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Genetic Connectivity Amongst New Zealand's Open Sandy Shore and Estuarine Coastal Taxa

New Zealand Aquatic Environment and Biodiversity Report No. 172

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EXECUTIVE SUMMARY

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This report summarises the main findings of research conducted to quantify patterns of genetic structure and rates of gene flow (connectivity) amongst populations of five species of New Zealand coastal marine taxa and to identify the location of likely barriers to gene flow (New Zealand Ministry for Primary Industries (MPI) contract ZBD2009-10). The size and complexity of New Zealand's marine environment creates challenges for the management of marine species. Therefore, identifying consistencies in the genetic population structure amongst species has the potential to confirm ecologically important barriers to gene flow, supporting the management of a wider array of species with similar life-history traits.

We focussed on five study species, three shellfish (tuatua, *Paphies subtriangulata*; pipi, *P. australis*; New Zealand scallop, *Pecten novaezelandiae)* and two finfish (sand flounder, *Rhombosolea plebeia*; yellow belly flounder, *R. leporina*). These species were chosen because they are all currently managed under New Zealand's quota management system (QMS), and represent taxa from estuarine and open sandy coastal habitats that have been poorly surveyed in past studies for genetic population structure. Microsatellite DNA markers were developed for all five species and used to characterise the genetic structure of samples collected from 10 to 19 locations per species throughout New Zealand.

All five species demonstrated unique patterns of genetic population structure. *Paphies subtriangulata* was generally characterised by large scale panmixia but some populations showed evidence that they may be genetically differentiated. At a large spatial scale, *P. australis* could be differentiated into three genetically distinct groups (northern, south eastern, south western) but at a smaller spatial scale there was evidence for genetic differentiation amongst populations separated by only tens to hundreds of kilometres. *Rhombosolea plebeia* showed evidence of north-south differentiation with a break between northern and southern localities at around 42°S. There was evidence of east-west differentiation for *R. leporina* and three genetically distinct groups were detected. *Pecten novaezelandiae* could be divided into five genetically differentiated regions: North, Central, Fiordland, Stewart Island and Chatham Is.

However some commonalities in genetic population structure and the location of barriers to dispersal were also observed. This study provided further evidence that the region just south of Cook Strait is an historical phylogeographic break and a contemporary barrier to gene flow for some species. It also showed that populations in the region can exhibit spatial and temporal genetic variability, and that there is not necessarily a barrier to gene flow for all species. This study highlighted the importance of physical oceanography, reproductive characteristics and ecology on population connectivity, and how the evolutionary history of a species (e.g., time of origin, exposure to glaciation and sea level fluctuations) can play an important role in shaping contemporary genetic population structure.

Whilst it is possible to identify common genetic patterns and barriers to dispersal amongst species, the species-specific genetic patterns observed in this study necessitate identification of genetic population structure of individual species, rather than assuming that all shellfish or fish (even if they share biological characteristics) have the same stock structure.

1. BACKGROUND

This report summarises the main findings of research conducted to quantify patterns of genetic structure and the rates of gene flow (connectivity) amongst populations of five New Zealand coastal marine taxa, and to identify the location of barriers to gene flow (New Zealand Ministry for Primary Industries (MPI) contract ZBD2009-10). The species chosen for this study were primarily based on a review of earlier literature describing the population genetic structure of New Zealand coastal taxa that identified gaps in our understanding of species from open sandy shores and estuarine environments (Gardner et al. 2010). For this study we selected two surf clam species (one open sandy coast, the other estuarine) and two flounder species (one open sandy coast, the other estuarine) as suitable research candidates. Subsequently, the New Zealand scallop (shallow subtidal, soft substrate environment) was added to the study.

2. INTRODUCTION

An understanding of genetic population structure and connectivity is an important component of understanding marine ecosystem function and can be used to enhance resource management and conservation measures (Laikre et al. 2005; Palsbøll et al. 2007; Taylor et al. 2005; Waples et al. 2008). Such knowledge is important as the world's marine ecosystems come increasingly under pressure from numerous anthropogenic activities, including fishing, pollution, debris, ocean acidification and invasive species (Scavia et al. 2002; Hewitt & Willing 2004; Halpern et al. 2007). The geographic distribution of individuals within a species' range (i.e., population structure) is in part determined by the rate at which individuals are exchanged between geographically separated locations, termed 'population connectivity' (Cowen et al. 2007). Determining the rates and routes by which populations are connected can inform the management of commercial fish stocks (Shaklee & Currens 2003), contribute to ensuring the effectiveness of marine protected areas (Palumbi 2003; Berumen et al. 2012) and identify sources of biosecurity threats (Geller et al. 2010).

2.1 The link between genetic population structure, connectivity and larval dispersal

Many marine species are characterised by a bi-phasic life history, whereby adults are relatively sedentary compared to their larvae, which are released into the ocean environment. Therefore, the processes operating at the larval stage largely determine the ability of the species to disperse between geographically distinct locations and the population structure that will form amongst adult populations. Dispersal ability is related to both the life history characteristics and the behaviour of the larvae, and the physical features of the ocean environment (Cowen & Sponaugle 2009). The small size of larvae relative to the large ocean environment poses a challenge when it comes to tracking dispersal routes amongst geographic locations. Direct- and indirect-sampling methods have been used to investigate population structure and connectivity (Levin 2006), of which population genetics is an increasingly powerful tool for defining reproductive units and revealing population processes operating at a range of spatial and temporal scales.

In the marine environment a continuum of genetic population structures exist, which range from 'open' to 'closed' (or genetically indistinct to genetically distinct) and are attributed to relative levels of connectivity. In 'open' populations, individuals are received and exported amongst populations, usually at wide spatial scales, gene flow is high, and random genetic drift is limited. Typically in such situations there will be little opportunity for the accumulation of genetic differences among populations. In 'closed' populations there is some sort of 'barrier', either physical or biological, that limits the exchange of individuals amongst geographically separated populations, leading to reduced gene flow and greater differentiation of populations.

Given that many marine species have a pelagic dispersal stage followed by a demersal adult stage (Nybakken & Bertness 2005), it has been suggested that the duration of the pelagic stage may provide a proxy for how connected populations are, and hence the type of genetic population structure that forms (Cowen & Sponaugle 2009; Shanks 2009). Species with a long pelagic larval duration (PLD), on the scale of months to years, may have greater potential for being transported further away from their location of origin, connecting spatially separated populations demographically and genetically. Species with a shorter PLD may have, on average, greater genetic differentiation between populations due to their more limited time for dispersal (Shanks 2009 and references therein). We would expect a positive relationship between pelagic larval duration and dispersal distance, however, there are too many exceptions to this rule of thumb for this hypothesis to be a reliable predictor of structure (Shanks 2009; Weersing & Toonen 2009).

For example, the New Zealand rock lobster (*Jasus edwardsii*) has one of the longest known PLDs of any marine species (more than two years), yet has two distinct populations within New Zealand, over 1100 km (Thomas & Bell 2013). In a review of New Zealand marine fauna, Ross et al. (2009) noted that larval duration had a positive correlation with population connectivity, however this pattern was highly variable in organisms with shorter larval durations (less than 10 days). Weersing & Toonen (2009) reviewed 300 peer reviewed studies and found no relationship with PLD and genetic structure after species with no larval duration were removed from the analysis. This means that there is no simple indicator that can be used to predict or understand how life history characters in general or PLD itself may be related to gene flow and population genetic structure. In addition, there is increasing awareness of the distinction between potential and realised larval dispersal (Reisser et al. 2014). Population connectivity is not as simple as how long a larva can potentially float as a passive particle in the ocean currents; instead localised oceanographic conditions may promote or prevent gene flow.

2.2 Models of genetic population structure in the marine environment

Due to the challenges of measuring genetic population connectivity in the marine environment, genetic population structure is often conceptualised using a number of models based on simplified and stable population states. Two models that are commonly referred to in the literature include the ‘stepping-stone model’ and ‘panmixia’.

Due to the inter-connected nature of the marine environment and perceived lack of barriers to larval dispersal, it has been argued that most species should be characterised by ‘open’ population structure (i.e., panmixia). Panmixia has been demonstrated over large geographic areas in many species, such as the European eel *Anguilla anguilla* (Palm et al. 2009), the Japanese eel *Anguilla japonic*a (Han et al. 2010), the Indo-Pacific sleek unicorn fish *Naso hexacanthus* (Horne & van Herwerden 2013), the triplefin *Grahamina gymnota* (Hickey et al. 2009) and the black nerite (Reisser et al. 2014).

The ‘stepping-stone’ model has also been proposed as a useful representation of the ‘linear’ distribution of marine populations along a coastline (Slatkin 1993). Under the stepping-stone model, populations exchange migrants with neighbouring populations at a greater rate than they do with more geographically distant populations, resulting in an isolation by distance (IBD) pattern, where distance among populations becomes a barrier to connectivity and geographically proximate populations are more genetically similar. This type of spatial pattern has been reported for the purple sea urchin *Stronglyocentrotus purpuratus* in the Indo-West Pacific (Palumbi, 1996), the barnacle *Balanus glandula* along the California coast (Sotka et al. 2004) and copper rockfish *Sebastes caurinus* along the West Coast of Canada and the United States (Buonaccorsi et al. 2002).

2.3 A new direction

With the increasing number of published genetic studies, it is evident that simple conceptual models do not sufficiently account for the complexity of processes and genetic patterns that are observed. A greater understanding of the physical and ecological processes experienced by marine species has highlighted the stochastic and unpredictable nature of connectivity processes in the marine environment (Siegel et al. 2008). Whilst the distance that larvae are able to disperse through the environment can certainly have an effect on the genetic structure of populations, the connectivity of marine populations can also be strongly affected by biology (Scheltema 1986; Rocha et al. 2007), larval behaviour (Kingsford et al. 2002), currents (Galindo et al. 2006; White et al. 2010), the spatial arrangement of suitable habitats for recruitment (Parsons 1996; Bilton 2002), and historical events, such as past glaciations or changes in sea level, which can leave a detectable signature in the genetic population structure of a species for many generations after the event has occurred (Hewitt 1996; Apte & Gardner 2002). This latter point is particularly important in the context of, for example, sea level change during the last one million years and the pattern of genetic structure that is still visible amongst many of New Zealand's coastal species (Gardner et al. 2010).

An additional complicating factor is that, whilst examining the genetic structure of a species at a point in time may identify patterns of differentiation amongst marine populations and the location of potential barriers to connectivity, these patterns or barriers are not always stable over the long term. A feature of barriers in the marine environment is that they may be temporally variable. By temporally variable, we mean that the barriers may restrict connectivity (i.e., have limited permeability) for much of the time but occasionally, with the right combination of physical and biological conditions, they can have increased permeability, allowing larger numbers of larvae to cross the barrier (Selkoe et al. 2010; Ovenden 2013). Over time these infrequent connectivity events may lead to a degree of homogenisation of genetic diversity amongst populations on a large spatial scale. This phenomenon may be responsible for the weak genetic population structure that is observed in many marine species (Waples 1987; Hedgecock et al. 2007).

In contrast, on a short time scale the variability of oceanographic processes can also cause small scale patterns of genetic heterogeneity, termed chaotic genetic patchiness (Johnson & Black 1982), where populations in close geographic proximity may show significant genetic differentiation. This may be a result of limited 'windows of opportunity' for larval survival, meaning that by chance, some genetically similar cohorts may have an increased chance of surviving and recruiting to the adult population. This may have a significant impact on a population's genetic structure in the short term, resulting in an increase in genetic differentiation with other nearby populations. Hedgecock (1994) reported this type of "sweepstake recruitment" effect in the Pacific oyster *Crassostrea gigas*. The implication of temporal variation in population connectivity is that even when a species appears to be genetically homogenous over a large spatial scale, barriers to larval dispersal may exist and on a smaller spatial scale populations may be reliant on self-recruitment for periods of time (Swearer et al. 2002).

2.4 The New Zealand context

New Zealand is an archipelago of over 700 islands, stretching from the subtropical Kermadec Islands at 29° S to the sub-Antarctic Campbell Island at 52° S (Figure 1). The oceanography of the region is complex; it straddles the subtropical convergence and is influenced by the inflow of both warm sub-tropical and cold sub-Antarctic water masses (Chiswell et al. 2015). Major westerly flowing current systems reach the continental shelf and are divided into numerous coastal currents and eddies. The latitudinal extent of the country means that significant north-south gradients exist in the environmental variables that characterise the physical ocean environment (Francis & Nelson 2003). It is difficult to predict the effect this complexity might have on the populations of marine organisms inhabiting the coastal marine environment (Ross et al. 2009). However, it is the complexity of the

region that makes it interesting for investigating the influence of oceanographic and environmental variation on genetic structure and connectivity in coastal marine species. New Zealand's large exclusive economic zone (EEZ) contains high levels of marine diversity and of endemism, and many commercially valuable species (Gordon et al. 2010). Understanding the patterns of connectivity that exist amongst coastal marine species in the New Zealand region (that is, separate stocks or connected populations), and how they might arise, is useful information to support sustainable fisheries management, conservation and biosecurity work.

To understand the effect that complex coastal marine environments can have on genetic population structure it is often useful to compare population genetic patterns across many species in the same region. This can be a powerful way to identify consistent, or unexpected patterns of connectivity, and geographic barriers to gene flow, and it can provide insight about the processes that form such patterns. Studies from the north-eastern Pacific have demonstrated the merit of this approach. For example, Kelly & Palumbi (2010) compared the genetic population structure of 50 rocky intertidal species from Alaska to California and found evidence for unexpected regional patterns of genetic variation. Furthermore, they found that habitat (specifically shore height in the intertidal zone) had a strong influence on levels of genetic subdivision within a species. A further example from Pacific reef fish demonstrates how, by comparing connectivity patterns amongst several species, unexpected cases of gene flow may be found across a well-established biogeographic barrier that was previously thought to be largely impermeable by migrants (Lessios & Robertson 2006). These studies demonstrate the value of comparing patterns of genetic population structure between species to understand connectivity at the ecosystem-wide level.

The attributes of the New Zealand coastal marine environment make it an ideal region in which to repeat similar multi-species investigations. Patterns of genetic population structure observed in New Zealand coastal marine species have been reviewed elsewhere (Gardner et al. 2010; Ross et al. 2009). Gardner et al. (2010) identified common patterns of genetic population structure by reviewing 58 studies of 42 coastal marine organisms, and summarising the patterns of observed genetic structure into five categories. Sixteen of these studies reported no genetic structure amongst populations, nine reported isolation by distance, 12 reported divergence within and/or amongst populations, one reported west-east divergence and 19 north-south divergence. In studies that reported genetic breaks amongst populations, sampling effort was often insufficient to determine the geographic location of the barrier but studies that described north-south differentiation commonly reported the location of that break at around 41–42° S, just south of Cook Strait. The review also identified habitat types (such as estuarine, soft substrate and open coast habitats) and geographic locations that had been poorly surveyed, and recommended that these knowledge gaps be filled.

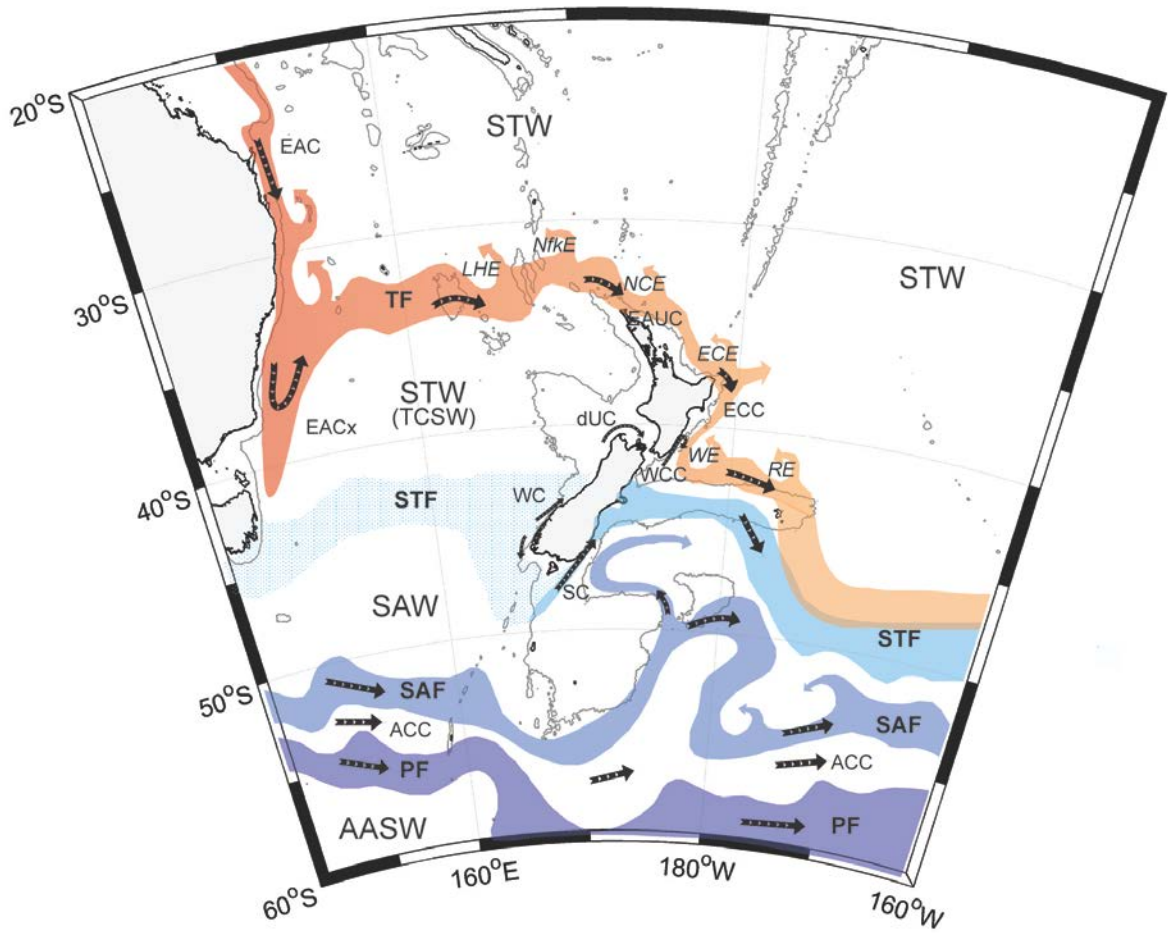

Figure 1: The New Zealand marine environment, showing the location of major surface current patterns water masses, and bathymetry. Abbreviations used for currents and water masses are: Subtropical Water (STW), Tasman Sea Central Water (TSCW), Subantarctic Water (SAW) and Antarctic Surface Water (AASW). Ocean fronts are Tasman Front (TF), Subtropical Front (STF), Subantarctic Front (SAF) and Polar Front (PF). Ocean currents are East Australia Current (EAC), East Australia Current extension (EACx), East Auckland Current (EAUC), East Cape Current (ECC), d'Urville Current (dUC), Wairarapa Coastal Current (WCC), Westland Current (WC), Southland Current (SC) and Antarctic Circumpolar Current (ACC). Eddies are Lord Howe Eddy (LHE), Norfolk Eddy (NfkE), North Cape Eddy (NCE), East Cape Eddy (ECE), Wairarapa Eddy (WE) and Rekohu Eddy (RE). Reproduced from Chiswell et al. (2015) with permission.

2.5 Background to the study species

Five species were used in this study of genetic population structure and connectivity in New Zealand coastal marine species. These species are representative of soft shore and estuarine environments that have been poorly surveyed in past studies of genetic population structure of New Zealand coastal marine species. In addition, all five species are currently managed within the QMS.

2.5.1 Paphies spp.

Paphies subtriangulata (commonly known as tuatua; Wood 1928) and *P. australis* (commonly known as pipi; Gmelin 1791) are bivalve molluscs belonging to the family Mesodesmatidae. In New Zealand the genus *Paphies* is comprised of large, edible 'surf clams', which also include the toheroa (*P. ventricosa*) and deep water tuatua (*P. donacina*). All four species form important recreational and customary fisheries and some support modest commercial catches (under 205 tonnes; Redfearn, 1987; Hooker, 1997; New Zealand Ministry for Primary Industries 2015a, 2015b).

2.5.1.1 Reproductive biology and ecology

Both *P. subtriangulata* and *P. australis* have widespread distributions around the coast of New Zealand and its offshore islands, including the Chatham Islands (both species) and Auckland Island (*P. australis*) (Powell 1979). *Paphies subtriangulata* is one of the most abundant infaunal bivalves on fine-sand, open coast beaches and can be found from the low intertidal zone to depths of about four metres (Redfearn 1987). *Paphies australis* is characteristic of coarse shell and sand substrates in sheltered harbour and estuarine environments, and can be found from the intertidal zone to depths of seven metres in channel areas (Morton & Miller 1968). The two species are known to coexist where these two habitats overlap, such as harbour entrances or sheltered sandy beaches (Grant et al. 1998).

Both species are gonochoristic and reproduce sexually by free-spawning of gametes, followed by external fertilisation. In the north-east of the North Island *P. subtriangulata* is known to spawn from February to April, followed by regeneration of the gonad from May to late August and resumption of spawning activity from September to November (Grant & Creese 1995). *Paphies australis* has a similar reproductive cycle to *P. subtriangulata*; gametes begin to form in autumn and by late winter gonads are mature and ready to spawn (Hooker & Creese 1995). The gametes are released over an extended spawning period from late winter to late summer (Hooker & Creese 1995). Due to similarities in their reproductive behaviour, *P. subtriangulata* and *P. australis* have been observed spawning synchronously in areas where their habitat overlaps, suggesting the possibility of hybridisation (Grant et al. 1998).

For both species the pelagic larval period lasts two to three weeks before settlement to the adult habitat (Redfearn 1987; Hooker 1997). Adults of both species are suspension feeders and can be found wedged a few centimetres below the surface of the sand (Richardson et al. 1982; Williams et al. 2007). Little is known about mortality and longevity in both species, but it has been suggested that *P. subtriangulata* can live five or more years and *P. australis* can live up to 10 years (Williams et al. 2007; New Zealand Ministry for Primary Industries 2015a).

There has been some confusion over the status of tuatua in New Zealand due to the morphological similarity of the tuatua (*P. subtriangulata*) and the deep water tuatua (*P. donacina*). Initially it was proposed that the two species represented two ends of a geographic cline as *P. subtriangulata* is more commonly found on northern beaches and *P. donacina* more common on southern beaches (Beu & Rooij-Schuiling 1982). Their status was resolved by examination of shell shape and colour, adductor muscle colour, and an electrophoretic marker that clearly revealed that they were separate species (Richardson et al. 1982). In addition, specimens from the Chatham Islands have sometimes been

considered to be a third species of tuatua, *P. porrecta* (Beu & Rooij-Schuiling 1982). An investigation of allozyme polymorphism at four loci from 13 locations around New Zealand indicated the presence of three geographical groups of *P. subtriangulata*: north, central and Chatham Island (Smith et al. 1989). Samples from Stewart Island may represent a fourth southern group owing to this population's geographical isolation and genetic differences with the central group (Smith et al. 1989).

2.5.1.2 Fisheries management

Paphies subtriangulata and *P. australis* are both managed under the QMS but there is little information on the stock structure of these species. The fisheries are primarily recreational and customary with shellfish being gathered by hand, but the level of recreational and customary harvest is unknown and there is no minimum legal size (New Zealand Ministry for Primary Industries 2015a, 2015b). The recreational daily catch limit for both species is 150 per person. Many stocks are assumed to be near virgin biomass but there is concern for depletion of some local stocks, especially in the Auckland-Coromandel region, where the limit has been reduced to 50 clams per person per day. High variability in larval recruitment is likely to mean that biomass is also highly variable (New Zealand Ministry for Primary Industries 2015a, 2015b).

Commercial harvest of *P. subtriangulata* is a dredge fishery restricted to the Kaipara Harbour in quota management area (QMA) 9 (Fishstock TUA 9) with a total allowable commercial catch (TACC) of 43 tonnes per year (New Zealand Ministry for Primary Industries 2015b). Commercial harvest of *P. australis* primarily comes from Mair Bank, Whangarei, in PPI 1A, with a TACC of 200 tonnes per year (New Zealand Ministry for Primary Industries 2015a). It has been estimated that *P. australis* is harvested below the maximum sustainable yield but it is unknown if these levels of harvest are sustainable in the long term (New Zealand Ministry for Primary Industries 2015a). The status of all *P. subtriangulata* stocks is unknown (New Zealand Ministry for Primary Industries 2015b).

2.5.1.3 Research needs and predictions

Whilst a previous study of allozyme variation in *P. subtriangulata* suggested that population differentiation might be present in this species (Smith et al. 1989), it is unknown whether genetically distinct populations of *P. australis* exist and how patterns of genetic variation might be geographically structured. Microsatellite DNA can be used to conduct a more detailed investigation of *P. subtriangulata* genetic population structure because microsatellites are a marker that can provide more resolution than allozymes. Patterns and rates of genetic connectivity amongst populations are unknown for both species. Such knowledge can inform fisheries management for these species, as stock structure is presently unknown.

Whilst recognising that ecology and reproductive traits are not definitive approaches for predicting a species' genetic population structure, nonetheless these characteristics can provide useful information to help formulate hypotheses for the most likely types of genetic population structure. Both *P. subtriangulata* and *P. australis* have pelagic larval durations of approximately three weeks, allowing larvae to potentially disperse amongst sites separated by several hundred kilometres (Shanks 2009; Shanks et al. 2003). The primary difference between these two species is in their habitat preferences. The open sandy coastlines inhabited by *P. subtriangulata* are thought to be characterised by fewer barriers to larval dispersal compared to *P. australis*. Several studies of bivalves inhabiting similar environments, such as the soft-shell clam (*Mya arenaria*; Strasser & Barber 2008), the Arctic surf clam (*Mactromeris polynyma*; Cassista & Hart 2007) and the surf clams *Donax serra* and *D. deltoides* (Murray-Jones & Ayre 1997; Laudien et al. 2003) have revealed limited genetic differences amongst populations separated by thousands of kilometres. Other studies have however, revealed some structure amongst populations but usually associated with a biogeographic break (*Merceneria*

merceneria; Baker et al. 2008), a physical barrier (*Coelomactra antiquate*; Kong et al. 2007) or isolation by distance (*Mactra veneriformis*; Hou et al. 2006).

In comparison, the estuarine habitats favoured by *P. australis* often represent discrete environments separated from each other by ecological and geographic barriers. It is expected that the exchange of individuals between estuaries will be limited, leading to the genetic differentiation of populations (Bilton 2002). However, on a geological timescale estuaries can be ephemeral. The origin of many present day estuaries is likely to be recent, so it is possible that there has been relatively little time for the accumulation of genetic differences amongst populations in such habitats (Williams et al. 2008). Presently the only study of a New Zealand estuarine bivalve has been of the common cockle (*Austrovenus stutchburyi*), which reported genetic differentiation amongst populations consistent with the isolation by distance model (Ross et al. 2011). There are also examples of genetic differentiation amongst populations of estuarine bivalves outside New Zealand waters, such as the lagoon cockle *Cerastoderma glaucum* (Tarnowska et al. 2010), the oyster *Crassostrea ariakensis* (Xiao et al. 2010), as well as amongst estuarine amphipods (Kelly et al. 2006) and fish (Bradbury et al. 2008; McCraney et al. 2010).

Based on the habitat differences between the two species and the results of previous studies, we hypothesise that connectivity amongst *P. australis* populations may be limited in comparison to connectivity amongst *P. subtriangulata* populations. This may result in higher levels of genetic differentiation amongst *P. australis* populations compared to *P. subtriangulata* populations. However, whilst habitat considerations are important, previous studies highlight the role that physical oceanographic features, environmental variability, historical events and natural selection can have on shaping the genetic population structure of marine species. These factors must be kept in mind when interpreting results.

2.5.2 Rhombosolea spp.

Flatfish of the genus *Rhombosolea* (Richardson 1843) inhabit sandy bottom estuarine and coastal communities. In New Zealand *Rhombosolea* consists of four recognised species, *R. plebeia* (Sand Flounder), *R. leporina* (Yellow Belly Flounder), *R. retiaria* (Black Flounder) and *R. tapirina* (Greenback Flounder). All are endemic except *R. tapirina* which has a limited range in New Zealand, but is widely distributed in southeast Australia. To date, the taxonomic status of Rhombosoleinae remains a Family (Nelson 2006) and the relationships therein have not been examined at the genus or species level.

2.5.2.1 Reproductive biology and ecology

Adults of *R. plebeia* and *R. leporina* are distributed throughout mainland New Zealand (Ayling & Cox 1982) and their juveniles are two of the most abundant species in estuarine systems (Morrison et al. 2002). *Rhombosolea plebeia* is widely distributed in shallow coastal waters throughout New Zealand to 100 m. The sister species, *R. leporina*, is less common and distributed to 50 m; both rely heavily on estuaries for early life history stages (Ayling & Cox 1982). The PLD has only been studied for *R. tapirina*. It was measured from fertilisation to metamorphosis as an average of 69 days (Crawford 1984) and from hatching to metamorphosis as 35 days (Jenkins 1987), although this varies widely with temperature (Chambers & Leggett 1992). Juvenile *R. plebeia* have been observed recruiting to estuaries at 4.5 mm total length (Roper 1986). At two years of age, flounders move out of estuaries onto shallow areas on the continental shelf (Mundy 1968; Colman 1978). Adults form large spawning aggregations offshore of harbours and estuaries during the austral winter and spring (September to December) (Mundy 1968; Ayling & Cox 1982). Females of *R. tapirina* can ovulate multiple times

during a spawning season (Barnett & Pankhurst 1999), which means that there can be several pulses of recruits within a season.

Dispersal of *Rhombosolea* spp. is most likely to occur during the pelagic larval stage, given that adult movements are somewhat restricted on the scale of the New Zealand region. Tagging studies of adult *R. leporina* and *R. plebeia* in the Hauraki Gulf, Canterbury Bight and at Banks Peninsula revealed that adult movement was localised (Colman 1974, 1978), and that fish have rarely been recorded moving more than 200 km (Colman 1978). Colman (1976) also found variation in the number of fin ray counts in sand flounder, with higher numbers in the west of the North Island and lowest numbers in the southeast of the South Island. Based on this he concluded that there were five discrete regions or populations, however it has been widely reported that number of vertebrae, fin and dorsal rays are strongly affected by temperature (Hubbs 1922; Barlow, 1961; Kinoshita et al. 2000) rather than genetic variation.

2.5.2.2 Fisheries management

Colman (1972) studied several hundred samples of *Rhombosolea* from the Hauraki Gulf and found that male sand and yellowbelly flounder were mature at 12 cm and 15 cm respectively, based on the presence of milky fluid in the testes. Fifty percent of female *R. plebeia* were found to be mature at 18 cm and 95% at 23 cm. Fifty percent of *R. leporina* were mature at 27 cm. Colman (1972) highlighted that at the minimum legal size (MLS) of 25 cm, only 15% of *R. leporina* females are sexually mature, later confirmed by Mutoro (2001). Currently the MLS remains at 25 cm (New Zealand Ministry for Primary Industries 2015c), which allows for males to spawn more than once in their lifetime, but females would be subject to heavy fishing pressure before their second spawning season (Colman 1985). Therefore, females might only spawn once during their lifetime and abundance is closely linked to year class strength (Colman 1985). The complex of eight flatfish species are managed as one unit (New Zealand Ministry for Primary Industries 2015c) which supports commercial and customary fisheries, with total flatfish landings of 2856 tonnes in 2011–2012 worth an estimated $6,852,860 (Clements et al. 2013).

2.5.2.3 Research needs and predictions

The assessment of the current state of flatfish stocks is based on tagging studies and morphology (Colman 1974, 1976 1978). There have been no further studies of connectivity amongst populations, with the result that the population dynamics and stock structure of these fishes are poorly understood (New Zealand Ministry for Primary Industries 2015c). Of the two species of flatfish examined for this study, one (*R. plebeia*) is widely distributed in shallow water around New Zealand; the other (*R. leporina*) is less widely distributed and uses estuaries as breeding grounds. It is unknown how these important ecological differences may contribute to species-specific differences in population genetic structure and to levels of genetic connectivity, despite the ecological and economic importance of these two species. Based on the habitat preference differences between the two species and consistent with general hypotheses about continuously distributed species versus species with fragmented distributions, we hypothesise that connectivity amongst *R. leporina* populations may be limited in comparison to connectivity amongst *R. plebeia* populations. This may result in higher levels of genetic differentiation amongst *R. leporina* populations compared to *R. plebeia* populations. Consistent with the predictions made above for the surf clams, we note that whilst habitat considerations are important, physical oceanographic features, environmental variability, historical events and natural selection can all play important roles in the shaping of genetic population structure of marine species.

