Diversity of New Zealand Deep-sea Amphipoda

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ABSTRACT

Biodiversity and the ecological and evolutionary processes which influence faunal distributions are poorly understood in deep-sea habitats. This thesis assesses diversity of deep-sea amphipod crustaceans at three taxonomic levels (family, species, genetic) on continental margins of New Zealand relative to environmental variables. Sampling was undertaken at 20 stations located on Chatham Rise and Challenger Plateau, two major geomorphic features with contrasting environmental conditions.

In Chapter 1, total diversity of the >12,500 amphipods assessed at the family-level revealed high abundance (range: 44 – 2074 individuals 1000 m^{-2}) and taxonomic richness (27 families). Amphipod assemblages at all stations were largely dominated by the same families. Chatham Rise stations were mostly similar in family composition to one another and to the two closest Challenger Plateau stations. Overall, amphipod community composition correlated most strongly with surface chlorophyll *a*, suggesting strong benthic-pelagic coupling.

In Chapter 2, I used molecular methods (COI DNA sequence thresholds of 6%) to estimate species diversity in the dominant amphipod family identified from Chapter 1 - the Phoxocephalidae. Analyses revealed 49 putative species-level taxa, which greatly exceeds the current number (n=17) of formally described New Zealand phoxocephalid species. A cluster of stations with highly similar taxa was identified, broadly distributed over the crest of the Chatham Rise, in association with elevated food availability. Similar to the family-level analyses of Chapter 1 multivariate analysis of phoxocephalid assemblages and environmental parameters revealed a strong correlation with measures of food supply.

Analyses of inter-station assemblages revealed a major split between regions, indicating minimal overlap in taxon distributions on eastern and western continental margins.

Chapter 3 examined genetic connectivity among deep-sea habitats. A phylogenetic analyses of three relatively abundant and widespread taxa; cf. *Ampelisca chiltoni,* cf. *Oediceroides apicalis* and Phoxocephalidae sp., was combined with available genetic data from a further 61 taxa collected from the same stations, to examine mean levels of inter-station genetic divergences. Nearly identical levels of genetic divergence were found between eastern and western regions for all three taxa. Furthermore, inter-station analysis of the wider amphipod community revealed similar patterns of genetic structure in most taxa. Based on molecular clock estimates, genetic divergences most likely corresponded to isolation following landmass changes during the Pleistocene.

This thesis research has revealed a biodiversity hotspot on New Zealand continental margins. I conclude that Cook Strait and the subtropical front are important structures responsible for shaping benthic communities on New Zealand continental margins and that vicariance is likely to have played an important role in the evolutionary radiation of the New Zealand deep-sea fauna.

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THESIS INTRODUCTION

The deep-sea floor is the single largest biome on earth and is also one of the least studied. Only 5% of the deep-sea has been explored with remote instruments and less than 0.01% of the deep-sea floor (the equivalent of a few football fields) has been sampled and studied in any detail (Ramirez-Llodra et al., 2010). As a result, knowledge of biodiversity and factors controlling the spatial distribution of fauna is limited for this ecosystem (Danovaro et al., 2008). However, studies to date have shown that the deep-sea supports one of the highest levels of biodiversity on the planet (Hessler and Sanders, 1967; Grassle and Macioleck, 1992; Snelgrove and Smith, 2002). Exactly how diverse is unknown, with estimates ranging from 500,000 (May, 1992) to 10 million deep-sea species (Grassle and Maciolek, 1992). Equally poorly understood are the evolutionary mechanisms which promote and maintain biodiversity in deep-sea habitats. Populations for deep-sea taxa are thought to be large and to exhibit only modest genetic structure, with little potential for the formation of new species (Zardus et al., 2006; Etter et al., 2011).

Despite inherent difficulties in sampling such a remote habitat, life in the deep-sea has long fascinated naturalists. Early researchers postulated that life ceases to exist at depth (>550 m) for 'want of necessary conditions' (De La Beche, 1834) and subsequent sampling of the Aegean Sea by Edward Forbes appeared to support this view, which became known as the azoic hypothesis (Forbes, 1844) (reviewed in Anderson and Rice, 2006). For the next few years it was generally accepted that the low temperature, lack of light, enormous pressure and stagnancy of the environment precluded the existence of life in the deep-sea. This was eventually falsified by Charles Wyville Thomson and William Carpenter who discovered hundreds of novel deep-sea species while dredging the deep waters (up to 1200 m) off the

British Isles and Spain, and in the Mediterranean (Thomson, 1873). With renewed interest in deep-sea species, Thomson and the HMS Challenger departed on a 3.5 year voyage, with the purpose of gathering scientific information. The expedition discovered (among many other things) 4000 new species (Thomson and Murray, 1911) and set the foundation for modern deep-sea biology.

Today, the lack of information on deep-sea ecosystems is still mostly attributable to the remoteness of the habitat; it is expensive and logistically difficult to sample even relatively shallow bathyal (200 – 2000 m) depths, so few samples have been taken. In addition, processing benthic samples is labour intensive and time consuming owing to high species abundance and diversity, a large proportion of which are undescribed (Blake and Grassle, 1994). This is unfortunate as benthic deep-sea habitats play a crucial role in global biogeochemical cycles (Canals et al., 2006), host a large proportion of Earth's as yet undiscovered biodiversity (e.g., Brandt et al., 2007) and are vulnerable to anthropogenic influences, the effects of which are almost completely unknown (Danovaro et al., 2009). Until the twentieth century, deep-sea ecosystems were relatively un-impacted by human activities, as they are buffered by >200 m water. However, in recent times, offshore oil and gas, pollution, over-harvesting, and global warming are all known to influence deep-sea communities.

To address the general lack of knowledge on New Zealand deep-sea fauna and to promote better understanding of marine process, in 2006 the New Zealand government initiated the Ocean Survey 20/20 (OS20/20) programme of seabed mapping and biodiversity surveys. As part of OS2020, and for the first time in SW Pacific waters (Lörz, 2010), marine invertebrates in the benthic boundary layer (BBL) were sampled at 20 stations on the

Chatham Rise (n=15) and Challenger Plateau (n=5). Previous studies have discovered very high abundance and diversity of BBL macrofauna elsewhere (Brandt, 1997; Brandt and Berge, 2007). However, with an estimated 91% of all marine species undescribed (Mora et al., 2011), many BBL macrofauna taxa on New Zealand's continental margins are likely to require lengthy species descriptions. Baseline knowledge of biodiversity is desperately needed in order predict diversity in unexplored areas as well as monitor and remediate future anthropogenic impacts on biodiversity in the deep-sea.

Clearly, greater emphasis on taxonomic development is needed to describe the biodiversity of the deep-sea. However, the supply of funding and the number of taxonomists continues to dwindle (Wilson, 2000). In contrast, the field of phylogenetics has seen rapid advancement over the past 20 years and has complimented numerous taxonomic studies (e.g., Pilgrim et al., 2002; Hou et al., 2009; Heethoff et al., 2011). The advent of high throughput DNA sequencing has armed taxonomists and other scientists with molecular tools to aid in species identification, delineation and discovery (Hebert et al., 2003a; Vernooy et al., 2010). The use of molecular markers, in combination with traditional morphological methods has added to the accuracy with which species can be identified and reliably compared to one another both within and between studies. Genetic data is unaffected by the common pitfalls of morphological identifications such as phenotypic plasticity, sexual dimorphism and life stage morphology differences (Packer et al., 2009). Moreover, DNA sequences have also revealed previously unrecognised, potentially cryptic species in several animal groups (Hebert et al., 2004, Witt et al., 2006, Zemlak et al., 2009).

