

Cryptic Kelp

Identifying and describing a new species in
the southern bull-kelp genus *Durvillaea*



by

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**Australian
National
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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.

A handwritten signature in black ink, consisting of a series of loops and a final horizontal stroke.

Xénia Weber

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Abstract

Genetic analyses offer unprecedented insight into the evolutionary history of species and can reveal a wealth of previously cryptic biodiversity. Species showing no obvious morphological or ecological differences, despite genetic differentiation, can offer opportunities to understand the processes underlying divergence. The Australian endemic brown macroalgae, *Durvillaea potatorum* (*Phaeophyceae*), is an ideal organism to study how divergence can arise and eventually lead to speciation in highly-disturbed environments. This study builds on a previous genetic investigation of *D. potatorum* that revealed two genetically differentiated, reproductively isolated lineages, potentially representing ‘cryptic species’. The aim was to identify any differences that correspond with the genetic division within *D. potatorum* by using a combined genetic, morphological, ecological and histological analytical approach. A total of 228 individuals from seven sites around coastal Tasmania were collected and measured *in situ* for a range of morphological and ecological characteristics (e.g. depth below mean sea level or stipe length). Tissue samples were also collected for each individual to allow genetic analyses using mitochondrial (COI) and nuclear (28S) markers as well as histological examination of reproductive structures. Differences between the two lineages were examined by a combination of univariate and multivariate modelling analyses (including non-metric multidimensional scaling, analysis of similarity, Adonis and partial least squares). Genetic analyses confirmed the presence of two deeply divergent monophyletic clades, representing genetically distinct species: *Durvillaea potatorum* (*sensu stricto*) and *Durvillaea lentus* *sp. nov.* Despite highly variable phenotypes, morphological and ecological differences were found. *Durvillaea lentus* was typically found in deeper waters and had a relatively thinner, more slender stipe. *Durvillaea lentus* was also less responsive to ecological conditions but appeared to invest more heavily in repair structures such as stipitate lateral branches. Spatial distributions and patterns of genetic diversity in contemporary populations of both species support allopatric speciation coinciding with the onset of glaciation cycles in the late Pliocene. The current sympatric distribution around Tasmania’s east and west-coasts can be explained by a combination of ecological divergence and interglacial range expansion, facilitated by changes to land connectivity and ocean currents. This study reveals how both geography and ecology can drive divergence, and eventually result in speciation. These findings suggest that high levels of disturbance can promote cryptic diversity and highlight the importance of long-term changes and short-term fluctuations in ecological conditions as drivers of biodiversity.

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List of acronyms and abbreviations

ANOSIM: Analysis of similarities

COI: Cytochrome c oxidase subunit I

DNA: Deoxyribonucleic acid

GLM: Generalised linear model

ITS2: Internal transcribed spacer

Kya: Thousand years ago

LGM: Last glacial maximum

ML: Maximum likelihood

Mya: Million years ago

NMDS: Non-metric multidimensional scaling

PCR: Polymerase chain reaction

PLS: Partial least squares

Glossary and Terms

Allele – the variant of a gene as determined by the nucleotides (adenine, cytosine, guanine or thymine) present at a particular locus

Allelic frequency – the occurrence of particular variants of a gene within a population

Allopatric – occurring in distinct geographical locations

Clade – a phylogenetic group of organisms including a common ancestor and all its descendants

Convergent evolution – a form of natural selection where distinct populations independently acquire similar adaptations

Cryptic species – species showing no obvious morphological or ecological differences, despite genetic differentiation

Dispersal – the movement of gametes or individuals between geographical locations

Divergence – the accumulation of genetic differences between groups via mutation, selection or drift resulting in genetically distinct populations

Divergent selection – a form of natural selection leading to the accumulation of different adaptations within distinct groups

Ecosystem engineer – an organism that affects the ecology of other organisms with the same community by altering biotic or abiotic conditions

Effective population size – the number of actively reproducing individuals in a population

Gene flow – exchange of genetic information between populations

Genotype – the combination of an individual's genes

Heterozygosity – different nucleotides found at the same locus on homologous chromosomes within the same individual

Hybridisation – the creation of new variants of a gene from sexual reproduction between genetically different individuals

Incomplete lineage sorting – incongruence between gene trees and species trees as a result of divergence occurring before alleles have reached fixation within the ancestral population

Interspecific – between species

Intraspecific – within species

Introgression – repeated gene flow between distinct populations or species

Locus – the specific site on a chromosome at which a gene is found

Marker – a specific genetic sequence from a chosen region of the genome

Neutral traits – characteristics of an organism that are not under selection

Niche – the set of specific conditions for which an organism is adapted to, typically referring to environmental or ecological conditions

Phenotype – the external characteristics of an individual as an expression of its genotype and interaction with the environment

Phenotypic plasticity – degree of changes to the external characteristics of an individual in response to environmental or ecological conditions without requiring any genetic alteration

Phylogeny – evolutionary history of taxa, often depicted as a tree to illustrate evolutionary relatedness

Pre and post-zygotic barriers – mechanisms that occur before or after fertilisation that prevent successful sexual reproduction

Range – spatial distribution of a population or species

Recombination – crossing over of chromosomes during meiosis resulting in new variants of the genes

Refugial population – relatively small groups of organisms restricted in range to sites of favourable environmental conditions

Sexual selection – a form of natural selection either occurring due to mate choice or competition for mating opportunities

Spatial autocorrelation - differences in environmental conditions and population dynamics co-vary with geographical differences

Speciation – the creation of species arising from population divergence

Stabilising selection – a form of natural selection which maintains the average trait and selects against extremes

Stand – a group of plants, or macroalgae, growing together

Sympatric – occurring in the same geographical area

Vicariance – geographical separation of populations by physical barriers

Chapter 1: Introduction

Biological diversity encompasses all life forms across terrestrial and aquatic habitats, for which there is enormous variation in individual, species, community and ecosystem characteristics (Sala *et al.*, 2000). Understanding the underlying processes of biodiversity generation, retention and loss provides explanations for patterns of richness, abundance and diversity in organisms existing in the past, present and future (Noda, 2004; Hooper *et al.*, 2005). However, even fundamental aspects of biodiversity such as the influence of ecological and geographical processes on divergence, are not fully understood and require further theoretical and empirical investigation (Schluter, 2001; Whittaker and Fernández-Palacios, 2007).

Various theories, models and studies have tried, and continue to attempt, to unravel the relative roles of geographical and ecological separation in initiating and maintaining divergence between organisms or populations. Geographical divergence can result from physical barriers separating populations, or large distances between populations relative to their dispersal capacity (Slatkin, 1987; Whittaker and Fernández-Palacios, 2007). In comparison, divergence by ecological separation arises and is reinforced by a variety of mechanisms including reproductive isolation as well as different responses to abiotic conditions, disturbance regimes and interactions between organisms (Via, 2001; Tellier *et al.*, 2011). Irrespective of whether divergence is due to geographical and/or ecological processes, separation limits opportunities for interbreeding, thereby reducing gene flow and allowing populations to follow unique evolutionary paths and become increasingly different (Dobzhansky, 1950).

Once sufficient differences have accumulated between populations, they can be considered distinct species. The criteria used to assign specific status can sometimes be conflicting between different species concepts, but commonly include morphological, ecological, behavioural and genetic traits considered characteristic of distinct species. Interestingly, in certain cases these distinctions may not be obvious or may never emerge. For example, ‘cryptic species’ are those for which genetic dissimilarity would support recognition of distinct species, and yet there is no discernible difference in appearance or behaviour (review Bickford *et al.*, 2007; Verbruggen, 2014). These cases have only recently become apparent with the development of molecular genetics as an increasing number of genes and genomes can be examined and analysed (*e.g.* Hebert *et al.*, 2004). In addition to the discovery of numerous cryptic species, genetic tools have also revealed the spatial distribution of genetic diversity, both within and between species (phylogeography). Consequently, genetics offers new and exciting opportunities to develop our understanding of important biological processes, such as the importance of ecology and geography in divergence, diversification and speciation. Brown macroalgae (*Phaeophyceae*) are a particularly interesting group to study, given their diversity, high levels of cryptic speciation, plastic morphology and ability to inhabit a wide variety of coastal habitats around Australia (Edgar, 1984; Cheshire and Hallam, 1989b; McDevit and Saunders, 2010; Verbruggen, 2014).

Durvillaea potatorum (Labillardière) J. E. Areschoug, is a prolific, commercially harvested *Phaeophyceae* that also acts as a keystone species and major ecosystem engineer, at highly exposed, rocky sites around Tasmanian and south-east Australian coasts (Millar, 2007). Findings from Cheshire and Hallam (1989b)'s examination of *D. potatorum* suggested the presence of two groups within the species, with slightly different morphological and ecological characteristics. Based on geographical distributions, these two groups were referred to as the 'eastern' and 'western' lineages. Since then, preliminary genetic analyses by Fraser *et al.* (2009h; 2010e) have confirmed the presence of both the 'eastern' and 'western' lineages within *D. potatorum*. The degree of genetic differentiation and lack of hybridisation between these two lineages suggests that they represent distinct species despite being currently recognised as only one. However, any characteristics that differentiate the two species are yet to be determined.

This study aims to integrate molecular, ecological, morphological and histological techniques to investigate and describe the two lineages, suspected to be cryptic species, within *D. potatorum*. This project will address a single key research question: *Is there ecological and morphological variation that corresponds with the genetic divergence of two lineages within Durvillaea potatorum?* The combination of a detailed phylogeny with morphological, geographical and ecological information will also develop our understanding of the processes triggering and maintaining divergence in Australia's coastal communities, and marine environments more broadly. This research presents three main hypotheses. Firstly, it is hypothesised that genetic findings will confirm the existence of two, reproductively isolated species, currently recognised as *D. potatorum*. Secondly, it is predicted that within field-sites the two lineages will show small-scale spatial variation in distribution along either depth or wave-disturbance gradients reflecting different environmental adaptations or responses. Thirdly, it is also hypothesised that both histological and morphological differences will be found between the two lineages, which will enable the identification of either species in the field.

The next chapter contains a literature review focused on the geographical and ecological processes of divergence, the use of genetics in studying these processes and provides a background to Australia's coastal ecosystems and *D. potatorum*'s biology and ecology. The third chapter describes the methodology and contains the methods employed to collect and analyse the genetic, histological, morphological and ecological data used in this study. The results from each of these approaches, as well as their integrated analyses, are presented in the fourth chapter. The fifth chapter contains a discussion of the results that examines the evidence for the recognition of separate species, as well as the morphological and ecological differences within *D. potatorum* offering potential evolutionary and ecological explanations for the divergence and speciation. A full species description of the new species is provided at the end of the discussion. The sixth chapter provides a summary of the findings and their implications for our understanding of species, evolution and the environment.

Chapter 2: Literature Review

This chapter examines the role of geography and ecology in driving divergence. It explains how divergence can be maintained through reproductive isolation and outlines how genetic analyses can offer insights into past divergence or speciation events. Finally, this chapter provides an overview of the biology and ecology of Australia's coastal ecosystems, with a particular focus on *D. potatorum* as the species considered in this study.

2.1 The process of evolutionary divergence

Populations diverge through evolutionary time by accumulating genetic differences via mutation, selection or drift (Gavrilets, 2003; Via, 2009). As populations' gene pools become increasingly different, it becomes more likely that traits will arise which limit interbreeding and gene flow, potentially to the point of complete reproductive isolation (Funk *et al.*, 2006; Via, 2009). The initiation, maintenance and reinforcement of genetic differentiation can be due to numerous, but not necessarily mutually exclusive, ecological and geographical processes.

2.2 Geographical separation and divergence

Gene flow reduces the accumulation of genetic differences between populations by altering the diversity and frequency of alleles (Silva *et al.*, 2014). Therefore, any condition that reduces the ability for groups to successfully interbreed will aid divergence between those groups (Dobzhansky, 1950; Doherty *et al.*, 1995). Geographical separation is one such condition, as populations which are isolated by distance or physical barriers (allopatry) will be less likely to exchange individuals and interbreed, than neighbouring (parapatric) or co-occurring (sympatric) populations (Losos and Glor, 2003). Populations which are further apart are the most likely to differ in population demographics (Léotard *et al.*, 2009), genetic diversity and allelic frequencies (Epps *et al.*, 2005; Crispo *et al.*, 2006).

A correlation between geographical separation and neutral genetic differences between populations indicates that geographical separation is affecting divergence (Slatkin, 1993; Davis *et al.*, 1998; *e.g.* Harris *et al.*, 2014; *e.g.* Bayha *et al.*, 2015). The distance or barrier required to limit, or prevent, gene flow depends on a species' dispersal capacity (*e.g.* Dennis and Shreeve, 1997). Changing conditions and dispersal capacities can allow previously separated populations to come back into contact following range contraction or expansion (*e.g.* Gravlund *et al.*, 1998; Hewitt, 2000).

Stochastic processes can change the allelic frequencies within separate populations and eventually result in populations following distinct evolutionary paths (review Lanfear *et al.*, 2014). New alleles can enter the gene pool through random mutations (Lachmann and Jablonka, 1996) and the rate at which these mutations arise limits novel trait development and affects a population's overall fitness (Lanfear *et al.*, 2014). Existing alleles may reach fixation or be lost

from the gene pool via genetic drift, which is the fluctuation in allelic frequency over time due to chance survival and reproductive success (Hellberg *et al.*, 2002). Although genetic drift can influence alleles independent of selection, it most commonly fixes neutral or nearly-neutral traits (Lanfear *et al.*, 2014) and occurs more quickly when effective population sizes are small (Kimura and Ohta, 1969; Slatkin, 1987) or generation times are short (Dominguez *et al.*, 2014; Martins *et al.*, 2015).

2.2.1 Dispersal: its effect on distribution and gene flow

Limited dispersal of individuals and gametes can also trigger allopatric divergence and enable ongoing population differentiation. The distance, rate and direction of dispersal can vary according to chance (Patarnello *et al.*, 2007), a species' dispersal biology and abiotic ecological factors (Harris *et al.*, 2014). Potential biotic factors include the mobility, size (Hughes *et al.*, 1994), reproductive age (Crispo *et al.*, 2006), developmental characteristics (Doherty *et al.*, 1995; Teske *et al.*, 2014) and territorial behaviour (Riley *et al.*, 2006) of the population, as well as competition (Weiher *et al.*, 1999) and habitat connectivity (Fraser *et al.*, 2010a; Martins *et al.*, 2015). In passively, rather than actively-dispersed organisms, dispersal is particularly dependent on ecological factors (Teller *et al.*, 2014); for example marine larvae are affected by ocean currents (Collins *et al.*, 2010; Bayha *et al.*, 2015) and wind dynamics can influence fungal spore dispersal (*e.g.* Rieux *et al.*, 2013). Human activities can also alter dispersal, for example the spread of *Rattus spp.* by ships (Theuerkauf *et al.*, 2015), *Mnemiopsis spp.* in ballast water (Bayha *et al.*, 2015) and the blocking of *Ovis canadensis nelson* movement by highways (Epps *et al.*, 2005).

A species' distribution is partly constrained by its dispersal capacity (Endler, 1973; Crispo *et al.*, 2006), however within a population, differences between individuals' realised and potential dispersal can create fine-scale spatial patterning and facilitate population divergence (Slatkin, 1987). Individuals with the greatest dispersal capacity are most commonly involved in population expansions and are clustered at range edges (Shine *et al.*, 2011). Range expansions often alter genetic diversity and the spatial distribution of alleles (*review* Excoffier *et al.*, 2009), with the potential to reduce genetic diversity (Provan and Bennett, 2008; *e.g.* White *et al.*, 2013; *e.g.* Bulgarella *et al.*, 2014) and effective population size at the expansion front (Bayha *et al.*, 2015; Theuerkauf *et al.*, 2015). However allelic frequency and genetic diversity may remain largely unchanged (*e.g.* Austerlitz *et al.*, 2000; Hallatschek *et al.*, 2007; *e.g.* Pluess, 2011) particularly when population expansions are slow due to low population growth rates, delayed maturity, unsuitable surrounding habitat (Roques *et al.*, 2012) or limited dispersal capacity (Goodsman *et al.*, 2014).

The effect of contact between previously separate populations depends on the size and frequency of dispersal events (Rius and Darling, 2014), hybridisation success (Hellberg *et al.*, 2002), selective pressures (Endler, 1973) and existing population characteristics (Waters *et al.*,

2013). Following invasive arrivals, local species may become excluded or driven to extinction (Nash *et al.*, 2013; *e.g.* Bertolino *et al.*, 2014), or alternatively, the introduction of genetic material may offer new advantageous alleles (*e.g.* Slatkin, 1987; Newman and Tallmon, 2001), improve adaptive capacity (Rius and Darling, 2014) and prevent the accumulation of deleterious mutations (*e.g.* Epps *et al.*, 2005; Monkkonen *et al.*, 2014). Assuming that species sharing similar niches experience equivalent selective pressures (Kostikova *et al.*, 2014), dispersal will be most beneficial when surrounding areas are ecologically similar (Lenormand, 2002).

2.2.2 Vicariance: physical barriers to dispersal

Population separation is often greater than linear geographic distance alone due to the presence of barriers and restricted connective pathways (McRae, 2006; *e.g.* Riley *et al.*, 2006; *e.g.* Harris *et al.*, 2014; Warren *et al.*, 2014) which can explain allopatric divergence and speciation (Nelson, 1981; Zink *et al.*, 2000). Through vicariance, previously continuous population distributions become disjunct due to the emergence of physical barriers such as waterfalls (*e.g.* Crispo *et al.*, 2006), rivers (*e.g.* Goedbloed *et al.*, 2013), roads (*e.g.* Riley *et al.*, 2006) or seasonal sea ice (*e.g.* Harris *et al.*, 2014). Barriers are often highly species and context specific (Slatkin, 1987; Patarnello *et al.*, 2007), for example steep-sided valleys and slopes obstruct *Rattus norvegicus*'s dispersal but not that of other species within the same genus on the island of Futuna (Theuerkauf *et al.*, 2015).

For a vicariant event to be linked to divergence, the estimated timing of historic population separation and contemporary spatial patterns of diversity should correspond to the suspected timing and location of any proposed barrier (Rull, 2015). Changing environmental conditions over time and place have affected contemporary population genetics, commonly seen in taxa which have persisted through the Pleistocene glaciations. Fluctuating climatic conditions changed habitat availability and connectivity for numerous taxa, resulting in range contraction to isolated climatic refuges and subsequent expansion under favourable conditions (*review* Hewitt, 2004; *review* Beheregaray, 2008; *review* Ludt and Rocha, 2015). The northern hemisphere, in particular, showed strong trends in latitudinal and altitudinal range shifts over the period, although refugial populations varied greatly in size, location and persistence (Mee and Moore, 2014). Small refugial populations, with higher genetic drift and inbreeding (Harris *et al.*, 2014), were most vulnerable to extinction, fixation of detrimental alleles and loss of genetic diversity (*review* Charlesworth and Charlesworth, 1987; *e.g.* Martins *et al.*, 2015).

2.3 Ecological processes of divergence

In addition to geographical isolation, populations can also separate and gradually accumulate differences in response to ecological conditions (Shafer and Wolf, 2013; Ellegren, 2014), often as a result of divergent or disruptive selection (Rice, 1987; Althoff *et al.*, 2014; Kostikova *et al.*, 2014). Classical examples include adaptive radiations of Darwin's finches

(Caspari, 1952) or sticklebacks (Schluter and McPhail, 1992). Selection acts on an individual's phenotype and results in traits, along with their underlying genetic basis, being favoured according to their effect on an individual's survival and reproductive success (Wright, 1932). Selective pressures are always changing, requiring continual adaptation, acclimation or migration as different traits are favoured over time and space (Coelho *et al.*, 2013). During divergence, certain traits may experience differentiation under strong divergent or sexual selection, while others may remain highly conserved, especially when influenced by stabilising selection (Bickford *et al.*, 2007; Ackerly, 2009; Preston *et al.*, 2011). However, convergent evolution can also result in morphological or ecological similarities between populations which do not reflect recent common ancestry (Gatesy *et al.*, 2001; Kostikova *et al.*, 2014).

2.3.1 Abiotic conditions and species distributions

Population and species diversification can be driven by local adaptations to abiotic conditions such as temperature, light and water availability (*e.g.* Riesch *et al.*, 2011; *e.g.* Vargas-Salinas *et al.*, 2014). Spatial patterning of populations can arise from spatial variation in abiotic conditions according to the population's physiology, fitness and adaptive capacity (Endler, 1973; Voda *et al.*, 2015). For example, many sessile species are found at different depths in coastal marine ecosystems depending on their ability to tolerate abiotic conditions such as swell disturbance (Benes and Carpenter, 2015), light, temperature and aerial exposure, all of which vary with depth (Bennett and Pope, 1960). Abiotic conditions are especially influential for the distribution of sessile species (Soininen, 2014) whose responses are limited to plastic growth (Harder *et al.*, 2004), or adaptations in morphology and dispersal capacity (Rice, 1987; Zhen and Ungerer, 2008). Spatial differences in ecological conditions mean that consideration of ecologically-driven divergence must correct for spatial and ecospatial autocorrelation (Meirmans, 2012; *review* Warren *et al.*, 2014).

The type, range, extremes and duration of abiotic conditions contributing to ecological divergence differ between species, sites and over time (Crispo and Chapman, 2010; Guenther and Martone, 2014). Differences in abiotic conditions can vary at a broad spatial scale [*e.g.* sea surface temperature (Edgar, 1984; Sommer *et al.*, 2013)] (*review* Willig *et al.*, 2003), or at a much smaller, microhabitat scale (*e.g.* kelp stands Kennelly and Underwood, 1993; *e.g.* coralline algae communities Guenther and Martone, 2014; Guisan *et al.*, 2014; *e.g.* aridland birds Zink, 2014). Inferences of species' ecological niches, adaptations and distributions are likely to be misguided unless both large and microgeographic scales are considered (Warren *et al.*, 2014). Yet, many studies have insufficient fine-scale resolution as a result of logistical challenges (Doherty *et al.*, 1995; Fowler-Walker *et al.*, 2005a; Hand *et al.*, 2015) or assumptions that gene flow will prevent small-scale variations (Richardson *et al.*, 2014). Abiotic conditions also change over time, for example global temperature and ocean circulation trends emerging over millennia (*review* Rahmstorf, 2002), increasing ocean temperature and acidity

over decades (IPCC, 2014), and daily fluctuations in wind or tides. Generally species respond to changing conditions through acclimation, gradual adaptation or migration but abrupt regime shifts can occur when an ecological threshold has been exceeded or numerous stressors combine (*e.g.* Seddon *et al.*, 2014; *e.g.* Rocha *et al.*, 2015). Models are commonly used to reconstruct and predict range contractions, expansions or shifts by integrating knowledge of abiotic conditions and population dynamics (Bulgarella *et al.*, 2014). However, models are limited by their ability to consider a species' realised niche (Kearney, 2006; *review* Warren, 2012; Warren *et al.*, 2014), discontinuous habitats (Hellberg *et al.*, 2002), species interactions and dispersal capacity (Davis *et al.*, 1998).

2.3.2 Disturbance and its impact on diversity

Environmental disturbances disrupt ecosystems processes and challenge the survival of organisms (*review* Banks *et al.*, 2013). Disturbances include events as diverse as human visitation (*e.g.* Kury and Gochfeld, 1975), volcanism (*e.g.* *Mt St Helens* Franklin *et al.*, 2000) or wave action (Gaylord, 1999). The scale, frequency and intensity of disturbance can affect species richness and distributions. At high levels of disturbance, ecological stress is greatest, taxonomic diversity is poor and populations ordinarily experience slower adaptation and longer generation times (Prinzing *et al.*, 2014). Despite high disturbance, certain species persist, or even thrive (*e.g.* Monkkonen *et al.*, 2014; *review* Van Dover, 2014). In contrast, when disturbance is rare the stable ecological state can encourage competitive exclusion and the dominance of only a few, highly adapted, species (Connell, 1978). Communities are considered most diverse and species-rich at 'intermediary' levels of disturbance (Connell, 1978; *e.g.* Marra *et al.*, 2014; *e.g.* Bogyó *et al.*, 2015), although this theory is far from universal (*e.g.* Stohlgren *et al.*, 1999; *e.g.* Pastro *et al.*, 2014).

Any change to the disturbance regime can affect community composition if species are unable to quickly and effectively respond or adapt. For example, following pollution events species-rich brown algae assemblages are replaced by homogeneous communities of stress tolerant species (Doblin and Clayton, 1995). Morphological or behavioural plasticity allows species to rapidly respond to ecological conditions without genetic changes (*review* Sultan, 2000). Plastic phenotypes can result in different dispersal modes (Teller *et al.*, 2014), predator avoidance behaviour (Relyea, 2001) or growth habits (Monro and Poore, 2009). As both adaptation and plasticity alter a species' phenotype, environmentally-controlled experiments, such as cross-breeding or transplantation, are often used to determine if trait variants are heritable or due to ecological responses (*e.g.* Feder *et al.*, 1994; *e.g.* Williams *et al.*, 1995; *e.g.* Fowler-Walker *et al.*, 2006; *review* Leimu and Fischer, 2008; Lynch and Kemp, 2014; Richardson *et al.*, 2014).

2.3.3 Competition facilitating and preventing co-occurrence

Competition between and within species can drive divergence, as individuals seek alternative ways of acquiring resources or adapt to novel and underutilised resources (Dieckmann and Doebeli, 1999). For competition to occur individuals or populations must co-occur and influence each other. Therefore, competition is assumed to be positively correlated with the geographical or ecological overlap between groups (Fritschie *et al.*, 2013; Benes and Carpenter, 2015), meaning that species from the same functional group, of a similar body size, or which are closely related, should experience the strongest competitive pressure and lowest rates of co-occurrence (*review* Violle *et al.*, 2011; Nash *et al.*, 2013). For example, competition between algae for light and nutrients results in limited growth of understorey foliose species when overstorey canopy cover is dense (Kennelly and Underwood, 1993; Benes and Carpenter, 2015). A lack of co-occurrence for similar or closely related taxa can also be the result of allopatric speciation, historical colonisation patterns or time since divergence (Warren *et al.*, 2014).

Conversely, similar taxa may be more likely to co-occur despite competition (Fritschie *et al.*, 2013) due to landscape filtering (Poff, 1997), niche conservatism (*review* Wiens and Graham, 2005) or selection for the competitively ‘inferior’ population as a result of frequency-dependent selection, intraspecific competition (Chesson, 2000) or environmental variability (Sommer *et al.*, 2013; Aiken and Navarrete, 2014). Modelling of marine communities suggests that co-occurrence of populations despite identical niches and fitness, can easily be maintained with stochastic larvae dispersal or low self-recruitment rates (Aiken and Navarrete, 2014). In addition to competition, numerous other inter and intraspecific interactions can alter the adaptive landscape of sympatric species and sustain divergence, for example herbivory, predation or parasitism (Montoya *et al.*, 2006).

2.4 Maintaining reproductive isolation

Reproductive isolation, from either pre-zygotic or post-zygotic barriers, allows ongoing divergence, potentially even complete speciation, as populations become increasingly differentiated (Noor and Feder, 2006). Pre-zygotic barriers include assortative mating through sexual selection (Chan and Levin, 2005), poor migrant survival (Hereford, 2009), gamete competition (Chan and Levin, 2005) or differences in the timing, location or method of reproduction between populations (Rice, 1987; Feder *et al.*, 1994). In contrast, post-zygotic mechanisms include outbreeding depression (Rice, 1987; Via *et al.*, 2000; Richardson *et al.*, 2014), unviable or sterile hybrids (Orr, 1995) and embryo mortality (Scopece *et al.*, 2008).

Reproductive isolation can arise gradually as the outcome of long-term divergence between populations, or can quickly create barriers which drive rapid divergence. Reproductive barriers may be selected for directly if gene flow negatively affects population fitness. Direct selection is common for pre-zygotic barriers at sites of population contact (*e.g.* Lemmon, 2009;

e.g. Runquist and Moeller, 2014). Alternatively, reproductive barriers can arise through stochastic generation, pleiotropy (Caspari, 1952; Via, 2009) or linkage to ecologically favourable traits (Rice, 1987; Richardson *et al.*, 2014). Gene flow between species is surprisingly common despite the variety and prevalence of reproductive barriers (Chan and Levin, 2005; *e.g.* Baums *et al.*, 2014; Warren *et al.*, 2014; *e.g.* Fontaine *et al.*, 2015), suggesting strong natural selection, habitat choice or sexual selection can overcome incomplete reproductive isolation (Via, 2009; Richardson *et al.*, 2014). In some cases, genetic diversity from outbreeding may confer fitness benefits and reproductively isolating traits may be selected against (*e.g.* Ebert *et al.*, 2002; Crispo *et al.*, 2006).

2.5 Genetics: phylogenetics and phylogeography

Divergence has population and species-level consequences, but the underlying processes occur on a molecular level and begin with a change at a single locus before spreading throughout the genome (Via, 2009). Genetic analyses allow this process to be followed by detecting the molecular differences between individuals and populations (*e.g.* Noor and Feder, 2006; Preston *et al.*, 2011; Arnegard *et al.*, 2014). Traditional genetic studies amplify and sequence a small number of selected markers which can be reliably aligned and exhibit variation at the desired taxonomic level (Hellberg *et al.*, 2002; Cristescu, 2014). Continual technological developments have enabled the analysis of an increasing number of samples, a greater variety of markers (Noor and Feder, 2006; Ho, 2014; Verbruggen, 2014) and more recently, whole genomes with next-generation sequencing.

Despite the enormous potential of molecular analyses, there are important limitations to consider. Phylogenetic analyses may be unable to detect phylogenetic signals when hidden behind recent events (Patarnello *et al.*, 2007; Fontaine *et al.*, 2015) or confined to certain regions of the genome (Kostikova *et al.*, 2014). Constructing accurate phylogenies to identify relationships and delimit species can also be complicated by misleading evidence arising from processes such as introgression, incomplete lineage sorting, genome reorganisation (*review* Naciri and Linder, 2015), selective sweeps (Brookfield, 2001; Hellberg *et al.*, 2002), or mitochondrial capture (Chan and Levin, 2005; Teske *et al.*, 2014). To minimise these problems, studies can increase the number and diversity of markers and samples (Chan and Levin, 2005; Ellegren, 2014; Bayha *et al.*, 2015; Naciri and Linder, 2015) or combine genetics with ecological modelling, natural history records as well as traditional morphological, ecological and chemical analyses (Manoylov, 2014; Tripp and Lendemer, 2014; Naciri and Linder, 2015).

As populations become increasingly differentiated they are more likely to form separate species, however speciation is not the inevitable outcome of any isolating event (*review* Nosil *et al.*, 2009). The likelihood of divergence resulting in speciation varies according to population size, fitness (Lanfear *et al.*, 2014), reproductive strategy (Chan and Levin, 2005), and external selective pressures (Crispo *et al.*, 2006). Typically speciation is a highly variable and lengthy

process that includes numerous phases of stasis or reversal (Shafer and Wolf, 2013) as populations shift throughout a fluid adaptive landscape (*e.g.* Bell, 2001). The point at which diverging lineages can be considered distinct species is arbitrary and depends on species concepts. Numerous species concepts exist that seek to define and provide criteria for species delimitation, for example the Biological or Phylogenetic Species Concepts (*review* De Queiroz, 2007). The importance of criteria such as reproductive isolation, adaptive environment, or unique morphologies varies according to the concept, and can often lead to different decisions about species status (*review* De Queiroz, 2007). Since the advent of molecular genetics, debates surrounding species concepts have been revived and reoriented (Naciri and Linder, 2015) and a growing number of species have been described based solely on genetic information (*e.g.* Shaw *et al.*, 2010; *review* Tripp and Lendemer, 2014).

Population genetics, when combined with knowledge of species distributions, has enabled phylogeographical studies to reveal important patterns of population structuring, and suggest the underlying environmental and ecological drivers (*review* Soininen, 2014). For marine systems, traditional assumptions of weak ecological gradients and minimal barriers to dispersal have been rejected (Patarnello *et al.*, 2007). Rather, there is growing evidence of the importance of climate, tides (Neiva *et al.*, 2014), sea levels (Doherty *et al.*, 1995), ocean currents (Poulin *et al.*, 2014) and continental drift (Tronholm *et al.*, 2012) in altering population connectivity and genetic diversity. For example, numerous intertidal species in south-east Australia show strong spatial patterning concordant with historic changes in ocean currents, connectivity and sea levels (Waters, 2008c; York *et al.*, 2008).

2.6 The biology and ecology of Australia's coasts

The diversity of Australia's marine and coastal ecosystems make these sites ideal for studying fundamental ecological and evolutionary processes. Rocky, highly wave-disturbed subtidal and intertidal communities found across the south-east (Connell and Irving, 2008; DNP, 2013) are dominated by large, dense stands of highly prolific, canopy forming brown macroalgae belonging to the *Fucales* and *Laminariales* (Cheshire and Hallam, 1988; Schiel *et al.*, 1995; Mansilla *et al.*, 2012). These macroalgae affect the distribution, diversity, abundance and behaviour of other species by providing habitat (Edmunds *et al.*, 2010; Smith *et al.*, 2014), affecting nutrient and light availability (Schiel *et al.*, 1995; Kirkman and Kendrick, 1997), altering water flow and creating physical disturbances from sweeping fronds (Edgar, 1984; Benes and Carpenter, 2015).

These coastal communities experience high levels of wave-disturbance, are strongly influenced by water flow dynamics (Dring, 1982; Denny, 1988) and must endure strong forces and mechanical stress (Cheshire and Hallam, 1988; Harder *et al.*, 2004). Wave intensity and frequency fluctuates throughout the day (Stevens *et al.*, 2004; Mansilla *et al.*, 2012) depending on winds (Hill *et al.*, 2010), water depth (Denny, 1988), currents, tides, underlying geology and

broader ocean circulation (Stevens *et al.*, 2004). Tasmania's coastline experiences great spatial variability in wave-disturbance and water temperatures, offering a diverse range of habitats for algae communities and endemic species (Bennett and Pope, 1960) (Fig. 1). Tasmania's west and south coasts are subject to intense westerly winds, storms and swells from the Southern Ocean (Bennett and Pope, 1960; Hill *et al.*, 2010). In comparison, the north-coast is calm and sheltered (Edgar, 1984). Conditions along the east-coast vary depending on location and season (Bennett and Pope, 1960; Edgar, 1984; Hill *et al.*, 2010).

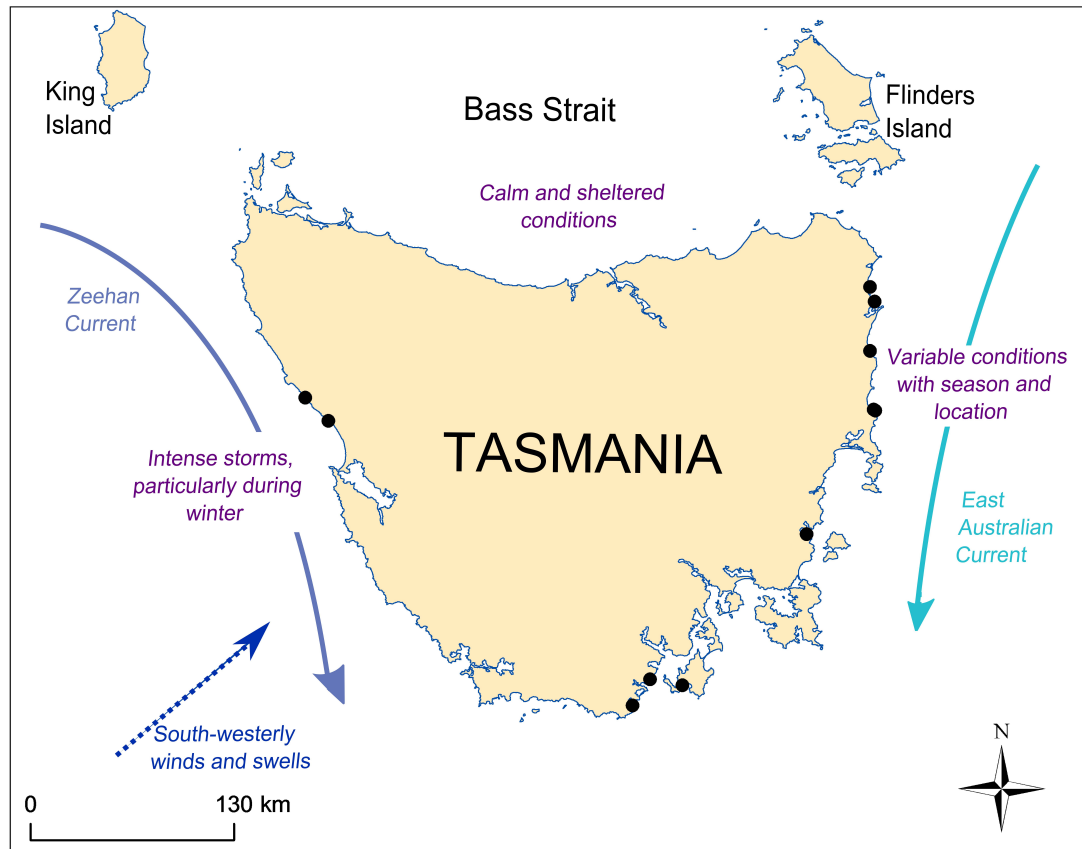


Figure 1 Map of Tasmania showing major ocean currents and variation in exposure between the north, east and west-coasts.

2.6.1 *Durvillaea potatorum*: biology and ecology

Studying the characteristics, environmental responses and ecological interactions of every species is logistically impossible, however inferences about common evolutionary and ecological processes can be based on representative species (Manoylov, 2014). *D. potatorum* was chosen as a model species in this study to explore processes of divergence, speciation and the evolution of biodiversity for Australia's temperate coastal communities. *Durvillaea potatorum* is an Australian endemic brown alga (*Phaeophyceae*) that serves as an ecosystem engineer and keystone species, is commercially harvested, has a wide distribution, large population size, morphological diversity, limited dispersal capacity, and molecular evidence of cryptic diversification (Schiel *et al.*, 1995; Fraser *et al.*, 2009a; Hill *et al.*, 2010).

Durvillaea is a genus of southern hemisphere brown macroalgae found primarily in the subtidal and intertidal zones of rocky, highly exposed coastal platforms and outcrops in cool-temperate waters (Bennett and Pope, 1960; Edgar, 1984; Cheshire and Hallam, 1988; Hay, 1994; Fraser *et al.*, 2010e). *Durvillaea potatorum* is the only species found in Australia (Bennett and Pope, 1960) and is distributed across numerous small islands, Tasmania's east, south and west coasts and south-east mainland coast from Robe (South Australia) (Hay, 1994; Kirkman and Kendrick, 1997) to Tathra (New South Wales) (Millar, 2007). Warming of water temperatures along the east-coast has been linked to recent southward range contractions of approximately 80 km (Edmunds *et al.*, 2009; Wernberg *et al.*, 2012), with projections of continued changes in distribution (Foster *et al.*, 2014). Records of populations outside Australia (i.e. Auckland Islands (Papenfuss, 1964) and Chile (Santelices, 1991)) remain unsupported (Fraser *et al.*, 2009h). *Durvillaea potatorum* typically forms a dense (4-108 kg m⁻² (Cheshire and Hallam, 1988)), narrow band extending anywhere from several metres away from the shoreline (Cheshire and Hallam, 1989b) to a maximum of 30 m (Kirkman and Kendrick, 1997) at southerly sites (Edgar, 1984).

The morphology of *D. potatorum* is well suited to withstand high wave-disturbance with thick, tough, yet flexible vegetative tissue that can easily regenerate (Cheshire and Hallam, 1985; 1988), a large holdfast for secure attachment to the substrate and a rigid zygote wall to prevent desiccation, herbivore or bacterial attack (Kevekordes and Clayton, 1999) (Fig. 2). Peak reproduction occurs over winter and early spring when gametes are released into the water column from dioecious gametophytes embedded within the blade of mature vegetative sporophytes (Clayton *et al.*, 1987; Clayton, 1990; Hay, 1994). Individuals can grow to over ten metres in length, weighing up to 75 kg and often exclude other algae and invertebrate species from persisting directly under the canopy by physically disturbing their settlement or through competitive exclusion (Dring, 1982; Hay, 1994; Schiel *et al.*, 1995; Underwood, 2007) (Fig. 3). As with other canopy-forming algae (Reed and Foster, 1984; e.g. *Eisenia* Benes and Carpenter, 2015), the dense canopy can shade out other organisms, restrict abundance and lower recruitment rates (Hay, 1994) but can also offer shelter and protection from heat or desiccation at low-tide in the intertidal zone (Schiel *et al.*, 2006; Underwood, 2007).

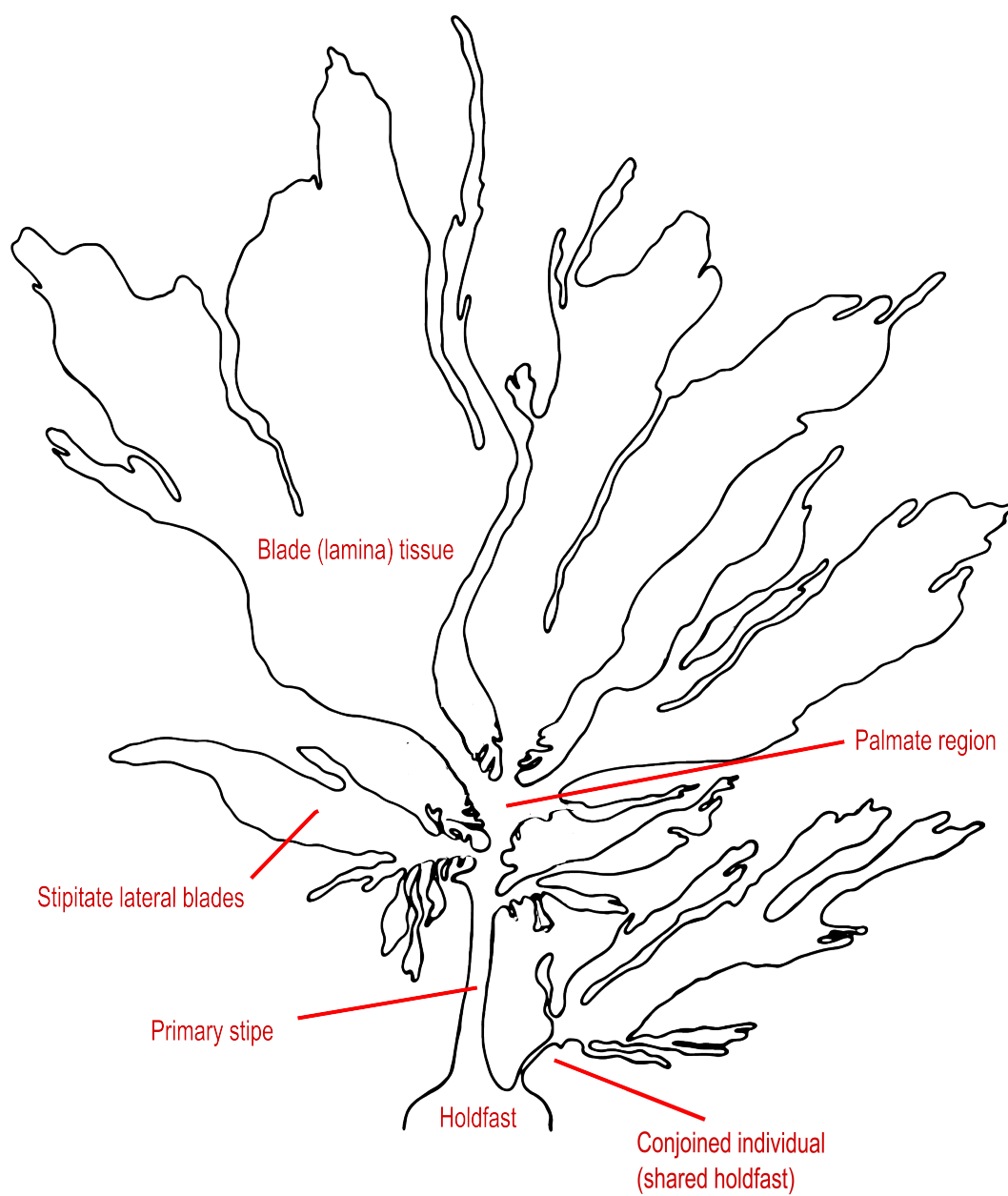


Figure 2 Sketch of *D. potatorum* showing key morphological features labelled in red

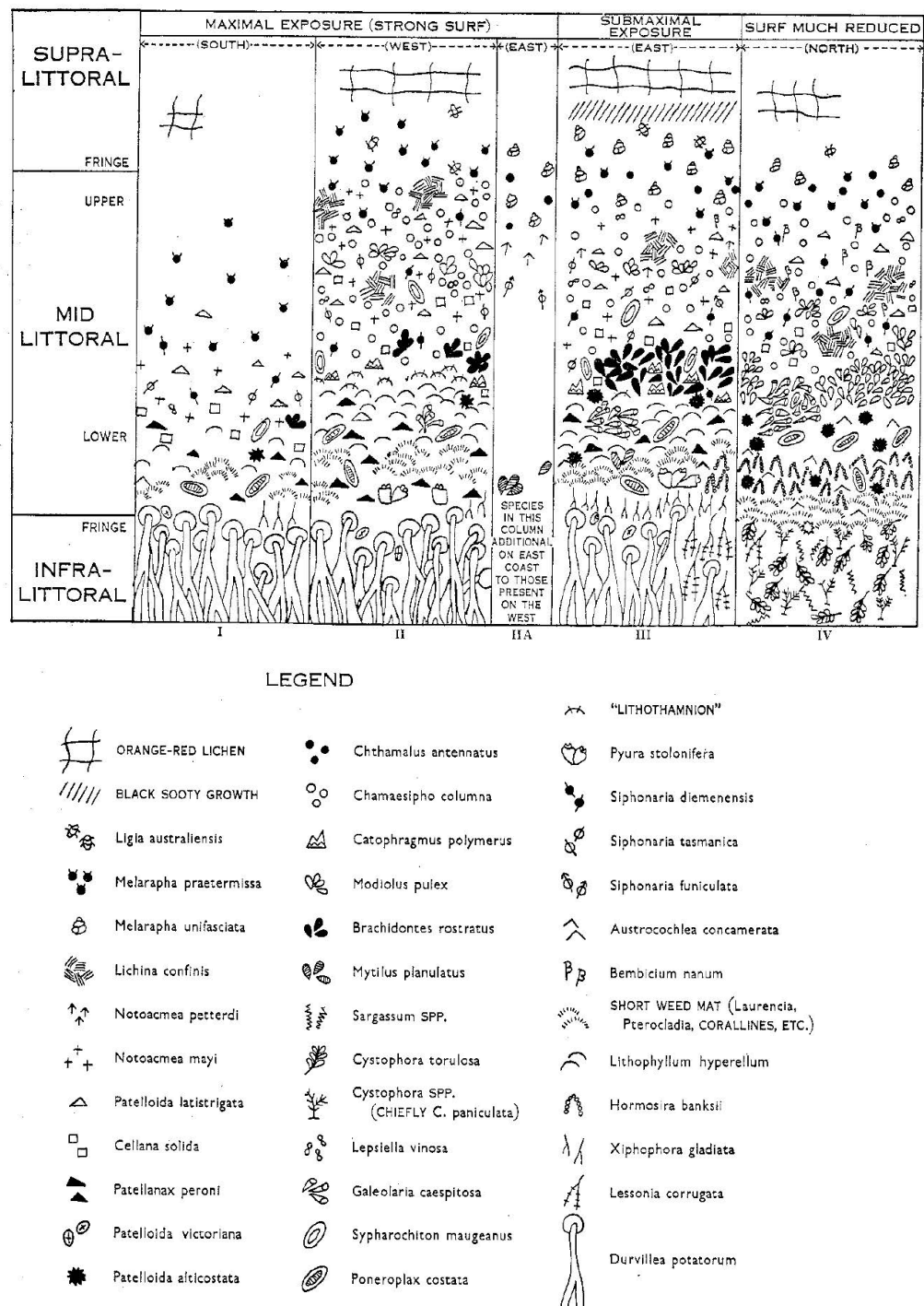


Figure 1 Schematic representation of species zoning patterns for coastal Tasmania according to wave exposure gradient (horizontal gradient) and depth (vertical gradient) taken from Bennett and Pope (1960). *Durvillaea potatorum* can be observed as a monospecific stand in all but the most sheltered sites.

Alternatively, *D. potatorum* can be beneficial to the survival of other organisms by offering complex and varied habitats in the intertidal and subtidal zone (Underwood, 2007; Aiken and Navarrete, 2014). Detached individuals can also offer habitat and food, either out at sea or when washed ashore, however unlike *D. antarctica*, the holdfasts remain intact and do not provide habitat for small invertebrates such as isopods (Hay, 1979). Given the massive size and prolific growth of *D. potatorum*, the species is also an extremely important primary producer that can increase the overall productivity and carrying capacity of the surrounding coastal community by maximizing light interception and the capture of dissolved nutrients (Hatcher, 1994) from Australia's comparatively nutrient-poor waters (Carter, 1994).