In addition, the role of individual mobility may also be important for the flatfish species, whereas individual mobility is not a consideration for the surf clam species. Larval flatfish spawned offshore must find their way back into estuaries, and some have specific habitat requirements, including temperature, sediment size and preferred prey (Bailey et al. 2005). However, most larval flatfish have poor swimming ability compared to other larval fish (Fukuhara 1988). In the process of recruitment, oceanographic events may dictate which estuaries juveniles are able to recruit to, explaining variability in the amount of recruitment between cohorts. Conversely, if adults return to the same localities to spawn, gene flow between estuaries may be low and family structure may be evident in juveniles within estuaries, which, when combined with habitat differences, could lead to local adaptation.

2.5.3 Pecten novaezelandiae

2.5.3.1 Reproductive biology and ecology

The New Zealand scallop, *Pecten novaezelandiae* (Reeve, 1853) is a large endemic bivalve species supporting important commercial, recreational and customary fisheries. It is characterised by a flat upper left valve and a convex lower right valve. It is an exceptionally fecund hermaphroditic species that reaches full maturity at about 65 mm shell length in the Hauraki Gulf, but the smallest mature individuals reported are 55 mm shell length (Williams & Babcock 2005). This species generally breeds in early summer even though partial spawning may occur from August to February (Williams et al. 2010) and individual populations are usually synchronous, i.e. individuals spawn at the same time (Shumway & Parsons 2006). The maximum reported age in unexploited populations is seven years. Growth to 100 mm shell length takes between 1.5 and 3.5 years, although growth rates are spatially and temporally variable. Morrison (1999) observed that mean size and growth rate decreases with increasing depth.

As with other scallops, adults of *P. novaezelandiae* are able to move by quickly clapping their valves, which propels the animal forward. However, most species become rapidly exhausted after moving short distances (Morrison 1999), which suggests that they are incapable of moving large distances. Tagging studies of adult scallops in the Hauraki Gulf suggest that movement of individuals is spatially limited and no animals moved between neighbouring patches of similar habitat type in a 100 m scale (Morrison 1999). In general, the PLD of scallops ranges from 6 to 70 days (Shumway & Parsons 2006) and has been shown to vary within species depending on temperature (Beaumont & Barnes 1992). *Pecten novaezelandiae* has a veliger larval stage that is estimated to persist for approximately three to four weeks (Bull 1976) and this is its main dispersal mechanism.

2.5.3.2 Fisheries management

The New Zealand scallop is a highly valued species, supporting important commercial, recreational and customary fisheries. However, its exploitation faces several problems mainly because its recruitment is highly variable and the processes that result in large periodic population fluctuations are poorly understood (New Zealand Ministry for Primary Industries 2013). Variability in spat numbers may be the result of human-induced processes such as fishing-related changes to habitat which affect juvenile scallop survival and increases in sedimentation input, which can affect adults and consequently reduce the likelihood of future larval settlement (Szostek et al. 2013; Talman et al. 2004). In New Zealand, evidence of declining stocks is found: (1) in the Coromandel fishery, which has been declining in terms of recruited biomass since 2005–06 (Williams et al. 2010); (2) in Golden Bay, which was effectively closed to commercial fishing in 2011 and where, in 2012, two scallop stocks or sub-stocks were considered to be overfished; and (3) in Tasman Bay, which has undergone a

voluntary closure to commercial fishing since 2006 due to fishery collapse (New Zealand Ministry for Primary Industries 2012).

Currently, the New Zealand scallop is managed under the QMS. MPI works closely with stakeholders to establish a sustainable and cautious TACC for each quota management area. Additionally, there is also a size limit, measured in terms of shell length, which varies accordingly to each QMA (Williams et al. 2010). However, there is little information on the stock structure and population dynamics of this species and this substantial uncertainty may lead to inappropriate estimates of sustainable yields.

2.5.3.3 Research needs

Despite their high value, the scallop fis faces several problems mainly because recruitment is highly variable and the processes that result in large periodic population fluctuations are not fully understood. Additionally, there is still substantial uncertainty about stock status and there are no previous studies on the population genetic structure to help elucidate the population dynamics of this species. This study will help to address these stock management issues.

One third of the genetic marine connectivity studies in New Zealand report a genetic break across the Cook Strait region (reviewed by Gardner et al. 2010) which is a waterway known to have disappeared during geological periods of pronounced global sea level change (Wei et al. 2013). However, the recent evolutionary origin of *P. novaezelandiae* might suggest a lack of genetic break across the Strait because the species' evolution in New Zealand is thought to post-date the period of global sea level change. As a reference, for *Perna canaliculus* and *Patiriella regularis* the reported divergence time between North and South Island populations dates to approximately 1.3 Ma ago (Apte & Gardner 2002; Ayers & Waters 2005). Fossil records document the origin of the genus *Pecten* in New Zealand in the middle to late Pleistocene, only approximately 450 000 (±0.09) years ago (Fleming 1979). Because *Pecten novaezelandiae* is an evolutionarily young species it is ideal for investigating the effects of recent processes on shaping population genetic structure.

2.6 Aims of the present study

The primary goal of this study was to use a standardised genetic methodology to determine patterns of population structure and genetic connectivity in five New Zealand coastal marine species, and to compare these patterns across species to identify any common patterns and the location of any potential barriers to connectivity. Tests were conducted to determine whether the patterns of genetic population structure observed for other New Zealand coastal marine species, as reported by Gardner et al. (2010), were present for the five study species (i.e., no structure, IBD, divergence within and/or amongst populations, north-south divergence, west-east divergence). At an individual species level, this information will be useful for fisheries management as it may enable stock structure to be identified. This study will contribute to a growing body of literature on patterns of genetic population structure and connectivity in the New Zealand marine environment, in this case for species that are characteristic of habitats that have been under-represented in past studies (Gardner et al. 2010).

To date, studies of genetic population structure in New Zealand marine species have primarily focussed on determining the geographic patterns of genetic diversity, and we now have a good understanding of the types of genetic structure that can be found in New Zealand marine organisms (Ross et al. 2009; Gardner et al. 2010). However, there is a poor understanding of the rates of connectivity amongst populations; with the exception of the work of Wei et al. (2013) on the New Zealand greenshell mussel, no attempts have been made to quantify rates or patterns of migration amongst populations. Instead, population connectivity is implied based on genetic similarities or differences amongst populations. Recent advances in computational power and theoretical

frameworks have made it possible to estimate levels of connectivity amongst populations on a variety of temporal and spatial scales, allowing for a better understanding of the genetic population structure uncovered by more traditional population genetic methods (Pearse & Crandall 2004; Hauser & Carvalho 2008). The value of these methods has been demonstrated by studies from outside the New Zealand region (e.g., Weetman et al. 2006; Fraser et al. 2007; Jolly et al. 2009; Harris et al. 2012) and are only just starting to be applied in the New Zealand context. Therefore this study aims to apply these methods to quantify the genetic connectivity of New Zealand coastal marine species. By being the first study to routinely apply these methods across a number of New Zealand species we can begin to establish the rates and routes by which marine populations are connected in the New Zealand coastal marine environment. This information may be useful to enhance resource management and conservation measures in the New Zealand coastal marine environment.

3. METHODS

3.1 Sample collection

Samples of *Paphies subtriangulata* and *P. australis* were collected between June 2010 and January 2013 from 10 and 13 locations, respectively (Figure 2, Table 1, Table 6). Samples were gathered by hand at low tide, photographed, shell length measured to the nearest 0.5 cm and a sub-sample of foot tissue taken and preserved in 80% ethanol.

Sample collection of *Rhombosolea plebeia* and *R. leporina* took place during the spring and summer of 2010/2011 and 2011/2012 (Figure 2, Table 11, Table 16). Flounders were collected using a 4 m wide beach seine with 9 mm mesh at low tide or incoming tide, yielding predominantly juvenile samples. Juveniles were euthanised and whole animals preserved in 80% ethanol for later processing. Total length, head length and a photograph were recorded for each individual to confirm juvenile species identification by morphometric analysis (Eldon & Smith, 1986). Larger fish caught using the beach seine were photographed, a fin clip was taken and preserved in 80% ethanol, and the animal was returned live to the estuary.

Adult flounder tissue samples were also collected on board inshore commercial vessels by New Zealand Ministry for Primary Industries fishery observers. A fin clip of approximately 2 cm^2 was cut from the pectoral fin on the blind side and preserved in 80% EDTA DMSO (Seutin et al. 1991). Total fish length, site location and latitude/longitude were recorded for spatial analysis. Remaining adult specimens were sampled from fresh catch directly from fishers or at fish markets. Adult specimens were identified on the basis of morphological characteristics and photographed. The total length was recorded, and a fin clip was sampled and fixed in 80% ethanol.

Individuals of *Pecten novaezelandiae* were sampled between 2012 and 2014. Samples were collected from 15 populations (Figure 2, Table 21) by dredging at depths between 15 and 50 m and SCUBA diving at depths between 7 and 15 m. Shell length of the left valve was recorded for all specimens and sub-samples of the adductor muscle were preserved in 80% ethanol at -20°C for later processing.

3.2 DNA extraction and genotyping

To estimate genetic diversity, population structure and connectivity in the study species microsatellite markers were developed for each species. Microsatellite markers consist of short motifs, typically two to six base pairs in length, which are repeated in tandem. These characteristics mean that they have a high mutation rate, making them ideal for high resolution, fine-scale measurements of genetic heterogeneity amongst populations (Avise 2004). Microsatellite loci were developed using massively parallel DNA sequencing techniques, following the methods described by Hannan et al. (2014) for *P.*

subtriangulata and *P. australis*, Constable (2014) for *R. plebeia* and *R. leporina*, and Silva & Gardner (2014) for *P. novaezelandiae*.

For each sample a piece of tissue approximately 25 mm^2 was used for DNA extraction, using one of the following methods:

a) A standard proteinase K digestion followed by phenol-chloroform purification and ethanol precipitation;
b) A Qiagen DNeasy Blood and Tissue extraction kit, following the manufacturer's protocols;
c) A Geneaid Genomic DNA Mini Kit, following the manufacturer's protocols;
d) A Zygem prepGEM extraction kit, following the manufacturer's protocols.

Various combinations of these standard DNA extraction techniques were trialled and applied to all five species.

DNA extracts were quantified using an Implen Nanophotometer and diluted in ddH_2O to a concentration of approximately 100 ng/µl for use in a PCR reaction. PCR reactions for each species followed the specific protocols described in Hannan et al. (2014), Constable (2014) and Silva & Gardner (2014). PCR products were size separated and the fluorescently labelled amplicons detected using an ABI3730XL (Life Technologies™) automated capillary sequencer. Allele sizes were determined and binned using the software GeneMarker version 2.2.0 (SoftGenetics).

3.3 Genetic diversity analyses

Genotype data were checked for scoring errors and large allele drop out using MICROCHECKER 2.2.3 (Van Oosterhout et al. 2004). Loci were checked for linkage disequilibrium and conformance to Hardy-Weinberg equilibrium (or HWE, the underlying assumption of many population genetics analyses, that allele and genotype frequencies in a population will remain constant from generation to generation in the absence of other evolutionary influences) using GENEPOP 4.2 (Rousset 2008; Markov-chain parameters: 10 000 dememorization steps, 1000 batches and 10 000 iterations per batch). Comparisons were considered significant if $p < 0.05$ after false discovery rate (FDR) correction for multiple tests (Verhoeven et al. 2005). LOSITAN (Antao et al. 2008) was used to detect outlier loci (50 000 simulations using a stepwise mutation model). The F_{ST} outlier method is based on the relationship between F_{ST} and expected heterozygosity (H_E) to identify loci that have excessively high or low F_{ST} compared to neutral expectations. A 95% confidence interval was used to determine which loci were outliers.

For each population the mean number of alleles (N_a) and allelic richness (R_a) were calculated using FSTAT 2.9.3.2 (Goudet 1995) or HPRare (Kalinowski 2005). Allelic richness is a measure of allele diversity corrected for the smallest sample size. Observed (H_O) and expected (H_E) heterozygosity were calculated using ARLEQUIN 3.5 (Excoffier & Lischer 2010) or GenAlEx 6.5 (Peakall & Smouse 2012). The inbreeding coefficient F_{IS} was calculated using GENEPOP 4.2 (Rousset 2008) or GenAlEx 6.5. Private alleles for each location were calculated by hand or using HPRare (Kalinowski 2005).

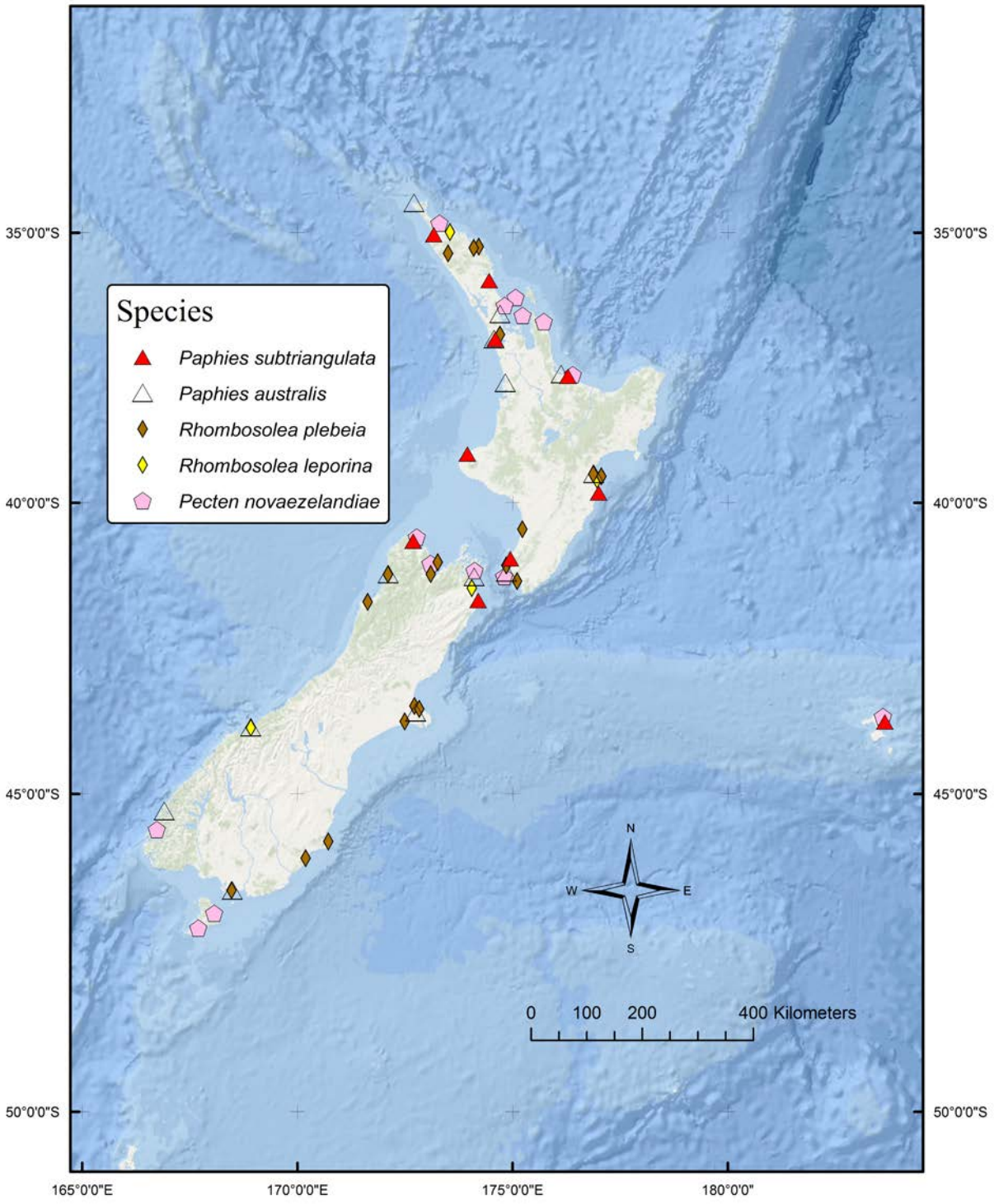

Figure 2: Collection locations used in the present study.

3.4 Population genetic analyses

Allele frequency differences between populations were measured using F_{ST} (Wright 1931, 1951), which is a measure of allele frequency divergence from idealised HWE proportions due to subpopulation structure. The Weir & Cockerham (1984) F_{ST} analogue for multiallelic loci was calculated between locations using GENEPOP 4.2. An exact test of population differentiation using the G log likelihood ratio test (Goudet et al. 1996) was performed in GENEPOP 4.2 (Markov-chain parameters: 10 000 dememorization steps, 1000 batches and 10 000 iterations per batch). Comparisons were considered significant if $p < 0.05$ after implementing the FDR correction. F_{ST} ranges from 0 to 1, where 0 indicates no genetic differentiation between populations and 1 indicates no sharing of alleles or complete differentiation of populations.

To determine which (if any) patterns of genetic population structure observed were present for the five study species (i.e., no structure, divergence within and/or amongst populations, north-south divergence, west-east divergence) qualitative assessments of genetic structure were made. In addition, Mantel tests were used to determine if an IBD pattern was present for each species, using FSTAT 2.9.3.2, ISOLDE as implemented in GENEPOP 4.2 or GenAlEx 6.5. Correlation between genetic differentiation (F_{ST}) and geographic distance (km) was assessed using 10 000 randomisations and was considered significant if $p < 0.05$. The shortest distance between sampling sites was determined using Google Maps.

Patterns of genetic differentiation amongst locations were visualised for each species using principal component analysis (PCA) and neighbour joining dendrograms. PCA was implemented in PCA-Gen 1.2 (http://www2.unil.ch/popgen/softwares/pcagen.htm) or GenAlEx 6.5. Axes were considered significant if $p < 0.05$. Neighbour joining dendrograms were generated using the software package POPTREE2 (Takezaki et al. 2010) based on F_{ST} values with 1000 bootstrap replicates. Analysis of Molecular Variance (AMOVA) tests were conducted in ARLEQUIN 3.5 (Excoffier & Lischer 2010) with 10 000 permutations or GenAlEx 6.5 with 99 permutations (significant if $p < 0.05$ after FDR correction). For *P. subtriangulata* and *P. australis* AMOVA tests were used to determine if the groupings observed in PCAs were significant and to test for north-south/west-east differentiation. For *R. plebeia* and *R. leporina* sampling locations were grouped by northern versus southern groups, and western versus eastern groups based on F_{ST}. For *P. novaezelandiae* AMOVA was implemented to test the significance of the differentiation between five groups: north, central, Fiordland, Stewart Island and Chatham Island. An AMOVA was also conducted for two Wellington Harbour samples to test whether the temporal sampling differences (2012 versus 2013) explained any of the variation in the dataset. Subsequent analyses were conducted using Wellington Harbour as one single location because temporal differences did not explain any of the variation between the two samples.

The genetic structure of locations was assessed for each species using two clustering methods: STRUCTURE 2.3.4 (Pritchard et al. 2000) and AWclust (Gao & Starmer 2008). STRUCTURE is a Bayesian clustering algorithm that attempts to assign each individual into *K* number of clusters to minimise departures from HWE and gametic disequilibrium. The following parameters were used:

a) for *P. subtriangulata* 10 independent STRUCTURE runs were performed for values of *K* ranging from 1 to 10, using 100 000 iterations and a burn-in length of 10 000;
b) for *P. australis* 10 independent STRUCTURE runs were performed for values of *K* ranging from 1 to 13, using 100 000 iterations and a burn-in length of 10 000;
c) for *R. plebeia* and *R. leporina* 5 independent STRUCTURE runs were performed for values of *K* ranging from 1 to 8, using 1 000 000 iterations and a burn-in length of 100 000; and
d) for *P. novaezelandiae* 5 independent STRUCTURE runs were performed for values of *K* ranging from 1 to 14, using 100 000 interactions and a burn-in length of 10 000.

The 'admixture' model and 'correlated allele frequencies' options were used (Falush et al. 2003), and sampling locations were used as a prior (i.e., as a known geographical input for the model) because this improves the performance of the program when genetic population structure is weak (Hubisz et al. 2009). The Evanno method (Evanno et al. 2005) as implemented in STRUCTURE HARVESTER

(Earl & VonHoldt 2012) was used to determine the value of *K* that best fit the data. This method is based on the rate of change in the log probability of data between successive *K* values, i.e., the most likely *K* value precedes the greatest rate of decline in the log probability.

The non-parametric clustering algorithm AWclust was used to determine genetic population structure without relying on the assumptions of HWE and linkage disequilibrium (Gao & Starmer 2008). Microsatellite data were converted into a presence/absence matrix where each allele was treated as a locus and allocated a 0, 1 or 2 to indicate the number of copies of that allele for each individual, following the method of Wei et al. (2013). The AWclust package was implemented in R (R core team 2012) to firstly calculate an allele sharing distance matrix then assign each individual to one cluster. A gap statistic that compares the pooled within-cluster sum of squares with expectations from a null reference distribution was calculated for values of *K* ranging from 1 to 8 for *P. subtriangulata*, *P. australis*, *R. plebeia* and *R. leporina* and from 1 to 14 for *P. novaezelandiae*, using 100 null simulations. The *K* value with the largest deviation from the expected distribution was determined to be the optimal value (Gao & Starmer 2008). Spearman rank correlation tests were used to determine whether there were any significant latitudinal or longitudinal gradients in the distribution of clusters identified by STRUCTURE and AWclust ($p < 0.05$ after FDR correction).

3.5 Estimation of migration rates

Several methods exist for estimating species-specific migration rates. Different methods make different assumptions about the data, including, for example, whether or not pairwise population genetic differences are small (low F_{ST} values) or large (high F_{ST} values), whether populations are in Hardy Weinberg equilibrium or not, and the presence or absence of linkage between loci. As a consequence, outcomes of some of the tests described previously influence the appropriateness of subsequent tests, with the result that one "standard" methodology cannot be employed.

3.5.1 Paphies spp.

For all migration rate analyses, sampling locations were amalgamated into groups that were significantly differentiated based on the results of population genetic analyses. This is based on the assumption that sampling locations that were not significantly differentiated are highly connected, which would make it difficult to accurately quantify migration. These analyses work best when there are moderate levels of genetic differentiation and connectivity amongst populations.

Contemporary migration amongst locations was estimated for each species using the Bayesian assignment approach implemented in BAYESASS 3.0.3 (Wilson & Rannala 2003). BAYESASS detects the temporary genetic disequilibrium that recent migrant genotypes are expected to show relative to their sampled population and uses this information to infer rates of migration amongst populations. BAYESASS detects migrants within the past few generations, typically equivalent to the number of cohorts present in the population. Approximately 10 runs of BAYESASS were conducted to determine the number of iterations required for the Markov chain Monte Carlo (MCMC) to converge and appropriate values for the mixing parameters Δa, Δf and Δm, which determine the rates at which parameters are accepted for each iteration. Final results were based on the mean of three independent runs using 1×10^7 iterations, the first 1×10^6 iterations being discarded as burn-in. Samples were taken every 500 iterations. The mixing parameters used for each run were Δa 0.8, Δf 0.8 and Δm 0.6.

Long-term migration rates amongst locations were estimated for each species using MIGRATE 3.5.1 (Beerli 2006). MIGRATE estimates two parameters: the population size parameter θ, which is equivalent to four times the effective population size scaled by mutation rate ($4N_e\mu$) and a mutation

scaled migration rate M ($m\mu$). A Bayesian MCMC strategy was used with a static heating scheme (temperatures: 1.0, 1.5, 3.0, 100 000; swapping interval 1) and uniform prior distributions for θ and M. Preliminary runs determined that the best prior intervals to use were 0–10 for θ and 0–10 000 for M. Three replicates were run for 1×10^5 iterations with a sample taken every 100 steps, resulting in a total of 30 000 recorded steps over the three replicate runs. The first 10 000 steps of each replicate were discarded as burn-in. Values of θ, M and their credible intervals (CI) were averaged over the three runs. θ and M were multiplied to give the effective number of migrants per generation (N_em) between each pair of sampling locations. The effective size of each population (N_e) can also be estimated from θ if the mutation rate of the markers used is known. An estimated mutation rate per generation for mollusc microsatellite loci of 1×10^{-4} was used (Ellegren 2000, 2004).

3.5.2 Rhombosolea spp. *and Pecten novaezelandiae*

Recent migration rates for each species of the flatfish and scallop species were estimated using an assignment test approach, as implemented in GeneClass2 (Piry et al. 2004). This method uses a Bayesian approach that does not assume HWE, or that all potential populations have been sampled. GeneClass2 was used to assign individuals to the most likely population of origin and estimate numbers of recent migrants between sampling locations by detecting individuals in the current generation (F0) that were in genotypic disequilibrium relative to their sampled population, and are therefore likely to have originated from a population other than where they were sampled. For *R. plebeia* and *R. leporina* all sampling locations were assumed to be individual populations, whereas for *P. novaezelandiae* sampling locations were amalgamated based on the four groups identified in previous population genetic analyses.

The test statistic *Lh/Lhmax* (ratio of the likelihood of drawing an individual's genotype from the population in which it was sampled to the maximum likelihood observed for this genotype in any population; Paetkau et al. 2004) and the Bayesian approach of (Rannala & Mountain 1997) were used to detect migrants. The probability of an individual's multilocus genotype originating from each location was calculated using the MCMC resampling method of Paetkau et al. (2004) with a 0.01 rejection level and a simulated population size of 10 000 individuals per site. Individuals were assigned to the group with the highest probability.

4. RESULTS

4.1 *Paphies subtriangulata*

A total of 517 *Paphies subtriangulata* individuals from ten locations were genotyped at 11 microsatellite loci (Table 1). Despite reports that *P. subtriangulata* is distributed throughout the South Island (Powell 1979), it was not possible to find this species further south than 42° S on the New Zealand mainland in the present study. Therefore the majority of *P. subtriangulata* locations sampled in this study were from the North Island except for two locations at the top of the South Island (Collingwood and Marfell's Beach) and one Chatham Island location, 660 km east of mainland New Zealand.

MICROCHECKER analyses revealed no evidence for genotype scoring errors or large allele drop out and there was no significant linkage disequilibrium amongst loci. Only two loci were in HWE for the total sample (*Psub_3* and *Psub_4*) and two loci (*Psub_5* and *Psub_7*) were not in HWE for any of the locations tested (data not shown). For most loci observed heterozygosity was lower than expected, but for *Psub_3* observed heterozygosity was similar to expected (Range H_O: 0.105 to 0.551; H_E: 0.121 to 0.791; data not shown). LOSITAN detected one outlier locus (*Psub_2*), which was excluded from further analysis; all subsequent analyses are based on 10 loci.

Diversity statistics calculated for each location showed that the mean number of alleles ranged from 6.000 to 8.091, allelic richness ranged from 5.290 to 6.566 and private alleles ranged from 0 to 13 (Table 2). The frequency of private alleles was generally low (less than 2%); i.e., these alleles can be considered 'rare'. CHA had a much larger number of private alleles than other *P. subtriangulata* locations and some of these alleles were observed in high frequency (up to 14%). Observed heterozygosity was lower than expected for all locations (Range H_O: 0.298 to 0.399; H_E: 0.521 to 0.588) and no locations were in HWE (Table 2). F_{IS} was above zero for all locations due to an excess of homozygotes (Range: 0.242 to 0.442; Table 2).

Table 1: Location, number of samples and geographical co-ordinates for *Paphies subtriangulata* samples used in this study.

Location	Abbreviation	Sample Size	Latitude	Longitude
Ruakaka	RUA	53	-35.91° S	174.46° E
Papamoa	PAP	51	-37.70° S	176.29° E
Waimarama	WMR	51	-39.82° S	177.00° E
Marfell's Beach	MAR	56	-41.72° S	174.20° E
Paekakariki	PKR	55	-40.99° S	174.95° E
Collingwood	COL	52	-40.68° S	172.69° E
Oakura	OAK	40	-39.12° S	173.95° E
Kakamatua	KAK	57	-37.01° S	174.60° E
Waipapakauri	WPK	54	-35.04° S	173.17° E
Chatham Island	CHA	48	-43.80° S	-176.35° W

Table 2: Genetic diversity statistics for each location and total for *Paphies subtriangulata* (N_a: mean number of alleles, R_a: allelic richness, PA: private alleles, H_O: observed heterozygosity, H_E: expected heterozygosity, F_{IS}: inbreeding co-efficient). F_{IS} values in bold show significant departure from Hardy Weinberg equilibrium (HWE) expectations after false discovery rate correction ($p < 0.05$). Location abbreviations are as described in Table 1.

Location	Statistic					
	N_a	R_a	PA	H_O	H_E	F_{IS}
RUA	7.818	6.489	5	0.353	0.588	**0.388**
PAP	7.091	5.928	4	0.358	0.559	**0.352**
WMR	7.182	6.044	2	0.352	0.553	**0.356**
MAR	7.636	5.797	5	0.322	0.541	**0.405**
PKR	7.636	5.647	3	0.399	0.550	**0.275**
COL	7.000	5.797	2	0.298	0.543	**0.442**
OAK	6.273	5.485	0	0.368	0.542	**0.318**
KAK	8.091	6.566	3	0.394	0.569	**0.301**
WPK	7.361	5.943	4	0.357	0.558	**0.339**
CHA	6.000	5.290	13	0.390	0.521	**0.242**
Total	14.455	6.542	40	0.355	0.553	**0.348**

The homozygote excess and departures from HWE observed in *P. subtriangulata* are also commonly reported in other bivalve species (e.g., Cassista & Hart 2007; Kong et al. 2007; Varela et al. 2009; Wei et al. 2013). A likely cause of HWE departures is null alleles, which for unknown reasons are particularly common in bivalves (e.g., Launey & Hedgecock 2001; Varela et al. 2009; Zhan et al. 2009). Simulations suggest that if effective population size (N_e) and gene flow are high, and frequency of null alleles is moderate (5–20%) then F_{ST} estimates should be unbiased by null alleles (Chapuis & Estoup 2007). With this in mind results should be interpreted with caution, especially for analyses where HWE is an underlying assumption.

Pairwise F_{ST} estimates amongst *P. subtriangulata* locations ranged from 0.000–0.153 with the highest F_{ST} values observed between CHA and mainland New Zealand locations (Table 3). The CHA population was significantly differentiated from all other locations. Amongst mainland locations, RUA and WMR were significantly differentiated from all other locations, and KAK and WPK were differentiated from most locations.

Mantel tests using all sampling locations revealed a significant pattern of IBD ($R^2 = 0.302$, $p < 0.001$; Figure 3A) but when the CHA population was excluded the IBD pattern amongst mainland locations was weaker and not significant ($R^2 = 0.085$, $p = 0.084$; Figure 3B). A PCA plot showed that the X axis explains 40.20% of variation ($p = 0.001$) and the Y axis 22.69% of variation ($p = 0.021$; Figure 4). The CHA and RUA populations appeared distinct from the remainder of the locations. A Neighbour Joining (NJ) dendrogram of *P. subtriangulata* locations (Figure 5) also clearly showed that CHA is differentiated from the other mainland locations. Amongst mainland locations there is evidence of differentiation of the RUA and WMR populations (87% support), but the remaining locations were not differentiated from each other (71% support).

An AMOVA (Table 4) of all sampling locations showed that 63.8% of variation was partitioned amongst individuals within the total sample and 32.7% of variation amongst individuals within populations. There were no significant north-south ({RUA, PAP, WMR, KAK, WPK} vs {MAR, PKR, COL, OAK}, $p = 0.055$) or west-east groupings ({RUA, PAP, WMR, MAR} vs {WPK, KAK, OAK, PKR, COL}, $p = 0.490$). Note that CHA was excluded from these analyses because its high degree of genetic differentiation from other locations meant that it had a disproportionate effect on results. The arrangement of populations that explained the most variation amongst groups was {CHA} {RUA} {WMR} {KAK} {PAP, MAR, PKR, COL, OAK, WPK} ($p = 0.001$).

