**PATTERNS OF GENETIC CONNECTIVITY IN
DEEP-SEA VULNERABLE MARINE
ECOSYSTEMS AND IMPLICATIONS FOR
CONSERVATION**

BY

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Abstract

Knowledge about and understanding of population structure and connectivity of deep-sea fauna decreases with increasing depth, but such information is crucial for the management of vulnerable marine ecosystems (VMEs) in particular. As such, research using genetic markers, which does not require knowledge of ecological or environmental processes as a prerequisite for the analysis, is a practical method to investigate population connectivity of VME indicator taxa. However, population genetics studies are yet to be broadly conducted in the deep sea around New Zealand.

To provide background information and develop hypotheses for this research, 196 population genetic studies of deep-sea fauna were reviewed and analysed. Based on the collected studies, four different patterns of spatial genetic structure were observed: global homogeneous, oceanic, regional, and fine structure. These different structures were reported that they were related to depth, topography, distance between populations, temperature and other biological factors. Quantification of the relationship between these factors and the detection of barriers to gene flow (barrier detection) showed that depth, currents and topography contributed significantly to barrier detection and depth and topography were acting as a barrier to gene flow in the deep sea. Furthermore, different sampling strategies and different genetic marker types significantly influenced genetic barrier detection. Comparison amongst different habitats suggested that different conservation strategies should be developed for different habitat types (Chapter 2).

This study used different genetic markers to assess the genetic connectivity amongst VME indicator taxa Vulnerable Marine Ecosystems (VME). Seven VME indicator taxa were selected: 4 sponges (*Neaulaxinia persicum*, *Penares* sp., *Pleroma menoui* and *Poecillastra laminaris*) and 3 corals (*Goniocorella dumosa*, *Madrepora oculata* and *Solenosmilia variabilis*), at different spatial scales. Due to lack of genetic information for these species, genetic markers were developed for *Poecillastra laminaris* (0) and *S. variabilis* (Chapter 4).

A geographic province (northern-southern province), region (north-central-south), and geomorphic feature hierarchical testing framework was employed to examine species-specific

genetic variation in mitochondrial (*COI*, *Cytb* and *12S*) and nuclear markers (microsatellites) amongst populations of four deep-sea sponges within the New Zealand region. For *Poecillastra laminaris*, significant mitochondrial and nuclear DNA genetic differences were revealed amongst biogeographic provinces. In contrast, no significant structure was detected across the same area for *Penares* sp. Both *Neoaulaxinia persicum* and *Pleroma menoui* were only available from the northern province, in which *Pleroma menoui* showed no evidence of genetic structure, but *N. persicum* exhibited a geographic differentiation in *12S*. No depth-related isolation was observed for any of the four species at the mitochondrial markers, nor at the microsatellite loci for *Poecillastra laminaris*. Genetic connectivity in *Poecillastra laminaris* is likely to be influenced by oceanic sub-surface currents that generate routes for gene flow and may also act as barriers to dispersal. Although data are limited, these results suggest that the differences in patterns of genetic structure amongst the species can be attributed to differences in life history and reproductive strategies. The results are discussed in the context of existing marine protected areas, and the future design of spatial management measures for protecting VMEs in the New Zealand region (Chapter 5).

To better understand the vulnerability of stony corals (*Goniocorella dumosa*, *Madrepora oculata* and *Solenosmilia variabilis*) to disturbance within the New Zealand region, and to guide marine protected area design, genetic structure and connectivity were determined using microsatellite loci and DNA sequencing. Analyses compared population genetic differentiation between two biogeographic provinces, amongst three sub-regions (north-central-south), and amongst geomorphic features. Population genetic differentiation varied amongst species and between marker types. For *G. dumosa*, genetic differentiation existed amongst regions and populations on geomorphic features, but not between provinces. For *M. oculata*, only a north-central-south regional structure was observed. For *S. variabilis*, genetic differentiation was observed between provinces, amongst regions and amongst geomorphic features based on microsatellite variation. Multivariate analyses indicated that populations on the Kermadec Ridge were genetically different from Chatham Rise populations in all three coral species. Furthermore, a significant isolation-by-depth pattern was observed for both marker types in *G. dumosa*, and also in *ITS* of *M. oculata*. An isolation-by-distance pattern was found in microsatellites of *S. variabilis*. Migrate analysis showed that medium to high self-recruitment were detected in all geomorphic feature populations, and different species presented different genetic connectivity patterns. These different patterns of population genetic structure and connectivity at a range of spatial scales

indicate that flexible spatial management is required for the conservation of deep-sea corals around New Zealand (Chapter 6).

Understanding the deep-sea ecological processes that shape spatial genetic patterns of species is critical for predicting evolutionary dynamics and defining significant evolutionary and/or management units. In this study, the potential role of environmental factors in shaping the genetic structure of the 7 deep-sea habit-providing study species was investigated using a seascape genetics approach. The genetic data were acquired from nuclear and mitochondrial sequences and microsatellite genotype data, and 25 environmental variables (5 topographic, 17 physiochemical and 3 biological variables). The results indicated that environmental factors affected genetic variation differently amongst the species. However, factors related to current and food source explained the north-central-south genetic structure in sponges and corals, and environmental variation in these parameters may be acting as a barrier to gene flow. At the geomorphic feature level, the DistLM and dbRDA analysis showed that factors related to the food source and topography were most related to genetic variation in microsatellites of sponge and corals. This study highlights the utility of seascape genetic studies to better understand the processes shaping the genetic structure of organisms (Chapter 7).

The outcomes of this study provide vital information to assist in effective management and conservation of VME indicator taxa and contribute to an understanding of evolutionary and ecological processes in the deep sea (Chapter 8).

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Chapter 6

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Specimen Collection

Sponges

Specimens included in this study were collected on various research cruises, details of those projects responsible for the collection of these valuable samples are provided as follow: “Seamounts: their importance to fisheries and marine ecosystems”, undertaken by the NIWA and funded by the former New Zealand Foundation for Research, Science and Technology (FRST) with additional funding from the former Ministry of Fisheries (MFISH TAN0104,TAN0905), NOAA Satellite Operations Facility (TAN0413 cruise), and Census of Marine Life (CoML) field programme on seamounts, CenSeam (TAN0604 cruise).; Specimens collected under the Scientific Observer Program funded by the New Zealand

Ministry for Primary Industries (MPI); New Zealand Cold Vents: New Vents program (RV Sonne voyage SO191) conducted by IFM-GEOMAR Leibniz-Institut für Meereswissenschaften an der Universität Kiel and partners; Fisheries research trawl surveys (TAN0501, TAN0813, TAN0901) conducted by NIWA and funded by MFish (now MPI); RENEWZ I (TAN0616) voyage, were components of the project ‘Exploration of Chemosynthetic Habitats of the New Zealand Region’, funded by NOAA Ocean Exploration and NIWA, with co-funding from Woods Hole Oceanographic Institution (WHOI), Scripps Oceanographic Institution, and the University of Hawaii; Ocean Survey 20/20 Chatham/Challenger Biodiversity and Seabed Habitat Project, jointly funded by MFish, Land Information New Zealand (LINZ), NIWA, and Department of Conservation; New Zealand-Australian “MacRidge 2” research voyage (TAN0803), the biological component of which was part of NIWA’s research project “Seamounts: their importance to fisheries and marine ecosystems” funded by FRST and CSIRO’s Division of Marine and Atmospheric Research project “Biodiversity Voyages of Discovery” funded by the CSIRO Wealth from Oceans Flagship; Kermadec Arc Minerals (KARMA, TAN1007) voyage, funded by the Ministry of Business, Innovation & Employment (MBIE), in collaboration with Auckland University, GNS Science, and WHOI; Ocean Survey 20/20 Mapping the Mineral Resources of the Kermadec Arc (voyage TAN1104), funded by LINZ, GNS, NIWA and WHOI; Biogenic Habitats on the Continental Shelf (voyages TAN1105 & TAN1108), funded by MFish, FRST, NIWA and Oceans Survey 20/20 R/V Tangaroa days funded by LINZ; ‘Impact of resource use on vulnerable deep-sea communities’ project (TAN1206), funded by MBIE; Nascent Inter-Ridge Volcanic And Neotectonic Activity (NIRVANA, TAN1213) voyage, funded by MPI, in collaboration with Auckland University, GNS, and the University of New Hampshire (USA) and with funding from the program ‘Impact of resource use on vulnerable deep-sea communities’ project, funded by MBIE; South Pacific Vulnerable Marine Ecosystems Project funded by MBIE.

Corals

Specimens and data were provided by the NIWA Invertebrate Collection including material from the following projects: “Seamounts: their importance to fisheries and marine ecosystems”, undertaken by the NIWA and funded by the former New Zealand Foundation for Research, Science and Technology (FRST) with additional funding from the former Ministry of Fisheries (MFish), NOAA Satellite Operations Facility (TAN0413) and the Census of Marine Life field programme on seamounts, CenSeam (TAN0604); RENEWZ I

(TAN0616) voyage, were components of the project ‘Exploration of Chemosynthetic Habitats of the New Zealand Region’, funded by NOAA Ocean Exploration and NIWA, with co-funding from Woods Hole Oceanographic Institution (WHOI), Scripps Oceanographic Institution, and the University of Hawaii; Ocean Survey 20/20 Chatham/Challenger Biodiversity and Seabed Habitat Project, jointly funded by MFish, Land Information New Zealand (LINZ), NIWA, and Department of Conservation; New Zealand-Australian “MacRidge 2” research voyage (TAN0803), the biological component of which was part of NIWA’s research project “Seamounts: their importance to fisheries and marine ecosystems” funded by FRST, and CSIRO’s Division of Marine and Atmospheric Research project “Biodiversity Voyages of Discovery” funded by the CSIRO Wealth from Oceans Flagship; ‘Impact of resource use on vulnerable deep-sea communities’ (DSCA: TAN1004, TAN1206, TAN1503), funded by the Ministry of Business, Innovation & Employment (MBIE); Kermadec Arc Minerals (KARMA) voyage, funded by MBIE, in collaboration with Auckland University, GNS Science, and WHOI; Ocean Survey 20/20 Mapping the Mineral Resources of the Kermadec Arc Project, funded by LINZ, Institute of Geological and Nuclear Science, NIWA, and WHOI; Biogenic Habitats on the Continental Shelf project (voyages TAN1105), funded by MPI, FRST, NIWA and Oceans Survey 20/20 shiptime (LINZ); Nascent Inter-Ridge Volcanic And Neotectonic Activity (NIRVANA, TAN1213) voyage, funded by the Ministry for Primary Industries (MPI), in collaboration with Auckland University, GNS, and the University of New Hampshire (USA) and with funding from the DSCA project, funded by MBIE; NIWA/GNS Oceans Survey 20/20 Reinga voyage (TAN1312), funded by LINZ and New Zealand Petroleum & Minerals; South Pacific Vulnerable Marine Ecosystems Project (C01X1229) funded by MBIE; Scientific Observer Program funded by MFish/MPI; NIWA trawl surveys funded by MFish/MPI; various campaigns and cruises of the New Zealand Oceanographic Institution.

Chapter 1. Introduction

1.1. The deep sea

The deep sea, usually defined as the region beyond the continental shelf break and at depths greater than 200 m, has a diverse topography and oceanography and encompasses a wide range of habitat types and depths. It is the single largest biome on the planet (Ramirez-Llodra *et al.*, 2011). Compared with shallow-water habitats and their biotas, which are relatively well studied, information from the deep sea around the world is still scarce. Despite being largely unknown, the deep sea is nevertheless extremely important with unique ecosystems, and contains many marine resources of a biological and non-biological nature of high potential commercial value (Ramirez-Llodra *et al.*, 2011).

In the deep-sea biome, different seafloor habitats are patchily distributed, including the continental slope, abyssal plain, trenches and troughs, seamounts, hydrothermal vents and cold seeps (Oldham *et al.*, 2014). These habitats represent different environments, and can contain different taxa. The water column above these seafloor habitats, is the largest habitat in the ocean, and comprises all the open ocean waters and is divided into pelagic zones at different depths (Oldham *et al.*, 2014), where most larval dispersal of deep-sea organisms is thought to occur. Some deep-sea habitats are much more studied than others (Oldham *et al.*, 2014). The abyssal plain, the most common benthic habitat in the deep sea, covers approximately half of the Earth's surface. However, the understanding of this habitat is limited due to the spatial scale of the area, and its inaccessibility (Ebbe *et al.*, 2010; Webb *et al.*, 2010). Trenches and troughs are long, narrow, steep-sided depressions, and the extreme depth keeps this area largely unexplored, such that most of the fauna at these deepest depths are still unknown (Oldham *et al.*, 2014). By comparison, the continental slope, seamounts, hydrothermal vents and cold seeps are relatively well studied (details see Sibuet & Olu 1998; van Dover *et al.*, 2002; Shank 2010; Vrijenhoek 2010; Levin *et al.*, 2016), but even including all of these habitats, only a small proportion of the deep-seafloor has been well studied.

In the past decades, as human activities have extended, anthropogenic impacts have increased with direct disturbances on deep-sea communities, and also indirect disturbances through changes to the chemistry of the oceans (Davies *et al.*, 2007; Halpen *et al.*, 2008; Pitcher *et al.*, 2008). In terms of direct effects, extractive resource exploitation is currently the main reason for degradation of deep-sea ecosystems (Davies *et al.*, 2007). Deep-sea fisheries have not only caused the number and diversity of fishes to decline (Baum *et al.*, 2003; Devine *et al.*, 2006; Worm *et al.*, 2005), but have also damaged structure-forming organisms (Hall-Spencer *et al.*, 2002; Koslow *et al.*, 2001; Pitcher *et al.*, 2008; Roberts, 2002), reduced

fisheries-associated species (by-catch) (Hareide *et al.*, 2005) and have removed epifauna and modified the substratum (Gage *et al.*, 2005). Re-suspension of large quantities of sediment by bottom trawling has also caused reductions in benthic abundance in deep-sea communities (Palanques *et al.*, 2001; Palanques *et al.*, 2006; Palanques *et al.*, 2014). Recently, increasing demands for, and diminishing reserves of, hydrocarbons and minerals on land and in shallow water are pushing exploration into the deeper ocean (Hallock *et al.*, 2004), with associated negative effects on deep-sea animals (Gass & Roberts, 2006; van Dover, 2014). Moreover, the negative effects of waste disposal and pollution in the deep sea are still not well understood or widely studied. In terms of indirect effects, both physical and ecological changes caused by climate change and ocean acidification are expected to have negative implications for deep-sea habitats and organisms (Levin *et al.*, 2001; Ruhl & Smith, 2004; Hansen *et al.*, 2004; Danovaro *et al.*, 2004; Guinotte *et al.*, 2006).

1.2. Vulnerable Marine Ecosystems (VMEs) and VME indicator taxa

With the ever increasing pressure of adverse impacts and their consequences, concerns for deep-sea conservation are increasingly being raised. However, it is impossible to protect everything in the vast area of the deep sea, and conservation priorities have been set. In 2008, the United Nations Food and Agriculture Organisation (FAO) defined particular vulnerable marine ecosystems (VMEs) where organisms and their associated taxa are easily disturbed, are slow to recover or may not recover at all. Identified VMEs include seamounts, hydrothermal vents, cold seeps, deep-sea coral reefs and sponge gardens (FAO, 2009). VMEs can be associated with marine resources, for example they maybe sites of interest for particular fisheries (seamounts) and mining (vents), and are thus subject to high current or future levels of exploitation (Clark *et al.*, 2010; Shank 2010; Boschen *et al.*, 2013; Levin *et al.*, 2016). As a consequence, the United Nations General Assembly Resolution 61/105 calls for states to protect VMEs. However, the first challenge is how to identify and locate VMEs because there is not much information about the distribution of VMEs in the deep sea. As such, VME indicator taxa have been identified to help locate VMEs.

The guidelines for the management of deep-sea fisheries provide examples of VME indicator taxa, (e.g. habitat-forming species of stony corals and sponges) (FAO, 2009), but regionally-specific VME indicator taxa are more relevant. Currently, different Regional

Fisheries Management Organisations (RFMOs) have identified regional-specific indicators (Table 1.1).

Table 1.1 VME indicator taxa amongst different Regional Fisheries Management Organisations

Common name	Taxonomic name	CCAMLR	NAFO	NEAFC	SPRFMO	GFCM
Sponges	Porifera	Y	Y	Y	Y	Y
Anemones	Actiniaria	Y	Y	Y	Y	Y
Soft corals	Alcyonacea	Y		Y	Y	Y
Gorgonian	Gorgonacea	Y		Y	Y	Y
Sea pens	Pennatulacea	Y	Y	Y	Y	Y
Stony corals	Scleractinia	Y	Y	Y	Y	Y
Black corals	Antipatharia	Y		Y	Y	Y
Stylasterid corals	Anthoathecata	Y			Y	Y
Bryozoans	Bryozoa	Y	Y			Y
Protozoans	Protozoa	Y		Y		
Foraminifera	Foraminifera	Y		Y		
Sea lilies	Stalked crinoids	Y	Y	Y	Y	Y
Armless stars	Euryalida	Y			Y	
Sea squirts	Ascidacea	Y	Y			

CCAMLR, Commission for the Conservation of Antarctic Marine Living Resources; NAFO, Northwest Atlantic Fisheries Organization; NEAFC, North East Atlantic Fisheries Commission; SPRFMO, South Pacific Regional Fisheries Management Organisation; GFCM, General Fisheries Commission for the Mediterranean. The information of this table was summarized from FAO (<http://www.fao.org/in-action/vulnerable-marine-ecosystems/definitions/en/>).

Following their identification, with the aid of distribution records or habitat suitability models for VME indicator taxa, the question of how to manage VMEs poses a major challenge. The design of effective spatial management measures to conserve VMEs requires information that can guide, for example, the distribution, size and spacing of marine protected areas (MPAs) (Agardy, 2000; O’Leary *et al.*, 2012). Therefore, as well as studies of the distribution of VMEs, it is also important that research is carried out that examines the evolutionary and ecological processes that influence the distribution of species and populations. Such research includes the genetic structure and connectivity of populations of VME indicator taxa, and the processes that influences these patterns.

1.3. Study area

The deep sea around New Zealand is physiographically complex, comprising a series of parallel ridges and troughs in the north west (Lord Howe Rise, New Caledonia Basin and

Norfolk Ridge), two island arcs of volcanic origin (Kermadec and Macquarie Ridge) in the north and south respectively, the Campbell Plateau and Chatham Rise in the southeast and east, and the Challenger Plateau and Tasman Basin in the west (Cullen, 1970) (Figure 1.1). As well as the complex topography, circulation around New Zealand is also complicated and it is mainly dominated by Subtropical Water (STW) and Subantarctic Water (SAW) (Chiswell *et al.*, 2015) (Figure 1.2). These and other environmental differences across the region are thought to account for the deep-sea fauna of the seas around New Zealand being separated into two biogeographical provinces (Watling *et al.*, 2013).

Currently, there are 17 seamount closures and 17 Benthic Protection Areas (BPAs) throughout the New Zealand EEZ, established in 2001 and 2007, respectively (Brodie & Clark, 2002; Helson *et al.*, 2010) (Figure 1.3). These areas were selected to protect the seafloor fauna, including VMEs, from bottom trawling, but the design of these BPAs did not consider the genetic connectivity of deep-sea populations.

Amongst the few deep-sea genetic studies, research illustrates a complex pattern for deep-sea connectivity at different spatial scales in the New Zealand deep sea. No population genetic structure has been found in fish, crustaceans or demosponges off New Zealand (Smith *et al.*, 1996; Bors *et al.*, 2012). However, significant population genetic structure exists amongst distinct populations on the Chatham Rise, Hikurangi Margin and Challenger Plateau for a polychaete (Bors *et al.*, 2012). Furthermore, Miller *et al.* (2010) found that there were non-overlapping 16S and *ITS* haplotype distributions in antipatharians, suggesting limited connections between seamounts in the north of New Zealand. They, and other authors, have also pointed out that ocean expanses of 500-1000 km may be effective barriers to gene flow for deep-sea coral species, because of topographically controlled current patterns which promote within-ridge rather than among-ridge connectivity (de Forges *et al.*, 2000; Miller *et al.*, 2010). In the Bay of Plenty, six populations of the antipatharian *Stichopathes filiformis* from six different seamounts also shared *ITS* haplotypes, suggesting that at scales of 50-100 km (Miller *et al.*, 2010), these seamounts may be connected by gene flow through surface and near-surface current flow (Sutton & Chereskin, 2002). Apparently, no consistent pattern for the connectivity of deep-sea fauna has yet emerged amongst different taxa and at different scales, within the New Zealand region.

Figure 1.1 Distribution of major geomorphic features and deep-sea lower bathyal provinces around
New Zealand

Figure 1.2 Summary of the major currents in the New Zealand region

EAUC, East Auckland Current; NCE, North Cape Eddy; ECE, East Cape Eddy; WE, Wairarapa Eddy (Leathwick *et al.*, 2008, Bors *et al.*, 2012).

Figure 1.3 Offshore protected areas within the New Zealand EEZ

1.4. Study species

Species of structure or habitat-forming sponges and corals are widely recognised as VME indicator taxa (Table 1.1), including within the New Zealand region (Parker *et al.*, 2009). However, population genetic connectivity knowledge about deep-sea sponges is very limited. In the present study, four demosponges, *Neaulaxinia persicum*, *Poecillastra laminaris*, *Pleroma menoui* and *Penares* sp., were selected to be tested for patterns of genetic structure (Figure 1.4).

Poecillastra laminaris, known as the fibreglass cup sponge, is very “hairy” with siliceous spicules, and has a morphological shape that is relatively variable (common as shallow cup-like or large ragged fan sponge with rippled edges) (Tracey *et al.*, 2007). Size of *Poecillastra laminaris* is often about 5-10 cm in diameter, and it is widely distributed around New Zealand across large range of a latitudinal range (~30°S - ~60°S) and depth (119-1615 m) (Database of the National Institute of Water & Atmospheric Research Invertebrate Collection (NIC), 2015).

Penares sp., known as the golden brown crater sponge, is a spherical sponge with a smooth, waxy surface indented all over with groups of oscules, and is compressible with a size of up to 15 cm in diameter (Tracey *et al.*, 2007). *Penares* sp. is often free-living and the distribution depth around New Zealand ranges from 350-1408 m (NIC database, 2015).

Neaulaxinia persicum is egg-shaped with a thin skirt base or encompassing branches of scleractinian corals. It is often attached to small pieces of scleractinian coral rubble and rock surfaces on the sides of seamounts. The size of *N. persicum* is about 5 cm in diameter (Kelly, 2007). The collected depth ranges from 581 to 1680 m (NIC database, 2015).

Pleroma menoui is a highly irregular multilobate encrusting plate, occasionally tubular or a thick fan composed of fused lobes. Immature sponges are thick flattened ear-shaped plates (Kelly, 2007). *P. menoui* is usually attached to hard substrata on continental shelf banks, and found with a range of other lithistid sponges, molluscs, bryozoans, brachiopods, echinoderms, hydroids, and stylasterid hydrocorals (Kelly, 2007). The maximum dimensions of this specimen are recorded as 21 cm from New Zealand and New Caledonia (Kelly, 2007). The depth distribution of this species is from 179 to 945 m, and all collected species are from north of the North Island (NIC database, 2015).

Amongst stony corals in the deep sea, *Goniocorella dumosa*, *Madrepora oculata* and *Solenosmilia variabilis*, are the three most common species within the New Zealand

Exclusive Economic Zone (EEZ) that can form habitat structures (Tracey *et al.*, 2011), and were this chosen for the present study.

Figure 1.4 Specimens of *Poecillastra laminaris* (A), *Neoaulaxinia persicum* (B), *Penares* sp. (C) and *Pleroma menoui* (D)

Madrepora oculata forms branched colonies, and is typically a secondary framework builder, often being associated with other reef-building corals such as *Lophelia pertusa* (in the Northern hemisphere) and *S. variabilis* (in the Southern hemisphere) (Hain & Corcoran, 2004; Hourigan *et al.*, 2007). Morphologically, *M. oculata* is quite variable in its tendency to branch and in its texture (Pawson *et al.*, 1983) (Figure 1.5), and it is usually bushy, forming small fan-shaped colonies, with thick skeletal parts that grow in a lamellar pattern (Hourigan *et al.*, 2007). Unlike *M. oculata* with less extensive structures, *G. dumosa* and *S. variabilis* can form single species structures that cover larger areas. Both these species develop complex 3D branching structures, but *G. dumosa* tends to occur as discrete colonies in high density thickets, whilst *S. variabilis* colonies can coalesce to form larger and continuous reef-like structures (Figure 1.5). *S. variabilis* is widely distributed in the South Pacific and occasionally found in the Atlantic Ocean, whereas *G. dumosa* is mainly reported from the

Pacific Ocean (Yabe & Eguchi, 1936; Pawson *et al.*, 1983; González-Romero *et al.*, 2009). Compared to the deeper distribution of *S. variabilis*, *G. dumosa* is frequently found in shallow water at depths of 300 to 400 m (Hain & Corcoran, 2004). *S. variabilis* prefers to settle on hard substrata (such as seamount summits), whereas *G. dumosa* predominantly forms colonies on soft bottom (O'Hara *et al.*, 2008). The collected depth ranges in NIC of *G. dumosa*, *M. oculata* and *S. variabilis* were 198-1270 m, 236-1537 m, and 322-1805 m, respectively.

Figure 1.5 Morphology and branching of *Goniocorella dumosa*, *Madrepora oculata* and

Solenastrea variabilis

In Figure 1.5 above, specimens labelled A (NIWA ID 54236) and G (3936) are easily misidentified when only a small proportion of skeleton is available; B (88224) is typical *G. dumosa* with smooth tubular bridges intersecting the branches (key feature); C (25319) and D (99727) are showed the variable branching of *M. oculata*, and the texture of D is much more fragile than that of E. G and H (72307) show the branching and polyps of *S. variabilis*. The colour of dry-preserved and ethanol-preserved specimens has faded out in all three corals. *In situ* image of *G. dumosa* (C), *M. oculata* (F), and *S. variabilis* (I) on the Chatham Rise (TAN1503 voyage, DTIS Camera). The white bars in C, F and I are 20 cm.

1.5. Aims and thesis structure

This PhD research is a part of the project “Predicting the occurrence of vulnerable marine ecosystems for planning spatial management in the South Pacific region”, which is funded by the Ministry for Business, Innovation and Employment (MBIE), New Zealand. The aim of this project is to produce scientific information that that can assist New Zealand and the South Pacific Regional Fisheries Management Organisation (SPRFMO) initiatives on spatial management in the South Pacific region, and potentially the New Zealand EEZ.

The overall aim of this PhD thesis was to investigate the patterns of genetic structure and connectivity of VME indicator taxa. The specifics were to:

1. Define patterns of genetic population structure and genetic connectivity in demosponges and corals, and identify barriers to dispersal.
2. Identify environmental variables that might explain the observed structure/connectivity and dispersal barriers for demosponges and corals.
3. Consider how observed patterns of population genetic structure and genetic connectivity can inform deep-sea conservation for VMEs around New Zealand.

Thesis Structure

Each chapter of this thesis (except Chapter 1 and Chapter 8) has its own specific objectives and individual sections of Introduction, Methods, Results and Discussion. The purpose of Chapter 2 is to give an overview on the development of the genetic connectivity field and develop suitable hypotheses for the data testing in Chapters 5 and 6. Chapter 3 describes the mitochondrial and microsatellites markers developed for *Poecillastra laminaris* and corresponds to publications in the journal *Mitochondrial DNA* (Zeng *et al.*, 2014a) and *Marine Biodiversity* (Zeng *et al.*, 2016). Chapter 4 describes the application of mitochondrial, microsatellite and single-nucleotide polymorphism markers developed for *Solenosmilia variabilis*, and corresponds to a publication in *Mitochondrial DNA* (Zeng *et al.*, 2014b). In Chapter 5, the genetic structures of four sponges are described, the null hypothesis of panmixia (i.e., no genetic structure) is separately tested in these species using mitochondrial sequence markers and the factors that shape the patterns observed are discussed. This chapter has been submitted for publication in the journal *Biodiversity and Conservation*. In Chapter 6, the genetic structures of three stony corals are described. The null hypothesis is also separately tested in these species using mitochondrial and nuclear sequences markers and microsatellites and the factors that shape the patterns observed are discussed. This chapter has

been drafted for submission to the journal *Molecular Ecology*. In Chapter 7, a seascape genetics approach is used to identify associations between the genetic structures of collected sponges and corals across New Zealand and environmental variables. Finally, Chapter 8 summarises the key findings, indicates future directions and provides recommendations about the management and conservation of New Zealand's VMEs.

**Chapter 2. Patterns of genetic connectivity in the deep sea:
challenges for, and applications to, the conservation of
Vulnerable Marine Ecosystems**

2.1. Introduction

Habitats, such as trenches and troughs, seamounts, hydrothermal vents and cold seeps, which have distinctive hydrodynamic, topographic and geomorphic features, are patchily distributed in the deep sea. Therefore, genetic connectivity amongst populations could be complex for spatially fragmented populations in different habitats (Hilário *et al.*, 2015). For the conservation of deep-sea habitats, connectivity amongst populations is an important consideration for marine protected area (MPA) designation and MPA network design (Cowen *et al.*, 2002; Palumbi, 2003; Anadón *et al.*, 2013). Connectivity is a critical component of marine ecology to answer the questions ‘where, how far and how much do larvae dispersal?’, and this provides information about identifying source and sink populations and characterising spatial units for conservation (Lowe & Allendorf, 2010). However, it is impossible to physically monitor the movement of larval migrants in marine ecosystems, because the migrants are spread in an open and wide marine medium and are very small (< 500 µm in diameter) making them impossible to track (Cowen & Sponaugle, 2009; Buston & D’Aloia, 2013). Estimating connectivity indirectly using molecular biology is an alternative and powerful approach using genetic metrics to provide an indirect measurement of connectivity by sampling the adults without the constraint of directly monitoring larvae (Hellberg *et al.*, 2002). Population connectivity describes the exchange of individuals of the same species between populations and, in the case of genetic connectivity (gene flow), the extent of gene exchange caused by immigrants (Boschen *et al.*, 2016). In other words, when populations are reproductively isolated and do not exchange genes or experience limited gene exchanges, genetic differences between them will accumulate and eventually form the population genetic structure. In practice, the analysis of genetic variation components at different spatial scale provides a solution for measuring patterns of population connectivity (Hedgecock *et al.*, 2007), often in the absence of much information about the ecosystem in question and also without large sample size from the populations in question.

Analysing the genetic connectivities in an ecosystem is not a simple task. Because patterns of genetic connectivity can operate on scales from organisms living at a special habitat, such as a vent chimney, seamount or trench, to within an ocean or even across multiple oceans, connectivity surveys should consider a nested design to investigate the multiple scales of connectivity of different species (Thaler *et al.*, 2011; Hilário *et al.*, 2015; Boschen *et al.*, 2016). In addition, the dynamics of populations change over time in response to changes in the environment and the supply of recruits (Excoffier *et al.*, 2009). Within a

network of connected populations, source populations that contribute most recruits to subsequent generations, help maintain the genetic diversity of the regional metapopulation, and these populations are viewed as being of more importance than other (sink) populations (Boschen *et al.*, 2016). In a management context, genetic studies are typically conducted with the ultimate objective of identifying genetic connectivity patterns to locate source populations, and with the aim of identifying factors that contribute to genetic isolation (reduced or zero genetic connectivity) between populations (Young, 2000). However, in a vast and largely unknown ecosystem such as the deep sea, perhaps the first question to ask is how to develop different spatial frames for testing genetic variation components because there is a big difference between genetic connectivity at the oceanic and at a fine (e.g., between two vent chimneys) spatial scale.

Recently, increasing physical oceanographic modelling, predictive habitat mapping, ground-truth surveys and identification of different ecosystem biota/communities (e.g., biogeographic provinces) have all contributed to an improved understanding of the scales of genetic connectivity in the deep sea. This understanding arises from new knowledge about species-specific habitat requirements, distributions and types of substrata within habitat types, as well as factors such as currents and localised topography that may act as a barrier to gene flow. Previous studies have all proposed schemes for biogeographic provinces within the deep sea (Zezina, 1997; Vinogradova, 1997; Briggs & Bowen, 2012). Subsequently, Watling *et al.* (2013) defined 14 lower bathyal and 14 abyssal provinces for the deep-sea benthos combined with the results of previous studies. This new scheme mainly considers regional variations in temperature, salinity and particulate organic carbon (POC) flux. As such, distinctive environmental conditions at the proposed province boundaries may act as barriers to gene flow (in a neutral genetic sense this type of environmental discontinuity is the most commonly invoked explanation of a barrier to gene flow). On the other hand, the boundaries between provinces provide an informative framework for testing the scales of genetic connectivity and whether the provinces may be considered as management units.

Despite the recognition of the importance of connectivity and the need to identify source and sink populations, not many genetic connectivity studies have been published for deep-sea ecosystems. This lack of study does not reflect a lack of appreciation of the deep sea itself, but arises because of the many technical limitations (logistics, sampling difficulties, but also including poor state of knowledge about the taxonomy of many deep-sea taxa and the historically poor state of preservation of archived samples) associated with working in the deep sea (Wandeler *et al.*, 2007; Miller *et al.*, 2011). In earlier deep-sea studies, much focus

has understandably been on the determination of taxonomic identity and evolutionary history, which are critical prerequisites for the identification of intraspecific populations for the pursuit of population genetic studies. However, a lack of biological (taxonomical identification) and distributional information for most species in deep-sea ecosystems means that genetic analyses are difficult to conduct and interpret at spatial scales that are meaningful to conservation.

To provide background information and to build hypotheses for this PhD project, in this chapter, a large number of deep-sea genetic studies were summarised and a large database of papers was compiled detailing population genetic connectivity of deep-sea species, from all regions of the world's oceans. The database was utilised, applying a meta-analysis approach, to determine if a general pattern of genetic structure exists and identify causative agents (factors) as barriers to gene flow amongst deep-sea taxa. Furthermore, comparisons of genetic connectivity amongst different habitats and between VME and non-VME indicator taxa were employed to help inform management decisions about the measures that may be most efficient for protecting VMEs.

2.2. Methods

2.2.1. Literature survey

Search terms “deep sea” and “genetic” were used in Google Scholar to compile a database of studies that documented studies of population genetics in the deep sea. Papers that were identified but that were not relevant research were manually excluded. Additional papers were added from the literature collections of colleagues and from the reference sections of the papers obtained through the search. All papers available for deep-sea taxa at the time of compilation (15 May 2016) were included, regardless of life-history strategy, marker type, habitat, or ecosystem. Species were considered to be deep-sea taxa if their depth range began or most samples were collected below 200 m depth. Microorganisms (not considered for marine conservation) and mammals (covered by a different conservation strategy) were not considered in this study.

In total, 196 population genetic or phylogeographic studies including the data from the present thesis, published from 1972 to 2016, were included in this review (publications listed in Table S2.1). When more than one species was included in a single publication (multi species cases) or when one species was examined by two or more different genetic markers

(multi marker cases), each case was considered separately. This selection procedure resulted in a total of 503 data lines in the database. Data in this database were included in a meta-analysis to answer the following questions about the population genetic structure and connectivity of deep-sea taxa: 1) What are the patterns of genetic structure that are reported? 2) Which factors are most likely to act as barriers to gene flow in the deep sea? 3) Which factors were identified as limiting the detection of barriers to gene flow? and 4) Which deep-sea habitats were genetically most isolated and therefore require the greatest attention in terms of future management and conservation?

2.2.2. Database compilation and variable selection

The database contained four sections - sampling, genetic markers, genetic results and factors contributing to the identification of barriers to gene flow as discussed by the paper authors (for details refer to subsections below). Information about these sections was extracted from the selected papers. If the information was not available (i.e., not given in the paper or could not be deduced or calculated from information in the paper) the parameter was recorded as a missing value. Information about DNA sequence length (e.g., the length in base pairs (bp) of a gene or DNA region) and the sample location (e.g., latitude and longitude) was checked in the National Center for Biotechnology Information (NCBI) database and on Google Earth, respectively, if the information was not stated in the paper.

Multiple factors compiled from the literature review present complex relationships, and do not work in isolation. That is, they are often interdependent such that changes in one factor may contribute to changes in another, producing cascading effects. Additionally, the interactions between environment and biology and research limitation generate obstacles to an in-depth appreciation of patterns of genetic connectivity and the identification of barriers to gene flow in the deep sea. With this in mind the data set was constructed to permit ease of identification of the most common patterns of genetic structure and also the major causes of barriers to gene flow.

2.2.2.1. Sampling

In the sampling section, minimum and maximum sampling depth, number of examined specimens (total number of specimens), number of sampled populations, and sample locations were recorded for each data line. Additionally, the code for the deep-sea

biogeographic province(s) relevant to the site sampling locations was introduced from Watling *et al.* (2013) to supplement the sampling location data, and the total number of provinces covered by the study was also included to test if the presence of a province boundary acted as a barrier gene flow, that is, to test the association between genetic structure and provinces boundary (Figure 2.1). Sampled area habitat types were classified for the deep sea as described by Oldham *et al.* (2014). Studies that did not describe the sampling habitat type were split into two categories, either continental slope if sampled at < 3000 m depth, or abyssal plain. Sampled habitat types were classified as abyssal plain (including plateaus), cold seep, hydrothermal vent, seamount, slope, and water column; there were too few studies dealing with trenches for this category to be included. Two records from food fall studies were assigned to where the whale carcasses (bones) were actually found, abyssal plain and seamount. When different habitat types were sampled within anyone study, the habitat type from which most samples were collected was used to represent all samples. The reason for this was that based on the limited information provided in many publications it was not possible to allocate individual samples to specific habitat types.

2.2.2.2. Genetic markers

In this section the type of marker, number of markers, the name of the marker (e.g. *COI*, *ITS*) and the length (base pairs, bp) of DNA sequence were recorded. Markers were sorted into type as allozyme, nuclear DNA sequence, mitochondrial DNA sequence, Amplified Fragment Length Polymorphism (AFLP), Randomly Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), Single Nucleotide Polymorphism (SNP), Single Strand Conformation Polymorphism (SSCP), and Microsatellite (SSR).

2.2.2.3. Genetic results

In this section the detection of a barrier to gene flow (binomial, yes or no), minimum and maximum values of nucleotide diversity (π), minimum and maximum values of genetic differentiation (Φ_{ST} (sequence marker) or F_{ST} (all other marker types)) were obtained from the studies. For the detection of a barrier to gene flow, if a publication reported a genetic barrier (the authors reported genetic structure(s) present), the barrier detection classification was “Yes”, otherwise the record was “No” (barrier undetected) – note, this variable is referred to hereafter as “genetic barrier”. When multiple values for genetic diversity and/or

differentiation were available in one publication by the same species, only the minimum and maximum values were recorded.

When only one value of these parameters was available this value was used as both the minimum and the maximum. Statistically non-significant values of F_{ST} (regardless of the size of the value) were set to zero because the focus here is on significant genetic differentiation. Factors that acted as genetic barriers as discussed in the publications were identified, and ranked according to their effects as either -1 or 1, with -1 standing for the factor was not considered as a genetic barrier and 1 meaning that it was considered as a genetic barrier.

Figure 2.1 Proposed lower bathyal (upper) and abyssal (below) provinces from Watling *et al* (2013)

2.2.2.4. Contributing factors

To identify factors that contributed to genetic isolation or connectivity (i.e., each factor has a binomial response, “Yes” or “No”, as to whether the study authors identified it as acting as a barrier to gene flow) currents (surface and deep currents, including eddies and gyres), distance, depth, seafloor topography, and temperature (mainly caused by hydrothermal systems and latitudinal variation) were categorised as environmental factors based on a review of the collected papers. In the database, for each factor, the value was set to 1 if it was considered as acting as a barrier to gene flow to explain the genetic structure, and to -1 when the factor when promoted genetic connectivity, and to 0 when the factor did not contribute to genetic differentiation or it was not discussed.

Factors related to biological aspects, broadly defined here to include, for instance, adult mobility potential, larval dispersal potential, reproductive strategy, evolutionary and population dynamic histories, were recorded. Because there were not enough published data to cover these individual categories, they were recorded as one lumped category. Very early on in the review it became apparent that sampling constraints, including factors such as insufficient specimens and invariant genetic markers, were closely related to the failure to detect barriers to gene flow. Accordingly, research limitations as described in the individual papers were identified as a separate category.

To provide general knowledge for management, vulnerable marine ecosystems (VMEs) were also considered. Criteria of VME indicator taxa (FAO, 2009) were employed to classify the investigated organism in each published study as being indicator taxa or not based on CCAMLR VME indicator taxa Classification Guide (CCAMLR, 2009). In VME determinations, if the sampling site contained seamount, hydrothermal vent, cold seep and Canyon (NAFO, <https://www.nafo.int/>), it was recorded as ‘Yes’, otherwise ‘No’. The same scoring approach was employed for the presence/absence of VME indicator taxa as recorded in each paper.

2.2.2.5. Data quality control and coding

All species’ scientific names were checked in the World Register of Marine Species database (WoRMS, <http://www.marinespecies.org>), and the synonymised name was replaced by the corrected name where necessary. In addition, information about the phylum of the study organism(s) of each published paper was included to complement the biological information. Finally, any additional information that appeared to be relevant (e.g., about

dispersal scale or reproductive mode) was recorded as a comment in the database to provide background knowledge for discussion, but this information was not included in the analyses, due to insufficient data.

In the database, phylum, location, deep-sea biogeographic province code, marker type, marker detail, and sampled area were all defined as categorical variables. Factors related to the detection of a barrier to gene flow (distance, depth, current, topography, temperature, biological factors, limitation), were coded as binary variables, as were VME indicator taxa (this category includes taxa such as corals and sponges that are typically associated with VMEs but in this case occur in non-VME habitats such as areas on the slope or abyss) and VME habitat. Variables such as minimum and maximum depth, nucleotide diversity, F_{ST} value, Φ_{ST} value, number of specimens, number of populations and genetic markers, and sequence length were coded as continuous variables.

2.2.3. Data analysis

Data checking, preliminary calculations (such as depth range which was calculated as maximum minus minimum depth), basic analyses (such as means, standard deviations and summary values) and the figures (plots) were all completed in Excel 2013. The density distribution charts were completed in R by package ‘ggplot2’. The meta-analysis was undertaken as described below.

2.2.3.1. Correlation analysis

Correlation analysis was conducted to reveal whether genetic barrier occurrence was different amongst different habitats between VME (hydrothermal vent, cold seep and seamount) and non-VME sites, and VME indicator taxa and non-VME indicator taxa (taxa list sees Chapter 1). Because the data for genetic barrier detection (binomial variable), habitat type (categorical variable), VME (binomial variable) and VME indicator taxa (binomial variable) were not numeric variables, cross tabulation (non-parametric) analysis was conducted to investigate whether barriers to gene flow occurred at different frequencies in different habitat types. All cross tabulation analyses were run in the software package IBM SPSS Statistics (v 22.0), and the χ^2 and Cramer’s V statistics were employed to test the relationships.

2.2.3.2. Logistic regression analysis

To quantify the contribution of multiple causative factors to the presence/absence of a genetic barrier, logistic regression analysis was employed to test for genetic barrier detection (barrier detected as 1 and undetected as 0) as an independent variable and factors (distances, depth, currents, topography, temperature, biological factors) as predictive variables. In this analysis the variable called “research limitation” (which includes factors such as low genetic marker variability, small sample sizes, restricted range of sampling, etc) was excluded because it does not help to explain how biological and physico-chemical and geological variables contribute to barrier detection. Sequential testing was employed to predict the relative contribution of factors to explain the presence of a genetic barrier (detection). Significant factors were more likely to contribute to a barrier for genetic connectivity in the deep-sea, whilst non-significant factors may promote genetic connectivity or be independent of barrier detection. Due to their non-significance in the primary analysis, joint effects (interactions) between variables were not considered in the logistic regression, which was run in R (v3.2.2).

2.2.3.3. General linear model (GLM) analyses

For all categories of genetic marker types, a general linear model was built to investigate the effects of sampling regime on genetic barrier detection. The categorical and continuous variables phylum, marker type, number of populations, number of biogeographic provinces, sample sizes, length for sequencing markers or number of loci for genotypic markers, minimum depth and depth range were defined as independent variables, and genetic barrier detection (Yes/No) was the dependent variable.

F_{ST} and Φ_{ST} values (dependent variables) were analysed separately using GLM with the independent variables being sample size, number of populations, number of genetic markers as covariates and phylum and marker type as fixed factors to determine the contribution of these independent variables to explaining variation in the genetic differentiation (F_{ST} or Φ_{ST}). This analysis is a supplementary test of genetic barrier presence based on a continuously distributed dependent value which is an estimate of genetic distance to the analysis described in the previous paragraph which is a categorical variable based on the presence or absence of a genetic barrier.

To determine if increased genetic variation is associated with a higher rate of barrier detection, Spearman (non-parametric) correlation analysis between standardised nucleotide

diversity and barrier detection ratios amongst different phyla was employed. To obtain the standardised nucleotide diversity, phylum and marker type as fixed factors and sample size (number of specimens examined in the study) and the length of DNA sequence (bp) as covariates were utilised to build the GLM to generate the standardised data.

In the present study, it was hypothesised that the larger depth ranges covered would result in a higher probability of genetic barrier detection. Minimum, maximum and depth range (subtraction of maximum and minimum) were compared between barrier detected and undetected groups. Standardised datasets of minimum, maximum and cover depth produced by GLM analysis with phylum and marker type as fixed factors and sample size and number of populations as covariates were compared to compensate for the variations between different studies. A *t-test* was used in the comparisons between detected and undetected groups, and the GLM analysis was based on the subset data that excluded the records with “invariance markers categorised in research limitations”.

Joint effects (interactions) between variables were considered in all GLM analyses, and all GLM and *t-test* analyses were run using the software package IBM SPSS Statistics (v 22.0).

2.3. Results and Discussion

2.3.1. Summary results

Published investigations covered all oceans, but more than one third of all papers was from the Pacific Ocean (188 of 503 records, 37.38%). The Indian Ocean was the least studied area (Figure 2.1). According to the deep-sea biogeographic province scheme of Watling *et al.* (2013), BY4 (North Atlantic Ocean including the Mediterranean Sea), and especially the Mid-Atlantic Ridge region, was the most researched biogeographic province (Figure 2.2) (25.05% of all records). The region with the lowest representation was the Southeast Pacific Ridges area, BY5 (1 of 503 records, 0.20 %). Research from other, deeper water provinces (AB1-14, 32 of 503 records, 6.36%) was much less common than research from shallow water provinces (BY1-14, 471 of 503 studies). None of the population genetics studies was conducted in the hadal provinces (HD 1-10).

Figure 2.2 The distribution of research covered by areas in deep-sea provinces (bar charts) and at an oceanic scale (pie chart)

In terms of vertical distribution (depth), most studies covered the mesopelagic and bathypelagic zones, and published reports for taxa below 2500 m were sharply reduced in number (Figure 2.3). More than 200 taxa belonging to 10 phyla were covered in these studies the Annelida (56), Arthropoda (164), Chaetognatha (2), Chordata (86), Cnidaria (75), Echinodermata (28), Foraminifera (14), Mollusca (67), Nemertea (1), and Porifera (10) (Table 2.1, Figure 2.4). As both genetic marker and ship-based sampling technologies have developed profoundly during the period from 1972 to 2016, and there has been a substantial increase in the number of studies published related to population genetic connectivity in the deep sea (Figure 2.5). Early studies used allozyme electrophoresis, and as methods have developed, later studies have used DNA sequencing and fragment analysis of DNA. More recently, still studies have used microsatellites and even single nucleotide polymorphisms (SNPs), as these techniques have become increasingly available and cost-effective.

Table 2.1 Number of species for which genetic connectivity estimates have been established

Phylum	Allozymes	Sequencing		Microsatellite	Others	Total
		Nuclear	Mitochondrial			
Nemertea	1					1
Chaetognatha		1	1			2
Porifera		2	8			10
Foraminifera		8	6			14
Echinodermata	9	1	18			28
Annelida	8	12	32	2	2	56
Mollusca	10	10	41	3	3	67
Cnidaria	3	23	36	13		75
Chordata	5		47	27	7	86
Arthropoda	14	31	106	9	4	164
Total	49	88	296	54	16	503

Figure 2.3 Covered depths for each record (lines from minimum to maximum) (top) and accumulated density of examined studies (bottom)

Figure 2.4 Genetic barrier detection distributions across different phyla

Blue is number of cases detected and orange is number of cases not detected

Figure 2.5 Summary of different marker types and the chronological distribution of their usage according to collected records

Numbers indicate number of records per marker type.

2.3.2. Patterns of genetic structure reported amongst deep-sea taxa

It has often been pointed out that in theory global hydrodynamic currents could promote the dispersal ability of larvae (even adults) to broadcast all around the world and thereby form a global pattern of genetic connectivity (McClain & Hardy, 2010). In addition, deep-sea species may be widely distributed because of strong adult mobility or a long larval dispersal period to overcome oceanographic and topographic barriers, and these factors may generate genetic exchange across long distance over time (Shank, 2010). Despite potential long-distance dispersal and gene flow amongst different populations, it is now recognised that the full genetic exchange of many species amongst different geographic populations is not achieved for several different reasons (this is the difference between potential versus realised dispersal (Reisser *et al.*, 2014).

Various patterns of genetic structures amongst different species have been reported. These patterns of genetic structure at different spatial were tested based on the sampled area, that is, at different spatial scales that mainly including global, oceanic, regional (within ocean amongst provinces) and fine (within province) scale (Figure 2.6).

Figure 2.6 Testing structures at different spatial scales amongst collected studies

Global scale, samples collected from at least four oceans; Oceanic Scale, samples collected from 2 to 3 oceans; Regional scale, samples collected from the same ocean but in different biogeographic provinces; Fine scale, samples collected within the same province.

2.3.2.1. Globally homogeneous - panmixia

To test presence/absence of population genetic structure at a global scale (at least four oceans) requires a large number of samples that cover all, or at least most of, the world's oceans. Practically, this is difficult to achieve because of the challenges of sampling logistics and because of the uneven distribution of organisms at a global scale (whilst some taxa are cosmopolitan in their distribution, most are not). In the database, only 11 records were examined for genetic structure at a global scale, and all employed DNA sequencing. Three of these records (*Hippasteria phrygiana* revealed by *ATPS* α , *Epistominella exigua* revealed by *12S*, *Epistominella exigua* revealed by *ITS*) did not report evidence of genetic structuring at a global scale (Lecroq *et al.*, 2009; Foltz *et al.*, 2013). However, Foltz *et al.* (2013) did detect evidence of significant genetic structure in *H. phrygiana* for *COI*, suggesting that lack of genetic structure at *ATPS* α at the global scale was because the latter genetic marker was insufficiently informative. For *E. exigua*, no structure was also detected at an oceanic scale in other studies (Brandt *et al.*, 2007; Pawlows *et al.*, 2007), but all of them used *12S* or *ITS* as the genetic markers. Overall, 9 records detected genetic differentiation at the global scale, and only 2 of 11 did not detect global structure, but more samples and different markers are required to prove globally homogeneous status, that is, panmixia.

2.3.2.2. Oceanic scale

Seventy-one records related to analyses conducted at the oceanic scale, across two or three oceans. Of these, 43 records (60.56%) reported significant genetic differentiation across a range of phyla, including the Arthropoda, Chaetognatha, Chordata, Echinodermata, Foraminifera, Mollusca and Porifera. Amongst these studies, the population genetic structure at the oceanic scale was always reported as arising from evolutionary isolation.

Comparing between different oceans, 28 records reported found no genetic structure between oceans across various taxa and different types of markers. This pattern might reflect the fact that deep-sea animals can maintain genetic connectivity at very broad scales. However, more samples and different markers are required to exclude the possibility that the absence of genetic structure was not caused by technical issues such as invariant markers and/or limited sample sizes.

2.3.2.3. Regional scale

At the regional scale, that is, amongst biogeographic provinces but within the same ocean, of the total 95 records, 58 (61.05%) reported evidence of genetic structure between provinces. Although 37 records reported no genetic structure, of these studies 15 records reported potential issues with small sample sizes, which potentially prevented the detection of genetic structure if it existed.

2.3.2.4. Fine scale structure within biogeographic provinces

Amongst the four different spatial scales examined, studies within biogeographic provinces were the most abundant, representing nearly two of every three records (65.61 %). Significant genetic structure was reported in more than half (54.85%) of these records. Amongst the records of no genetic structure (149 in total), 78 records of them indicated that the absence of structure might be attributable to small sample sizes.

2.3.3. Factors effecting genetic connectivity

Different genetic structural patterns amongst different taxa are likely to be influenced by the number and different types of habitats at different scales through a mass environmental effect (Shmida & Wilson, 1985). It is, however, not clear how numerous environmental factors act to influence genetic connectivity in deep-sea communities.

The existence of one or more genetic barriers (Figure 2.7) was reported in 43.34% of the 503 records that were reviewed. Of these, in 31.65% of the cases the barrier was caused by depth, 23.39% by biological factors, 20.18% by distance, 19.26% by seafloor topography, and 16.51% by currents. Amongst the genetic barrier undetected records, more than one third of the records (39.30%) revealed that small sample sizes and/or non-informative genetic marker type may have contributed to the failure to reveal population genetic differentiation and a barrier to gene flow. About one third (31.58%) of all of the records indicated that pelagic larval duration (PLD) contributed to genetic connectivity over different spatial scales, whilst 3.86% reported that currents assisted larval dispersal (Figure 2.7). Furthermore, in the genetic barrier detected records, 10.49% reported that distance was not a genetic isolating factor and 7.49% indicated that depth was not a barrier to gene flow.

Figure 2.7 Summary of contributions of individual effects amongst categorised factors for genetic barriers detection

The number indicates the percentage of barriers detected (middle pie chart) and the colour represents the detected (blue) and undetected barrier (orange). The number indicates the percentage of each categorised factors contributing to genetic barrier absence (left pie chart) and presence (right pie chart).

2.3.4. Validation of factors

As summarised and discussed above, the effects of each factor on genetic connectivity were complex. Whilst currents and life-history characteristics could both weaken and strengthen genetic connectivity, other factors were also reported that may cause genetic isolation or independence from shaping genetic connectivity. However, whether these factors were related to genetic barriers and how important these factors are as barriers have not been tested yet.

In logistical regression analysis, the whole model was significant ($p < 0.0001$) and the estimates for each factor except life-history characteristic were positive. This means that the factors identified in this study were significantly related with genetic barriers. The quantifying of the contribution to barrier detection indicated that depth, currents and topography were the variables that contributed to the formation of a barrier for genetic connectivity and that biological factors tended to promote genetic connectivity at different spatial. In the sequential test for the regression, depth (after adding depth, the residual deviance was greatly reduced) contributed most in the model amongst the reviewed studies, implying that depth was the most common barrier for deep-sea organismal gene flow. Although temperature and biological factors did not contribute significantly to the barrier detection, each of their residual deviances was reduced after its addition to the model (Table 2.2). Further and detailed discussions are given in the following paragraphs.

Logistic regression analysis results indicated that currents were significant ($p = 0.037$), whereas sequential testing indicated that the contribution of currents to the model was borderline non-significant ($p = 0.062$) (Table 2.3). Overall, these results suggest that currents do not play a major role as barriers to gene flow amongst deep-sea taxa.

Table 2.2 Logistical regression analyses of the contribution of different factors to the detection of barriers to gene flow amongst multiple deep-sea taxa

Variables	Estimate	Std. Error	z value	p
Intercept	1.443	0.125	11.523	< 0.001 ***
Distances	0.437	0.290	1.509	0.131
Depth	0.937	0.306	3.060	0.002 **
Currents	0.722	0.346	2.089	0.037 *
Topography	0.908	0.397	2.285	0.022 *
Temperature	0.351	1.098	0.319	0.749
Biological factors	-0.340	0.219	-1.551	0.121

Significant codes were <0.001 '***', 0.001 '**', 0.01 '*'.

Table 2.3 Sequential test for factors to barrier detection

Adding variables	Df	Deviance	Resid. Df	Resid. Dev	p
Null			502	447.310	
Distance	1	3.300	501	444.020	0.069
Depth	1	9.882	500	434.130	0.002*
Currents	1	3.495	499	430.640	0.062
Topography	1	5.820	498	424.820	0.016*
Temperature	1	0.106	497	424.710	0.745
Biological factors	1	2.419	495	421.590	0.120

Significant codes were <0.001 ‘***’ 0.001 ‘**’ 0.01 ‘*’.

2.3.4.1. Horizontal distance

Of the 95 records that discussed distance effects, 28 records reported that genetic connectivity patterns did not conform to an isolation by distance pattern (i.e., distance was not a genetic barrier) (Figure 2.7). The logistical regression and sequential test showed that distance was non-significant in the model, which indicated that distance was not a main factor acting as a barrier to gene flow (Table 2.2 and Table 2.3).

Isolation by distance (IBD) is the tendency of populations that are geographically closer to be more genetically similar than populations that are further apart. Both dispersal variance and migration probabilities may contribute to local genetic differentiation (Ishida, 2009). Genetic similarity across distance for deep-sea organisms is most likely to be attributable to currents, especially deep currents (or near-bottom flow), which have pervasive effects because they can influence deep-sea larval and adult dispersal directly. This may explain why the distance was not a significant contributor for barrier detection in logistical and sequential tests. Unlike many shallow water invertebrates, large horizontal distances (100s of km) do not act as barriers to gene flow for several deep-sea species, such as *Argyropelecus hemigimnus* (Chordata) (Gordeeva, 2014), *Austrolaenilla pelagica* (Annelida) (Neal *et al.*, 2014), *Callogorgia americana delta* (Cnidaria) (Quattrini 2014), *Gigantopelta chessoia* (Mollusca) (Roterman *et al.*, 2016) and *Kiwa tyleri* (Arthropoda) (Roterman *et al.*, 2016). This observation may be related to the longer PLD for deep-sea species compared to shallow and eurybathic species (Hilário *et al.*, 2015).

In the present review, 18 studies revealed an IBD pattern within a single deep-sea biogeographic province (i.e., within an environmentally similar region). The reason that fewer deep-sea than shallow water species exhibited a pattern of IBD may be explained by the double constraint of small habitat patch sizes in the deep sea (e.g., seamount, vent and seep sites) and different reproduction and dispersal strategies (Selkoe *et al.*, 2014).

2.3.4.2. Depth and associated gradients

In the present study, 87 records reported depth-associated genetic differentiation in various habitats, including the slope, seamounts, and hydrothermal vents. Amongst collected studies that detected genetic barriers, depth was the most common factor acting as a barrier for gene flow (solely for about 24% and jointly for about 9% of all studies) (Figure 2.7). Both the results of the logistical and sequential test agreed that depth was a significant contributing explanatory factor to the detection of a barrier to gene flow ($p < 0.01$) (Table 2.2 and Table 2.3).

In the deep sea, as depth increases, so the pressure, salinity and density also increase, but the temperature, dissolved oxygen and alkalinity decrease. Eventually, at hadal depths (> 6000 m) the environment is more stable, with the cold temperature, high pressure and less alkaline pH (Pibernat *et al.*, 2007). This different environment from shallow seas puts deep-sea organisms under strong ecological pressure, which may lead to a change in the composition of genes within a species and between shallow and deep populations. For example, whole genome comparison between a shallow-water urchin *Strongylocentrotus purpuratus* and a deep-sea urchin *Alloccentrotus fragilis* has illustrated that genes have undergone selection for the deep sea (Oliver *et al.*, 2010), supporting the hypothesis that depth-derived selection may cause divergence of morphology and genetic mutations.

Except for different environment genetic selection, the gradients associated with depth were restricted to the vertical distributions of deep-sea organisms, and this discontinuous distribution indirectly affected gene flow. For example, deep-sea stony coral survival and colonisation potential may be related to the concentrations of O_2 and CO_3^{2-} (Thiagarajan *et al.*, 2013, *Desmophyllum dianthus*), and to temperature (Brooke *et al.*, 2013, *Lophelia pertusa*). The ongoing genetic divergence and larval dispersal disconnection caused by depth and associated deep-sea environmental gradients ultimately separate the gene pools of taxa at different depths.

The depth at which genetic breaks occurred for different species varied a lot across taxa. There were two patterns of depth-associated barriers: a single barrier present and multi breaks across the vertical distribution. In both the North and South Atlantic populations of the deep-sea bivalve *Deminucula atacellana* above and below approximately 3,300 m were observed to be genetically distinct, whilst within these depth regimes the populations showed little divergence (Chase *et al.*, 1998). Similar cases of depth differentiation have been detected in bathyal brittle-stars (O'Hara *et al.*, 2014), the coral *D. dianthus* (Miller *et al.*,

2011), the amphipod *Eurythenes gryllus* (France & Kocher, 1996; Havermans *et al.*, 2013), and the seastar *Zoroaster fulgens* (Howell *et al.*, 2004). For multi barriers patterns, significant differences were found amongst echinoderms at 250-m depth intervals for the *Asteroschema clavigera* and at 100-m depth intervals for *Ophiocreas oedipus* (Cho & Shank 2010).

However, depth does not appear to be an important structuring factor in several large-scale studies, including those of *Paragorgia arborea* (Herrera *et al.*, 2012), *Bathybembix bairdii* (Siebenaller, 1978) and *Frigidoalvania brychia* (Quattro *et al.*, 2001), all of which exhibited high genetic similarity amongst different depth classes. As far as it is possible to state at the moment, the relationship between depth and genetic differentiation is species-specific. Besides, amongst those studies that revealed that depth was not a barrier to gene flow, most had some sort sampling bias (all of them covered a sampling depth range of <1,400 m). To verify whether sampling bias caused failures to reveal depth-associated barriers, a further investigation was conducted in research limitations.

2.3.4.3. Oceanic currents

In the reviewed literature, 64 records considered that currents affected connectivity patterns, with currents having both positive and negative effects on population connectivity (Figure 2.7). The positive (promoting gene flow, 14 records) and negative (acting as a barrier to gene flow, 26 records) aspects of currents may explain why the factor currents was found to contribute significantly to barrier detection (logistical regression) but not contribute significantly to being a barrier (sequential test) to genetic connectivity in the present analysis (Table 2.2 and Table 2.3).

Oceanic currents are complex, derived from many factors, and it is this complexity that makes assessing genetic connectivity very difficult. Currents may not only bring food, nutrients and other resources to deep-sea taxa, but currents may also directly contribute to the dispersal of species through larval supply, and also at sufficiently high speeds will erode the sediments and many of the organisms on or within them (Levin *et al.*, 2001; Watanabe *et al.*, 2009). Furthermore, in the Horizontal Distances section, currents were considered as the factors that assisted long-distance larval dispersal. Also, ocean currents may, through their influence on larval transport and recruitment, interact with the genetic consequences of the large variance in individual reproductive success to generate patterns of fine-scale patchy genetic structure (Banks *et al.*, 2007).

This two-sided aspect of currents (promoting and hindering the connectivity) may be demonstrated by two examples, the Antarctic Circumpolar Current (ACC) and equatorial currents in East Pacific. The ACC is an example of how a current may act as a conveyor belt for connectivity. In the present study, 57.14% records were reported as being genetically homogeneous in the region of the Antarctic Convergence. ACC connects the southern regions of the Atlantic, Pacific and Indian oceans, and serves as a principal pathway of exchange amongst them, promoting larval dispersal. Therefore, it is not surprising that with assistance of the ACC the taxon-specific populations were found to be genetically homogeneous within this region (biogeographic province BY9). In contrast, in the deep-sea province BY7 (the Cocos Plate), extensive genetic differentiation along the East Pacific Rise has been reported that is related to equatorial currents (Bucklin, 1988; France *et al.*, 1992; Craddock *et al.*, 1997; Won *et al.*, 2003; Hurtado *et al.*, 2004; Matabos *et al.*, 2008; Plouviez *et al.*, 2009, 2010, 2013; Coykendall *et al.*, 2011). In other words, the equatorial currents impeded the bilateral connection of larval from north and south of the equator.

Arellano *et al.* (2014) provided direct evidence that the long-lived larvae of two cold-seep molluscs (*Bathymodiolus childressi* and *Bathynnerita naticoidea*) migrate hundreds of meters above the ocean floor. In this way, both deep water and shallow water currents may transport the larvae of deep-sea taxa, highlighting the interactions between biological factors such as larval vertical behaviour and physical oceanography. For example, larvae may exhibit behavioural adaptations that allow them to move up into surface waters thereby taking advantage of the faster moving surface currents (compared to deeper currents) that assist deep-sea organisms in their dispersal.

2.3.4.4. Seafloor topography

In the database, 39 records (33 solely and 6 with other factors) revealed that topography may act as a barrier to gene flow (Figure 2.7). In the logistical and sequential analyses, a significant contribution of topography was detected in both analyses (Table 2.2 and Table 2.3), which indicated that topography is an important factor acting as a genetic barrier.

Topography may shape directly the deep-sea animals' settlement and colonisation, as well as indirectly through modification of other environmental factors (such as sediments) (Gage, 1996). From the collected publications, 3 studies conducted within any one basin reported that study populations were all genetically homogeneous (but note that number of

sampled populations and number of sampled individuals may be small): not surprisingly, populations from different basins were more likely to be genetically differentiated (but no significant IBDs detected). This result may be interpreted, at least in part, as indicating a lack of topographic features as barriers to larval dispersal within any one deep-sea basin. In contrast, topographic features, especially pronounced changes in bathymetry (e.g., ridges) are associated with barriers to genetic connectivity for deep-sea animals. For instance, the Strait of Gibraltar with its shallow depth acts as a barrier for genetic connectivity between the deeper basins either side of it, the North Atlantic Ocean and the Mediterranean Sea (e.g., Catarino *et al.*, 2015), and the Florida Straits also act to constrict animal movements northward (e.g., Portnoy *et al.*, 2014).