However, several other studies have highlighted the potential pitfalls and problems of a molecular based approach to species identification (e.g. Moritz and Cicero, 2004; Will

and Rubinoff, 2004). For example, “pseudogenes” may be a potential confounding factor. Pseudogenes are non-functional duplications of a portion of the mtDNA, located in the nuclear genome which can vary from actual mtDNA by up to 18% (Williams and Knowlton, 2001) and appear to be prevalent across a wide range of taxa (Bensasson et al., 2001). Unrecognised pseudogenes in phylogenetic or population studies may result in spurious results. Fortunately, psuedogenes can usually be detected by screening DNA sequences for “stop codons” which are frequently present in pseudogene sequences (Williams and Knowlton, 2001). Differential rates of evolution among taxa may also confound attempts to identify species based on molecular data, especially across broad taxonomic groups. For example, it is known that slower rates of mtDNA evolution in Cnidaria result in lower intraspecific divergences (<2%) compared with other phyla (Hebert et al., 2003b). However, in the majority of studies of other animal phyla, interspecific greatly exceeds intraspecific genetic variation and generally corresponds to known species boundaries. The natural history of mtDNA may present problems for molecular approaches to species identification that are based on mtDNA. These include reduced effective population size, maternal inheritance, recombination, inconsistent mutation rate, heteroplasmy, compounding evolutionary processes and inherited symbionts (Hurst and Jiggins, 2005; Rubinoff et al., 2006) and could limit the application and utility of molecular based efforts. Species level paraphyly and polyphyly (Funk and Omland, 2003) resulting from imperfect taxonomy or incomplete lineage sorting (Meyer and Paulay, 2005) can also distort interpretations of DNA data. In particular, regional based studies can under-sample the most closely related species, which are often allopatric, and thus overestimate average interspecific differences (Meyer and Paulay, 2005; Bergsten et al., 2012). These limitations can be mitigated by improved coverage of interspecific variation by broadening the spatial scale of sampling.

Recently, the effect of geographic scale has also been recognised as a confounding influence on molecular-based species identification, particularly when calculating intraspecific divergence limits. Early studies (e.g. Hebert et al., 2003a; Barrett and Hebert, 2005) were criticised (e.g. Meyer and Paulay, 2005) for underestimating the extent of intraspecific divergence by only sampling a limited number of geographically clumped populations. Specifically, intraspecific variation is significantly correlated with the geographical scale of sampling (Bergsten et al., 2012). Thus, to avoid underestimation of intraspecific genetic variation, sampling should encompass the widest possible extent of a taxon's range. Despite these possible limitations, molecular data hold tremendous potential for taxon identification in ecological studies.

In habitats such as the deep-sea, when the majority of species are undescribed, molecular data can provide the taxonomic information necessary for ecological investigation. Genetic data also allow analyses of biodiversity at the intraspecific level which can investigate evolutionary processes (Heads, 2005). Little is currently known about how evolution has unfolded in the vast stretches of the deep ocean (Etter et al., 2005). Even basic information such as which processes are most important and the geographic and bathymetric scales over which they operate are poorly understood. Assessing genetic diversity of deep-sea species will improve knowledge in these areas.

To investigate deep-sea biodiversity and assess the efficacy of genetic methods for species identifications in unknown taxa, I selected amphipods as they are among the most abundant and diverse of marine taxa and particularly dominant in continental margin habitats (Brandt, 1993). New Zealand is known to have a diverse marine amphipod fauna (Barnard, 1972), although prior to the present study, this was largely represented by

recordings of coastal taxa (Webber et al., 2010), which are more easily sampled than those of the deep-sea. The New Zealand continental shelf (<1000 m) greatly exceeds the total land area of New Zealand and studies to date indicate a highly diverse fauna (McKnight and Probert, 1997; Probert and Grove, 1998). However, due to a lack of sampling effort targeted at the BBL, biodiversity of Amphipoda inhabiting New Zealand's continental margins was largely unknown prior to beginning my thesis research in 2007. Accordingly, my thesis assesses the biodiversity of BBL Amphipoda and investigates the ecological and evolutionary factors that control spatial distributions of animals inhabiting the deep-sea.

ORGANISATION OF THESIS

This thesis investigated amphipod biodiversity at a different level of biological organisation (family / species / population) in each of the three chapters. In Chapter 1, I assess the abundance and diversity of epibenthic amphipods (Crustacea) from two regions; the Chatham Rise and Challenger Plateau. This chapter also investigated correlations between amphipod families and environmental variables such as depth, average autumn surface chlorophyll *a* concentration, total organic matter, $CaCO_3$, clay and mud content. Determining and understanding changes in community structure and composition across environmental gradients is an essential element of community ecology (Levin, 1992). The multidisciplinary nature of the OS2020 voyages meant that environmental variables were measured concurrently at many of the stations included in this thesis. By measuring the correlation between amphipod biodiversity and a suite of environmental variables, I was able to reveal the factors which are most influential on the spatial distribution and abundance of deep-sea amphipod taxa.

Due to high species diversity, most of which is currently undescribed, comparisons of deep-sea communities are frequently limited to a relatively small group of easily identified megafaunal taxa or to the analysis of higher taxonomic levels within indicator groups (i.e. Chapter 1). In Chapter 2, I utilised molecular data (COI sequences) to assess species level biodiversity in a numerically dominant amphipod family (Phoxocephalidae). In previous cases where a more extensive taxonomic framework exists, studies have found that COI sequences form discrete clusters corresponding to morphological species (Witt et al., 2006; Costa et al., 2009; Hou et al., 2009; Radulovici et al., 2009) with interspecific variation on average 25 times greater than intraspecific. Consequently, COI divergences can be used to

demarcate species identities in taxonomically difficult taxa and accurately compare biodiversity among regions. As with Chapter 1, the correlation of environmental variables with amphipod communities was investigated. I tested the hypothesis that the diversity of deep-sea macrofauna is correlated with increased food supply associated with higher surface productivity.

My final research chapter examined genetic structure among populations of several amphipod taxa with ranges that include undersea ridges, oceanic convergences and historical land barriers; all putative obstructions to gene flow. Here, my aim was to assess levels of gene flow within and between Chatham Rise and Challenger Plateau and to test the hypothesis that genetically distinct populations will occur as a consequence of isolation caused by past hydrogeographic barriers following landmass changes during the Pleistocene. In other marine habitats, geological events such as uplift and landmass changes can disrupt gene flow and isolate formerly interbreeding populations, leading to deeply divergent, geographically sorted lineages (Stevens and Hogg, 2004; Kelly et al., 2006). I predicted that, similar to coastal taxa of New Zealand, genetically distinct populations would be found among populations inhabiting eastern and western stations.

The thesis concludes with a summary of the main findings of my research, as well as suggestions for future research. Additionally, I include as an appendix a manuscript on the vicariant evolution of species within *Paracorophium*, a coastal amphipod genus in New Zealand partly based on data collected during my MSc research. However, further sampling and manuscript preparation and publication was undertaken during the course of my PhD. This manuscript provides further information on evolutionary processes described in Chapter 3.