Table 3: Pairwise F_{ST} values amongst *Paphies subtriangulata* locations. Values in bold represent significant differentiation after false discovery rate correction ($p < 0.05$) as assessed by an exact G test (Goudet et al. 1996). Location abbreviations are as described in Table 1.

	RUA	PAP	WMR	MAR	PKR	COL	OAK	KAK	WPK
PAP	**0.038**								
WMR	**0.021**	0.031							
MAR	**0.041**	0.000	**0.028**						
PKR	**0.031**	0.006	**0.012**	0.003					
COL	**0.046**	0.000	**0.025**	0.000	**0.004**				
OAK	**0.045**	0.005	**0.014**	0.002	0.000	0.003			
KAK	**0.035**	**0.021**	**0.003**	**0.014**	0.004	**0.014**	**0.005**		
WPK	**0.023**	**0.012**	**0.008**	**0.007**	**0.005**	0.006	**0.007**	**0.004**	
CHA	**0.074**	**0.129**	**0.108**	**0.133**	**0.117**	**0.144**	**0.153**	**0.130**	**0.116**

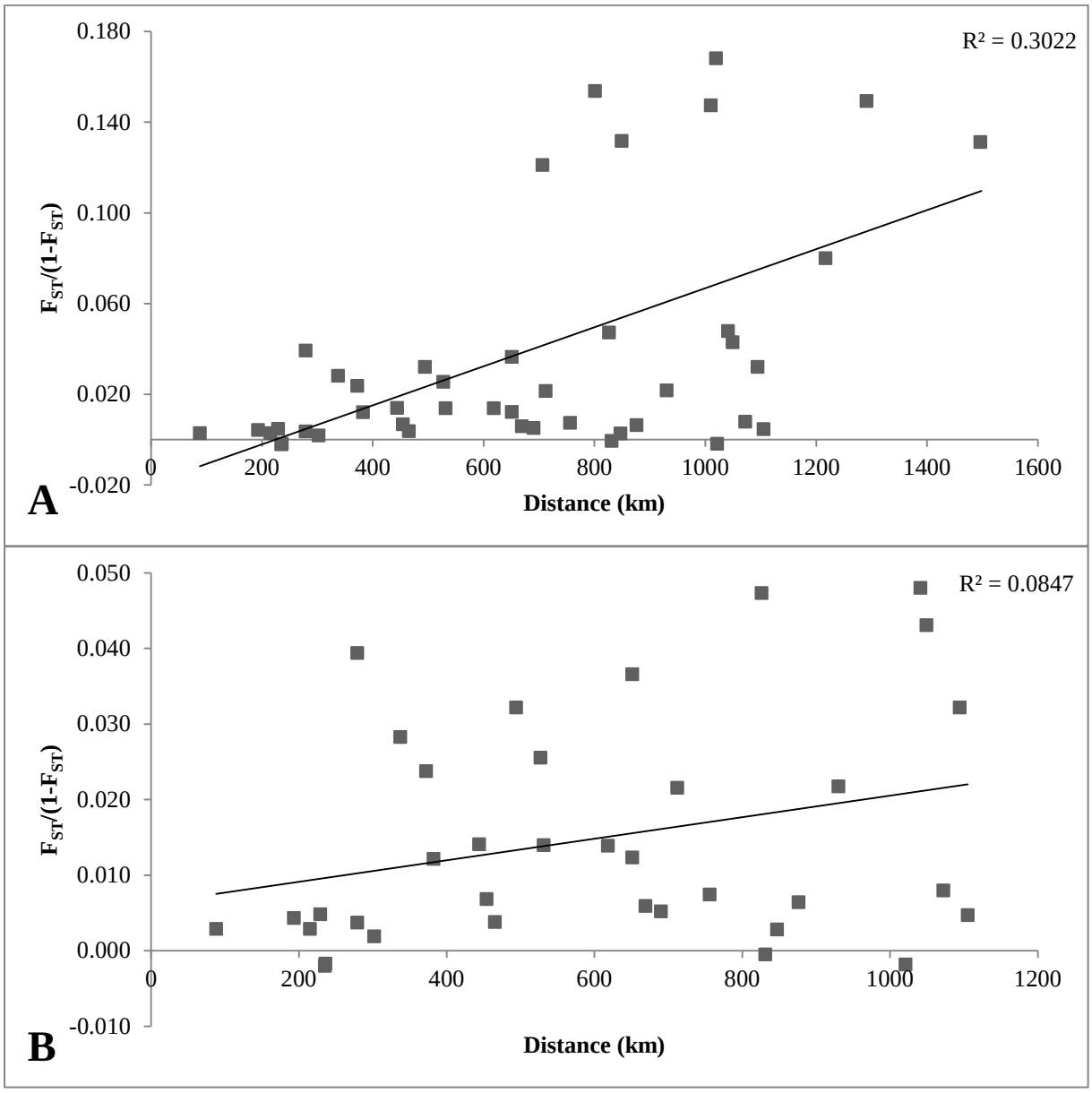

Figure 3: Mantel tests for isolation by distance for A: all *Paphies subtriangulata* locations ($p < 0.001$); and B: mainland *Paphies subtriangulata* locations, excluding CHA ($p = 0.084$).

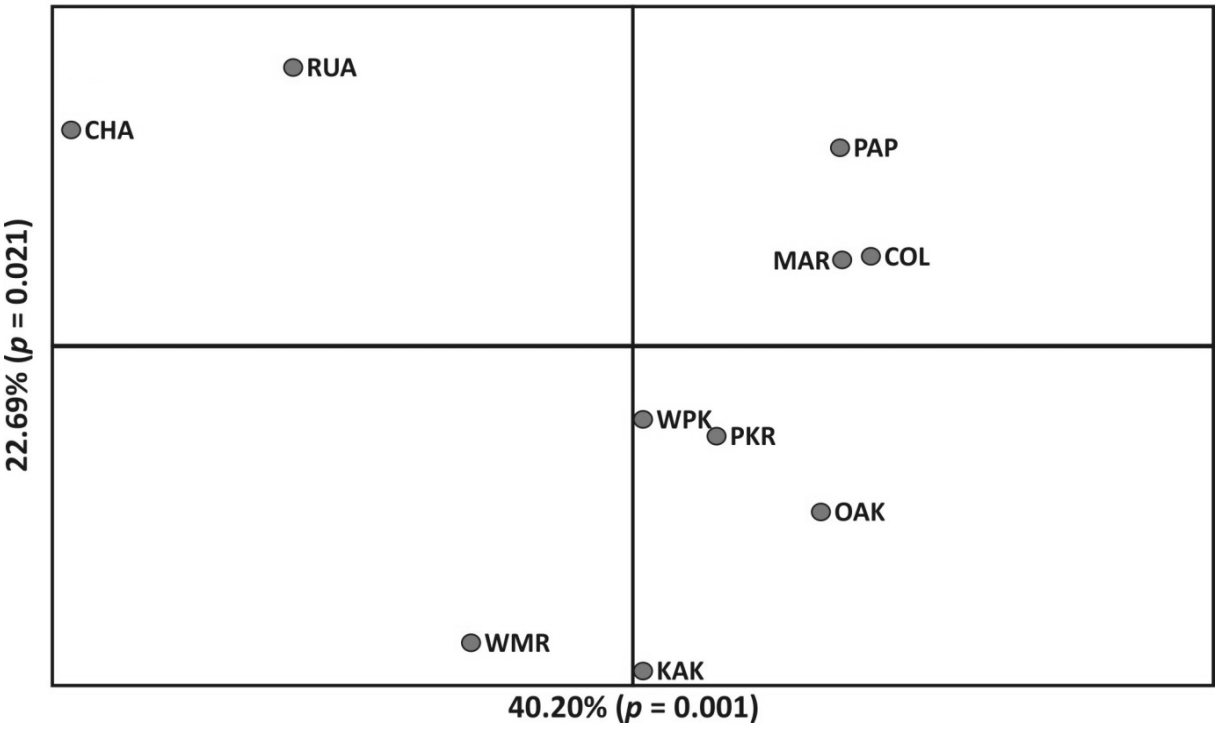

Figure 4: Principal component analysis for *Paphies subtriangulata* showing patterns of genetic population differentiation. The percentage of inertia explained by each axis and significance of the axis are displayed. An axis was considered significant if $p < 0.05$. Location abbreviations are as described in Table 1.

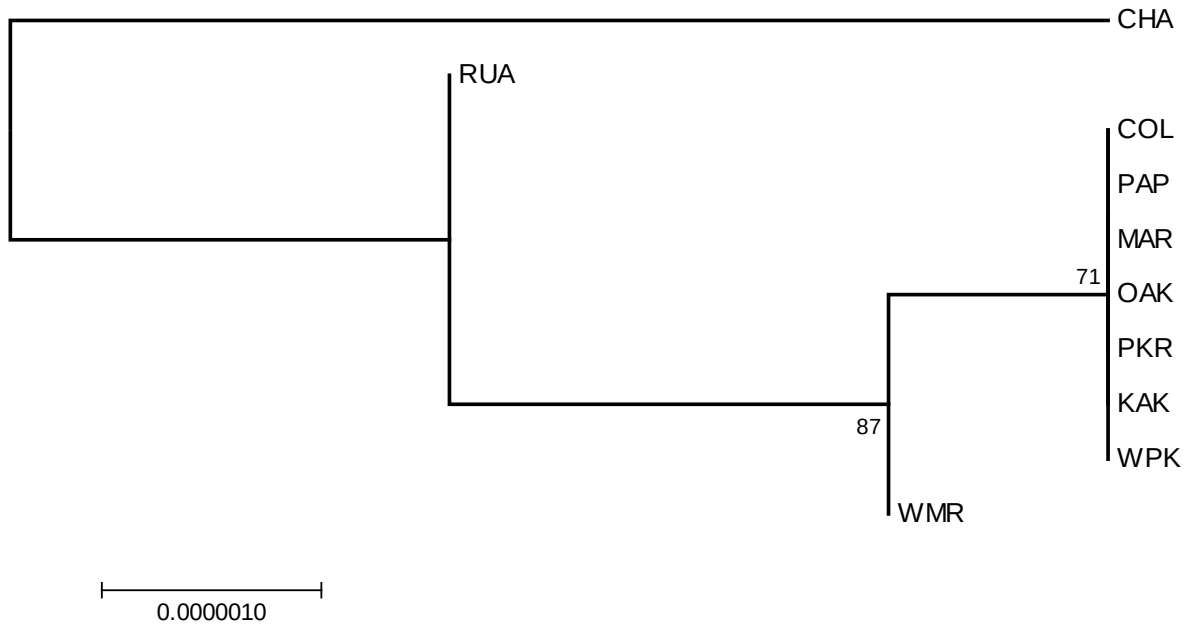

Figure 5: Neighbour Joining dendrogram based on F_{ST} values for *P. subtriangulata*. Location abbreviations are as described in Table 1.

Table 4: AMOVA analyses for *Paphies subtriangulata*. Population structure was considered significant if $p < 0.05$ after false discovery rate correction for multiple tests.

Groupings Tested	Group Configuration	Source of Variance											
		Amongst groups			Amongst populations within groups			Amongst individuals within populations			Amongst individuals within the total sample		
		% var.	F_{CT}	*p*-value	% var.	F_{SC}	*p*-value	% var.	F_{IS}	*p*-value	% var.	F_{IT}	*p*-value
1 group		-	-	-	3.493	0.035	**0.001**	32.697	0.339	**0.001**	63.81	0.362	**0.001**
North-South Division	{RUA, PAP, WMR, KAK, WPK}{MAR, PKR, COL, OAK}	0.403	0.004	0.055	1.297	0.013	**0.001**	34.038	0.346	**0.001**	64.262	0.357	**0.001**
West-East Division	{RUA, PAP, WMR, MAR}{WPK, KAK, OAK, PKR, COL}	-0.023	0.000	0.490	1.536	0.015	**0.001**	34.103	0.346	**0.001**	64.383	0.356	**0.001**
Differentiated Pop. Groups	{CHA}{RUA}{WMR}{KAK}{PAP, MAR, PKR, COL, OAK, WPK}	4.48	0.045	**0.001**	0.409	0.004	**0.021**	32.224	0.339	**0.001**	62.886	0.371	**0.001**

Results from the STRUCTURE analyses have been transferred to maps of New Zealand to show the extent of genetic differentiation amongst populations and between regions (e.g. Figure 6A). The genetic diversity contained within each population is displayed within a circle specific to that population. When STRUCTURE analyses revealed two or more genetic groups across New Zealand for any one species the circle is divided by use of different colours into however many groups were identified. These plots represent a semi-quantitative way of visualising the genetic diversity within a location, but within these plots there is no testing of the extent of genetic differences between pairs of locations or across regions or of genetic exchange (such formal testing is carried out by analysis of F_{ST} values for population pairwise contrasts, by way of AMOVA to test for regional differences in genetic diversity, and using MIGRATE to estimate the number of genetic exchanges between populations). Nonetheless, a quick inspection of the plot may give a sense of the geographic distribution of genetic diversity within a species. Thus, locations which share colours are likely to also share gene flow between them as a function of the proportions of the different colours.

STRUCTURE analysis of all locations revealed two genetic clusters (Figure 6A). Cluster one was most prevalent amongst the mainland New Zealand locations and cluster two was most prevalent in the CHA population. The strong genetic structure between CHA and mainland New Zealand locations was supported by a Spearman rank correlation test, which showed a significant longitudinal difference in the distribution of clusters (Spearman's r = -0.72 to 0.72, p = 0.018 to 0.022). AWclust analysis revealed five genetic clusters (Figure 7A). Cluster one was dominant in the CHA population but rare amongst mainland locations. There was no significant correlation amongst the proportion of clusters and latitude or longitude. STRUCTURE and AWclust analyses both detected a difference in population structure between mainland New Zealand locations and Chatham Island, but AWclust also detected much more structure within the mainland New Zealand populations.

When STRUCTURE and AWclust analyses were repeated for mainland New Zealand locations STRUCTURE detected four genetic clusters (Figure 6B) and AWclust detected three clusters (Figure 7B). Apart from some evidence that RUA might have a different genetic make-up compared to other locations there was little evidence for any significant genetic structure amongst mainland locations. This was supported by Spearman rank correlation tests, which found no significant correlation between cluster proportion and latitude/longitude were found for mainland New Zealand locations with both STRUCTURE and AWclust analyses.

For all migration analyses locations were grouped into the five genetically differentiated groups identified by the above population structure analyses. Recent migration rates amongst the five genetically differentiated *P. subtriangulata* groups, as estimated by BAYESASS, were consistent with the pattern of genetic population structure that was observed (Figure 8). Migration rates were highly variable, ranging from 0% to 31%. Self-recruitment rates were high and ranged from 67% to 99%. Migration amongst the groups was unidirectional, with all migrants sourced from the grouping of the six genetically undifferentiated locations (PAP, MAR, PKR, COL, OAK, WPK). There were high levels of migration to WMR (29%) and KAK (31%), and lower levels of migration to RUA (8%). No migration to CHA was detected.

Historical migration rates amongst the five genetically differentiated *P. subtriangulata* groups, estimated by MIGRATE, displayed a large range of values but showed that migration has occurred amongst all groups when averaged over longer periods of time. Migration rates ranged from 1.83 to 49.50 migrants per generation (Table 5). The θ values ranged from 0.05 to 0.18, which equated to effective population sizes ranging from 125.00 to 441.68 individuals. RUA was identified as a large source of migrants in the long term, followed by the grouping of PAP, MAR, PKR, COL, OAK and WPK (referred to as 'REST' in results tables). A moderate level of migration from mainland New Zealand to CHA was detected, but few migrants were sourced from CHA.

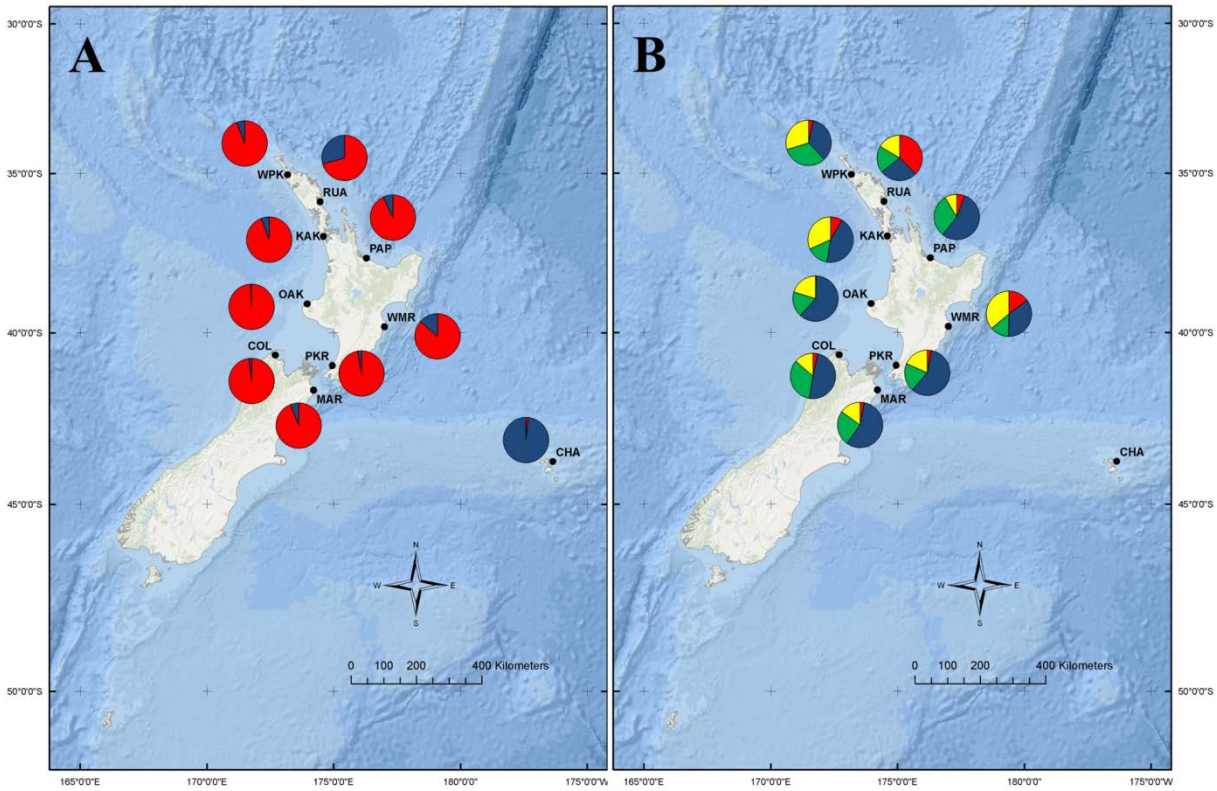

Figure 6: STRUCTURE results showing cluster proportion assigned to each location for A: all *Paphies subtriangulata* locations (*K* = 2) and B: mainland *Paphies subtriangulata* locations, excluding CHA (*K* = 4). Each colour denotes a different genetic cluster.

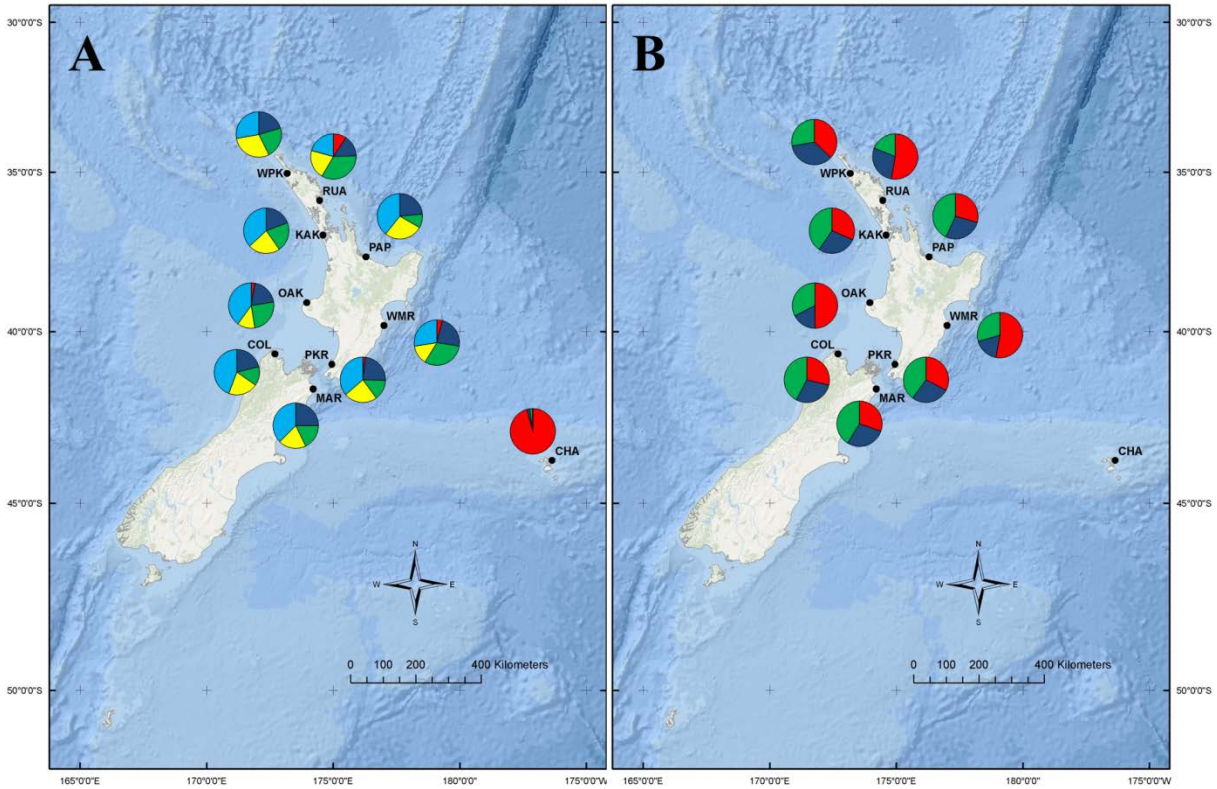

Figure 7: AWclust results showing cluster proportion assigned to each location for A: all *Paphies subtriangulata* locations (*K* = 5) and B: mainland *Paphies subtriangulata* locations, excluding CHA (*K* = 3). Each colour denotes a different genetic cluster.

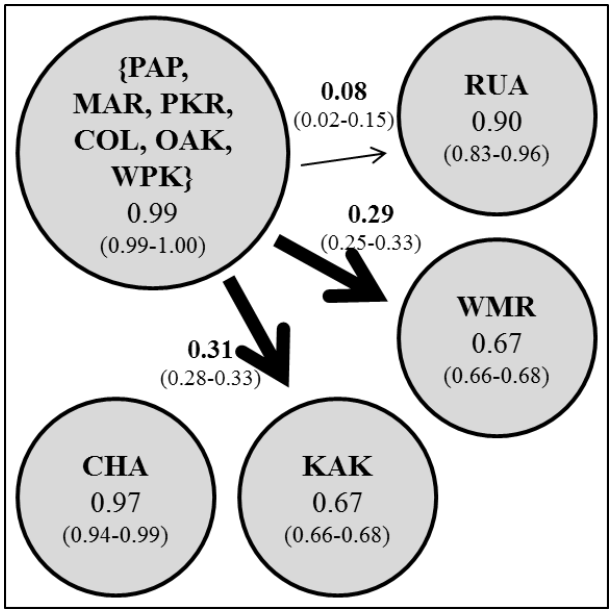

Figure 8: Recent migration rates amongst five genetically differentiated population groups of *Paphies subtriangulata*, as determined by BAYESASS. Values in grey circles represent self-recruitment rates for each location (95% confidence intervals in brackets). Arrows represent migration amongst locations with the proportion of migrants indicated in bold (95% confidence interval in brackets). Arrow thickness indicates the relative contribution of migrants from each putative source location.

Table 5: Results of MIGRATE analysis, estimating the number of migrants per generation (N_em), the population size parameter theta (θ), their credible intervals (in brackets) and effective population size (N_e, using the mutation rate 1×10^{-4}) for five genetically differentiated *Paphies subtriangulata* population groups as determined by exact G and AMOVA tests. 'REST' refers to the grouping of PAP, MAR, PKR, COL, OAK and WPK.

Receiving Location	Source Location					θ	N_e
	RUA	WMR	CHA	KAK	REST		
RUA		4.03	2.32	1.83	4.90	0.18	441.68
		(0–53.33)	(0–45.33)	(0–41.24)	(0–56.00)	(0–0.35)	
WMR	10.01		2.32	2.17	5.83	0.11	275.00
	(0–76.27)		(0–45.33)	(0–44.09)	(0–57.56)	(0–0.27)	
CHA	13.55	4.77		1.83	4.90	0.06	158.33
	(0–83.02)	(0–55.11)		(0–42.67)	(0–54.44)	(0–0.26)	
KAK	11.19	49.50	4.86		7.23	0.05	125.00
	(0–78.58)	(0–177.78)	(0–54.40)		(0–63.78)	(0–0.21)	
REST	11.19	4.77	2.32	3.17		0.07	175.00
	(0–85.51)	(0–55.11)	(0–45.33)	(0–49.78)		(0–0.23)	

In summary, a major genetic break occurs between *P. subtriangulata* at the Chatham Islands and populations found on mainland New Zealand (Figure 9). Over much of mainland New Zealand *P. subtriangulata* forms one largely undifferentiated population, suggesting regular genetic connectivity within this area. However, analyses of genetic population structure show that in some areas (i.e., RUA, WMR and KAK) populations may be differentiated to varying extents, suggesting that there may be some limited restrictions to full genetic connectivity. However, migration analyses only showed support for restricted connectivity to RUA in recent times and varying levels of connectivity were detected amongst all populations over longer time scales.

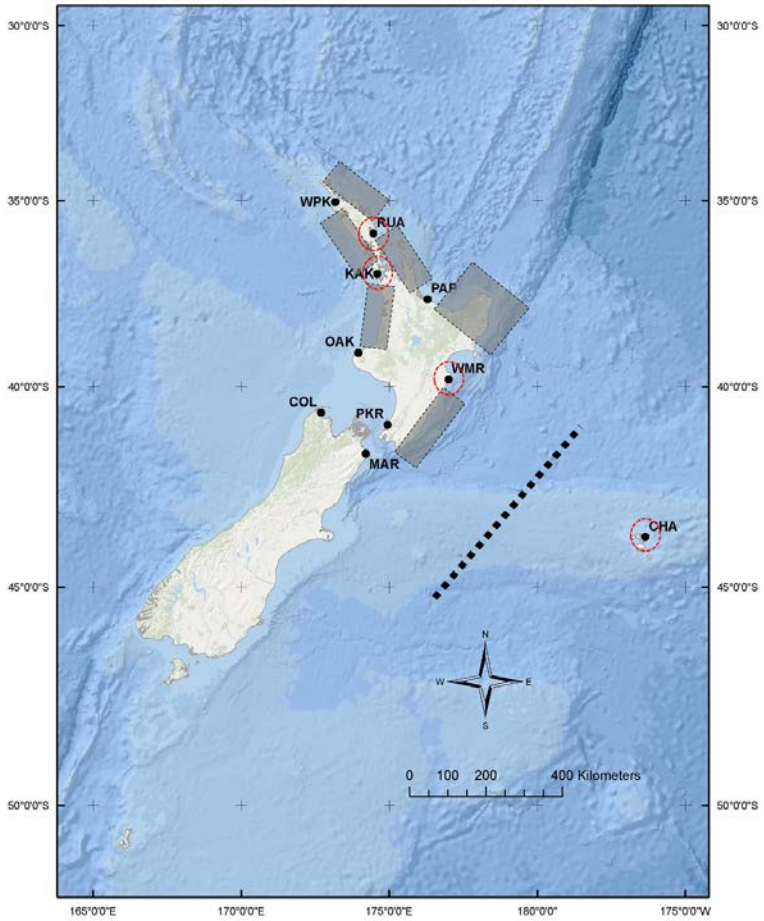

Figure 9: Location of genetically differentiated populations of *Paphies subtriangulata* and barriers to genetic connectivity. Genetically differentiated populations are those sampling locations enclosed by red dashed lines. The geographic areas where barriers to genetic connectivity are assumed to occur are indicated by shaded grey boxes on mainland New Zealand (these boxes cover large sections of coastline because it was not possible to pinpoint the exact location of barriers; it is assumed the barrier lies somewhere within the shaded area) and by the thicker dashed line which indicates the major barrier to dispersal between Chatham Island and mainland New Zealand populations. The remainder of the *Paphies subtriangulata* locations not encompassed by red dashed lines are part of one genetically undifferentiated 'panmictic' population; the populations surrounded by dashed red lines show a level of genetic differentiation from the 'panmictic' grouping.

4.2 *Paphies australis*

A total of 674 *Paphies australis* individuals from 13 locations were genotyped at 14 microsatellite loci (Table 6). *Paphies australis* was found to be common and widespread around the main islands of New Zealand; seven North Island and six South Island locations were sampled for this study.

MICROCHECKER found no evidence for genotype scoring errors or large allele drop out and there was no significant linkage disequilibrium amongst loci. One locus was in Hardy Weinberg Equilibrium (HWE), meaning it met the assumptions for the statistical analyses to follow, for the total sample (*Paus_5*) and seven loci were not in HWE for any of the locations tested (*Paus_2*, *Paus_3*, *Paus_4*, *Paus_8*, *Paus_9*, *Paus_11*, *Paus_14*; data not shown). For most loci, observed was lower than expected heterozygosity, except for one locus (*Paus_5*) where it was higher than expected (Range H_O: 0.240 to 0.660; H_E: 0.411 to 0.871; data not shown). LOSITAN detected one outlier locus (*Paus_9*) and one locus (*Paus_6*) was unable to be consistently genotyped so was excluded from all analyses; subsequent analyses are based on 12 loci.

Diversity statistics calculated for each location showed that the mean number of alleles at each location ranged from 8.231 to 11.231, allelic richness ranged from 7.072 to 8.259 and the number of private alleles ranged from 1 to 10 (Table 7). In general the frequency of these private alleles was low (less than 2%), suggesting that they represent 'rare alleles' that have not contributed significantly to genetic population variation. Observed heterozygosity was lower than expected heterozygosity for all locations (Range H_O: 0.378 to 0.497; H_E: 0.635 to 0.719) and no locations were in HWE (Table 7). F_{IS} was above zero for all locations due to an excess of homozygotes (range of 0.284 to 0.456; Table 7).

Table 6: Location, number of samples and geographical co-ordinates for *Paphies australis* samples used in this study.

Location	Abbreviation	Sample Size	Latitude	Longitude
Raglan	RAG	55	-37.82° S	174.83° E
Huia	HUI	54	-37.01° S	174.57° E
Tapotupotu Bay	TAP	55	-34.43° S	172.71° E
Waiwera	WAI	54	-36.54° S	174.71° E
Tauranga	TAU	53	-37.66° S	176.13° E
Napier	NAP	50	-39.48° S	176.89° E
Petone	PET	56	-41.23° S	174.86° E
Hakahaka Bay	HAK	55	-41.31° S	174.11° E
Lyttelton	LYT	52	-43.64° S	172.75° E
Bluff	BLU	51	-46.57° S	168.49° E
Doubtful Sound	DBT	53	-45.28° S	166.91° E
Okuru	OKU	35	-43.89° S	168.92° E
Karamea	KAR	51	-41.26° S	172.11° E

Table 7: Genetic diversity statistics for each location and total for *Paphies australis* (N_a: mean number of alleles, R_a: allelic richness, PA: private alleles, H_O: observed heterozygosity, H_E: expected heterozygosity, F_{IS}: inbreeding co-efficient). F_{IS} values in bold show significant departure from Hardy Weinberg equilibrium (HWE) after false discovery rate correction ($p < 0.05$). Location abbreviations are as described in Table 6.