2.3.4.5. Temperature

In the present study, only 7 records made mention of the influence of temperature on genetic connectivity and the logistical and sequential tests indicated that temperature does not explain or contribute to the presence of a barrier to gene flow amongst deep-sea taxa (Figure 2.7, Table 2.2 and Table 2.3).

Temperature, which is not independent of latitude, hydrothermal system or depth, has important implications for species' distributions and genetic barrier formation (Jorde *et al.*, 2015). In ophiuroid populations, genetic connectivity may be adapted to, or at least related to different temperatures, with phylogeographical breaks occurring between tropical, temperate and polar regions (O'Hara *et al.*, 2014). However, a recent modelling approach focusing on ophiuroid diversity has suggested that this pattern might be driven by energy availability (Woolley *et al.*, 2016), which in turn, may not be completely independent of sea surface temperature. These indirect contributions to genetic connections might explain why temperature was non-significant in both analyses.

2.3.4.6. Biological factors

In this study, 116 records pointed out that biological factors contribute directly to promoting genetic connectivity amongst populations at different scales. Meanwhile, 39 records reported that life-history characteristics limited the genetic connections between populations (Figure 2.7). Both the logistical and sequential tests supported the hypothesis that biological factors related to life-history characteristics were not likely to be a genetic barrier ($p > 0.05$) (Table 2.2 and Table 2.3).

Although information about mode of reproduction and larval dispersal strategies is important to help interpret the connectivity of different populations, biological knowledge of this sort for most deep-sea species is still unknown. Although a further categorised analysis of the variables in the group “biological factor” was not possible in the present study, three aspects were identified from the collected studies to explain positive and negative effects on genetic connectivity: larval dispersal and reproduction, population history, and adult mobility and body size.

2.3.4.6.1. Larval dispersal and reproduction

Biogeographic patterns are generally considered to be linked to larval ecology, because organisms with low dispersal capabilities (and hence reduced gene flow) are expected to have restricted distributions. In the present study, a longer life-duration and a prolonged pelagic larval phase amongst most teleost fishes suggested a high potential for connectivity and gene flow between different geographic populations through dispersal (e.g., *Aphanopus carbo* (Longmore *et al.*, 2014), and *Beryx decadactylus* (Friess & Sedberry, 2011)). Lecithotrophic species have relatively few eggs and a short larval period compared to planktotrophic species, but planktotrophic larvae are constrained by the availability of food (Young, 2003), and it is expected that species that possess these two different larval types will ultimately have different connectivity patterns. This presumption has subsequently been demonstrated by O’Hara *et al.* (2014) for five deep-sea ophiurids. Factors such as inbreeding, self-recruitment and asexual reproduction all help to maintain low genetic diversity within subpopulations and contribute to high genetic variability from site to site. For example, in *Lophelia pertusa*, local recruitment of larvae leads to limited gene flow amongst populations (Le Goff-Vitry *et al.*, 2004). Also, higher levels of genetic differentiation (80%) were detected in Annelida compared with other phyla. This finding for Annelida may be related to their trochophore larval stage, and it also could explain why distance was reported to be a genetic barrier for most annelids.

2.3.4.6.2. Population history

In the evolutionary origin of deep-sea taxa, genetic subdivision may arise across ocean expanses following radiation and dispersal events and also during major geological and mass extinction events (Herrera *et al.*, 2012, 2015). These different evolutionary histories are important in explaining various forms of pronounced differentiation between populations in

close geographic proximity. In addition, a relatively recent population bottleneck or speciation event followed by limited migration and the beginnings of regional divergence may result in limited differentiation or even genetic homogeneity. For example, hydrothermal shrimp, *Rimicaris exoculata*, was reported to have undergone a recent population expansion and connectivity along the Mid-Atlantic Ridge (Creasey *et al.*, 1996; Petersen *et al.*, 2010; Teixeira *et al.*, 2012).

2.3.4.6.3. Adult mobility and body size

Mobility of adults affects genetic connectivity. In giant squid (*Architeuthis dux*), a cosmopolitan species with high adult mobility, there was no detectable phylogenetic structure at the mitochondrial level (Winkelmann *et al.*, 2013). Similarly, low levels of global genetic differentiation were reported in the deep-sea teleost *Hoplostethus atlanticus* (Smith *et al.*, 1996b; Varela *et al.*, 2012, 2013). Great swimming ability of this species is expected to achieve population exchange and the formation of a metapopulation.

Small body size may make for easily transported propagules that contribute to high levels of connectivity and a global distribution. For instance, foraminifera are a common and cosmopolitan member of the microfauna in the deep sea. *Epistominella vitrea* was collected from a depth range of 30 to 4803 m, but no genetic differentiation was detected at different depths or amongst geographic populations based on data from different marker types (Pawlowski *et al.*, 2007b). Similar results were found in other foraminifera, including *Epistominella exigua*, *Cibicides wuellerstorfi* and *Oridorsalis umbonatus* (Brandt *et al.*, 2007; Pawlowski *et al.*, 2007b; Lecroq *et al.*, 2009), suggesting that small body size contributes to easy transport and high genetic connectivity.

2.3.5. Research limitations

The GLM analyses of sampling regime (Table 2.4) showed that the variables depth range, number of bio geographic provinces, number of populations, phylum, marker type and sample size were significant in explaining variation in genetic barrier detection ($p < 0.05$) (Table 2.4). The GLM analyses of F_{ST} (Table 2.5) and Φ_{ST} (Table 2.6) showed that not all the sampling parameters influenced the minimum value of genetic differentiation (F_{ST} for genotypic markers and Φ_{ST} for sequencing markers). However, the maximum value of F_{ST} was significantly affected by phylum, marker types and their interactions, and Φ_{ST} was

significantly affected by sample size, number of populations and phylum. As discussed below, these parameters were mainly related to two aspects of genetic markers and sampling.

Table 2.4 General linear model analyses of sampling regime on genetic barrier detection

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected model	22.774	48	0.474	2.279	0.000
Intercept	1.920	1	1.920	9.225	0.003
Minimum depth	0.019	1	0.019	0.093	0.761
Depth range	0.892	1	0.892	4.286	0.039
Total Samples	0.146	1	0.146	0.700	0.404
Number of populations	0.232	1	0.232	1.114	0.292
Number of provinces	1.405	1	1.405	6.751	0.010
DNA sequenced length/number of loci	0.106	1	0.106	0.507	0.477
Phylum	6.287	8	0.786	3.775	0.000
Marker type	0.471	2	0.236	1.132	0.324
Habitat	1.288	6	0.215	1.032	0.405
Phylum * Marker type	2.858	7	0.408	1.961	0.060
Phylum * Habitat	3.831	12	0.319	1.533	0.112
Marker type * Habitat	0.546	4	0.136	0.655	0.624
Phylum * Marker type * Habitat	0.581	3	0.194	0.930	0.427
Error	58.706	282	0.208		
Total	186.000	331			
Corrected Total	81.480	330			

R Squared = 0.280 (Adjusted R Squared = 0.157).

2.3.5.1.1. Genetic markers

Choice of appropriate markers is always a concern for genetic studies. In studies involving assessments of patterns of multi-species genetic variability, concerns about the comparability of genetic data have arisen where analyses of different markers in a single species have produced conflicting results (Ayala & Valentine 1974; Craddock *et al.*, 1995; Hensley *et al.*, 1995; Jollivet *et al.*, 1995; Hurtado *et al.*, 2004; Plouviez *et al.*, 2009, 2013) or inconsistent results (Abbiati *et al.*, 1993; Costantini *et al.*, 2007, 2010). These different patterns of response may arise because of differences in sample size and/or with the variability of the different marker types (i.e., the inherent properties of specific marker types).

Amongst the collected studies, no significant Spearman correlation coefficients were observed between nucleotide diversity (neither minimum nor maximum, standardised or unstandardised) and barrier detection ratios (Table 2.7). This result might indicate that genetic structures varied across the different species because the standardised nucleotide diversity values excluded sampling effects.

Table 2.5 Different sampling parameter effects on F_{ST} measures

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	F_{ST} Min	0.162 ^a	21	0.008	1.994	0.025
	F_{ST} Max	2.281 ^b	21	0.109	4.422	0.000
Intercept	F_{ST} Min	0.002	1	0.002	0.632	0.431
	F_{ST} Max	0.026	1	0.026	1.054	0.310
Minimum depth	F_{ST} Min	0.000	1	0.000	0.064	0.802
	F_{ST} Max	0.002	1	0.002	0.082	0.775
Depth range	F_{ST} Min	0.002	1	0.002	0.525	0.472
	F_{ST} Max	0.001	1	0.001	0.031	0.862
Sample size	F_{ST} Min	0.000	1	0.000	0.017	0.898
	F_{ST} Max	0.003	1	0.003	0.111	0.740
Number of populations	F_{ST} Min	0.000	1	0.000	0.006	0.941
	F_{ST} Max	0.007	1	0.007	0.301	0.586
Number of provinces	F_{ST} Min	0.001	1	0.001	0.168	0.684
	F_{ST} Max	0.006	1	0.006	0.236	0.629
Number of loci	F_{ST} Min	0.002	1	0.002	0.588	0.447
	F_{ST} Max	0.023	1	0.023	0.944	0.336
Phylum	F_{ST} Min	0.025	4	0.006	1.613	0.186
	F_{ST} Max	0.449	4	0.112	4.574	0.003
Marker type	F_{ST} Min	0.048	5	0.010	2.470	0.045
	F_{ST} Max	1.038	5	0.208	8.455	0.000
Phylum * Marker type	F_{ST} Min	0.049	6	0.008	2.121	0.068
	F_{ST} Max	0.381	6	0.063	2.582	0.030
Error	F_{ST} Min	0.186	48	0.004		
	F_{ST} Max	1.179	48	0.025		
Total	F_{ST} Min	0.406	70			
	F_{ST} Max	5.368	70			
Corrected Total	F_{ST} Min	0.349	69			
	F_{ST} Max	3.460	69			

a. R Squared = 0.466 (Adjusted R Squared = 0.232). b. R Squared = 0.659 (Adjusted R Squared = 0.510).

Table 2.6 Different sampling parameter effects on Φ_{ST} measures

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	Φ_{ST} Min	.546 ^a	18	0.030	0.653	0.845
	Φ_{ST} Max	3.071 ^b	18	0.171	2.821	0.001
Intercept	Φ_{ST} Min	0.045	1	0.045	0.977	0.326
	Φ_{ST} Max	0.747	1	0.747	12.348	0.001
Minimum depth	Φ_{ST} Min	0.000	1	0.000	0.009	0.926
	Φ_{ST} Max	0.069	1	0.069	1.141	0.289
Depth range	Φ_{ST} Min	0.000	1	0.000	0.001	0.973
	Φ_{ST} Max	0.008	1	0.008	0.133	0.716
Sample size	Φ_{ST} Min	0.096	1	0.096	2.067	0.155
	Φ_{ST} Max	0.720	1	0.720	11.901	0.001
Number of populations	Φ_{ST} Min	0.077	1	0.077	1.650	0.203
	Φ_{ST} Max	0.790	1	0.790	13.068	0.001
Number of provinces	Φ_{ST} Min	0.038	1	0.038	0.810	0.371
	Φ_{ST} Max	0.022	1	0.022	0.360	0.550
Sequence length	Φ_{ST} Min	0.001	1	0.001	0.011	0.917
	Φ_{ST} Max	0.001	1	0.001	0.013	0.909
Phylum	Φ_{ST} Min	0.190	6	0.032	0.679	0.667
	Φ_{ST} Max	0.860	6	0.143	2.370	0.038
Marker type	Φ_{ST} Min	0.000	1	0.000	0.008	0.929
	Φ_{ST} Max	0.002	1	0.002	0.025	0.874
Phylum * Marker type	Φ_{ST} Min	0.009	5	0.002	0.037	0.999
	Φ_{ST} Max	0.390	5	0.078	1.290	0.278
Error	Φ_{ST} Min	3.394	73	0.046		
	Φ_{ST} Max	4.416	73	0.060		
Total	Φ_{ST} Min	5.817	92			
	Φ_{ST} Max	20.669	92			
Corrected Total	Φ_{ST} Min	3.940	91			
	Φ_{ST} Max	7.487	91			

a. R Squared = 0.139 (Adjusted R Squared = -0.074). b. R Squared = 0.410 (Adjusted R Squared = 0.265).

Table 2.7 Average nucleotide diversity of mitochondrial and nuclear genes

Phylum	Size	Reported		Standardised		Barrier detection rate (%)
		Min	Max	Min	Max	
Annelida	20	0.0004	0.0584	0.008±0.002	0.011±0.006	80.95
Arthropoda	75	0.0000	0.1568	0.004±0.001	0.020±0.004	52.90
Chaetognatha	1	0.0220	0.0540	0.022±0.009	0.054±0.023	100.00
Chordata	41	0.0002	0.0728	0.009±0.001	0.011±0.004	55.32
Cnidaria	30	0.0000	0.1680	0.004±0.002	0.020±0.004	40.68
Echinodermata	17	0.0000	0.0125	0.004±0.005	0.008±0.012	84.21
Foraminifera	6	0.0000	0.0019	0.000±0.004	0.000±0.009	50.00
Mollusca	33	0.0000	0.0315	0.004±0.002	0.008±0.005	44.00
Porifera	7	0.0000	0.0758	0.001±0.004	0.043±0.009	50.00

2.3.5.1.2. Sampling

It is appropriate to accept evidence of no genetic structure only when appropriate molecular markers are employed, when the sample size is large enough, and when the location of sampled populations encompasses the full extent of the known distribution of the taxon in question. In practice, these criteria are rarely met in most deep-sea studies. In the collected data, the mean and median sample sizes were 24.63 and 16.00 individuals per population, respectively (Figure 2.8). Interestingly, 84 of 216 records (38.89%) that revealed a genetic differentiation were based on less than 10 individuals per population. Sample size as an independent variable did not explain significant variation in either F_{ST} minimum or F_{ST} maximum values (Table 2.5), and nor did it explain significant variation in Φ_{ST} minimum although it did explain significant variation in Φ_{ST} maximum values (Table 2.6).

Figure 2.8 Average sample size with depth distribution of reviewed studies

Histogram on top is density of the average sample size, and histogram on the right is density of the average depth.

For deep-sea genetic studies, samples from different depth collections were important for barrier detection. In the reviewed studies, a greater depth range of sampling resulted in a greater detection rate of genetic differentiation, i.e., depth range is strongly associated with an ability to detect a barrier to gene flow (Table 2.8). The GLM analysis showed that the records of detected barriers had a greater depth range than undetected records ($p < 0.01$) (Table 2.8). In addition, the number of bio geographic provinces sampled was also significantly associated with barrier detection ($p < 0.01$), that is, more provinces sampled the greater the chance to detect one or more genetic barriers (Table 2.8). This suggests that genetic barriers may be associated with province boundaries (rather than with distance *per se* because, as noted previously, distance did not explain significant variation in barrier detection).

Table 2.8 Detection of a barrier to gene flow as a function of depth

Depth barriers	Unstandardised (SD)		Standardised (SE)	
	Detected	Undetected	Detected	Undetected
Min	741.579±751.928	615.163±654.051	734.011±56.269**	826.930±78.731
Max	2051.842±1154.352	2480.07±1467.091	2817.380±109.970**	2656.391±153.869
Depth Range	1310.263±1240.331	1864.907±1283.325	2083.368±113.658**	1829.461±159.028

For population genetic studies, a greater sample size and more populations sampled would tend, on average, to provide more information and to provide more spatially explicit detail. However, a major challenge in studying population genetics in the deep sea is to obtain material that can be used for genetic analysis, given the costs and constraints of sampling. This is especially true for microfauna that are extremely small, difficult to separate from sediment, and are typically fixed in formalin which degrades DNA (Chase *et al.*, 1998). The other disappointing aspect of sampling limitation is that most old specimens are not suitable for DNA extraction. For example, Miller *et al.* (2010) were unable to get sequence data from scleractinians more than 5-6 years old unless the specimens had been ultra-frozen. Similarly, Boschen *et al.* (2015) were only able to obtain reasonable quality DNA from 150 of 942 (~16%) archived vent mussels. Although sampling has been problematic in the past, new technologies for both sampling, such as new remotely operated vehicles (ROVs) and autonomously underwater vehicles (AUVs), and genetic research such as next-generation sequencing, may eventually help to elucidate patterns of genetic connectivity in the deep sea.

2.3.6. Barrier occurrences amongst habitat types

Amongst different habitat types, the continental slope was a relatively well-studied area (48.7% of all studies), and the water column was the least researched area (3.4%) (Figure 2.9). The χ^2 test results for barrier detection and the sampled areas showed that barrier detection was significantly different amongst the different habitat types ($p < 0.01$), which indicated that the occurrence of genetic difference was at least in part a function of different sampled efforts within the different habitat types. The rate of barrier detection decreased sequentially according to habitat type, from cold seep, water column, hydrothermal vent, slope, seamount and abyssal plain. All studies in the review reported that the high percentage of barriers to gene flow detected in cold seep and water column habitats were caused by biological factors that were presumably evolutionary adaptations to the habitat in question (e.g. Cunha *et al.*, 2012; Laakmann *et al.*, 2012; Cowart *et al.*, 2013; Faure *et al.*, 2015). Because limited research has been conducted in these habitats, it was difficult to draw further conclusions about the mechanisms that may result in isolation for these two open ecosystems.

In the present review, it was found that 66.32% of cases from hydrothermal vents and 75.00% of cases from seeps were genetically isolated. However, amongst 109 hydrothermal vent records in this study, only 6 records reported that the thermal system was the main factor that contributed to the isolation. The high variability of environmental conditions at hydrothermal vents (steep thermal gradients and high chemical heterogeneity) and the constant modification of the hydrothermal vent landscape due to the fluid displacement has led to recurrent ‘crash and flush’ in populations that is likely to promote genetic structure and cause localised low genetic diversity (Faure *et al.*, 2009). In other studies of vents, distance and currents were more important than habitat type in explaining genetic differentiation. However, some vent-associated species seem to have a long larval dispersal duration, which may help to achieve genetic connection between patches of habitat. For instance, Kojima *et al.* (2003, 2006) found that tube worms, *Arcovestia ivanovi* and *Lamellibrachia juni*, had migrated from hydrothermal fields in the Eastern Pacific Rise to those in the back-arc basins in the western Pacific, which indicates that long distance genetic connectivity exists. A similar result was also obtained for other hydrothermal species, including *Riftia pachyptila* (Bucklin 1988; Black *et al.*, 1994; Coykendall *et al.*, 2011), *Bathymodiolus thermophilus* (Craddock *et al.*, 1995), and *Tevnia jerichonana* and *Oasisia alvinae* (Black *et al.*, 1998). In addition, *Austinograea rodriguezensis*, *Rimicaris kairei*, *Alviniconcha* sp. and populations of

the scaly-foot gastropod were all reported as having high connectivity and therefore low levels of genetic structure along the central Indian Ocean Ridge (Beedessee *et al.*, 2013).

Seamounts constitute a distinct deep-sea habitat characterised by accelerated currents, exposed hard substrata, and a patchy structure in an otherwise continuous oceanic habitat. In the reviewed studies, seamount as a habitat type does not appear to be important in explaining the presence of barriers to gene flow. Distance is also not reported as being a barrier that isolates different seamount populations for all species. What is important, as noted previously, is depth, as recorded for corals and echinoderms (Miller *et al.*, 2010; Miller *et al.*, 2011; Miller & Gunasekara, 2016; Ayala *et al.*, 1975; Foltz *et al.*, 2013). Although currents and topography are important physical elements that influence seamount ecosystems (White & Mohn, 2004), neither appears to impede genetic connectivity and they may even promote it.

Figure 2.9 Comparisons of the presence of barriers to gene flow as a function of habitat type

2.3.7. Barrier occurrence difference between VMEs and non-VMEs

Both the χ^2 test ($p > 0.05$) and Cramer's coefficient values ($V > 0.15$) agreed that VMEs and VME indicator taxa were not associated with genetic barrier detection. In other words, barriers to genetic connectivity were reported at similar rates for all habitat types as they were for VME habitats, and also for indicator taxa (such as corals and sponges) that occurred at non-VME sites when compared with such taxa at VME sites (Table 2.9). Only temperature explained significant variation in barrier detection between VME and non-VME sites (Table 2.9), whilst in the comparison of indicator taxa and non-indicator taxa, both distance and topography explained significant variation in barrier detection. In the examined indicator taxa, topography was a genetic barrier amongst different populations, and distance genetically isolated most of VME indicator taxa populations. These results indicate that different habitats have different genetic connectivity patterns, and therefore that conservation and management plans need to be adapted to suit different habitat types.

Table 2.9 Comparisons of barrier detection between VMEs and non-VMEs and between indicator taxa and non-indicator taxa

Variables	VMEs and non-VMEs		Indicator taxa and non-indicator taxa	
	Cramer's V	χ^2 (p value)	Cramer's V	χ^2 (p value)
Barrier detection	0.010	0.829	0.060	0.180
Distance	0.117	0.254	0.212	0.039
Depth	0.012	0.895	0.172	0.068
Current	0.025	0.835	0.053	0.664
Topography	0.119	0.350	0.262	0.039
Temperature	1.000	0.003	-	-
Life	0.001	0.994	0.160	0.845

-, insufficient temperature data were not available.

2.4. Implications for management

Understanding patterns of deep-sea genetic connectivity is essential to provide information and recommendations for management and conservation to protect or minimise negative effects from anthropological disturbances in the deep-sea ecosystem. Currently, in the World Database on Protected Areas (WDPA), 175 countries have established marine protected areas of different types, which cover about 12.34% (44,643,023.23 km²) of the global ocean. This includes 3,345,306.37 km² of No-Take areas, which cover about 0.92% of

the global ocean (WDPA database, <http://www.unep-wcmc.org/wdpa>). Amongst these protected areas, less than 0.5% of the global surface has been protected, and most of the areas are located in shallow water (Chape *et al.*, 2003). In the few deep-sea protection areas, all are associated with seamounts and hydrothermal vents, or cold-water corals (see summary in Davies *et al.*, 2007). Based on the aforementioned results and discussions, patterns of genetic connectivity patterns varied amongst different species and spatial scales, and were caused by different factors. Specific conservation strategies should be adopted depending on the VME location and community composition.

2.4.1. Spatial management

From the summary results, it was very difficult to draw a clear pattern: the genetic connectivity varied a lot amongst different species, different habitats and at different scales. To address these complex patterns, spatial management would be the optimal solution, which means different management strategies are adapted to address different patterns of genetic connectivity. In a broad context, large spatial scale genetic connectivity should consider oceanic-scale protection (e.g. giant squid), and regional protection should be considered for moderate genetic connectivity (e.g. fish *Sebastes mentella*), and local protection should be set in place for small-scale structured populations (e.g. antipatharians).

Of those factors that affected genetic connectivity, depth is very important as a genetic barrier for most deep-sea animals, indicating that vertical protection is essential for deep-sea conservation. However, protection sites should not be simply placed by depth, because genetic diversity or differentiation is not simply correlated with depth. Significant differences in barrier detection amongst different habitats indicated that more reserves should place on cold seep and hydrothermal vent sites. Non-benthic protection should be considered for organisms inhabited the water column, because a greater level of genetic isolation was found in this habitat. More factors, such as seafloor topography and oceanic dynamics, included in the design would be considered appropriate.

2.4.2. Management amongst habitats

In the deep sea, geological variability (e.g., substratum type, topographic complexity), hydrography (e.g., prevailing current, water density) and their complex interactions form and shape several different habitats (Oldham *et al.*, 2014). These environmentally distinct habitats

may give rise to different selection regimes for different populations, which indirectly shape genetic differentiation and form barriers to gene flow.

The results of the present study showed that barriers to gene flow were detected in all different habitats. Except for populations on the abyssal plain, more half records in the populations associated with other habitats were revealed genetic structures, and organisms associated with hydrothermal vents and cold seeps exhibited more population genetic differentiation compared to organisms that inhabited other habitats (Figure 2.9). This suggests that the priorities of placing protection areas should consider the different habitat types, and that areas that are more isolated be allocated to higher conservation needs, such as hydrothermal vents and cold seeps. Different habitats are presently facing different levels and types of anthropologic disturbance affects: the continental slope is the most affected at present (Ramirez-Llodra *et al.*, 2011). Although it is the most disturbed, these areas have received the most protection from fisheries (e.g. fisheries closure areas, marine protected areas and no-take marine reserves). However, hydrothermal vents, cold seeps and seamounts are also directly affected by human resource exploitation, such as fishing and the prospect of mining in the very near future. Recently, the Regional Fisheries Management Organizations (RFMOs) and some countries have closed a range of seamounts, hydrothermal vents and slope areas to bottom fisheries (Ramirez-Llodra *et al.*, 2011), but for such decisions, most have not included connectivity information when the closure areas were designed or established. Based on the findings, protection areas in these habitats would lessen human impacts on the deep sea.

2.4.3. Management of VMEs and VME indicator taxa

Distance and topography were significantly related to VME indicator taxon genetic connectivity (Table 2.9). When designing protection areas, the distance to neighbourhood protection and bathymetric variables should be preferentially considered. Reasonable distance between protected areas will help to maintain population connectivity, and provide sources for the threatened populations. Because different species have different scales of genetic connectivity, the distance range would be established on a species by species basis. Because all of the VME indicator taxa are benthic, it is not surprising that topography was an important factor in helping to explain genetic barrier detection. This finding indicates that populations inhabiting in or on different topographic features should be considered as different management units. But other biological factors, such as growth rate and ability to

recover after artificial disturbance, should be included in the design as well, because they are important for management but could not be reflected by the genetic markers.

2.4.4. Management Units

The model indicated that there were genetic barriers between deep-sea biogeographic provinces, which suggests that management should be applied at the spatial scale of the provinces. Genetic differentiation was significantly related to number of provinces covered (Figure 2.2, Table 2.4). Studies from both the BY7 (Cocos Plate) and BY9 (Antarctica) provinces reported that currents shaped genetic connectivity. Equatorial currents in BY7 caused a north-south differentiation (82.22% of records from this province had a barrier) (e.g. Coykendall *et al.*, 2011; Craddock, Lutz & Vrijenhoek 1997; Hurtado, Lutz & Vrijenhoek 2004; Jollivet *et al.*, 1995; Matabos *et al.*, 2008; Plouviez *et al.*, 2009; Shank & Halanynch 2007; Won *et al.*, 2003), whereas the Antarctic Circumpolar Current (ACC) in BY9 (59.10% of records did not detect a barrier) promoted circular connectivity (e.g. Arango, Soler-Membrives & Miller 2011; Baird, Miller & Stark 2011; Fevolden & Schneppenheim 1989; Neal *et al.*, 2014; Pawlowski, Bowser & Gooday 2007; Raupach *et al.*, 2010; Riehl & Kaiser 2012; Roterman *et al.*, 2016; Wilson *et al.*, 2007). Whilst it is difficult to provide general suggestions for management or conservation in all provinces, different features of each province might provide a basic idea for management. Biogeographic provinces could be considered as a *de facto* management unit if not much information is available to help make informed management decisions.

2.5. Future work

To obtain a better understanding of genetic connectivity in the deep sea, there are still many gaps that exist in my collected data and analyses. Firstly, data are absent from the Arctic Ocean and most of the Indian, SE Pacific, NW Pacific, and S Atlantic oceans, and few records exist from hadal depths and most of the abyssal depths are also lacking data on genetic connectivity. Secondly, increasing awareness of new species and a lack of information about the reproduction modes and larval dispersal strategies for most species creates difficulties when attempting to explain the connectivity patterns. This, in turn, directly hampers protection design. Thirdly, highly polymorphic genetic markers (i.e., markers that are most informative for gene flow) are still unavailable for most deep-sea species, making it

difficult to uncover the population genetic structure and barriers to gene flow. Although highly polymorphic co-dominant markers that are ideally suited for deep-sea studies, such as microsatellites and SNPs, can now be generated quickly and reasonably cheaply, most studies are currently still using DNA sequence data for species from the deep sea. These limitations or problems are the most important areas to be addressed in future analyses. In addition, *in situ* investigations of VME resilience to anthropogenic disturbance and recovery from damage are also required to identify appropriate management units and to aid in the design of protection networks for deep-sea species.

Appendix

Table S2.1 Details of publications included in the database

ID	Species	Depth cover (m)	Sample size	Provinces Code	Genetic Marker	Barrier Detection	Factors	Reference
1	<i>Abyssorhynchomene spp</i>	1013	419	BY3	Alloenzyme	Yes	Topography	France <i>et al.</i> , (1994)
2	<i>Acanella</i>	3660	21	BY4	ND6	No	Limitations	Thoma <i>et al.</i> , (2009)
3	<i>Acanthaspidea drygalskii</i>	3433	36	BY9 BY10	16s	No	Current	Raupach <i>et al.</i> , (2006)
4	<i>Acanthephyra pelagica</i>		815	BY4	SSR	Yes	Distance	Jorquera-Paegelow <i>et al.</i> , (2014)
5	<i>Adipicola pacifica</i>	277	128	BY12	ND4	No	Biological Factors	Fukasawa <i>et al.</i> , (2015)
6	<i>Aetideidae</i>	2000	17	BY1 BY9	COI	No	Current	Laakmann <i>et al.</i> , (2012)
7	<i>Aetideidae</i>	2000	17	BY1 BY9	18s	Yes	Biological Factors	Laakmann <i>et al.</i> , (2012)
8	<i>Aetideidae</i>	2000	17	BY1 BY9	28s	Yes	Biological Factors	Laakmann <i>et al.</i> , (2012)
9	<i>Aetideidae</i>	2000	17	BY1 BY9	ITS2	Yes	Biological Factors	Laakmann <i>et al.</i> , (2012)
10	<i>Allopathes robillardii</i>	0	54	BY11 BY6 BY10	ITS	Yes	Topography	Miller <i>et al.</i> , (2010)
11	<i>Alvinella caudata</i>	625	176	BY7	Alloenzyme	Yes	Temperature	Jollivet <i>et al.</i> , (1995)
12	<i>Alvinella pompejana</i>	625	185	BY7	Alloenzyme	Yes	Temperature	Jollivet <i>et al.</i> , (1995)
13	<i>Alvinella pompejana</i>	340	246	BY7	COI	Yes	Biological Factors & Ocean current	Plouviez <i>et al.</i> , (2009)
14	<i>Alvinella pompejana</i>	826	93	BY7	COI	No	Biological Factors	Hurtado <i>et al.</i> , (2004)
15	<i>Alvinella pompejana</i>	346	67	BY7	COI	Yes	Current	Plouviez <i>et al.</i> , (2010)
16	<i>Alvinella pompejana</i>	346	17	BY7	SAHH	Yes	Current	Plouviez <i>et al.</i> , (2010)
17	<i>Alvinella pompejana</i>	346	20	BY7	PGM	Yes	Current	Plouviez <i>et al.</i> , (2010)
18	<i>Alvinella pompejana</i>	346	20	BY7	GlobX	Yes	Current	Plouviez <i>et al.</i> , (2010)
19	<i>Alvinella pompejana</i>	346	40	BY7	Alloenzyme	Yes	Current	Plouviez <i>et al.</i> , (2010)
20	<i>Alvinella pompejana</i>	346	74	BY7	RFLP	Yes	Current	Plouviez <i>et al.</i> , (2010)
21	<i>Alviniconcha</i>	0	44	BY11	COI	No	Biological Factors	Beedessee <i>et al.</i> , (2013)
22	<i>Alviniconcha hessleri</i>	2130	100	BY12	ATPS β	No	Temperature	Hidaka <i>et al.</i> , (2015)
23	<i>Alviniconcha hessleri</i>	2130	40	BY13	COI	No	Temperature	Hidaka <i>et al.</i> , (2015)
24	<i>Alviniconcha spp.</i>	250	28	BY12	Alloenzyme	Yes	Biological Factors	Denis <i>et al.</i> , (1993)
25	<i>Alvinocaris dissimilis</i>	0	17	BY12	COI	No	Topography	Yahagi <i>et al.</i> , (2015)
26	<i>Alvinocaris longirostris</i>	723	66	BY12	COI	No	Topography	Yahagi <i>et al.</i> , (2015)
27	<i>Alvinocaris sp</i>	264	38	BY12	COI	No	Topography	Yahagi <i>et al.</i> , (2015)
28	<i>Anoplopoma fimbria</i>	414	57	BY3	COI	No	Limitations	Tripp-Valdez <i>et al.</i> , (2012)
29	<i>Anoplopoma fimbria</i>	414	183	BY3	SSR	Yes	Biological Factors	Tripp-Valdez <i>et al.</i> , (2012)
30	<i>Antimora rostrata</i>	1825	393	BY4	SSR	No	Limitations	White <i>et al.</i> , (2011)
31	<i>Aphanopus carbo</i>	670	651	BY4	SSR	No	Biological Factors	Longmore <i>et al.</i> , (2014)
32	<i>Architeuthis dux</i>	700	43	BY13 BY6 BY4 BY11 BY12 BY2 BY14	mtDNA genome	No	Biological Factors	Winkelmann <i>et al.</i> , (2013)
33	<i>Argyropelecus hemigymnus</i>	550	12	BY4 BY 10 BY13	COI	Yes	Depth & Distance	Gordeeva <i>et al.</i> , (2014)
34	<i>Aristaeomorpha foliacea</i>		317	BY4 BY11	COI	Yes	Biological Factors & Topography	Fernández <i>et al.</i> , (2013)
35	<i>Aristeus antennatus</i>	821	230	BY4	SSR	No	Biological Factors	Sacco <i>et al.</i> , (2011)
36	<i>Aristeus antennatus</i>		175	BY4	D-loop	No	Biological Factors	Maggio <i>et al.</i> , (2009)

37	<i>Aristeus antennatus</i>		236	BY4	D-loop	No	Limitations	Lo Brutto <i>et al.</i> , (2012)
38	<i>Aristeus antennatus</i>		192	BY4	AFLP	No	Limitations	Lo Brutto <i>et al.</i> , (2012)
39	<i>Aristeus antennatus</i>	220	319	BY4	COI(500)+16s(447)	No	Biological Factors	Marra <i>et al.</i> , (2015)
40	<i>Aristeus antennatus</i>	1150	321	BY4	16s	No	Depth	Sarda <i>et al.</i> , (2010)
41	<i>Aristeus antennatus</i>		137	BY4	COI514+16s547	No	Biological Factors	Roldan <i>et al.</i> , (2009)
42	<i>Aristeus antennatus</i>	100	175	BY4	D-loop	No	Biological Factors	Maggio <i>et al.</i> , (2009)
43	<i>Aristeus antennatus</i>	821	227	BY4	SSR	No	Biological Factors	Cannas <i>et al.</i> , (2011)
44	<i>Aristeus antennatus</i>		497	BY4	COI	Yes	Topography	Fernández <i>et al.</i> , (2011)
45	<i>Aristeus antennatus</i>		506	BY4	16s	Yes	Topography	Fernández <i>et al.</i> , (2011)
46	<i>Asteroschema clavigerum</i>	1000	91	BY4	16s	Yes	Depth	Cho <i>et al.</i> , (2010)
47	<i>Asteroschema clavigerum</i>	1000	37	BY4	COI	Yes	Depth	Cho <i>et al.</i> , (2010)
48	<i>Atlantoserolis vema</i>	1185	17	AB5	18s	Yes	Topography	Brandt <i>et al.</i> , (2014)
49	<i>Atlantoserolis vema</i>	1185	20	AB5	16s	Yes	Topography	Brandt <i>et al.</i> , (2014)
50	<i>Atlantoserolis vema</i>	1185	17	AB5	COI	Yes	Topography	Brandt <i>et al.</i> , (2014)
51	<i>Austinograea rodriguezensis</i>	0	36	BY11	COI	No	Current	Beedessee <i>et al.</i> , (2013)
52	<i>Austrolaenilla antarctica</i>	2252	19	BY9	COI	Yes	Biological Factors	Neal <i>et al.</i> , (2014)
53	<i>Austrolaenilla pelagica</i>	2253	19	BY9	COI	No	Limitations	Neal <i>et al.</i> , (2015)
54	<i>Bathybembix bairdii</i>	577	21	BY3	Alloenzyme	Yes	Depth	Siebenaller <i>et al.</i> , (1978)
55	<i>Bathymodiolus</i>	416	120	BY7	COI	Yes	Distance	Won <i>et al.</i> , (2003)
56	<i>Bathymodiolus</i>	416	10	BY7	Alloenzyme	Yes	Distance	Won <i>et al.</i> , (2003)
57	<i>Bathymodiolus</i>	1140	22	BY6	COI	Yes	Current	Smith <i>et al.</i> , (2004)
58	<i>Bathymodiolus</i>	1140	22	BY6	Alloenzyme	Yes	Current	Smith <i>et al.</i> , (2004)
59	<i>Bathymodiolus</i>	3310	67	BY4 BY12	ND4	No	Topography	Kyuno <i>et al.</i> , (2009)
60	<i>Bathymodiolus</i>			BY4	SNP	Yes	Distance	Corinna <i>et al.</i> , (2015)
61	<i>Bathymodiolus brevior</i>	2413	241	BY6 BY11 BY12	COI	Yes	Distance	Breusing <i>et al.</i> , (2015)
62	<i>Bathymodiolus brevior</i>	2413	241	BY6 BY11 BY12	ND4	Yes	Distance	Breusing <i>et al.</i> , (2015)
63	<i>Bathymodiolus brevior</i>	2413	241	BY6 BY11 BY12	Col-1	No	Current & Biological Factors	Breusing <i>et al.</i> , (2015)
64	<i>Bathymodiolus brevior</i>	2414	241	BY6 BY11 BY13	Val tRNA	No	Current & Biological Factors	Breusing <i>et al.</i> , (2015)
65	<i>Bathymodiolus brevior</i>	2414	241	BY6 BY11 BY13	Met tRNA	No	Current & Biological Factors	Breusing <i>et al.</i> , (2015)
66	<i>Bathymodiolus brevior</i>	2414	241	BY6 BY11 BY13	EF1 α	No	Current & Biological Factors	Breusing <i>et al.</i> , (2015)
67	<i>Bathymodiolus brevior</i>	2414	241	BY6 BY11 BY14	Cat	Yes	Current & Biological Factors	Breusing <i>et al.</i> , (2015)
68	<i>Bathymodiolus brevior</i>	2414	241	BY6 BY11 BY14	H3	No	Current & Biological Factors	Breusing <i>et al.</i> , (2015)
69	<i>Bathymodiolus brevior</i>	2414	241	BY6 BY11 BY14	Alloenzyme	Yes	Distance	Breusing <i>et al.</i> , (2015)
70	<i>Bathymodiolus brooksi</i>	2213	67	BY4	COI	Yes	Biological Factors & Vent	Faure <i>et al.</i> , (2015)
71	<i>Bathymodiolus brooksi</i>	2213	67	BY4	ND4	No	Biological Factors & Vent	Faure <i>et al.</i> , (2015)
72	<i>Bathymodiolus childressi</i>	1828	99	BY4	COI	Yes	Biological Factors & Vent	Faure <i>et al.</i> , (2015)
73	<i>Bathymodiolus childressi</i>	1828	76	BY4	ND4	No	Biological Factors & Vent	Faure <i>et al.</i> , (2015)
74	<i>Bathymodiolus geikotsucola</i>	31	40	BY12	ND4	No	Biological Factors	Fukasawa <i>et al.</i> , (2015)
75	<i>Bathymodiolus heckerae</i>	0	32	BY4	COI	Yes	Biological Factors & Vent	Faure <i>et al.</i> , (2015)
76	<i>Bathymodiolus heckerae</i>	0	32	BY4	ND4	No	Limitations	Faure <i>et al.</i> , (2015)

77	<i>Bathymodiolus platifrons</i>	258.7	52	BY12	COI+Cytb+16s	No	Biological Factors	Shen <i>et al.</i> , (2016)
78	<i>Bathymodiolus platifrons</i>	280	28	BY12	SNP	Yes	Temperature	Xu <i>et al.</i> , (2016)
79	<i>Bathymodiolus</i> sp. nov.	0	5	BY4	COI	Yes	Biological Factors & Vent	Faure <i>et al.</i> , (2015)
80	<i>Bathymodiolus</i> sp. nov.	0	5	BY4	ND4	No	Limitations	Faure <i>et al.</i> , (2015)
81	<i>Bathymodiolus</i> spp	885	161	BY14	Alloenzyme	Yes	Distance	Moraga <i>et al.</i> , (1994)
82	<i>Bathymodiolus thermophilus</i>	170	58	BY7	RFLP	No	Biological Factors	Craddock <i>et al.</i> , (1995)
83	<i>Bathymodiolus thermophilus</i>	170	58	BY7	Alloenzyme	No	Biological Factors	Craddock <i>et al.</i> , (1995)
84	<i>Bathymodiolus thermophilus</i>	340	309	BY7	COI	Yes	Biological Factors & Ocean current	Plouviez <i>et al.</i> , (2009)
85	<i>Bathymodiolus thermophilus</i>	600	346	BY7	Alloenzyme	Yes	Distance	Plouviez <i>et al.</i> , (2013)
86	<i>Bathymodiolus thermophilus</i>	600	347	BY7	COI	Yes	Depth & Distance	Plouviez <i>et al.</i> , (2013)
87	<i>Bathymodiolus thermophilus</i>	600	83	BY7	Sulfo1	Yes	Depth & Distance	Plouviez <i>et al.</i> , (2013)
88	<i>Bathymodiolus thermophilus</i>	600	97	BY7	Lyso	Yes	Depth & Distance	Plouviez <i>et al.</i> , (2013)
89	<i>Bathymodiolus thermophilus</i>	600	94	BY7	SAHH	Yes	Depth & Distance	Plouviez <i>et al.</i> , (2013)
90	<i>Bathymodiolus thermophilus</i>	600	45	BY7	EF1 α	Yes	Depth & Distance	Plouviez <i>et al.</i> , (2013)
91	<i>Bathypathes</i>	3660	18	BY4	COI	No	Limitations	Thoma <i>et al.</i> , (2009)
92	<i>Bathypathes</i>	3660	18	BY4	ND2	No	Limitations	Thoma <i>et al.</i> , (2009)
93	<i>Bathypathes</i>	3660	18	BY4	ND1	No	Limitations	Thoma <i>et al.</i> , (2009)
94	<i>Benthodytes typica</i>	2050	86	BY4	Alloenzyme	No	Biological Factors	Bisol <i>et al.</i> , (1984)
95	<i>Benthogone rosea</i>	2050	51	BY4	Alloenzyme	No	Biological Factors	Bisol <i>et al.</i> , (1984)
96	<i>Benthoosema suborbitale</i>	640	10	BY4 BY 10 BY13	COI	Yes	Depth	Gordeeva <i>et al.</i> , (2014)
97	<i>Beryx decadactylus</i>		305	BY4	D-loop	No	Biological Factors	Fries <i>et al.</i> , (2011)
98	<i>Beryx splendens</i>		204	BY4 BY11 BY6 BY10 BY12	Cytb	Yes	Current	Lévy-Hartmann, <i>et al.</i> , (2011)
99	<i>Beryx splendens</i>	596	238	BY12	Cytb	Yes	Distance	Hoarau <i>et al.</i> , (2000)
100	<i>Betamorphia fusiformis</i>	3864	50	BY9 AB6	16s	Yes	Topography	Raupach <i>et al.</i> , (2007)
101	<i>Betamorphia fusiformis</i>	3864	50	BY9 AB6	18s	No	Limitations	Raupach <i>et al.</i> , (2007)
102	<i>Bolinichthys indicus</i>	625	10	BY4 BY 10 BY13	COI	Yes	Depth	Gordeeva <i>et al.</i> , (2014)
103	<i>Branchipolynoe symmytilida</i>	340	207	BY7	COI	Yes	Biological Factors & Ocean current	Plouviez <i>et al.</i> , (2009)
104	<i>Branchipolynoe symmytilida</i>	826	61	BY7	COI	No	Biological Factors	Hurtado <i>et al.</i> , (2004)
105	<i>Brosme brosme</i>	900	764	BY2 BY4	SSR	Yes	Depth & Biological Factors	Knutsen <i>et al.</i> , (2009)
106	<i>Caecosagitta macrocephala</i>	3999	52	BY3 BY13	COI	Yes	Depth & Current	Miyamoto <i>et al.</i> , (2010)
107	<i>Caecosagitta macrocephala</i>	3999	52	BY3 BY13	ITS1	Yes	Depth & Current	Miyamoto <i>et al.</i> , (2010)
108	<i>Calanus finmarchicus</i>		182	BY2 BY1	16s	Yes	Biological Factors	Bucklin <i>et al.</i> , (1996)
109	<i>Calanus finmarchicus</i>		92	BY2 BY1	COI	Yes	Current	Bucklin <i>et al.</i> , (2000)
110	<i>Calanus finmarchicus</i>		83	BY2 BY1	PGI	Yes	Current	Bucklin <i>et al.</i> , (2000)
111	<i>Callogorgia a. delta</i>	415	113	BY4	SSR	Yes	Depth	Quattrini <i>et al.</i> , (2014)
112	<i>Carcharhinus acronotus</i>	0	257	BY4	D-loop	Yes	Current & Topography	Portnoy <i>et al.</i> , (2014)
113	<i>Carcharhinus acronotus</i>	0	650	BY4	SSR	Yes	Current & Topography	Portnoy <i>et al.</i> , (2014)
114	<i>Centroscymnus coelolepis</i>	1825	155	BY4 BY10 BY11	D-loop	Yes	Topography	Catarino <i>et al.</i> , (2015)
115	<i>Centroscymnus coelolepis</i>	1825	503	BY4 BY10 BY11	SSR	Yes	Topography	Catarino <i>et al.</i> , (2015)
116	<i>Centroscymnus crepidater</i>	550	92	BY6 BY4	D-loop	Yes	Current	Cunha <i>et al.</i> , (2012)
117	<i>Centroscymnus crepidater</i>	550	61	BY6 BY4	COI	Yes	Current	Cunha <i>et al.</i> , (2012)

118	<i>Centroscyrnus crepidater</i>	550	160	BY6 BY4	SSR	Yes	Current	Cunha <i>et al.</i> , (2012)
119	<i>Ceratoscopelus warmingii</i>	725	20	BY4 BY 10 BY13	COI	Yes	Depth & Distance	Gordeeva <i>et al.</i> , (2014)
120	<i>cf. Ampelisca chiltoni</i>	1000	28	BY6	COI	Yes	Topography	Knox <i>et al.</i> , (2012)
121	<i>cf. Oediceroides apicalis</i>	1000	25	BY6	COI	Yes	Topography	Knox <i>et al.</i> , (2012)
122	<i>Chaceon notialis</i>	750	35	BY13	16s	No	Limitations	Mantelatto <i>et al.</i> , (2014)
123	<i>Chaceon quinquedens</i>	616	37	BY4	16s	Yes	Biological Factors	Weinberg <i>et al.</i> , (2003)
124	<i>Chaceon quinquedens</i>	616	15	BY4	ITS	Yes	Biological Factors	Weinberg <i>et al.</i> , (2003)
125	<i>Chaceon ramosae</i>	750	35	BY13	COI	No	Limitations	Mantelatto <i>et al.</i> , (2014)
126	<i>Chelator aequabilis</i>	368	3	BY13	16s	No	Limitations	Brix <i>et al.</i> , (2015)
127	<i>Chelator aequabilis</i>	368	4	BY13	COI	No	Limitations	Brix <i>et al.</i> , (2015)
128	<i>Chelator aequabilis</i>	368	2	BY13	18s	No	Limitations	Brix <i>et al.</i> , (2015)
129	<i>Chelator insignis</i>	2537	47	BY2	COI	Yes	Depth	Brix <i>et al.</i> , (2014)
130	<i>Chelator insignis</i>	2537	36	BY2	16s	Yes	Depth	Brix <i>et al.</i> , (2014)
131	<i>Chelator insignis</i>	2537	11	BY2	18s	Yes	Depth	Brix <i>et al.</i> , (2014)
132	<i>Chelator insignis</i>	2563	3	BY13	COI	No	Limitations	Brix <i>et al.</i> , (2015)
133	<i>Chelator insignis</i>	2563	2	BY13	16S	No	Limitations	Brix <i>et al.</i> , (2015)
134	<i>Chelator rugosus</i>	1	6	BY13	COI	No	Limitations	Brix <i>et al.</i> , (2015)
135	<i>Chelator rugosus</i>	1	4	BY13	16S	No	Limitations	Brix <i>et al.</i> , (2015)
136	<i>Chelator rugosus</i>	1	3	BY13	18S	No	Limitations	Brix <i>et al.</i> , (2015)
137	<i>Chorismus antarcticus</i>	1968	178	BY9	COI	No	Biological Factors	Raupach <i>et al.</i> , (2010)
138	<i>Chorismus antarcticus</i>	1968	178	BY9	16s	No	Limitations	Raupach <i>et al.</i> , (2010)
139	<i>Chorismus antarcticus</i>	1968	178	BY9	28s	No	Limitations	Raupach <i>et al.</i> , (2010)
140	<i>Chorismus antarcticus</i>	1968	178	BY9	16s	No	Limitations	Raupach <i>et al.</i> , (2010)
141	<i>Chorismus antarcticus</i>	1968	178	BY9	28s	No	Limitations	Raupach <i>et al.</i> , (2010)
142	<i>Chorocaris</i> sp. 2	650	179	BY12	COI	Yes	Topography	Thaler <i>et al.</i> , (2014)
143	<i>Chorocaris</i> sp. 2	650	273	BY12	SSR	Yes	Topography	Thaler <i>et al.</i> , (2014)
144	<i>Chrysogorgia</i>	3660	24	BY4	MutS	No	Limitations	Thoma <i>et al.</i> , (2009)
145	<i>Chrysomallon squamiferum</i>	0	93	BY11	COI	Yes	Biological Factors	Chen <i>et al.</i> , (2015)
146	<i>Cibicides wuellerstorfi</i>	4403	37	AB2 AB6	12s	No	Biological Factors	Pawlowski <i>et al.</i> , (2007)
147	<i>Cibicides wuellerstorfi</i>	4403	37	AB2 AB6	ITS	No	Biological Factors	Pawlowski <i>et al.</i> , (2007)
148	<i>Cibicides wuellerstorfi</i>	4403	12	BY9 BY10	ITS	Yes	Biological Factors	Brandt <i>et al.</i> , (2007)
149	<i>Cibicides wuellerstorfi</i>	808	23	BY7 BY4	12s	No	Limitations	Burkett <i>et al.</i> , (2014)
150	<i>Clencharia abyssorum</i>	2106	43	BY2 AB2	16s	No	Limitations	Etter <i>et al.</i> , (2007)
151	<i>Corallium lauense</i>	190	134	BY14	SSR	Yes	Current	Baco <i>et al.</i> , (2005)
152	<i>Corallium rubrum</i>	20	2	BY4	Alloenzyme	Yes	Biological Factors	Abbiati <i>et al.</i> , (1993)
153	<i>Corallium rubrum</i>	234	12	BY4	SSR	Yes	Depth	Costantini <i>et al.</i> , (2009)
154	<i>Corallium rubrum</i>	234	12	BY4	MutS	Yes	Depth	Costantini <i>et al.</i> , (2009)
155	<i>Corallium rubrum</i>	234	12	BY4	ITS1	Yes	Depth	Costantini <i>et al.</i> , (2009)
156	<i>Corallium rubrum</i>	20	398	BY4	SSR	Yes	Biological Factors	Costantini <i>et al.</i> , (2007)
157	<i>Coryphaenoides armatus</i>	3479	210	BY4 BY11	SSR	Yes	Biological Factors	Ritchie <i>et al.</i> , (2013)
158	<i>Coryphaenoides armatus</i>	750	10	BY4 BY7 BY2 BY3 BY8	Alloenzyme	No	Biological Factors	Wilson <i>et al.</i> , (1984)
159	<i>Coryphaenoides rupestris</i>	1033	417	BY4	SSR	Yes	Depth & Distance	White <i>et al.</i> , (2010)
160	<i>Coryphaenoides yaquinae</i>	2400	11	BY7 BY3	Alloenzyme	No	Biological Factors	Wilson <i>et al.</i> , (1983)

161	<i>Desmophyllum dianthus</i>	826	112	BY4	ITS	Yes	Depth & Distance	Addamo <i>et al.</i> , (2012)
162	<i>Desmophyllum dianthus</i>	826	98	BY4	28s	No	Biological Factors	Addamo <i>et al.</i> , (2012)
163	<i>Desmophyllum dianthus</i>	826	57	BY4	16s	No	Biological Factors	Addamo <i>et al.</i> , (2012)
164	<i>Desmophyllum dianthus</i>	826	51	BY4	COI	No	Biological Factors	Addamo <i>et al.</i> , (2012)
165	<i>Desmophyllum dianthus</i>	700	58	BY11 BY6 BY10	ITS	Yes	Depth & Topography	Miller <i>et al.</i> , (2010)
166	<i>Desmophyllum dianthus</i>	700	30	BY11 BY6 BY10	D-loop	Yes	Depth & Topography	Miller <i>et al.</i> , (2010)
167	<i>Desmophyllum dianthus</i>	1400	162	BY6 BY8 BY10	D-loop	Yes	Depth	Miller <i>et al.</i> , (2011)
168	<i>Desmophyllum dianthus</i>	1400	162	BY6 BY8 BY10	ITS	Yes	Depth	Miller <i>et al.</i> , (2011)
169	<i>Desmophyllum dianthus</i>	1400	162	BY6 BY8 BY10	16s	Yes	Depth	Miller <i>et al.</i> , (2011)
170	<i>Desmophyllum dianthus</i>	1906	313	BY10	SSR	Yes	Depth	Miller <i>et al.</i> , (2016)
171	<i>Diaphus mollis</i>	565	12	BY4 BY 10 BY13	COI	Yes	Depth	Gordeeva <i>et al.</i> , (2014)
172	<i>Diogenichthys atlanticus</i>	550	11	BY4 BY 10 BY13	COI	Yes	Depth & Distance	Gordeeva <i>et al.</i> , (2014)
173	<i>Diplopteraster multipes</i>	1800	31	BY3	Alloenzyme	No	Limitations	Ayala <i>et al.</i> , (1975)
174	<i>Dissostichus eleginoides</i>	1800	450	BY13 BY10	SSR	Yes	Current	Shaw <i>et al.</i> , (2004)
175	<i>Dissostichus eleginoides</i>	1800	396	BY13 BY10	ND2	Yes	Current	Shaw <i>et al.</i> , (2004)
176	<i>Dissostichus eleginoides</i>	1800	446	BY13 BY10	D-loop	Yes	Current	Shaw <i>et al.</i> , (2004)
177	<i>Dissostichus eleginoides</i>		151	BY10	12s	Yes	Distance & Depth & Topography	Rogers <i>et al.</i> , (2006)
178	<i>Dissostichus eleginoides</i>		151	BY10	16s	No	Limitations	Rogers <i>et al.</i> , (2006)
179	<i>Dissostichus eleginoides</i>		274	BY10	SSR	Yes	Distance & Depth & Topography	Rogers <i>et al.</i> , (2006)
180	<i>Enallopsammia rostrata</i>	0	75	BY11 BY6 BY10	ITS	Yes	Topography	Miller <i>et al.</i> , (2010)
181	<i>Enallopsammia rostrata</i>	0	90	BY11 BY6 BY10	D-loop	Yes	Topography	Miller <i>et al.</i> , (2010)
182	<i>Ennucula similis</i>	1650	53	BY2 AB2	16s	Yes	Depth	Etter <i>et al.</i> , (2007)
183	<i>Epistominella exigua</i>	4403	37	AB2 AB6	12s	Yes	Depth	Pawlowski <i>et al.</i> , (2007)
184	<i>Epistominella exigua</i>	4403	37	AB2 AB6	ITS	Yes	Depth	Pawlowski <i>et al.</i> , (2007)
185	<i>Epistominella exigua</i>	4403	42	BY9 BY10	ITS	Yes	Biological Factors	Brandt <i>et al.</i> , (2007)
186	<i>Epistominella exigua</i>	2580	23	BY12 BY2 BY4 BY13	12s	No	Biological Factors	Lecroq <i>et al.</i> , (2009)
187	<i>Epistominella exigua</i>	2580	118	BY12 BY2 BY4 BY13	ITS	No	Biological Factors	Lecroq <i>et al.</i> , (2009)
188	<i>Epistominella vitrea</i>	4773	7	BY9	12s	No	Limitations	Pawlowski <i>et al.</i> , (2007)
189	<i>Epistominella vitrea</i>	4773	9	BY9	ITS	No	Limitations	Pawlowski <i>et al.</i> , (2007)
190	<i>Escarpia laminata</i>	1752	17	BY4	COI	Yes	Distance	Cowart <i>et al.</i> , (2013)
191	<i>Escarpia laminata</i>	1752	16	BY4	16s	Yes	Distance	Cowart <i>et al.</i> , (2013)
192	<i>Escarpia laminata</i>	1752	17	BY4	Cytb	Yes	Distance	Cowart <i>et al.</i> , (2013)
193	<i>Escarpia laminata</i>	1752	17	BY4	Haemoglobin subunit B2	Yes	Distance	Cowart <i>et al.</i> , (2013)
194	<i>Escarpia laminata</i>	1752	129	BY4	SSR	Yes	Distance	Cowart <i>et al.</i> , (2013)
195	<i>Escarpia southwardae</i>	1752	17	BY4	COI	Yes	Distance	Cowart <i>et al.</i> , (2013)
196	<i>Escarpia southwardae</i>	1752	19	BY4	16s	Yes	Distance	Cowart <i>et al.</i> , (2013)
197	<i>Escarpia southwardae</i>	1752	12	BY4	Cytb	Yes	Distance	Cowart <i>et al.</i> , (2013)
198	<i>Escarpia southwardae</i>	1752	16	BY4	Haemoglobin subunit B2	Yes	Distance	Cowart <i>et al.</i> , (2013)
199	<i>Escarpia southwardae</i>	1752	80	BY4	SSR	Yes	Temperature	Cowart <i>et al.</i> , (2013)
200	<i>Escarpia spicata</i>	1752	8	BY4	COI	Yes	Distance	Cowart <i>et al.</i> , (2013)
201	<i>Escarpia spicata</i>	1752	10	BY4	16s	Yes	Distance	Cowart <i>et al.</i> , (2013)
202	<i>Escarpia spicata</i>	1752	10	BY4	Cytb	Yes	Distance	Cowart <i>et al.</i> , (2013)
203	<i>Escarpia spicata</i>	1752	10	BY4	Haemoglobin subunit B2	Yes	Distance	Cowart <i>et al.</i> , (2013)

204	<i>Etelis "marshi"</i>	290	770	BY14	Cytb	No	Biological Factors	Andrews <i>et al.</i> , (2014)
205	<i>Etelis "marshi"</i>	290	770	BY14	SSR	No	Biological Factors	Andrews <i>et al.</i> , (2014)
206	<i>Etelis carbunculus</i>		90	BY12	D-loop	No	Biological Factors & Limitations	Loeun <i>et al.</i> , (2014)
207	<i>Etelis coruscans</i>	290	787	BY14	Cytb	No	Biological Factors	Andrews <i>et al.</i> , (2014)
208	<i>Etelis coruscans</i>	290	787	BY14	SSR	No	Biological Factors	Andrews <i>et al.</i> , (2014)
209	<i>Etelis coruscans</i>		90	BY12	D-loop	No	Biological Factors & Limitations	Loeun <i>et al.</i> , (2014)
210	<i>Euchaetidae</i>	2000	22	BY1 BY9	COI	No	Current	Laakmann <i>et al.</i> , (2012)
211	<i>Euchaetidae</i>	2000	22	BY1 BY9	18s	Yes	Biological Factors	Laakmann <i>et al.</i> , (2012)
212	<i>Euchaetidae</i>	2000	22	BY1 BY9	28s	Yes	Biological Factors	Laakmann <i>et al.</i> , (2012)
213	<i>Euchaetidae</i>	2000	22	BY1 BY9	ITS2	Yes	Biological Factors	Laakmann <i>et al.</i> , (2012)
214	<i>Eulepetopsis vitrea</i>	115	559	BY7	Alloenzyme	Yes	Limitations	Craddock <i>et al.</i> , (1997)
215	<i>Eulepetopsis vitrea</i>	340	297	BY7	COI	Yes	Biological Factors & Ocean current	Plouviez <i>et al.</i> , (2009)
216	<i>Eumunida annulosa</i>	1348	34	BY12	COI	No	Limitations	Samadi <i>et al.</i> , (2006)
217	<i>Eumunida sternomaculata</i>	1348	20	BY12	COI	No	Limitations	Samadi <i>et al.</i> , (2006)
218	<i>Euphausia superba</i>		880	BY9	Alloenzyme	No	Biological Factors	Fevolden <i>et al.</i> , (1989)
219	<i>Euphausia superba</i>	2500	1185	BY9 BY10	Alloenzyme	No	Current	MacDonald <i>et al.</i> , (1986)
220	<i>Euphausia superba</i>		249	BY9	ND1	Yes	Current	Zane <i>et al.</i> , (1998)
221	<i>Euphausia superba</i>	910	585	BY13	SNP	No	Biological Factors	Batta-Lonaa <i>et al.</i> , (2011)
222	<i>Eurythenes gryllus</i>	4648	95	BY1 BY4 AB2 AB12	16s	Yes	Depth	France <i>et al.</i> , (1996)
223	<i>Eurythenes gryllus</i>	3854	47	AB6 AB3 AB5 AB2 BY4 BY14 AB11 AB12 BY1 BY9 BY10	COI	Yes	Depth	Havermans <i>et al.</i> , (2013)
224	<i>Eurythenes gryllus</i>	3855	47	AB6 AB3 AB5 AB2 BY4 BY14 AB11 AB12 BY1 BY9 BY10	16s	Yes	Depth	Havermans <i>et al.</i> , (2013)
225	<i>Eurythenes gryllus</i>	3856	47	AB6 AB3 AB5 AB2 BY4 BY14 AB11 AB12 BY1 BY9 BY10	28s	Yes	Depth	Havermans <i>et al.</i> , (2013)
226	<i>Eurythenes gryllus</i>	0	107	BY14	Alloenzyme	Yes	Topography	Bucklin <i>et al.</i> , (1987)
227	<i>Eusirus giganteus</i>	636	52	BY9	COI	Yes	Biological Factors & Limitations	Baird <i>et al.</i> , (2011)
228	<i>Eusirus giganteus</i>	636	57	BY9	Cytb	Yes	Biological Factors & Limitations	Baird <i>et al.</i> , (2011)
229	<i>Eusirus giganteus</i>	636	21	BY9	ITS2	No	Biological Factors & Limitations	Baird <i>et al.</i> , (2011)
230	<i>Eusirus perdentatus</i>	635	65	BY9	COI	Yes	Biological Factors & Limitations	Baird <i>et al.</i> , (2011)
231	<i>Eusirus perdentatus</i>	635	52	BY9	Cytb	Yes	Biological Factors & Limitations	Baird <i>et al.</i> , (2011)
232	<i>Eusirus perdentatus</i>	635	12	BY9	ITS2	No	Biological Factors & Limitations	Baird <i>et al.</i> , (2011)
233	<i>Frigidoalvania brychia</i>	645	63	BY4	16s	Yes	Depth	Quattro <i>et al.</i> , (2001)
234	<i>Gigantidas gladius</i>	693	150	BY6	COI	No	Limitations	Boschen <i>et al.</i> , (2015)
235	<i>Gigantopelta chessoia</i>	250	84	BY9	COI	No	Biological Factors	Roterman <i>et al.</i> , (2016)
236	<i>Gigantopelta chessoia</i>	250	84	BY9	SSR	No	Biological Factors	Roterman <i>et al.</i> , (2016)
237	<i>Glycera cf kerguelensis</i>	3237	38	BY13	COI	Yes	Depth	Schuller <i>et al.</i> , (2011)
238	<i>Glycera cf kerguelensis</i>	3237	11	BY13	28s	Yes	Depth	Schuller <i>et al.</i> , (2011)