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CHAPTER 1

ABUNDANCE AND DIVERSITY OF EPIBENTHIC AMPHIPODS (CRUSTACEA) FROM CONTRASTING BATHYAL HABITATS[†]

Keywords: Challenger Plateau – Chatham Rise – Community composition – Macrofauna – New Zealand – Subtropical Front

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ABSTRACT

To investigate relationships between epibenthic macrofauna and bathyal habitat characteristics, we examined the abundance, diversity and community composition of amphipod crustaceans relative to environmental variables on two major bathymetric features of New Zealand - the Chatham Rise and Challenger Plateau. An epibenthic (Brenke) sledge was used to sample depths ranging from 200 – 1200 m. Fifteen stations were sampled on the Chatham Rise, which is an extensive submarine ridge, east of New Zealand, characterised by high productivity in surface waters, associated with the Subtropical Front. Five stations were sampled on the Challenger Plateau, west of New Zealand, a region with a similar depth range, but less topographical relief and lower pelagic productivity relative to the Chatham Rise. Over 12,500 amphipods were recovered and identified. We found high abundance (range: 44 – 2074 individuals 1000 m^{-2}) and taxonomic richness (27 families) in both regions. Amphipod assemblages at all stations were largely dominated by the same families, particularly the Phoxocephalidae. Chatham Rise stations were mostly similar in family composition to one another and to the two closest Challenger Plateau stations. However, the remaining three, more distal, western Challenger Plateau stations were highly differentiated from other stations and from one another, despite being relatively similar habitats. Overall, amphipod community composition correlated most strongly with surface chlorophyll *a*, suggesting strong benthic-pelagic coupling and emphasising the importance of benthic-pelagic links in bathyal ecosystems.

INTRODUCTION

Epibenthic communities of bathyal habitats are structured by both biotic and abiotic factors (Levin et al., 2001; McClain and Etter, 2005). However, knowledge of their biodiversity and factors controlling the spatial distribution of fauna is limited for most of these deep-sea habitats (Danovaro et al., 2008). This is unfortunate, as inhabitants of bathyal ecosystems represent a large portion of Earth's as yet undiscovered biodiversity (Brandt et al., 2007; Williams et al., 2010). They also play crucial roles in global biogeochemical cycles as well as local ecological processes (Canals et al., 2006; Danovaro et al., 2008, respectively). The present study focuses on the bathyal habitats of New Zealand and explores the relationship between spatial patterns in biodiversity with environmental characteristics.

Many factors contribute to the distribution of biota in bathyal ecosystems (Snelgrove and Butman, 1994; Ellingsen, 2002). Over large spatial scales, depth and latitude act as indirect surrogates for temperature and pressure, which control species distributions by means of physiological constraints (Gappa et al., 2006; Brandt et al., 2007, McArthur et al., 2010). Bathymetric variation in the form of geomorphic features, such as submarine canyons, gullies, terraces and hills, are also influential in structuring the composition and functioning of continental margin biota (Levin and Dayton, 2009; Rathburn et al., 2009). Depth also influences food availability at the seafloor, since organic matter decays during its passage through the water column. At smaller scales, spatially variable inputs of organic matter may attract specialised fauna, and increase local and regional biodiversity (Snelgrove et al., 1992, 1996). Diversity and biomass are known to increase with elevated pelagic productivity in bathyal habitats (Brandt, 1995), suggesting close benthic-pelagic coupling.

Sediment type may also influence community composition by favouring different habitat and feeding preferences among benthic species (Stransky and Brandt, 2010).

In order to examine the influence of these environmental variables on bathyal fauna, we focussed on the amphipod crustaceans (Amphipoda). We selected amphipods as they are among the most abundant and diverse of marine taxa and particularly dominant in bathyal habitats (Brandt, 1993; Brokeland et al., 2007). Indeed, in our material they comprised 3.1 to 35.5% (average 18.4%) of the total individuals sampled (Lörz, 2010). Amphipods provide a significant food source to higher trophic levels (Dauby et al., 2003), and their burrowing behaviour in the upper sediment layers may influence benthic carbon cycling (Brandt, 1995). However, little is known of the taxonomic richness of bathyal amphipods from the South Pacific Ocean, or the factors that influence their abundance and community composition.

The lack of taxonomic information is largely due to the small body size (<10 mm for most species) and relatively high mobility of amphipods, both of which limit collection with commonly used sampling equipment such as box grabs, multicores or epibenthic sleds with mesh size >10mm. To redress this gap, we sampled using a small-meshed benthic trawl (Brenke, 2005), designed to semi-quantitatively sample the benthic boundary layer. We examined the relationships between abundance and taxonomic composition of amphipods and key environmental variables at water depths ranging from 200 – 1200 m on two major bathymetric features of the New Zealand continental margin: the Chatham Rise and Challenger Plateau. Both features occupy similar depth and latitudinal ranges, but exhibit large differences in the degree of topographic variability and pelagic productivity within each region.

Chatham Rise is a broad submarine ridge stretching eastwards for over 800 km from east of New Zealand's South Island near Christchurch to the Chatham Islands and beyond (Fig. 1.1). The rise is generally flat-topped at 300 – 400 m depth and descends into deeper waters (>2500 m) to the north and south. The Subtropical Front, which marks the mixing of subtropical and subantarctic surface waters, appears to be bathymetrically locked to the southern flank of the rise near 44°S (Uddstrom and Oien, 1999; Sutton, 2001). This region represents a zone of elevated primary production (chlorophyll *a* 0.2 – 0.8 mg m^{-3}; Fig. 1.1), with a production peak in the austral spring (Murphy et al., 2001). As a result, the southern flanks of the Chatham Rise receive a higher proportion of sinking organic matter (Nodder and Northcote, 2001), and elevated phytodetritus deposition has been observed during bloom periods (Nodder et al., 2007). Consequently, higher macro- and meio-benthic biomass tends to predominate on the southern flank of the Chatham Rise (Probert and McKnight, 1993; Grove et al., 2006; Berkenbusch et al., 2011). In contrast, the Challenger Plateau is a largely homogenous, flat-lying continental block, submerged beneath the Tasman Sea, west of New Zealand (Fig. 1.1). The plateau is bathed by relatively warm, oligotrophic subtropical water masses, and overall surface productivity of the Challenger Plateau is comparatively low, with chlorophyll *a* values ranging from <0.2 – 0.5 mg m^{-3} (Murphy et al., 2001; Fig. 1.1). Phytoplankton blooms do not occur with the frequency or magnitude of those on Chatham Rise. However, sporadic blooms associated with seasonal terrigenous inputs and upwelling events are known to occur off the west coast of New Zealand's South Island, and to influence benthic communities along the eastern, landward margin of the Challenger Plateau (Moore and Murdoch, 1993; Bradford-Grieve et al., 2006).

While previous studies have demonstrated correlations between bathyal amphipod communities and various environmental factors (e.g., food supply, Brandt, 1996; sediment

type, Stransky and Brandt, 2010), studies in regions with such contrasting physical settings are rare. We compared data across bathyal habitats with naturally variable environmental characteristics and predicted that amphipod communities would be more variable in areas with greater topographic relief and higher productivity in overlying waters. However, our results are contrary to this prediction and require an alternative explanation.