Durvillaea potatorum, is also of commercial importance as one of many macroalgae species collected for phycocolloid extraction (McHugh, 2003). Phycocolloids are used as thickeners, gels and films (McHugh, 2003) in food, beauty products, and pharmaceuticals (Dhargalkar and Verlecar, 2009) as well as enabling heavy metal decontamination due to biosorbent properties (e.g. Matheickal and Yu, 1999; e.g. Yu and Kaewsarn, 2000). Approximately 2500 tonnes (dry weight) of beach cast *D. potatorum* is sustainably harvested each year from King Island, Tasmania, before being exported as dried chips to Scotland for phycocolloid extraction (Virtue and Nichols, 1994; Kirkman and Kendrick, 1997; Kelp Industries, 2004; 2005).

2.6.2 *Durvillaea potatorum*: taxonomy

Accurate phylogenies, species delimitation and measurements of intraspecific variation are needed to inform management decisions, answer ecological questions (Chan and Levin, 2005; Kress *et al.*, 2015) and understand the processes behind diversification (Slaton, 2015). Phylogenetic analyses are especially difficult when species are rare, or lack clear defining characters due to either extremely high, or virtually no, morphological variability (Manoylov, 2014). Macroalgae are notoriously difficult to identify and classify given their highly variable and plastic morphologies (Hay, 1994; Fowler-Walker *et al.*, 2005c; e.g. Fowler-Walker *et al.*, 2006) and frequent instances of cryptic species complexes (Robba *et al.*, 2006; Mateo-Cid *et al.*, 2014; Verbruggen, 2014).

The taxonomy of *Durvillaea*, initially based on morphological and ecological characters, has been controversial and repeatedly modified (Hay, 1979; 1994; Fraser *et al.*, 2010e). The genus currently belongs to the order Fucales (Rousseau and De Reviers, 1999). Hay (1979) ascribed four species (including *D. potatorum*) to the monogeneric family Durvillaeaceae but recent genetic analyses by Fraser *et al.* (2010e) support the existence of at least six species (Fig 4), including the newly described *D. poha* (Fraser *et al.*, 2012b).

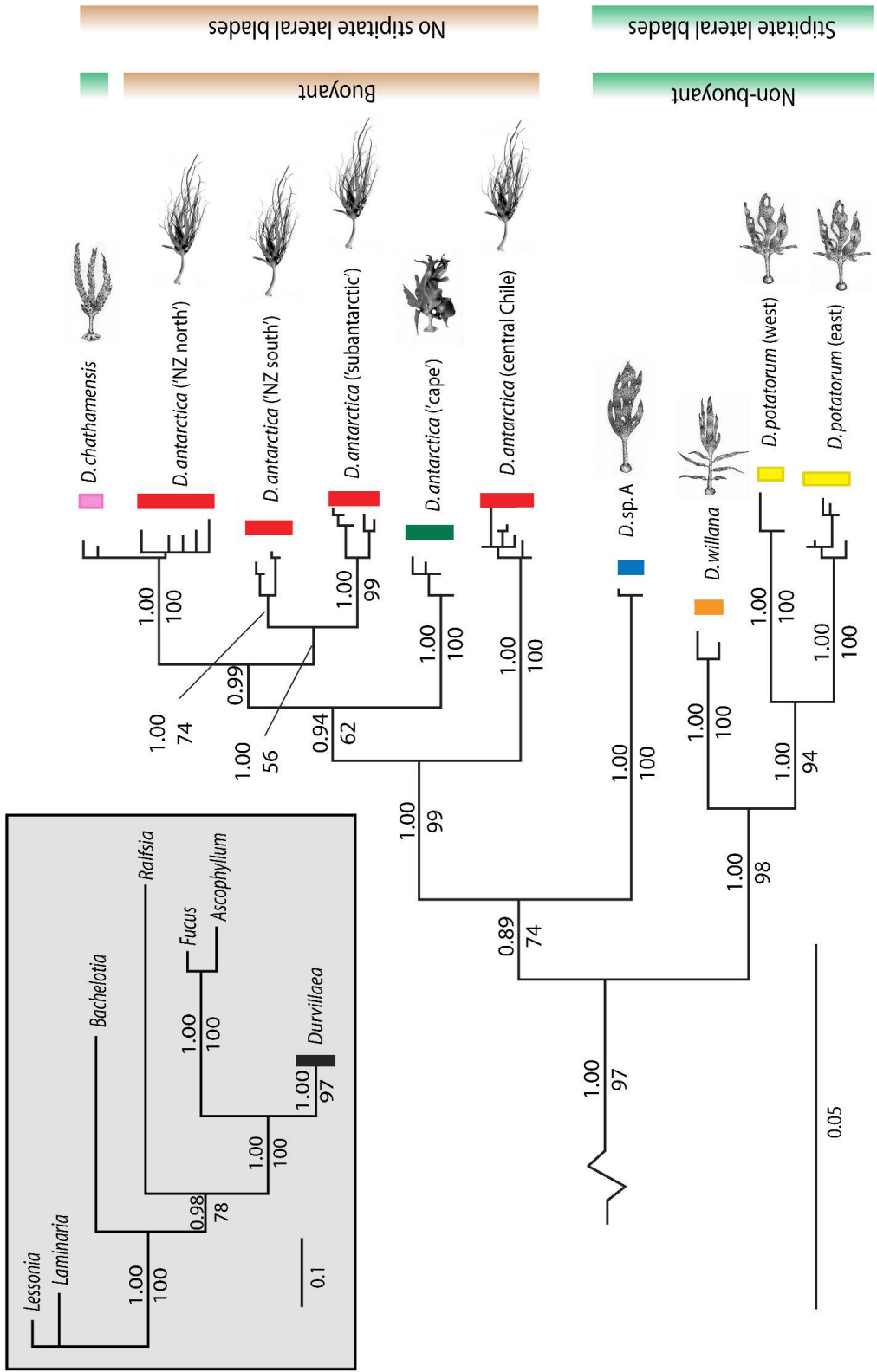


Figure 4 *Durvillaea* phylogeny based on the concatenated chloroplast, nuclear and mitochondria dataset from Fraser et al. (2010e).

Cheshire and Hallam (1985; 1988; 1989b) completed the most recent and thorough morphological and ecological examinations of *D. potatorum*. They determined that morphology varied due to wave-disturbance over small distances of several metres as well as according to latitudinal gradients. Furthermore, Cheshire and Hallam (1989b) suggested differences in maximum width, maximum length, wet weight, number of perforations and vulnerability to slime mould could be attributed to ‘eastern’ and ‘western’ intraspecific forms, but were unable to determine if this represented discrete or clinal variation. Fraser *et al.* (2009h; 2010e)’s mitochondrial, nuclear and chloroplast phylogenetic analyses supported two reproductively isolated ‘eastern’ and ‘western’ lineages based on intra-lineage genetic diversity and geographical patterning. Although the existence of both lineages has been confirmed, much remains to be determined, such as the exact range for each lineage, distinctive morphological or ecological characteristics, the effect of co-occurrence at sites of sympatry and the relative role of ecology and geography in divergence.

Much remains to be discovered about the evolution of biodiversity and the processes responsible for both diversification and speciation. The importance of geographical, ecological and abiotic factors in generating divergence has been acknowledged, but their relative contributions are far more challenging to determine. Existing research on *D. potatorum*’s ecology, spatial distribution and genetic diversity supports the presence of an ‘eastern’ and ‘western’ lineage yet how these two groups differ, and the processes involved in their divergence remains unknown. This study aims to resolve current uncertainty by undertaking a comprehensive, integrated analysis of the species’ morphology, ecology, histology and genetics.

Chapter 3: Methods and Methodology

3.1 Sample collection and site characteristics

Fieldwork was conducted on 2nd-6th February 2015 to sample *D. potatorum* from seven coastal field-sites around Tasmania. Five sites were chosen on the east-coast ('Spring Beach', 'The Gardens', 'Secret Spot', 'Lady's Bay' and 'The Whale') and two on the west-coast ('Trial Harbour' and 'Granville Harbour'). To minimise confounding differences between sites in ecological and geographical conditions this study focused on the east-coast, where both the 'eastern' and 'western' lineages are known to co-occur (Fraser *et al.*, 2009h). West-coast sites, where only the 'western' lineage has previously been found (Fraser *et al.*, 2009h), were included to observe if morphological or ecological characteristics varied depending on whether a stand was monotypic or sympatric. Tasmania's north-coast was excluded, as the sheltered conditions do not support large stands of *D. potatorum* (Hay, 1994). The south-east, and other remote locations that were not easily accessible by car or on foot, were also excluded.

In total, 228 individuals were sampled, with at least 30 individuals collected at each location (Fig. 5). Every individual was given a unique identification code to relate the ecological and morphological characters recorded in the field with the individual's photograph and tissue samples. Tissue samples of approximately 50 mm² were taken from frond tips for both histological and genetic analyses and placed in pre-labelled 1.5 mL safelock plastic vials. For histology, tissue pieces were fixed in 7% formalin:seawater solution and stored in the dark to prevent oxidation. For genetic analyses, tissue pieces were kept in ethanol, which was replaced after 24 hours. On returning to the laboratory, vials were stored at -20°C until frond tissue was removed, dried at 60°C for 20-30 minutes and stored on silica beads. The 2015 dataset of 228 individuals was supplemented with 103 samples previously collected from four east-coast sites ('Waubas Bay', 'Governor Island', 'Binalong Bay' and 'Cloudy Bay') (Fig. 5) in 2010-2011 by Dr. Ceridwen Fraser, Prof. Jon Waters and Prof. Graham Edgar.

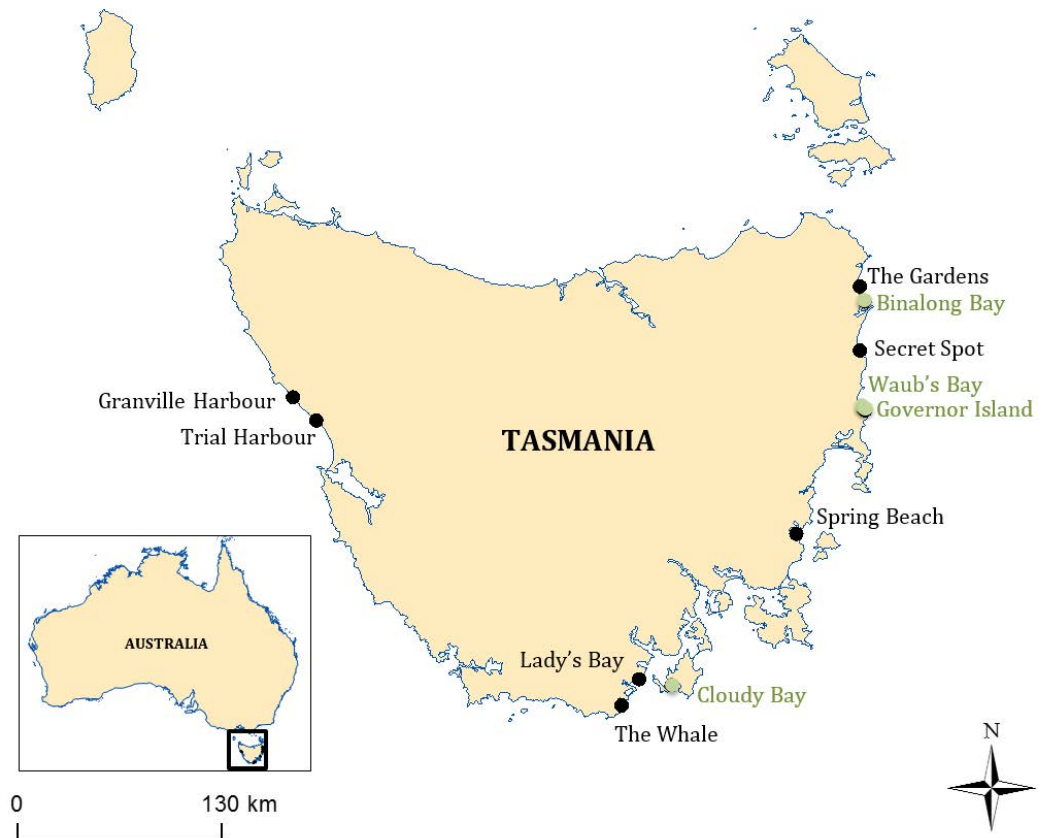
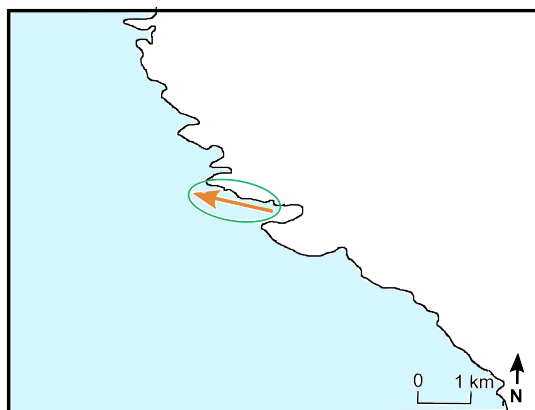


Figure 5 Map of Tasmania showing all eleven field sites considered in this study. The seven sites visited as part of the February 2015 sampling effort are shown in black, and the additional four sites from previous collections in 2010-2011 are shown in green.

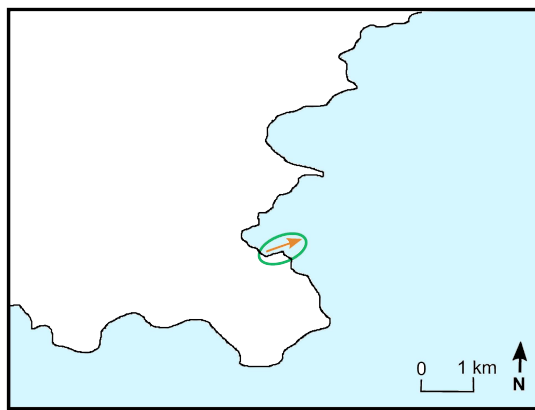
3.1.1 Ecological observations and site characteristics

Field observations were complemented by pre-existing environmental data to determine the influence of ecological conditions on divergence within *D. potatorum*. At each site the date, time, and GPS coordinates were recorded. Observations of water clarity, sand cover and stand density were made qualitatively during diving efforts. Photographs and sketches were taken of the local coastline to show where individuals were sampled (Fig 6). Using ArcGIS10.3.1 (ESRI, 2015) and Geoscience Australia's publicly-available National Marine Bioregionalisation of Australia GIS datasets (Heap *et al.*, 2005) an additional 12 ecological conditions were evaluated for each site: bathymetry, seabed oxygen, phosphate, silicate, nitrate and carbonate, mean sediment grain size, proportion of sand and gravel and mode of sediment movement.

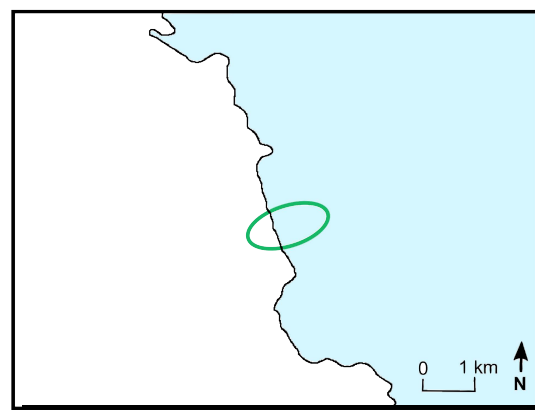
Granville Harbour



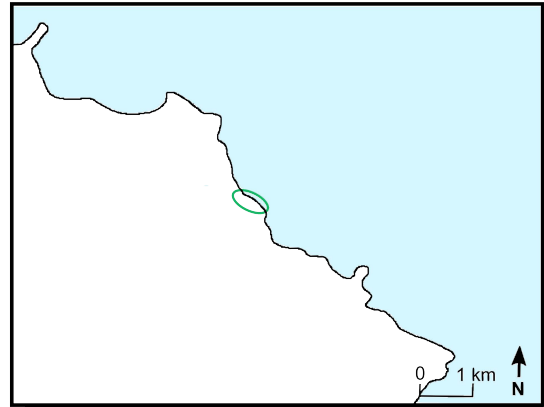
Lady's Bay



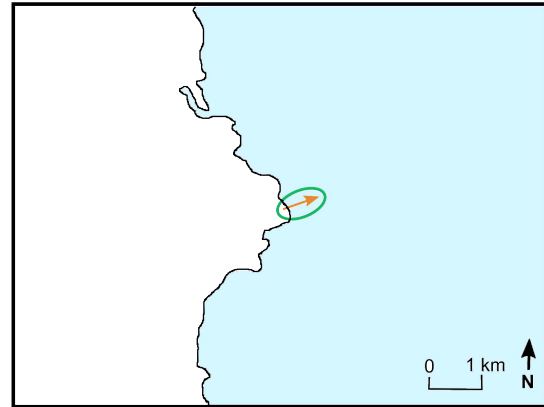
Secret Spot



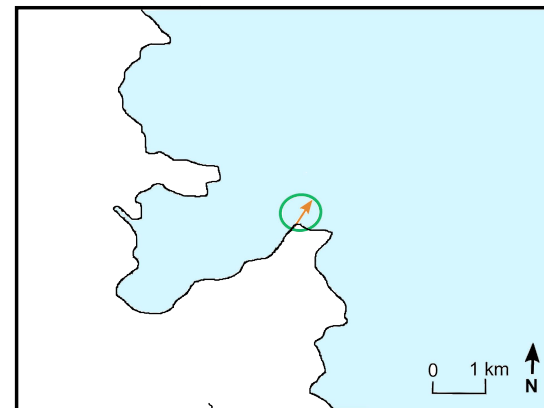
Spring Beach



The Gardens



The Whale



Trial Harbour

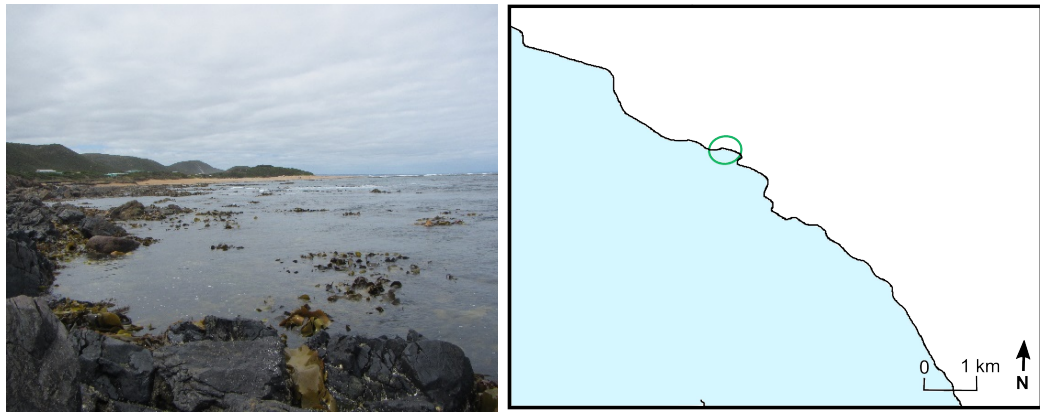


Figure 6 Photographs (left) and fieldsite sketches (right) showing the location of sampled individuals for each of the seven field sites visited during the 2015 sampling effort. Individuals were collected from the region circled in green. Orange arrows represent increasing ‘exposure gradients’ (where applicable).

For every individual the ‘depth’ from mean sea level and within-site wave-disturbance were recorded. Where possible, sampling followed both depth and wave-disturbance gradients, but at some sites disturbance did not vary (e.g. ‘Trial Harbour’), and at others *D. potatorum* did not extend into the subtidal zone (e.g. ‘Spring Beach’). To compare sites, as well as individuals within-sites, two separate measures of wave-disturbance were used. The first measure, hereafter ‘exposure’, was a subjective categorisation of wave-disturbance for each individual relative to others from the same site (A: least exposed to D: most exposed). The second measure, hereafter ‘openness’, indicated the overall wave-disturbance of a site based on Hill *et al.* (2010)’s openness index. This standardised index calculates the distance wind can travel uninterrupted from a given point along 48 evenly spaced 650 km transects, and is a more accurate alternative to hand-drawn calculations of the percentage of open ocean (e.g. Fowler-Walker *et al.*, 2006; Leaper *et al.*, 2014). ‘Openness’ was calculated retrospectively for each site as the mean of the two nearest scores contained within the Australian Ocean Data Network Portal’s publically available GIS dataset (Hill *et al.*, 2011) and viewed using ArcGIS.

3.1.2 Morphological characterisation of individuals

A total of fifteen morphological characteristics were recorded either directly in the field or calculated as derived measures for every individual (Fig. 7 & Table 1). Characteristics selected are known to vary within *D. potatorum* and/or are commonly informative in delimitating species among *Durvillaea*. As differences between the two lineages may occur only under specific developmental or environmental conditions, individuals across the range of development, size and physiological forms were collected. Extremely large specimens exceeding five metres could not be safely brought ashore and could therefore not be included.

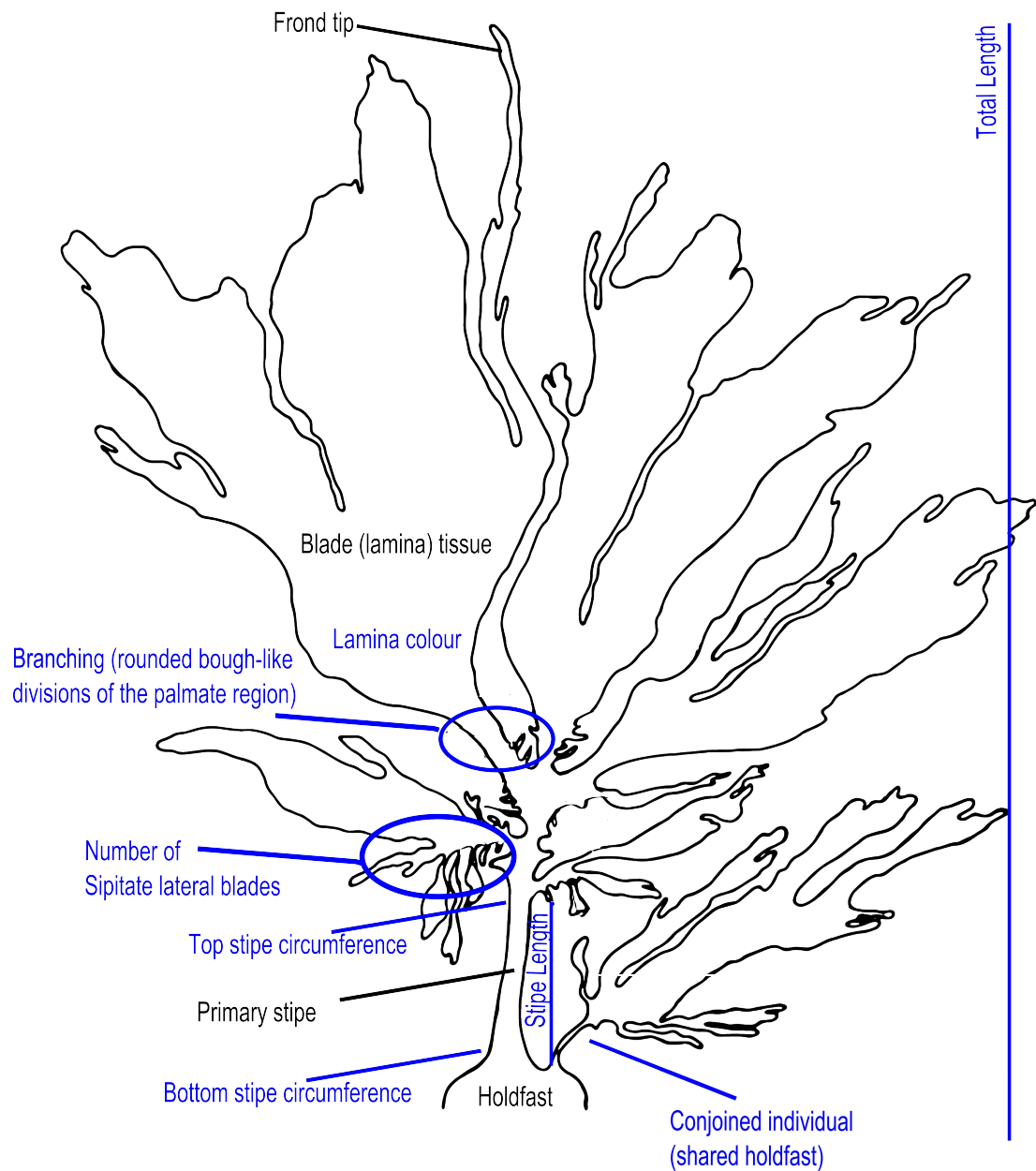


Figure 7 Sketch of an adult *D. potatorum* showing the eight morphological characteristics collected in the field (labelled in blue). Additional labels of the basic physiological structures are also included.

Table 1 Definitions and explanations of how the seven derived morphological characters were calculated.

Derived morphological character	Definition	Calculation
'Stipe proportion'	Ratio of stipe length to total length of the individual	$\log(\text{total length}) - \log(\text{stipe length})$
'Stipe stoutness (base)'	Ratio of stipe circumference measured at base of top to stipe length	$\log(\text{stipe length}) - \log(\text{stipe base circumference})$
'Stipe stoutness (top)'	Ratio of stipe circumference measured at top of stipe to stipe length	$\log(\text{stipe length}) - \log(\text{stipe top circumference})$
'Stipe stoutness (mean)'	Ratio of stipe circumference measured as the mean of the stipe circumference (mean of base and top circumference) to stipe length	$\log(\text{stipe length}) - \log((\text{stipe base circumference} + \text{stipe top circumference})/2)$
'Tapering'	Ratio of circumference at the base of the stipe to the circumference at the top of the stipe	$\log(\text{stipe base circumference}) - \log(\text{stipe top circumference})$
'Lateral number'	Number of stipitate lateral blades on both the left and right side of the palmate region	number of stipitate lateral blades on left + number on right
'Lateral asymmetry'	Difference between the number of stipitate lateral blades on the left and right side of the palmate region	number of stipitate laterals on the side of the palmate region with the highest count – number on the other side

At low-tide, intertidal specimens were photographed and measured *in situ*, however at high tide and in the subtidal zone, individuals were destructively harvested by cutting just above the holdfast and photographed ashore. For each individual, ‘total length’, ‘stipe length’ and circumference at the base and top of the stipe (‘stipe base’ and ‘stipe top’) were measured (± 0.1 cm) using a ten metre tape. Stipe circumference was also used to define individuals as either adults or juveniles, based on Cheshire and Hallam (1989a)’s 40 mm delimitation for adults. ‘Lamina colour’ from the tip of the longest frond was determined using a Benjamin Moore paint colour chart (Rorabaugh n.d.). Other measures included the number of stipitate lateral blades either side of the palmate region, the shape of the palmate region, if the holdfast was shared with another individual (‘conjoined’), if the palmate region was divided into several large, rounded boughs [‘branched’ (*sensu* Hay, 1977)] and additional comments (e.g. lamina margins and parasitic load). An additional seven derived morphological characters were calculated to compare growth patterns across individuals of different ages and sizes. The derived characters were ‘stipe proportion’ ‘stipe stoutness’, ‘tapering’, ‘lateral number’ and ‘lateral asymmetry’ (Table 1). The accuracy of all field measurements was confirmed by comparing values for each individual to their corresponding photo. Ten modifications were made (e.g. correcting ‘total length’ from 192 cm to 19.2 cm) and two individuals had to be excluded as one was missing ‘total length’ and the second did not have a corresponding tissue sample. When combined with the 2010-2011 collections, the total dataset comprised 329 individuals. However previous sampling efforts had only a subset of ecological and morphological variables meaning sample size for certain characteristics was reduced (exposure 226, top stipe 280, lateral number 299, conjoined 279 and branched 279).

3.2 Histological examination of reproductive structures

Cross-sections of frond tissue were examined by light microscopy to determine any differences in reproductive structures between the ‘eastern’ and ‘western’ lineages that may reveal a pre-zygotic isolating mechanism. Particular attention was given to conceptacle characteristics used as diagnostic taxonomic traits in other *Phaeophyceae* and known to vary among other species of *Durvillaea*, including conceptacle wall incursions, structure of male antheridia and attachment of female oogonia to branched paraphyses and/or directly on conceptacle walls (Nizamuddin, 1968; Hay, 1979; Clayton *et al.*, 1987; Hay, 1994) (Fig 8). Thin slices of formalin-fixed frond tissue were hand-sectioned by scalpel, kept moist in a synthetic seawater solution and examined for conceptacles using a Nikon SMZ 1000 stereomicroscope (Nikon Instruments Inc., Melville, United States of America). Cross-sections found with conceptacles were mounted on slides with a 10% karo solution and left to dry for several weeks (Clayton, 2015, pers. comm., 27 April). Where possible the presence/absence of conceptacles, maturity, sex and structural observations were recorded. Images were recorded using a Leica DMLB light microscope, Leica MC120 HD camera and Leica EZ software 3.0

(Leica Camera AG, Wetzlar, Germany). To corroborate interpretations, a subset of images were sent to algal physiologist Prof. Clayton, Emeritus Professor in Biological Sciences (Monash University).

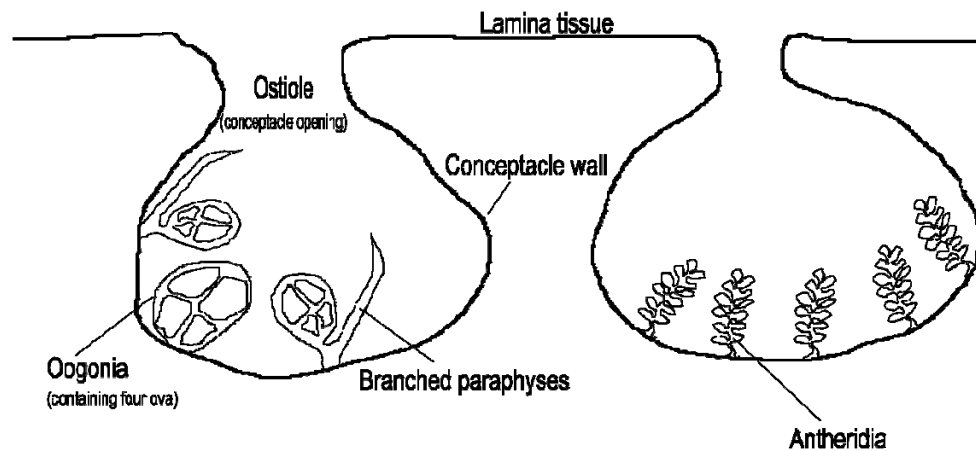


Figure 8 Diagram showing a simplified male (left) and female (right) *D. potatorum* conceptacle taken in cross-section showing key morphological structures. Note that although shown together, *D. potatorum* is dioecious with only female or male conceptacles found in any individual's blade tissue.

3.3 Genetic analyses to assign lineages

The genotype of every individual had to be determined before any ecological or morphological differences between the 'eastern' and 'western' lineages could be determined. DNA was extracted following Walsh *et al.* (1991)'s Chelex-based protocol. Small pieces of dried frond (<1 mm²) were incubated overnight at 60°C in 800 µL of 5% Chelex solution (Bio-Rad Laboratories, Hercules, United States of America) with 10mg mL⁻¹ proteinase K, followed by eight minutes at 90°C to denature the enzyme. The final extraction was diluted one hundred-fold in MilliQ water.

Following extraction, a mitochondrial (COI) marker commonly used for species identification (Hebert *et al.*, 2004; Macaya and Zuccarello, 2010; McDevit and Saunders, 2010; Saunders and McDevit, 2014) and a nuclear (28S) marker successful in revealing intraspecific variation within *D. potatorum* (e.g. Fraser *et al.*, 2009g; e.g. Fraser *et al.*, 2010a) were amplified by Polymerase Chain Reaction (PCR). Including both a mitochondrial and nuclear marker provides higher confidence in determining phylogenetic relationships, offers different levels of resolution (Lowe and Allendorf, 2010) and can signal hybridisation or processes such as incomplete lineage sorting (Hellberg *et al.*, 2002). PCR amplification followed a modified protocol from (Fraser *et al.*, 2009a) with a master-mix of 3 µL Perfect Taq buffer (5Prime GmbH, Hilden, Germany), 3 µL dNTPs (1 mM), 1.5 µL forward primer (10 mM), 1.5 µL reverse primer (10mM), 0.68 U Perfect Taq polymerase (5Prime GmbH, Hilden, Germany) and 19.35 µL MilliQ H₂O. Minor variations to this master-mix were made if samples amplified poorly or could not be successfully sequenced, (e.g. increasing volume of DNA to 2.5 µL).

Primers for COI were GazF₁ (5' TCAACAAATCATAAAGATATTGG 3') and GazR₁ (5' ACTTCTGGATGTCCAAAAAYCA 3') (Saunders, 2005). Primers for 28S were DurvLSUF₂ (5' AAGAAACTAACCAGGATTCCC 3') and DurvLSUFR₁ (5' GCGACGACACGCRARGGC 3') (Fraser *et al.*, 2010e). An Eppendorf Mastercycler (Eppendorf S, Hamburg, Germany) was used to amplify extractions with marker-specific profiles. For COI: 94°C for two minutes; 40 cycles of 94°C for 15 seconds, 45°C (annealing temperature) for 30 seconds, 72°C for one minute and then a final four minute extension at 72°C. The same profile was used for 28S with the annealing temperature raised to 55°C. Trials were conducted with primers 5.8S_F (5'CGATGAAGAACGCAGCGAAATGC-GAT-3') and 25BR2 (5'-TCCTCCGCTTAG-TATATGCTTAA-3') (Yoshida *et al.*, 2000) to amplify a second nuclear marker (ITS2) using the following profile; 93°C for one minute; 35 cycles of 94°C for 15 seconds, 55°C (annealing temperature) for 30 seconds, 72°C for 45 seconds and a final 10°C hold. All trials were unsuccessful despite modifications (e.g. altering annealing temperature 35-55°C).

PCR products underwent gel electrophoresis to identify which samples had been successfully amplified. For each DNA extraction and amplification batch, control blanks were included to check for contamination; any concerns resulted in samples being re-extracted and/or re-amplified. Successfully amplified samples were purified using a QIAquick PCR Purification Kit (QIAGEN, Venlo, Netherlands). Most samples were sequenced with forward primers on an Applied Biosystems 3730 capillary sequencer (Thermo Fisher Scientific Inc., Waltham, United States of America) by the Australian Genomic Research Facility (Sydney, Australia). Remaining samples of low-concentration PCR products were sequenced on an Applied Biosystems 3730xl capillary sequencer (Thermo Fisher Scientific Inc., Waltham, United States of America) by the University of Otago's Genetic Analysis Services (Otago, New Zealand), as they required a smaller quantity of amplified DNA.

The genotype of the type specimen, *Fucus potatorum* (basionym of *D. potatorum*) was determined by the same extraction, amplification and sequencing methods as outlined above. Type material was obtained from Museo di Storia Naturale (University of Florence, Italy).

3.4 Data analysis

3.4.1 Phylogenetic and genetic distance analyses

Bayesian and Maximum Likelihood (ML) phylogenetic analyses were conducted on both mitochondrial and nuclear sequences to assign individuals to lineages and determine phylogenetic relationships. Sequences were viewed, trimmed, aligned and ambiguities manually corrected using Geneious 6.1.8 (Kearse *et al.*, 2012). Informative sites were examined by eye for base-call ambiguities (two different nucleotides at the same loci) to record any instances of heterozygosity, potentially signalling hybridisation. Aligned sequences were exported to MEGA6.06 (Tamura *et al.*, 2013) along with 21 Genbank sequences (Appendix A) which

included an ‘eastern’ and ‘western’ representative for each marker and closely related species to root phylogenetic trees (e.g. *Durvillaea antarctica* and *Cystoseira geminata*).

The models of DNA sequence evolution which best fit both COI and 28S were determined by MrModelTest2.3 (Nylander, 2004) run through PAUP4.0 (Swofford, 2002) according to Akaike’s Information Criterion. Both Bayesian and ML analyses were based on the same model parameters: HKY+G for COI (gamma shape 0.1005, proportion invariable sites 0) and GTR+I for 28S (proportion invariable sites 0.8345). Bayesian phylogenetic analyses were run with MrBayes3.2.0 (Ronquist and Huelsenbeck, 2003), with Markov chain Monte Carlo (MCMC) searches of two separate runs and four heated chains. Trees were sampled every 500 generations for a total of 2.5×10^6 generations. As confirmed by Tracer 1.5 (Rambaut and Drummond, 2004) the first 25% of sampled trees were discarded as ‘burn-in’ until the point of chain convergence had been exceeded. The remaining trees were summarised as a final strict consensus tree with Posterior Probability support values. ML phylogenetic analyses were run using PhyML (Guindon *et al.*, 2010) using the Geneious plug-in. Tree topology was improved by subtree pruning and regrafting (SPR) and the starting tree was determined by BIONJ. Support was determined by bootstrapping with 1000 replicates. All trees were viewed in FigTree1.4.2 (Rambaut, 2014). Evolutionary divergence as uncorrected pairwise distances between the ‘eastern’ and ‘western’ lineages for both COI and 28S were estimated in MEGA6.06, using maximum composite likelihood modelling and bootstrapping with 1000 replicates.

3.4.2 Combined morphological and ecological analyses

Once individuals were assigned to either the ‘western’ or ‘eastern’ lineage, relationships with morphological and ecological variables could be determined by preliminary data exploration, univariate regression and multidimensional modelling analyses (Box 1), all conducted in RStudio (R Development Core Team, 2015) (R script Electronic Appendix B). Summary statistics, boxplots and column graphs were created to determine if differences in ecological and morphological variables coincided with differences between sites, lineages and either adults or juveniles (n.b. ‘heterozygotes’ as discussed in section 4.3 were excluded unless otherwise specified). Additionally, physiological responses to ecological conditions were plotted. Outliers (*sensu* O’Hara and Kotze, 2010), were examined and one individual from the 2010-2011 sampling effort was excluded as the unusual stipe measurements could not be confirmed due to a lack of photographic evidence (i.e. ‘stipe base’ 20 cm, ‘stipe length’ 4 cm, ‘total length’ 30 cm).

Box 1: Chosen univariate and multivariate statistical analyses

Regression and GLMs– test hypotheses about relationships between variables and provide estimates of the individual contribution of variables (Dunteman and Ho., 2006; Legendre and Legendre, 2012). GLMs can be used where the response variable is categorical or binary (Dunteman and Ho., 2006) and are considered relatively robust against variable normality and independence (Dunteman and Ho., 2006).

Mantel test – compares two matrices relating to the same set of objects and determines if these are significantly more or less similar than a pair of randomly permuted matrices (Mantel, 1967). Mantel tests are commonly employed to test spatial autocorrelation to minimise Type I statistical errors (O'Hara and Kotze, 2010).

NMDS –a multivariate ordination method that standardises data (Legendre and Legendre, 2012) to allow comparison of variables with different measurement types and scales (Borcard *et al.*, 2011). NMDS ordination plots individuals in reduced-dimensional space with grouping of points according to similarity (Legendre and Legendre, 2012).

Gower dissimilarity matrix – calculates similarity of range normalised values. Gower matrices can include qualitative, quantitative, semi-quantitative and binary variables (Legendre and Legendre, 2012).. They are also tolerant of missing data (Borcard *et al.*, 2011).

ANOSIM –hypotheses testing of NMDS ordination (Anderson, 2001) to determine the degree of group dissimilarity (Venn *et al.*, 2014). ANOSIM based on NMDS output consider the rank, rather than absolute value, of objects (Legendre and Legendre, 2012). ANOSIM assumes that the differences between groups is less than those between groups and is therefore vulnerable to differences in the dispersion of variables (Legendre and Legendre, 2012).

Adonis –a variant of permutational multivariate analysis of variance (PERMANOVA) which compares within and between group similarities (Oksanen *et al.*, 2015). It is based upon any dissimilarity matrix, and can test hypotheses that involve multiple, interacting factors (Anderson, 2001). It is less sensitive to variable dispersion than ANOSIM (Buttigieg and Ramette, 2014) and is tolerant of non-independent variables (Anderson, 2001).

Two-block PLS – tests for covariance between two multivariate blocks of explanatory variables for the same set of individuals (Hammer, 2012). PLS is based on correlation matrices and is tolerant of both data type and distribution (MacLeod, 2007).

Univariate analyses were performed to determine the effect of lineage and site for each of the morphological and ecological variables. All quantitative measurements (e.g. ‘total length’) were tested with linear regression, counts (e.g. ‘lateral number’) with quasi-Poisson regression and binary or categorical variables (e.g. ‘branched’) with logistic regression. These were performed for the complete dataset and repeated with the exclusion of ‘heterozygotes’. Data were left untransformed and in their original form and analyses. Transformations were not found to alter the effect of lineage or site on any morphological variable, and although converting observations to differences from the site mean (to control for site differences) increased the number of significant findings, it also reduced model fit in subsequent multivariate analyses and was therefore less powerful in understanding the major differences between lineages and sites. Mantel tests (Dray and Dufour, 2007) assessed spatial autocorrelation for each morphological variable by comparing the site mean to coastal distance between sites. Coastal distances were calculated by manually tracing the coastline using Google Earth (2013) ‘path ruler’ tool, beginning at ‘Granville Harbour’ and moving between sites in an anticlockwise direction.

Multivariate analyses were conducted to identify potential underlying and emergent differences in morphological and ecological variables between lineages. Non-metric multidimensional scaling (NMDS) analyses (Oksanen *et al.*, 2015) were run to visualise similarity between individuals and lineages as clustering in reduced-dimension ordination space. NMDS analyses were based on pairwise Gower dissimilarity matrices (Maechler *et al.*, 2015) for total, adult and juvenile datasets, as well as with or without ‘heterozygotes’. Shephard plots and stress values were used to determine model fit. To improve explanatory power, each NMDS was repeated three times with the previous best solution as the starting point taken and only those variables found by univariate analysis to have a significant lineage effect were included ($\alpha=0.01$). To avoid collinearity between variables and erroneous ecological conclusions (Peters, 1991; O’Hara and Kotze, 2010; Legendre and Legendre, 2012) whenever numerous variables were significant and showed strong linear relationships between each other (e.g. ‘top’ and ‘base stipe stoutness’), only the variable with the strongest explanatory power was included. Using a subset of ecological and morphological variables is appropriate as the multivariate analyses were aimed at identifying key differences between the genetic lineages and including a greater number of variables lowered ordination fit and explanatory power. As NMDS tolerates missing data (Hammer, 2012) all individuals could be included irrespective of the number of measurements available. To quantify the degree of dissimilarity observed in NMDS ordinations Analysis of Similarities (ANOSIM) were run with 999 permutations of Euclidean dissimilarity matrices (Oksanen *et al.*, 2015). ANOSIMs were repeated to test both the difference between the two lineages excluding ‘heterozygotes’, and all three genotypes. To test the underlying assumption of consistency in variable dispersion, plots for the combined, adult and juvenile data were created (Oksanen *et al.*, 2015). Adonis, also run with 999 permutations of Euclidean

dissimilarity matrices (Oksanen *et al.*, 2015), considered the degree of dissimilarity between ‘eastern’ and ‘western’ lineages with site as a factor.

To test the explanatory power of the same suite of variables plotted in NMDs, Generalised Linear Models (GLM) were run that considered lineage (with and without ‘heterozygotes’) as the binary response variable. However only data from the 2015 sampling effort were included, as GLMs cannot tolerate missing values. GLMs were conducted both with and without juveniles. Goodness of fit was assessed by the change in deviance (O’Hara and Kotze, 2010) and R^2 value. Predicted probability graphs were created for any variables found to contribute significantly to the model.

Finally, two-block Partial Least Squares Regression (PLS) analyses were conducted (Sanchez *et al.*, 2012) to determine the degree of covariance between depth and openness to all morphological variables. The six PLS analyses used Tucker’s Inter-Battery method of factor analysis to the complete 2015 sampling effort, as well as each lineage separately and when ‘heterozygotes’ were excluded.

Chapter 4: Results

4.1 Field observations and site characteristics

Field observations and supplementary ecological datasets revealed differences in ecological and environmental characteristics between the seven Tasmanian sites visited in 2015. As compared with most sites along the east-coast, both ‘Granville Harbour’ and ‘Trial Harbour’ (west-coast) had greater water clarity, sand cover and density of *Durvillaea potatorum* in the intertidal region (Table 2). The two west-coast sites were also the most wave-disturbed, as determined by high ‘openness’ (Fig 9). Each site, including those visited during earlier sampling efforts (2010-2011), had a different ‘openness’ value which was not found to correlate with the proportion of ‘eastern’ and ‘western’ individuals across sites (see section 4.3). Indeed, sites with similar ‘openness’ were found to have markedly different lineage proportions (e.g. ‘Spring Beach’ and ‘Lady’s Bay’).

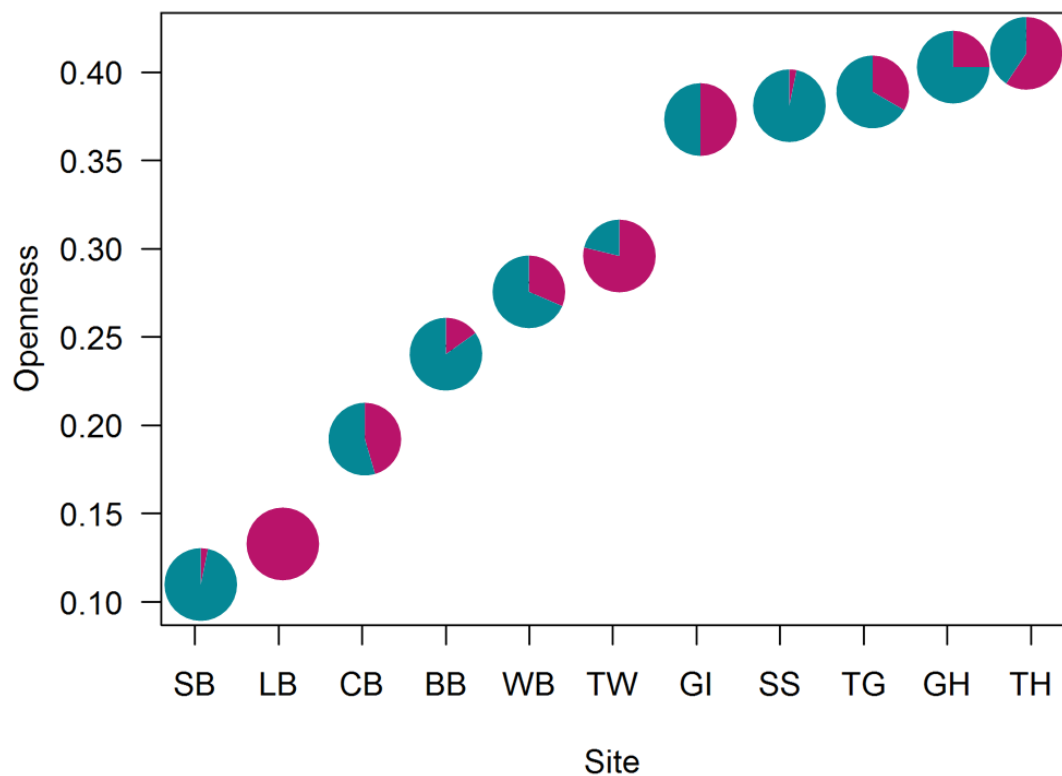


Figure 9 Graph of the ‘openness’ values for all eleven sites from the combined sampling efforts of 2010, 2011 and 2015. All sites were found to have different ‘openness values’, with those on the west coast the two highest. Pie graphs indicate the percentage of each lineage at each site, with ‘western’ represented in blue and ‘eastern’ in pink. There does not appear to be any pattern between the spatial distribution of lineages and ‘openness’. ‘Openness’ values were calculated based on Hill *et al.* (2011)’s dataset.

Table 2: Ecological observations, site characteristics and sampling details of the seven field sites visited during the 2015 sampling effort.

Field Site	Time & Date	Openness Score	Latitude & Longitude	Water Clarity	Sand Cover	Stand Density
Granville Harbour	15 ⁰⁰ 02.02.2015	0.398	S41°48.339' E145°01.785'	Clear	Patchy coarse sand	Moderate
Lady's Bay	11 ³⁰ 05.02.2015	0.132	S43°25.447' E147°00.894'	Moderately clear	No sand	Dense to 1 m
Secret Spot	15 ⁰⁰ 03.02.2015	0.382	S41°32.265' E148°17.058'	Moderately clear	No sand	
Spring Beach	15 ⁰⁰ 01.02.2015	0.099	S42°34.978' E147°54.877'	Moderate-poor	No sand	Patchy
The Gardens	11 ³⁰ 03.02.2015	0.392	S41°09.709' E148°17.036'	Moderately clear	No sand	Patchy
The Whale	14 ⁴⁵ 05.02.2015	0.287	S43°34.131' E146°55.042'	Moderately clear	No sand	Dense to 2.5 m
Trial Harbour	12 ⁰⁰ 02.02.2015	0.421	S41°55.648' E145°10.265'	Clear	Sandy patches at depth	Dense

The proportion of ‘eastern’ and ‘western’ individuals at each site was found to correspond to spatial patterns in ocean nutrient levels and sediment properties obtained from Heap *et al.* (2005)’s dataset (Table 3). In particular, ‘eastern’ individuals were more commonly found where seabed nitrate was higher and carbonate levels were lower. Sediment size was consistently < 0.5 mm for all east-coast sites, whereas west-coast sites, particularly ‘Trial Harbour’, had larger mean particle size and hence also higher proportion of gravel. Observations of individuals collected from both ‘Granville Harbour’ and ‘Trial Harbour’ attest to increased sediment abrasion of lamina tissue, with higher levels of tissue loss than at other sites. Measurement of wave and tidal effect, as determined by the proportion of time when forces are strong enough to shift sediments, were found to be high ($> 80\%$) for west-coast sites but data from the east-coast were missing. Mapping of below-surface bathymetry showed no difference between sites within 20 km off-shore, but certain sites had shoals, valleys or banks beyond the continental shelf (Fig 10).