239	<i>Goniocorella dumosa</i>	1072	133	BY6 BY10	ITS	Yes	Depth	Zeng <i>et al.</i> , (2016)
240	<i>Goniocorella dumosa</i>	1072	70	BY6 BY10	D-loop	No	Limitations	Zeng <i>et al.</i> , (2016)
241	<i>Goniocorella dumosa</i>	1072	78	BY6 BY10	SSR	Yes	Depth	Zeng <i>et al.</i> , (2016)
242	<i>Helicolenus dactylopterus</i>	800	212	BY4	Cytb	Yes	Current	Aboim <i>et al.</i> , (2005)
243	<i>Helicolenus dactylopterus</i>	800	208	BY4	D-loop	Yes	Current	Aboim <i>et al.</i> , (2005)
244	<i>Hesiolyra bergi</i>	340	235	BY7	COI	No	Biological Factors	Plouviez <i>et al.</i> , (2009)
245	<i>Hexadella dedritifera</i>	673	30	BY4	COI	Yes	Current	Reveillaud <i>et al.</i> , (2010)
246	<i>Hexadella dedritifera</i>	674	21	BY4	28s	Yes	Current	Reveillaud <i>et al.</i> , (2010)
247	<i>Hexadella dedritifera</i>	675	20	BY4	ATPs Intron	Yes	Current	Reveillaud <i>et al.</i> , (2010)
248	<i>Hippasteria phrygiana</i>	557	140	BY3 BY4 BY6 BY8 BY10 BY11 BY12 BY13	COI	Yes	Biological Factors	Foltz <i>et al.</i> , (2013)
249	<i>Hippasteria phrygiana</i>	557	250	BY3 BY4 BY6 BY8 BY10 BY11 BY12 BY13	ATPS α	No	Distance	Foltz <i>et al.</i> , (2013)
250	<i>Holothuria (Holothuria) mammata</i>		177	BY4	16s	Yes	Current	Borrero-Pérez <i>et al.</i> , (2011)
251	<i>Holothuria (Holothuria) mammata</i>		177	BY4	COI	Yes	Current	Borrero-Pérez <i>et al.</i> , (2011)
252	<i>Hoplostethus atlanticus</i>		294	BY4 BY13	SSR	No	Biological Factors	White <i>et al.</i> , (2009)
253	<i>Hoplostethus atlanticus</i>	1000	388	BY4	SSR	Yes	Topography	Carlsso <i>et al.</i> , (2011)
254	<i>Hoplostethus atlanticus</i>		546	BY4 BY8 BY11 BY6 BY10 BY13	COI	No	Biological Factors	Varela <i>et al.</i> , (2012)
255	<i>Hoplostethus atlanticus</i>		546	BY4 BY8 BY11 BY6 BY10 BY13	Cytb	No	Biological Factors	Varela <i>et al.</i> , (2012)
256	<i>Hoplostethus atlanticus</i>	1730	95	BY6 BY10	Cytb	No	Biological Factors	Varela <i>et al.</i> , (2013)
257	<i>Hoplostethus atlanticus</i>	1730	546	BY6 BY10	COI	No	Biological Factors	Varela <i>et al.</i> , (2013)
258	<i>Hoplostethus atlanticus</i>	1730	812	BY6 BY10	SSR	No	Biological Factors	Varela <i>et al.</i> , (2013)
259	<i>Hoplostethus atlanticus</i>		246	BY6 BY10	RFLP	Yes	Distance	Smith <i>et al.</i> , (1996)
260	<i>Hyalinoecia longibranchiata</i>	326	61	BY6	COI	Yes	Current	Bors <i>et al.</i> , (2012)
261	<i>Hyalinoecia longibranchiata</i>	326	61	BY6	16S	Yes	Current	Bors <i>et al.</i> , (2012)
262	<i>Hyperoglyphe antarctica</i>		680	BY6 BY10	Alloenzyme	No	Limitations	Bolch <i>et al.</i> , (1993)
263	<i>Ifremeria nautilei</i>	350	52	BY12	COI	Yes	Distance	Kojima <i>et al.</i> , (2000)
264	<i>Ifremeria nautilei</i>	1320	158	BY12	COI	Yes	Topography	Thaler <i>et al.</i> , (2011)
265	<i>Ifremeria nautilei</i>	1320	275	BY12	SSR	Yes	Topography	Thaler <i>et al.</i> , (2011)
266	<i>Iridogorgia</i>	3660	15	BY4	MutS	No	Limitations	Thoma <i>et al.</i> , (2009)
267	<i>Jasus paulensis</i>		193	BY13	COII	No	Current	von der Heyden <i>et al.</i> , (2007)
268	<i>Keratoisidinae</i>	2910	66	BY6 BY10	16s	No	Biological Factors & Limitations	Smith <i>et al.</i> , (2004)
269	<i>Keratoisidinae</i>	2910	66	BY6 BY10	NCR	No	Biological Factors & Limitations	Smith <i>et al.</i> , (2004)
270	<i>Kiwa tyleri</i>	239	90	BY9	COI	No	Temperature	Roterman <i>et al.</i> , (2016)
271	<i>Kiwa tyleri</i>	239	91	BY9	SSR	No	Temperature	Roterman <i>et al.</i> , (2016)
272	<i>Lamellibrachia juni</i>	1337	34	BY6 BY12	COI	Yes	Topography	Kojima <i>et al.</i> , (2006)
273	<i>Lampadena chavesi</i>	725	5	BY4 BY 10 BY13	COI	Yes	Depth	Gordeeva <i>et al.</i> , (2014)
274	<i>Lampadena speculigera</i>	850	3	BY4 BY 10 BY13	COI	Yes	Depth & Distance	Gordeeva <i>et al.</i> , (2014)
275	<i>Ledella ultima</i>	1056	81	BY2 AB2	16s	No	Limitations	Etter <i>et al.</i> , (2007)
276	<i>Ledella ultima</i>	2581	176	AB5 AB2 AB4 BY4	16s	Yes	Topography	Etter <i>et al.</i> , (2011)
277	<i>Lepetodrilus elevatus</i>	115	251	BY7	Alloenzyme	No	Limitations	Craddock <i>et al.</i> , (1997)
278	<i>Lepetodrilus elevatus</i>		330	BY7	Alloenzyme	Yes	Biological Factors	Matabos <i>et al.</i> , (2007)

279	<i>Lepetodrilus elevatus</i>		77		BY7		COI	Yes	Biological Factors	Matabos <i>et al.</i> , (2007)
280	<i>Lepetodrilus elevatus</i>		334		BY7		Alloenzyme	Yes	Biological Factors	Matabos <i>et al.</i> , (2007)
281	<i>Lepetodrilus elevatus</i>	340	439		BY7		COI	Yes	Biological Factors & Ocean current	Plouviez <i>et al.</i> , (2009)
282	<i>Lepetodrilus elevatus</i>	1703	157		BY3		COI	Yes	Topography	Johnson <i>et al.</i> , (2006)
283	<i>Lepetodrilus elevatus</i>	1703	157		BY3		PGM-I	Yes	Topography	Johnson <i>et al.</i> , (2006)
284	<i>Lepetodrilus elevatus</i>	1703	157		BY3		Alloenzyme	Yes	Topography	Johnson <i>et al.</i> , (2006)
285	<i>Lepetodrilus galriftensis</i>	115	40		BY7		Alloenzyme	No	Limitations	Craddock <i>et al.</i> , (1997)
286	<i>Lepetodrilus nux</i>	950	154		BY12		COI	Yes	Biological Factors & Vent & Current	Nakamura <i>et al.</i> , (2014)
287	<i>Lepetodrilus ovalis</i>	340	187		BY7		COI	Yes	Biological Factors & Ocean current	Plouviez <i>et al.</i> , (2009)
288	<i>Lepetodrilus pustulosus</i>	115	128		BY7		Alloenzyme	Yes	Limitations	Craddock <i>et al.</i> , (1997)
289	<i>Lepetodrilus</i> sp.	1211	140		BY9		COI	Yes	Current	Roterman <i>et al.</i> , (2016)
290	<i>Lepetodrilus</i> sp.	1211	140		BY9		SSR	Yes	Current	Roterman <i>et al.</i> , (2016)
291	<i>Liocarcinus depurator</i>	750	115		BY4		COI	Yes	Topography	García-Merchána <i>et al.</i> , (2012)
292	<i>Lophelia pertusa</i>		190		BY4		SSR	Yes	Distance	Morrison <i>et al.</i> , (2008)
293	<i>Lophelia pertusa</i>	1392	401		BY4		SSR	Yes	Current & Distance	Morrison <i>et al.</i> , (2011)
294	<i>Lophelia pertusa</i>		77		BY4		ITS	Yes	Biological Factors	Le Goff-Vitry <i>et al.</i> , (2004)
295	<i>Lophelia pertusa</i>		388		BY4		SSR	Yes	Biological Factors	Le Goff-Vitry <i>et al.</i> , (2004)
296	<i>Lophelia pertusa</i>		142		BY4		SSR	Yes	Biological Factors	Dahl <i>et al.</i> , (2012)
297	<i>Lophelia pertusa</i>	515	10		BY4		ITS2	No	Limitations	Flot <i>et al.</i> , (2013)
298	<i>Lophelia pertusa</i>	515	2		BY4		mtDNA genome	No	Limitations	Flot <i>et al.</i> , (2013)
299	<i>Lycodes japonicus</i>	103	53		BY3		COI	No	Biological Factors	Sakuma <i>et al.</i> , (2015)
300	<i>Lycodes japonicus</i>	103	53		BY3		Cytb	No	Biological Factors	Sakuma <i>et al.</i> , (2015)
301	<i>Lycodes matsubarai</i>	189	90		BY3 BY12		D-loop	No	Limitations	Sakuma <i>et al.</i> , (2014)
302	<i>Lycodes matsubarai</i>	189	90		BY3 BY12		Cytb	Yes	Biological Factors & Topography	Sakuma <i>et al.</i> , (2014)
303	<i>Lycodes ocellatus</i>	102	37		BY3		COI	No	Biological Factors	Sakuma <i>et al.</i> , (2015)
304	<i>Lycodes ocellatus</i>	102	37		BY3		Cytb	No	Biological Factors	Sakuma <i>et al.</i> , (2015)
305	<i>Macropipus tuberculatus</i>	750	115		BY4		COI	Yes	Topography	García-Merchána <i>et al.</i> , (2012)
306	<i>Macroscapha</i> spp.	2809	219		BY9		COI	Yes	Depth & Distance	Brandãoa <i>et al.</i> , (2010)
307	<i>Macroscapha</i> spp.	2809	219		BY9		ITS	Yes	Depth & Distance	Brandãoa <i>et al.</i> , (2010)
308	<i>Macrostylis roaldi</i>	937	22		BY9		COI	No	Depth	Riehl <i>et al.</i> , (2012)
309	<i>Macrostylis roaldi</i>	937	39		BY9		12s	No	Depth	Riehl <i>et al.</i> , (2012)
310	<i>Macrostylis roaldi</i>	937	35		BY9		16s	No	Depth	Riehl <i>et al.</i> , (2012)
311	<i>Macruronus novaezelandiae</i>		105		BY6 BY10		RFLP	No	Biological Factors	Smith <i>et al.</i> , (1996)
312	<i>Madrepore oculata</i>	0	42		BY11 BY6 BY10		16s	No	Limitations	Miller <i>et al.</i> , (2010)
313	<i>Madrepore oculata</i>	1301	74		BY6 BY10		ITS	Yes	Depth	Zeng <i>et al.</i> , (2016)
314	<i>Madrepore oculata</i>	1301	134		BY6 BY10		SSR	No	Limitations	Zeng <i>et al.</i> , (2016)
315	<i>Metallogorgia</i>	3660	39		BY4		MutS	No	Limitations	Thoma <i>et al.</i> , (2009)
316	<i>Microstomus pacificus</i>		110		BY3		D-loop	Yes	Biological Factors	Stepien <i>et al.</i> , (1999)
317	<i>Moroteuthis ingens</i>	1355	73		BY9 BY10		RAPD	No	Biological Factors	Sands <i>et al.</i> , (2003)
318	<i>Munida acantha</i>	1348	21		BY12		COI	No	Limitations	Samadi <i>et al.</i> , (2006)

319	<i>Munida acantha</i>	1348	35	BY12	COI	No	Limitations	Samadi <i>et al.</i> , (2006)
320	<i>Munida gracilis</i>	325	52	BY6	COI	No	Biological Factors	Bors <i>et al.</i> , (2012)
321	<i>Munida intermedia</i>	750	113	BY4	COI	No	Current	García-Merchána <i>et al.</i> , (2012)
322	<i>Munida thoe</i>	1348	35	BY12	COI	No	Limitations	Samadi <i>et al.</i> , (2006)
323	<i>Munida zebra</i>	1348	36	BY12	COI	No	Limitations	Samadi <i>et al.</i> , (2006)
324	<i>Munidopsis lauensis</i>	650	111	BY12	COI	Yes	Topography	Thaler <i>et al.</i> , (2014)
325	<i>Munidopsis lauensis</i>	650	95	BY12	SSR	Yes	Topography	Thaler <i>et al.</i> , (2014)
326	<i>Munidopsis scobina</i>	100	421	BY11	Alloenzyme	Yes	Distance & Biological Factors	Creasey <i>et al.</i> , (2000)
327	<i>Myxoderma sacculatum</i>	1800	31	BY3	Alloenzyme	No	Limitations	Ayala <i>et al.</i> , (1975)
328	<i>Narella</i>	3056	41	BY3 BY14	MutS	Yes	Depth	Baco <i>et al.</i> , (2012)
329	<i>Narella</i>	3056	41	BY3 BY14	ND2	Yes	Depth & Distance	Baco <i>et al.</i> , (2012)
330	<i>Narella</i>	3056	41	BY3 BY14	ND6	Yes	Depth	Baco <i>et al.</i> , (2012)
331	<i>Narella</i>	3056	41	BY3 BY14	NCR1	Yes	Depth & Distance	Baco <i>et al.</i> , (2012)
332	<i>Narella</i>	3056	41	BY3 BY14	COI	Yes	Depth	Baco <i>et al.</i> , (2012)
333	<i>Nassaria problematica</i>	1348	24	BY12	COI	Yes	Biological Factors	Samadi <i>et al.</i> , (2006)
334	<i>Nearchaster aciculatus</i>	1800	31	BY3	Alloenzyme	No	Limitations	Ayala <i>et al.</i> , (1975)
335	<i>Nematocarcinus lanceopes</i>	1968	187	BY9	COI	No	Biological Factors	Raupach <i>et al.</i> , (2010)
336	<i>Nematocarcinus lanceopes</i>	1968	187	BY9	16s	No	Limitations	Raupach <i>et al.</i> , (2010)
337	<i>Nematocarcinus lanceopes</i>	1968	187	BY9	28s	No	Limitations	Raupach <i>et al.</i> , (2010)
338	<i>Nematocarcinus lanceopes</i>	1968	187	BY9	16s	No	Limitations	Raupach <i>et al.</i> , (2010)
339	<i>Nematocarcinus lanceopes</i>	1968	187	BY9	28s	No	Limitations	Raupach <i>et al.</i> , (2010)
340	<i>Neaulaxinia persicum</i>	660.5	34	BY6 BY10	COI	No	Limitations	Zeng <i>et al.</i> , (2015)
341	<i>Neaulaxinia persicum</i>	660.5	12	BY6 BY10	12s	No	Limitations	Zeng <i>et al.</i> , (2015)
342	<i>Notoscopelus resplendens</i>	700	9	BY4 BY 10 BY13	COI	Yes	Depth & Distance	Gordeeva <i>et al.</i> , (2014)
343	<i>Nucula ataccellana</i>	2732	91	BY2 AB2	16s	Yes	Depth	Etter <i>et al.</i> , (2007)
344	<i>Nucula ataccellana</i>	2808	130	BY4 BY13	16s	Yes	Depth	Zardus <i>et al.</i> , (2006)
345	<i>Nucula ataccellana</i>	2700	89	BY4	16s	Yes	Depth & Distance	Chase <i>et al.</i> , (1998)
346	<i>Nucula ataccellana</i>	2865	188	BY4 BY13	COI	Yes	Depth	Jennings <i>et al.</i> , (2014)
347	<i>Nucula ataccellana</i>	2865	188	BY4 BY13	16s	Yes	Depth	Jennings <i>et al.</i> , (2014)
348	<i>Nucula ataccellana</i>	2865	188	BY4 BY13	CAL	Yes	Depth	Jennings <i>et al.</i> , (2014)
349	<i>Nucula ataccellana</i>	2200	93	BY4	COI	Yes	Depth	Jennings <i>et al.</i> , (2013)
350	<i>Nucula ataccellana</i>	2200	93	BY4	CAL	Yes	Depth	Jennings <i>et al.</i> , (2013)
351	<i>Nucula ataccellana</i>	2200	84	BY4	MAC	Yes	Depth	Jennings <i>et al.</i> , (2013)
352	<i>Nucula ataccellana</i>	2200	91	BY4	DAC3	Yes	Depth	Jennings <i>et al.</i> , (2013)
353	<i>Nucula ataccellana</i>	2200	91	BY4	DAC6	Yes	Depth	Jennings <i>et al.</i> , (2013)
354	<i>Nymphon australe</i>	1032	131	BY9	COI	Yes	Biological Factors	Arangoa <i>et al.</i> , (2010)
355	<i>Nymphon australe</i>	1032	81	BY9	16s	No	Biological Factors	Arangoa <i>et al.</i> , (2010)
356	<i>Oasisia alvinae</i>	747	4	BY3 BY7	Alloenzyme	No	Distance & Biological Factors	Black <i>et al.</i> , (1998)
357	<i>Oasisia alvinae</i>	747	8	BY3 BY7	COI	No	Distance & Biological Factors	Black <i>et al.</i> , (1998)
358	<i>Oasisia alvinae</i>	826	43	BY7	COI	Yes	Topography	Hurtado <i>et al.</i> , (2004)
359	<i>Ophiacantha vivipara</i>	3300	72	BY9	COI	Yes	Depth & Temperature	O'Hara <i>et al.</i> , (2014)

360	<i>Ophiactis abyssicola</i>	1627	160	BY4 BY11 BY6	COI	Yes	Depth & Distance & Temperature	O'Hara <i>et al.</i> , (2014)
361	<i>Ophiocreas oedipus</i>	1000	25	BY4	16s	Yes	Depth & Distance	Cho <i>et al.</i> , (2010)
362	<i>Ophiocreas oedipus</i>	1000	25	BY4	COI	Yes	Depth & Distance	Cho <i>et al.</i> , (2010)
363	<i>Ophiomusium lymani</i>	792	75	BY4	Alloenzyme	No	Limitations	Hensley <i>et al.</i> , (1995)
364	<i>Ophiomusium lymani</i>	1000	233	BY3	Alloenzyme	No	Distance	Doyle <i>et al.</i> , (1972)
365	<i>Ophiomusium lymani</i>	0	257	BY3	Alloenzyme	No	Limitations	Ayala <i>et al.</i> , (1974)
366	<i>Ophiomyxa vivipara</i>	3300	102	BY6 BY11	COI	Yes	Depth & Temperature	O'Hara <i>et al.</i> , (2014)
367	<i>Ophioplinthaca abyssalis</i>	1000	113	BY4	16s	Yes	Topography	Cho <i>et al.</i> , (2010)
368	<i>Ophioplinthaca chelys</i>	1000	68	BY4	16s	Yes	Topography	Cho <i>et al.</i> , (2010)
369	<i>Ophiothrix aristulata</i>	3300	82	BY6	COI	Yes	Depth & Temperature	O'Hara <i>et al.</i> , (2014)
370	<i>Ophiura ooplax</i>	3300	73	BY6	COI	Yes	Depth & Temperature	O'Hara <i>et al.</i> , (2014)
371	<i>Oridorsalis umbonatus</i>	4403	37	AB2 AB6	12s	Yes	Distance	Pawlowski <i>et al.</i> , (2007)
372	<i>Oridorsalis umbonatus</i>	4403	37	AB2 AB6	ITS	Yes	Distance	Pawlowski <i>et al.</i> , (2007)
373	<i>Oridorsalis umbonatus</i>	4403	18	BY9 BY10	ITS	Yes	Biological Factors	Brandt <i>et al.</i> , (2007)
374	<i>Ostracoda</i>	4403	21	BY9	Sequencing	Yes	Limitations	Brandt <i>et al.</i> , (2007)
375	<i>Ostracoda</i>	4403	21	BY9	Sequencing	Yes	Limitations	Brandt <i>et al.</i> , (2007)
376	<i>Ostracoda</i>	4403	21	BY9	12s	Yes	Limitations	Brandt <i>et al.</i> , (2007)
377	<i>Pagellus bogaraveo</i>	700	19	BY4	Cytb	No	Limitations	Stockley <i>et al.</i> , (2005)
378	<i>Pagellus bogaraveo</i>	700	140	BY4	D-loop	Yes	Biological Factors	Stockley <i>et al.</i> , (2005)
379	<i>Pagellus bogaraveo</i>	700	350	BY4	SSR	Yes	Biological Factors	Stockley <i>et al.</i> , (2005)
380	<i>Pagellus bogaraveo</i>		123	BY4	SSR	No	Biological Factors	Piñera <i>et al.</i> , (2007)
381	<i>Pagurus alatus</i>	750	93	BY4	COI	No	Distance	García-Merchána <i>et al.</i> , (2012)
382	<i>Pagurus excavatus</i>	750	114	BY4	COI	No	Distance	García-Merchána <i>et al.</i> , (2012)
383	<i>Pandalus borealis</i>	500	2100	BY1 BY2 BY4	SSR	Yes	Temperature	Jorde <i>et al.</i> , (2015)
384	<i>Pandalus borealis</i>	450	1893	BY1	SSR	No	Current	Knutsen <i>et al.</i> , (2014)
385	<i>Pandalus borealis</i>	564	3865	BY4	Alloenzyme	Yes	Biological Factors	Drengstig <i>et al.</i> , (2000)
386	<i>Paragorgia arborea</i>	1480	92	BY4 BY3 BY12 BY6 BY8 BY10	mtDNA genome	Yes	Distance	Herrera <i>et al.</i> , (2012)
387	<i>Paragorgia arborea</i>	1480	48	BY4 BY3 BY12 BY6 BY8 BY10	ITS2	Yes	Distance	Herrera <i>et al.</i> , (2012)
388	<i>Paralvinella grasslei</i>	625	182	BY7	Alloenzyme	No	Biological Factors	Jollivet <i>et al.</i> , (1995)
389	<i>Paramuricea</i>	3660	85	BY4	COI	No	Limitations	Thoma <i>et al.</i> , (2009)
390	<i>Paramuricea</i>	3660	85	BY4	ND1	No	Limitations	Thoma <i>et al.</i> , (2009)
391	<i>Paramuricea</i>	3660	85	BY4	ND2	No	Limitations	Thoma <i>et al.</i> , (2009)
392	<i>Parantipathes</i>	3660	32	BY4	ND2	No	Limitations	Thoma <i>et al.</i> , (2009)
393	<i>Parantipathes</i>	3660	32	BY4	ND1	No	Limitations	Thoma <i>et al.</i> , (2009)
394	<i>Parantipathes</i>	3660	32	BY4	COI	No	Limitations	Thoma <i>et al.</i> , (2009)
395	<i>Parapenaeus longirostris</i>	750	101	BY4	COI	No	Distance	García-Merchána <i>et al.</i> , (2012)
396	<i>Parapenaeus longirostris</i>		240	BY4	AFLP	Yes	Distance	Brutto <i>et al.</i> , (2013)
397	<i>Parapenaeus longirostris</i>		107	BY4	D-loop	Yes	Distance	Brutto <i>et al.</i> , (2013)
398	<i>Parbolasia corrugatus</i>		115	BY9	Alloenzyme	Yes	Biological Factors & Ocean current	Rogers <i>et al.</i> , (1998)
399	<i>Pareledone aequipapillae</i>		350	BY9	COI	Yes	Current	Allcock <i>et al.</i> , (2010)
400	<i>Pareledone turqueti</i>	1091	82	BY9	Alloenzyme	Yes	Depth	Allcock <i>et al.</i> , (1997)

401	<i>Parvochelus russus</i>	0	5	BY13	COI	No	Limitations	Brix <i>et al.</i> , (2015)
402	<i>Parvochelus russus</i>	0	2	BY13	16S	No	Limitations	Brix <i>et al.</i> , (2015)
403	<i>Parvochelus russus</i>	0	1	BY13	18S	No	Limitations	Brix <i>et al.</i> , (2015)
404	<i>Penares palmatoclada</i>	1055	22	BY6 BY10	COI	No	Limitations	Zeng <i>et al.</i> , (2015)
405	<i>Penares palmatoclada</i>	1055	18	BY6 BY10	12s	No	Limitations	Zeng <i>et al.</i> , (2015)
406	<i>Phoxocephalidae</i>	1000	297	BY6	COI	Yes	Biological Factors	Knox <i>et al.</i> , (2012)
407	<i>Phoxocephalidae sp</i>	1000	12	BY6	COI	Yes	Topography	Knox <i>et al.</i> , (2012)
408	<i>Pleraster jordani</i>	1800	31	BY3	Alloenzyme	No	Limitations	Ayala <i>et al.</i> , (1975)
409	<i>Pleroma menoui</i>	734	19	BY6 BY10	COI	No	Limitations	Zeng <i>et al.</i> , (2015)
410	<i>Plesionika heterocarpus</i>	750	118	BY4	COI	No	Distance	García-Merchána <i>et al.</i> , (2012)
411	<i>Pocillopora damicornis</i>		16	BY14	CALM	No	Limitations	Flot <i>et al.</i> , (2008)
412	<i>Pocillopora damicornis</i>		16	BY14	EF1 α	No	Limitations	Flot <i>et al.</i> , (2008)
413	<i>Pocillopora damicornis</i>		16	BY14	ATPS β	No	Limitations	Flot <i>et al.</i> , (2008)
414	<i>Pocillopora ligulata</i>		6	BY14	ITS2	No	Limitations	Flot <i>et al.</i> , (2008)
415	<i>Pocillopora molokensis</i>		5	BY14	D-loop	No	Limitations	Flot <i>et al.</i> , (2008)
416	<i>Pocillopora molokensis</i>		5	BY14	ORF	No	Limitations	Flot <i>et al.</i> , (2008)
417	<i>Poecillastra laminaris</i>	1385	54	BY6 BY10	COI	Yes	Current	Zeng <i>et al.</i> , (2015)
418	<i>Poecillastra laminaris</i>	1385	49	BY6 BY10	Cytb	Yes	Current	Zeng <i>et al.</i> , (2015)
419	<i>Polyprion americanus</i>	560	422	BY4	SSR	Yes	Temperature & Current	Ball <i>et al.</i> , (2000)
420	<i>Polyprion americanus</i>	200	179	BY4 BY6 BY13	ND1	No	Biological Factors	Sedberry <i>et al.</i> , (1996)
421	<i>Promachocrinus kerguelensis</i>	1054	37	BY9	COI	No	Biological Factors	Wilson <i>et al.</i> , (2007)
422	<i>Promachocrinus kerguelensis</i>	1054	37	BY9	Cytb	No	Biological Factors	Wilson <i>et al.</i> , (2007)
423	<i>Pseudopentaceros wheeleri</i>		10	BY14	COI	No	Topography	Martin <i>et al.</i> , (1992)
424	<i>Pseudopentaceros wheeleri</i>		17	BY14	RFLP	No	Topography	Martin <i>et al.</i> , (1992)
425	<i>Riftia pachyptila</i>	610	235	BY3 BY7	Alloenzyme	Yes	Distance	Black <i>et al.</i> , (1994)
426	<i>Riftia pachyptila</i>		100	BY7	Alloenzyme	Yes	Biological Factors	Bucklin <i>et al.</i> , (1988)
427	<i>Riftia pachyptila</i>	754	352	BY7	Rpt 46.1	Yes	Distance	Coykendall <i>et al.</i> , (2011)
428	<i>Riftia pachyptila</i>	754	380	BY7	Rpt 84.1	Yes	Distance	Coykendall <i>et al.</i> , (2011)
429	<i>Riftia pachyptila</i>	754	171	BY7	Cytb	Yes	Distance	Coykendall <i>et al.</i> , (2011)
430	<i>Riftia pachyptila</i>	754	10	BY7	ATPS α	Yes	Distance	Coykendall <i>et al.</i> , (2011)
431	<i>Riftia pachyptila</i>	826	135	BY7	COI	Yes	Topography	Hurtado <i>et al.</i> , (2004)
432	<i>Riftia pachyptila</i>	618	29	BY7	AFLP	Yes	Biological Factors	Shank <i>et al.</i> , (2007)
433	<i>Rimicaris exoculata</i>		312	BY4	Alloenzyme	No	Biological Factors	Creasey <i>et al.</i> , (1996)
434	<i>Rimicaris exoculata</i>	1300	12	BY4	COI	No	Limitations	Petersen <i>et al.</i> , (2010)
435	<i>Rimicaris exoculata</i>	1300	12	BY4	Cytb	No	Limitations	Petersen <i>et al.</i> , (2010)
436	<i>Rimicaris exoculata</i>	1450	152	BY4	COI	No	Biological Factors	Teixeira <i>et al.</i> , (2011)
437	<i>Rimicaris exoculata</i>	1450	687	BY4	SSR	No	Biological Factors	Teixeira <i>et al.</i> , (2012)
438	<i>Rimicaris kairei</i>	0	54	BY11	COI	No	Biological Factors	Beedessee <i>et al.</i> , (2013)
439	<i>Sassia remensa</i>	1348	24	BY12	COI	No	Biological Factors	Samadi <i>et al.</i> , (2006)
440	<i>Scaphander bathymophilus</i>	4400	68	BY4	COI	Yes	Topography	Eilertsen <i>et al.</i> , (2015)
441	<i>Scaphander bathymophilus</i>	4401	68	BY4	16s	Yes	Topography	Eilertsen <i>et al.</i> , (2015)
442	<i>Scaphander lignarius</i>	784	112	BY4	COI	No	Limitations	Eilertsen <i>et al.</i> , (2015)

443	<i>Scaphander lignarius</i>	784	111	BY4	16s	No	Limitations	Eilertsen <i>et al.</i> , (2015)
444	<i>Scaphander lignarius</i>	784	92	BY4	28s	No	Limitations	Eilertsen <i>et al.</i> , (2015)
445	<i>Scaphander nobilis</i>	2800	9	BY4	16s	No	Biological Factors	Eilertsen <i>et al.</i> , (2015)
446	<i>Scaphander punctostriatus</i>	2530	150	BY4	COI	Yes	Biological Factors	Eilertsen <i>et al.</i> , (2015)
447	<i>Scaphander punctostriatus</i>	2530	150	BY4	16s	Yes	Biological Factors	Eilertsen <i>et al.</i> , (2015)
448	<i>Scaphander punctostriatus</i>	2530	150	BY4	28s	No	Biological Factors	Eilertsen <i>et al.</i> , (2015)
449	<i>Scaphander watsoni</i>	400	32	BY4	COI	No	Biological Factors	Eilertsen <i>et al.</i> , (2015)
450	<i>Scaphander watsoni</i>	400	32	BY4	16s	No	Biological Factors	Eilertsen <i>et al.</i> , (2015)
451	<i>Scaphander watsoni</i>	400	32	BY4	28s	Yes	Biological Factors	Eilertsen <i>et al.</i> , (2015)
452	<i>Scyliorhinus canicula</i>	446	431	BY4	COI	Yes	Topography	Kousteni <i>et al.</i> , (2015)
453	<i>Scyliorhinus canicula</i>	446	834	BY4	SSR	Yes	Topography	Kousteni <i>et al.</i> , (2015)
454	<i>Sebastes fasciatus</i>	295	596	BY4	SSR	Yes	Limitations	Valentin <i>et al.</i> , (2014)
455	<i>Sebastes marinus</i>		130	BY2	ND3	Yes	Depth	Schmidt <i>et al.</i> , (2005)
456	<i>Sebastes mentella</i>	700	2931	BY2	Cytb	Yes	Depth	Ingimarsdóttir <i>et al.</i> , (2008)
457	<i>Sebastes mentella</i>		98	BY2	SSR	Yes	Depth	Pampoulie <i>et al.</i> , (2008)
458	<i>Sebastes mentella</i>		130	BY2	SSR	Yes	Biological Factors	Schmidt <i>et al.</i> , (2005)
459	<i>Sebastes mentella</i>	659	1763	BY2	Alloenzyme	Yes	Depth	Danielsdóttir <i>et al.</i> , (2008)
460	<i>Sebastes mentella</i>	295	495	BY4	SSR	Yes	Depth	Valentin <i>et al.</i> , (2014)
461	<i>Sebastes mentella</i>		4	BY2	16s	No	Limitations	Sundt <i>et al.</i> , (1998)
462	<i>Sebastes mentella</i>	580	1946	BY2 BY1	Alloenzyme	Yes	Depth	Johansen <i>et al.</i> , (2000)
463	<i>Sebastes mentella</i>		1901	BY2	SSR	Yes	Depth	Stefánsson <i>et al.</i> , (2009)
464	<i>Sebastes mentella</i>	502	1240	BY2	SSR	Yes	Depth	Stefánsson <i>et al.</i> , (2009)
465	<i>Sebastes mentella</i>		973	BY2 BY4	SSR	Yes	Current	Roques <i>et al.</i> , (2002)
466	<i>Sebastolobus macrochir</i>	781	66	BY12	D-loop	No	Biological Factors	Sakaguchi <i>et al.</i> , (2014)
467	<i>Shinkaia crosnieri</i>	377.7	64	BY12	COI+Cytb+16s	Yes	Temperature	Shen <i>et al.</i> , (2016)
468	<i>Shinkaia crosnieri</i>	355	66	BY12	COI	Yes	Temperature	Yang <i>et al.</i> , (2016)
469	<i>Shinkaia crosnieri</i>	355	76	BY12	ANT	No	Limitations	Yang <i>et al.</i> , (2016)
470	<i>Shinkaicaris leurokolos</i>	1071	53	BY12	COI	No	Topography	Yahagi <i>et al.</i> , (2015)
471	<i>Sicyopus commensalis</i>	0	55	BY4	Alloenzyme	No	Biological Factors	Bronsdon <i>et al.</i> , (1997)
472	<i>Sigmops elongatus</i>	701	7	BY4 BY 10 BY13	COI	Yes	Depth	Gordeeva <i>et al.</i> , (2014)
473	<i>Solenosmilia variabilis</i>	0	33	BY11 BY6 BY10	16s	No	Limitations	Miller <i>et al.</i> , (2010)
474	<i>Solenosmilia variabilis</i>	0	12	BY11 BY6 BY10	D-loop	No	Biological Factors	Miller <i>et al.</i> , (2010)
475	<i>Solenosmilia variabilis</i>	1483	186	BY6 BY10	ITS	No	Limitations	Zeng <i>et al.</i> , (2016)
476	<i>Solenosmilia variabilis</i>	308	568	BY10	SSR	Yes	Biological Factors	Miller <i>et al.</i> , (2016)
477	<i>Solenosmilia variabilis</i>	1483	139	BY6 BY10	D-loop	No	Limitations	Zeng <i>et al.</i> , (2016)
478	<i>Solenosmilia variabilis</i>	1483	198	BY6 BY10	SSR	Yes	Distance	Zeng <i>et al.</i> , (2016)
479	<i>Stephanauge inornata</i>	0	75	BY4	Alloenzyme	No	Biological Factors	Bronsdon <i>et al.</i> , (1997)
480	<i>Stephanocyathus spiniger</i>	0	18	BY11 BY6 BY10	ITS	No	Current	Miller <i>et al.</i> , (2010)
481	<i>Stephanocyathus spiniger</i>	0	5	BY11 BY6 BY10	16s	No	Limitations	Miller <i>et al.</i> , (2010)
482	<i>Stereomastis suhmi</i>	2620	14	BY10 & BY13	COI	No	Current	Farias <i>et al.</i> , (2015)
483	<i>Sternoptyx diaphana</i>	300	11	BY4 BY 10 BY13	COI	Yes	Distance	Gordeeva <i>et al.</i> , (2014)
484	<i>Stichopathes filiformis</i>	0	17	BY11 BY6 BY10	ITS	No	Limitations	Miller <i>et al.</i> , (2010)
485	<i>Stichopathes variabilis</i>	0	41	BY11 BY6 BY10	16s	Yes	Topography	Miller <i>et al.</i> , (2010)

486	<i>Stichopathes variabilis</i>	0	57	BY11 BY6 BY10	ITS	Yes	Topography	Miller <i>et al.</i> , (2010)
487	<i>Terebellides gingko</i>	5152.5	15	BY13	16s	No	Biological Factors	Schuller <i>et al.</i> , (2012)
488	<i>Tevnia jerichonana</i>	747	4	BY3 BY7	Alloenzyme	No	Distance & Biological Factors	Black <i>et al.</i> , (1998)
489	<i>Tevnia jerichonana</i>	747	8	BY3 BY7	COI	No	Distance & Biological Factors	Black <i>et al.</i> , (1998)
490	<i>Tevnia jerichonana</i>	512	24	BY5 BY7	COI	Yes	Distance	Zhang <i>et al.</i> , (2015)
491	<i>Tevnia jerichonana</i>	512	27	BY5 BY7	Cytb	Yes	Distance	Zhang <i>et al.</i> , (2015)
492	<i>Tevnia jerichonana</i>	512	38	BY5 BY7	HSP	Yes	Distance	Zhang <i>et al.</i> , (2015)
493	<i>Tevnia jerichonana</i>	512	20	BY5 BY7	ATPS α	No	Limitations	Zhang <i>et al.</i> , (2015)
494	<i>Tevnia jerichonana</i>	826	114	BY7	COI	Yes	Topography	Hurtado <i>et al.</i> , (2004)
495	<i>Tokoprymno maia</i>	1350	80	BY6 BY9 BY10	ITS2+28s	Yes	Current	Dueñas <i>et al.</i> , (2016)
496	<i>Valenciennellus tripunctulatus</i>	200	11	BY4 BY 10 BY13	COI	No	Current	Gordeeva <i>et al.</i> , (2014)
497	<i>Ventiella sulfuris</i>	180	310	BY7	Alloenzyme	Yes	Topography	France <i>et al.</i> , (1992)
498	<i>Vestimentiferan</i>	3118	37	BY14 BY5	COI	Yes	Depth	Mcmullin <i>et al.</i> , (2003)
499	<i>Vestimentiferan</i>	3118	37	BY14 BY6	16s	Yes	Depth	Mcmullin <i>et al.</i> , (2003)
500	<i>Vinciguerrria nimbaria</i>	300	10	BY4 BY 10 BY13	COI	Yes	Depth	Gordeeva <i>et al.</i> , (2014)
501	<i>Vulcanolepas</i>	2226	96	BY11	SNP	Yes	Current	Herrera <i>et al.</i> , (2015)
502	<i>Zoroaster fulgens</i>	3556	25	BY4	16s	Yes	Depth	Howell <i>et al.</i> , (2004)
503	<i>Zoroaster fulgens</i>	3556	19	BY4	COI	Yes	Depth	Howell <i>et al.</i> , (2004)

Chapter 3. Data mining from *Poecillastra laminaris* (Demosponge) 454 sequencing data

3.1. Introduction

Poecillastra laminaris (Sollas) is a common demosponge found in the New Zealand region, and is widely distributed on seamounts and other habitats from 100 to 1500 m water depth (van Soest *et al.*, 2005). Extensive deep-sea trawl fisheries occur on seamounts throughout the New Zealand Exclusive Economic Zone (EEZ) (e.g., Clark & O'Driscoll, 2003), and benthic fauna such as demosponges are known to be vulnerable to damage from such fishing activities (e.g., Clark & Tittensor, 2010). Because this species, like other structure-forming sponges, may provide shelter and habitat (e.g., places for reproduction) for other species (Murillo *et al.*, 2012), it can be considered as an indicator taxon for Vulnerable Marine Ecosystems (VMEs) in the South Pacific Ocean (Parker *et al.*, 2009). Knowledge of population differentiation and connectivity of sponge species in the South Pacific is generally lacking, despite the recognition that such information is useful for spatial management planning (Almany *et al.*, 2009). As such, knowledge about the population genetic structure of *Poecillastra laminaris* has the potential to contribute to improved spatial management measures, including the identification or re-design of marine protection areas (MPAs) in the New Zealand EEZ and the wider South Pacific.

Next-generation sequencing (NGS), also known as high-throughput sequencing, is now very popular in genetic studies and allows researchers to sequence DNA and RNA much more quickly and cheaply than the previously used Sanger sequencing. However, the power of discovering genetic markers in deep-sea animals, and especially in deep-sea sponges, is still in its infancy and remains a challenge (Sharma *et al.*, 2012). In this study, to develop suitable markers for *Poecillastra laminaris*, next generation sequencing was utilised to obtain genomic information and also to generate new genetic markers for population studies.

3.2. Materials and methods

3.2.1. Samples, DNA extractions and pyrosequencing

The *Poecillastra laminaris* used in this study, NIWA ID 86172, was collected on 18 October 2012 during cruise TAN1213, from a seamount on the Colville Ridge, New Zealand (30.177 S, 179.743 E), between 387 and 422 m depth. After collection, the specimen was kept in absolute ethanol at the National Institute of Water and Atmospheric Research (NIWA) invertebrate collection. DNA from this individual was extracted using the guanidinium

thiocyanate method (Boom *et al.*, 1990), and was then quantified by Nanodrop 1000 (Thermo Scientific) and 0.5% agar electrophoresis on 80 V for 2 h. Before 454 GS-FLX Pyrosequencing, a genomic DNA barcoded library was constructed (Macrogen Inc., Korea). The 454 GS-FLX Titanium platform was utilised to sequence the library (Macrogen Inc., Korea).

3.2.2. Microsatellite discovery and characterisation

MSATCOMMANDER 0.8 was used to identify microsatellite sequences and to design primers for genetic analysis. Microsatellites, also called simple sequence repeats (SSR), with a minimum of 5 repeats for di-, tri-, tetra-, penta-, and hexanucleotides were selected, and MSATCOMMANDER with default settings, except for the minimum and maximum PCR product size (set to 100–400 bp), was used to design PCR primers. After primers were chosen with similar annealing temperatures and low penalty scores for both forward and reverse primers, the top 50 primer pairs were used for further PCR amplification test.

The PCR successfully-amplified loci were chosen to genotype to test for their levels of polymorphism. M13 (fluorescent labelling) was used as an economic tool to test the polymorphism of PCR-succeeded candidate loci, and the forward primers of these loci were subsequently tagged with M13 sequences (Schuelke, 2000). To test whether these loci were the polymorphic, 30 individuals were selected to conduct the PCR amplification. PCR amplifications were carried out in 30 µl reactions consisting of 15 µl PCR Red Mixture (MyTaq™ Red Mix), 0.5 µl of forward primer and labelled M13, 1 µl of reverse primer and genomic DNA, and 12 µl of water. The thermal cycler was programmed for touchdown PCR, in which denaturation at 94 °C for 5 min was followed by 20 cycles of three 45 s steps: denaturation at 94°C, annealing at 65–55 °C and extension at 72 °C; the initial annealing temperature of 65 °C was reduced by 0.5 °C every cycle to reach 55 °C, and then followed by 10 cycles of annealing at 55 °C. After the first 30 cycles, another 8 cycles of annealing at 53 °C were employed to amplify the M13. The PCR ended with an extra extension of 30 min at 72 °C.

Fluorescently labelled PCR products were run on an ABI 3730 DNA Sequencer (Macrogen Inc., Korea), scored using the GeneScan-500 (LIZ) size standard, and analysed with GeneMarker software (GeneMarker, USA). Allelic frequencies, number and size of alleles, and observed and expected heterozygosities were estimated in GenAlEx 6.5 (Peakall

& Smouse, 2012). Departures from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were tested in GENEPOP on the web v4.0 with default settings. Polymorphic information content (PIC) was calculated using CERVUS 3.0 (Marshall *et al.*, 1998).

3.2.3. Mitochondrial genome assembly and marker design

Using a DNA alignment methodology similar to that described by Elbrecht *et al.* (2015), the mitogenome of *Geodia neptuni* (GenBank No. NC_006990, Lavrov *et al.*, 2005) was used as a reference to identify sequences of mitochondrial origin in the quality-trimmed 454 data using the discontinuous megablast algorithm BLASTn within Geneious R7 (max e-values = 1e-20) (Drummond *et al.*, 2011). After extracting the relevant reads, the genome was assembled by mapping to the reference mitogenome of *G. neptuni* using Geneious software. The first draft genome was subsequently used as a search query to identify additional reads in the 454 read database. The mitogenome was annotated for coding DNA sequence (CDS), tRNA and rRNA by comparison with all available Demospongiae mitogenomes in GenBank (n=8). Thereafter, the ORFs finder and Annotation (Invertebrate Mitochondrial) functions in Geneious were used to check the position and length of each CDSs.

After assembled genome, the genes, *Cytochrome c oxidase subunit I (COI)*, *Cytochrome b (Cytb)* and *12 Ribosomal RNA (12S)*, were selected to design specific primers in Geneious (v 7.0). To obtain cross-amplified primers for other study sponges, the primers binding areas were restricted to the shared sequences in three genes between *G. neptuni* and *Poecillastra laminaris* mitogenomes. Three different pairs of primers for each gene were designed, and three *Poecillastra laminaris* were used to test the primers performance (PCR amplification and polymorphism).

3.3. Results

3.3.1. Next Generation Sequencing

For 454 pyrosequencing of *Poecillastra laminaris* genomic DNA, the total number of base pairs was 171,248,552 with 257,561 reads and an average sequence length of 665 bp. Because there is no published genome information for closely related species, it was difficult

to estimate the coverage of this sequencing data, and it is impossible to compare the distribution patterns of microsatellites.

3.3.2. Mitogenome assembly

After assembly, the complete mitogenome of *Poecillastra laminaris* was 18,413 bp in length. The final assembly contained 530 reads from the 454 pyrosequencing data base, with a minimum and maximum coverage of 7 and 46 sequences, respectively, and an average sequence coverage of 22.7 (SD=6.1). The mitochondrial genome included 14 protein-coding genes, 2 ribosomal RNA genes and 24 transfer RNA genes. The base composition of the genome was A (29.1%), T (35.2%), C (14.0%) and G (21.7%) with a GC content of 35.7% (Table 3.1). The annotated mitogenome is available online in NCBI database (GenBank accession number KM362735).

Table 3.1 Characterisation of the mitochondrial genome of *Poecillastra laminaris*

Name	Location5'-3'	Size	Start	Stop	Name	Location5'-3'	Size	Start	Stop
<i>rnl rRNA</i>	32–2449	2418			<i>nad3</i>	9442–9798	357	ATG	TAG
<i>COII</i>	2474–3232	759	ATG	TAA	<i>trnR(ucg) tRNA</i>	9811–9881	71		
<i>trnK(uuu) tRNA</i>	3268–3340	73			<i>nad4L</i>	9882–10,211	301	GTG	TAA
<i>ATP8</i>	3342–3533	192	ATG	TAA	<i>COI</i>	10,199–11,761	1563	GTG	TAA
<i>ATP6</i>	3533–4267	735	ATG	TAA	<i>trnS(uga) tRNA</i>	11,795–11,879	75		
<i>trnR(ucu) tRNA</i>	4289–4361	73			<i>trnI(cau) tRNA</i>	11,915–11,987	72		
<i>COIII</i>	4380–5168	789	ATG	TAG	<i>trnD(guc) tRNA</i>	12,001–12,072	72		
<i>trnQ(uug) tRNA</i>	5192–5264	73			<i>trnC(gca) tRNA</i>	12,073–12,144	72		
<i>trnW(uca) tRNA</i>	5283–5353	71			<i>nad1</i>	12,165–13,148	984	ATG	TAG
<i>trnN(guu) tRNA</i>	5371–5442	72			<i>trnL(uaa) tRNA</i>	13,181–13,261	81		
<i>trnL(uag) tRNA</i>	5443–5516	74			<i>trnI(gau) tRNA</i>	13,286–13,359	74		
<i>Cytb</i>	5519–6664	1146	GTG	TAA	<i>trnY(gua) tRNA</i>	13,409–13,481	73		
<i>trnT(ugu) tRNA</i>	6675–6747	73			<i>trnM(cau) tRNA</i>	13,489–13,559	71		
<i>ATP9</i>	6788–7024	237	ATG	TAA	<i>nad2</i>	13,590–14,987	1398	ATG	TAA
<i>trnS(gcu) tRNA</i>	7062–7146	85			<i>nad5</i>	14,991–16,835	1845	ATG	TAG
<i>trnP(ugg) tRNA</i>	7176–7248	73			<i>trnA(ugc) tRNA</i>	16,826–16,898	73		
<i>nad4</i>	7270–8697	1428	ATG	TAG	<i>trnF(gaa) tRNA</i>	16,917–16,989	73		
<i>trnH(gug) tRNA</i>	8703–8774	72			<i>rns rRNA</i>	17,020–18,254	1235		
<i>trnE(uuc) tRNA</i>	8802–8873	72			<i>trnG(ucc) tRNA</i>	18,255–18,326	72		
<i>nad6</i>	8876–9433	558	GTG	TAA	<i>trnV(uac) tRNA</i>	18,341–18,413	73		

The complete mitochondrial genome of *Poecillastra laminaris* (18,413 bp) was 2.2% longer than the genome of *G. neptuni* (18,020 bp), mainly due to insertions in non-coding regions. There were however, 6 insertions in coding regions, including 12 bp and 21 bp in the

nad5, 9 bp in the *nad4L*, 9 bp in the *nad4*, 18 bp in the *COII* and 10 bp in the *ATP9* genes. The number and order of predicted genes in the *Poecillastra laminaris* mitogenome were identical according to *G. neptuni*, and the pairwise sequence identity was 92.0%.

In three designed markers, all genes were successfully amplified in *Poecillastra laminaris* (details see Table 3.2). Furthermore, these three pairs of primers were used to amplify in *Penares* sp., *Pleroma menoui* and *Neoaulaxinia persicum*, and only *COI* was amplified in all other three species. The *Cytb* was failed to obtain PCR products from other three species, but *I2S* was succeeded to amplify in *Penares* sp. and *N. persicum*. These three markers were used in following chapters to investigate genetic connectivity patterns of sponges and reveal the environmental factors that explained genetic structures.

Table 3.2 Details of PCR primers and conditions used to amplify three DNA regions

DNA regions	Primer names	Primers 5'-3'	Annealing temperature (°C)	Trimmed size (bp)
<i>I2S</i>	Rnl F	GACACAGGGATGTCGCAGAA	50	~700
	Rnl R	TGACGGGCGATTTGTACGAA		
<i>COI</i>	19F	TGGTGCTTTTTCGGGGATGA	50	400-470
	570R	ATCGCACCAGCCAATACAGG		
<i>Cytb</i>	<i>Cytb</i> F	CGCTAATGGGGCGTCTATGT	50	402
	<i>Cytb</i> R	AAAGTTTTCTGCGTCCCCCA		

3.3.3. Microsatellite distribution

In total, 48,938 putative microsatellites were discovered in the *Poecillastra laminaris* DNA sequence data, of which the di-, tri-, tetra-, penta- and hexanucleotide repeats numbered 46,254 (94.51%), 640 (1.31%), 1,764 (3.61%), 110 (0.22%) and 170 (0.35%), respectively. The abundance of dinucleotide repeats was much higher than other repeat types, with a dominance of AC repeats (89.45% in dinucleotide repeats).

3.3.4. Microsatellite characterisation

After primary exclusion of PCR-failed and monomorphic loci, only 10 polymorphic loci were amplified in most specimens. These 10 microsatellites were highly polymorphic, with 10 to 27 alleles per locus (mean $\pm$ SD of 16.2 ± 5.1). Expected and observed heterozygosities of those loci were 0.786-0.952 and 0.550-0.926, respectively. Except PNL8, the other 9 loci showed significant deviations from Hardy–Weinberg Equilibrium; significant linkage disequilibrium was only observed between PLN21 and PLN48 ($P = 0.0465$).

Sequences containing microsatellites identified for *Poecillastra laminaris* have been deposited in GenBank (accession numbers in Table 3.3).

Table 3.3 Characterisation of 10 microsatellites for *Poecillastra laminaris*

Locus	Primer sequences (5'-3')	Motif	N	Size range	Na	Ho	He	GenBank No.
PLN4	F: TGT TAG CGC CTG TGT TGA AC R: TGC CCG GAG AGT TAT TGT GG	[ACAG]7 ...[ACAG]]17	23	124-244	21	0.783	0.936	KT372680
PLN7	F: CAG TGG ATG TGC ATT GTG GG R: TAC ACA TGC CCT GGT CTC TG	[ACAT]6 ...[ACAT]]6	20	392-488	16	0.550	0.920	KT372681
PLN8	F: GCA AAC GGT TTC CCT TGT TG R: CTG TGT CCA CCT GCA ATA CG	[ACAT]7	21	414-478	16	0.857	0.913	KT372682
PLN17	F: CTC TCT TTG TCC TCT CCG GG R: AGG ATG CCG ACC CAT TCT AC	[AG]8...[AG]10	24	267-315	13	0.917	0.880	KT372683
PLN21	F: TGA AGC CCT ACA GTG GAC AG R: CTG CCA ACC GTA CCA AGA AC	[AGG]12 ...[AGG] 8	27	262-358	19	0.556	0.921	KT372684
PLN26	F: TGG TCA CTT GAG ATC GAG CC R: CAG GTG AAC TCA ATG GCT GC	[AT]11	27	126-212	15	0.926	0.824	KT372685
PLN27	F: GAT GCA GCA CAA GAC CTT CC R: TCC ATT CCT CAG TAG CTC GC	[AT]11	26	336-376	10	0.692	0.786	KT372686
PLN34	F: CAT GAC AGT TGT GCT CAC CC R: ATC AAT CAC CTG AGG ACC CG	[AT]13	34	172-238	15	0.735	0.817	KT372687
PLN39	F: CAT CCA TAT CTG TCG CAC GC R: CAG GAG AGG AGG TCA TCA CG	[AT]15	28	151-285	27	0.786	0.952	KT372688
PLN48	F: CAA GAA CCA ACA ACT CCG GG R: TCG CAT TAA TTT CCG ACC GC	[CG]8	29	311-359	10	0.172	0.788	KT372689

N successfully scored individuals, Na No. of different alleles, Ho observed heterozygosity, and He expected heterozygosity.

3.4. Discussion and conclusion

Testing showed that all loci exhibited significant heterozygote deficits except PLN17 and PLN26, explaining the fact that most loci deviated from Hardy-Weinberg Equilibrium. Details about whether these deviations result from technical artefacts of the markers themselves (e.g. null alleles) and/or with the examined population were analysed discussed in Chapter 5.

These microsatellite markers are now being applied to multiple populations of *Poecillastra laminaris* sampled from the New Zealand EEZ to answer questions about genetic structure and barriers to gene flow which will inform current initiatives to develop options for the increased protection of VMEs in the South Pacific Ocean.

Chapter 4. Data mining from *Solenosmilia variabilis* (Scleractinia) Next Generation Sequencing data

4.1. Introduction

Due to limited research, the deep-sea ecosystems on the planet remain poorly understood. Invisible geographic and oceanographic barriers bring a challenge for explaining population differentiation and connectivity on different scales. Although there has been rapid growth in developing new and highly informative marker types, sampling constraints and lack of reference genomes have often limited the study of evolutionary questions and ability to address conservation issues (Sharma *et al.*, 2012).

Stony coral *Solenosmilia variabilis* is included in the family Caryophylliidae, within the Scleractinia. It has a cosmopolitan distribution and occurs in the upper bathyal zone (151-2165 m from WoRMS and NIWA Invertebrate Collection database). *S. variabilis* is a branching, matrix-forming stony coral that builds up a similar (repeating) and complex 3-dimensional structure that forms ecological niches for a large diversity of associated micro- and macrofauna (Rogers 1999; Henry & Roberts 2007; Althaus *et al.*, 2009; Rogers *et al.*, 2014). Stony coral reef habitat is fragile and easily damaged by near-bottom trawling (Clark & Rowden, 2009), and the coral is potentially vulnerable to the effects of climate change and ocean acidification as well (Fallon *et al.*, 2014). Owing to its important ecological function, this coral is an indicator for Vulnerable Marine Ecosystem (VME) in the South Pacific (Parker *et al.*, 2009). Unfortunately, the current knowledge of deep-sea species is limited: reproduction, larval dispersal and recruitment information for *S. variabilis* are still not well-studied. Using genetic tools to understand patterns of population connectivity of this species will help to provide valuable information for deep-sea conservation.

Despite its importance, no DNA markers have yet been developed for this species. As reported in a previous study (Addamo *et al.*, 2015), microsatellites have been developed in less than 3% of Scleractinian species. These limited genetic resources for Scleractinia have impeded population genetic studies, especially for deep-sea stony corals. However, recent publications reported that Scleractinia showed a degree of microsatellite polymorphism that is transferable, with high levels of polymorphism detected in one species also being found at the corresponding locus of another species for many loci across genus, family and order level (Nakajima *et al.*, 2009; Wang *et al.*, 2009; Tang *et al.*, 2010; Casado-Amezua *et al.*, 2011; 2012; Davies *et al.*, 2013; Shinzato *et al.*, 2014; Zilberberg *et al.*, 2014; Montoya-Maya *et al.*, 2014; Addamo *et al.*, 2015). Microsatellite transferability (cross-species amplification) amongst related species is allowed for by the homologous nature of the DNA sequence in the microsatellite flanking regions. Therefore, transferability can be a very important factor in

facilitating the use of microsatellites because it reduces costs when fresh samples are difficult to obtain or reference genomes are not available.

Next-generation sequencing (NGS), also known as high-throughput sequencing, has become very popular in genetic studies after its development in the mid to late 1990s. With ongoing technological development, it allows researchers to sequence DNA and RNA much more quickly and cheaply than the previously used Sanger sequencing, and as such has revolutionised the study of genomics and molecular biology. There are a number of distinctive modern sequencing technologies, including Illumina (Solexa), Roche 454, Ion torrent (Proton / PGM) and SOLiD sequencing (Liu *et al.*, 2012). Roche 454 sequencing, also known as pyrosequencing, as used in this study, produces long reads with high accuracy and relatively low cost, and the longer reads are beneficial for genome assembly and microsatellite primer design. Illumina (sequencing by synthesis), which is the sequencing method for the restriction-site-associated DNA (RAD) sequencing data, generates shorter reads but the depth of sequence coverage is much greater than for 454 sequencing. Theoretically, 454 sequencing data with longer reads is better to develop microsatellites, and RAD-sequencing data with higher depth has advantages for discovering SNPs. However, the power of these methodologies to discover markers in non-model species (i.e. with no reference genome), and especially in taxa from the deep-sea benthos, remains largely unexplored and is a challenge (Sharma *et al.*, 2012).

In this study, to develop suitable markers for *S. variabilis*, next generation sequencing was utilised to obtain genomic information. Markers were developed to assess genetic connectivity and to identify barriers to gene flow for *S. variabilis* for use in the context of increased protection of New Zealand's VMEs, and cross-amplification in other common stony coral in New Zealand (*Goniocorella dumosa*, *Desmophyllum dianthus* and *Madrepora oculata*) were also tested, and cross-amplifications were compared between species. These developed markers and cross-amplification results were considered and utilized in genetic connectivity assessment if feasible.

4.2. Materials and methods

4.2.1. Samples, DNA extraction and pyrosequencing

Three live *S. variabilis* (NIWA ID number 94287 (37.707 S, 169.015 W, 1244 m); 94550 (41.581 S, 164.255 W, 1223 m); 94590 (41.365 S, 164.419 W, 1220 m)) were

collected from the Louisville Seamount Chain off NE New Zealand during cruise TAN1402 in February, 2014. Specimens were initially preserved in absolute ethanol and stored at -80 °C on board, and then transferred from NIWA to the VUW laboratory under permit from Biosecurity New Zealand. Genomic DNA (gDNA) for pyrosequencing was extracted using the phenol-chloroform method. After extraction, DNA of two species was quantified by Nanodrop 1000 (Thermo Scientific) and 0.5% agar electrophoresis at 80 V for 2 h. Before 454 GS-FLX pyrosequencing, the three genomic DNA samples were barcoded as three separate libraries (barcoding and libraries construction were conducted by Macrogen Inc., Korea). The 454 GS-FLX Titanium platform was utilised to sequence these libraries (Macrogen Inc., Korea).

4.2.2. Mitochondrial genome assembly and marker design

Following (Elbrecht *et al.*, 2013) the mitogenome of *Lophelia pertusa* (GenBank No. KC875349 (Flot *et al.*, 2013), with the highest pairwise identity at the *COI* region, was used to identify DNA sequences and extract all mitogenome reads from the *S. variabilis* libraries using the discontinuous megablast algorithm BLASTn in the Geneious software package (max e-values 1e-20) (Drummond *et al.*, 2013). Extracted reads were assembled by mapping to the reference mitogenome of *L. pertusa*. The first draft genome was manually checked and then used as a search query to identify additional reads in the 454 read database. The mitogenome was annotated for coding DNA sequence (CDS), tRNA and rRNA by comparison with all available scleractinian mitogenomes in GenBank (n=30). The ORFs finder and Annotation (Invertebrate Mitochondrial) functions in Geneious were used to check the position, start and stop codons and length of each CDS. The same method was applied to construct three mitochondrial genomes from the three libraries but using the mitogenome constructed above as the reference. After assembling the mitogenomes from three libraries, multiple alignment (ClustalW) was utilised to compare differences amongst the three mitogenomes in Geneious.

To develop the polymorphic sequencing markers, the two assembled mitogenomes were aligned to identify the unidentical sites. Candidate genes which the sequences were different from each other in two mitogenomes were selected to design primers in Geneious (v7.0). For *ITS* marker, the available *ITS* sequences Scleractinia were used to extract *ITS* reads in 454 data and extracted reads were assembled with the same setting as described above and

multiple alignment was completed by ClustalW in Geneious (v7). The primers were designed based on the alignment with default settings.

4.2.3. Microsatellite discovery and characterisation

MSATCOMMANDER 0.8 was used to isolate microsatellites and design primers for genetic analysis on all library data, respectively. Microsatellites, also called simple sequence repeats (SSR), with a minimum of 5 repeats for di-, tri-, tetra-, penta-, and hexanucleotides were selected, and MSATCOMMANDER with default settings, except for the minimum and maximum PCR product size (set to 100–400 bp), was used to design PCR primers for *S. variabilis*.

To identify polymorphic microsatellites directly in *S. variabilis*, Microsoft Excel (v 2013) was used to identify the locus which are shared same motif (exactly same repeated motif and primers pair) but having a different number of repeats amongst different libraries. This is to waive the experimental test of whether the locus was polymorphic, and the only experiment is to verify PCR amplification. After successfully-amplified, the selected microsatellites were subsequently characterised by genotyping.

The gDNA of 3 specimens (collected off New Zealand in the SW Pacific Ocean) were initially used to test for successful PCR amplification by visualising amplified products on 2% agarose gels. Primers that successfully amplified a product (putative microsatellite locus) were allocated to different multiplex systems by Multiplex Manager 1.0 (Holleley & Geerts, 2009).

The forward primers from each primer pair were fluorescently 5' end labelled with 6-FAM, NED, VIC or PET following the results of the multiplex. For genotyping, PCRs were carried out in a total volume of 40 µl with 20 µl PCR Red Mixture MyTaq™ Red Mix), 1 µl forward and reverse primers, 2 µl of template DNA and topped up with dd H₂O. PCR amplifications were performed with an initial denaturing step of 94 °C for 10 min, followed by 32 cycles of 30 s at 94 °C, 45 s annealing at 55 °C, and 45 s extension at 72 °C, and a final extension of 30 min at 72 °C.

Nine microsatellite loci of *S. variabilis* from Miller & Gunasekera (2016) were also introduced in this study, and the amplification process of these 9 loci followed the method described above. The same PCR conditions for the newly developed and the introduced microsatellites were employed for cross-species amplification in three other stony corals

(most-common stony corals in New Zealand deep-sea coral reef), *G. dumosa*, *D. dianthus* and *M. oculata*.

All microsatellites characterisation in *S. variabilis*, *G. dumosa*, *D. dianthus* and *M. oculata* were based on randomly selected 30 individuals for each species. Fluorescently labelled PCR products were run on an ABI 3730 DNA Sequencer (Macrogen Inc., Korea), scored using the GeneScan-500 (LIZ) size standard, and analysed with GeneMarker software (GeneMarker, USA). Allelic frequencies, number and size of alleles, and observed and expected heterozygosities were estimated in GenAlEx 6.5 (Peakall & Smouse, 2012). Departures from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were tested in GENEPOP on the web v4.0 with default settings. Polymorphic information content (PIC) was calculated using CERVUS 3.0 (Marshall *et al.*, 1998).

4.2.4. Single nucleotide polymorphisms (SNPs) discovery

Three 454 sequencing datasets from the present study and a RAD sequencing dataset from a collaborator's lab (Karen Miller, AIMS, Australia) were combined to develop SNPs for *S. variabilis* population genetics studies. Three RAD samples were pooled as one sample for subsequent analysis due to the lack of RAD index information. To combine 454 and RAD sequencing data, all four datasets were mapped to a reference genome. Because there is no reference genome for *S. variabilis* the only available Scleractinian genome in NCBI, that for *Acropora digitifera*, was employed. After data filtering, the clean data from the four datasets were mapped onto the *A. digitifera* genome for data alignment. BWA (0.7.12), with the number of threads (-t) was 4 and minimum seed length (-k) was 32 in BWA-MEM algorithm, were used for reads mapping to gain the initial alignment result, and (Li & Durbin, 2009). SAMtools (0.1.18) was used for sorting, merging and PCR duplication based on the initial alignment result (Li *et al.*, 2009). Due to the low mapping rate, a lower standard for SNP detection and filtering were considered, and the SNP and INDEL calling, filtering details were alignment score ≥ 10 and variation quality score ≥ 10 .

4.2.5. Statistical analysis

4.2.5.1. Microsatellites

To detect whether different sequencing approaches caused markers detection issues, after calculation of the percentage of each type of motif, hierarchical cluster analysis (cluster method used between-group linkage) was employed to reveal the similarity of microsatellite distributions. Additionally, a χ^2 test was used to investigate the distributions difference of microsatellites amongst different sequencing methods in the software package IBM SPSS Statistics (v 22.0).

The cross amplification was investigated amongst four species, and whether any differences between the amplifications amongst different species (*D. dianthus*, *G. dumosa*, *M. oculata* and *S. variabilis*) was analysed by two different multivariates analyses, Structure (Bayesian) and Discriminant Analysis of Principal Components (DAPC). The software package Structure (v 2.3.4, K from 1 to 5 and the length of burnin period and number of MCMC reps after burnin were 5,000 and 10,000, respectively), and DAPC analysis was completed in R projected by Package ‘adgenet’. Both analyses were run with all 27 microsatellite locus data and then with data from 12 loci that amplified in all four species, including B106, C107, D5, B1, B123, SVA1, SVA3, SVA2, SVA4, SVA12, SVA13 and SVA11. Structure Harvester (<http://taylor0.biology.ucla.edu/structureHarvester/>) was used to estimate the K value. The wilcoxon signed-rank test by IBM SPSS Statistics (v 22.0) was employed to investigate the cross-amplification differences in previous studies.