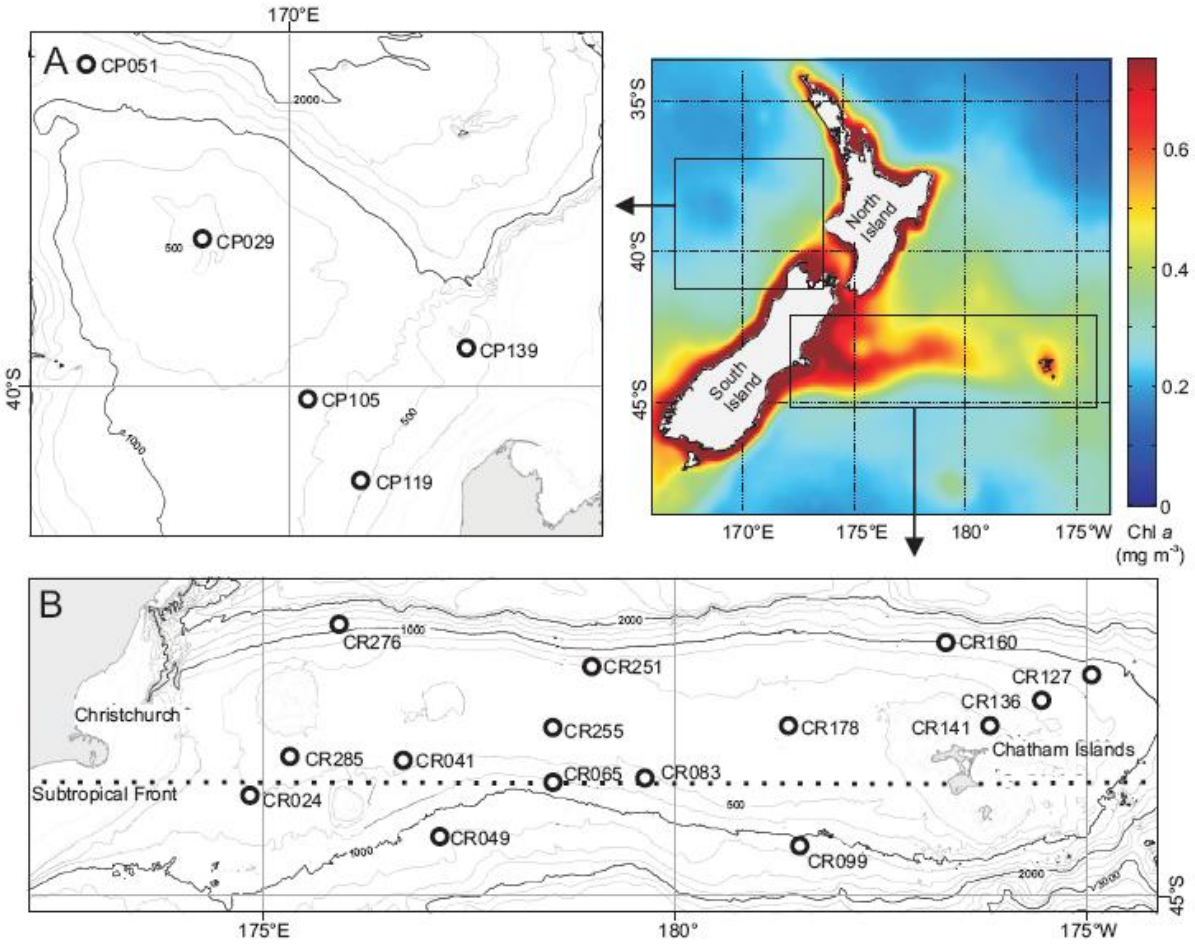


FIGURE 1.1. Mean surface chlorophyll *a* (mg m^{-3}) concentrations off New Zealand from 1998 – 2007 derived from SeaWiFS data (courtesy of NASA/Orbimage, USA). Expanded map sections show bathymetry and Brenke sledge sampling locations on (A) Challenger Plateau (TAN0707) and (B) Chatham Rise (TAN0705). The approximate location of the Subtropical Front (44° S) is shown as a dotted line on the Chatham Rise map.

METHODS

Station selection and sampling protocol

Sampling of the Chatham Rise was undertaken on a cruise from 29 March to 30 April 2007, and on Challenger Plateau from 26 May to 7 June 2007 (National Institute of Water and Atmospheric Research [NIWA] cruises TAN0705 and TAN0707 respectively). Station positions were randomly located within environmentally homogenous strata identified using oceanographic variables (see Nodder, 2007 for details), and a multivariate classification scheme (Legendre et al., 2002) that was based on seabed parameters derived from a multibeam survey conducted in 2006 (NIWA cruise TAN0610). In total, 20 stations were sampled (15 from the Chatham Rise and five from Challenger Plateau) spanning a similar depth range (200 – 1200 m) at both locations (Table 1.1). The greater number of samples taken from the Chatham Rise reflects an attempt to capture the greater number of environmental strata, relative to the Challenger Plateau.

An epibenthic sledge, built after Brenke (2005) and hereafter referred to as a "Brenke sledge", was deployed once at each station. This approach was undertaken because of the large amount of biotic data (100s to 1000s of individuals) representative of a wide area (~1000 m^{-2}) that can be generated from a single Brenke sledge tow. The seemingly small number of samples and lack of replication was a logistic constraint associated the long tow distances (>340 m in all cases), the time involved with the processing of large samples and the expense of sampling at bathyal depths. Prior to sampling, multibeam surveys assigned a bottom continuity score to each station, all of which were smooth or undulating, suggesting consistent topographic conditions. The same weighting / floatation devices were attached to the sledge throughout this study and the gear deployed successfully at all

stations included in this survey (see Nodder, 2007 for further details). Trawl distances (estimated according to equation 2 in Brenke, 2005), ranged from 340 – 1204 m with an average of 847 m (Table 1.1). The Brenke sledge carries two stacked sampling boxes: lower (epi) and upper (supra), each with an opening of 100 cm width by 35 cm height and a 500 µm mesh capture net. Closing doors prevented pelagic by-catch during Brenke sledge descent and retrieval. Upon retrieval, net contents were immediately rinsed with surface seawater into containers. To avoid damaging delicate specimens, samples were then elutriated, a process that allowed lighter organic matter to pass onto 500 µm sieves using gentle water agitation. Following elutriation, remaining material was also sieved. All material was immediately preserved in 95% ethanol for later analyses and is stored at the NIWA invertebrate collection in Wellington, New Zealand.

We used ocean colour estimates of surface chlorophyll *a* concentrations (http://oceancolor.gsfc.nasa.gov/) as a proxy for inter-station variability in primary production. For each station we averaged daily surface chlorophyll *a* estimates for the three months (March-June) prior to the cruise to obtain an inter-annual range and seasonal (autumn) average for the past ten years (1998 – 2007) (Table 1.1). Sediment samples were collected using an Ocean Instruments deep-ocean multi-corer at all but five stations (CR099, CR127, CR141, CR255 and CR285). Samples were analysed for physical (grain size) and chemical properties (total organic matter (TOM), calcium carbonate ($CaCO_3$), total pigments (chlorophyll *a* + phaeopigments)) using standard techniques (see Nodder et al., 2011). Chemical data were derived from the uppermost 0 – 0.5 cm and grain size data were averaged over the top 0 – 5 cm (ten samples at 0.5 cm intervals). Bottom temperature estimates were provided from a Seabird SBE37 MicroCat attached to other devices deployed at the same sites.

Sample sorting and taxonomic identification

We chose to use only the supranet samples for inter-station analyses owing to the more manageable sample volumes. However, to examine potential differences between epi- and supranets, three epinet samples (CR083, CR136 and CR285) from a similar depth range (400 – 600 m) on the Chatham Rise were also analysed. Although the two Brenke sledge openings (epi- and supranet) are designed to sample separate depth strata, observations have shown that water below the supranet opening is also sampled due to turbulence in front of the gear and the tow wire stirring up sediment and fauna ahead of the box (Linse et al., 2002).