Location						Statistic
	N_a	R_a	PA	H_O	H_E	F_{IS}
RAG	9.077	7.210	4	0.449	0.635	**0.289**
HUI	10.308	8.253	5	0.380	0.691	**0.439**
TAP	10.000	7.912	3	0.426	0.689	**0.376**
WAI	9.615	7.360	5	0.453	0.646	**0.284**
TAU	9.538	7.582	2	0.390	0.704	**0.436**
NAP	9.308	7.714	3	0.389	0.680	**0.416**
PET	9.462	7.659	4	0.400	0.699	**0.415**
HAK	11.231	8.259	10	0.463	0.672	**0.303**
LYT	9.769	7.789	6	0.482	0.699	**0.298**
BLU	10.692	8.270	10	0.497	0.700	**0.285**
DBT	9.000	7.072	3	0.448	0.669	**0.315**
OKU	8.231	7.498	3	0.380	0.719	**0.456**
KAR	9.154	7.700	1	0.378	0.709	**0.450**
Total	19.538	8.202	59	0.462	0.688	**0.372**

Pairwise F_{ST} amongst *P. australis* locations was low, ranging from 0.000 to 0.024 (Table 8). Exact G tests indicated that 62 of 78 pairwise comparisons had significant genetic differentiation. In general, pairwise F_{ST} values were highest when comparing North Island locations to South Island locations. F_{ST} values were also higher within North Island locations than within South Island locations. This finding suggests genetic differentiation between northern and southern populations, as well as a larger amount of genetic differentiation amongst northern populations, compared to amongst southern populations.

Despite F_{ST} values suggestive of north-south differentiation, Mantel tests were unable to find evidence of a significant IBD pattern (R^2 = 0.036, p = 0.104; Figure 10). A PCA showed that the X axis explained 16.46% of variation (p = 0.036) and the Y axis 25.57% of variation (p = 0.001; Figure 11). There were two obvious groupings of southern populations on the left side of the plot: {HAK, LYT, BLU, DBT} and {PET, KAR, OKU}. The remaining northern populations (RAG, HUI, TAP, WAI, TAU and NAP) were dispersed on the right side of the plot. Whilst a NJ dendrogram of *P. australis* locations (Figure 12) showed some indication of northern and southern population groupings, this analysis was unable to detect any statistically significant population groupings.

Table 8: Pairwise F_{ST} values amongst *Paphies australis* locations. Values in bold represent significant differentiation after false discovery rate correction ($p < 0.05$) as assessed by an exact G test (Goudet et al. 1996). Location abbreviations are as described in Table 6.

	RAG	HUI	TAP	WAI	TAU	NAP	PET	HAK	LYT	BLU	DBT	OKU
HUI	**0.017**											
TAP	**0.009**	**0.013**										
WAI	**0.003**	**0.020**	0.005									
TAU	**0.023**	**0.007**	**0.008**	**0.022**								
NAP	**0.005**	0.000	**0.004**	**0.011**	0.003							
PET	**0.012**	0.001	**0.009**	**0.017**	**0.011**	0.001						
HAK	**0.011**	**0.021**	**0.013**	**0.010**	**0.024**	**0.011**	**0.014**					
LYT	**0.006**	**0.010**	**0.006**	**0.006**	**0.018**	0.006	0.001	**0.008**				
BLU	**0.006**	**0.015**	0.008	**0.012**	**0.022**	**0.009**	**0.005**	**0.007**	**0.002**			
DBT	**0.006**	**0.015**	**0.010**	**0.013**	**0.022**	**0.011**	**0.007**	**0.007**	**0.004**	**0.004**		
OKU	**0.011**	0.001	**0.006**	**0.016**	**0.006**	0.000	0.000	**0.008**	0.000	**0.002**	**0.005**	
KAR	**0.019**	**0.008**	**0.012**	**0.023**	**0.013**	0.006	0.000	**0.016**	0.003	**0.012**	**0.007**	0.001

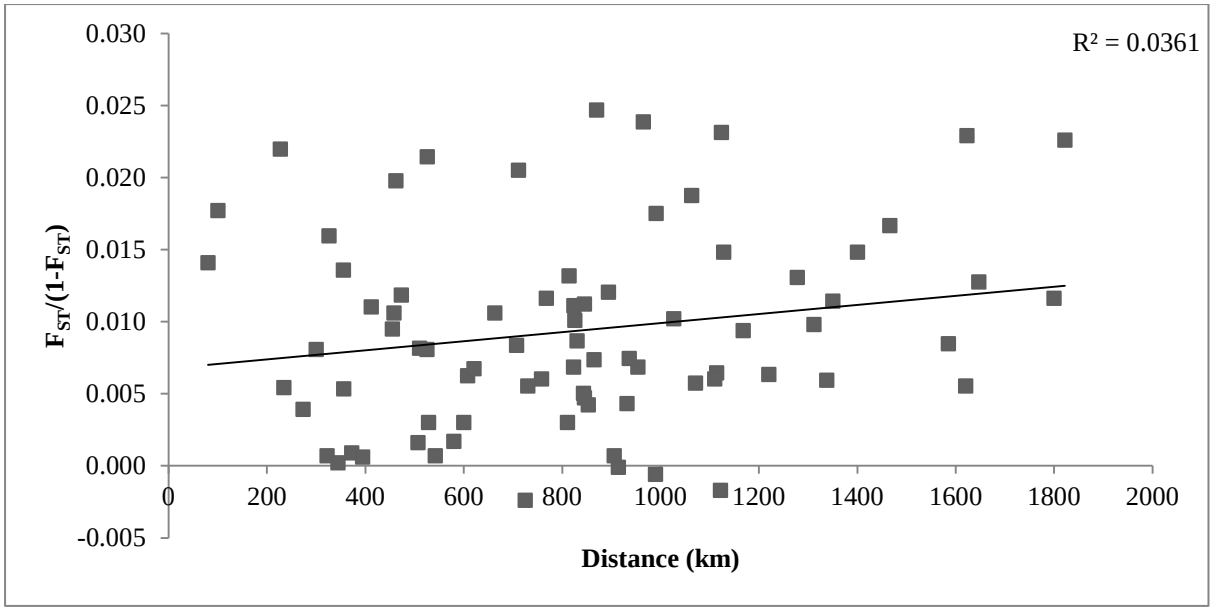

Figure 10: Mantel test for isolation by distance for *Paphies australis* locations (p = 0.104).

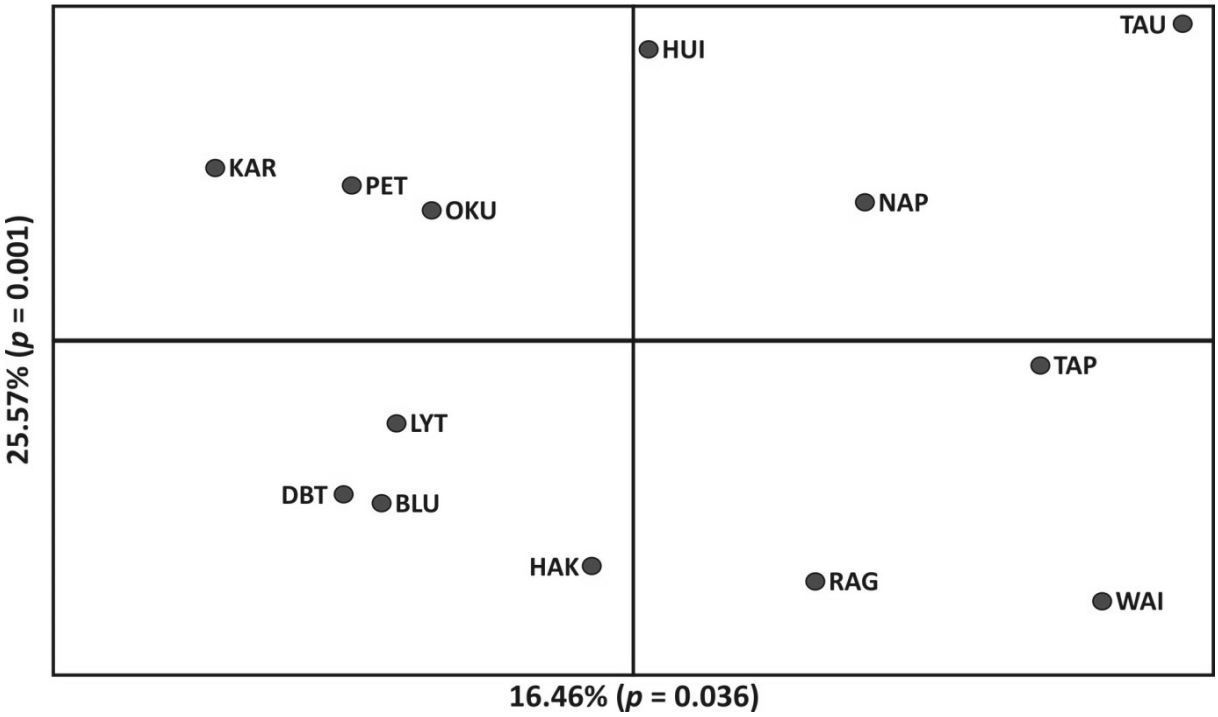

Figure 11: Principal component analysis for *Paphies australis* showing patterns of genetic population differentiation. The percentage of inertia explained by each axis and significance of the axis are displayed. An axis was considered significant if $p < 0.05$. Location abbreviations are as described in Table 6.

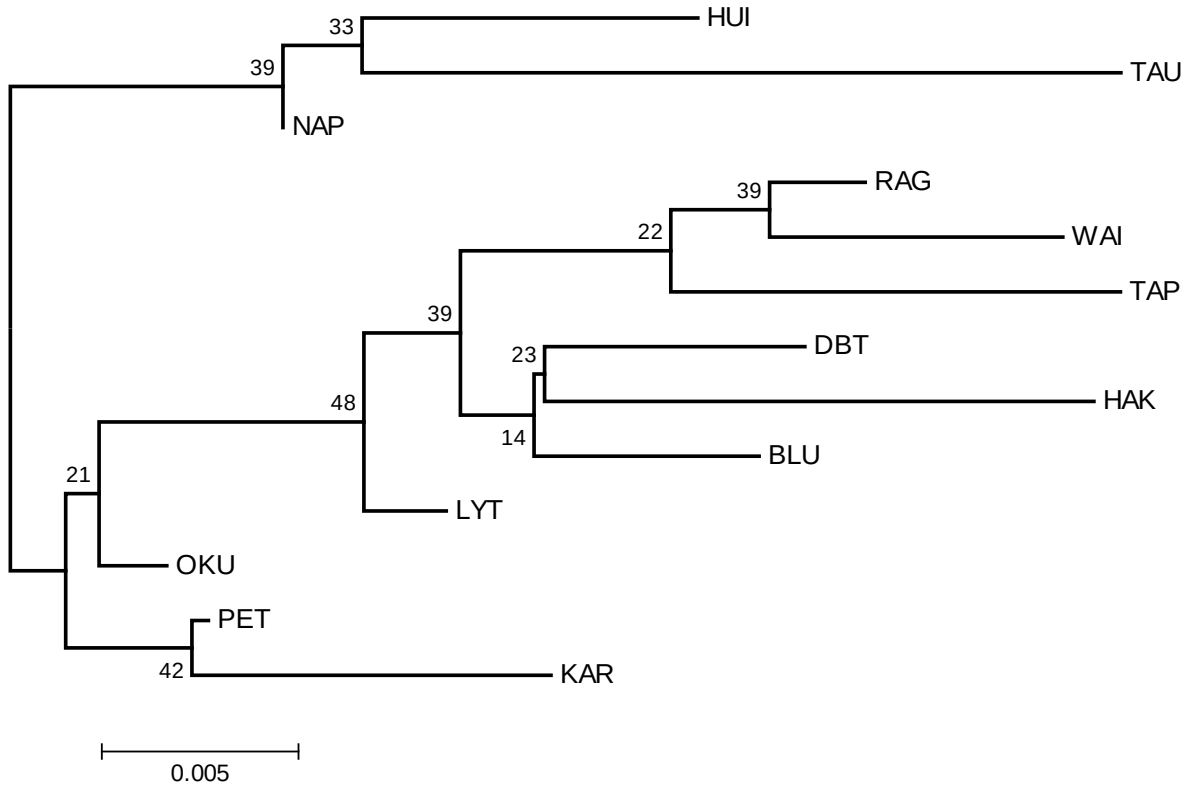

Figure 12: Neighbour Joining dendrogram based on F_{ST} values for *Paphies australis*. Location abbreviations are as described in Table 6.

AMOVA (Table 9) of all locations showed that 64% of variation was partitioned amongst individuals within the total samples and 35% of variation was partitioned amongst individuals within populations. There was significant support for a north-south grouping ({RAG, HUI, TAP, WAI, TAU, NAP} vs {PET, HAK, LYT, BLU, DBT, OKU, KAR}, p = 0.007) but not for a west-east grouping ({HUI, RAG, PET, KAR, OKU, DBT} vs {TAP, WAI, TAU, NAP, HAK, LYT, BLU}, p = 0.218). There was also strong support for further division of the southern locations; the arrangement of locations that explained the most variation amongst groups was {RAG, HUI, TAP, WAI, TAU, NAP} {HAK, LYT, BLU, DBT} {PET, KAR, OKU} (p = 0.001).

Table 9: AMOVA analyses for *Paphies australis*. Population structure was considered significant if $p < 0.05$ after false discovery rate correction for multiple tests.

Groupings Tested	Group Configuration	Source of Variance											
		Amongst groups			Amongst populations within groups			Amongst individuals within populations			Amongst individuals within the total sample		
		% var.	F_{CT}	*p*-value	% var.	F_{SC}	*p*-value	% var.	F_{IS}	*p*-value	% var.	F_{IT}	*p*-value
1 group		-	-	-	0.940	0.006	**0.005**	34.963	0.313	**0.001**	64.098	0.318	**0.001**
North-South Division	{RAG, HUI, TAP, WAI, TAU, NAP}{PET, HAK, LYT, BLU, DBT, OKU, KAR}	0.325	0.003	**0.007**	0.762	0.008	**0.001**	34.911	0.353	**0.001**	64.002	0.356	**0.001**
West-East Division	{HUI, RAG, PET, KAR, OKU, DBT}{TAP, WAI, TAU, NAP, HAK, LYT, BLU}	0.075	0.001	0.218	0.899	0.009	**0.001**	34.951	0.353	**0.001**	64.075	0.359	**0.001**
3 groups	{RAG, HUI, TAP, WAI, TAU, NAP}{HAK, LYT, BLU, DBT}{PET, KAR, OKU}	0.387	0.004	**0.001**	0.674	0.007	**0.001**	34.920	0.353	**0.001**	64.019	0.360	**0.001**

STRUCTURE analyses revealed two clusters but there was no obvious geographic pattern to the distribution of clusters as Spearman rank correlation tests were unable to detect any significant latitudinal or longitudinal correlations (Figure 13). AWclust analyses produced similar results to SRUCTURE; gap statistics revealed two clusters and again there was no significant correlation in the geographical distribution of these clusters (Figure 14).

For the purposes of migration analyses *P. australis* locations were grouped into three genetically differentiated groups: a northern group (I) consisting of RAG, HUI, TAP, WAI, TAU and NAP, a south eastern group (II) consisting of HAK, LYT, BLU and DBT, and a south western group (III) consisting of PET, KAR and OKU. BAYESASS estimates of recent migration showed that group I was the largest source of migrants, with the highest levels of migration occurring from group I to III (27%; Figure 15). Groups I and II exchanged similar amounts of migrants (5–7%) and 6% of group III individuals originated from group II. Self-recruitment was high for groups I and II (93–95%) but was lower for group III (67%).

Long-term migration rates for *P. australis*, estimated by MIGRATE showed that migration has occurred amongst all three groups, at rates ranging from 24.83–72.41 migrants per generation (Table 10). Group I was the largest source of migrants, supplying 72.41 and 57.63 migrants per generation to groups II and III, respectively. Large number of migrants were also exchanged amongst the two southern groups (II→III: 42.17; III→II: 34.77) but there was lower migration from the two southern groups to the north (II→I: 29.39, III→I: 24.83). The θ values ranged from 0.38–0.50, which equated to effective population sizes ranging from 958.33 to 1241.68 individuals.

In summary, population genetic analyses provided some evidence for three genetically differentiated populations of *P. australis* (Figure 16). This finding suggests two major barriers to connectivity: one around the Cook Strait region (although the exact latitude of this barrier may differ from west to east coast) and one around the Fiordland region on the west coast of the South Island. Migration analyses suggest regular connectivity amongst these three groups, although predominantly in a north to south direction.

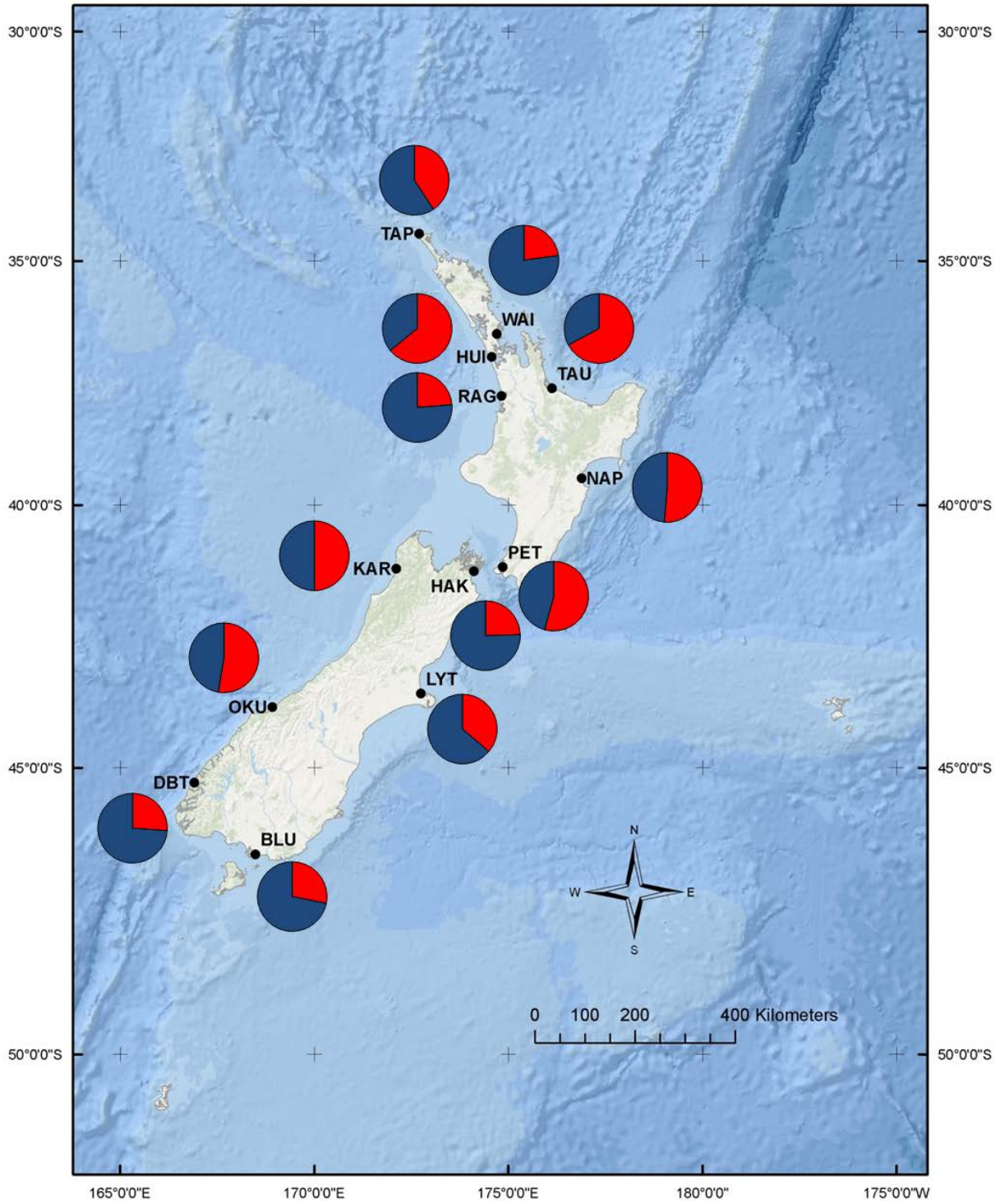

Figure 13: Output from STRUCTURE analyses for *Paphies australis* showing the proportion of each cluster assigned to each location (*K* = 2). Each colour denotes a different genetic cluster. Spearman rank correlation tests were unable to detect any significant correlations between cluster proportion and latitude or longitude.

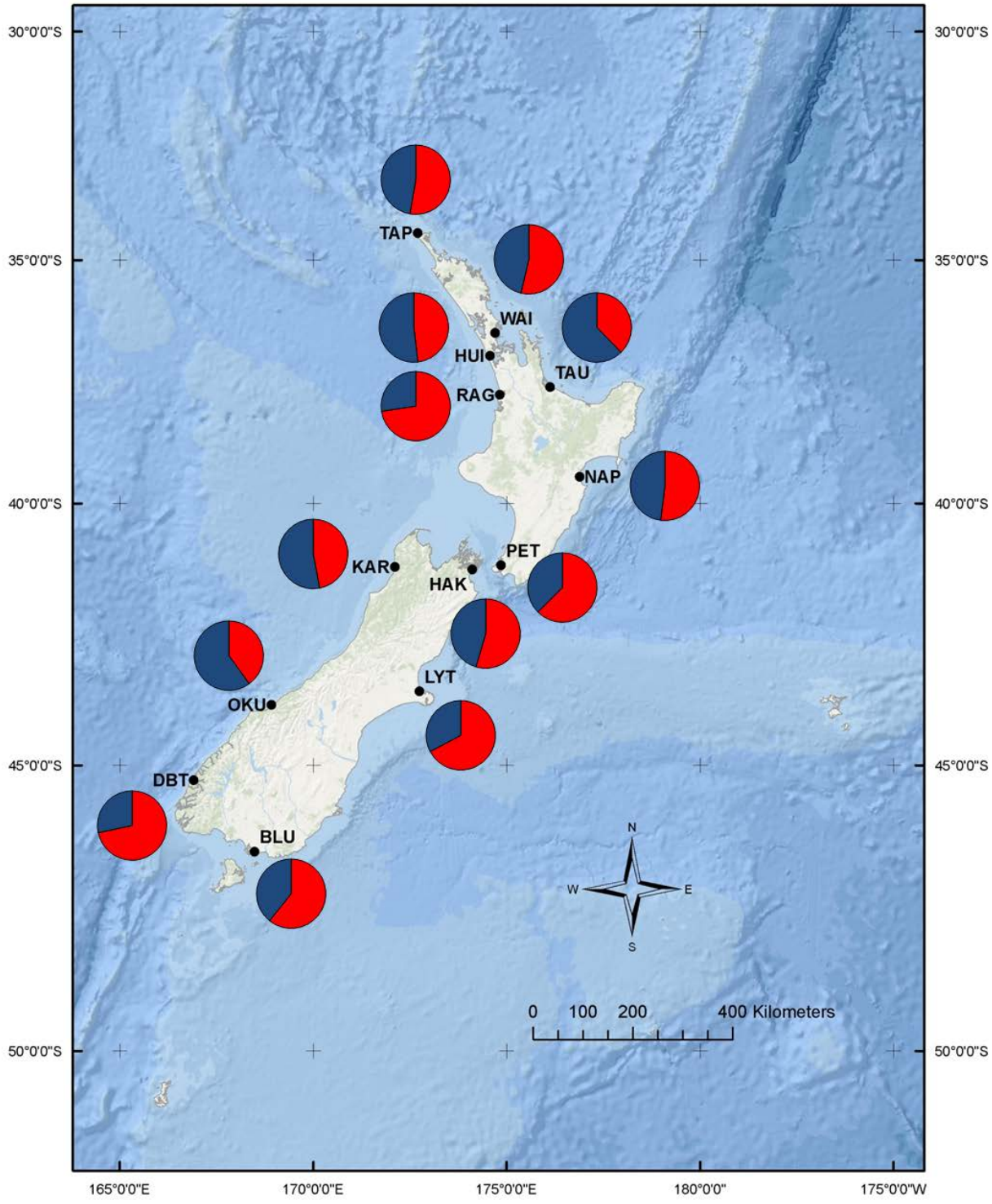

Figure 14: Output from AWclust analyses for *Paphies australis* showing the proportion of each cluster assigned to each location (*K* = 2). Each colour denotes a different genetic cluster. Spearman rank correlation tests were unable to detect any significant correlations between cluster proportion and latitude or longitude.

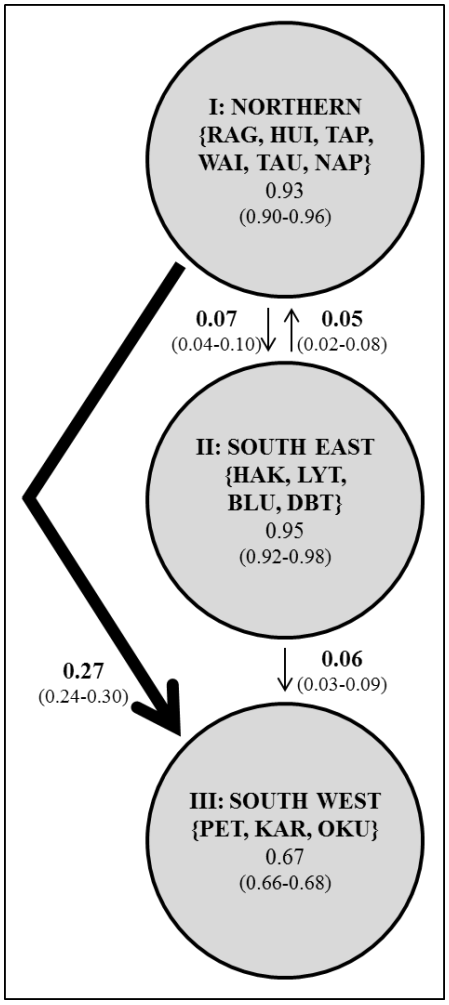

Figure 15: Recent migration rates amongst three genetically differentiated population groups of *Paphies australis*, as determined by BAYESASS. Values in grey circles represent self-recruitment rates for each location (95% confidence intervals in brackets). Arrows represent migration amongst locations with the proportion of migrants indicated in bold (95% confidence interval in brackets). Arrow thickness indicates the relative contribution of migrants from each putative source location.

Table 10: Results of MIGRATE analysis, estimating the number of migrants per generation (N_em), the population size parameter theta (θ), their credible intervals (in brackets) and effective population size (N_e, using the mutation rate 1×10^{-4}) for three genetically differentiated *Paphies australis* population groups as determined by exact G and AMOVA tests.

Receiving Location	Source Location			θ	N_e
	I: Northern	II: South East	III: South West		
I: Northern		29.39	24.83	0.44	1108.33
		(0–139.20)	(0–150.75)	(0.27–0.61)	
II: South East	72.41		34.77	0.38	958.33
	(0–198.18)		(0–164.88)	(0.17–0.58)	
III: South West	57.63	42.17		0.50	1241.68
	(0–186.05)	(0–162.40)		(0.26–0.71)	

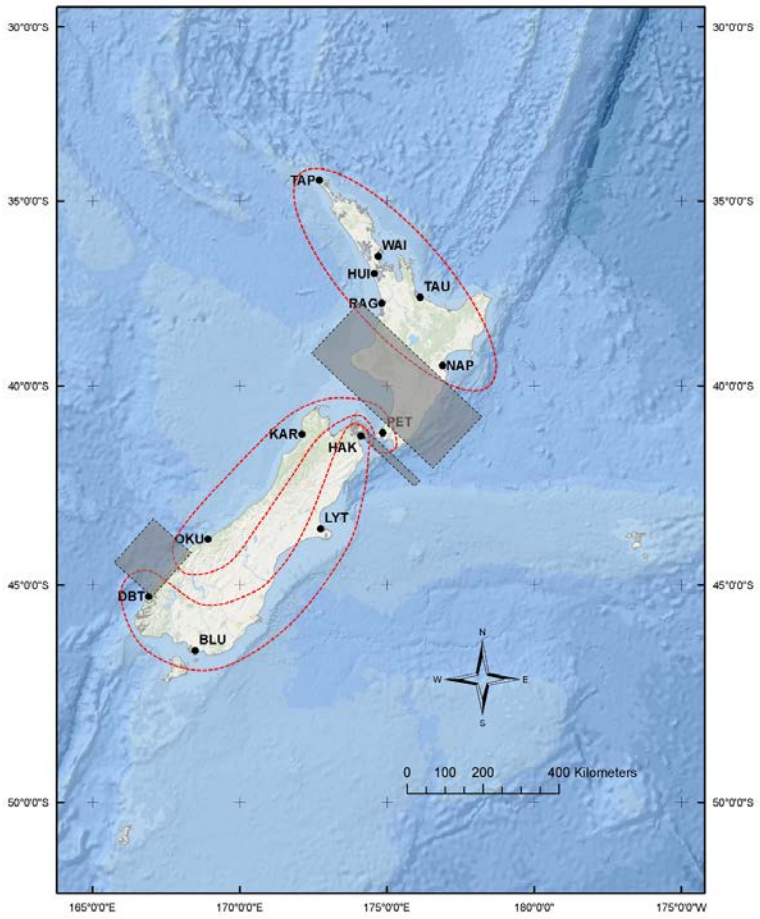

Figure 16: Location of genetically differentiated populations of *Paphies australis* and barriers to genetic connectivity. Populations are those sampling locations enclosed by red dashed lines. The geographic areas where barriers to genetic connectivity are assumed to occur are indicated by shaded grey boxes (these boxes cover large sections of coastline because it was not possible to pinpoint the exact location of barriers; it is assumed the barrier lies somewhere within the shaded area).

4.3 *Rhombosolea plebeia*

A total of 858 *Rhombosolea plebeia* adults and juveniles from 19 locations were genotyped at 12 loci (Table 11). MICROCHECKER identified six loci (*Rpleb5*, 25, *65*, *79*, *124* and *145*) as possibly having null alleles due to an overall homozygote excess, but no scoring errors due to stutter or large allele drop out were detected. Tests for HWE at each locus identified loci *Rpleb25*, *57*, *65*, *79* and *145* as being significantly out of HWE for more than half of the sampled locations after false discovery rate (FDR) correction for multiple tests (data not shown).

Outlier analysis performed in LOSITAN identified locus *Rpleb65* as a candidate for strong positive selection, *Rpleb57* as a candidate for weak positive selection and *Rpleb131* as a candidate for weak balancing selection when samples were split into 22 localities (years separated). When all localities were combined, locus *Rpleb145* was a candidate for strong positive selection. No consistent patterns of linkage disequilibrium were found across localities or loci. *Rpleb65* and *145* were excluded from further analysis for possibly being under selection, missing data and being significantly out of HWE across many populations.

Table 11: Location, number of samples and geographic co-ordinates of *Rhombosolea plebeia* samples used in this study.

Location	Abbreviation	Sample Size	Latitude	Longitude
Dicks Bay 2010	DB10	38	-35.27° S	174.22° E
Dicks Bay 2011	DB11	51	-35.27° S	174.22° E
Paihia	PA	24	-35.29° S	174.10° E
Hokianga Harbour	HK	48	-35.40° S	173.51° E
Manukau Harbour	MK	23	-36.93° S	174.71° E
Hawke Bay	HB	45	-39.52° S	177.06° E
Ahuriri Estuary	AR	47	-39.48° S	176.88° E
Foxton Estuary	FX	48	-40.47° S	175.23° E
Porirua Harbour	PH	42	-41.11° S	174.85° E
Lake Ferry	LF	30	-41.38° S	175.11° E
Tasman Bay	TB	48	-41.05° S	173.27° E
Mapua Harbour	MH	47	-41.26° S	173.10° E
Karamea River Mouth	KM	23	-41.26° S	172.10° E
Westport	WS	20	-41.74° S	171.63° E
Avon Heathcote Estuary 2011	AH11	49	-43.53° S	172.72° E
Avon Heathcote Estuary 2012	AH12	49	-43.56° S	172.71° E
Lyttelton Harbour	LH	50	-43.58° S	172.83° E
Lake Ellesmere	LE	49	-43.79° S	172.50° E
Otago Harbour	OT	22	-45.78° S	170.72° E
Taieri Mouth	TE	50	-46.05° S	170.19° E
Awarua Bay 2011	AB11	29	-46.57° S	168.47° E
Awarua Bay 2012	AB12	26	-46.56° S	168.43° E

Locality-specific genetic diversity statistics for *R. plebeia* (Table 12) showed slightly lower levels of observed heterozygosity (H_o range = 0.365 to 0.480) than expected heterozygosity (H_e range = 0.433 to 0.508). However, 16 of 19 populations were significantly out of HWE after FDR correction for multiple tests. Allelic richness ranged from 3.84 to 4.82, F_{IS} ranged from -0.028 at KM to 0.190 at PH (mean 0.119), and seven private alleles were found at seven locations.