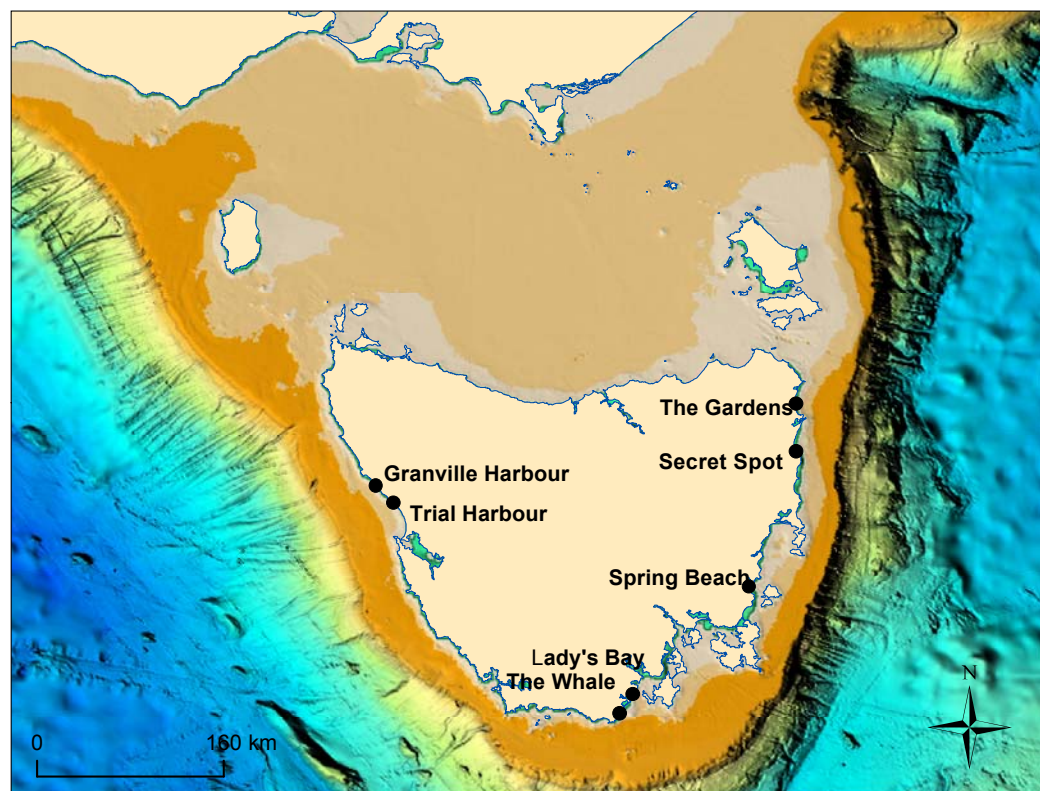


Figure 10 Bathymetry map from Heap *et al.* (2005)’s publicly available National Marine Bioregionalisation of Australia GIS dataset. Differences in bathymetry between study sites only occur at least 20 km from shore where the continental shelf drops away. Modification to include site locations. Colour gradient of orange to deepening blue at depth.

Table 3 Ecological conditions for the seven field sites from the 2015 sampling effort obtained from National Marine Bioregionalisation of Australia GIS datasets (Heap *et al.*, 2005). The proportion of 'eastern' and 'western' individuals at each site collected during the 2015 sampling effort is included for reference.

Field Site	Lineage (% East:West)	Seabed Nitrate	Carbonate (%)	Mean grain size (mm)	Sand:Gravel	Sediment movement
Granville Harbour	25:75	Moderate	20-40	0.5-1	4:1	Waves and tidal
Lady's Bay	100:0	High	20-40	0.25-0.5	4:1	Waves only
Secret Spot	3:97	Low-Moderate	20-40	0.25-0.5	4:1	Waves only
Spring Beach	3:97	Low	40-60	0.25-0.5	4:1	Waves only
The Gardens	33:67	Low-Moderate	20-40	0.25-0.5	4:1	Waves only
The Whale	79:21	High	0-20	0.25-0.5	4:1	Waves and tidal
Trial Harbour	59:41	Moderate	40-60	1-2	1:4	Waves only

4.2 Histological examination of reproductive structures

Histological examination offered limited insight into differences of reproductive structures between ‘eastern’ and ‘western’ individuals as only 14 specimens (total 227) were found to contain mature conceptacles. Microscopic examination revealed 12 mature females (containing oogonia), two mature males (containing antheridia) and 19 specimens with mature, but empty, conceptacles (Fig 11). All remaining specimens had no, or only immature, conceptacles for which sex or structural characteristics could not be determined. The presence of mature conceptacles was not found to differ between ‘eastern’ and ‘western’ individuals, and the low number is most likely attributable to tissue collection outside of the winter reproductive period (Clayton *et al.*, 1987). There was also no apparent difference between the two lineages and the occurrence of conceptacle wall incursions. Additionally, there were no differences in oogonia attachment between ‘eastern’ and ‘western’ females (Table 4). Two individuals found to display heterozygosity for the nuclear marker (28S) (see section 4.2) had relatively high numbers of aborted oogonia (Fig 12), suggesting failed gamete release during the preceding reproductive season (Clayton *et al.*, 1987).

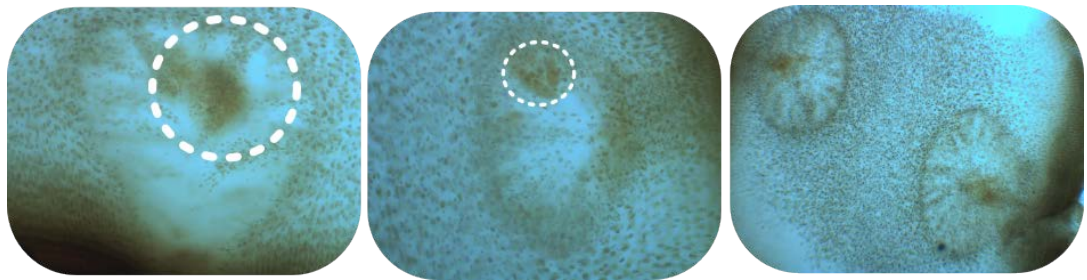


Figure 11 Images taken using a light showing mature male (left 1000x), female (centre 1000x) and empty (right 400x) mature conceptacles of *D. potatorum*. The male antheridia are shown attached to filaments (circled in white) and the female oogonia can either be attached to the wall (circled in white), to filaments or detached. Wall incursions can be seen in the empty conceptacle.

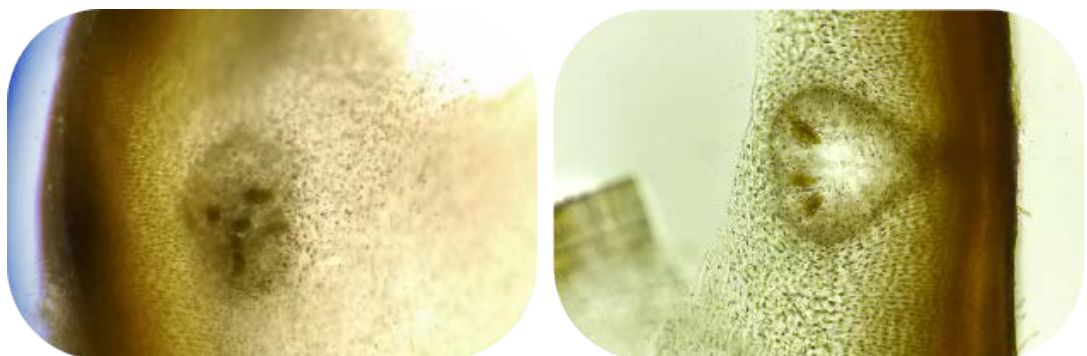


Figure 12 Images taken using a light microscope (magnification 400x) showing aborted oogonia found in the mature conceptacles of ‘heterozygotes’ from ‘The Gardens’ (ID173 and TG171).

Table 4 Results from histological examination of conceptacles showing the sex and visible reproductive structures for each mature specimen.

Site and ID code	Genotype	Sex	Oogonia attachment (females only)	Antheridia attachment (males only)	Wall incursions
Lady's Bay 280	Eastern	Female	Wall borne and detached		No
Spring Beach 03	Western	Female	Wall borne		No
Spring Beach 19	Eastern	Female	Wall borne		Yes
The Gardens 163	Hybrid	Female	Unclear		Yes
The Gardens 171	Hybrid	Female	Wall borne and filaments		Yes
Trial Harbour 66	Eastern	Male		Unclear	Yes
Trial Harbour 75	Eastern	Female	Wall borne		Yes
Trial Harbour 77	Western	Female	Wall borne and filaments		No
The Whale 317	Western	Male		Unclear	Yes
The Whale 319	Eastern	Female	Detached		Yes
The Whale 321	Eastern	Female	Detached		Yes
The Whale 323	Western	Female	Wall borne and filaments		Yes
The Whale 324	Eastern	Male		Unclear	Yes
The Whale 328	Eastern	Female	Wall borne, filaments and detached		Yes
The Whale 329	Eastern	Female	Wall borne and detached		Yes

4.3 Genetic analyses to assign lineages

Genetic analyses enabled individuals collected in 2015 to be assigned to either the ‘eastern’ or ‘western’ lineage and for cases of heterozygosity to be identified. From 227 samples, a total of 227 nuclear sequences (28S) and 151 mitochondrial sequences (COI) were successfully obtained (Table 5). Despite repeated attempts at amplification, sequencing success was lower in COI than 28S, presumably due to low PCR amplicon concentration prior to sequencing. Sequencing success for COI was particularly low among ‘eastern’ individuals collected from ‘Lady’s Bay’ and ‘The Whale’. This pattern of poor sequencing success may indicate a nucleotide substitution in the primer binding region among these south-east coast populations, however this seems unlikely as COI is a commonly used marker for species identification as its primer region is highly conserved across taxa (Hellberg *et al.*, 2002; Hebert *et al.*, 2004). Additionally, poor amplification and short sequence read lengths prevented sequencing of the second nuclear marker, ITS2, despite primers having previously been successful for *Sargassum* spp. (Yoshida *et al.*, 2000).

Table 5 The number of successful sequences for both nuclear (28S) and mitochondrial (COI) markers, included when separated according to lineage. All 28S sequencing attempts were successful. The number of base pairs indicates the average alignment length for each marker. The percentage of successful sequences obtained for each lineage for each marker is also included.

Marker	Alignment Length (mean number base pairs)	Successful sequences (%)	
		Eastern	Western
28S	443	100	100
COI	629	56	74

Twelve loci across 28S were found to vary between ‘eastern’ and ‘western’ individuals (parsimony-informative sites). Sixty-eight individuals were found to be heterozygous for at least one of these loci by displaying both the ‘eastern’ and ‘western’ alleles (Table 6). Within these heterozygous individuals, five from ‘Granville Harbour’ and four from ‘The Gardens’ were found to be heterozygous at more than two parsimony-informative sites (Table 7). Individuals from ‘Granville Harbour’ had 4-12 heterozygous sites that were concentrated at the 3’ end of the sequence, whereas individuals from ‘The Gardens’ displayed at least ten. As heterozygosity at informative sites can represent hybridisation (see section 5.1), all nine individuals (hereafter ‘heterozygotes’) were considered a separate genotype or excluded from all subsequent analyses. No instances of heterozygosity were found for COI informative sites, as expected for haploid mitochondria with uniparental inheritance (Palumbi *et al.*, 2001; Hellberg *et al.*, 2002).

Table 6 Simplified nuclear sequence (28S) showing only the 12 parsimony-informative sites used in differentiating ‘eastern’ and ‘western’ individuals. The allelic states for each lineage are displayed for each informative site, and the percentage of heterozygotes is also included.

Lineage	Site Number											
	5	14	17	36	108	123	329	347	366	409	410	411
Eastern	A	C	G	T	C	T	T	C	T	T	C	G
Western	G	T	T	G	C	G	C	T	G	C	G	A
Hybrids	1.3	3.1	2.2	3.1	22.5	4.0	5.7	4.0	4.4	2.2	1.3	2.7

Table 7 Nuclear sequences (28S) of the nine individuals found to be heterozygous at more than two informative sites. The number of heterozygous sites varied among individuals and between sites. Individuals from Granville Harbour had fewer heterozygous sites on average, and these were concentrated towards the 3’ end of the region. Individuals from The Gardens displayed at least ten heterozygous sites. Heterozygous sites highlighted

Site and ID	Site Number											
	5	14	17	36	108	123	329	347	366	409	410	411
Granville Harbour												
ID:116	G	T	T	G	C	G	Y	Y	K	C	G	R
Granville Harbour												
ID:117	G	T	T	G	C	G	Y	T	K	C	G	R
Granville Harbour												
ID:124	G	T	T	G	C	R	Y	Y	K	Y	S	R
Granville Harbour												
ID:125	G	T	T	G	C	R	Y	Y	K	Y	S	R
Granville Harbour												
ID:126	?	Y	K	?	Y	R	Y	Y	K	Y	S	R
The Gardens												
ID:161	R	Y	K	K	Y	R	Y	Y	T	Y	S	R
The Gardens												
ID:163	R	Y	?	K	Y	R	Y	Y	T	Y	S	R
The Gardens												
ID:171	R	Y	T	K	Y	R	Y	Y	K	Y	S	R
The Gardens												
ID:176	R	Y	?	K	Y	R	Y	Y	T	Y	C	R

When phylogenetic trees were created based on nuclear 28S sequences, all samples of *D. potatorum* fell into two clades, corresponding to the ‘eastern’ and ‘western’ lineages. There was complete agreement between both Bayesian and Maximum Likelihood analyses in assigning individuals to either clade irrespective of whether ‘heterozygotes’ were included (Fig 13 & Appendix C). The ‘western’ clade of 123 individuals received strong support from both Maximum Likelihood bootstrap and Bayesian posterior probability values irrespective of whether ‘heterozygotes’ were included. The ‘eastern’ clade of 95 individuals was also well supported by posterior probability values but not as well by bootstrap values, particularly when ‘heterozygotes’ were included. The ‘eastern’ and ‘western’ clades (excluding ‘heterozygotes’) were 1.4-2.4% divergent for 28S and individuals within lineages showed lower genetic distance

(0-0.3% ‘western’ and 0-0.7% ‘eastern’) (uncorrected p value). Mitochondrial COI sequences supported the assigning of individuals to monophyletic ‘eastern’ and ‘western’ clades (Fig 14 & Appendix D). The monophyletic ‘western’ clade received strong support from both Bayesian and Maximum Likelihood analyses. Unlike all other phylogenetic analyses, the Maximum Likelihood COI tree did not support a monophyletic *D. potatorum* and instead placed the poorly supported ‘eastern’ clade as sister to other *Durvillaea* spp. For mitochondrial DNA, the ‘eastern’ and ‘western’ clades were 4.3-4.7% divergent, and the intra-lineage genetic distances were < 0.1% and 0-0.6% respectively (uncorrected p value). Individuals collected in 2010-2011 had previously been sequenced and assigned to either the ‘western’ or ‘eastern’ lineage.

The ‘eastern’ and ‘western’ lineages were found to co-occur on both the east and west-coast of Tasmania (Fig 15). The proportion of either lineage at each site cannot be compared quantitatively given the sampling approach but overall ‘western’ individuals were more common on the west and north-east coasts and ‘eastern’ individuals at southern sites. Mitochondrial sequences revealed six unique ‘eastern’, and eight unique ‘western’ haplotypes. Between two to eight haplotypes were found at each site with lower diversity on the west-coast among ‘western’ individuals, but higher diversity among ‘eastern’ individuals (Fig 16).

Durvillaea potatorum type specimen was collected by Labillardière (1806). The individual is small, less than 30 cm in length, has many branches, crenate lamina margins, numerous perforations and a circular holdfast (Fig 17 & 18). Genetic characterisation revealed the type as belonging to the ‘western’ lineage with a partial nuclear sequence (28S, 350 base pairs) revealing four ‘western’ alleles at parsimony informative sites.

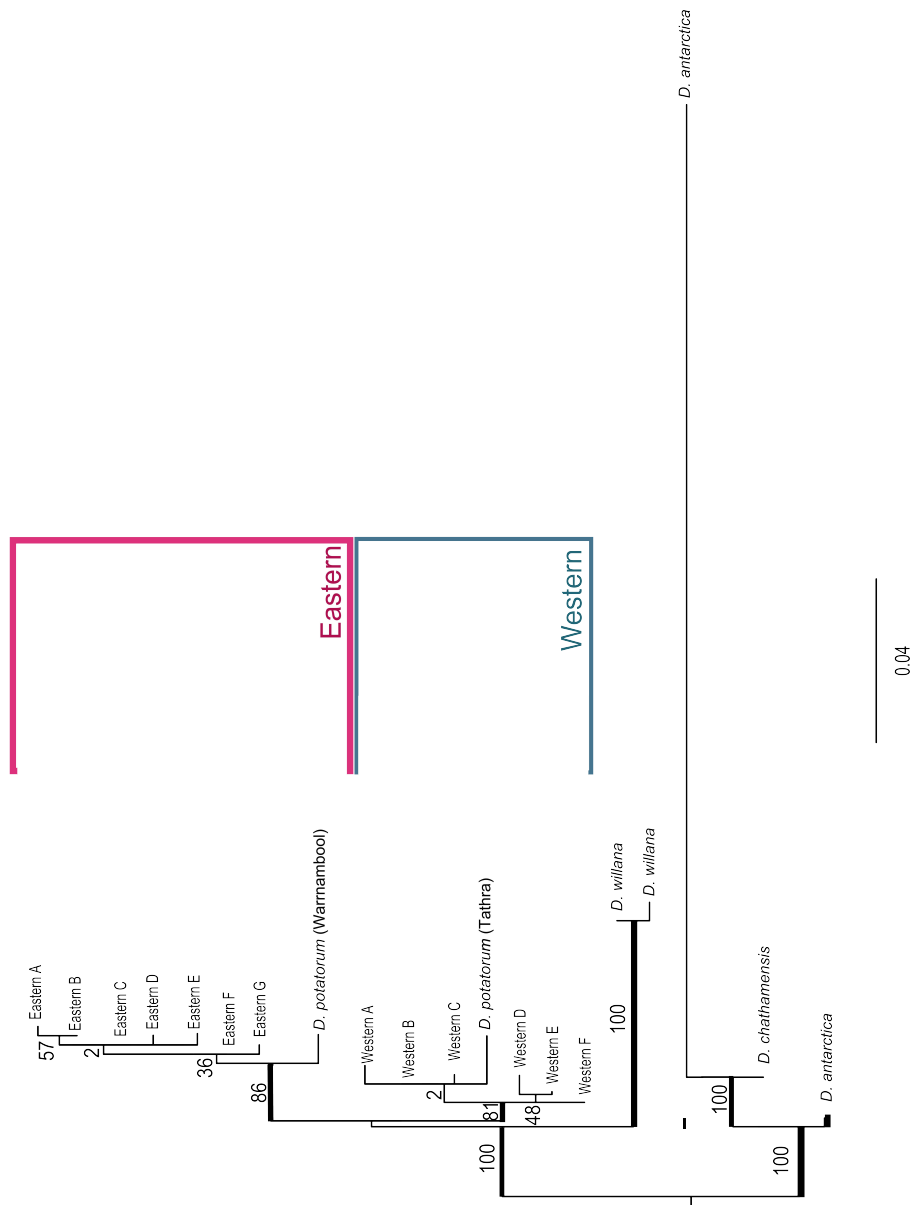


Figure 13 Phylogeny of *D. potatorum* for 232 nuclear 28S sequences as determined by Maximum Likelihood analysis. Labels above branches represent bootstrap values (excluding 'heterozygotes'). Bayesian posterior probability (excluding 'heterozygotes') greater than 0.95 are represented by solid bold branches. The 'western' clade was distinguished by the Genbank sample from Warrnambool (Victoria) and the 'eastern' clade was assigned by the Genbank sample from Tathra (New South Wales).

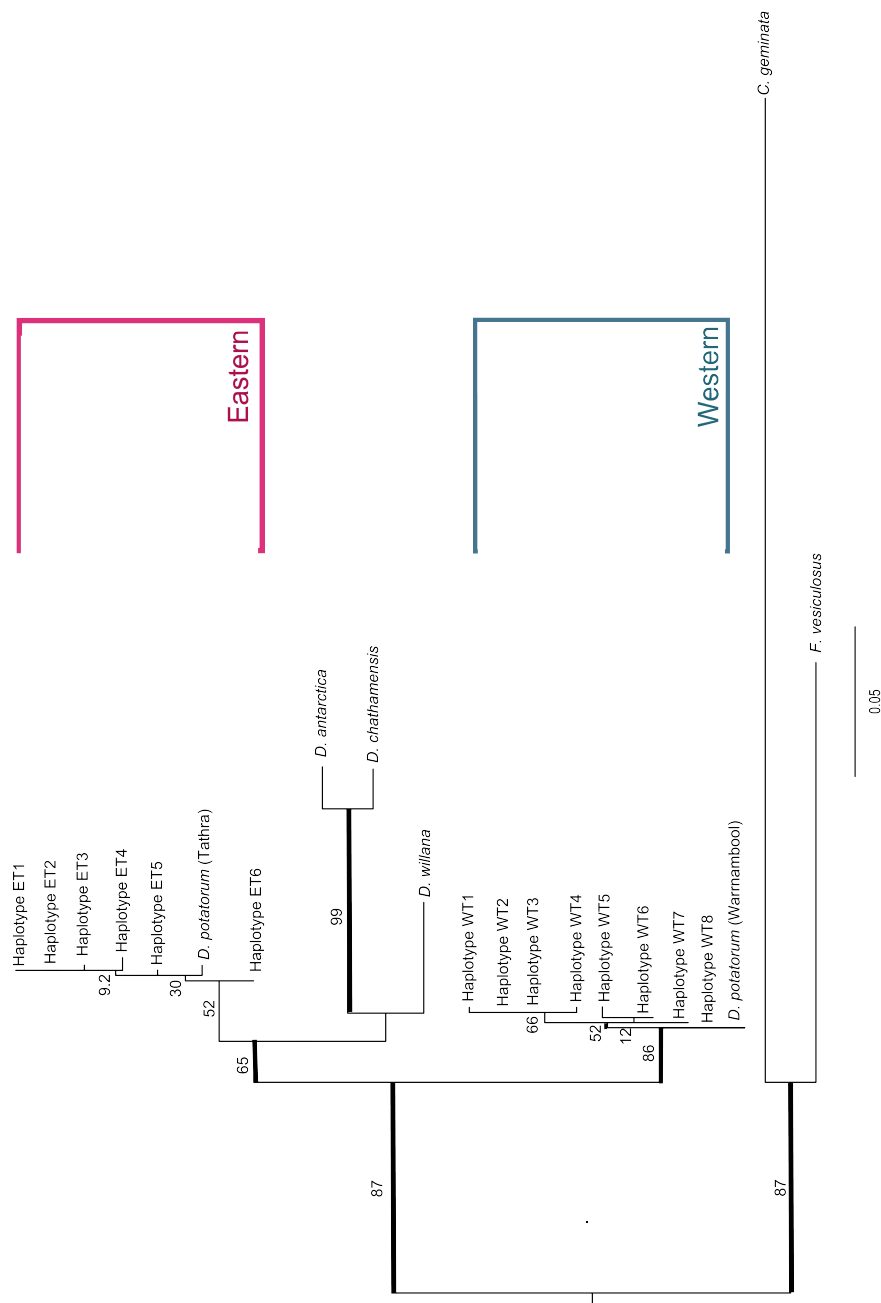


Figure 14 Phylogeny of *D. potatorum* for 232 nuclear COI sequences as determined by Maximum Likelihood analysis. Labels above branches represent bootstrap values (excluding 'heterozygotes'). Bayesian posterior probability (excluding 'heterozygotes') greater than 0.75 are indicated by dashed branches and values above 0.95 are represented by solid bold branches. The 'western' clade was distinguished by the Genbank sample from Warrnambool (Victoria) and the 'eastern' clade was assigned by the Genbank sample from Tathra (New South Wales).



Figure 15 Map of Tasmania showing the total dataset of eleven field sites with the proportion of individuals from each lineage (determined by 28S) represented as pie graphs. The 'western' lineage is represented in blue and the 'eastern' in pink.

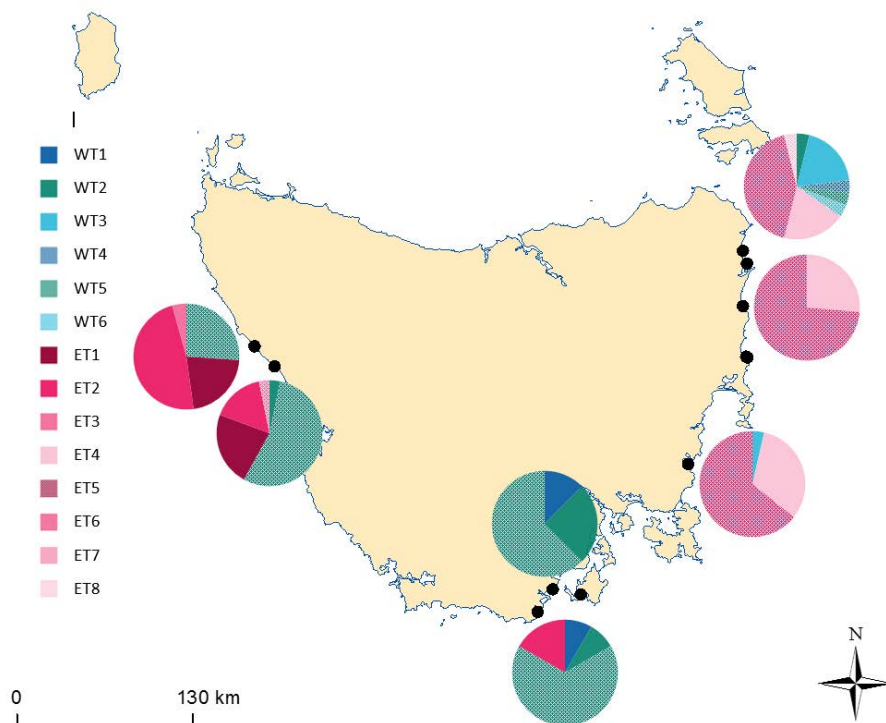


Figure 16 Map of Tasmania showing mitochondrial (COI) haplotypes from the 2015 sampling collection. Pie graphs represent the distribution of haplotypes for each of the seven sites, with haplotypes of the 'western' lineage represented as shades of blue and the 'eastern' as shades of pink. In total, six unique haplotypes were found within the 'western' lineage and eight within the 'eastern'

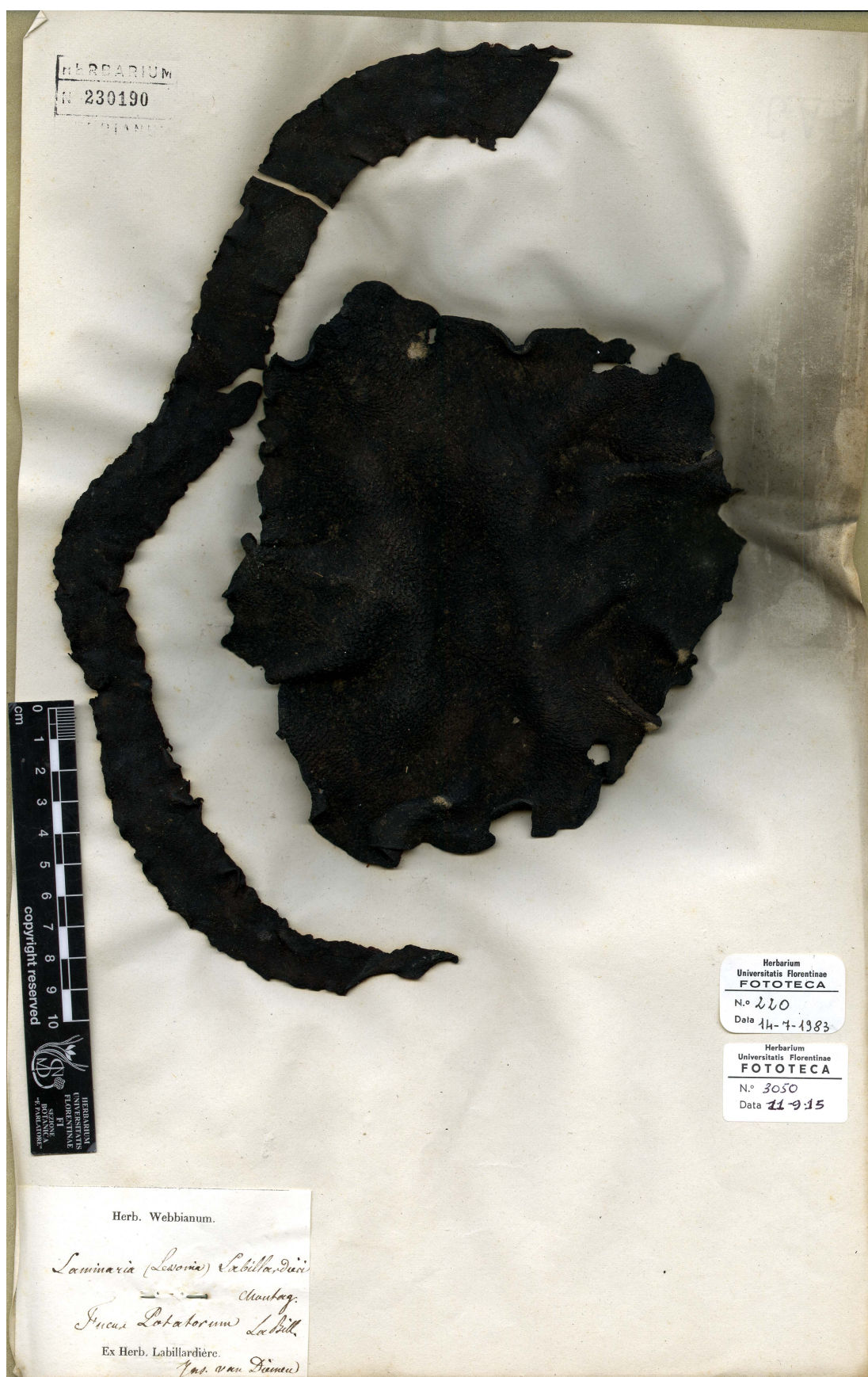


Figure 18 Photograph of the *D. potatorum* type specimen, sheet two showing the holdfast (basioynm: *Fucus potatorum*). Obtained from Museo di Storia Naturale (University of Florence, Italy)

4.4 Morphological and ecological characterisation

4.4.1 Morphological differences between lineages

Eight morphological variables were found to vary significantly between ‘eastern’, ‘western’ and ‘heterozygotes’: ‘stipe base’ ($p<0.001$), ‘stipe stoutness’ ($p<0.001$), ‘stipe proportion’ ($p=0.001$), ‘total length’ ($p=0.010$), ‘tapering’ ($p=0.014$), ‘conjoined’ ($p=0.025$), ‘branched’ ($p=0.032$) and ‘lateral number’ ($p=0.049$) (Table 8 & Fig. 19). These morphological differences were strongest among adults and were rarely observed in juveniles. As compared with ‘eastern’ and ‘western’ individuals, the nine ‘heterozygotes’ had higher ‘stipe proportion’ ($p<0.001$), ‘stipe stoutness’ ($p=0.002-0.01$) and lower ‘tapering’ ($p=0.05$) (Fig 19). There was a noticeable difference in morphology between ‘heterozygotes’ according to site (Fig 20). As compared with ‘heterozygotes’ from ‘The Gardens’, those found at ‘Granville Harbour’ were lighter in colour, had thinner lamina tissue, poorly defined palmate regions and no stipitate lateral blades or mature conceptacles. Both populations had straight, slender stipes and rough lamina margins. To avoid confounding differences between ‘eastern’ and ‘western’ lineages, ‘heterozygotes’ were excluded from the following results unless otherwise stated.

Table 8 The eight morphological variables found to vary significantly between ‘eastern’, ‘western’ and ‘heterozygotes’. Ranges indicate the second and third quartile values to provide an indication of common values and the extent of variability for each characteristic. Single values are the population mean.

Morphological character	Values for each genotype			Significance (p value)
	Eastern	Western	Heterozygotes	
Stipe base (cm)	3.2 – 7.0	4.5 – 11.0	3.0 – 5.0	<0.001
Stipe stoutness	0.14 – 0.36	0.40 – 0.57	0.25 – 0.37	<0.001
Stipe proportion	0.66 – 0.95	0.85 – 1.17	1.11 – 1.24	0.001
Total length (cm)	56.3 – 156.3	90.0 – 210.5	96.0 – 170.0	0.010
Tapering	0.02 – 0.15	0.03 – 0.15	0.02 – 0.03	0.014
Conjoined (%)	54	37	56	0.025
Branched (%)	14	6	0	0.032
Lateral number	0.0 – 11.3	0.0 – 9.0	0.0 – 14.0	0.049

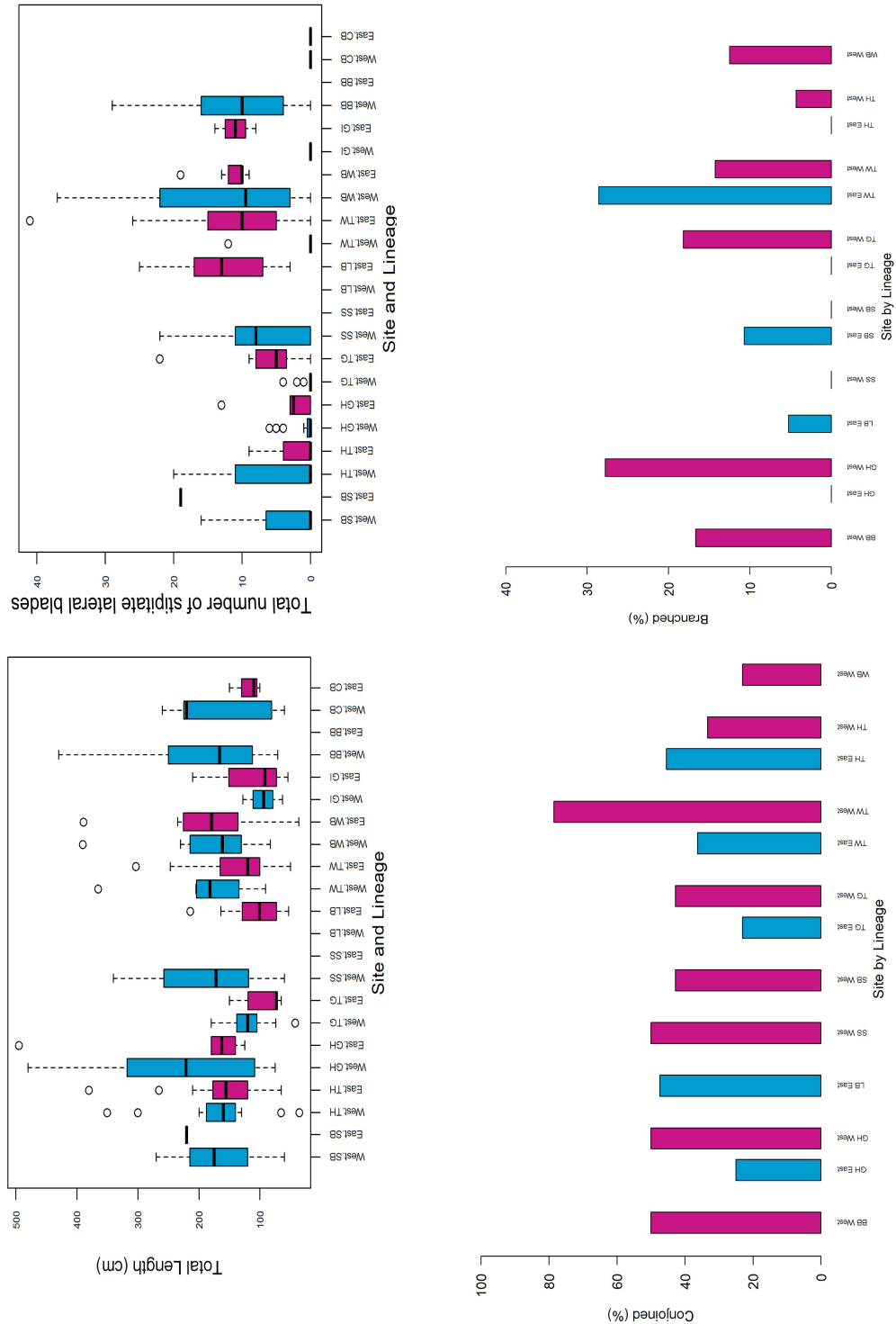


Figure 4 Compilation of boxplots for the eight morphological variables found to vary significantly between ‘eastern’ and ‘western’ lineages. In all cases juveniles and ‘heterozygotes’ have been excluded. Groups have been assigned on the basis of lineage and site, with ‘western’ populations depicted as blue and ‘eastern’ populations in pink. Continued over page.

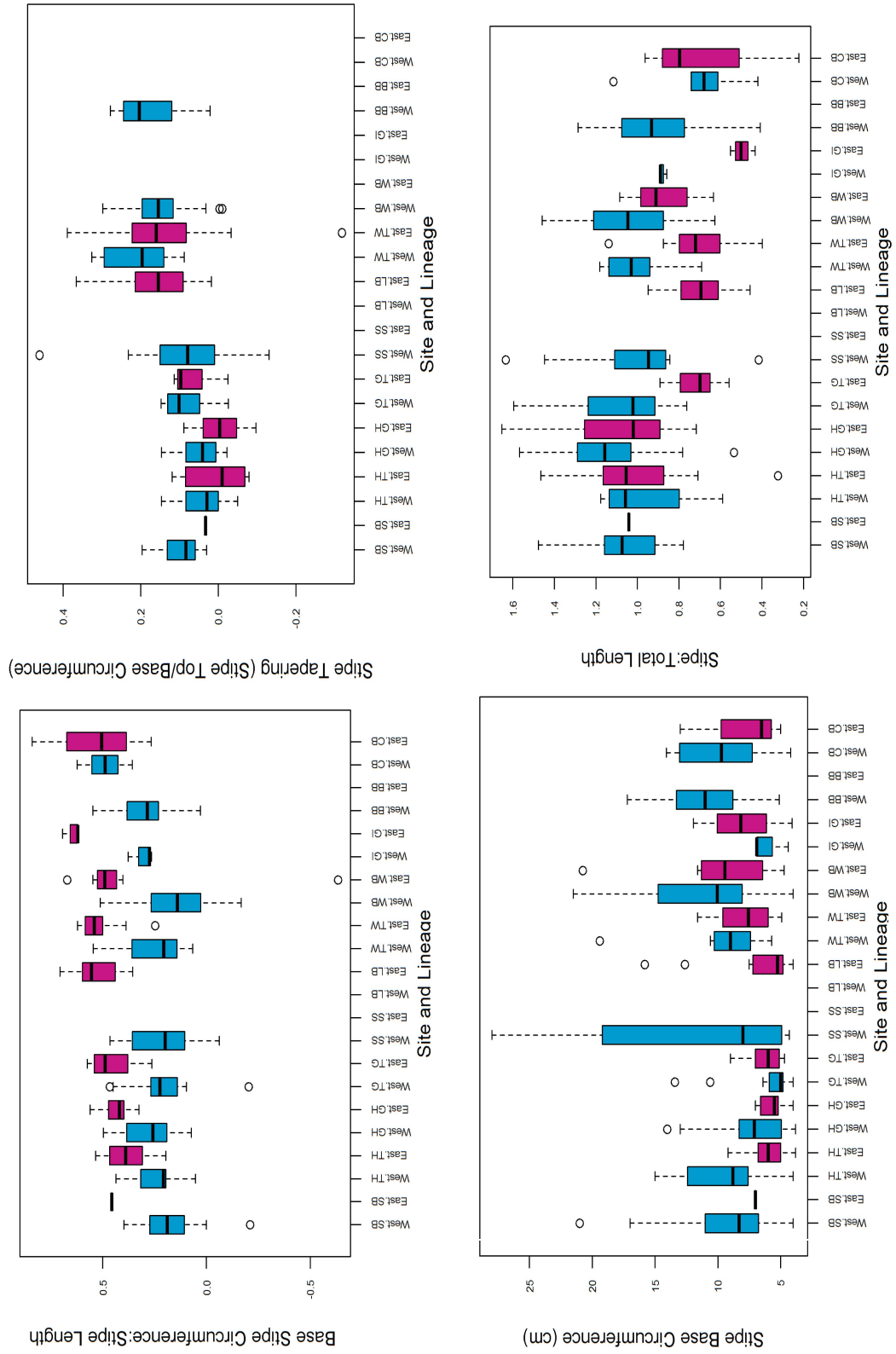




Figure 20 Photos of characteristic ‘heterozygotes’ from ‘The Gardens’ (left) and ‘Granville Harbour’ (right) illustrating the differences in morphology. The four individuals from ‘The Gardens’ appear relatively healthy and mature. The five individuals from ‘Granville Harbour’ appear immature, have simple physiology and seem to have limited structural protection from wave disturbance.

‘Western’ individuals had a thicker, stouter stipe than ‘eastern’ individuals. The difference in stipe circumference was most noticeable for ‘stipe base’. ‘Stipe base’ measurements exceeding 12 cm, a ‘stipe stoutness’ value above 0.62, and ‘stipe proportion’ above 1.4, were characteristic of the ‘western’ lineage (Fig 21). Any negative value of ‘stipe stoutness’ was highly characteristic of ‘eastern’ individuals. ‘Western’ stipes were also longer relative to ‘total length’, but did not differ from ‘eastern’ individuals in ‘tapering’. A difference in stipe ‘tapering’ was noticeable for the very straight stipes of ‘heterozygotes’. ‘Western’ individuals were typically longer than ‘eastern’, although ‘total length’ also varied significantly according to site ($p=0.031$). Individuals of more than three metres were most common at highly wave-disturbed sites, which also had fewer ‘branched’ individuals. Although ‘branched’ individuals were never more than 30% of any population, they were more common for the ‘eastern’ lineage.

‘Conjoined’ pairs or triplets sharing the same holdfast were always of the same lineage and were more common among ‘eastern’ individuals. The ‘eastern’ lineage was also characterised by higher counts of stipitate lateral blades for all populations, except adults at ‘Trial Harbour’. Juveniles rarely had stipitate lateral blades, although when present these were only found on ‘eastern’ individuals. High counts of stipitate lateral blades also become less common at greater depths, especially among ‘eastern’ individuals, no ‘eastern’ individual with more than five stipitate lateral blades was found below 0.5 m depth (Fig 22).

Any differences between sites for morphological and ecological variables are unlikely to be the result of spatial autocorrelation, as coastal distance between sites did not have a significant effect.

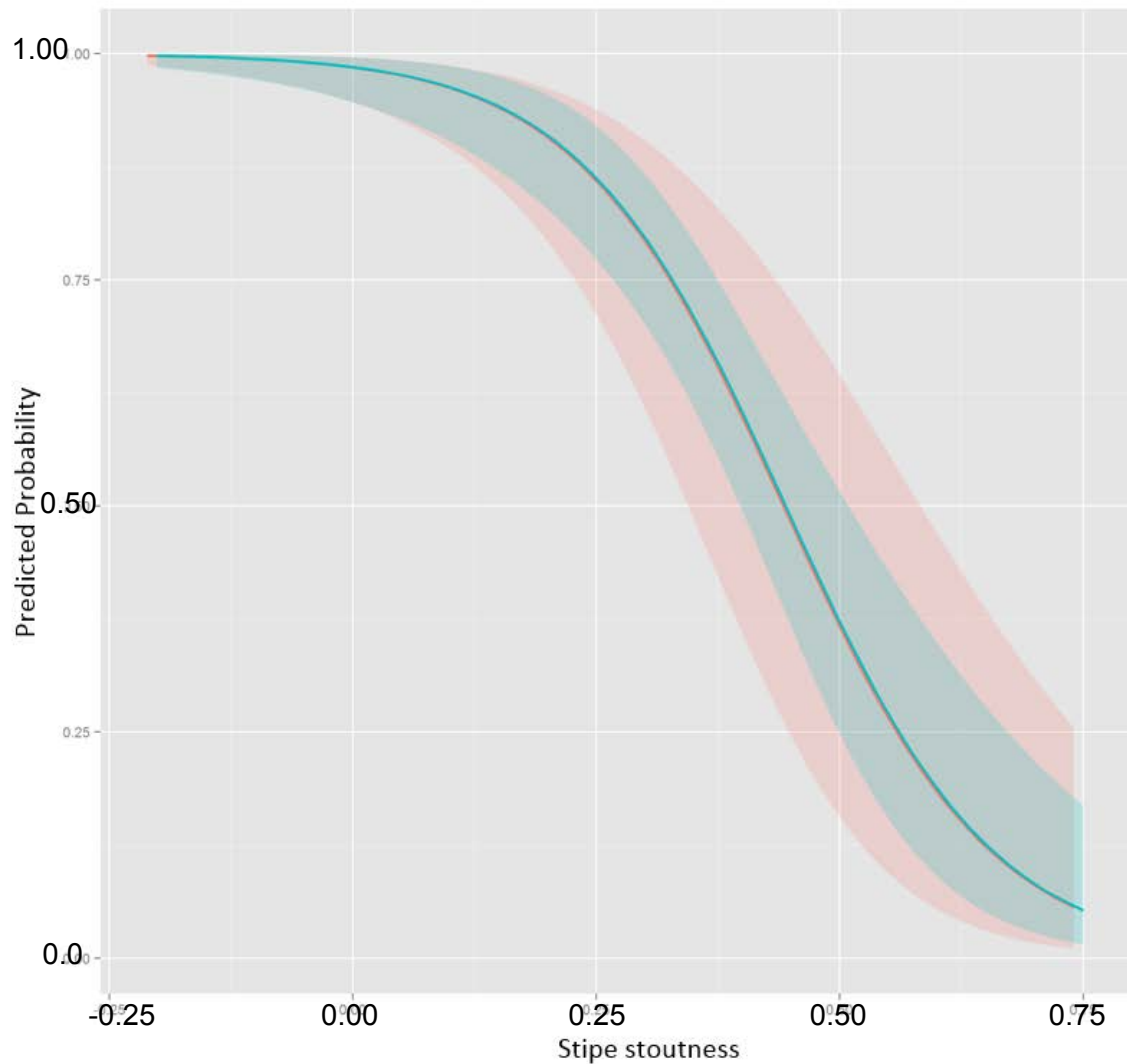


Figure 21 Predicted probability graph from Generalised Linear Modelling depicts the probability of an individual originating from the 'western' or 'eastern' lineage for a given 'stipe stoutness'. The vertical axis indicates probability of the individual being 'western' (i.e. 'stipe stoutness' above 0.6 would have extremely low probability of being from a 'western' individual, indicating more slender stipes among 'eastern' individuals). The red shaded area represents juveniles and the smaller blue shaded area represents adults.

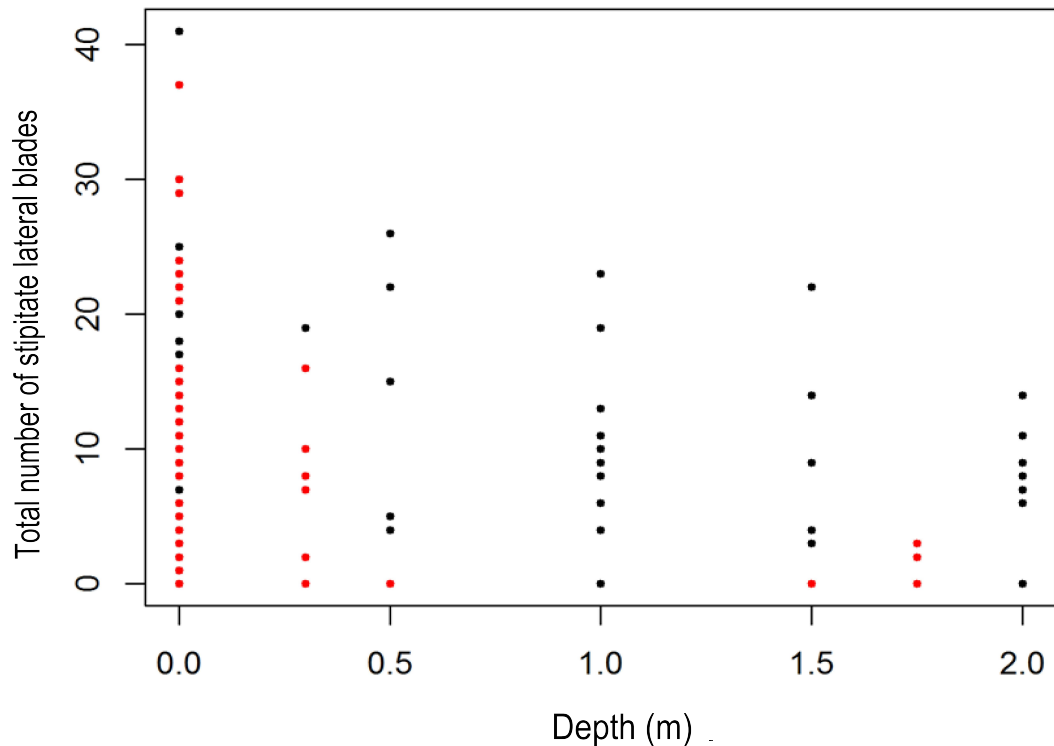


Figure 22 Scatter plot of 'lateral number' across depths for all collection efforts. 'Eastern' and 'western' individuals can be distinguished based on dot colour, represented as red and black respectively. The absence of high lateral numbers with depth is most pronounced among 'eastern' individuals.

