as the genotypic diversity for a range of protists- including Phytomyxea- remain largely unknown (Weisse, 2008). Phytomyxean organisms are severely under-represented in genetic databases and environmental samplings (Bulman *et al.*, 2007; Neuhauser *et al.*, 2014; Burki *et al.*, 2010), and organisms closely related to *Maullinia* such as *Plasmodiophora brassicae* are known to present high numbers of introns that complicate genetic analyses (Bulman *et al.*, 2007). As a result, it has been difficult to determine whether distinct species occupy geographical areas, as levels of genetic variation within and between species are largely unknown.

2.4 *Literature review conclusion*

The biogeography and infection dynamics of an organism or pathosystem are shaped by a number of factors. Morphological, behavioural and genetic factors interact with the environment to shape infections on local scales, with changes in these conditions along gradients shaping the distribution and intensity of infection. These trends may be analysed through both ecological and genetic data, providing insight into contemporary and historical events shaping where the organisms are found today, and allowing predictions as to where they may be found in the future. Despite the potentially devastating effects of large-scale *Maullinia* infections on the keystone genus *Durvillaea*, many of these data remain lacking for components of the DM pathosystem, pointing to the need for contemporary research.

Chapter 3: Methods

3.1 *Field work*

Sampling for this research was undertaken in Australia and Chile to replicate latitudinal effects on three closely-related species from continents within the same ocean basin, seeking to analyse the dynamics of the DM pathosystem.

3.1.1 Site selection

Australia

Based on the distribution of *Durvillaea potatorum* as described in Millar (2007), Cheshire and Hallam (1988), and the Atlas of Life in the Coastal Wilderness (Atlas of Life in the Coastal Wilderness, 2014), several areas were identified for potential sampling. Millar (2007) describes the northern range limit of the species existing at Tathra (NSW), and this was selected as the first sampling location. Jahnke (1978) discussed infections likely to be *Maullinia* spp. on *D. potatorum* at Sorrento (Vic) (Table 1), and this was designated as the final sampling location.

Sites were selected using Google Earth, to locate rocky platforms exposed to high wave energy. Visual examinations were conducted in situ at low tide to determine the presence or absence of accessible kelp beds at ten sites: Tathra, Merimbula, Green Cape, Mallacoota, Cape Conran, Cape Liptrap, Wilson's Promontory, Cape Paterson, Cape Schanck, and Sorrento back beach (latitudinal range: -36.72 to -38.49) (Figure 9). No kelp beds were visible from the shore at Merimbula, Cape Liptrap, Wilson's Promontory, and Cape Paterson, and sampling was not feasible at Sorrento back beach due to large swell. As such, intertidal sampling was limited to Tathra, Green Cape, Mallacoota, Cape Conran and Cape Schanck (Table 2; Figure 9).

Table 2 Site descriptions: Australia

Site	State	Latitude	Longitude	Description
Tathra	NSW	-36.72	149.99	Flat, rocky platform below Tathra Hotel- high wave exposure.
City Rock	NSW	-37.25	150.014	Flat rocky platform along edge of large bay.
Mallacoota	Vic	-37.57	149.764	Series of rocky reefs near boat ramp- more exposed towards point/ outer edge.
Cape Conran	Vic	-37.8	148.743	Series of parallel rocky reefs- moderate wave exposure.
Cape Schanck	Vic	-38.49	144.889	Large flat rocky platform with gulley, moderate wave exposure.

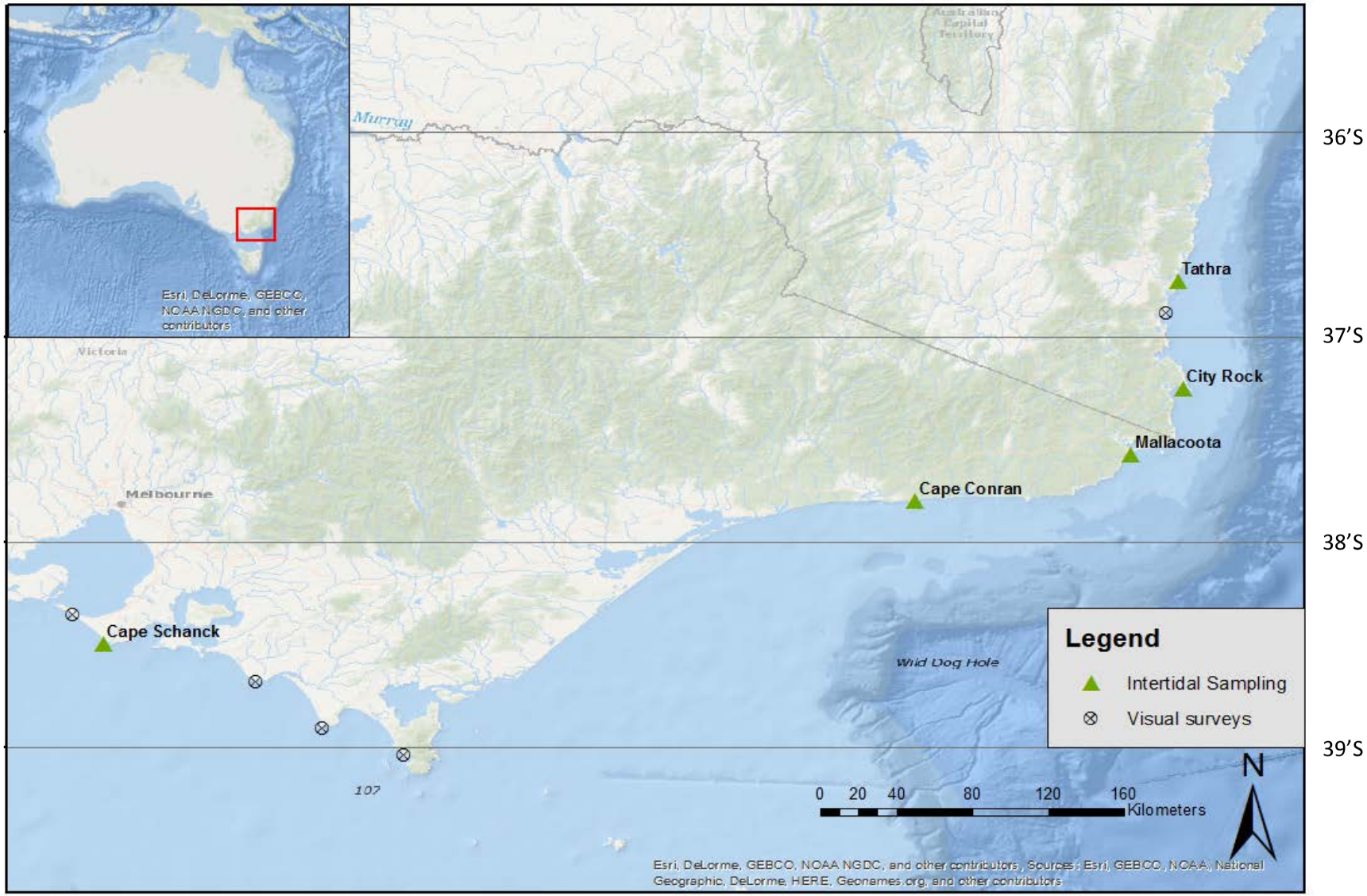


Figure 9 Map of locations for intertidal sampling and visual surveying in Australia

Research permit conditions

Site selection was constrained by conditions set out in research permits for NSW and Victoria (Appendix 1). These included sampling outside marine protected areas, proper sterilisation of equipment to reduce risk of transferring pathogens between sites, and prompt and proper notification of fieldwork commencement dates to local regulatory authorities. Delays in receiving the permit for Victoria forced sampling to occur at sites outside marine protected areas and below the low tide mark of terrestrial parks, where permits are not required for tissue collection (Appendix 1) (Hadden, S., 2015, personal communication, 15/10/2015).

Engagement of citizen scientists

In an effort to increase the efficiency of site selection, and due to the lack of information on the presence of *Maullinia* spp. in Australia, citizen scientists were engaged to identify areas with galls on *D. potatorum* and *Durvillaea* sp.nov. A description of the characteristics of *Maullinia* spp. infections, based on the work of Goecke *et al.* (2012), was posted in an article on the Atlas of Life in the Coastal Wilderness, requesting anyone who had seen similar infections on Australian bull-kelp to report a sighting (see Hepburn, 2015).

Complementary to this, a radio interview was conducted on ABC South East NSW (10/8/15), in which listeners on the NSW south-coast were requested to read the article posted online and report possible infections. The SCUBA divers federation of Victoria was also contacted via the DiveOz forums (Blake, 2015) in order to identify potential sites accessible for sampling, and to determine if any members had noticed infections on populations of bull-kelp.

Several researchers from a range of other geographic locales including the sub-antarctic region were also contacted and asked to report infections and collect samples if they suspected the parasite was infecting local *Durvillaea* populations (Table 3).

Table 3 Individuals contacted to establish sampling locations and request sample collection

Name	**Geographical area**	**Affiliation**
Simon Bulman	New Zealand	Scientist, The New Zealand Institute for Plant & Food Research, Ltd.
Norman Glass	Tristan da Cunha/ Gough Islands	Data manager/ observer, Tristan Da Cunha Fisheries Department.
Dr. Sigrid Neuhauser	Austria	Researcher, Institute of Microbiology, University of Innsbruck. *Note: not asked to sample, contacted to determine areas where other researchers may be able to sample.*
Libby Hepburn	NSW	Atlas of Life in the Coastal Wilderness region coordinator.
n/a	Victoria, Australia	SCUBA divers federation of Victoria

Chile

Intertidal sampling

Sites for intertidal sampling in Chile were selected based on the incidence of *Maullinia* sp. infections in the literature (see Table 1), and positive amplification of samples of diseased tissue from beach-cast kelp collected in Chile by Professor Martin Thiel and colleagues prior to this thesis' sampling period.

DNA barcoding of 90 samples collected in 2014/15, including sequencing of several samples, confirmed the presence of *Maullinia* sp. infections on beach-cast kelp at Mar Brava, Quidico, Queule, Itata Norte, Pucatrihue, Chaihuin, San Pedro de la Paz, Cucao, Carelmapu, Hua-Huar, and Curinanco (Electronic Appendix 1). It was assumed they represented infections from local populations for further sampling.

Sites were selected based on three criteria: latitudinal spread, safety and accessibility of samples, and prior identification of potential *Maullinia* sp. infections. Intertidal sampling occurred at Pichicuy, Quintay, Pichilemu, Curanipe, Queule, Chaihuin, Hua Huar and Mar Brava (Table 4; Figure 10). While a visit to a site between Curanipe and Queule had been planned, time constraints resulted in a gap between the latitudes of -36'S and -39'S (Figure 10).

Table 4 Site descriptions: Chile

Site	Latitude	Longitude	Description
Pichicuy	-32.3263	-71.4718	Small bay with lots of rocky reefs. Evidence of seaweed harvest (*Macrocystis*). Lots of isopod damage.
Quintay	-33.2049	-71.7008	Recreational area- lots of people. Small bay with central reef.
Pichilemu	-34.3937	-72.0266	Site with evidence of kelp harvest
Curanipe	-35.8956	-72.6880	Rock platform approx. 10m. off beach.
Queule	-39.3132	-73.2273	Small bay accessible by dirt path. Rocky platforms along bay edge and in centre.
Chaihuin	-39.8901	-73.4837	Rocky reefs running parallel to shore. Evidence of kelp harvest
Hua Huar	-41.3117	-73.8401	Series of rocky platforms. Isopod damage; 'spotty' disease on kelp.
Mar Brava	-41.9458	-74.0399	Remote (dirt road). Evidence of kelp harvest- *Macrocystis* and Cochayuyo.

Research conditions

Where possible samples were collected in areas outside of traditional management zones (‘Parcelas de Cochayuyo’) where the harvest of bull-kelp is locally regulated. Where sampling did occur in such zones, Chaihuin and Pichicuy, the work was verbally agreed upon with members of the traditional management groups.

Beach sampling

Beach sampling occurred at sites pre-designated in the project of Prof. Martin Thiel. In total, 34 beaches were sampled (Figure 10).

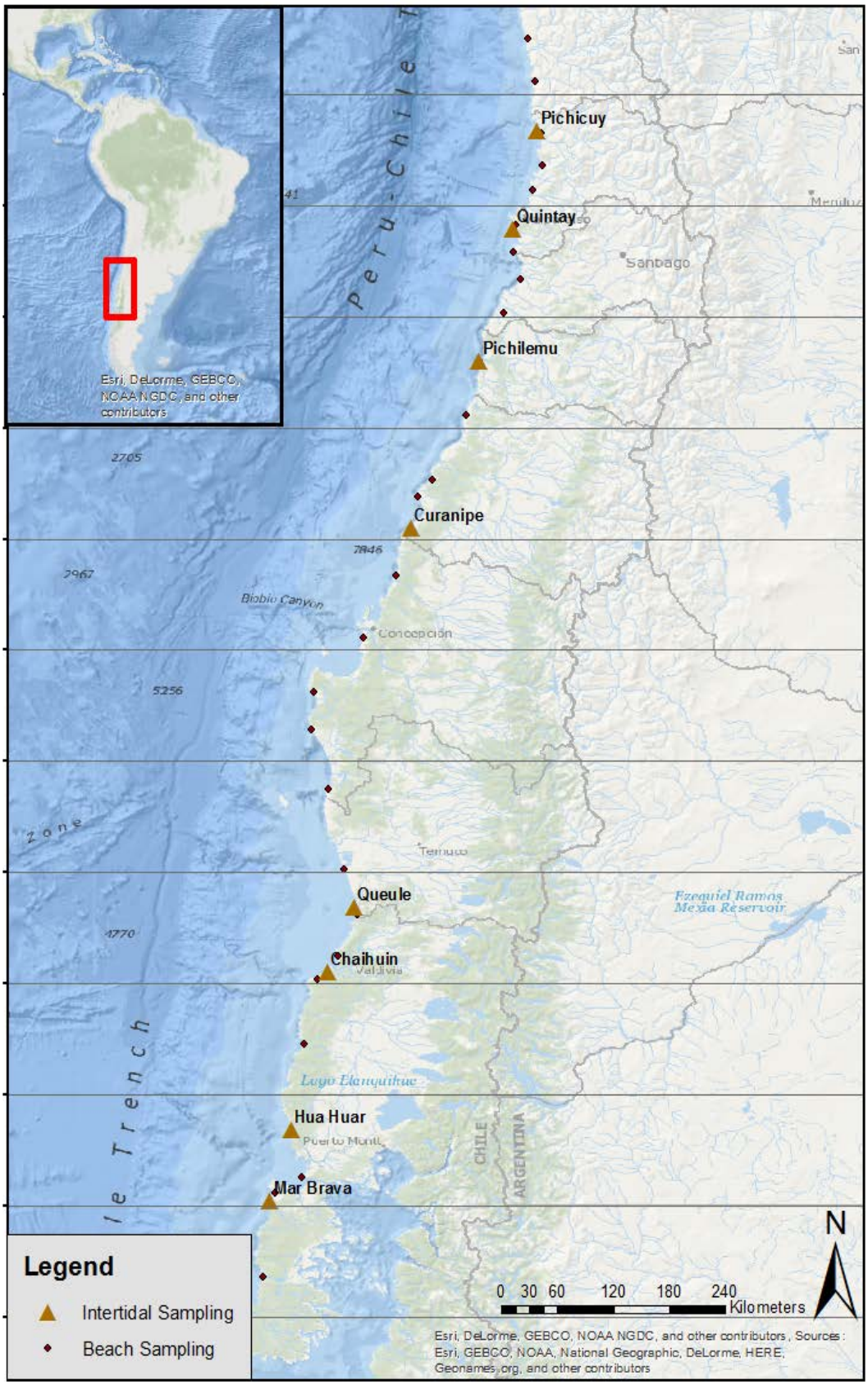


Figure 10 Map of intertidal and beach sampling locations in Chile

3.1.2 Data collection

Sampling period

To reduce the risk of seasonality influencing results, all intertidal sampling occurred in late spring and summer 2015/16. I collected Australian data over seven days in November and December 2015. I travelled to Chile to collect data over two two-week periods in December 2015 and January 2016.

Complementary to the work of this thesis, data from summer and winter 2014-2016 on the proportion of infected beach-cast kelp at all sites were made available by the research group of Prof. Martin Thiel.

Infection identification

Morphological characteristics considered likely to represent *Maullinia* spp. infections on *Durvillaea* spp. hosts were based on descriptions in Aguilera (1988), Jahnke (1978) and Goecke *et al.* (2012). Infections were recorded in the field by sighting *'Discoloured, yellowish warty tissue, forming circular or elliptical raised galls, or holes surrounded by such tissue'* (Appendix 2), and photographs were taken to determine variability in size, colour, and texture of infected tissue (Electronic Appendix 2).

Intertidal sampling

Sampling sought to quantify elements of importance for algal infectious diseases as described in Correa and Sánchez (1996): the incidence of disease, population affected (age or size of host), differential susceptibility between hosts, and severity of the disease.

Infection incidence was quantified along a series of 10m transects. While three transects were measured per site in Australia, the number of transects varied from three to five between sites in Chile based on the extent of kelp beds, safety considerations, and the availability of researchers. As this research was concerned with proportional incidences of infection per population, variability in number of transects does not affect the representativeness of data as sampling along each transect remained consistent.

Transects were laid on rocky platforms partially or completely emergent at low tide. As *Durvillaea* species often inhabit fragmented rocky platforms composed of a number of reefs separated by water, it was not always possible to lay transects parallel to one another. Rather, they were laid on the edges of such platforms, separated by at least two metres if on the same platform, where kelp are likely to experience similar exposure between sites (Appendix 3).

The number of healthy and diseased kelp were counted on each transect, with stipe intercepts considered an individual hit. Morphological measurements were limited to length as

this was the fastest and safest characteristic able to be measured in high wave energy environments without harvesting entire kelps. As a method of randomisation, length measurements were taken for every fifth healthy individual, and every third diseased individual, as it was assumed the prevalence of the latter would prove lower than the former. Length was measured from the base of the holdfast to the end of the longest frond. Multiple stipes from the same holdfast were considered multiple intercepts, as these likely represent distinct individuals (Figure 11).

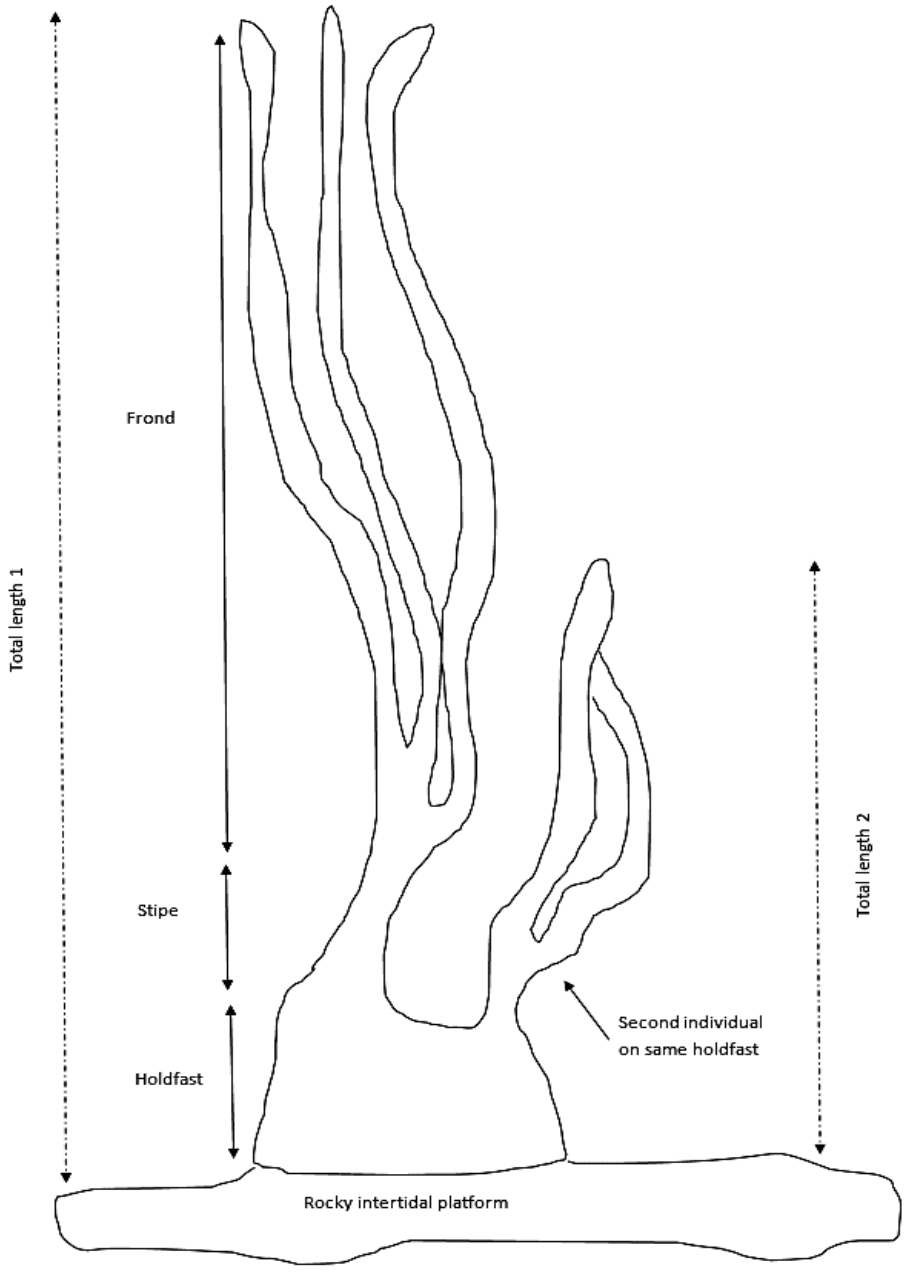


Figure 11 Diagram of 'typical' kelp showing length measured

For each diseased individual measured, a sample of infected tissue was excised and placed in a 50ml Eppendorf tube and labelled with the site name and an individual code to match samples with field-recorded morphological characteristics. Tubes were filled with 70% ethanol, with the alcohol replaced once after several hours. After 24-48 hours, samples were either air-dried on clean paper towel or in an oven at 60°C for several hours, then placed in sealed and labelled sample bags with silica gel as desiccation is considered effective for preserving algal tissue for molecular work.

Approximately 1700 kelp individuals were visually surveyed across Chile and Australia. Tissue samples and length measurements were taken from 149 diseased individuals, and length measurement from 256 healthy individuals.

Beach sampling

As beach-cast sampling was a component of the larger project of Professor Martin Thiel, methods were adjusted to fit into the project's research design. The length of infected beach-cast kelp with their holdfast attached were measured, and diseased tissue excised, preserved, and labelled for laboratory extraction as per the method used for intertidal sampling. Diseased tissue was sampled on kelp showing little signs of decomposition, as DNA from decomposed individuals would likely not amplify.

Approximately 50 samples were collected from 67 beach-cast kelp individuals, alongside morphological measurements of 1170 healthy individuals.

3.2 *Laboratory work*

3.2.1 Sample selection: FSES *Durvillaea* spp. gall collection

Samples of diseased *Durvillaea* spp. tissue from the FSES collection were selected for DNA barcoding based on the characteristics of *Maullinia* sp. infections described in section 3.1.2. Holes with smooth edges, or evidence of 'furry' growths, were not extracted as they were likely to represent infections of *Herpodiscus* sp. or other epiphytic algae.

Approximately 200 samples were visually checked, with 38 of these- from Gough, Marion and Kerguelen islands (Electronic Appendix 1)- tested via DNA barcoding (using *Maullinia*-specific primers: see below).

3.2.2 Sample extraction, amplification, sequencing

Extraction protocol

Samples were extracted following the Chelex© protocol of Walsh *et al.* (1991). Small (<2mm) pieces of tissue were excised using a scalpel and tweezers (sterilised between samples with 70% ethanol and flame) and placed in 800µl of 5% Chelex© solution (Bio-Rad Laboratories,

Hercules, USA). Extractions also included 1µl of 10mg mL^{-1} proteinase K for Australian samples, however this reagent proved unavailable in Professor Thiel's laboratory in Chile and solutions there thus contained only Chelex© resin. Extractions of Chilean samples could not be performed in Australia due to import restrictions (see Appendix 4).

Samples were incubated at 60°C overnight, raised to 90°C for 8 minutes for those containing Proteinase K in order to denature the enzyme before Polymerase Chain Reaction (PCR). Samples were finally centrifuged for 3 minutes at 13,000 rpm, and the supernatant diluted 1:100 in MilliQ water to reduce the possibility of alginates blocking PCR processes (see Wilson *et al.*, 2016).