Pairwise F_{ST} values amongst all *R. plebeia* locations over 10 loci (Table 13) ranged from 0.000 to 0.039. Of the 171 tests of allelic differentiation, 93 were significant after FDR corrections for multiple tests, where the highest *p*-value of statistical significance was 0.028. LE and HK had the greatest number of significant comparisons.

Table 12: Genetic diversity statistics for each location and total for *Rhombosolea plebeia* (N_a: mean number of alleles, R_a: allelic richness, PA: private alleles, H_O: observed heterozygosity, H_E: expected heterozygosity, F_{IS}: inbreeding co-efficient). F_{IS} values in bold show significant departure from HWE expectations after false discovery rate correction ($p < 0.05$). Location abbreviations are as described in Table 11.

Location	Statistic					
	N_a	R_a	PA	H_O	H_E	F_{IS}
DB	1.82	4.27	1	0.405	0.475	**0.170**
PA	2.54	3.84	1	0.399	0.468	**0.162**
HK	1.58	4.13	1	0.422	0.464	**0.143**
MK	2.57	4.00	0	0.390	0.463	**0.185**
HB	1.62	4.01	0	0.365	0.433	**0.154**
AR	1.74	4.50	1	0.456	0.486	**0.099**
FX	1.73	4.38	0	0.424	0.467	**0.044**
PH	1.86	4.27	0	0.398	0.469	**0.190**
LF	2.40	4.53	0	0.431	0.488	**0.134**
TB	1.67	4.66	0	0.432	0.496	**0.188**
MH	1.70	4.54	0	0.417	0.473	**0.152**
KM	2.91	4.62	1	0.480	0.477	-0.028
WS	3.30	4.60	0	0.420	0.464	0.133
AH	1.59	4.49	0	0.463	0.503	**0.070**
LH	1.38	4.23	0	0.423	0.477	**0.124**
LE	1.47	4.42	1	0.454	0.457	0.013
OT	3.00	4.34	0	0.453	0.466	0.043
TE	1.66	4.82	1	0.441	0.479	**0.164**
AB	2.69	4.63	0	0.438	0.508	**0.112**
Total	2.06	4.38	0.368	0.427	0.474	**0.119**

Table 13: Pairwise F_{ST} values amongst *Rhombosolea plebeia* locations. Values in bold represent significant differentiation after false discovery rate correction ($p < 0.05$) as assessed by an exact G test (Goudet et al. 1996). Location abbreviations are as described in Table 11.

	DB	PA	HK	MK	HB	AR	FX	PH	LF	TB	MH	KM	WS	AH	LH	LE	OT	TE
PA	0.000																	
HK	**0.008**	0.000																
MK	0.001	0.000	0.000															
HB	**0.005**	0.000	**0.004**	0.000														
AR	**0.003**	0.000	**0.004**	0.000	0.001													
FX	**0.015**	0.005	**0.013**	0.001	0.007	0.008												
PH	**0.011**	0.000	**0.011**	0.001	**0.009**	0.003	0.001											
LF	0.004	0.000	0.002	0.000	0.007	0.001	0.000	0.000										
TB	**0.006**	0.000	**0.009**	0.000	0.002	0.000	0.007	**0.006**	0.003									
MH	**0.010**	0.000	**0.017**	0.011	0.010	0.007	0.010	**0.007**	0.006	0.000								
KM	**0.009**	0.000	**0.002**	0.000	**0.001**	0.001	0.000	0.000	0.002	0.000	0.009							
WS	**0.002**	**0.008**	**0.017**	**0.008**	**0.013**	0.010	**0.021**	**0.010**	**0.016**	0.000	0.005	0.004						
AH	**0.023**	**0.007**	**0.018**	**0.012**	**0.015**	**0.012**	**0.011**	**0.012**	0.008	0.000	**0.003**	**0.012**	**0.021**					
LH	**0.015**	0.000	**0.014**	0.003	**0.009**	**0.009**	0.003	0.002	0.001	0.000	0.001	**0.013**	**0.019**	0.001				
LE	**0.031**	**0.019**	**0.030**	**0.031**	**0.034**	**0.024**	**0.022**	**0.021**	**0.019**	**0.009**	**0.001**	**0.031**	**0.030**	**0.006**	**0.004**			
OT	**0.014**	0.000	**0.008**	0.006	**0.011**	**0.013**	**0.012**	**0.010**	0.006	0.001	0.004	**0.015**	**0.016**	0.000	0.000	0.006		
TE	**0.028**	**0.011**	**0.026**	**0.022**	**0.024**	**0.020**	**0.022**	**0.019**	**0.016**	0.001	0.001	**0.020**	**0.018**	0.001	0.004	0.003	0.000	
AB	**0.033**	**0.016**	**0.029**	**0.023**	**0.026**	**0.019**	**0.022**	**0.022**	**0.020**	0.007	0.008	**0.014**	**0.024**	0.006	0.015	**0.019**	0.017	0.007

A Mantel test indicated a weak IBD signal ($R^2 = 0.141$, $p < 0.001$; Figure 17). PCA results showed that Axis 1 explained 50.08% of the variation in the data and Axis 2 explained 17.09% of the variation (Figure 18). The plot showed a tight grouping of the North Island populations on the right side of axis 1 in addition to WS and KM, and the remaining South Island populations were on the left of axis 1. There was one particularly close grouping of HB and AH, which geographically are only 10 km apart. A NJ dendrogram showed most of the southern populations grouping together with the exception of WS and KM (Figure 19), but with low support (50–55%). LE and TE grouped together (63% support), and DB and WS grouped together (51% support).

AMOVA analysis (Table 14) for *R. plebeia* locations grouped into northern and southern groups showed that 79.5% of the variation was explained amongst individuals within the total sample), 19.2% was explained amongst individuals within populations, 0.6% was explained amongst populations within groups and 0.616% was explained amongst groups; all of these percentages were significant at the 0.05 level. Results were similar when locations were grouped into eastern and western groups, and were also significant at the 0.05 level.

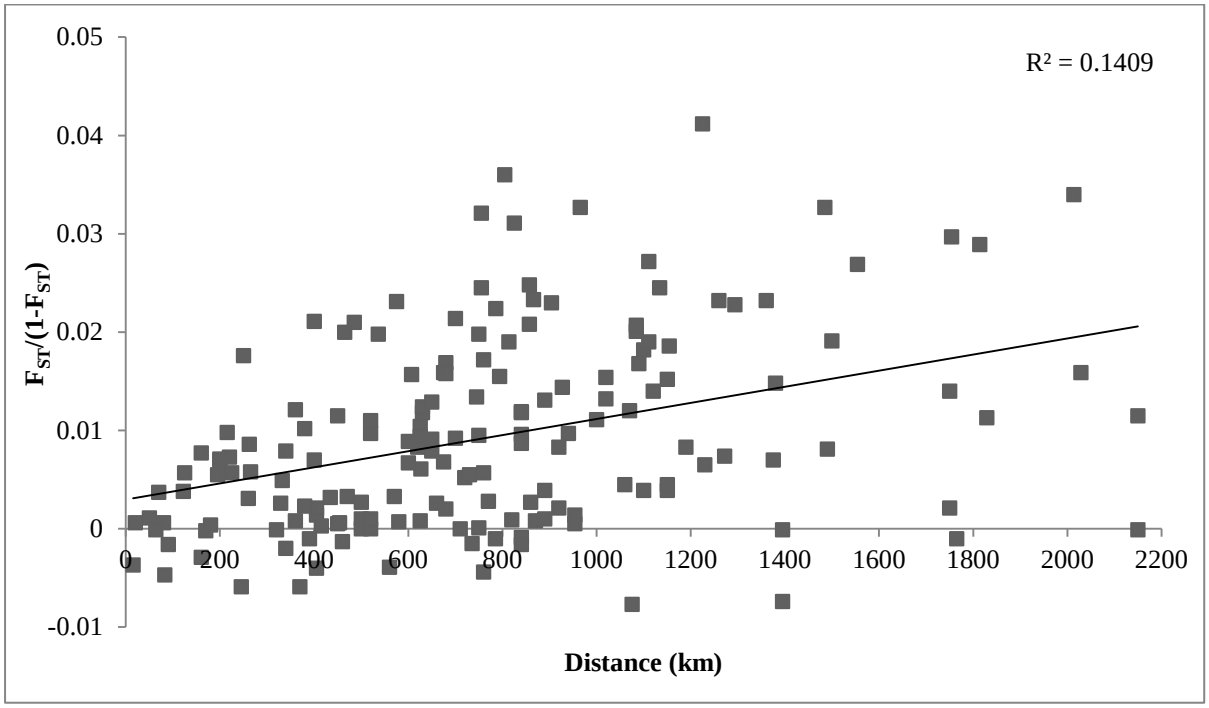

Figure 17: Mantel test for isolation by distance for *Rhombosolea plebeia* locations ($p < 0.001$).

Figure 18: Principal component analysis for *Rhombosolea plebeia* showing patterns of genetic population differentiation. The percentage of inertia explained by each axis is displayed. Location abbreviations are as described in Table 11.

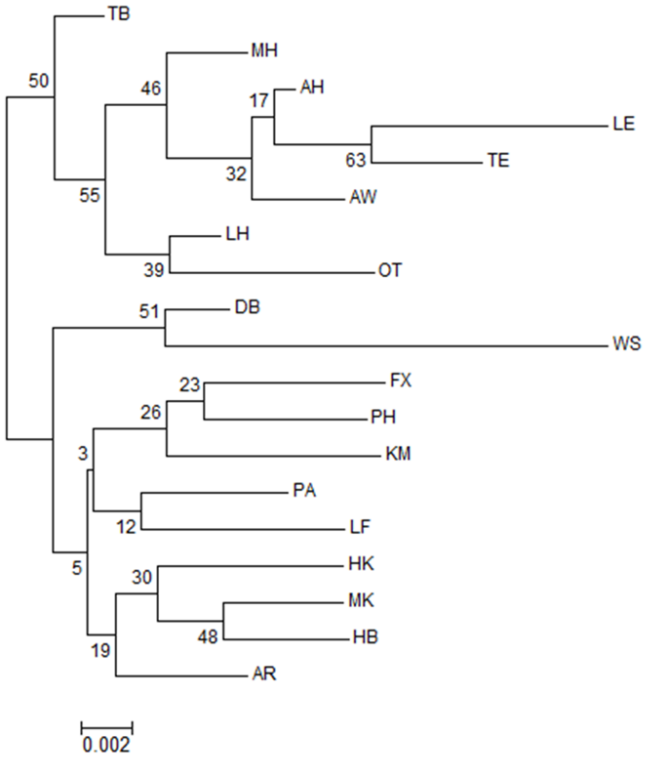

Figure 19: Neighbour Joining dendrogram based on F_{ST} values for *Rhombosolea plebeia*. Location abbreviations are as described in Table 11.

Table 14: AMOVA results for *Rhombosolea plebeia* using F_{ST} with North versus South and West versus East groupings.

North versus South

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Variance	Estimated Variance	% Variation	Differentiation Indices	*p*-value
Amongst groups North (DB, PA, HK, MG, HB, AR, FX, PR, LF, TB, MH) South (KM, WS, AH, LH, LE, OT, TE, AB)	1	18.6	18.564	0.016	0.616%	F_{RT} = 0.006	**0.010**
Amongst populations within groups	17	77.5	4.556	0.017	0.630%	F_{SR} = 0.006	**0.010**
Amongst individuals within populations	843	2606.8	3.092	0.504	19.220%	F_{ST} = 0.012	**0.010**
Amongst individuals within the total sample	862	1797.0	2.085	2.085	79.534%	F_{IS} = 0.195	**0.010**
Total	1723	4499.8		2.621	100%	F_{IT} = 0.205	**0.010**

West versus East

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Variance	Estimated Variance	% Variation	Differentiation Indices	*p*-value
Amongst groups West (HK, MG, KM, WS, FX, PH, TB, MH) East (DB, PA, AR, HB, LF, AH, LH, LE, OT, TE, AB)	1	7.0	7.043	0.002	0.085%	F_{RT} = 0.001	**0.030**
Amongst populations within groups	17	87.9	5.173	0.023	0.889%	F_{SR} = 0.009	**0.010**
Amongst individuals within populations	843	2607.8	3.093	0.504	19.292%	F_{ST} = 0.010	**0.010**
Within populations within the total sample	862	1797.0	2.085	2.085	79.734%	F_{IS} = 0.195	**0.010**
Total	1723	4499.8		2.615	100%	F_{IT} = 0.203	**0.010**

A cluster analysis in STRUCTURE showed that the most likely *K* value was two clusters (Figure 20). The AWclust gap analysis showed that the most likely number of clusters was five; the analysis had widely overlapping margins of error that did not reveal a clear geographic pattern (Figure 21). A Spearman rank correlation test of the geographic distribution of STRUCTURE clusters supported a relationship with latitude and longitude. The results showed a statistically significant decrease in cluster one (red) from north to south (Spearman's $r = 0.874$, $p < 0.001$), and a corresponding increase in cluster two (dark blue) from north to south (Spearman's $r = -0.894$, $p < 0.001$). There was also a significant decrease in cluster one (red) from east to west (Spearman's $r = -0.663$, $p = 0.002$). A Spearman rank correlation test of the geographic distribution of AWclust clusters showed some correlation with longitude. There was a statistically significant increase in cluster one (dark blue) from north to south (Spearman's $r = -0.617$, $p = 0.005$), and decrease in cluster three (red) from north to south (Spearman's $r = 0.616$, $p = 0.005$).

Assignment analysis, implemented in GENECLASS2, showed a mean correct assignment of 35.69% for the localities (Table 15). The locality with the highest level of correct assignment was KM at 60.00% and the lowest was AH at 21.65%. The individuals from AH were most likely to assign to locations further south. The locality OT received 6.52% of the total number of individuals assigned to populations other than the ones they were collected from.

In summary, *R. plebeia* shows differentiation between northern and southern localities, with a break around 42°S (south of Westport (WS) on the west coast and Lake Ferry (LF) on the east coast; Figure 22). However, because no samples were collected south of Westport on the west coast, or between Lake Ferry and Avon Heathcote on the east coast, it was not possible to determine the exact location of the break. Further, these sampling gaps, combined with a weak IBD signal, means that it was not possible to differentiate IBD population structure from hierarchical structure. Assignment tests were inconclusive; they showed moderate levels of self-recruitment within each location, but assignments between locations appeared to be randomly distributed with no evidence of source or sink populations.

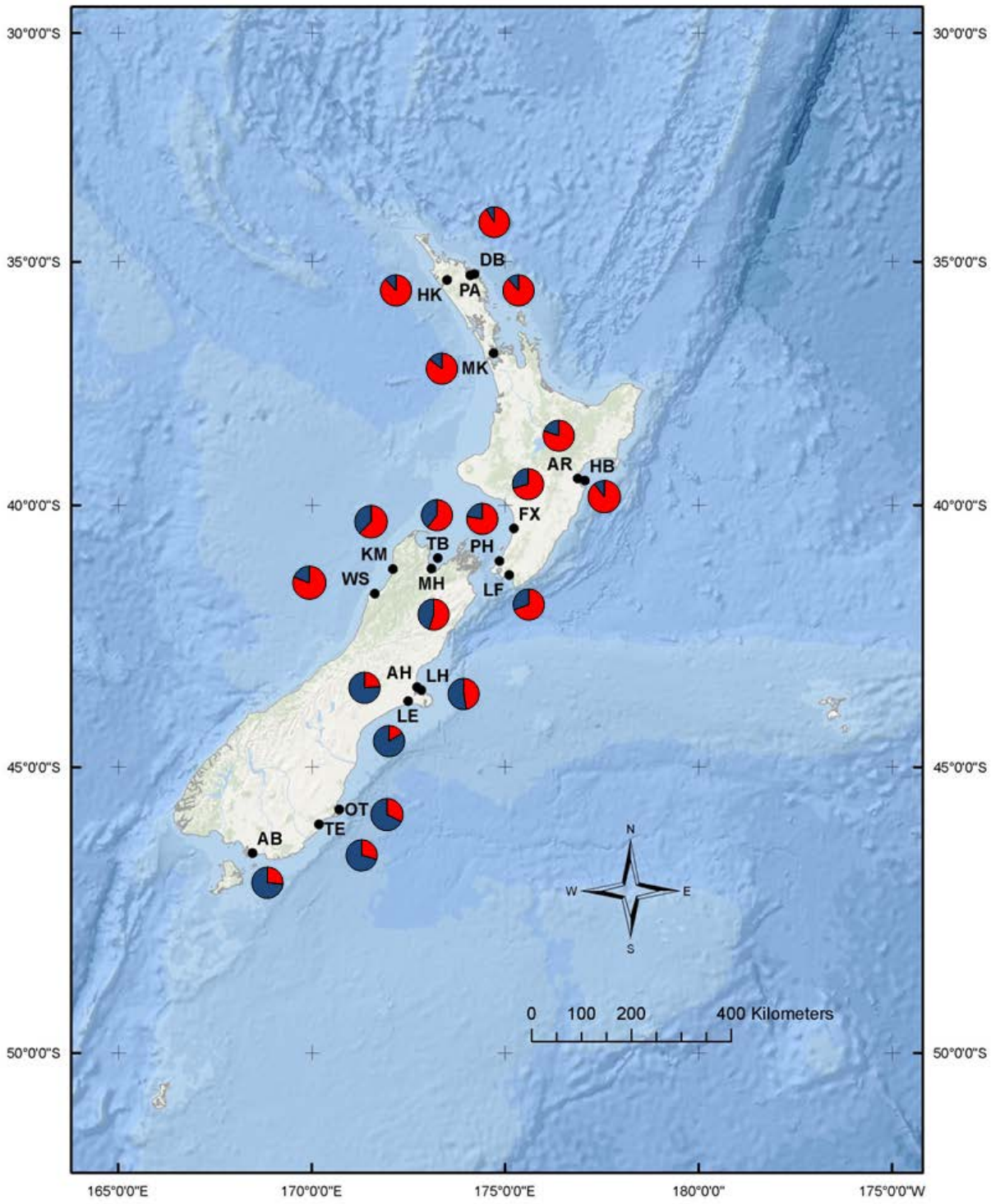

Figure 20: Output from STRUCTURE analyses for *Rhombosolea plebeia* showing the proportion of each cluster assigned to each location (*K* = 2). Each colour denotes a different genetic cluster. A Spearman rank correlation test of the geographic distribution of clusters supported a correlation with latitude and longitude.

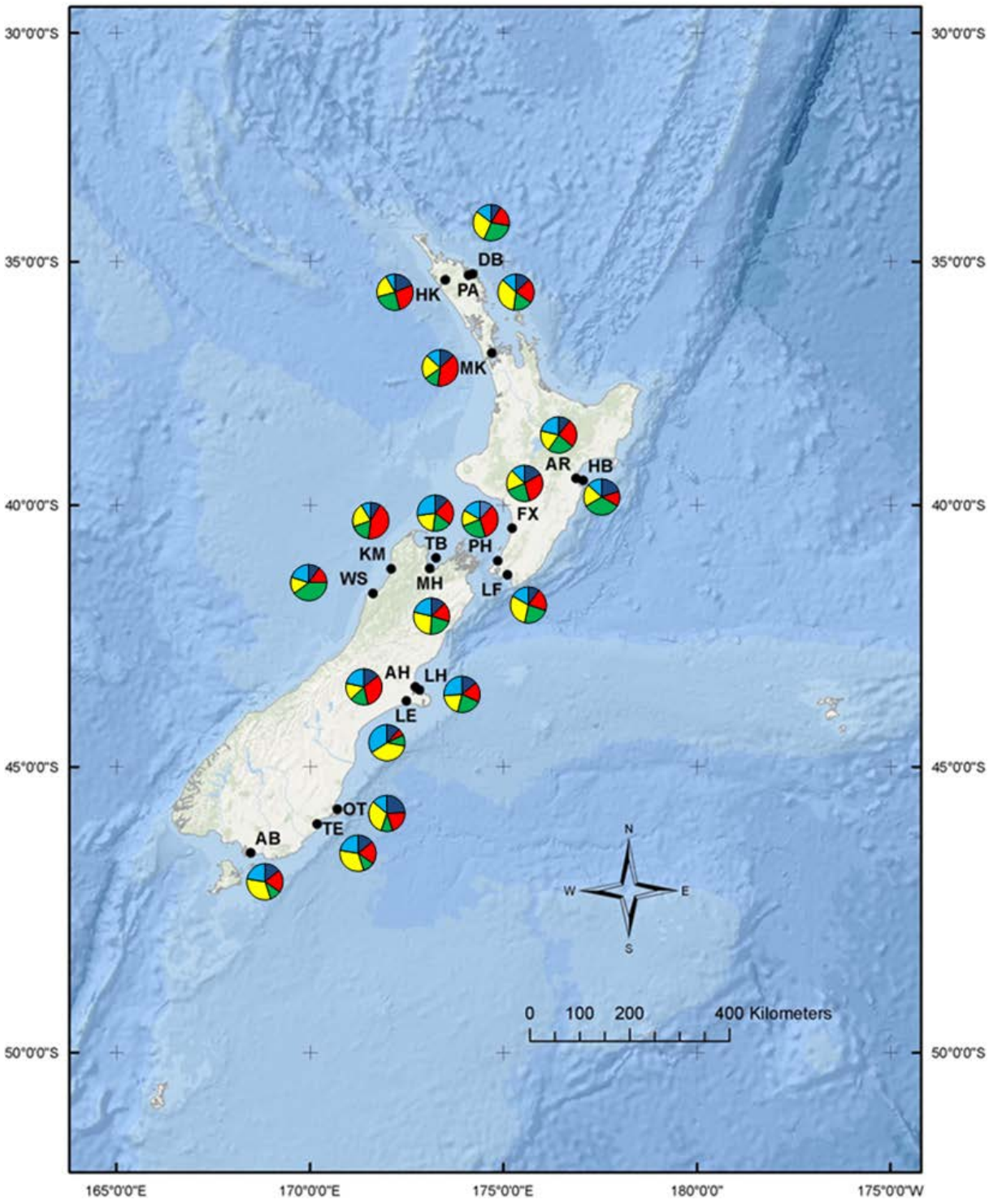

Figure 21: Output from AWclust analyses for *Rhombosolea plebeia* showing the proportion of each cluster assigned to each location (*K* = 5). Each colour denotes a different genetic cluster. A Spearman rank correlation test of the geographic distribution of clusters showed some correlation with longitude for clusters 1 and 3, but there is limited evidence of genetic differentiation amongst populations.

Table 15: GENECLASS2 assignment results for *Rhombosolea plebeia* showing exchange of first generation (F0) migrants amongst localities. Location abbreviations are as described in Table 11.

Receiving location	Source of F0 migrants of *R. plebeia*																			Total F0 Received
	DB	PA	HK	MK	HB	AR	FX	PH	LF	TB	MH	KM	WS	AH	LH	LE	OT	TE	AB	
DB		1							1		1		1		1					5
PA					1															1
HK										1					2		1	1		5
MK			1														1			2
HB				2							1									3
AR		1											1					1	1	4
FX		2	1													1				4
PH						1					1				1					3
LF	1										1		2							4
TB			1									1					1		1	4
MH																				0
KM						1	1							1						3
WS									1	1				1						3
AH									1											1
LH												1					1			2
LE														1						1
OT															1					1
TE											1			1	1					3
AB										1						1	2			4
Total F0 supplied	1	4	3	2	1	2	1	0	3	2	5	2	4	4	6	2	6	2	2	

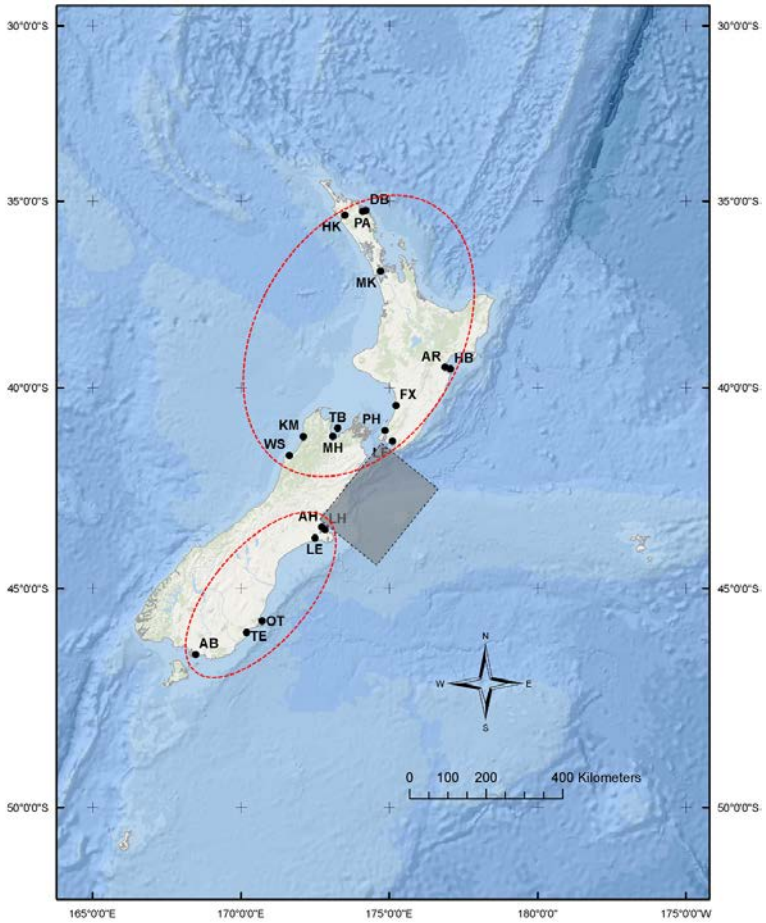

Figure 22: Location of genetically differentiated populations of *Rhombosolea plebeia* and barriers to genetic connectivity. Populations are those sampling locations enclosed by red dashed lines. The geographic areas where barriers to genetic connectivity are assumed to occur are indicated by shaded grey boxes (these boxes cover large sections of coastline because it was not possible to pinpoint the exact location of barriers; it is assumed the barrier lies somewhere within the shaded area).

4.4 *Rhombosolea leporina*

A total of 404 *Rhombosolea leporina* adults and juveniles from ten locations were genotyped at 14 loci (Table 16). No loci were identified as candidates for selection and no patterns of linkage disequilibrium were found across populations or loci. MICROCHECKER indicated that locus *Rlep1* had an excess of homozygotes and null alleles, and *Rlep22* had a homozygote excess and null alleles, but no scoring errors due to stutter or large allele drop out were detected. Tests for HWE at each locus identified *Rlep20, 22, 23* and *37* as being significantly out of HWE for more than half of the locations sampled after FDR correction for multiple tests (data not shown). Locus *Rlep22* was excluded from further analysis due to homozygote excess, null alleles and missing data.

Locality-specific genetic diversity statistics for *R. leporina* (Table 17) showed slightly lower levels of observed heterozygosity (H_o range 0.485 to 0.542) than expected heterozygosity (H_e range = 0.534 to 0.568), and eight out of ten locations were significantly out of HWE. Mean allelic richness ranged from 4.71 to 6.36, F_{IS} ranged from -0.012 at WS to 0.149 at AH, with a mean of 0.075. The number of private alleles was 16 across six populations.

Pairwise F_{ST} comparisons amongst all *R. leporina* locations over 13 loci (Table 18) ranged from 0.000 to 0.046. Of the 45 tests, 36 were significant after FDR correction for multiple tests where the highest *p*-value of statistical significance was 0.042. F_{ST} values for HB, TB and BL were highly differentiated at all nine comparisons with other locations.

Table 16: Location, number of samples and geographic co-ordinates of *Rhombosolea leporina* samples used in this study.

Location	Abbreviation	Sample Size	Latitude	Longitude
Mangonui Harbour	MG	50	-34.99° S	173.55° E
Hokianga Harbour	HK	26	-35.40° S	173.51° E
Hawke Bay	HB	49	-39.59° S	176.97° E
Tasman Bay	TB	47	-41.05° S	173.27° E
Blenheim	BL	50	-41.50° S	174.06° E
Karamea River Mouth	KM	11	-41.26° S	172.10° E
Westport	WS	27	-41.74° S	171.63° E
Avon Heathcote Estuary	AH	46	-43.53° S	172.72° E
Lyttelton Harbour	LH	48	-43.58° S	172.83° E
Haast	HS	50	-43.90° S	168.92° E

Table 17: Genetic diversity statistics for each location and total for *Rhombosolea leporina* (N_a: mean number of alleles, R_a: allelic richness, PA: private alleles, H_O: observed heterozygosity, H_E: expected heterozygosity, F_{IS}: inbreeding co-efficient). F_{IS} values in bold show significant departure from HWE expectations after false discovery rate correction ($p < 0.05$). Location abbreviations are as described in Table 16.

Location	Statistic					
	N_a	R_a	PA	H_O	H_E	F_{IS}
MG	1.88	5.79	4	0.525	0.548	0.025
HK	3.31	5.79	0	0.525	0.567	**0.072**
HB	1.96	5.8	3	0.498	0.535	**0.060**
TB	2.04	5.72	0	0.535	0.548	**0.017**
BL	1.90	4.71	0	0.497	0.556	**0.070**
KM	6.36	n/a	1	0.485	0.534	**0.107**
WS	3.04	5.26	0	0.542	0.534	-0.012
AH	2.26	6.36	5	0.497	0.561	**0.149**
LH	2.27	6.22	1	0.500	0.554	**0.066**
HS	1.92	5.81	2	0.520	0.568	**0.072**
Total	2.69	5.17	1.080	0.484	0.522	**0.075**

Table 18: Pairwise F_{ST} values amongst *Rhombosolea leporina* locations. Values in bold represent significant differentiation after false discovery rate correction ($p < 0.05$) as assessed by an exact G test (Goudet et al. 1996). Location abbreviations are as described in Table 16.

	MG	HK	HB	TB	BL	KM	WS	AH	LH
HK	**0.011**								
HB	**0.006**	**0.012**							
TB	**0.012**	**0.013**	**0.002**						
BL	**0.019**	**0.018**	**0.008**	0.003					
KM	0.023	0.015	**0.032**	**0.045**	**0.043**				
WS	**0.007**	0.000	**0.008**	**0.010**	**0.019**	0.025			
AH	**0.024**	**0.017**	**0.011**	**0.012**	**0.013**	0.032	**0.010**		
LH	**0.018**	**0.015**	**0.010**	**0.005**	**0.010**	**0.046**	**0.010**	0.000	
HS	**0.012**	0.000	**0.011**	**0.009**	**0.016**	0.020	0.000	**0.016**	**0.013**

A Mantel test detected no IBD relationship for *R. leporina* ($R^2 = 0.002$, $p = 0.308$; Figure 23). A PCA plot showed that Axis 1 explained 44.81% of the variation and Axis 2 28.55% of the variation (Figure 24). East coast locations, except for TB, were grouped on the right side of axis 1. West coast localities, in addition to MG, were grouped on the left side of axis 1. A NJ dendrogram showed 78% support for most of the east coast locations grouping together, with the exception of MG, but also included the TB location (Figure 25). There was 68% support for a grouping with HK and HS. The main structure of the tree did not change when KM was removed. There was very high bootstrap support (96%) for a grouping between AH and LH, which are approximately 8 km apart.

AMOVA analysis (Table 19) of *R. leporina* locations grouped into northern and southern groups showed that 85.53% of variation was explained amongst individuals in the total sample, 12.74% amongst individuals within populations, 1.46% amongst populations within groups and 0.27% amongst groups, with all percentages significant at the 0.05 level. Eastern and western groupings showed similar results with 85.55% of variation explained amongst individuals within the total samples, 12.75% amongst individuals within populations, 1.50% amongst populations within groups and 0.21% amongst groups, with all percentages significant at the 0.05 level.