4.4.2 Ecological differences between lineages

The occurrence of 'eastern', 'western' and 'heterozygote' individuals was found to vary according to within and between-site ecological conditions. Between sites the degree of wave-disturbance and 'openness' experienced by the three groups was significantly different ($p < 0.001$). The difference in 'openness' between 'eastern' and 'western' lineages was not significant when 'heterozygotes' were excluded ($p = 0.12$) ('heterozygotes' were found at highly wave-disturbed sites). However, there was a difference for juveniles as 'western' juveniles were more common at highly wave-disturbed sites (Fig 23). Within-sites, the 'western' lineage was significantly more likely to occur in shallow water than the 'eastern' lineage ($p < 0.001$), irrespective of whether juveniles or 'heterozygotes' were included (Fig 24).

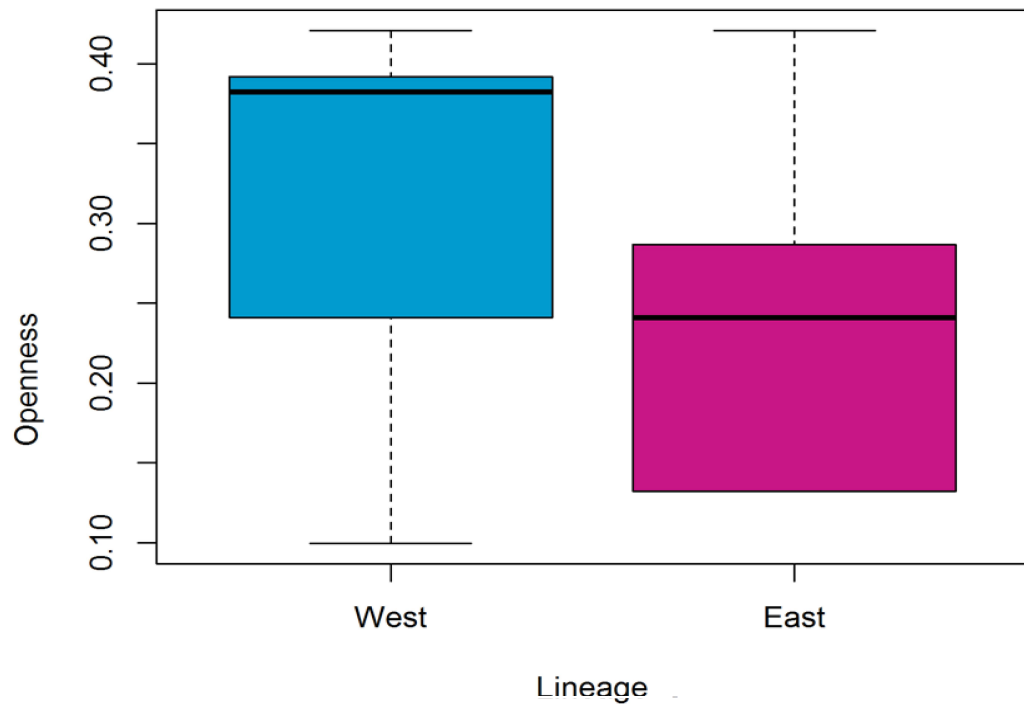


Figure 23 Boxplot of juveniles separated according to lineage and 'openness' values for sites. 'Western' juveniles were more commonly found at sites with greater wave disturbance

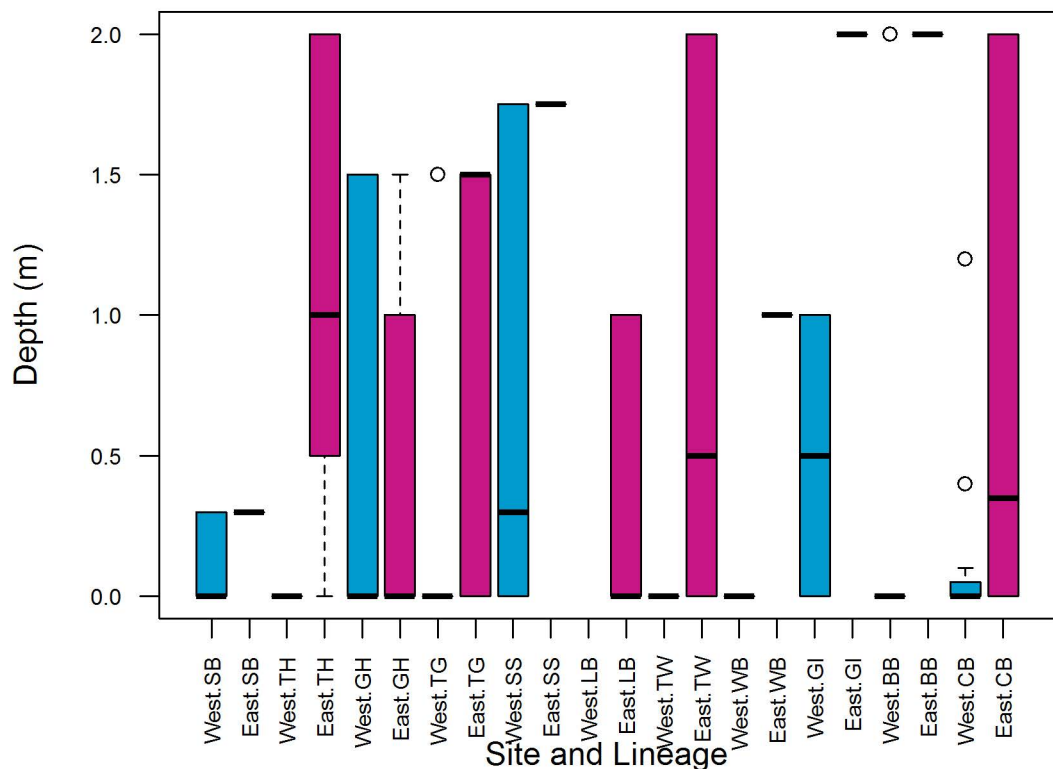


Figure 24 Boxplots for adult and juvenile *D. potatorum* separated according to lineage (excluding 'heterozygotes') and depth of occurrence. 'Western' individuals were more commonly found in shallow water, associated with the intertidal region. For all sites bar 'Granville Harbour' there was a pattern of one lineages occurring in a single, concentrated depth band, while the other occupied the complete range of depths. However, there was no pattern for either 'eastern' or 'western' individuals to be associated with the restricted distribution.

4.4.3 Combined ecological and morphological differences between lineages

All three multivariate analyses (NMDS, ANOSIM, Adonis) supported a significant difference between ‘eastern’ and ‘western’ individuals when ecological and morphological characteristics were combined (Fig 25). The difference between ‘eastern’ and ‘western’ individuals was greatest when both ‘heterozygotes’ and juveniles were excluded. Dissimilarity of lineages was apparent from the NMDS ordinations. When either ‘heterozygotes’ or juveniles were included the overlap between ‘eastern’ and ‘western’ confidence interval ellipses increased (Fig 26). A decrease in the degree of dissimilarity between the two lineages when ‘heterozygotes’ and/or juveniles were included was also supported quantitatively by a lower, yet still significant ($p=0.001$), ANOSIM R value (Table 9). The difference between ‘eastern’ and ‘western’ lineages was not significant according to ANOSIM when only juveniles were included ($p=0.344$) and there was almost complete overlap of confidence intervals for the two lineages in NMDS (Fig 27) (n.b. ANOSIM results for juveniles should be treated with caution as the underlying assumption of variable dispersion was violated). All NMDS analyses were run in three dimensions to maximise model fit, as determined by stress values (0.08-0.11) and Shepard plots (non-metric R^2 fit 0.988-0.994). Model fit decreased when ‘heterozygotes’ and/or juveniles were included. Furthermore, Adonis revealed that the lineage of individuals explained 23% of the differences between adults ($p=0.001$), but did not significantly explain the differences between juveniles. Significant effects according to Adonis did not change even when individuals were compared within-sites.

Table 9 The degree of dissimilarity between ‘eastern’, ‘western’ and ‘heterozygotes’ as calculated based on ANOSIM R values, significance levels and Adonis significance tests. Higher ANOSIM R values indicate greater differences between genotypes.

	Dissimilarity (ANOSIM R)	ANOSIM (p value)	Adonis (p value)	NMDS stress value
Adults only	0.36	0.001	0.001	0.09
Adults with ‘heterozygotes’	0.32	0.001	0.001	0.10
Juveniles only	0.08	0.014	0.344	0.08
Juveniles with ‘heterozygotes’	0.06	0.05	0.18	0.11

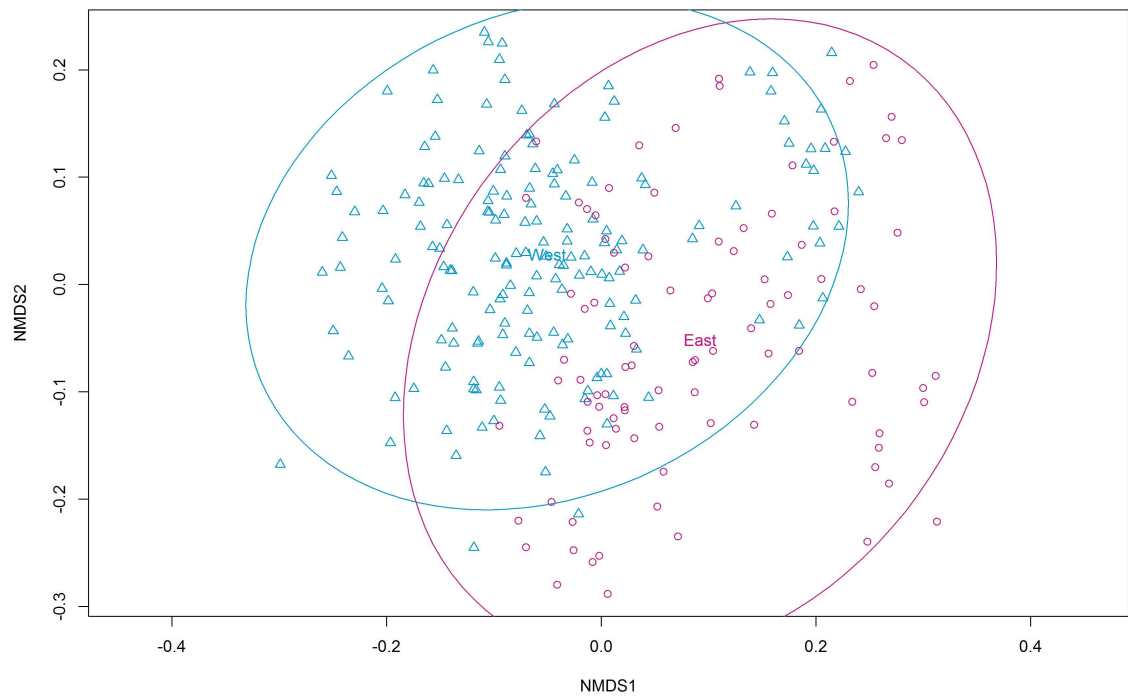


Figure 25 Non-metric multidimensional scaling ordination of adult *D. potatorum* (excluding 'heterozygotes') showing the clustering of 'western' (blue) individuals, and 'eastern' (pink) individuals in reduced dimensional space. The coloured ellipses represent 95% confidence intervals for each group and with the area of overlap indicating the extent of similarity between the two groups.

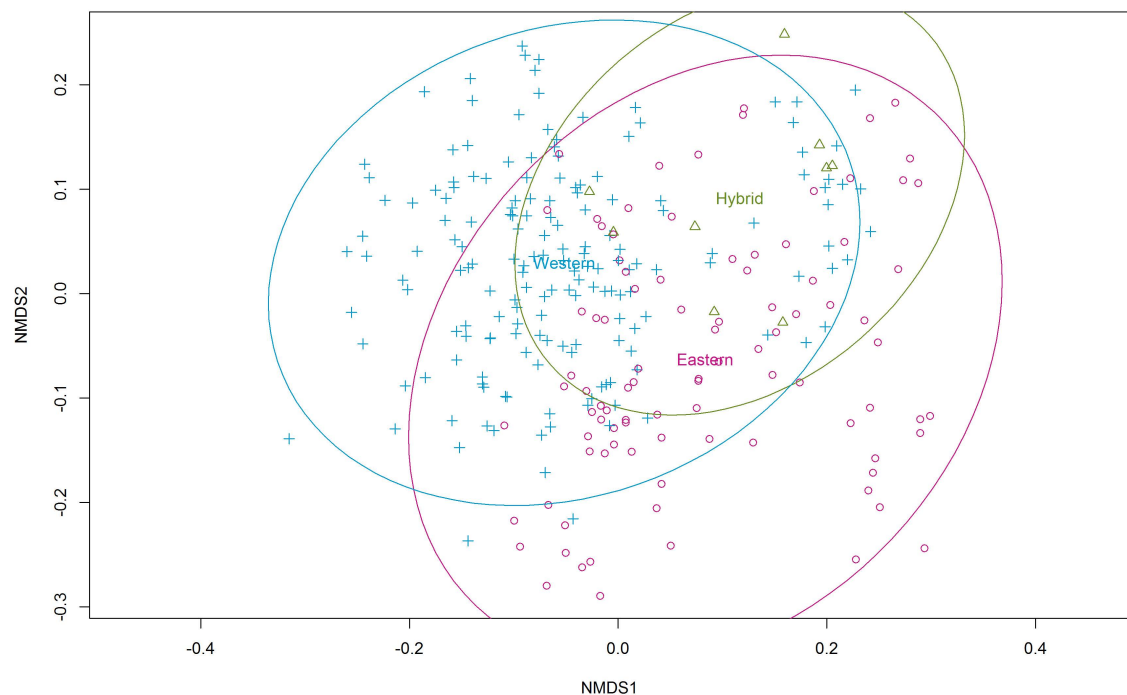


Figure 26 Non-metric multidimensional scaling ordination of adult *D. potatorum* (including 'heterozygotes') showing the clustering of 'western' (blue) individuals, 'eastern' (pink) and 'heterozygotes' (green), in reduced dimensional space. The coloured ellipses represent 95% confidence intervals for each group and with the area of overlap indicating the extent of similarity between the two groups.

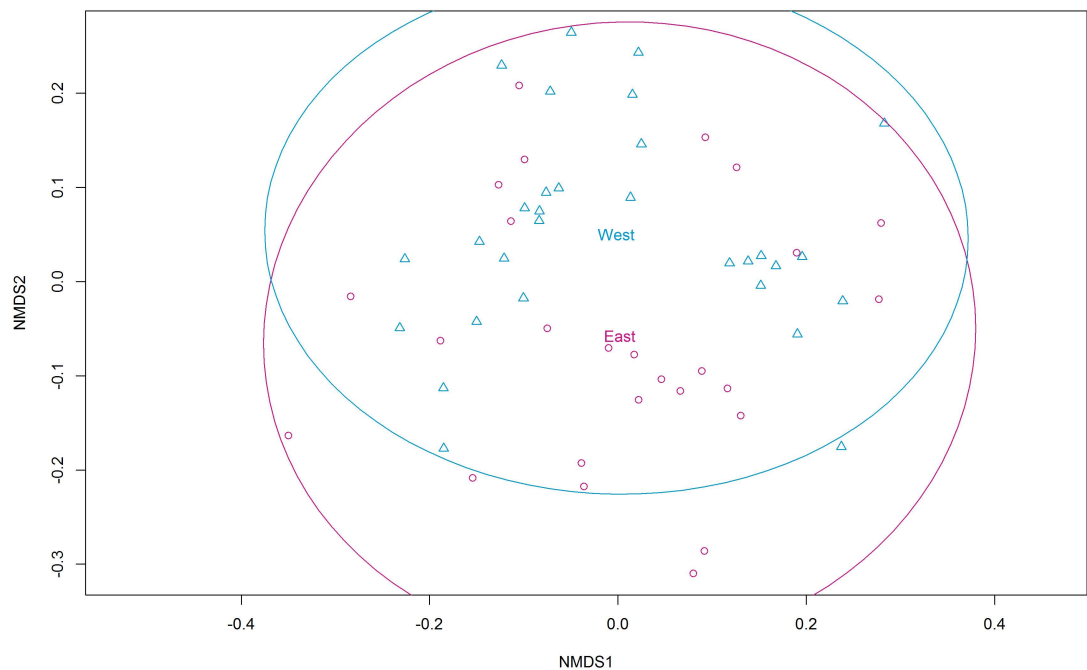


Figure 27 Non-metric multidimensional scaling ordination of juvenile *D. potatorum* showing the clustering of ‘western’ (blue) individuals, ‘eastern’ (pink) and ‘heterozygotes’ (green), in reduced dimensional space. The coloured ellipses represent 95% confidence intervals for each group, with the area of overlap indicating the extent of similarity between the two groups.

Four ecological and morphological variables were found to significantly explain the difference between ‘eastern’ and ‘western’ individuals according to GLM. These variables were ‘stipe proportion’ ($p=0.017$), ‘stipe base’ ($p=0.013$), ‘depth’ ($p=0.001$), and ‘stipe stoutness’ ($p<0.001$) and they remained significant irrespective of whether juveniles or ‘heterozygotes’ were excluded (Fig 26).

According to PLS analyses there was a significant correlation ($p=0.003$) between ecological and morphological characters for individuals collected in 2015 (Table 10). PLS plots showed a positive correlation between ‘openness’, ‘total length’ and ‘stipe top’, and a negative correlation between ‘openness’ and ‘stipe stoutness’. Depth was positively correlated with ‘tapering’, ‘lateral number’ and ‘branched’. These relationships were not affected when ‘heterozygotes’ were included, but the overall correlation between ecological and morphological characters was weaker. When PLS analyses were conducted on the two lineages separately, only the ‘western’ lineage was found to have a significant correlation between morphological and ecological characteristics ($p=0.0004$). For the ‘western’ lineage there was a positive correlation between ‘openness’, ‘tapering’ and ‘branched’ and a negative correlation between ‘openness’ and ‘stipe stoutness’. Depth was positively correlated with ‘stipe length’, ‘lateral number’, ‘stipe top’ and ‘total length’.

Table 10 Partial Least Square results indicating the degree of correlation between ecological and morphological variables for the 2015 sampling effort, as well as the 'eastern' and 'western' lineages separately. Results are presented when 'heterozygotes' were both included and excluded.

	PLS Correlation value	Significance (p value)
Excluding 'heterozygotes'	0.305	0.003
Including 'heterozygotes'	0.226	0.002
'Western' lineage	0.27	<0.001
'Eastern' lineage	0.119	0.298

Chapter 5: Discussion

The genetic, morphological and ecological results of this study support two distinct species within *Durvillaea potatorum* in its current circumscription. The ‘eastern’ and ‘western’ lineages are revised in this thesis as *Durvillaea lentus* and *Durvillaea potatorum* respectively. Morphological and ecological differences between the two species were found, and suggest alternative strategies to persisting in highly wave-disturbed habitats. The divergence and eventual speciation of *D. lentus* and *D. potatorum* can be explained from historic geographical isolation and subsequent ecological differentiation. The findings from this study have broader implications for understanding the role of geography and ecology in divergence, as well as the creation and evolution of biodiversity.

5.1 Genetic analyses confirm two distinct species

Genetic analyses unequivocally support the splitting of *D. potatorum* into two distinct species based on two well-supported, deeply divergent monophyletic clades (Fig 13). This study found complete concordance across markers and phylogenetic approaches in assigning individuals to either the ‘western’ or ‘eastern’ lineage proposed by Cheshire and Hallam (1988) and confirmed by Fraser *et al.* (2009h). Although the Maximum Likelihood COI phylogeny showed a paraphyletic *D. potatorum*, the Bayesian phylogeny on the same data was congruent with the previously resolved monophyly of the species (Fig 4) [see concatenated phylogeny (Fraser *et al.*, 2010e)].

The ‘eastern’ and ‘western’ clades were 4.3-4.7% divergent (uncorrected p distance) for COI, which is comparable to interspecific distances for other brown macroalgae; for example, 3-3.8% for *D. antarctica* and *D. poha* (Fraser *et al.*, 2009a). Interspecific distances for other genera, once cryptic species complexes have been resolved, include 3% for *Macrocystis* (Macaya and Zuccarello, 2010), and 4-4.8% for *Laminariaceae* (McDevit and Saunders, 2010; Balakirev *et al.*, 2012; Saunders and McDevit, 2014). Distances between COI haplotypes within both ‘eastern’ and ‘western’ lineages (< 0.6%) were also comparable to intraspecific distances in other brown macroalgae, for example, 0.1-0.9% within *D. poha* and *D. antarctica* (Fraser *et al.*, 2009a), and < 0.5% for most species within the *Laminariaceae* (McDevit and Saunders, 2010; Saunders and McDevit, 2014). However, intraspecific distances as great as 1.4% have been reported within the *Ectocarpales* (Peters *et al.*, 2015). Uncorrected genetic distances were lower for nuclear 28S than mitochondrial COI, which is unsurprising given faster substitution rates in mitochondrial versus nuclear DNA (Hudson and Turelli, 2003). Intra-lineage (< 0.7%) and inter-lineage (1.4-2.4%) 28S distances for *D. potatorum* are comparable to intraspecific (0-0.4%) and interspecific (1.1-5.8%) distances for other *Durvillaea*. (Fraser *et al.*, 2010e). To date, there have been few other phaeophyceae studies that have included 28S, but generally variation is low, even between orders (Rousseau *et al.*, 2001).

Although phylogenetic analyses separated all individuals into two distinct clades, nuclear (28S) sequences revealed nine ‘heterozygotes’ with both ‘eastern’ and ‘western’ alleles at molecular sites known to differentiate the two lineages. Five of these ‘heterozygotes’ appear to be early generation hybrids with widespread heterozygosity over the informative sites. The morphology of these hybrids appears consistent with other *D. potatorum* and three adults had mature conceptacles. Two females were found with aborted oogonia in their conceptacles, indicating poor gamete release during the preceding winter reproductive period (Clayton *et al.*, 1987). The third mature hybrid had no visible antheridia or oogonia in its conceptacles but it cannot be determined whether this was because gametes were never produced, or because they had been successfully released. Even if gamete release was successful, fertilisation by gametes from ‘eastern’ or ‘western’ individuals may not have been possible due to asynchronous timing of gamete release or genetic incompatibilities (Coyer *et al.*, 2002). The small number of hybrids and restriction to two fieldsites suggests low reproductive potential of hybrids, although cross-breeding experiments could resolve uncertainty about hybridisation potential and hybrid viability. The other four ‘heterozygotes’ were only heterozygous for informative sites at the 3’ end of 28S, which may have arisen from nuclear recombination, retained ancestral polymorphism or recent mutations. Nuclear recombination in such a short region of the genome would only be expected after numerous generations of hybridisation, but widespread introgression between ‘eastern’ and ‘western’ lineages was not found. Alternatively, these four ‘heterozygotes’ may have retained ancestral polymorphism due to incomplete lineage sorting or gained recent mutations from stochastic or adaptive processes. Future studies could include additional nuclear markers or genomic sequencing to gain more information about levels of heterozygosity across the genome and to draw more definitive conclusions about introgression between the two lineages.

The deep genetic divergence between the monophyletic ‘eastern’ and ‘western’ lineages, with limited hybridisation, supports long-term reproductive separation between the two lineages. Taxonomic revision is required to recognise two separate species within *D. potatorum* under the Phylogenetic Species Concept (*sensu* Cracraft, 1983), COI barcoding (Hebert *et al.*, 2004) and De Queiroz (2007)’s universal concept of species. Other species concepts, such as strict interpretations of the Biological Species Concept, are not suitable for groups such as the *Phaeophyceae* (Balakirev *et al.*, 2012) as reproductive isolation is rarely absolute between species (*e.g.* Bolwell *et al.*, 1977; *e.g.* Coyer *et al.*, 2002) and even higher order taxa (*e.g.* Lewis and Neushul, 1995). Genetic analyses are now commonly used in species delimitation, with some studies even using molecular information as the sole source of evidence (Rousseau *et al.*, 2001; Leliaert *et al.*, 2014). Molecular approaches are especially powerful for cases of cryptic species or highly variable morphotypes, frequently found in both *Rhodophyceae* and *Phaeophyceae* (Leliaert *et al.*, 2014). Following molecular and morphological identification of the type specimen (Fig. 17 & 18) of *D. potatorum* as belonging to the ‘western’ lineage, the

‘western’ lineage will hereafter be referred to as *D. potatorum* (*sensu stricto*) and the ‘eastern’ lineage as *Durvillaea lentus* (see section 5.5).

5.2 Morphological and ecological differences between *D. lentus* and *D. potatorum*

The splitting of *D. lentus* from *D. potatorum* is supported by genetic findings, however there were also numerous morphological and ecological characters which differed between the two species, particularly stipe morphology and distribution along depth gradients. *Durvillaea potatorum* individuals were generally longer, had proportionately longer yet thicker stipes, fewer stipitate lateral blades and were less likely to be ‘branched’ than *D. lentus* (Fig. 28 & 29). Additionally, *D. potatorum* was less often ‘conjoined’ and found in shallower water. As for many brown macroalgae with highly plastic morphology (*e.g.* Cheshire and Hallam, 1989b; *e.g.* Macaya and Zuccarello, 2010; *e.g.* McDevit and Saunders, 2010; *e.g.* Balakirev *et al.*, 2012; Verbruggen, 2014) and simple body plans (Niklas, 1992), no single discriminatory trait was found to enable easy, reliable field identification. It is unlikely that collector bias has prevented morphological differences between the two species from being identified; collectors from the three sampling efforts have worked together on the species in the field, and analysis using data from collection efforts separately was not found to dramatically alter results.

The most informative character for species identification was stipe morphology, with significant differences in relative and absolute dimensions between the two species. Stipe length and thickness have previously been used to distinguish species within *Durvillaea* (Hay, 1979; Fraser *et al.*, 2009a). Traits such as stipe morphology aid in species identification, but they also affect an individual’s fitness including vulnerability to wave-disturbance. As wave size increases, so do the forces algae must withstand (Stevens *et al.*, 2002). Drag, pressure and abrasion (Denny, 1988; Stevens *et al.*, 2002) can result in tissue damage (Dring, 1982) stress and detachment from the substrate (Fowler-Walker *et al.*, 2005a; Hill *et al.*, 2010). Therefore, to persist despite high wave-disturbance algae modify the flexibility of their structures. Greater flexibility minimises drag and dissipates energy by reorientating streamlined shapes to suit the prevailing flow (Denny, 1988; Harder *et al.*, 2006). In contrast, rigidity restricts stipe movement, thereby reducing blade acceleration (Stevens *et al.*, 2002) and protecting against strain-induced damage (Denny, 1988). Rigid stipes are also less vulnerable to repeated torsional strain which occurs when blades flip over as a wave passes (Harder *et al.*, 2006).

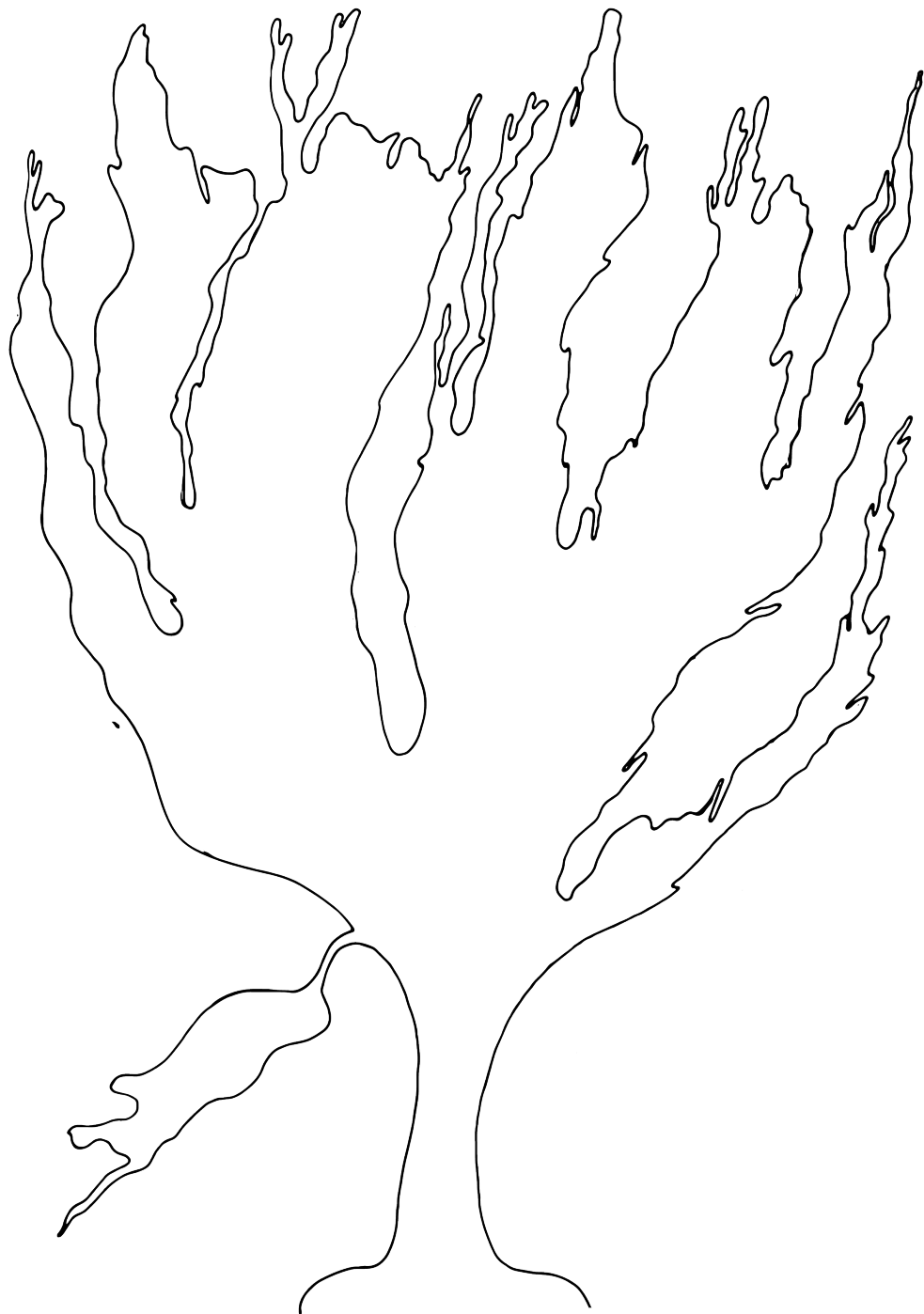


Figure 28 Illustration of a typical *D. potatorum* (*sensu stricto*) showing the characteristic stout, thick stipe, limited stipitate lateral blade development and an intact palmate region without branching.



Figure 29 Illustration of a typical *D. lentus* showing the characteristic slender, shorter stipe, prolific stipitate lateral blade development, branching of the palmate region into distinct boughs, conjoined holdfast and stipitate lateral blades on juveniles.

The thinner, more flexible stipes of *D. lentus* are expected to be more responsive to strong changes in water flow. Maximum wave-induced acceleration and drag typically occurs at the zone of breaking waves (Stevens *et al.*, 2002), coinciding with the slightly deeper distribution of *D. lentus*. In comparison *D. potatorum*'s thicker, more rigid stipes may offer greater protection against detachment in the highly turbulent intertidal zone and minimise tissue abrasion against the rocky shore in the shallow water. Rigid stipes should also be able to bear more weight, which would benefit the larger, heavier *D. potatorum* (Cheshire and Hallam, 1989b) experiencing prolonged periods of aerial exposure when their body weight is no longer supported by water. The 'flexible' and 'rigid' strategies of *D. lentus* and *D. potatorum* in response to environmental conditions could be enhanced by differences in stipe tissue

composition, particularly the concentration and structure of mannuronate and guluronate acids (Vreeland *et al.*, 1990), this requires further research.

In addition to differences in stipe flexibility and rigidity, multivariate analyses support different responses to disturbance-induced damage between *D. lentus* and *D. potatorum*. According to PLS analyses, *D. potatorum*'s correlated with ecological conditions, indicating a more plastic phenotype that minimises damage by altering morphological structures to suit environmental conditions. In contrast, there was no significant correlation between the morphology of *D. lentus* and ecological conditions. Without such a responsive physiology to provide protection, *D. lentus* may experience greater stress and tissue loss, potentially explaining shorter *total lengths*. To compensate, *D. lentus* appears to place greater investment in repair mechanisms, with more stipitate lateral blades and 'branching' to replace tissue lost from seasonal shedding, environmental damage, herbivory and disease (Niklas, 1992). The restorative role of 'branching' and stipitate lateral growth has been established in storm-damaged *D. potatorum (sensu lato)* (Hay, 1994) as well as intertidal *D. antarctica* (Fraser *et al.*, 2009a). Interestingly, stipitate lateral blades were never found on juvenile *D. potatorum* and may be an early expression of the 'repair' or 'prevent' approaches damage of the two species.

The different distributions of *D. lentus* and *D. potatorum* within-sites along depth gradients also results in the two species experiencing different ecological stresses (Fig 30). Intertidal individuals, as compared with those found subtidally, experience longer aerial exposure (Dring, 1982), greater desiccation risk (Guenther and Martone, 2014), reduced nutrient uptake and are subjected to extreme light and temperature fluctuations (Womersley and King, 1990). In brown macroalgae, as depth increases total length is also expected to increase as longer individuals experience less drag and maximum light acquisition (Fowler-Walker *et al.*, 2005c). However, this study revealed longer 'total lengths' for the shallower *D. potatorum*. Longer 'total lengths' may offer advantages in the intertidal zone by providing greater shading to minimise excessive radiation (Womersley and King, 1990) and by increasing surface area for nutrient acquisition and reproductive output. Nitrate availability may be an important factor in the distribution of *D. potatorum* and *D. lentus* both within and between-sites. Nitrate is a well-known limiting factor in marine ecosystems (Hatcher, 1994), especially Australia's nutrient-poor coastal waters (Jeffrey *et al.*, 1990) and affects *D. potatorum (sensu lato)*'s growth (Rolley, 1980). The correlation between available nitrate (Fig 31) and the spatial distribution of both species suggests differences in nutrient use or requirements but further studies are needed.

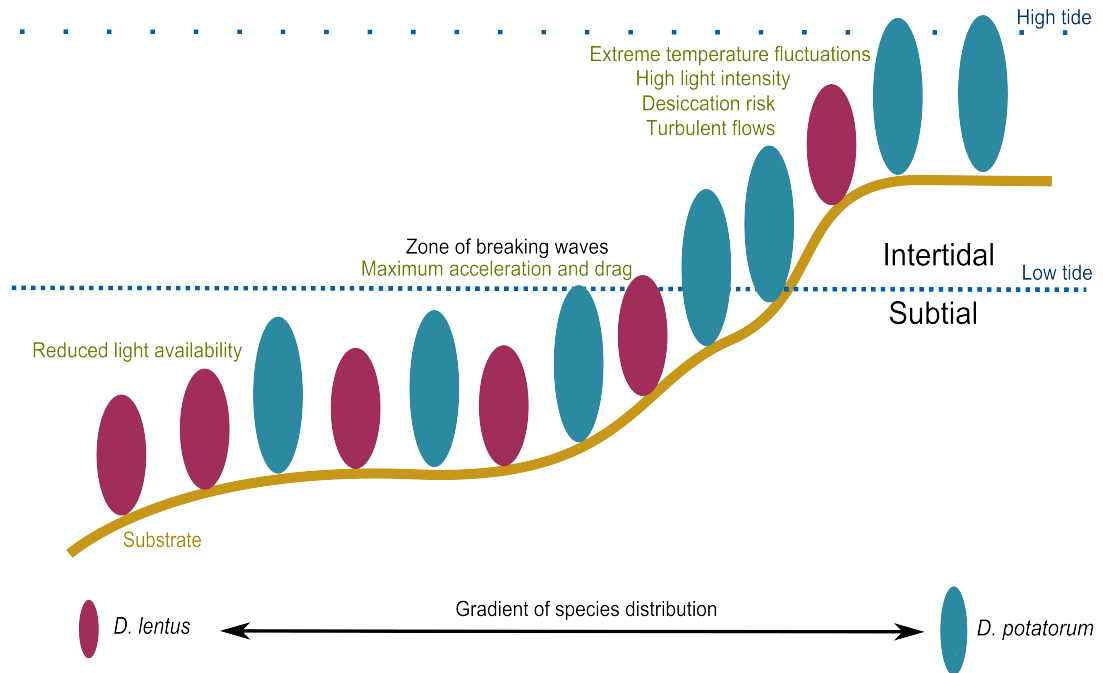


Figure 30 Depiction of changes along a depth gradient in the distribution and ecological stress experienced by *D. lentus* and *D. potatorum*. The relative distribution of both lineages is indicated, along with the intertidal, subtidal and zone of breaking waves. Abiotic stresses and conditions are written in green. The more common distribution of *D. potatorum* in the intertidal increases exposure to extreme light, temperature and desiccation. *Durvillaea lentus* with a slightly deeper distribution experiences maximum acceleration and drag forces, as well as greater light competition at depth.

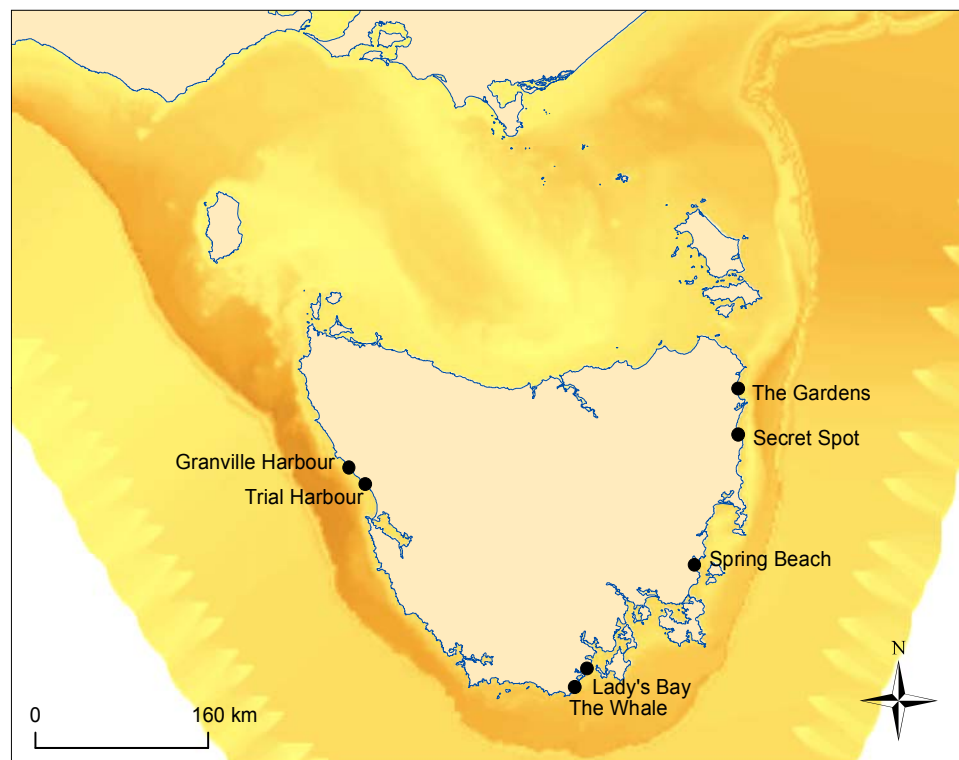


Figure 31 Map of seabed nitrate from Heap *et al.* (2005)'s NMBA dataset. Modified to show the seven field sites visited during the 2015 sampling effort. Darker yellow-orange represent higher levels of seabed nitrate.

5.3 Speciation of *D. potatorum* and *D. lentus*

5.3.1 Allopatric speciation and the role of glaciations

The spatial distributions of *D. lentus* and *D. potatorum* and the clear east-west split in mainland populations (Fraser *et al.*, 2009h) supports geographical isolation resulting in allopatric divergence. Glaciation cycles beginning at the end of the Pliocene and early Pleistocene affected climatic and oceanic conditions and had a substantial impact on the range and genetic diversity of species (review Hewitt, 2004; review Beheregaray, 2008; Silva *et al.*, 2014; review Ludt and Rocha, 2015). Molecular clock dating of a *Durvillaea* phylogeny based on four markers estimated the divergence between *D. lentus* and *D. potatorum* to have occurred between 2.2 and 8.4 mya (Fraser *et al.*, 2010e) (Fig 32). However, these figures must be interpreted with caution as molecular dating of brown algae is notoriously imprecise due to uncertainty surrounding mutation rates and a lack of fossil records for calibration (Price, 1990; Brookfield, 2001). If accepted, the estimated divergence time corresponds with the end of the Miocene, all of the Pliocene and the early Pleistocene, which coincides with the onset of glaciation cycles. During glaciation periods, cooler temperatures and increased glacial ice lowered sea levels, altered ocean currents and created large terrestrial glaciers (McKinnon *et al.*, 2004). Warmer interglacials saw sea levels rise and air temperatures increase (Wells and Okada, 1996). Fluctuating climatic conditions affected many species by altering habitat availability, suitability and connectivity (Endler, 1973; McKinnon *et al.*, 2004; McRae, 2006; Patarnello *et al.*, 2007; Teske *et al.*, 2014).

In south-east Australia, falling sea levels during glaciation periods resulted in land bridges forming between Tasmania and the mainland (Fig 33-34). Although now submerged, the eastern and western sills either side of Bass Strait were exposed whenever sea levels dropped by 55 and 75 m respectively (relative to contemporary sea level) (Blom and Alsop, 1988; Cole *et al.*, 1989; McKinnon *et al.*, 2004). Therefore, during periods of intense glaciation ancestral *D. potatorum* (*sensu lato*) populations on the west and east coasts of Tasmania would have been connected to the mainland via the King Island Basement Ridge and Bassian Isthmus (Jones and Holdgate, 1980) (Fig 34). During weaker glaciations, the higher eastern sill would have maintained east-coast connectivity, but King Island and western Victoria would have been separated by shallow water (Porch and Allen, 1995). The presence of this water passage allowed a sheltered embayment to form in Bass Basin (Blom and Alsop, 1988; Porch and Allen, 1995) which is unlikely to have supported species such as *D. potatorum* (*sensu lato*) which depend on rocky, wave-disturbed sites. Depending on the dispersal capacity of ancestral *D. potatorum* (*sensu lato*) and ocean currents, some gene flow between mainland and Tasmanian populations may have been possible. Coinciding with lower sea levels, sea currents changed during glaciations. Approximately 20kya during the Last Glacial Maximum (LGM) (McKinnon *et al.*, 2004), the Leeuwin current, which brings warmer tropical water eastwards towards Tasmania,

weakened and no longer reached beyond the Great Australian Bight or fed into the Zeehan current (York *et al.*, 2008; Colgan and Schreiter, 2011). The resulting drop in sea surface temperatures off Tasmania's west-coast was intensified by a 2-5° northward expansion of the subtropical convergence (Wells and Okada, 1996). These rapidly cooling temperatures may have exceeded the temperature tolerance of *D. potatorum* (*sensu lato*) and led to the temporary loss of populations from south-western Tasmania (Fig 36). Unlike *D. antarctica* which is able to persist in cold, sub-Antarctic waters (Fraser *et al.*, 2010a), *D. potatorum*'s (*sensu lato*) growth (Rolley, 1980) and reproductive output (Fraser *et al.*, 2010e) is sensitive to temperature. In contrast, east-coast populations may not contracted northward, as warmer sea surface temperatures could have been maintained by an intensifying East Australian Current (Fraser *et al.*, 2009h).

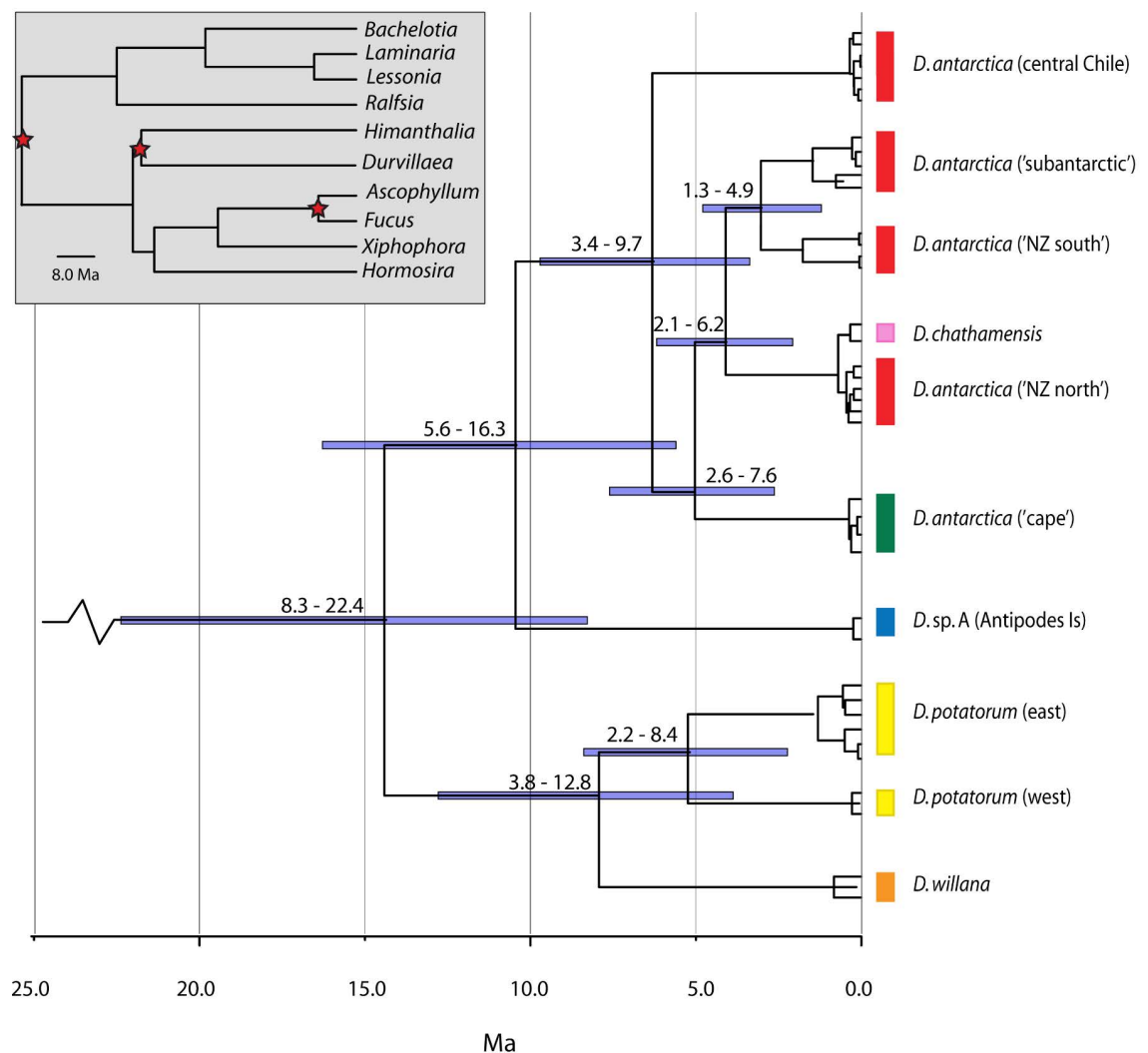


Figure 32 Bayesian phylogeny with molecular clock estimates of divergence times taken from (Fraser *et al.*, 2010e). Shows a generic origin approximately 8.4-2.2mya and a split between *D. lentus* and *D. potatorum* (shown as *D. potatorum* 'east' and 'west') 22.4-8.3mya.