PCR protocol

Sample amplification was conducted in a 20µl PerfectTaq solution, comprising 12.5µl MilliQ water, 0.2µl each of forward and reverse 10mM primers, 2µl of PerfectTaq buffer (5Prime GmbH, Hilden, Germany), 4µl of 1mMol dNTPS, 0.1µl of PerfectTaq polymerase (5Prime GmbH, Hilden, Germany) and 1µl of 1:100 sample supernatant.

PCRs were run with an Eppendorf Mastercycler (Epgradient S, Hamburg, Germany) using the *Maullinia* Touchdown protocol of Goecke *et al.* (2012), comprising: 96°C for 4 minutes, two cycles of 96°C for 25 seconds, 65°C for 25s and 72°C for 1.5 minutes, two cycles each with an annealing temperature of 60°C and 58°C, and 30 cycles with an annealing temperature of 54°C and then 72°C for 10 minutes. Total PCR length was 2 hours 17 minutes.

This research used the genus-specific primers Mau2F (5'ACGGGTACGAGGGACGTGGG) and Mau9R (TGCATCAGTGTAGCGAGCGT) (Goecke *et al.*, 2012). Primers amplified the 18S nuclear ribosomal gene, which is of particular use for the taxonomic classification of organisms (DNA barcoding), descriptions of variation between populations of differing geographical origin, and analyses of protist phylogenetic relationships (Hadziavdic *et al.*, 2014; Wang *et al.*, 2014; Pawlowski *et al.*, 2012). This marker is regularly used for protist research, being easily amplified through PCR and relatively robust in forming single-gene phylogenies (Boenigk *et al.*, 2012). I designed new primers for a range of alternative molecular markers, but none proved effective (see section 3.2.3).

Gel electrophoresis

In order to establish which samples had successfully amplified, PCR products were run on a 1% agarose gel at 100 volts for approximately 25 minutes. Gel electrophoreses included PCR and extraction negatives for each sample batch, in order to confirm no contamination from procedures or reagents had caused sample amplification. Bands of the right length (approx. 1800bp) were considered a 'positive hit' for the organism of interest.

DNA purification and sequencing

PCR products showing signs of positive amplification were purified using a QIAQuick PCR Purification Kit (QIAGEN, Venlo, Netherlands) to be sent for sequencing at the University of Otago's Genetic Analysis Services (Otago, New Zealand), using an Applied Biosystems 3730xl capillary sequencer (Thermo Fisher Scientific, Inc., Waltham, USA). Samples were sent with approximately 18ng of DNA (1ng per 100 bp of product) and 1µl of 3.2mM forward primer (Mau2F) in a total volume of 5µl/ sample.

3.2.3 Alternative primer design

Several other primers were developed following the method of Fraser and Waters (2013) in order to sequence the Cox3, COI, and 5.8s markers, as analyses of multiple nuclear and mitochondrial genes provide a greater understanding of biogeographic and phylogenetic processes (Tian *et al.*, 2013). Iterative testing of primers with various alterations to procedures and reagents did not lead to successful amplification (Table 5), and due to time and budget restraints the remainder of this research project concentrated on the use of the existing primers Mau2F/ Mau9R.

It is highly likely that new primers failed to amplify *Maullinia* sequences due to the limited availability of *Maullinia* and closely related organism sequences for other markers in Genbank. As a result, new primers were developed using information from quite distantly related taxa.

Table 5 Alternative primer design and testing

Marker	Reference sequences	Primer (F: Forward, R: Reverse)	Test Date	Conditions	Amplification
5.8s	*Fucus vesiculosus* #EU525661 *Maullinia ectocarpii* (partial) #AF405547	Mau5.8sF4:CATACCRATATGAAACAAGG Mau5.8sF5:CTTTGGGTGATTACCCTTTC Mau5.8sF6:GGTGGGTGTTGCTTGTGAGAAG Mau5.8sR4:CATCGACAAAACTCAATAAAGAG Mau5.8sR5:CTTTATGCATTGGRTATTCGTTTC Mau5.8sR6:GCCTGATCTGAGGTCTTTATG	3/8/15	All combinations. Standard 45.	Smeary and multiple bands. Combinations H+ I may have worked but way too many bands. Bright band seen in G4 may be ITS1+5.8s+ITS2 (around 400bp)
			5/8/15	Combination F6+ R4, R5, R6. Standard 60	Possible faint band combination A. Contamination- band in negative.
			6/8/15	As above- standard 55.	Contamination in negative again
			7/8/15	Replaced reagents. Combo F6+ R4, standard 55.	One smeary band
			14/8/15	F6+R4+ magnesium, standard 55.	Smeared
			24/8/15	R4+F6, R6+F6, magnesium, standard 50.	No bands.
COI	*Durvillaea antarctica* #EU919398 *Cyphoderia ampulla*	MauCOIF1: TAATTGGGACGACATTATCTG MauCOIF2: ATTATCTGTTTTAATTAGGG MauCOIF3: TAGGGCAGAATTAGCTTATC MauCOIR1: CTGATATAAAATAGGATCTCC MauCOIR2: TAGGATCTCCTCCTCCAGCC	23/7/15	Trying combinations. Standard 50.	No obvious bands/ smearing. Very little success.
			24/7/15	Retry Standard 45.	Standard 45 seems better. Smearing in some wells. Combination B fully smeared, combination E quite smeared. Combination I promising.

	#HM187673	MauCOIR3: TATAAAATAGGATCTCCTC	6/8/15	Retry combination F3+R3- standard 45.	No bands.
			12/8/15	‘’ ‘’. More samples.	Very faint bands.
			13/8/15	Retry F3+ R3 with magnesium. Also try F3+R2 combination.	Some faint bands- gel purified for sequencing.
Cox3	*Durvillaea potatorum* host included as reference to make primers parasite-specific) #EU681446 *Spongospora subterranea* # KF738139	SponCoxF1:AGTAGATGCTAGCCCTTGACC SponCoxF2:CAATTTTTTCTTCGTTTTCCG SponCoxF3:CGCTTTAGTTTTAACTAGCGGG SponCoxR1:TTTCGTTTCCGATTAAATTAAAG SponCoxR2:GTAGTTACAAAGTAGGTGAAAC SponCoxR3:GATTAAATTAAAGTAGTTAC	27/7/15	Try all primer combinations- standard 45.	Mostly smeared. Negatives clear. Possible bands in A3/A4.
			28/7/15	F1+R1, F1+R2, F1+R3, standard 50 + magnesium.	Nothing visible. Primer dimer bright but no bands/ smears.

3.3 *Statistical analyses*

3.3.1 Phylogenetic analyses

Sequences were aligned, trimmed, and ambiguities were corrected in Geneious 6.1.8 (Kearse et al., 2012) using, where possible, IUPAC ambiguity codes for base-call ambiguities (two possible nucleotides at a locus). The alignment of 58 sequences, including two outgroups of related organisms and two published sequences of *M ectocarpii* and *Maullinia* sp. was exported to MEGA6.06 (Tamura *et al.*, 2013) for preliminary examination of phylogenetic relationships.

Haplotype networks were developed in TCS1.21 (Clement *et al.*, 2000), with one representative from each unique sequence used to construct phylogenetic trees. Haplotype diversity was analysed using a simple linear regression of number of sequences vs. number of unique sequences in Chile and Australia.

The most appropriate model of DNA evolution was determined using jModeltest2 (Darriba *et al.*, 2012) according to Akaike's Information Criterion (AICc). Model parameters were: TrN+G (gamma shape 0.4240, proportion inv sites 0). The Maximum Likelihood (ML) phylogenetic tree was constructed in PhyML 3.0 (Guindon *et al.*, 2010) with support for nodes determined using 1000 bootstrap iterations. The Bayesian phylogenetic tree was constructed in MrBayes 3.2.0 (Ronquist and Huelsenbeck, 2003) with Markov Chain Monte Carlo (MCMC) searches of four chains and burn-in of 10,000 trees. Trees were sampled every 100 generations for a total of 5000000 generations.

Box 1: Phylogenetic analyses

Maximum likelihood (ML) and Bayesian analyses: phylogenetic trees, constructed using ML and Bayesian techniques, are widely used to test evolutionary relationships. ML analyses seek to quantify how likely it is that the relationships observed are 'true' based on iterative testing (bootstrapping), with larger results showing greater support for relationships. Bayesian analyses produce posterior probability values for nodes in phylogenetic trees by combining tree likelihood measures and prior phylogeny probability (Douady *et al.*, 2003). Using the two techniques in concert can provide greater confidence in estimated phylogenetic relationships.

Haplotype analysis: determines the number of, and relationships between, unique genetic lineages at a population-scale (Clement *et al.*, 2000). Haplotype networks represent prevalence, connectivity, and difference between haplotypes, using visual cues such as nodes to represent missing haplotypes or evolutionary steps between groups.

3.3.2 Morphological and ecological analyses

Analyses of ecological and morphological data were carried out in Genstat 18.1 (VSNi, 2011). Latitudinal effect on infection incidence, as well as effect of infection status on host length, were tested for using Generalised Linear Modelling (GLM), while the effect of latitude on host length was quantified using grouped linear regression. A two-sample binomial test was used to compare infection incidence between seasons in Chilean beach-cast kelp. For all analyses p values <0.05 was considered significant.

Testing for latitudinal effects in infection incidence using a binomial model showed over-dispersion, suggesting the distribution was inappropriate or possible data clustering. A negative binomial distribution with a logarithm link function was used instead, as such models are of particular use for count data and are able to deal with over-dispersion (Lawless, 1987). Modelling used number of infected kelp per transect per latitudinal group, offset by the natural log of total kelp due to large variability in total numbers of kelp per transect. Data from each country were tested individually, and in a single model using Wald tests to determine the effect of dropping terms, to determine if any country-level effects altered results.

Length difference based on host infection status was modelled for intertidal data with a binomial distribution and logit link function. Due to the large difference in numbers of infected and non-infected beach-cast kelp, a binomial GLM proved inappropriate and a two-sample t-test was used. Large variability in length measurements was accounted for using log10(length) in analyses.

Latitudinal effect on host length was tested for using a linear regression grouped by infection status. Seasonal effects were tested for using a two-sample binomial analysis of numbers of infected and non-infected kelp in beach sampling for summer/ winter 2014/15 and 2015/16.

Box 2: Statistical analyses

Linear regression: tests the relationship between variables and the strength of this relationship; concerned with dependence (Weisberg, 2005) and primarily used for continuous data.

Generalised Linear Modelling (GLM): GLMs are a robust method for analysing a range of data types, including categorical or binary. They are able to cope with non-normal distributions, and can point to the relative contribution of variables on an outcome (Dunteman and Ho, 2006). Binomial modelling is often used to determine how factors affect the number of ‘successes’ in a dataset (i.e. number of infected kelp).

Chapter 4: Results

4.1 *Infection geography*

Samples collected in this research indicate the presence of *Maullinia* spp. infections at all intertidal sites visited, and a majority of beaches (Electronic Appendix 1). DNA barcoding confirms, for the first time, infections of *Maullinia ectocarpii* on populations of *Durvillaea potatorum* and *Durvillaea* sp. nov in south-eastern continental Australia. Infections of *Maullinia sp*. were also confirmed throughout central Chile on *Durvillaea antarctica*. No infections were identified by other scientists (Table 3) or confirmed on samples from sub-antarctic locales.

4.2 *Infection morphology*

Qualitative observations of samples collected in this research showed several morphological characteristics were shared between infections in Australia and Chile, with some differences evident between hosts. Novel data emerged regarding the possible progression of infections in the DM pathosystem.

While some infections of *Maullinia* sp. in Chile were similar in shape, size, and colour to those in Goecke *et al.* (2012) and Aguilera (1988), infected tissue showed a broader range of characteristics than previously described. Diameter of galls ranged from 2cm to 15cm. Galls were found from the centre (Figure 12a) to the edges (Figure 12b) of kelp fronds, with rarer infections occurring on the stipe (Figure 12c). Infected tissue had a warty appearance, being raised and tougher than surrounding areas and often showing concentric rings of discolouration (Figure 12a).

Reviewing images of infected tissue in Chile cast doubt over the accuracy of gall identification by researchers in Quintay (site 2), being one of the earlier sites visited during sampling where methodology was still under development (Electronic Appendix 2). As other organisms are known to produce gall-like structures on *D. antarctica* in Chile (Saavedra, 2011), quantitative data were omitted for this site in analyses to reduce the risk of trends being skewed by alternative species.

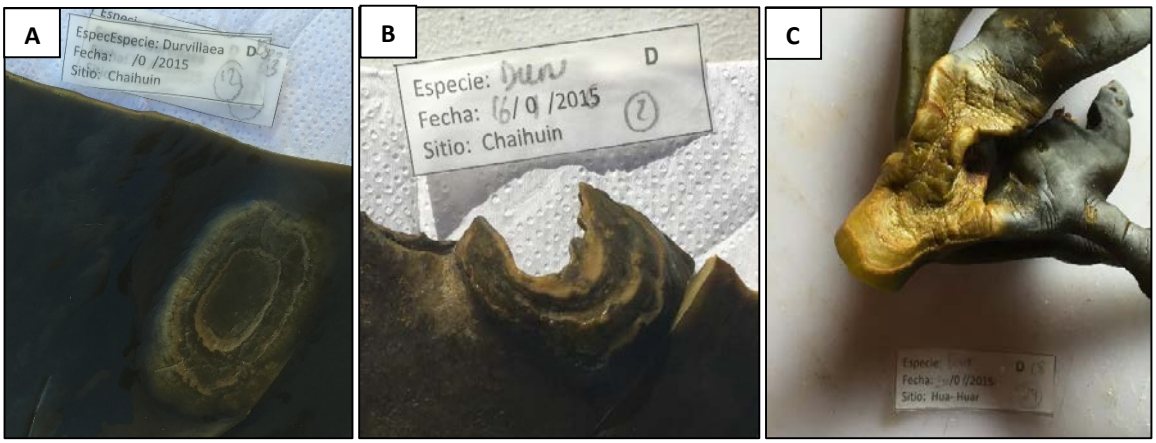


Figure 12 'Typical' infections amplifying positively for <u>Maullinia</u> sp. on <u>D.antarctica</u> in Chile (paper length: 5cm) showing A) a gall with concentric rings, B) an infected frond edge, C) an infection spread along the stipe and lower frond

Infections in Australia were smaller than their Chilean counterparts, ranging in size from 1cm to 5cm. Tissue showed a more uniform yellow colour, being warty and tough, similar to infections found in Chile (Figure 13). Infections along the edges of kelp fronds were less common than in Chilean populations, being more often identified as warty holes and small raised galls in central tissue.

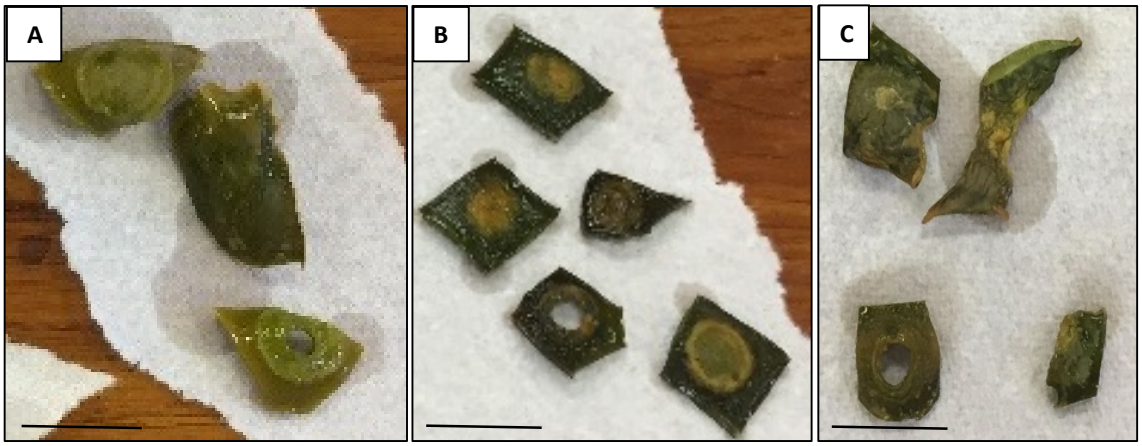


Figure 13 Typical' infections amplifying positively for <u>M. ectocarpii</u> on <u>Durvillaea</u> species in Australia (scale bar: 2cm) (A and B: Mallacoota; C: Cape Conran)

Tissue infected by *Maullinia* spp. in both locales suggested similar infection progression. Galls begin relatively small, and can grow to substantial size. At a certain point, tissue in the centre of the gall begins to slough away, likely due to necrosis, eventually forming a hole in the infected frond. Once large enough, this hole appeared to break open, either becoming the new edge of the frond, or leading to the infected tissue breaking off (Figure 14).

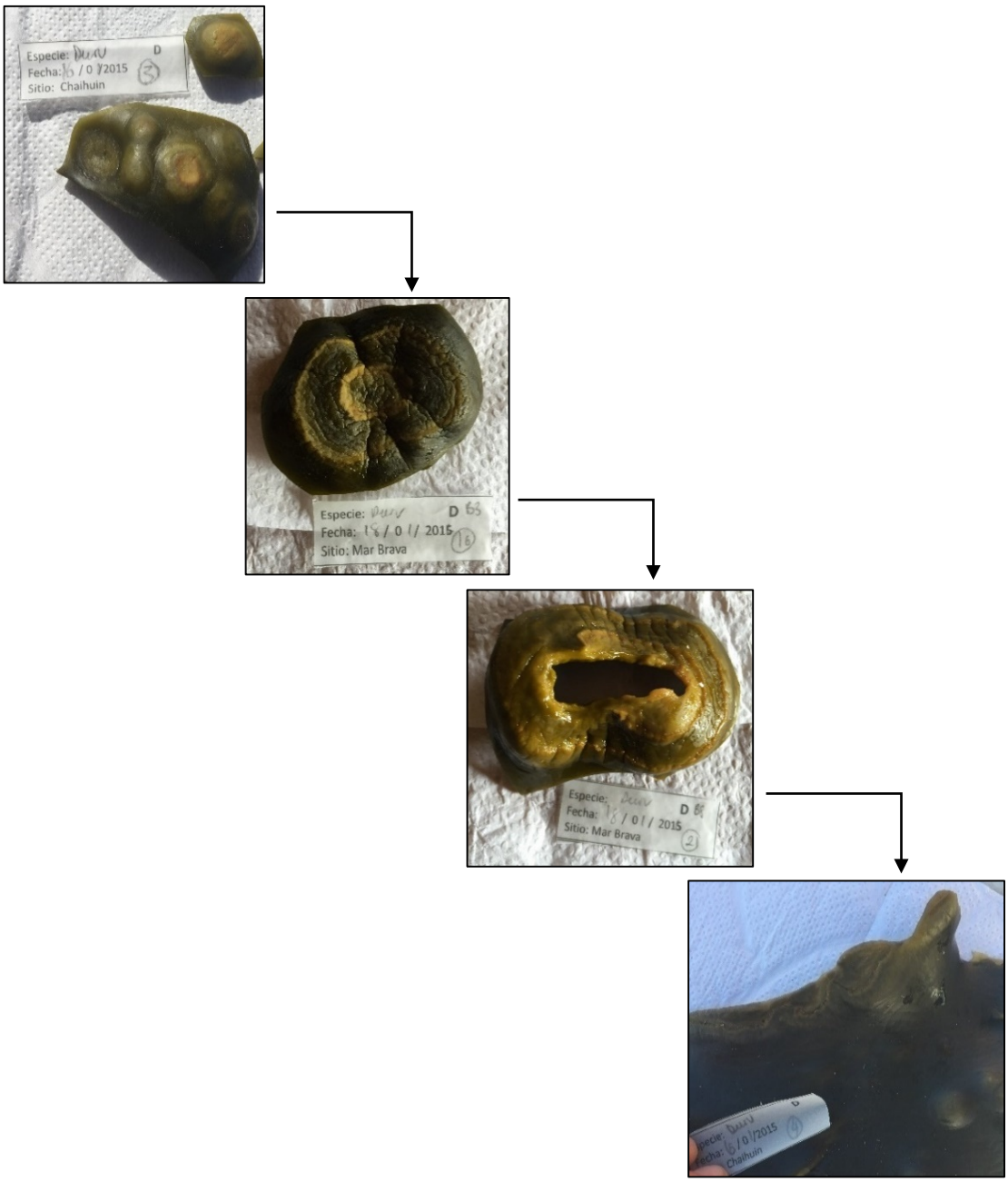


Figure 14 Proposed progression of infections of <u>Maullinia</u> sp. on <u>Durvillaea antarctica</u> in Chile, from left to right: gall formation, gall enlargement, necrosis, gall rupture and new edge formation (paper length: 5cm).

4.3 *Genetic analyses*

Samples from Australia and Chile showed similar rates of amplification from single-attempt PCRs (approx. 60%), with sequenced products being a similar length (approx. 800bp). Amplification failure rates are often high for Polymerase Chain Reactions (PCRs), particularly for those using tissue from kelp and seaweed (Wilson *et al.*, 2016). Acknowledging this, and the similarity of infection morphology between sites (omitting Quintay, see section 4.1), it was assumed all samples collected represented the organism of interest and all quantitative field data could be analysed with relative certainty of representing DM pathosystem trends.

4.3.1 Haplotype analyses

Sequencing of 54 samples from eight intertidal sites in Chile and four intertidal sites in Australia yielded 12 new, unique sequences. No sequences were shared between countries, although the published *M. ectocarpii* sequence of Maier *et al.* (2000), from Chile, was resolved as closely-related to Australian *Maullinia*.

Chile

Eight unique sequences were identified in the 31 samples sequenced. While a greater number of samples per site may have provided a better understanding of the distribution of genetic variation, preliminary findings suggest little geographic patterning of *Maullinia* sp. lineages along the Chilean coast (Figure 15). The most abundant sequence, MC1, occurred from the southernmost site to the second northernmost locale. The least abundant and most genetically distinct, MC8, appeared at only one site (Pichilemu) (Figure 15). Sequence MC4, which matched the published sequence from *Maullinia* sp. (Goecke *et al.*, 2012), was found from the southernmost to northernmost site (Figure 15).

Haplotype diversity appeared to relate to number of sequences obtained at each site (Figure 17a), i.e. where more samples were sequenced, more unique sequences were found, suggesting high genetic diversity within *Maullinia* sp. along the Chilean coast. This raises the possibility that the sampling regime of this research could not give a full picture of genetic diversity in Chilean *Maullinia*, however it was not a goal of the study to do so.

Australia

Four unique sequences were found in the 22 samples sequenced for Australian sites (Figure 16). Preliminary findings from the small number of samples appeared to show separation into eastern and western groups, with sequence MA1 being present at all eastern sites and MA4 being most abundant at Cape Schanck (Figure 16). No sequences were shared between eastern and western areas, corresponding to the break in host species as described by Weber (2015) (see

section 2.3, Figure 6), with groups showing dissimilarity from each other and published sequences for *M. ectocarpii*.

There was no relationship between the number of samples sequenced and the number of unique sequences obtained in Australia (Figure 17b).