4.2.5.2. SNPs

Considering the different types of sequencing data (genomic assembled data for *A. digitifera*, 454 datasets for 94287, 94550 and 94590 and RAD-sequencing dataset for L008), and the composition percentage of each type of SNP was calculated. These new data were used for χ^2 testing and hierarchical cluster analysis to reveal the relations by SPSS (v 22.0). A Venn diagram, made using the package “VennDiagram” in R, was used to examine the SNPs distributional relationships amongst the different taxa. After transforming into numeric variants the SNPs that were detected in the 5 species-specific datasets, Structure (v 2. 3. 4) was used to reveal the differentiations of those SNPs amongst taxa.

4.3. Results and Discussion

4.3.1. Next Generation Sequencing

In *S. variabilis*, the average length of sequences from each of the three libraries varied from 607 to 727 bp, and total compiled library length ranged from 17,479,912 to 239,596,471 bp. This accounts for approximately 3.97 to 54.40% of the genome, assuming a genome size of 420 Mb (estimated from the size of *Acropora digitifera* (Shinzato *et al.*, 2011). In Rad-seq, the mean length of sequence reads was 151 bp with a total sequence length of 478,110,330 bp. After mapping to the *A. digitifera* genome, the successful mapping rate of the 454 data was about 20%, but the RAD-seq data was only 13.6% (Table 4.1 and Table 4.2). The alignment results showed that the mapping rate for each sample was low, and this may be due to the great difference between the reference and the sequenced samples. Given the data were mapped to the reference genome of *A. digitifera*, but the annotation file of the reference genome is not available, and the sequence could not be therefore annotated except mitochondrial sequences.

The choice of a sequencing strategy must take into account the research goals, ability to store and analyse data, the ongoing changes in performance parameters, and the cost of NGS platforms (Kumar *et al.*, 2012). Developing suitable genetic markers for *S. variabilis* to reveal population genetic structuring is the main target. Currently few genetic marker resources exist for *S. variabilis*, wherein 454 pyrosequencing and Rad-sequencing in the present study were shown to be excellent methods for large scale prediction of molecular markers for future genetic studies in non-model organisms. There are many different aspects of these two next generation sequencing methods, but only mitochondrial genome assemble, microsatellite development and single nucleotide polymorphism discovery were compared in this study. In mitochondrial genome assembly, 454 sequencing provided better performance than Rad-sequencing, with a higher coverage and greater depth. Although Rad-sequencing data produced many more microsatellites, the percentage of useful microsatellites was much lower than that derived from the 454 sequencing data. However, Rad-sequencing, as the main stream of developing SNP markers, was much more powerful than 454 sequencing in SNP identifications. The Rad-sequencing generated more SNPs in total and also a greater number of unique SNPs. Both sequencing methods have had a lot of successful examples of SNPs discovery and utilisations in genetic studies (e.g. Emerson *et al.*, 2010; Hohenlohe *et al.*,

2010). But one doubt about using 454 sequencing to identify SNPs is whether the depth of sequences is sufficient to exclude involute ones, because deep sequence coverage leading to dense and confident SNPs. In the present study, the average coverage depth of sequencing per SNP in 454 sequencing was not significantly different from RAD sequencing using χ^2 test. Additionally, mapping to reference genomes reduced the risk of invalid SNP discovery, although the mapping ratio was low.

Table 4.1 Summary information for sequencing data for *Solenosmilia variabilis*

Sample ID	Sequencing Method	Source	Total Reads	Average length (bp)
L008	Rad-seq	Australia	3,635,820	151.0
94550	454	New Zealand	242,903	713.1
94590	454	New Zealand	19,610	607.5
94287	454	New Zealand	218,657	727.3

Table 4.2 Summary information for sample alignment mapping to *Acropora digitifera*

Sample ID	Total bases	Mapped bases	Mapping Rate (%)
L008	478,110,330	65,043,497	13.60
94550	239,596,471	48,869,961	20.40
94590	17,479,912	3,555,465	20.34
94287	221,635,258	44,454,467	20.06

4.3.2. Mitogenome assembly and marker development

The first assembled draft contained 506 reads from three pooled databases, with good overall coverage (minimum coverage was 7 sequences, maximum coverage was 38 sequences, average coverage was 22.0 (SD=6.8)). Having assembled all relevant reads from each library, two *S. variabilis* mitogenomes were successfully obtained, one from a coral from Anvil Seamount and one from an individual collected from Valerie Guyot. The two mitogenomes were almost identical, the length of each being 15,968 bp. Both mitochondrial genomes included 13 protein-coding genes, 2 ribosomal RNA genes, 2 transfer RNA genes and a *D-loop* region (Table 4.3). The base composition of the genome was A (24.2%), T (41.1%), C (13.7%) and G (21.0%). There were 5 nucleotide differences (0.03%) between the two mitogenomes, including synonymous substitutions in the genes *nad1*, *nad4L* and *nad2* (at 1276 bp, 6596 bp and 10,502 bp, respectively) and two 1 bp differences (G/T transversions at 8701 and 8768 bp) in the *D-loop* region. The annotated mitogenomes are available online in the NCBI database (GenBank accession numbers KM609293 and KM609294).

The full mitochondrial genome of *S. variabilis*, at 15,968 bp, was slightly shorter than that of a branching habitat-forming species not found in the New Zealand region, *L. pertusa* (16,149 bp). The small difference between the species is primarily due to a 186 bp deletion in the *D-loop* region of *S. variabilis*. The number and order of the predicted genes in the *S. variabilis* mitogenome were identical to *L. pertusa*, and the pairwise sequence identity between the two taxa was 97.5%.

After successful assembly of the mitogenome of the New Zealand specimens, 1593 reads of Rad-sequencing data of an Australian specimen were mapped to the assembled genome with coverage from 0 to 112-fold (12.20 ± 13.00 (Mean $\pm$ SD)), but they failed to assemble another complete genome (coverage was 98.3%). Pairwise identity between mapped sequences and a reference genome (above assembled genome) was 99.20%, which indicated that the mitochondrial genomes were much closer within species (*S. variabilis*) than between species (*S. variabilis* - *L. pertusa*).

Although unable to assemble the full mitogenome of the third New Zealand specimen, there was no differences in the *D-loop* region between the two specimens from Valerie Guyot, suggesting that genetic variation in the mitochondrial genome is low. Similarly, limited genetic differences in the mitogenomes of two *L. pertusa* collected from the Mediterranean and Barents seas have been reported, which differed only by a single non-synonymous substitution (Flot *et al.*, 2013). The variation information of the mitogenome will be beneficial for future phylogenetic and phylogeographic studies of Scleractinia, particularly for *S. variabilis*, and useful where there may be some confusion with specimen identification.

Table 4.3 Characterisation of the mitochondrial genome of *Solenosmilia variabilis*

Name	Location5'-3'	Size (bp)	Start	Stop	Name	Location5'-3'	Size (bp)	Start	Stop
<i>nad5</i>	1-8295	1836	ATG	TAA	<i>D-loop</i>	8296-8818	523		
<i>nad5 intron</i>	715-7173	6459			<i>Cytb</i>	8819-9979	1161	ATG	TAG
<i>nad1</i>	824-1771	948	ATG	TAA	<i>nad2</i>	9975-11291	1317	ATT	TAA
<i>ATP6</i>	1824-2522	699	ATG	TAA	<i>nad6</i>	11293-11847	555	GTG	TAA
<i>nad4</i>	2522-3967	1446	ATG	TAG	<i>tRNA-Trp</i>	12250-12319	70		
<i>12S rRNA</i>	3968-4991	1023			<i>ATP8</i>	12323-12490	168	ATG	TAA
<i>COIII</i>	4992-5771	780	ATG	TAG	<i>COI</i>	12480-14045	1566	ATG	TAA
<i>COII</i>	5759-6484	726	GTG	TAA	<i>tRNA-Met</i>	14041-14111	71		
<i>nad4L</i>	6474-6773	300	ATG	TAA	<i>16S rRNA</i>	14112-15968	1857		
<i>nad3</i>	6775-7119	345	ATG	TAA					

High similarities in mitochondrial genes of Scleractinia had been proved in this study, and a slow evolutionary rate in mitogenome would explain this (Shearer *et al.*, 2002). The

low variation between individuals and species indicated that mitochondrial genes might not be suitable for *S. variabilis* population genetic studies.

After compared the alignments, *D-loop*, as the most variable area in mitogenome, was selected to design primers. Details of *ITS* and *D-loop* primers were present in Table 4.4. After testing in *D. dianthus*, *G. dumosa*, *M. oculata* and *S. variabilis*, *ITS* genes were successfully amplified in all species and *D-loop* was only amplified in *G. dumosa* and *S. variabilis*. These two markers were used in following chapters to investigate genetic connectivity patterns of corals and reveal the environmental factors that explained genetic structures.

Table 4.4 Details of PCR primers and conditions used to amplify two DNA regions

DNA regions	Primer names	Primers 5'-3'	Annealing temperature (°C)	Trimmed size (bp)
<i>ITS</i>	348 F	TTGACGGTGGATCTCTTGGC	50	~400
	854 R	CAAATTTGAGCTGGTCCCGC		
<i>D-loop</i>	74F	CCCAGGGGCCTTGTTCAATTCTA	50	~400
	834R	TGGAGGGAGAGGGCAAATTCATA		

4.3.3. Microsatellite distribution

After searching, MSATCOMMANDER identified totals of 45,389, 17,289, 19,906 and 6,236 microsatellites from specimens L008, 94287, 94550 and 94590, respectively. Because the results for 94590 were much less than other two New Zealand samples, 94590 was excluded from microsatellite distribution comparisons. In the 94287 and 94550 datasets, 37.99% of microsatellites were dinucleotide, 29.88% trinucleotide, 24.80% tetranucleotide, 3.05% pentanucleotide and 4.28% hexanucleotide repeats. χ^2 testing showed that there were no significant differences between the numbers of different repeat motif types for samples 94287 and 94550 (Table 4.5) ($p > 0.05$). Also, the χ^2 test showed the distributions between the 454 and RAD sequencing methodologies were not significantly different ($p > 0.05$). Dinucleotide repeats were the most common type in all datasets except that for 94590, with penta- and hexanucleotide repeats being present at much lower frequencies. Although compositions of trinucleotide and tetranucleotide varied a lot amongst different species, the distributions of microsatellites showed a taxonomically related pattern, with *D. dianthus* and *S. variabilis* grouped together (they are in the same family, Figure 4.1). The placement of sample 94590 as an outlier is related to the reduced genome coverage of this sample.

To develop useful microsatellites, sequences containing > 50-bp sequence on both sides of the microsatellite repeat were considered sufficient for primer design. After this filter, percentages of those microsatellites were 11.73%, 73.61%, 32.64% and 73.58% in L008, 94550, 94590 and 94287, respectively. It is also worth noting that tetranucleotide and trinucleotide repeat compositions were different in different sequencing methods, with tetranucleotide repeats having a higher proportion than triplet repeats in Rad-sequences, but 454 sequencing was the opposite (Table 4.5). A previous study has noted that tetranucleotide repeats occur at a lower proportion in invertebrate genomes than triplet repeats (Tóth *et al.*, 2000). If true, then this might imply that 454 sequencing was more accurate than Rad-sequencing. Present study also provided evidences that different sequencing methods were likely to cause sequences abundant and distribution basis, but the difference caused by different sequencing methods was still within species level if no sequencing-libraries construction issues.

Table 4.5 Microsatellite distributions (%) information for three species

Repeats	<i>Acropora digitifera</i>	<i>Desmophyllum dianthus</i> *	<i>Solenosmilia variabilis</i>				
			Average	94287	94550	94590	L008
Dinucleotide	42.33	43.31	37.99	38.34	37.64	23.00	35.65
Trinucleotide	48.45	9.19	29.88	29.78	29.99	55.18	24.60
Tetranucleotide	7.17	35.43	24.80	25.74	23.86	12.99	25.18
Pentanucleotide	1.21	9.68	3.05	3.09	3.01	2.28	2.81
Hexanucleotide	0.84	2.38	4.28	3.06	5.50	6.56	11.76

* Data from Addamo *et al.* (2015). Average was the mean of 94278 and 94550.

Figure 4.1 Dendrogram of sample similarity based on microsatellite variation amongst three corals

* Data were obtained from Addamo *et al.* (2015); Distance interval was Squared Euclidean distance. *Private alleles were calculated amongst *Goniocorella dumosa*, *Desmophyllum dianthus*, *Madrepora oculata*, *Solenosmilia variabilis*.

4.3.4. Microsatellite characterisation

After comparison of primer pairs and motifs, 22 potential polymorphic microsatellites were identified. After primary amplification, 2 homozygote loci and 2 failures were excluded from further characterisations (Table 4.6). Locus SVA20 amplified more than 2 alleles, and it was therefore excluded from scoring for further analysis. In those 27 microsatellites (including 9 loci from Miller & Gunasekera (2016)), polymorphism information content (PIC) estimates showed that 23 loci were reasonably to highly informative ($PIC > 0.5$) (Table 4.7). Observed (H_o) and expected (H_e) heterozygosities averaged 0.724 and 0.731, respectively. Tests for linkage disequilibrium (LD) yielded 56 (18.98%) significant P values out of 295 pairwise comparisons ($p < 0.05$), and only SVA10 and SVA14 were totally unlinked from other markers. 17 markers exhibited significant deviations from HWE (Table 4.7).

Table 4.6 Primer information for *Solenosmilia variabilis* microsatellites

Name	Forward	Reward	Motif
SVA1	ACTTGACGAGAAGCTTTGG	CAGAGTCCCAGTACAAAGTCG	[AAAC]6
SVA2	TTGCCTACATGACAACCTGC	TGCGTGAGTCCAGAGATCAG	[AAAC]15
SVA3	GACAGCAGGAACGTTACAGC	ATGGATTCTGTCGTGTTCTC	[AAC]9
SVA4	GGTAGTGGAGGTAGGCATCG	GCCGCTAATGAACAGGATCG	[AAC]9
SVA5	GGTGAGCAAGGCCATACAAC	TGTCCCGTATGCCTTGTTG	[AAC]9
SVA7	ATGGTACCGTAAACTGCCGC	CAGCTCTCCTCTGGTATGCC	[AAT]12
SVA8	GGCCGTGGTTATAGATCACAG	CCAAACTCGCCTGATATGCC	[AATAC]23
SVA10	TGGTGGAAGCAAGTCATGG	TTGGCCATGTCTGCTCAAAG	[AATC]11
SVA11	AGGAGACGACACTTACGCTG	GCGATCCTGTTCCAACGTTT	[AATG]11
SVA12	TCTGGTCACCTGGCTAATGG	TGTAGACAGCCATACAATCCG	[AATG]10
SVA13	GCAGGCAGTCAGTTAGTTCG	CTGACAACACAAGGCACCTG	[ACAG]6
SVA14	TTGTAACCAGGCTCAACTCG	GAACGTTTCGCTGTCTTCATG	[ACATC]27
SVA15	ACTAGAGCCCAGTCTGTTCG	TACTGCTCTGTGTGGTTTCGG	[AG]11
SVA16	TATTCCTCCGTGCCAGTAC	GAGTCCAACACGCTAACCAC	[AG]13
SVA17	AAATTCTGTATGCCTCGCGG	AGTTTGTGGAAATAAGGCGTG	[AT]8
SVA18	AGCCATGTTGTTTCGAGGTC	TGCATCTGAAGCCCTGTCAG	[AT]9
SVA19	ACTGCTACCGTTCCTTGAGG	AATGCAACGATGGTATAGGCC	[ATC]15
SVA20	GGGACGCTTATTTGGTCTAGC	AAACGCCATCAACACAGAGG	[ATC]17

In this study, 27 primer pairs produced reliable PCR amplicons that were scorable with the number of alleles (NA) per locus varying from 3 to 22 (average 10.96) (Table 4.7). The NA s found in the present study were similar to those reported in *Lophelia pertusa* (12.6 - Le Goff & Rogers, 2002) and 17.92 (Morrison *et al.*, 2008), but higher than *D. dianthus* (9.13 - Addamo *et al.*, 2015), *Seriatopora hystris* (8.8 - Underwood *et al.*, 2006), and *Acropora millepora* (8.9 - van Oppen *et al.*, 2007). Amongst examined loci, a high proportion of

markers not in Hardy-Weinberg equilibrium in every species might explain poor primer annealing (Kwok *et al.*, 1990), differential amplification of size-variant alleles (Wattier *et al.*, 1998), or poor quality DNA causing PCR failure (Gagneux *et al.*, 1997). On the other hand, the Wahlund effect, inbreeding, asexual reproduction and the complex genetic structure of *S. variabilis* have possibly contributed to the departure from equilibrium (Chakraborty *et al.*, 1992).

Table 4.7 Characterisation of 27 microsatellite loci for *Solenosmilia variabilis*

Loci	Range	No	Private*	success	PIC	Ho	He	HW Equilibrium
B106	253-268	6	4	33	0.547	0.970	0.621	***
C107	201-237	9	3	33	0.718	0.879	0.747	ns
D5	166-257	12	4	33	0.799	0.727	0.817	*
B1	119-183	14	12	33	0.807	0.758	0.821	*
B116	227-240	6	4	33	0.624	0.697	0.684	ns
D103	197-206	4	1	33	0.444	0.394	0.479	ns
B123	159-185	5	2	33	0.337	0.242	0.360	**
D213	105-180	11	5	33	0.652	1.000	0.699	***
C4	203-227	3	0	33	0.111	0.121	0.115	ns
SVA1	209-257	9	4	33	0.539	0.545	0.564	***
SVA2	270-304	12	5	32	0.847	0.563	0.862	***
SVA3	395-418	10	2	33	0.730	0.515	0.766	***
SVA4	261-312	15	5	33	0.820	0.909	0.837	ns
SVA5	197-345	15	14	33	0.870	1.000	0.881	***
SVA7	341-398	11	4	33	0.821	1.000	0.841	ns
SVA8	260-383	15	12	32	0.838	0.594	0.850	***
SVA10	404-472	13	6	33	0.879	1.000	0.889	ns
SVA11	380-484	16	13	33	0.757	1.000	0.782	***
SVA12	357-453	15	12	33	0.763	0.455	0.786	***
SVA13	185-266	10	3	33	0.683	0.848	0.702	ns
SVA14	284-418	22	19	33	0.939	0.818	0.942	**
SVA15	197-221	10	4	33	0.679	1.000	0.720	*
SVA16	336-370	10	4	33	0.633	0.545	0.660	***
SVA17	329-403	10	10	8	0.854	0.750	0.867	ns
SVA18	316-390	13	4	33	0.823	0.697	0.839	**
SVA19	338-437	9	6	5	0.868	0.800	0.880	ns
SVA20	Multicopies							

Ho, Observed Heterozygosity; He, Expected Heterozygosity; ns, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. *Private alleles were calculated amongst *Goniocorella dumosa*, *Desmophyllum dianthus*, *Madrepora oculata*, *Solenosmilia variabilis*. Success – number of individuals for which successful PCR amplifications were obtained (n= 30 individuals tested)

4.3.5. Microsatellite transferability

To develop population genetic markers for other corals, especially for *G. dumosa*, these 27 microsatellites were tested for their transferability in three other closely related taxa, *G. dumosa*, *D. dianthus* and *M. oculata*. The details of locus-specific cross amplification in the different species was as follows.

Table 4.8 Cross-amplification information of 27 loci in *Goniocorella dumosa*

Loci	Range	No	Private*	success	PIC	Ho	He	HW Equilibrium
B106	241-256	5	3	29	0.625	0.690	0.673	**
C107	177-234	9	2	29	0.712	0.517	0.749	**
D5	177-298	17	9	29	0.869	0.724	0.878	***
B1	104-206	6	5	29	0.508	1.000	0.589	*
B116	237-262	6	5	15	0.732	0.800	0.769	**
D103	173-203	5	2	20	0.686	0.700	0.734	***
B123	158-194	7	3	29	0.670	0.483	0.716	***
D213	105-216	10	4	27	0.801	0.815	0.822	***
C4	106-239	9	8	24	0.796	0.583	0.818	***
SVA1	221-249	7	1	26	0.656	0.346	0.643	***
SVA2	236-336	7	3	23	0.625	0.435	0.612	**
SVA3	302-431	16	10	23	0.835	0.565	0.847	***
SVA4	201-300	7	2	23	0.818	0.462	0.787	ns
SVA5	187-250	9	9	10	0.753	0.400	0.715	**
SVA7	311-488	18	13	25	0.787	0.440	0.797	***
SVA8	200-320	10	7	16	0.803	0.688	0.820	***
SVA10	400-472	9	2	25	0.796	0.840	0.817	ns
SVA11			Monomorphic			1.000	0.500	***
SVA12	301-427	9	9	16	0.787	0.625	0.811	***
SVA13	218-251	8	1	28	0.724	0.964	0.756	**
SVA14	360-492	6	3	18	0.603	0.500	0.664	**
SVA15	183-249	11	8	23	0.793	0.609	0.816	***
SVA16	336-380	7	3	10	0.714	0.400	0.740	*
SVA17			Monomorphic & Homozygotes					
SVA18	316-400	14	5	21	0.807	0.667	0.828	ns
SVA19			Monomorphic			1.000	0.500	***
SVA20	359-440	5	5	14	0.760	0.500	0.793	***

Ho, Observed heterozygosity; He, Expected heterozygosity; ns, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. *Private alleles were calculated amongst *Goniocorella dumosa*, *Desmophyllum dianthus*, *Madrepora oculata*, *Solenosmilia variabilis*. Success – number of individuals for which successful PCR amplifications were obtained (n= 29 individuals tested)

4.3.5.1. *Goniocorella dumosa*

In *G. dumosa*, all 27 loci were successfully amplified, but SVA11, SVA17 and SVA19 were monomorphic. The *NA* of 24 polymorphic microsatellite loci varied from 5 to 18, with an average of 7.64 (Table 4.8). PIC estimates showed all locus were highly informative. *Ho* and *He* were 0.630 and 0.748, respectively. LD generated 78 (29.21%) significant P values out of 267 pairwise comparisons ($p < 0.05$), and none of them were totally unlinked from other markers. Only three markers showed no significant deviation from HWE (Table 4.8).

4.3.5.2. *Desmophyllum dianthus*

21 of 28 loci were polymorphic and produced 3-16 alleles per locus with an average of 7.64 in *D. dianthus* (Table 4.9). Loci D103, D213, SVA8, SVA10 and SVA20 were unsuccessful amplifications and locus B116 was monomorphic. Lower values of *Ho* and *He* were found in *D. dianthus* than *S. variabilis*, which were 0.460 and 0.681, respectively. PIC levels? Following testing for LD, only 5 (2.87%) significant P values were found in 174 pairwise comparisons ($p < 0.05$), and only B1 & SVA3, SVA5 & SVA7, D5 & SVA18, SVA4 & SVA13 and C4 & SVA16 formed linkage pairs. Eight loci exhibited significant deviations from HWE (Table 4.9).

4.3.5.3. *Madrepora oculata*

Less than half (13 of 27 loci) of locus were successfully amplified and scored with *NA* varying from 3 to 9, with an average of 4.6 in *M. oculata* (Table 4.10). The average values of *Ho* and *He* were 0.709 and 0.609, which were similar to *S. variabilis*. LD generated 3 (5.56%) significant P values out of 54 pairwise comparisons ($p < 0.05$), and all loci were totally unlinked from each other, except C107 & D5, C107 & SVA13 and D5 & SVA13. Only 2 markers exhibited significant deviations from HWE ($p < 0.01$) (Table 4.10).

Table 4.9 Cross-amplification information of 27 loci in *Desmophyllum dianthus*

Loci	Range	No	Private*	success	PIC	Ho	He	HW Equilibrium
B106	247-274	10	6	22	0.856	0.909	0.870	ns
C107	197-239	9	9	22	0.787	0.556	0.809	ns
D5	219-263	11	11	19	0.783	0.652	0.802	ns
B1	119-149	10	9	14	0.778	0.600	0.801	ns
B116				Monomorphic & Homozygotes				
D103				No amplification				
B123	169-177	3	2	12	0.545	0.000	0.500	**
D213				No amplifications				
C4	185-227	4	3	21	0.182	0.190	0.178	ns
SVA1	175-223	3	3	4	0.714	0.000	0.625	*
SVA2	268-292	6	1	21	0.735	0.571	0.771	**
SVA3	385-421	12	10	22	0.859	0.636	0.872	ns
SVA4	279-342	12	9	15	0.852	0.667	0.864	ns
SVA5	320-341	5	4	9	0.589	0.222	0.630	**
SVA7	365-380	6	0	15	0.685	0.533	0.662	ns
SVA8				No amplification				
SVA10				No amplification				
SVA11	338-498	9	9	23	0.371	0.304	0.380	*
SVA12	361-392	5	4	16	0.708	0.250	0.752	***
SVA13	206-281	16	6	23	0.83	0.696	0.840	ns
SVA14	157-298	12	12	15	0.849	0.467	0.860	**
SVA15	199-245	8	5	10	0.832	0.700	0.850	ns
SVA16	330-342	6	3	23	0.706	0.348	0.749	***
SVA17	353-387	3	3	12	0.150	0.167	0.156	ns
SVA18	379-383	3	3	20	0.371	0.300	0.454	ns
SVA19	314-389	15	13	18	0.906	0.889	0.881	ns
SVA20				No amplification				

Ho, Observed heterozygosity; He, Expected heterozygosity; ns, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. *Private alleles were calculated amongst *Goniocorella dumosa*, *Desmophyllum dianthus*, *Madrepora oculata*, *Solenosmilia variabilis*. Success – number of individuals for which successful PCR amplifications were obtained (n= 23 individuals tested)

Table 4.10 Cross-amplification information of 27 loci in *Madrepora oculata*

Loci	Range	No	Private*	success	PIC	Ho	He	HW Equilibrium
B106	220-256	9	7	16	0.683	1.000	0.723	ns
C107	198-243	7	3	16	0.712	0.563	0.750	ns
D5	210-272	6	6	12	0.643	0.833	0.681	ns
B1			Monomorphic			1.000	0.500	**
B116				No amplification				
D103				No amplification				
B123	168-198	5	3	5	0.701	0.400	0.740	ns
D213	105-144	3	0	7	0.465	0.857	0.561	ns
C4				No amplification				
SVA1	225-237	3	0	7	0.325	0.286	0.357	ns
SVA2	276-292	3	0	5	0.410	0.200	0.460	ns
SVA3	344-416	7	1	6	0.765	1.000	0.792	ns
SVA4	248-288	3	2	2	0.555	0.500	0.625	ns
SVA5				No amplification				
SVA7				No amplification				
SVA8				No amplification				
SVA10	380-452	5	1	6	0.644	1.000	0.694	ns
SVA11	420-448	3	1	14	0.424	1.000	0.533	**
SVA12	421-437	3	0	3	0.593	0.667	0.667	ns
SVA13	221-230	3	0	16	0.371	0.625	0.447	ns
SVA14				No amplification				
SVA15				No amplification				
SVA16				No amplification				
SVA17				No amplification				
SVA18				No amplification				
SVA19				No amplification				
SVA20				No amplification				

Ho, Observed heterozygosity; He, Expected heterozygosity; ns, not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. *Private alleles were calculated amongst *Goniocorella dumosa*, *Desmophyllum dianthus*, *Madrepora oculata*, *Solenosmilia variabilis*. Success – number of individuals for which successful PCR amplifications were obtained (n= 16 individuals tested)

4.3.5.4. Differences in cross-species amplifications

Not all of the 27 microsatellite loci worked in every species, and the amplification rate decreased from *G. dumosa* (100%), to *D. dianthus* (81.48%) and on to *M. oculata* (51.85%), in accordance with the increasing genetic divergence amongst the species (Primmer & Merila, 2002). The successfully-amplified ratio in this study also showed that the cross-amplification is closed related to taxonomical and morphological relations. The lowest was found in *M. oculata*, which is in the family Oculinidae. Although *G. dumosa*, *D. dianthus* and *S. variabilis* belong to the Caryophylliidae, *G. dumosa* and *S. variabilis*

(branching and bushy colonies) are more morphologically similar than they are to *D. dianthus* (cup-shape and solitary colonies). This might explain the higher success rate of successful cross amplification in *G. dumosa* than *D. dianthus*. Only 12 loci were successfully amplified in all four species, and the allele ranges of each locus were very similar amongst species. Both Structure and DAPC analysis (Figure 4.2 and Figure 4.3) agreed that either all loci (27) or the loci (12) amplified in all species indicated that there were four groups (K=4).

Figure 4.2 Structure analyses of all loci (A) and 12 loci that amplified for all four species (B)

GDU, *Goniocorella dumosa*; DDI, *Desmophyllum dianthus*; MOC, *Madrepora oculata* and SVA, *Solenosmilia variabilis*.

Here, 153 microsatellite loci from ten publications including this study, all of which have reported cross-amplification in Scleractinia, have been summarized in Table S4.1. From these microsatellites, over half of the loci could amplify in at least two different species of the same genus. The proportion of loci that cross-amplified decreased as taxonomic level increased. Trinucleotide repeats are the most common microsatellite motifs that could amplify in other species, and analysis of G/C (%) content showed that most cross-amplified loci were composed of A/T rich repeats (Figure 4.4). A Wilcoxon signed-rank test showed that NA, range and He did not elicit a statistically significant change in cross amplification between the species that microsatellites developed from and the species that the microsatellites could cross amplify ($Z = -1.871$, $p = 0.061$; $Z = -0.022$, $p = 0.983$; $Z = -1.454$, $p = 0.146$, respectively), but fragment size range (low and upper boundary) and Ho were significantly different between the two species ($Z = -2.261$, $p = 0.024$; $Z = -1.987$, $p = 0.047$; $Z = -4.811$, $p < 0.001$). This result meant that the differences of the same loci in different

species were distinguished between the species even sharing some alleles. In the present study, structure and DAPC were revealed a relatively clear separation amongst species, although a few misidentified in Structure analysis (Figure 4.2) and DAPC complot (Figure 4.3) were because the PCR amplification failures.

Figure 4.3 DAPC scatterplot (above) and complot (below) of all loci (A, 27 loci) and part (B, 12 loci) amongst four species

GDU, *Goniocorella dumosa*; DDI, *Desmophyllum dianthus*; MOC, *Madrepora oculata* and SVA, *Solenosmilia variabilis*. Both charts (A and B) were based on 60 principal components.

Figure 4.4 Distribution features of cross-amplified microsatellites of motifs (A), transferability level (B), G/C content (C)

Di, Tri, Tetra, Penta and Hexa were Dinucleotide, Trinucleotide, Tetranucleotide, Pentanucleotide and Hexanucleotide repeat motifs. Order, Family and Genus represent transferability in different order, family and genus.

4.3.6. Single nucleotide polymorphisms (SNPs)

After filtering the datasets, 102,678 reads were generated, and 62655, 8618, 63643 and 152023 SNPs were found, with average depth of 51.27, 43.21, 3.38 and 38.84, in samples L008, 94550, 94590 and 94287, respectively. A total of 846 insertions or deletions (indels) were discovered, and 715, 677, 221 and 711 were discovered in L008, 94550, 94590 and 94287, respectively (Table 4.11).

Table 4.11 SNPs distributions amongst different samples

SNPs		<i>Acropora digitifera</i> D=1	<i>Solenosmilia variabilis</i>			
			L008 D=51.27±66.08	94550 D=43.21±59.50	94590 D=3.38±4.91	94287 D=38.84±54.62
Homogeneity	A	41419	24342	14592	2087	14954
	C	34770	23421	14695	2132	14962
	G	34129	23423	14646	2015	14803
	T	41705	24491	14849	1984	15079
Transition*	A-G		2127	1168	110	1217
	C-T		2190	1215	132	1180
Transversion*	A-C		650	369	52	364
	G-C		459	282	23	254
	G-T		723	348	29	364
	T-A		852	491	54	466
Indel		846	715	677	221	711
Total		152869	103393	63332	8839	64354

D means sequences depth (Mean $\pm$ SD). * Transition and Transversion in L008 are including within heterozygosity a genome and within a population, Transition and Transversion in 94550, 94590 and 94287 are heterozygosity within the genome.

A Venn diagram showed the relation and distribution of the SNPs that excluded indels amongst the four *S. variabilis* samples. In total, 64,352 (62.67%), 20,888 (33.34%), 1,708 (19.82%) and 20,554 (32.30%) unique loci were found in samples L008, 94550, 94590 and 94287, respectively (Figure 4.5). Only 1,983 SNPs were identical amongst *S. variabilis* samples but different from *A. digitifera*, which were small proportion in L008 (1.93%), 94550 (3.17%) and 94287 (3.12%). Hierarchical cluster analysis provided a further illustration of the relationship of SNPs amongst samples (Figure 4.6). Samples 94287 and 94550 firstly joined together, and then grouped with L008. The limited coverage of sample 94590 might explain why it grouped with *A. digitifera* rather than with the other three *S. variabilis* samples. However, STRUCTURE analysis of SNPs also failed to tell the difference between species which was same as microsatellites (Figure 4.7). This might be related to the methods used to develop these SNPs, because these SNPs were from the cross-genus shared sequences, where might be very conserved. The common SNPs between or amongst samples might cause this blurring of species boundaries by the SNPs markers, and a further examination of those loci was expected to solve this problem.

Figure 4.5 Venn diagram of SNPs in four *Solenosmilia variabilis* samples

Distance interval was Squared Euclidean distance. SVA, *Solenosmilia variabilis* (94287, 94550 and 94590 were 454 sequencing, and L008 was Illumina sequencing), and ADI, *Acropora digitifera*.

Figure 4.6 Dendrogram of 5 samples revealed by SNPs that were detected in all datasets

Distance interval was Squared Euclidean distance. SVA, *Solenosmilia variabilis* (94287, 94550 and 94590 were 454 sequencing, and L008 was Illumina sequencing), and ADI, *Acropora digitifera*.

Figure 4.7 Structures results of sample coral samples based on SNPs (K=2)

SVA, *Solenosmilia variabilis* (94287, 94550 and 94590 were 454 sequencing, and L008 was Illumina sequencing), and ADI, *Acropora digitifera*.

4.4. Conclusion

Three marker types, mitochondrial genes from full mitogenome sequence, microsatellites and SNPs, were generated from next generation sequencing data for *S. variabilis*. Mitochondrial genes have slow evolutionary rates and are relatively conserved, making them not particularly suitable for population genetic studies in stony corals. An exception here is the non-coding mtDNA *D-loop* region. Microsatellites are informative markers that may be useful in further studies, and their cross-amplification showed high and informative levels of polymorphism in other stony coral species. These markers are good candidates for the genetic studies, and performance and details information, such as deviation from HBW and neutrality of the markers, were discussed in Chapter 6 when applying their to test different populations. SNPs are supposed to be good markers for population studies. A further selection of SNPs needs to be examined, and then potential loci could be applied for genetic studies of single taxa.

Appendix

Table S4.1 Information on the cross-amplification of microsatellite loci in Scleractinia

Locus	Origin species	Motif	Range	Cross species	Range	Cross level	Reference
Amil2-07	<i>Acropora millepora</i>	[TG]7AG	123-129	<i>Acropora austera</i>	96-126	Genus	(Montoya-Maya, Macdonald, & Schleyer, 2014)
Amil2-10	<i>Acropora millepora</i>	TA[TG]11	156-192	<i>Acropora austera</i>	100-156	Genus	
Amil2-23	<i>Acropora millepora</i>	[AG]7	129-137	<i>Acropora austera</i>	127-133	Genus	
Acr181	<i>Acropora palmata</i>	[AAT]10	152	<i>Acropora austera</i>	118-205	Genus	
Acr182	<i>Acropora palmata</i>	[AAT]18	165	<i>Acropora austera</i>	132-228	Genus	
EST014	<i>Acropora millepora</i>	[TCT]13	143-173	<i>Acropora austera</i>	124-166	Genus	
Mhi1	<i>Mussismilia hispida</i>	[GT]8	170-204	<i>Mussismilia braziliensis</i>		Genus	
Mhi2	<i>Mussismilia hispida</i>	CACAGA]3[GA][CA]3[GACA][GACT][CA]2[CGCAGA][CA]5	176-404	<i>Mussismilia braziliensis</i>		Genus	
Mhi4	<i>Mussismilia hispida</i>	[CAA]5[CATCGT][CA]3[CAT][CAA]5[CAT][CAA]3[CTGAA][CA]3	86-239	<i>Mussismilia braziliensis</i>		Genus	
Mhi5	<i>Mussismilia hispida</i>	[CAA]2[CAC]3[CAA]4[CAT][CAA]2[CAC][CAA]5[CAT][CAA]2[CA]C	163-393	<i>Mussismilia braziliensis</i>		Genus	
Mhi14	<i>Mussismilia hispida</i>	[GTT]2[GCT][GTT][GCT]3[GTT]3[GCT]3[GT]4[GCT][GTT][TAT][GTT]8	219-295	<i>Mussismilia braziliensis</i>		Genus	(Zilberberg, Peluso, Marques, & Cunha, 2014)
Mhi16	<i>Mussismilia hispida</i>	[GTT]2[CTT][GTT]7	174-197	<i>Mussismilia braziliensis</i>		Genus	
Mhi17	<i>Mussismilia hispida</i>	[CACAA]4	168-184	<i>Mussismilia braziliensis</i>		Genus	
Mhi18	<i>Mussismilia hispida</i>	[GT]17	164-306	<i>Mussismilia braziliensis</i>		Genus	
Mhi20	<i>Mussismilia hispida</i>	[CA]3[CG][CAA]12	172-231	<i>Mussismilia braziliensis</i>		Genus	
Mhi21	<i>Mussismilia hispida</i>	[CAA]7[TAA][CGA][CAA]6[CGA]2[CAA]7	192-221	<i>Mussismilia braziliensis</i>		Genus	
Mhi23	<i>Mussismilia hispida</i>	[CAA]16[CAT][CAA]2	217-257	<i>Mussismilia braziliensis</i>		Genus	
Mhi24	<i>Mussismilia hispida</i>	[CAA]12	113-160	<i>Mussismilia braziliensis</i>		Genus	
Mhi26	<i>Mussismilia hispida</i>	[GTT]12[TACAG][CAT][CATAT]3[AT]2[CAT][ATCA]	164-220	<i>Mussismilia braziliensis</i>		Genus	
Ac-7E-AC	<i>Astroides calycularis</i>	[AC]13	210-220	<i>Tubastrea micrantha, sp1, sp2, sp3</i>		Family	(Casado-Amezua, Goffredo, Templado, & Machordom, 2012)
Ac-L7	<i>Astroides calycularis</i>	[GAT]15	154-196	<i>Tubastrea micrantha, sp1, sp2, sp3</i>		Family	
Ac-L10	<i>Astroides calycularis</i>	[GAT]11GGT[GAT]4	260-278	<i>Tubastrea micrantha, sp1, sp2, sp3</i>		Family	
Ac-L11	<i>Astroides calycularis</i>	[TCA]14	260-308	<i>Tubastrea micrantha, sp1, sp2, sp3</i>		Family	

Ac-L18	<i>Astroides calycularis</i>	[CA]17CG[CA]4	270-302	<i>Tubastrea micrantha,sp2</i>	Family	
Ac-L20	<i>Astroides calycularis</i>	[TG]23	356-386	<i>Tubastrea micrantha,sp1,s p2,sp3</i>	Family	
Ac-L22	<i>Astroides calycularis</i>	[AGAC]9	215-219	<i>Tubastrea micrantha,sp1,s p3</i>	Family	
Ac-L23	<i>Astroides calycularis</i>	[AGAC]4AGTC[AGAC]2AGAAA[AGAC]2	291-299	<i>Tubastrea micrantha,sp1,s p2,sp3</i>	Family	
Ac-L25	<i>Astroides calycularis</i>	[CAT]18	123-144	<i>Tubastrea micrantha,sp1,s p2,sp3</i>	Family	
Ac-L31	<i>Astroides calycularis</i>	[GT]4GC[GT]2AT[GT]5GC[GT]2ATGTCT[GT]11	265-267	<i>Tubastrea micrantha,sp1,s p2,sp3</i>	Family	
Ac-L33	<i>Astroides calycularis</i>	[GAYA]11GACGCAC A[GAYA]4CAGTA[GY A][TACAYA]11	336-412	<i>Tubastrea micrantha,sp1,s p2,sp3</i>	Family	
Ac-L34	<i>Astroides calycularis</i>	[GAT]10N19[GAT]4	221-224	<i>Tubastrea micrantha,sp2</i>	Family	
Ac-L37	<i>Astroides calycularis</i>	[GRT]10GAC[GRT]2 GTTGAC[GRT] GTT 2 [GRT]12	210-231	<i>Tubastrea micrantha,sp2</i>	Family	
8346m3	<i>Acropora enuis</i>	[ATT]12	162-213	<i>Acropora digitifera</i>	186-210	Genus
7961m4	<i>Acropora enuis</i>	[AAAG]7	192-208	<i>Acropora digitifera</i>	174-190	Genus
11745m3	<i>Acropora enuis</i>	[AAT]16 GATAATGAT[AAT]11	218-269	<i>Acropora digitifera</i>	171-183	Genus
12406m3	<i>Acropora enuis</i>	[AAC]12	163-175	<i>Acropora digitifera</i>	164-188	Genus
11543m5	<i>Acropora enuis</i>	[AAAAG]5	132-142	<i>Acropora digitifera</i>	125-140	Genus
530m4	<i>Acropora enuis</i>	[AATG]3GATG[AATG]7	269-405	<i>Acropora digitifera</i>	382-398	Genus
11401m4	<i>Acropora enuis</i>	[ATTT]8	382-418	<i>Acropora digitifera</i>	364-400	Genus
441m6	<i>Acropora enuis</i>	[CTCGGT]4	288-312	<i>Acropora digitifera</i>	268-298	Genus
11292m4	<i>Acropora enuis</i>	[AATG]4	443-495	<i>Acropora digitifera</i>	466-502	Genus
8499m4	<i>Acropora enuis</i>	[CGGT]5	340-356	<i>Acropora digitifera</i>	341-361	Genus
7203m5	<i>Acropora enuis</i>	[AAAAT]4	279-319	<i>Acropora digitifera</i>	293-298	Genus
10366m5	<i>Acropora enuis</i>	[AAAAC]5	222-232	<i>Acropora digitifera</i>	216-226	Genus
12130m5	<i>Acropora enuis</i>	[AAAAC]6	248-283	<i>Acropora digitifera</i>	265-295	Genus
4546m2	<i>Acropora enuis</i>	[AT]26	250-282	<i>Acropora digitifera</i>	231-255	Genus
9079m3	<i>Acropora enuis</i>	[TAA]11	269-287	<i>Acropora digitifera</i>	219-303	Genus
11192m4	<i>Acropora enuis</i>	[AAAC]7	127-139	<i>Acropora digitifera</i>	104-120	Genus
8010m6	<i>Acropora enuis</i>	[AAAGGG]4	204-228	<i>Acropora digitifera</i>	189-213	Genus
12198m3	<i>Acropora enuis</i>	[TAA]14	304-346	<i>Acropora digitifera</i>	294-339	Genus
7850m4	<i>Acropora enuis</i>	[AATC]9	225-265	<i>Acropora digitifera</i>	229-253	Genus
M_fav29	<i>Montastraea faveolata</i>	[CAT]16	417-477	<i>Montastraea franksib, annularisb</i>	Genus	(Davies et al., 2013)
M_fav3	<i>Montastraea</i>	[TTTTG]8	227-247	<i>Montastraea</i>	Genus	

(Chuya
Shinzato et
al., 2014)

0C	<i>faveolata</i>			<i>franksib,</i> <i>annularisb</i>		
M_fav3 A	<i>Montastraea</i> <i>faveolata</i>	[ATG]25	161-218	<i>Montastraea</i> <i>franksib,</i> <i>annularisb</i>	Genus	
M_fav4 B	<i>Montastraea</i> <i>faveolata</i>	[TTTG]17	365-417	<i>Montastraea</i> <i>franksib,</i> <i>annularisb</i>	Genus	
M_fav5 A	<i>Montastraea</i> <i>faveolata</i>	[CGA]17	340-394	<i>Montastraea</i> <i>franksib,</i> <i>annularisb</i>	Genus	
M_fav6 B	<i>Montastraea</i> <i>faveolata</i>	[CA]33	401-445	<i>Montastraea</i> <i>franksib,</i> <i>annularisb</i>	Genus	
M_fav7 A	<i>Montastraea</i> <i>faveolata</i>	[CAT]24	465-519	<i>Montastraea</i> <i>franksib,</i> <i>annularisb</i>	Genus	
M_fav8 C	<i>Montastraea</i> <i>faveolata</i>	[CAA]38	289-355	<i>Montastraea</i> <i>franksib,</i> <i>annularisb</i>	Genus	
M_fav9 B	<i>Montastraea</i> <i>faveolata</i>	[CAAT]21	278-322	<i>Montastraea</i> <i>franksib,</i> <i>annularisb</i>	Genus	
Acr166	<i>Acropora</i> <i>palmata</i>	[AAT]28		<i>Acropora</i> <i>digitifera</i>	96-162	Genus
Acr181	<i>Acropora</i> <i>palmata</i>	[AAT]10		<i>Acropora</i> <i>digitifera</i>	122- 239	Genus
Acr182	<i>Acropora</i> <i>palmata</i>	[AAT]18		<i>Acropora</i> <i>digitifera</i>	123- 195	Genus
Acr8	<i>Acropora</i> <i>digitifera</i>	[CT]3GT[CT]5	83-88	<i>Acropora sp1</i>	83-88	Genus
A.mill2- 8	<i>Acropora</i> <i>millepora</i>	[CA]]9	97-113	<i>Acropora</i> <i>digitifera</i>	92-96	Genus
A.mill2- 8	<i>Acropora</i> <i>millepora</i>	[CA]]9	97-113	<i>Acropora sp1</i>	66-94	Genus
A.mill2- 22	<i>Acropora</i> <i>millepora</i>	TA[TG]11	156-192	<i>Acropora</i> <i>digitifera</i>	160- 192	Genus
A.mill2- 22	<i>Acropora</i> <i>millepora</i>	TA[TG]11	156-192	<i>Acropora sp1</i>	162- 190	Genus
Cc-L5	<i>Cladocora</i> <i>caespitosa</i>	[AC]16	149-179	<i>Oculina</i> <i>patagonica</i>		Order
Cc-L13	<i>Cladocora</i> <i>caespitosa</i>	[AAG]7 AAA [AAG]8 GAG [AAC]5	300-350	<i>Oculina</i> <i>patagonica</i>		Order
Cc-L16	<i>Cladocora</i> <i>caespitosa</i>	[GGGGGT]5	236-269	<i>Oculina</i> <i>patagonica</i>		Order
Cc-L19	<i>Cladocora</i> <i>caespitosa</i>	[AAC]7ACCAGCTAC[AAC]2	199-211	<i>Oculina</i> <i>patagonica</i>		Order
Cc-L27	<i>Cladocora</i> <i>caespitosa</i>	[AAC]8	192-201	<i>Oculina</i> <i>patagonica</i>		Order
Cc-L37	<i>Cladocora</i> <i>caespitosa</i>	[AAC]3[AAT]4[AAC]4 [AAT]4[AAC]4 [AAT]3[AAC]7	243-246	<i>Oculina</i> <i>patagonica</i>		Order
Cc-L38	<i>Cladocora</i> <i>caespitosa</i>	[AAC]13	340-361	<i>Oculina</i> <i>patagonica</i>		Order
EST007	<i>Acropora</i> <i>millepora</i>	[TTTC]5	99-107	<i>Acropora</i> <i>hyacinthus</i>		Genus
EST014	<i>Acropora</i> <i>millepora</i>	[TCT]13	143-173	<i>Acropora</i> <i>hyacinthus</i>		Genus
EST016	<i>Acropora</i> <i>millepora</i>	[AAC]7	97-122	<i>Acropora</i> <i>hyacinthus</i>		Genus
EST032	<i>Acropora</i> <i>millepora</i>	[TTA]21	138-187	<i>Acropora</i> <i>hyacinthus</i>		Genus
EST062	<i>Acropora</i> <i>millepora</i>	[GAT]9	110-126	<i>Acropora</i> <i>hyacinthus</i>		Genus
EST063	<i>Acropora</i> <i>millepora</i>	[TC]8	96-104	<i>Acropora</i> <i>hyacinthus</i>		Genus
EST097	<i>Acropora</i>	[TGA]7	123-135	<i>Acropora</i>		Genus

(Nakajima,
Nishikawa,
Iguchi, &
Sakai,
2009)

(Casado-
Amezua *et*
al., 2011)

(Wang,
Zhang, &
Matz, 2009)

	<i>millepora</i>			<i>hyacinthus</i>		
EST098	<i>Acropora millepora</i>	[TG]12	98-118	<i>Acropora hyacinthus</i>	Genus	
EST121	<i>Acropora millepora</i>	[ATGCCG]4	100-112	<i>Acropora hyacinthus</i>	Genus	
EST122	<i>Acropora millepora</i>	[TTA]18	99-141	<i>Acropora hyacinthus</i>	Genus	
EST149	<i>Acropora millepora</i>	[GAT]9	118-130	<i>Acropora hyacinthus</i>	Genus	
EST181	<i>Acropora millepora</i>	[ATG]10	145-157	<i>Acropora hyacinthus</i>	Genus	
EST196	<i>Acropora millepora</i>	[TAA]9	117-145	<i>Acropora hyacinthus</i>	Genus	
EST245	<i>Acropora millepora</i>	[CA]10	115-124	<i>Acropora hyacinthus</i>	Genus	
EST254	<i>Acropora millepora</i>	[CA]12	86-100	<i>Acropora hyacinthus</i>	Genus	
WGS051	<i>Acropora millepora</i>	[GATA]8	151-216	<i>Acropora hyacinthus</i>	Genus	
WGS092	<i>Acropora millepora</i>	[ATT]12	111-200	<i>Acropora hyacinthus</i>	Genus	
WGS101	<i>Acropora millepora</i>	[TTA]14[GTA]10	134-190	<i>Acropora hyacinthus</i>	Genus	
WGS112	<i>Acropora millepora</i>	[AAT]9	166-184	<i>Acropora hyacinthus</i>	Genus	
WGS134	<i>Acropora millepora</i>	[GATA]6	105-133	<i>Acropora hyacinthus</i>	Genus	
WGS152	<i>Acropora millepora</i>	[AT]9	87-110	<i>Acropora hyacinthus</i>	Genus	
WGS153	<i>Acropora millepora</i>	[AATC]7	106-126	<i>Acropora hyacinthus</i>	Genus	
WGS189	<i>Acropora millepora</i>	[ATCT]7	158-194	<i>Acropora hyacinthus</i>	Genus	
WGS196	<i>Acropora millepora</i>	[ATAC]6	128-234	<i>Acropora hyacinthus</i>	Genus	
WGS211	<i>Acropora millepora</i>	[TAA]8	181-199	<i>Acropora hyacinthus</i>	Genus	
Acr181	<i>Acropora palmata</i>	[AAT]10		<i>Acropora muricata</i>	125-197	Genus
Acr182	<i>Acropora palmata</i>	[AAT]18		<i>Acropora muricata</i>	129-183	Genus
Acr180	<i>Acropora palmata</i>	[AAT]19		<i>Acropora muricata</i>	92-158	Genus
Acr207	<i>Acropora palmata</i>	[AAT]20		<i>Acropora muricata</i>	101-188	Genus
Acr166	<i>Acropora palmata</i>	[AAT]28		<i>Acropora muricata</i>	87-162	Genus
Acr45	<i>Acropora muricata</i>	[CT]17		<i>Acropora digitifera</i>		Genus
Acr1-60	<i>Acropora muricata</i>	[GT]10	182-213	<i>Acropora digitifera</i>	171-233	Genus
Acr53	<i>Acropora muricata</i>	[GT]10	159-171	<i>Acropora digitifera</i>	161-193	Genus
Acr3-27	<i>Acropora muricata</i>	[GT]13		<i>Acropora digitifera</i>		Genus
Acr1-4	<i>Acropora muricata</i>	[GT]20	137-201	<i>Acropora digitifera</i>	129-243	Genus
Acr181	<i>Acropora palmata</i>	[AAT]10		<i>Acropora humilis</i>		Genus
Acr182	<i>Acropora palmata</i>	[AAT]18		<i>Acropora humilis</i>		Genus
Acr180	<i>Acropora palmata</i>	[AAT]19		<i>Acropora humilis</i>		Genus
Acr207	<i>Acropora palmata</i>	[AAT]20		<i>Acropora humilis</i>		Genus
Acr166	<i>Acropora palmata</i>	[AAT]28		<i>Acropora humilis</i>		Genus

(Tang, Wei,
Chen,
Wallace, &
Chen, 2010)

Acr1-60	<i>Acropora muricata</i>	[GT]10	182-213	<i>Acropora humilis</i>	Genus	
Acr53	<i>Acropora muricata</i>	[GT]10	159-171	<i>Acropora humilis</i>	Genus	
Acr1-4	<i>Acropora muricata</i>	[GT]20	137-201	<i>Acropora humilis</i>	Genus	
Acr181	<i>Acropora palmata</i>	[AAT]10		<i>Acropora hyacinthus</i>	Genus	
Acr182	<i>Acropora palmata</i>	[AAT]18		<i>Acropora hyacinthus</i>	Genus	
Acr180	<i>Acropora palmata</i>	[AAT]19		<i>Acropora hyacinthus</i>	Genus	
Acr207	<i>Acropora palmata</i>	[AAT]20		<i>Acropora hyacinthus</i>	Genus	
Acr166	<i>Acropora palmata</i>	[AAT]28		<i>Acropora hyacinthus</i>	Genus	
Acr45	<i>Acropora muricata</i>	[CT]17		<i>Acropora hyacinthus</i>	Genus	
Acr1-60	<i>Acropora muricata</i>	[GT]10	182-213	<i>Acropora hyacinthus</i>	Genus	
Acr53	<i>Acropora muricata</i>	[GT]10	159-171	<i>Acropora hyacinthus</i>	Genus	
Acr3-27	<i>Acropora muricata</i>	[GT]13		<i>Acropora hyacinthus</i>	Genus	
Acr1-4	<i>Acropora muricata</i>	[GT]20	137-201	<i>Acropora hyacinthus</i>	Genus	
Acr181	<i>Acropora palmata</i>	[AAT]10		<i>Acropora valida</i>	Genus	
Acr182	<i>Acropora palmata</i>	[AAT]18		<i>Acropora valida</i>	Genus	
Acr180	<i>Acropora palmata</i>	[AAT]19		<i>Acropora valida</i>	Genus	
Acr207	<i>Acropora palmata</i>	[AAT]20		<i>Acropora valida</i>	Genus	
Acr166	<i>Acropora palmata</i>	[AAT]28		<i>Acropora valida</i>	Genus	
Acr1-60	<i>Acropora muricata</i>	[GT]10	182-213	<i>Acropora valida</i>	Genus	
Acr53	<i>Acropora muricata</i>	[GT]10	159-171	<i>Acropora valida</i>	Genus	
Acr1-4	<i>Acropora muricata</i>	[GT]20	137-201	<i>Acropora valida</i>	Genus	
Acr181	<i>Acropora palmata</i>	[AAT]10		<i>Acropora palmata</i>	Genus	
Acr181	<i>Acropora palmata</i>	[AAT]10		<i>Acropora sp1</i>	146-234	Genus
Acr182	<i>Acropora palmata</i>	[AAT]18		<i>Acropora palmata</i>		Genus
Acr182	<i>Acropora palmata</i>	[AAT]18		<i>Acropora sp1</i>	137-208	Genus
Acr180	<i>Acropora palmata</i>	[AAT]19		<i>Acropora palmata</i>		Genus
Acr180	<i>Acropora palmata</i>	[AAT]19		<i>Acropora digitifera</i>	86-167	Genus
Acr207	<i>Acropora palmata</i>	[AAT]20		<i>Acropora palmata</i>		Genus
Acr207	<i>Acropora palmata</i>	[AAT]20		<i>Acropora digitifera</i>	110-188	Genus
Acr166	<i>Acropora palmata</i>	[AAT]28		<i>Acropora palmata</i>		Genus
Acr166	<i>Acropora palmata</i>	[AAT]28		<i>Acropora sp1</i>	124-157	Genus
DdL100	<i>Desmophyllum dianthus</i>	[TCA]9	128-140	<i>Scleractinia</i>	Order	(Addamo et
DdL102	<i>Desmophyllum dianthus</i>	[TGT]6	123-152	<i>Scleractinia</i>	Order	al., 2015)

DdL107*	<i>Desmophyllum dianthus</i>	[TTG]7	143-179	<i>Scleractinia</i>	Order
DdL109	<i>Desmophyllum dianthus</i>	[TGT]6	128-140	<i>Scleractinia</i>	Order
DdL13	<i>Desmophyllum dianthus</i>	[GTT]15	86-122	<i>Scleractinia</i>	Order
DdL16	<i>Desmophyllum dianthus</i>	[CAA]11	104-161	<i>Scleractinia</i>	Order
DdL22*	<i>Desmophyllum dianthus</i>	[ACA]13	119-168	<i>Scleractinia</i>	Order
DdL24	<i>Desmophyllum dianthus</i>	[TGTT]10	162-212	<i>Scleractinia</i>	Order
DdL34	<i>Desmophyllum dianthus</i>	[GAT]14	176-202	<i>Scleractinia</i>	Order
DdL41	<i>Desmophyllum dianthus</i>	[GAT]11	150-183	<i>Scleractinia</i>	Order
DdL50	<i>Desmophyllum dianthus</i>	[TTG]11[CTGTTG]3	194-281	<i>Scleractinia</i>	Order
DdL56	<i>Desmophyllum dianthus</i>	[GTTT]8	116-148	<i>Scleractinia</i>	Order
DdL57	<i>Desmophyllum dianthus</i>	[TTAC]5	117-137	<i>Scleractinia</i>	Order
DdL58*	<i>Desmophyllum dianthus</i>	[TCTT]6	105-153	<i>Scleractinia</i>	Order
DdL7*	<i>Desmophyllum dianthus</i>	[CAA]CAG[CAA]10	214-281	<i>Scleractinia</i>	Order
DdL73	<i>Desmophyllum dianthus</i>	[TTTG]7	158-178	<i>Scleractinia</i>	Order
DdL82	<i>Desmophyllum dianthus</i>	GTT]12	116-134	<i>Scleractinia</i>	Order
DdL83	<i>Desmophyllum dianthus</i>	[TGT]8[GGT]3	230-260	<i>Scleractinia</i>	Order
DdL84*	<i>Desmophyllum dianthus</i>	[CAA]6CAGCAA	135-210	<i>Scleractinia</i>	Order
DdL86	<i>Desmophyllum dianthus</i>	[CAA]7	146-152	<i>Scleractinia</i>	Order
DdL90	<i>Desmophyllum dianthus</i>	[ATC]5GTC[ATC]GTC	104-1119	<i>Scleractinia</i>	Order
DdL91	<i>Desmophyllum dianthus</i>	[GGT]9	205-223	<i>Scleractinia</i>	Order
DdL96	<i>Desmophyllum dianthus</i>	[AAC]7	102-156	<i>Scleractinia</i>	Order
DdL98	<i>Desmophyllum dianthus</i>	[TCA]8	135-150	<i>Scleractinia</i>	Order

**Chapter 5. Patterns of genetic connectivity amongst four
deep-sea demosponges in the New Zealand region:
implications for the protection of vulnerable marine
ecosystems**

5.1. Introduction

Larval dispersal and genetic connectivity are poorly understood in the deep sea, despite the fact that they shape patterns of biodiversity and biogeography, and may provide insights to help guide the protection of rare, threatened and endangered species (Hilário *et al.*, 2015; Baco *et al.*, 2016). Analysis of genetic variation and quantification of the extent of genetic connectivity for multiple taxa can be used to plan and evaluate the suitability of the location, size and spacing of marine protected areas (MPAs) and networks (reviewed by Palumbi, 2003; Lotterhos *et al.*, 2014; van Der Meer *et al.*, 2014; Jackson *et al.*, 2015; Truelove *et al.*, 2015). Whilst the identification of source and sink populations, as well as genetic hotspots, has long been achievable in shallow-water biota, this has not been the case for the deep sea. In general, this is because the high cost and technical challenge of sampling in the deep sea make it difficult to acquire specimens at large spatial scales, and across a wide range of depths (Hilário *et al.*, 2015). In addition, whilst analysis of existing (e.g. archived) material may provide opportunities for the examination of genetic variation and population genetic structure, much of the material presently held in many collections is unlikely to be suitable for such work (Boschen *et al.*, 2015). However, given the ongoing anthropogenic threats to the deep sea that include fishing, dumping, oil and gas exploration and potential deep-sea mining (Ramirez-Llodra *et al.*, 2010), there is an increasing need to better understand patterns of population connectivity amongst vulnerable marine ecosystems (VMEs) so that protection can be put in place.

The relatively recent concept of VMEs has presented major challenges for the protection of deep-sea areas. VMEs are defined as “assemblages of marine benthic organisms which are susceptible to anthropogenic disturbance, especially that arising from the impact of fishing gear used in bottom fishing” (FAO, 2009). The protection of VMEs is called for in resolutions of the United Nations General Assembly (UNGA), and such protection is the responsibility of nation states within their Exclusive Economic Zones (EEZs) as well as bodies that manage resource use in the area beyond national jurisdiction (ABNJ) (e.g. Regional Fisheries Management Organisations, RFMOs) (Ardron *et al.*, 2013). VMEs are typically characterised by taxa that are fragile and susceptible to damage (in particular from fishing gear), have high longevity and slow growth rates, and have a limited ability (or complete inability) to recover from major disturbance events (FAO, 2009). Such indicator taxa have been identified for particular regions (e.g. Parker *et al.*, 2009 for the South Pacific Ocean; Parker & Bowden, 2010 for Antarctica). VMEs are often associated with seamounts,

which can be sites of intense fishing activity because some fish species aggregate around seamounts for breeding or feeding (Clark & Rowden, 2009). A variety of different measures have been enacted to help protect VMEs, including spatial and temporal fishing gear restrictions, move-on rules, as well as areas closed to fishing, but the efficacy of these protection measures has been questioned (Auster *et al.*, 2011; Clark & Dunn, 2012; Penney & Guinotte, 2013). If VMEs are to be protected adequately by spatial closures then further research is required to improve knowledge of the distribution of VME indicator taxa, to better understand how such VMEs are connected in terms of sources and sinks of larval recruits, and to quantify the spatial scale(s) at which genetic populations are structured.

Sponges are considered to be VME indicator taxa in the South Pacific Ocean (Parker *et al.*, 2009), but studies of their distribution, life history and population connectivity are rare. In shallow-water sponges, genetic structure has been observed in most studies, and across a range of sponges at different spatial scales using different markers. This structure has been attributed to hydrodynamic forces and limited dispersal capabilities (Duran *et al.*, 2004abc; Whalan *et al.*, 2005; Bentlage & Wörheide, 2007; Whalan *et al.*, 2008a; Blanquer *et al.*, 2009; López-Legentil & Pawlik, 2009; Blanquer & Uriz, 2010; DeBiasse *et al.*, 2010; Xavier *et al.*, 2010; Dailianis *et al.*, 2011; Guardiola *et al.*, 2012). This small-scale structure may be explained by limited dispersal ability, because for most sponges the planktonic larvae settle within a few days (Maldonado & Bergquist, 2002). This information from shallow-water sponges may provide some indication of the genetic structure of deep-sea sponges. However, the environment is very different between shallow and deep water (Watling *et al.*, 2013), and these differences are expected to shape different patterns of population genetic structure. For instance, previous studies have pointed out that lower temperatures increase larval duration and dispersal capabilities (Whalan *et al.*, 2008b), and this longer larval duration may assist sponges to achieve large-scale population connectivity in the deep sea (Hilário *et al.*, 2015). Therefore, information from shallow-water sponges is probably of limited value, and studies of genetic structure and connectivity of deep-sea species are required.

Whilst genetic differentiation amongst many species in New Zealand's coastal environment is well documented (Gardner *et al.*, 2010), patterns of population genetic structure of deep-sea taxa are largely unknown. To date, studies have not revealed any consistent patterns of genetic connectivity or structuring across a range of deep-sea VME indicator taxa in the New Zealand region. A study of bamboo corals (Keratoisidinae) reported common mitochondrial haplotypes occurring over a wide geographical range (27 °S - 50 °S) around New Zealand and no indication of genetic barriers to dispersal (Smith *et al.*, 2004a).