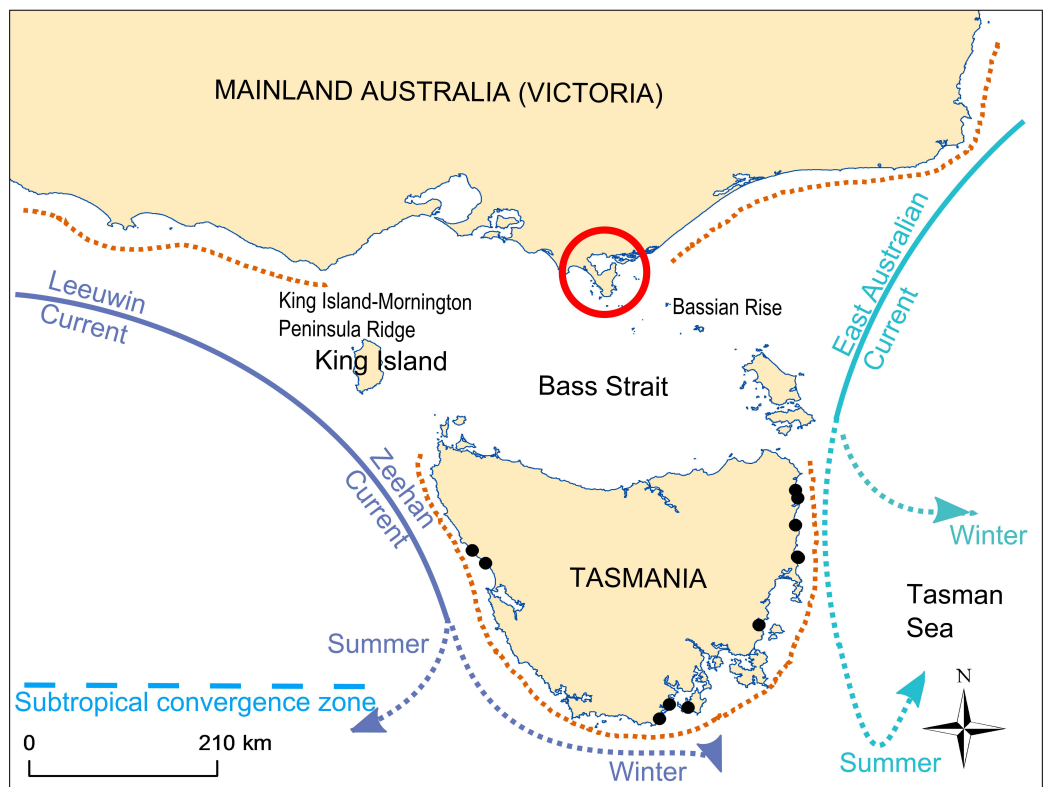


Figure 33 Map of south-east Australia showing major ocean currents, contemporary shoreline and the current distribution of *D. potatorum* (*sensu lato*) (shown as orange dotted line following the coastline). Wilson's Promontory, site of numerous population disjunctions across taxa, is circled in red.

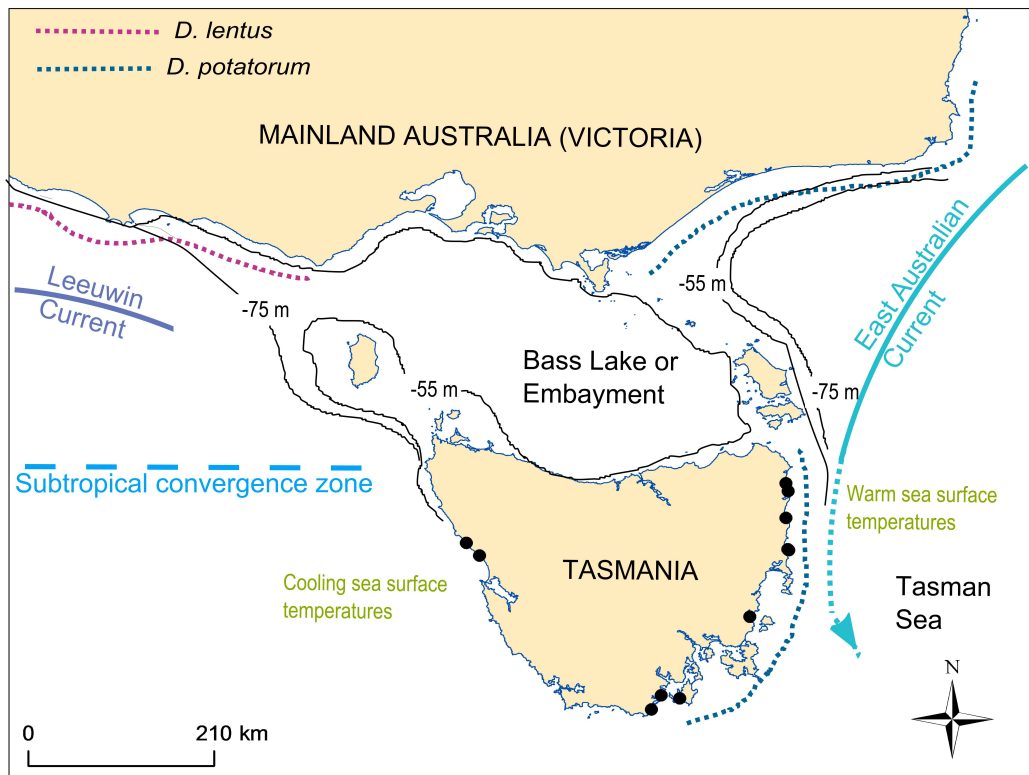


Figure 34 Map of south-east Australia showing changes in land connectivity between Tasmanian and the mainland. Falling sea levels, the presence of land bridges and changes in ocean currents under glaciation conditions could have isolated east-coast and west-coast ancestral populations of *D. potatorum*.

Ocean dynamics and land exposure around Tasmania before the LGM are poorly understood; however, similar processes are likely to have occurred during earlier glaciations. Although the quaternary is considered the key period of climatic changes altering species biogeography, other geological periods saw equally, or even more extreme, changes to ocean currents, water connectivity, thermal gradients (Hommersand, 2007; Whittaker and Fernández-Palacios, 2007), radiation and sea levels (Carter, 1994). During any of these glaciations the emergence of land bridges and cold sea surface temperatures along the west-coast of Tasmania could have isolated ancestral populations of *D. potatorum* (*sensu lato*). The process may even have occurred repeatedly during numerous glaciations until the LGM. The first major glaciation in the early Pleistocene (approx. 1.5-1.8 mya) (Markgraf *et al.*, 1995; McKinnon *et al.*, 2004) coincides with the estimated divergence time of *D. potatorum* (*sensu stricto*) and *D. lentus*. Although not a significantly cold period globally, Tasmania experienced more extreme climatic cooling and ice coverage than during more recent Pleistocene glaciations (McKinnon *et al.*, 2004). Following a period of subsidence, faulting and sedimentation forming Bass Basin, the end of the Pliocene was also a time when the entire western sill was exposed (Corbett *et al.*, 2014). Instead of vicariance triggered by global climate cycles, allopatric divergence between *D. potatorum* and *D. lentus* could have arisen from smaller barriers to dispersal, such as long sandy beaches or stretches of sheltered coast. Low levels of wave-disturbance in Bass Strait, sparse rocky outcrops and long sandy beaches (e.g. Ninety mile beach near Wilson's Promontory) (Hay, 1994; York *et al.*, 2008) are considered contemporary barriers to admixture between mainland *D. lentus* and *D. potatorum* (Fig 35). Similarly, population connectivity of other *Phaeophyceae* and *Rhodophyceae* show that sandy beaches can be important barriers to gene flow (e.g. Fraser *et al.*, 2010a; e.g. Fraser *et al.*, 2013). Unfortunately, available geological evidence does not provide sufficient resolution of Australia's historical coastlines to determine whether any such barrier existed, or its precise location. Irrespective of whether geographical separation arose from land bridge formation, changing ocean conditions or stretches of unsuitable habitat, reproductive isolation of *D. potatorum* and *D. lentus* would have allowed morphological and genetic differences to accumulate.

5.3.2 Sympatric divergence and convergent evolution

Changing climatic conditions or the loss of dispersal barriers following geographical isolation would have allowed range expansion and secondary contact between *D. lentus* and *D. potatorum* (Fig 35). As divergence was maintained despite secondary contact, pre or post-zygotic reproductive barriers must have already been present or arose quickly thereafter. Dispersal of *D. potatorum* along the west-coast could have occurred quickly by following the Zeehan current, whereas the subsequent range expansion of both species northwards on opposite coasts is likely to have been more gradual. For *D. lentus* colonisation events along the west-coast of Tasmania are most likely to have coincided with strong southerly swells (Bennett and

Pope, 1960; Hill *et al.*, 2010) or during summer and spring, when the Zeehan and Leeuwin currents are weakest (Jeffrey *et al.*, 1990). On the east-coast, *D. potatorum*'s progressive northerly range expansion would have been most likely during winter, when the East Australian Current weakened and strong anticlockwise eddies formed (Jeffrey *et al.*, 1990; Colgan and Schreiter, 2011). Counter-current eddies have previously been shown to affect phylogeographical patterns along Australia's south-east coast (*e.g.* York *et al.*, 2008; Smith *et al.*, 2015). As temperatures warmed and the Bassian Isthmus became completely submerged, mainland and Tasmanian populations would have become isolated. Bass Strait therefore allowed mainland sites to remain monospecific, with only *D. lentus* on the east and *D. potatorum* on the west.

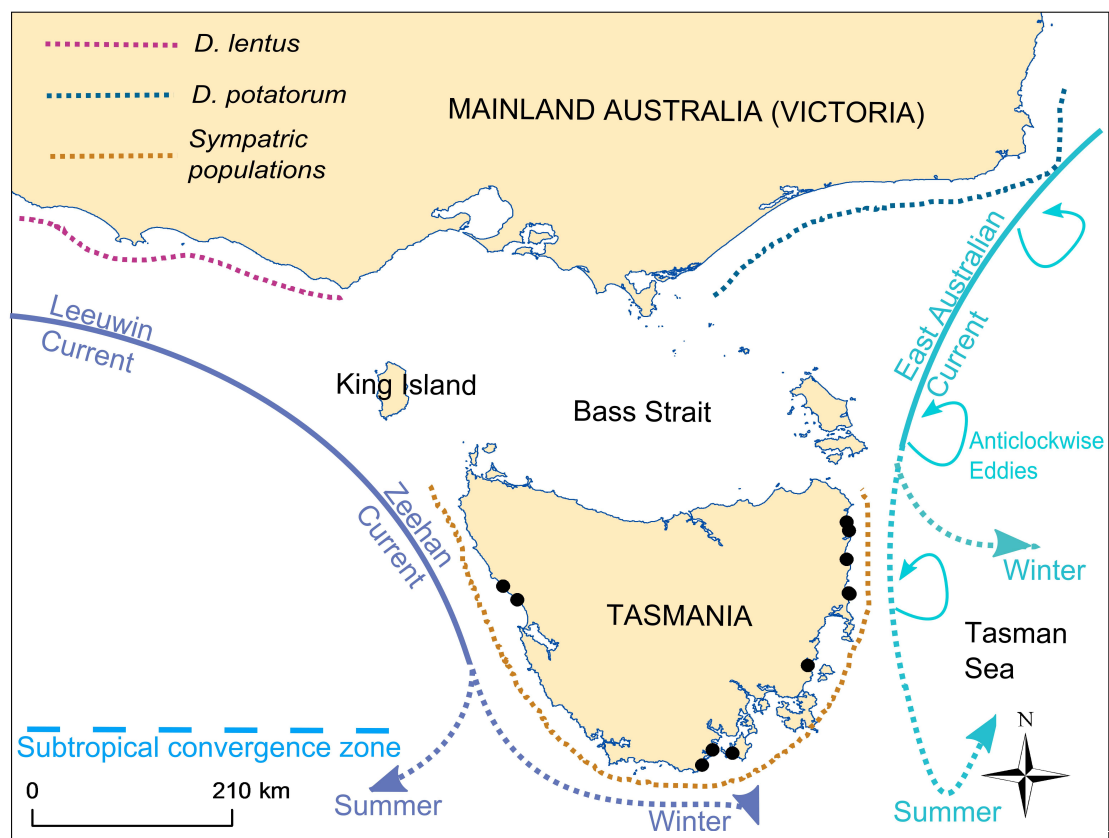


Figure 35 Map of south-east Australia showing sea levels and ocean currents during interglacial periods and since the Last Glacial Maximum. The range expansion and subsequent secondary contact of *D. lentus* and *D. potatorum* (*sensu lato*) is shown in blue and pink respectively.

Substantial range changes may seem unlikely as contemporary populations of *D. potatorum* (*sensu lato*) are considered to be poor dispersers with solid blades (Harder *et al.*, 2004) and passive gametes that rapidly attach to the substrate (Clayton and King, 1990; Clayton and Ashburner, 1994). However growing evidence shows inferring dispersal capacity from morphology and life history is often a poor predictor of long-distance migration (Johannesson, 1988; Winston, 2012; Fraser *et al.*, 2013). For example, solid blades in *D. lentus* and *D. potatorum* does not exclude the possibility that an ancestral form had buoyant blades and could quickly colonise new habitats. Indeed, internal blade structure appears to be a labile trait among

Durvillaea. Fraser *et al.* (2010e) proposed that buoyant, rather than solid, blades could be the ancestral state. Following range expansion, both *D. lentus* and *D. potatorum* could have quickly lost buoyancy, as has been inferred for the solid-bladed *D. chathamensis* (Fraser *et al.*, 2010e). Loss of buoyancy may not have occurred simultaneously in both species, as selection for greater dispersal capacity afforded by buoyant blades may have weakened earlier for *D. lentus*. If *D. lentus* was able to persist along the warmer east-coast of Tasmania during glaciations, range expansion in subsequent interglacials would have been much smaller than for *D. potatorum*, making the investment in honeycomb lamina no longer advantageous.

Sympatric divergence seems unlikely for *D. lentus* or *D. potatorum* given the strong overlap in the morphological and ecological characteristics of both species. A possible scenario involves adaptive loss of buoyancy in the ancestral population occurring in one species, before the other. Loss of buoyancy at different times may have temporarily resulted in ecological niche partitioning, allowing divergence at the same site. Fraser *et al.* (2010e) proposed an adaptive benefit of solid blades in the subtidal environment for *D. chathamensis*. Given *D. lentus*' contemporary subtidal distribution, ancestral populations of *D. lentus* may have been the first to lose buoyancy, given the species' contemporary subtidal distribution. The solid blades in both *D. lentus* and *D. potatorum* may be a product of convergent evolution with a loss of buoyancy occurring independently in both species. However, sympatric divergence does not explain the strong phylogeographical patterning apparent today and cannot account for monospecific mainland stands. Although outside this study's scope, more comprehensive examination of mainland genetic diversity could be undertaken to confirm these sites are monospecific. Mainland sampling would also reveal whether differences in morphology and ecology between the two species are more or less apparent in presumably monospecific populations. Loss of buoyancy seems more likely to have maintained, rather than triggered, divergence between the two species.

5.4 Evolutionary implications beyond *D. potatorum*

5.4.1 Buoyant blades and the origin of *Durvillaea*

The potential for buoyant blades to be the ancestral state within *Durvillaea* (Fraser *et al.*, 2010e) offers new explanations for the origin of the genus. Currently, *Durvillaea* is thought to have originated in the south-west Pacific approximately 20-30 mya, with current diversity concentrated around New Zealand (Fraser *et al.*, 2010e). In contrast, an Australian origin offers a more parsimonious explanation of diversification within the genus and is consistent with the known marine floristic diversity of Australia (Hommersand, 2007). Dispersal and colonisation patterns of marine species along strong westerly currents, such as the Antarctic Circumpolar Current, have been well established in the Southern Hemisphere (Waters, 2008a). The presence of *D. potatorum* (*sensu lato*) in Tasmania, to the west of New Zealand, is challenging to explain with a south-west Pacific origin for the genus. To correspond with prevailing currents, *D.*

potatorum (*sensu lato*)’s ancestor would have had to traverse large distances to reach Tasmania. Evidence from the widespread circumpolar distribution of *D. antarctica* (Fraser *et al.*, 2012a), and the deposition of wrack material on Tasmania’s west-coast (Waters, 2008a) shows that long-distance transoceanic transport is possible. However, *D. potatorum* (*sensu lato*) is not derived from *D. antarctica*, but rather forms a distinct clade with *D. willana* (Fig 4). Furthermore, it is difficult to explain northward expansion along Tasmanian and mainland coasts during the warm conditions of the Miocene and Pliocene when sea levels were much higher and connectivity between Tasmania and Australia was limited (Markgraf *et al.*, 1995). If instead Australia was the origin of *Durvillaea*, buoyant ancestral populations on the east-coast of Tasmania or the mainland could have rafted along westerly offshoots of the East Australian Current (Colgan and Schreiter, 2011) to disperse and diversify throughout the south-west Pacific. Furthermore, a buoyant Australian ancestor would resolve the inconsistency between estimated speciation dates for the solid-bladed *D. sp. A.* and the period of geological formation of its habitat, the Antipodes Islands (Hay, 1994). A buoyant ancestor from Australia or surrounding islands could have colonised the Antipodes after their emergence.

5.4.2 Phylogeographical patterns and shared barriers

The allopatric divergence scenario for *D. potatorum* and *D. lentus* explored above (see section 5.3.1) adds weight to a growing body of phylogeographical studies that demonstrate the importance of major climatic oscillations as drivers of biodiversity. Although glaciation cycles, particularly during the Pleistocene, have dramatically altered the genetic diversity and spatial distribution of a wide range of species (review Hewitt, 2000; review Hewitt, 2004), comparatively little is known about the southern hemisphere (Beheregaray, 2008). Studying the phylogeography of species across south-eastern Australia allows the effect of fluctuating climatic conditions, sea levels, ocean currents and land connectivity to be compared between marine and terrestrial taxa. The distributions of *D. potatorum* and *D. lentus* show an east-west separation, which has also been observed for other species inhabiting rocky, wave-disturbed habitats including barnacles, shrimps and echinoderms (review York *et al.*, 2008). Other marine species, even those with vastly different dispersal capacities and habitat requirements, show similar phylogeographical structuring around Wilson’s Promontory (Waters, 2008c; review York *et al.*, 2008) (Fig 35). The area surrounding Wilson’s Promontory is likely to have affected population dispersal as it represents the last point of connection between mainland Australia and Tasmania, and also has long stretches of sheltered sandy beaches (review York *et al.*, 2008). The importance of major climatic oscillations on land connectivity between Tasmania and the mainland has also been supported by phylogeographical studies of terrestrial species (*e.g.* *Eucalyptus* McKinnon *et al.*, 2004; *e.g.* *Thylogale* Macqueen *et al.*, 2009). Concordance between different species with contrasting abiotic and biotic niches strengthens

the hypothesis that major glaciation cycles have had global impacts on biodiversity since the end of the Pliocene.

5.4.3 Processes sustaining sympatry

The lack of a definitive morphological or ecological character separating *D. potatorum* and *D. lentus* indicates strong overlap in niche space. As both species are found sympatrically on both Tasmania's east and west coasts they are also likely to share traits not examined in this study, such as temperature or salinity tolerance (Fraser *et al.*, 2009h). Overall, the shared environmental and ecological niches of the two species appear to be constraining morphology, with plastic phenotypes able to respond to the fluctuating environmental conditions instead of adaptive diversification. The persistence of sister species within a common niche space challenges assumptions that interspecific competition will lead to competitive exclusion or diversifying selection (Whittaker and Fernández-Palacios, 2007; Zink, 2014). *Durvillaea lentus* and *D. potatorum* may sustain high niche overlap by exploiting fine-scale ecological or environmental differences not detected in this study, for example rock platform aspect or wind conditions (Dring, 1982). Alternatively, sympatry might be sustained by fluctuating conditions or extreme disturbance events (Underwood, 2007) resulting in continual changes to the proportion of each species at a site. Factors may include seasonal changes in sea surface temperatures (Hay, 1994), variable herbivory pressure (Whittaker and Fernández-Palacios, 2007) or major storms. Although no measures of population dynamics were made in this study, the proportion of individuals from either species often differed between juveniles and adults at each site. If the distribution of juveniles is representative of future population trends, then sites such as 'Binalong Bay', with very few *D. lentus* juveniles, may change in composition and become dominated by *D. potatorum*. To explore population dynamics and connectivity in greater detail, future research could expand on the two genetic markers chosen for this study and utilise microsatellites or genome-wide single nucleotide polymorphisms.

The ability for species to display limited phenotypic differentiation despite having experienced markedly different evolutionary histories suggests that environmentally and geologically dynamic regions, such as Australia's south-east, may harbour high levels of cryptic biodiversity. This study supports differentiation of *D. potatorum* and *D. lentus* in their strategies to withstand disturbance, rather than any single morphological or ecological trait. When combined, the slight differences between the two species supports *D. lentus* having more 'flexible' structures but a phenotype which is less responsive to ecological conditions and requires greater investment in repair mechanisms to withstand high levels of wave-disturbance. In contrast, *D. potatorum*'s strategy to withstanding the same high levels of disturbance involves more rigid structures but a phenotype which is more responsive to ecological conditions. This raises an intriguing question about whether species subject to ongoing, high

levels of disturbance experience a trade-off between short-term structures capable of withstanding strong forces or the plastic capacity of that phenotype.

5.5 *Species description: Durvillaea lentus* sp. nov.

A single partial nuclear sequence supports the *D. potatorum* (*sensu lato*) type specimen as belonging to the ‘western’ lineage. Despite the presence of certain morphological traits more typical of ‘eastern’ individuals (i.e. ‘branching’, short ‘total length’ and ‘stipe length’), the ‘stipe stoutness’, ‘stipe proportion’ and absence of stipitate lateral blades, supports the type as ‘western’. No information can be determined from type locality as the specified collection location ‘Cape van Diemen’ cannot be determined. There are no historical records of Cape van Diemen in Tasmania. Furthermore, Labillardière (1806) does not provide details of the site’s ecology but collection from the easily accessible intertidal zone would be consistent with the ‘western’ lineage.

5.5.1 *Durvillaea lentus* sp. nov.

Perennial individuals rarely exceeding five metres in total length (Fig 30). Thick lamina tissue at base of palmate region, becoming increasingly thin towards frond tips. Lamina commonly divided, blades becoming narrower towards tips. Frequently greeny-yellow to brown lamina colour. Infrequent crenate or serrate blade margins. Commonly found with damage including tears and perforations. Internal lamina solid, blades non-buoyant. Single primary stipe, up to half a metre in length. Stipe straight, or slightly narrowing at top. Palmate region infrequently divided into distinct rounded boughs. Holdfast solid and not found to support burrowing invertebrates. Holdfasts infrequently shared by two to three coalesced individuals.

Dioecious and reproductively mature in winter. Oogonia containing four ova, borne on conceptacle walls or branched paraphyses. Infrequently remain in conceptacles outside reproductive period. Antheridia attached to branched paraphyses.

Found in the intertidal and subtidal zones of rocky coastal sites. Able to withstand high levels of wave-disturbance. Often found in sympatry with *D. potatorum* in Tasmania, although more common in deeper subtidal waters.

ETYMOLOGY: The specific name ‘*lentus*’ refers to the slender, flexible stipes characteristic of the species. ‘*Lentus*’ also has meanings of toughness, tenacity and xxx, well suited to ability of the species to withstand wave-disturbance.

TYPE SPECIMENS: holotype and isotype to be collected, sequenced and deposited with Tasmanian Herbarium, (Hobart, Tasmania). Molecular sequences to be made available on GenBank. **TYPE LOCALITY:** to be determined.

KEY DISTINGUISHING TRAITS: *D. lentus* compared with other species within the genus: (I) *Durvillaea potatorum*: narrow, slender stipe, stipe base circumference rarely exceeds 12cm, more commonly branched (clear divisions of the palmate region), higher counts of

stipitate lateral blades emerging from the palmate region, particularly among juveniles, occurs more commonly in the subtidal zone (II) *Durvillaea willana*: found in the intertidal zone, oogonia also attached to paraphyses (III) *Durvillaea antarctica*: stipitate lateral blade development emerging from the palmate region, solid lamina blades, solid holdfast does not support burrowing invertebrates (IV) *Durvillaea poha*: stipitate lateral blades emerging from the palmate region, solid lamina blades, solid holdfast does not support invertebrates, found at greater depths in the subtidal zone and more wave-exposed sites (V) *Durvillaea chathamensis*: found in the intertidal zone, branching (clear divisions of the palmate region), solid holdfast does not support burrowing invertebrates (VI) Undescribed *Durvillaea* endemic to the Antipodes Islands (see Hay, 1994): found in the intertidal zone, oogonia also borne directly on conceptacle walls.

DISTRIBUTION: Endemic to south-east Australia including Tasmania. Found along the east-coast of New South Wales and Victoria as well as the east and north-west coasts of Tasmania. Suspected to also occur on Tasmania's south-west coast. No known populations on the north-coast of Tasmania. Mainland populations found as far north as Tathra (New South Wales) but not extending further west than Wilson's Promontory (Victoria).

Chapter 6: Conclusion

Genetic analyses performed in this study support the existence of two distinct species within *Durvillaea potatorum* (*sensu lato*), with levels of genetic distance comparable to those found between other species of brown macroalgae. Therefore, the ‘eastern’ and ‘western’ lineages proposed by Cheshire and Hallam (1989b) actually represent two distinct species, hereafter *D. potatorum* (*sensu stricto*) and *D. lentus* sp. nov. Despite the potential for hybridisation between *D. potatorum* (*sensu stricto*) and *D. lentus*, the small number of putative hybrids, and doubts surrounding their reproductive viability, suggest any gene flow is limited enough not to affect species differentiation and ongoing divergence. The genetic and histological findings from this study therefore support the first hypothesis that there are two distinct species within *D. potatorum* (*sensu lato*), however reproductive isolation between the two species is not absolute. Taxonomic revision of *D. potatorum* (*sensu lato*), to recognise *D. lentus* as a distinct species, will inform conservation planning, sustainable harvesting assessments and predictions of how these species may respond to future environmental and ecological changes.

Along with genetic differences, the recognition of *D. lentus* as a separate species was supported by differences in morphological and ecological characteristics. *Durvillaea potatorum* (*sensu stricto*) was typically found to occur in shallower water and display a proportionately longer, thicker and stouter stipe than *D. lentus*. Although no single distinguishing trait could be identified to separate the species, overall, the differences suggest that *D. lentus* and *D. potatorum* have acquired unique ecological strategies to persist on the highly wave-disturbed rocky coasts of Tasmania. Specifically, in contrast to *D. potatorum*, *D. lentus* exhibits a less responsive phenotype to ecological conditions, a greater investment in repair mechanisms and a thinner, more flexible stipe suited to the slightly deeper water in which it is found. Therefore, the morphological and ecological findings only partially support the second and third hypotheses. With respect to within-site distribution, *D. potatorum* was more common at shallower depths than *D. lentus*; however, this was a relative difference, as both species were found at all depths. The presence of morphological and histological differences was less pronounced than anticipated, with overlapping phenotypes preventing definitive characteristics from being identified between the two species. Although further differences may be found (e.g. temperature tolerance), these are not required to recognise *D. potatorum* and *D. lentus* as separate species and are not, traits that would allow easy identification of either species in the field.

The findings from this study also allowed inferences to be made about the processes involved in the divergence and speciation of *D. potatorum* and *D. lentus*. The current spatial distribution of genetic diversity across coastal south-east Australia, and estimated divergence timing, suggest these species separated due to the geological and oceanic conditions that

occurred at the end of the Pliocene and throughout the Pleistocene. Allopatric speciation during glaciation periods when sea levels and ocean currents changed, was reinforced by ecological and stochastic diversification within the two isolated species. Despite subsequent range expansion and strong ecological and morphological similarities, gene flow remained limited and eventually resulted in speciation.

Studying the divergence of *D. potatorum* and *D. lentus* has also offered insight into the processes underlying both the generation and retention of biodiversity. Specifically, this research has highlighted how environmental conditions, geographical isolation and small-scale variation in disturbance regimes drive divergence and speciation. Species subject to high levels of disturbance may experience an investment trade-off between phenotypic durability and plasticity, as seen in the different strategies of *D. potatorum* and *D. lentus* to withstand high wave-disturbance. Additionally, this research demonstrated that both long-term gradual changes in climate and geology, along with short-term fluctuations in disturbance and environmental stress, can support sympatric species, constrain phenotypes and maintain cryptic diversity. The full potential of environmental and ecological variability across spatial and temporal scales in creating biodiversity is likely to have been underestimated.

Decoding the genetics, morphology and ecology of a seemingly ‘cryptic’ species of brown macroalgae has revealed a new species and offered insights into the hidden mechanisms of evolution.

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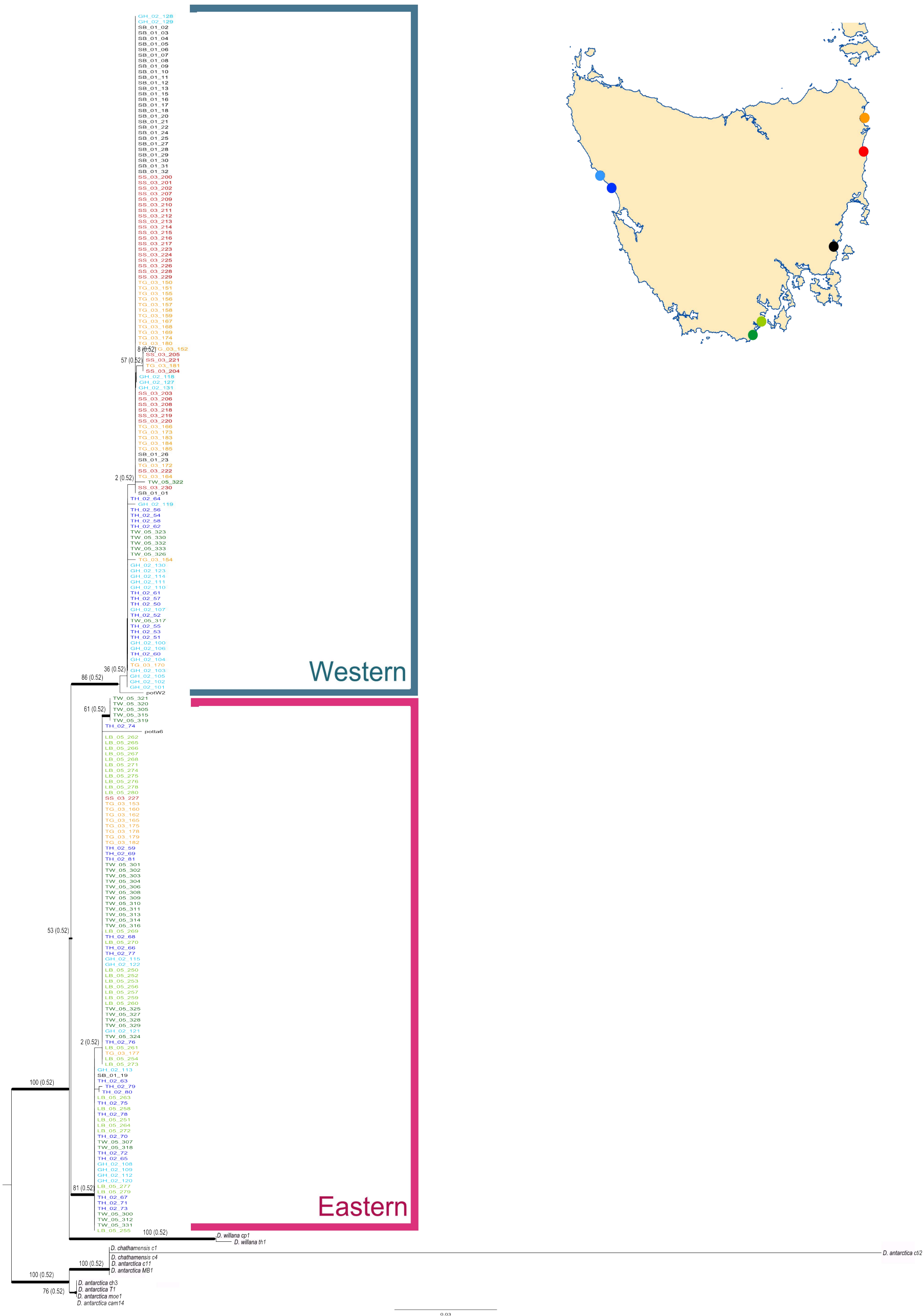
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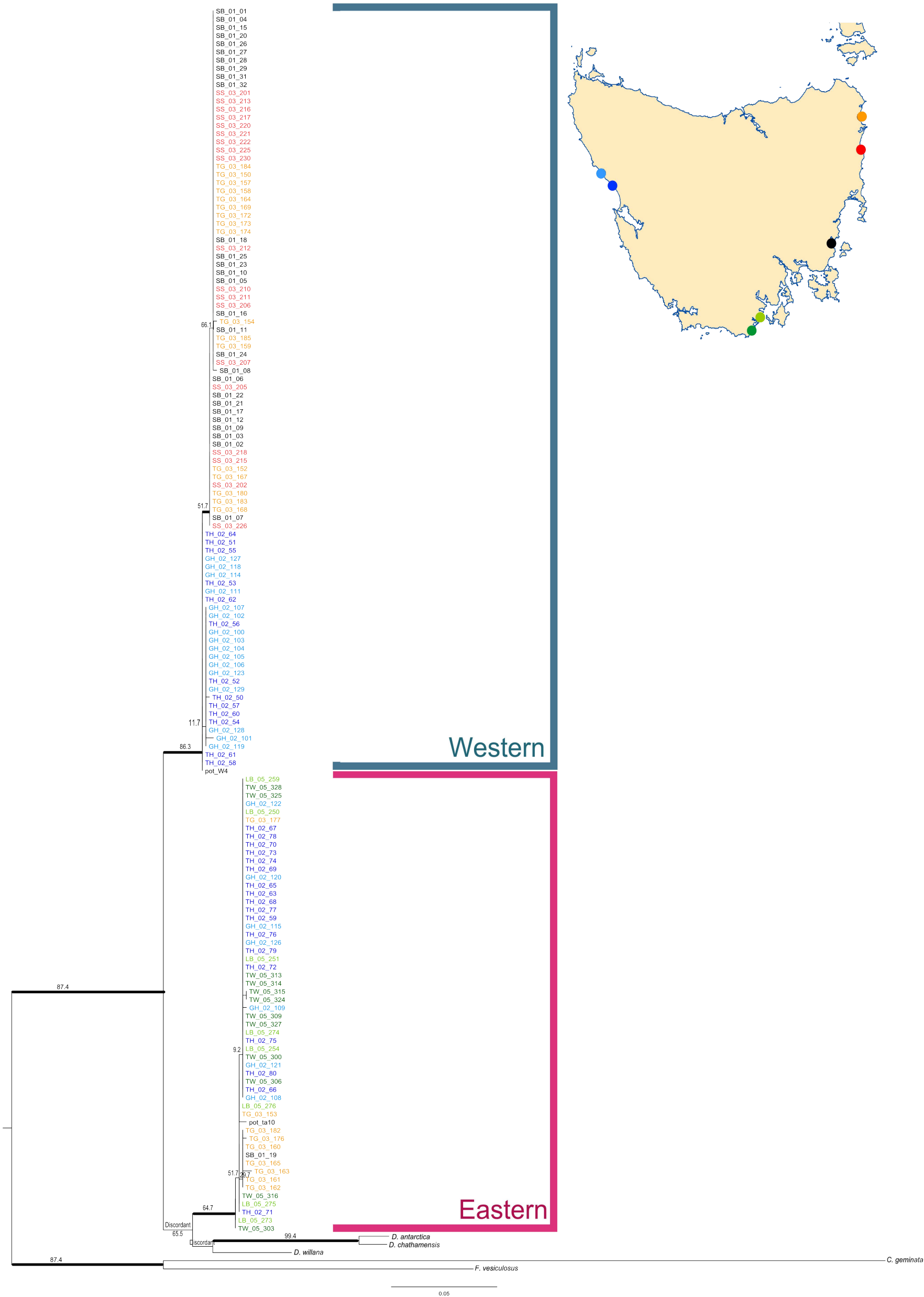
Appendix A: Genbank sequences

Species	Marker	Accession number
<i>Cystoseira geminata</i>	COI	FJ409137.1
<i>Durvillaea antarctica</i>	COI	FJ550109.1
	28S	HQ386055.1
		HQ386056.1
		HQ386057.1
		HQ386058.1
		HQ386060.1
		HQ386061.1
		HQ386062.1
<i>Durvillaea chathamensis</i>		HQ386063.1
	COI	HQ386080.1
	28S	HQ386064.1
<i>Durvillaea potatorum</i> (‘eastern’)		HQ386065.1
	COI	FJ873095.1
<i>Durvillaea potatorum</i> (‘western’)	28S	HQ386072.1
	COI	FJ873098.1
<i>Durvillaea willana</i>	28S	HQ386074.1
	COI	HQ386132.1
	28S	HQ386078.1
<i>Fucus vesiculosus</i>		HQ386079.1
	COI	EU646754.1

Appendix B: Complete 28S phylogeny



Appendix C: Complete COI phylogeny



Electronic Appendix A: R script

```
#Required packages
library(aod)
library(SoDA)
library(plyr)
library(psych)
library(ade4)
library(vegan)
library(cluster)
library(gclus)
library(FD)
library(RColorBrewer)
library(labdsv)
library(vegan3d)
library(ggplot2)
library("plspm")
library("plsdepot")
library(geomorph)
library(scatterplot3d)

#Load and prepare data
data<-read.csv("RData.csv")
nonedata<-read.csv("RDatanone.csv")
head(nonedata)

#Summary data
xtabs(~Lineage+Site+Development, data=data)
xtabs(~Lineage+Exposure, data=data)
xtabs(~Lineage+Development, data=data)
xtabs(~Lineage+Conjoin, data=data)
xtabs(~Lineage+Branch, data=data)
xtabs(~Lineage+Depth, data=data)

#Boxplots
bpdata<-nonedata[,c(1:22)]
bpdata$Latitude<-bpdata$Latitude*(-1)
colourscheme<-c("#009ACD", "#C71585", "#D73027")
palette(colourscheme)
bpadult<-bpdata[bpdata$Development=="Adult",]
bpjuv<-bpdata[bpdata$Development=="Juvenile",]
totwest<-bpdata[bpdata$Lineage=="West",]
toteast<-bpdata[bpdata$Lineage=="East",]
SB<-bpdata[bpdata$Site=="Spring Beach",]
SBwest<-totwest[totwest$Site=="Spring Beach",]
SBeast<-toteast[toteast$Site=="Spring Beach",]
TH<-bpdata[bpdata$Site=="Trial Harbour",]
THwest<-totwest[totwest$Site=="Trial Harbour",]
THEast<-toteast[toteast$Site=="Trial Harbour",]
GH<-bpdata[bpdata$Site=="Granville Harbour",]
GHwest<-totwest[totwest$Site=="Granville Harbour",]
GHeast<-toteast[toteast$Site=="Granville Harbour",]
TG<-bpdata[bpdata$Site=="The Gardens",]
TGwest<-totwest[totwest$Site=="The Gardens",]
TGeast<-toteast[toteast$Site=="The Gardens",]
SS<-bpdata[bpdata$Site=="Secret Spot",]
```

```

SSwest<-totwest[totwest$Site=="Secret Spot",]
SSeast<-toteast[toteast$Site=="Secret Spot",]
LB<-bpdata[bpdata$Site=="Lady's Bay",]
LBwest<-totwest[totwest$Site=="Lady's Bay",]
LBeast<-toteast[toteast$Site=="Lady's Bay",]
TW<-bpdata[bpdata$Site=="The Whale",]
TWwest<-totwest[totwest$Site=="The Whale",]
TWeast<-toteast[toteast$Site=="The Whale",]
WB<-bpdata[bpdata$Site=="Waubs Bay",]
WBwest<-totwest[totwest$Site=="Waubs Bay",]
WBeast<-toteast[toteast$Site=="Waubs Bay",]
GI<-bpdata[bpdata$Site=="Governor Island",]
GIwest<-totwest[totwest$Site=="Governor Island",]
Gleast<-toteast[toteast$Site=="Governor Island",]
BB<-bpdata[bpdata$Site=="Binalong Bay",]
BBwest<-totwest[totwest$Site=="Binalong Bay",]
BBeast<-toteast[toteast$Site=="Binalong Bay",]
CB<-bpdata[bpdata$Site=="Cloudy Bay",]
CBwest<-totwest[totwest$Site=="Cloudy Bay",]
CBeast<-toteast[toteast$Site=="Cloudy Bay",]
totwestad<-bpadult[bpadult$Lineage=="West",]
toteastad<-bpadult[bpadult$Lineage=="East",]
SBad<-bpadult[bpadult$Site=="Spring Beach",]
SBwestad<-totwestad[totwestad$Site=="Spring Beach",]
SBeastad<-toteastad[toteastad$Site=="Spring Beach",]
THad<-bpadult[bpadult$Site=="Trial Harbour",]
THwestad<-totwestad[totwestad$Site=="Trial Harbour",]
THEastad<-toteastad[toteastad$Site=="Trial Harbour",]
GHad<-bpadult[bpadult$Site=="Granville Harbour",]
GHwestad<-totwestad[totwestad$Site=="Granville Harbour",]
GHEastad<-toteastad[toteastad$Site=="Granville Harbour",]
TGad<-bpadult[bpadult$Site=="The Gardens",]
TGwestad<-totwestad[totwestad$Site=="The Gardens",]
TGeastad<-toteastad[toteastad$Site=="The Gardens",]
SSad<-bpadult[bpadult$Site=="Secret Spot",]
SSwestad<-totwestad[totwestad$Site=="Secret Spot",]
SSeastad<-toteastad[toteastad$Site=="Secret Spot",]
LBad<-bpadult[bpadult$Site=="Lady's Bay",]
LBwestad<-totwestad[totwestad$Site=="Lady's Bay",]
LBeastad<-toteastad[toteastad$Site=="Lady's Bay",]
TWad<-bpadult[bpadult$Site=="The Whale",]
TWwestad<-totwestad[totwestad$Site=="The Whale",]
TWeastad<-toteastad[toteastad$Site=="The Whale",]
WBad<-bpadult[bpadult$Site=="Waubs Bay",]
WBwestad<-totwestad[totwestad$Site=="Waubs Bay",]
WBeastad<-toteastad[toteastad$Site=="Waubs Bay",]
Glad<-bpadult[bpadult$Site=="Governor Island",]
GIwestad<-totwestad[totwestad$Site=="Governor Island",]
Gleastad<-toteastad[toteastad$Site=="Governor Island",]
BBad<-bpadult[bpadult$Site=="Binalong Bay",]
BBwestad<-totwestad[totwestad$Site=="Binalong Bay",]
BBeastad<-toteastad[toteastad$Site=="Binalong Bay",]
CBad<-bpadult[bpadult$Site=="Cloudy Bay",]
CBwestad<-totwestad[totwestad$Site=="Cloudy Bay",]
CBeastad<-toteastad[toteastad$Site=="Cloudy Bay",]
totwestjuv<-bpjuv[bpjuv$Lineage=="West",]
toteastjuv<-bpjuv[bpjuv$Lineage=="East",]

```

```

SBjuv<-bpjuv[bpjuv$Site=="Spring Beach",]
SBwestjuv<-totwestjuv[totwestjuv$Site=="Spring Beach",]
SBeastjuv<-toteastjuv[toteastjuv$Site=="Spring Beach",]
THjuv<-bpjuv[bpjuv$Site=="Trial Harbour",]
THwestjuv<-totwestjuv[totwestjuv$Site=="Trial Harbour",]
THEastjuv<-toteastjuv[toteastjuv$Site=="Trial Harbour",]
GHjuv<-bpjuv[bpjuv$Site=="Granville Harbour",]
GHwestjuv<-totwestjuv[totwestjuv$Site=="Granville Harbour",]
GHeastjuv<-toteastjuv[toteastjuv$Site=="Granville Harbour",]
TGjuv<-bpjuv[bpjuv$Site=="The Gardens",]
TGwestjuv<-totwestjuv[totwestjuv$Site=="The Gardens",]
TGeastjuv<-toteastjuv[toteastjuv$Site=="The Gardens",]
SSjuv<-bpjuv[bpjuv$Site=="Secret Spot",]
SSwestjuv<-totwestjuv[totwestjuv$Site=="Secret Spot",]
SSeastjuv<-toteastjuv[toteastjuv$Site=="Secret Spot",]
LBjuv<-bpjuv[bpjuv$Site=="Lady's Bay",]
LBwestjuv<-totwestjuv[totwestjuv$Site=="Lady's Bay",]
LBeastjuv<-toteastjuv[toteastjuv$Site=="Lady's Bay",]
TWjuv<-bpjuv[bpjuv$Site=="The Whale",]
TWwestjuv<-totwestjuv[totwestjuv$Site=="The Whale",]
TWeastjuv<-toteastjuv[toteastjuv$Site=="The Whale",]
WBjuv<-bpjuv[bpjuv$Site=="Waubs Bay",]
WBwestjuv<-totwestjuv[totwestjuv$Site=="Waubs Bay",]
WBeastjuv<-toteastjuv[toteastjuv$Site=="Waubs Bay",]
GIjuv<-bpjuv[bpjuv$Site=="Governor Island",]
GIwestjuv<-totwestjuv[totwestjuv$Site=="Governor Island",]
Gleastjuv<-toteastjuv[toteastjuv$Site=="Governor Island",]
BBjuv<-bpjuv[bpjuv$Site=="Binalong Bay",]
BBwestjuv<-totwestjuv[totwestjuv$Site=="Binalong Bay",]
BBeastjuv<-toteastjuv[toteastjuv$Site=="Binalong Bay",]
CBjuv<-bpjuv[bpjuv$Site=="Cloudy Bay",]
CBwestjuv<-totwestjuv[totwestjuv$Site=="Cloudy Bay",]
CBeastjuv<-toteastjuv[toteastjuv$Site=="Cloudy Bay",]
bpdata$Lineage=factor(bpdata$Lineage, c("West", "East"))
tiff("1OpennessLineageBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw",
res=300)
boxplot(Openness~Lineage, data=bpdata, col=(c("#009ACD", "#C71585")), xlab="Lineage",
ylab="Openness (Fetch Score)")
bpdata$Latitude<-bpdata$Latitude*(-1)
dev.off()
tiff("2LatitudeLineageBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw",
res=300)
boxplot(Latitude~Lineage, data=bpdata, col=(c("#009ACD", "#C71585")), xlab="Lineage",
ylab="Latitude")
dev.off()
bpdata$Latitude<-bpdata$Latitude*(-1)
levels(bpdata$Site)<-
c(levels(bpdata$Site), "SB", "TH", "GH", "TG", "SS", "LB", "TW", "WB", "GI", "BB", "CB")
bpdata$Site[bpdata$Site=="Spring Beach"]<-"SB"
bpdata$Site[bpdata$Site=="Trial Harbour"]<-"TH"
bpdata$Site[bpdata$Site=="Granville Harbour"]<-"GH"
bpdata$Site[bpdata$Site=="The Gardens"]<-"TG"
bpdata$Site[bpdata$Site=="Secret Spot"]<-"SS"
bpdata$Site[bpdata$Site=="Lady's Bay"]<-"LB"
bpdata$Site[bpdata$Site=="The Whale"]<-"TW"
bpdata$Site[bpdata$Site=="Waubs Bay"]<-"WB"
bpdata$Site[bpdata$Site=="Governor Island"]<-"GI"

```

```

bpdata$Site[bpdata$Site=="Binalong Bay"]<-"BB"
bpdata$Site[bpdata$Site=="Cloudy Bay"]<-"CB"
levels(bpdata$Lineage)<-c(levels(bpdata$Lineage),"West","East")
bpdata$Lineage[bpdata$Lineage=="West"]<-"West"
bpdata$Lineage[bpdata$Lineage=="East"]<-"East"
bpdata<-droplevels(bpdata)
bpadult<-bpdata[bpdata$Development=="Adult",]
bpjuv<-bpdata[bpdata$Development=="Juvenile",]
tiff("3LongitudeLineageBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw",
res=300)
boxplot(Longitude~Lineage, data=bpdata, col=(c("#009ACD","#C71585")), xlab="Lineage",
ylab="Longitude")
dev.off()
tiff("4DepthLineageBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Depth~Lineage*Site, data=bpdata, col=(c("#009ACD","#C71585")), xlab="Site and
Lineage", ylab="Depth (m)", las=2, cex.axis=0.7)
dev.off()
tiff("5TlengthLineageBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw",
res=300)
boxplot(Tlength~Lineage*Site, data=bpdata, col=(c("#009ACD","#C71585")), xlab="Site and
Lineage", ylab="Total Length (cm)", las=2, cex.axis=0.55)
dev.off()
tiff("6SlengthLineageBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw",
res=300)
boxplot(Slength~Lineage*Site, data=bpdata, col=(c("#009ACD","#C71585")), xlab="Site and
Lineage", ylab="Stipe Length (cm)", las=2, cex.axis=0.55)
dev.off()
tiff("7StlengthLineageBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw",
res=300)
boxplot(Stlength~Lineage*Site, data=bpdata, col=(c("#009ACD","#C71585")), xlab="Site and
Lineage", ylab="Stipe:Total Length", las=2, cex.axis=0.55)
dev.off()
tiff("8BcirLineageBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Bcir~Lineage*Site, data=bpdata, col=(c("#009ACD","#C71585")), xlab="Site and
Lineage", ylab="Stipe Base Circumference (cm)", las=2, cex.axis=0.55)
dev.off()
tiff("9TcirLineageBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Tcir~Lineage*Site, data=bpdata, col=(c("#009ACD","#C71585")), xlab="Site and
Lineage", ylab="Stipe Top Circumference (cm)", las=2, cex.axis=0.55)
dev.off()
tiff("10BcirLineageBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Bcirlen~Lineage*Site, data=bpdata, col=(c("#009ACD","#C71585")), xlab="Site and
Lineage", ylab=" Base Stipe Circumference:Stipe Length", las=2, cex.axis=0.55)
dev.off()
tiff("11TcirlenLineageBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw",
res=300)
boxplot(Tcirlen~Lineage*Site, data=bpdata, col=(c("#009ACD","#C71585")), xlab="Site and
Lineage", ylab=" Top Stipe Circumference:Stipe Length", las=2, cex.axis=0.55)
dev.off()
tiff("12McirlenLineageBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw",
res=300)
boxplot(Mcirlen~Lineage*Site, data=bpdata, col=(c("#009ACD","#C71585")), xlab="Site and
Lineage", ylab=" Mean Stipe Circumference:Stipe Length", las=2, cex.axis=0.55)
dev.off()
tiff("13TaperLineageBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Taper~Lineage*Site, data=bpdata, col=(c("#009ACD","#C71585")), xlab="Site and
Lineage", ylab="Stipe Tapering (Stipe Top/Base Circumference)", las=2, cex.axis=0.55)