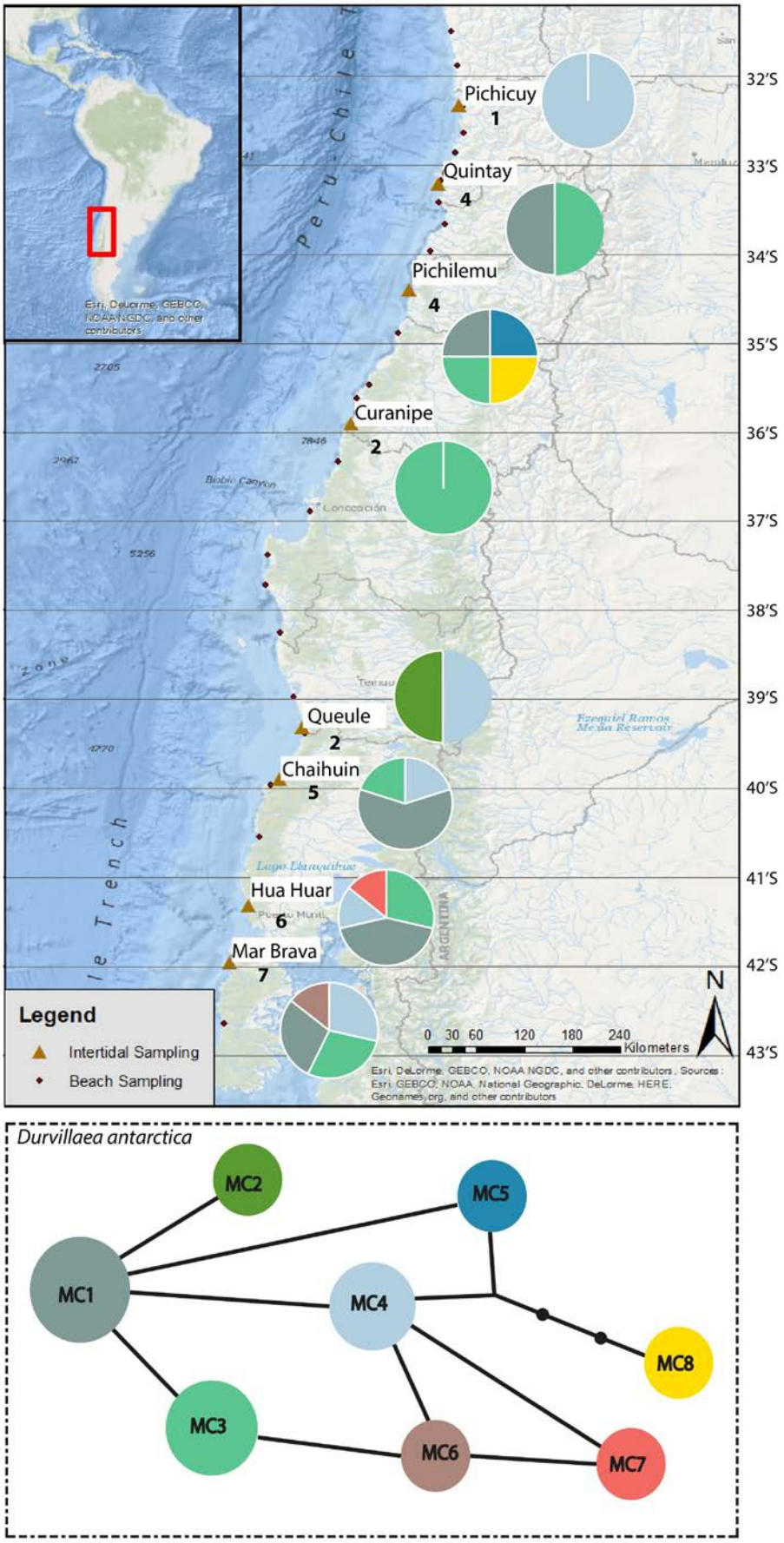


Figure 15 Above: proportion of <u>Maullinia sp.</u> haplotypes at each intertidal sampling location in Chile (number of sequences represented under site name). Below: haplotype network showing host species (dashed box), relative prevalence (circle size), linkage, and dissimilarity between haplotypes (black nodes).

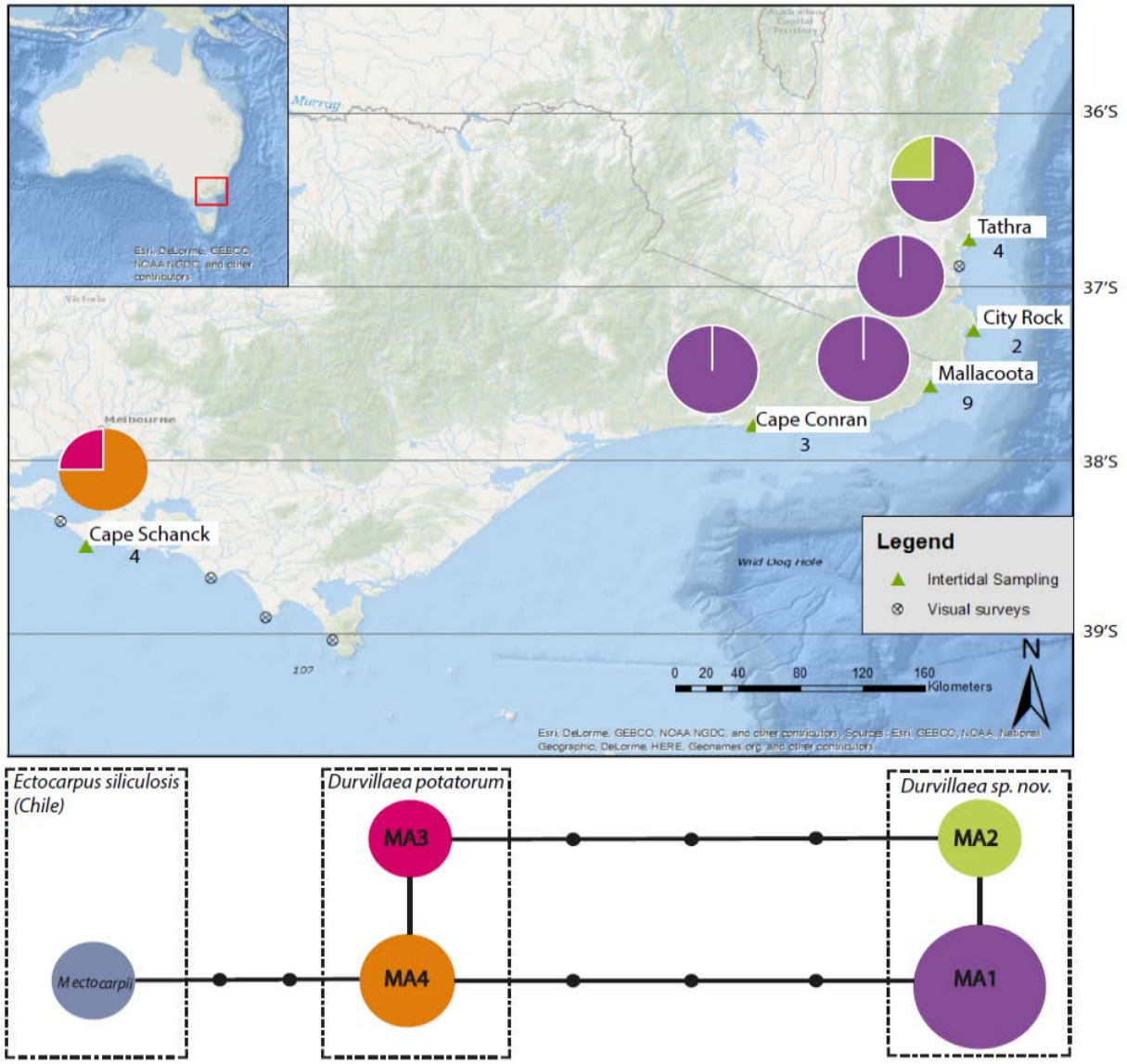
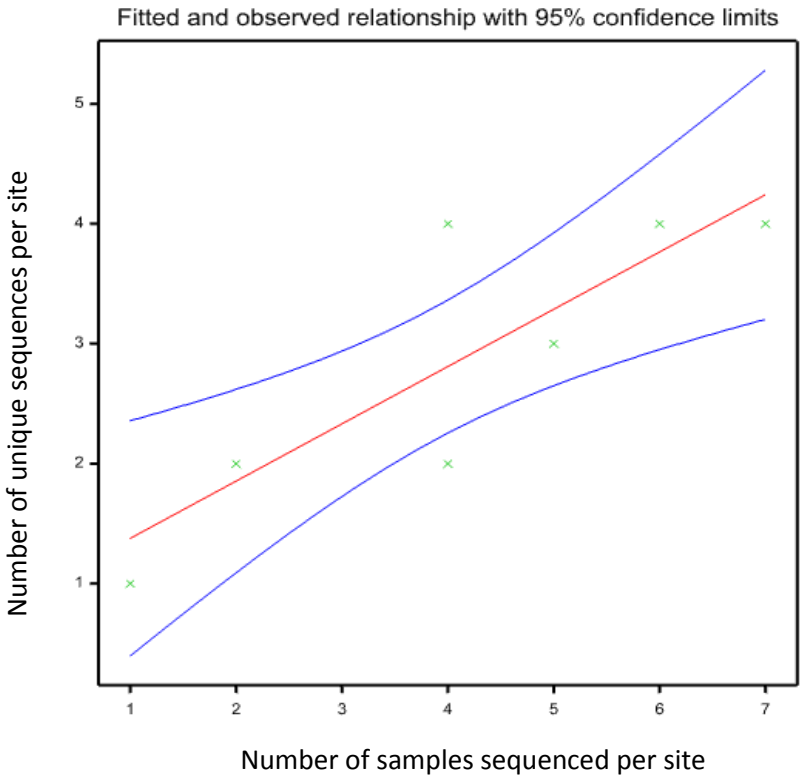


Figure 16 Above: proportion of <u>Maullinia ectocarpii</u> haplotypes at each intertidal sampling location in Australia (number of sequences represented under site name). Below: haplotype network showing host species (dashed box), relative prevalence (circle size), linkage, and dissimilarity between haplotypes (black nodes).

A



B

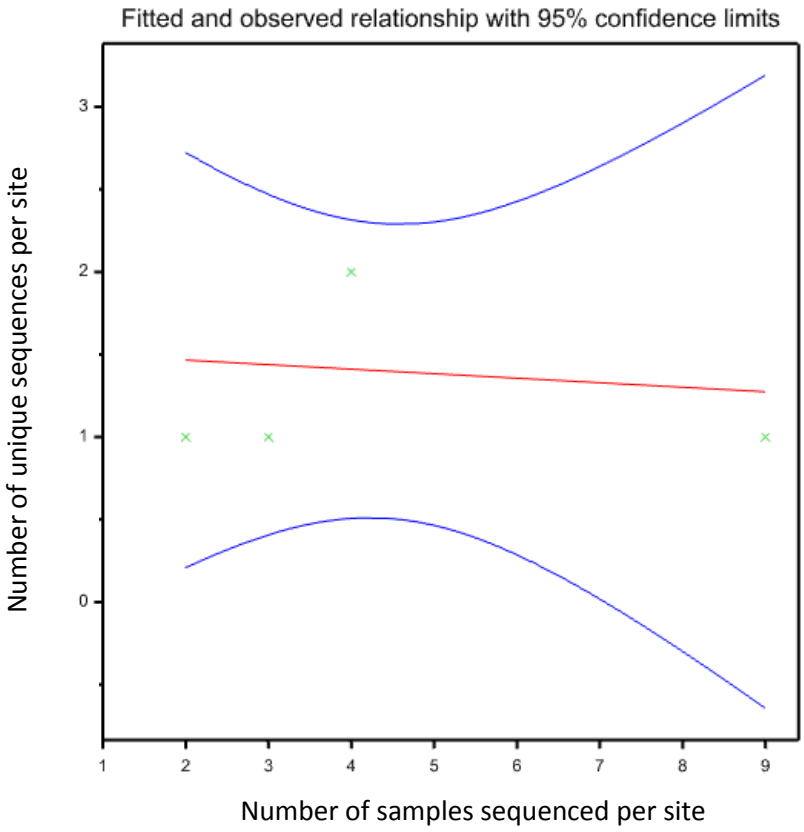


Figure 17 Linear regression of haplotype diversity (number of sequences) vs. number of samples sequenced in A) Chile and B) Australia.95% confidence intervals are represented in blue.

4.3.2 Phylogenetic structure

The 18S ML and Bayesian phylogenetic trees showed strong consistency in overall topology and branch support (Figure 18). Samples were separated into clades representing continental groups (Australia and Chile) with strong bootstrap and Bayesian PP support, and robust separation from related outgroups.

Australian samples showed similarity to published sequences of *M. ectocarpii* (Table 6), and likely represent individuals of this species. The separation of samples into 'eastern' (MA1, MA2) and 'western' (MA3, MA4) groups was strongly supported (Figure 18, 0.3% divergence Table 6), with 'western' individuals at Cape Schanck showing greater similarity to *M. ectocarpii*. Little phylogeographic structure was evident in the Chilean samples (Figure 18), with samples showing strong similarity to the *Maullinia* sp. sequence of Goecke *et al* (2012) (Table 6).

Table 6 Percentage sequence divergence values

	Chilean samples	**Australian Samples**	***Maullinia ectocarpii*** (Maier *et al.*, 2000)	***Maullinia* sp.** (Goecke *et al.*, 2012)
Chilean samples	0.1%-0.3%	2.4%-2.6%	2.5-2.7%	0.1%
Australian samples	2.4%-2.6%	0.3%	0.1%-0.4%	2.3%-2.5%

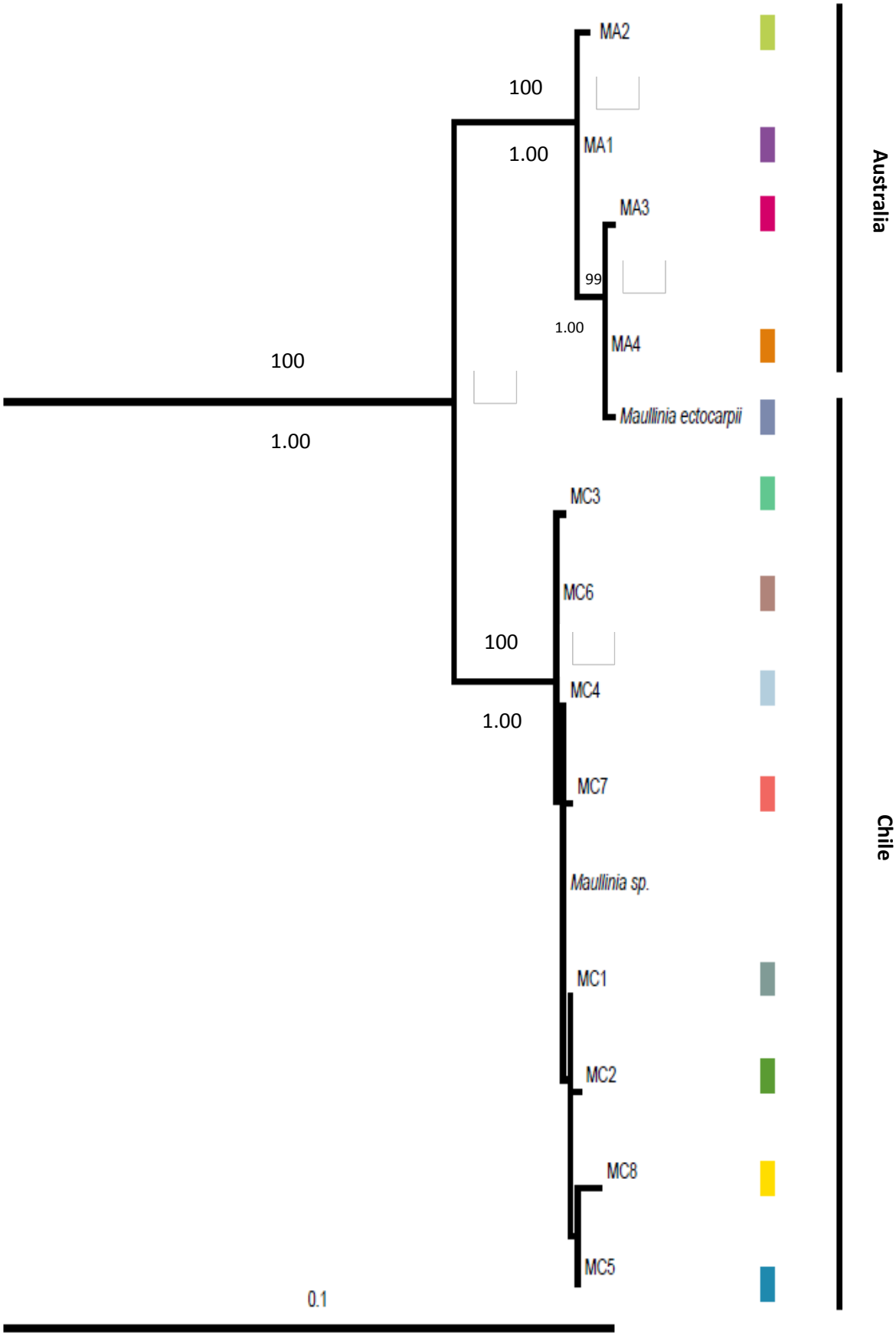


Figure 18 Phylogeny of <u>Maullinia</u> spp. samples collected in Australia and Chile. Coloured rectangles represent unique sequences. Percentage maximum likelihood scores above 75% are shown above branches and Bayesian posterior probability below. Outgroups have been trimmed for clarity. Included are two previously published sequences from Maier et al. (2000) and Goecke et al. (2012)

4.4 *Latitudinal effects*

4.4.1 Australia

Generalised linear modelling for Australia supported latitude being a significant predictor of infection incidence (p=0.015) (Table 7), showing numbers of infected kelp increasing towards higher latitudes (Figure 19a). High numbers of infected kelp were recorded at sites towards the centre of the sampling range, corresponding to Mallacoota and Cape Conran. The lowest numbers of infected kelp were recorded at site two (City Rock), with next lowest incidence occurring at the host's range limit and the northernmost site, Tathra (Figure 19a).

4.4.2 Chile

Generalised linear modelling for Chile did not support latitude being a significant predictor of infection incidence when data for Quintay were included in analyses (p=0.130) (Electronic Appendix 3). Omitting these data, due to the possibility of inaccurate sampling (see section 4.1), showed latitude being a significant predictor of infection incidence in Chile (p<0.001) (Figure 19b; Table 7).

Much like Australia, the linear trend showed numbers of infected individuals increasing towards higher latitudes with both the inclusion and exclusion of data from Quintay. Variability was evident within the dataset, with southern sites such as Queule (site 4, Figure 19b) showing low incidences. Infection incidence did not increase towards the host's northern latitudinal range limit.

4.4.3 Countries combined

Testing for country effects using grouped country data showed country and latitude were significant predictors of infection incidence (country: p=0.011; Latitude: p<0.001) (Table 7), with Australian data showing a steeper trend in infection incidences (Figure 19c), likely due to the smaller latitudinal range.

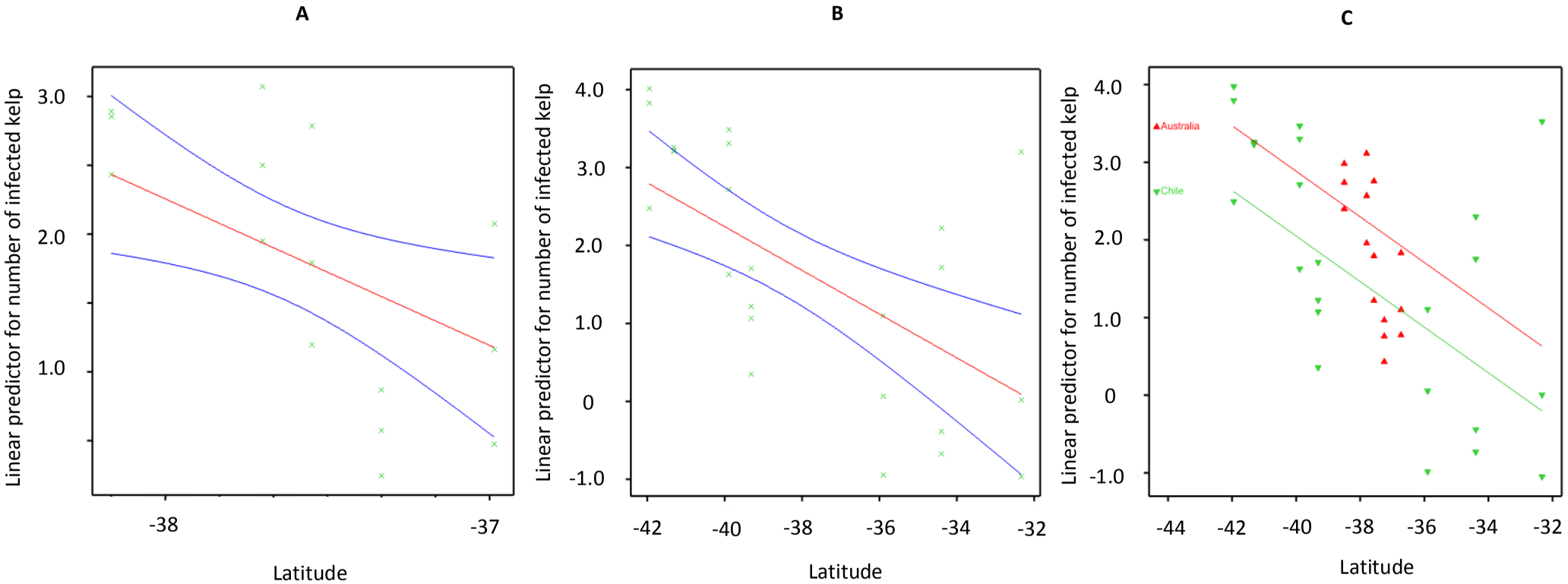


Figure 19 Generalised Linear Modelling of latitudinal effect on infection incidence (offset by the natural log of total kelp) in: A) Australia, B) Chile, and C) Countries combined. Data from Quintay are ommitted. Linear trendlines are shown in red with 95% confidence intervals represented in blue for A) and B). Linear trendlines are colour-coded based on country in C).

Table 7 Significant (p<0.05) GLM output statistics

	Model	p value	Wald statistic	d.f.	Slope	SE	Residual deviance
Latitude: Australia	Negative binomial	0.015	5.883	1	-0.71	0.29	1.532
Latitude: Chile	Negative binomial	<0.001	13.68	1	-0.28	0.07	1.287
Latitude: Countries combined	Negative binomial	Latitude: <0.001	19.32	1	-0.29	0.07	1.336
		Country: 0.011	6.55	1	-0.83	0.33	

4.5 *Host length*

4.5.1 Latitude and length

Regression analysis showed that kelp length (both infected and uninfected) increased with increasing latitude in Australia (R^2=0.34; p=0.003) (Figure 20a). No significant effect was found in Chile (R^2=0.03; p=0.102) (Figure 20b).

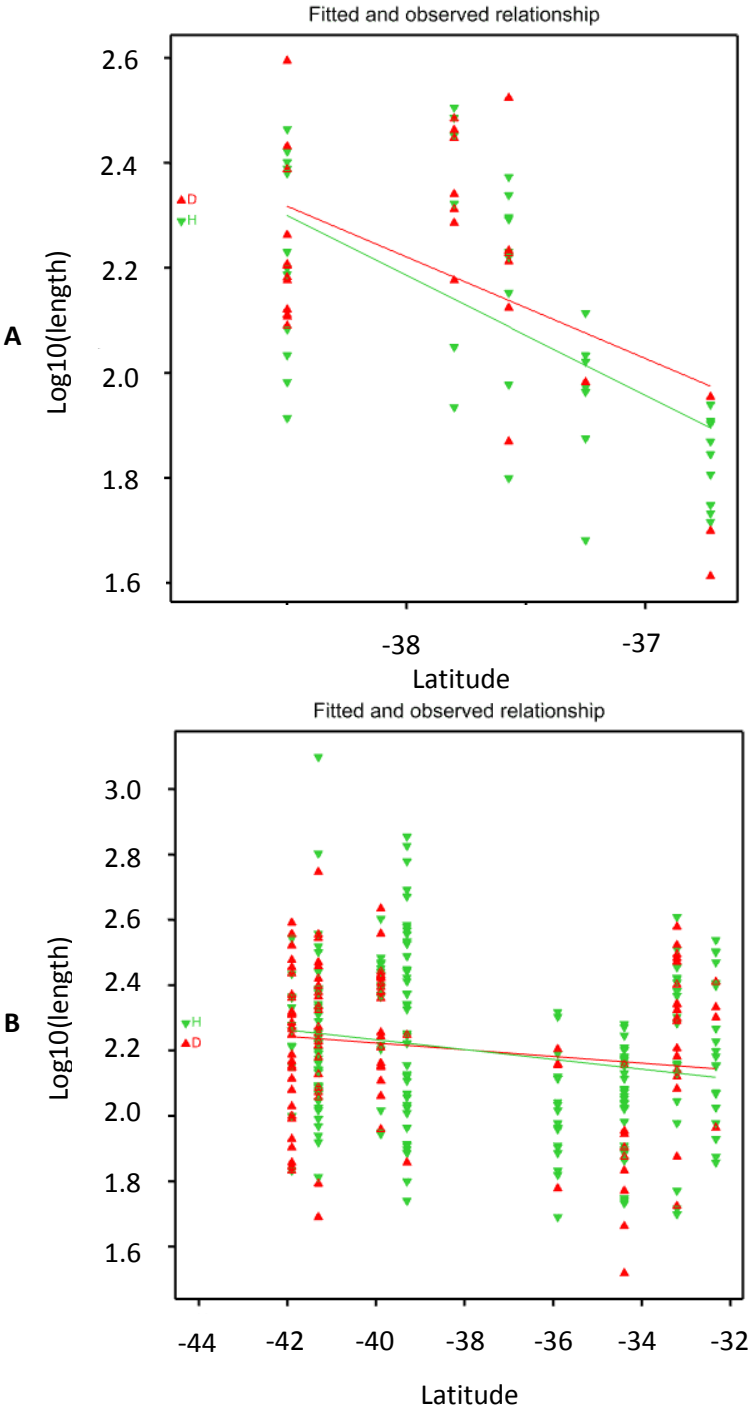


Figure 20 Linear regression with groups of log10(length) for infected (D, red) and healthy (H, green) intertidal kelp along latitudinal gradients in A) Australia and B) Chile.