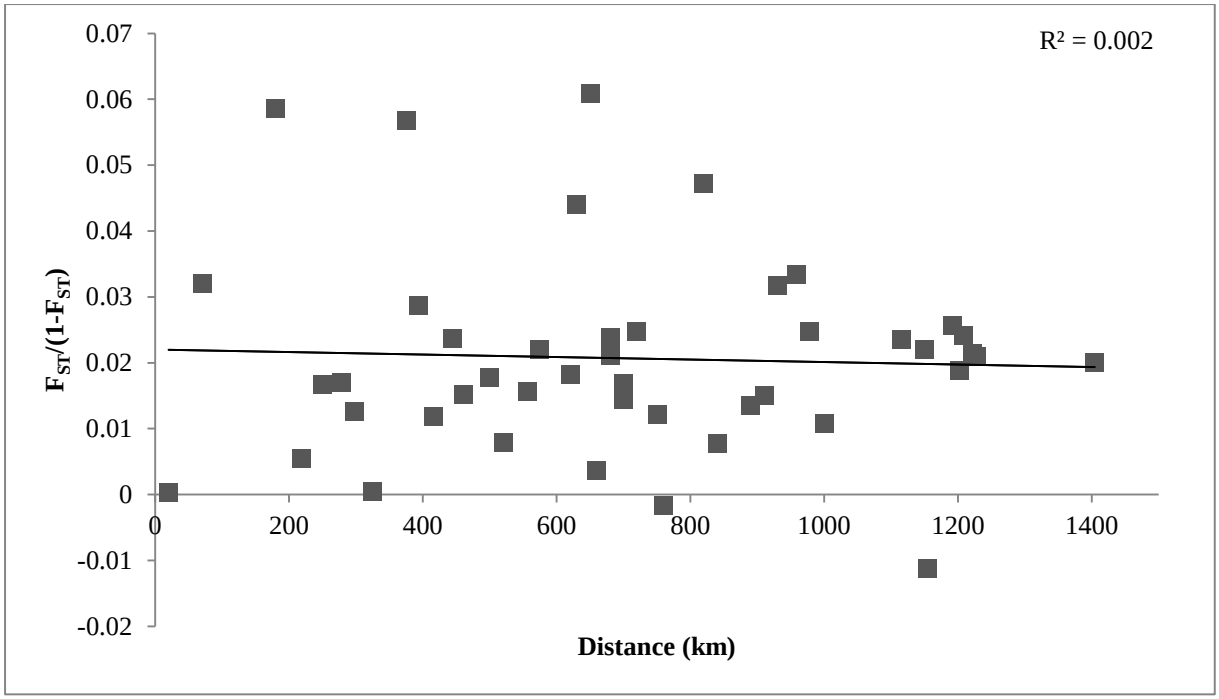

Figure 23: Mantel test for isolation by distance for *Rhombosolea leporina* locations (p = 0.308).

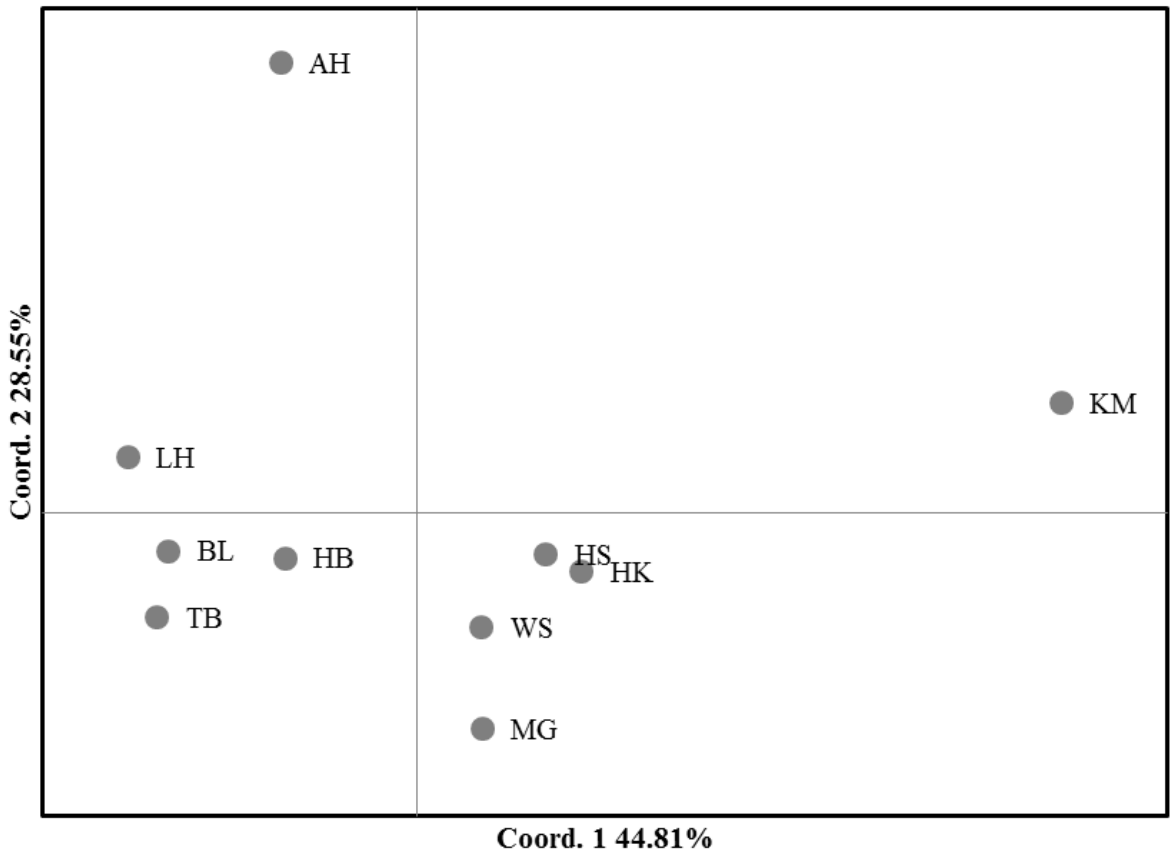

Figure 24: Principal component analysis for *Rhombosolea leporina* showing patterns of genetic population differentiation. The percentage of inertia explained by each axis is displayed. Location abbreviations are as described in Table 16.

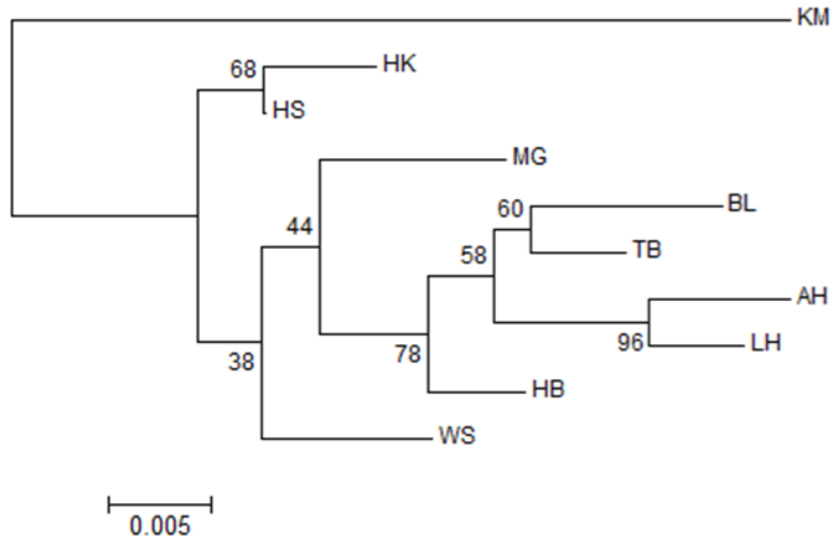

Figure 25: Neighbour Joining dendrogram based on F_{ST} values for *Rhombosolea leporina*. Location abbreviations are as described in Table 16.

Table 19: AMOVA results for *Rhombosolea leporina* using F_{ST} with North versus South and East versus West groups.

North versus South

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Variance	Estimated Variance	% Variation	Differentiation Indexes	*p*-value
Between Regions North (MG, HK, HB, TB) and South (KM, WS, BL, AH, LH, HS)	1	14.2	14.242	0.011	0.266%	F_{RT} = 0.003	**0.010**
Amongst Populations	8	74.3	9.293	0.060	1.461%	F_{SR} = 0.015	**0.010**
Amongst Individuals	393	1795.5	4.569	0.524	12.744%	F_{ST} = 0.017	**0.010**
Within Populations	403	1418.5	3.520	3.520	85.529%	F_{IS} = 0.130	**0.010**
Total	805	3302.6		4.115	100.000%	F_{IT} = 0.145	**0.010**

East versus West

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Variance	Estimated Variance	% Variation	Differentiation Indexes	*p*-value
Between Regions West (HK, TB, KM, WS, HS) and East (MG, HB, BL, AH, LH)	1	13.1	13.136	0.009	0.209%	F_{RT} = 0.002	**0.010**
Amongst Populations	8	75.4	9.431	0.062	1.497%	F_{SR} = 0.015	**0.010**
Amongst Individuals	393	1795.5	4.569	0.524	12.747%	F_{ST} = 0.017	**0.010**
Within Populations	403	1418.5	3.520	3.520	85.548%	F_{IS} = 0.130	**0.010**
Total	805	3302.6		4.114	100.000%	F_{IT} = 0.145	**0.010**

Cluster analysis in STRUCTURE showed that the most likely value of *K* was four clusters (Figure 26). Cluster one (red) was most prevalent amongst eastern and central locations, whereas cluster two (green) was most prevalent amongst west coast locations. Cluster three (dark blue) was largely observed at TB and locations on the eastern part of the South Island (AH, LH). AWclust analysis revealed three clusters but there was no obvious geographical distribution of these clusters (Figure 27). This was supported by a Spearman rank correlation analysis, which did not show any significant correlations between cluster proportion and latitude or longitude for both STRUCTURE and AWclust analyses.

Assignment analysis, implemented in GENECLASS2 (Table 20), had a mean of 66.81% correct assignment to the original locations; the highest percentage of correct assignment was at KM (81.82%) and the lowest was at HS (38.00%). The majority of individuals assigned to a population other than where they were collected were from HK and WS (6.19%). KM was the most likely to receive first generation migrants with 36.36% of the total sample size possibly being immigrants, and was also the most likely to donate first generation migrants (18.18%). HK and WS were composed of about 15% immigrants, and HK was the second most likely to provide emigrants at 15.38% of the total sample size for that location.

In summary, population genetic analyses provided evidence for three groups for *R. leporina* (Figure 28). There was genetic connectivity amongst all locations sampled on the western side of New Zealand, suggesting that MG, HK, KM, WS and HS form one population. Connectivity was also high along the east coast but there appeared to be two population groups; Avon Heathcote and Lyttelton Harbour grouped together across several analyses and were differentiated from the other three eastern locations (HB, TB, BL). Assignment tests showed moderate levels of self-recruitment within each location and moderate levels of gene flow between all locations, but assignments between locations appeared to be randomly distributed with no evidence of source or sink populations. There was no evidence of an isolation by distance signal providing evidence for hierarchical structure and barriers to connectivity amongst these groups.

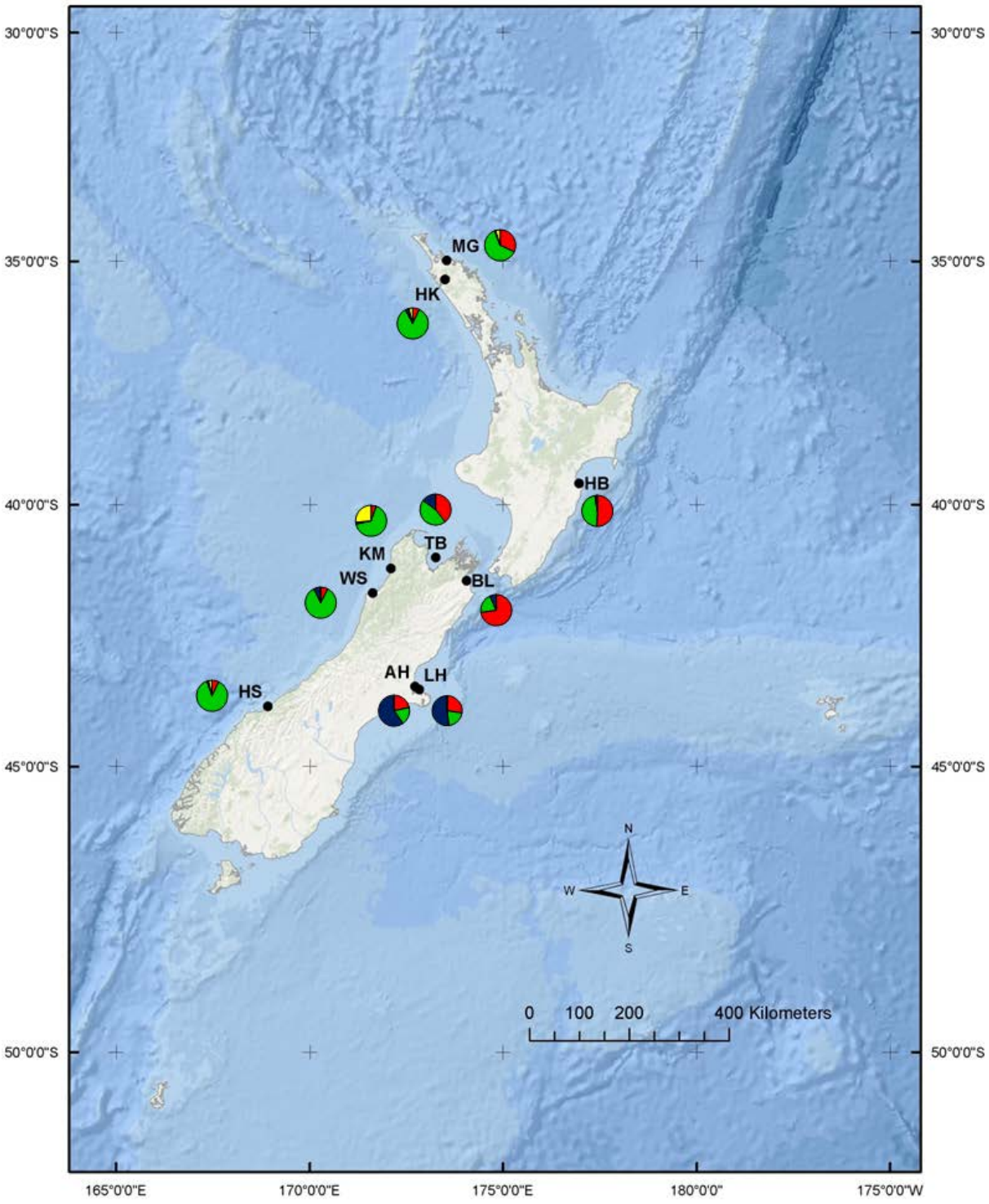

Figure 26: Output from STRUCTURE analyses for *Rhombosolea leporina* showing the proportion of each cluster assigned to each location (*K* = 4). Each colour denotes a different genetic cluster. Spearman rank correlation analysis did not show any significant correlations between cluster proportion and latitude or longitude.

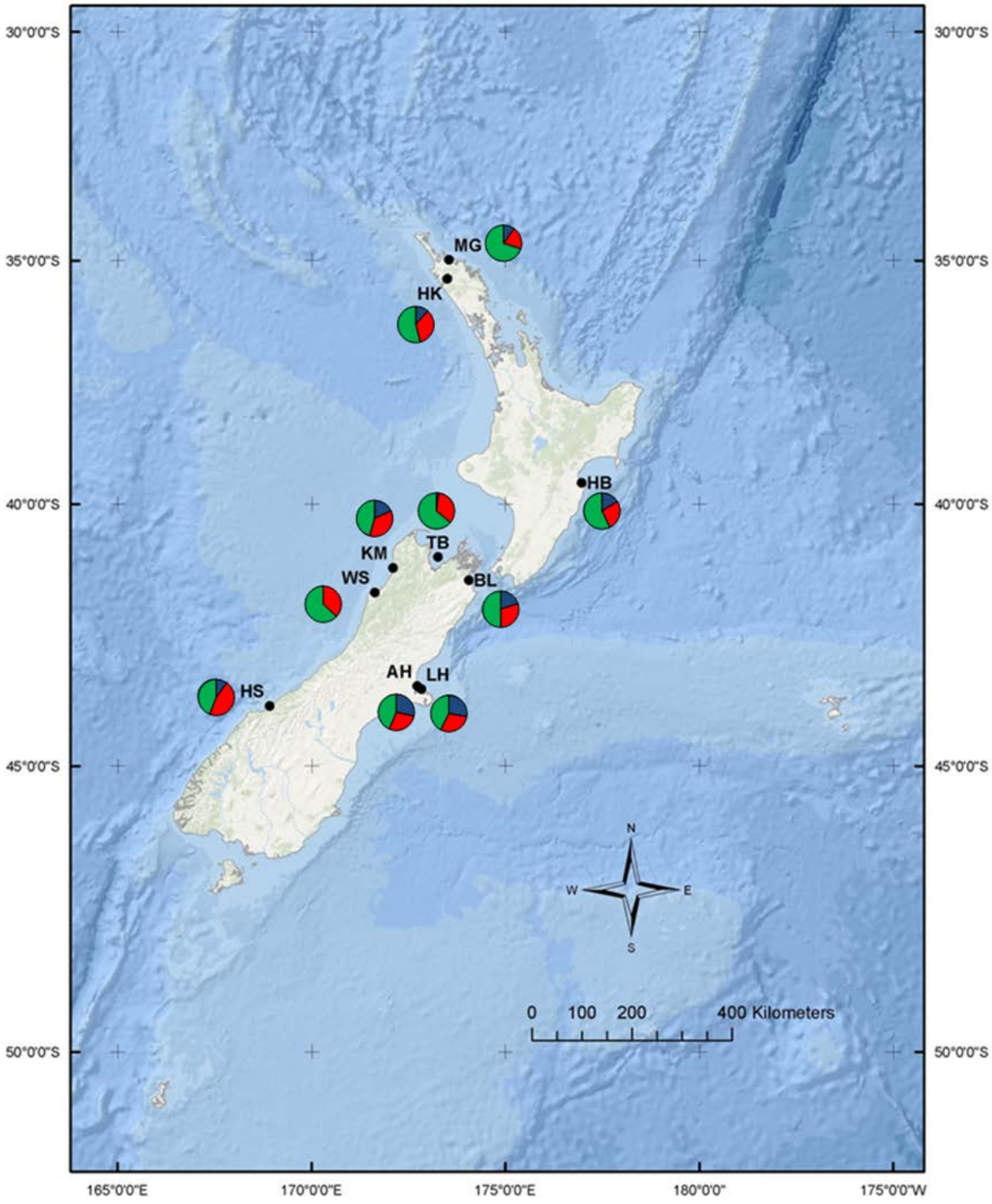

Figure 27: Output from AWclust analyses for *Rhombosolea leporina* showing the proportion of each cluster assigned to each location (*K* = 3). Each colour denotes a different genetic cluster. Spearman rank correlation analysis did not show any significant correlations between cluster proportion and latitude or longitude.

Table 20: GENECLASS2 assignment results for *Rhombosolea leporina* showing exchange of first generation migrants amongst localities. Location abbreviations are as described in Table 16.

Receiving location	Source of F0 migrants of *R. leporina*										Total F0 Received
	MG	HK	HB	TB	BL	KM	WS	AH	LH	HS	
MG			1	2	4	1	4	1			13
HK	1			1		1	3			4	10
HB	3	1		5			4				13
TB	2	1	4		4		2	2	3	2	20
BL	2	2	1	4			1	1	4		15
KM		1					1				2
WS		3	1						1	1	6
AH	1	3	3		2		2		3	2	16
LH	2	1		4			1	3		2	13
HS	1	13	2	5		2	7				30
Total F0 supplied	12	25	12	21	10	4	25	7	11	11	

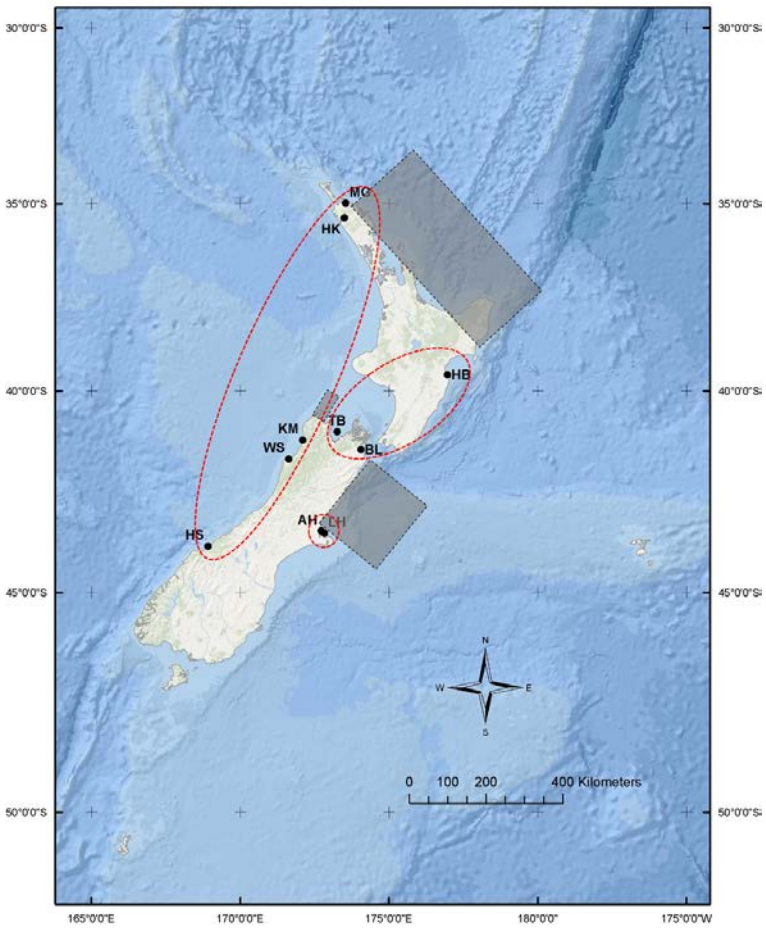

Figure 28: Location of genetically differentiated populations of *Rhombosolea leporina* and barriers to genetic connectivity. Populations are those sampling locations enclosed by red dashed lines. The geographic areas where barriers to genetic connectivity are assumed to occur are indicated by shaded grey boxes (these boxes cover large sections of coastline because it was not possible to pinpoint the exact location of barriers; it is assumed the barrier lies somewhere within the shaded area).

4.5 *Pecten novaezelandiae*

A total of 952 *Pecten novaezelandiae* adults from 15 locations were genotyped at 12 loci (Table 21). MICROCHECKER identified putative null alleles at *Pnova_02*, *Pnova_05*, *Pnova_10*, *Pnova_24*, *Pnova_27* and *Pnova_33* due to homozygote excess at each locus but no pattern of null allele frequency as a function of either sampling location or locus was identified. No large allele dropout was detected. Loci *Pnova_24* and *Pnova_33* were identified as being significantly out of HWE at more than half of the sampled locations (data not shown). No evidence of significant linkage disequilibrium was detected between loci. *Pnova_24* and *Pnova_31* were identified by LOSITAN as F_{ST} outliers and candidates for positive selection under the infinite allele and stepwise mutation models. Due to this combination of factors these two loci (*Pnova_24* and *Pnova_31*) were excluded from the following analyses.

Locality-specific genetic variation for *P. novaezelandiae* over 10 loci is summarised in Table 22. Mean number of alleles per locus ranged from 5.6 in STE12 and 12.9 in RAN and LBI. Allelic richness ranged from 4.91 in FIO to 5.89 in RAN. In total, there were 27 private alleles across all 15 locations; RAN had the highest number (8). Overall, there were lower levels of observed heterozygosity (ranging from 0.564 in HAU to 0.695 in STE12), than expected heterozygosity (ranging from 0.635 in FIO to 0.680 in STE14). F_{IS} ranged from -0.090 at STE12 to 0.146 at HAU.

Table 21: Location, number of samples and geographical co-ordinates for *Pecten novaezelandiae* samples used in this study.

Location	Abbreviation	Sample Size	Latitude	Longitude
Rangaunu Bay	RAN	99	-34.67° S	173.30° E
Little Barrier Is	LBI	99	-36.23° S	175.07° E
Jones Bay	JON	40	-36.38° S	174.82° E
Hauraki Gulf	HAU	99	-36.57° S	175.23° E
Mercury Is	MER	71	-36.68° S	175.73° E
Tauranga	TAU	99	-37.67° S	176.40° E
Wellington Harbour 2012	WEL12	74	-41.30° S	174.80° E
Wellington Harbour 2013	WEL13	48	-41.30° S	174.80° E
Marlborough Sounds	MAR	50	-41.19° S	174.11° E
Tasman Bay	TAS	48	-41.07° S	173.09° E
Golden Bay	GOL	48	-40.60° S	172.77° E
Fiordland	FIO	36	-45.58° S	166.73° E
Stewart Is, Port Pegasus 2013	STE12	13	-47.15° S	167.70° E
Stewart Is, Paterson Inlet 2014	STE14	48	-46.93° S	168.07° E
Chatham Is	CHA	99	-43.70° S	-176.38° W

Table 22: Genetic diversity statistics (based on 10 loci) for each location and total for *Pecten novaezelandiae*. (N_a: mean number of alleles, R_a: allelic richness, PA: private alleles, H_O: observed heterozygosity, H_E: expected heterozygosity, F_{IS}: inbreeding co-efficient). F_{IS} values in bold show significant departure from HWE expectations after false discovery rate correction ($p < 0.05$). Location abbreviations are as described in Table 21.

Location	Region						Statistic
		N_a	R_a	PA	H_O	H_E	F_{IS}
RAN	North	12.9	5.89	8	0.616	0.676	**0.078**
LBI	North	12.9	5.83	2	0.605	0.665	**0.088**
JON	North	10.4	5.76	1	0.596	0.668	**0.115**
HAU	North	12.8	5.85	2	0.564	0.671	**0.146**
MER	North	12.2	5.87	5	0.662	0.670	0.006
TAU	North	12.8	5.70	1	0.596	0.658	**0.083**
WEL12	Central	11.6	5.73	1	0.639	0.648	-0.001
WEL13	Central	11.4	5.75	1	0.608	0.651	**0.058**
MAR	Central	10.8	5.69	0	0.609	0.661	**0.095**
TAS	Central	10.1	5.45	1	0.575	0.642	**0.088**
GOL	Central	10.8	5.66	0	0.583	0.641	**0.062**
FIO	South	7.5	4.91	0	0.593	0.635	0.059
STE12	South	5.6	5.28	0	0.695	0.650	-0.090
STE14	South	9.5	5.41	4	0.689	0.680	-0.013
CHA	Chatham	11.3	5.50	1	0.611	0.663	**0.074**
Total		10.84	5.62	27	0.616	0.659	**0.056**

Pairwise F_{ST} analyses amongst all locations ranged from 0.000 to 0.027 (Table 23). Of the 105 pairwise comparisons between locations, 44 were significant after FDR corrections. FIO, STE14 and CHA had the highest values of F_{ST} and the highest number of statistically significant pairwise comparisons. There were six significant comparisons between locations in northern and central New Zealand (WEL12–MER, WEL13–TAU, MAR–MER, MAR–TAU, TAS–HAU, TAS–MER), but there were no significant comparisons between locations within the northern and central regions. HAU had the lowest mean F_{ST} per location (0.004) and FIO had the highest (0.015).

As temporal differences did not explain any of the variation in the datasets of WEL12 and WEL13 (AMOVA, $p = 0.05$); the following analyses were conducted using WEL as one location. Mantel tests revealed a weak but significant IBD signal when using all the mainland locations ($R^2 = 0.315$, $p < 0.010$; Figure 29). A PCA showed that Axis 1 explained 39.15% of the variation and Axis 2 explained 24.56% of the variation (Figure 30). Locations sampled from the top of the North Island formed one group (RAN, LBI, JON, HAU, MER, TAU) and the locations sampled in central New Zealand formed another group (WEL, MAR, TAS, GOL) on the left side of axis 1. Fiordland (FIO), Stewart (STE12, STE14) and Chatham Islands (CHA) were plotted on the right side of axis 1.

A NJ dendrogram (Figure 31) grouped locations STE12 and STE14 together with 70% support. FIO grouped separately and CHA grouped together with the north and central group but with low support (50%). All the locations from the north and central groups (RAN, LBI, JON, HAU, MER, TAU, WEL, MAR, TAS, GOL) were grouped together with 30% support. GOL and TAS were grouped together with 45% support and there was high support (70%) for the grouping of MER and TAU. AMOVA analysis of five groups (North, Central, Fiordland, Stewart Island and Chatham Island) showed 86.2% of the variation was explained amongst individuals within the total samples, 12.8% explained amongst individuals within populations, 0.2% explained amongst populations within groups and 0.8% explained amongst groups; the model was significant ($p = 0.001$; Table 24).

Table 23: Pairwise F_{ST} values amongst *Pecten novaezelandiae* locations using 10 loci. Values in bold represent significant differentiation after false discovery rate correction ($p < 0.05$) as assessed by exact G test (Goudet et al. 1996). Location abbreviations are as described in Table 21.

	RAN	LBI	JON	HAU	MER	TAU	WEL 12	WEL 13	MAR	TAS	GOL	FIO	STE 12	STE14
LBI	0.000													
JON	0.000	0.0010												
HAU	0.000	0.001	0.001											
MER	0.002	0.003	0.002	0.000										
TAU	0.002	0.001	0.002	0.002	0.000									
WEL12	0.002	0.001	0.002	0.002	**0.007**	0.006								
WEL13	0.001	0.004	0.000	0.001	0.006	**0.008**	0.000							
MAR	0.001	0.004	0.002	0.002	**0.008**	**0.009**	0.002	0.001						
TAS	0.001	0.000	0.000	**0.003**	**0.006**	0.003	0.001	0.000	0.001					
GOL	0.003	0.000	0.005	0.001	0.007	0.006	0.000	0.002	0.003	0.000				
FIO	**0.017**	**0.018**	**0.018**	**0.014**	**0.016**	**0.017**	**0.012**	**0.019**	**0.012**	**0.013**	**0.010**			
STE12	0.005	0.011	0.004	0.000	0.009	0.008	0.011	0.003	0.004	**0.008**	0.015	**0.015**		
STE14	**0.020**	**0.022**	**0.023**	**0.021**	**0.027**	**0.024**	**0.018**	**0.017**	**0.019**	**0.018**	**0.022**	**0.016**	0.000	
CHA	**0.005**	**0.005**	**0.007**	**0.005**	**0.011**	**0.008**	**0.005**	**0.009**	**0.008**	**0.008**	**0.008**	**0.018**	0.002	**0.018**

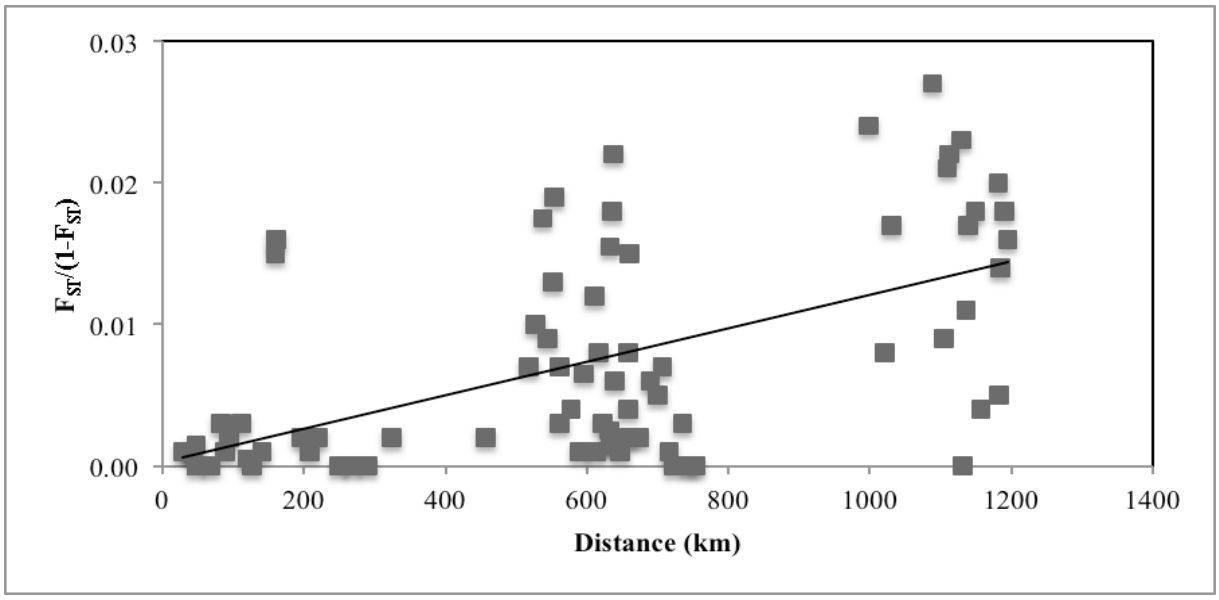

Figure 29: Mantel test for isolation by distance for all mainland *Pecten novaezelandiae* locations (R^2 = 0.324, $p < 0.01$).