Due to the high numbers of undescribed, morphologically complex and potentially cryptic species found in similar studies (e.g. Lörz, 2010, Williams et al., 2011), we identified Amphipoda to the family-level using Lowry and Springthorpe (2001). In this monograph, calliopiids, eusirids and pontogeneiids are all considered to be synonymous with the Eusiridae (*sensu lato*). Previous research has shown that family-level analyses provide an effective assessment of biodiversity for under-described or poorly understood assemblages (e.g. Olsgard et al., 1997). Damaged, unidentifiable amphipods were included in abundance calculations, but not in diversity or multivariate analyses.

Statistical analyses

To account for variable trawl distances (see Table 1.1), numbers of individuals within supranet samples are presented as densities per 1000 m^{-2}. In order to determine any potential confounding effects of trawl distance on univariate results, we measured the Pearson's correlation of trawling distance against standardised abundance and diversity and found no correlation ($p>0.45$ in both cases). Taxon diversity at each station was estimated using family richness. To correct for differing amphipod abundance among stations, family

diversity was also measured using the Shannon-Wiener diversity index ($H'=\Sigma i$ pi (log$e$ pi), where pi is the proportional abundance of taxon i in the sample), which assumes that all taxa in the community are represented in the sample. This assumption is addressed by the large number of individuals taken per sample and the family-level analyses used for comparisons.

Normality of environmental data was determined using Shapiro-Wilks tests. All variables were normally distributed with the exception of depth, which was corrected by a $\log^{10}$ transformation. Linear correlations between each of the environmental variables and our abundance and diversity measures were analysed with Pearson's correlation. These correlations were conducted using the 15 stations where we had multi-corer deployments to estimate sediment characteristics (Table 1.1).

Raw taxon composition data from each station were 4^{th} root-transformed to lessen the influence of dominant taxa before Bray-Curtis similarity calculations. Resulting linkages were visualised in a cluster diagram and multidimensional scaling (MDS) plot (PRIMER 6). The contribution of individual families to dissimilarity between groupings identified from cluster and MDS analyses was examined using the similarity percentages (SIMPER) procedure (Clarke, 1993). Correlations between community structure and environmental variables were examined using the BIOENV procedure (Clarke and Ainsworth, 1993). For this, we used normalised environmental variables from the 15 available stations.

Abundance and diversity for three epinet samples were calculated using the same methods used for supranets. Additionally, we compared taxon abundance in epi- and supranet using Pearson's correlation. To determine the degree of similarity in community composition of epi- and supranets relative to interstation differences, we ran an additional

multivariate analysis (PRIMER 6) using percent taxon composition from the epi- and supranets of stations CR083, CR136 and CR285. These data were 4^{th} root-transformed, before Bray-Curtis similarity calculations, and resulting linkages were visualised in an MDS plot.

Table 1.1.

Epibenthic sled trawl distance, depth range and environmental variables of study stations on the Chatham Rise and Challenger Plateau. Average autumn surface chlorophyl a concentration derived from SeaWiFS data (1998 – 2007) (range of annual values in brackets) and environmental variables used in statistical analyses. Organic sediment measurements and mud and clay content are derived from multicore samples (Nodder et al., 2011).

Station	Trawl dist (m)	Depth range (m)	Surface chl *a* (mg m^{-3})	Temp (°C)	Total (ug/g dw)	TOM (%)	$CaCO_3$ (%)	Clay content (% <4 µm)	Mud (% <63 µm)
Chatham Rise (Voyage TAN0705)									
CR024	340	512 – 513	0.55 (0.40 – 0.68)	6.4	9.0	3.5	9.7	8.2	89.8
CR041	926	478 – 479	0.49 (0.27 – 0.72)	7.1	7.2	4.0	14.7	10.2	85.8
CR049	463	1235 – 1239	0.49 (0.27 – 0.72)	3.2	3.1	1.8	37.6	8.5	82.9
CR065	370	769 – 771	0.45 (0.26 – 0.62)	5.6	4.6	2.8	29.5	16.5	45.8
CR083	1204	529 – 530	0.42 (0.24 – 0.59)	7.2	5.2	2.5	19.5	10.6	87.3
CR099	586	1076 – 1103	0.31 (0.21 – 0.39)	3.0					
CR127	923	933 – 940	0.48 (0.39 – 0.61)	6.4					
CR136	926	638 – 644	0.41 (0.33 – 0.54)	7.7	3.5	2.5	23.0	15.1	63.8
CR141	889	196 – 218	0.41 (0.33 – 0.54)	11.8					
CR160	1037	1023 – 1026	0.36 (0.29 – 0.41)	5.3	4.0	5.0	61.6	14.6	37.5
CR178	963	424 – 425	0.40 (0.28 – 0.51)	7.9	5.6	3.9	60.9	31.1	44.9
CR251	1111	520 – 530	0.37 (0.29 – 0.48)	8.5	3.8	3.1	49.8	27.9	44.2
CR255	945	346 – 346	0.40 (0.28 – 0.51)	8.8					
CR276	945	1194 – 1199	0.40 (0.32 – 0.48)	3.9	6.3	3.3	13.1	4.2	94.1
CR285	923	418 – 422	0.55 (0.40 – 0.68)	7.5					
Challenger Plateau (Voyage TAN0707)									
CP029	1074	480 – 480	0.18 (0.15 – 0.22)	10.4	1.4	1.9	89.5	18.4	35.5
CP051	963	1207 – 1213	0.16 (0.13 – 0.18)	4.4	1.1	1.8	86.1	18.7	41.8
CP105	926	803 – 805	0.22 (0.18 – 0.27)	6.6	0.8	5.7	68.6	6.4	83.5
CP119	883	529 – 534	0.27 (0.22 – 0.37)	9.6	1.3	2.0	56.9	10.5	67.6
CP139	926	264 – 266	0.31 (0.25 – 0.41)	13.1	3.6	1.9	27.4	4.5	90.1

RESULTS

Amphipod biodiversity: univariate

The 20 Brenke sledge samples yielded 12,513 individuals from 27 benthic amphipod families. Inter-station abundance was highly variable and ranged from 44 to 2074 individuals (ind.) 1000 m^{-2} (Table 1.2). Mean amphipod abundance on Chatham Rise (868±595 (±1SD) ind. 1000 m^{-2}) was approximately double that found on Challenger Plateau (390±257 ind. 1000 m^{-2}). Ten families (Phoxocephalidae, Urothoidae, Eusiridae, Exoedicerotidae, Ischyroceridae, Aoridae, Oedicerotidae, Liljeborgiidae, Lysianassoidae and Synopiidae), comprising 86% of the total amphipod composition, were present at 18 or more of the 20 stations (Table 1.3; see Appendix 1.1 for individual station compositions). Of note was the discovery of a widespread group that could not be identified to family with available literature (full description will be provided elsewhere). This previously unknown family was found in 14 of the 20 stations, although it occurred in relatively low numbers (overall composition=2.7%). Low numbers (n<5) of Hyperiidea and Vibiliidae (likely of pelagic origin) were identified but later removed from the dataset. Despite sampling fewer stations on the Challenger Plateau relative to Chatham Rise, a similar number of families was recorded in both regions. Of all families, 23 occurred on both the Chatham Rise and Challenger Plateau. Three families occurred exclusively on the Chatham Rise (Amarillidae, Amphilochidae and Ochlesidae) and one on Challenger Plateau (Corophiidae), although in low numbers (n<10 per family). Shannon-Wiener diversity did not vary greatly between stations and ranged from 1.60 – 2.56 (Table 1.2).