4.5.2 Infection and length

Generalised linear modelling for intertidal data in Chile and Australia found infection status was not a significant predictor of length (Ch: p=0.605; Au: p=0.076). (Figure 21a, b). No significant length difference was found between healthy and diseased beach-cast kelp along the Chilean coast using a two–sample two-tailed t-test ($t_{(1232)}$=-1.77; p=0.077) (Figure 21c).

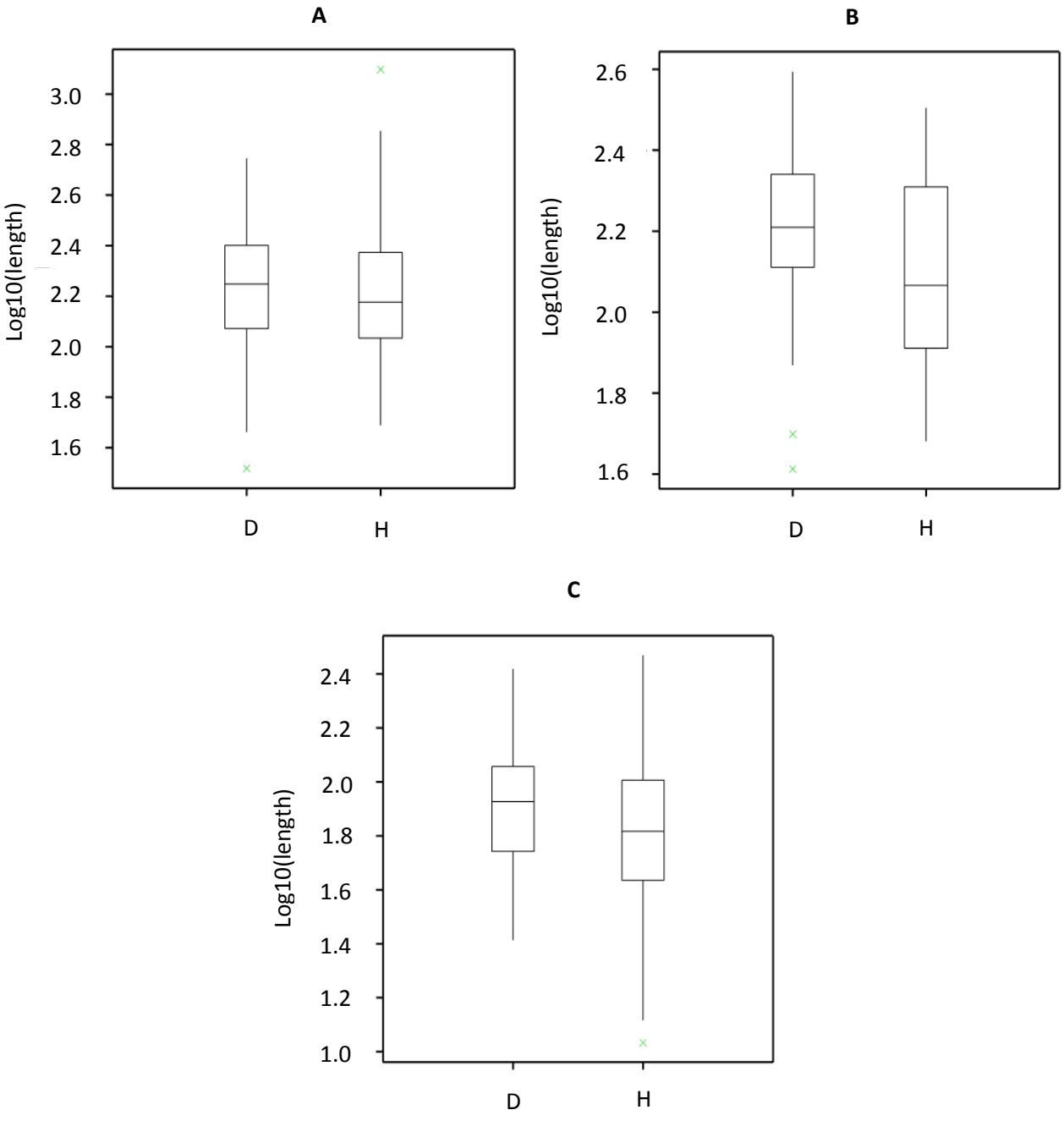


Figure 21 Boxplots of log10(length) for infected (D) and uninfected (H) intertidal kelp in A) Chile, B) Australia, and C) Chilean beaches.

4.6 *Seasonal effects: Chile*

Two-sample binomial testing of numbers of infected kelp in summer and winter for 2014/15 and 2015/16 showed a significant probability of season affecting infection incidence on beach-cast kelp (p=0.026). Winter appeared to show a greater proportion of infected kelp across years. Variability was evident between seasons and years, however, with number of infections proving particularly low in Summer 2014/15 (Figure 22).

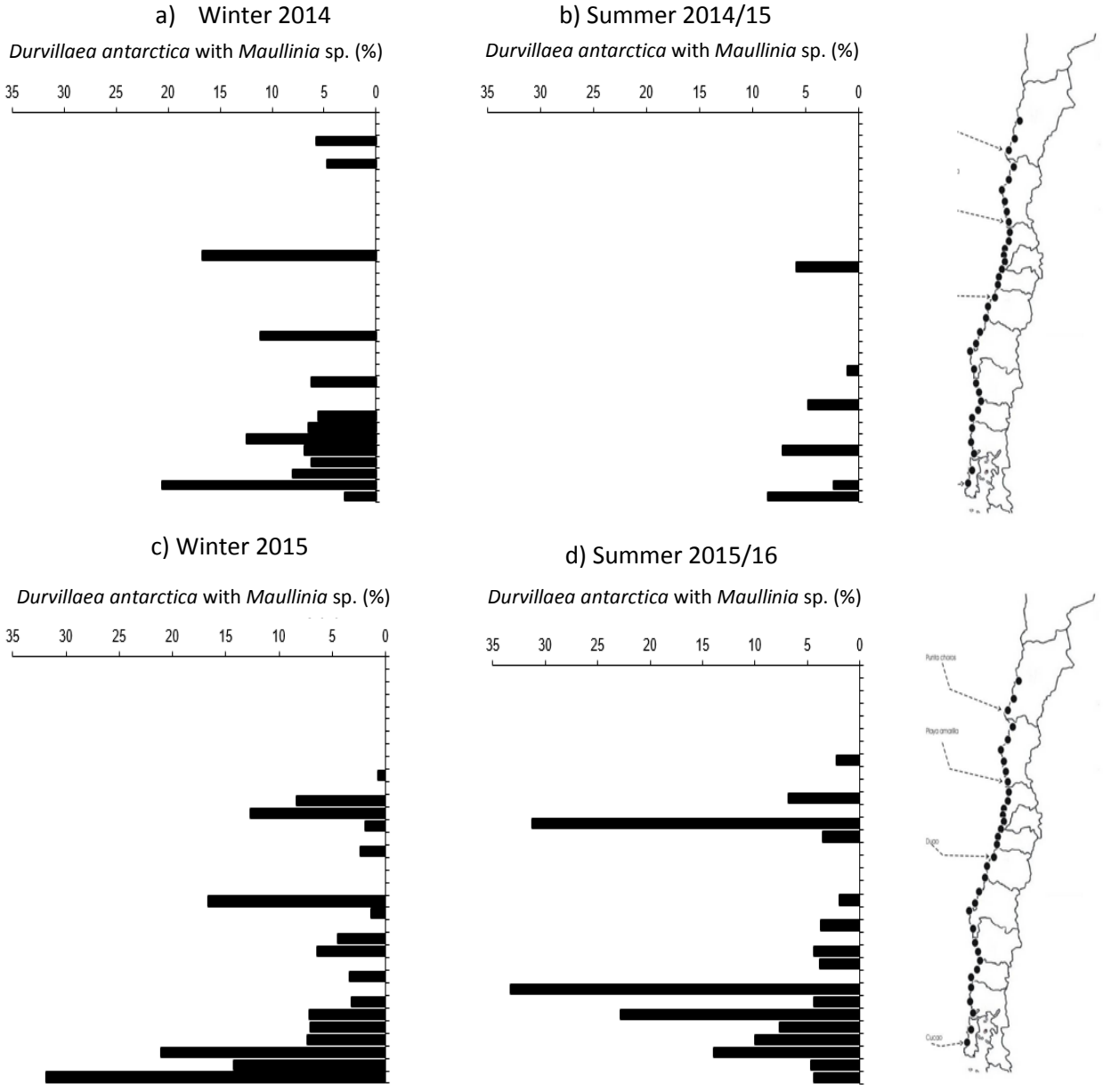


Figure 22 Percentage of infected kelp per beach sampling site along the Chilean coast for Winter/ Summer 2014/15 and 2015/16 (figure provided by Boris Lopez, UCN)

Chapter 5: Discussion

This research provides novel insights into the biogeography and infection dynamics of a marine algal pathosystem in Australia and Chile. Genetic data confirm, for the first time, the presence of *Maullinia ectocarpii* on populations of *Durvillaea potatorum* and *Durvillaea* sp. nov in south-eastern Australia, as well as extensive infections of *Maullinia* sp. on *Durvillaea antarctica* along the central and southern Chilean coast. Despite the presence of a rafting host (*D. antarctica*) in the Durvillaea-Maullinia (DM) pathosystem, trans-continental *Maullinia* sp. connectivity was not inferred from the results of this study. Similarity of *M. ectocarpii* populations between countries, however, indicates the possibility of connectivity outside pathosystem boundaries. Parasite genetic differentiation between the three host species in this research suggests an element of host specificity, while increasing host stress towards northern range limits does not appear to promote greater infection incidence. Rather, a consistent pattern of increased parasite prevalence towards higher latitudes in Chile and Australia suggests alternative processes shape pathosystem trends. While infections do not appear to relate to host size, understanding the seasonality and morphology of infections may assist in monitoring and managing this parasite into the future as *Durvillaea* spp. are placed under increasing stress from anthropogenic and environmental influences.

5.1 *Genetic data indicate differences between Maullinia species on Durvillaea hosts in Australia versus Chile.*

Australian and Chilean parasite populations were consistently separated into two deeply divergent clades within the genus *Maullinia*, with the 18S phylogeny showing strong Bayesian and ML support (Figure 18). Continental sample divergence ranged from 2.4-2.6%, aligning with the between-organism results of Kupper *et al.* (2006) and Goecke *et al.* (2012). While this marker has been used for protist species delineation before, similarities around 95% are believed to represent congeneric species for Cercozoan organisms such as the Phytomyxea (Pawlowski *et al.*, 2012), and further analyses of a range of genetic and morphological characters are needed to confirm species classification (Boenigk *et al.*, 2012; Pawlowski *et al.*, 2012). Within-country divergence ranged from 0.1%-0.3% in Chile and was 0.3% in Australia, with genetic diversity appearing to relate to host species (Table 6, Figures 15 & 16). Genetic analyses in this thesis are relatively simple compared to the complex phylogenetic and phylogeographical approaches available to modern biogeographers, however they provide preliminary insights into a range of dynamics potentially shaping DM pathosystem trends. Differences within and between species point to the potential role of large and small-scale dispersal, host shifts, and host specificity in maintaining or eroding genetic stratification within this parasite genus.

Dispersal in the DM pathosystem

The strong separation of *Maullinia* sequences between countries in this research (Figure 18) does not appear to support the hypothesis that the dispersal ability of *D. antarctica* promotes the genetic homogenisation of parasite populations across the Southern Ocean. While infections were confirmed through DNA barcoding of samples in Australia and Chile, none were found in samples collected in other sub-antarctic locales. A more directed sampling effort in those regions may reveal infections into the future, as the presence of this parasite on beach-cast kelp shows it is able to disperse at least small distances on rafting hosts. While analyses of the slowly-evolving 18S marker do not allow robust conclusions regarding contemporary connectivity (Hillis and Dixon, 1991), these data suggest the possibility of interesting dispersal trends. Differentiation between countries may point to population connectivity outside of pathosystem boundaries, and patterning of haplotypes within country may point to small-scale dispersal dynamics shaping the biogeography of the DM pathosystem.

The similarity of samples from the western Pacific to that obtained from *Ectocarpus siliculosus* in Chile by Maier *et al.* (2000) (0.1%-0.4% divergence) (Table 6, Figure 18) suggests that connectivity may be maintained by alternative processes than rafting on *D. antarctica*. While *E. siliculosus* is not recognised as a species able to disperse over long distances through rafting, long-term survival of floating individuals has been shown close to shore (Macaya *et al.*, 2005). The species is considered a 'cosmopolitan' alga, with an extremely broad distribution in a wide range of environments (Charrier *et al.*, 2008). Several species within the genus grow epiphytically on other rafting algal species (Macaya *et al.*, 2016), while others are known to grow as free-floating organisms (Macaya *et al.*, 2005), suggesting infected individuals may show a passive ability to transport pathogens long-distance. I propose that connectivity of populations of *M. ectocarpii* may be promoted through dispersal on vectors outside the DM pathosystem, such as other algae or anthropogenic elements (e.g. ship ballast), or through zoospores produced on such vectors. Such 'cryptic' linkages highlight the complexity inherent in pathosystem analyses.

The high genetic diversity and lack of phylogeographic structure along the Chilean coast (Figure 15) may point to the role of short-distance dispersal and coastal current systems in shaping connectivity. Geographic separation would lead to particular sequences becoming 'fixed' in certain locations, yet sequences in this research are largely spread across the entire sampling range (Figure 15). As this parasite's dispersal likely occurs through both rafting of infected tissue and larval/ zoosporic stages (Neuhauser *et al.*, 2011b), marine transport systems such as currents may promote genetic exchange between populations. The Chilean coast is affected by a number of such systems, in particular the Humboldt Current, which transports cool water towards the equator, and the Peru Countercurrent, which transports warmer subtropical water to the south (Silva *et al.*, 2009) (Figure 23). These currents are known to affect a range of organisms along the

Chilean coast (Peters and Breeman, 1993) including kelp rafts (Rothä usler *et al.*, 2011), with similar currents affecting organismal larval stages (Gaylord *et al.*, 2000). As such, the broad geographical spread of genotypes seen across sites in Chile (Figure 15) may be attributable to local genetic mixing through passive dispersal mechanisms.

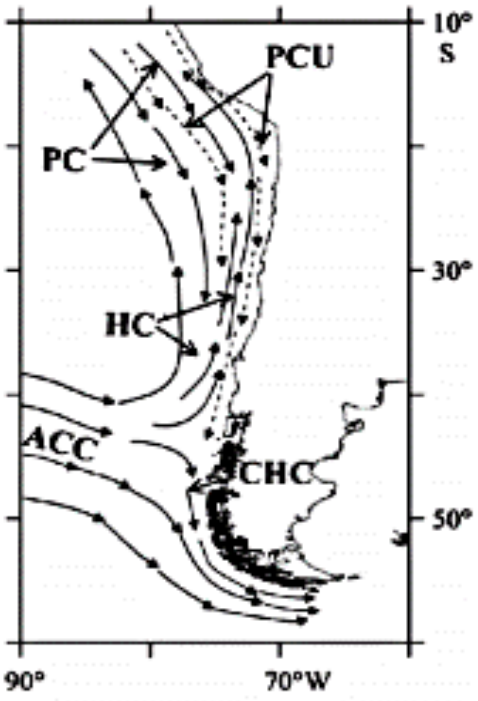


Figure 23 Current systems acting along the Chilean coast (HC: Humboldt Current, PCU: Peru Chile Underercurrent, ACC: Antarctic Circumpolar Current, PC:Peru Countercurrent, CHC: Cape Horn Current). (Silva et al., 2009)

In contrast, coastal current systems in south-eastern Australia are likely to maintain the separation of pathogen populations to the east and west of southern Victoria, leading to the genetic differentiation seen in the results of this research (0.3% divergence) (Table 6, Figure 16). Genetic and morphological differences have been shown for a range of biota across the biogeographical break at Wilson's Promontory in southern Victoria (O'Hara and Poore, 2000; Waters, 2008), including for the genus *Durvillaea* (Fraser *et al.*, 2009b; Weber, 2015). While this differentiation is broadly understood to be an historical outcome of vicariant processes related to the Bassian Isthmus land bridge during the last glacial maximum (Fraser *et al.*, 2009b) (Figure 24), it has been maintained due to modern oceanographic processes. The East Australian (EAC) and Zeehan (ZC) currents are implicated in the separation of highly dispersive organisms such as *Nerita* species across an east-west disjunction at Wilson's Promontory (Waters *et al.*, 2005), leading to genetic and morphological differences between populations. The EAC, which runs down the eastern Australian coast, forms currents and eddies towards southern NSW that may trap organisms in local areas or limit dispersal down the south-eastern coasts of the mainland and Tasmania (Smith

et al., 2015). The ZC runs southward down the western side of Tasmania, transporting water and organisms around the southern edge of the island (Weber, 2015). As such, differences seen in the genetic composition of *M. ectocarpii* populations across the break at Wilsons Promontory in Australia (Figure 16), and the lack of any sequences shared across the break, suggest dispersal remains constrained to within eastern and western areas, with isolation promoting ongoing molecular differentiation. This study examined five sites, with only one site west of Wilson's Promontory and less than ten sequences per site, suggesting further analyses of a greater number of samples may confirm whether this pattern reflects ecological reality.

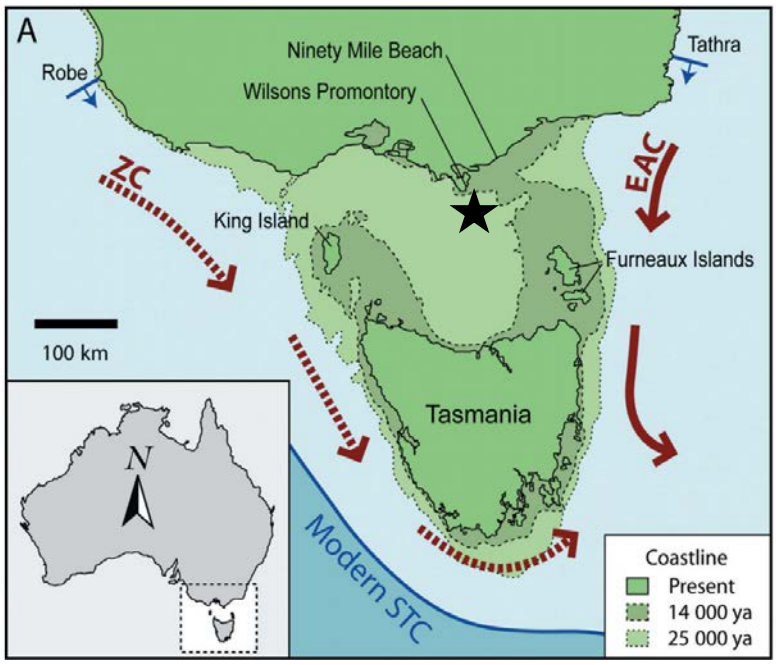


Figure 24 Map of Southeastern Australia showing the Zeehan (ZC) and East Australian (EAC) currents, historical coastlines of the Bassian Isthmus, and the proposed biogeographical break at Wilson's Promontory (star) (adapted from Fraser et al., 2009b).

Host range and host shifting

The separation of *M. ectocarpii* and *Maullinia* sp. haplotypes between hosts in south-eastern Australia and Chile (Figures 15 & 16) also suggest important implications for host ranges, shifting, and specificity within this protist genus.

It is highly likely that *M. ectocarpii* underwent a host shift in Australia, rather than arriving on *Durvillaea* spp. material from another locale, as infections have been recorded on *E. siliculosis* in previous research (Maier *et al.*, 2000). Species within the same genus often show differential host ranges and specificity (Staats *et al.*, 2005), from highly specialised to generalist interactions, and so it is possible that the organism found in Chile has specialised to its *Durvillaea* host, whereas that in Australia transferred from an alternative host. Host shifting may allow

parasites to survive long-term in environments with ephemeral hosts or adverse environmental conditions (Neuhauser *et al.*, 2014), as infections on one species supplement those on another. This research did not seek to understand how infections on multiple hosts alter infection trends for the DM pathosystem. These preliminary findings provide a tantalising glimpse, however, of how host-shifting may affect the emergence of host-specific genetic lineages in Australia and Chile.

Host specificity: co-evolution vs. host shift differentiation

Congruence in host and parasite phylogeographic structure can reveal the molecular implications of symbiotic interactions over a range of timescales. Host species-scale congruence was found for sequences obtained in this research (Figures 15 & 16), suggesting possible implications regarding the molecular specialisation of parasites.

Due to the often obligate nature of parasitic interactions, evidence has shown that host speciation events may lead to concurrent speciation of pathogens (co-evolution) due to the 'evolutionary arms race' necessary for hosts to resist, and parasites to promote, infection (Carius *et al.*, 2001). Alternative pathways for genetic differentiation arise from novel host-parasite interactions, such as the movement of pathogens onto new hosts and subsequent biotic and behavioural specialisation (host-shift differentiation) (de Vienne *et al.*, 2013) (Figure 25). While the concept of host-parasite co-speciation has long been of interest to parasitologists, increasing evidence has suggested the incidence of such events has been overestimated and that host shifts more regularly shape patterns of parasite genetic specificity (de Vienne *et al.*, 2013). For example, comparisons of phylogenies for the plant pathogenic genus *Botrytis* and a range of angiosperms revealed differences in divergence times that indicated host-shifting as the primary factor shaping molecular host specificity (Staats *et al.*, 2005). Research on *Eurychasma dicksonii* suggested algal host genetic factors have a strong impact on infection success (Gachon *et al.*, 2009), confirming pathogens need to adapt to specific hosts for pathosystem proliferation.

The relatively simple molecular approaches of this research do not allow phylogenetic tracking of *Durvillaea* and *Maullinia* species to look for long-term congruence. Furthermore, difficulties in the molecular dating of protist species due to a lack of fossil records and a poor understanding of baseline levels of genetic differentiation (Weisse, 2008) make it difficult to draw conclusions regarding molecular links between parasite and host clades. The host-tracking of parasite sequences in the DM pathosystem (Figure 26) suggest, however, that molecular characteristics of parasites may act to shape infection trends.