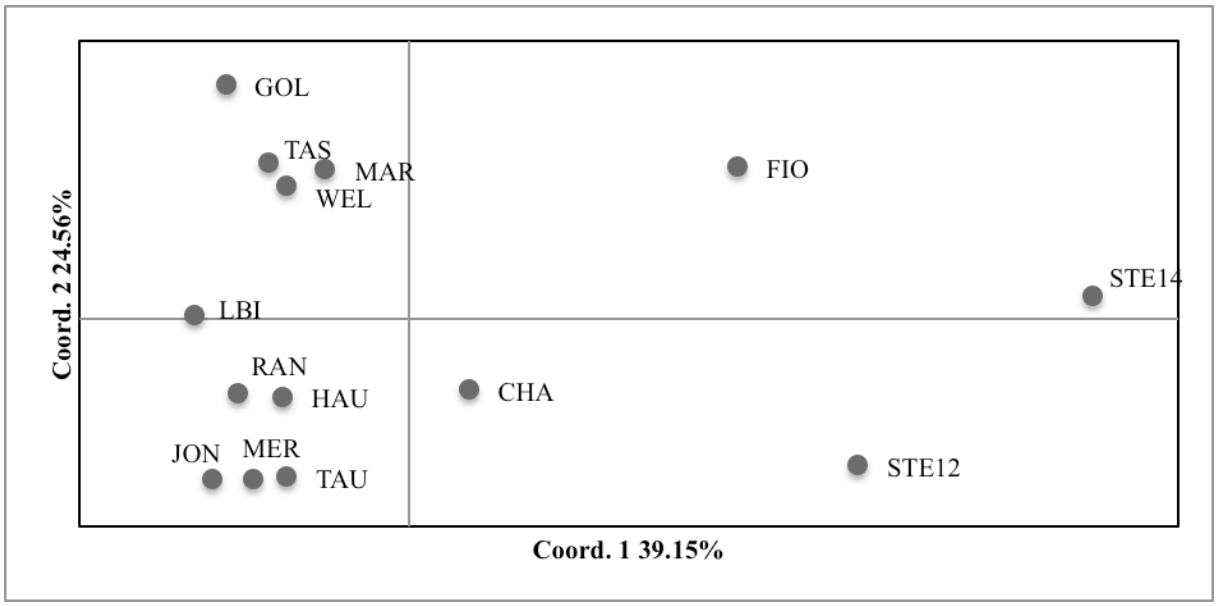

Figure 30: Principal component analysis for *Pecten novaezelandiae* showing patterns of genetic population differentiation. The percentage of inertia explained by each axis is displayed. Location abbreviations are as described in Table 21.

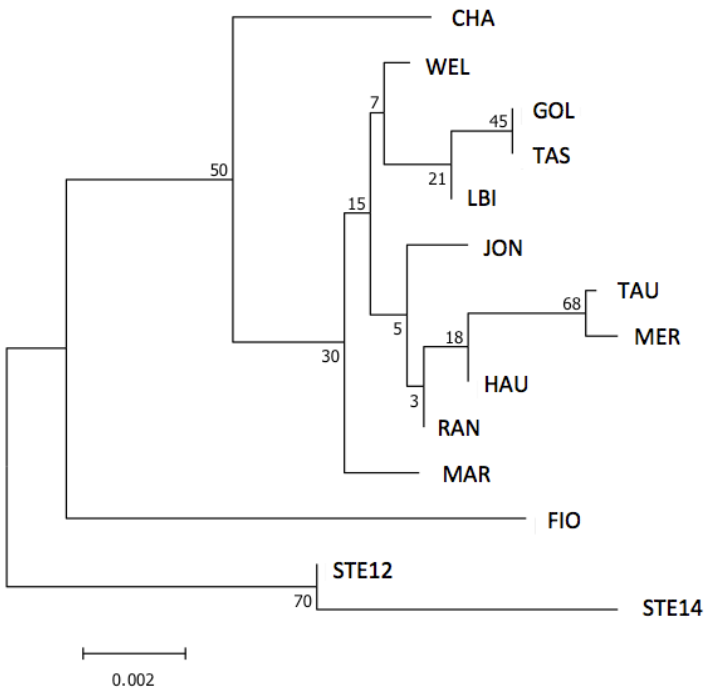

Figure 31: Neighbour Joining dendrogram based on F_{ST} values for *Pecten novaezelandiae*. Location abbreviations are as described in Table 21.

Table 24: AMOVA results for *Pecten novaezelandiae* with sampling locations arranged into five groups (North, Central, Fiordland, Stewart Island and Chatham Is) as determined by PCA and NJ analyses.

Source of variation	Degrees of Freedom	Sum of Squares	Mean Variance	Estimated Variance	% Variation	Differentiation Indexes	*p*-value
Amongst groups	4	53.012	13.253	0.028	0.8%	F_{RT} = 0.008	**0.001**
Amongst populations within groups	10	47.825	4.782	0.008	0.2%	F_{SR} = 0.002	**0.001**
Amongst individuals within populations	937	3586.13	3.827	0.437	12.8%	F_{ST} = 0.010	**0.001**
Amongst individuals within the total samples	952	2811.00	2.953	2.953	86.2%	F_{IS} = 0.129	**0.001**
Total	1903	6497.97		3.425	100.0%	F_{IT} = 0.138	**0.001**

STRUCTURE analysis showed that the most likely vale of *K* was two clusters (Figure 32) and there was a north-south cline in the proportions of clusters. This cline was highly supported by a Spearman rank correlation test, which showed a significant decline in the proportion of cluster one (dark blue) with increasing latitude (Spearman's r = -0.890, $p < 0.001$) and an increase of cluster two (red) with increasing latitude (Spearman's r = 0.890, $p < 0.001$). The non-parametric analysis AWclust was unable to detect any meaningful population structure, showing a large gap value at $K = 1$ and an increase towards $K = 14$ (data not shown).

Assignment analysis, implemented in GENECLASS2, detected a low number (9) of first generation (F0) migrants for all four regions (Table 25). Locations in the northern region were not likely to receive F0 migrants whilst CHA was likely to receive the most F0 migrants (4). The low number of all F0 migrants was probably an underestimation due to the low levels of differentiation that result when migrant individuals have similar genotypes to the individuals from the location of origin. The region with the highest level of correct assignment was the north (85%), whilst the region with the lowest level was CHA (17.3%). There was a southwards increase in the percentage of individuals recruited from regions other than the collection site and CHA was the region with the highest percentage (82.7%; Table 26).

In summary, *P. novaezelandiae* can be divided into five genetically differentiated regions (North, Central, Fiordland, Stewart Island and Chatham Is; Figure 33). Within regions, locations appeared to be well connected by gene flow with little evidence of genetic differentiation, but some level of population structure was found amongst regions. A significant IBD signal and a degree of differentiation from north to south were apparent, but this finding may represent hierarchical structure rather than true IBD. Locations sampled in the southern margin of this species' distribution in Fiordland and Stewart Island were the most differentiated. Assignment tests revealed high levels of self-recruitment within the northern region and suggest that this region might be playing an important role as a source of larvae.

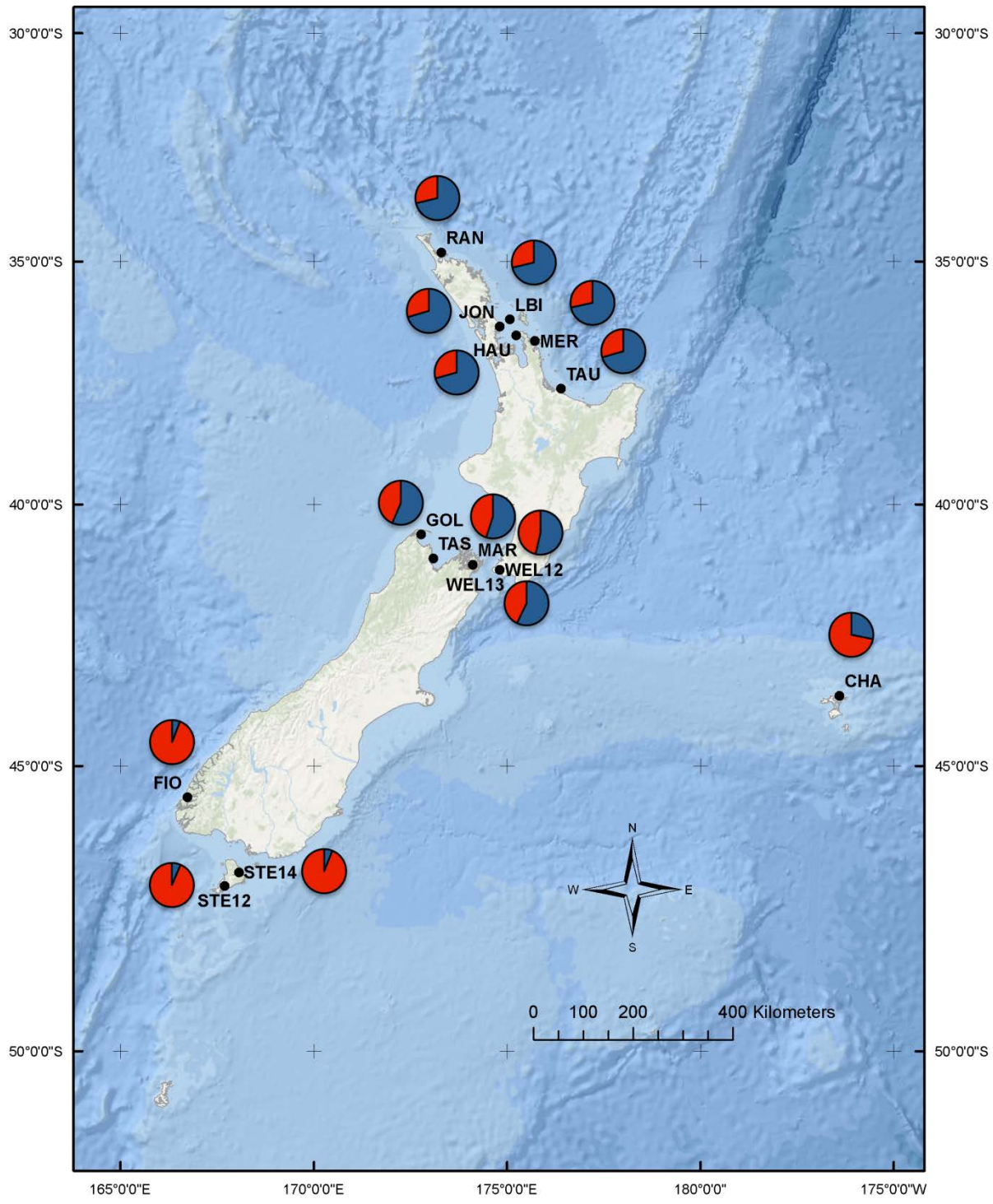

Figure 32: Output from STRUCTURE analyses for *Pecten novaezelandiae* showing the proportion of each cluster assigned to each location (K = 2). Each colour denotes a different cluster. A Spearman rank correlation test showed a significant relationship between cluster proportion and latitude.

Table 25: GENECLASS2 results for *Pecten novaezelandiae* showing the number of first generation (F0) migrants detected for each region.

Receiving location	Source of F0 migrants				Total F0 migrants
	North	Central	South	CHA	
North					
Central	2			1	3
South	1			1	2
CHA	2	2			4

Table 26: GENECLASS2 assignment test results showing the percent of *Pecten novaezelandiae* individuals collected from each sampled region, assigned to each sampled potential source region. "Total other regions" is the percent of individuals recruited from regions other than the collection site. Italicised values represent percent self-recruitment.

Collection region	Assigned region					Total other regions
	North	Central	South	CHA	Unassigned	
North	*85.0*	13.8	0.8	0.2	0.2	14.8
Central	51.3	*46.7*	1.2	0.8		53.3
South	43.0	20.4	*36.6*			63.4
CHA	58.2	20.4	4.1	*17.3*		82.7

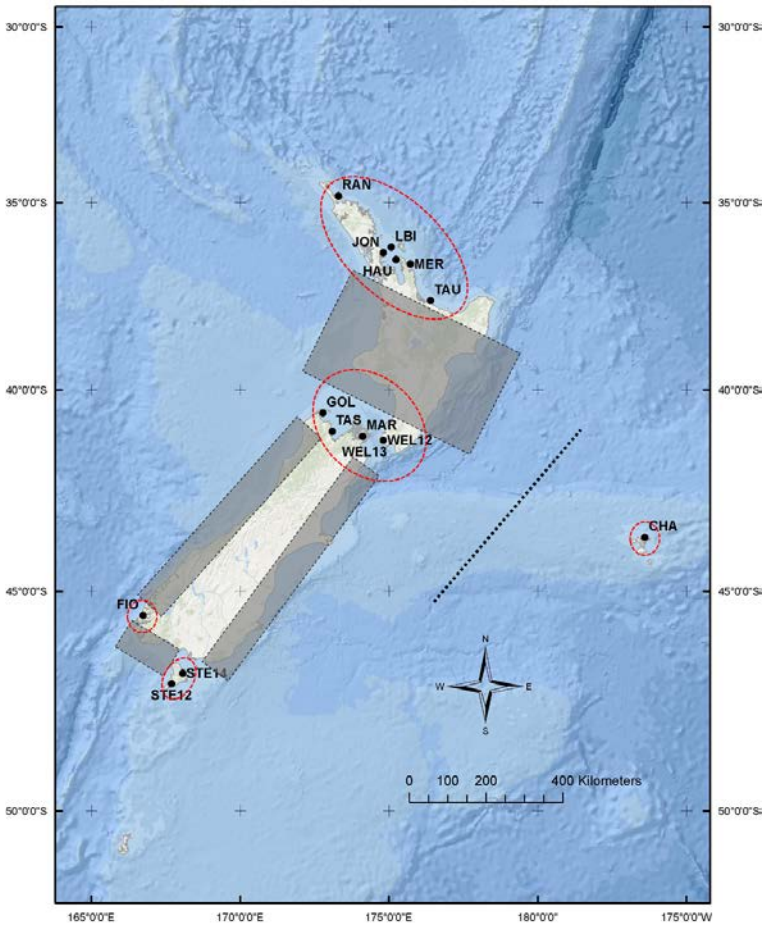

Figure 33: Location of genetically differentiated populations of *Pecten novaezelandiae* and barriers to genetic connectivity. Populations are those sampling locations enclosed by red dashed lines. The geographic areas where barriers to genetic connectivity are assumed to occur are indicated by shaded grey boxes (these boxes cover large sections of coastline because it was not possible to pinpoint the exact location of barriers; it is assumed the barrier lies somewhere within the shaded area) and by the thicker dashed line between mainland New Zealand and Chatham Island.

5. DISCUSSION

In this study population genetic techniques were applied to five New Zealand coastal marine species from soft sediment habitats for the purposes of determining patterns of genetic population structure and genetic connectivity, and to compare these patterns across species to identify common patterns and barriers to connectivity. This study addresses a gap in our knowledge of connectivity patterns amongst shallow water, soft sediment coastal and estuarine species.

5.1 Major findings

5.1.1 Paphies subtriangulata

Fitting the pattern of genetic population structure observed for *P. subtriangulata* into the categories described for other New Zealand coastal marine species (no genetic structure amongst populations, isolation by distance, divergence within and/or amongst populations, west-east divergence and north-south divergence; Gardner et al. 2010) was not straight forward because different types of structure were observed in different parts of the sampling area. There was strong genetic differentiation between the Chatham Island (CHA) population and the other mainland New Zealand populations, suggesting that distance between mainland New Zealand and the Chatham Islands is a barrier to dispersal in this species. This is supported by estimates of recent migration, which showed high self-recruitment for the Chatham Island population and no detectable connectivity with mainland populations. Long-term migration rates provide support for past gene flow, although these are likely to be from rare events as migration rates to and from Chatham Island were low compared to migration rates amongst mainland populations.

Smith et al. (1989) also showed that Chatham Island *P. subtriangulata* were differentiated from mainland populations based on allozyme markers and morphological analyses, providing support for the suggestion that this population may represent a separate species of tuatua (Beu & Rooij-Schuiling 1982), although species status has never been formally proposed or verified. The high estimates of self-recruitment and rare gene flow events to Chatham Island are conditions under which speciation is likely to occur. Genetic differentiation of Chatham Island populations has been reported for other coastal marine species (e.g., Goldstien et al. 2009; Hickey et al. 2009; Will et al. 2011) but for others there appears to be little to no restriction on gene flow to mainland New Zealand (e.g., Ross et al. 2009; Buchanan & Zuccarello 2012 and see Section 5.1.5 on *Pecten novaezelandiae*). These species-specific differences could be related to the specific reproductive and life history characteristics of each species.

Amongst mainland locations it was difficult to reject the null hypothesis of panmixia as many of the sampled locations formed a genetically undifferentiated group (PAP, MAR, PKR, COL, OAK and WPK) suggesting few restrictions to connectivity amongst these locations over both short and long timescales. This pattern is what might be expected for a broadcast spawning coastal invertebrate, where there are likely to be few restrictions to larvae entering coastal currents that can potentially disperse them over long distances. However, in other parts of the sampled range there was modest support for divergence amongst populations (RUA, WMR and KAK), suggesting that barriers to dispersal exist in northern and eastern parts of the North Island of New Zealand. Cases of genetic population differentiation are not unexpected for coastal marine species along the mainland of New Zealand, and the areas of restricted dispersal identified for *P. subtriangulata* in this study have also been reported for other species (Ross et al. 2011; Will et al. 2011; Buchanan & Zuccarello 2012). There was no support for the ‘northern’ and ‘central’ groups reported by Smith et al. (1989); the genetically undifferentiated group observed in the present study included locations in both northern and central New Zealand. However, differences in population structure inferred by different markers are common (e.g., Apte & Gardner 2001, 2002; Star et al. 2003; Ross et al. 2011).

There are several mechanisms that could be responsible for the genetic differentiation of these three populations. Ruakaka (RUA) is the most highly differentiated population with high self-recruitment, suggesting that eddies in this area (i.e., the North Cape Eddy; Stanton et al. 1997) may be entraining larvae and thereby promoting self-recruitment and preventing larval exchange with other populations. However, long term migration estimates show Ruakaka to be a significant source of migrants, suggesting variability in rates of gene flow between Ruakaka and other parts of mainland New Zealand over time. Similarly, genetic differentiation of the Waimarama (WMR) population could also be interpreted as evidence for variable migration and self-recruitment rates over time. This population is located in the vicinity of the Wairarapa Eddy (Chiswell & Roemmich 1998; Chiswell 2003) and depending on oceanographic conditions, could allow for local larval retention in some years with sporadic connectivity events to other populations in some years. Differentiation of the Kakamatua (KAK) population could be due to its location inside the Manukau Harbour, where it is possible that tidal circulation is retaining larvae, limiting connectivity and promoting genetic differentiation (Bilton 2002). Overall, the lower degree of support for genetic differentiation amongst mainland locations suggests that any barriers to connectivity are transitory or highly permeable.

5.1.2 Paphies australis

Overall, genetic population structure in *P. australis* was weak and not all analyses were able to detect genetic population structure. However, some analyses provided evidence for reduced larval exchange between *P. australis* populations, which have led to genetic differentiation over both small (hundreds of kilometres) and regional (thousands of kilometres) scales. There was evidence for three genetically distinct populations: northern, south-eastern and south-western. The north-south pattern of genetic differentiation that is commonly reported amongst New Zealand coastal marine species (Gardner et al. 2010) was detected in this species, and there was further evidence for division of southern locations into eastern and western groupings, which has also been reported for the greenshell mussel (Apte & Gardner 2002). Barriers resulting in reduced dispersal of larvae are likely to be present around the Cook Strait area and on the west coast of the South Island, located approximately in the Fiordland region. Furthermore, some locations within the northern population showed evidence of genetic differentiation, often at small spatial scales of 100–200 km. In comparison, genetic divergence amongst southern locations was lower, which implies that connectivity is more restricted amongst northern locations and higher amongst southern locations.

These results are consistent with the habitat preference of *P. australis*. The estuarine habitat of this species may be driving genetic differentiation by promoting self-recruitment and creating barriers to dispersal amongst populations. However, more consistent estimates of migration over long time scales suggest that there is variation in the effectiveness of these barriers: they may be overcome sometimes leading to gene flow amongst populations and the overall weak population structure that was observed. In particular, the small scale and geographically unstructured patterns of genetic differentiation seen amongst northern locations is suggestive of 'chaotic genetic patchiness', which can arise when connectivity amongst populations is not consistent over time (Johnson & Black 1982; Hellberg et al. 2002; Selkoe et al. 2006) and is often a feature of broadcast spawning invertebrates (Varela et al. 2009; Hedgecock & Pudovkin 2011). One interpretation is that connectivity appears more consistent when averaged over longer time frames and at larger spatial scales (hundreds to thousands of kilometres), but at small spatial scales (tens to hundreds of kilometres) short term fluctuations in larval supply can result in a very different pattern of genetic structuring – it is chaotic. These contrasting spatial patterns show how both historical and contemporary events can be evident in the genetic population structure of a species.

Current direction and velocity are likely to explain much of the genetic population structure and migration patterns that were observed for *P. australis*. The predominant direction of current flow around New Zealand is west to east. The southward flowing West Auckland Current may transport

larvae from northern locations to the west coast of the South Island and to the east coast via the D'Urville Current through Cook Strait, explaining the asymmetrical migration rates from north to south. The genetic demarcation observed around the Cook Strait region may be influenced by current patterns in this area, which may restrict connectivity amongst populations of *P. australis* and many other marine species (e.g. Apte & Gardner 2002; Star et al. 2003; Waters & Roy 2004; Ayers & Waters 2005; Goldstien et al. 2006; Ross et al. 2011). These patterns are described in more detail in Section 5.2.1.

South of Fiordland (in a similar location to where a genetic demarcation was observed in *P. australis*) the predominant current direction changes to a southerly flow, joining the Southland Current which flows along the bottom of the South Island then northwards up the east coast (Laing & Chiswell 2003). This current is one of the least variable and most predictable of the coastal currents (Chiswell & Rickard 2011). In comparison, the east coast of the North Island is characterised by a more complex situation: the East Auckland Current and East Cape Current create a series of complex coastal eddies, which act to retain larvae and limit dispersal (Stanton et al. 1997; Chiswell & Roemmich 1998). These differences in current patterns could explain why some northern locations were characterised by small-scale genetic differentiation and high self-recruitment, whereas self-recruitment and genetic differentiation amongst southern locations was low.

Other factors that may contribute to genetic population structure and connectivity of *P. australis* include glaciation and human mediated dispersal. Long-term genetic signals from range restrictions and population fragmentation associated with the last glacial maximum (LGM) are still thought to influence genetic structure of New Zealand coastal marine organisms (Wallis & Trewick 2009). Genetic demarcations have been described for several marine and terrestrial species around the Fiordland region, similar to that observed in *P. australis*, which have been attributed to past glaciations (e.g. Hickey et al. 2009; Trewick & Wallis 2001). More recently, Wei et al. (2013) have suggested that the pronounced north-south split seen in many, but not all, coastal taxa in New Zealand dates to approximately 1.0 to 1.3 M years before the present, at a time of major global sea level change. This form of disturbance and its associated loss and gain of suitable coastal habitat, with subsequent effects on genetic connectivity, is thought to have given rise to the two lineage pattern of structure observed for many taxa.

Patterns of genetic population structure are often explained in light of natural dispersive processes and human-mediated dispersal is often overlooked, but it can result in patterns of genetic structure that are independent of natural routes and barriers to dispersal (Carlton 2003). Species living in harbours and near ports are known to be affected by human-mediated dispersal (Marins et al. 2010; Torkkola et al. 2013) and it has been suggested as an explanation for unusual or inconsistent patterns of genetic population structure seen in other New Zealand coastal marine invertebrates (Apte & Gardner 2001; Ross et al. 2011). Whilst this study was unable to specifically test the hypothesis that genetic population structure in *P. australis* is influenced by human mediated dispersal, based on observations in the studies cited above, it is possible that human mediated dispersal could explain the instances of geographically unstructured genetic similarity that were observed amongst some populations in this study. For example, Raglan (RAG) and Napier (NAP) are genetically undifferentiated despite being significantly different from geographically proximate populations, suggesting recent connectivity between these two locations but not to locations in between.

Interestingly, the only other study of an estuarine bivalve in New Zealand (*Austrovenus stutchburyi*) reported similar genetic population structure to *P. australis*. Seven genetically differentiated groups were reported and there was evidence for some connectivity amongst these groups (Ross et al. 2011). Similar genetic patterns have also been observed in estuarine bivalves from outside the New Zealand region (e.g., Tarnowska et al. 2010; Xiao et al. 2010), suggesting that there are features of the estuarine habitat that globally may play a role in driving the type of genetic population structure that was observed in *P. australis* and other estuarine bivalves.

5.1.3 Rhombosolea plebeia

There appear to be two genetically distinct groups of *R. plebeia*: a northern group and a south-eastern group. Whilst some analyses suggested slight differentiation amongst northern groups, overall the results showed connectivity around the North Island, the top of the South Island and with western locations (DB, PA, HK, MK, AR, HB, FX, PH, LF, TB, MH, WS and KM). Southern-eastern locations (AH, LH, LE, OT, TE and AW) appeared to for a second group and these locations were differentiated from the northern and western localities.

In meristic studies of *R. plebeia*, five distinct groups based on significant differences in anal and dorsal ray counts were observed (Colman 1976, 1985). Coleman observed that the south-eastern group on the South Island was clearly differentiated from the west coast populations on the South Island and central populations. The genetic data also support a south-east group. Colman concluded from his earlier tagging studies (Colman 1974, 1978), that some populations may be their own distinct groups, such as Tasman Bay, Hawke Bay and the Hauraki Gulf (Colman 1985). In contrast, the genetic data show connectivity amongst these locations and around the North Island.

Rhombosolea plebeia shows significant differentiation between a northern and southern group of localities, but these data were not clear on whether the north - south break proposed by Shears et al. (2008) across the Cook Strait, or the differentiation found in the review by Gardner et al. (2010) at about 42°S, exist in this species. Samples from the Karamea River estuary mouth (KM) and the Orowaiti Lagoon, Westport (WS), were significantly differentiated from a few populations on the North Island, but are not significantly different from Tasman Bay (TB) or Mapua Harbour (MH). Furthermore, PCA and STRUCTURE analysis showed Karamea River and Orowaiti Lagoon samples grouping with northern populations, suggesting they are part of a northern group. However, we were unable to collect *R. plebeia* samples south of Orowaiti Lagoon, so it is unknown whether a genetic break occurs at this location, or whether there is connectivity with locations further south on the west coast, as with *R. leporina*.

On the east coast a break between Lake Ferry (LF) and Avon Heathcote Estuary (AH)/Lyttelton Harbour (LH) is consistent with the break proposed by Shears et al. (2008) and Gardner et al. (2010) but the absence of sampling locations along this section of coast means it is not possible to pinpoint the location of this break. A weak but significant IBD signal further complicates the situation; further sampling along this section of the east coast, and south of Orowaiti Lagoon would be required to determine if there is truly a barrier to dispersal, which may be causing the differentiation of northern and southern groups. Alternatively, further sampling in these areas could reveal a genetic cline and confirmation of IBD genetic population structure.

The genetic data show that the Lake Ellesmere (LE) population is strongly differentiated from most other localities, even neighbouring Avon Heathcote Estuary and Lyttelton Harbour. There appears to be some connectivity with Otago Harbour (OT) and Taieri Mouth (TE) to the south. Lake Ellesmere is a brackish, eutrophic lagoon, which is periodically opened manually to the sea to prevent the flooding of surrounding farmland. How this practice affects genetic connectivity of flatfish and other species within Lake Ellesmere is unknown.

Despite strong currents in the Cook Strait area (i.e., the D'Urville Current channelling through the Cook Strait) there appears to be high connectivity across this area. Adult movements, rather than currents, may explain connectivity between the North and South Islands on the west coast. There is a wide sandy, shallow (0–150 m) plateau along the west side of the Cook Strait. *Rhombosolea plebeia* is known to occur to 100 m depth and to travel up to 400 km, so even if larval fish were swept away by the D'Urville Current, adult fish could easily move between the islands, promoting gene flow on the west coast. On the east side of Cook Strait there is a steep increase in depth, as well as the Hikurangi Trough further south, which may limit adult movements.

5.1.4 *Rhombosolea leporina*

Rhombosolea leporina appears to consist of three genetically distinct groups: a west coast group that includes the top of the North Island (MG, HK, KM, WS and HS), a central group that includes Hawke Bay (TB, MH and HB), and a Banks Peninsula group (AH and LH). Whilst the STRUCTURE analysis showed four clusters, one of those clusters was mostly attributed to the small sample size (n = 11) of the Karamea River estuary mouth (KM) location. AMOVA analysis showed both significant west to east differentiation and north to south differentiation. The north to south signal may be driven by the strong differentiation of two southeast locations, Avon Heathcote Estuary (AH) and Lyttelton Harbour (LH), which appear to be a distinct group. The west to east differentiation seems to be driven by strong connectivity along the west coast. There was no evidence of IBD, most likely because of the structuring within subgroupings, rather than a genetic cline.

These data do not support a north-south genetic split just south of Cook Strait (Shears et al. 2008; Gardner et al. 2010). *Rhombosolea leporina* were collected as far south as Haast (HS), and the west coast populations seem to exhibit strong genetic connectivity along approximately 1200 km of coastline. The Tasman Bay (TB) population, which is in the centre of the sampled range, is differentiated from the west coast cluster. Tasman Bay shows connectivity with Blenheim (BL) and Hawke Bay (HB). Therefore, our analyses support these three central locations being a connected group of populations, separate from the west and from the southeast.

The major currents are not likely to explain differentiation between the east and west localities on the North Island. The prevailing south-easterly flowing Tasman Front splits over the top of the North Island into the West Auckland Current and the East Auckland Current, however there is some connectivity between populations across Cape Reinga. The Hawke Bay population is genetically connected with the Tasman Bay and Blenheim populations, which might be expected to be separated by the D'Urville Current. Smaller coastal currents may play a greater role in dispersal and retention for this species, or adult movements across suitable shallow habitat could mean that connectivity is not entirely dependent on currents acting at the larval stage.

The Banks Peninsula population was differentiated from all northern and western populations. There was gene flow between the Avon Heathcote Estuary and Lyttelton Harbour, which could be due to the geographic proximity of these locations and is supported by tagging experiments (Colman 1978). This pattern is also similar to that described for the triplefin fish *Grahamina capito*, which showed a distinct Banks Peninsula clade (Hickey et al. 2009). In this area, the Southland Current flows north along the Banks Peninsula, and breaks into eddies around the Avon Heathcote Estuary, which may retain larvae (Mundy 1968). Another possible explanation for the genetic differentiation between the Banks Peninsula populations and the rest of the mainland is the historical geography of the area. In the Pliocene (about 5 million years ago), sea levels were at their highest in New Zealand, leaving the Banks Peninsula as an isolated island (Wallis & Trewick 2009), possibly segregating these localities from others. The genetic signal of this separation may still exist in contemporary populations. The absence of *R. leporina* collected further south may support this explanation.