Three species of deep-sea corals showed evidence of genetic subdivision on seamounts and slopes within the Australia–New Zealand region, but a further six coral species exhibited either low genetic diversity or no evidence of genetic structure at scales of tens to hundreds of kilometres (Miller *et al.*, 2010). In contrast, a later and broader study of the stony coral *Desmophyllum dianthus*, reported regional genetic differentiation as well as genetic differences amongst populations with depth across the region (Miller *et al.*, 2011). Along the Kermadec Volcanic Arc (north of New Zealand), Smith *et al.* (2004b) reported heterogeneity in allozyme gene frequencies between vent mussels collected from two seamounts only 50 km apart. However, high levels of gene flow in the region amongst sites 10s to 100s km apart and limited genetic structure were reported by a later study of the same vent mussel based on *COI* variation (Boschen *et al.*, 2015). The role of currents as either promoters of, or barriers to, gene flow is likely to be region-specific and requires much more consideration at a variety of different spatial and temporal scales. For example, in waters south of New Zealand, the Antarctic Circumpolar Current is believed to act as an oceanographic barrier to gene flow of deep-sea octocorals, separating populations to the north (around New Zealand and Tasmania) and south (Antarctica) at approximately 55 °S on the Macquarie Ridge (Dueñas *et al.*, 2016). In addition, deep water (1000 m depth) currents flow from north and south off New Zealand’s east coast and meet along the Chatham Rise (Chiswell *et al.*, 2015), and this might act as a barrier to larval dispersal in some regions but also act to promote mixing and thereby enhance diversity in others.

As noted by Miller *et al.* (2010) and Boschen *et al.* (2015), in the New Zealand region, understanding of the degree of isolation of deep-sea populations with respect to gene flow and connectivity is important for management planning, and the design of a network of marine protected areas. Whilst deep-sea genetic studies in the New Zealand region span a range of VME indicator taxa, the spatial coverage is patchy and therefore difficult to interpret holistically in terms of management recommendations for protection. In the present study, mitochondrial DNA sequence variation was examined to assess genetic connectivity amongst populations of four deep-sea demosponge species in the New Zealand region to provide information for management of VMEs in the South Pacific region.

5.2. Materials and methods

5.2.1. Samples

Ten sponges (*Poecillastra laminaris*, *Penares* sp., *Pleroma menoui*, *Neoaulaxinia persicum*, *Ecionemia novaezealandiae*, *Hyalascus maui*, *Geodia vestigifera*, *Herengeria vasiformis*, *Reidispongia coerulea*, *Neoschrammeniella fulvodesmus*) were selected as potential candidates for study, based on their adherence to VME criteria (FAO, 2009), wide distribution in the New Zealand region, and the availability of samples. After initial assessment of the available specimens for these ten species in the NIWA Invertebrate Collection, and the results of preliminary screening analysis, samples from four demosponges (*Neoaulaxinia persicum*, *Penares* sp., *Pleroma menoui* and *Poecillastra laminaris*) were analysed. The total number of samples across all four species was 117 (Table 5.1), and most were collected from within the New Zealand EEZ (Figure 5.1). The majority of specimens were from seamount and slope habitats from 122-1507 m water depth: most were preserved in ethanol, with the rest dry-preserved. Due to patchy deep-sea sampling and the different distributions of the species, samples for all species were not available throughout the study region. *Penares* sp. and *Poecillastra laminaris* samples were found in both biogeographic provinces, but *N. persicum* and *P. menoui* samples were not collected from the waters south of New Zealand. The full distributions of the latter two species are still unknown, but they have not yet been found in the southern province and are currently considered species with a northern restricted distribution.

In the New Zealand region, the deep sea is comprised of various bathymetries, topographies and hydrodynamics (Snelder *et al.*, 2007). In the present study, because sampling at each site recovered only one individual sponge, all specimens within 1° of latitude and longitude were grouped into one population: *N. persicum*, *P. palmatoclada*, *P. menoui* and *Poecillastra laminaris* were assigned into 8, 7, 8 and 19 populations for plotting of haplotype distributions, respectively. Samples for all species were categorised into 100 m depth range intervals based on their depth of collection.

Within the New Zealand region, two deep ocean floor biogeographic provinces are presently recognised, a northern (BY6) and a southern (BY10) province (Figure 5.1), each with its own characteristics (e.g., bottom water temperature and particulate organic carbon (POC) flux) (Watling *et al.*, 2013). To investigate genetic differentiation amongst

populations, a hierarchical testing framework based on features (provinces, regions, geomorphic characters) at different spatial scales (Figure 5.1) was employed. First, samples from all 4 species were assigned to the northern and southern biogeographic provinces following Watling *et al.* (2013) with a boundary at 45 °S. Second, samples were divided into northern (north of the Chatham Rise), central (the Chatham Rise) and southern (south of the Chatham Rise) regions with boundaries at latitudes 42 °S and 45 °S. For *N. persicum* and *P. menoui*, a further division at latitude 34 °S was to separate the species into an upper (Fiji Basin) and lower (New Zealand EEZ) group in the northern province. Third, samples were grouped based on geomorphic features, including the Fiji Basin, Kermadec Ridge, Challenger Plateau, Hikurangi Margin, Chatham Rise, Campbell Plateau and Macquarie Ridge. Full collection details are summarised in Table S5.1.

Table 5.1 Number of partial sequences plus length of sequence (bp) for three mitochondrial genes

Species	<i>COI</i>		<i>12S</i>		<i>Cytb</i>	
	n	length	n	length	n	length
<i>Poecillastra laminaris</i>	54	402			49	402
<i>Penares</i> sp.	22	423	18	694		
<i>Neaulaxinia persicum</i>	34	462	12	704		
<i>Pleroma menoui</i>	19	431				

5.2.2. Molecular methods

Total DNA was recovered using a guanidine thiocyanate extraction methodology (Boom *et al.*, 1990) and DNA was stored at 4 °C in 0.1X TE buffer. To identify informative population genetic markers in demosponges, three mitochondrial DNA regions with different rates of mutation, the *ribosomal subunit* (*12S*), *cytochrome oxidase subunit I* (*COI*) and *cytochrome b* (*Cytb*), were used to estimate genetic connectivity and population genetic differentiation. Sponge-specific primers for *12S*, *COI* and *Cytb* were designed and utilised to amplify these genes in this study; primers and annealing temperatures used for each species are summarised in (Chapter 3). PCR reactions (30 µl) for all three genes contained 1 µl DNA template, 15 µl 2 × MyTaq™ Mix (Bioline, UK), 1 µl of each primer (Macrogen, Korea), and 12 µl ddH₂O. The thermal cycling profile consisted of an initial denaturation step at 94 °C for 5 min, then 32 cycles of a three-step program (95 °C for 30 s, 50 °C for 30 s and 72 °C for 45 s), with a 20-min final extension at 72 °C. PCR products were sent to Macrogen (Macrogen Inc, Korea) for purification and sequencing on an ABI3730 sequencer. Total species-specific sample size was large enough for only

Poecillastra laminaris to permit assessment of nuclear DNA variation at 10 polymorphic microsatellite loci (described in Chapter 3).

Figure 5.1 Map showing the locations of samples for the study species.

Hierarchical testing of regional genetic differentiation is employed: (1) blue (northern) and green (southern) backgrounds represent the two biogeographic lower bathyal provinces (BY6 and BY10) of Watling *et al.* (2013) in the New Zealand region; (2) red dashed lines at 42°S and 45°S indicate boundaries between north (< 42°S), central (42°S to 45°S) and south (>45°S) regions for *Poecillastra laminaris* and *Penares* sp. and black dashed line at 32°S shows boundary between the north and south regions for *Neoaulaxinia persicum* and *Pleroma menoui*; (3) major geomorphic features are named on the map.

5.2.3. Data analysis

All sequences were checked and edited using Geneious (v 7, Biomatters Ltd, New Zealand). Sequences with HQ values (percentage of untrimmed bases in a sequence that is high quality) of < 80% were discarded. Multiple sequences were aligned using the plugin ClustalW Alignment (Gap Open Cost=100 and Gap Extend Cost=10) in Geneious, and then all alignments were viewed by eye. Neighbour-Joining (NJ) phylogenetic trees were constructed with default settings and highest similarity sequence in NCBI as an out-group by using the Tree Builder option in Geneious v7. For all populations with two or more individuals' intra-specific genetic diversity was evaluated by computing the number of haplotypes, the number of polymorphic sites, haplotypic diversity (h), and nucleotide diversity (π). A t -test was used to compare the difference of haplotypes diversity between markers.

AMOVA of sequence variation between/amongst regions (northern versus southern biogeographic provinces; northern versus central versus southern regions; amongst geomorphic features) and amongst populations was tested. Because small sample size will influence the accuracy of the results, AMOVA amongst populations on geomorphic features was employed only when population-specific sample sizes were larger than five. The AMOVA of sequence variation amongst populations on geomorphic feature with sample sizes > 5 showed the same pattern of results as when sample sizes were less than five. Therefore, to maximise information return the AMOVA results from all geomorphic feature populations are presented, but conclusions about these population were only drawn when there was general agreement between the two different approaches based on sample size.

Mismatch Distribution, Tajima's D (Tajima, 1989) and the Ramos-Onsins-Rozas Test (R_2) of Neutrality were analysed using the R-package software program PEGAS v0.6 (Paradis, 2010). Pairwise comparisons of population differentiation were performed in Arlequin and significance values estimated after 1000 permutations (Excoffier & Lischer, 2010). Between-province or within-province Φ_{ST} statistics were calculated to test for genetic differentiation amongst populations. If significant differentiation amongst populations was detected, the location of the genetic discontinuity was identified using Barrier v2.2 (Manni *et al.*, 2004). SAMOVA, using SPADS V1.0 (Dellicour & Mardulyn, 2014), was used to detect the number of potential populations per species with non-significant variant components identified amongst populations in AMOVA.

In order to visualise the spatial patterns of genetic variation for each marker, the specimens were colour-coded according to haplotype, their geographic coordinates of collection were plotted using ArcGIS (ESRI, USA), and their collection depth was plotted on an X-Y scatter plot. To assess the amount of variability in the populations of each species that was represented in the samples, haplotype accumulation curves were generated (Gotelli & Colwell, 2001) by calculating estimates of the mean and variance for the number of accumulated haplotypes through 1000 random permutations, using the R package SPIDER v1.1 (Brown *et al.*, 2012). The Mantel test (Mantel, 1967) was employed to test for isolation-by-depth by comparing the matrix of Φ_{ST} values to the matrix of depth (m) values and for isolation-by-distance by comparing the matrix of Φ_{ST} values to the matrix of shortest actual distances (km) between pairs of sites (Isolation by Distance, web service Jensen *et al.*, 2005).

The program MIGRATE (Beerli & Felsenstein, 1999) was used to estimate θ (theta, the effective population size $\times$ the mutation rate per site), and migration (M) under the sequence model. A full migration matrix model was employed as the migration estimating model, and each Markov chain Monte Carlo (MCMC) run consisted of 10 short chains (sampling 50,000 trees) and 1 long chain (sampling 500,000 trees) with a burn-in period of 10,000 trees. All runs were repeated five times to verify consistency of results, and the data presented are the average of the five runs.

For microsatellite data analysis, allelic frequencies, number of alleles, departures from Hardy-Weinberg equilibrium (HWE), linkage disequilibrium (LD) and observed and expected heterozygosities were estimated in GenAlEx (v 6.5). Arlequin was used to estimate Weir and Cockerham's (1984) unbiased estimator of Wright's F statistic (F_{ST}). Micro-Checker (v2.2.1, van Oosterhout *et al.*, 2004) was used to identify possible genotyping errors such as stuttering, large allele dropout, and null alleles within the data set by performing 10,000 randomisations (threshold was set at 0.10 then raised to 0.15 when insufficient loci were present). Loci under selection and/or with null alleles were removed and a second (neutral loci) data set was created. Both the full (n=10 loci) and the reduced (n=4 loci) data sets were tested for population genetic differentiation using Arlequin (AMOVA, 10,000 permutations) for differentiation between provinces and amongst regions. The Mantel test (GenAlEx) was employed to test for isolation-by-depth by comparing the matrix of Nei's unbiased genetic distance values to the matrix of depth (m) values and isolation-by-distance by comparing the matrix of Nei's unbiased genetic distance values to the matrix of shortest actual distances (km) between pairs of sites.

5.3. Results

After trimming and checking, *COI* sequence data were obtained for all 4 species, *12S* sequence data for two species, and *Cytb* sequence data for one species (Table 5.2). Overall haplotypic diversity for all populations for *COI* was greater than that for *12S* of *P. palmatoclada*, *12S* of *N. persicum* or *Cytb* of *Poecillastra laminaris*, but *t*-test showed that none reached significance ($p > 0.05$) (Table 5.3 -Table 5.5).

Table 5.2 Number of partial sequences (n) plus length of sequence (bp) for three mitochondrial genes for each of four sponge species

Genes	Species	n	Trimmed length (bp)	NCBI Accession ID
<i>COI</i>	<i>Poecillastra laminaris</i>	54	402	Hap_1-18: KX685272-KX685289
	<i>Penares</i> sp.	16	423	Hap_1-5: KX685302-KX685306
	<i>Neoaaulaxinia persicum</i>	30	462	Hap_1-7: KX685290-KX685296
	<i>Pleroma menoui</i>	16	431	Hap_1-5: KX685297-KX685301
<i>CytB</i>	<i>Poecillastra laminaris</i>	49	402	Hap_1-18: KX685307-KX685324
<i>12S</i>	<i>Penares</i> sp.	14	694	Hap_1-2: KX685325-KX685326
	<i>Neoaaulaxinia persicum</i>	11	704	Hap_1-2: KX685327-KX685328

Table 5.3 Nucleotide diversity statistics for *Poecillastra laminaris* at three different spatial scales

Grouping		<i>COI</i>			<i>Cytb</i>		
		n	Hd	π	n	Hd	π
Province	Northern	34	0.848	0.0145	30	0.800	0.0120
	Southern	19	0.749	0.0156	19	0.811	0.0152
Region	North	15	0.619	0.0037	14	0.681	0.0054
	Central	19	0.895	0.0185	17	0.721	0.0142
	South	19	0.749	0.0156	19	0.811	0.0152
Geomorphic feature	Kermadec Ridge	9	0.556	0.0021	9	0.583	0.0017
	Challenger Plateau	2	0	0	2	0	0
	Hikurangi Margin	2	1	0.0075	2	1	0.0100
	Chatham Rise	19	0.895	0.0185	17	0.721	0.0142
	Bounty Trough	2	1	0.0473	2	1	0.0274
	Campbell Plateau	3	0	0	3	0	0
	Macquarie Ridge	13	0.628	0.0121	14	0.758	0.0137
Total		53	0.893	0.0185	49	0.889	0.0152

n is the number of sequences for analysis from the site, s is the number of polymorphic nucleotide sites in the sequence, h is the number of haplotypes represented at the site, Hd is haplotype diversity, and π is nucleotide diversity.

5.3.1. Geographic distribution of haplotypes

In total, 11 unique *COI* haplotypes were observed for *Poecillastra laminaris* in the northern province, only 4 haplotypes were restricted to the southern province, and 8 haplotypes were restricted to individuals (Figure 5.2A). Hap 1 was the numerically dominant *COI* haplotype, with all occurrences except one individual (in S17) occurring in the north. For *Cytb*, 9 haplotypes were only distributed in the northern province and 7 haplotypes were only found in the southern province. Hap 5 was the numerically most abundant haplotype and it was only found in the northern province.

Table 5.4 Nucleotide diversity statistics for *Penares* sp. at three different spatial scales

Grouping		<i>COI</i>			<i>12S</i>		
		n	Hd	π	n	Hd	π
Province	Northern	14	0.396	0.0025	12	0.167	0.0002
	Southern	2	1	0.0047	2	0	0
Region	North	7	0.524	0.0041	6	0.333	0.0005
	Central	7	0.286	0.0007	6	0	0
	South	2	1	0.0047	2	0	0
Geomorphic feature	Kermadec Ridge	5	0.7	0.0052	4	0.5	0.0004
	Challenger Plateau	1	-	-	1	-	-
	Hikurangi Margin	1	-	-	1	-	-
	Chatham Rise	7	0.286	0.0007	6	0	0
	Macquarie Ridge	2	1	0.0047	2	0	0
	Total	30	0.411	0.0025	26	0.148	0.0002

n is the number of sequences for analysis from the site, s is the number of polymorphic nucleotide sites in the sequence, h is the number of haplotypes represented at the site, Hd is haplotype diversity, and π is nucleotide diversity.

A numerically dominant *COI* haplotype (Hap1) was observed for *Penares* sp. across the northern and southern provinces: other haplotypes were only found in single individuals (Figure 5.2B). For *12S*, Hap1 was shared across both provinces, and other haplotypes were only found in single individuals. There was no support for the hypothesis of geographic genetic structure by province for haplotypic diversity distributions of *COI* or *12S* for this species.

Eleven *COI* haplotypes for *N. persicum* were found within the northern province, but only Hap1 was widely spread across this province. There was limited evidence of *12S* haplotypic diversity within the northern province. The haplotypic distributions of *COI* and *12S* did not reveal patterns of spatial genetic differentiation (Figure 5.2C).

Only five *COI* haplotypes of *Pleroma menoui* were found in the northern province: Hap2 was distributed across the region (Figure 5.2D). There was limited evidence to suggest the presence of spatial genetic structure.

5.3.2. Population genetic diversity

For *Poecillastra laminaris* the *COI* haplotypic diversity of the northern province was greater than in the southern province, whereas nucleotide diversity was lower. The middle region was the highest haplotypic and nucleotide diversity amongst three regions. Testing of differences in haplotypic diversity between geomorphic features, the highest haplotypic diversity was found in Chatham Rise, but the highest nucleotide diversity was in Campbell Plateau. For *Cytb*, both haplotypic and nucleotide diversity were greater in the southern than in the northern province, and the haplotypic and nucleotide diversity of south region was higher than other two regions. The greatest haplotypic and nucleotide diversity values of *Cytb* were all found in Macquarie amongst the geomorphic groups (Figure 5.2A and Table 5.3).

Table 5.5 Nucleotide diversity statistics for *Neoaulaxinia persicum* and *Pleroma menoui* at two different spatial scales

Grouping		<i>Neoaulaxinia persicum</i>						<i>Pleroma menoui</i>		
		<i>COI</i>			<i>12S</i>			<i>COI</i>		
		n	Hd	π	n	Hd	π	n	Hd	π
Region	North	2	0	0	1	0	0	7	0.524	0.002
	South	28	0.537	0.0019	10	0	0	9	0.417	0.0026
Geomorphic feature	Norfolk Ridge	-	-	-	-	-	-	4	0.833	0.0035
	Kermadec Ridge	7	0.288	0.0006	4	0.5	0.02547	7	0.286	0.0007
	Three Kings Ridge	12	0.318	0.0011	4	0	0	2	0	0
	NE Continental Slope	-	-	-	-	-	-	3	0.667	0.0062
	Challenger Plateau	1	-	-	-	-	-	-	-	-
	Hikurangi Margin	2	0	0	1	-	-	-	-	-
	Chatham Rise	8	0.75	0.003	2	1	0	-	-	-

n is the number of sequences for analysis from the site, s is the number of polymorphic nucleotide sites in the sequence, h is the number of haplotypes represented at the site, Hd is haplotype diversity, and π is nucleotide diversity.

Figure 5.2 Haplotype map for *Poecillastra laminaris* COI (above line) and *Cytb* (below line) (A), *Penares* sp. COI (above line) and *12S* (below line) (B), *Neoaulaxinia persicum* COI (above line) and *12S* (below line) (C), *Pleroma menoui* COI (D)

For *Penares* sp. the *COI* nucleotide diversity of the southern province was greater than that of the northern province. For the three regions the south had the highest values, mainly contributed by samples from Macquarie Ridge. For *12S*, nucleotide diversity was greater in the northern than the southern province. The north region had the greatest nucleotide diversity amongst the regions, which was closely related to high diversity associated with populations from the Kermadec Ridge (Figure 5.2B and Table 5.4)

For *N. persicum* the *COI* haplotypic and nucleotide diversity values were greater in the south than north region: populations on the Three Kings Ridge had the highest nucleotide and Chatham Rise populations had the highest haplotype diversity values. For *12S* the haplotype number, the number of polymorphic sites, haplotype diversity and nucleotide diversity values could only be calculated for the south region. The Kermadec Ridge population had the highest nucleotide and haplotypic diversity (Figure 5.2C and Table 5.5).

For *Pleroma menoui*, the highest *COI* nucleotide diversity was in populations from the Norfolk Ridge, and the nucleotide diversity in the north was greater than in the south region. The haplotypic diversity for *Pleroma menoui* followed the same pattern (Figure 5.2D and Table 5.5).

5.3.3. Population genetic structure

Amongst the *Poecillastra laminaris* populations, Φ_{ST} values for both *COI* and *Cytb* were highly variable (Tables S5.2-5.5), but were often based on small sample sizes (sometimes 2 individuals per population). The AMOVA of genetic structure across *Poecillastra laminaris* populations (Table 5.6) revealed significant differentiation between populations of the northern and southern provinces, amongst the three regions and also amongst the different geomorphic features for both *COI* and *Cytb* ($p < 0.01$). Except for a weak significant result for in *Cytb*, populations within the provinces, regions or geomorphic features were not statistically significantly different ($p > 0.05$), whereas significant variation existed within all populations ($p < 0.01$). Whilst this non-significant differentiation amongst populations might result from an under-estimated number of populations, the SAMOVA based on all *COI* and *Cytb* sequences revealed that the potential number of populations was 18 (Figure S5.1), which was very close to the actual number of sampling sites (19), although 8 sites (N2, N3, N6, N10, S11, S13, S16 and S19) had only one sequence present for one or the other marker. The NJ phylogenetic trees revealed evidence of geographic structure for the *Poecillastra laminaris* populations (Figure S5.2). Whilst not all sequences were grouped

according to sampling location, most were, providing support for a north and south differentiation.

Table 5.6 Hierarchical AMOVA results for *Poecillastra laminaris* when tested for genetic variation at mitochondrial genes

Source of variation	<i>COI</i>			<i>Cytb</i>		
	df	ss	%V	df	ss	%V
Between provinces	1	40.201	32.34**	1	22.481	21.9**
Within provinces between populations	16	60.618	9.53	16	54.323	11.87
Within populations	35	92.068	58.13**	33	76	66.23**
Total	52	192.887		50	152.804	
Amongst regions	2	59.329	36.17**	2	35.06	25.42**
Within regions between populations	15	41.49	1.32	15	41.744	5.80*
Within populations	35	92.068	62.51**	33	76	68.7**
Total	52	192.887		50	152.804	
Amongst geomorphic features	4	68.37	38.58**	4	50.037	34.53**
Within geomorphic features between populations	11	26.702	-2.15	12	24.596	-3.03
Within populations	34	90.568	63.58**	32	74	68.50**
Total	49	185.64		48	148.633	

Significant values of $p < 0.05$ are marked as *, and $p < 0.01$ are marked as **.

Table 5.7 Hierarchical AMOVA results for *Penares* sp. when tested for genetic variation at mitochondrial genes

Source of variation	<i>COI</i>			<i>12S</i>		
	df	ss	%V	df	ss	%V
Between provinces	1	0.768	10.53	1	0.012	-22.35
Within provinces between populations	3	1.6	-0.01	3	0.167	-18.82
Within populations	11	6.257	91.47	9	0.75	141.18
Total	15	8.625		13	0.929	
Amongst regions	2	1.625	18.14	2	0.095	32.24
Within regions between populations	4	1.767	16.08	4	0.083	-89.32
Within populations	9	5.233	97.94	7	0.75	157.08
Total	15	8.625		13	0.929	
Amongst geomorphic features	4	2.368	8.06	4	0.179	60.04
Within geomorphic features between populations	2	1.024	-8.89	2	0	-119.88
Within populations	9	5.233	100.83	7	0.75	159.85
Total	15	8.625		13	0.929	

Significant values of $p < 0.05$ are marked as *, and $p < 0.01$ are marked as **.

In contrast, analysis of Φ_{ST} values revealed no genetic structure within each of the *Penares* sp., *N. persicum* and *Pleroma menoui* samples. Species-specific pairwise population Φ_{ST} values were all below 0.1 with no statistically significant differences between pairs of populations. AMOVA revealed no significant ($p > 0.05$) hierarchical population genetic

structure for *Penares* sp. and *P. menoui* across the sampled area (Table 5.7 and Table 5.8). Similarly, NJ phylogenetic trees and haplotypes distribution did not support geographic structure between and within provinces (Figures S5.3-5.6).

Table 5.8 Hierarchical AMOVA results for *Neoaulaxinia persicum* and *Pleroma menoui* when tested for genetic variation at the *COI* mitochondrial gene

Source of variation	<i>Neoaulaxinia persicum</i>			<i>Pleroma menoui</i>		
	df	ss	%V	df	ss	%V
Between regions	1	0.133	-25.68	1	0.484	-5.51
Within regions between populations	6	3.564	17.05	6	3.849	25.99
Within populations	22	8.436	108.63	8	3.167	79.53
Total	29	12.133		15	7.5	
Amongst geomorphic features	3	1.44	-18.41	3	1.726	-6.04
Within geomorphic features between populations	4	2.258	27.47	4	2.607	27.45
Within populations	22	8.436	90.94	8	3.167	78.59
Total	29	12.133		15	7.5	

Significant values of $p < 0.05$ are marked as *, and $p < 0.01$ are marked as **. AMOVA analysis for 12S in *Neoaulaxinia persicum* was not possible.

5.3.4. Isolation by distance and depth

The seven tests across all 4 species revealed no significant isolation by depth relationship, but one significant isolation by distance relationship, which was for *COI* of *Poecillastra laminaris* (Table 5.9).

Table 5.9 Mantel tests of isolation by depth and isolation by distance amongst different markers and species

Species	Depth				Distance			
	<i>COI</i>	<i>Cytb</i>	<i>12S</i>	Microsatellites	<i>COI</i>	<i>Cytb</i>	<i>12S</i>	Microsatellites
<i>Poecillastra laminaris</i>	0.025 (0.212)	0.121 (0.052)		0.009 (0.400)	0.411 (<0.001)	0.403 (1.000)		-0.019 (0.400)
<i>Penares</i> sp.	0.169 (0.060)		0.114 (0.240)		0.103 (0.280)		-0.092 (0.610)	
<i>Neoaulaxinia persicum</i>	-0.049 (0.039)		-0.202 (0.170)		0.156 (0.060)		0.554 (0.070)	
<i>Pleroma menoui</i>	0.001 (0.460)				-0.032 (0.520)			

Data are shown as R^2 (p value). Numbers in bold indicate significant correlations.

5.3.5. Tajima's D and R2 tests of neutrality and mismatch distribution

Tajima's D was negative for all four species for all mtDNA markers, but only statistically significant ($p < 0.05$) in populations of *N. persicum* (*COI* and *12S*), *P. menoui* (*COI*) and *Penares* sp. (*12S*). The Ramos-Onsins-Rozas test of neutrality revealed that all R2 values were low and not statistically significant (Table S5.6). The mismatch distribution analyses showed no evidence of rapid population expansion and indicated that the empirical and stable expectations were not in agreement for all four species (Figures S5.7-5.9), which implied that the different species had different population histories. Also, such negative values in the absence of evidence for rapid expansion may signify an excess of low frequency polymorphisms of the mtDNA markers of *P. palmatoclada*, *N. persicum* and *P. menoui*.

5.3.6. Genetic barriers

Genetic barriers to gene flow between/amongst populations of *Poecillastra laminaris* based on the *COI* pairwise population Φ_{ST} values and geographical coordinates were predicted to occur around the north of the North Island and the south of the South Island. Based on the *Cytb* Φ_{ST} values the analysis indicated that barriers may also exist to the west and north of the North Island (Figure S5.10).

5.3.7. Larval migration

Because of small sample sizes, only *Poecillastra laminaris* had enough datasets to predict the larval migrations amongst populations. The average θ values estimated by the MIGRATE software for two loci (*COI* and *Cytb*) data set ranged from 0.0001 (Kermadec Ridge) to 0.0079 (Hikurangi Margin) (Table 5.10). Estimates of per generation immigrants were also variable amongst samples (only migrate values >1 are shown in Table 5.10). For northern populations (Kermadec Ridge, Challenger Plateau and Hikurangi Margin) there was no contribution to the southern populations (Campbell Plateau and Macquarie Ridge), but the Macquarie Ridge population contributed immigrants to the Kermadec Ridge and Challenger Plateau populations. The Kermadec Ridge was the only source of immigrants to the Hikurangi Margin population, and the Chatham Rise population (in the central region) contributed to all populations except the Hikurangi Margin and received immigrants from all populations except those in the south (Table 5.10).

5.3.8. Microsatellite variation

The null alleles and selection information of all microsatellites present in Table S5.7. Number of alleles per locus varied from 6 to 16 (average = 12.53), excepted heterozygosity (H_e) ranged from 0.665 to 0.893 per locus, and significant heterozygote deficiencies (5) slightly outnumbered significant heterozygote excesses (3) (Table S5.8). Diversity index values (N , N_a , N_e) were greater in the northern than in the southern province, whereas diversity index values were greater in the south region than in the central or north regions (Table S5.8). For the full (10 loci) data set, AMOVA revealed no significant genetic structure amongst the north-central-south regions or between the northern-southern provinces. However, for the neutral data set (4 loci) AMOVA revealed significant genetic structure amongst the north-central-south regions but not between the northern-southern provinces (Table S5.9). At the geomorphic population level, the full data set revealed significant structure amongst population, but the neutral loci data set did not reveal the structure. Examination of F_{ST} values further illustrated that the north region was isolated from the central and south regions (Tables S5.10-11). There was no isolation by distance or by depth patterns observed for microsatellite variation in *Poecillastra laminaris*.

Table 5.10 Theta and number of effective migrants per generation amongst pooled populations from 6 locations based on *COI* and *Cytb* sequences for *Poecillastra laminaris*

	To Kermadec	To Challenger	To Hikurangi	To Chatham	To Campbell	To Macquarie
From Kermadec Ridge (North)	0.0001	156.8	2360	470.37	0.00	0.00
From Challenger Plateau (North)	1010.00	0.0027	0.00	417.08	0.00	0.00
From Hikurangi Margin (North)	22.39	0.00	0.0079	89.57	0.00	0.00
From Chatham Rise (Central)	464.27	2230.00	0.00	0.0067	650.04	557.16
From Campbell Plateau (South)	0.00	0.00	0.00	0.00	0.0063	582.17
From Macquarie Ridge (South)	314.76	366.92	0.00	0.00	1420.00	0.0042

For immigration occurring between population pairs, the upper row is the receiving population and the left column is the broadcasting population. θ values are given in the diagonal. Populations were pooled: N1, N3, and N4 are Kermadec Ridge; N5 and N6 are Challenger Plateau; N7 is Hikurangi Margin; N8, N9 and N10 are Chatham Rise; S11, S13, S14 and S16 are Campbell Plateau; S12, S15, S17, S18 and S19 are Macquarie Ridge.

5.4. Discussion

This study is one of only a few to have examined genetic connectivity of deep-sea invertebrate populations in the New Zealand region, and also one of a very limited number of world-wide studies to focus on deep-sea sponge population genetics. The potential reasons for the observed patterns of population genetic structure of the four study demosponges are discussed below, as well the implications of these genetic connectivity patterns for the design of MPAs to protect VMEs in the region. First, the issues relating to sampling and marker selection are considered.

5.4.1. Sampling effect, marker selection and genetic diversity

A multi-species approach has been employed to understand the extent of genetic connectivity, to identify the location of putative barriers to gene flow within the New Zealand region, and to identify congruent and incongruent patterns of connectivity and dispersal amongst four demosponges. As noted in previous studies, one major limitation of studies of deep-sea genetic connectivity is often the limited availability of material, both numbers of individuals per population and numbers of populations (Cho & Shank, 2010; Miller *et al.*, 2010; Boschen *et al.*, 2015). In this study, because the sample sizes for all sponge populations inhabiting different sites were small, this may result in a failure to reveal the full extent of genetic diversity within such populations (Figure S5.11). For example, (1) the sample size was > 30 individuals for only *Poecillastra laminaris* and *N. persicum*, (2) the haplotype accumulation analysis showed that all four species did not have enough specimens to recover all expected haplotypic diversity, and (3) SAMOVA results indicated that a better understanding of population structure within the northern and southern provinces required more samples (Figure S5.1). In reality, an understanding of the population genetics of many deep-sea taxa has to be pursued with less than perfect sample sets because of the current management need to protect biodiversity, and particularly VMEs. Because of the recognised need to protect VMEs, studies such as that of Boschen *et al.* (2015) and the present one attempt to minimise the biases of small sample sizes by using conservative analytical approaches and qualitative interpretations of genetic structure and patterns.

Studies of the population genetic variation of sponges are uncommon, but typically report that the genetic diversity of the mitochondrial genome in demosponges is low and that rates of evolution are slow (Duran *et al.*, 2004; Lavrov *et al.*, 2005; Wörheide, 2006).

Consistent with these previous findings, *12S* in *Penares* sp. and *N. persicum* exhibited low levels of nucleotide polymorphism (usually $\pi < 0.01$), whereas the nucleotide diversity of *COI* was generally > 0.01 in all four species, which is much higher than in the above studies. In *Poecillastra laminaris*, both *COI* and *Cytb* exhibited significant genetic differentiation within populations from the New Zealand region even allowing for small sample sizes, which indicates that both markers are appropriate for population genetic research for this species. Geographically widespread haplotypes may possibly be explained by mutation rates that are too low to be sensitive to recent population change events (Smith *et al.*, 2004b). Whilst *12S* for *Penares* sp. and *N. persicum* and *COI* for *P. menoui* are not informative enough to reveal species-specific population structure, sufficient genetic variation within and amongst the four sponge species was observed to reveal discordant patterns of genetic structure of sufficient magnitude to be useful to inform management options.

5.4.2. Patterns of genetic connectivity

Several patterns of connectivity and dispersal amongst the four demosponges were revealed by mtDNA markers within and between the two lower bathyal biogeographic provinces in the New Zealand region. *Penares* sp. exhibited an apparent absence of regional genetic differentiation between the provinces, consistent with results for highly mobile deep-sea species, including fish (Bolch *et al.*, 1993; Varela *et al.*, 2012, 2013) and giant squid (Winkelmann *et al.*, 2013). Sponges are sessile and one possible explanation for panmixia is that *Penares* sp. larvae may have a long dispersal duration which would allow the species to overcome the physical barrier of distance between the two provinces (see section below). In addition, the present day distribution of lithistids probably reflects palaeoenvironmental change around the time of the Eocene, whereas astrophorina such as *Poecillastra laminaris* and other non-lithistids are not so constrained (Kelly *et al.*, 2003; Kelly, 2003).

Between-province genetic structure was observed for *Poecillastra laminaris* which is consistent with a putative barrier to gene flow between the northern and southern provinces (indicated by both *COI* and *Cytb* markers). Similar large-scale north-south genetic population structure has also been reported previously in the region for corals (Miller *et al.*, 2010), fish (Smith *et al.*, 1996a; Lévy-Hartmann *et al.*, 2011), and a sea star (Foltz *et al.*, 2013). Within the biogeographic provinces, gene flow was evident in all four sponge species, even at scales of 1000s of kilometres. Similar levels of gene flow, with little or no evidence of within-province structure in the same region, have been observed amongst populations of fish (e.g.

(Sedberry & Carlin, 1996; Hoarau & Borsa, 2000), ophiuroids (e.g., (O'Hara *et al.*, 2014) and crustaceans (Bors *et al.*, 2012). Other studies in the region have revealed evidence of genetic structure at smaller spatial scales within provinces, for example for deep-sea amphipods (Knox *et al.*, 2012) and polychaetes (Bors *et al.*, 2012). SAMOVA results (Figure S1) indicated that there was potentially genetic structure for *Poecillastra laminaris* within provinces, and barriers analysis of *COI* sequences for this species suggests a west-east barrier to population dispersal (Figure S6). However, with limited samples it is not possible to be certain that such within province population structure exists for *Poecillastra laminaris*.

5.4.3. Life history and dispersal strategies

Species-specific differences in reproductive strategies may contribute to differences in patterns of gene flow and genetic structure observed across taxa (e.g. Samadi *et al.*, 2006; Bors *et al.*, 2012). Populations of *Poecillastra laminaris* exhibited significant genetic structure amongst the geomorphic features, consistent with patterns of genetic structure exhibited by shallow-water sponges. Small-scale genetic structure was detected in *Crambe crambe* (Duran *et al.*, 2004; Calderón *et al.*, 2007), *Phyllospongia lamellose*, *Phyllospongia alcicornis*, *Carterospongia flabellifera* and *Collospongia auris* (Benzie *et al.*, 1994), *Paraleucilla magna* (Guardiola *et al.*, 2012), *Scopalina lophyropoda* (Blanquer *et al.*, 2009), *Xestospongia muta* (López-Legentil & Pawlik, 2008), and *Xestospongia testudinaria* (Bell *et al.*, 2014), and they reported that asexual reproduction (Calderón *et al.*, 2007), larval dispersal (Blanquer *et al.*, 2009; López-Legentil & Pawlik, 2008), inbreeding (Duran *et al.*, 2004; Guardiola *et al.*, 2012; Bell *et al.*, 2014), and self-recruitment (Bell *et al.*, 2014) all played important roles in structuring populations. In the present study, strong self-recruitment was observed in *Poecillastra laminaris* (Figure S5.11), further supporting the suggestion that self-recruitment is an explanation for some patterns of genetic structure detected in sponges. Deep-sea demosponges have a wide-range of reproductive strategies (Witte, 1996; Lemoine *et al.*, 2007), but no studies have determined empirically the reproductive strategies or mode of larval dispersal for the four species examined in the present study. Studies of other demosponges have reported asexual and sexual reproduction, and dispersal by larvae and by floating propagules, to the extent that these markedly different strategies corresponded directly with the different genetic connectivity patterns amongst different species (Maldonado, 2006; Mariani *et al.*, 2006; Lemoine *et al.*, 2007). This limited information

brought challenges to assessing the importance of reproductive and dispersal strategies on population structure and genetic connectivity.

Nonetheless, results of some of the analyses suggest that the study sponges are likely to have larvae capable of wide dispersal. Haplotype networks for *Penares* sp., *Pleroma menoui* and *N. persicum* revealed rapid population expansion (Slatkin & Hudson, 1991). Also, statistically significant negative Tajima's D values for all species' populations and widespread distributions of haplotypes in each of the three species also might provide further support for the recent expansion. Mismatch distributions analysis accepted the hypothesis of rapid expansion of these populations in their examined provinces, although such an interpretation must be viewed with caution given the small sample sizes. This recent expansion may explain the panmixia of populations in the examined areas.

5.4.4. Oceanographic dynamics

The northern and southern geographical genetic differentiation reported for *Poecillastra laminaris*, and the within-province pattern of genetic homogeneity reported for *Poecillastra laminaris*, *P. menoui* and *N. persicum*, may be attributable to a relationship between sponge distribution and oceanic current systems. Currents may promote the long-distance transport of larvae, but may also act as potential barriers to gene flow or agents of retention of larvae (Rogers *et al.*, 1998; Zane *et al.*, 1998; Sutton & Chereskin, 2002; Shaw *et al.*, 2004; Plouviez *et al.*, 2013). In the New Zealand region, the major currents with the greatest potential influence on population connectivity are associated with the Tasman Front, the Subtropical Front and the Subantarctic Front (Figure 5.3). The different currents and frontal systems give rise to different environments in the north and south of the region, and may impede larval dispersal and reduce or perhaps even block gene flow across the Chatham Rise. The haplotypic distribution of *Poecillastra laminaris* (which was sampled across the entire study region) supports the contention that gene flow into and through the study region could be affected by ocean current dynamics, and indicates that two main population connection routes may exist: (1) A northern route from the northwest of North Island with the southerly flow of the East Auckland Current down the east coast of North Island with the East Cape Current, and then eastwards immediately north of the Chatham Rise and the Subtropical Front, and (2) a southern route from south of South Island northwards with the Southland Current, then eastwards immediately south of Chatham Rise and the Subtropical Front. The unique *Poecillastra laminaris* haplotypes for *COI* and *Cytb* found at the most

southerly sampling location in the region (Site S19) may indicate that populations in this area are isolated by the Antarctic Circumpolar Current in the Subantarctic Front. Further evidence is needed to support this hypothesis for *Poecillastra laminaris*, but a recent study of octocorals indicates that this current is capable of separating and structuring octocoral populations, and it acts as a ‘soft’ barrier (Dueñas *et al.*, 2016). This hypothesis is also supported by studies of the present day versus palaeoenvironmental distribution of some Recent and fossil lithistid sponges (Kelly *et al.*, 2003; Kelly, 2003; Kelly and Buckeridge, 2005).

Amongst deep-sea species found off New Zealand, genetic differentiation between the Challenger Plateau and the Chatham Rise has been found for orange roughy (Smith *et al.*, 1996a), amphipods (Knox *et al.*, 2012) and quill worms (Bors *et al.*, 2012), some of which was attributed to ocean current dynamics. In this study, differentiation was not detected between populations at these two locations, although the mitochondrial marker used may be insensitive to recent population events in sponges (Becerro *et al.*, 2012). More powerful markers, such as microsatellites and SNPs, might be able to clarify the possible existence of genetic barriers within/between provinces.

5.4.5. Depth and associated environmental gradients

Depth is an important factor contributing to and explaining patterns of genetic connectivity for benthic fauna, particularly for taxa that are distributed across a wide depth range (McMullin *et al.*, 2003; Brandão *et al.*, 2010a; Miller *et al.*, 2010; Schüller 2011; O’Hara *et al.*, 2014). In the present study, there was no evidence for isolation by depth. However, the small samples sizes will have reduced the power to detect such a relationship. In addition, the deepest specimen was collected from ~1500 m, whilst depth-related distinctions in population structure reported in most previous studies were deeper: 3000 m for the amphipod *Eurythenes gryllus* (Havermans *et al.*, 2013), 2500 m for the bivalve *Deminucula atacellana* (Chase *et al.*, 1998), and 1700 m for ophiuroids (O’Hara *et al.*, 2014). However, Miller *et al.* (2011) reported that the stony coral *Desmophyllum dianthus* from different depth strata (<600 m, 1000–1500 m, >1500 m) were strongly differentiated, indicating limited vertical larval dispersal. These authors suggested that this genetic differentiation with depth was consistent with the stratification of different water masses in the region. Further sampling across a greater depth range within the New Zealand region is

required to test the existence of a relationship between depth and genetic differentiation for the four sponge species examined in the present study.

Figure 5.3 Haplotype map for *Poecillastra laminaris* COI (above line) and *Cytb* (below line) overlain with a summary of the major currents in the New Zealand region

Pie charts indicate haplotypic composition of each location and numbers indicate the total number of sequences from each location. Note EAUC, East Auckland Currents; NCE, North Cape Eddy; ECE, East Cape Eddy; WE, Wairarapa Eddy. (Leathwick *et al.*, 2008, Bors *et al.*, 2012).

5.4.6. Conservation and management implications

In order to protect the seafloor fauna from the effects of bottom trawling, 17 seamount closures and 17 Benthic Protection Areas (BPAs) were established throughout the New

Zealand EEZ in 2001 and 2007, respectively (Brodie & Clark, 2002; Helson *et al.*, 2010) (Figure 5.4). These areas protect the benthos and water column fauna up to a height of 50 m off the substratum, from fishing-related activities. The total area protected amounts to ~1.2 million km² of seabed that is ~ 30% of New Zealand's EEZ. Amongst the criteria for the selection of these protected areas was that they include fauna vulnerable to disturbance from bottom trawling (i.e. VMEs) and are representative of the benthic fauna and habitats found throughout the EEZ. However, genetic connectivity of the fauna within and between these areas was not initially considered. Since their instigation, only two studies have examined the efficacy of the BPA design, and both have suggested some of the current BPAs could be re-located to provide more effective protection of biodiversity (Leathwick *et al.*, 2008; Bors *et al.*, 2012).

Understanding connectivity amongst areas is fundamental to designing an effective MPA network, or for modifying the existing distribution of MPAs to create a network (Palumbi, 2003). To maintain genetic diversity, protection of genetically distinct populations should also be considered as an integral component of MPA area design (Bors *et al.*, 2012). The results of the present study indicate that populations of *Penares* sp., *Pleroma menoui* and *N. persicum* are likely to be well connected amongst the presently protected areas within their distribution. Data for *Poecillastra laminaris*, the most widely sampled species, provides more useful information for assessing the effectiveness of current protection measures and future MPA design in the EEZ as a proxy for VME indicator species with similar patterns of genetic connectivity (Figure 5.4). Populations associated with the Kermadec Ridge, Challenger Plateau Chatham Rise and the Macquarie Ridge may be the main genetic sources for populations around New Zealand. The Kermadec BPA provides protection for the northern source of genetic population connectivity, and this large BPA has recently been proposed as a full ocean sanctuary (<http://www.mfe.govt.nz/marine/kermadec-ocean-sanctuary/question-answers-kermadec-ocean-sanctuary>). The Challenger Plateau is partly covered by a large BPA that will protect, at least in part, the genetic source populations on that part of the Plateau. However, there are no BPAs on the Macquarie Ridge, although two seamount closures to the west and east of the ridge, and a BPA to the south, may provide some protection for the southern source of genetic connectivity. There are protected areas in relatively close proximity to the remainder of the study sites that represent the range of genetic variation observed for *Poecillastra laminaris* across the region. However, if these areas protect this diversity will depend on whether they provide suitable habitat for VME indicator taxa such as sponges.

Figure 5.4 Haplotype map for *Poecillastra laminaris* COI (above line) and *Cytb* (below line) overlain with protected areas within the New Zealand EEZ

Pie charts indicate haplotypic composition of each location and numbers indicate the total number of sequences from each location.

The findings of the present study indicate that new BPAs on the Chatham Rise should be considered for sponges. The current two BPAs on the Rise cover a very limited depth range *along* the Rise, and this may not be sufficient to protect the genetic variation *across* the

Rise. Whilst there are no population genetic data for *Poecillastra laminaris* from within these two BPAs, the haplotypic distributions showed that the populations sampled on the Chatham Rise had the highest genetic diversity. This result suggests that further protected areas, or an enlarging of the current BPAs, could be considered to afford greater protection to the benthic biodiversity associated with the Chatham Rise. This was also a finding of Bors *et al.* (2012) for other benthic taxa studied on the Chatham Rise. The results of the present study, and those of previous genetic connectivity studies around New Zealand (Smith *et al.*, 2004b; Arango *et al.*, 2011; Bors *et al.*, 2012; Knox *et al.*, 2012; Foltz *et al.*, 2013; Boschen *et al.*, 2015; Dueñas *et al.*, 2016), are now beginning to provide information that can be used to improve the design (location, size and spacing) of the BPAs and any other similar protection measures in the EEZ, and demonstrate the need for a flexible spatial management system that can be periodically adjusted to accommodate increased understanding about the connectivity of a range of benthic taxa.

Appendix

Table S5.1 Details of collections of four demosponges and haplotypes codes

NIWA Accession	Date	Latitude	Longitude	Depth (m)	Taxon	Group	COI	Cytb/12S
18223	6/12/1989	-37.135	177.297	1050	<i>Neaulaxinia persicum</i>	5	Hap_3	
18224	-	-31.98	174.265	1178.5	<i>Neaulaxinia persicum</i>	3	Hap_1	
18226	14/04/2002	-34.118	174.15	735	<i>Neaulaxinia persicum</i>	5	Hap_1	Hap_1
18227	17/04/2002	-34.163	173.962	786	<i>Neaulaxinia persicum</i>	4	Hap_1	Hap_1
18228	17/04/2002	-34.163	173.962	786	<i>Neaulaxinia persicum</i>	4	Hap_1	
18229	17/04/2002	-34.163	173.962	786	<i>Neaulaxinia persicum</i>	4	Hap_1	
18230	17/04/2002	-34.163	173.962	786	<i>Neaulaxinia persicum</i>	4	Hap_5	Hap_1
18231	17/04/2002	-34.162	173.962	795	<i>Neaulaxinia persicum</i>	4	Hap_1	
18232	17/04/2002	-34.162	173.962	795	<i>Neaulaxinia persicum</i>	4	Hap_1	
18233	17/04/2002	-34.162	173.962	795	<i>Neaulaxinia persicum</i>	4	Hap_1	Hap_1
18234	18/04/2002	-34.159	173.963	790	<i>Neaulaxinia persicum</i>	4	Hap_1	Hap_1
18235	18/04/2002	-34.164	173.964	812.5	<i>Neaulaxinia persicum</i>	4	Hap_1	
18236	18/04/2002	-34.164	173.964	812.5	<i>Neaulaxinia persicum</i>	4	Hap_2	
18238	19/04/2002	-34.042	174.817	836	<i>Neaulaxinia persicum</i>	4	Hap_1	
18239	16/04/2001	-42.786	180.015	946.5	<i>Neaulaxinia persicum</i>	9	Hap_4	Hap_1
18241	15/04/2001	-42.767	180.011	816	<i>Neaulaxinia persicum</i>	9	Hap_5	
18243	18/04/2001	-42.785	180.002	961	<i>Neaulaxinia persicum</i>	9	Hap_1	
18246	21/04/2001	-42.761	180.007	845.5	<i>Neaulaxinia persicum</i>	9	Hap_6	Hap_1
18247	15/04/2001	-42.767	180.011	816	<i>Neaulaxinia persicum</i>	9	Hap_1	
18248	15/04/2001	-42.767	180.011	816	<i>Neaulaxinia persicum</i>	9	Hap_1	
24705	21/04/2001	-42.761	180.007	845.5	<i>Neaulaxinia persicum</i>	9	Hap_1	
25136	29/05/2006	-42.755	180.006	945	<i>Neaulaxinia persicum</i>	9	Hap_6	
25613	25/03/1967	-37.392	177.2	602	<i>Neaulaxinia persicum</i>	5	Hap_1	Hap_1
25647	-	-37.484	167.685	904	<i>Neaulaxinia persicum</i>	6	Hap_6	
25650	22/03/1982	-28.55	182.167	800	<i>Neaulaxinia persicum</i>	2	Hap_1	Hap_2
27033	6/11/2006	-39.544	178.332	817	<i>Neaulaxinia persicum</i>	7	Hap_1	Hap_1
27035	10/11/2006	-41.295	176.559	740.5	<i>Neaulaxinia persicum</i>	8	Hap_7	
82328	18/04/2012	-36.452	177.846	1143	<i>Neaulaxinia persicum</i>	5	Hap_1	
82712	22/04/2012	-36.822	177.46	903.5	<i>Neaulaxinia persicum</i>	5	Hap_1	Hap_1
82859	24/04/2012	-36.45	177.84	856	<i>Neaulaxinia persicum</i>	5	Hap_1	
32057	6/03/2007	-40.03	178.19	659	<i>Penares</i> sp.	5	Hap_1	Hap_1
40143	9/04/2008	-52.486	160.412	455	<i>Penares</i> sp.	12	Hap_3	Hap_1
50584	19/12/2007	-43.85	185.643	802	<i>Penares</i> sp.	7	Hap_1	Hap_1
52637	5/04/2008	-51.05	162.022	443.5	<i>Penares</i> sp.	11	Hap_1	Hap_1
53591	26/06/2009	-44.148	185.308	520	<i>Penares</i> sp.	7	Hap_1	Hap_1
53776	26/06/2009	-44.158	185.443	585	<i>Penares</i> sp.	7	Hap_1	Hap_1
54032	27/06/2009	-44.145	185.275	790.5	<i>Penares</i> sp.	7	Hap_2	Hap_1
54053	27/06/2009	-44.15	185.241	564	<i>Penares</i> sp.	7	Hap_1	Hap_1
54239	28/06/2009	-44.16	185.446	551.5	<i>Penares</i> sp.	7	Hap_1	
64513	2/06/2010	-35.355	178.524	1187.5	<i>Penares</i> sp.	1	Hap_4	Hap_2
64903	7/06/2010	-35.355	178.538	1305	<i>Penares</i> sp.	1	Hap_1	Hap_1
65028	8/06/2010	-35.285	178.863	1260	<i>Penares</i> sp.	1	Hap_5	Hap_1
66051	24/11/2009	-44.468	181.416	710	<i>Penares</i> sp.	9	Hap_1	Hap_1

66087	16/07/2009	-37.333	168	935.5	<i>Penares</i> sp.	4	Hap_1	Hap_1
72415	8/03/2011	-35.355	178.531	1315.5	<i>Penares</i> sp.	1	Hap_1	
72476	9/03/2011	-35.351	178.54	1232.5	<i>Penares</i> sp.	1	Hap_1	Hap_1
18347	18/06/1981	-34.66	172.218	270	<i>Pleroma menoui</i>	7	Hap_2	
18348	28/11/1989	-36.162	176.806	945	<i>Pleroma menoui</i>	8	Hap_4	
18354	17/04/2002	-34.146	173.963	812.5	<i>Pleroma menoui</i>	6	Hap_2	
18355	14/04/2002	-34.109	174.134	581	<i>Pleroma menoui</i>	6	Hap_2	
18522	11/08/1999	-24.88	168.363	529	<i>Pleroma menoui</i>	1	Hap_2	
18523	11/08/1999	-24.88	168.363	529	<i>Pleroma menoui</i>	1	Hap_2	
18524	11/08/1999	-24.778	168.139	268.5	<i>Pleroma menoui</i>	1	Hap_3	
25054	12/11/2004	-37.472	177.224	211.5	<i>Pleroma menoui</i>	9	Hap_2	
25606	18/06/1981	-34.66	172.218	270	<i>Pleroma menoui</i>	7	Hap_5	
31160	15/11/2004	-37.474	176.91	252	<i>Pleroma menoui</i>	9	Hap_2	
44566	23/07/1975	-29.413	168.22	570	<i>Pleroma menoui</i>	2	Hap_1	
62490	4/03/2011	-36.448	177.839	838	<i>Pleroma menoui</i>	8	Hap_2	
73762	4/04/2011	-38.154	173.789	294	<i>Pleroma menoui</i>	10	Hap_2	
86173	18/10/2012	-30.178	179.74	404.5	<i>Pleroma menoui</i>	4	Hap_2	
86174	18/10/2012	-30.178	179.74	404.5	<i>Pleroma menoui</i>	4	Hap_2	
86180	18/10/2012	-30.178	179.74	404.5	<i>Pleroma menoui</i>	4	Hap_2	
24692	15/04/2001	-42.767	180.011	816	<i>Poecillastra laminaris</i>	8	Hap_5	Hap_7
24694	16/04/2001	-42.786	180.015	946.5	<i>Poecillastra laminaris</i>	8	Hap_4	Hap_5
24701	20/04/2001	-42.768	180.078	922.5	<i>Poecillastra laminaris</i>	8	Hap_4	Hap_5
24702	21/04/2001	-42.729	180.11	1030	<i>Poecillastra laminaris</i>	8	Hap_4	Hap_5
25102	28/05/2006	-42.758	180.011	805	<i>Poecillastra laminaris</i>	8	Hap_6	Hap_8
25103	28/05/2006	-42.758	180.011	805	<i>Poecillastra laminaris</i>	8	Hap_6	Hap_9
25105	28/05/2006	-42.758	180.011	805	<i>Poecillastra laminaris</i>	8	Hap_7	Hap_5
25106	28/05/2006	-42.758	180.011	805	<i>Poecillastra laminaris</i>	8	Hap_6	Hap_8
25193	4/06/2006	-42.787	180.018	998	<i>Poecillastra laminaris</i>	8	Hap_8	
25653	4/06/2006	-42.716	180.036	1017.5	<i>Poecillastra laminaris</i>	8	Hap_4	Hap_5
26995	4/11/2006	-40.04	178.143	738.5	<i>Poecillastra laminaris</i>	7	Hap_4	Hap_6
28596	29/05/2007	-38.025	168.442	572.5	<i>Poecillastra laminaris</i>	5	Hap_4	Hap_5
31184	16/11/2004	-37.334	177.08	466	<i>Poecillastra laminaris</i>	4	Hap_1	Hap_1
31192	14/11/2004	-37.355	177.102	270	<i>Poecillastra laminaris</i>	4	Hap_3	Hap_1
31201	16/11/2004	-37.352	177.099	353	<i>Poecillastra laminaris</i>	4	Hap_1	
39835	1/04/2008	-50.098	163.471	1096.5	<i>Poecillastra laminaris</i>	15	Hap_14	Hap_13
40107	9/04/2008	-52.486	160.412	455	<i>Poecillastra laminaris</i>	17	Hap_15	Hap_9
40169	9/04/2008	-52.498	160.491	122	<i>Poecillastra laminaris</i>	17	Hap_15	Hap_9
40308	9/04/2008	-52.398	160.663	444.5	<i>Poecillastra laminaris</i>	17	Hap_1	Hap_16
40406	9/04/2008	-52.392	160.685	515	<i>Poecillastra laminaris</i>	17	Hap_14	Hap_13
40734	15/04/2008	-55.381	158.43	570.5	<i>Poecillastra laminaris</i>	18	Hap_14	Hap_17
40794	15/04/2008	-55.362	158.432	565.5	<i>Poecillastra laminaris</i>	18	Hap_14	Hap_13
40794	15/04/2008	-55.362	158.432	565.5	<i>Poecillastra laminaris</i>	18	Hap_14	Hap_13
40872	15/04/2008	-55.369	158.385	539	<i>Poecillastra laminaris</i>	18	Hap_14	Hap_13
48103	2/12/2008	-48.972	171.679	526.5	<i>Poecillastra laminaris</i>	13	Hap_15	Hap_9
51814	19/04/2002	-34.053	174.808	865	<i>Poecillastra laminaris</i>	2	Hap_1	
51910	21/04/2001	-42.721	180.039	951	<i>Poecillastra laminaris</i>	8	Hap_9	
51914	21/04/2001	-42.721	180.039	951	<i>Poecillastra laminaris</i>	8	Hap_10	Hap_9
52473	3/06/2007	-38.204	168.584	519	<i>Poecillastra laminaris</i>	5	Hap_4	Hap_5
52500	16/11/2004	-37.352	177.099	353	<i>Poecillastra laminaris</i>	4	Hap_3	Hap_1

52632	5/04/2008	-51.05	162.022	443.5	<i>Poecillastra laminaris</i>	15	Hap_17	Hap_15
52634	30/03/2008	-48.535	164.948	1086	<i>Poecillastra laminaris</i>	12	Hap_5	Hap_12
52639	19/04/2008	-59.049	158.908	1507.5	<i>Poecillastra laminaris</i>	19	Hap_18	Hap_18
52966	4/11/2006	-40.04	178.147	765	<i>Poecillastra laminaris</i>	7	Hap_1	Hap_1
53408	22/06/2009	-42.835	180.013	822.5	<i>Poecillastra laminaris</i>	8	Hap_11	Hap_5
53777	26/06/2009	-44.158	185.443	585	<i>Poecillastra laminaris</i>	9	Hap_12	Hap_10
53778	26/06/2009	-44.158	185.443	585	<i>Poecillastra laminaris</i>	9	Hap_13	Hap_5
53985	27/06/2009	-44.149	185.307	553	<i>Poecillastra laminaris</i>	9	Hap_4	Hap_5
54352	28/06/2009	-44.03	185.409	812	<i>Poecillastra laminaris</i>	9	Hap_4	Hap_5
58816	11/10/2002	-48.403	174.341	896	<i>Poecillastra laminaris</i>	11	Hap_15	Hap_9
62135	27/06/2009	-44.15	185.241	564	<i>Poecillastra laminaris</i>	9	Hap_14	Hap_11
65027	8/06/2010	-35.284	178.864	1265	<i>Poecillastra laminaris</i>	3		Hap_1
70623	30/11/1998	-48.618	164.885	940	<i>Poecillastra laminaris</i>	12	Hap_14	Hap_13
70624	30/11/1998	-48.618	164.885	940	<i>Poecillastra laminaris</i>	12	Hap_14	Hap_13
70625	17/03/1978	-44.16	180.763	320	<i>Poecillastra laminaris</i>	10	Hap_1	Hap_1
73767	4/04/2011	-38.154	173.789	294	<i>Poecillastra laminaris</i>	6	Hap_1	Hap_1
76254	14/11/2004	-37.355	177.101	276.5	<i>Poecillastra laminaris</i>	4	Hap_1	Hap_1
76474	5/09/2006	-51.68	169.914	370	<i>Poecillastra laminaris</i>	16	Hap_15	Hap_9
76823	22/04/2003	-49.612	178.776	318.5	<i>Poecillastra laminaris</i>	14	Hap_15	Hap_9
81658	18/10/2012	-30.187	179.72	410	<i>Poecillastra laminaris</i>	1	Hap_1	Hap_2
86091	18/10/2012	-30.187	179.72	410	<i>Poecillastra laminaris</i>	1	Hap_1	Hap_3
86172	18/10/2012	-30.178	179.74	404.5	<i>Poecillastra laminaris</i>	1	Hap_2	
86178	18/10/2012	-30.177	179.737	404	<i>Poecillastra laminaris</i>	1		Hap_4
86182	18/10/2012	-30.178	179.74	404.5	<i>Poecillastra laminaris</i>	1	Hap_1	Hap_1
86710	13/11/2008	-49.308	176.328	1040	<i>Poecillastra laminaris</i>	14	Hap_16	Hap_14

Table S5.2 *Poecillastra laminaris*
Pairwise Φ_{ST} values for *COI* (below diagonal) and *Cytb* (above diagonal) amongst populations

Population	Kermadec	Three Kings	Challenger	NW Slope	Hikurangi	Chatham	Campbell	Bounty	Macquarie
Kermadec		-	0.891**	-1	0.41	0.371**	0.937**	0.700*	0.400**
Three Kings	-0.875		-	-	-	-	-	-	-
Challenger	0.780*	1		1	0.5	-0.058	1.000*	0.421	0.432**
NW Slope	-0.875	0	1		-1	-0.063	1	-0.467	-0.039
Hikurangi	0.264	-1	0	-1		-0.012	0.848	0.063	0.154
Chatham	0.329**	-0.101	-0.104	-0.101	-0.072		0.474**	0.144	0.226**
Campbell	0.95**	1	1	1	0.919	0.469		0.250	0.434*
Bounty	0.652*	-0.727	0.136	-0.727	0	0.148*	0.25		0.058
Macquarie	0.650**	0.444	0.502*	0.444	0.462*	0.210**	0.467**	0.238	

Significant values of $p < 0.05$ are marked as *, and $p < 0.01$ are marked as **. - means data is unavailable.

Table S5.3 *Penares* sp.
Pairwise Φ_{ST} values for *COI* (below diagonal) and *12S* (above diagonal) amongst populations

Population	Macquarie	Kermadec	Challenger	Hikurangi	Chatham
Macquarie	0	-0.263	0	0	0
Kermadec	0.029		-1.000	-1.000	0.111
Challenger	-1.000	-0.571		0	0
Hikurangi	-1.000	-0.571	0		0
Chatham	0.281	0.224	-1.000	-1.000	

Significant values of $p < 0.05$ are marked as *, and $p < 0.01$ are marked as **.

Table S5.4 *Neoaulaxinia persicum*
Pairwise Φ_{ST} values for *COI* (below diagonal) and *12S* (above diagonal) amongst populations

Population	Kermadec	Three Kings	Chatham	Challenger	Hikurangi
Kermadec	0	0	-0.263	-	-1
Three Kings	-0.016	0		-	0
Chatham	0.16	0.01	0	-	0
Challenger	0.867	0.76	0.143	0	-
Hikurangi	0.273	0.052	-0.262	0.333	0

Significant values of $p < 0.05$ are marked as *, and $p < 0.01$ are marked as **.

Table S5.5 *Pleroma menoui*
Pairwise Φ_{ST} values for *COI* amongst populations

Population	Norfolk	Kermadec	Three Kings	NW Slope
Norfolk	0			
Kermadec	0.095	0		
Three Kings	-0.263	-0.313	0	
NW Slope	0.029	0.225	-0.2	0

Significant values of $p < 0.05$ are marked as *, and $p < 0.01$ are marked as **.

Table S5.6 Tajima's (D) and the Ramos-Onsins-Rozas Test of Neutrality (R2) test results for different markers across four different species

Species	Marker	D	R2
<i>Poecillastra laminaris</i>	<i>COI</i>	-0.3025 (0.7623)	0.0958 (0.4354)
<i>Poecillastra laminaris</i>	<i>Cytb</i>	-0.3757 (0.7071)	0.0940 (0.4180)
<i>Penares</i> sp.	<i>COI</i>	-0.7804 (0.4352)	0.1117 (0.2054)
<i>Penares</i> sp.	<i>12S</i>	-1.1552 (0.2480)	0.2575 (0.9838)
<i>Neoaulaxinia persicum</i>	<i>COI</i>	-0.9194 (0.3579)	0.0862 (0.1822)
<i>Neoaulaxinia persicum</i>	<i>12S</i>	-3.0332 (0.0242)	0.2890 (1.0000)
<i>Pleroma menoui</i>	<i>COI</i>	-2.1098 (0.0349)	0.1326 (0.4366)

Note: Data expressed as value (p value)

Table S5.7 *Poecillastra laminaris*
Tests for detection of null alleles and selection for all ten microsatellite loci across all samples

Locus	NullAlleles?	Oosterhout	Selection?
PLN4	yes	0.0865	No
PLN7	yes	0.1826	Positive
PLN8	no	0.0341	No
PLN17	no	-0.059	Balancing
PLN21	yes	0.2173	Positive
PLN26	no	-0.0791	No
PLN27	no	0.0795	No
PLN34	no	0.0385	No
PLN39	yes	0.0623	No
PLN48	yes	0.3459	No

Oosterhout – estimate of frequency of null alleles (from MicroChecker software)

Table S5.8 Microsatellite diversity statistics for *Poecillastra laminaris* at two different spatial scales

Group	Loci	ong26	ong34	ong27	ong17	ong8	Mean	SE
Northern province	N	19	39	20	31	14	24.600	4.545
	Na	11.000	14.000	6.000	16.000	13.000	12.000	1.703
	Ne	5.270	5.783	3.433	8.430	9.333	6.450	1.076
	Ho	0.947	0.769	0.600	0.903	1.000	0.844	0.072
	He	0.810	0.827	0.709	0.881	0.893	0.824	0.033
	HWE	***	*	*	***	ns		
North region	N	10	18	12	16	8	12.800	1.855
	Na	6.000	9.000	6.000	12.000	9.000	8.400	1.122
	Ne	2.985	3.600	3.600	8.393	7.529	5.222	1.132
	Ho	0.900	0.778	0.667	0.875	1.000	0.844	0.057
	He	0.665	0.722	0.722	0.881	0.867	0.771	0.043
	HWE	*	ns	ns	*	ns		
Central region	N	9	21	8	15	6	11.800	2.746
	Na	7.000	12.000	4.000	11.000	9.000	8.600	1.435
	Ne	5.400	6.255	3.122	6.250	8.000	5.805	0.793
	Ho	1.000	0.762	0.500	0.933	1.000	0.839	0.095
	He	0.815	0.840	0.680	0.840	0.875	0.810	0.034
	HWE	ns	*	ns	ns	ns		
Southern province/ South region	N	12	23	8	16	6	13.000	3.033
	Na	10.000	15.000	8.000	7.000	8.000	9.600	1.435
	Ne	7.024	7.399	6.095	4.655	7.200	6.475	0.507
	Ho	1.000	0.826	0.875	0.938	0.500	0.828	0.087
	He	0.858	0.865	0.836	0.785	0.861	0.841	0.015
	HWE	ns	*	ns	ns	ns		

N=number of individuals successfully PCR amplified, Na = No. of Different Alleles, Ne = No. of Effective Alleles, Ho = Observed Heterozygosity, HWE=significances of deviation from Hardy–Weinberg equilibrium. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, $p > 0.05$.