Many of the environmental variables co-varied. The average autumn surface chlorophyll *a* concentration was strongly correlated with total sediment pigments (r=0.84, p<0.001) and latitude (r=0.93, p<0.001) suggesting a close link between surface production,

latitude and benthic food availability. Temperature and depth were also correlated, as were many of the sediment grain size categories. Consequently, we used a reduced set of weakly correlated ($r<0.8$) environmental variables (depth, average autumn surface chlorophyll *a*, TOM, $CaCO_3$, clay and mud content) in subsequent uni- and multivariate statistical analyses. Performing Pearson's correlations of environmental variables against both abundance and diversity, we found only diversity and depth ($r=0.57$, $p=0.03$), were significantly correlated.

Amphipod biodiversity: multivariate

Multivariate analyses revealed five highly disparate stations located on both the Challenger Plateau (CP029, CP051, CP105) and the Chatham Rise (CR127 and CR141), which were highly dissimilar to each other (except CP105 and CR127), and to the remaining stations (Fig. 1.2A). These five stations had low abundance (44 – 473 ind.1000 m^{-2}), relative to the remaining stations (369 – 2074 ind.1000 m^{-2}). However, despite this lower abundance, univariate diversity measures were similar (Table 1.2). Our analyses also revealed a grouping, comprised of 15 stations from both regions, which clustered together above 75% similarity (Fig. 1.2A). Within this cluster, three sub-groupings could be discerned (Fig. 1.2B). Group I comprised stations running along the southern side of the Chatham Rise crest, near the Subtropical Front. Group II consisted of stations located on the northern crest and slopes (CR251, CR255 and CR276) as well as southern slopes (CR049, CR099). Finally, group III was made up of a geographically disparate set of stations from the landward Challenger Plateau (CP119, CP139), stations from the crest of the Chatham Rise (CR041, CR285) and two north-eastern Chatham Rise stations (CR136, CP160) (Fig. 1.2C, D).

SIMPER analyses revealed that the dissimilarity between groups I – III was caused by collective differences in relative abundances of several taxa from each group. This result was possibly a consequence of the widespread distribution of most amphipod families. For

example, the highest contribution of any family to between-group dissimilarity was only 9.4% (unknown family: between groups II and II). By comparing the average abundance of families from within groups, we identified those which characterised each grouping. Lower proportions of Oedicerotidae, Ampeliscidae, Liljeborgiidae and Melitidae differentiated group I. Group II was characterised by higher proportions of Gammaridae, and the unknown taxon. Group III stations typically had higher proportions of Pardaliscidae and lower proportions of Urothoidae and Phoxocephalidae. Using the BIOENV procedure, the best explanatory variables of amphipod community composition were surface chlorophyll *a* (49% variability) and $CaCO_3$ (29%). All other environmental variables were only weakly correlated with biotic patterns (<11%).

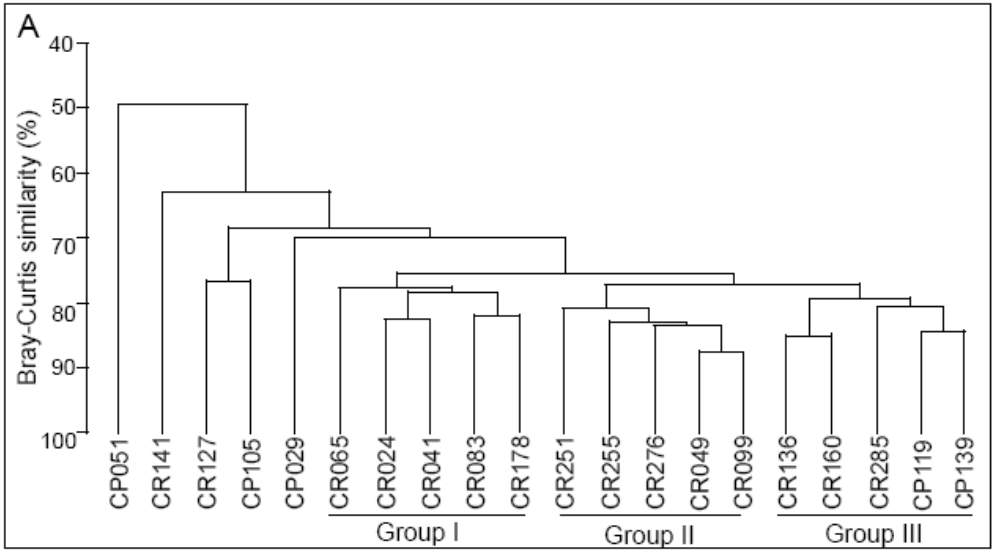
A further 6695 individuals were sorted and identified from three epinet samples on the Chatham Rise. Epinet samples were found to harbour approximately equal (e.g. CR136) or far greater (e.g. CR083, CR285) numbers of amphipods than the corresponding supranet samples (Table 1.2). Despite such differences in total abundance between epi- and supranets from the same station, taxonomic richness and measures of diversity were similar (Table 1.2). Comparison of taxon abundance from epi- and supranet samples revealed strong linear correlations at each of the three stations (CR083 r=0.93, p=<0.01; CR136 r=0.88, p=<0.01; CR285 r=0.90, p=<0.01). An MDS plot was generated using per cent taxon composition to examine community similarity of nets from each station (Fig. 1.3). All three epinet samples were closely related to their supranet counterparts, with an average similarity of 89%. In comparison, differences between stations were higher, with only 78 to 82% community similarity.

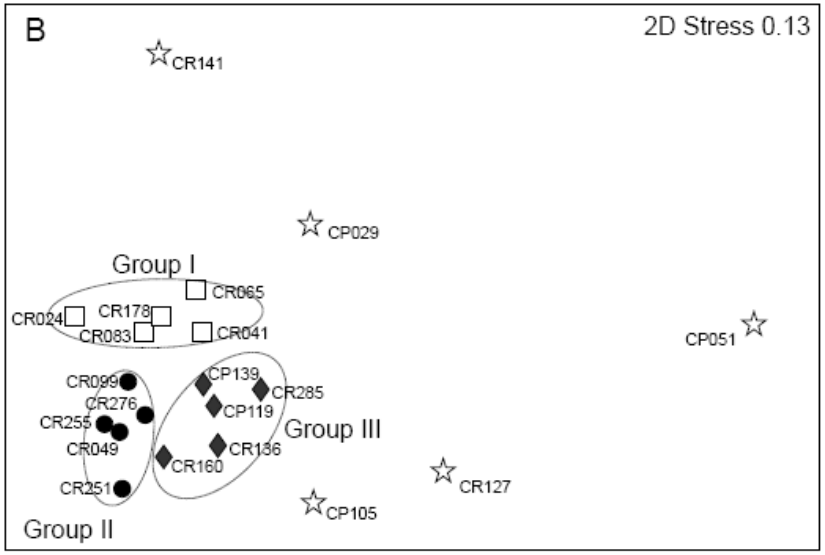
Table 1.2.
Amphipod abundance and diversity from 20 suprabenthic and three epibenthic samples on Chatham Rise and Challenger Plateau (see Appendix 1.1 for station composition).