Philopatry and site fidelity may explain some of the genetic structure observed in *R. plebeia* and *R. leporina* as it may provide a mechanism to limit dispersal and promote genetic differentiation amongst populations. Many species of pleuronectids are known to have some site fidelity between breeding and foraging sites (Gibson 1997). Spawning behaviour of adults may play a role in the local retention of larvae by aggregating at the mouths of harbours and estuaries to promote the transport of eggs into the nursery grounds (Frisk et al. 2013). Juvenile *R. plebeia* have been seen recruiting into estuaries (Roper 1986), which may be facilitated by the juveniles changing their position in the water column, migrating up to catch an incoming tide into the estuary and then moving deeper to be retained (Epifanio & Garvine 2001). These patterns may be reinforced over generations as parents return to these same estuaries to spawn (Bailey 1997). Adult movements may play a larger than expected role in population structure (Frisk et al. 2013). For example, the winter flounder (*Pseudopleuronectes americanus*) has resident and migratory populations within the same bays (Crivello et al. 2004).

Quantifying movements between nursery grounds and adult habitats could clarify some of these relationships (Gillanders et al. 2003).

5.1.5 Pecten novaezelandiae

The low overall level of genetic differentiation detected in *P. novaezelandiae* is not unsurprising given the large reproductive potential, the pelagic larval duration (approximately three weeks) and the recent evolutionary history of this species. Despite this weak population genetic structure, significant differences between northern, central and southern regions were observed. *Pecten novaezelandiae* did not have a continuous distribution in some parts of New Zealand, for example around the southern margin of the 42°S parallel, so it was not relevant to test for specific genetic breaks around the Cook Strait region as the absence of populations is in itself likely to be contributing to any genetic differentiation between regions.

As a result, a hierarchical degree of differentiation from north to south was apparent. A weak but significant IBD signal was detected but further examination of Mantel test results suggests that it was influenced by hierarchical structure. The overall weak genetic population structure and hierarchical structure meant that clustering analyses were difficult to resolve, as they are known to perform poorly in the presence of hierarchical structure. AWclust revealed no evidence of structure (K = 1; i.e., panmixia) but STRUCTURE was able to detect a north-south cline in the proportions of two clusters. There was no evidence that the distance between mainland New Zealand and the Chatham Islands results in a strong barrier to gene flow in this species.

Generally, the patterns of genetic differentiation observed between regions were supported by the assignment analyses. There is evidence that the northern group persists mainly through self-recruitment but it is also a possible important source of larvae as all other regions had a high percentage of individuals recruited from the northern group. Migration between the mainland populations and the Chatham Islands was both recent and historically frequent. Over the long-term, exchange of migrants was mainly unidirectional with a high percentage of individuals recruited from populations on the mainland (82.7%), which may explain the low level of differentiation found at the Chatham Islands despite its geographic isolation and provides evidence of larval transport flowing eastward in the predominant current direction.

Several mechanisms could be responsible for the genetic differentiation observed in *P. novaezelandiae*. The population in Fiordland (FIO) showed the lowest levels of diversity and high differentiation from the remaining populations. As reviewed in Gardner et al. (2010), this pattern has been observed in other species (e.g. Mladenov et al. 1997) and it might reflect the unusual conditions of the fiord habitat, with semi-closed estuarine circulation. Also, the Stewart Island site (STE14) was the most differentiated population, which could be due to its location inside Paterson Inlet where tidal circulation may allow for local larval retention. In addition, Chiswell & Rickard (2011) estimated eddy diffusivity to be higher in the south than in the north of New Zealand. This is because as the Subtropical Front flows across the Tasman Sea and turns into the Westland Current, it becomes weak and highly variable. Therefore, eddies in these areas may be preventing larval exchange with other populations. In contrast, whilst the Chatham Islands are 660 km southeast of the North Island, *P. novaezelandiae* collected at this location had genetic similarities with mainland populations. This suggests that larvae of *P. novaezelandiae* have the potential to disperse over large distances and are strongly influenced by ocean currents. The subtropical convergence flowing eastward towards the Chatham Islands may be facilitating larval transport.

5.2 Common patterns

When devising management or conservation strategies for a marine region it is useful to identify any common patterns of genetic population structure and connectivity across multiple species, along with the common factors that drive these patterns. This information can be used to make predictions about patterns and drivers of genetic population structure for species that have not been studied directly. However, making generalisations must be done with caution as it is important to remember that species-specific patterns can arise.

It is now widely established that marine species can show significant genetic population structure despite the apparent connected nature of the marine realm. In fact very few studies of coastal marine species with a New Zealand-wide sampling range failed to report some sort of genetic population subdivision (Gardner et al. 2010), and the present study is no exception. Although all five study species displayed unique patterns of genetic population structure, a common observation was that this structure was weak, which is frequently seen in marine species (Hedgecock et al. 2007). This observation implies relatively high connectivity for these species but the lack of New Zealand-wide panmixia means that barriers to larval dispersal are present in the New Zealand marine environment. The result was that genetic disjunctions between populations were observed, ranging in scale from regional (thousands of kilometres) to small scale and geographically unstructured (tens to hundreds of kilometres). Where similar patterns of weak but significant genetic population structure have been reported in the literature, these are sometimes attributed to temporal variability in the effectiveness of the barrier to limit larval dispersal (Selkoe et al. 2006; Ovenden 2013). This can be interpreted to mean that most of the time the permeability of the barrier is low and larvae are restricted from crossing the barrier but under the right combination of physical and biological conditions larger numbers of larvae may be able to cross. When these barriers restrict connectivity they facilitate genetic differentiation amongst populations via genetic drift, mutation and selection, but on the occasions when the barriers are overcome the resulting gene flow events between populations can erode the genetic differentiation that has accumulated between connectivity events.

Whilst it was not possible to directly test for temporal variation in genetic connectivity amongst populations in the present study (this would require repeat sampling over several decades), the patterns of genetic population structure that were observed are consistent with what we would expect to see if levels of connectivity between populations varied over time. The long term versus short term estimates of migration for *P. australis* and *P. subtriangulata* provide further indirect evidence for temporal variation in connectivity: restricted connectivity was detected in the short term whereas more consistent migration was detected when averaged over longer periods of time. An increasing number of studies in the literature report similar patterns of weak but significant population structure, attributed to temporal variation in larval connectivity, leading to the term ‘crinkled connectivity’ being coined to describe this phenomenon (Ovenden 2013).

Most of the patterns of genetic population structure described for other New Zealand coastal marine species by Gardner et al. (2010) were observed in the present study and were often shared between two or more of the study species. These patterns, including the location of possible common barriers and the mechanisms behind them, are discussed below.

5.2.1 North-South differentiation

A variant of a north-south pattern of genetic differentiation was observed in all study species, except *P. subtriangulata*. This study provides further evidence that a barrier to dispersal may exist in the region of Cook Strait for some marine organisms (note that Cook Strait itself may not necessarily be the barrier). A division at approximately 39° S on the east coast of the North Island has been reported for some species, e.g., amphipods (Stevens & Hogg 2004), snapper (Bernal-Ramírez & Adcock 2003) and cockles (Ross et al. 2011). However, for other species a division at approximately 42° S (location

of Cape Campbell on the east coast of the South Island) has been reported, e.g., greenshell mussels (Apte & Gardner 2002; Star et al. 2003), cushion stars (Waters & Roy 2004; Ayers & Waters 2005), and limpets (Goldstien et al. 2006) The exact location of this barrier at fine spatial scales (e.g. tens of metres) is hard to identify, in large part because ecologically this area is species depauperate. However, the application of cline analysis (i.e., shape and rate of change of individual gene frequencies as a function of latitude) has revealed that the centre location of the genetic discontinuity (barrier to gene flow) for the greenshell mussel is geospatially consistent regardless of allele or locus and occurs at 41.7° S ± 0.6 (mean ± s.d.) (Gardner & Wei 2015). The general, but not universal, existence of this genetic discontinuity across multiple taxa in this region suggests that distinct stocks of many species exist on either side (north versus south) on both the east and west coasts of New Zealand. From a management perspective, this further suggests that treating these stocks as distinct (or at the very least recognising that there is limited gene flow between them) is an appropriate strategy.

In our study it was not possible to directly test for the geographic location of a genetic break in the vicinity of Cook Strait for *P. novaezelandiae* due to the absence of samples from this region. Despite this, we did identify evidence of a genetic transition zone across the centre of New Zealand (in the vicinity of 42° S) from northern to southern stocks. There was however, strong evidence for a division at 42° S on the east coast of New Zealand for *R. plebeia* and *R. leporina*, but little evidence for a division on the west coast. For *P. australis* the division is likely to occur somewhere above 41° S due to the inclusion of Petone (PET) with other South Island locations. The inability to find *P. subtriangulata* populations further south than 42° S in the present study could in itself be taken as evidence for a barrier to southward dispersal in this species.

Current patterns around the Cook Strait area are often implicated in driving these patterns of genetic population differentiation. On the west coast of New Zealand current flow is weaker than along the east coast, is less predictable and is often influenced by wind-driven events (Chiswell & Rickard 2011). As a consequence the north-south division that is often strong on the east coast can be weaker, difficult to pinpoint and sometimes absent, such as in the case of *R. plebeia* and *R. leporina*. As described previously, the ability for adult flounder to move across the shallow Taranaki Bight may contribute to weaker genetic differentiation amongst west coast locations.

On the east coast of New Zealand, the mixing of the East Cape Current, Wairarapa Eddy, Southland Current and strong Cook Strait currents forces current flow offshore across the Chatham Rise, which is likely to transport larvae into unsuitable open ocean habitat and create a significant barrier to the north-south dispersal of marine organisms (Laing & Chiswell 2003). This area of current mixing is associated with a known division between biogeographic provinces (Shears et al. 2008; Walls 2009) where the warmer northern water mass meets the cooler southern water mass. The difference in water temperature regimes may also form a barrier, preventing locally adapted populations from becoming established outside their biogeographic province. Wei et al. (2013) describe a locus in the greenshell mussel *Perna canaliculus* with strong north-south differentiation at about 41 °S on the west coast and at about 42 °S on the east coast of the South Island that is significantly correlated with sea surface temperature, suggesting that this locus is under selection for different water temperature regimes. A similar pattern was observed for a *P. australis* locus, which was discarded as an outlier in the present study, suggesting that this locus may also be under selection for water temperature (Hannan 2014).

5.2.2 West-East differentiation

West-east differentiation is an unusual pattern of genetic population structure amongst New Zealand coastal marine species, with Gardner et al. (2010) identifying only one other study reporting this pattern. This was for an allozyme assessment of snapper, which by its natural distribution is restricted to warmer waters in central and northern New Zealand (Smith et al. 1978), and more recent microsatellite analysis of snapper populations has indicated the existence of a north-south split

(Bernal-Ramírez & Adcock 2003). There was some evidence in both *P. australis* and *R. leporina* for a degree of genetic differentiation between west and east coast populations, suggesting some restriction of dispersal between the west and east coasts of New Zealand for these species. For *P. australis* this west-east differentiation was limited to South Island locations only, with North Island locations showing a different pattern of genetic population structure. For *R. leporina*, evidence for north-south differentiation was weak. Instead, there were indications of high connectivity along the west coast, and significant genetic differentiation between this west coast population and eastern locations around central New Zealand and the Banks Peninsula area further south.

West-east genetic differentiation could be driven by the geography of mainland New Zealand; the latitudinal extent of the land mass means that there is substantial distance between some populations on either side of the New Zealand mainland, which may pose a barrier to connectivity. During the last glacial maximum a lower sea level meant that the North and South Islands comprised a single landmass, eliminating the ability for marine organisms to disperse via Cook Strait. The genetic population structure of many marine and terrestrial species inhabiting southern New Zealand is theorised to still be influenced by the signature of past glaciation (Wallis & Trewick 2009). It could be argued that the west-east differentiation observed in the present study can be attributed to this historical signal, generated by the recurring fluctuations in sea level during the Pleistocene. In the present study we propose that glaciation could be responsible for some of the genetic population structure observed in *P. australis* and *R. leporina*, so it is possible that it also contributes to the west-east differentiation that was observed.

A further contributing factor may be habitat availability. Both *P. australis* and *R. leporina* are largely estuarine species, and parts of the South Island and west coast are lacking in suitable estuarine habitat, compared to the northern and eastern parts of New Zealand (Hume et al. 2007). The Fiordland region is characterised by discrete fiords and deep bathymetry. These physical features, in conjunction with a sudden change in current direction to the north of the region, could retain larvae in the region and create a barrier of unsuitable habitat around the bottom of the South Island for some species. It is notable that we were unable to collect *R. leporina* from the southern part of the South island in the present study, which provides further support that non-availability of habitat creates a barrier to dispersal between the west and east coasts for this species. This finding is supported by the differentiation of many marine populations in the Fiordland region (including *P. novaezelandiae* from the present study) and provides evidence that this region presents a barrier to dispersal for marine species.

5.2.3 Divergence amongst populations

Whilst most of the study species were characterised by some sort of regional-level genetic population structure, there were also several instances where divergence of populations was observed on a smaller spatial scale. For example, overall genetic population structure in mainland *P. subtriangulata* was very weak, but three populations (Ruakaka, Waimarama and Kakamatua) were significantly differentiated from this panmictic group. Divergence amongst populations was particularly evident in *P. australis*, which showed significant genetic differentiation over small geographic distances amongst many North Island populations. Most putative populations of flounder included more than one discrete sampling locality, but Lake Ellesmere samples of *R. plebeia* were anomalous as they were significantly differentiated from all other sampled populations. For *P. novaezelandiae* sampled locations within north and central regions showed little evidence of genetic differentiation but significant differences were observed with Fiordland and Stewart Island.

Most of these cases of divergence amongst populations coincide with an ocean current that may promote larval retention and restrict connectivity amongst populations, or some unique characteristic of the sampling location. For example, complex currents and eddies in north-eastern New Zealand seem to have had a similar influence on *P. subtriangulata*, *P. australis* and *P. novaezelandiae*

resulting in significant differentiation of some populations in northern New Zealand. The same pattern was not seen in the two flounder species but the ability for adult flounder movements to also contribute to genetic connectivity may mean that these currents do not restrict connectivity in the same way as they do for bivalves, where connectivity is limited to the larval stage. Genetic differentiation of *P. subtriangulata* from the Manukau Harbour could be related to its isolation within the harbour habitat and similarly, a population of *R. plebeia* that was isolated in the brackish lagoon of Lake Ellesmere also shows genetic differentiation. In both cases it is possible that low connectivity and local adaptation to extreme physical conditions are driving differentiation of these populations.

What these findings demonstrate is that, whilst it might be useful to make generalisations about the likely genetic population structure of a species based on its life history characteristics or known barriers to dispersal, it must be kept in mind that in some cases, the unique life history characteristics of a species or unique physical features of a habitat can result in unexpected patterns of genetic divergence.

5.2.3.1 The Chatham Islands

The most significant example of divergence amongst populations observed in this study was that of Chatham Island *P. subtriangulata* from mainland New Zealand populations. In comparison, *P. novaezelandiae* collected from the Chatham Islands showed only weak differentiation from mainland populations. This is not an uncommon finding: as discussed previously, divergence of Chatham Islands populations has been reported for some coastal marine species, whereas for other species there appears to be little to no differentiation from mainland New Zealand populations. Often this is attributed to the different life history characteristics of the species, but in this case the question remains: why do two bivalve molluscs with similar pelagic larval duration show such different patterns of genetic population structure?

The observed difference could result from the evolutionary age of each species. The recent evolutionary history (about 1 Million years ago (Mya); Beu & Maxwell 1990) of *P. novaezelandiae* could be one of the factors responsible for the weak genetic population structure observed between Chatham Island and the mainland, as there has not been a long period of time over which differentiation could occur. A similar lack of differentiation between mainland New Zealand and Chatham Island was observed in the bivalve *Austrovenus stutchburyi*, a species which is estimated to have originated approximately 5 Mya (Beu & Maxwell 1990; Ross et al. 2011) and also in the blue mussel *Mytilus galloprovincialis* (originated approximately 1 Mya ago; Beu & Maxwell 1990; Westfall 2011).

In comparison, the origin of *P. subtriangulata* is estimated at approximately 20 Mya (Beu & Maxwell 1990), which may explain why the Chatham Island population shows much greater differentiation from mainland New Zealand populations as there has been a longer period of time over which differentiation can occur. There has even been a suggestion (Beu & Rooij-Schuiling 1982; Smith et al. 1989), that this population may represent a different species of tuatua, although more focussed research would be required to answer this question.

5.3 Species-specific patterns of genetic structure: the importance of biological and ecological life history characteristics

Whilst the physical properties of the marine environment can affect the genetic population structure of marine organisms in similar ways (e.g., by creating common barriers to dispersal or by promoting long distance dispersal), many species-specific contemporary and historical factors can also affect genetic population structure. The present study has demonstrated that unexpected patterns of genetic

population structure often arise, making it difficult to generalise between species. The fundamental challenge is to identify the relative influence of common processes against species-specific processes that contribute to the genetic population structure of all species.

The influence of habitat can be clearly seen when comparing the genetic population structure of *P. subtriangulata* and *P. australis*. Despite having similar reproductive strategies and pelagic larval duration, the open coast habitat of *P. subtriangulata* means that this species experiences fewer impediments to larval connectivity compared to *P. australis*, where connectivity is restricted by the features of its estuarine habitat. As a consequence, connectivity was higher and genetic population structure weaker in *P. subtriangulata* compared to *P. australis*. In fact, of the five study species, *P. subtriangulata* is the only one that is considered to be a truly 'open coast' species and was the only species to show a predominantly 'panmictic' population structure over the sampled populations.

The distinction in habitat preference between the two flatfish species is less clear cut than that between the two surf clam species. Whilst *R. plebeia* is more estuarine in its habitat preference than *R. leporina*, both use estuaries for breeding. *Rhombosolea plebeia* and *R. leporina* have some similarities in their genetic patterns, but with some notable differences that may be related to life history. *Rhombosolea plebeia*, which showed more genetic connectivity amongst localities, is geographically more widely distributed around New Zealand and found to a depth of 100 m (Ayling & Cox 1982). *Rhombosolea leporina* had more significant differences amongst localities, less connectivity and higher levels of self-recruitment. *Rhombosolea leporina* is restricted to 50 m and mainly concentrated around the North Island and at Banks Peninsula (www.NABIS.gov.nz). This restriction to shallower depths in *R. leporina* may reduce the amount of habitat available and thereby limit migration, thus restricting gene flow. Both species showed a break in connectivity between south-eastern populations and populations to the north and west, which may be related to strong currents through the Cook Strait or the sharp increase in depth around the Hikurangi Trough. It is important to note however, that only the PLD of *R. tapirina* has been measured (Crawford 1984; Jenkins 1987), therefore there may be differences in PLD between the species that may help explain some of the patterns of genetic structuring.

Whilst all species showed isolated cases of divergence amongst populations, this was most striking amongst northern populations of *P. australis*, where significant differentiation was seen amongst populations separated by as little as 100–200 km. Whilst the estuarine habitat of *P. australis* is expected to promote the genetic divergence of populations, *P. novaezelandiae*, *R. plebeia* and *R. leporina* are also considered to be estuarine species and do not show the same degree of population differentiation. However, *P. australis* is an intertidal species, whereas the other species are subtidal, which raises the possibility that local adaptation plays a more significant role in driving genetic differentiation amongst *P. australis* populations. Intertidal areas are known for their extreme range of environmental conditions, and selection and adaptation are widely recognised as playing important roles in structuring populations of intertidal organisms (see Schmidt et al. 2008 and references therein).

A further contributing factor to small scale genetic differentiation seen in *P. australis* could be the complex current features and physical geography of northern New Zealand. Numerous eddies and coastal landforms can retain larvae and create unpredictable barriers to dispersal. This mechanism could also explain the genetic divergence seen amongst some northern *P. subtriangulata* populations and the genetic temporal instability observed for some northern populations of *P. novaezelandiae* (Silva 2015). Similar patterns of genetic differentiation are reported for north-eastern populations in the amphipod *Paracorophium excavatum* (Stevens & Hogg 2004) and the alga *Gracilarias chilensis* (Intasuwan et al. 1993). In comparison, genetic diversity and differentiation amongst southern populations of *P. australis* were found to be lower, possibly because of more consistent southern currents, regular coastline geography and possible glacial influences. This is a pattern that has been noted for other New Zealand coastal marine species (e.g., Hickey et al. 2009; Ross et al. 2011).

Finally, differences in genetic population structure between the bivalve species featured in this study (i.e., *Paphies* spp. versus *P. novaezelandiae*) highlights the role that evolutionary history can play in determining contemporary genetic population structure. This was particularly evident in the level of differentiation of Chatham Island populations. As described above, the more evolutionarily ancient *P. subtriangulata* showed a high degree of genetic differentiation between Chatham Island and mainland populations, compared to the more evolutionarily recent *P. novaezelandiae*, which still shows genetic connection between Chatham Island and mainland populations. Given their longer evolutionary history, it is also not unexpected that the genetic population structure of the *Paphies* species will show evidence of past glaciation events in comparison to the evolutionarily recent *P. novaezelandiae*.

5.4 Comparison to other coastal marine species in New Zealand and internationally

Ross et al. (2009) and Gardner et al. (2010) summarised the patterns of genetic population structure seen in New Zealand coastal marine species. This study has contributed to this body of knowledge and we have already highlighted specific patterns of genetic population structure observed in the present study that have also been reported for other species (e.g., a barrier around Cook Strait, differentiation of Chatham Island and mainland populations). How do the findings of this study compare more generally to what is already known about the genetic population structure of New Zealand's coastal marine organisms, and internationally where regional scale population genetic studies have been undertaken?

There are very few studies of estuarine and soft sediment species in New Zealand which have sampled the entire country for comparison (Gardner et al. 2010) and these studies reported a variety of genetic population structure. For example, seagrass (*Zostera muelleri*) shows low levels of gene flow between adjacent estuaries, but strong differentiation between the North and South Islands (Jones et al. 2008). Jones et al. (2008) did not include any samples from the south part of the North Island, but the authors identify the Cook Strait as a possible barrier and major currents as the vector for gene flow. A study of *Gracilaria chilensis* found a population grouping in the north of the North Island, and a second group that was composed of the rest of New Zealand (Intasuwan et al. 1993). More complex patterns were observed for populations of *Paracorophium excavatum* and *P. lucasi*, which showed differentiation between the north and south locations, as well as some evidence for west-east differentiation (Schnabel et al. 2000; Stevens & Hogg 2004). The estuarine triplefin *Grahamina nigripenne* and common cockle *Austrovenus stutchburyi* also showed complex patterns of genetic population structure, which included evidence for IBD and north-south differentiation (*G. nigripenne*; Hickey et al. 2009), and differentiation into multiple population groups (*A. stutchburyi*; Ross et al. 2011).

In comparison, the majority of New Zealand-wide rocky intertidal studies summarised by Gardner et al. (2010) report north-south differentiation. This includes a wide a wide variety of taxa such as Ophiuroidea, Asteroidea, Gastropoda, Bivalvia and Polyplacophora (Apte & Gardner 2002; Star et al. 2003; Waters & Roy 2004; Goldstien et al. 2006; Veale & Lavery 2012). Smaller numbers of studies report IBD (Hickey et al. 2009; Veale & Lavery 2012) or no structure (Waters et al. 2005; Del Mundo 2009). These studies suggest that major barriers to dispersal in the New Zealand marine environment (i.e., the north-south barrier near Cook Strait) exist independently of a species' habitat type. These studies also suggest that estuarine species can display a larger variety of genetic population structures, compared to rocky intertidal species, which have shown a predominantly north-south differentiation pattern over a variety of taxa.

It is not unexpected that estuarine/soft sediment species may show more complex patterns of genetic population structure given the aforementioned characteristics of estuaries. In comparison, the predominant pattern of north-south differentiation seen in rocky intertidal species suggests that there is an aspect of this habitat type that drives this pattern. However, it is difficult to speculate on why this might be or draw any firm conclusions from these data given the inconsistent methodologies amongst

the different studies (e.g., sample sizes, sampling locations and ranges, marker types). Of further note are the studies that have focussed on fiord species, almost all of which show genetic divergence amongst fiords (Miller 1997, 1998; Miller et al. 2004; Ostrow 2004). Similar to the findings of the present study, these studies provide evidence that there are significant barriers to the dispersal of marine organisms in the Fiordland region.

In the Gardner et al. (2010) review, 9 of the 38 studies (24%) that had a New Zealand wide sampling structure showed no genetic structure across the range sampled. Studies that showed a lack of genetic structure were diverse in respect to habitat type, ecology and PLD and no single biological or ecological factor ties these species together to explain the lack of structure. However, these studies tended to be characterised by a small number of sampling locations or the use of low resolution markers, such as allozymes, suggesting that methodological factors are responsible for the apparent lack of genetic population structure. This is demonstrated in species such as greenshell mussels and blackfoot paua, where subsequent studies with higher sample number and different markers revealed genetic structure that was not detected in earlier studies (Apte & Gardner 2002; Apte et al. 2003; Star et al. 2003; Will & Gemmell 2008). As with this present study, even species in the same genus can have different genetic patterns. Each species has an individual evolutionary history, and although shaped by similar physical forces as their contemporaries, may have widely different biotic and environmental interactions over time.

There are few international studies which have attempted a region-wide multi-species comparison of genetic population structure in marine species to which the present study can be compared. A review of genetic connectivity in 50 invertebrate species along the eastern Pacific showed that very few species experienced a disjunction in connectivity over a well-known biogeographic boundary between northern and southern California (Kelly & Palumbi 2010). Whilst California is an area with abrupt changes in current, water temperature and species distributions, the occasional transport of larvae during El Niño conditions may be enough to erase a signal of genetic differentiation (Shanks & Eckert 2005). On the other hand, a study of 27 taxonomically and ecologically diverse species across the Hawaiian Archipelago showed concordant barriers to gene flow concentrated in four regions of the Archipelago indicating that these species with different taxonomy, life history, and ecology are responding to common factors that limit dispersal and define independent genetic units (Toonen et al. 2011).

These international region-wide studies show that although there may be real and consistent boundaries to dispersal that could lead to genetic differentiation, a low level of gene flow through occasional long distance dispersal is theoretically sufficient to reduce a weak signal of population structure (Wright 1931; Mills & Allendorf 1996). Similarly, our study detected (and reaffirmed) one common barrier to dispersal that has an effect on the genetic population structure of many New Zealand species, including most of the species used in the present study (i.e. the north-south barrier in the region of Cook Strait). The weak patterns of genetic differentiation seen in this study (and other studies of New Zealand marine species) are consistent with what we would expect to see if there was temporal (and possibly spatial) variation in its ability to restrict dispersal of marine organisms across the Cook Strait region.

5.5 Implications of the research for marine biodiversity

Biodiversity is often described at three levels (Féral 2002): (i) infra-specific (genetic level), (ii) organismal (species level), and (iii) ecosystem level. A fourth level, the land- (or sea-) scape level integrates the condition, pattern, and connectivity of natural ecosystems. A further aspect of biodiversity is its dynamic nature – it encompasses a complex set of relationships that change in space and time (Féral 2002). Genetic diversity underpins the ability of a population (and ultimately a species') ability to respond to its dynamic environment. Therefore, it is important to understand the levels of genetic diversity within a species and how it is dispersed amongst populations in order to

sustainably manage or conserve the biodiversity of a species, and hence its ability to persist in a dynamic environment.

This study has provided further evidence that barriers to connectivity exist in the New Zealand coastal marine environment, although these barriers are not completely preventing larval dispersal in any species. Some of these barriers (e.g., the barrier in the Cook Strait region) affect a large number of species that are diverse in terms of their habitat preferences, life history characteristics and taxonomic groupings. When managing marine biodiversity it is important to recognise that there is a high likelihood that such a barrier will influence the diversity levels of a species and how it responds to environmental change. For example, the presence of such barriers will influence a population's ability to recover from local-scale extinctions or massive population declines. However, this study has also shown how species specific barriers to connectivity can arise, which can be difficult to predict until the genetic population structure of individual species can be investigated.

In this study we have attempted to suggest some factors that might help indicate whether a species is likely to be characterised by fragmented genetic population structure or a more panmictic population structure, which may help inform biodiversity management decisions in the absence of an adequate knowledge of population structure. For example evidence from the present study and others. (Tarnowska et al. 2010; Xiao et al. 2010; Ross et al. 2011) suggests that the habitat characteristics of estuarine species seem to make them more susceptible to dispersal barriers and they are often characterised by a greater number genetically different groups. In comparison, we have pointed out that in studies of rocky intertidal species, they seem to be characterised by fewer genetically different groups and in this study the only 'open coast' species (*P. subtriangulata*) was found to have weaker genetic population structure, suggesting that open coast species are subject to fewer barriers to connectivity.

In the absence of any knowledge of a species' genetic population structure it may be possible to make predictions about how this structure will be influenced by connectivity restricting barriers based on the known genetic population structure of species inhabiting similar habitats or with similar life history characteristics. However generalisations such as this are unlikely to provide a robust framework upon which to manage biodiversity. Whilst these generalisations can form the basis of hypotheses about the genetic population structure of a species, they cannot replace an investigation of species specific genetic population structure. We have stressed the role that individual evolutionary history can play in shaping the genetic population structure of a species and producing unexpected patterns of genetic population structure. A good example is provided in the present study, where *R. plebeia* and *R. leporina* were found to have quite different patterns of genetic population structure, which would have been difficult to predict given their similar biological and ecological characteristics.

Despite evidence for genetically differentiated groups in all five study species, overall population structure was also weak for all five species, indicating a 'crinkled' model of connectivity (Ovenden 2013). An implication of crinkled connectivity is that the forces that determine genetic population structure (gene flow, drift, mutation, selection) may take a long time to reach any sort of balance or equilibrium. This raises the possibility as to whether a stable equilibrium state could ever be reached for some marine species, especially those with a recent evolutionary origin, large population sizes and temporal variation in recruitment (Boileau et al. 1992).

A further implication of crinkled connectivity for the biodiversity of a species is that populations may be genetically connected (i.e. genes are exchanged amongst populations over extended periods of time) but in the short term populations may not be demographically connected at the rates required to maintain a viable breeding population, and therefore may not be resilient to any sudden ecosystem changes (e.g. human mediated events such as climate change or overfishing). Demographic connectivity is crucial to maintaining biodiversity if populations become locally extinct and the on-going persistence of a population becomes dependent on external recruitment.

5.6 Future directions

High resolution genetic markers have greatly improved our knowledge of population structure in marine species. Patterns that were previously hidden using allozymes have become clearer with more discriminatory genetic markers (Apte & Gardner 2001, 2002). Single nucleotide polymorphisms (SNPs) are another genetic marker that will not only show neutral variation, but can identify regions of the genome that are under selection. With the further advances and drop in cost of genome-wide sequencing, gene coding regions can be identified to help identify functional differences between populations, allowing the possibility of more focussed management of marine populations.

Genetic methods of determining population structure and connectivity could be complemented through the use of oceanographic or Lagrangian particle modelling. Where Lagrangian modelling has been used to track larvae of New Zealand marine invertebrates it has shown that self-recruitment is likely to be high and sporadic, with larvae travelling no more than tens of kilometres from their natal sites (Broekhuizen et al. 2011; Stephens et al. 2006). High-resolution biophysical models, integrating life history and oceanographic data, can also be very informative. For example, modelling larval dispersal of the snapper *Pagrus auratus* suggested that larval vertical behaviour and El Niño-Southern Oscillation (ENSO) cycles can affect the dispersal direction and settlement of larvae (Le Port et al. 2014).

Population genetic data is a significant component of the combined scientific knowledge of the species. Just as morphological and ecological traits of congeneric species can differ, so can the population structure of closely related species. These long term patterns have developed over separate evolutionary trajectories. As is often the way with biological systems, the picture is complicated and is much better served in a holistic view rather than piecemeal with extrapolation. The more information we have about each individual species, the better prepared we are to manage it effectively. The unique characteristics of the marine environment mean that future investigations of population genetic structure in marine species are likely to delve further into the temporal nature of population structure, the interactions with and influence of landscape features, and the role that adaptation plays in driving this structure.

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