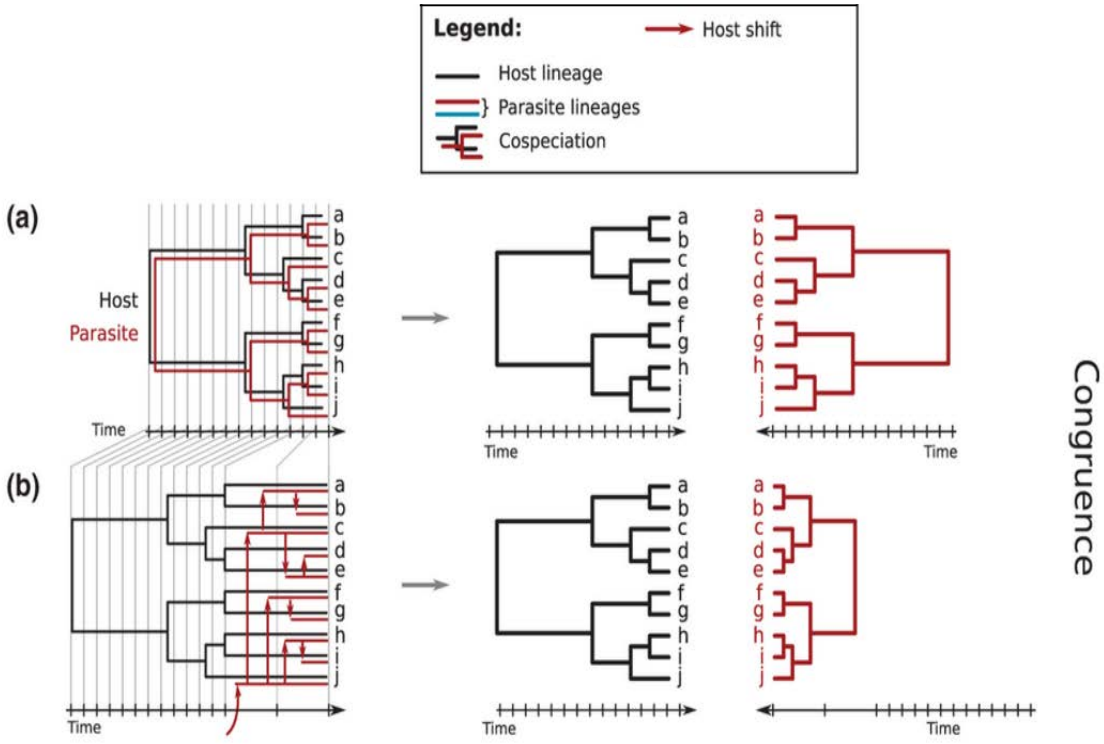


Figure 25 Illustration of the processes of a) host-parasite co-evolution, and b) host shift genetic differentiation, resulting in pathosystem molecular congruence (de Vienne et al., 2013)

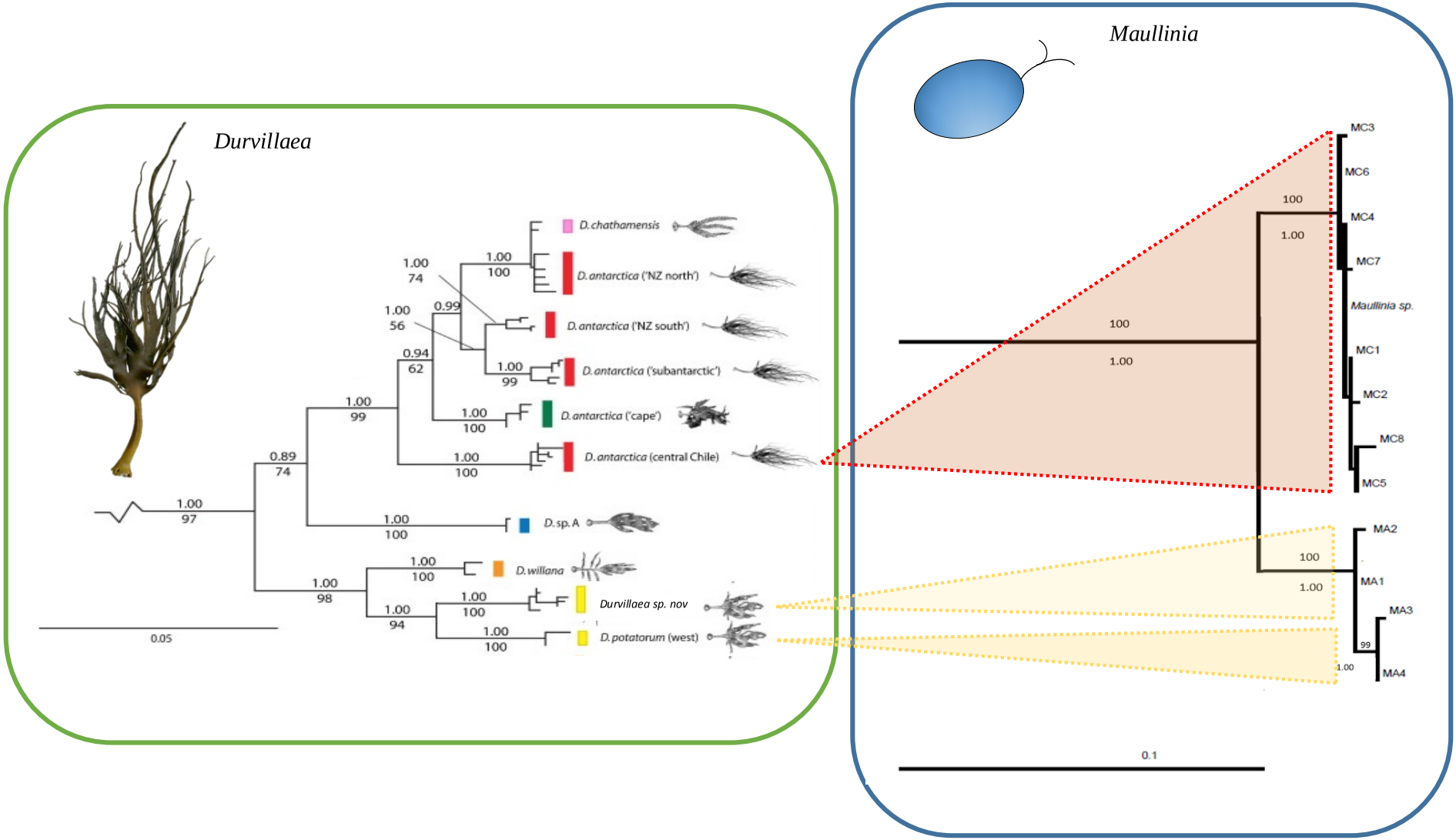


Figure 26 Multi-gene maximum likelihood phylogeny of <u>Durvillaea</u> species (adapted from Fraser et al., 2010b), and 18S phylogeny of <u>Maullinia</u> samples showing congruence in host-parasite genetic structure.

5.2 *Infection incidence increases towards hosts' southern latitudinal range limits.*

My hypothesis that infection incidence would increase towards hosts' northern latitudinal range limit due to increasing physiological stress was not supported, with infection incidence instead found to increase towards higher latitudes (Figure 19). This result supports the postulation of Aguilera (1988) that infections within this pathosystem intensify towards the south in Chile. Variability in infection incidence suggests parasite susceptibility to local conditions (Poulin *et al.*, 2012), and as latitude was found to be a significant factor shaping the distribution of infections (Figure 19, Table 7), it likely represents an element of the environment implicated in pathosystem dynamics.

Central abundance supporting infection

Pathogen transmission is known to increase with higher densities and greater connectivity between hosts (Izhar and Ben-Ami, 2015; Poulin *et al.*, 2012). Central Abundance Theory, which postulates an organism will be found in greatest numbers and densities at the centre of its geographical range (Samis and Eckert, 2007), would thus suggest greater risk of infection towards the centre of species' latitudinal ranges (Figure 27).

Durvillaea species in Australia are known to show strong morphological relationships to latitude and wave exposure (Cheshire and Hallam, 1989), transitioning from a relatively rare intertidal element at Tathra to a dominant habitat forming species on rocky intertidal platforms in Tasmania (Millar, 2007). While kelp density was not quantified in this research, morphological data indicated a significant increase in the length of Australian *Durvillaea* spp. with increasing latitude (Figure 20), suggesting greater environmental suitability. While no sampling was undertaken in the true centre of host species' ranges in Australia (northern Tasmania) (Figure 27), this trend supports the concept of greater density and connectivity between populations occurring at higher latitudes.

D. antarctica in Chile experiences competition from other algal species including *Lessonia* spp. towards its northern latitudinal range limit, reducing its density and prevalence (Santelices *et al.*, 1980). Throughout the centre of its distribution in both Chile and other sub-antarctic locales, it reaches high densities and biomass, becoming a major component of most rocky intertidal zones (Taylor and Schiel, 2005). While genetic data have suggested low connectivity between coastal areas from rafting (see Fraser *et al.*, 2010a), organisms able to disperse through zoospores and on

algal rafts may show greater connectivity with the greater abundance of hosts. This trend may be further strengthened through the directionality of dispersal in coastal current systems.

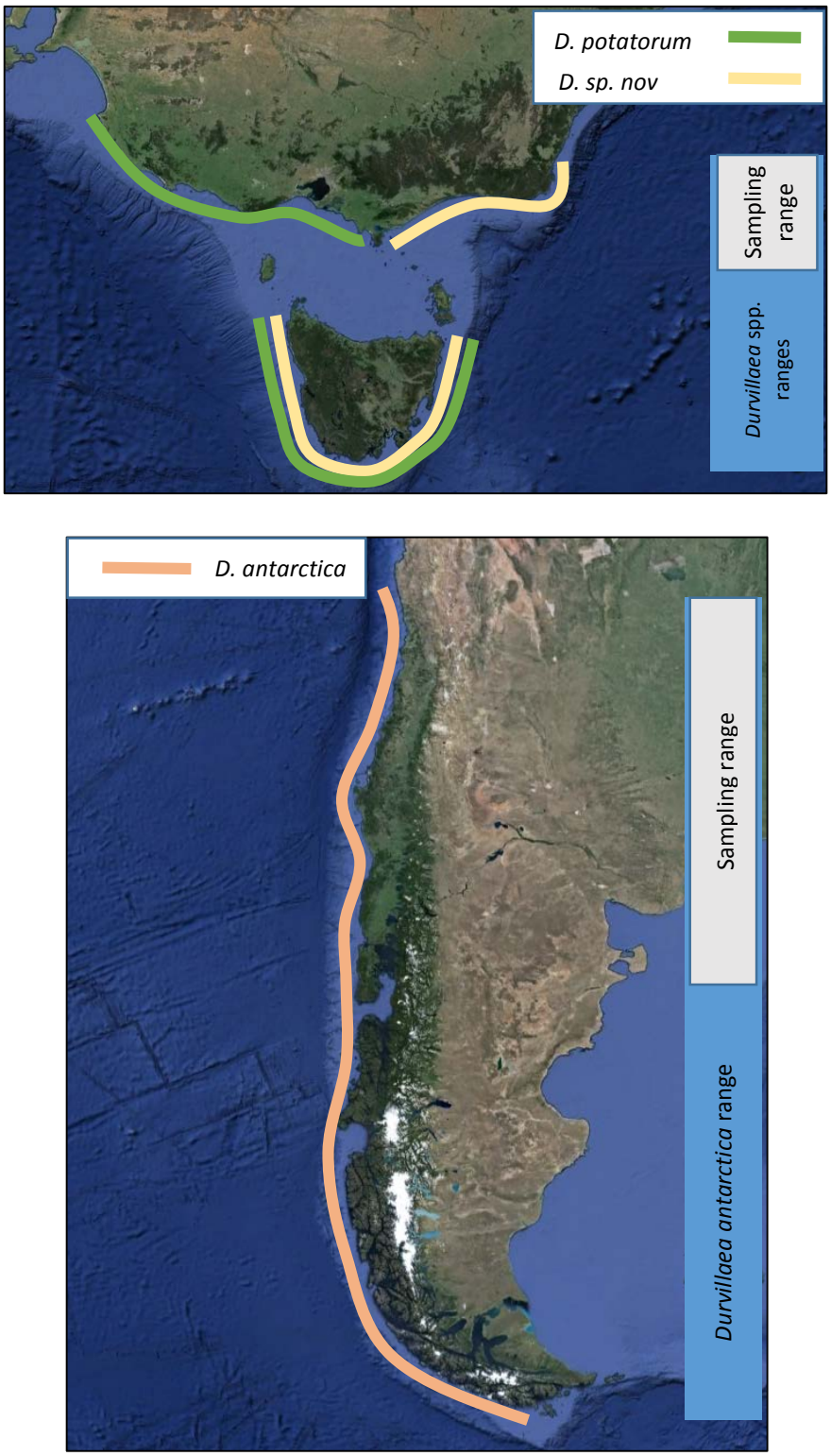


Figure 27 Maps of Australia (Google Earth, 2015b) and Chile (Google Earth, 2015a) showing geographical ranges of <u>Durvillaea</u> species and sampling ranges of this thesis. In both cases sampling was undertaken to the approximate centre of host ranges.

Coastal current systems

The ZC and EAC run in a primarily southern direction (Figure 24), promoting the dispersal of rafting and larval stages of organisms down the south-eastern coast of mainland Australia, and the western and eastern coasts of Tasmania. While the EAC has been shown to promote small-scale northward mixing of some species along the southern NSW coast due to seasonal eddy formation (Smith *et al.*, 2015), its flow throughout the rest of the year likely transports organisms southward. This would also explain the genetic mixing seen within the NSW and eastern Victorian coastlines (Figure 16) as eddies transport small numbers of dispersive organisms between regional populations.

The Humboldt Current runs primarily northward along the Chilean coast (Figure 23). Along with westerly winds, is believed to transport organisms to lower latitudes; however, research has shown patterns of dispersal and accumulation can operate in a range of directions due to interactions between wind, oceanographic features, coastal geomorphology, and dispersal mechanism (i.e. floating on kelp) (Hinojosa *et al.*, 2011). Preferential transport and accumulation of a greater number of infected hosts or parasite larval stages to higher latitudes (central abundance) would increase the connectivity of southerly hosts, intensifying infection incidence away from northern latitudinal range limits. If a parasite were able to arrive at such northern populations, the possibility also exists that underlying characteristics of hosts such as resistant ecotypes may reduce risk of infection.

Ecotype effects

While it was originally hypothesised hosts would prove most susceptible to infection towards their range limits due to higher physiological stress, a contrasting body of literature explored the possibility of these hosts showing greater resistance due to their ability to survive in marginal conditions. As data for the DM pathosystem do not support highest infection incidences occurring towards host range limits (Figure 19), it is possible that *Durvillaea* ecotypes show differential abilities to resist infection along the latitudinal gradients sampled.

Ecotypes remain a factor of particular interest to pathosystem research, as it is increasingly suggested that genotype-by-environment interactions shape broad-scale parasite trends (Wolinska and King, 2009). Host and parasite fitness (i.e. resistance and virulence) are differentially affected by the environment in which they operate (Wolinska and King, 2009), with intraspecific genetic differentiation often reflecting the response of an organism to its local conditions (Kaltz and Shykoff, 1998). *Durvillaea* ecotypes able to survive at range limits may, due to underlying genetic or morphological characters, prove more resistant to pathogenic infection than those under comparatively less stress towards the centre of their range. Ecotype effects have been shown for other algal species including *Undaria pinnitifida* (Gao *et al.*, 2012), whose populations varied in

temperature resistance depending on the latitude from which samples were extracted. Individuals at range limits often show genetic and morphological differences allowing them to resist adverse environmental and biological interactions (e.g. Pereira *et al.*, 2015), suggesting genotype-by-environment interactions may shape *Durvillaea* spp. pathogen infection rates. Such ecotypes often develop due to strong environmental patterns, the most pervasive of which being temperature gradients in the marine intertidal realm (Cruces *et al.*, 2012).

Temperature effects

The relationship between latitude and water temperature is a well-recognised phenomenon along a range of coastlines (Tuya *et al.*, 2012), with both Australia and Chile showing a steady decrease in mean water temperature towards higher latitudes (Figure 28). Temperature effects have been shown in a range of terrestrial and marine pathosystems (Case *et al.*, 2011), acting on both host and parasite. Thermal properties are well-recognised for *Durvillaea* species, with increasing temperature associated with increased physiological stress (Cruces *et al.*, 2012), and while such effects remain unquantified for *Maullinia* species (Neuhauser *et al.*, 2011b), data from this research suggest the possibility of thermal factors playing a role in the DM pathosystem.

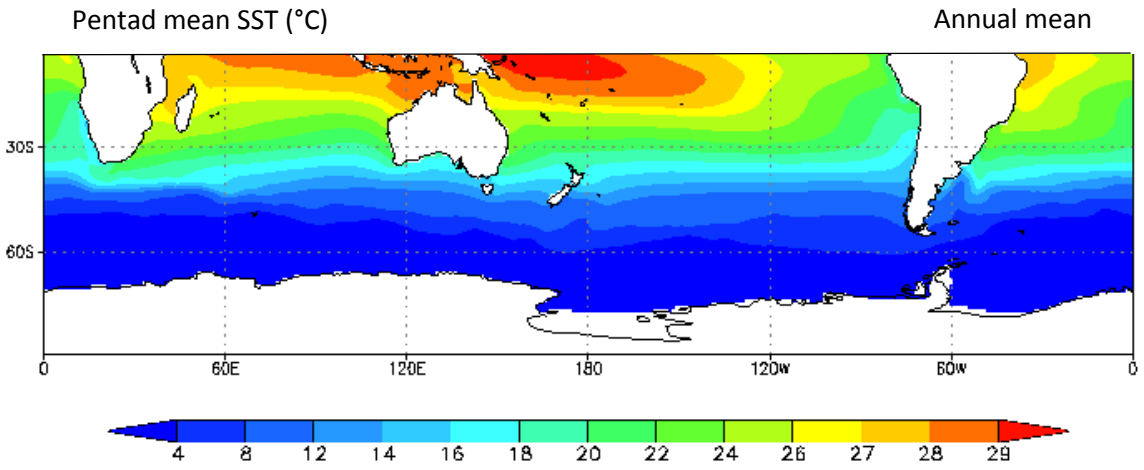


Figure 28 Annual mean sea-surface temperature in the Southern Hemisphere, showing decreasing temperature with increasing latitude (adapted from Voelker, 2002)

Similarity in the temperature requirements of host and parasite have been found in previous pathosystem research (Molina *et al.*; Studer *et al.*, 2010), suggesting moderate temperatures in which hosts grow best may also support greater virulence, and higher infection incidence, for parasites. A greater body of literature, however, has supported temperature extremes (often at species range limits) being fundamental to increased infection incidence (Mitchell *et al.*, 2005; Granke and Hausbeck, 2009; Eggert *et al.*, 2010), particularly when the thermal tolerance of parasitic species is larger than that of its host. Consistency in latitudinal trends for this research

would suggest the DM pathosystem may be most active in ‘moderate’ temperatures towards the centre of the host species’ latitudinal range. Thermal tolerance depends on a species’ life history, as larvae have been found to persist for shorter periods at sea when exposed to heat (Bradbury *et al.*, 2008; Cowen and Sponaugle, 2009), limiting ability to disperse and, in the case of parasites, infect new hosts. As such, the possibility exists that temperature effects for the DM pathosystem act on a number of scales. Cooler temperatures towards higher latitudes may hasten the development of parasites on their host, or may promote the dispersal and long-term survival of larval stages.

The consistency of the southward infection intensification between countries (Figure 19) and over time (see Aguilera, 1988), despite differing host and parasite species, would suggest large-scale environmental characteristics such as temperature may be shaping the pathosystem trend. While a reduction in temperature towards higher latitudes would support the linear trend of the GLMs, outliers in infection incidence point to how alternative factors may shape variability in pathosystem trends.

Alternative environmental variables

Analysing outliers for infection incidence alongside qualitative observations reveals how alternative factors may shape DM pathosystem infection dynamics. While this research was not concerned with small-scale variables involved in infection incidence along latitudinal gradients, previous data have suggested the possibility of anthropogenic impacts such as pollutants (Lafferty and Kuris, 1999) and kelp harvesting (Bustamante and Castilla, 1990; Castilla and Bustamante, 1989; Castilla *et al.*, 2007) shaping overall trends.

Pollution is a factor increasingly implicated in outbreaks of marine disease (Li *et al.*, 2010; Saavedra, 2011), including on species of algae (Buschmann *et al.*, 2014). Pollutants may be considered as having greatest impacts closest to large population centres or areas of industry, and being least impactful in zones that experience ‘flushing’ from water movement or are at a distance from dense population centres. The lowest infection incidence in Australia was measured at City Rock (site 2, Figure 19a), an area proximate to the Ben Boyd terrestrial National Park and approximately 15km from the closest town. Water quality is likely to be higher here than sites such as Cape Schanck, the site closest to Melbourne and other towns along the Mornington Peninsula, where large numbers of infected kelp were encountered (site 5, Figure 19a). While data from Quintay were omitted from quantitative analyses due to uncertainty regarding infection identification (see section 4.1), this sampling location is the most proximate site to Valparaiso and Santiago de Chile, the most densely populated areas in Chile. Anthropogenic impacts at this site may therefore be highest, leading to greater disease incidence on local kelp populations.

Another element that was unaccounted for in research and analytical design was the cultural and economic significance of *D. antarctica* in Chile, and the implications of kelp harvesting on overall parasite loads. Cochayuyo (*D. antarctica*) is heavily harvested along the southern-Chilean coast, more so than in central/ northern locales. As such, healthy individuals may be preferentially removed for sale, leaving a greater number of infected individuals on rocky intertidal platforms in the south, and altering incidence measures. While kelp harvest was evident at a number of sites (see Table 4, Figure 29), visual surveying of removed individuals at such sites suggested infected and uninfected kelp were collected equally, without clear avoidance of those with galls.

Figure 29 Evidence of kelp harvest at Mar Brava

5.3 *Infection morphology suggests ecological implications and a need for further research*

Infection morphology remained largely consistent between hosts (Figures 12 & 13). Rates of amplification for DNA barcoding suggests field-based identification of this parasite based on morphology may be undertaken with relatively high confidence (see section 4.3), allowing long-term monitoring of infection trends. The infection progression of this parasite on *Durvillaea* hosts described in Figure 14 adds to the conjecture of Neuhauser *et al.* (2011b) and Goecke *et al.* (2012) regarding the potential impacts of *Maullinia* and other Phytomyxean parasites in marine environments. Analyses of the relationship between host length and infection, while revealing no pattern for this pathosystem (Figure 21), may suggest host genus characteristics swamp out morphological impacts of infection. Seasonal fluctuation in numbers of infected kelp (Figure 22) would suggest a need for continued research on the dynamics of the DM system, and monitoring

of infections over short (seasonal) and long (yearly/ decadal) timescales in light of predicted increases in infection intensity under future climate scenarios (Eggert *et al.*, 2010).

Infection morphology and impacts

The hypothesis that infected kelp would show decreased length due to resource partitioning in galls was not supported in this research (Figure 21). This is likely to be an outcome of the morphological plasticity of the host genus, whose variability in length is already affected by a number of factors including wave exposure, age and stress (Cheshire and Hallam, 1989; Fraser *et al.*, 2009a; Weber, 2015). The morphology of *Maullinia* spp. galls on *Durvillaea* hosts suggest alternative impacts, however, as infected tissue is prone to necrotic breakage, leading to potentially large numbers of infected fronds entering the marine environment.

Neuhauser *et al.* (2011b), Gleason *et al.* (2014) and Goecke *et al.* (2012) propose broad-scale impacts for parasites including Phytomyxea in marine food webs. These impacts operate on a range of scales, with dispersing larvae providing food for low trophic levels (Sime-Ngando and Niquil, 2011), and alterations to the metabolism and reproductive potential of hosts through tissue hypertrophy increasing organic matter entering food webs (Neuhauser *et al.*, 2011a). Frond damage by *Maullinia* spp. infections was previously only hypothetically linked to increases in tissue breakage, and beach-cast data for this research confirm this trend. Large numbers of entire kelp and fragments showed signs of infection and evidence of breakage at the site of galls (Figure 30), supporting the postulation that infections of organisms such as *Maullinia* spp. input large volumes of host tissue into the environment. Data suggest, however, that the impacts of this input may vary throughout the year.

Figure 30 Evidence of tissue breakage on infected kelp

Infection seasonality

The significant effect of season on infection incidence for beach cast kelp (Figure 22) suggests two possibilities for temporal change in the DM pathosystem: firstly, infection incidence increases in cooler months, and secondly, a greater proportion of infected kelp are detached and transported during winter. While temperature effects were discussed as possible factors shaping infection trends in the DM pathosystem (section 5.2), I propose preferential winter detachment is most likely, due to well-known patterns of seasonal intertidal pathogen shedding in Chile.

Winter storms along the Chilean coast are a seasonal occurrence known to dislodge and deposit intertidal organisms, including kelp, on beaches. The high frequency and violence of such storms has been linked to the preferential removal of dead or diseased organisms (Correa *et al.*, 1993), and proposed as a factor acting to control pathogen population size. Seasonal fluctuations in the incidence of bacterial infections were found on *Iridaea laminarioides*, and linked to temperature effects and winter dislodgement of kelp during storms (Correa *et al.*, 1993). As such, it is highly likely that hosts weakened by *Maullinia* spp. or other gall-forming parasites are removed in greater numbers during winter months, as healthy individuals show greater structural resistance. This would suggest the parasites dispersal on host tissue spikes during such events, which may have implications for between-population connectivity as dispersal is promoted during winter months on diseased *D. antarctica* tissue. The seasonality of this pathosystem will need to be determined through similar long-term sampling in Australia to determine if this represents a large-scale trend.

Much previous research regarding *Maullinia* spp. in marine intertidal zones has hypothesised broad-scale impacts on algal assemblages. This thesis confirms these suspicions, showing impacts from infections on intertidal and beach-cast kelp. The reduction in frond structural integrity from gall formation and necrosis, and associated increase in frond detachment, combined with the prevalence of winter storms along the Chilean coast would suggest this pathosystem experiences cyclical dispersal spikes. Determining any temporal patterns in the Australian DM pathosystem may provide interesting insights into how the organisms of interest react to environmental change, and the quantitative impacts of tissue loss from infections.

5.4 *Limitations, implications, and future research*

Limitations

This research did not aim to collect comprehensive phylogenetic and ecological data, but rather describe broad trends for the DM pathosystem over a large geographical area. The unexpected molecular findings of this thesis suggest a need for more complex phylogeographical analyses. The small number of sequences obtained provide a tantalising glimpse into molecular interactions between the organisms of interest. However, the rigour of molecular work would be

improved through analyses more samples, and sequencing of a greater range of markers, to confirm genetic trends.