Station	Net	Abundance (ind. 1000 m^{-2})	No. of families	*H'* diversity (log *e*)
CR024	Supra	1476	14	1.87
CR041	Supra	447	15	2.11
CR049	Supra	1320	18	2.41
CR065	Supra	508	14	2.13
CR083	Supra	697	17	1.99
CR083	Epi	1861	19	2.09
CR099	Supra	974	17	2.23
CR127	Supra	125	17	2.35
CR136	Supra	391	20	2.46
CR136	Epi	449	19	2.56
CR141	Supra	316	12	1.60
CR160	Supra	795	19	2.55
CR178	Supra	551	15	1.99
CR251	Supra	1918	23	2.04
CR255	Supra	2074	19	2.16
CR276	Supra	1057	19	2.43
CR285	Supra	369	20	2.29
CR285	Epi	4376	22	2.30
CP029	Supra	473	14	2.05
CP051	Supra	44	12	2.25
CP105	Supra	239	21	2.54
CP119	Supra	717	19	2.09
CP139	Supra	477	19	2.31

Table 1.3.
List of amphipod taxa, their distributions on Chatham Rise and Challenger Plateau and percent contribution to total amphipod composition, overall and within each region (see Appendix 1.1 for individual station abundance data).

Family	Distribution (# of occurrances)			% composition		
	Chatham Rise (/15)	Challenger Plateau (/5)	Total (/20)	Chatham Rise	Challenger Plateau	Total
Phoxocephalidae	15	5	20	25.6	20.9	25.0
Urothoidae	15	4	19	15.4	4.9	14.1
Eusiridae	15	5	20	8.6	18.5	9.8
Exoedicerotidae	15	5	20	7.8	8.5	7.9
Ischyroceridae	15	5	20	6.2	6.2	6.2
Aoridae	15	5	20	6.0	5.8	6.0
Oedicerotidae	14	5	19	4.5	5.5	4.6
Liljeborgiidae	14	4	18	4.0	5.3	4.2
Lysianassoidea	14	4	18	4.9	5.1	4.9
Synopiidae	14	5	19	3.1	4.6	3.3
Unidentified family	11	3	14	2.9	1.6	2.7
Gammaridae	10	4	14	1.9	1.6	1.9
Pardaliscidae	9	3	12	1.0	0.6	1.0
Caprellidae	15	2	17	1.1	0.2	1.0
Stenothoidae	14	3	17	1.0	0.9	1.0
Ampeliscidae	11	5	16	0.7	0.6	0.7
Cyphocarididae	2	4	6	0.1	1.7	0.3
Melitidae	6	3	9	0.3	0.6	0.3
Stegocephalidae	8	4	12	0.3	0.7	0.3
Dexaminidae	9	2	11	0.3	0.3	0.3
Epimeridae	2	2	4	0.0	0.1	0.0
Hyperiidea	1	1	2	0.0	0.2	0.0
Cyproideidae	2	1	3	0.0	0.1	0.0
Amphilochidae	1	0	1	0.0	0.0	0.0
Ochlesidae	1	0	1	0.0	0.0	0.0
Corophiidae	0	1	1	0.0	0.1	0.0
Vibilidae	0	1	1	0.0	0.0	0.0


A
Bray-Curtis similarity (%)
40
50
60
70
80
90
100
CP051
CR141
CR127
CP105
CP029
CR065
CR024
CR041
CR083
CR178
CR251
CR255
CR276
CR049
CR099
CR136
CR160
CR285
CP119
CP139
Group I
Group II
Group III


B
2D Stress 0.13
CR141
CP029
Group I
CR065
CR024
CR178
CR083
CR041
CP051
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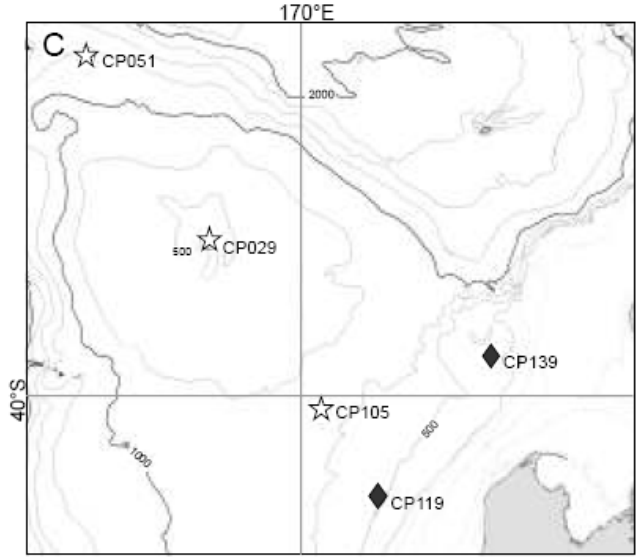
CR049
CR136
CR160
CR251
CR127
CP105
Group II


170°E
C
CP051
2000
500
CP029
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CP119
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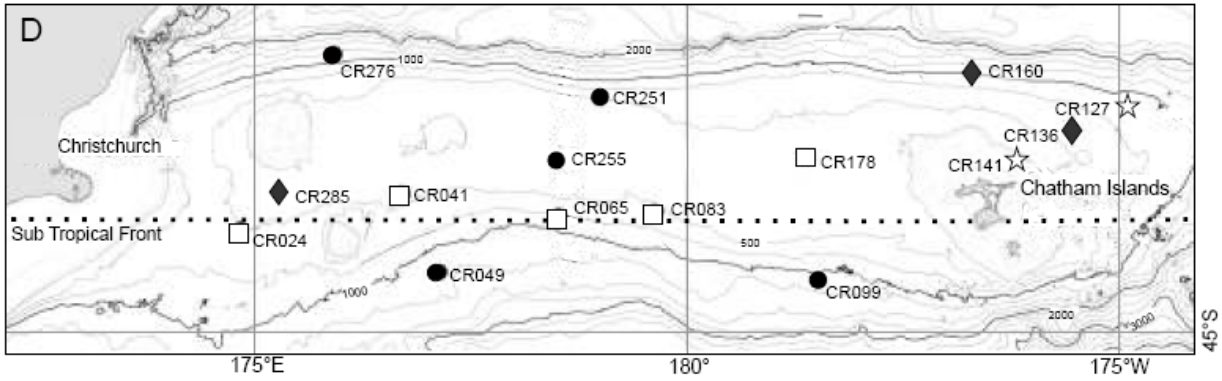
D
CR276
1000
2000
CR160
CR251
CR127
CR136
Christchurch
CR255
CR178
CR141
Chatham Islands
CR285
CR041
CR065
CR083
Sub Tropical Front
CR024
500
CR049
1000
CR099
2000
3000
45°S
175°E
180°
175°W

Previous page: FIGURE 1.2. (A) Dendrogram based on fourth-root transformed percent composition station data of amphipod families. Bars at the bottom indicate groupings based on Bray-Curtis similarity. (B) MDS representation of amphipod abundance data. Shapes correspond to groupings identified in (A) and are shown on maps of the Challenger Plateau (C) and Chatham Rise (D). The approximate location of the subtropical front (44° S) is shown as a dotted line on the Chatham Rise map.