```

```

dev.off()
tiff("14SdiffLineageBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Sdiff~Lineage*Site, data=bpdata, col=(c("#009ACD", "#C71585")), xlab="Site and Lineage", ylab="Difference in # Secondary Stipes", las=2, cex.axis=0.55)
dev.off()
tiff("15StotLineageBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Stot~Lineage*Site, data=bpdata, col=(c("#009ACD", "#C71585")), xlab="Site and Lineage", ylab="Total number of secondary stipes", las=2, cex.axis=0.55)
dev.off()
tiff("16OpennessAdultBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Openness~Lineage, data=bpadult, col=(c("#009ACD", "#C71585")), xlab="Lineage", ylab="Openness (Fetch Score)")
dev.off()
tiff("17LatitudeAdultBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
bpadult$Latitude<-bpadult$Latitude*(-1)
boxplot(Latitude~Lineage, data=bpadult, col=(c("#009ACD", "#C71585")), xlab="Lineage", ylab="Latitude")
dev.off()
bpadult$Latitude<-bpadult$Latitude*(-1)
tiff("19LongitudeAdultBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Longitude~Lineage, data=bpadult, col=(c("#009ACD", "#C71585")), xlab="Lineage", ylab="Longitude")
dev.off()
tiff("20DepthAdultBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Depth~Lineage*Site, data=bpadult, col=(c("#009ACD", "#C71585")), xlab="Site and Lineage", ylab="Depth (m)", las=2, cex.axis=0.55)
dev.off()
tiff("21TlengthAdultBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Tlength~Lineage*Site, data=bpadult, col=(c("#009ACD", "#C71585")), xlab="Site and Lineage", ylab="Total Length (cm)", las=2, cex.axis=0.55)
dev.off()
tiff("22SlengthAdultBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Slength~Lineage*Site, data=bpadult, col=(c("#009ACD", "#C71585")), xlab="Site and Lineage", ylab="Stipe Length (cm)", las=2, cex.axis=0.55)
dev.off()
tiff("23StlengthAdultBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Stlength~Lineage*Site, data=bpadult, col=(c("#009ACD", "#C71585")), xlab="Site and Lineage", ylab="Stipe:Total Length", las=2, cex.axis=0.55)
dev.off()
tiff("24BcirAdultBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Bcir~Lineage*Site, data=bpadult, col=(c("#009ACD", "#C71585")), xlab="Site and Lineage", ylab="Stipe Base Circumference (cm)", las=2, cex.axis=0.55)
dev.off()
tiff("25TcirAdultBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Tcir~Lineage*Site, data=bpadult, col=(c("#009ACD", "#C71585")), xlab="Site and Lineage", ylab="Stipe Top Circumference (cm)", las=2, cex.axis=0.55)
dev.off()
tiff("26BcirlenAdultBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Bcirlen~Lineage*Site, data=bpadult, col=(c("#009ACD", "#C71585")), xlab="Site and Lineage", ylab="Base Stipe Circumference:Stipe Length", las=2, cex.axis=0.55)
dev.off()
tiff("27TcirlenAdultBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)

```

```

boxplot(Tcirlen~Lineage*Site, data=bpadult, col=(c("#009ACD", "#C71585")), xlab="Site and
Lineage", ylab=" Top Stipe Circumference:Stipe Length", las=2, cex.axis=0.55)
dev.off()
tiff("28McirlenAdultBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Mcirlen~Lineage*Site, data=bpadult, col=(c("#009ACD", "#C71585")), xlab="Site and
Lineage", ylab=" Mean Stipe Circumference:Stipe Length", las=2, cex.axis=0.55)
dev.off()
tiff("29TaperAdultBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Taper~Lineage*Site, data=bpadult, col=(c("#009ACD", "#C71585")), xlab="Site and
Lineage", ylab="Stipe Tapering (Stipe Top/Base Circumference)", las=2, cex.axis=0.55)
dev.off()
tiff("30SdiffAdultBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Sdiff~Lineage*Site, data=bpadult, col=(c("#009ACD", "#C71585")), xlab="Site and
Lineage", ylab="Difference in # Secondary Stipes", las=2, cex.axis=0.55)
dev.off()
tiff("31StotAdultLineageBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw",
res=300)
boxplot(Stot~Lineage*Site, data=bpadult, col=(c("#009ACD", "#C71585")), xlab="Site and
Lineage", ylab="Total number of secondary stipes", las=2, cex.axis=0.55)
dev.off()
tiff("32OpennessJuvBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Openness~Lineage, data=bpjuv, col=(c("#009ACD", "#C71585")), xlab="Lineage",
ylab="Openness (Fetch Score)")
bpjuv$Latitude<-bpjuv$Latitude*(-1)
dev.off()
tiff("33LatitudejuvBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Latitude~Lineage, data=bpjuv, col=(c("#009ACD", "#C71585")), xlab="Lineage",
ylab="Latitude")
bpjuv$Latitude<-bpjuv$Latitude*(-1)
dev.off()
tiff("34LongitudejuvBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Longitude~Lineage, data=bpjuv, col=(c("#009ACD", "#C71585")), xlab="Lineage",
ylab="Longitude")
dev.off()
tiff("35DepthjuvBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Depth~Lineage*Site, data=bpjuv, col=(c("#009ACD", "#C71585")), xlab="Site and
Lineage", ylab="Depth (m)", las=2, cex.axis=0.55)
dev.off()
tiff("36TlengthjuvBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Tlength~Lineage*Site, data=bpjuv, col=(c("#009ACD", "#C71585")), xlab="Site and
Lineage", ylab="Total Length (cm)", las=2, cex.axis=0.55)
dev.off()
tiff("37SlengthjuvBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Slength~Lineage*Site, data=bpjuv, col=(c("#009ACD", "#C71585")), xlab="Site and
Lineage", ylab="Stipe Length (cm)", las=2, cex.axis=0.55)
dev.off()
tiff("38StlengthjuvBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Stlength~Lineage*Site, data=bpjuv, col=(c("#009ACD", "#C71585")), xlab="Site and
Lineage", ylab="Stipe:Total Length", las=2, cex.axis=0.55)
dev.off()
tiff("39BcirjuvBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Bcir~Lineage*Site, data=bpjuv, col=(c("#009ACD", "#C71585")), xlab="Site and
Lineage", ylab="Stipe Base Circumference (cm)", las=2, cex.axis=0.55)
dev.off()
tiff("40TcirjuvBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Tcir~Lineage*Site, data=bpjuv, col=(c("#009ACD", "#C71585")), xlab="Site and
Lineage", ylab="Stipe Top Circumference (cm)", las=2, cex.axis=0.55)

```



```

dev.off()
tiff("41BcirlenjuvBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Bcirlen~Lineage*Site, data=bpjuv, col=(c("#009ACD", "#C71585")), xlab="Site and
Lineage", ylab=" Base Stipe Circumference:Stipe Length", las=2, cex.axis=0.55)
dev.off()
tiff("42TcirlenjuvBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Tcirlen~Lineage*Site, data=bpjuv, col=(c("#009ACD", "#C71585")), xlab="Site and
Lineage", ylab=" Top Stipe Circumference:Stipe Length", las=2, cex.axis=0.55)
dev.off()
tiff("43McirlenjuvBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Mcirlen~Lineage*Site, data=bpjuv, col=(c("#009ACD", "#C71585")), xlab="Site and
Lineage", ylab=" Mean Stipe Circumference:Stipe Length", las=2, cex.axis=0.55)
dev.off()
tiff("44TaperjuvBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Taper~Lineage*Site, data=bpjuv, col=(c("#009ACD", "#C71585")), xlab="Site and
Lineage", ylab="Stipe Tapering (Stipe Top/Base Circumference)", las=2, cex.axis=0.55)
dev.off()
tiff("45SdiffjuvBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Sdiff~Lineage*Site, data=bpjuv, col=(c("#009ACD", "#C71585")), xlab="Site and
Lineage", ylab="Difference in # Secondary Stipes", las=2, cex.axis=0.55)
dev.off()
tiff("46StotjuvBoxPlot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
boxplot(Stot~Lineage*Site, data=bpjuv, col=(c("#009ACD", "#C71585")), xlab="Site and
Lineage", ylab="Total number of secondary stipes", las=2, cex.axis=0.55)
dev.off()
SBwestadexp<-as.data.frame(prop.table(table(SBwestad$Exposure)))
colnames(SBwestadexp)<-c("exposure", "SBwestpercent")
SBeastadexp<-as.data.frame(prop.table(table(SBeastad$Exposure)))
colnames(SBeastadexp)<-c("exposure", "SBeastpercent")
THwestadexp<-as.data.frame(prop.table(table(THwestad$Exposure)))
colnames(THwestadexp)<-c("exposure", "THwestpercent")
THEastadexp<-as.data.frame(prop.table(table(THeastad$Exposure)))
colnames(THeastadexp)<-c("exposure", "THeastpercent")
GHwestadexp<-as.data.frame(prop.table(table(GHwestad$Exposure)))
colnames(GHwestadexp)<-c("exposure", "GHwestpercent")
GHeastadexp<-as.data.frame(prop.table(table(GHeastad$Exposure)))
colnames(GHeastadexp)<-c("exposure", "GHeastpercent")
TGwestadexp<-as.data.frame(prop.table(table(TGwestad$Exposure)))
colnames(TGwestadexp)<-c("exposure", "TGwestpercent")
TGeastadexp<-as.data.frame(prop.table(table(TGeastad$Exposure)))
colnames(TGeastadexp)<-c("exposure", "TGeastpercent")
SSwestadexp<-as.data.frame(prop.table(table(SSwestad$Exposure)))
colnames(SSwestadexp)<-c("exposure", "SSwestpercent")
SSeastadexp<-as.data.frame(prop.table(table(SSeastad$Exposure)))
colnames(SSeastadexp)<-c("exposure", "SSeastpercent")
LBwestadexp<-as.data.frame(prop.table(table(LBwestad$Exposure)))
colnames(LBwestadexp)<-c("exposure", "LBwestpercent")
LBeastadexp<-as.data.frame(prop.table(table(LBeastad$Exposure)))
colnames(LBeastadexp)<-c("exposure", "LBeastpercent")
TWwestadexp<-as.data.frame(prop.table(table(TWwestad$Exposure)))
colnames(TWwestadexp)<-c("exposure", "TWwestpercent")
TWeastadexp<-as.data.frame(prop.table(table(TWeastad$Exposure)))
colnames(TWeastadexp)<-c("exposure", "TWeastpercent")
THwestadexp<-as.data.frame(prop.table(table(THwestad$Exposure)))
colnames(THwestadexp)<-c("exposure", "THwestpercent")
THeastadexp<-as.data.frame(prop.table(table(THeastad$Exposure)))
colnames(THeastadexp)<-c("exposure", "THeastpercent")

```

```

exposureprop<-merge(GHeastadexp,GHwestadexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,LBeastadexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,SSwestadexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,SBeastadexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,SBwestadexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,TGeastadexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,TGwestadexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,TWeastadexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,TWwestadexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,THEastadexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,THwestadexp, by="exposure", all=TRUE)
exposureprop[is.na(exposureprop)]<-0
exposurepercent<-exposureprop[-1,-1]
droplevels(exposurepercent)
tiff("47ExposureAdultPlot.tiff", height=6, width=8.4, units='in', compression="lzw", res=300)
par(mar=c(5.5,5.5,4.1,2.1),mgp=c(4,1,0))
barplot(as.matrix(exposurepercent), las=2, col=(c("slateblue4","steelblue3","skyblue","snow")),
legend.text=(c(1,2,3,4)), cex.names=0.555, xlab="Site", ylab="Proportion", args.legend = list(x
= "topright", bty = "n", inset=c(-0.05, 0)), names=c("GH East","GH West","LB East","SS
West","SB East","SB West", "TG East","TG West", "TW East","TW West", "TH East", "TH
West"), space=c(1,0.5,1.5,1.5,1.5,0.5,1.5,0.5,1.5,0.5,1.5,0.5)),
dev.off()
# insert names and spaces names=c("GH East","GH West","LB East","SS West","SB East","SB
West", "TG East","TG West", "TW East","TW West","TH East", "TH West")
space=c(1,0.5,1.5,1.5,1.5,0.5,1.5,0.5,1.5,0.5,1.5,0.5),
SBwestjuvexp<-as.data.frame(prop.table(table(SBwestjuv$Exposure)))
colnames(SBwestjuvexp)<-c("exposure","SBwestpercent")
THwestjuvexp<-as.data.frame(prop.table(table(THwestjuv$Exposure)))
colnames(THwestjuvexp)<-c("exposure","THwestpercent")
THEastjuvexp<-as.data.frame(prop.table(table(THeastjuv$Exposure)))
colnames(THeastjuvexp)<-c("exposure","THEastpercent")
GHwestjuvexp<-as.data.frame(prop.table(table(GHwestjuv$Exposure)))
colnames(GHwestjuvexp)<-c("exposure","GHwestpercent")
GHeastjuvexp<-as.data.frame(prop.table(table(GHeastjuv$Exposure)))
colnames(GHeastjuvexp)<-c("exposure","GHeastpercent")
TGwestjuvexp<-as.data.frame(prop.table(table(TGwestjuv$Exposure)))
colnames(TGwestjuvexp)<-c("exposure","TGwestpercent")
TGeastjuvexp<-as.data.frame(prop.table(table(TGeastjuv$Exposure)))
colnames(TGeastjuvexp)<-c("exposure","TGeastpercent")
SSwestjuvexp<-as.data.frame(prop.table(table(SSwestjuv$Exposure)))
colnames(SSwestjuvexp)<-c("exposure","SSwestpercent")
SSeastjuvexp<-as.data.frame(prop.table(table(SSeastjuv$Exposure)))
colnames(SSeastjuvexp)<-c("exposure","SSeastpercent")
LBwestjuvexp<-as.data.frame(prop.table(table(LBwestjuv$Exposure)))
colnames(LBwestjuvexp)<-c("exposure","LBwestpercent")
LBeastjuvexp<-as.data.frame(prop.table(table(LBeastjuv$Exposure)))
colnames(LBeastjuvexp)<-c("exposure","LBeastpercent")
TWeastjuvexp<-as.data.frame(prop.table(table(TWeastjuv$Exposure)))
colnames(TWeastjuvexp)<-c("exposure","TWeastpercent")
THwestjuvexp<-as.data.frame(prop.table(table(THwestjuv$Exposure)))
colnames(THwestjuvexp)<-c("exposure","THwestpercent")
THeastjuvexp<-as.data.frame(prop.table(table(THeastjuv$Exposure)))
colnames(THeastjuvexp)<-c("exposure","THeastpercent")
exposureprop<-merge(GHeastjuvexp,GHwestjuvexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,LBeastjuvexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,SSwestjuvexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,SBwestjuvexp, by="exposure", all=TRUE)

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exposureprop<-merge(exposureprop,TGeastjuvexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,TGwestjuvexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,TWeastjuvexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,THEastjuvexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,THwestjuvexp, by="exposure", all=TRUE)
exposureprop[is.na(exposureprop)]<-0
exposurepercent<-exposureprop[-1,-1]
droplevels(exposurepercent)
tiff("48ExposureJuvPlot.tiff", height=6, width=8.4, units='in', compression="lzw", res=300)
par(mar=c(5.5,5.5,4.1,2.1),mgp=c(4,1,0))
barplot(as.matrix(exposurepercent), las=2, col=(c("slateblue4","steelblue3","skyblue","snow")),
legend.text=(c(1,2,3,4)), cex.names=0.555, xlab="Site", ylab="Proportion", args.legend = list(x
= "topright", bty = "n", inset=c(-0.05, 0)), names=c("GH East","GH West","LB East","SS
West","SB West", "TG East","TG West", "TW East", "TH East", "TH West"),
space=c(1,0.5,1.5,1.5,1.5,1.5,0.5,1.5,1.5,0.5))
dev.off()
# insert names and spaces names=c("GH East","GH West","LB East","SS West","SB East","SB
West", "TG East","TG West", "TW East","TW West","TH East", "TH West")
space=c(1,0.5,1.5,1.5,1.5,0.5,1.5,0.5,1.5,0.5,1.5,0.5),
SBwestexp<-as.data.frame(prop.table(table(SBwest$Exposure)))
colnames(SBwestexp)<-c("exposure","SBwestpercent")
SBeastexp<-as.data.frame(prop.table(table(SBeast$Exposure)))
colnames(SBeastexp)<-c("exposure","SBeastpercent")
THwestexp<-as.data.frame(prop.table(table(THwest$Exposure)))
colnames(THwestexp)<-c("exposure","THwestpercent")
THEastexp<-as.data.frame(prop.table(table(THeast$Exposure)))
colnames(THeastexp)<-c("exposure","THeastpercent")
GHwestexp<-as.data.frame(prop.table(table(GHwest$Exposure)))
colnames(GHwestexp)<-c("exposure","GHwestpercent")
GHeastexp<-as.data.frame(prop.table(table(GHeast$Exposure)))
colnames(GHeastexp)<-c("exposure","GHeastpercent")
TGwestexp<-as.data.frame(prop.table(table(TGwest$Exposure)))
colnames(TGwestexp)<-c("exposure","TGwestpercent")
TGeastexp<-as.data.frame(prop.table(table(TGeast$Exposure)))
colnames(TGeastexp)<-c("exposure","TGeastpercent")
SSwestexp<-as.data.frame(prop.table(table(SSwest$Exposure)))
colnames(SSwestexp)<-c("exposure","SSwestpercent")
SSeastexp<-as.data.frame(prop.table(table(SSeast$Exposure)))
colnames(SSeastexp)<-c("exposure","SSeastpercent")
LBwestexp<-as.data.frame(prop.table(table(LBwest$Exposure)))
colnames(LBwestexp)<-c("exposure","LBwestpercent")
LBeastexp<-as.data.frame(prop.table(table(LBeast$Exposure)))
colnames(LBeastexp)<-c("exposure","LBeastpercent")
TWwestexp<-as.data.frame(prop.table(table(TWwest$Exposure)))
colnames(TWwestexp)<-c("exposure","TWwestpercent")
TWeastexp<-as.data.frame(prop.table(table(TWeast$Exposure)))
colnames(TWeastexp)<-c("exposure","TWeastpercent")
THwestexp<-as.data.frame(prop.table(table(THwest$Exposure)))
colnames(THwestexp)<-c("exposure","THwestpercent")
THeastexp<-as.data.frame(prop.table(table(THeast$Exposure)))
colnames(THeastexp)<-c("exposure","THeastpercent")
exposureprop<-merge(GHeastexp,GHwestexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,LBeastexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,SSwestexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,SBeastexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,SBwestexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,TGeastexp, by="exposure", all=TRUE)

```

```

exposureprop<-merge(exposureprop,TGwestexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,TWeastexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,TWwestexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,THEastexp, by="exposure", all=TRUE)
exposureprop<-merge(exposureprop,THwestexp, by="exposure", all=TRUE)
exposureprop[is.na(exposureprop)]<-0
exposurepercent<-exposureprop[-1,-1]
droplevels(exposurepercent)
tiff("49ExposurePlot.tiff", height=6, width=8.4, units='in', compression="lzw", res=300)
par(mar=c(5.5,5.5,4.1,2.1),mgp=c(4,1,0))
barplot(as.matrix(exposurepercent), las=2, col=(c("slateblue4","steelblue3","skyblue","snow")),
legend.text=(c(1,2,3,4)), cex.names=0.555, xlab="Site", ylab="Proportion", args.legend = list(x
= "topright", bty = "n", inset=c(-0.05, 0)), names=c("GH East","GH West","LB East","SS
West","SB East","SB West", "TG East","TG West", "TW East","TW West", "TH East", "TH
West"), space=c(1,0.5,1.5,1.5,1.5,0.5,1.5,0.5,1.5,0.5,1.5,0.5))
dev.off()

# insert names and spaces names=c("GH East","GH West","LB East","SS West","SB East","SB
West", "TG East","TG West", "TW East","TW West","TH East", "TH West")
space=c(1,0.5,1.5,1.5,1.5,0.5,1.5,0.5,1.5,0.5,1.5,0.5),
conjoin<-function(x) {
  conjoinprop<-as.data.frame(prop.table(table(x[,21])))
  conjoinprop<-conjoinprop[2,2]*100
}
SBwestconjoin<-conjoin(SBwest)
SBeastconjoin<-conjoin(SBeast)
THwestconjoin<-conjoin(THwest)
THEastconjoin<-conjoin(THeast)
GHwestconjoin<-conjoin(GHwest)
GHeastconjoin<-conjoin(GHeast)
TGwestconjoin<-conjoin(TGwest)
TGeastconjoin<-conjoin(TGeast)
LBwestconjoin<-conjoin(LBwest)
LBeastconjoin<-conjoin(LBeast)
SSwestconjoin<-conjoin(SSwest)
SSeastconjoin<-conjoin(SSeast)
TWwestconjoin<-conjoin(TWwest)
TWeastconjoin<-conjoin(TWeast)
WBwestconjoin<-conjoin(WBwest)
BBwestconjoin<-conjoin(BBwest)
sitesconjoined<-
c(GHeastconjoin,GHwestconjoin,LBeastconjoin,SSeastconjoin,SSwestconjoin,SBeastconjoin,S
Bwestconjoin,TGeastconjoin,TGwestconjoin,TWeastconjoin,TWwestconjoin,THeastconjoin,T
Hwestconjoin, WBwestconjoin,BBwestconjoin)
conjoined<-t(as.data.frame(sitesconjoined))
colnames(conjoined)<-c("BB West", "GH East", "GH West", "LB East", "SS East", "SS West", "SB
East", "SB West", "TG East", "TG West", "TW East", "TW West", "TH East", "TH West", "WB
West")
conjoined[is.na(conjoined)]<-0
tiff("50ConjoinPlot.tiff", height=6, width=8.4, units='in', compression="lzw", res=300)
barplot(conjoined, las=2, beside=TRUE, cex.names=0.555, xlab="Site by Lineage",
ylab="Conjoined (%)", ylim=c(0,100),
col=c("#C71585","#009ACD","#C71585","#009ACD","#009ACD","#C71585","#009ACD","#
C71585","#009ACD","#C71585","#009ACD","#C71585","#009ACD","#C71585","#C71585"),
space=c(1,1.5,0.5,1.5,1.5,0.5,1.5,0.5,1.5,0.5,1.5,0.5,1.5,0.5,1.5))
dev.off()
conjoin<-function(x) {

```

```

conjoinprop<-as.data.frame(prop.table(table(x[,21])))
conjoinprop<-(conjoinprop[2,2])*100
}
SBwestconjoin<-conjoin(SBwestad)
SBeastconjoin<-conjoin(SBeastad)
THwestconjoin<-conjoin(THwestad)
THEastconjoin<-conjoin(THeastad)
GHwestconjoin<-conjoin(GHwestad)
GHeastconjoin<-conjoin(GHeastad)
TGwestconjoin<-conjoin(TGwestad)
TGeastconjoin<-conjoin(TGeastad)
LBwestconjoin<-conjoin(LBwestad)
LBeastconjoin<-conjoin(LBeastad)
SSwestconjoin<-conjoin(SSwestad)
SSeastconjoin<-conjoin(SSeastad)
TWwestconjoin<-conjoin(TWwestad)
TWeastconjoin<-conjoin(TWeastad)
WBwestconjoin<-conjoin(WBwestad)
BBwestconjoin<-conjoin(BBwestad)
sitesconjoined<-
c(GHeastconjoin,GHwestconjoin,LBeastconjoin,SSwestconjoin,SBwestconjoin,TGeastconjoin,
TGwestconjoin,TWwestconjoin,TWeastconjoin,
                                THEastconjoin,THwestconjoin,
WBwestconjoin, BBwestconjoin)
conjoined<-t(as.data.frame(sitesconjoined))
colnames(conjoined)<-c("BB West", "GH East", "GH West", "LB East", "SS West", "SB
West","TG East","TG West","TW East", "TW West", "TH East", "TH West", "WB West")
conjoined[is.na(conjoined)]<-0
tiff("51ConjoinAdultPlot.tiff", height=6, width=8.4, units='in', compression="lzw", res=300)
barplot(conjoined, las=2, cex.names=0.555, beside=TRUE, xlab="Site by Lineage",
ylab="Conjoined (%)", col=c("#C71585","#009ACD","#C71585","#009ACD","#C71585",
"#C71585",
                                "#009ACD","#C71585",
"#009ACD","#C71585","#009ACD","#C71585","#C71585"), ylim=c(0,100), space=c(1,1.5,
0.5,1.5,1.5,1.5,1.5,0.5,1.5,0.5,1.5,0.5,1.5) )
dev.off()
branch<-function(x) {
  branchprop<-as.data.frame(prop.table(table(x[,22])))
  branchprop<-branchprop[2,2]*100
}
SBwestbranch<-branch(SBwest)
SBeastbranch<-branch(SBeast)
THwestbranch<-branch(THwest)
THEastbranch<-branch(THeast)
GHwestbranch<-branch(GHwest)
GHeastbranch<-branch(GHeast)
TGwestbranch<-branch(TGwest)
TGeastbranch<-branch(TGeast)
LBeastbranch<-branch(LBeast)
SSwestbranch<-branch(SSwest)
SSeastbranch<-branch(SSeast)
TWwestbranch<-branch(TWwest)
TWeastbranch<-branch(TWeast)
WBwestbranch<-branch(WBwest)
BBwestbranch<-branch(BBwest)
sitesbranch<-c(BBwestbranch,
GHeastbranch,GHwestbranch,LBeastbranch,SSeastbranch,SSwestbranch,SBeastbranch,SBwest
branch,TGeastbranch,TGwestbranch,TWeastbranch,TWwestbranch,THEastbranch,THwestbranc
h, WBwestbranch)

```

```

branched<-t(as.data.frame(sitesbranch))
colnames(branched)<-c("BB West","GH East","GH West","LB East","SS East","SS West","SB
East","SB West","TG East","TG West","TW East","TW West","TH East","TH West","WB
West")
branched[is.na(branched)]<-0
tiff("52BranchPlot.tiff", height=6, width=8.4, units='in', compression="lzw", res=300)
barplot(branched, las=2, cex.names=0.555, beside=TRUE, xlab="Site by Lineage",
ylab="Branched (%)", ylim=c(0,40),
col=c("#C71585","#009ACD","#C71585","#009ACD","#009ACD","#C71585","#009ACD","#
C71585","#009ACD","#C71585","#009ACD","#C71585","#009ACD","#C71585","#C71585"),
space=c(1,1.5,0.5,1.5,1.5,0.5,1.5,0.5,1.5,0.5,1.5,0.5,1.5,1.5,1.5) )
dev.off()
branch<-function(x) {
  branchprop<-as.data.frame(prop.table(table(x[,22])))
  branchprop<-branchprop[2,2]*100
}
SBwestbranch<-branch(SBwestad)
SBeastbranch<-branch(SBeastad)
THwestbranch<-branch(THwestad)
THEastbranch<-branch(THEastad)
GHwestbranch<-branch(GHwestad)
GHeastbranch<-branch(GHeastad)
TGwestbranch<-branch(TGwestad)
TGeastbranch<-branch(TGeastad)
LBwestbranch<-branch(LBwestad)
LBeastbranch<-branch(LBeastad)
SSwestbranch<-branch(SSwestad)
SSeastbranch<-branch(SSeastad)
TWwestbranch<-branch(TWwestad)
TWeastbranch<-branch(TWeastad)
WBwestbranch<-branch(WBwestad)
BBwestbranch<-branch(BBwestad)
sitesbranched<-
c(GHeastbranch,GHwestbranch,LBeastbranch,SSwestbranch,SBeastbranch,SBwestbranch,TGe
astbranch,TGwestbranch,TWeastbranch,TWwestbranch,THEastbranch,THwestbranch,BBwestbr
anch,WBwestbranch)
branched<-t(as.data.frame(sitesbranched))
colnames(branched)<-c("BB West","GH East","GH West","LB East","SS West","SB East","SB
West","TG East","TG West","TW East","TW West","TH East","TH West","WB West")
branched[is.na(branched)]<-0
tiff("53BranchAdultPlot.tiff", height=6, width=8.4, units='in', compression="lzw", res=300)
barplot(branched, las=2, cex.names=0.555, beside=TRUE, xlab="Site by Lineage",
ylab="Branched (%)", ylim=c(0,40),
col=c("#C71585","#009ACD","#C71585","#009ACD","#C71585","#009ACD","#C71585","#0
09ACD","#C71585","#009ACD","#C71585","#009ACD","#C71585","#C71585"),
space=c(1,1.5,0.5,1.5,1.5,0.5,1.5,0.5,1.5,0.5,1.5,0.5,1.5,1.5,1.5) )
dev.off()

#Univariate analyses using original data
univdata<-nonedata
sitefactor<-as.factor(univdata$Site)
tiff("54VariableCorrelationPlot.tiff", height=11.8, width=11.8, units='in', compression="lzw",
res=300)
pairs(univdata[,c(7,9:19)])
dev.off()

#Transform long and lat

```

```

geotrans<-geoXY(univdata$Latitude, univdata$Longitude)
univdata$Latitude<-geotrans[,1]
univdata$Longitude<-geotrans[,2]

#Linear regression Lineage+Site (Longitude, Latitude, Openness, Exposure, Detph, Tlength,
Slength, Stlength, Bcir, Tcir,Bcirln, Tcirln, Mcirlen, Taper)
sitereg<-univdata[,c(2:5,7,9:20)]
layout(matrix(1:4,2,2))
mfrow=c(2,2)
siteopen<-lm(Openness~Lineage+Site, data=sitereg)
summary(siteopen)
logLik(siteopen)
tiff("55AOpenRegression.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(siteopen)
dev.off()
wald.test(b=coef(siteopen), Sigma=vcov(siteopen), Terms=c(3))
sitedepth<-lm(Depth~Lineage+Site, data=sitereg)
summary(sitedepth)
logLik(sitedepth)
tiff("55DepthRegression.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitedepth)
dev.off()
wald.test(b=coef(sitedepth), Sigma=vcov(sitedepth), Terms=c(3))
sitetlen<-lm(Tlength~Lineage+Site, data=sitereg)
summary(sitetlen)
logLik(sitetlen)
tiff("56TlenRegression.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitetlen)
dev.off()
wald.test(b=coef(sitetlen), Sigma=vcov(sitetlen), Terms=c(3))
siteslen<-lm(Slength~Lineage+Site, data=sitereg)
summary(siteslen)
logLik(siteslen)
tiff("57SlengRegression.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(siteslen)
dev.off()
wald.test(b=coef(siteslen), Sigma=vcov(siteslen), Terms=c(3))
sitestlen<-lm(Stlength~Lineage+Site, data=sitereg)
summary(sitestlen)
logLik(sitestlen)
tiff("58StlenRegression.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitestlen)
dev.off()
wald.test(b=coef(sitestlen), Sigma=vcov(sitestlen), Terms=c(3))
sitebsc<-lm(Bcir~Lineage+Site, data=sitereg)
summary(sitebsc)
logLik(sitebsc)

```

```

tiff("59BscirRegression.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitebsc)
dev.off()
wald.test(b=coef(sitebsc), Sigma=vcov(sitebsc), Terms=c(3))
sitetsc<-lm(Tcir~Lineage+Site, data=sitereg)
summary(sitetsc)
logLik(sitetsc)
tiff("60TscirhRegression.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitetsc)
dev.off()
wald.test(b=coef(sitetsc), Sigma=vcov(sitetsc), Terms=c(3))
sitebscl<-lm(Bcirlen~Lineage+Site, data=sitereg)
summary(sitebscl)
logLik(sitebscl)
tiff("61BSclRegression.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitebscl)
dev.off()
wald.test(b=coef(sitebscl), Sigma=vcov(sitebscl), Terms=c(3))
sitestcl<-lm(Tcirlen~Lineage+Site, data=sitereg)
summary(sitestcl)
logLik(sitestcl)
tiff("62TclRegression.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitestcl)
dev.off()
wald.test(b=coef(sitestcl), Sigma=vcov(sitestcl), Terms=c(3))
sitescl<-lm(Mcirlen~Lineage+Site, data=sitereg)
summary(sitescl)
logLik(sitescl)
tiff("63MSclRegression.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitescl)
dev.off()
wald.test(b=coef(sitescl), Sigma=vcov(sitescl), Terms=c(3))
sitestap<-lm(Taper~Lineage+Site, data=sitereg)
summary(sitestap)
logLik(sitestap)
tiff("64TaperRegression.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitestap)
dev.off()
wald.test(b=coef(sitestap), Sigma=vcov(sitestap), Terms=c(3))
#Quasi-poisson glm (Sdiff, Stot)
siteSdiff<-glm(Sdiff~Lineage+Site, data=sitereg, family=quasipoisson(log))
summary(siteSdiff)
logLik(siteSdiff)
tiff("65SdiffRegression.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
layout(matrix(1:4,2,2))

```



```

mfrow=c(2,2)
plot(siteSdiff)
dev.off()
wald.test(b=coef(siteSdiff), Sigma=vcov(siteSdiff), Terms=c(3))
sitestot<-glm(Stot~Lineage+Site, data=sitereg, family=quasipoisson(log))
summary(sitestot)
logLik(sitestot)
tiff("66StotRegression.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitestot)
dev.off()
wald.test(b=coef(sitestot), Sigma=vcov(sitestot), Terms=c(3))
#Logistic (Conjoin, Branch)
spotreg<-univdata[,c(2,5,8,21:22)]
sitecon<-glm(Site~Conjoin, data=spotreg, family=binomial)
summary(sitecon)
sitebran<-glm(Site~Branch, data=spotreg, family=binomial)
summary(sitebran)
linereg<-univdata[,c(5,8,21:22)]
lineexp<-glm(Lineage~Exposure, data=linereg, family=binomial)
summary(lineexp)
linecon<-glm(Lineage~Conjoin, data=linereg, family=binomial)
summary(linecon)
linebran<-glm(Lineage~Branch, data=linereg, family=binomial)
summary(linebran)

#Linear regression Lineage+Site TRANSFORMED (Longitude, Latitude, Openness, Exposure,
Detph, Tlength, Slength, Stlength, Bcir, Tcir,Bcirln, Tcirln, Mcirlen, Taper)
sitereg<-univdata[,c(2:5,7,9:20)]
totrans<-c("Tlength","Slength","Bcir","Tcir","Stot","Sdiff")
siteregtrans<-sitereg[,c(7,8,10,11,16,17)]
trans<-log1p(siteregtrans)
excluddata<-sitereg[,c(1:6,9,12:15)]
siteregtrans2<-cbind(excluddata,trans)
siteregtrans<-siteregtrans2
layout(matrix(1:4,2,2))
mfrow=c(2,2)
sitetlen<-lm(Tlength~Lineage+Site, data=siteregtrans)
summary(sitetlen)
logLik(sitetlen)
tiff("56TlenRegressiontrans.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitetlen)
dev.off()
wald.test(b=coef(sitetlen), Sigma=vcov(sitetlen), Terms=c(3))
siteslen<-lm(Slength~Lineage+Site, data=siteregtrans)
summary(siteslen)
logLik(siteslen)
tiff("57SlengRegressiontrans.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(siteslen)
dev.off()

```

```

wald.test(b=coef(siteslen), Sigma=vcov(siteslen), Terms=c(3))
sitebsc<-lm(Bcir~Lineage+Site, data=siteregtrans)
summary(sitebsc)
logLik(sitebsc)
tiff("59BscirRegressiontrans.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitebsc)
dev.off()
wald.test(b=coef(sitebsc), Sigma=vcov(sitebsc), Terms=c(3))
sitetesc<-lm(Tcir~Lineage+Site, data=siteregtrans)
summary(sitetesc)
logLik(sitetesc)
tiff("60TscirRegressiontrans.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitetesc)
dev.off()
wald.test(b=coef(sitetesc), Sigma=vcov(sitetesc), Terms=c(3))
#Quasi-poisson glm (Sdiff, Stot) TRANSFORMED
siteSdiff<-glm(Sdiff~Lineage+Site, data=siteregtrans, family=quasipoisson(log))
summary(siteSdiff)
logLik(siteSdiff)
tiff("65SdiffRegressiontrans.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(siteSdiff)
dev.off()
wald.test(b=coef(siteSdiff), Sigma=vcov(siteSdiff), Terms=c(3))
sitestot<-glm(Stot~Lineage+Site, data=siteregtrans, family=quasipoisson(log))
summary(sitestot)
logLik(sitestot)
tiff("66StotRegressiontrans.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitestot)
dev.off()
wald.test(b=coef(sitestot), Sigma=vcov(sitestot), Terms=c(3))

#Linear regression Lineage (Longitude, Latitude, Openness, Exposure, Detph, Tlength, Slength,
Stlength, Bcir, Tcir, Bcirlen, Tcirlen, Mcirlen, Taper)
univdata<-data
sitefactor<-as.factor(univdata$Site)
geotrans<-geoXY(univdata$Latitude, univdata$Longitude)
univdata$Latitude<-geotrans[,1]
univdata$Longitude<-geotrans[,2]
sitereg<-univdata[,c(2:5,7,9:20)]
layout(matrix(1:4,2,2))
mfrow=c(2,2)
siteopen<-lm(Openness~Lineage, data=sitereg)
summary(siteopen)
logLik(siteopen)

```

```

tiff("55AOpenRegressionLineage.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(siteopen)
dev.off()
sitedepth<-lm(Depth~Lineage, data=sitereg)
summary(sitedepth)
logLik(sitedepth)
tiff("55DepthRegressionLineage.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitedepth)
dev.off()
sitetlen<-lm(Tlength~Lineage, data=sitereg)
summary(sitetlen)
logLik(sitetlen)
tiff("56TlenRegressionLineage.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitetlen)
dev.off()
siteslen<-lm(Slength~Lineage, data=sitereg)
summary(siteslen)
logLik(siteslen)
tiff("57SlengRegressionLineage.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(siteslen)
dev.off()
sitestlen<-lm(Stlength~Lineage, data=sitereg)
summary(sitestlen)
logLik(sitestlen)
tiff("58StlenRegressionLineage.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitestlen)
dev.off()
sitebsc<-lm(Bcir~Lineage, data=sitereg)
summary(sitebsc)
logLik(sitebsc)
tiff("59BscirRegressionLineage.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitebsc)
dev.off()
sitetsc<-lm(Tcir~Lineage, data=sitereg)
summary(sitetsc)
logLik(sitetsc)
tiff("60TscirhRegressionLineage.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))

```

```

mfrow=c(2,2)
plot(sitetsc)
dev.off()
sitebscl<-lm(Bcirlen~Lineage, data=sitereg)
summary(sitebscl)
logLik(sitebscl)
tiff("61BSclRegressionLineage.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitebscl)
dev.off()
sitestcl<-lm(Tcirlen~Lineage, data=sitereg)
summary(sitestcl)
logLik(sitestcl)
tiff("62TclRegressionLineage.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitestcl)
dev.off()
sitescl<-lm(Mcirlen~Lineage, data=sitereg)
summary(sitescl)
logLik(sitescl)
tiff("63MSclRegressionLineage.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitescl)
dev.off()
sitestap<-lm(Taper~Lineage, data=sitereg)
summary(sitestap)
logLik(sitestap)
tiff("64TaperRegressionLineage.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitestap)
dev.off()

#Quasi-poisson glm (Sdiff, Stot)
siteSdiff<-glm(Sdiff~Lineage, data=sitereg, family=quasipoisson(log))
summary(siteSdiff)
logLik(siteSdiff)
tiff("65SdiffRegressionLineage.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(siteSdiff)
dev.off()
sitestot<-glm(Stot~Lineage, data=sitereg, family=quasipoisson(log))
summary(sitestot)
logLik(sitestot)
tiff("66StotRegressionLineage.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)

```

```

plot(sitestot)
dev.off()

#Site regression Site (Longitude, Latitude, Openness, Exposure, Detph, Tlength, Slength,
Stlength, Bcir, Tcir, Bcir, Tcir, Mcir, Tcir, Mcir, Tcir, Taper)
sitereg<-univdata[,c(2:5,7,9:20)]
layout(matrix(1:4,2,2))
mfrow=c(2,2)
sitedepth<-lm(Depth~Site, data=sitereg)
summary(sitedepth)
logLik(sitedepth)
tiff("55DepthRegressionSite.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitedepth)
dev.off()
wald.test(b=coef(sitedepth), Sigma=vcov(sitedepth), Terms=c(3))
sitetlen<-lm(Tlength~Site, data=sitereg)
summary(sitetlen)
logLik(sitetlen)
tiff("56TlenRegressionSite.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitetlen)
dev.off()
wald.test(b=coef(sitetlen), Sigma=vcov(sitetlen), Terms=c(3))
siteslen<-lm(Slength~Site, data=sitereg)
summary(siteslen)
logLik(siteslen)
tiff("57SlengRegressionSite.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(siteslen)
dev.off()
wald.test(b=coef(siteslen), Sigma=vcov(siteslen), Terms=c(3))
sitestlen<-lm(Stlength~Site, data=sitereg)
summary(sitestlen)
logLik(sitestlen)
tiff("58StlenRegressionSite.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitestlen)
dev.off()
wald.test(b=coef(sitestlen), Sigma=vcov(sitestlen), Terms=c(3))
sitebsc<-lm(Bcir~Site, data=sitereg)
summary(sitebsc)
logLik(sitebsc)
tiff("59BscirRegressionSite.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitebsc)
dev.off()
wald.test(b=coef(sitebsc), Sigma=vcov(sitebsc), Terms=c(3))

```

```

sitetsc<-lm(Tcir~Site, data=sitereg)
summary(sitetsc)
logLik(sitetsc)
tiff("60TscirhRegressionSite.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitetsc)
dev.off()
wald.test(b=coef(sitetsc), Sigma=vcov(sitetsc), Terms=c(3))
sitebscl<-lm(Bcirlen~Site, data=sitereg)
summary(sitebscl)
logLik(sitebscl)
tiff("61BSclRegressionSite.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitebscl)
dev.off()
wald.test(b=coef(sitebscl), Sigma=vcov(sitebscl), Terms=c(3))
sitestcl<-lm(Tcirlen~Site, data=sitereg)
summary(sitestcl)
logLik(sitestcl)
tiff("62TclRegressionSite.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitestcl)
dev.off()
wald.test(b=coef(sitestcl), Sigma=vcov(sitestcl), Terms=c(3))
sitescl<-lm(Mcirlen~Site, data=sitereg)
summary(sitescl)
logLik(sitescl)
tiff("63MSclRegressionSite.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitescl)
dev.off()
wald.test(b=coef(sitescl), Sigma=vcov(sitescl), Terms=c(3))
sitestap<-lm(Taper~Site, data=sitereg)
summary(sitestap)
logLik(sitestap)
tiff("64TaperRegressionSite.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))
mfrow=c(2,2)
plot(sitestap)
dev.off()
wald.test(b=coef(sitestap), Sigma=vcov(sitestap), Terms=c(3))

#Quasi-poisson glm (Sdiff, Stot)
siteSdiff<-glm(Sdiff~Site, data=sitereg, family=quasipoisson(log))
summary(siteSdiff)
logLik(siteSdiff)
tiff("65SdiffRegressionSite.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
layout(matrix(1:4,2,2))

```

```

mfrow=c(2,2)
plot(siteSdiff)
dev.off()
wald.test(b=coef(siteSdiff), Sigma=vcov(siteSdiff), Terms=c(3))
sitestot<-glm(Stot~Site, data=sitereg, family=quasipoisson(log))
summary(sitestot)
logLik(sitestot)
tiff("66StotRegressionSite.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
layout(matrix(1:4,2,2))
plot(sitestot)
mfrow=c(2,2)
dev.off()
wald.test(b=coef(sitestot), Sigma=vcov(sitestot), Terms=c(3))

#Spatial autocorrelation checks
distdata<-read.csv("Distances.csv")
spatdata<-distdata[,c(2:3,8:19)]
meansumm<-ddply(spatdata, "Site", summarise,
  depth=mean(Depth),
  total=mean(Tlength),
  stipe=mean(Slength),
  stipetotal=mean(Stlength),
  base=mean(Bcir),
  top=mean(Tcir),
  bcirlength=mean(Bcirlen),
  tcirlength=mean(Tcirlen),
  cirlength=mean(Mcirlen),
  tapering=mean(Taper),
  diff=mean(Sdiff))
meansumm2<-ddply(spatdata, "Site", summarise,
  tottwo=mean(Stot),
  distance=mean(Distance),
  tapering=mean(Taper))
meansumm<-cbind(meansumm,meansumm2)
meansumm<-meansumm[,-11]
indiv<-(dist(meansumm$distance))
Tlength<-(dist(meansumm$total))
Tlength[is.na(Tlength)]<-0
mantel.rtest(Tlength, indiv, nrep=9999)
Slength<-(dist(meansumm$stipe))
Slength[is.na(Slength)]<-0
mantel.rtest(Slength, indiv, nrep=9999)
Stlength<-(dist(meansumm$stipetotal))
Stlength[is.na(Stlength)]<-0
mantel.rtest(Stlength, indiv, nrep=9999)
bcircum<-(dist(meansumm$base))
bcircum[is.na(bcircum)]<-0
mantel.rtest(bcircum, indiv, nrep=9999)
tcircumlength<-(dist(meansumm$top))
tcircumlength[is.na(tcircumlength)]<-0
mantel.rtest(tcircumlength, indiv, nrep=9999)
taper<-(dist(meansumm$tapering))
taper[is.na(taper)]<-0
mantel.rtest(taper, indiv, nrep=9999)
Sdiff<-(dist(meansumm$diff))
Sdiff[is.na(Sdiff)]<-0
mantel.rtest(Sdiff, indiv, nrep=9999)

```

```

stipetot<-(dist(meansumm$tottwo))
stipetot[is.na(stipetot)]<-0
mantel.rtest(stipetot, indiv, nrepet=9999)
bscirlength<-(dist(meansumm$bscirlength))
bscirlength[is.na(bscirlength)]<-0
mantel.rtest(bscirlength, indiv, nrepet=9999)
tscirlength<-(dist(meansumm$tscirlength))
tscirlength[is.na(tscirlength)]<-0
mantel.rtest(tscirlength, indiv, nrepet=9999)
scirlength<-(dist(meansumm$scirlength))
scirlength[is.na(scirlength)]<-0
mantel.rtest(scirlength, indiv, nrepet=9999)
spatdata<-data[,c(2:4,9:22)]
freqsumm<-ddply(spatdata, "Site", summarise,
                conjoin=((sum(Conjoin==1))/(length(Conjoin))),
                branch=((sum(Branch==1))/(length(Branch)))
)
Conjoin<-(dist(freqsumm$conjoin))
Conjoin[is.na(Conjoin)]<-0
mantel.rtest(Conjoin, indiv, nrepet=9999)
Branch<-(dist(freqsumm$branch))
Branch[is.na(Branch)]<-0
mantel.rtest(Branch, indiv, nrepet=9999)

#NMDS and ANOSIM 2 Dimensions
nadata<-data
geotrans<-geoXY(nadata$Latitude, nadata$Longitude)
nadata$Latitude<-geotrans[,1]
nadata$Longitude<-geotrans[,2]
total<-nadata[,c(1:275),c(1:22)]
totalwest<-total[total$Lineage=="West",]
totaleast<-total[total$Lineage=="East",]
totalhybrid<-total[total$Lineage=="Hybrid",]
totaljuv<-total[total$Develop=="Juvenile",]
totaladult<-total[total$Develop=="Adult",]
#then select relevant columns for meaningful variables
nmds<-c(9, 12, 13, 15, 18)
nmdstotal<-total[,nmds]
nmdstotalwest<-totalwest[,nmds]
nmdstotaleast<-totaleast[,nmds]
nmdstotalhybrid<-totalhybrid[,nmds]
nmdstotaljuv<-totaljuv[,nmds]
nmdstotaladult<-totaladult[,nmds]
#ensure Gower's categories of ordratio for a ratio, asymm for binary refer to the appropriate
columns
total.gow<-daisy(nmdstotal, metric="gower", stand=TRUE, type=list(ordratio=c(2,4,5)))
head(total.gow)
tiff("70Dissimilarity.tiff", height=16, width=23.6, units='in', compression="lzw", res=300)
coldiss(total.gow)
dev.off()
total.nmdsg<-metaMDS(total.gow, distance="gower")
total.nmdsgo<-metaMDS(total.gow, distance="gower", previous.best = total.nmdsg)
total.nmdsgow<-metaMDS(total.gow, distance="gower", previous.best=total.nmdsgo)
total.nmdsgow$stress
tiff("71Shepardplot.tiff", height=8, width=11.8, units='in', compression="lzw", res=300)
par(mfrow=c(1,1))
stressplot(total.nmdsgow)