Similarly, approaches used to understand environmental trends limit the scope of conclusions. As latitude can be considered a proxy for a range of environmental variables, a number of factors may affect the proposed trend of increased infection incidence with increasing latitude. Further, the large difference in latitudinal ranges between continents (two degrees in Australia vs. nine degrees in Chile), and associated difference in numbers of sampling sites, may have contributed to the significant effect found for latitude between-countries. This is particularly true as Australian latitudes fit within the sampling gap in Chile (see Figure 19c). The overall trend from these data is of greater interest, however, than the significance found through modelling.

For the purposes of this thesis it was assumed field-based identification of *Maullinia* infections was rigorous due to similar morphology and amplification rates for samples collected (section 4.3). While randomisation of gall sampling improved the rigour of conclusions, the possibility exists that trends represent the dynamics of a range of gall-forming diseases, rather than just *Maullinia*, as not every gall was able to be collected. Further, multiple pathogens may infect a single host, suggesting several samples may need to be taken for DNA barcoding.

While seasonal information suggests interesting implications for temporal patterns in infection incidence, the paucity of data (i.e. only two seasons) limits the strength of conclusions regarding long-term trends. These data do, however, represent a first step towards developing a system for monitoring the effects of this pathosystem interaction into the future.

Implications

Any disease on keystone organisms such as *Durvillaea* species will inevitably have flow-on effects to other trophic levels. This thesis has shown infections of *Maullinia* are common on this algal genus in Australia and Chile, supporting its description as a cosmopolite. Damage caused to hosts may not directly lead to mortality, but affect the distribution of algal matter in the marine environment. If predictions are correct and infections increase into the future, this may have implications for the population structure and harvest of the genus in sub-antarctic and cold-temperate regions.

Future research

More research will be needed to quantify long-term effects. Due to time and budget restraints, attempts to develop primers for alternative molecular markers were abandoned. Such data will be integral to elucidating the genetic interactions between *Maullinia* spp. and *Durvillaea* spp. hosts. Determining contemporary patterns of connectivity will require concurrent studies of host and parasite, using molecular approaches such as microsatellite analyses.

Extending sample collection to the coasts of Tasmania, Patagonia (Chile), and sub-antarctic locales will be central to confirming whether host-specific sequences are an exception or a rule for the parasite genus, and whether infection incidence continues with the same trend south of continental Australia and central Chile. More complex environmental analyses, including measurements of variables such as water quality, and modelling of factors such as temperature over time, may reveal the link between latitude and infection incidence.

Qualitative observations of gall morphology are inherently subjective, and marking infected kelp and monitoring the progression of gall size, shape, and necrosis over time (similar to Aguilera, 1988) may provide greater insight into how infections develop.

Chapter 6: Conclusions

Understanding the factors implicated in outbreaks of marine disease is essential to appropriate monitoring and management of marine ecosystems into the future. This research has shown the importance of combining molecular and ecological approaches in order to describe pathosystem dynamics, particularly when the system includes a micro-organism whose identification can prove challenging. Analysing the *Durvillaea-Maullinia* (DM) pathosystem has provided insights into marine protist parasitology and algal pathology, increasing knowledge regarding the biogeography and infection dynamics of component species.

Previous research limited the distribution of *Maullinia* spp. on *Durvillaea* spp. hosts to central Chile. This thesis has shown that the parasitic genus is infecting Australian populations of *Durvillaea potatorum* and *Durvillaea* sp. nov, and has probably been doing so since the 1970s (see Jahnke, 1978). The existence of this parasite on brown algal hosts across the Pacific would suggest a high likelihood of the pathosystem extending to other locales. While long-distance rafting on *D. antarctica* was not supported by data in this study, alternative dispersal vectors outside pathosystem boundaries may explain genetic similarity between *M. ectocarpii* in Australia and Chile. The presence of the parasite on beach-cast kelp, and low genetic structure along the Chilean coast, suggest rafting on *D. antarctica* is possible, and likely occurs over short distances alongside dispersal through zoospores. The molecular differentiation found between Australia and Chile suggest two species within this parasite genus may infect *Durvillaea* hosts, with one, *Maullinia ectocarpii*, proposed to have undergone a host shift in the western Pacific.

Alongside suggestions of host shifting, genetic data point to the possibility of unique lineages infecting *Durvillaea* species. This specificity may increase virulence on different hosts, affecting infection trends, however further research will be needed to confirm such effects. Overall, ecological data from this research show a similar infection trend along the two latitudinal gradients sampled. Infection incidence increased towards higher latitudes, going against ecological theory regarding the effects of range stress on host resistance. Several possibilities may explain this phenomenon, including interactions between biotic and environmental factors such as ecotypes and temperature.

Further research will be needed to determine what factors shape infection trends. This thesis provides a preliminary description of interactions between organisms in the DM pathosystem. More complex phylogenetic and environmental analyses will enable researchers to tease out what elements of the organisms and environment in which they operate alter infection incidence. Molecular analyses using a greater number of markers, and modelling of a range of environmental factors will be crucial to understanding the impacts of *Maullinia* spp., the ‘Sinister Southerners’, on *Durvillaea* spp. populations under future climate scenarios.

References

Abbott, I. A., 1996. Ethnobotany of seaweeds: clues to uses of seaweeds, *Hydrobiologia,* **326-327**(1): 15-20. Available at: http://dx.doi.org/10.1007/BF00047782

Abbott, K. C., 2011. A dispersal-induced paradox: synchrony and stability in stochastic metapopulations, *Ecology Letters,* **14**(11): 1158-1169. Available at: http://dx.doi.org/10.1111/j.1461-0248.2011.01670.x

Aguilera, M., Rivera, P.J., Westermeier, R., 1988. Presencia de hongos plasmodiophorales en plantas de *Durvillaea antarctica* (Cham.) Hariot (Phaeophyta, Durvilleaceae) del sur de Chile, *Gayana Botanica,* **45**(1): 337-343.

Alberto, F., Raimondi, P. T., Reed, D. C., Coelho, N. C., Leblois, R., Whitmer, A. and Serrão, E. A., 2010. Habitat continuity and geographic distance predict population genetic differentiation in giant kelp, *Ecology,* **91**(1): 49-56. Available at: http://dx.doi.org/10.1890/09-0050.1

Anderson, R. M. and Gordon, D. M., 1982. Processes influencing the distribution of parasite numbers within host populations with special emphasis on parasite-induced host mortalities, *Parasitology,* **85**(02): 373-398. Available at: http://dx.doi.org/10.1017/S0031182000055347

Andrews, J. H., 1976. The pathology of marine algae, *Biological Reviews,* **51**(2): 211-252. Available at: http://dx.doi.org/10.1111/j.1469-185X.1976.tb01125.x

Atlas of Life in the Coastal Wilderness, 2014. *Durvillaea potatorum (Labillardiere) Areschoug,* Available at: http://alcw.ala.org.au/bdrs-core/alcw/home.htm (accessed 20/11/15).

Avise, J. C., 2009. Phylogeography: retrospect and prospect, *Journal of Biogeography,* **36**(1): 3-15. Available at: http://dx.doi.org/10.1111/j.1365-2699.2008.02032.x

Blake, A., 2015. *Shore Dive Sites- Wilsons Prom,* Available at: http://www.diveoz.com.au/index.php/forum/dive-destinations-australian-diving-destinations/27405-shore-dive-sites-wilsons-prom.html (accessed 6/11/15).

Blakeslee, A. M. H., Byers, J. E. and Lesser, M. P., 2008. Solving cryptogenic histories using host and parasite molecular genetics: the resolution of *Littorina littorea*'s North American origin, *Molecular Ecology,* **17**(16): 3684-3696. Available at: http://dx.doi.org/10.1111/j.1365-294X.2008.03865.x

Boenigk, J., Ereshefsky, M., Hoef-Emden, K., Mallet, J. and Bass, D., 2012. Concepts in protistology: Species definitions and boundaries, *European Journal of Protistology,* **48**(2): 96-102. Available at: http://www.sciencedirect.com/science/article/pii/S0932473911000824

Bradbury, I. R., Laurel, B., Snelgrove, P. V. R., Bentzen, P. and Campana, S. E., 2008. *Global patterns in marine dispersal estimates: the influence of geography, taxonomic category and life history*, Proceedings of the Royal Society of London B: Biological Sciences, London, Available at: http://rspb.royalsocietypublishing.org/royprsb/275/1644/1803.full.pdf

Bulman, S., Ridgway, H. J., Eady, C. and Conner, A. J., 2007. Intron-rich gene structure in the intracellular plant parasite *Plasmodiophora brassicae*, *Protist,* **158**(4): 423-433. Available at: http://www.sciencedirect.com/science/article/pii/S1434461007000296

Burki, F., Kudryavtsev, A., Matz, M. V., Aglyamova, G. V., Bulman, S., Fiers, M., Keeling, P. J. and Pawlowski, J., 2010. Evolution of Rhizaria: new insights from phylogenomic analysis of uncultivated protists, *BMC Evolutionary Biology,* **10**(377): 1-18.

Buschmann, A. H., Prescott, S., Potin, P., Faugeron, S., Vásquez, J. A., Camus, C., Infante, J., Hernández-González, M. C., Gutíerrez, A. and Varela, D. A., 2014. The status of kelp exploitation and marine agronomy, with emphasis on *Macrocystis pyrifera* in Chile, *Advances in Botanical Research,* **71**: 161-188.

Bustamante, R. H. and Castilla, J. C., 1990. Impact of human exploitation on populations of the intertidal southern bull-kelp *Durvillaea antarctica* (Phaeophyta, Durvilleales)

in central Chile, *Biological Conservation,* **52**. Available at: http://dx.doi.org/10.1016/0006-3207(90)90126-A

Carius, H. J., Little, T. J. and Ebert, D., 2001. Genetic variation in a host-parasite association: potential for coevolution and frequency-dependent selection, *Evolution,* **55**(6): 1136-1145. Available at: http://dx.doi.org/10.1111/j.0014-3820.2001.tb00633.x

Case, R. J., Longford, S. R., Campbell, A. H., Low, A., Tujula, N., Steinberg, P. D. and Kjelleberg, S., 2011. Temperature induced bacterial virulence and bleaching disease in a chemically defended marine macroalga, *Environmental Microbiology,* **13**(2): 529-537. Available at: http://dx.doi.org/10.1111/j.1462-2920.2010.02356.x

Castilla, J. C. and Bustamante, R. H., 1989. Human exclusion from rocky intertidal of Las Cruces, central Chile - effects on *Durvillaea antarctica* (Phaeophyta, Durvilleales), *Marine Ecology Progress Series,* **50**: 203-214. Available at: http://dx.doi.org/10.3354/meps050203

Castilla, J. C., Campo, M. A. and Bustamante, R. H., 2007. Recovery of *Durvillaea antarctica* (Durvilleales) inside and outside Las Cruces marine reserve, Chile, *Ecological Applications,* **17**(5): 1511-1522. Available at: http://dx.doi.org/10.1890/06-1285.1

Charrier, B., Coelho, S. M., Le Bail, A., Tonon, T., Michel, G., Potin, P., Kloareg, B., Boyen, C., Peters, A. F. and Cock, J. M., 2008. Development and physiology of the brown alga *Ectocarpus siliculosus*: two centuries of research, *New Phytologist,* **177**(2): 319-332. Available at: http://dx.doi.org/10.1111/j.1469-8137.2007.02304.x

Cheshire, A. C. and Hallam, N. D., 1988. Biomass and density of native stands of *Durvillaea potatorum* (southern bull-kelp) in south eastern Australia, *Marine ecology progress series. Oldendorf,* **48**(3): 277-283.

Cheshire, A. C. and Hallam, N. D., 1989. Morphological Differences in the Southern Bull-Kelp (*Durvillaea potatorum*) throughout South-Eastern Australia, *Botanica Marina,* **32**(3): 191. Available at: http://www.degruyter.com/view/j/botm.1989.32.issue-3/botm.1989.32.3.191/botm.1989.32.3.191.xml

Clement, M., Posada, D. and Crandall, K. A., 2000. TCS: a computer program to estimate gene genealogies, *Molecular Ecology,* **9**(10): 1657-1659.

Collinge, S. K., Ray, C., Cully Jr, J. F., Ostfeld, R., Keesing, F. and Eviner, V., 2008. Effects of disease on keystone species, dominant species, and their communities, In *Infectious disease ecology*, pp. 129-144.

Correa, J. A., 1996. Diseases in seaweeds: an introduction, In *Fifteenth International Seaweed Symposium: Proceedings of the Fifteenth International Seaweed Symposium held in Valdivia, Chile, in January 1995* (Eds, Lindstrom, S. C. and Chapman, D. J.) Springer Netherlands, Dordrecht, pp. 87-88.

Correa, J. A., Flores, V. and Sánchez, P., 1993. Deformative disease in *Iridaea laminarioides* (Rhodophyta): gall development associated with an endophytic cyanobacterium, *Journal of Phycology,* **29**(6): 853-860. Available at: http://dx.doi.org/10.1111/j.0022-3646.1993.00853.x

Correa, J. A. and Sánchez, P. A., 1996. Ecological aspects of algal infectious diseases, In *Fifteenth International Seaweed Symposium: Proceedings of the Fifteenth International Seaweed Symposium held in Valdivia, Chile, in January 1995* (Eds, Lindstrom, S. C. and Chapman, D. J.) Springer Netherlands, Dordrecht, pp. 89-95.

Cowen, R. K. and Sponaugle, S., 2009. Larval dispersal and marine population connectivity, *Annual Review of Marine Science,* **1**(1): 443-466. Available at: http://www.annualreviews.org/doi/abs/10.1146/annurev.marine.010908.163757

Criscione, C. D., Poulin, R. and Blouin, M. S., 2005. Molecular ecology of parasites: elucidating ecological and microevolutionary processes, *Molecular Ecology,* **14**(8): 2247-2257. Available at: http://onlinelibrary.wiley.com/store/10.1111/j.1365-294X.2005.02587.x/asset/j.1365-294X.2005.02587.x.pdf?v=1&t=icwyczsd&s=597bfa314f86adb942672180e7d2d70134936709

Crowe, T. P., Thompson, R. C., Bray, S. and Hawkins, S. J., 2000. Impacts of anthropogenic stress on rocky intertidal communities, *Journal of Aquatic Ecosystem Stress and Recovery,* **7**(4): 273-297. Available at: http://dx.doi.org/10.1023/A%3A1009911928100

Cruces, E., Huovinen, P. and Gómez, I., 2012. Interactive effects of UV radiation and enhanced temperature on photosynthesis, phlorotannin induction and antioxidant activities of two sub-Antarctic brown algae, *Marine Biology,* **160**(1): 1-13. Available at: http://dx.doi.org/10.1007/s00227-012-2049-8

Cumming, R. A., Nikula, R., Spencer, H. G. and Waters, J. M., 2014. Transoceanic genetic similarities of kelp-associated sea slug populations: long-distance dispersal via rafting?, *Journal of Biogeography,* **41**(12): 2357-2370. Available at: http://dx.doi.org/10.1111/jbi.12376

Darriba, D., Taboada, G. L., Doallo, R. and Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing, *Nat Methods,* **9**(8): 772.

de Vienne, D. M., Refrégier, G., López-Villavicencio, M., Tellier, A., Hood, M. E. and Giraud, T., 2013. Cospeciation vs host-shift speciation: methods for testing, evidence from natural associations and relation to coevolution, *New Phytologist,* **198**(2): 347-385. Available at: http://dx.doi.org/10.1111/nph.12150

Douady, C. J., Delsuc, F., Boucher, Y., Doolittle, W. F. and Douzery, E. J. P., 2003. Comparison of Bayesian and Maximum Likelihood Bootstrap Measures of Phylogenetic Reliability, *Molecular Biology and Evolution,* **20**(2): 248-254. Available at: http://mbe.oxfordjournals.org/content/20/2/248.abstract

Dunteman, G. H. and Ho, M. H. R., 2006. *An Introduction to Generalized Linear Models,* SAGE Publications, Thousand Oaks, California, USA.

Eggert, A., Peters, A. F. and Küpper, F. C., 2010. The potential impact of climate change on endophyte infections in kelp sporophytes, In *Seaweeds and their Role in Globally Changing Environments*, Vol. 15 (Eds, Seckbach, J., Einav, R. and Israel, A.) Springer Netherlands, pp. 139-154.

Emerson, B. C. and Hewitt, G. M., 2005. Phylogeography, *Current Biology,* **15**(10): R367-R371. Available at: http://www.sciencedirect.com/science/article/pii/S0960982205005038

Foissner, W., 2006. Biogeography and dispersal of micro-organisms: a review emphasizing protists, *Acta Protozoologica,* **45**(2): 111-136.

Foissner, W., 2008. Protist diversity and distribution: some basic considerations, *Biodiversity and Conservation,* **17**(2): 235-242. Available at: http://dx.doi.org/10.1007/s10531-007-9248-5

Fraser, C. I., Hay, C. H., Spencer, H. G. and Waters, J. M., 2009a. Genetic and morphological analyses of the southern bull kelp *Durvillaea antarctica* (Phaeophyceae: Durvillaeales) in New Zealand reveal cryptic species, *Journal of Phycology,* **45**. Available at: http://dx.doi.org/10.1111/j.1529-8817.2009.00658.x

Fraser, C. I., Spencer, H. G. and Waters, J. M., 2009b. Glacial oceanographic contrasts explain phylogeography of Australian bull kelp, *Molecular Ecology,* **18**(10): 2287-2296. Available at: http://dx.doi.org/10.1111/j.1365-294X.2009.04201.x

Fraser, C. I., Thiel, M., Spencer, H. G. and Waters, J. M., 2010a. Contemporary habitat discontinuity and historic glacial ice drive genetic divergence in Chilean kelp, *BMC Evolutionary Biology,* **10**(1): 1-12. Available at: http://dx.doi.org/10.1186/1471-2148-10-203

Fraser, C. I. and Waters, J. M., 2013. Algal parasite *Herpodiscus durvillaeae* (Phaeophyceae: Sphacelariales) inferred to have traversed the Pacific ocean with its buoyant host, *Journal of Phycology,* **49**(1): 202-206. Available at: http://dx.doi.org/10.1111/jpy.12017

Fraser, C. I., Winter, D. J., Spencer, H. G. and Waters, J. M., 2010b. Multigene phylogeny of the southern bull-kelp genus *Durvillaea* (Phaeophyceae: Fucales), *Molecular Phylogenetics and Evolution,* **57**(3): 1301-1311. Available at: http://www.sciencedirect.com/science/article/pii/S1055790310004185

Gachon, C. M. M., Sime-Ngando, T., Strittmatter, M., Chambouvet, A. and Kim, G. H., 2010. Algal diseases: spotlight on a black box, *Trends in Plant Science,* **15**(11): 633-640. Available at: http://www.sciencedirect.com/science/article/pii/S1360138510001640

Gachon, C. M. M., Strittmatter, M., Müller, D. G., Kleinteich, J. and Küpper, F. C., 2009. Detection of differential host susceptibility to the marine oomycete pathogen *Eurychasma dicksonii* by real-time PCR: not all algae are equal, *Applied and Environmental Microbiology,* **75**(2): 322-328. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2620704/pdf/1885-08.pdf

Gao, X., Endo, H., Taniguchi, K. and Agatsuma, Y., 2012. Genetic differentiation of high-temperature tolerance in the kelp *Undaria pinnatifida* sporophytes from geographically separated populations along the Pacific coast of Japan, *Journal of Applied Phycology,* **25**(2): 567-574. Available at: http://dx.doi.org/10.1007/s10811-012-9891-4

Gaylord, B., Gaines, S. D. and Associate Editor: Hugh, P., 2000. Temperature or transport? Range limits in marine species mediated solely by flow, *The American Naturalist,* **155**(6): 769-789. Available at: http://www.jstor.org/stable/10.1086/303357

Gillespie, R. G., Baldwin, B. G., Waters, J. M., Fraser, C. I., Nikula, R. and Roderick, G. K., 2012. Long-distance dispersal: a framework for hypothesis testing, *Trends in Ecology & Evolution,* **27**(1): 47-56. Available at: http://www.sciencedirect.com/science/article/pii/S0169534711002606

Gleason, F. H., Lilje, O., Marano, A. V., Sime-Ngando, T., Sullivan, B. K., Kirchmair, M. and Neuhauser, S., 2014. Ecological functions of zoosporic hyperparasites, *Frontiers in Microbiology,* **5**: 244. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4035849/

Gleason, F. H., van Ogtrop, F., Lilje, O. and Larkum, A. W. D., 2013. Ecological roles of zoosporic parasites in blue carbon ecosystems, *Fungal Ecology,* **6**(5): 319-327. Available at: http://www.sciencedirect.com/science/article/pii/S1754504813000718

Goecke, F., Wiese, J., Núñez, A., Labes, A., Imhoff, J. F. and Neuhauser, S., 2012. A novel phytomyxean parasite associated with galls on the bull-kelp *Durvillaea antarctica* (Chamisso) Hariot, *PLoS ONE,* **7**(9): e45358. Available at: http://dx.doi.org/10.1371%2Fjournal.pone.0045358

Google Earth, 2015a. *38°57'53.88"S, 72°49'03.28"W, elevation 475m.,* Available at: http://www.google.com/earth/index.html (accessed 12/5/2016).

Google Earth, 2015b. *40°23'48.97"S, 146°26'19.99"E, elevation 495m.,* Available at: http://www.google.com/earth/index.html (accessed 12/5/2016).

Granke, L. L. and Hausbeck, M. K., 2009. Effects of temperature, concentration, age, and algaecides on *Phytophthora capsici* zoospore infectivity, *Plant Disease,* **94**(1): 54-60. Available at: http://dx.doi.org/10.1094/PDIS-94-1-0054

Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W. and Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0, *Systematic Biology,* **59**(3): 307-321.

Gupta, S., Hill, A. V., Kwiatkowski, D., Greenwood, A. M., Greenwood, B. M. and Day, K. P., 1994. Parasite virulence and disease patterns in *Plasmodium falciparum* malaria, *Proceedings of the National Academy of Sciences,* **91**(9): 3715-3719. Available at: http://www.pnas.org/content/91/9/3715.abstract

Hadziavdic, K., Lekang, K., Lanzen, A., Jonassen, I., Thompson, E. M. and Troedsson, C., 2014. Characterization of the 18S rRNA gene for designing universal Eukaryote specific primers, *PLoS ONE,* **9**(2): e87624. Available at: http://dx.doi.org/10.1371%2Fjournal.pone.0087624

Hajibabaei, M., Singer, G. A. C., Hebert, P. D. N. and Hickey, D. A., 2007. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics, *Trends in Genetics,* **23**(4): 167-172. Available at: http://www.sciencedirect.com/science/article/pii/S0168952507000364

Hedgecock, D., 1986. Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates?, *Bulletin of Marine Science,* **39**(2): 550-564. Available at: http://www.ingentaconnect.com/content/umrsmas/bullmar/1986/00000039/00000002/art00033

Hepburn, L., 2015. *Can you help find Kelp Galls?,* Available at: http://www.atlasoflife.org.au/can-you-help-find-kelp-galls/ (accessed 1/1/2016).

Hillis, D. M. and Dixon, M. T., 1991. Ribosomal DNA: molecular evolution and phylogenetic inference, *The Quarterly Review of Biology,* **66**(4): 411-453. Available at: http://www.jstor.org/stable/2831326

Hinojosa, I. A., Rivadeneira, M. M. and Thiel, M., 2011. Temporal and spatial distribution of floating objects in coastal waters of central–southern Chile and Patagonian fjords, *Continental Shelf Research,* **31**(3–4): 172-186. Available at: http://www.sciencedirect.com/science/article/pii/S0278434310001524

Hoberg, E. P. and Klassen, G. J., 2002. Revealing the faunal tapestry: co-evolution and historical biogeography of hosts and parasites in marine systems, *Parasitology,* **124**: S3-S22. Available at: http://dx.doi.org/10.1017/S0031182002001841

Hochberg, M. E. and Ives, A. R., 1999. Can natural enemies enforce geographical range limits?, *Ecography,* **22**(3): 268-276. Available at: http://dx.doi.org/10.1111/j.1600-0587.1999.tb00502.x

Holt, R. D. and Barfield, M., 2009. Trophic interactions and range limits: the diverse roles of predation, *Proceedings of the Royal Society of London B: Biological Sciences,* **276**(1661): 1435-1442. Available at: http://rspb.royalsocietypublishing.org/royprsb/276/1661/1435.full.pdf

Hopper, J. V., Kuris, A. M., Lorda, J., Simmonds, S. E., White, C. and Hechinger, R. F., 2014. Reduced parasite diversity and abundance in a marine whelk in its expanded geographical range, *Journal of Biogeography,* **41**(9): 1674-1684. Available at: http://dx.doi.org/10.1111/jbi.12329

Izhar, R. and Ben-Ami, F., 2015. Host age modulates parasite infectivity, virulence and reproduction, *Journal of Animal Ecology,* **84**(4): 1018-1028. Available at: http://dx.doi.org/10.1111/1365-2656.12352

Jahnke, R., 1978. 'A study of gall diseased laminae of the marine brown alga *Durvillaea potatorum* (Labilladiere) Areschoug.', Honours, Department of Botany, La Trobe University, Melbourne.