Table S5.9 Hierarchical AMOVA results for *Poecillastra laminaris* when tested for neutral microsatellite variation (n=4 loci)

Source of variation	All				Neutral			
	df	ss	Var. comp.	%V	df	ss	Var. comp.	%V
Between provinces	1	4.66	0.000	0	1	1.981	0.002	0
Amongst individuals	61	314.586	1.618	46**	61	112.542	0.466	34**
Within individuals	63	121.000	1.921	54**	63	57.500	0.913	66**
Total	125	440.246	3.539		125	172.024	1.381	
Amongst regions	2	12.312	0.029	1	2	5.503	0.022	2*
Amongst individuals	60	306.634	1.595	45**	60	109.021	0.452	33**
Within individuals	63	121.000	1.921	54**	63	57.500	0.913	66**
Total	125	440.246	3.544		125	172.024	1.387	
Amongst geomorphic populations	4	26.333	0.083	2**	4	8.032	0.012	1
Amongst individuals	51	249.836	1.521	44**	51	89.656	0.446	34**
Within individuals	56	104.000	1.857	54	56	48.500	0.866	65**
Total	111	380.170	3.461		111	146.188	1.324	

*, ** indicates significant level at $p < 0.05$ and $p < 0.01$, respectively.

**Table S5.10 *Poecillastra laminaris*
Pairwise F_{ST} values for neutral microsatellites amongst north, central and south regions**

	North	Central	South
North			
Central	0.029**		
South	0.023**	0.002	

** indicates significant level at $p < 0.001$.

**Table S5.11 *Poecillastra laminaris*
Pairwise F_{ST} values for all microsatellites amongst geomorphic populations**

	Campbell Plateau	Chatham Rise	NE Slope	Kermadec Ridge	Macquarie Ridge
Campbell Plateau					
Chatham Rise	-0.024				
NE Slope	-0.005	0.003			
Kermadec Ridge	0.143*	0.096**	0		
Macquarie Ridge	-0.008	-0.008	-0.022	0.06*	

*, ** indicates significant level at $p < 0.05$ and $p < 0.01$, respectively.

Figure S5.1 Potential number of populations of *Poecillastra laminaris* based on *COI* and *Cytb* variation

Figure S5.2 NJ phylogenetic tree of *COI* (left) and *CytB* (right) for *Poecillastra laminaris*

Figure S5.3 NJ phylogenetic tree of *COI* (left) and *12S* (right) for *Penares* sp.

Figure S5.4 NJ phylogenetic tree of *COI* (left) and *12S* (right) for *Neoaulaxinia persicum*

Figure S5.5 NJ phylogenetic tree of *COI* for *Pleroma menoui*

Figure S5.6 Mismatch distribution analysis of *COI* variation for *Poecillastra laminaris* (A), *Penares sp.* (B), *Neoaulaxinia persicum* (C), and *Pleroma menoui* (D)

Figure S5.7 Mismatch distribution analysis of *Cytb* for *Poecillastra laminaris*

Figure S5.8 Mismatch distribution analysis of *12S* for *Penares* sp. (left) and *Neoaulaxinia persicum* (right)

Figure S5.9 Predicted genetic barriers (red lines) for *Poecillastra laminaris* populations based on *COI* (A) and *Cytb* (B) from Barriers software

Figure S5.10 Sampling effect test for all species on different markers. A. *COI* of *Poecillastra laminaris*, B. *Cytb* of *Poecillastra laminaris*, C. *COI* of *Penares* sp., D. *12S* of *Penares* sp., E. *COI* of *Neoaulaxinia persicum*, F. *12S* of *Neoaulaxinia persicum*, G. *COI* of *Pleroma menoui*

Figure S5.11 Circular plot of migration (gene flow) between and within populations of *Poecillastra laminaris* on geomorphic features based *COI* and *Cytb* sequences

**Chapter 6. Population structure and genetic connectivity
of stony corals (Scleractinia) in the New Zealand region
based on analysis of microsatellite and sequence data:
implications for conservation and management**

6.1. Introduction

Knowledge of the deep sea is still very limited because of the logistical limitations and costs of sampling such a large and inaccessible ecosystem (Ramirez-Llodra *et al.*, 2011). Whilst deep-sea communities and their ecological structures and functions are still being described, they have already faced considerable threats from anthropogenic activities such as fishing, mining, dumping, pollution and climate change (Glover & Smith, 2003; Roberts *et al.*, 2006; Turley *et al.*, 2007; Ramirez-Llodra *et al.*, 2011; Penney, 2014). Because it is impossible to protect everything in the deep sea (defined here as areas below 200 m depth), Vulnerable Marine Ecosystems (VMEs) have been selected as a protection priority under United Nations General Assembly Resolutions (61/105, and 59/25). VMEs are habitats and ecosystems characterised by uniqueness or rarity of their species, their significant ecological function, that are easily disturbed by anthropogenic activities and that may exhibit slow or even no recovery from disturbance (FAO, 2009). Based on the FAO characteristics, Parker *et al.* (2009) identified sponges, anemones, soft corals, sea fans, sea pens, stony corals, black corals, hydro corals, sea lilies and armless seastars as VME indicator taxa for the South Pacific Ocean. The presence of these taxa can be used to identify VMEs that may be vulnerable to impacts from fishing activities in the South Pacific Ocean, and guide management measures designed to protect them (Penny *et al.*, 2009).

If VMEs are to be protected adequately by spatial closures then further research is required to improve knowledge of the distribution of VME indicator taxa, to better understand how such VMEs are connected in terms of sources and sinks of larval recruits, and to quantify the spatial scale(s) at which genetic populations are structured.

Habitat-forming stony corals are considered to be VME indicator taxa (e.g., Parker *et al.*, 2009), and population genetics studies of these corals have reported that structure patterns vary by different species, spatial scale and different markers. Genetic structure in *Lophelia pertusa*, which is a common and well-studied reef-forming coral in the Northern Hemisphere, was detected at fine-spatial scales using microsatellites (Le Goff-Vitry *et al.*, 2004; Morrison *et al.*, 2008, 2011; Dahl *et al.*, 2012). Populations of *Desmophyllum dianthus*, which is a cosmopolite cup coral, have been detected to be isolated by depth using different markers (Miller *et al.*, 2010, 2011, 2016; Addamo *et al.*, 2012). Recently, Miller *et al.* (unpublished) found a fine-scale genetic structure in *Solenosmilia variabilis* using microsatellites. Studies of other coral species, *Enallopsammia rostrata* (Miller *et al.*, 2010), *Madrepora oculata* (Miller *et al.*, 2010), *Pocillopora damicornis* (Flot *et al.*, 2008), *Pocillopora ligulata* (Flot *et al.*,

2008), *Pocillopora molokensis* (Flot *et al.*, 2008), *Solenosmilia variabilis* (Miller *et al.*, 2010), and *Stephanocyathus spiniger* (Miller *et al.*, 2010), have not detected any genetic structure, possibly due to limited samples and invariant genetic markers. Interestingly, the use of mitochondrial markers did not reveal any genetic structure in reef-forming corals such as *E. rostrata* (Miller *et al.*, 2010), *L. pertusa* (Flot *et al.*, 2013), *M. oculata* (Miller *et al.*, 2010) and *S. variabilis* (Miller *et al.*, 2010), and this might indicate that to detect genetic structures in these corals requires more sensitive markers.

In the New Zealand EEZ, the distribution of habitat-forming corals has been evaluated, and three of these species have an affinity for seamount habitat; *S. variabilis*, *M. oculata*, and *Goniocorella dumosa* (Tracey *et al.*, 2011). Seamounts are the focus of several deep-sea fisheries in New Zealand waters (Clark & O'Driscoll, 2003), and both *G. dumosa* and *S. variabilis* has been recorded in large quantities as bycatch from seamount fisheries (Probert *et al.*, 1997, Anderson & Clark, 2003). As well as an association with seamounts *G. dumosa* is also associated with phosphorite nodules on soft sediments on the Chatham Rise (Kudrass & Rad, 1984), which suggests that *G. dumosa* may be impacted by any future mining of this resource (Dawson, 1984). Unfortunately, knowledge of the mode of larval development of these three corals is still poorly understood, and information about patterns of larval movement and gene flow is sparse.

These data are, however, important for management decisions related to the conservation of corals because if extensive gene exchange exists between populations then the loss of areas of reef may not be damaging to the overall genetic diversity of the species. In this chapter, efforts to elucidate the species-specific patterns of genetic connectivity amongst populations are described. Newly developed microsatellite markers were genotyped and a nuclear gene (internally transcribed spacer, *ITS2*) and a mitochondrial region (the control region, *D-loop*) were sequenced for the three VME indicator species, *G. dumosa*, *M. oculata* and *S. variabilis*. A province, region, and geomorphic feature hierarchical framework was employed to test the hypotheses that: (1) the water mass characteristics of two biogeographic provinces (bottom water temperature, salinity, oxygen, and particulate organic matter flux) will influence the north-south population distributions of the three species across the New Zealand region, which in turn will be reflected in a province-scale pattern of genetic structure; (2) current flows from north and south off New Zealand that meet along the Chatham Rise to the east and that form the Sub-tropical Front will act as conduits and barriers (respectively) to larval dispersal, which will be reflected in a north-central-south regional-scale pattern of genetic structure, and higher genetic diversity in the central putative mixing

region on the Chatham Rise; and (3) hydrodynamic conditions (e.g., current flows, eddies, turbulent mixing) associated with particular topographic features (such as slopes, seamounts, plateau, rises, ridges, troughs, basins) influence the dispersal of larvae, potentially restricting dispersal amongst these features, and thereby generating genetic structure between pairs of populations of corals found on these features. Tests were also conducted to examine the potential influence of geographic distance and depth on genetic population structure. *Post hoc* analyses were conducted on data to examine patterns of genetic population structure and potential barriers to gene flow independent of the hypothesis testing framework, and to identify potential larval migration patterns. Results of the genetic analyses are interpreted in terms of their significance for management of these VME indicator species around New Zealand and the possible establishment of new offshore marine protected areas.

6.2. Materials and methods

6.2.1. Samples

Three structure-forming corals (*Goniocorella dumosa*, *Madrepora oculata*, *Solenosmilia variabilis*) were selected based on their adherence to VME criteria, wide distribution in the New Zealand region, and availability of samples. The total number of specimens across all three species was 410 (78 *M. oculata*, 134 *G. dumosa*, 198 *S. variabilis*). Samples were mainly sourced from the NIWA Invertebrate Collection, which archives specimens obtained from a range sampling expeditions since the 1950s. Most specimens were preserved in ethanol, with the rest dry-preserved. Additional samples were collected during a research survey to Louisville Seamount Chain in 2014 (details see Clark *et al.*, 2015). Most of the samples were from within the New Zealand EEZ (Figure 6.1), and the majority of specimens were from seamount and slope habitats from 200-1300 m water depth (range from 198-1805 m).

In the present study, because sampling at any one sampling station recovered only one sample (which may contain one or more specimens), the specimens within 1° of latitude and longitude were grouped into one preliminary group for plotting the haplotype distributions, and the preliminary groups were further assigned into different populations at different scales that reflect the various bathymetric, topographic and hydrodynamic features that could influence connectivity amongst populations. Specifically, these groups are based on the two lower-bathyal biogeographic provinces that cover the study region (northern and southern

provinces), three regions delineated by the approximate boundaries of the subtropical front region over the Chatham Rise (north-central-south regions), and on geomorphic features (named features such as seamounts, rises, plateau etc). Due to patchy deep-sea sampling and the different distributions of the species, samples for all species were not available from all locations throughout the region. Meanwhile, to achieve a balance between the validation of results and extracting maximum information content from the specimens, the minimum sample size was set at 4 for population analysis.

Figure 6.1 Map showing the distribution of samples amongst biogeographic provinces, regions, and geomorphic features used for the analysis of population structure for three species of deep-sea stony corals

Provinces, BY06 in blue, BY10 in green (Watling *et al.*, 2013), red dashed lines indicate the boundaries for the north-central-south regions, and geomorphic features were labelled with feature names.

6.2.2. Molecular methods

Total DNA was extracted using a guanidinium thiocyanate extraction methodology (Boom *et al.*, 1990) and DNA was stored at 4 °C in 0.1X TE buffer. To identify informative population genetic markers in Scleractinia, one mitochondrial DNA region (*D-loop*, also called the control region) and one nuclear region (partial sequence of the internal transcribed spacer, *ITS*) were used to estimate genetic connectivity and population genetic differentiation. Coral-specific primers for *ITS* and *D-loop* were designed and utilised to amplify these products; primers and annealing temperatures used for all species are summarised in Chapter 4. Only *ITS* consistently amplified DNA from most specimens for the three species; *D-loop* failed to amplify in *M. oculata* due to its absence in the mitochondrial genome of this species (Lin *et al.*, 2012). PCR reactions (30 µl) for all three genes contained 1 µl DNA template, 15 µl 2 × MyTaq™ Mix (Bioline, UK), 1 µl of each primer (Macrogen, Korea), and 12 µl ddH₂O. The thermal cycling profile consisted of an initial denaturation at 94 °C for 5 min, then 32 cycles of a three-step programme (95 °C for 30 s, 50 °C for 30 s and 72 °C for 45 s), with a 20-min final extension at 72 °C. PCR products were sent to Macrogen (Macrogen Inc, Korea) for purification and sequencing on an ABI3730 sequencer.

In total, 27 microsatellite loci (described in Chapter 4) were utilised in *G. dumosa* and *S. variabilis*, and 11 microsatellite loci from a collaborator's laboratory were used for *M. oculata* (see External Contributionss). The forward primers from each primer pair were fluorescently 5' end labelled with 6-FAM, NED, VIC, or PET for multiplexing (details in Chapter 4). For genotyping, PCRs were carried out in a total volume of 40 µl with 20 µl PCR Red Mixture MyTaq™ Red Mix), 1 µl forward and reverse primers, 2 µl of template DNA and topped up with dd H₂O. PCR amplifications were performed with an initial denaturing step of 94 °C for 10 min, followed by 32 cycles of 30 s at 94 °C, 45 s annealing at 55 °C, and 45 s extension at 72 °C, and a final extension of 30 min at 72 °C.

Fluorescently labelled PCR products were run on an ABI 3730 DNA Sequencer (Macrogen Inc, Korea), scored using the GeneScan-500 (LIZ) size standard, and analysed with the GeneMarker software (GeneMarker, USA).

6.2.3. Data analysis

6.2.3.1. *D-loop* and ITS

All sequences were checked and edited using Geneious v7 (Biomatters Ltd, New Zealand). Sequences with HQ values of < 80% were discarded. Multiple sequences were aligned using the plugin ClustalW Alignment (Gap Open Cost=100 and Gap Extend Cost=10) in Geneious, and then all alignments were viewed by eye. Neighbour-Joining (NJ) phylogenetic trees were constructed with default settings and by using the highest similarity sequence in NCBI as an out-group by using the Tree Builder option in Geneious v7. For all populations with two or more individuals' intra-specific genetic diversity was evaluated by computing the number of haplotypes, the number of polymorphic sites, haplotypic diversity (h), and nucleotide diversity (π).

Analyses of molecular variance (AMOVA) between locations (between north and south biogeographic provinces, or amongst northern, central and southern regions, or amongst geomorphic features as appropriate) and amongst populations were tested using the Arlequin software package. Pairwise comparisons of population differentiation were also performed in Arlequin and significance values estimated after 1000 permutations (Excoffier & Lischer, 2010). Between-location or within-location Φ_{ST} statistics were calculated to test for genetic differentiation amongst populations.

To visualise the spatial patterns of genetic variation for each marker, the specimens were colour-coded according to haplotype, and their geographic coordinates of collection were plotted using ArcGIS (ESRI, USA). The Mantel test (Mantel, 1967) was employed to test for isolation-by-depth by comparing the matrix of Φ_{ST} values to the matrix of depth (m) values. This test was also used to assess isolation-by-distance by comparing the matrix of Φ_{ST} values to the matrix of shortest actual distances (km) between pairs of sites using the software GenAlEx 6.5 (Peakall & Smouse, 2012).

6.2.3.2. Microsatellites

Micro-Checker 2.2.1 software (van Oosterhout *et al.*, 2004) was used to identify possible genotyping errors such as stuttering, large allele dropout, and null alleles within the data set by performing 10,000 randomisations, where the threshold was set at 0.10 then raised to 0.15 when insufficient loci were present. Loci found to be under selection were removed and a second reduced data set was created. Both the full and the reduced data sets were tested,

where appropriate, for evidence of population genetic structure and differentiation. Allelic frequencies, number of alleles, departures from Hardy–Weinberg equilibrium (HWE), linkage disequilibrium (LD) and observed and expected heterozygosities were estimated in GenAlEx 6.5. The Arlequin software package was used to estimate Weir and Cockerham’s (1984) unbiased estimator of Wright’s F_{ST} and for hierarchical F_{ST} analyses (AMOVA, 10,000 permutations) on the microsatellite data set. If significant differentiation amongst populations was detected, the location of the genetic discontinuity was identified using BARRIER v2.2 (Manni *et al.*, 2004). The Mantel test was employed to test for isolation-by-depth by comparing the matrix of F_{ST} values to the matrix of depth (m) values. This test was also used to test for isolation-by-distance by comparing the matrix of F_{ST} values to the matrix of shortest actual distances (km) between pairs of sites by software GenAlEx 6.5. The software package LOSITAN was employed to test the selected loci for whether they were neutral or under selection (Antao *et al.*, 2008). Additionally, multilocus matches in GenAlex were employed to detect the asexual/colonial relationship between individuals.

Discriminant analysis of principal components (DAPC) (Jombart *et al.*, 2010) was used to identify population structure amongst the microsatellite multi-locus genotypes of all individuals. This method entails no assumptions regarding the cause of structure (i.e., island model versus isolation-by-distance (IBD)). Analysis was implemented in R 3.0.2 (R Development Core Team 2015) using the package “adeigenet” 1.4-2 (Jombart, 2008). The optimal number of genetic clusters (K) was chosen when the Bayesian Information Criterion (BIC) values were the lowest. Scatterplots of microsatellite genotypes in relation to discriminant functions were created in “adeigenet”. Principal components analysis (PCA) in the software package GenAlex was also used to examine population structure.

STRUCTURE (v2.3.4) was employed to infer the number of distinct genetic groups based on Bayesian assignment analysis (Pritchard *et al.*, 2000; Falush *et al.*, 2007). A total run length of 50,000 generations with a burn-in period of 5,000 generations was employed. The models were run in three iterations for K (number of distinct genetic clusters) values to evaluate likelihood scores and consistency amongst runs (Falush *et al.*, 2007). The optimal number of K was chosen as the maximum number of clusters based on preliminary analyses, which yielded likelihoods lower than those observed at lower values of K. This number was computed in the online program Structure Harvester (Earl & vonHoldt, 2012) (available: <http://taylor0.biology.ucla.edu/structureHarvester/>).

Where any significant F_{ST} or Φ_{ST} had detected at the scale of geomorphic features, MIGRATE (Beerli & Felsenstein, 1999; Beerli, 2006) was used to estimate both theta (θ),

which is equal to $4Ne\mu$ (where Ne is the long-term effective population size, and μ is the mutation rate) and M , which is equal to the ratio m/μ (where m is the immigration rate). Analyses were performed under the allele model: each Markov chain Monte Carlo (MCMC) run consisted of 10 short chains (sampling 10,000 trees) and three long chains (sampling 100,000 trees) with a burn-in period of 10,000 trees. All runs were repeated five times to verify consistency of results, and the data presented are the average of the five runs. The results were visualised using the circlize package in R (Gu *et al.*, 2014).

6.3. Results

Working on historic (sample age: *G. dumosa* 1~57 years, average 23 y; *M. oculata* 1~55 y, average 15 y; *S. variabilis* 1~50 y, average 9 y) and also on dry-preserved (31.56% of *G. dumosa* and 55.49% of *M. oculata* and 58.70% of *S. variabilis*) samples is a challenge for any genetic study. Before DNA extraction, only 28.09% of *G. dumosa*, 20.13% of *M. oculata*, and 52.62% of *S. variabilis* passed the initial selection for the following molecular experiments (Table S6.1). After several tests of efficiency of DNA extraction (salt, CTAB, phenol-chloroform, and some commercial kits), the method described above in the previous section was the best performing. Nevertheless, none of the extraction methods worked particularly well for *M. oculata*. Although the species-specific collections had wide-ranging distributions around the New Zealand EEZ and beyond, DNA sample availability was ultimately limited in some regions for each species.

6.3.1. Population structure based on *ITS* and *D-loop* variation

After trimming and checking, *ITS* sequence data were obtained for all 3 species and *D-loop* sequence data for *G. dumosa* and *S. variabilis*. For *ITS*, haplotypic diversity was relatively low, with only 6 haplotypes in 83 *G. dumosa* specimens, 4 haplotypes in 40 *M. oculata* and 12 haplotypes in 118 *S. variabilis*. For *D-loop*, haplotypic diversity in *S. variabilis* was much lower than that in *G. dumosa*, with 12 haplotypes in 26 *G. dumosa* and 4 haplotypes in 141 *S. variabilis* specimens (Figure S6.1).

6.3.1.1. Population genetic diversity

For *G. dumosa*, greater *ITS* haplotypic diversity was found in the southern province than in the northern province, but nucleotide diversity in the northern province was greater

than in the southern province. *D-loop* showed that both haplotypic and nucleotide diversity of the southern province was greater than in the northern province. Samples from the Kermadec Ridge had the highest haplotypic and nucleotide diversity for both *ITS* and *D-loop* (Table 6.1 and Figure 6.2).

For *M. oculata*, the *ITS* haplotypic and nucleotide diversity of the northern province were greater than those of the southern province, and the *ITS* haplotypic and nucleotide diversity values decreased from the northern to the southern region. The highest haplotypic and nucleotide diversity values were found on the Kermadec Ridge amongst the geomorphic features (Table 6.2 and Figure 6.2).

Table 6.1 *ITS* and *D-loop* diversity statistics for *Goniocorella dumosa*

Populations	<i>ITS</i>						<i>D-loop</i>				
	N	n	s	h	Hd	π	n	s	h	Hd	π
Northern province	125	80	22	5	0.636	0.023	26	51	11	0.825	0.028
Southern province	8	3	1	2	0.667	0.002	2	25	2	1.000	0.048
North region	31	25	21	4	0.600	0.021	6	30	5	0.933	0.028
Central region	94	55	22	5	0.640	0.023	20	49	8	0.805	0.029
South region	8	3	1	2	0.667	0.002	2	25	2	1.000	0.048
Kermadec Ridge	14	11	21	4	0.709	0.026	3	22	2	0.667	0.028
Northeast continental slope	7	3	0	1	0.000	0.000					
Challenger Plateau	8	8	12	2	0.571	0.021	2	12	2	1.000	0.023
Hikurangi Margin	2	2	13	2	1.000	0.040	1	0	1	0.000	0.000
Chatham Rise	94	55	22	5	0.640	0.023	20	49	8	0.805	0.029
Campbell Plateau	6	2	0	1	0.000	0.000					
Macquarie Ridge	2	1	0	1	0.000	0.000	1	0	1	0.000	0.000
Total	133	83	22	6	0.629	0.022	70	51	12	0.788	0.027

N is the total number of individuals sampled for a site, n is the number of sequences for analysis from the site, s is the number of polymorphic nucleotide sites in the sequence, h is the number of haplotypes represented at the site, Hd is haplotype diversity, and π is nucleotide diversity.

For *S. variabilis* *ITS* and *D-loop* variation, both the haplotypic and nucleotide diversities of the southern province were greater than those in the northern province. The haplotypic and nucleotide diversity values of *ITS* and *D-loop* decreased from the southern to the northern region. Amongst the geomorphic features, the greatest haplotypic and nucleotide diversity values of *ITS* were found on the Chatham Rise, whereas the greatest haplotypic and nucleotide diversity values of *D-loop* were found on the Macquarie Ridge (Table 6.3 and Figure 6.3).

Table 6.2 ITS diversity statistics for *Madrepora oculata*

Populations	N	n	s	h	Hd	π
Northern province	58	26	78	3	0.557	0.122
Southern province	16	10	1	2	0.200	0.001
North region	15	8	78	3	0.679	0.177
Central region	43	16	41	2	0.458	0.082
South region	16	10	1	2	0.200	0.001
Kermadec Ridge	7	5	64	2	0.600	0.168
Challenger Plateau	5	2	0	1	0.000	0.000
Norfolk Ridge	2	1	0	1	0.000	0.000
Hikurangi Margin	1	1	0	0	0.000	0.000
Chatham Rise	43	16	41	2	0.458	0.082
Campbell Plateau	9	4	0	1	0.000	0.000
Bounty Plateau	5	4	1	2	5.000	0.002
Bounty Trough	2	3	0	1	0.000	0.000
Total	74	34	78	4	0.476	0.095

N is the total number of individuals sampled for a site, n is the number of sequences for analysis from the site, s is the number of polymorphic nucleotide sites in the sequence, h is the number of haplotypes represented at the site, Hd is haplotype diversity, and π is nucleotide diversity.

Table 6.3 ITS and D-loop diversity statistics for *Solenosmilia variabilis*

Population	ITS						D-loop				
	N	n	s	h	Hd	π	n	s	h	Hd	π
Northern province	148	96	155	9	0.287	0.023	119	4	4	0.098	0.000
Southern province	36	25	149	5	0.420	0.055	22	2	2	0.173	0.001
North region	56	48	2	3	0.121	0.000	75	3	3	0.079	0.000
Central region	77	48	155	8	0.432	0.045	44	3	3	0.132	0.001
South region	36	25	149	5	0.420	0.055	22	2	2	0.173	0.001
Kermadec Ridge	46	32	2	3	0.179	0.001	49	3	3	0.119	0.000
Challenger Plateau	1	1	0	1	0.000	0.000					
Hikurangi Margin	9	2	0	1	0.000	0.000	8	0	1	0.000	0.000
Chatham Rise	77	48	155	8	0.432	0.045	44	3	3	0.132	0.001
Campbell Plateau	13	5	0	1	0.000	0.000	6	0	1	0.000	0.000
Bollons Seamount	2	2	0	1	0.000	0.000	4	0	1	0.000	0.000
Bounty Plateau	6	2	18	2	1.000	0.050					
Bounty Trough	6	1	0	1	0.000	0.000	2	0	1	0.000	0.000
Macquarie Ridge	19	12	147	4	0.636	0.103	8	2	2	0.429	0.002
Tasman Basin	3	2	0	1	0.000	0.000					
Louisville Seamount Chain	15	13	0	1	0.000	0.000	18	0	1	0.000	0.000
Total	186	104	156	11	0.299	0.031	139	3	3	0.097	0.000

N is the total number of individuals sampled for a site, n is the number of sequences for analysis from the site, s is the number of polymorphic nucleotide sites in the sequence, h is the number of haplotypes represented at the site, Hd is haplotype diversity, and π is nucleotide diversity.

Figure 6.2 Haplotype map for *Goniocorella dumosa* (left) and *Madrepora oculata* (right) ITS (above line) and D-loop (below line)

Pie charts indicate haplotypic composition of each location and numbers indicate the total number of sequences from each location.

6.3.1.2. Geographic distribution of haplotypes

A single dominant *ITS* haplotype was observed for each of *G. dumosa* (Hap1), *M. oculata* (Hap3) and *S. variabilis* (Hap2) across the sampled area: other haplotypes were mainly found in a single individual (Figure S6.1). Common haplotypes of *D-loop* were found in most of the sampled areas for both *S. variabilis* and *G. dumosa*. The occurrence of common haplotypes across the whole region for all three species indicated that there was limited geographic genetic structure for haplotypic diversity distributions.

Figure 6.3 Haplotype map for *Solenosmilia variabilis* *ITS* (above line) and *D-loop* (below line)

Pie charts indicate haplotypic composition of each location and numbers indicate the total number of sequences from each location.

6.3.1.3. Population genetic structure

In the three corals, amongst the populations, Φ_{ST} values for both *ITS* and *D-loop* were variable but relatively low. In *G. dumosa* only one significant Φ_{ST} value was observed, between the Northeast Continental Slope and Chatham Rise populations for *ITS*. No significant pairwise population Φ_{ST} values were observed for *M. oculata* for *ITS*. These pairwise population Φ_{ST} values were calculated based on small sample sizes (~ 2 individuals/population), and more robust results would be generated from larger sample sizes. However, pairwise population Φ_{ST} values of *S. variabilis* showed a significant genetic difference between the Macquarie Ridge and Louisville Seamount Chain populations in both the *ITS* and *D-loop* markers, and at the *ITS* marker the Macquarie Ridge population was significantly different from the Kermadec Ridge population.

Table 6.4 Pairwise Φ_{ST} values for *ITS* of *Madrepora oculata* (above diagonal) and *Solenosmilia variabilis* (below diagonal) amongst geomorphic feature populations

	Bounty Plateau	Campbell Plateau	Chatham Rise	Kermadec Ridge	Louisville Seamount Chain	Macquarie Ridge
Bounty Plateau		0.063	0.148	0.447	-	-
Campbell Plateau	-		0.175	0.5	-	-
Chatham Rise	-	-0.087		0.457**	-	-
Kermadec Ridge	-	-0.094	0.019		-	-
Louisville Seamount Chain	-	0	-0.014	-0.018		-
Macquarie Ridge	-	-0.018	0.032	0.223*	0.100*	

Significant values of $p < 0.05$ are marked as *, and $p < 0.01$ are marked as **. Data for *G. dumosa* not included because all Φ_{ST} values are non-significant.

The AMOVA test of genetic structure for both *ITS* and *D-loop* across *G. dumosa* populations (Table 6.5) did not detect any large-scale structure (between northern/southern provinces, or amongst north, centre, south regions, or amongst geomorphic feature populations) ($p > 0.05$). In contrast, *ITS* variation revealed significant differentiation amongst the three regions (north, centre, south) for *M. oculata* ($p < 0.05$) (Table 6.6). In *S. variabilis*, both *ITS* and *D-loop* variation across the populations (Table 6.7) showed no large-scale structure. The apparent absence of differentiation amongst populations might be caused by low levels of variability across the markers.

Table 6.5 Hierarchical AMOVA results for *Goniocorella dumosa* when tested for genetic variation at the *ITS* and *D-loop* regions

Source of variation	<i>ITS</i>				<i>D-loop</i>			
	df	ss	Var. comp.	%V	df	ss	Var. comp.	%V
Between provinces	1	14.472	1.329	16.77	1	14.050	0.941	7.17
Within provinces between populations	23	202.969	0.976	12.32*	15	191.759	0.931	7.09
Within populations	58	325.872	5.618	70.91*	11	123.833	11.258	85.74
Total	82	543.313	7.923		27	329.643	13.130	
Amongst regions	2	29.294	0.298	4.36	2	18.976	-0.701	-5.91
Within provinces between populations	22	188.147	0.925	13.52*	14	186.833	1.300	10.97
Within populations	58	325.872	5.618	82.12*	11	123.833	11.258	94.94
Total	82	543.313	6.841		27	329.643	11.857	
Amongst geomorphic features	7	50.141	-0.296	-4.51	4	47.310	-0.438	-3.64
Within geomorphic features between populations	17	167.301	1.246	18.97*	12	158.500	1.202	10
Within populations	58	325.872	5.618	85.54*	11	123.833	11.258	93.65
Total	82	543.313	6.582		27	326.643	12.021	

Significant values of $p < 0.05$ are marked as *, and $p < 0.01$ are marked as **.

6.3.1.4. Isolation by distance and by depth

Amongst the ten tests, there was no statistically significant evidence supporting isolation by distance ($p > 0.05$), but *ITS* sequences revealed a significant isolation by depth for *M. oculata* and *G. dumosa* (Table 6.8).

Table 6.6 Hierarchical AMOVA results for *Madrepora oculata* when tested for genetic variation at the *ITS* and Microsatellites

Source of variation	<i>ITS</i>				Microsatellites			
	df	ss	Var. comp.	%V	df	ss	Var. comp.	%V
Between provinces	1	45.818	1.304	9.25	1	1.595	0.020	2.01**
Within provinces between populations	16	312.917	6.520	46.24**	2	1.163	-0.014	-1.38
Within populations	21	131.778	6.255	44.50**	182	183.78	1.010	99.37
Total	38	490.513	14.100		185	186.538		
Amongst regions	2	118.46	3.275	22.62*	2	2.070	0.015	1.49
Within provinces between populations	15	240.275	4.928	34.04*	1	0.688	-0.017	-1.68
Within populations	21	131.778	6.275	43.34**	182	183.78	1.010	100.19
Total	38	490.513	14.478		185	186.538	1.008	
Amongst geomorphic features	6	184.796	3.369	23.68				
Within geomorphic features between populations	11	173.939	4.581	32.21*	3	2.758	-0.002	-0.24
Within populations	21	131.778	6.275	44.11*	182	183.780	1.01	100.24
Total	38	490.513	14.225		185	186.538	1.007	

Significant values of $p < 0.05$ are marked as *, and $p < 0.01$ are marked as **.

6.3.2. Population structure based on microsatellites

The software MICRO-CHECKER detected null alleles in three corals: loci with high frequencies of null alleles should be theoretically excluded from population genetic analysis. For *G. dumosa*, *M. oculata* and *S. variabilis*, 4 (Table S6.2), 5 (Table S6.3) and 5 (Table S6.4) loci exhibited null alleles, respectively. The software Lositan revealed that 11 loci in *G. dumosa*, 1 locus in *M. oculata*, and 8 loci in *S. variabilis* were under balancing selection. In *M. oculata*, a higher proportion of genotyping failures was detected than for the other two species. Twenty European samples of *M. oculata* were genotyped to verify the ability of the loci to distinguish between the New Zealand and the European samples (Figure S6.2). To investigate the effects of loci under selection, the AMOVA test and Migrate were run twice, with the neutral only and the all loci dataset.

Table 6.7 Hierarchical AMOVA results for *Solenosmilia variabilis* when tested for genetic variation at the ITS and D-loop

Source of variation	ITS				D-loop			
	df	ss	Var. comp.	%V	df	ss	Var. comp.	%V
Between provinces	1	17.656	0.345	4.88	1	0.125	-0.000	-0.24
Within provinces between populations	30	154.985	-0.628	-8.87	21	2.684	0.007	6.8
Within populations	89	654.855	7.358	103.99	118	10.667	0.090	91.44
Total	120	827.496	7.075		140	13.475	0.097	
Amongst regions	2	32.042	0.407	5.84	2	0.163	-0.003	-2.87
Within provinces between populations	29	140.599	-0.791	-11.34	20	2.645	0.008	8.69
Within populations	89	654.855	7.358	105.5	118	10.667	0.090	94.18
Total	120	827.496	6.974		140	13.475	0.093	
Amongst geomorphic features	10	4.277	0.162	2.35	8	0.863	-0.004	-3.82
Within geomorphic features between populations	21	108.364	-0.637	-9.25	14	1.945	0.010	9.99
Within populations	89	654.855	7.358	106.89	118	10.667	0.090	93.83
Total	120	827.496	6.883		140	13.475	0.096	

Significant values of $p < 0.05$ are marked as *, and $p < 0.01$ are marked as **.

Table 6.8 Mantel tests of isolation by distance and isolation by depth amongst different markers in *Goniocorella dumosa*, *Madrepora oculata* and *Solenosmilia variabilis*

Loci	<i>Goniocorella dumosa</i>		<i>Madrepora oculata</i>		<i>Solenosmilia variabilis</i>	
	Distance	Depth	Distance	Depth	Distance	Depth
Microsatellites	0.0002 (0.28)	0.0115 (0.01)	0.0024 (0.22)	0.0113 (0.06)	0.0108 (0.01)	0.0017 (0.09)
ITS	0.0004 (0.37)	0.0132 (0.05)	0.0015 (0.32)	0.1023 (0.01)	0.0005 (0.27)	0.0047 (0.08)
D-loop	0.0003 (0.20)	0.0022 (0.29)	NA	NA	0.0119 (0.10)	-0.070 (0.08)

Expressed as R^2 values above and p values (in brackets) below the diagonal: significant values were bold. NA - not available.

6.3.2.1. Genotype identity test

To screen out any genetically identical individuals (clones or fragments of individuals), all genotyped loci were utilised. In *S. variabilis* (24 loci), only one pair of specimens (NIWA ID 25489, two different fragments) was exactly the same (i.e., shared the same multi-locus genotype) across all amplified loci. These two samples were considered to be from the same colony, and one of them was therefore excluded for population genetic analysis. In *G. dumosa* (20 loci), no specimens with the same multi-locus genotype were

detected amongst 108 individuals. In *M. oculata* (11 loci), 2 individuals (NIWA ID 46492 & 47791) from Campbell Plateau, 2 individuals (ID 4004 & 4005) from Chatham Rise and 2 individuals (ID 42734 & 42830) from Chatham Rise were 2-loci identical (the other loci failed to be genotyped), for which it was difficult to distinguish whether the pairs were from the same colony or not. Due to the sample size for *M. oculata*, those pairs were considered as non-colony individuals, and both of the pairs were kept for the analyses. Except for these 3 pairs, the other 87 were unique genotypes and were considered to be individual specimens.

6.3.2.2. Population genetic diversity

For *G. dumosa*, the number of alleles per locus varied from 7 to 27 (average = 8.20). Observed heterozygosity (H_O) ranged from 0.500 to 1.000 per locus and most H_O values were greater in all populations than expected. There were 66 instances of departures from HWE in 140 tests (Table 6.9). For *M. oculata*, the number of alleles per locus ranged from 5 to 19 (average = 9.96). H_O values ranged from 0.273 to 0.944 per locus, and unlike the other two coral species, were lower in all populations than expected. Approximately two thirds (68.33%) of all loci exhibited significant departures from HWE (Table 6.10). For *S. variabilis*, the number of alleles varied from 7 to 31 (average = 14.42). H_O ranged from 0.211 to 1.000, and most loci exhibited significant departures from HWE (Table 6.11).

Table 6.9 Basic population genetic information of microsatellite data at three spatial scales for***Goniocorella dumosa***

Population	Locus	B106	B1	C107	D5	B123	D213	SVA13	C4	SVA15	SVA10
Northern province	N	104	104	95	91	85	81	73	75	70	63
	Na	7	7	15	16	12	14	12	15	25	9
	Ne	4.258	2.125	4.926	8.658	3.898	5.295	4.786	4.987	6.644	5.005
	Ho	0.808	0.904	0.547	0.769	0.706	0.79	0.877	0.613	0.629	0.73
	He	0.765	0.529	0.797	0.884	0.743	0.811	0.791	0.799	0.849	0.8
	HWE	***	***	***	**	***	***	***	***	***	***
Southern Province	N	4	4	4	4	3	3	3	3	2	2
	Na	4	3	7	5	3	3	2	4	3	2
	Ne	2.909	2.462	6.400	3.200	2.571	2.571	1.800	3.600	2.667	2.000
	Ho	0.500	1.000	1.000	0.500	1.000	1.000	0.667	1.000	1.000	0.000
	He	0.656	0.594	0.844	0.688	0.611	0.611	0.444	0.722	0.625	0.500
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
North Region	N	27	27	26	24	25	23	18	23	21	17
	Na	7	5	10	13	10	10	10	11	17	6
	Ne	4.942	2.236	4.012	7.024	4.209	5.264	6.353	4.301	7.056	5.352
	Ho	0.778	0.963	0.500	0.708	1.000	0.739	0.833	0.652	0.714	0.824
	He	0.798	0.553	0.751	0.858	0.762	0.810	0.843	0.767	0.858	0.813
	HWE	*	**	***	ns	*	**	ns	ns	ns	ns
Central Region	N	77	77	69	67	60	58	55	52	49	46
	Na	6	6	13	16	11	11	10	12	17	8
	Ne	3.836	2.084	4.972	8.960	3.752	4.745	4.133	5.026	6.141	4.575
	Ho	0.818	0.883	0.565	0.791	0.583	0.810	0.891	0.596	0.592	0.696
	He	0.739	0.520	0.799	0.888	0.733	0.789	0.758	0.801	0.837	0.781
	HWE	***	***	***	***	***	***	***	***	***	***
South Region	N	4	4	4	4	3	3	3	3	2	2
	Na	4	3	7	5	3	3	2	4	3	2
	Ne	2.909	2.462	6.400	3.200	2.571	2.571	1.800	3.600	2.667	2.000
	Ho	0.500	1.000	1.000	0.500	1.000	1.000	0.667	1.000	1.000	0.000
	He	0.656	0.594	0.844	0.688	0.611	0.611	0.444	0.722	0.625	0.500
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Chatham Rise	N	77	77	69	67	60	58	55	52	49	46
	Na	6	6	13	16	11	11	10	12	17	8
	Ne	3.836	2.084	4.972	8.960	3.752	4.745	4.133	5.026	6.141	4.575
	Ho	0.818	0.883	0.565	0.791	0.583	0.810	0.891	0.596	0.592	0.696
	He	0.739	0.520	0.799	0.888	0.733	0.789	0.758	0.801	0.837	0.781
	HWE	***	***	***	***	***	***	***	***	***	***
Kermadec Ridge	N	12	12	12	11	12	12	8	12	9	8
	Na	6	2	9	9	9	6	5	8	11	6
	Ne	4.114	1.986	3.840	4.939	4.571	4.114	3.879	4.114	8.100	4.923
	Ho	0.750	0.917	0.500	0.455	1.000	0.750	0.750	0.750	0.889	0.750
	He	0.757	0.497	0.740	0.798	0.781	0.757	0.742	0.757	0.877	0.797
	HWE	ns	**	*	**	ns	ns	ns	ns	ns	ns
Challenger Plateau	N	7	7	7	7	6	4	5	5	5	5
	Na	5	4	4	7	5	4	7	7	6	6
	Ne	4.455	2.579	2.513	5.444	3.600	3.200	6.250	5.556	4.167	5.556
	Ho	0.714	1.000	0.571	1.000	1.000	0.500	1.000	0.600	0.400	1.000
	He	0.776	0.612	0.602	0.816	0.722	0.688	0.840	0.820	0.760	0.820
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	*	ns
NE continental slope	N	4	4	3	3	4	4	3	4	4	3
	Na	5	3	4	4	4	6	3	3	7	3
	Ne	4.000	2.462	3.600	3.000	2.909	5.333	2.571	1.684	6.400	2.571
	Ho	1.000	1.000	0.333	0.667	1.000	1.000	1.000	0.500	1.000	0.667
	He	0.750	0.594	0.722	0.667	0.656	0.813	0.611	0.406	0.844	0.611
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	*	ns

Continues

Population	Locus	SVA2	SVA1 ₈	SVA8	D103	SVA1	B116	SVA1 ₁	SVA4	SVA7	SVA2 ₀	Mean	SE
Northern province	N	66	63	60	64	56	63	54	23	39	34	68.15	4.841
	Na	13	22	20	10	11	13	7	16	15	17	13.8	1.085
	Ne	3.546	6.005	4.343	6.015	3.942	5.682	2.633	11.5	8.895	6.404	5.477	0.494
	Ho	0.652	0.667	0.75	0.703	0.339	0.651	1	0.913	0.641	0.824	0.726	0.033
	He	0.718	0.833	0.77	0.834	0.746	0.824	0.62	0.913	0.888	0.844	0.788	0.02
	HWE	***	***	***	***	***	***	***	ns	***	ns		
Southern Province	N	2	2	1	2	1	1	3	0	0	1	2.25	0.289
	Na	2	2	2	3	1	2	2	0	0	2	2.6	0.358
	Ne	2.000	1.600	2.000	2.667	1.000	2.000	2.000	0.000	0.000	2.000	2.272	0.298
	Ho	1.000	0.500	1.000	1.000	0.000	1.000	1.000	0.000	0.000	1.000	0.708	0.091
	He	0.500	0.375	0.500	0.625	0.000	0.500	0.500	0.000	0.000	0.500	0.490	0.053
	HWE	ns	ns	ns	ns	Mono	ns	ns	Mono	Mono	ns		
North Region	N	18	18	19	20	16	22	9	8	12	15	48.75	3.754
	Na	10	12	14	8	10	10	3	9	9	11	11.5	0.838
	Ne	5.945	6.231	4.813	5.634	5.505	5.068	2.219	7.529	5.538	6.818	5.074	0.486
	Ho	0.889	0.778	0.842	0.800	0.625	0.636	1.000	1.000	0.500	0.933	0.698	0.037
	He	0.832	0.840	0.792	0.823	0.818	0.803	0.549	0.867	0.819	0.853	0.768	0.022
	HWE	***	ns	ns	ns	ns	ns	*	ns	**	ns		
Central Region	N	48	45	41	44	40	41	45	15	27	19	19.4	1.238
	Na	8	19	15	10	9	12	6	15	14	12	9.75	0.695
	Ne	2.753	5.556	3.979	5.779	2.747	5.827	2.682	10.465	8.526	4.945	5.303	0.321
	Ho	0.563	0.622	0.707	0.659	0.225	0.659	1.000	0.867	0.704	0.737	0.786	0.034
	He	0.637	0.820	0.749	0.827	0.636	0.828	0.627	0.904	0.883	0.798	0.790	0.020
	HWE	***	***	***	***	***	***	***	ns	***	ns		
South Region	N	2	2	1	2	1	1	3	0	0	1	2.25	0.289
	Na	2	2	2	3	1	2	2	0	0	2	2.6	0.358
	Ne	2.000	1.600	2.000	2.667	1.000	2.000	2.000	0.000	0.000	2.000	2.272	0.298
	Ho	1.000	0.500	1.000	1.000	0.000	1.000	1.000	0.000	0.000	1.000	0.708	0.091
	He	0.500	0.375	0.500	0.625	0.000	0.500	0.500	0.000	0.000	0.500	0.490	0.053
	HWE	ns	ns	ns	ns	Mono	ns	ns	Mono	Mono	ns		
Chatham Rise	N	48	45	41	44	40	41	45	15	27	19	48.75	3.754
	Na	8	19	15	10	9	12	6	15	14	12	11.5	0.838
	Ne	2.753	5.556	3.979	5.779	2.747	5.827	2.682	10.465	8.526	4.945	5.074	0.486
	Ho	0.563	0.622	0.707	0.659	0.225	0.659	1.000	0.867	0.704	0.737	0.698	0.037
	He	0.637	0.820	0.749	0.827	0.636	0.828	0.627	0.904	0.883	0.798	0.768	0.022
	HWE	***	***	***	***	***	***	***	ns	***	ns		
Kermadec Ridge	N	10	9	10	11	9	12	3	3	5	7	9.35	0.658
	Na	10	10	9	7	6	8	2	5	4	7	6.905	0.569
	Ne	7.143	5.786	3.509	4.654	3.682	4.114	2.000	4.500	2.941	5.765	4.434	0.331
	Ho	0.800	0.889	0.700	0.818	0.556	0.667	1.000	1.000	0.200	1.000	0.757	0.047
	He	0.860	0.827	0.715	0.785	0.728	0.757	0.500	0.778	0.660	0.827	0.747	0.022
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	*	ns		
Challenger Plateau	N	3	4	4	4	5	5	3	3	1	4	4.7	0.356
	Na	4	5	6	6	7	4	3	4	1	5	5	0.348
	Ne	3.600	4.000	4.571	4.571	6.250	3.846	2.571	3.600	1.000	3.200	4.026	0.303
	Ho	1.000	0.750	1.000	1.000	0.800	0.600	1.000	1.000	0.000	1.000	0.797	0.062
	He	0.722	0.750	0.781	0.781	0.840	0.740	0.611	0.722	0.000	0.688	0.705	0.041
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	Mono	ns		
NE continental slope	N	3	4	3	3	1	3	3	0	4	3	3.15	0.233
	Na	3	2	5	3	1	4	2	0	5	4	3.55	0.366
	Ne	2.571	2.000	4.500	3.000	1.000	3.600	2.000	0.000	4.571	3.000	3.039	0.328
	Ho	1.000	0.500	1.000	0.667	0.000	0.333	1.000	0.000	1.000	0.667	0.717	0.077
	He	0.611	0.500	0.778	0.667	0.000	0.722	0.500	0.000	0.781	0.667	0.595	0.052
	HWE	ns	ns	ns	ns	Mono	ns	ns	Mono	ns	ns		

N = number of individuals successfully amplified, Na = number of different alleles, Ne = number of effective alleles, Ho = observed heterozygosity, He = expected heterozygosity, HWE = test of deviation from Hardy–Weinberg equilibrium. Geomorphic feature groups were only included in this table when the number of individuals successfully amplified was larger than 4. Significant values of $p > 0.05$ are marked as ns, $p < 0.05$ are marked as *, $p < 0.01$ are marked as **, and $p < 0.001$ are marked as ***. Mono means the locus is monomorphic.

Table 6.10 Basic population genetic information of microsatellite data at three spatial scales for *Madrepora oculata*

Population	Locus	c016	g028	c7	g006	g004	g25	c013	g016	g022	a5	g010	Mean	SE
Northern province	N	46	37	20	42	45	22	54	24	23	41	45	36.27 3	3.583
	Na	14	18	13	11	16	5	11	14	15	18	13	13.45 5	1.107
	Ne	9.51	8.322	5.442	5.219	10.38 5	4.19	2.602	5.62	5.75	4.344	6.853	0.814	0.024
	Ho	0.891	0.757	0.7	0.738	0.622	0.273	0.463	0.708	0.478	0.488	0.689	0.619	0.053
	He	0.895	0.88	0.816	0.808	0.904	0.761	0.616	0.822	0.826	0.77	0.854	0.814	0.024
	HW	**	***	***	***	***	***	***	**	***	***	***	***	
Southern Province	N	15	7	6	10	15	1	14	4	5	11	12	9.091	1.443
	Na	11	6	5	4	13	1	4	4	5	9	9	6.455	1.082
	Ne	6.818	4.261	3.789	3.39	8.036	1	2.78	2.909	3.125	5.902	4.364	0.683	0.072
	Ho	0.867	0.857	0.833	0.7	0.733	0	0.643	0.75	0.4	0.455	0.667	0.628	0.078
	He	0.853	0.765	0.736	0.705	0.876	0	0.64	0.656	0.68	0.831	0.771	0.683	0.072
	HW	*	ns	ns	ns	*	Mono	*	ns	ns	***	**		
North Region	N	18	15	6	13	15	9	16	8	9	15	14	12.54 5	1.171
	Na	14	14	8	8	11	5	7	8	6	13	11	9.545	0.966
	Ne	9.127	9.184	6.545	5.2	7.5	4.378	2.197	4.923	3.375	6.522	6.323	5.934	0.664
	Ho	0.944	0.8	0.667	0.615	0.733	0.111	0.438	0.625	0.444	0.533	0.786	0.609	0.068
	He	0.89	0.891	0.847	0.808	0.867	0.772	0.545	0.797	0.704	0.847	0.842	0.801	0.03
	HW	ns	ns	ns	**	ns	**	ns	ns	*	***	ns		
Central Region	N	30	23	15	30	32	13	39	16	15	28	33	24.90 9	2.692
	Na	12	14	9	8	16	5	10	12	11	14	11	11.09 1	0.929
	Ne	8.145	6.491	4.018	4.945	9.752	3.25	2.873	5.447	6.338	3.401	6.6	5.569	0.652
	Ho	0.867	0.739	0.733	0.8	0.563	0.385	0.462	0.75	0.467	0.464	0.636	0.624	0.049
	He	0.877	0.846	0.751	0.798	0.897	0.692	0.652	0.816	0.842	0.706	0.848	0.793	0.025
	HW	**	ns	*	***	***	***	***	ns	**	***	***		
South Region	N	13	6	5	9	13	1	13	4	4	9	10	7.909	1.261
	Na	8	6	5	4	12	1	3	4	5	8	9	5.909	0.939
	Ne	6.818	4.261	3.789	3.39	8.036	1	2.78	2.909	3.125	5.902	4.364	0.683	0.072
	Ho	0.846	0.833	0.8	0.667	0.769	0	0.692	0.75	0.5	0.444	0.7	0.637	0.074
	He	0.825	0.778	0.74	0.698	0.876	0	0.589	0.656	0.75	0.84	0.815	0.688	0.073
	HW	ns	ns	ns	ns	*	Mono	ns	ns	ns	***	*		
Chatham Rise	N	30	23	15	30	32	13	39	16	15	28	33	4.727	0.648
	Na	12	14	9	8	16	5	10	12	11	14	11	5.364	0.812
	Ne	8.145	6.491	4.018	4.945	9.752	3.25	2.873	5.447	6.338	3.401	6.6	5.569	0.652
	Ho	0.867	0.739	0.733	0.8	0.563	0.385	0.462	0.75	0.467	0.464	0.636	0.623	0.106
	He	0.877	0.846	0.751	0.798	0.897	0.692	0.652	0.816	0.842	0.706	0.848	0.689	0.053
	HW	**	ns	*	***	***	***	***	ns	**	***	***		
Campbell Plateau	N	14	7	6	9	15	1	14	4	4	10	11	8.636	1.403
	Na	11	6	5	4	13	1	4	4	5	8	9	6.364	1.064
	Ne	7.259	4.261	3.789	3.176	8.036	1.000	2.780	2.909	4.000	5.128	3.967	4.210	0.604
	Ho	0.857	0.857	0.833	0.667	0.733	0	0.643	0.750	0.500	0.500	0.636	0.634	0.074
	He	0.862	0.765	0.736	0.685	0.876	0	0.640	0.656	0.750	0.805	0.748	0.684	0.072
	HW	*	ns	ns	ns	*	Mono	*	ns	ns	**	*		
Challenger Plateau	N	8	6	3	9	6	6	6	4	5	9	8	6.364	0.592
	Na	9	7	6	6	6	3	6	4	2	11	8	6.182	0.784
	Ne	5.333	6.000	6.000	4.765	4.500	2.571	2.667	2.909	1.471	10.12 5	4.129	4.588	0.712
	Ho	1.000	0.833	1.000	0.444	0.500	0	0.500	1.000	0.400	0.556	0.750	0.635	0.095
	He	0.813	0.833	0.833	0.790	0.778	0.611	0.625	0.656	0.320	0.901	0.758	0.720	0.049
	HW	ns	ns	ns	*	ns	**	ns	ns	ns	*	ns		
Kermadec Ridge	N	8	7	2	3	6	3	8	4	3	4	4	4.727	0.648
	Na	10	10	2	5	6	3	3	5	4	4	7	5.364	0.812
	Ne	8.533	8.167	2.000	4.500	4.000	2.571	1.471	4.571	3.000	2.286	6.400	4.318	0.736
	Ho	0.875	0.857	0	1.000	1.000	0.333	0.375	0.250	0.667	0.500	1.000	0.623	0.106
	He	0.883	0.878	0.500	0.778	0.750	0.611	0.320	0.781	0.667	0.563	0.844	0.689	0.053
	HW	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns		

N = number of individuals successfully amplified, Na = number of different alleles, Ne = number of effective alleles, Ho = observed heterozygosity, He = expected heterozygosity, HW = test of deviation from Hardy–Weinberg equilibrium. Geomorphic feature groups were only included in this table when the number of individuals successfully amplified was larger than 4. Significant values of $p > 0.05$ are marked as ns, $p < 0.05$ are marked as *, $p < 0.01$ are marked as **, and $p < 0.001$ are marked as ***. Mono means the locus is monomorphic.

6.3.2.3. Population genetic structure

For all three coral species there was very strong concordance in AMOVA results for the reduced and for the all loci data sets, indicating that the presence or absence of a signal of genetic differentiation is conserved across all surveyed loci (Table 6.12). The AMOVA of genetic structure for *G. dumosa* detected large-scale structure amongst north, central, south regions and amongst the geomorphic features, but not between northern and southern provinces. The only AMOVA evidence of genetic structure in *M. oculata* was amongst the north, central and south regions (Table 6.13). The AMOVA for *S. variabilis* detected large-scale structure between northern and southern provinces, amongst north, central, south regions and amongst the geomorphic features. Notably, a north-central-south regional differentiation was found in all three species for both data sets (Table 6.14).

For all three coral species, tests of differentiation (F_{ST} values) amongst populations from the geomorphic features for both the reduced and all loci data sets showed similar patterns (Tables 6.14-6.16). In *G. dumosa*, the Kermadec Ridge population was significantly different from the Chatham Rise population. In *M. oculata*, no significant pairwise F_{ST} values were observed for pairwise testing amongst all (Campbell Plateau, Challenger Plateau, Chatham Rise and Kermadec Ridge) populations (data not shown). For *S. variabilis* pairwise population F_{ST} values revealed no significant genetic differences between the Bounty Trough population and all other populations, and the Kermadec Ridge and Chatham Rise populations.