```



```

dev.off()
colourscheme<-c("#C71585", "#698B22", "#009ACD")
palette(colourscheme)
nmdsforgow<-data.frame(total$Lineage, nmdstotal)
head(nmdsforgow)
#rename according to columns present
colnames(nmdsforgow)<-c("Lineage", "Depth", "Stlength", "Bcir", "Bcirlen", "Taper")
head(nmdsforgow)
tiff("72NMDStotal.tiff", height=8, width=11.8, units='in', compression="lzw", res=300)
plot(total.nmdsgow, type="n")
points(total.nmdsgow, display="sites", col=as.numeric(nmdsforgow$Lineage),
pch=as.numeric(nmdsforgow$Lineage))
colourcat<-sort(unique(nmdsforgow$Lineage))
for (i in seq(colourcat)){
  ordiellipse(total.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=FALSE, col=i, show.groups=colourcat[i])
}
for (i in seq(colourcat)){
  ordiellipse(total.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=TRUE, col=i, show.groups=colourcat[i])
}
dev.off()
tiff("73NMDStotalnumber.tiff", height=8, width=11.8, units='in', compression="lzw", res=300)
ordiplot(total.nmdsgow, type="n")
orditorp(total.nmdsgow, display="sites")
colourcat<-sort(unique(nmdsforgow$Lineage))
for (i in seq(colourcat)){
  ordiellipse(total.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=FALSE, col=i, show.groups=colourcat[i])
}
for (i in seq(colourcat)){
  ordiellipse(total.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=TRUE, col=i, show.groups=colourcat[i])
}
dev.off()
anosim.gow<-anosim(total.nmdsgow$points, c(total$Lineage, total$Lineage),
permutations=999, distance="euclidean")
summary(anosim.gow)
tiff("74ANOSIM.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
plot(anosim.gow)
dev.off()
adonis(total.gow~Lineage+Site, total, permutations=999)
totaladult.gow<-daisy(nmdstotaladult, metric="gower", stand=TRUE,
type=list(ordratio=c(2,4,5)))
head(totaladult.gow)
tiff("75Dissimilarityadult.tiff", height=16, width=23.6, units='in', compression="lzw", res=300)
coldiss(totaladult.gow)
dev.off()
totaladult.nmdsg<-metaMDS(totaladult.gow, distance="gower")
totaladult.nmdsgo<-metaMDS(totaladult.gow, distance="gower", previous.best =
totaladult.nmdsg)
totaladult.nmdsgow<-metaMDS(totaladult.gow, distance="gower",
previous.best=totaladult.nmdsgo)
totaladult.nmdsgow$stress
tiff("76ShepardAdult.tiff", height=8, width=11.8, units='in', compression="lzw", res=300)
par(mfrow=c(1,1))
stressplot(totaladult.nmdsgow)

```

```

dev.off()
nmdsforgow<-data.frame(totaladult$Lineage, nmdstotaladult)
head(nmdsforgow)
#rename according to columns present
colnames(nmdsforgow)<-c("Lineage", "Depth", "Stlength", "Bcir", "Bcirlen", "Taper")
head(nmdsforgow)
tiff("77NMDSAdult.tiff", height=8, width=11.8, units='in', compression="lzw", res=300)
plot(totaladult.nmdsgow, type="n")
points(totaladult.nmdsgow, display="sites", col=as.numeric(nmdsforgow$Lineage),
pch=as.numeric(nmdsforgow$Lineage))
colourcat<-sort(unique(nmdsforgow$Lineage))
for (i in seq(colourcat)){
  ordiellipse(totaladult.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=FALSE, col=i, show.groups=colourcat[i])
}
for (i in seq(colourcat)){
  ordiellipse(totaladult.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=TRUE, col=i, show.groups=colourcat[i])
}
dev.off()
tiff("78NMDSAdultnumb.tiff", height=8, width=11.8, units='in', compression="lzw", res=300)
ordiplot(totaladult.nmdsgow, type="n")
orditorp(totaladult.nmdsgow, display="sites")
colourcat<-sort(unique(nmdsforgow$Lineage))
for (i in seq(colourcat)){
  ordiellipse(totaladult.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=FALSE, col=i, show.groups=colourcat[i])
}
for (i in seq(colourcat)){
  ordiellipse(totaladult.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=TRUE, col=i, show.groups=colourcat[i])
}
dev.off()
anosim.gow<-anosim(totaladult.nmdsgow$points, c(totaladult$Lineage, totaladult$Lineage),
permutations=999, distance="euclidean")
summary(anosim.gow)
tiff("79ANOSIMadult.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
plot(anosim.gow)
dev.off()
adonis(totaladult.gow~Lineage+Site, totaladult, permutations=999)
totaljuv.gow<-daisy(nmdstotaljuv, metric="gower", stand=TRUE, type=list(ordratio=c(2,4,5)))
head(totaljuv.gow)
tiff("80Dissimilarityjuv.tiff", height=16, width=23.6, units='in', compression="lzw", res=300)
coldiss(totaljuv.gow)
dev.off()
totaljuv.nmdsg<-metaMDS(totaljuv.gow, distance="gower")
totaljuv.nmdsgo<-metaMDS(totaljuv.gow, distance="gower", previous.best = totaljuv.nmdsg)
totaljuv.nmdsgow<-metaMDS(totaljuv.gow, distance="gower", previous.best=totaljuv.nmdsgo)
totaljuv.nmdsgow$stress
tiff("81Shepardjuv.tiff", height=8, width=11.8, units='in', compression="lzw", res=300)
par(mfrow=c(1,1))
stressplot(totaljuv.nmdsgow)
dev.off()
nmdsforgow<-data.frame(totaljuv$Lineage, nmdstotaljuv)
head(nmdsforgow)
#rename according to columns present
colnames(nmdsforgow)<-c("Lineage", "Depth", "Stlength", "Bcir", "Bcirlen", "Taper")

```

```

head(nmdsforgow)
tiff("82NMDSjuv.tiff", height=8, width=11.8, units='in', compression="lzw", res=300)
plot(totaljuv.nmdsgow, type="n")
points(totaljuv.nmdsgow, display="sites", col=as.numeric(nmdsforgow$Lineage),
pch=as.numeric(nmdsforgow$Lineage))
colourcat<-sort(unique(nmdsforgow$Lineage))
for (i in seq(colourcat)){
  ordiellipse(totaljuv.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=FALSE, col=i, show.groups=colourcat[i])
}
for (i in seq(colourcat)){
  ordiellipse(totaljuv.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=TRUE, col=i, show.groups=colourcat[i])
}
dev.off()
tiff("83NMDSjuvnum.tiff", height=8, width=11.8, units='in', compression="lzw", res=300)
ordiplot(totaljuv.nmdsgow, type="n")
orditorp(totaljuv.nmdsgow, display="sites")
colourcat<-sort(unique(nmdsforgow$Lineage))
for (i in seq(colourcat)){
  ordiellipse(totaljuv.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=FALSE, col=i, show.groups=colourcat[i])
}
for (i in seq(colourcat)){
  ordiellipse(totaljuv.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=TRUE, col=i, show.groups=colourcat[i])
}
dev.off()
anosim.gow<-anosim(totaljuv.nmdsgow$points, c(totaljuv$Lineage, totaljuv$Lineage),
permutations=999, distance="euclidean")
summary(anosim.gow)
tiff("84ANOSIMjuv.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
plot(anosim.gow)
dev.off()
adonis.gow<-adonis(totaljuv.gow~Lineage+Site, totaljuv, permutations=999)
adonis.gow
#NMDS, ANOSIM Three dimensions
nadata<-data
geotrans<-geoXY(nadata$Latitude, nadata$Longitude)
nadata$Latitude<-geotrans[,1]
nadata$Longitude<-geotrans[,2]
total<-nadata[c(1:275),c(1:22)]
totalwest<-total[total$Lineage=="West",]
totaleast<-total[total$Lineage=="East",]
totaljuv<-total[total$Develop=="Juvenile",]
totaladult<-total[total$Develop=="Adult",]
#then select relevant columns for meaningful variables
nmds<-c(9, 12, 13, 15, 18)
nmdstotal<-total[,nmds]
nmdstotalwest<-totalwest[,nmds]
nmdstotaleast<-totaleast[,nmds]
nmdstotaljuv<-totaljuv[,nmds]
nmdstotaladult<-totaladult[,nmds]
#ensure Gower's categories of ordratio for a ratio, asymm for binary refer to the appropriate
columns
total.gow<-daisy(nmdstotal, metric="gower", stand=TRUE, type=list(ordratio=c(2,4,5)))
head(total.gow)

```

```

tiff("70Dissimilarity3d.tiff", height=16, width=23.6, units='in', compression="lzw", res=300)
coldiss(total.gow)
dev.off()
total.nmdsg<-metaMDS(total.gow, distance="gower", k=3)
total.nmdsgo<-metaMDS(total.gow, distance="gower", k=3, previous.best = total.nmdsg)
total.nmdsgow<-metaMDS(total.gow, distance="gower", k=3, previous.best=total.nmdsgo)
total.nmdsgow$stress
tiff("71Shepardplot3d.tiff", height=8, width=11.8, units='in', compression="lzw", res=300)
par(mfrow=c(1,1))
stressplot(total.nmdsgow)
dev.off()
colourscheme<-c("#C71585", "#698B22", "#009ACD")
palette(colourscheme)
nmdsforgow<-data.frame(total$Lineage, nmdstotal)
head(nmdsforgow)
#rename according to columns present
colnames(nmdsforgow)<-c("Lineage", "Depth", "Stlength", "Bcir", "Bcirlen", "Taper")
head(nmdsforgow)
tiff("72NMDStotal3d.tiff", height=8, width=11.8, units='in', compression="lzw", res=300)
plot(total.nmdsgow, type="n")
points(total.nmdsgow, display="sites", col=as.numeric(nmdsforgow$Lineage),
pch=as.numeric(nmdsforgow$Lineage))
colourcat<-sort(unique(nmdsforgow$Lineage))
for (i in seq(colourcat)){
  ordiellipse(total.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=FALSE, col=i, show.groups=colourcat[i])
}
for (i in seq(colourcat)){
  ordiellipse(total.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=TRUE, col=i, show.groups=colourcat[i])
}
dev.off()
tiff("73NMDStotalnumber3d.tiff", height=8, width=11.8, units='in', compression="lzw",
res=300)
ordiplot(total.nmdsgow, type="n")
orditorp(total.nmdsgow, display="sites")
colourcat<-sort(unique(nmdsforgow$Lineage))
for (i in seq(colourcat)){
  ordiellipse(total.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=FALSE, col=i, show.groups=colourcat[i])
}
for (i in seq(colourcat)){
  ordiellipse(total.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=TRUE, col=i, show.groups=colourcat[i])
}
dev.off()
anosim.gow<-anosim(total.nmdsgow$points, c(total$Lineage, total$Lineage),
permutations=999, distance="euclidean")
summary(anosim.gow)
tiff("74ANOSIM3d.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
plot(anosim.gow)
dev.off()
adonis(total.gow~Lineage, total, permutations=999, strata=total$Site)
totaladult.gow<-daisy(nmdstotaladult, metric="gower", stand=TRUE,
type=list(ordratio=c(2,4,5)))
head(totaladult.gow)

```

```

tiff("75Dissimilarityadult3d.tiff", height=16, width=23.6, units='in', compression="lzw",
res=300)
coldiss(totaladult.gow)
dev.off()
totaladult.nmdsg<-metaMDS(totaladult.gow, k=3, distance="gower")
totaladult.nmdsgo<-metaMDS(totaladult.gow, k=3, distance="gower", previous.best =
totaladult.nmdsg)
totaladult.nmdsgow<-metaMDS(totaladult.gow, k=3, distance="gower",
previous.best=totaladult.nmdsgo)
totaladult.nmdsgow$stress
tiff("76ShepardAdult3d.tiff", height=8, width=11.8, units='in', compression="lzw", res=300)
par(mfrow=c(1,1))
stressplot(totaladult.nmdsgow)
dev.off()
nmdsforgow<-data.frame(totaladult$Lineage, nmdstotaladult)
head(nmdsforgow)
#rename according to columns present
colnames(nmdsforgow)<-c("Lineage","Depth","Stlength","Bcir","Bcirlen","Taper")
head(nmdsforgow)
tiff("77NMDSAdult3d.tiff", height=8, width=11.8, units='in', compression="lzw", res=300)
plot(totaladult.nmdsgow, type="n")
points(totaladult.nmdsgow, display="sites", col=as.numeric(nmdsforgow$Lineage),
pch=as.numeric(nmdsforgow$Lineage))
colourcat<-sort(unique(nmdsforgow$Lineage))
for (i in seq(colourcat)){
ordiellipse(totaladult.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=FALSE, col=i, show.groups=colourcat[i])
}
for (i in seq(colourcat)){
ordiellipse(totaladult.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=TRUE, col=i, show.groups=colourcat[i])
}
dev.off()
tiff("78NMDSAdultnumb3d.tiff", height=8, width=11.8, units='in', compression="lzw",
res=300)
ordiplot(totaladult.nmdsgow, type="n")
orditorp(totaladult.nmdsgow, display="sites")
colourcat<-sort(unique(nmdsforgow$Lineage))
for (i in seq(colourcat)){
ordiellipse(totaladult.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=FALSE, col=i, show.groups=colourcat[i])
}
for (i in seq(colourcat)){
ordiellipse(totaladult.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=TRUE, col=i, show.groups=colourcat[i])
}
dev.off()
anosim.gow<-anosim(totaladult.nmdsgow$points, c(totaladult$Lineage, totaladult$Lineage),
permutations=999, distance="euclidean")
summary(anosim.gow)
tiff("79ANOSIMadult3d.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
plot(anosim.gow)
dev.off()
adonis(totaladult.gow~Lineage, totaladult, permutations=999, strata=totaladult$Site)
totaljuv.gow<-daisy(nmdstotaljuv, metric="gower", stand=TRUE, type=list(ordratio=c(2,4,5)))
head(totaljuv.gow)
tiff("80Dissimilarityjuv3d.tiff", height=16, width=23.6, units='in', compression="lzw", res=300)

```

```

coldiss(totaljuv.gow)
dev.off()
totaljuv.nmdsg<-metaMDS(totaljuv.gow, k=3, distance="gower")
totaljuv.nmdsgo<-metaMDS(totaljuv.gow, k=3, distance="gower", previous.best =
totaljuv.nmdsg)
totaljuv.nmdsgow<-metaMDS(totaljuv.gow, k=3, distance="gower",
previous.best=totaljuv.nmdsgo)
totaljuv.nmdsgow$stress
tiff("81Shepardjuv3d.tiff", height=8, width=11.8, units='in', compression="lzw", res=300)
par(mfrow=c(1,1))
stressplot(totaljuv.nmdsgow)
dev.off()
nmidsforgow<-data.frame(totaljuv$Lineage, nmids$totaljuv)
head(nmidsforgow)
#rename according to columns present
colnames(nmidsforgow)<-c("Lineage","Depth","sTlength","Bcir","Bcirlen","Taper")
head(nmidsforgow)
tiff("82NMDSjuv3d.tiff", height=8, width=11.8, units='in', compression="lzw", res=300)
plot(totaljuv.nmdsgow, type="n")
points(totaljuv.nmdsgow, display="sites", col=as.numeric(nmidsforgow$Lineage),
pch=as.numeric(nmidsforgow$Lineage))
colourcat<-sort(unique(nmidsforgow$Lineage))
for (i in seq(colourcat)){
ordiellipse(totaljuv.nmdsgow, nmidsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=FALSE, col=i, show.groups=colourcat[i])
}
for (i in seq(colourcat)){
ordiellipse(totaljuv.nmdsgow, nmidsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=TRUE, col=i, show.groups=colourcat[i])
}
dev.off()
tiff("83NMDSjuvnum3d.tiff", height=8, width=11.8, units='in', compression="lzw", res=300)
ordiplot(totaljuv.nmdsgow, type="n")
orditorp(totaljuv.nmdsgow, display="sites")
colourcat<-sort(unique(nmidsforgow$Lineage))
for (i in seq(colourcat)){
ordiellipse(totaljuv.nmdsgow, nmidsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=FALSE, col=i, show.groups=colourcat[i])
}
for (i in seq(colourcat)){
ordiellipse(totaljuv.nmdsgow, nmidsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=TRUE, col=i, show.groups=colourcat[i])
}
dev.off()
anosim.gow<-anosim(totaljuv.nmdsgow$points, c(totaljuv$Lineage, totaljuv$Lineage),
permutations=999, distance="euclidean")
summary(anosim.gow)
tiff("84ANOSIMjuv3d.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
plot(anosim.gow)
dev.off()
adonis.gow<-adonis(totaljuv.gow~Lineage, totaljuv, permutations=999, strata=totaljuv$Site)
adonis.gow
tiff("Totdispersal.tiff", height=8, width=16, units='in', compression="lzw", res=300)
totdis<-betadisper(total.gow, group=total$Site)
boxplot (totdis)
dev.off()
tiff("Adispersal.tiff", height=8, width=16, units='in', compression="lzw", res=300)

```

```

addis<-betadisper(totaladult.gow, group=totaladult$Site)
boxplot (addis)
dev.off()
tiff("Juvdispersal.tiff", height=8, width=16, units='in', compression="lzw", res=300)
juvdis<-betadisper(totaljuv.gow, group=totaljuv$Site)
boxplot (juvdis)
dev.off()

#NMDS, ANOSIM Three dimensions Excluding
nadata<-nonedata
geotrans<-geoXY(nadata$Latitude, nadata$Longitude)
nadata$Latitude<-geotrans[,1]
nadata$Longitude<-geotrans[,2]
total<-nadata[c(1:266),c(1:22)]
totalwest<-total[total$Lineage=="West",]
totaleast<-total[total$Lineage=="East",]
totaljuv<-total[total$Develop=="Juvenile",]
totaladult<-total[total$Develop=="Adult",]
#then select relevant columns for meaningful variables
nmlds<-c(9, 12, 13, 15, 18)
nmldstotal<-total[,nmlds]
nmldstotalwest<-totalwest[,nmlds]
nmldstotaleast<-totaleast[,nmlds]
nmldstotaljuv<-totaljuv[,nmlds]
nmldstotaladult<-totaladult[,nmlds]
#ensure Gower's categories of ordratio for a ratio, asymm for binary refer to the appropriate
columns
total.gow<-daisy(nmldstotal, metric="gower", stand=TRUE, type=list(ordratio=c(2,4,5)))
head(total.gow)
tiff("70Dissimilarity3d.tiff", height=16, width=23.6, units='in', compression="lzw", res=300)
coldiss(total.gow)
dev.off()
total.nmdsg<-metaMDS(total.gow, distance="gower", k=3)
total.nmdsgo<-metaMDS(total.gow, distance="gower", k=3, previous.best = total.nmdsg)
total.nmdsgow<-metaMDS(total.gow, distance="gower", k=3, previous.best=total.nmdsgo)
total.nmdsgow$stress
tiff("71Shepardplot3dnohybrid.tiff", height=8, width=11.8, units='in', compression="lzw",
res=300)
par(mfrow=c(1,1))
stressplot(total.nmdsgow)
dev.off()
colourscheme<-c("#C71585", "#009ACD", "#698B22")
palette(colourscheme)
nmldsfor Gow<-data.frame(total$Lineage, nmldstotal)
head(nmldsfor Gow)
#rename according to columns present
colnames(nmldsfor Gow)<-c("Lineage", "Depth", "Stlength", "Bcir", "Bcirlen", "Taper")
head(nmldsfor Gow)
tiff("72NMDS total3dnohybrid.tiff", height=8, width=11.8, units='in', compression="lzw",
res=300)
plot(total.nmdsgow, type="n")
points(total.nmdsgow, display="sites", col=as.numeric(nmldsfor Gow$Lineage),
pch=as.numeric(nmldsfor Gow$Lineage))
colourcat<-sort(unique(nmldsfor Gow$Lineage))
for (i in seq(colourcat)){
ordiellipse(total.nmdsgow, nmldsfor Gow$Lineage, display="sites", kind="sd", conf=0.95,
label=FALSE, col=i, show.groups=colourcat[i])

```

```

}
for (i in seq(colourcat)){
  ordiellipse(total.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=TRUE, col=i, show.groups=colourcat[i])
}
dev.off()
tiff("73NMDStotalnumber3dnohybrid.tiff", height=8, width=11.8, units='in',
compression="lzw", res=300)
ordiplot(total.nmdsgow, type="n")
orditorp(total.nmdsgow, display="sites")
colourcat<-sort(unique(nmdsforgow$Lineage))
for (i in seq(colourcat)){
  ordiellipse(total.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=FALSE, col=i, show.groups=colourcat[i])
}
for (i in seq(colourcat)){
  ordiellipse(total.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=TRUE, col=i, show.groups=colourcat[i])
}
dev.off()
anosim.gow<-anosim(total.nmdsgow$points, c(total$Lineage, total$Lineage),
permutations=999, distance="euclidean")
summary(anosim.gow)
tiff("74ANOSIM3dnohybrid.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
plot(anosim.gow)
dev.off()
adonis(total.gow~Lineage, total, permutations=999, strata=total$Site)
totaladult.gow<-daisy(nmdstotaladult, metric="gower", stand=TRUE,
type=list(ordratio=c(2,4,5)))
head(totaladult.gow)
tiff("75Dissimilarityadult3dnohybrid.tiff", height=16, width=23.6, units='in',
compression="lzw", res=300)
coldiss(totaladult.gow)
dev.off()
totaladult.nmdsg<-metaMDS(totaladult.gow, k=3, distance="gower")
totaladult.nmdsgo<-metaMDS(totaladult.gow, k=3, distance="gower", previous.best =
totaladult.nmdsg)
totaladult.nmdsgow<-metaMDS(totaladult.gow, k=3, distance="gower",
previous.best=totaladult.nmdsgo)
totaladult.nmdsgow$stress
tiff("76ShepardAdult3dnohybrid.tiff", height=8, width=11.8, units='in', compression="lzw",
res=300)
par(mfrow=c(1,1))
stressplot(totaladult.nmdsgow)
dev.off()
nmdsforgow<-data.frame(totaladult$Lineage, nmdstotaladult)
head(nmdsforgow)
#rename according to columns present
colnames(nmdsforgow)<-c("Lineage","Depth","Stlength","Bcir","Bcirlen","Taper")
head(nmdsforgow)
tiff("77NMDSAdult3dnohybrid.tiff", height=8, width=11.8, units='in', compression="lzw",
res=300)
plot(totaladult.nmdsgow, type="n")
points(totaladult.nmdsgow, display="sites", col=as.numeric(nmdsforgow$Lineage),
pch=as.numeric(nmdsforgow$Lineage))
colourcat<-sort(unique(nmdsforgow$Lineage))

```



```

for (i in seq(colourcat)){
  ordiellipse(totaladult.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=FALSE, col=i, show.groups=colourcat[i])
}
for (i in seq(colourcat)){
  ordiellipse(totaladult.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=TRUE, col=i, show.groups=colourcat[i])
}
dev.off()
tiff("78NMDSAdultnumb3dnohybrid.tiff", height=8, width=11.8, units='in', compression="lzw",
res=300)
ordiplot(totaladult.nmdsgow, type="n")
orditorp(totaladult.nmdsgow, display="sites")
colourcat<-sort(unique(nmdsforgow$Lineage))
for (i in seq(colourcat)){
  ordiellipse(totaladult.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=FALSE, col=i, show.groups=colourcat[i])
}
for (i in seq(colourcat)){
  ordiellipse(totaladult.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=TRUE, col=i, show.groups=colourcat[i])
}
dev.off()
anosim.gow<-anosim(totaladult.nmdsgow$points, c(totaladult$Lineage, totaladult$Lineage),
permutations=999, distance="euclidean")
summary(anosim.gow)
tiff("79ANOSIMadult3dnohybrid.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
plot(anosim.gow)
dev.off()
adonis(totaladult.gow~Lineage, totaladult, permutations=999, strata=totaladult$Site)
totaljuv.gow<-daisy(nmdstotaljuv, metric="gower", stand=TRUE, type=list(ordratio=c(2,4,5)))
head(totaljuv.gow)
tiff("80Dissimilarityjuv3dnohybrid.tiff", height=16, width=23.6, units='in', compression="lzw",
res=300)
coldiss(totaljuv.gow)
dev.off()
totaljuv.nmdsg<-metaMDS(totaljuv.gow, k=3, distance="gower")
totaljuv.nmdsgo<-metaMDS(totaljuv.gow, k=3, distance="gower", previous.best =
totaljuv.nmdsg)
totaljuv.nmdsgow<-metaMDS(totaljuv.gow, k=3, distance="gower",
previous.best=totaljuv.nmdsgo)
totaljuv.nmdsgow$stress
tiff("81Shepardjuv3dnohybrid.tiff", height=8, width=11.8, units='in', compression="lzw",
res=300)
par(mfrow=c(1,1))
stressplot(totaljuv.nmdsgow)
dev.off()
nmdsforgow<-data.frame(totaljuv$Lineage, nmdstotaljuv)
head(nmdsforgow)
#rename according to columns present
colnames(nmdsforgow)<-c("Lineage", "Depth", "sTlength", "Bcir", "Bcirlen", "Taper")
head(nmdsforgow)
tiff("82NMDSjuv3dnohybrid.tiff", height=8, width=11.8, units='in', compression="lzw",
res=300)
plot(totaljuv.nmdsgow, type="n")

```

```

points(totaljuv.nmdsgow, display="sites", col=as.numeric(nmdsforgow$Lineage),
pch=as.numeric(nmdsforgow$Lineage))
colourcat<-sort(unique(nmdsforgow$Lineage))
for (i in seq(colourcat)){
  ordiellipse(totaljuv.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=FALSE, col=i, show.groups=colourcat[i])
}
for (i in seq(colourcat)){
  ordiellipse(totaljuv.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=TRUE, col=i, show.groups=colourcat[i])
}
dev.off()
tiff("83NMDSjuvnum3dnohybrid.tiff", height=8, width=11.8, units='in', compression="lzw",
res=300)
ordiplot(totaljuv.nmdsgow, type="n")
orditorp(totaljuv.nmdsgow, display="sites")
colourcat<-sort(unique(nmdsforgow$Lineage))
for (i in seq(colourcat)){
  ordiellipse(totaljuv.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=FALSE, col=i, show.groups=colourcat[i])
}
for (i in seq(colourcat)){
  ordiellipse(totaljuv.nmdsgow, nmdsforgow$Lineage, display="sites", kind="sd", conf=0.95,
label=TRUE, col=i, show.groups=colourcat[i])
}
dev.off()
anosim.gow<-anosim(totaljuv.nmdsgow$points, c(totaljuv$Lineage, totaljuv$Lineage),
permutations=999, distance="euclidean")
summary(anosim.gow)
tiff("84ANOSIMjuv3dnohybrid.tiff", height=10, width=11.8, units='in', compression="lzw",
res=300)
plot(anosim.gow)
dev.off()
adonis.gow<-adonis(totaljuv.gow~Lineage, totaljuv, permutations=999, strata=totaljuv$Site)
adonis.gow
tiff("Totdispersalnohybrid.tiff", height=8, width=16, units='in', compression="lzw", res=300)
totdis<-betadisper(total.gow, group=total$Site)
boxplot (totdis)
dev.off()
tiff("Adispersalnohybrid.tiff", height=8, width=16, units='in', compression="lzw", res=300)
addis<-betadisper(totaladult.gow, group=totaladult$Site)
boxplot (addis)
dev.off()
tiff("Juvdispersalnohybrid.tiff", height=8, width=16, units='in', compression="lzw", res=300)
juvdis<-betadisper(totaljuv.gow, group=totaljuv$Site)
boxplot (juvdis)
dev.off()

#GLM
glmdata<-distdata
glmdata$Site<-factor(glmdata$Site)
glmdata$Exposure<-factor(glmdata$Exposure)
glmdata$Development<-factor(glmdata$Development)
glmdata$Development<-revalue(glmdata$Development, c("Adult"="2", "Juvenile"="1"))
glmdata$Lineage<-revalue(glmdata$Lineage, c("Eastern"="1", "Western"="2"))
#select variables to include and modify data frame length appropriately
glmdata<-glmdata[c(1:266),c(4,5,8,11,12,14,17)]

```

```

mylogit<-glm(Lineage~Depth+Development+Stlength+Bcir+Bcirlen+Taper,      data=glmdata,
family="binomial")
summary(mylogit)
with(mylogit, null.deviance-deviance)
with(mylogit, df.null-df.residual)
with(mylogit,pchisq(null.deviance-deviance,df.null-df.residual,lower.tail=FALSE))
logLik(mylogit)
#include in wald.test term length the ranked variable i.e.exposure/development
wald.test(b=coef(mylogit), Sigma=vcov(mylogit), Terms=c(3))
exp(cbind(OR=coef(mylogit),confint(mylogit)))
#run GLM for predicted probabilities with only one rank i.e. development
rankglm<-glmdata[c(1:266),]
head(rankglm)
ranklogit<-glm(Lineage~Development+Depth+Stlength+Bcir+Bcirlen+Taper,      data=rankglm,
family="binomial")
summary(ranklogit)
glmdata1<-with(rankglm,
data.frame(Depth=mean(Depth),Tlength=mean(Tlength),Stlength=mean(Stlength),Bcir=mean(
Bcir),Bcirlen=mean(Bcirlen),Taper=mean(Taper),Branch=mean(Branch),
Development=factor(1:2)))
glmdata1$DevelopmentP<-predict(ranklogit, newdata=glmdata1, type="response")
glmdata1
#plot any meaningful variable by replacing name of variable and excluding from glata list
minfrom<-min(rankglm$Depth)
maxto<-max(rankglm$Depth)
glmdata2<-with(rankglm,      data.frame(Depth=rep(seq(from=minfrom,      to      =maxto,
length.out=100),4),Tlength=mean(Tlength),Stlength=mean(Stlength),Bcir=mean(Bcir),Bcirlen=
mean(Bcirlen),Taper=mean(Taper),Branch=mean(Branch), Development=factor(1:2)))
glmdata3<-cbind(glmdata2, predict(ranklogit, newdata=glmdata2, type="link", se=TRUE))
glmdata3<-within(glmdata3, {
  PredictedProb<-plogis(fit)
  LL<-plogis(fit-(1.96*se.fit))
  UL<-plogis(fit+(1.96*se.fit))
})
head(glmdata3)
tiff("85GLMpp.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
ggplot(glmdata3, aes(x=Depth, y=PredictedProb))+geom_ribbon(aes(ymin=LL, ymax=UL,
fill=Development), alpha=0.2)+geom_line(aes(colour=Development), size=1)
dev.off()
minfrom<-min(rankglm$Stlength)
maxto<-max(rankglm$Stlength)
glmdata2<-with(rankglm,      data.frame(Stlength=rep(seq(from=minfrom,      to      =maxto,
length.out=100),4),Tlength=mean(Tlength),Branch=mean(Branch),Bcir=mean(Bcir),Bcirlen=m
ean(Bcirlen),Taper=mean(Taper),Depth=mean(Depth), Development=factor(1:2)))
glmdata3<-cbind(glmdata2, predict(ranklogit, newdata=glmdata2, type="link", se=TRUE))
glmdata3<-within(glmdata3, {
  PredictedProb<-plogis(fit)
  LL<-plogis(fit-(1.96*se.fit))
  UL<-plogis(fit+(1.96*se.fit))
})
head(glmdata3)
tiff("85GLMStlengthpp.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
ggplot(glmdata3, aes(x=Stlength, y=PredictedProb))+geom_ribbon(aes(ymin=LL, ymax=UL,
fill=Development), alpha=0.2)+geom_line(aes(colour=Development), size=1)
dev.off()
minfrom<-min(rankglm$Bcir)
maxto<-max(rankglm$Bcir)

```

```

glmdata2<-with(rankglm, data.frame(Bcir=rep(seq(from=minfrom, to =maxto,
length.out=100),4),Tlength=mean(Tlength),Stlength=mean(Stlength),Branch=mean(Branch),Bc
irlen=mean(Bcirlen),Taper=mean(Taper),Depth=mean(Depth), Development=factor(1:2)))
glmdata3<-cbind(glmdata2, predict(ranklogit, newdata=glmdata2, type="link", se=TRUE))
glmdata3<-within(glmdata3, {
  PredictedProb<-plogis(fit)
  LL<-plogis(fit-(1.96*se.fit))
  UL<-plogis(fit+(1.96*se.fit))
})
head(glmdata3)
tiff("85GLMBcirpp.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
ggplot(glmdata3, aes(x=Bcir, y=PredictedProb))+geom_ribbon(aes(ymin=LL, ymax=UL,
fill=Development), alpha=0.2)+geom_line(aes(colour=Development), size=1)
dev.off()
minfrom<-min(rankglm$Bcirlen)
maxto<-max(rankglm$Bcirlen)
glmdata2<-with(rankglm, data.frame(Bcirlen=rep(seq(from=minfrom, to =maxto,
length.out=100),4),Tlength=mean(Tlength),Stlength=mean(Stlength),Bcir=mean(Bcir),Branch=
mean(Branch),Taper=mean(Taper),Depth=mean(Depth), Development=factor(1:2)))
glmdata3<-cbind(glmdata2, predict(ranklogit, newdata=glmdata2, type="link", se=TRUE))
glmdata3<-within(glmdata3, {
  PredictedProb<-plogis(fit)
  LL<-plogis(fit-(1.96*se.fit))
  UL<-plogis(fit+(1.96*se.fit))
})
head(glmdata3)
tiff("85GLMBcirlenhpp.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
ggplot(glmdata3, aes(x=Bcirlen, y=PredictedProb))+geom_ribbon(aes(ymin=LL, ymax=UL,
fill=Development), alpha=0.2)+geom_line(aes(colour=Development), size=1)
dev.off()

#for adults
adglmdata<-glmdata[glmdata$Development=="2",]
mylogit<-glm(Lineage~Depth+Stlength+Bcir+Bcirlen+Taper, data=adglmdata,
family="binomial")
summary(mylogit)
with(mylogit, null.deviance-deviance)
with(mylogit, df.null-df.residual)
with(mylogit,pchisq(null.deviance-deviance,df.null-df.residual,lower.tail=FALSE))
logLik(mylogit)
exp(cbind(OR=coef(mylogit),confint(mylogit)))

#Two Block Partial Least Squares - analysis of correlation between two multivariate data sets
#Tucker's inter-battery factor analysis
plsdata<-distdata[1:217,c(6,8:21)]
head(plsdata)
str(plsdata)
summary(plsdata)
cor(plsdata)
morphpls<-plsdata[,c(3:15)]
enviroppls<-plsdata[,c(1:2)]
plsinterbat<-interbat(morphpls,enviroppls, scaled=TRUE)
plsinterbat
plsinterbat$values
head(round(plsinterbat$x.scores, 12))
head(round(plsinterbat$y.scores, 4))
plsinterbat$cor.xt

```

```

plsinterbat$cor.yu
plsinterbat$cor.xu
plsinterbat$cor.yt
plsinterbat$R2X
plsinterbat$R2Y
plsinterbat$com.xu
plsinterbat$com.yt
plsinterbat$statistic
tiff("87PLS.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
plot(plsinterbat)
dev.off()
tiff("89PLSobserv.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
plot(plsinterbat, what="observations", show.names=TRUE)
dev.off()
pls2data<-distdata[1:266,c(6,8:12,14,16,18,19)]
head(pls2data)
str(pls2data)
summary(pls2data)
cor(pls2data)
morphpls2<-pls2data[,c(3:10)]
enviroppls2<-pls2data[,c(1:2)]
pls2interbat<-interbat(morphpls2,enviroppls2, scaled=TRUE)
pls2interbat
pls2interbat$values
head(round(pls2interbat$x.scores, 8))
head(round(pls2interbat$y.scores, 4))
pls2interbat$cor.xt
pls2interbat$cor.yu
pls2interbat$cor.xu
pls2interbat$cor.yt
pls2interbat$R2X
pls2interbat$R2Y
pls2interbat$com.xu
pls2interbat$com.yt
pls2interbat$statistic
tiff("90PLS2.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
plot(pls2interbat)
dev.off()
tiff("92PLS2observ.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
plot(pls2interbat, what="observations", show.names=TRUE)
dev.off()
tiff("93PLSplot.tiff", height=8, width=11.8, units='in', compression="lzw", res=300)
two.b.pls(morphpls,enviroppls, iter=999, ShowPlot=TRUE)
dev.off()
#Two Block Partial Least Squares - analysis of correlation between two multivariate data sets
WESTERN
#Tucker's inter-battery factor analysis
plsdatawest<-distdata[1:217,c(4,6,8:21)]
plsdatawest<-plsdatawest[plsdatawest$Lineage=="Western",]
plsdatawest<-plsdatawest[,-1]
head(plsdatawest)
str(plsdatawest)
summary(plsdatawest)
cor(plsdatawest)
morphpls<-plsdatawest[,c(3:15)]
enviroppls<-plsdatawest[,c(1:2)]
plsinterbat<-interbat(morphpls,enviroppls, scaled=TRUE)

```

```

plsinterbat
plsinterbat$values
head(round(plsinterbat$x.scores, 12))
head(round(plsinterbat$y.scores, 4))
plsinterbat$cor.xt
plsinterbat$cor.yu
plsinterbat$cor.xu
plsinterbat$cor.yt
plsinterbat$R2X
plsinterbat$R2Y
plsinterbat$com.xu
plsinterbat$com.yt
plsinterbat$statistic
tiff("87PLSwest.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
plot(plsinterbat)
dev.off()
tiff("89PLSobservwest.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
plot(plsinterbat, what="observations", show.names=TRUE)
dev.off()
pls2datawest<-distdata[1:266,c(4,6,8:12,14,16,18,19)]
pls2datawest<-pls2datawest[pls2datawest$Lineage=="Western",]
pls2datawest<-pls2datawest[,-1]
head(pls2datawest)
str(pls2datawest)
summary(pls2datawest)
cor(pls2datawest)
morphpls2<-pls2datawest[,c(3:10)]
enviroppls2<-pls2datawest[,c(1:2)]
pls2interbat<-interbat(morphpls2,enviroppls2, scaled=TRUE)
pls2interbat
pls2interbat$values
head(round(pls2interbat$x.scores, 8))
head(round(pls2interbat$y.scores, 4))
pls2interbat$cor.xt
pls2interbat$cor.yu
pls2interbat$cor.xu
pls2interbat$cor.yt
pls2interbat$R2X
pls2interbat$R2Y
pls2interbat$com.xu
pls2interbat$com.yt
pls2interbat$statistic
tiff("90PLS2west.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
plot(pls2interbat)
dev.off()
tiff("92PLS2observwest.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
plot(pls2interbat, what="observations", show.names=TRUE)
dev.off()
tiff("93PLSplotwest.tiff", height=8, width=11.8, units='in', compression="lzw", res=300)
two.b.pls(morphpls,enviroppls, iter=999, ShowPlot=TRUE)
dev.off()
#Two Block Partial Least Squares - analysis of correlation between two multivariate data sets
East
#Tucker's inter-battery factor analysis
plsdataeast<-distdata[1:217,c(4,6,8:21)]
plsdataeast<-plsdataeast[plsdataeast$Lineage=="Eastern",]
plsdataeast<-plsdataeast[,-1]

```

```

head(plsdataeast)
str(plsdataeast)
summary(plsdataeast)
cor(plsdataeast)
morphpls<-plsdataeast[,c(3:15)]
enviroppls<-plsdataeast[,c(1:2)]
plsinterbat<-interbat(morphpls,enviroppls, scaled=TRUE)
plsinterbat
plsinterbat$values
head(round(plsinterbat$x.scores, 12))
head(round(plsinterbat$y.scores, 4))
plsinterbat$cor.xt
plsinterbat$cor.yu
plsinterbat$cor.xu
plsinterbat$cor.yt
plsinterbat$R2X
plsinterbat$R2Y
plsinterbat$com.xu
plsinterbat$com.yt
plsinterbat$statistic
tiff("87PLSeast.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
plot(plsinterbat)
dev.off()
tiff("89PLSobserveast.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
plot(plsinterbat, what="observations", show.names=TRUE)
dev.off()
pls2dataeast<-distdata[1:266,c(4,6,8:12,14,16,18,19)]
pls2dataeast<-pls2dataeast[pls2dataeast$Lineage=="Eastern",]
pls2dataeast<-pls2dataeast[,-1]
head(pls2dataeast)
str(pls2dataeast)
summary(pls2dataeast)
cor(pls2dataeast)
morphpls2<-pls2dataeast[,c(3:10)]
enviroppls2<-pls2dataeast[,c(1:2)]
pls2interbat<-interbat(morphpls2,enviroppls2, scaled=TRUE)
pls2interbat
pls2interbat$values
head(round(pls2interbat$x.scores, 8))
head(round(pls2interbat$y.scores, 4))
pls2interbat$cor.xt
pls2interbat$cor.yu
pls2interbat$cor.xu
pls2interbat$cor.yt
pls2interbat$R2X
pls2interbat$R2Y
pls2interbat$com.xu
pls2interbat$com.yt
pls2interbat$statistic
tiff("90PLS2east.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
plot(pls2interbat)
dev.off()
tiff("92PLS2observeast.tiff", height=10, width=11.8, units='in', compression="lzw", res=300)
plot(pls2interbat, what="observations", show.names=TRUE)
dev.off()
tiff("93PLSploteast.tiff", height=8, width=11.8, units='in', compression="lzw", res=300)
two.b.pls(morphpls,enviroppls, iter=999, ShowPlot=TRUE)

```

```

dev.off()

#Ecological correlations
ecdata<-data
head(ecdata)
ecdata<-ecdata[ecdata$Development=="Adult",]
tiff("94LatTcir.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
plot(ecdata$Latitude,ecdata$Tcir, ylab="Top Stipe Circumference (cm)", xlab="Latitude",
col=ecdata$Lineage, pch=20, cex=0.75)
dev.off()
tiff("95LatBcir.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
plot(ecdata$Latitude,ecdata$Bcir, ylab="Base Stipe Circumference (cm)", xlab="Latitude",
col=ecdata$Lineage, pch=20, cex=0.75)
dev.off()
tiff("96LatSlength.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
plot(ecdata$Latitude,ecdata$Slength,ylab="Stipe Length (cm)", xlab="Latitude",
col=ecdata$Lineage, pch=20, cex=0.75)
dev.off()
tiff("97DepthSlength.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
plot(ecdata$Depth,ecdata$Slength, ylab="Stipe Length (cm)", xlab="Depth (m)",
col=ecdata$Lineage, pch=20, cex=0.75)
dev.off()
tiff("98DepthTcir.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
plot( ecdata$Depth, ecdata$Tcir,ylab="Top Stipe Circumference (cm)", xlab="Depth (m)",
col=ecdata$Lineage, pch=20, cex=0.75)
dev.off()
tiff("99DepthBcir.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
plot(ecdata$Depth, ecdata$Bcir, ylab="Base Stipe Circumference (cm)", xlab="Depth (m)",
col=ecdata$Lineage, pch=20, cex=0.75)
dev.off()
tiff("100OpennessStot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
plot(ecdata$Openness, ecdata$Stot, ylab="Total number of Secondary Stipes", xlab="Openness
Scores (Fetch)", col=ecdata$Lineage, pch=20, cex=0.75)
dev.off()
tiff("101OpennessTlength.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
plot(ecdata$Openness,ecdata$Tlength, ylab="Total Length (m)", xlab="Openness Scores
(Fetch)", col=ecdata$Lineage, pch=20, cex=0.75)
dev.off()
tiff("102ExposureTlength.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
plot( ecdata$Exposure,ecdata$Tlength, ylab="Total Length (m)", xlab="Exposure Class",
col=ecdata$Lineage, pch=20, cex=0.75)
dev.off()
tiff("103OpennessSlength.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
plot(ecdata$Openness,ecdata$Slength, ylab="Stipe Length (cm)", xlab="Openness Scores
(Fetch)", col=ecdata$Lineage, pch=20, cex=0.75)
dev.off()
tiff("104ExposureSlength.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
plot( ecdata$Exposure,ecdata$Slength, ylab="Stipe Length (cm)", xlab="Exposure Class",
col=ecdata$Lineage, pch=20, cex=0.75)
dev.off()
tiff("105DepthStot.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
plot(ecdata$Depth,ecdata$Stot, ylab="Total number of Secondary Stipes", xlab="Depth(m)",
col=ecdata$Lineage, pch=20, cex=0.75)
dev.off()
sitefactor<-as.factor(ecdata$Site)
meansumm<-ddply(ecdata, "Site", summarise,
                branchmean=mean(Branch),

```



```

        openmean=mean(Openness)
    )
    branchsumm<-meansumm[c(4:10),]
    tiff("106OpennessBranch.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
    plot(branchsumm$openmean,branchsumm$branchmean, ylab="Branching", xlab="Openness
    Scores (Fetch)", col=ecdata$Lineage, pch=20, cex=0.75)
    dev.off()
    #Openness graph
    opendata<-data[,c(2,5:7)]
    meansumm<-ddply(opendata, "Site", summarise,
        open=mean(Openness))
    open<-meansumm[order(meansumm$open),]
    tiff("107OpennessSite.tiff", height=5, width=5.9, units='in', compression="lzw", res=300)
    plot(open$open, xlab="Site", ylab="Openness", las=2, xaxt="n")
    axis(1, at=1:11, labels=c("SB", "LB", "CB", "BB", "WB", "TW", "GI", "SS", "TG", "GH", "TH"))
    dev.off()
    #Exposure GLM
    exposedata<-read.csv("Exposure.csv")
    exposedata
    exposureglm<-glm(Percentage~Exposure+Lineage+Site, data=exposedata,
    family=quasipoisson(log))
    summary(exposureglm)
    logLik(exposureglm)
    tiff("108ExposureRegression.tiff", height=10, width=11.8, units='in', compression="lzw",
    res=300)
    layout(matrix(1:4,2,2))
    mfrow=c(2,2)
    plot(siteSdiff)
    dev.off()
    wald.test(b=coef(exposureglm), Sigma=vcov(exposureglm), Terms=c(3))

```

Electronic Appendix B: Total data collection

Sample ID	Site	Latitude	Longitude	Lineage	Development	Openness	Exposure	Depth
SB.01.01	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0
SB.01.02	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0
SB.01.03	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0
SB.01.04	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0
SB.01.05	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0
SB.01.06	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0
SB.01.07	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0
SB.01.08	Spring Beach	-42.838333	148.143611	Western	Juvenile	0.0995005	A	0
SB.01.09	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0
SB.01.10	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0
SB.01.11	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0
SB.01.12	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0
SB.01.13	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0
SB.01.15	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0
SB.01.16	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0
SB.01.17	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0
SB.01.18	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0.3
SB.01.19	Spring Beach	-42.838333	148.143611	Eastern	Adult	0.0995005	A	0.3
SB.01.20	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0.3
SB.01.21	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0.3
SB.01.22	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0.3
SB.01.23	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0.3
SB.01.24	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0.3
SB.01.25	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0.3
SB.01.26	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0.3
SB.01.27	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0
SB.01.28	Spring Beach	-42.838333	148.143611	Western	Juvenile	0.0995005	A	0
SB.01.29	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0
SB.01.30	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0
SB.01.31	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0
SB.01.32	Spring Beach	-42.838333	148.143611	Western	Adult	0.0995005	A	0
TH.02.00	Trial Harbour	-41.93	145.240277	Western	Adult	0.4208755	A	0
TH.02.01	Trial Harbour	-41.93	145.240277	Western	Juvenile	0.4208755	A	0
TH.02.02	Trial Harbour	-41.93	145.240277	Western	Adult	0.4208755	A	0
TH.02.03	Trial Harbour	-41.93	145.240277	Western	Adult	0.4208755	A	0
TH.02.04	Trial Harbour	-41.93	145.240277	Western	Adult	0.4208755	A	0
TH.02.05	Trial Harbour	-41.93	145.240277	Western	Adult	0.4208755	A	0
TH.02.06	Trial Harbour	-41.93	145.240277	Western	Adult	0.4208755	A	0
TH.02.07	Trial Harbour	-41.93	145.240277	Western	Juvenile	0.4208755	A	0
TH.02.08	Trial Harbour	-41.93	145.240277	Western	Adult	0.4208755	A	0
TH.02.09	Trial Harbour	-41.93	145.240277	Eastern	Adult	0.4208755	A	0
TH.02.10	Trial Harbour	-41.93	145.240277	Western	Adult	0.4208755	A	0
TH.02.11	Trial Harbour	-41.93	145.240277	Western	Adult	0.4208755	A	0
TH.02.12	Trial Harbour	-41.93	145.240277	Western	Adult	0.4208755	A	0
TH.02.13	Trial Harbour	-41.93	145.240277	Eastern	Adult	0.4208755	A	0
TH.02.14	Trial Harbour	-41.93	145.240277	Western	Adult	0.4208755	A	0
TH.02.15	Trial Harbour	-41.93	145.240277	Eastern	Adult	0.4208755	A	0.5
TH.02.16	Trial Harbour	-41.93	145.240277	Eastern	Adult	0.4208755	A	0.5
TH.02.17	Trial Harbour	-41.93	145.240277	Eastern	Adult	0.4208755	A	0.5
TH.02.18	Trial Harbour	-41.93	145.240277	Eastern	Adult	0.4208755	A	0.5
TH.02.19	Trial Harbour	-41.93	145.240277	Eastern	Adult	0.4208755	A	0.5
TH.02.20	Trial Harbour	-41.93	145.240277	Eastern	Juvenile	0.4208755	A	0.5