Johannesson, K., 1988. The paradox of Rockall: why is a brooding gastropod (*Littorina saxatilis*) more widespread than one having a planktonic larval dispersal stage (*L. littorea*)?, *Marine Biology,* **99**(4): 507-513. Available at: http://dx.doi.org/10.1007/BF00392558

Kaltz, O. and Shykoff, J. A., 1998. Local adaptation in host-parasite systems, *Heredity,* **81**(4): 361-370. Available at: http://dx.doi.org/10.1046/j.1365-2540.1998.00435.x

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. and Drummond, A., 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data, *Bioinformatics,* **28**(12): 1647-1649. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3371832/

Kennedy, C. R. and Bush, A. O., 1994. The relationship between pattern and scale in parasite communities: a stranger in a strange land, *Parasitology,* **109**(02): 187-196. Available at: http://dx.doi.org/10.1017/S0031182000076290

Kennelly, S. J., 1987. Physical disturbances in an Australian kelp community. II. Effects on understorey species due to differences in kelp cover., *Marine Ecology Progress Series,* **40**: 155-165.

Koehl, M. A. R., 1979. Stiffness or extensibility of intertidal algae: a comparative study of modes of withstanding wave action, *Journal of Biomechanics,* **12**(8): 634. Available at: http://www.sciencedirect.com/science/article/pii/0021929079901283

Krasnov, B. R., Shenbrot, G. I., Khokhlova, I. S., Mouillot, D. and Poulin, R., 2008. Latitudinal gradients in niche breadth: empirical evidence from haematophagous ectoparasites, *Journal of Biogeography,* **35**(4): 592-601. Available at: http://dx.doi.org/10.1111/j.1365-2699.2007.01800.x

Kupper, F., Maier, I., Muller, D., Loiseaux-de Goer, S. and Guillou, L., 2006. Phylogenetic affinities of two eukaryotic pathogens of marine macroalgae, *Eurychasma dicksonii* (Wright) Magnus and *Chytridium polysiphoniae* Cohn, *Cryptogamie-Algologie,* **27**(2): 165-184.

Lafferty, K. D. and Kuris, A. M., 1999. How environmental stress affects the impacts of parasites, *Limnology and Oceanography,* **44**(3): 925-931.

Lara, E., Heger, T. J., Scheihing, R. and Mitchell, E. A. D., 2011. COI gene and ecological data suggest size-dependent high dispersal and low intra-specific diversity in free-living terrestrial protists (Euglyphida: *Assulina*), *Journal of Biogeography,* **38**(4): 640-650. Available at: http://dx.doi.org/10.1111/j.1365-2699.2010.02426.x

Lawless, J. F., 1987. Negative binomial and mixed Poisson regression, *Canadian Journal of Statistics,* **15**(3): 209-225. Available at: http://dx.doi.org/10.2307/3314912

Li, W., Zhang, T., Tang, X. and Wang, B., 2010. Oomycetes and fungi: important parasites on marine algae, *Acta Oceanologica Sinica,* **29**(5): 74-81. Available at: http://dx.doi.org/10.1007/s13131-010-0065-4

Lindenfors, P., Nunn, C. L., Jones, K. E., Cunningham, A. A., Sechrest, W. and Gittleman, J. L., 2007. Parasite species richness in carnivores: effects of host body mass, latitude, geographical range and population density, *Global Ecology and Biogeography,* **16**(4): 496-509. Available at: http://dx.doi.org/10.1111/j.1466-8238.2006.00301.x

Macaya, E. C., Boltaña, S., Hinojosa, I. A., Macchiavello, J. E., Valdivia, N. A., Vásquez, N. R., Buschmann, A. H., Vásquez, J. A., Alonso Vega, J. M. and Thiel, M., 2005. Presenec of sporophylls in floating kelp rafts of *Macrocystis* spp. (Phaeophyceae) along the Chilean Pacific coast, *Journal of Phycology,* **41**(5): 913-922. Available at: http://dx.doi.org/10.1111/j.1529-8817.2005.00118.x

Macaya, E. C., López, B., Tala, F., Tellier, F. and Thiel, M., 2016. Float and raft: role of buoyant seaweeds in the phylogeography and genetic structure of non-buoyant associated flora, In *Seaweed Phylogeography: Adaptation and Evolution of Seaweeds under Environmental Change* (Eds, Hu, Z.-M. and Fraser, C.) Springer Netherlands, Dordrecht, pp. 97-130.

Maier, I., Parodi, E., Westermeier, R. and Müller, D. G., 2000. *Maullinia ectocarpii* gen. et sp. nov. (Plasmodiophorea), an intracellular parasite in *Ectocarpus siliculosus* (Ectocarpales, Phaeophyceae) and other filamentous brown algae, *Protist,* **151**(3): 225-238. Available at: http://www.sciencedirect.com/science/article/pii/S1434461004700218

Millar, A. J., 2007. The Flindersian and Peronian Provinces, in *Algae of Australia: introduction* (Eds, McCarthy, P.M. and Orchard, A.E.) CSIRO Publishing, Collingwood, Australia.

Mitchell, S. E., Rogers, E. S., Little, T. J. and Read, A. F., 2005. Host-parasite and genotype-by-environment interactions: temperature modifies potential for selection by a sterlizing pathogen, *Evolution,* **59**(1): 70-80. Available at: http://dx.doi.org/10.1111/j.0014-3820.2005.tb00895.x

Molina, F. I., Hughes, G. C. and Craigie, J. S., Temperature-influenced infection rates in the *Chondrus crispus—Petersenia pollagaster* pathosystem: a regression analysis, *Marine Biology,* **97**(3): 431-433. Available at: http://dx.doi.org/10.1007/BF00397774

Moore, L. B. and Cribb, A. B., 1952. The brown alga *Durvillea antarctica* in Australian waters, *Nature,* **169**(4313): 1100-1101. Available at: http://dx.doi.org/10.1038/1691100a0

Moritz, C. and Cicero, C., 2004. DNA Barcoding: Promise and Pitfalls, *PLoS Biol,* **2**(10): e354. Available at: http://dx.doi.org/10.1371%2Fjournal.pbio.0020354

Mouritsen, K. N., Tompkins, D. M. and Poulin, R., 2005. Climate warming may cause a parasite-induced collapse in coastal amphipod populations, *Oecologia,* **146**(3): 476-483. Available at: http://dx.doi.org/10.1007/s00442-005-0223-0

Müller, D. G., Küpper, F. C. and Küpper, H., 1999. Infection experiments reveal broad host ranges of *Eurychasma dicksonii* (Oomycota) and *Chytridium polysiphoniae* (Chytridiomycota), two eukaryotic parasites in marine brown algae (Phaeophyceae), *Phycological Research,* **47**(3): 217-223. Available at: http://dx.doi.org/10.1046/j.1440-1835.1999.00165.x

Neuhauser, S., Kirchmair, M., Bulman, S. and Bass, D., 2014. Cross-kingdom host shifts of phytomyxid parasites, *BMC Evolutionary Biology,* **14**: 33-33. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4016497/

Neuhauser, S., Kirchmair, M. and Gleason, F., 2011a. The ecological potentials of Phytomyxea ("plasmodiophorids") in aquatic food webs, *Hydrobiologia,* **659**(1): 23-35. Available at: http://dx.doi.org/10.1007/s10750-010-0508-0

Neuhauser, S., Kirchmair, M. and Gleason, F. H., 2011b. Ecological roles of the parasitic phytomyxids (plasmodiophorids) in marine ecosystems – a review, *Marine and Freshwater Research,* **62**(4): 365-371. Available at: http://www.publish.csiro.au/paper/MF10282

Nikula, R., Spencer, H. G. and Waters, J. M., 2011. Evolutionary consequences of microhabitat: population-genetic structuring in kelp- vs. rock-associated chitons, *Molecular Ecology,* **20**(23): 4915-4924. Available at: http://dx.doi.org/10.1111/j.1365-294X.2011.05332.x

O'Hara, T. D. and Poore, G. C. B., 2000. Patterns of distribution for southern Australian marine echinoderms and decapods, *Journal of Biogeography,* **27**(6): 1321-1335. Available at: http://dx.doi.org/10.1046/j.1365-2699.2000.00499.x

Padilla, D. K., Chotkowski, M. A. and Lucy, A. J. B., 1996. Predicting the spread of Zebra Mussels (*Dreissena polymorpha*) to inland waters using boater movement patterns, *Global Ecology and Biogeography Letters,* **5**(6): 353-359. Available at: http://www.jstor.org/stable/2997590

Parodi, E. R., Cáceres, E. J., Westermeier, R. and Müller, D. G., 2010. Secondary zoospores in the algal endoparasite *Maullinia ectocarpii* (Plasmodiophoromycota), *Biocell,* **34**: 45-52. Available at: http://www.scielo.org.ar/scielo.php?script=sci_arttext&pid=S0327-95452010000100006&nrm=iso

Pawlowski, J., Audic, S., Adl, S., Bass, D., Belbahri, L., Berney, C., Bowser, S. S., Cepicka, I., Decelle, J., Dunthorn, M., Fiore-Donno, A. M., Gile, G. H., Holzmann, M., Jahn, R., Jirk, M., Keeling, P. J., Kostka, M., Kudryavtsev, A., Lara, E., Luke, J., Mann, D. G., Mitchell, E. A. D., Nitsche, F., Romeralo, M., Saunders, G. W., Simpson, A. G. B., Smirnov, A. V., Spouge, J. L., Stern, R. F., Stoeck, T., Zimmermann, J., Schindel, D. and de Vargas, C., 2012. CBOL protist working group: barcoding Eukaryotic richness beyond the animal, plant, and fungal kingdoms, *PLoS Biol,* **10**(11): e1001419. Available at: http://dx.doi.org/10.1371%2Fjournal.pbio.1001419

Pereira, T. R., Engelen, A. H., Pearson, G. A., Valero, M. and Serrão, E. A., 2015. Response of kelps from different latitudes to consecutive heat shock, *Journal of Experimental Marine Biology and Ecology,* **463**: 57-62. Available at: http://www.sciencedirect.com/science/article/pii/S0022098114002834

Peters, A. F. and Breeman, A. M., 1993. Temperature tolerance and latitudinal range of brown algae from temperate Pacific South America, *Marine Biology,* **115**(1): 143-150. Available at: http://dx.doi.org/10.1007/BF00349396

Poulin, R., Blanar, C. A., Thieltges, D. W. and Marcogliese, D. J., 2012. Scaling up from epidemiology to biogeography: local infection patterns predict geographical distribution in fish parasites, *Journal of Biogeography,* **39**(6): 1157-1166. Available at: http://dx.doi.org/10.1111/j.1365-2699.2011.02667.x

Poulin, R., Krasnov, B. R. and Mouillot, D., 2011a. Host specificity in phylogenetic and geographic space, *Trends in Parasitology,* **27**(8): 355-361. Available at: http://www.sciencedirect.com/science/article/pii/S147149221100095X

Poulin, R., Krasnov, B. R., Mouillot, D. and Thieltges, D. W., 2011b. The comparative ecology and biogeography of parasites, *Philosophical Transactions of the Royal Society of London B: Biological Sciences,* **366**(1576): 2379-2390. Available at: http://rstb.royalsocietypublishing.org/content/royptb/366/1576/2379.full.pdf

Robinson, R. A., 1977. Plant pathosystems, *Annals of the New York Academy of Sciences,* **287**(1): 238-242. Available at: http://dx.doi.org/10.1111/j.1749-6632.1977.tb34243.x

Rödder, D., Veith, M. and Lötters, S., 2008. Environmental gradients explaining the prevalence and intensity of infection with the amphibian chytrid fungus: the host's perspective, *Animal Conservation,* **11**(6): 513-517. Available at: http://dx.doi.org/10.1111/j.1469-1795.2008.00210.x

Rohde, K., 2002. Ecology and biogeography of marine parasites, In *Advances in Marine Biology*, Vol. Volume 43 Academic Press, pp. 1-83.

Ronquist, F. and Huelsenbeck, J. P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models, *Bioinformatics,* **19**(12): 1572-1574.

Rothä usler, E., Gómez, I., Hinojosa, I. A., Karsten, U., Miranda, L., Tala, F. and Thiel, M., 2011. Kelp rafts in the Humboldt Current: Interplay of abiotic and biotic factors limit their floating persistence and dispersal potential, *Limnology and Oceanography,* **56**(5): 1751-1763. Available at: http://dx.doi.org/10.4319/lo.2011.56.5.1751

Saavedra, F. G., 2011. 'Associations Between Microbes and Macroalgae: Host, Epiphyte and Environmental Effects', Dissertation, Mathematisch-Naturwissenschaftlichen Fakultät, Christian-Albrechts-Universität zu Kiel, Kiel.

Samis, K. E. and Eckert, C. G., 2007. Testing the abundant center model using range-wide demographic surveys of two coastal dune plants, *Ecology,* **88**(7): 1747-1758. Available at: http://dx.doi.org/10.1890/06-1153.1

Santelices, B., Castilla, J. C., Cancino, J. and Schmiede, P., 1980. Comparative ecology of *Lessonia nigrescens* and *Durvillaea antarctica* (phaeophyta) in Central Chile, *Marine Biology,* **59**(2): 119-132. Available at: http://dx.doi.org/10.1007/BF00405461

Sexton, J. P., McIntyre, P. J., Angert, A. L. and Rice, K. J., 2009. Evolution and ecology of species range limits, *Annual Review of Ecology, Evolution, and Systematics,* **40**(1): 415-436. Available at: http://www.annualreviews.org/doi/abs/10.1146/annurev.ecolsys.110308.120317

Silva, N., Rojas, N. and Fedele, A., 2009. Water masses in the Humboldt Current System: Properties, distribution, and the nitrate deficit as a chemical water mass tracer for Equatorial Subsurface Water off Chile, *Deep Sea Research Part II: Topical Studies in Oceanography,* **56**(16): 1004-1020. Available at: http://www.sciencedirect.com/science/article/pii/S0967064508004220

Sime-Ngando, T. and Niquil, N., 2011. Editorial: 'Disregarded' microbial diversity and ecological potentials in aquatic systems: a new paradigm shift ahead, *Hydrobiologia,* **659**(1): 1-4. Available at: http://dx.doi.org/10.1007/s10750-010-0511-5

Smith, L. M., Hutchings, P. and Fraser, C. I., 2015. Molecular evidence supports coastal dispersal among estuaries for two benthic marine worm (Nephtyidae) species in southeastern Australia, *Marine Biology,* **162**(6): 1319-1327. Available at: http://dx.doi.org/10.1007/s00227-015-2671-3

Smith, S. D. A., 2002. Kelp rafts in the Southern Ocean, *Global Ecology and Biogeography,* **11**(1): 67-69. Available at: http://dx.doi.org/10.1046/j.1466-822X.2001.00259.x

Sogin, M. L. and Silberman, J. D., 1998. Evolution of the protists and protistan parasites from the perspective of molecular systematics, *International Journal for Parasitology,* **28**(1): 11-20. Available at: http://www.sciencedirect.com/science/article/pii/S0020751997001811

Staats, M., van Baarlen, P. and van Kan, J. A. L., 2005. Molecular phylogeny of the plant pathogenic genus *Botrytis* and the evolution of host specificity, *Molecular Biology*

and Evolution, **22**(2): 333-346. Available at: http://mbe.oxfordjournals.org/content/22/2/333.abstract

Stigall, A. L., 2012. Speciation collapse and invasive species dynamics during the Late Devonian "Mass Extinction", *GSA Today,* **22**(1): 4-9.

Studer, A., Thieltges, D. and Poulin, R., 2010. Parasites and global warming: net effects of temperature on an intertidal host-parasite system, *Marine Ecology Progress Series,* **415**: 11-22.

Suzuki, R. O., Degawa, Y., Suzuki, S. N. and Hosoya, T., 2015. Local-and regional-scale spatial patterns of two fungal pathogens of *Miscanthus sinensis* in grassland communities, *Mycoscience,* **56**(1): 42-48. Available at: http://www.sciencedirect.com/science/article/pii/S1340354014000278

Tala, F., Gómez, I., Luna-Jorquera, G. and Thiel, M., 2013. Morphological, physiological and reproductive conditions of rafting bull kelp (*Durvillaea antarctica*) in northern-central Chile (30°S), *Marine Biology,* **160**(6): 1339-1351. Available at: http://dx.doi.org/10.1007/s00227-013-2186-8

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S., 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0, *Molecular Biology and Evolution,* **30**(12): 2725-2729. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3840312/

Taylor, D. I. and Schiel, D. R., 2005. Self-replacement and community modification by the southern bull kelp *Durvillaea antarctica*, *Marine Ecology Progress Series,* **288**: 87-102. Available at: http://www.int-res.com/abstracts/meps/v288/p87-102/

Thiel, M. and Gutow, L., 2005. The ecology of rafting in the marine environment. II. The rafting organisms and community, In *Oceanography and Marine Biology: an annual review*, Vol. 43, pp. 279-418.

Tian, Z., Liu, G., Yin, H., Luo, J., Guan, G., Xie, J., Luo, J., Zheng, J., Tian, M., Yuan, X., Wang, F., Chen, R. and Wang, H., 2013. Cytochrome c oxidase subunit III (COX3) gene, an informative marker for phylogenetic analysis and differentiation of *Babesia* species in China, *Infection, Genetics and Evolution,* **18**: 13-17. Available at: http://www.sciencedirect.com/science/article/pii/S1567134813001408

Tuya, F., Cacabelos, E., Duarte, P., Jacinto, D., Castro, J. J., Silva, T., Bertocci, I., Franco, J. N., Arenas, F. and Coca, J., 2012. Patterns of landscape and assemblage structure along a latitudinal gradient in ocean climate, *Marine Ecology Progress Series,* **466**: 9-19.

Underwood, A. J. and Kennelly, S. J., 1990. Ecology of marine algae on rocky shores and subtidal reefs in temperate Australia, *Hydrobiologia,* **192**(1): 3-20. Available at: http://dx.doi.org/10.1007/BF00006224

Voelker, D., 2002. *Meeresgeologie im Internet: Die Oberflächentemperatur der Ozeane (SST)* Available at: http://meeresgeo.geoinf.fu-berlin.de/inhalt/seatemp02.php?print=true&js=1&sg=9 (accessed 23/5/2016).

VSNi, 2011. *Genstat for Windows 14th Edition,* VSN International, Hemel Hempstead, UK.

Walsh, P. S., Metzger, D. A. and Higuchi, R., 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material, *BioTechniques,* **10**(4): 506-513. Available at: http://europepmc.org/abstract/MED/1867860

Wang, Y., Tian, R. M., Gao, Z. M., Bougouffa, S. and Qian, P.-Y., 2014. Optimal eukaryotic 18S and universal 16S/18S ribosomal RNA primers and their application in a study of symbiosis, *PLoS ONE,* **9**(3): e90053. Available at: http://dx.doi.org/10.1371%2Fjournal.pone.0090053

Waters, J. M., 2008. Marine biogeographical disjunction in temperate Australia: historical landbridge, contemporary currents, or both?, *Diversity and Distributions,* **14**(4): 692-700. Available at: http://dx.doi.org/10.1111/j.1472-4642.2008.00481.x

Waters, J. M., King, T. M., O'Loughlin, P. M. and Spencer, H. G., 2005. Phylogeographical disjunction in abundant high-dispersal littoral gastropods, *Molecular Ecology,* **14**(9): 2789-2802. Available at: http://dx.doi.org/10.1111/j.1365-294X.2005.02635.x

Weber, X., 2015. 'Cryptic Kelp: Identifying and describing a new species in the southern bull-kelp *Durvillaea*', Honours, Fenner School of Environment and Society, Australian National University, Canberra.

Weisberg, S., 2005. *Applied linear regression,* John Wiley & Sons, Hoboken, New Jersey.

Weisse, T., 2008. Distribution and diversity of aquatic protists: an evolutionary and ecological perspective, *Biodiversity and Conservation,* **17**(2): 243-259. Available at: http://dx.doi.org/10.1007/s10531-007-9249-4

Wernberg, T., Thomsen, M. S., Tuya, F., Kendrick, G. A., Staehr, P. A. and Toohey, B. D., 2010. Decreasing resilience of kelp beds along a latitudinal temperature gradient: potential implications for a warmer future, *Ecology Letters,* **13**(6): 685-694. Available at: http://dx.doi.org/10.1111/j.1461-0248.2010.01466.x

Whipps, C. M. and Kent, M. L., 2006. Phylogeography of the cosmopolitan marine parasite *Kudoa thyrsites* (Myxozoa: Myxosporea), *Journal of Eukaryotic Microbiology,* **53**(5): 364-373. Available at: http://dx.doi.org/10.1111/j.1550-7408.2006.00114.x

Wilson, L. J., Weber, X. A., King, T. M. and Fraser, C. I., 2016. DNA extraction techniques for genomic analyses of macroalgae, In *Seaweed Phylogeography: adaptation and evolution of seaweeds under environmental change* (Eds, Hu, Z.-M. and Fraser, C.) Springer Netherlands, Dordrecht, pp. 363-386.

Wolinska, J. and King, K. C., 2009. Environment can alter selection in host–parasite interactions, *Trends in Parasitology,* **25**(5): 236-244. Available at: http://www.sciencedirect.com/science/article/pii/S1471492209000622

Appendix 1: Australian Research Permits

NSW GOVERNMENT Department of Primary Industries

Mr Callum Blake
Australian National University
GEO02.07, Building 48A
CANBERRA ACT 2600

Our Reference: P15/0097-1.0 & OUT15/27876

21 October 2015

Dear Mr Callum Blake

I refer to your request for a scientific collection permit to count and excise samples of diseased tissue (galls) on Bull Kelp.

I am pleased to advise that your request has been approved. The permit is enclosed and is subject to the conditions as specified therein. Please note that the permit **will expire on 21 October 2018**.

The permit authorises activities under the *Fisheries Management Act 1994* only and does not in any way affect your obligations under the *Animal Research Act 1985*. You are advised that if you are using animals for research then you may require accreditation and/or licensing. It is your responsibility to ensure that you are not in breach of the *Animal Research Act 1985,* and this permit is conditional on that requirement. For further information you should contact the Licensing Clerk, Animal Welfare Unit, NSW DPI on 02 6391 3725 or fax 02 6391 3570.

The Director, Aquaculture & Aquatic Environment reserves the right to request information of the activities conducted under this permit either during or at the expiry of the permit.

Please be advised that if you wish to amend your permit, an amendment request must be submitted in writing or via email. Also note that any amendments will incur an amendment fee.

Should you wish to renew an existing permit or apply for a new permit, please submit a completed application, REF, maps and relevant fee, as well as any required information listed above. Please allow approximately 4-6 weeks for assessment. The latest application forms and fee schedule are available on the department's website www.dpi.nsw.gov.au

Should you have any further enquiries regarding this matter please do not hesitate to contact me on (02) 4916 3824.

Yours sincerely

Ryan Jefferson
Aquaculture Officer

Fisheries NSW
Port Stephens Fisheries Institute
Locked Bag 1, NELSON BAY NSW 2315
Tel: 02 4982 1232 Fax: 02 4982 1107
ABN 72 189 919 072 www.dpi.nsw.gov.au

Department of Primary Industries

SCIENTIFIC COLLECTION PERMIT

SCIENTIFIC COLLECTION PERMIT
Section 37 *Fisheries Management Act 1994*
Part II of the *Marine Estate Management Regulation 2009*

PERMIT HOLDER DETAILS

Mr Callum Blake
Australian National University
GEO02.07, Building 48A
CANBERRA ACT 2600

Permit No: P15/0097-1.0
Issued Date: 21/10/2015
Expiry Date: 21/10/2018

Additional People:

Any personnel under the direct authority of the permit holder.

In accordance with the *Fisheries Management Act 1994* the permit holder listed on this permit and additional people are hereby authorised to:

- Count and excise samples of diseased tissue (galls) on Bull Kelp.