Table 6.11 Basic population genetic information of microsatellite data at three spatial scales for *Solenosmilia variabilis*

Population	Locus	B106	C107	D5	B1	B116	D103	B123	D213
Northern Province	N	163	159	160	162	148	135	161	160
	Na	9	17	19	19	14	7	13	22
	Ne	2.632	4.28	5.411	4.661	3.932	2.331	1.979	5.156
	Ho	0.957	0.736	0.788	0.901	0.682	0.548	0.304	0.988
	He	0.62	0.766	0.815	0.785	0.746	0.571	0.495	0.806
	HWE	***	***	***	***	***	***	***	***
Southern Province	N	38	37	38	39	35	32	38	39
	Na	5	12	13	11	7	5	9	12
	Ne	2.283	5.099	6.639	3.441	3.895	3.205	1.807	4.5
	Ho	0.895	0.946	0.737	0.744	0.514	0.625	0.289	0.949
	He	0.562	0.804	0.849	0.709	0.743	0.688	0.447	0.778
	HWE	***	***	*	***	***	**	***	***
North Region	N	80	79	79	79	70	68	80	77
	Na	8	13	18	17	13	7	12	21
	Ne	3.197	4.411	5.33	7.048	4.048	2.169	2.162	6.566
	Ho	0.925	0.709	0.785	0.962	0.729	0.5	0.313	0.987
	He	0.687	0.773	0.812	0.858	0.753	0.539	0.538	0.848
	HWE	***	***	***	***	***	***	***	***
Central Region	N	83	80	81	83	78	67	81	83
	Na	7	14	14	13	9	6	10	9
	Ne	2.176	4.057	5.166	3.191	3.672	2.483	1.808	4.135
	Ho	0.988	0.763	0.79	0.843	0.641	0.597	0.296	0.988
	He	0.54	0.754	0.806	0.687	0.728	0.597	0.447	0.758
	HWE	***	***	***	***	***	***	***	***
South Region	N	38	37	38	39	35	32	38	39
	Na	5	12	13	11	7	5	9	12
	Ne	2.283	5.099	6.639	3.441	3.895	3.205	1.807	4.5
	Ho	0.895	0.946	0.737	0.744	0.514	0.625	0.289	0.949
	He	0.562	0.804	0.849	0.709	0.743	0.688	0.447	0.778
	HWE	***	***	*	***	***	**	***	***
Chatham Rise	N	83	80	81	83	78	67	81	83
	Na	7	14	14	13	9	6	10	9
	Ne	2.176	4.057	5.166	3.191	3.672	2.483	1.808	4.135
	Ho	0.988	0.763	0.79	0.843	0.641	0.597	0.296	0.988
	He	0.54	0.754	0.806	0.687	0.728	0.597	0.447	0.758
	HWE	***	***	***	***	***	***	***	***
Kermadec Ridge	N	40	39	39	39	35	33	39	40
	Na	7	9	15	15	8	7	10	17
	Ne	2.576	4.702	4.061	7.141	2.984	2.679	2.227	4.769
	Ho	0.925	0.795	0.744	0.923	0.629	0.515	0.282	0.975
	He	0.612	0.787	0.754	0.86	0.665	0.627	0.551	0.79
	HWE	*	***	***	***	**	***	***	***
Louisville Seamount Chain	N	35	35	35	35	30	30	36	32
	Na	6	9	13	12	10	5	8	15
	Ne	4.077	3.137	5.889	5.53	4.455	1.654	1.799	9.143
	Ho	0.943	0.571	0.8	1	0.833	0.433	0.278	1
	He	0.755	0.681	0.83	0.819	0.776	0.396	0.444	0.891
	HWE	***	ns	***	***	***	ns	***	***
Macquarie Ridge	N	15	13	15	15	13	12	14	15
	Na	2	8	10	6	5	5	5	7
	Ne	2	4.39	7.143	2.813	3.976	3.31	1.867	4.245
	Ho	1	0.923	0.867	1	0.462	0.583	0.286	1
	He	0.5	0.772	0.86	0.644	0.749	0.698	0.464	0.764
	HWE	***	*	ns	***	**	*	ns	***
Bounty Trough	N	5	5	5	5	5	5	5	5
	Na	3	5	5	5	4	4	2	5
	Ne	2.381	3.571	3.571	3.846	3.333	2.381	1.22	3.571
	Ho	1	1	0.6	0.6	0.6	0.4	0.2	1
	He	0.58	0.72	0.72	0.74	0.7	0.58	0.18	0.72
	HWE	ns	ns	ns	ns	ns	ns	ns	ns
Bounty Plateau	N	4	4	4	4	4	4	4	4
	Na	3	3	4	4	2	4	2	2
	Ne	2.909	2.462	3.2	2.286	1.6	3.556	1.28	2
	Ho	0.75	1	0.5	0.5	0.5	0.75	0.25	1
	He	0.656	0.594	0.688	0.563	0.375	0.719	0.219	0.5
	HWE	ns	ns	ns	ns	ns	ns	ns	ns
Hikurangi Margin	N	4	4	4	4	4	4	4	4
	Na	2	4	3	6	3	3	5	3
	Ne	2	3.2	2.462	4.571	2.909	2.133	4	2.462
	Ho	1	1	1	1	0.75	0.75	0.75	1
	He	0.5	0.688	0.594	0.781	0.656	0.531	0.75	0.594
	HWE	ns	ns	ns	ns	ns	ns	ns	ns
Campbell Plateau	N	8	8	8	8	7	7	8	8
	Na	2	5	6	4	4	4	4	7
	Ne	1.969	3.556	2.723	2.246	3.63	2.882	1.488	5.12
	Ho	0.875	0.875	0.75	0.375	0.857	0.857	0.25	0.875
	He	0.492	0.719	0.633	0.555	0.724	0.653	0.328	0.805
	HWE	ns	ns	ns	ns	ns	ns	ns	ns

Continues

Population	Locus	C4	SVA1	SVA3	SVA7	SVA2	SVA14	SVA15	SVA4	SVA12
Northern Province	N	159	132	132	123	133	88	159	117	70
	Na	11	19	22	18	12	22	22	26	20
	Ne	1.398	3.242	6.333	5.887	6.676	14.821	3.956	8.197	5.847
	Ho	0.245	0.598	0.659	0.943	0.677	0.727	0.887	0.846	0.471
	He	0.285	0.692	0.842	0.83	0.85	0.933	0.747	0.878	0.829
	HWE	***	***	***	***	***	***	***	***	***
Southern Province	N	36	34	28	24	37	15	37	33	17
	Na	6	12	11	10	9	13	13	18	12
	Ne	1.624	2.953	6.817	7.432	4.265	9.375	5.024	10.573	7.049
	Ho	0.25	0.588	0.75	0.917	0.595	0.667	0.892	0.909	0.588
	He	0.384	0.661	0.853	0.865	0.766	0.893	0.801	0.905	0.858
	HWE	***	***	ns	ns	ns	ns	***	***	**
North Region	N	80	71	77	74	72	53	80	60	38
	Na	8	18	16	16	10	21	14	16	14
	Ne	1.298	4.211	5.01	5.77	7.175	13.872	3.514	7.385	7.166
	Ho	0.213	0.648	0.623	0.959	0.708	0.868	0.9	0.783	0.553
	He	0.23	0.763	0.8	0.827	0.861	0.928	0.715	0.865	0.86
	HWE	***	***	***	***	***	***	***	***	***
Central Region	N	79	61	55	49	61	35	79	57	32
	Na	7	12	18	12	12	18	18	22	13
	Ne	1.505	2.442	8.013	5.965	5.742	11.557	4.292	8.483	4.163
	Ho	0.278	0.541	0.709	0.918	0.639	0.514	0.873	0.912	0.375
	He	0.335	0.59	0.875	0.832	0.826	0.913	0.767	0.882	0.76
	HWE	***	**	***	***	***	***	***	***	***
South Region	N	36	34	28	24	37	15	37	33	17
	Na	6	12	11	10	9	13	13	18	12
	Ne	1.624	2.953	6.817	7.432	4.265	9.375	5.024	10.573	7.049
	Ho	0.25	0.588	0.75	0.917	0.595	0.667	0.892	0.909	0.588
	He	0.384	0.661	0.853	0.865	0.766	0.893	0.801	0.905	0.858
	HWE	***	***	ns	ns	ns	ns	***	***	**
Chatham Rise	N	79	61	55	49	61	35	79	57	32
	Na	7	12	18	12	12	18	18	22	13
	Ne	1.505	2.442	8.013	5.965	5.742	11.557	4.292	8.483	4.163
	Ho	0.278	0.541	0.709	0.918	0.639	0.514	0.873	0.912	0.375
	He	0.335	0.59	0.875	0.832	0.826	0.913	0.767	0.882	0.76
	HWE	***	**	***	***	***	***	***	***	***
Kermadec Ridge	N	38	35	36	35	34	28	39	31	21
	Na	7	10	12	12	9	18	12	14	7
	Ne	1.282	1.989	4.662	6.447	6.165	13.635	3.723	7.364	4.594
	Ho	0.211	0.486	0.639	1	0.735	0.857	0.949	0.839	0.476
	He	0.22	0.497	0.785	0.845	0.838	0.927	0.731	0.864	0.782
	HWE	***	***	***	***	ns	ns	***	**	***
Louisville Seamount Chain	N	37	31	36	34	34	23	36	25	13
	Na	4	16	13	11	10	15	11	9	10
	Ne	1.284	9.856	3.216	4.817	5.766	9.281	3.256	6.25	5.729
	Ho	0.189	0.871	0.583	0.912	0.735	0.87	0.833	0.72	0.692
	He	0.221	0.899	0.689	0.792	0.827	0.892	0.693	0.84	0.825
	HWE	***	***	***	***	***	ns	ns	*	***
Macquarie Ridge	N	13	11	7	6	15	2	14	12	6
	Na	5	6	7	6	6	3	9	10	8
	Ne	1.965	2.521	5.444	4.8	3.516	2.667	5.851	8.727	6.545
	Ho	0.385	0.636	0.714	1	0.467	0.5	0.786	0.833	0.667
	He	0.491	0.603	0.816	0.792	0.716	0.625	0.829	0.885	0.847
	HWE	ns	ns	ns	ns	*	ns	***	**	ns
Bounty Trough	N	5	5	5	4	5	4	5	5	3
	Na	3	5	6	5	4	5	2	6	3
	Ne	1.515	2.5	4.545	4	3.846	3.2	2	5.556	2.571
	Ho	0.4	0.6	0.8	1	0.4	0.75	1	1	0.333
	He	0.34	0.6	0.78	0.75	0.74	0.688	0.5	0.82	0.611
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	ns
Bounty Plateau	N	4	4	4	4	4	3	4	4	3
	Na	1	3	5	6	5	3	4	4	5
	Ne	1	2.462	4.571	5.333	4	2.571	3.556	3.556	4.5
	Ho	0	0.5	0.75	1	0.75	0.333	1	1	1
	He	0	0.594	0.781	0.813	0.75	0.611	0.719	0.719	0.778
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	ns
Hikurangi Margin	N	4	4	4	4	3	2	4	3	3
	Na	2	2	6	4	3	4	3	3	3
	Ne	1.28	1.28	5.333	3.2	2.571	4	2.462	2.571	2
	Ho	0.25	0.25	0.75	1	0.333	1	1	0.667	0.667
	He	0.219	0.219	0.813	0.688	0.611	0.75	0.594	0.611	0.5
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	ns
Campbell Plateau	N	8	8	8	8	8	4	8	7	4
	Na	2	5	6	8	7	7	6	8	5
	Ne	1.133	2	3.2	5.818	4.923	6.4	3.282	6.125	4
	Ho	0.125	0.375	0.75	0.75	0.875	0.75	1	1	0.5
	He	0.117	0.5	0.688	0.828	0.797	0.844	0.695	0.837	0.75
	HWE	ns	ns	ns	ns	ns	ns	ns	ns	ns

Continues

Population	Locus	SVA16	SVA13	SVA11	SVA10	SVA5	SVA18	SVA8	Mean	SE
Northern Province	N	102	153	126	119	102	133	101	133.208	5.414
	Na	13	15	22	23	12	31	26	18.083	1.21
	Ne	2.459	2.689	3.967	9.824	7.368	7.125	8.064	5.343	0.612
	Ho	0.539	0.732	0.984	0.882	0.98	0.639	0.545	0.719	0.043
	He	0.593	0.628	0.748	0.898	0.864	0.86	0.876	0.748	0.031
	HWE	***	***	***	***	***	***	***		
Southern Province	N	24	36	30	28	23	28	23	31.208	1.455
	Na	10	7	8	14	10	14	17	10.75	0.695
	Ne	3.282	2.374	2.932	8.909	7.149	6.73	10.373	5.322	0.556
	Ho	0.583	0.778	1	0.857	1	0.643	0.696	0.725	0.042
	He	0.695	0.579	0.659	0.888	0.86	0.851	0.904	0.75	0.029
	HWE	***	***	***	*	***	***	ns		
North Region	N	58	76	64	69	56	72	53	69.375	2.282
	Na	11	15	18	21	11	23	23	15.167	0.959
	Ne	2.475	3.447	5.543	10.206	7.024	7.145	8.946	5.63	0.585
	Ho	0.534	0.737	0.984	0.899	0.964	0.681	0.604	0.732	0.043
	He	0.596	0.71	0.82	0.902	0.858	0.86	0.888	0.762	0.032
	HWE	***	***	***	***	***	***	***		
Central Region	N	44	77	62	50	46	61	48	63.833	3.343
	Na	11	8	13	17	11	23	21	13.25	0.984
	Ne	2.344	2.103	2.815	8.803	7.425	6.615	6.847	4.825	0.543
	Ho	0.545	0.727	0.984	0.86	1	0.59	0.479	0.702	0.045
	He	0.573	0.524	0.645	0.886	0.865	0.849	0.854	0.721	0.032
	HWE	***	***	***	***	***	***	***		
South Region	N	24	36	30	28	23	28	23	31.208	1.455
	Na	10	7	8	14	10	14	17	10.75	0.695
	Ne	3.282	2.374	2.932	8.909	7.149	6.73	10.373	5.322	0.556
	Ho	0.583	0.778	1	0.857	1	0.643	0.696	0.725	0.042
	He	0.695	0.579	0.659	0.888	0.86	0.851	0.904	0.75	0.029
	HWE	***	***	***	*	***	***	ns		
Chatham Rise	N	44	77	62	50	46	61	48	63.833	3.343
	Na	11	8	13	17	11	23	21	13.25	0.984
	Ne	2.344	2.103	2.815	8.803	7.425	6.615	6.847	4.825	0.543
	Ho	0.545	0.727	0.984	0.86	1	0.59	0.479	0.702	0.045
	He	0.573	0.524	0.645	0.886	0.865	0.849	0.854	0.721	0.032
	HWE	***	***	***	***	***	***	***		
Kermadec Ridge	N	31	37	28	33	30	36	28	34.333	0.992
	Na	8	12	9	16	11	18	17	11.667	0.768
	Ne	2.379	3.271	2.872	8.25	7.004	6.529	8.385	4.987	0.571
	Ho	0.548	0.811	1	0.933	0.667	0.667	0.571	0.73	0.047
	He	0.58	0.694	0.652	0.879	0.857	0.847	0.881	0.73	0.033
	HWE	***	***	***	***	ns	***	**		
Louisville Seamount Chain	N	23	34	32	32	23	31	22	30.583	1.231
	Na	8	11	15	15	9	15	15	11.042	0.703
	Ne	2.625	3.676	8.569	5.721	5.688	6.744	6.769	5.205	0.495
	Ho	0.522	0.676	0.969	0.781	1	0.71	0.591	0.73	0.045
	He	0.619	0.728	0.883	0.825	0.824	0.852	0.852	0.744	0.035
	HWE	***	ns	**	***	ns	ns	***		
Macquarie Ridge	N	7	14	10	8	6	8	4	10.625	0.823
	Na	6	3	3	7	8	7	3	6.042	0.452
	Ne	4.667	2.074	2.198	5.333	6.545	6.095	2.667	4.223	0.391
	Ho	0.571	0.857	1	0.75	1	0.75	0	0.71	0.054
	He	0.786	0.518	0.545	0.813	0.847	0.836	0.625	0.709	0.027
	HWE	ns	*	*	ns	ns	ns	*		
Bounty Trough	N	5	5	4	4	5	5	4	4.708	0.112
	Na	7	3	3	5	6	6	5	4.458	0.276
	Ne	4.545	1.852	2.462	4	5	4.167	4.571	2.381	0.233
	Ho	0.8	0.6	1	1	1	0.6	0.5	1	0.054
	He	0.78	0.46	0.594	0.75	0.8	0.76	0.781	0.58	0.032
	HWE	ns	ns	ns	ns	ns	ns	ns		
Bounty Plateau	N	4	4	4	4	4	4	4	3.917	0.058
	Na	2	2	5	7	6	5	5	3.833	0.317
	Ne	1.28	1.28	4.571	6.4	4.571	4	4.571	3.23	0.295
	Ho	0.25	0.25	1	1	1	0.5	1	0.691	0.065
	He	0.219	0.219	0.781	0.844	0.781	0.75	0.781	0.602	0.047
	HWE	ns	ns	ns	ns	ns	ns	ns		
Hikurangi Margin	N	3	4	3	3	3	4	2	3.542	0.134
	Na	2	3	4	5	5	3	4	3.542	0.241
	Ne	1.385	1.684	3.6	4.5	4.5	1.684	4	2.908	0.239
	Ho	0.333	0.5	1	1	1	0.5	1	0.771	0.056
	He	0.278	0.406	0.722	0.778	0.778	0.406	0.75	0.592	0.037
	HWE	ns	ns	ns	ns	ns	ns	ns		
Campbell Plateau	N	7	8	8	8	7	8	8	7.458	0.233
	Na	5	5	6	10	8	7	9	5.833	0.411
	Ne	2.227	2.783	3.368	8	7	4.741	6.737	3.973	0.39
	Ho	0.571	0.875	1	0.875	1	0.625	0.875	0.736	0.051
	He	0.551	0.641	0.703	0.875	0.857	0.789	0.852	0.676	0.037
	HWE	ns	ns	ns	ns	ns	ns	ns		

N = number of individuals successfully amplified, Na = number of different alleles, Ne = number of effective alleles, Ho = observed heterozygosity, He = expected heterozygosity, HWE = test of deviation from Hardy–Weinberg equilibrium. Geomorphic feature groups were only included in this table when the number of individuals successfully amplified was larger than 4. Significant values of $p > 0.05$ are marked as ns, $p < 0.05$ are marked as *, $p < 0.01$ are marked as **, and $p < 0.001$ are marked as ***. Mono means the locus is monomorphic.

Table 6.12 Hierarchical AMOVA results for *Goniocorella dumosa* when tested for microsatellite genetic variation

Source of variation	All loci			Neutral loci only		
	df	ss	Var. comp.	df	ss	Var. comp.
Between Provinces	1	9.373	0.000	1	3.629	0.000
Amongst Individuals	106	1058.678	2.390**	106	422.144	0.769**
Within Individuals	108	562.500	5.208**	108	264.000	2.444**
Total	215	1630.551	7.598	215	689.773	3.213
Amongst Regions	2	27.947	0.088*	2	10.180	0.025*
Amongst Individuals	105	1040.104	2.349**	105	415.593	0.757**
Within Individuals	108	562.500	5.208**	108	264.000	2.444**
Total	215	1630.551	7.645	215	689.773	3.226
Amongst Geomorphic Features	3	0.196	0.196**	3	16.653	0.063**
Amongst Individuals	96	2.318	2.318**	96	377.757	0.740**
Within Individuals	100	5.240	5.240**	100	245.500	2.455**
Total	199	7.754	7.754	199	639.910	3.258

Significant values of $p < 0.05$ are marked as *, and $p < 0.01$ are marked as **.

Table 6.13 Hierarchical AMOVA results for *Madrepora oculata* when tested for microsatellite genetic variation

Source of variation	All loci			Neutral loci only		
	df	ss	Var. comp.	df	ss	Var. comp.
Between Provinces	1	6.420	0.013	1	3.234	0.004
Amongst Individuals	91	507.634	1.926**	91	269.846	0.934**
Within Individuals	93	160.500	1.726**	93	102	1.097**
Total	185	674.554	3.665	185	375.081	2.035
Amongst Regions	2	13.474	0.022*	2	7.581	0.016*
Amongst Individuals	90	500.580	1.918**	90	265.5	0.927**
Within Individuals	93	160.500	1.726**	93	102	1.097**
Total	185	674.554	3.666	185	375.081	2.039
Amongst Geomorphic Features	3	18.763	0.017	3	11.377	0.029
Amongst Individuals	55	324.898	1.886**	55	174.724	0.893**
Within Individuals	59	126.000	2.136**	59	82.000	1.390**
Total	117	469.661	4.038	117	268.102	2.312

Significant values of $p < 0.05$ are marked as *, and $p < 0.01$ are marked as **.

Table 6.14 Hierarchical AMOVA results for *Solenosmilia variabilis* when tested for microsatellite genetic variation

Source of variation	All			Neutral only		
	df	ss	Var. comp.	df	ss	Var. comp.
Between Provinces	1	16.070	0.038*	1	11.039	0.044**
Amongst Individuals	206	2322.236	2.207**	206	1112.833	0.700**
Within Individuals	208	1426.500	6.858**	208	832.500	4.002**
Total	415	3764.805	9.103	415	1956.373	4.747
Amongst Regions	2	52.773	0.115**	2	32.454	0.083**
Amongst Individuals	205	2285.532	2.145**	205	1091.419	0.661**
Within Individuals	208	1426.500	6.858**	208	832.500	4.002**
Total	415	3764.805	9.119	415	1956.373	4.746
Amongst Geomorphic Features	7	151.751	0.259**	7	80.076	0.150**
Amongst Individuals	192	2080.067	1.971**	192	989.109	0.570**
Within Individuals	200	1378.500	6.893**	200	802.500	4.013**
Total	399	3610.318	9.122	399	1871.685	4.732

Significant values of $p < 0.05$ are marked as *, and $p < 0.01$ are marked as **.

Table 6.15 Pairwise F_{ST} values for all (below diagonal) and neutral (above diagonal) loci amongst *Goniocorella dumosa* populations

	NE Slope	Challenger Plateau	Chatham Rise	Kermadec Ridge
NE Slope		-0.018	0.033	0.009
Challenger Plateau	-0.047		-0.006	0.015
Chatham Rise	0.001	-0.014		0.044*
Kermadec Ridge	-0.012	0.003	0.024*	

Table 6.16 Pairwise population genetic differentiation (F_{ST}) of *Madrepora oculata* in microsatellites

	Chatham Rise	Kermadec Ridge	Challenger Plateau	Campbell Plateau
Chatham Rise	0.000	-0.016	-0.011	0.007
Kermadec Ridge		0.000	-0.025	0.021
Challenger Plateau			0.000	-0.002
Campbell Plateau				0.000

Significant values of $p < 0.05$ are marked as *, and $p < 0.01$ are marked as **.

Table 6.17 Pairwise F_{ST} values for all (below diagonal) and neutral (above diagonal) amongst *Solenosmilia variabilis* populations

	Bollons Seamount	Bounty Plateau	Bounty Trough	Campbell Plateau	Chatham Rise	Hikuran gi Margin	Kermade c Ridge	Louisville Seamount	Macquari e Ridge	Tasman Basin
Bollons Seamount	0	-0.11	0.007	-0.046	-0.057	0.049	-0.001	0.038	-0.077	-0.086
Bounty Plateau	-0.121	0	-0.045	-0.083	-0.058	-0.038	-0.016	0.027	-0.076	-0.067
Bounty Trough	-0.023	-0.070	0	-0.024	0.015	-0.055	-0.026	0.046*	0.028**	0.045
Campbell Plateau	-0.025	-0.073	-0.041	0	-0.015	-0.045	0.003	0.058**	-0.024	-0.007
Chatham Rise	-0.038	-0.053	-0.006	-0.013	0	0.034	0.024**	0.070**	-0.013	-0.005
Hikurangi Margin	0.062	-0.031	-0.057	-0.056	0.022*	0	-0.011	0.068*	0.045*	0.070
Kermadec Ridge	-0.008	-0.027	-0.031	-0.001	0.016**	-0.004	0	0.036**	0.036**	0.039
Louisville Seamount	0.035	0.020	0.037	0.057**	0.055**	0.077*	0.029**	0	0.076**	0.042
Macquarie Ridge	-0.049	-0.069	-0.005	-0.030	-0.013	0.014*	0.020*	0.061**	0	-0.028
Tasman Basin	-0.091	-0.092	-0.010	-0.037	-0.019	0.022	0.005	0.041*	-0.047	0

Significant values of $p < 0.05$ are marked as *, and $p < 0.01$ are marked as **.

6.3.2.4. Isolation by distance and isolation by depth

Tests for isolation by distance and by depth identified only two significant results - in *S. variabilis* for distance and in *G. dumosa* for depth (Table 6.8).

6.3.3. Post-hoc analyses

6.3.3.1. Geographic distribution

Microsatellite variation revealed the presence of multiple genetic groups within each of the three corals. In *G. dumosa*, the DAPC scatter plot (Figure 6.4) and the Structure Harvester plot (Figure S6.3) provided evidence of more than one population, but the number of putative genetic clusters was inconsistent between Structure ($K=2$) and DAPC ($K=3$, Northeast Slope, Kermadec Ridge and the rest of populations). In *M. oculata*, DAPC revealed that the Campbell Plateau and Chatham Rise populations were genetically similar to each other (Figure 6.5). The Structure Harvester plot indicated the presence of three groups (Figure S6.3). For *S. variabilis*, the DAPC differentiated *S. variabilis* into 5 genetic clusters, Campbell Plateau, Bounty Plateau, Bounty Trough, Louisville Seamount Chain, and all other populations (Figure 6.6). Depending on the Structure Harvester results used to determine the

number of populations, $K=3$ (based on ΔK) was the best grouping cluster number (Figure S6.3). All analyses provided support for the existence of a differentiated Louisville Seamount Chain population.

Figure 6.4 Scatterplot (above) and complot (below) of DAPC of *Goniocorella dumosa* based on variation at all microsatellite loci (60 PCs and 2 DAs)

Figure 6.5 Scatterplot (above) and complot (below) of DAPC of *Madrepora oculata* based on variation at all microsatellite loci (60 PCs and 2 DAs)

Figure 6.6 Scatterplot (above) and complot (below) of DAPC of *Solenosmilia variabilis* based on variation at all microsatellite loci (154 PCs and 2 DAs)

6.3.3.2. Barriers to gene flow

For *G. dumosa*, BARRIER software predicted that barriers to gene flow may exist for the Challenger Plateau and for the Chatham Rise populations (Figure 6.7A). In *M. oculata*, because there were no significant pairwise F_{ST} values calculated from the microsatellite data sets, the barriers to gene flow were predicted from the Φ_{ST} values of the *ITS* sequence, which indicated that the Kermadec Ridge (to the north) and Chatham Rise populations are isolated (Figure 6.7B). There was generally very good agreement for the location of barriers to gene flow amongst populations of *S. variabilis* based on Φ_{ST} values of the *ITS* sequence (Figure 6.7C) and F_{ST} values of the full microsatellite data set (Figure 6.7D). In both instances, four barriers to gene flow were predicted to occur, isolating the far north (Kermadec Ridge), the far east (Louisville Seamount Chain), the east (Chatham Rise), the south central (Campbell Plateau, Bounty Plateau, Bounty Trough), and the far south (Macquarie Ridge) populations. Comparisons of the locations of barriers to gene flow amongst species are somewhat limited by differences in sampling coverage for the three corals, but where spatial coverage overlaps, barriers to gene flow are common (Figure 6.7). In particular, regions such as the Kermadec Ridge and the Chatham Rise are identified as being bounded by barriers to gene flow, and

other regions such as the south central (Campbell Plateau, Bounty Plateau, Bounty Trough) and also the far south (Macquarie Ridge) are also bounded by barriers. The status of populations on the Louisville Seamount Chain (to the east, outside the NZ EEZ) is hard to determine given that there only have samples of *S. variabilis* from this region, but there is strong evidence of its isolation based on two marker types (Figure 6.7).

Figure 6.7 Barriers to gene flow indicated by software *Barriers* based on the full microsatellite data set for *Goniocorella dumosa* (A), *ITS* data for *Madrepora oculata* (B), *ITS* data for *Solenosmilia variabilis* (C) and the full microsatellite data set for *Solenosmilia variabilis* (D)

6.3.3.3. Larval migration

For *G. dumosa*, θ values estimated from MIGRATE ranged from 0.012 (Chatham Rise) to 0.962 (NE Slope) for all loci, and from 0.102 (NE Slope) to 0.931 (Challenger Plateau) for the neutral loci data set. All populations showed evidence of high levels of self-

recruitment and there was evidence of only low levels of between-population gene flow in both microsatellite data sets. In both cases, populations on the Kermadec Ridge and the NE Slope were identified as source populations because they contributed but did not receive migrants. Additionally, both sets of analyses agreed that the populations on the Chatham Rise and the Challenger Plateau were not genetically connected with each other (Figure 6.8).

Figure 6.8 Circular plots of migration (gene flow) between and within populations of *Goniocorella dumosa* on geomorphic features based on all microsatellite loci (left) and neutral microsatellite loci (right)

For *M. oculata*, θ values estimated from the *ITS* data set ranged from 0.004 (Chatham Rise) to 0.265 (Bounty Plateau). Estimates of per generation migrants were variable amongst sample locations and suggested low to moderate gene flow. All populations showed evidence of some self-recruitment. The populations on the Kermadec Ridge and on the Chatham Rise did not exchange migrants with other populations. The Campbell Plateau and Bounty Plateau populations were well connected with each other (Figure 6.9).

Figure 6.9 Circular plots of migration (gene flow) between and within populations of *Madrepora oculata* on geomorphic features based on *ITS* sequence data

For *S. variabilis*, θ values ranged from 0.076 (Bounty Trough) to 0.136 (Hikurangi Margin) in the full microsatellite dataset, and from 0.183 (Hikurangi Margin) to 1.880 (Macquarie Ridge) in the neutral microsatellite data set. Estimates of numbers of migrants per generation were variable amongst samples, and all populations showed evidence of medium to high levels of self-recruitment in both data sets (Figure 6.9). The Macquarie Ridge population made no contribution to the populations in the north (Kermadec Ridge, Louisville Seamount Chain and Hikurangi Margin), and the Louisville Seamount Chain population did not contribute migrants to the Macquarie Ridge population. Populations such as those on the Kermadec Ridge and the Bounty Trough were identified as being sources that contributed migrants to other populations but received limited or no migrants, whereas the Louisville Seamount Chain population was identified as a sink that received migrants from other populations but did not contribute to other populations.

Figure 6.10 Circular plots of migration (gene flow) between and within populations of *Solenosmilia variabilis* on geomorphic features based on *ITS* sequence data (top), all microsatellite loci (bottom left) and neutral microsatellite loci (bottom right)

6.4. Discussion

Three habitat-forming deep-sea corals, *G. dumosa*, *M. oculata* and *S. variabilis*, were examined for genetic connectivity and gene flow amongst populations in the New Zealand region for the purpose of contributing to effective spatial management planning options to protect VMEs. An *a priori* hierarchical and spatially explicit approach was employed to hypothesis testing for population genetic structure, and identified barriers to gene flow as

well as source and sink populations, all of which may contribute significantly to management decisions with conservation outcomes in the deep sea (e.g., Boschen *et al.*, 2016). The testing framework is based on knowledge of environmental, and at times biological, differences between regions within and beyond the New Zealand EEZ, and also the possible influence of physical oceanographic currents that may influence genetic connectivity amongst geomorphic features such as ridges, plateaux and troughs. In addition, to maximise genetic information return, an approach that uses both a subset of putatively neutral microsatellite loci and also all loci was employed, regardless of possible causes of deviation from neutrality. This approach, whilst not common practice, has been shown to be of value (e.g., Wei *et al.*, 2013; Gagnaire *et al.*, 2015), in particular when loci that would ordinarily be discarded are informative, despite not meeting neutral expectations. The results for the full locus data set and for neutral loci subset are very much in agreement, and therefore provide some degree of independent confirmation of the findings.

6.4.1. Influence of sample sizes and markers on structures detected

As previously discussed for sponges in Chapter 5, small sample sizes for the three corals examined here are a challenge for population genetics studies. In practice, an understanding of the population genetics of many deep-sea taxa has to be pursued with less than perfect sample sets (resulting from logistic and cost difficulties of sample collection) because of the current management need to protect biodiversity, and particularly sensitive habitats such as VMEs. Although the limited sampling made it difficult to reveal small-scale population genetic structure, the approach employed here provides a broad spatial-scale view on the connectivity of these coral populations. Small samples sizes and an absence of fine spatial scale sampling are widely acknowledged as inevitable constraints in studies of deep-sea organisms (Boschen *et al.*, 2015, 2016). Although the sample sizes are acknowledged as a limitation that could fail to realise the full extent of genetic diversity within and between populations, they can still reveal information about patterns of genetic structure of sufficient magnitude to be useful to inform management decisions.

Due to low genetic diversity of nuclear (*ITS*) and mitochondrial regions (*16s* and *D-loop*), it found that they had limited application in *S. variabilis* and *M. oculata* population genetics study (Miller *et al.*, 2010). In the present research, genetic diversities of *ITS* and *D-loop* regions were also low, but both of them provided evidence of population structure in all three examined species. While focusing on small-scale structures, sequencing markers were

problematic to draw high resolution patterns. On the other hand, microsatellites, compared to sequences, provide better resolution of genetic structures, connectivity and isolation, but they require a larger sample size. Therefore, in the present study, to obtain a comprehensive understanding of the three examined corals both DNA sequence and microsatellite marker types were considered, and robust results were highlighted when both marker results were in agreement.

As the hierarchical spatial testing increased in definition, so the sample sizes decreased. Thus, the analyses have small sample sizes for most geomorphic features but larger sample sizes for the north-south biogeographic province level of testing. Estimates of, for example, migrants between populations on geomorphic features should be interpreted with care and are best viewed in combination with other analyses. However, larger-scale population groups (provinces and regions) and populations on some geomorphic features (such as the Chatham Rise) had large enough sample sizes to reveal patterns of genetic differentiation and connectivity. Overall, the analyses are probably best viewed as a conservative interpretation of patterns of population genetic differentiation.

Use of the software package STRUCTURE (Falush *et al.*, 2007) to help identify the number of distinct genetic groups (i.e., identification of K) is now widespread in population genetics analyses (e.g., Ruiz-Ramos *et al.*, 2015). STRUCTURE is a parametric analysis, and is known to perform best when sample sizes are equal, when null alleles are absent and when populations are in HWE (Putman & Carbone, 2014; Puechmille, 2016). The analyses using STRUCTURE with no *a priori* groupings did not provide reliable results, most probably because of large differences in population/region sample sizes and the absence of HWE in several samples. Absence of HWE amongst deep-sea corals, in particular the presence of significant heterozygote deficiencies, is routinely reported (Le Goff & Rogers, 2002; Baco *et al.*, 2006; Lunden *et al.*, 2014). One interpretation of multi-locus heterozygote deficiencies is that recruitment in many populations is locally derived with infrequent long-distance dispersal events. Such an interpretation is consistent with the results in this study for the three coral species were examined (see below). Because of the uncertainty associated with the STRUCTURE results, present study has focussed on other analyses for which there is greater robustness and more confirmation from other approaches.

Miller *et al.* (2010) noted that low genetic diversity of nuclear (*ITS*) and mitochondrial regions (*16s* and *D-loop*) limits the utility of these markers in population genetics studies of *S. variabilis* and *M. oculata*. In the present study, genetic diversities of *ITS* and *D-loop* regions were low, and neither marker provided evidence of population structure

in *G. dumosa* or *S. variabilis*, but *ITS* in *M. oculata* revealed a north-central-south regional pattern of differentiation. There were no unique haplotypes in any of the geomorphic feature populations and only four significant Φ_{ST} values were observed between pairs of populations on geomorphic features. These findings for the DNA sequences indicate that insufficient variation exists for these markers types to be informative at the scale of my work. In contrast, microsatellite variation provided much greater resolution of species-specific genetic structure, connectivity and isolation. Where significant results exist for both marker types for the same species they are in agreement (e.g., location of putative barriers to gene flow and estimation of exchange of migrants). In the present study, to obtain a comprehensive understanding of the population genetic structure of the three corals both DNA sequence and microsatellite marker types are considered, and robust results are highlighted when both marker results are in agreement.

6.4.2. Patterns of genetic connectivity

The DNA sequence markers provided only limited evidence of population genetic structure based on the hierarchical hypothesis testing framework, examination of microsatellite variation (full and reduced data sets) for all three species revealed structure at different spatial scales. Populations of *M. oculata* exhibited regional structure, populations of *G. dumosa* were differentiated at the regional and geomorphic features scale, and populations of *S. variabilis* exhibited structure between provinces, amongst regions and also amongst geomorphic features. In other comparable studies little or no evidence of province scale structure has been observed amongst populations of crustaceans and sponges in the New Zealand region (Bors *et al.*, 2012; Chapter 5). Thus, it is concluded that there is little support for the hypothesis that water mass characteristics associated with biogeographic provinces within the New Zealand region result in genetic population structure of *G. dumosa* and *M. oculata*. However, a north-central-south regional pattern of structure was observed in another deep-sea Scleractinian, the cup coral, *Desmophyllum dianthus*, based on *ITS* variation (Miller *et al.*, 2010) and also in the deep-sea sponge *Poecillastra laminaris* based *COI*, *Cytb* and microsatellite variation (Chapter 5). In combination, these findings provide evidence in support of the hypothesis that currents along the Chatham Rise act as a barrier to gene flow for several species across multiple phyla. Significant genetic differentiation has already been reported at the geomorphic feature population level in non-coral species between, for example, the Challenger Plateau and the Chatham Rise (Bors *et al.*, 2012; Knox *et al.*, 2012).

These results, combined with those for the deep-sea corals, support the hypothesis that oceanic dynamics contribute to the formation of fine-scale population structure amongst populations on different geomorphic features within, and most likely also outside, the New Zealand EEZ.

6.4.3. Oceanographic dynamics

The patterns of multi-species genetic differentiation detected by the various markers may be attributable to a relationship between population distributions and oceanic current systems. Currents not only play a role in promoting long-distance transport of larvae, but may also act as barriers to gene flow (Rogers *et al.*, 1998; Zane *et al.*, 1998; Roques *et al.*, 2002; Sutton & Chereskin, 2002; Shaw *et al.*, 2004; Plouviez *et al.*, 2013). For example, currents acting as a ‘soft’ barrier to population connectivity have been indicated for octocorals in a recent study around New Zealand (Dueñas *et al.*, 2016).

In this study, it was hypothesised that currents associated with the Subtropical Front would influence north-central-south regional scale population connectivity, which was indeed observed for all three corals. Surface and intermediate currents flow from northwest New Zealand in a southerly direction to the Chatham Rise following the eastern coastline of the North Island. Currents also flow from the southeast of New Zealand northward along the east coast of the South Island towards the Chatham Rise. The result is that currents from the warmer north and the cooler south meet and mix along the Chatham Rise, forming the Subtropical Front, before heading east into the Pacific Ocean (Chiswell *et al.*, 2015). These two contrasting sets of currents, their associated eddies and the Subtropical Front may therefore impede larval migration between the south and north regions, and provide an explanation for the increased genetic diversity values observed amongst populations on the Chatham Rise (central region) for all three corals and also for some sponges (Chapter 5).

At the geomorphic features level, our analyses of the three corals provided specific evidence for a lack of genetic connectivity between populations on the Kermadec Ridge and the Chatham Rise (significant F_{ST} , clear split in DAPC plots and few if any migrants between these populations). No previous study has investigated connectivity between these two areas, but significant genetic subdivision between populations of *D. dianthus* was detected between the Auckland Island slope (far south) and seamounts on the northern Chatham Rise (Miller *et al.*, 2010). This differentiation is consistent with patterns of flow at depth, with water from the far south flowing north until it reaches the southern edge of the Chatham Rise, before it is

deflected eastward (Chiswell *et al.*, 2015). In other words, migrants from the Auckland Island slope are not expected to reach the northern edge of the Chatham Rise, which will result in some degree of genetic differentiation, which is indeed what is observed. For *S. variabilis*, the most apparent difference between populations on geomorphic features was that between the Louisville Seamount Chain and all other populations. The general lack of current flow from the Louisville Seamount Chain towards most topographic features elsewhere in the New Zealand region (Chiswell & Rickard, 2006) is likely to be the reason that populations of *S. variabilis* on this feature are differentiated genetically from those in the rest of the region.

6.4.4. Depth and associated environmental gradients

The influence of currents on population connectivity is expected to vary with depth, but generally we lack sufficient knowledge about the detail of depth-stratified flow to make robust predictions about how patterns of gene flow may vary with depth, in particular over large spatial scales. Nonetheless, depth is an important factor contributing to patterns of genetic connectivity for some benthic fauna, particularly for taxa that are distributed across a wide depth range (Chapter 2). In the present study, there was no evidence for isolation by depth (across the depth range 322-1805 m) for *S. variabilis*. Populations of *S. variabilis* from Australian waters were also not differentiated by depth, but populations were sampled within a more restricted depth range (1000-1400 m) (Miller & Gunasekera, 2016). For populations of both *G. dumosa* (from 300-600 m) and *M. oculata* (from 700-1200 m) at least one marker revealed the existence of a pattern of isolation by depth. As neither *G. dumosa* nor *M. oculata* populations were genetically isolated by distance, this implies that depth and related environmental gradients may play an important role in the larval migration of these species. Within the New Zealand region Miller *et al.* (2011) reported that the stony cup coral *D. dianthus* from different depth strata (<600 m, 1000–1500 m, >1500 m) are strongly differentiated based on DNA sequence variation. Unfortunately, the limited sample sizes of the present study precluded detailed analysis to determine at what depth(s) genetic differentiation for *G. dumosa* and *M. oculata* populations takes place (i.e., the response may just as easily be a gradient as an abrupt break).

6.4.5. Life history and dispersal strategies

Species-specific differences in reproductive strategies may contribute to differences in patterns of gene flow and genetic structure observed across taxa in the deep sea (e.g., Bors *et al.*, 2012; Samadi *et al.*, 2006; Hilário *et al.*, 2015). All three showed pronounced patterns of self-recruitment which doubtless have contributed to the patterns of genetic structure detected in all three species, as well as the limited connectivity that exist amongst regions. Interestingly, the migration estimates amongst populations on different geomorphic features for the three corals may be related to maximum oocyte size i.e., there is a positive relationship between increasing oocyte size and increasing connectivity (Table 6.18 summarises data for the three corals). *M. oculata* has the largest mean oocyte size (2~3 times larger than other two species) and was the only species for which significant differentiation amongst populations on geomorphic features was not observed, whereas *G. dumosa* and *S. variabilis* with a smaller mean oocyte diameter exhibited less connectivity (i.e., significant structure existed amongst populations on geomorphic features). Based on previous studies, *S. variabilis* and *M. oculata* are lecithotrophic, and they feed only after settlement and metamorphosis (Waller *et al.*, 2005; Pires *et al.*, 2014). Given its similarity to *S. variabilis* in terms of morphology and reproduction, the larvae of *G. dumosa* are likely to share the same characteristics. Based on this assumption, the larger oocyte size, providing more nutritional support, may promote a longer larval life and greater dispersal potential for *M. oculata*. Such a case would explain the MIGRATE result that *M. oculata* had a greater number of migrants amongst the geomorphic populations than either *G. dumosa* or *S. variabilis*, but will, of course, require testing. This result provides further support for the idea that life history characteristics may vary even between closely related deep-sea corals (Burgess & Babcock, 2005) and therefore can influence patterns of population connectivity.

In the present study, none of the three coral species showed strong evidence of asexual reproduction when all specimens were assessed using multi-locus microsatellite genotypes. Whilst there was no direct evidence to indicate asexual reproduction in any of the three species, the smaller effective population size with heterozygote deficits at most loci of *S. variabilis* suggests that this species has a higher asexual or inbreeding rate in populations on geomorphic features than the other two species. Miller & Gunasekera (2016) predicted that clonal reproduction may account for as much as 76% of recruitment in Australian populations of *S. variabilis*, and if applicable in the New Zealand context then this high asexual recruitment rate would explain the self-recruitment result in this study. Asexual reproduction

has also been observed in *G. dumosa*, but the proportion of clonal reproduction in recruitment is unknown, whilst for *M. oculata* there is no evidence for asexual reproduction (Burgess & Babcock, 2005). However, it has been reported that mixed clonal/sexual reproduction is nearly indistinguishable from strict sexual reproduction as long as the proportion of clonal reproduction is not large (Balloux *et al.*, 2003).

Table 6.18 Information about reproductive life-history characteristics for *Goniocorella dumosa*, *Madrepora oculata* and *Solenosmilia variabilis*

Species	Mode of Sexual reproduction	Max Oocyte diameter	Fecundity (oocytes per polyp)	Methods	Production	Larvae
<i>Goniocorella dumosa</i>	Gonochoric (1)	135 µm (1)	480 (1)	Spawner (1)	Seasonal (1)	-
<i>Madrepora oculata</i>	Functional Gonochoric (1,2,3)	350 µm (1) 650 µm (2)	10 (1)	Broadcast Spawner (1,2)	Periodic (1) / Continuous (2)	Lecithotrophic (1,2)
<i>Solenosmilia variabilis</i>	Gonochoric (1,2)	165 µm (1) 337µm (2)	290 (1)	Broadcast Spawner (1,2)	Seasonal (1) / Continuous (2, 4)	Lecithotrophic (1,2)

1. Waller (2005). 2. Pires *et al.* (2014). 3. Having a few hermaphroditic colonies. 4. A reproductive peak between April and September (Autumn–Spring).

6.4.6. Conservation and management implications

Seventeen seamount closure areas and 17 Benthic Protection Areas (BPAs) were established to protect benthic fauna, including VMEs such as coral reefs, from bottom trawl fisheries throughout the New Zealand EEZ in 2001 and 2007, respectively (Brodie & Clark, 2002; Helson *et al.*, 2010). These seamount closures and BPAs were not designed with population connectivity within and between these areas in mind, beyond the general principle that such protected areas should be large and be distributed amongst different environments throughout the EEZ. Understanding connectivity amongst areas is fundamental to designing an effective protected area network, or for modifying the existing distribution of protected areas to create a network (Palumbi, 2003). The maintenance of genetic diversity, and the protection of genetically distinct populations should also be considered as an integral component of protected area design (Bors *et al.*, 2012).

The results of the present study for *G. dumosa*, *M. oculata* and *S. variabilis* provide new information useful for assessing the effectiveness of existing protection measures, and the design of future protected area networks in the EEZ as a proxy for VME indicator species with similar patterns of genetic population connectivity. The populations of the three coral species all showed evidence of pronounced self-recruitment within the scale of the

geomorphic features examined. This finding suggests that additional protected areas will be required to maintain genetic diversity (through self-recruitment) of populations at features that do not already receive some protection. For *S. variabilis*, populations on all the geomorphic features examined were to some extent source populations for other populations, but those from the Kermadec Ridge and the Chatham Rise seem to be particularly important. Of particular significance as a migrant source for *G. dumosa* and *S. variabilis* populations was the Kermadec Ridge.

Currently, most geomorphic features examined contain one or more BPAs or seamount closures. The exceptions are the Hikurangi Margin, Bounty Trough and Macquarie Ridge, as well as the Lord Howe Rise and the Louisville Seamount Chain both of which are outside the New Zealand EEZ. The Hikurangi Margin is an area subject to bottom trawling (Clark & O'Driscoll, 2003) and populations of VME indicator taxa, such as reef-forming corals, are therefore at risk from disturbance. The Bounty Trough is a geomorphic feature where deep-water drilling for hydrocarbons may occur in the future (Wood & Davy, 2008), making populations of VME indicator taxa that exist there also at risk from anthropogenic activities. Although there are no BPAs on the Macquarie Ridge, two seamount closures to the west and east of the ridge, and a BPA to the south, may provide some protection for this southern source of genetic connectivity. However, whether these areas protect this genetic diversity will depend on whether they provide suitable habitat for the corals. Source populations of corals on the Kermadec Ridge are mainly protected from bottom trawling by the Kermadec and Tectonic Reach BPAs, and will likely receive additional protection should a proposed Kermadec Ocean Sanctuary be established in the near future (<http://www.mfe.govt.nz/marine/kermadec-ocean-sanctuary>). However, outside of the sanctuary area corals and other VME indicator taxa are still vulnerable to disturbance from future seabed mining of sulphide deposits in the region (Boschen *et al.*, 2015). While there are two BPAs on the Chatham Rise, they do not cover the depth range of the two deeper-distributed species, in particular *S. variabilis*, nor do they provide much protection along the axis of the Rise, including some areas where *G. dumosa* forms its most dense thickets in an area of interest for phosphorite nodule mining. Considering the relative importance of the Chatham Rise as a sink population and as a hotspot area with the highest genetic diversity for the three coral species, additional protected areas should be considered here for the future management of human activities on this feature. This recommendation is the same as that made for deep-sea sponges in the New Zealand region (Chapter 5).

The results of the present study, and those of previous genetic connectivity studies for various species around New Zealand (Smith *et al.*, 2004b; Miller *et al.*, 2010; Bors *et al.*, 2012; Knox *et al.*, 2012; Foltz *et al.*, 2013; Boschen *et al.*, 2015; Dueñas *et al.*, 2016) provide genetic connectivity information at different scales. Not surprisingly, their connectivity patterns differ for the range of taxa with differing ecological characteristics. These variable patterns demonstrate the need for a flexible spatial management system that can be periodically adjusted to accommodate increased understanding about the connectivity of a range of benthic taxa at a variety of spatial scales.

Appendix

Table S6.1 Success ratios of three corals for molecular experiments

	Total	Sampled	ITS	D-loop	SSR
<i>Goniocorella dumosa</i>	244	134 (28.09%)	133 (27.85%)	70 (14.68%)	108 (22.64%)
<i>Madrepora oculata</i>	164	96 (20.13%)	74 (15.51%)	-	93 (19.50%)
<i>Solenosmilia variabilis</i>	477	251 (52.62%)	186 (38.99%)	139 (29.14%)	208 (43.61%)

Table S6.2 Locus by locus information for *Goniocorella dumosa*

Locus	Success	F_{IS}	F_{ST}	Oosterhout	Selection
B106	100.00%	-0.241	0.335	-0.0278	Neutral
B1	100.00%	-0.794	0.031	-0.4978	Balancing
C107	91.67%	0.088	0.222	0.148	Neutral
D5	87.96%	-0.066	0.203	0.073	Neutral
B123	81.48%	-0.474	0.148	0.013	Balancing
D213	77.78%	-0.297	0.265	-0.0046	Neutral
SVA13	70.37%	-0.419	0.331	-0.0807	Neutral
C4	72.22%	-0.235	0.273	0.1009	Positive
SVA15	66.67%	-0.089	0.258	0.1327	Neutral
SVA10	60.19%	-0.172	0.482	0.0562	Balancing
SVA2	62.96%	-0.524	0.208	0.0314	Balancing
SVA18	60.19%	-0.276	0.434	0.0952	Positive
SVA8	56.48%	-0.417	0.364	0.0166	Neutral
D103	61.11%	-0.229	0.401	0.0737	Neutral
SVA1	52.78%	0.046	0.629	0.2778	Positive
B116	59.26%	-0.156	0.352	0.0917	Balancing
SVA11	52.78%	-0.853	0.459	-0.4397	Balancing
SVA4	43.52%	-0.43	0.553	-0.0002	Balancing
SVA7	36.11%	0.185	0.602	0.1387	Positive
SVA20	57.41%	-0.358	0.464	0.0137	Neutral

Table S6.3 Locus by locus information for *Madrepora oculata*

Locus	Success	F_{IS}	F_{ST}	Oosterhout	Selection
c016	65.59%	-0.445	0.247	0.0018	Neutral
g028	47.31%	-0.392	0.460	0.0630	Neutral
c7	27.96%	-0.539	0.604	0.0515	Neutral
g006	55.91%	-0.423	0.417	0.0361	Neutral
g004	64.52%	-0.182	0.358	0.1405	Neutral
g25	24.73%	0.572	0.850	0.3126	Positive
c013	73.12%	-0.319	0.463	0.1130	Neutral
g016	30.11%	-0.263	0.648	0.0718	Neutral
g022	30.11%	-0.044	0.678	0.2185	Neutral
a5	55.91%	0.009	0.434	0.2003	Neutral
g010	61.29%	-0.333	0.311	0.1030	Neutral

Table S6.4 Locus by locus information for *Solenosmilia variabilis*

Locus	Success	F_{IS}	F_{ST}	Oosterhout	Selection
B106	96.63%	-0.559	0.188	-0.3679	Neutral
C107	94.23%	-0.330	0.180	-0.0057	Neutral
D5	95.19%	-0.127	0.221	0.0257	Neutral
B1	96.63%	-0.259	0.178	-0.0799	Neutral
B116	87.98%	-0.067	0.284	0.0523	Positive
D103	80.29%	-0.138	0.285	0.0221	Neutral
B123	95.67%	0.062	0.234	0.1865	Neutral
D213	95.67%	-0.420	0.162	-0.1265	Balance
C4	93.75%	-0.115	0.346	0.0819	Positive
SVA1	79.81%	-0.176	0.181	0.0869	Neutral
SVA3	76.92%	-0.090	0.217	0.1007	Neutral
SVA7	70.67%	-0.350	0.249	-0.0666	Neutral
SVA2	81.73%	0.057	0.215	0.1084	Neutral
SVA14	49.52%	-0.045	0.363	0.1137	Positive
SVA15	94.23%	-0.380	0.160	-0.1153	Neutral
SVA4	72.12%	-0.215	0.232	0.0131	Neutral
SVA12	41.83%	0.195	0.444	0.2038	Neutral
SVA16	60.58%	-0.140	0.391	0.0598	Balance
SVA13	90.87%	-0.444	0.221	-0.1311	Neutral
SVA11	75.00%	-0.532	0.248	-0.2239	Balance
SVA10	70.67%	-0.263	0.206	0.0137	Balance
SVA5	60.10%	-0.256	0.359	-0.0728	Balance
SVA18	77.40%	-0.047	0.276	0.1269	Neutral
SVA8	59.62%	-0.076	0.236	0.1823	Neutral

Figure S6.1 Haplotype distributions *Goniocorella dumosa* D-loop (A) ITS (B), *Madrepora oculata* ITS (C), *Solenosmilia variabilis* D-loop (D) and ITS (E) across populations on geomorphic features

Figure S6.2 Scatterplot of DAPC of *Madrepora oculata* with New Zealand populations and the European population

Fig S6.3 Optimal genetic clusters predicted by BIC for *Goniocorella dumosa* (A), *Madrepora oculata* (B) and *Solenosmilia variabilis* (C) and by Structure Harvester for *Goniocorella dumosa* (D), *Madrepora oculata* (E) and *Solenosmilia variabilis* (F)

Figure S6.4 The 1st and 2nd coordinates of PCoA in *Solenosmilia variabilis* (A), *Madrepora oculata* (B) and *Goniocorella dumosa* (C)

Fig S6.5 Re-assignments for *Goniocorella dumosa* (A), *Madrepora oculata* (B), *Solenosmilia variabilis* (C) based on genotyping results

Inf means the potential population based on K indicated from BIC in DAPC.

**Chapter 7. Identifying environmental factors associated
with the genetic structure of Vulnerable Marine
Ecosystem indicator taxa in the New Zealand region**

7.1. Introduction

Currently, deep sea biological and environmental processes are still poorly understood, especially in areas such as how spatially arrayed environmental and habitat features influence evolutionary processes and population genetic connectivity. The deep-sea environment experiences increasing pressure and acidity, and generally decreasing temperature with increasing depth (Amils *et al.*, 2007). Such gradients of physicochemical properties may strongly influence dispersal, settlement and recruitment patterns of deep-sea taxa. Additionally, oceanic currents (e.g. bottom currents (Chevaldonné *et al.*, 1997) and surface currents (Arellano *et al.*, 2014)) play an important role in shaping the patterns of genetic connectivity amongst populations in many deep-sea species (Miller *et al.*, 2010; Miller, 2011; Baco & Cairns, 2012), with the result that several different factors may act as barriers to gene flow in the deep sea (see Chapter 2).

Vulnerable marine ecosystems (VMEs) are those that are both easily disturbed by anthropologic activities and very slow to recover, or may never recover, and which are physically or functionally fragile (FAO, 2009). Coral reefs and sponge gardens are two recognised VMEs in the deep sea (FAO, 2009). Indicator taxa of these VMEs include scleractinian coral species that build reef frameworks (e.g. *Lophelia pertusa* Freiwald *et al.*, 2004) and demosponges (e.g. *Geodiidae* NEAFC, 2014). Although there are no studies that have quantified the relationships between environmental factors and the maintenance of genetic structure in the deep sea, there are some studies from other research areas that might provide information about this interaction for VME indicator taxa of coral reefs and sponge gardens. Generally, depth, temperature, salinity, aragonite saturation state and particulate organic carbon (POC) have been recognised as influencing the formation of deep sea coral and sponge habitat (Adkins *et al.*, 2003; Kiriakoulakis *et al.*, 2005; Davies *et al.*, 2008; Dullo *et al.*, 2008; Dodds *et al.*, 2009; Guinan *et al.*, 2009; Roberts *et al.*, 2009; Case *et al.*, 2010). In addition, topography and sediment are also regarded as important factors influencing the distribution of coral and sponge habitat-forming deep-sea species (Dolan *et al.*, 2008; Brooke *et al.*, 2009). At the small scale, internal tidal mixing and pH variation may have significant effects on corals larvae and their ability to connect different populations (Frederiksen *et al.*, 1992; McCulloch *et al.*, 2012). However, the relative influence (weak, strong) and the mechanism of action by which these environmental variables moderate the interaction between habitat and population genetic structure and connectivity are still unknown.

The discipline of seascape genetics is extended from landscape genetics, and attempts to explicitly integrate spatial ecological information with population genetic data to characterise marine spatial environmental factors that contribute to genetic connectivity and/or population genetic isolation (Manel *et al.*, 2003). Selkoe *et al.* (2016) summarised seascape genetics studies over the last 10 years and found that temperature, oceanography and geography showed equal prevalence of influence on spatial genetic patterns of shallow-sea species. However, no such studies have been conducted on deep-sea organisms. Within the New Zealand region, many species have distributions that encompass a range of environments, and are therefore amenable to quantification of the effect of environmental variation on genetic variation and population genetic structuring (e.g. Silva & Gardner, 2016; Wei *et al.*, 2013). Complex patterns of species-specific genetic variation in VME indicator taxa in New Zealand region have been described previously (Chapter 5 and Chapter 6). Such patterns may be caused by a number of different factors, including historical processes (phylogeography), connectivity (high versus low gene flow within and amongst populations), and environmental variation (contemporary selection pressure). Whilst there is no indication that this multi-taxon genetic structuring is attributable to local or regional adaptation, the recognition of its existence as a pre-existing condition is important. This recognition does not preclude the existence of other structuring, perhaps at a different spatial scale, which results from environmental variation across the New Zealand region.

Because of its highly variable seafloor topography and bathymetry and its complex patterns of oceanic dynamics, New Zealand presents a rare opportunity to test the possible effect of multi-factorial environmental influences on deep-sea genetic variation. In a global assessment of the biogeography of the deep sea based on depth, temperature, salinity, dissolved oxygen, particulate organic carbon (POC), hydrographic and bathymetric parameters (Watling *et al.*, 2013), the New Zealand region was divided into two provinces, a northern (BY6) and a southern (BY10) province with a boundary at latitude $\sim 45^{\circ}\text{S}$. The northern province includes plateaus around the North Island of New Zealand, and extends northward along the Kermadec and Colville Ridges almost to Tonga, as well as including the seamounts of the Louisville Seamount Chain (beyond New Zealand's Exclusive Economic Zone, the EEZ). The water mass is dominated by Antarctic Intermediate Water with temperatures averaging 2.8°C and with moderately high POC flux ($4\text{--}6\text{ g m}^{-2}\text{ yr}^{-1}$). The southern province includes the Campbell Plateau, Bounty Trough, and the Macquarie Ridge that extends to $\sim 60^{\circ}\text{S}$. This province is characterised by Circumpolar Deep Water with a lower mean temperature ($1\text{--}2^{\circ}\text{C}$) and lower POC flux ($1.5\text{--}3\text{ g m}^{-2}\text{ yr}^{-1}$) than the northern

province (Watling *et al.*, 2013). The water mass characteristics of these two provinces is likely to influence the population distribution of deep-sea species across the New Zealand region, which in turn could be reflected in province-scale patterns of genetic structure. Three major currents and their associated fronts around New Zealand, the Tasman Front, the Subtropical Front and the Subantarctic Front, are expected to have the greatest influence on population connectivity of deep-sea populations in the region. These currents and frontal systems differentiate the water mass environments in the north and the south of the region, and also influence the direction of north and south currents across the Chatham Rise that may reduce or perhaps even block gene flow across this feature. For this reason, we identified a north, central and south region separated at 42°S and 45°S (above 42°S is north region, between 42-45°S is central region and below 45°S is south region) in this study. The seafloor of the New Zealand region is distinguished by different geomorphic features (such as seamounts, plateaux and ridges) which are characterised by different topographies and hydrodynamics that could influence genetic differentiation between populations on these geomorphic features (e.g. Bucklin *et al.*, 1987; Yang *et al.*, 2016). These three environmental-based classifications provide a background framework against which to test genetic differentiation amongst deep-sea populations of VME indicator taxa.

In the present study, data for 7 VME indicator taxa, 4 sponges (*Neoaulaxinia persicum*, *Penares* sp., *Pleroma menoui* and *Poecillastra laminaris*) and 3 corals (*Goniocorella dumosa*, *Madrepora oculata* and *Solenosmilia variabilis*), collected around New Zealand across ~34° of latitude and ~1750 m of depth, have been examined to achieve an understanding of the seascape genetics of these species. In total, 25 environmental variables and two different types of genetic data (sequencing and genotyping) were employed to quantify the relationships between environmental and genetic variation at different spatial scales. The results provide information about which environmental factors shape the genetic structure of the populations, and this information may directly contribute to understanding connectivity amongst VMEs and inform appropriate management measures.

7.2. Materials and methods

7.2.1. Sample collection and molecular experiments

All 4 sponges (*Neoaulaxinia persicum*, *Penares* sp., *Pleroma menoui* and *Poecillastra laminaris*) and 3 corals (*Goniocorella dumosa*, *Madrepora oculata* and *Solenosmilia*

variabilis) were sampled from the NIWA invertebrate collection (NIC); details are presented in Chapter 5 and Chapter 6. Tissue samples from any one collection site were based on only one fragment or piece to avoid duplication of individuals because of colonial or asexual reproduction.

7.2.2. Data collection and analyses

7.2.2.1. Data collection

7.2.2.1.1. DNA sequences

A total of 480 samples (*G. dumosa* n=108, *M. oculata* n=93, *N. persicum* n=30, *Penares* sp. n=15, *P. menoui* n=16, *Poecillastra laminaris* n=63 and *S. variabilis* n=208) were sequenced for mitochondrial and/or nuclear DNA regions (Table 7.1). The protocols for and basic analyses of sequencing and genotyping of data are reported in the previous chapters. Individual pair-wise distances data from DNA sequences were generated from software MEGA (v 6).

Table 7.1 Numbers of specimens used for each genetic marker

Species	12S	COI	Cytb	D-loop	ITS	Microsatellites
<i>Goniocorella dumosa</i>	-	-	-	27	83	108
<i>Madrepora oculata</i>	-	-	-	-	40	93
<i>Solenosmilia variabilis</i>	-	-	-	141	121	208
<i>Neoaulaxinia persicum</i>	11	30	-	-	-	-
<i>Pleroma menoui</i>	-	16	-	-	-	-
<i>Poecillastra laminaris</i>	-	53	51	-	-	63
<i>Penares</i> sp.	14	15	-	-	-	-

-, data not available

7.2.2.1.2. Microsatellite loci

All loci used in this study are the same as described in Chapter 5 and Chapter 6, including all 10 loci and 4 neutral loci (ong8, ong26, ong27 and ong34) for *Poecillastra laminaris*; all 20 loci and 8 neutral loci (B106, B123, D5, D103, D213, SVA8, SVA13 and SVA20) for *G. dumosa*; all 11 loci and 6 neutral loci (c7, c016, g006, g010, g016 and g028) for *M. oculata*; all 24 loci and 12 neutral loci (B1, B106, C107, D5, D103, SVA1, SVA2,

SVA3, SVA4, SVA7, SVA13 and SVA15) for *S. variabilis*. For each species, a pairwise individual-by-individual genotypic distance matrix was calculated from the microsatellite data using the Codom-Genotypic distance option within GenAlEx (Peakall & Smouse 2012). Allelic frequencies were calculated using GenAlEx.

Table 7.2 Detailed information of environmental variables included in this study

Type	Variable	Abbreviation	unit	Resolution	Source
Topographic variables	Bathymetric Position Index – Broad	bpi-broad	-	25 m radius	Wright <i>et al.</i> (2005), 1+25
	Bathymetric Position Index – Fine	bpi-fine	-	5 m radius	Wright <i>et al.</i> (2005), 1+5
	Seamount	smt	Binomial (yes/no)	1 km	Rowden <i>et al.</i> (2008),
	Slope in percent	slope-percent	%	0.00001	Jenness (2012)
	Standard Deviation of Slope	stdev-slope	-	3x3 window	Grohmann <i>et al.</i> (2011),
Physico-chemical variables	Sea surface temperature	sst	Degree	1 km	NOAA satellite data
	Sea surface temperature gradient	sstgrd	°C km-1	1 km	Uddstrom & Oien (1999),
	Bottom current speed	botspd	m s-1	1 km	Hadfield <i>et al.</i> (2002)
	Bottom water temperature	tempbot	°C	1 km	CARS (2009)
	Bottom water temperature residuals	tempres	°C km-1	1 km	(www.cmar.csiro.au/cars) CARS (2009), Leathwick <i>et al.</i> (2012)
	Temperature	temperature	°C	0.001	CARS (2009)
	Tidal current speed	tidcurr	ms-1	1 km	Walters <i>et al.</i> (2001), Hadfield <i>et al.</i> (2002)
	Density	sigma-theta	kg m-3	0.01	NIWA
	Dissolved organic matter	disorg	m-1	1 km	NASA SeaDas
	Dynamic topography	dynoc	m	1 km	AVISO (http://www.aviso.oceanobs.com)
	Nitrate	woanite	µmol l-1	1°	Garcia <i>et al.</i> (2006)
	Omega Aragonite	aragonite	ΩARAG	-	Bostock <i>et al.</i> (2014)
	Omega Calcite	calcite	ΩCALC	-	Bostock <i>et al.</i> (2014)
	Oxygen	oxygen	ml l-1	-	CARS (2009)
	Phosphate	woaphosc	µmol l-1	1°	Garcia <i>et al.</i> (2006)
	Salinity	salinity	psu	-	CARS (2009)
	Silicate	silicate	µmol l-1	1°	Garcia <i>et al.</i> (2006b)
Biological variables	Chromophoric dissolved organic matter	cdom	aDOM (443) m-1	1 km	Pinkerton <i>et al.</i> (2006)
	Particulate organic carbon export	POC	mg Corg.m-2 d-1	-	Lutz <i>et al.</i> (2007)
	Surface water primary productivity	vgpm	mg C m-2 d-1	1 km	Behrenfield & Falkowski (1997)

7.2.2.1.3. Environmental variables

Data for 25 environmental variables - 5 topographic, 17 physicochemical and 3 biological variables - were obtained from various sources (Table 7.2). All the environmental variables have been shown from previous studies to potentially influence the distribution of coral and sponge species (see references in Introduction and Tracey *et al.*, 2011). The variables calcite and aragonite were excluded from the analysis of sponge data, while silicate was excluded from the analysis of coral data.

7.2.2.2. Statistical analysis

Highly correlated environmental variables were identified and eliminated from subsequent analysis to reduce problems that arise for the interpretation of models that include a large number of collinear predictors (Graham, 2003). The Pearson correlation coefficients between all predictor variables were calculated for those data collected at the sponge and coral sample locations. Correlations were visualised, and where correlations were > 0.95 one of the predictor variables was eliminated. Correlation analyses of environmental parameters in the entire dataset and their visualisation were conducted using the R project (v3.2.2).

Following the spatially hierarchical testing framework described in the previous chapters, the same approach was employed here. Specimens were grouped according to (1) the northern versus southern biogeographic provinces of Watling *et al.* (2013), (2) the north-central-south regions, and (3) geomorphic features: NE continental slope, Bollons Seamount, Bounty Plateau, Bounty Trough, Campbell Plateau, Challenger Plateau, Chatham Rise, Hikurangi Margin, Kermadec Ridge, Louisville Seamount Chain, Macquarie Ridge and Tasman Basin. Not all species were collected from all geomorphic feature regions: spatial coverage was dictated by availability of archived material in the NIWA Invertebrate Collection and newly collected material from recent research cruises.

A generalised linear model (GLM) was employed to test for the effect of environmental variables on genetic distance values calculated at the individual level on DNA sequence markers for all 7 species and on genotyping markers (microsatellites) for *Poecillastra laminaris*, *G. dumosa*, *M. oculata* and *S. variabilis*. A mean genetic distance value for each individual was calculated from all individual pairwise values, to be used as a dependent variable. To remove the effects of multi-collinearity amongst environmental variables, GLM analyses were performed using a stepwise model (both forwards and backwards), with variable inclusion/exclusion based on the Akaike Information Criterion

(AIC) and Bayesian information criterion (BIC). The best fit model was chosen from the lowest AIC or BIC value model. All analyses were conducted using the package MASS in R (v3.2.2). Missing environmental values for the three species were replaced by means to reduce information loss. For all these analyses, the null hypothesis is that none of the environmental parameters (independent variables) explain significant variation in the genetic data set (dependent variable).

To test the null hypothesis from a population perspective, analyses were conducted using distance-based linear models (DistLM) using the statistical software PERMANOVA+ (Anderson *et al.*, 2008). The DistLM routine is a semi-parametric, permutation-based method that does not rely on the assumption of normally distributed data (Anderson *et al.*, 2008). The best-fitting relationship was chosen for the final regression models by comparing adjusted r^2 selection criterion. Relationships between environmental parameters (averaged for all sites within a population) were initially examined by analysing each predictor separately (marginal tests), and then sequentially using adjusted r^2 selection procedure. Similarity matrices in DistLM analyses were built using Bray-Curtis resemblance matrices of allele frequencies. The p-values for individual predictor variables were obtained using 9,999 permutations (Leduc *et al.*, 2012). Distance-based redundancy analysis (dbRDA) plots were also generated using PERMANOVA+ to visualise the results once the best DistLM model of each species was obtained.

Based on microsatellite variation results from Chapter 5 and Chapter 6, significant genetic structure was detected at the northern-southern province level for *S. variabilis*, north-central-south region level for all four species (*G. dumosa*, *M. oculata*, *P. laminaris* and *S. variabilis*), and at the level of geomorphic feature populations for *G. dumosa*, *P. laminaris* and *S. variabilis*. Only these structured populations were considered for the DistLM analysis. However, the province level was ultimately excluded from the analyses because the DistLM failed to build any models for *S. variabilis* for both the all loci and the neutral loci data sets.

Because the environmental variables data set is less complete beyond the New Zealand EEZ (no information for 10 variables, bottom speed, dissolved organic matter, sea surface temperature, sea surface temperature gradient, bottom water temperature, bottom water temperature residuals, tidal current speed and surface water primary productivity) for *S. variabilis*, averages of these 10 variables were used to replace the missing values in the Louisville Seamount Chain population (beyond the New Zealand EEZ) to complete the DistLM analyses at regional and geomorphic feature levels.

The difference between the individual level (GLM) and the population level (DistLM) procedures, as employed in this study, is that the former is conducted on mean values of individual pairwise genetic distance and the latter on raw allele frequency data at the population level (all three analyses use the same environmental data). These analyses are different but complementary in their approach to determining if and in what way the observed genetic variation is explained by the environmental variables.

7.3. Results

Bivariate correlations showed that several of the topographic, physico-chemical and biological parameters were significantly correlated, especially the variables bpi-broad, depth, temperature, seamount, sea surface temperature and surface water primary productivity that were correlated with one or more of the other variables. Amongst the correlations, several of the physico-chemical variables were significantly correlated with each other (Figure 7.1). Notably, calcite and aragonite were highly correlated ($r^2 > 0.99$), as were temperature, aragonite, sigma-theta and calcite ($r^2 > 0.95$); woanite and woaphosc ($r^2 > 0.95$); and tempbot and tempres ($r^2 > 0.95$). Therefore, aragonite was selected to represent calcite, temperature and sigma-theta in the coral models, and sigma-theta for temperature in the sponge models. Tempbot was selected to represent tempres, and woanite for woaphosc in models for both coral and sponge models. This selection procedure resulted in 20 variables (bpi-broad, bpi-fine, oxygen, POC, salinity, silicate, slope-percent, stdev-slope, sigma-theta, woanite, botspd, cdom, disorg, dynoc, smt, sstgrd, sst, tempbot, tidcurr and vgpm) for the sponge data analysis, and 19 variables (bpi-broad, bpi-fine, aragonite, oxygen, POC, salinity, slope-percent, stdev-slope, woanite, botspd, cdom, disorg, dynoc, smt, sstgrd, sst, tempbot, tidcurr and vgpm) for the coral analysis.