TH.02.21	Trial Harbour	-41.93	145.240277	Eastern	Adult	0.4208755	A	2
TH.02.22	Trial Harbour	-41.93	145.240277	Eastern	Juvenile	0.4208755	A	2
TH.02.23	Trial Harbour	-41.93	145.240277	Eastern	Juvenile	0.4208755	A	2
TH.02.24	Trial Harbour	-41.93	145.240277	Eastern	Adult	0.4208755	A	2
TH.02.25	Trial Harbour	-41.93	145.240277	Eastern	Adult	0.4208755	A	2
TH.02.26	Trial Harbour	-41.93	145.240277	Eastern	Adult	0.4208755	A	2
TH.02.27	Trial Harbour	-41.93	145.240277	Eastern	Adult	0.4208755	A	1.5
TH.02.28	Trial Harbour	-41.93	145.240277	Eastern	Juvenile	0.4208755	A	1
TH.02.29	Trial Harbour	-41.93	145.240277	Eastern	Juvenile	0.4208755	A	1
TH.02.30	Trial Harbour	-41.93	145.240277	Eastern	Adult	0.4208755	A	1
TH.02.31	Trial Harbour	-41.93	145.240277	Eastern	Adult	0.4208755	A	1
GH.02.100	Granville Harbour	-41.894166	145.342722	Western	Adult	0.3983615	A	0
GH.02.101	Granville Harbour	-41.894166	145.342722	Western	Adult	0.3983615	A	0
GH.02.102	Granville Harbour	-41.894166	145.342722	Western	Adult	0.3983615	A	0
GH.02.103	Granville Harbour	-41.894166	145.342722	Western	Adult	0.3983615	B	0
GH.02.104	Granville Harbour	-41.894166	145.342722	Western	Juvenile	0.3983615	B	0
GH.02.105	Granville Harbour	-41.894166	145.342722	Western	Adult	0.3983615	B	0
GH.02.106	Granville Harbour	-41.894166	145.342722	Western	Adult	0.3983615	C	0
GH.02.107	Granville Harbour	-41.894166	145.342722	Western	Adult	0.3983615	C	0
GH.02.108	Granville Harbour	-41.894166	145.342722	Eastern	Adult	0.3983615	C	0
GH.02.109	Granville Harbour	-41.894166	145.342722	Eastern	Adult	0.3983615	C	0
GH.02.110	Granville Harbour	-41.894166	145.342722	Western	Adult	0.3983615	C	0
GH.02.111	Granville Harbour	-41.894166	145.342722	Western	Adult	0.3983615	C	0
GH.02.112	Granville Harbour	-41.894166	145.342722	Eastern	Adult	0.3983615	C	0
GH.02.113	Granville Harbour	-41.894166	145.342722	Eastern	Adult	0.3983615	D	0
GH.02.114	Granville Harbour	-41.894166	145.342722	Western	Adult	0.3983615	D	0
GH.02.115	Granville Harbour	-41.894166	145.342722	Eastern	Adult	0.3983615	D	0.5
GH.02.116	Granville Harbour	-41.894166	145.342722	Hybrid	Adult	0.3983615	D	0.5
GH.02.117	Granville Harbour	-41.894166	145.342722	Hybrid	Adult	0.3983615	D	0.5
GH.02.118	Granville Harbour	-41.894166	145.342722	Western	Adult	0.3983615	D	0.5
GH.02.119	Granville Harbour	-41.894166	145.342722	Western	Adult	0.3983615	D	1.5
GH.02.120	Granville Harbour	-41.894166	145.342722	Eastern	Adult	0.3983615	D	1.5
GH.02.121	Granville Harbour	-41.894166	145.342722	Eastern	Juvenile	0.3983615	D	1.5
GH.02.122	Granville Harbour	-41.894166	145.342722	Western	Juvenile	0.3983615	D	1.5
GH.02.123	Granville Harbour	-41.894166	145.342722	Western	Juvenile	0.3983615	D	1.5
GH.02.124	Granville Harbour	-41.894166	145.342722	Hybrid	Juvenile	0.3983615	D	1.5
GH.02.125	Granville Harbour	-41.894166	145.342722	Hybrid	Juvenile	0.3983615	D	1.5
GH.02.126	Granville Harbour	-41.894166	145.342722	Hybrid	Juvenile	0.3983615	D	1.5
GH.02.127	Granville Harbour	-41.894166	145.342722	Western	Adult	0.3983615	D	1.5
GH.02.128	Granville Harbour	-41.894166	145.342722	Western	Adult	0.3983615	D	1.5
GH.02.129	Granville Harbour	-41.894166	145.342722	Western	Juvenile	0.3983615	D	1.5
GH.02.130	Granville Harbour	-41.894166	145.342722	Western	Adult	0.3983615	D	1.5
GH.02.131	Granville Harbour	-41.894166	145.342722	Western	Adult	0.3983615	D	0.5
TG.03.150	The Gardens	-41.346944	148.293333	Western	Adult	0.3917485	A	0
TG.03.151	The Gardens	-41.346944	148.293333	Western	Adult	0.3917485	A	0
TG.03.152	The Gardens	-41.346944	148.293333	Western	Adult	0.3917485	A	0
TG.03.153	The Gardens	-41.346944	148.293333	Eastern	Adult	0.3917485	A	0
TG.03.154	The Gardens	-41.346944	148.293333	Western	Adult	0.3917485	A	0
TG.03.155	The Gardens	-41.346944	148.293333	Western	Adult	0.3917485	A	0
TG.03.156	The Gardens	-41.346944	148.293333	Western	Juvenile	0.3917485	A	0
TG.03.157	The Gardens	-41.346944	148.293333	Western	Juvenile	0.3917485	A	0
TG.03.158	The Gardens	-41.346944	148.293333	Western	Juvenile	0.3917485	A	0
TG.03.159	The Gardens	-41.346944	148.293333	Western	Adult	0.3917485	A	0
TG.03.160	The Gardens	-41.346944	148.293333	Eastern	Adult	0.3917485	A	0
TG.03.161	The Gardens	-41.346944	148.293333	Hybrid	Adult	0.3917485	A	0
TG.03.162	The Gardens	-41.346944	148.293333	Eastern	Adult	0.3917485	A	0

TG.03.163	The Gardens	-41.346944	148.293333	Hybrid	Adult	0.3917485	B	1
TG.03.164	The Gardens	-41.346944	148.293333	Western	Juvenile	0.3917485	B	0
TG.03.165	The Gardens	-41.346944	148.293333	Eastern	Juvenile	0.3917485	B	0
TG.03.166	The Gardens	-41.346944	148.293333	Western	Juvenile	0.3917485	B	0
TG.03.167	The Gardens	-41.346944	148.293333	Western	Juvenile	0.3917485	B	0
TG.03.168	The Gardens	-41.346944	148.293333	Western	Adult	0.3917485	B	0
TG.03.169	The Gardens	-41.346944	148.293333	Western	Adult	0.3917485	B	0
TG.03.170	The Gardens	-41.346944	148.293333	Western	Juvenile	0.3917485	B	0
TG.03.171	The Gardens	-41.346944	148.293333	Hybrid	Adult	0.3917485	B	0
TG.03.172	The Gardens	-41.346944	148.293333	Western	Adult	0.3917485	B	0
TG.03.173	The Gardens	-41.346944	148.293333	Western	Adult	0.3917485	B	0
TG.03.174	The Gardens	-41.346944	148.293333	Western	Adult	0.3917485	B	0
TG.03.175	The Gardens	-41.346944	148.293333	Eastern	Adult	0.3917485	B	1.5
TG.03.176	The Gardens	-41.346944	148.293333	Hybrid	Adult	0.3917485	B	1.5
TG.03.177	The Gardens	-41.346944	148.293333	Eastern	Adult	0.3917485	B	1.5
TG.03.178	The Gardens	-41.346944	148.293333	Eastern	Adult	0.3917485	B	1.5
TG.03.179	The Gardens	-41.346944	148.293333	Eastern	Adult	0.3917485	B	1.5
TG.03.180	The Gardens	-41.346944	148.293333	Western	Juvenile	0.3917485	B	1.5
TG.03.181	The Gardens	-41.346944	148.293333	Western	Juvenile	0.3917485	B	1.5
TG.03.182	The Gardens	-41.346944	148.293333	Eastern	Juvenile	0.3917485	B	1.5
TG.03.183	The Gardens	-41.346944	148.293333	Western	Juvenile	0.3917485	B	1.5
TG.03.184	The Gardens	-41.346944	148.293333	Western	Adult	0.3917485	B	1.5
TG.03.185	The Gardens	-41.346944	148.293333	Western	Adult	0.3917485	B	1.5
SS.03.200	Secret Spot	-41.606944	148.299444	Western	Adult	0.3823715	A	0
SS.03.201	Secret Spot	-41.606944	148.299444	Western	Adult	0.3823715	A	0
SS.03.202	Secret Spot	-41.606944	148.299444	Western	Juvenile	0.3823715	A	0
SS.03.203	Secret Spot	-41.606944	148.299444	Western	Adult	0.3823715	A	0
SS.03.204	Secret Spot	-41.606944	148.299444	Western	Adult	0.3823715	A	0
SS.03.205	Secret Spot	-41.606944	148.299444	Western	Adult	0.3823715	A	0
SS.03.206	Secret Spot	-41.606944	148.299444	Western	Adult	0.3823715	A	0
SS.03.207	Secret Spot	-41.606944	148.299444	Western	Adult	0.3823715	A	0
SS.03.208	Secret Spot	-41.606944	148.299444	Western	Adult	0.3823715	A	0
SS.03.209	Secret Spot	-41.606944	148.299444	Western	Adult	0.3823715	A	0
SS.03.210	Secret Spot	-41.606944	148.299444	Western	Juvenile	0.3823715	A	0
SS.03.211	Secret Spot	-41.606944	148.299444	Western	Adult	0.3823715	A	0
SS.03.212	Secret Spot	-41.606944	148.299444	Western	Adult	0.3823715	A	0
SS.03.213	Secret Spot	-41.606944	148.299444	Western	Juvenile	0.3823715	A	0.3
SS.03.214	Secret Spot	-41.606944	148.299444	Western	Juvenile	0.3823715	A	0.3
SS.03.215	Secret Spot	-41.606944	148.299444	Western	Juvenile	0.3823715	A	0.3
SS.03.216	Secret Spot	-41.606944	148.299444	Western	Juvenile	0.3823715	A	0.3
SS.03.217	Secret Spot	-41.606944	148.299444	Western	Juvenile	0.3823715	A	0.3
SS.03.218	Secret Spot	-41.606944	148.299444	Western	Adult	0.3823715	A	0.3
SS.03.219	Secret Spot	-41.606944	148.299444	Western	Adult	0.3823715	A	0.3
SS.03.220	Secret Spot	-41.606944	148.299444	Western	Adult	0.3823715	A	0.3
SS.03.221	Secret Spot	-41.606944	148.299444	Western	Adult	0.3823715	A	1.75
SS.03.222	Secret Spot	-41.606944	148.299444	Western	Adult	0.3823715	A	1.75
SS.03.223	Secret Spot	-41.606944	148.299444	Western	Juvenile	0.3823715	A	1.75
SS.03.224	Secret Spot	-41.606944	148.299444	Western	Adult	0.3823715	A	1.75
SS.03.225	Secret Spot	-41.606944	148.299444	Western	Adult	0.3823715	A	1.75
SS.03.226	Secret Spot	-41.606944	148.299444	Western	Juvenile	0.3823715	A	1.75
SS.03.227	Secret Spot	-41.606944	148.299444	Eastern	Juvenile	0.3823715	A	1.75
SS.03.228	Secret Spot	-41.606944	148.299444	Western	Adult	0.3823715	A	1.75
SS.03.229	Secret Spot	-41.606944	148.299444	Western	Juvenile	0.3823715	A	1.75
SS.03.230	Secret Spot	-41.505944	148.299444	Western	Juvenile	0.3823715	A	1.75
LB.05.250	Lady's Bay	-43.540833	147.248333	Eastern	Adult	0.1322135	A	0
LB.05.251	Lady's Bay	-43.540833	147.248333	Eastern	Adult	0.1322135	A	0

[illegible]

TW.05.328	The Whale	-43.603055	146.928333	Eastern	Adult	0.2868665	A	0
TW.05.329	The Whale	-43.603055	146.928333	Eastern	Adult	0.2868665	A	0
TW.05.330	The Whale	-43.603055	146.928333	Western	Adult	0.2868665	A	0
TW.05.331	The Whale	-43.603055	146.928333	Eastern	Adult	0.2868665	A	0
TW.05.332	The Whale	-43.603055	146.928333	Western	Adult	0.2868665	A	0
TW.05.333	The Whale	-43.603055	146.928333	Western	Adult	0.2868665	A	0
Edg.bic.1	Waubs Bay	-41.868514	148.304092	Western	Adult	0.276810667		0
Edg.bic.2	Waubs Bay	-41.868514	148.304092	Western	Adult	0.276810667		0
Edg.bic.3	Waubs Bay	-41.868514	148.304092	Western	Adult	0.276810667		0
Edg.bic.4	Waubs Bay	-41.868514	148.304092	Western	Adult	0.276810667		0
Edg.bic.5	Waubs Bay	-41.868514	148.304092	Western	Adult	0.276810667		0
Edg.bic.6	Waubs Bay	-41.868514	148.304092	Western	Adult	0.276810667		0
Edg.bic.7	Waubs Bay	-41.868514	148.304092	Western	Adult	0.276810667		0
Edg.bic.8	Waubs Bay	-41.868514	148.304092	Western	Adult	0.276810667		0
Edg.bic.9	Waubs Bay	-41.868514	148.304092	Western	Adult	0.276810667		0
Edg.bic.10	Waubs Bay	-41.868514	148.304092	Western	Adult	0.276810667		0
Edg.bic.11	Waubs Bay	-41.868514	148.304092	Western	Adult	0.276810667		0
Edg.bic.12	Waubs Bay	-41.868514	148.304092	Western	Adult	0.276810667		0
Edg.bic.13	Waubs Bay	-41.868514	148.304092	Western	Adult	0.276810667		0
Edg.bic.14	Waubs Bay	-41.868514	148.304092	Western	Adult	0.276810667		0
Edg.bic.15	Waubs Bay	-41.868514	148.304092	Western	Adult	0.276810667		0
Edg.bic.16	Waubs Bay	-41.868514	148.304092	Western	Adult	0.276810667		0
Edg.bic.17	Waubs Bay	-41.868514	148.304092	Western	Adult	0.276810667		0
Edg.bic.18	Waubs Bay	-41.868514	148.304092	Western	Adult	0.276810667		0
Edg.bic.19	Waubs Bay	-41.868514	148.304092	Western	Adult	0.276810667		0
Edg.bic.20	Waubs Bay	-41.868514	148.304092	Western	Adult	0.276810667		0
Edg.bic.21	Waubs Bay	-41.868514	148.304092	Western	Adult	0.276810667		0
Edg.bic.22	Waubs Bay	-41.868514	148.304092	Western	Adult	0.276810667		0
Edg.bic.23	Waubs Bay	-41.868514	148.304092	Western	Adult	0.276810667		0
Edg.bic.24	Waubs Bay	-41.868514	148.304092	Western	Adult	0.276810667		0
Edg.bin.2	Binalong Bay	-41.24912	148.308448	Western	Adult	0.241105333		0
Edg.bin.3	Binalong Bay	-41.24912	148.308448	Western	Adult	0.241105333		0
Edg.bin.4	Binalong Bay	-41.24912	148.308448	Western	Adult	0.241105333		0
Edg.bin.5	Binalong Bay	-41.24912	148.308448	Western	Adult	0.241105333		0
Edg.bin.6	Binalong Bay	-41.24912	148.308448	Western	Adult	0.241105333		0
Edg.bin.7	Binalong Bay	-41.24912	148.308448	Western	Adult	0.241105333		0
Edg.bin.8	Binalong Bay	-41.24912	148.308448	Western	Adult	0.241105333		0
Edg.bin.9	Binalong Bay	-41.24912	148.308448	Western	Adult	0.241105333		0
Edg.bin.10	Binalong Bay	-41.24912	148.308448	Western	Adult	0.241105333		0
Edg.bin.11	Binalong Bay	-41.24912	148.308448	Western	Adult	0.241105333		0
Edg.bin.12	Binalong Bay	-41.24912	148.308448	Western	Juvenile	0.241105333		0
Edg.bin.16	Binalong Bay	-41.24912	148.308448	Western	Adult	0.241105333		0
Edg.bin.19	Binalong Bay	-41.24912	148.308448	Western	Adult	0.241105333		0
Edg.bin.21	Binalong Bay	-41.24912	148.308448	Western	Adult	0.241105333		0
Edg.bin.13	Binalong Bay	-41.24912	148.308448	Western	Adult	0.241105333		0
Edg.bin.14	Binalong Bay	-41.24912	148.308448	Western	Adult	0.241105333		0
Edg.bin.15	Binalong Bay	-41.24912	148.308448	Western	Adult	0.241105333		0
Edg.bin.17	Binalong Bay	-41.24912	148.308448	Western	Adult	0.241105333		0
Edg.bin.18	Binalong Bay	-41.24912	148.308448	Western	Adult	0.241105333		0
Edg.bin.20	Binalong Bay	-41.24912	148.308448	Western	Adult	0.241105333		0
Edg.bin.22	Binalong Bay	-41.24912	148.308448	Western	Adult	0.241105333		0
Edg.bin.23	Binalong Bay	-41.24912	148.308448	Western	Adult	0.241105333		0
Edg.bin.24	Binalong Bay	-41.24912	148.308448	Western	Adult	0.241105333		0
Edg.bin.25	Binalong Bay	-41.24912	148.308448	Western	Adult	0.241105333		0
Edg.bin.1	Binalong Bay	-41.24912	148.308448	Western	Adult	0.241105333		0
Edg.WB.1	Waubs Bay	-41.868514	148.304092	Eastern	Adult	0.276810667		1

Edg.WB.2	Waubus Bay	-41.868514	148.304092	Eastern	Adult	0.276810667	1
Edg.WB.3	Waubus Bay	-41.868514	148.304092	Eastern	Adult	0.276810667	1
Edg.WB.4	Waubus Bay	-41.868514	148.304092	Eastern	Juvenile	0.276810667	1
Edg.WB.5	Waubus Bay	-41.868514	148.304092	Eastern	Adult	0.276810667	1
Edg.WB.6	Waubus Bay	-41.868514	148.304092	Eastern	Adult	0.276810667	1
Edg.WB.7	Waubus Bay	-41.868514	148.304092	Eastern	Adult	0.276810667	1
Edg.WB.8	Waubus Bay	-41.868514	148.304092	Eastern	Juvenile	0.276810667	1
Edg.WB.9	Waubus Bay	-41.868514	148.304092	Eastern	Juvenile	0.276810667	1
Edg.gov.5	Waubus Bay	-41.868514	148.304092	Eastern	Adult	0.276810667	1
Edg.gov.6	Waubus Bay	-41.868514	148.304092	Eastern	Adult	0.276810667	1
Edg.gov.7	Governor Island	-41.87557	148.31241	Eastern	Juvenile	0.370963	2
Edg.gov.8	Governor Island	-41.87557	148.31241	Eastern	Adult	0.370963	2
Edg.gov.9	Governor Island	-41.87557	148.31241	Eastern	Adult	0.370963	2
Edg.gov.11	Governor Island	-41.87557	148.31241	Eastern	Adult	0.370963	2
Edg.bin.25	Binalong Bay	-41.24912	148.308448	Eastern	Juvenile	0.241105333	2
Edg.bin.27	Binalong Bay	-41.24912	148.308448	Eastern	Juvenile	0.241105333	2
Edg.bin.28	Binalong Bay	-41.24912	148.308448	Eastern	Juvenile	0.241105333	2
Edg.bin.31	Binalong Bay	-41.24912	148.308448	Eastern	Juvenile	0.241105333	2
Edg.bin.32	Binalong Bay	-41.24912	148.308448	Eastern	Juvenile	0.241105333	2
Edg.pot.1	Cloudy Bay	-43.44978	147.20385	Eastern	Adult	0.190196333	0.6
Edg.pot.3	Cloudy Bay	-43.44978	147.20385	Eastern	Adult	0.190196333	2
Edg.pot.4	Cloudy Bay	-43.44978	147.20385	Eastern	Juvenile	0.190196333	2
Edg.pot.5	Cloudy Bay	-43.44978	147.20385	Eastern	Adult	0.190196333	0.1
Edg.pot.6	Cloudy Bay	-43.44978	147.20385	Eastern	Juvenile	0.190196333	0.1
Edg.pot.9	Cloudy Bay	-43.44978	147.20385	Eastern	Juvenile	0.190196333	0
Edg.pot.11	Cloudy Bay	-43.44978	147.20385	Eastern	Juvenile	0.190196333	0
Edg.pot.13	Cloudy Bay	-43.44978	147.20385	Eastern	Juvenile	0.190196333	0
Edg.pot.14	Cloudy Bay	-43.44978	147.20385	Eastern	Juvenile	0.190196333	2
Edg.pot.15	Cloudy Bay	-43.44978	147.20385	Eastern	Juvenile	0.190196333	2
Edg.gov.1	Governor Island	-41.87557	148.31241	Western	Adult	0.370963	0
Edg.gov.2	Governor Island	-41.87557	148.31241	Western	Adult	0.370963	0
Edg.gov.3	Governor Island	-41.87557	148.31241	Western	Juvenile	0.370963	1
Edg.gov.4	Governor Island	-41.87557	148.31241	Western	Adult	0.370963	1
Edg.bin.29	Binalong Bay	-41.24912	148.308448	Western	Juvenile	0.241105333	2
Edg.bin.30	Binalong Bay	-41.24912	148.308448	Western	Juvenile	0.241105333	2
Edg.bin.33	Binalong Bay	-41.24912	148.308448	Western	Adult	0.241105333	2
Edg.pot.24	Cloudy Bay	-43.43963	147.23267	Western	Adult	0.190196333	0
Edg.pot.2	Cloudy Bay	-43.44978	147.20385	Western	Adult	0.190196333	1.2
Edg.pot.7	Cloudy Bay	-43.44978	147.20385	Western	Juvenile	0.190196333	0.1
Edg.pot.8	Cloudy Bay	-43.44978	147.20385	Western	Adult	0.190196333	0.4
Edg.pot.10	Cloudy Bay	-43.44978	147.20385	Western	Adult	0.190196333	0
Edg.pot.12	Cloudy Bay	-43.44978	147.20385	Western	Juvenile	0.190196333	0
Edg.pot.16	Cloudy Bay	-43.43963	147.23267	Western	Adult	0.190196333	0
Edg.pot.17	Cloudy Bay	-43.43963	147.23267	Western	Adult	0.190196333	0
Edg.pot.18	Cloudy Bay	-43.43963	147.23267	Western	Juvenile	0.190196333	0
Edg.pot.19	Cloudy Bay	-43.43963	147.23267	Western	Juvenile	0.190196333	0
Edg.pot.20	Cloudy Bay	-43.43963	147.23267	Western	Juvenile	0.190196333	0
Edg.pot.21	Cloudy Bay	-43.43963	147.23267	Western	Adult	0.190196333	0

Total length (cm)	Stipe length (cm)	Stipe Proportion	Stipe Stipe base top	Base stoutness	Top stoutness	Mean stoutness	Tapering
210	11	1.28	9.5	7	0.064	0.196	0.125
200	12	1.22	6.4	4.6	0.273	0.416	0.339
165	13	1.10	8.4	6	0.190	0.336	0.257
130	13	1.00	6.2	5.2	0.322	0.398	0.358
210	19	1.04	15.0	14	0.103	0.133	0.117
180	20	0.95	11.0	7.8	0.260	0.409	0.328
230	17	1.13	14.0	11	0.084	0.189	0.134
50	4	1.10	2.6	2.2	0.187	0.260	0.222
220	22	1.00	17.0	13	0.112	0.228	0.166
60	10	0.78	4.0	3	0.398	0.523	0.456
170	13	1.12	8.2	6.2	0.200	0.322	0.257
120	15	0.90	8.0	5.5	0.273	0.436	0.347
240	8	1.48	13.0	11	-0.211	-0.138	-0.176
220	9	1.39	6.5	6	0.141	0.176	0.158
200	14	1.15	11.0	7	0.105	0.301	0.192
190	15	1.10	8.0	5.6	0.273	0.428	0.344
210	15	1.15	10.0	8.8	0.176	0.232	0.203
220	20	1.04	7.0	6.5	0.456	0.488	0.472
230	30	0.88	21.0	17.5	0.155	0.234	0.193
100	10	1.00	4.6	4	0.337	0.398	0.367
110	13	0.93	5.8	4.4	0.351	0.470	0.406
100	13	0.89	6.0	5	0.336	0.415	0.374
130	13	1.00	8.0	7	0.211	0.269	0.239
120	17	0.85	9.0	7.6	0.276	0.350	0.311
250	9	1.44	7.0	6.2	0.109	0.162	0.135
270	17	1.20	11.0	9	0.189	0.276	0.230
110	3	1.56	3.0	2.8	0.000	0.030	0.015
120	9	1.12	8.0	7	0.051	0.109	0.079
145	10	1.16	10.0	7.4	0.000	0.131	0.060
110	14	0.90	8.0	7	0.243	0.301	0.271
120	15	0.90	10.0	8.5	0.176	0.247	0.210
160	13	1.09	8.4	9	0.190	0.160	0.174
33	8	0.62	2.4	2.1	0.523	0.581	0.551
35	9	0.59	4.0	3.2	0.352	0.449	0.398
160	14	1.06	8.8	8.6	0.202	0.212	0.207
200	14	1.15	12.4	11.6	0.053	0.082	0.067
150	14	1.03	10.2	9.9	0.138	0.150	0.144
175	24	0.86	15.0	11.5	0.204	0.320	0.258
25	3	0.92	1.8	1.5	0.222	0.301	0.260
130	24	0.73	13.8	15.5	0.240	0.190	0.214
210	15	1.15	6.0	7	0.398	0.331	0.363
350	24	1.16	8.8	9	0.436	0.426	0.431
300	20	1.18	12.4	11.2	0.208	0.252	0.229
65	13	0.70	5.6	4	0.366	0.512	0.433
175	13	1.13	6.4	7.5	0.308	0.239	0.272
170	13	1.12	6.8	5.8	0.281	0.351	0.315
177	18	0.99	6.8	5.6	0.423	0.507	0.463
141	12	1.07	6.5	6.2	0.266	0.287	0.276
120	11	1.04	7.0	8.2	0.196	0.128	0.161
172	23	0.87	7.0	8.4	0.517	0.437	0.475
87	17	0.71	5.8	4.6	0.467	0.568	0.514
61	8.5	0.86	3.0	2.8	0.452	0.482	0.467

161	11	1.17	4.6	5	0.379	0.342	0.360	-0.036
133	8	1.22	3.4	3.8	0.372	0.323	0.347	-0.048
94	5	1.27	2.8	3.4	0.252	0.167	0.208	-0.084
150	10	1.18	3.8	4.5	0.420	0.347	0.382	-0.073
265	10	1.42	5.6	5.4	0.252	0.268	0.260	0.016
65	31	0.32	9.2	7	0.528	0.646	0.583	0.119
380	13	1.47	6.0	5	0.336	0.415	0.374	0.079
69	4	1.24	2.6	2.6	0.187	0.187	0.187	0.000
172	11	1.19	3.8	3.2	0.462	0.536	0.497	0.075
80	12	0.82	5.0	4	0.380	0.477	0.426	0.097
121	13	0.97	3.8	4.5	0.534	0.461	0.496	-0.073
130	38	0.53	13.0	11.8	0.466	0.508	0.486	0.042
270	22	1.09	7.0	6.4	0.497	0.536	0.516	0.039
245	20	1.09	11.0	11	0.260	0.260	0.260	0.000
320	9	1.55	7.6	7	0.073	0.109	0.091	0.036
35	5	0.85	2.8	2.6	0.252	0.284	0.268	0.032
480	13	1.57	8.0	8	0.211	0.211	0.211	0.000
330	15	1.34	8.0	7.8	0.273	0.284	0.278	0.011
90	6	1.18	4.5	3.8	0.125	0.198	0.160	0.073
170	15	1.05	5.6	7	0.428	0.331	0.377	-0.097
125	24	0.72	6.6	6.4	0.561	0.574	0.567	0.013
315	10	1.50	4.5	4	0.347	0.398	0.372	0.051
247	18	1.14	14.0	10.4	0.109	0.238	0.169	0.129
140	18	0.89	7.0	6.4	0.410	0.449	0.429	0.039
180	10	1.26	4.0	4.2	0.398	0.377	0.387	-0.021
112	7	1.20	4.2	4.2	0.222	0.222	0.222	0.000
495	11	1.65	5.2	5.8	0.325	0.278	0.301	-0.047
122	15	0.91	4.8	4.6	0.495	0.513	0.504	0.018
160	11	1.16	4.7	4.3	0.369	0.408	0.388	0.039
105	10	1.02	3.8	4	0.420	0.398	0.409	-0.022
115	19	0.78	8.6	7.6	0.344	0.398	0.370	0.054
155	16	0.99	5.4	4.4	0.472	0.561	0.514	0.089
118	7	1.23	2.8	2.7	0.398	0.414	0.406	0.016
132	8	1.22	2.8	2.8	0.456	0.456	0.456	0.000
70	6	1.07	3.0	3.4	0.301	0.247	0.273	-0.054
85	5	1.23	2.4	2.2	0.319	0.357	0.337	0.038
90	5	1.26	2.8	2.6	0.252	0.284	0.268	0.032
96	6	1.20	3.0	2.8	0.301	0.331	0.316	0.030
75	12	0.80	7.0	5	0.234	0.380	0.301	0.146
198	13	1.18	7.2	7	0.257	0.269	0.263	0.012
60	6	1.00	3.2	3.1	0.273	0.287	0.280	0.014
88	8	1.04	5.4	4	0.171	0.301	0.231	0.130
327	19	1.24	6.2	5	0.486	0.580	0.531	0.093
42	6.7	0.80	4.0	2.9	0.224	0.364	0.288	0.140
120	9	1.12	5.9	4.2	0.183	0.331	0.251	0.148
104	18	0.76	6.4	5	0.449	0.556	0.499	0.107
73	16	0.66	5.2	4	0.488	0.602	0.541	0.114
130	14	0.97	4.8	5.1	0.465	0.439	0.452	-0.026
105	10	1.02	5.4	4	0.268	0.398	0.328	0.130
21	6.5	0.51	2.8	2.8	0.366	0.366	0.366	0.000
62	6	1.01	3.7	2.8	0.210	0.331	0.266	0.121
65	7	0.97	3.0	3	0.368	0.368	0.368	0.000
180	18	1.00	10.6	7.6	0.230	0.374	0.296	0.144
150	30	0.70	8.0	7	0.574	0.632	0.602	0.058
102	8	1.11	4.0	3.8	0.301	0.323	0.312	0.022
70	9	0.89	4.7	3.7	0.282	0.386	0.331	0.104

266	34	0.89	10.6	11	0.506	0.490	0.498	-0.016
57	6	0.98	3.0	2.8	0.301	0.331	0.316	0.030
50	6	0.92	2.8	2.8	0.331	0.331	0.331	0.000
59	3	1.29	2.8	2.4	0.030	0.097	0.062	0.067
18	2	0.95	1.8	1.8	0.046	0.046	0.046	0.000
120	18	0.82	13.4	12	0.128	0.176	0.151	0.048
105	9	1.07	5.0	4	0.255	0.352	0.301	0.097
50	8	0.80	3.0	2.6	0.426	0.488	0.456	0.062
175	10	1.24	5.8	5.4	0.237	0.268	0.252	0.031
145	8	1.26	5.0	4.2	0.204	0.280	0.240	0.076
118	3	1.59	4.8	4.6	-0.204	-0.186	-0.195	0.018
142	6	1.37	4.8	3.7	0.097	0.210	0.150	0.113
140	32	0.64	9.0	8.5	0.551	0.576	0.563	0.025
170	6	1.45	5.0	5.4	0.079	0.046	0.062	-0.033
65	18	0.56	6.0	4.8	0.477	0.574	0.523	0.097
73	11	0.82	6.0	4.7	0.263	0.369	0.313	0.106
99	17	0.77	5.0	5.3	0.531	0.506	0.519	-0.025
29	3	0.99	2.0	1.8	0.176	0.222	0.198	0.046
58	4	1.16	2.8	2.6	0.155	0.187	0.171	0.032
33	6	0.74	2.4	2.2	0.398	0.436	0.416	0.038
155	7	1.35	3.3	2.8	0.327	0.398	0.361	0.071
74	9	0.91	4.8	3.8	0.273	0.374	0.321	0.101
138	8	1.24	5.8	5.4	0.140	0.171	0.155	0.031
255	19	1.13	15.0	16.8	0.103	0.053	0.077	-0.049
112	14	0.90	4.8	4	0.465	0.544	0.503	0.079
136	4	1.53	3.0	2.4	0.125	0.222	0.171	0.097
260	37	0.85	28.0	29	0.121	0.106	0.113	-0.015
265	38	0.84	26.0	9	0.165	0.626	0.337	0.461
172	4	1.63	4.6	4.3	-0.061	-0.031	-0.046	0.029
136	11	1.09	8.6	6.8	0.107	0.209	0.155	0.102
270	38	0.85	25.0	19	0.182	0.301	0.237	0.119
125	48	0.42	19.4	25	0.393	0.283	0.335	-0.110
230	26	0.95	20.8	12.2	0.097	0.329	0.197	0.232
58	6	0.99	3.0	3	0.301	0.301	0.301	0.000
320	40	0.90	17.0	17	0.372	0.372	0.372	0.000
210	30	0.85	19.0	11.5	0.198	0.416	0.294	0.218
50	3	1.22	2.0	2	0.176	0.176	0.176	0.000
66	5	1.12	3.6	3.8	0.143	0.119	0.131	-0.023
34	2	1.23	1.4	1.4	0.155	0.155	0.155	0.000
104	7	1.17	3.6	3.4	0.289	0.314	0.301	0.025
25	5	0.70	2.8	1.6	0.252	0.495	0.357	0.243
70	6	1.07	5.0	3.3	0.079	0.260	0.160	0.180
196	7	1.45	8.0	6.8	-0.058	0.013	-0.024	0.071
340	21	1.21	7.4	10	0.453	0.322	0.383	-0.131
60	8	0.88	4.4	3.9	0.260	0.312	0.285	0.052
145	8	1.26	4.3	3.4	0.270	0.372	0.318	0.102
162	9	1.26	3.9	2.6	0.363	0.539	0.442	0.176
160	13	1.09	4.8	4.6	0.433	0.451	0.442	0.018
90	11	0.91	5.2	3.2	0.325	0.536	0.418	0.211
20	1	1.30	0.6	0.6	0.222	0.222	0.222	0.000
42	5	0.92	2.8	2.4	0.252	0.319	0.284	0.067
112	11	1.01	5.0	4	0.342	0.439	0.388	0.097
17	2	0.93	1.5	1.4	0.125	0.155	0.140	0.030
78	5	1.19	3.9	3.4	0.108	0.167	0.137	0.060
164	36	0.66	7.1	6	0.705	0.778	0.740	0.073
73	19	0.58	4.8	3.9	0.598	0.688	0.640	0.090

81	18	0.65	3.2	3.1	0.750	0.764	0.757	0.014
63	22	0.46	4.9	2.9	0.652	0.880	0.751	0.228
99	30	0.52	7.2	3.1	0.620	0.986	0.765	0.366
19.2	4.1	0.67	1.0	0.5	0.613	0.914	0.738	0.301
71	22	0.51	6.2	3.3	0.550	0.824	0.666	0.274
118	21	0.75	5.0	4	0.623	0.720	0.669	0.097
160	18	0.95	5.0	3.3	0.556	0.737	0.637	0.180
124	20	0.79	5.1	4.9	0.593	0.611	0.602	0.017
32	7	0.66	2.8	2.4	0.398	0.465	0.430	0.067
27	3	0.95	1.8	1.7	0.222	0.247	0.234	0.025
44	5	0.94	1.9	1.8	0.420	0.444	0.432	0.023
22	2	1.04	1.6	1.4	0.097	0.155	0.125	0.058
57	13	0.64	4.3	3	0.480	0.637	0.552	0.156
88	15	0.77	4.0	3.1	0.574	0.685	0.626	0.111
53	13	0.61	5.4	3.8	0.382	0.534	0.451	0.153
129	24	0.73	6.6	4.6	0.561	0.717	0.632	0.157
214	32	0.83	12.6	7.7	0.405	0.619	0.499	0.214
80	11	0.86	4.0	3.4	0.439	0.510	0.473	0.071
79	15	0.72	4.4	4	0.533	0.574	0.553	0.041
102	22	0.67	7.2	5.4	0.485	0.610	0.543	0.125
160	37	0.64	15.8	9	0.370	0.614	0.475	0.244
68	7	0.99	3.5	2.8	0.301	0.398	0.347	0.097
59	8	0.87	3.5	3	0.359	0.426	0.391	0.067
28	5	0.75	3.0	2	0.222	0.398	0.301	0.176
40	5	0.90	2.9	2.5	0.237	0.301	0.268	0.064
45	7	0.81	2.6	2	0.430	0.544	0.483	0.114
25	2	1.10	1.4	1.4	0.155	0.155	0.155	0.000
26	3	0.94	1.8	1.4	0.222	0.331	0.273	0.109
105	17	0.79	7.5	5.2	0.355	0.514	0.428	0.159
81	24	0.53	6.0	3.6	0.602	0.824	0.699	0.222
88	14	0.80	5.0	3	0.447	0.669	0.544	0.222
111	38	0.47	11.6	5.8	0.515	0.816	0.640	0.301
120	16	0.88	4.9	3.8	0.514	0.624	0.566	0.110
40	9	0.65	3.0	2.3	0.477	0.593	0.531	0.115
197	38	0.71	9.4	6.7	0.607	0.754	0.674	0.147
115	18	0.81	5.8	4.8	0.492	0.574	0.531	0.082
21	4	0.72	2.7	2.4	0.171	0.222	0.196	0.051
127	26	0.69	7.0	5	0.570	0.716	0.637	0.146
88	23	0.58	6.0	4	0.584	0.760	0.663	0.176
100	25	0.60	7.2	6.8	0.541	0.565	0.553	0.025
114	23	0.70	6.0	3.8	0.584	0.782	0.672	0.198
102	28	0.56	7.9	4.1	0.550	0.834	0.669	0.285
70	14	0.70	3.2	2.5	0.641	0.748	0.691	0.107
91	17	0.73	4.9	3.3	0.540	0.712	0.618	0.172
58	12	0.68	3.2	2.6	0.574	0.664	0.617	0.090
365	24	1.18	19.4	10.2	0.092	0.372	0.210	0.279
303	43	0.85	11.0	10	0.592	0.633	0.612	0.041
165	31	0.73	9.8	4	0.500	0.889	0.653	0.389
120	20	0.78	6.2	4.7	0.509	0.629	0.565	0.120
164	40	0.61	9.6	6.1	0.620	0.817	0.707	0.197
98	20	0.69	5.7	2.8	0.545	0.854	0.673	0.309
170	17	1.00	10.6	5	0.205	0.531	0.338	0.326
247	18	1.14	10.2	11	0.247	0.214	0.230	-0.033
175	28	0.80	8.6	6.9	0.513	0.608	0.558	0.096
203	14	1.16	9.0	6.3	0.192	0.347	0.262	0.155
164	22	0.87	9.0	18.8	0.388	0.068	0.199	-0.320

149	37	0.60	10.3	4.7	0.555	0.896	0.693	0.341
179	33	0.73	7.9	6.6	0.621	0.699	0.658	0.078
91	7	1.11	6.0	4.9	0.067	0.155	0.109	0.088
50	20	0.40	6.8	4.5	0.469	0.648	0.549	0.179
182	17	1.03	10.0	7.5	0.230	0.355	0.288	0.125
205	27	0.88	8.8	5.6	0.487	0.683	0.574	0.196
390	65.2	0.78	21.5	20	0.482	0.513	0.497	0.031
163	11.5	1.15	9.6	6.6	0.078	0.241	0.152	0.163
128	11.5	1.05	9.8	6.5	0.069	0.248	0.150	0.178
140	33	0.63	20.5	14	0.207	0.372	0.282	0.166
164	33	0.70	16.5	10	0.301	0.519	0.396	0.217
211	10	1.32	8.4	6.1	0.076	0.215	0.140	0.139
85	14.5	0.77	5.6	4	0.413	0.559	0.480	0.146
103	13	0.90	7.6	5.1	0.233	0.406	0.311	0.173
212	11	1.28	7.5	5.5	0.166	0.301	0.228	0.135
230	14.5	1.20	10.0	5.8	0.161	0.398	0.264	0.237
158	5.5	1.46	8.1	6.5	-0.168	-0.073	-0.123	0.096
153	16	0.98	16.5	11	-0.013	0.163	0.066	0.176
128	15	0.93	11.6	8.8	0.112	0.232	0.167	0.120
166	13	1.11	4.0	4.1	0.512	0.501	0.506	-0.011
217	9	1.38	13.0	7.5	-0.160	0.079	-0.056	0.239
158	9.5	1.22	7.0	5.4	0.133	0.245	0.185	0.113
220	14.5	1.18	17.7	13	-0.087	0.047	-0.025	0.134
230	23	1.00	17.1	11.2	0.129	0.313	0.211	0.184
171	11	1.19	11.5	7.1	-0.019	0.190	0.073	0.209
133	7.5	1.25	10.1	10.2	-0.129	-0.134	-0.131	-0.004
110	15.5	0.85	11.0	6	0.149	0.412	0.261	0.263
83	17	0.69	11.5	5.8	0.170	0.467	0.293	0.297
160	20	0.90	8.0	6	0.398	0.523	0.456	0.125
217	19.5	1.05	8.5	7.5	0.361	0.415	0.387	0.054
365	35	1.02	12.6	12	0.444	0.465	0.454	0.021
256	30	0.93	17.2	9.5	0.242	0.499	0.352	0.258
370	27	1.14	14.0	8	0.285	0.528	0.390	0.243
165	19	0.94	9.5	6	0.301	0.501	0.389	0.200
290	15	1.29	14.0	8.2	0.030	0.262	0.131	0.232
230	14	1.22	12.5	8.9	0.049	0.197	0.117	0.148
112	12.8	0.94	5.1	4.8	0.400	0.426	0.413	0.026
72	15	0.68	7.4	4.3	0.307	0.543	0.409	0.236
123	17.5	0.85	10.7	6	0.214	0.465	0.321	0.251
105	13	0.91	8.8	5	0.169	0.415	0.275	0.246
36	7.8	0.66	3.8	3	0.312	0.415	0.361	0.103
232	15	1.19	8.0	6.4	0.273	0.370	0.319	0.097
248	25	1.00	11.0	8.6	0.357	0.463	0.407	0.107
142	19	0.87	11.0	5.8	0.237	0.515	0.354	0.278
109	14	0.89	7.5	4.5	0.271	0.493	0.368	0.222
105	41	0.41	14.5	9	0.451	0.659	0.543	0.207
168	29	0.76	15.2	11.2	0.281	0.413	0.342	0.133
71	21	0.53	8.5	5.5	0.393	0.582	0.477	0.189
160	27	0.77	11.2	10.6	0.382	0.406	0.394	0.024
231	37	0.80	15.2	10	0.386	0.568	0.468	0.182
250	21	1.08	12.5	8.8	0.225	0.378	0.295	0.152
258	19	1.13	9.0	5	0.325	0.580	0.434	0.255
90	16.5	0.74	9.7	5.8	0.231	0.454	0.328	0.223
166	17	0.99	10.0	5.5	0.230	0.490	0.341	0.260
430	27	1.20	13.3	12	0.308	0.352	0.329	0.045
195	22	0.95	4.7		0.669		0.669	

36	4.8	0.88	20.7	-0.635	-0.635
216	34	0.80	11.0	0.490	0.490
67	14	0.68	3.8	0.570	0.570
162	31	0.72	10.1	0.489	0.489
235	24	0.99	7.5	0.503	0.503
164	13.5	1.08	5.3	0.403	0.403
53	13.2	0.60	3.1	0.623	0.623
57	9.4	0.78	2.2	0.631	0.631
110	25.6	0.63	8.8	0.464	0.464
389	41	0.98	11.6	0.547	0.547
54	6	0.95	2.2	0.436	0.436
54	17	0.50	4.1	0.619	0.619
92	34	0.43	8.2	0.619	0.619
210	59	0.55	11.9	0.694	0.694
45	9.6	0.67	3.5	0.444	0.444
52	6.8	0.88	2.5	0.432	0.432
68	11	0.79	3.8	0.465	0.465
61	8	0.88	1.9	0.628	0.628
45	9.4	0.68	2.2	0.631	0.631
110	12	0.96	6.5	0.266	0.266
150	90	0.22	13.0	0.840	0.840
36	6	0.78	2.0	0.477	0.477
100	16	0.80	5.0	0.505	0.505
31	2.2	1.15	0.6	0.564	0.564
51	8.5	0.78	3.0	0.452	0.452
43	11.2	0.58	2.8	0.602	0.602
53	9.5	0.75	2.5	0.580	0.580
39	18	0.34	3.8	0.675	0.675
36	6	0.78	1.8	0.523	0.523
94	13	0.86	6.9	0.274	0.274
63	8.1	0.89	4.4	0.265	0.265
41	8.7	0.67	3.8	0.363	0.363
128	16.5	0.89	6.9	0.378	0.378
55	9	0.79	3.5	0.416	0.416
45	9.6	0.67	2.8	0.531	0.531
145	24.4	0.77	6.9	0.548	0.548
100	38	0.42	14.1	0.431	0.431
230	55	0.62	13.1	0.623	0.623
98	12	0.91	3.8	0.499	0.499
220	40	0.74	13.0	0.488	0.488
62	13	0.68	4.2	0.491	0.491
59	15	0.59	3.8	0.596	0.596
260	20	1.11	8.8	0.357	0.357
60	15	0.60	5.7	0.420	0.420
150	12	1.10	3.5	0.535	0.535
74	10	0.87	2.8	0.553	0.553
88	16	0.74	3.8	0.624	0.624
220	40	0.74	9.7	0.615	0.615

Lateral asymmetry	Lateral number	Conjoined	Branched
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0	0	0	0
0	0	1	0
3	3	1	0
0	0	1	0
0	0	1	0
1	9	1	0
0	0	1	0
5	5	0	0
0	0	1	0
0	0	1	0
6	10	0	0
0	0	0	1
0	0	1	0
4	16	1	0
0	0	1	0
0	0	0	1
3	19	0	0
1	7	0	0
0	0	1	0
0	0	1	0
0	0	0	0
0	8	0	0
0	2	0	0
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0	0	1	0
0	0	1	0
0	0	0	0
0	0	0	0
1	11	1	0
7	11	1	0
6	20	0	0
0	0	0	0
5	11	0	0
1	7	0	0
0	0	0	0
8	8	1	0
0	0	1	0
1	9	1	1
0	0	0	0
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0	0	1	1
2	4	1	0
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0	0	1	0

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10	22	1	0
3	5	1	0

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3	11	1	0
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11	11	0	0
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3	17	0	0

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1	5	0	0
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2	12	1	0
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0	6	0	0
3	3	0	1
2	26	1	0
4	22	0	0
1	5	0	1
5	15	1	0
2	12	1	0
2	26	0	0
3	15	0	0
1	5	1	0
0	0	0	1
3	7	0	1
8	14	1	0
3	9	0	0
0	6	1	0
1	7	0	0
1	11	0	0
0	8	0	0
0	0	0	1
3	5	1	0
0	0	1	0
0	6	0	0
2	12	0	0
2	12	1	0
0	0	1	0
0	0	0	1
0	0	1	0
0	0	1	0
0	18	0	0

0	10	0	0
5	41	0	0
0	0	0	1
2	10	1	0
0	0	0	0
0	0	0	0
2	30	0	0
2	12	0	0
4	10	1	1
2	2	0	0
1	1	0	0
8	22	0	0
0	0	0	0
2	2	0	0
0	4	0	1
9	9	0	0
3	13	1	1
8	24	0	0
2	12	0	0
0	0	1	0
12	22	0	0
4	4	0	0
1	37	1	0
0	2	0	0
3	29	1	0
0	10	1	0
2	4	0	0
4	4	0	0
0	24	1	0
3	5	1	0
1	15	0	0
0	4	0	0
0	12	0	0
9	21	0	0
4	16	0	0
4	6	1	0
2	2	0	0
2	16	0	0
21	21	1	0
1	1	0	0
4	18	1	0
5	5	0	0
5	5	1	0
2	4	0	0
7	11		0
3	9		0
3	29		0
3	23		0
4	12		0
0	2		0
6	10		1
2	2		0
4	14		0
3	3		0
3	21		
3	13		

[illegible]