The specimens must only be taken from waters at the following locations:

- Coastal waters within NSW (excluding Marine Parks and Aquatic Reserves)

Methods

- Hand Collection

Species (total numbers/amounts to be collected over the term of the permit unless otherwise specified)

- Bull Kelp *Durvillea potatorum* 50 individual galls (~2cm^2 each)

Subject to the following conditions:

1. Specimens must only be taken in accordance with research being undertaken by the permit holder, the information provided by the permit holder in their application, and the conditions of this permit. In the event of an inconsistency between the conditions of this permit and any information provided by the permit holder, the conditions of this permit shall prevail to the extent of the inconsistency.

2. Unless otherwise specified within this permit, all restrictions (collecting, fishing and otherwise) under the *Fisheries Management Act 1994* apply and must be complied with.

3. No activity other than the collection activity specified in this permit, may be carried out by the permit holder or other nominated person/s when engaged in any collection activity authorised by this permit.

4. The permit holder or any additional person/s operating under this permit must display a clearly visible sign, in letters at least 15cm high, at the location of the activity during all collecting and field activities, identifying the name of the organisation conducting the research the permit number and the word 'Research'.

5. The permit holder must contact the local District Fisheries Officer, in the area where collecting is to be undertaken, **at least 72 hours prior to the commencement** of any collection activities (unless the District Fisheries Officer agrees to a local arrangement). The DFO should also be contacted when an activity relating to this permit (even if not physically collecting) is conducted in an area of public concern (i.e. Aquatic Reserve or Intertidal Protected Area). The following information must be provided: details of the permit number; collecting methods; specific locations and accurate times for any proposed activities; where vessels are to be utilised, registration details and descriptions, including boat ramps being used. Details of the person coordinating the field work, including their contact details for in the field, must also be provided. This person will be responsible for supervising all collecting activities.
For contact details see: http://www.dpi.nsw.gov.au/fisheries/recreational/contact

6. The permit holder must if required by a Fisheries Officer or Marine Park Officer to do so, immediately provide the name, address, date of birth and contact number for any or all additional people that have been nominated by the permit holder to carry out collection activities associated with this permit.

7. The permit holder and any additional person/s listed under this permit must carry this permit (or a copy thereof) at all times during collecting activities and must be produced to a Fisheries Officer or Marine Park Ranger on demand.

8. The permit holder must ensure that any additional person/s operating under the authority of this permit reads and understands all conditions relating to the permit.

9. This permit does not authorise collection activities from Marine Parks declared under Section 6 of the *Marine Estate Management Act 2014*, Aquatic Reserves declared under the Section 57(2)(b) of the *Marine Estate Management Act 2014* or the taking of fish from the waters of Lord Howe Island, unless otherwise specified within this permit.

10. No fish or invertebrate taken under this permit may be used for personal consumption, sale or public exhibition by any person, unless approved in writing by the Minister or a person who has the delegated authority of the Minister to issue this permit.

11. The permit holder and any additional person/s operating under this permit must behave in a professional, reasonable and lawful manner that is not likely to generate conflict and criticism with other waterway users.

12. The permit holder must implement one of the following actions to prevent the translocation of aquatic pests and diseases between waterways, either;
    - clean and disinfect all aquatic fieldwork equipment and vessels at the conclusion of field operations in each waterway **and** dry any equipment that has biofouling (such as oyster trays, moorings, other infrastructure) for at least 30 days **or**;
    - use separate equipment in each waterway

13. The permit holder must clean, disinfect and where possible dry all vehicles, boats, trailers and equipment (including PPE and clothing) at the conclusion of field operations in each waterway before moving to a new waterway.

14. The permit holder must not intentionally or inadvertently translocate any biological material and/or water between waterways unless authorised in this permit.

15. The permit holder must destroy any noxious fish, noxious marine vegetation or pests or parasites listed as Class B diseases (under Schedule 6B of the *Fisheries Management Act 1994*) collected, sampled or taken as by-catch in a humane manner and not return it to any waterway unless authorised in this permit. See Schedule 6B and 6C of the *Fisheries Management Act 1994* and www.dpi.nsw.gov.au/biosecurity/aquatic

16. The permit holder must avoid disturbing or interfering with any noxious fish, noxious marine vegetation or pests or parasites listed as Class B diseases (under Schedule 6B of the *Fisheries Management Act 1994*) during research and collection activities unless authorised in this permit. See Schedule 6B and 6C of the *Fisheries Management Act 1994* and www.dpi.nsw.gov.au/biosecurity/aquatic

17. The permit holder must notify a Fisheries Officer or the Aquatic Biosecurity and Risk Management unit (02 4982 1232) if they know or reasonably suspect that a declared disease (within the meaning of Division 4 , Part 6 of the *Fisheries Management Act 1994*) or new location of an aquatic pest is present or may be present.
    See
    http://www.legislation.nsw.gov.au/maintop/view/inforce/act+38+1994+cd+0+N

18. This permit does not authorise the taking of protected fish or threatened species, populations or ecological communities listed under the *Fisheries Management Act 1994* unless otherwise specified within this permit. For information see http://www.dpi.nsw.gov.au/fisheries/species-protection

Department of Primary Industries

SCIENTIFIC COLLECTION PERMIT

19. This permit does not give the right of entry to private property. Permission to enter private property must be obtained from the owner or occupier.

20. The Director Aquaculture & Aquatic Environment reserves the right to request information of the activities conducted under this permit during the life of the permit, at or after the expiry of the permit.

21. This permit is valid only if the permit holder has complied with the requirements of the *Animal Research Act 1985* in respect of the activities proposed. This permit only authorises activities under the *Fisheries Management Act 1994* and does not in any way negate your obligations under the *Animal Research Act 1985*.

22. This permit may be cancelled or suspended at any time prior to the expiry date for any reason.

Bob Creese
Director Fisheries Research

Fisheries NSW
Port Stephens Fisheries Institute
Locked Bag 1, NELSON BAY NSW 2315
Tel: 02 4982 1232 Fax: 02 4982 1107
ABN 72 189 919 072 www.dpi.nsw.gov.au

Permit No: **10007771**

File No: **FF383428**

Department of Environment, Land, Water and Planning

NATIONAL PARKS ACT 1975
RESEARCH PERMIT

Pursuant to the provisions of the **National Parks Act 1975**, permission is hereby granted to:

Mr Callum Blake
Fenner School of Environment and Society
Australian National University
Building 141, Linnaeus Way
Canberra 2601

And Alisdair Blake in order to

i) excise small tissue samples, equivalent to the size of a fish bite (approx. 2cm2) from diseased kelps using a knife and removed from the site for sequencing. Tissue samples will be collected for every 10th gall;

within Cape Liptrap Coastal Park, Mornington Peninsula National Park, Wilsons Promontory National Park, Wilsons Promontory Marine National Park, Wilsons Promontory Marine Park and Wilsons Promontory Marine Reserve for the purpose of investigating infection dynamics of *Maullinia* sp on *Durvillaea potatorum* (Southern Bull-kelp) in Australian waters.

Permission is given subject to the following particular conditions:

1. **Arrangements for access and authorisation to conduct research on land managed by Parks Victoria must be made at least five days in advance with the Area Chief Ranger or local delegate. He/ she is to be advised, in advance, of each visit to the park made in connection with the provisions of this permit. The permit holder should contact the Parks Victoria Information Centre on 13 19 63 and ask for the contact number of the Area Chief Ranger for the park where the research is to be undertaken. This contact is critical to:**
    a. **determine jointly, where necessary, specific fieldwork locations that do not overlap with existing research projects,**
    b. **ensure tracks are accessible and that track conditions are safe for vehicle access,**
    c. **establish emergency procedures and communications procedures in the event of an emergency,**
    d. **confirm there are no prescribed burns scheduled for your specific fieldwork location and any access points to that specific location,**
    e. **confirm there are no bush fires currently burning in or near your specific fieldwork location and any access points to that specific fieldwork location, and**
    f. **allow for an on-site induction by Parks Victoria staff if required.**
2. This permit does not allow for any taking of wildlife, plants or invertebrates from the Marine National Parks and Marine Sanctuaries. Species listed under the *Flora and Fauna Guarantee Act 1988* must not be collected.
3. The direction of any authorised officer of Parks Victoria or Fisheries Victoria, in relation to the application of this permit on land managed by Parks Victoria, must be followed.
4. At all times personnel should be easily recognisable as researchers with **Australian National University.** This must include boats and all other research equipment.
5. The researchers are required to notify the local Biodiversity officer of their proposed activities at least three days in advance. He/she is to be advised, in advance, of each visit made in connection with the provisions of this permit. Contact the Department of Environment, Land, Water and Planning (DELWP) Customer Service Centre on 136 186 and ask for the contact number for the Biodiversity officer of the nearest DELWP office where the research is to be undertaken.
6. Adherence to the Code of Practice for collecting diseased tissue samples from *Durvillaea potatorum* in Victorian waters must be followed.
7. All equipment (including knives used for excising) **MUST** be thoroughly cleaned with household bleach. If the research involves the use of boats, to avoid the spread of marine pests ensure the correct procedures for boat cleaning are followed. Please refer to "Aquatic Pests: Treat 'em mean - keep your boat clean", available from DELWP by calling 136 186 or via www.delwp.vic.gov.au/marinepests. Any equipment that has been left in the water for an extended period should also be doused with a dilute solution of household bleach in order to kill any attached marine biota.
8. During the course of research, if a suspected new marine pest for that area is collected or sighted, you must report the incident to DELWP by calling 136 186 or online or via www.delwp.vic.gov.au/marinepests.

.....

Bryan Welch
Acting Environment and Natural Resources, Regional Manager
(Delegate of the Secretary)…/2

9. Vehicle access past locked gates requires prior approval on each occasion. It is preferable that work requiring access past locked gates and/or walking off track is conducted between Monday and Friday. Weekends should be avoided wherever possible. Access behind closed gates on public holidays may not be possible.

10. It is essential that researchers ensure that vehicles travelling on management vehicle tracks are free of soil, gravel and plant material prior to travel/entry to the park. They must also ensure that when going off-track all boots and any tools used are free of soil, gravel and plant material, and must be disinfected. This can be done with either Phytoclean mixed 1 part Phytoclean to 10 parts water, or with domestic bleach mixed 1 part bleach to 4 parts water.

11. Vehicles must remain on existing public roads and tracks and, subject to agreement with local Parks Victoria staff, Management Vehicles Only roads and tracks. Applicant's vehicles and research equipment should be clearly identifiable as belonging to the research organisation. The associated project and permit number should also be identifiable to avoid misunderstanding by Rangers and the public.

12. Research activities are **not permitted** within Parks on Fire Danger Rating days of **CODE RED** where **the safest option is to leave the night before, or early in the morning. Notify the relevant Parks Victoria Office of your departure.** It is the researchers' responsibility to keep themselves aware of fire danger forecasts at all times during field work.

13. Provisions of the **National Parks Act 1975** and the National Parks Regulations 2013 are to be fully observed, except where exemption is specifically provided for in this permit. All signage in National Parks must be complied with, except where exemption is specifically provided for in this permit.

14. Any sites or artefacts or Aboriginal cultural significance located in the course of this research must not be disturbed. In addition, the location of any sites and/ or artefacts must be recorded and reported immediately to the Area Chief Ranger of the park in which they occur and to Aboriginal Affairs Victoria (ph 1800 762 003) to be recorded on the Victorian Aboriginal Heritage Register (VAHR). For more information on Aboriginal artefacts, please view the Aboriginal Affairs Victoria website.

15. The permit holder must provide specific reports and data identified and requested by the Environment and Natural Resources, Regional Manager, Department of Environment, Land, Water and Planning, within 7 working days of the request in both hard copy and electronic format.

16. A written report of all scientific work conducted is to be forwarded to the Environmental Research Co-ordinator Department of Environment, Land, Water and Planning within 30 days of the expiration of this permit. This report should include details of all specimens collected.

17. The data must be entered into to the Victorian Biodiversity Atlas (VBA). Your data may remain in the system as 'Draft' for up to 12 months from the date of expiration of this permit, at which point it must be submitted for expert review and subsequent publication. There are mandatory fields required to make a VBA record. Information about how to enter data into the VBA can be obtained from the DELWP website, Biodiversity pages at: http://www.delwp.vic.gov.au/environment-and-wildlife/biodiversity/victorian-biodiversity-atlas

18. **A short research summary of results outlining the key findings and completed on the attached template must be provided to both** the **Environmental Research Co-ordinator, DELWP**, PO Box 137, Heidelberg, 3084 and **Dr John Wright, Parks Victoria, Level 10, 5353 Bourke St, Melbourne VIC 3000 (john.wright@parks.vic.gov.au), within 30 days of the expiration of this research permit.** The publication of any results in connection with this research permit must refer to the fact that they were collected or obtained under the terms of this permit and copies of any reports, theses or published articles must also be provided.

19. Where any rare or threatened species, as listed in the *Flora and Fauna Guarantee Act* 1988, or listed on the DELWP website advisory lists (www.delwp.vic.gov.au), are identified, the exact location should be noted and reported to the biodiversity.info@delwp.vic.gov.au as well as the relevant Biodiversity Officer of the Department of Environment, Land, Water and Planning, and the Ranger In Charge of the Park, at the earliest possible convenience.

20. **The research permit must be carried by the permit holder** whilst undertaking any work in any area named on the permit and shown on demand to any Authorised Officer of the Department of Environment, Land, Water and Planning or any Authorised Officer of Parks Victoria.

21. Failure to comply with any condition of this permit may result in its cancellation, at the discretion of the Environment and Natural Resources, Regional Manager.

22. **Requests for the renewal of a research permit will be considered only after all other permit requirements are met.**

This permit shall, unless revoked, remain in force until **30 June 2016**.

.....

Bryan Welch
Acting Environment and Natural Resources, Regional Manager
(Delegate of the Secretary) **Date of issue: 1/12/15**

I, the permit holder (as named above) have read and fully understand the conditions of this permit.

..... Date of signature:
Permit holder

*** This permit is only valid when signed and dated by the permit holder*

Department of Environment, Land, Water and Planning

PO Box 137 Heidelberg
Victoria 3084 Australia
www.delwp.vic.gov.au
ABN 90 719 052 204

File: FF383428

Mr Callum Blake
Fenner School of Environment and Society
Australian National University
Building 141, Linnaeus Way
Canberra 2601

Dear Callum

I refer to your application for a research permit under the **National Parks Act 1975** to undertake research in Cape Liptrap Coastal Park,Mornington Peninsula National Park,Wilsons Promontory National Park. Permit number **10007771** (expiry date **30 June 2016**) has been granted. If this project is to continue after that date please write to me at the address below requesting a renewal. You are required to submit a copy of your final report. The permit was referred to Parks Victoria to ensure that this project is consistent with park management objectives.

The granting of this research permit is based on the premise that the work will potentially contribute to scientific knowledge, is supported by both the Department of Environment, Land, Water and Planning and broadly by the community. Researchers should note that the issue of this permit is a privilege not available to the general community and therefore the probity reflected in this research permit indicates the responsibility researchers have to properly undertake their authorised work. Close attention must be paid to all conditions on the permit, failure to do so can result in non-compliance.

You are reminded that you **must** contact the Area Chief Ranger or delegate (contactable via 13 19 63) at **least five days** prior to commencing any research in parks.

Please note this permit is only valid when signed and dated by the permit holder (see final page of permit).

Should you require further information, please email: environmental.research@delwp.vic.gov.au or telephone 9450 8746. Please quote the file number and permit number in all correspondence relating to this permit.

Yours sincerely,

Dr Sue Hadden
Environmental Research Co-ordinator

Appendix 2: Infection identification guidelines

Researchers' guide to identifying possible *Maullinia* sp. infections on *Durvillaea* species.

Above: a likely infection of *Maullinia* sp. (left) (image: Ceridwen Fraser); galls formed by infection on *Durvillaea Antarctica* in Chile (centre) (image: Goecke *et al*, 2012); infection on *D antarctica* in Chile (Aguilera, 1988).

Descriptions:

Goecke *et* al (2012): yellowish, protruded, wart-like growths, irregular in form and size. Tending to be circular or elliptic with a diameter between 0.5-4cm

Jahncke (1978): protruded growths, galls and holes.
'Discoloured, yellowish warty tissue, forming circular or elliptical raised galls, or holes surrounded by such tissue'

Update Dec 2015:
Confirmed infections on *Durvillaea potatorum* in NSW and Victoria, Australia.

Appendix 3: Example of sampling environment

Durvillaea species tend to grow on a series of rocky platforms separated by water, and as such transect placement was not always parallel or continuous. In the above image a transect was laid along the platform edge with the two researchers, and continued along to the same edge of the reef in the background to a total of 10m. In some areas transects were laid parallel to each other, separated by at least two meters, on either side of similar platforms.

Appendix 4: Import permit

Permit to Import Quarantine Material

This permit is issued under *Quarantine Act 1908 Section 13(2AA)*

Permit: 0000259546

Valid For: multiple consignments between 29 January 2016 and 29 January 2018

This permit is issued to: Mr Callum James Blake
34 Sturdee Cres
Monash ACT 2904
Australia

Attention: Mr Callum James Blake

This permit is issued for the import of Plant and Plant Products (Non-standard goods).

Exporter details:	Various exporters
Country of export:	Chile

This permit includes the following commodity (or commodities). Refer to the indicated page for details of the permit conditions:

1.	Plant pathogens (preserved or extracted)	Description:	Parasitic protozoan - Maullinia sp. (DNA only)
Country of origin:	Chile		
Permit Conditions(s):	Genetic material, purified and derived from microorganisms and viruses		Page 3

NOTE: Where a commodity has more than one set of permit conditions please read each set to determine which set of permit conditions applies to a specific consignment.

---------- End of Commodity List ----------

This Permit is granted subject to the condition that fees determined under Section 86E are paid.

Jutta Tuerck
Delegate of Director of Quarantine Date: 29 January 2016

T +61 2 6272 3933
F +61 2 6272 5161
18 Marcus Clarke Street
Canberra City ACT 2601
GPO Box 858
Canberra ACT 2601
agriculture.gov.au
ABN 24 113 085 695

Important Information about this Permit and the Import of Commodities

Note: This permit covers Department of Agriculture quarantine requirements. It is your responsibility to ensure all legal requirements relating to the commodities described in this Import Permit are met. While you should rely on your own inquiries regarding your legal obligations, the following information is provided to assist you in meeting your legal obligations in relation to the importation of the commodities described in this Import Permit.

Authority to Import

You are authorised to import the commodities described in this import permit under the listed conditions.

Compliance with Permit Conditions and Freedom from Contamination

All imports may be subject to quarantine inspection on arrival to determine compliance with the listed permit conditions and freedom from contamination. Imports not in compliance or not appropriately identified or packaged and labelled in accordance with the import conditions they represent may be subject to seizure, treatment, re-export or destruction at the importer's expense.

Compliance with Other Regulatory Provisions

Additionally, all foods imported into Australia must comply with the provisions of the Imported Food Control Act 1992, and may be inspected and/or analysed against the requirements of the Australia New Zealand Food Standards Code.

All imports containing or derived from Genetically Modified material must comply with the Gene Technology Act 2000.

It is the importer's responsibility to identify, and to ensure it has complied with, all requirements of any other regulatory organisations and advisory bodies prior to and after importation including The Department of Immigration and Border Protection, The Department of Health, Therapeutic Goods Administration, Australian Pesticides and Veterinary Medicines Authority, Department of the Environment, Food Standards Australia New Zealand and any state agencies such as Departments of Agriculture and Health and Environmental Protection authorities. Importers should note that this list is not exhaustive.

Change of Import Conditions

Import conditions are subject to change at the discretion of the Director of Quarantine. This permit may be revoked without notice.

Notification of Import

Notification of the import must be provided to Department of Agriculture for all imported goods other than goods imported as accompanied baggage or goods imported via the mail and not prescribed under the Customs Act 1901. Notification must be consistent with Quarantine Regulations 2000 (examples include a Quarantine Entry or a Quarantine declaration).

Valid Import Permit

The importer must hold a valid permit to import quarantine material for the goods being presented for clearance.

The importer must verify that an import permit has been issued in relation to the consignment by one of the following means:

i. The positive identification of the permit to Department of Agriculture at the time that the goods are being processed for quarantine clearance, such as by presenting the import permit.

OR

ii. Any form of physical, digital or verbal correspondence presented with information that allows an import permit to be identified.

Provision of Required Documentation

All required documentation must accompany each consignment. Alternatively, necessary documentation will need to be presented to Department of Agriculture at the time of clearance. In order to facilitate clearance, airfreight or mail shipments should have all documentation securely attached to the outside of the package, and clearly marked "Attention Quarantine". Documentation may include import permit (or import permit number), government certification and invoice.

If the product description on the import permit varies from the identifying documentation provided for clearance, the importer is responsible for providing evidence to the quarantine officer that the Import Permit covers the products in the consignment.

Any documentation provided must comply with the Department of Agriculture's Minimum Documentation Requirements Policy.

Permit Conditions

It is the Importer's responsibility to ensure that the following permit conditions are met in relation to each consignment. Where more than one set of permit conditions is shown for a commodity please read each set of conditions to determine which applies to a specific consignment.

1. Genetic material, purified and derived from microorganisms and viruses

This section contains permit conditions for the following commodity (or commodities):

1.	Plant pathogens (preserved or extracted) Product Description: Parasitic protozoan - Maullinia sp. (DNA only)

1.1. Biosecurity Pathway

a. This permit allows for the import of purified genetic material from Maullinia sp. (DNA only)

The material must be preserved in at least 70% or greater alcohol (e.g. ethanol, propanol), prior DNA extraction using a standard laboratory procedure that lyses cells, and/or removes lipids, proteins and other molecules from the solution and results in a purified DNA or RNA product. A declaration demonstrating this process must be presented to Quarantine at the time of clearance.

b. The goods must be clearly labelled with the name of the source microorganism or virus.

c. **Post entry/end use conditions**

1. These conditions allow for the importation of goods for *in vitro* laboratory studies (or *in vivo* use in laboratory organisms) only.
2. Laboratory organisms are those defined in the following list and must be contained under laboratory or animal house conditions: guinea pigs, hamsters, mice, rats, rabbits or microorganisms. Work in all other animals and plants is not permitted.
3. For all uses in non-laboratory organisms (e.g. chickens, sheep, cattle, etc.) or plants a separate application for *in vivo* use must be lodged with, and approved by the Department of Agriculture. This also applies if the product is to be used in veterinary vaccine or veterinary therapeutic manufacture.
4. These conditions do not permit the direct or indirect exposure of the imported materials or derivatives to non-laboratory organisms or plants.
5. These conditions do not permit the use of the samples for microbiological cultures or viral isolation.
6. It is the importer's responsibility to ensure that the goods are labelled "*in vitro* use or *in vivo* use in laboratory organisms only" on the smallest packaged unit prior to distribution.
7. It is the importer's responsibility to ensure compliance with all international (e.g. International Air Transport Association) and domestic requirements concerning the safe handling, transport and labelling of biological material.
8. It is the end user's responsibility to ensure that all laboratory products are used in accordance with the current AS/NZS 2243 Safety in Laboratories standards and Office

of the Gene Technology Regulator (OGTR) requirements.

d. If the consignment meets all documentation requirements at the time of clearance, it may be released from quarantine.

e. **Conditions of Administration**

1. Documents must be provided with each consignment which:

   1.1. identify the consignment e.g. entry number.

   1.2. identify all goods being imported as part of this consignment e.g. invoice or waybill or importer's manifest.

   1.3. describe the goods being imported (where not clear). Example 1: Product XRab = Purified protein derived from rabbits. Example 2: Product AX = Synthetic antibiotic. Example 3: Comte = Cheese.

2. For further information please contact:

   Regional - Clearance assistance:
   http://www.agriculture.gov.au/about/contactus/phone/regional

   Canberra - Administrative assistance or technical assistance: email imports@agriculture.gov.au (See Attachments) or phone 1800 900 090

It is the importer's responsibility to provide any additional information which is requested in order to demonstrate that the import permit covers all goods being imported.

f. Under the Quarantine Service Fees Determination 2005, fees are payable to the Department of Agriculture for all services. A list of all quarantine & export fees is available on the Department of Agriculture's website.

g. Non-commodity information requirements for imported cargo also apply, please refer to the BICON case Non-Commodity Cargo Clearance.

Timber packaging, pallets or dunnage associated with the consignment may be subject to inspection and treatment on arrival, unless sufficient evidence of a Department of Agriculture approved treatment is provided.

All documentation presented to the department to assist in determining the level of biosecurity risk posed by transportation pathways and packaging must also meet the requirements of the non-commodity case.

---------- End of Permit Conditions ----------

Import Services Team contact details

Import Services Team

Phone: 1800 900 090

Email: imports@agriculture.gov.au