7.3.1. GLM analysis

Amongst all populations, the GLM analysis established different models for different species and different genetic markers using 20 environmental variables for sponges and 19 variables for corals to explain the genetic variation at the individual level. BIC modelling was less successful at building models than AIC modelling, and AIC was therefore chosen to select environmental variables that explained the genetic variations. The AIC test removed the environmental variables that did not contribute to explaining genetic variation, such that

the final model for each marker in each species represented the best fit subset of environmental variables that explained local genetic variation within populations.

Figure 7.1 Heatmap of correlations between environmental variables

Colour represents the values of correlation coefficients: blue to red represents the value from -1 to 1.

7.3.1.1. GLM analyses for sponges

Due to small sample sizes and/or low variation within the genetic markers, the GLM analysis of *COI* sequence variation in individuals of *N. persicum* and *P. menoui* failed to reveal a relationship between genetic and environmental variables (that is, model building failed). The model based on *COI* of *Penares* sp. was non-significant ($p = 0.124$). Models for *N. persicum*, *P. menoui* and *Penares* sp. could not be built for the *12S* sequence datasets

because of low DNA sequence variation. Only the models for *Poecillastra laminaris* could be built that were statistically significant ($p < 0.05$) for both sequencing and genotyping datasets.

7.3.1.1.1. *Poecillastra laminaris*

The best fit model explaining variation in the *COI* sequence data contained 11 of the 20 variables, the best fit model for *Cytb* variation contained 16 variables, whilst the optimal model for variation in the microsatellite data set contained 9 and 12 variables for all and neutral microsatellites, respectively. Three variables, sea surface temperature (sst), sea surface temperature gradient (sstgrd) and nitrate (woanite), occurred in all three models (Table 7.3).

7.3.1.1.2. *Neoaulaxinia persicum*, *Pleroma menoui* and *Penares* sp.

Model fitting for these three sponge species was made difficult by small sample sizes and/or low levels of genetic variation. In the non-significant model for *Penares* sp., the environment variables POC and oxygen contributed to explaining variation in the *COI* sequence data set, whilst the model for *ITS* sequence variation could not be fitted for *Penares* sp. or for *N. persicum*.

7.3.1.2. GLM analyses for corals

Eleven models were developed and tested for the three coral species for the DNA sequence and microsatellite (genotype) data. The multiple R^2 values of the models were low (< 0.500) except for *ITS* of *M. oculata* (> 0.900). However, all models, except *D-loop* for *G. dumosa*, neutral microsatellites for *M. oculata* and for *S. variabilis*, were statistically significant ($p < 0.05$), indicating that a model with a low r^2 value can still help to predict how changes in the environmental variables may be associated with changes in genetic distance values amongst locations. Sea surface temperature gradient (sstgrd) was significant in explaining the genetic differentiation amongst individuals of all three corals for all models (except all microsatellite data for *G. dumosa*), even in non-significant models. This parameter may be considered as a common environmental variable that is related to genetic differentiation amongst individuals for the three stony coral species. The details of species-specific models are described below.

Table 7.3 General linear model analysis results of genetic variation at the individual level for *Poecillastra laminaris*

Variables	<i>COI</i>	<i>Cytb</i>	All SSR	Neutral SSR
Multiple R ²	0.544	0.803	0.44	0.441
Adjusted R ²	0.371	0.66	0.311	0.872
p-value	0.007	<0.001	0.004	0.023
Intercept	-1.544 ± 0.456**	-0.439 ± 0.339ns	1761.000 ± 662.700*	123.600 ± 55.850*
botspd	E	-0.068 ± 0.029*	E	E
bpi broad	0.000 ± 0.000*	E	-0.007 ± 0.004#	-0.008 ± 0.003**
bpi fine	E	0.000 ± 0.000#	E	E
cdom	E	-0.022 ± 0.008**	E	5.185 ± 1.813**
disorg	E	-0.660 ± 0.181**	E	111.400 ± 51.740*
dynoc	E	-0.053 ± 0.012***	E	E
oxygen	0.017 ± 0.005**	E	-15.120 ± 3.870***	-2.120 ± 1.025*
POC	0.002 ± 0.001*	0.000 ± 0.000ns	-0.950 ± 0.440*	-1.538 ± 0.431**
Salinity	0.041 ± 0.012**	E	-28.510 ± 11.910*	E
sigma theta	E	0.015 ± 0.011	-20.480 ± 7.410**	-2.747 ± 1.620#
silicate	0.001±0.000***	0.000 ± 0.000*	E	E
slope percent	0.000 ± 0.000#	0.000 ± 0.000ns	E	-0.121 ± 0.077ns
smt	E	-0.003 ± 0.002#	E	E
sst	1.980 ± 0.853*	-2.616 ± 0.551***	-15610.000 ± 472.600**	-794.200 ± 245.700**
sstgrd	-5.432 ± 2.254*	6.181 ± 1.348***	2810.000 ± 1152.000*	1337.000 ± 551.000*
stdev slope	E	-0.002 ± 0.001*	E	0.587 ± 0.286*
tempbot	-0.002 ± 0.001#	-0.002 ± 0.001#	E	E
tidcurr	-0.019 ± 0.014ns	E	37.430 ± 10.520***	E
vgpm	E	0.000 ± 0.000**	E	29.800 ± 7.295***
woanite	0.002 ± 0.001**	0.001 ± 0.000**	-1.090 ± 0.306***	-0.666 ± 0.168***

Significance levels at $p < 0.001$, $p < 0.01$, $p < 0.05$ and $p < 0.1$ are labelled as ***, **, *, #, and ns. Variable with “E” indicated that it was excluded from the modelling. Models for *Neoaulaxinia persicum*, *Pleroma menoui* and *Penares* sp. *COI* gene was failed to construct a model.

7.3.1.2.1. *Goniocorella dumosa*

The best fit model for the *D-loop* sequence data included 13 variables, but the whole model was not significant ($p > 0.05$). For the *ITS* sequence data the best fit model contained only 3 variables, whilst for microsatellite variation the best fit model contained 3 variables for all loci and 5 variables for neutral loci. Amongst these three best fit models, dynamic topography (dynoc) was always included and contributed significantly, and sea surface temperature gradient (sstgrd) was also a significant contributor to the models except for the model based on all microsatellite data (Table 7.4).

Table 7.4 General linear model analysis results of genetic variation for *Goniocorella dumosa*

Variables	D-loop	ITS	All SSR	Neutral SSR
Multiple R2	0.56	0.112	0.091	0.139
Adjusted R2	0.083	0.077	0.064	0.096
p-value	0.394	0.028	0.021	0.009
Intercept	-1.482 ± 0.702#	0.003 ± 0.008ns	30.917 ± 3.616***	-634.297 ± 219.031**
botspd	-0.349 ± 0.148*	E	E	E
bpi broad	E	E	E	E
bpi fine	0.000 ± 0.000#	E	E	E
cdom	E	E	-1.249 ± 0.749#	E
disorg	-1.276 ± 0.403**	E	46.423 ± 20.917*	E
dynoc	-0.484 ± 0.169*	0.035 ± 0.014*	18.578 ± 6.005**	11.412 ± 3.987**
oxygen	0.012 ± 0.009ns	E	E	5.885 ± 1.931**
POC	E	E	E	E
salinity	0.051 ± 0.023*	E	E	18.411 ± 6.249**
aragonite	E	E	E	-14.403 ± 4.623**
slope percent	-0.002 ± 0.001#	E	E	E
smt	-0.027 ± 0.023ns	E	E	E
sst	-1.378 ± 0.565*	-0.428 ± 0.199*	E	E
sstgrd	6.689 ± 2.098**	1.029 ± 0.382**	E	73.295 ± 31.874*
stdev slope	-0.013 ± 0.007#	E	E	E
tempbot	E	E	E	E
tidcurr	-0.222 ± 0.079*	E	E	E
vgpm	0.000 ± 0.000*	E	E	E
woanite	E	E	E	E

Significance levels at $p < 0.001$, $p < 0.01$, $p < 0.05$ and $p < 0.1$ are labelled as ***, **, *, #, and ns. Variable with “E” indicated that it was excluded from the modelling.

7.3.1.2.2. *Madrepora oculata*

For nuclear *ITS* DNA sequence data the best fit model contained 11 of the 19 environmental variables, whilst the best fit model for the microsatellite variation included 5 variables for all loci and 6 for the neutral loci. However, the model based on neutral microsatellites was not significant. For these nuclear DNA markers, 2 environmental variables (sea surface temperature gradient (sstgrd) and surface water primary productivity (vgpm)) were present in all models (Table 7.5).

7.3.1.2.3. *Solenosmilia variabilis*

For the *D-loop* analysis, the best fit model contained 14 variables, whilst a 7-variable model provided the best fit for variation for the *ITS* DNA sequence. The models for microsatellite variation included 5 variables for both the all loci and neutral loci datasets.

These three models all shared an environmental variable - sea surface temperature gradient (sstgrd). The model based on the neutral loci was not statistically significant (Table 7.6).

Table 7.5 General linear model analysis results of genetic variation for *Madrepora oculata*

Variables	ITS	All SSR	Neutral SSR
Multiple R ²	0.833	0.208	0.101
Adjusted R ²	0.767	0.161	0.048
p-value	<0.001	0.001	0.104
Intercept	0.765 ± 0.209**	22.070 ± 1.572***	9.771 ± 1.149***
botspd	-0.648 ± 0.240*	20.460 ± 12.03#	E
bpi broad	0.000 ± 0.000***	E	E
bpi fine	E	E	E
cdom	0.097 ± 0.043*	-6.219 ± 1.764***	E
disorg	-9.132 ± 2.022***	E	-83.280 ± 36.540*
dynoc	E	E	E
oxygen	-0.100 ± 0.039*	0.383 ± 0.141**	E
POC	E	E	0.213 ± 0.099*
salinity	E	E	E
aragonite	E	E	E
slope percent	E	E	E
smt	E	E	E
sst	-6.616 ± 5.270ns	E	E
sstgrd	15.000 ± 9.504ns	-504.200 ± 131.800***	-162.500 ± 72.320*
stdev slope	E	E	E
tempbot	-0.041 ± 0.011**	E	-0.694 ± 0.341*
tidcurr	-0.874 ± 0.199***	E	E
vgpm	0.001 ± 0.000***	0.019 ± 0.006**	0.007 ± 0.003*
woanite	-0.009 ± 0.002***	E	E

Significance levels at $p < 0.001$, $p < 0.01$, $p < 0.05$ and $p < 0.1$ are labelled as ***, **, *, #, and ns. Variable with “E” indicated that it was excluded from the modelling.

7.3.2. DistLM analyses

7.3.2.1. North-central-south region

Marginal tests resulting from DISTLM analysis at the regional scale showed that the genetic structure of the four species was related to different environmental factors (Table 7.7). After removal of the variables that did not explain genetic variation, through sequential testing, only one variable was retained in each model. In *G. dumosa*, the biological factor surface water primary productivity (vgpm) explained genetic structure ($r^2 > 0.66$) in both the all loci and the neutral loci data sets. Dynamic topography (dynoc) and seamount (smt) contributed to genetic differentiation in *M. oculata* in the all loci and the neutral loci data sets,

respectively. The variable dynamic topography explained variation in the all loci and neutral loci data sets of *S. variabilis*. Physicochemical variables (silicate and bottom current speed) explained variation in the all loci and neutral loci data sets of *Poecillastra laminaris*, respectively.

Table 7.6 General linear model analysis results of genetic variation for *Solenosmilia variabilis*

Variables	D-loop	ITS	All SSR	Neutral SSR
Multiple R2	0.383	0.145	0.103	0.048
Adjusted R2	0.298	0.082	0.081	0.019
p-value	<0.001	0.033	<0.001	0.154
Intercept	-1.002 ± 0.320**	-0.71 ± 0.264**	43.272 ± 1.470***	19.577 ± 1.093***
botspd	E	E	-17.009 ± 10.183#	E
bpi broad	0.000 ± 0.000*	E	E	E
bpi fine	0.000 ± 0.000#	0.000 ± 0.000#	E	E
cdom	0.001 ± 0.001ns	E	E	-2.200 ± 0.961*
disorg	0.062 ± 0.027*	E	E	49.257 ± 29.187#
dynoc	0.017 ± 0.004***	E	E	6.451 ± 2.328**
oxygen	0.023 ± 0.005***	0.074 ± 0.037*	E	E
POC	E	E	-0.175 ± 0.071*	E
salinity	0.027 ± 0.009**	E	E	E
aragonite	-0.046 ± 0.010***	E	2.281 ± 0.819**	E
slope percent	0.000 ± 0.000*	E	E	E
smt	-0.001 ± 0.000*	0.055 ± 0.026*	E	E
sst	0.171 ± 0.119ns	-13.960 ± 7.574#	E	-236.314 ± 154.622ns
sstgrd	-0.395 ± 0.264ns	41.980 ± 16.230*	353.840 ± 156.672*	605.165 ± 353.257#
stdev slope	E	0.015 ± 0.007*	E	E
tempbot	-0.001 ± 0.000*	E	0.919 ± 0.555#	E
tidcurr	0.007 ± 0.005ns	E	E	E
vgpm	E	E	E	E
woanite	E	0.010 ± 0.004*	E	E

Significance levels at $p < 0.001$, $p < 0.01$, $p < 0.05$ and $p < 0.1$ are labelled as ***, **, *, #, and ns. Variable with “E” indicated that it was excluded from the modelling.

Table 7.7 Sequential test results of the DistLM analysis for microsatellite data amongst the North-Central-South regions

Species	Loci	Variable	Adj R ²	SS(trace)	p	Proportion
<i>Goniocorella dumosa</i>	All	+vgpm	0.669	2429.8	0.169	0.834
	Neutral	+vgpm	0.739	2409.1	0.169	0.870
<i>Madrepora oculata</i>	All	+dynoc	0.342	1406.2	0.159	0.671
	Neutral	+smt	0.324	1319.2	0.162	0.662
<i>Solenosmilia variabilis</i>	All	+dynoc	0.284	353.02	0.167	0.642
	Neutral	+dynoc	0.394	360.18	0.167	0.697
<i>Poecillastra laminaris</i>	All	+silicate	0.178	1870.8	0.161	0.589
	Neutral	+botspd	0.249	1515.2	0.162	0.624

7.3.2.2. Geomorphic feature populations

Marginal tests resulting from DISTLM analysis showed that microsatellite variation of the sponge *Poecillastra laminaris* exhibited a significant relationship with nitrate, bottom water temperature, salinity and bottom current speed. When considered alone, nitrate explained 34.9% of the variability, bottom water temperature 29.3%, salinity 23.0% in the all loci data set, and in the neutral loci data set bottom water temperature explained 34.9% of the variation, nitrate 35.8%, and bottom current speed 18.8% (Table 7.8 and Figure 7.2). Despite the fact that each individual environmental factor was not significant, the selected best fit model from the sequential testing explained 87.2% and 89.5 % of the variation observed in the full microsatellite and the neutral loci data sets, respectively (Table 7.7). Both models built for the all loci and neutral loci data sets agreed that nitrate was the variable that explained most of the genetic structure at the geomorphic feature spatial scale. The dbRDA results illustrated that the populations from the Campbell Plateau and the NE Slope were genetically different from all other populations, and that nitrate and bottom water temperature were the main contributors to this pattern in the two data sets (Figure 7.2).

Figure 7.2 dbRDA analyses of all (left) and neutral (right) microsatellite variation for *Poecillastra laminaris*

Table 7.8 Sequential test results of the DistLM analysis for microsatellite data amongst geomorphic features for all species

Species	Loci	Variable	Adj R ²	SS(trace)	p	Proportion
<i>Goniocorella dumosa</i>	ALL	+woanite	0.222	1783.0	0.090	0.482
		+aragonite	0.350	1117.5	0.393	0.302
	Neutral	+woanite	0.233	1802.3	0.145	0.489
		+aragonite	0.477	1243.6	0.321	0.337
		+stdevslope	0.069	1242.1	0.103	0.202
<i>Solenosmilia variabilis</i>	ALL	+smt	0.187	1334.8	0.026	0.217
		+tempbot	0.286	1065.5	0.080	0.173
		+slopepercent	0.363	829.2	0.211	0.135
		+stdevslope	0.139	1345.4	0.028	0.262
	Neutral	+smt	0.238	999.0	0.049	0.194
		+tempbot	0.304	751.2	0.149	0.146
		+aragonite	0.339	589.1	0.345	0.115
		+tidcurr	0.401	576.1	0.342	0.112
		+salinity	0.518	526.2	0.394	0.102
		+woanite	0.131	3963.5	0.037	0.349
<i>Pocillostra laminaris</i>	ALL	+tempbot	0.284	3336.3	0.287	0.293
		+salinity	0.487	2612.4	0.338	0.230
		+tempbot	0.132	3675.6	0.073	0.349
	Neutral	+woanite	0.414	3768.4	0.083	0.358
		+botspd	0.578	1975.9	0.339	0.188

For *G. dumosa*, the DistLM analysis from the all loci and neutral loci data sets showed that nitrate and aragonite were the main factors that explained microsatellite variation: these variables explained 78.34% of the all microsatellites genetic variation and 82.57% of the neutral loci genetic variation. The greatest single contributor was nitrate at 48.16% for all loci and 48.86% for neutral loci. The dbRDA plot showed that populations from Kermadec Ridge and Chatham Rise grouped together with similar levels of nitrate and aragonite. The Challenger Plateau and NE Continental Slope population were separated each other, as well as Chatham Rise and Kermadec Ridge populations, associated with a variation in nitrate (Figure 7.3).

Figure 7.3 dbRDA analyses of all (above) and neutral (below) microsatellite variation for *Goniocorella dumosa*

Figure 7.4 dbRDA analyses of all (left) and neutral (right) microsatellite variation for *Solenosmilia variabilis*

Microsatellite variation of all loci of *S. variabilis* was partially explained by standard deviation of slope, seamount percentage, bottom water temperature and slope percentage.

These four variables explained 72.7% of the observed genetic variability, with the highest contribution being made by seamount percentage (Table 7.8). In the dbRDA plot, the Hikurangi Margin population was separated by standard deviation of slope from the other populations, and the other three environmental variables were separated populations into 6 groups. In the results of the neutral microsatellite loci analysis, standard deviation of slope, seamount percentage, bottom water temperature, aragonite, tidal current speed and salinity were the factors selected in the DistLM analysis, and these 6 variables accounted for 93.1% of the genetic variation. Amongst these variables, standard deviation of slope was the most significant variable in the DistLM analysis, and it explained 26.2% of the microsatellite genetic variation (Figure 7.4). The dbRDA plots revealed that the 8 geomorphic feature populations were genetically different from one another, and that this separation was influenced by the combination of these 6 environmental variables. Furthermore, the Bounty Plateau population was distinct from the other populations due to the influence of aragonite variation (Figure 7.4).

7.4. Discussion

Contrasting patterns of population genetic structure have been reported in previous chapters for the 4 sponge and 3 coral taxa throughout their distributional ranges in the New Zealand region. Here, environmental variability has been examined to help explain the taxon-specific genetic variation. Small sample size is however, a very big challenge that deep-sea researchers have to confront, and was a problem in the present study. Because sampling in the present study was based on availability of material collected by different research voyages over the years, rather than from a directed sampling programme, the distributions of the specimens analysed here had a spatial bias, with the highest density from the Chatham Rise (associated with opportunistic collecting during fisheries research surveys to the region). This bias may contribute to a failure to reveal seascape genetic patterns at a geomorphic feature population level. Moreover, because this is the first test of the role of environmental variability explaining genetic variability for deep-sea species, published information from previous studies for comparative purposes was entirely absent. Nonetheless, despite small population sample sizes in some cases (minimum n is 4) and a lack of comparative information about the relationships between environmental factors and genetic differentiation, the results generated here contribute to a more comprehensive understanding about environmental and genetic interactions at both small and large spatial scales.

7.4.1. Choices for markers and research scales

The selected environmental variables varied amongst the different models for each species according to the different genetic markers and different spatial scales. For DNA sequence markers, mitochondrial markers had a better performance than nuclear markers, as judged by higher r^2 values in the one sponge and three corals models. There was no evidence to support the hypothesis that the environmental variables explained in absolute terms increased levels of genetic variation *per se* (e.g. the nucleotide diversity of the mitochondrial *D-loop* region was lower than that of the nuclear *ITS* region in *S. variabilis*, but the R^2 value of the *D-loop* model was higher than that of the *ITS* model). One possible reason for this result is that the mitochondrial genome is more sensitive than the nuclear genome to environmental variability, perhaps because almost all of the mitogenome is coding whereas most of the nuclear genome is non-coding. For example, whole-organism metabolic rate has been shown to be affected by genetic polymorphism across mtDNA (Arnqvist *et al.*, 2010). In the present study, oxygen and temperature variation significantly explained mtDNA variation in both corals and sponges (oxygen and temperature are directly related to metabolic rate (Gillooly *et al.*, 2001; Seibel & Drazen, 2007)), and this may be indirect evidence supporting why the model had a better explanation for the mtDNA markers. Microsatellites, which had a better performance in population genetic studies, were less sensitive to environmental factors, as might be expected for markers that are assumed to be selectively neutral. Whether there is a direct selection-based association between genetic and environmental variation, or whether the environmental variables simply act as barriers to gene flow (a more neutral rather than selection-based explanation) remains to be determined.

In the present study, analyses of data grouped at different spatial scales produced very different results. Outcomes varied as a result of different marker types. For example, at the local scale (individual level) most significant results involved dominant markers (DNA sequences), but at a broader scale (geomorphic feature population level) it was the co-dominant markers (microsatellites) that revealed significant relationships with environmental variables.

Of note is the fact that a single environmental variable may be included in all models for a single species, but for one marker the variable's contribution was positive and for another marker within the same species the variable's contribution was negative. Examples include aragonite and dissolved organic matter. Determining how a variable may have both a positive and negative contribution to genetic variation within the same species but for

different markers will require further investigation. It may, however, be explained by direct selection pressure on one marker (e.g., the mitogenome) and an absence of selection pressure on another marker type (e.g., the nuclear genome). Given the linked nature of regions (genes) on the circular mitogenome, associations (either positive or negative) may be expected to be the same for two or more regions of mitochondrial DNA. The results here do not however, always support this expectation, indicating that different regions of the mitogenome may be responding differentially to the environmental variation, either as settled adults or as dispersive larvae.

7.4.2. Environmental effects and genetic variations

In general, topographic factors (such as bathymetric variation), physico-chemical factors (such as temperature, current speed, oxygen, aragonite saturation state, and nutrients), and biological factors (such as surface water Chl-a concentration) were examined directly in the present study. They have previously been identified as environmental drivers for controlling the large-scale distribution of deep-sea corals (Bryan & Metaxas, 2006, 2007; Davies *et al.*, 2008; Tittensor *et al.*, 2009; Woodby *et al.*, 2009; Fisher *et al.*, 2014; Arellano *et al.*, 2014).

At the north-central-south regional scale, the main variables driving the genetic structure in corals were dynamic topography, surface water primary productivity, seamount and bottom current speed. Dynamic topography, which is a measure of relative sea surface height, reflects the potential for large scale current movements (Tracey *et al.*, 2011). Significant contributions of dynamic topography to explain genetic variation in *M. oculata* and *S. variabilis* supported the hypothesis in Chapter 6 that large-scale currents associated with the Tropical and Subtropical fronts shaped the regional genetic structure. For *G. dumosa*, the regional structure was influenced by surface water primary productivity, which is related to the amount of food available to suspension-feeding corals (Tracey *et al.*, 2011). The importance of this variable for this species is also probably related to the influence of currents and fronts on genetic connectivity between the central region and the north and south region. That is, *G. dumosa* was more common in relatively shallower water (compared with *M. oculata* and *S. variabilis*) on the Chatham Rise of the central region, which is an area of particularly high sea surface primary productivity. In the sponge, *Poecillastra laminaris*, silicate was significant in explaining more half of the regional structure, but bottom current speed was the most important variable when tested against the neutral loci (it explained >

60%). This result might indicate that the genome of *Poecillastra laminaris* was under some sort of (unknown) selection pressure for silicate, and that the bottom current speed is acting as a genetic barrier by affecting larval settlement and/or the available food source. However, this hypothesis will require further evidence to test it. Overall, the current was an important factor that contributed to the formation of genetic structures for deep-sea corals and a sponge around New Zealand, and supports the primary explanation for the north-central-south regional genetic variation presented in Chapters 5 and 6.

At the geomorphic feature level, the factors that affected genetic connectivity patterns varied across the different species. In *G. dumosa*, nitrate (co-correlate = phosphate) and aragonite (co-correlates = calcite, temperature, sigma-theta) were the factors that shaped the structure. These two variables have been shown to modify coral growth and calcification in other stony coral species (Marubini & Atkinson, 1999; Leclercq *et al.*, 2000; Turley *et al.*, 2007; Silverman *et al.*, 2007). In addition, temperature and water density (sigma-theta) have been documented as restricting the occurrence deep-sea stony corals (Freiwald *et al.*, 2004; Roberts *et al.*, 2006; Davies *et al.*, 2008; Dullo *et al.*, 2008). In *S. variabilis*, standard deviation of slope and seamount were the most important factors that influenced the genetic variation of populations at the geomorphic features scale, in both the all loci and neutral loci microsatellite data set. This indicates that the topography of different geomorphic features may affect the genetic structure of this species. This might provide an explanation for genetic isolations of populations from Kermadec Ridge, Louisville Seamount Chain and Macquarie Ridge. Previous studies of corals have detected similar patterns: genetic differentiation was significantly different between slope and ridges in *Desmophyllum dianthus* (Miller *et al.*, 2010), and *Narella* (Baco & Cairns, 2012). However, neither of these studies discussed the relationship between genetic differentiation and topography: further studies are required to verify this relationship. In *Poecillastra laminaris*, nitrate (co-correlate = phosphate) and bottom water temperature (co-correlate = bottom water temperature residuals) were the crucial explanators of population genetic variation at the geomorphic features scale, possibly reflecting that factors relating to energy were involved in the connectivity amongst populations (see next paragraph for discussion on food availability). Generally, one possible interpretation for all of the aforementioned variables acting as barriers to population connectivity relates to them reflecting the absence of a suitable environment for larval settlement and development amongst different geomorphic features. That is, the patterns of genetic structure could be driven by different conditions amongst geomorphic features that challenged the larvae from other populations to survive, colonise and/or reproduce once

established as an adult, such that little or no genetic contribution amongst local gene pools induced genetic differentiation between local populations. However, there are no studies available for deep-sea species to provide evidence to support this suggestion.

At the individual level, the variables that elucidated the patterns of genetic variation differed not only amongst species but also by genetic marker. The important variables identified at this level were similar to those identified by habitat suitability or species distribution modelling, but the importance of each variable in the present study was different from those identified by these previous studies (Guinotte *et al.*, 2006; Tittensor *et al.*, 2009; Davies & Guinotte, 2011; Tracey *et al.*, 2011). In this study, sea surface temperature gradient was significant as an explainer of genetic variation in all three corals and the sponge *Poecillastra laminaris* for different markers, except the all loci data set of *G. dumosa*. In addition, a significant contribution of sea surface temperature was also detected as an explainer of genetic variation in *P. laminaris* and *S. variabilis*. Sea surface temperature and sea surface temperature gradient are both related to primary productivity, and the latter to the location of frontal systems (Hurst *et al.*, 2012). Primary productivity and frontal systems are proxies for food availability (Abelson *et al.*, 1993; Kaiser, 2011), and the genetic variation patterns of these two species might therefore be driven by food source availability. Food is closely related to an organism's growth, fecundity, larval dispersal and settlement (Thorson, 1950; Buss & Jackson, 1981; Bertness & Gaines, 1993; Qian, 1994; Pechenik *et al.*, 1996; Fouzai *et al.*, 2015), and thus food availability indirectly effects the genetic contribution made by one population to another population (by fecundity) and/or the genetic connectivity between populations (by larval dispersal and settlement). In *Poecillastra laminaris*, as well as sea surface temperature and sea surface temperature gradient, nitrate (co-correlate = phosphate) and particulate organic carbon export (POC) were included in all 4 individual-level models. The significant relationship between POC and genetic structure were also detected in a shallow-sea reef sponge (*Stylissa carteri*) (Giles *et al.*, 2015). A previous study has found that POC and nitrogen (PON) are very important food sources for sponges (Hadas *et al.*, 2009), further suggesting that food source availability might be the reason for the genetic differentiation observed in *Poecillastra laminaris*. However, in modelling the distribution of the sponge *Geodia*, surface temperature did not contribute to the prediction (Knudby *et al.*, 2013), indicating that this contradictory finding necessitates further investigation. Interestingly, dynamic topography was an important contribution to explaining the individual-level genetic variation of *G. dumosa* in all 4 models but not at large scales (regional and geomorphic feature level) for this species (it was important at these scales for

the other two coral species). As noted above, dynamic topography is related to large-scale current dynamics, which have been shown previously to be related to the distribution of suspension-feeding organisms through influencing food source availability and variability (Frederiksen *et al.*, 1992; Tittensor *et al.*, 2009), which provides more support for the explanation that food availability might explain the local genetic structure observed in *G. dumosa*. Generally, the results at the local scale from the present study suggest that food resource variability helps to explain small-scale genetic differentiation for both corals and sponges.

Interestingly, a previous study has found that deep-sea corals and sponges have similar environmental demands (Rooper *et al.*, 2014), but despite this, the variables that influenced the patterns of genetic structure at different scales were species-specific. Nevertheless, how these different variables modified these species-specific patterns of genetic connectivity is still unclear. Additionally, high correlation coefficients leave as yet unresolved the question of whether species-specific genetic variation is correlated with a single environmental variable or multiple variables. Investigations of genotypic, phenotypic and environmental variability, as well as of gene-environment interactions are required to resolve this.

7.4.3. Implications for management

Marine environment managers are increasingly incorporating genetic information into management planning (Waples *et al.*, 2008; McCook *et al.*, 2009; Haig *et al.*, 2016), and the results of seascape genetic modelling can help inform management options that need to respond to factors that influence genetic structure and connectivity. To maintain population-level genetic diversity and effective population size, it is essential to understand the patterns of genetic connectivity that exist amongst deep-sea populations. However, open spatial population structures, logistical sampling difficulties and unknown barriers to gene flow present challenges to connectivity research. To overcome these challenges, seascape genetic research may open another window to improve understanding of the linkages between genetics and the spatial ecology of populations (Selkoe *et al.*, 2008).

The genetic separation of some deep-sea coral and sponge populations into regions or locations that are characterised by particular temperature or food availability regimes illustrates the importance of protecting these taxa via spatial management options that include locations (e.g. marine protected areas) that represent these environmental regimes. Additionally, the identification of variables that help to explain coral and sponge genetic

variation indicates that networks of protected areas should include representatives of different geomorphic features, and conservation priorities could be allocated to sites that contain particularly complex topography. Related to the latter recommendation, the results from the present study indicate that the Kermadec region is important, because all examined species occurred there, and it has a complex bathymetry (slope, plateaux, seamounts, plains etc). Hence protecting this area serves to achieve a number of potential conservation objectives (representative populations and genetic diversity), something that currently being considered in the form of the Kermadec Ocean Sanctuary proposal (<http://www.mfe.govt.nz/marine/kermadec-ocean-sanctuary>).

Appendix

Table S7.1 Mean values of the environmental variables for the *Poecillastra laminaris* populations

Variable	Central Region	North Region	South Region	Campbell Plateau	Chatham Rise	Auckland Slope	Kermadec Ridge	Macquarie Ridge
bpi-broad	120.526	618.438	996.882	8.750	120.526	440.750	927.200	1300.923
bpi-fine	59.789	246.625	164.588	14.500	59.789	225.250	422.800	210.769
oxygen	4.627	4.539	5.134	5.298	4.627	4.617	4.546	5.021
POC	14.475	9.237	5.326	11.125	14.475	12.721	5.410	3.817
salinity	34.440	34.770	34.380	34.385	34.440	34.882	34.852	34.383
silicate	28.732	13.620	35.789	31.394	28.732	8.899	6.724	38.939
slope-percent	2.312	7.060	10.518	1.158	2.312	8.833	8.585	13.398
stdev-slope	1.269	3.191	3.841	0.357	1.269	3.184	4.725	4.913
sigma-theta	31.684	31.299	31.778	31.731	31.684	31.196	31.141	31.793
woanite	29.016	23.146	28.240	25.256	29.016	16.339	26.838	29.592
botspd	0.107	0.096	0.119	0.110	0.107	0.102	0.051	0.093
cdom	2.509	1.948	1.415	1.390	2.509	2.277	1.534	1.416
disorg	0.050	0.023	0.026	0.022	0.050	0.033	0.007	0.031
dynoc	0.524	0.693	0.361	0.329	0.524	0.597	0.813	0.438
smt	0.737	0.500	0.722	0.000	0.737	1.000	0.000	1.000
sstgrd	0.009	0.006	0.006	0.003	0.009	0.009	0.007	0.007
sst	0.017	0.014	0.010	0.006	0.017	0.021	0.013	0.013
tempbot	-0.114	0.599	-0.919	-1.466	-0.114	0.933	-0.069	-0.076
tidcurr	0.182	0.100	0.154	0.206	0.182	0.041	0.124	0.121
vgpm	640.344	538.315	317.396	284.656	640.344	672.843	351.955	355.189

Table S7.2 Mean values of the environmental variables for the *Goniocorella dumosa* populations

Variable	Central Region	North Region	South Region	NE Slope	Challenger Plateau	Chatham Rise	Kermadec Ridge
bpi-broad	45.435	267.375	274.600	124.500	79.800	45.435	399.083
bpi-fine	25.355	106.417	95.200	21.000	8.000	25.355	172.333
aragonite	1.627	1.739	1.468	1.954	1.939	1.627	1.582
oxygen	5.374	4.612	5.407	4.517	4.618	5.374	4.601
POC	25.135	15.711	10.278	15.824	16.279	25.135	14.003
salinity	34.518	34.817	34.443	34.994	34.978	34.518	34.703
slope-percent	1.665	8.757	5.343	2.266	3.250	1.665	12.132
stdev-slope	0.546	2.332	2.668	0.484	0.819	0.546	3.433
woanite	19.275	18.164	20.895	13.868	20.375	19.275	18.777
botspd	0.073	0.085	0.045	0.085	0.052	0.073	0.098
cdom	2.388	2.317	1.430	1.930	2.388	2.388	2.288
disorg	0.063	0.039	0.026	0.039	0.041	0.063	0.033
dynoc	0.480	0.593	0.448	0.623	0.555	0.480	0.606
smt	0.145	0.333	0.200	0.000	0.000	0.145	0.583
sstgrd	0.013	0.008	0.006	0.009	0.006	0.013	0.008
sst	0.024	0.020	0.013	0.021	0.017	0.024	0.021
tempbot	-0.371	1.349	-0.489	1.580	2.395	-0.371	0.950
tidcurr	0.382	0.094	0.266	0.147	0.205	0.382	0.048
vgpm	653.412	705.329	326.955	684.132	636.341	653.412	681.477

Table S7.3 Mean values of the environmental variables for the *Madrepora oculata* populations

Variable	Central Region	North Region	South Region
bpi-broad	61.048	373.533	-6.929
bpi-fine	8.476	123.067	0.571
aragonite	1.092	1.518	1.061
oxygen	4.375	4.424	4.773
POC	13.869	11.733	7.831
salinity	34.425	34.733	34.358
slope-percent	2.858	4.357	1.214
stdev-slope	0.765	2.010	0.106
woanite	27.707	24.164	30.024
botspd	0.113	0.085	0.119
cdom	2.392	2.118	1.365
disorg	0.050	0.032	0.022
dynoc	0.514	0.610	0.284
smt	0.238	0.400	0.000
sstgrd	0.009	0.005	0.007
sst	0.016	0.013	0.013
tempbot	-0.244	1.014	-1.658
tidcurr	0.173	0.123	0.120
vgpm	624.800	611.207	292.567

Table S7.4 Mean values of the environmental variables for the *Solenosmilia variabilis* populations at regional scale

Variable	Central Region	North Region	South Region
bpi-broad	92.912	1131.808	251.088
bpi-fine	9.147	315.673	58.853
aragonite	1.207	1.327	1.189
oxygen	4.319	4.117	4.580
POC	13.967	5.043	5.638
salinity	34.456	34.469	34.402
slope-percent	3.131	13.529	4.263
stdev-slope	0.820	5.077	1.785
woanite	28.835	32.498	31.602
botspd	0.120	0.080	0.093
cdom	2.504	1.902	1.382
disorg	0.052	0.024	0.024
dynoc	0.524	0.693	0.366
smt	0.265	0.868	0.176
sstgrd	0.008	0.003	0.006
sst	0.016	0.007	0.011
tempbot	-0.074	0.282	-0.874
tidcurr	0.162	0.092	0.120
vgpm	649.818	540.369	312.129

Table S7.4 Mean values of the environmental variables for the *Solenosmilia variabilis* geomorphic feature populations

Variable	Bounty Plateau	Bounty Trough	Campbell Plateau	Chatham Rise	Hikurangi Margin	Kermadec Ridge	Louisville Seamount Chain	Macquarie Ridge
bpibroad	27.000	74.800	6.750	92.912	603.500	909.429	2016.917	538.733
bpifine	-0.500	38.600	-2.625	9.147	53.250	344.171	330.833	122.067
aragonite	1.467	1.078	1.142	1.207	1.256	1.238	1.595	1.213
oxygen	4.851	4.355	4.698	4.319	4.604	4.004	4.323	4.484
POC	9.822	5.712	6.976	13.967	9.559	5.108	2.621	4.406
salinity	34.324	34.450	34.313	34.456	34.463	34.489	34.402	34.391
slopepercent	0.958	2.026	1.405	3.131	8.304	13.047	16.740	7.979
stdevslope	0.179	0.769	0.490	0.820	1.727	4.797	7.071	3.478
woanite	30.027	32.940	31.687	28.835	25.291	33.364	32.060	31.263
botspd	0.091	0.097	0.132	0.120	0.191	0.069	0.105	0.072
cdom	1.356	1.283	1.402	2.504	2.365	1.819	1.799	1.411
disorg	0.029	0.023	0.019	0.052	0.039	0.019	0.031	0.026
dynoc	0.223	0.221	0.308	0.524	0.589	0.713	0.466	0.484
smt	0.000	0.200	0.000	0.265	0.750	0.857	1.000	0.333
sstgrd	0.007	0.007	0.005	0.008	0.005	0.003	0.006	0.005
sst	0.015	0.012	0.009	0.016	0.011	0.006	0.011	0.010
tempbot	-2.315	-0.779	-1.559	-0.074	0.341	0.282	-0.478	-0.157
tidcurr	0.142	0.107	0.111	0.162	0.109	0.090	0.123	0.122
vgpm	343.394	318.312	252.408	649.818	730.177	503.669	462.871	333.582

Chapter 8. General discussion

New Zealand's exclusive economic zone (EEZ) covers approximately fifteen times the land area of the country, with most areas being greater than 200 m in depth, and is comprised of various topographical habitats and complex hydrodynamic systems. However, deep-sea organisms around the world are poorly researched and the New Zealand region is no exception. Meanwhile, the increasing exploitation of marine resources and human-induced environmental changes brings a threat to this relatively unknown system. As such, there are growing concerns about protection in the deep sea and the impacts of anthropological activities, mainly fisheries (Ramirez-Llodra *et al.*, 2011). The vulnerable marine ecosystem (VME) concept originated from discussions at the United Nations General Assembly (UNGA), as established in UNGA Resolution 61/105. The FAO International Guidelines for the Management of Deep-sea Fisheries in the High Seas expanded on the VME concept for fisheries management (FAO, 2009). VME indicator taxa are defined as those that are unique or rare, functionally significant, fragile, structurally complex, slow to recover or may never recover, exhibit slow growth, late age of maturity, low or unpredictable recruitment, and are long lived (e.g. Parker *et al.*, 2009). In New Zealand, it has been reported that VME indicator taxa such as deep-sea corals are impacted by commercial trawling, both within and outside New Zealand's EEZ (Anderson & Clark, 2003; Clark & Rowden, 2009). However, limited information about the genetic structure and population dynamics of deep-sea species, especially VME indicator taxa, constrains effective management and conservation of marine resources. Population genetics integrated with ecological data is considered to be a powerful approach to address questions about population structure, and gain an understanding of connectivity patterns amongst populations to better inform managers who make recommendations about spatial planning actions that may include marine protected areas (Palumbi, 2003).

The general aims of this thesis were to investigate the genetic structure of VME indicator taxa, specifically 4 sponges (*Neoaulaxinia persicum*, *Penares* sp., *Pleroma menoui* and *Poecillastra laminaris*) and 3 corals (*Goniocorella dumosa*, *Madrepora oculata* and *Solenosmilia variabilis*), and their relationships with environmental variation, and provide information for deep-sea conservation around New Zealand. For this first population genetics study of deep-sea sponges, and one of only a few studies of deep-sea stony corals, a multidisciplinary approach was used with different genetic markers (mainly developed in this study) and environmental data. This study provides important information for management of these VME indicator taxa, especially the poorly-understood sponges, it contributes to a

broad understanding of the patterns of gene flow around New Zealand and it identifies potential factors that shape the patterns of population genetic structure and connectivity.

In this general discussion, the major findings of this study are reviewed and summarised, and considered with respect to their contribution to the field of deep-sea population genetics. The management implications of the research are also highlighted, and finally future research needs are identified.

8.1. Major findings

8.1.1. Marker performance

In the present study, three different types of genetic markers were involved, sequence (*COI*, *Cytb*, *12S*, *D-loop*, *ITS*), genotype (microsatellites) and single nucleotide polymorphism (SNPs). Sequence markers, both mitochondrial and nuclear, were developed for *Poecillastra laminaris* (0) and *Solenosmilia variabilis* (Chapter 4) in this study. For microsatellites, 10 polymorphic microsatellite markers were developed for *Poecillastra laminaris*, and 18 polymorphic microsatellites were successfully generated from 454 next generation sequencing data for *S. variabilis*. Cross-amplification of these microsatellites and another 9 microsatellites from previous studies were tested in three stony corals (*Desmophyllum dianthus*, *Goniocorella dumosa*, *Madrepora oculata*). A significant number of SNPs (102,678) and indels (846) were identified based on specific filter criteria for *S. variabilis*, and the variations amongst individuals were tested.

In the sponges, although *COI* was successfully amplified in four species, only for *Poecillastra laminaris* was genetic structure detected. Surprisingly, the nucleotide diversities of *COI* were higher than have been reported for shallow water sponges (Duran *et al.*, 2004; Lavrov *et al.*, 2005; Wörheide, 2006). However, these higher genetic diversities did not contribute to structure discovery, because genetic structure has been detected in shallow water sponges with much lower nucleotide diversity levels (Duran *et al.*, 2003; Wörheide 2006; DeBiasse *et al.*, 2009; López-Legentil and Pawlik 2009). This result also supported the hypothesis in Chapter 2 that genetic variation is not related to the ability to detect barriers. In the corals, the sequence data did not reveal evidence of genetic structure in *G. dumosa* or *S. variabilis* for either *ITS* or *D-loop*. Variations of these two non-coding genes still could not provide enough resolution to show evidence of genetic structure, consistent with findings in a previous study (Miller *et al.*, 2011). In general, *COI* and *Cytb* in *P. laminaris* and *ITS* in *M.*

oculata provided enough resolution to reveal population structure, and they could be used for future studies. Additionally, *ITS* markers for sponges and *COI* markers for corals were tested at the beginning of this project, but multicopy PCR products and poor quality of sequencing meant that these markers were excluded from subsequent research.

For microsatellites, major issues for both sponges and corals were missing values in genotypes and small sample sizes. These issues resulted in inconsistent results between different analytical approaches, despite lots of effort for data quality control and post-hoc testing. Small sample size is a common challenge in deep-sea research (see Chapter 2), and the balance between accuracy and information loss from valued samples is now widely appreciated. Depending on the availability of samples from the archive for the present study, minimum sample size was set at 4 individuals. Meanwhile, many loci exhibited null alleles in all three corals, caused by missed alleles in the genotyping. Considering the DNA quality available from archived samples in a study such as this one, this phenomenon might be resolved by extensive (time consuming and expensive) testing of new PCR protocols. Although there were some disadvantages to the use of microsatellite data sets, different spatial structures were still revealed and their presence supported by different analytical approaches.

SNPs are a product of next generation sequencing, but so far have not been widely used in deep-sea population genetics studies. In the present study, the developed SNPs failed to reveal a clear split between different populations (Australasia and the Louisville Seamount Chain), and this indicated a further filter of these SNPs is required. When designing the primers, many SNPs exhibited polyadenylations of upstream and/or downstream sequences: these could be a technical challenge to the use of these SNPs. Further experiments are required to characterise these candidate SNPs.

8.1.2. Common patterns of genetic structure in the New Zealand deep sea

As reviewed for coastal species, there is a north-south genetic differentiation in New Zealand, and a putative barrier to gene flow at about 42°S (Gardner *et al.*, 2010). However, no reports from deep-sea organisms have discussed this pattern of differentiation. Based on the Watling *et al.* (2013) definition of deep-sea biogeographic provinces, there was potentially a north-south differentiation around 45°S, where the province boundary exists. In the present study, the patterns of genetic variation observed for collected species around New Zealand reflected different hierarchical degrees of differentiation from north to south and

different geomorphic feature populations (Table 8.1). The results showed that the patterns varied amongst different species, as well as with different genetic markers.

Table 8.1 Genetic differentiation patterns amongst different species in the present study

Species	Marker	Between Provinces	Amongst Regions	Amongst Geomorphic Feature Populations	Isolation by Distance	Isolation by Depth
<i>Poecillastra laminaris</i>	<i>COI</i>	Y	Y	Y	Y	N
	<i>Cytb</i>	Y	Y	Y	N	N
	All SSR	N	N	Y	N	N
	Neutral SSR	N	Y	N	N	N
<i>Penares</i> sp.	<i>COI</i>	N	N	N	N	N
	<i>12S</i>	N	N	N	N	N
<i>Neoaulaxinia persicum</i>	<i>COI</i>	N	N	N	N	N
	<i>12S</i>	N	N	N	N	N
<i>Pleroma menoui</i>	<i>COI</i>	N	N	N	N	N
	<i>ITS</i>	N	N	N	N	Y
<i>Goniocorella dumosa</i>	<i>D-loop</i>	N	N	N	N	N
	All SSR	N	Y	Y	N	Y
	Neutral SSR	N	Y	Y	N	N
<i>Madrepora oculata</i>	<i>ITS</i>	N	Y	N	N	Y
	All SSR	N	Y	N	N	N
	Neutral SSR	N	Y	N	N	N
<i>Solenosmilia variabilis</i>	<i>ITS</i>	N	N	N	N	N
	<i>D-loop</i>	N	N	N	N	N
	All SSR	Y	Y	Y	Y	N
	Neutral SSR	Y	Y	Y	N	N

Y means significant relationship at $p < 0.05$, and N means $p > 0.05$.

As hypothesised in Chapter 1, some common patterns were expected to be observed amongst the examined species. Due to issues with small sample sizes and uninformative markers, no spatial structure was observed in *Penares* sp., *Neoaulaxinium persicum* or *Pleroma menoui*, and these species are not involved in the following discussion. Indeed, variation in the mitochondrial DNA markers *COI* and *Cytb* for *P. laminaris*, nuclear DNA marker *ITS* for *M. oculata*, microsatellites for *Poecillastra laminaris*, *G. dumosa*, *M. oculata* and *S. variabilis* have all revealed the existence of pronounced north-central-south regional structure in deep-sea organisms (Table 8.1). However, despite this common pattern, there were more species-specific patterns discovered. Mitochondrial genes of *P. laminaris* and microsatellites of *S. variabilis* revealed a northern-southern provinces structure, and only *M. oculata* exhibited no evidence of genetic structure at the geomorphic feature level. Isolation by distance was observed in *P. laminaris* and *S. variabilis*, and isolation by depth was found in *G. dumosa* and *M. oculata* (Table 8.1). These shared and different patterns could be contributed to interaction between the species themselves and their environments, as discussed in the subsequent sections.

8.1.3. Connectivity amongst geomorphic feature populations

Overall, less population genetic structure was detected among geomorphic feature populations within regions (north region: Kermadec Ridge, Challenger Plateau and Hikurangi Margin and south region: Bounty Trough, Campbell Plateau, Macquarie Ridge) than amongst regions for both the sponge and corals (Table 8.2). This result indicates that more connection exists within regions and less or no connections exist amongst regions; this is also in agreement with the north-central-south region structure. Amongst the geomorphic feature populations, populations from the Kermadec and Macquarie ridges were genetically isolated (Table 8.2). Furthermore, populations of *S. variabilis* from the Louisville Seamount Chain were also genetically distinct from other populations. The Kermadec and Macquarie ridge populations are located at the northern-most and southern-most extremes of the New Zealand EEZ, and the Louisville Seamount Chain occurs to the northeast of New Zealand, beyond the EEZ.

Table 8.2 Summary of significant genetic differentiation amongst different geomorphic feature populations of sponges (above diagonal) and corals (below diagonal)

	Kermadec Ridge	Challenger Plateau	Hikurangi Margin	Chatham Rise	Bounty Trough	Campbell Plateau	Macquarie Ridge
Kermadec Ridge		<i>P. laminaris</i> (<i>Cytb</i>)		<i>P. laminaris</i> (<i>COI</i> , <i>Cytb</i> and all SSR)	<i>P. laminaris</i> (<i>COI</i>)	<i>P. laminaris</i> (all SSR)	<i>P. laminaris</i> (<i>COI</i> , <i>Cytb</i> and all SSR)
Challenger Plateau							<i>P. laminaris</i> (<i>COI</i>)
Hikurangi Margin							<i>P. laminaris</i> (<i>COI</i>)
	<i>M. oculata</i> (<i>ITS</i>)						
	<i>G. dumosa</i> (all and neutral SSR)						
Chatham Rise	<i>S. variabilis</i> (all and neutral SSR)		<i>S. variabilis</i> (all SSR)				<i>P. laminaris</i> (<i>COI</i> and <i>Cytb</i>)
Bounty Trough							
Campbell Plateau	<i>G. dumosa</i> (SSR)			<i>G. dumosa</i> (SSR)			
Macquarie Ridge	<i>S. variabilis</i> (<i>ITS</i> , all and neutral SSR)		<i>S. variabilis</i> (all and neutral SSR)		<i>S. variabilis</i> (all SSR)		
					<i>S. variabilis</i> (all and neutral SSR)		
Louisville Seamount	<i>S. variabilis</i> (all and neutral SSR)		<i>S. variabilis</i> (all and Neutral SSR)	<i>S. variabilis</i> (all and neutral SSR)	<i>S. variabilis</i> (all and neutral SSR)	<i>S. variabilis</i> (all and neutral SSR)	<i>S. variabilis</i> (<i>D-loop</i> , <i>ITS</i> , all and neutral SSR)

The significances of showed species with different markers (in the brackets) were less than 0.05.

Migration analysis of four species (3 corals, 1 sponge) showed that connectivity amongst geomorphic feature populations is very complicated. Chiefly, all populations had strong self-recruitment, and limited connections between populations (Chapter 5 and Chapter 6). On a broad scale, migration between the north and south regions was less than that within regions in all four species. The population from the Kermadec Ridge is acting as a source population (providing migrants to other populations but receiving few if any migrants from other populations), whilst the population from the Chatham Rise was both a source and a sink population. Populations on geomorphic features in the south region (such as Bounty Plateau, Campbell Plateau, Macquarie Ridge) were relatively well connected with each other.

8.1.4. Factors contributing to genetic differentiation

Understanding the deep-sea environmental and biological processes that shape spatial genetic patterns of species is critical for predicting evolutionary dynamics and defining significant evolutionary and/or management units. In this study, the potential role of environmental and biological factors in modifying the genetic structure of deep-sea habitat-forming species in the New Zealand region was first evaluated based on the findings of Chapters 5 and 6, and then the role of environmental factors was investigated using a seascape genetics approach in Chapter 7.

8.1.4.1. Depth and Distance

Depth was reported as the most important factor as a genetic barrier for deep-sea organisms in the meta-analysis (Chapter 2), but most of the species in this study did not exhibit a pattern of genetic isolation by depth. In the 7 examined species across different markers, only microsatellites and *ITS* of *G. dumosa* and *ITS* of *M. oculata* revealed a weak pattern of isolation by depth. The distribution of collected specimens showed that *G. dumosa* had a relatively narrow and comparatively shallow distribution and *M. oculata* had a moderate distribution (Figure 8.1): the depth distribution did not support that genetic isolation preferentially occurred in wide depth ranges. This result could indicate that the influence of depth on genetic connectivity is different amongst species, even taxonomically close species. Furthermore, variation in depth was significantly associated with nuclear marker type (*ITS* and microsatellites) but not mitochondrial marker (*D-loop*), which indicated that different marker types exhibit different results.

Figure 8.1 Sample collection depth distributions of all examined species

Distance is a barrier to gene flow in some deep-sea organisms (Chapter 2), however, isolation by distance (IBD) only occurred in *Poecillastra laminaris* (COI) and *S. variabilis* (microsatellites), and non-significant values were observed for the other marker types of the other species (Table 8.1). Previous study has showed that deep-sea organisms have a long planktonic larval duration (Baco *et al.*, 2016; Hilário *et al.*, 2015), and this might assist most deep-sea species to achieve large-scale genetic connectivity. Lack of distance as a barrier to gene flow for these species indicated that the studied species achieved colonisation and genetic connectivity in patchy habitats. However, whilst there was a possibility that the distances between two sampling sites might not be great enough to reveal the genetic isolation caused by distance, this explanation seems to be unlikely given the spatial scales involved and the fact that patterns of IBD were observed for *Poecillastra laminaris* and *S. variabilis*.

8.1.4.2. Currents and Topography

In the meta-analysis (Chapter 2) currents and topography were identified as significant barriers to genetic connectivity. In the present study, dynamic topography (a proxy for large-scale current flow) and standard deviation of slope were the explanators in the seascapes models for genetic variation in three corals at different spatial levels, providing direct evidence that currents and topography affect genetic connectivity.

The hydrodynamic system may not only act as a ‘soft’ barrier for genetic connectivity, but it also can promote larval dispersal and achieving long-distance genetic connection (Rogers *et al.*, 1998; Zane *et al.*, 1998; Roques *et al.*, 2002; Sutton & Chereskin, 2002; Shaw *et al.*, 2004; Plouviez *et al.*, 2013). Three major currents and their associated fronts around New Zealand, the Tasman Front, the Subtropical Front and the Subantarctic Front, are expected to have the greatest potential influence on population connectivity of deep-sea populations in the region. These currents and frontal systems differentiate the water mass environments in the north and the south of the region, and also influence the direction of north and south currents across the Chatham Rise that may reduce or perhaps even block gene flow across this feature. Populations of three coral species and the sponge, *Poecillastra laminaris*, from the Kermadec Ridge were shown to be genetically differentiated from other populations in the New Zealand region, and the East Auckland Current and its associated eddies may be responsible for this relative isolation (Chiswell & Roemmich, 1998). Significant genetic differentiations were detected between the populations from Louisville Seamount Chain and other populations within New Zealand EEZ (Chapter 6), and the general lack of current flow from the Louisville Seamount Chain towards New Zealand (Chiswell & Rickard, 2006) may act to separate populations on this feature from those in the rest of the region. The D’Urville Current (Chiswell, 2000) might assist *M. oculata* larvae to connect between geomorphic features to the west, and explain the lack of genetic differentiation between Challenger Plateau and Chatham Rise observed for *G. dumosa* and *M. oculata*. Furthermore, genetic differentiation between biogeographic provinces was found for *S. variabilis*, and this was possibly caused by the different currents and frontal systems in the north (Tropical Front) and south (Subtropical Front and Subantarctic Front) of the region (Chiswell *et al.*, 2015), which may impede larval dispersal and reduce or perhaps even block gene flow across the Chatham Rise.

On the role of currents in promoting gene flow, the haplotypic distribution of all species and panmixia patterns at different scales in different species could be affected by ocean current dynamics, and the results of this study indicate that two main population connection routes may exist. First, a northern route from the northwest of the North Island with the southerly flow of the East Auckland Current down the east coast of North Island with the East Cape Current, and then eastwards immediately north of the Chatham Rise and the Subtropical Front. Second, a southern route from south of the South Island northwards with the Southland Current, then eastwards immediately south of the Chatham Rise and the

Subtropical Front. However, hydrodynamic modelling that includes simulated larval dispersal is required to further substantiate this hypothesis.

The observed isolation of most populations on the Kermadec Ridge, Macquarie Ridge and Louisville Seamount Chain could possibly be explained by their relative spatial isolation. However, only the genetic structure of two study species (*P. laminaris* and *S. variabilis*) showed evidence for isolation by distance. Another explanation for the isolation of populations on the Louisville Seamount Chain, and Kermadec and Macquarie ridges relates to the populations being sampled from seamounts on these features. Although there was no direct evidence to show that there is a relationship between seamount and the presence of genetic barriers, topographic controlled current flows and other hydrodynamic features associated with seamounts are thought likely to influence the genetic isolation of populations (Shank, 2010). However, evidence for such patterns is equivocal (Chapter 2), and the influence of the interaction of topography and currents at the seamount-scale on genetic structure requires further investigation.

8.1.4.3. Life features

Reproduction and larval dispersal strategies are considered important factors in shaping genetic connectivity patterns in many deep-sea invertebrates (for examples see Hilário *et al.* (2015)), and species-specific differences in reproductive strategies may contribute to differences in patterns of gene flow and genetic structure observed across taxa (e.g. Bors *et al.*, 2012; Samadi *et al.*, 2006). Deep-sea demosponges and corals have a wide-range of reproductive strategies (Witte, 1996; Waller 2005; Lemoine *et al.*, 2007; Pires *et al.*, 2014), but very few studies have determined empirically the reproductive strategies or mode of larval dispersal for the four species examined in the present study. Studies of these species or closely related species have reported different reproduction and dispersal strategies, to the extent that these markedly different strategies corresponded directly with the different genetic connectivity patterns amongst different species (Waller, 2005; Lemoine *et al.*, 2007; Miller, 2011; Pires *et al.*, 2014).

Detailed information on the reproductive biology of the four species would be highly beneficial for assessing the importance of reproductive and dispersal strategies on population structure and genetic connectivity. Unfortunately, there is no reproduction information available for sponges in the present study. The three corals are thought to exhibit a similar

reproductive strategy - continuous reproduction, lecithotrophic larvae and feeding after settlement and metamorphosis (Waller, 2005; Pires *et al.*, 2014).

One of the unexpected results of the seascape analysis was the finding that food availability proxies (nitrate and surface water primary productivity) were related to genetic structure at regional and individual level. Food availability may influence population size through colonisation success, which in turn may influence the dispersal potential of a population. However, this study was the first to examine the relationships between genetic variation and environmental variation in the deep sea, and so there is limited context in which to place the finding.

8.2. Management implications and future directions

Findings of this study contribute to VME management, with significant new knowledge about deep-sea connectivity in New Zealand and important findings regarding the processes that can shape the population genetic structure of VME indicator taxa. New genetic markers were developed for sponges and corals, and these powerful tools were used to address ecological questions in the deep sea. The multidisciplinary approach was used to investigate the effects of environmental factors on population genetic connectivity. In general, these findings can be applied to the management of VMEs around New Zealand, and provide a background to other benthic species with similar life history characteristics and inhabiting similar habitats.

Examination of genetic population structure for sponge and coral species illustrates that the connectivity within regions is species-specific. Although connectivity patterns vary, this research provides strong evidence across multiple species that the Kermadec Ridge and Macquarie Ridge regions are genetically differentiated from other parts of the New Zealand EEZ. Moreover, the genetic differentiation between the Louisville Seamount Chain and other populations within the EEZ indicates that genetic disconnections exist between inside and outside of the New Zealand EEZ. These results indicate that areas on the Kermadec and Macquarie ridges and the Louisville Seamount Chain need to be represented in a New Zealand region-scale network of marine protected areas. The current Kermadec Benthic Protection Area (BPA) and the proposed establishment of the Kermadec Ocean Sanctuary (<http://www.mfe.govt.nz/marine/kermadec-ocean-sanctuary>) will contribute towards ensuring the protection of VME indicator taxa in that region. However, there are presently no large protection areas on the Macquarie Ridge or the Louisville Seamount Chain, which could

bring a risk that the populations from these two features might not recovery after major anthropogenic disturbance events. While bottom trawling on seamount features on the Macquarie Ridge is relatively limited, such disturbance is more evident on some seamounts on the Louisville Seamount Chain where only limited protection measures are in place (Penney & Guinotte 2013).

The Chatham Rise (central region) was identified as containing sink populations for two VME indicator taxa (*P. laminaris* and *S. variabilis*) and an area of high genetic diversity for 5 taxa (*P. laminaris*, *N. persicum*, *G. dumosa*, *M. oculata* and *S. variabilis*). Currently, there are two BPAs on the Chatham Rise, and they cover very limited areas *along* the Rise. Because of the relative importance of the Chatham Rise for genetic variation in the region, further protected areas, or an enlarging of the current BPAs (e.g. to extend *across* the rise), should be considered to afford greater protection to VMEs.

Amongst the examined species, the connectivity patterns, as well as the factors that influenced the patterns, varied from species and between spatial scales. Previous studies in the New Zealand region have also revealed different connectivity patterns in other species (Smith *et al.*, 2004b; Miller *et al.*, 2010; Bors *et al.*, 2012; Knox *et al.*, 2012; Foltz *et al.*, 2013; Boschen *et al.*, 2015; Dueñas *et al.*, 2016). Not only is more knowledge about genetic connectivity patterns required for the management of VME indicator taxa but the current findings and previous findings argue that a management system will need to be flexible to change with the advent of new information.

8.3. Future research directions

In the present study, a hierarchical province, region and geomorphic feature framework was employed to test spatial genetic variation of VME indicator taxa in the New Zealand region. For future research, it will be useful to use additional frameworks. For example, the Marine Environment Classification (MEC) of the New Zealand region (Snelder *et al.*, 2007). This classification could provide a more comprehensive spatial framework for understanding spatial genetic variation because it synthesises a greater deal of environmental variation than the framework employed in this study, and the hierarchical level that can be applied is flexible (from 1- 290 classes). Unfortunately, samples included in this study were sufficiently unbalanced to allow for a suitable coverage of classes, and thus the MEC framework was not adopted. In the future, directed sampling might allow for the use of the

MEC framework to examine genetic spatial structure across the New Zealand in a way that is more directly applicable to management.

As a new genetic marker type, SNPs have been developed for *S. variabilis*, although this is an unfinished part of this project. Characterisation of these markers is essential before they are usable. Primary characterisation found that polyadenylation was linked to many of the SNPs and this caused primer design issues. In Chapter 4, however, no clear genetic splits were observed between populations of *S. variabilis* if all SNPs were included in the analysis. Unfortunately, it is not financially feasible to genotype all SNPs across all individuals, and large SNP data sets bring other issues, such as computationally intensive analyses (for details see Helyar *et al.*, 2011). Despite obstacles to the use of this marker type, the low-scoring error rates, relative ease of calibration amongst laboratories compared to length-based markers and the associated ability to assemble combined temporal and spatial data sets from multiple laboratories make SNPs very attractive in population genetic studies (Brumfield *et al.*, 2003; Morin *et al.*, 2004). This is especially the case in terms of the advantages of using SNPs for poor quality DNA samples, which is common for deep-sea samples (Morin & McCarthy, 2007; Smith *et al.*, 2011). Thus, future genetic research in the deep sea around New Zealand should include this marker type and further development work is planned for the SNPs developed here.

Increasing attempts are made to understand the factors responsible for genetic connectivity in deep-sea populations (Hilário *et al.*, 2015; Baco *et al.*, 2016). In the present study, the use of seascape genetics aims to determine a spatially explicit correlation between genetic population parameters and environmental factors, and it provides a glimpse of the relations between genetic variation and environmental variation. This study identified that broad-scale current patterns were an important factor that contributes to genetic connectivity in the region. Integration of genetic information with hydrodynamic dispersal modelling (e.g. Viard *et al.*, 2006; Berry *et al.*, 2012) will yield a more comprehensive understanding of which currents, and how currents, influence genetic connectivity around New Zealand. Such a study should be considered as the next-step in the investigation of the genetic patterns determined by this study.

Building a genetic connectivity model to predict genetic variation in unsampled areas is advantageous from a management aspect (Epps *et al.*, 2007). However, genetic connectivity is a descriptive variable, and how to best introduce genetic parameters into predictive models that represent genetic connectivity has not been widely addressed. Dolgener *et al.* (2014) employed an environmental niche factor analysis (ENFA) to quantify

genetic diversity and its relationship with environmental factors, and found that the ENFA-based landscape genetics approach allows researchers to put various aspects of long-versus short-term effective population size and genetic connectivity into an ecological and spatially explicit context, enabling forecast assessments to be made under future environmental scenarios. However, they did not use this approach to make predictions into environmental space. Models that couple genetic and environmental information, and predict into environmental space, may not only guide the design of contemporary spatial protection measures, but may also predict long term changes, which is very informative for deep-sea management and conversation.

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