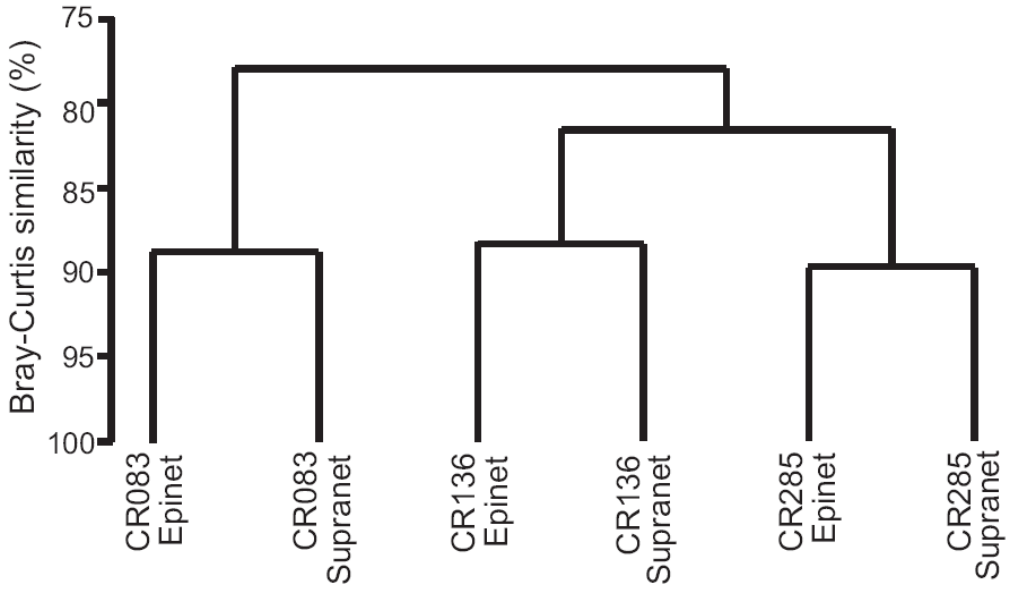

FIGURE 1.3. Dendrogram based on fourth-root transformed percent taxon composition data of amphipod families from epi- and supranets of three Chatham Rise stations.

DISCUSSION

Amphipod biodiversity

Overall, taxonomic diversity of New Zealand bathyal amphipods (mean number of families per station: 18.7, total 27) was similar to that recorded previously for Brenke sledge samples collected at polar latitudes (e.g. Brandt, 1997; Brandt and Berge, 2007). For example, 17 of the 34 Arctic families (Brandt, 1997), and 13 of the 17 Antarctic families (Lörz and Brandt, 2003) were present in our survey. The comparable family diversity of New Zealand amphipods to those from high latitude regions, suggests a similarly rich bathyal amphipod community. Other crustacean groups are known to be highly abundant and diverse on New Zealand continental margins. For example, 48 species (at least 37 of which are new to science) from 13 families were identified from 1,984 individuals of Tanaidacea (Lörz, 2010). Similar to previous work reporting a diversity peak for bathyal macrofauna at water depths of 1220 – 1800 m (Maciolek and Smith, 2009; MacDonald et al., 2010), we also found that amphipod diversity increased to our maximum sampling depth of 1200 m.

The focus on family-level amphipod diversity was necessitated due to the difficulties of species identifications in samples containing primarily undescribed taxa. While species-level data would potentially reveal additional patterns, family-level data have been found to be an effective proxy (Olsgard et al., 1997). For example, previous research has demonstrated that spatial patterns observed at the family-level provide similar results to those at species-level (Olsgard et al., 2003; Hirst, 2008; Tataranni et al., 2009). Studies have also shown that diversity in any ecosystem may be partitioned differently among the hierarchy of taxonomic units, according to the age or successional stage of the assemblage, even though total species diversity is equal (Warwick and Clarke, 1995). Taxonomic

distinctness has therefore been incorporated into several diversity estimates (e.g. Clarke and Warwick, 1998) to provide information on the 'relatedness' of species assemblages, thereby highlighting the value of higher-level taxonomic data.

We found very high numbers of amphipods in most of our samples, indicating their potential importance in bathyal benthic ecosystems. Abundances varied greatly among stations but were similar in range to those found in other studies using suprabenthic sampling equipment (e.g. Linse et al., 2002; Lörz and Brandt, 2003). Previous studies using pooled epi- and supranet samples have also shown considerable variability and even replicate Brenke sledge samples from the same area can differ greatly in abundance (Kaiser et al., 2008). These highly variable abundances likely reflect the naturally patchy spatial distribution of amphipods in bathyal habitats (Brokeland et al., 2007). Amphipod abundances on the Chatham Rise were expected to exceed those of the Challenger Plateau as a consequence of elevated pelagic production in the former (Murphy et al., 2001). Indeed, nine of the ten highest amphipod abundances were on stations on the Chatham Rise, suggesting strong pelagic-benthic linkages.

Vertical stratification of hyperbenthic amphipod communities, examined through comparison of epi- and supranets showed that abundances were usually greater in epinet samples, suggesting that most amphipod biomass occurs in close proximity to, or at, the seafloor. While abundance varied greatly between epi- and supranets, amphipod community composition did not. Previous studies (e.g. Linse et al., 2002) have speculated that supranets sample organisms from below the opening (i.e. epinet fauna) owing to disturbance from the towing wire and hydrodynamic effects. For these reasons, many studies using Brenke sledge combine the epi- and supranet samples (e.g. Brandt et al., 2007;

Kaiser et al., 2008) and do not report on the individual net contents. However, we found that diversity measures (Shannon-Wiener, taxon richness) and community composition in epi- and supranets were highly comparable at the amphipod family-level. Accordingly, supranets could provide a useful and relatively rapid proxy for total Brenke sledge family diversity and community composition.

Relationship between biodiversity and environmental factors

BIOENV analyses showed that surface chlorophyll *a* correlated most strongly with amphipod community composition, suggesting that elevated primary productivity associated with the Subtropical Front is an important determinant of bathyal amphipod community structure. Stations on the western Challenger Plateau (CP029 and CP051) had the lowest surface chlorophyll *a* and had highly dissimilar amphipod communities, which strongly influenced this finding. The correlation between primary productivity and amphipod community composition suggests that surface chlorophyll *a*, or other associated variables (e.g., total sediment pigments, latitude), may be used to make general predictions in areas where biological data are limited. However, some of the environmental variables we compared (e.g. sediment variables, $CaCO_3$, TOM), which did not correlate as strongly, can vary widely over relatively small scales due to their patchy spatial distribution on bathyal seafloor habitats. Since Brenke sledge trawl lengths were up to 1 km long, sweeping an area at least 100 cm wide, and a multi-corer sample covers only a fraction of this area (0.008 m^{-2}), we might expect lower correlations with variables collected in this way simply due to differences in spatial sampling coverage.

The greatest variability in amphipod community composition occurred on the western Challenger Plateau, whereas the other stations mostly grouped together. This

finding was somewhat surprising, as we had expected relatively uniform amphipod community composition, reflecting low productivity and less bathymetric variability on the Challenger Plateau, compared with the more productive and topographically complex Chatham Rise. Indeed, these disparate stations were characterised by low surface and benthic pigments, relative to remaining stations, and suggesting correlations with regional productivity. Amphipod abundances were also reduced, probably in response to lower food input. However, despite lower abundances, diversity was comparable to that of stations on Chatham Rise. One possible explanation is that in more productive areas, organic enrichment leads to larger populations and hypoxia can limit species richness (Levin and Gage, 1998). An alternative explanation is that the grouping of these disparate stations reflects the fact that they all yield relatively low numbers of amphipods. Specifically, greater variability in relative taxon proportions is more likely in samples of patchily distributed taxa with reduced abundance.

The remaining stations were located on the crest and slopes of the Chatham Rise and landward Challenger Plateau (CP119, CP139) and community composition was mostly similar, possibly due to the dominance of opportunistic taxa that can occur in association with episodic fluxes in phytodetritus (Corliss et al., 2009). Elevated primary production in waters overlying southern Chatham Rise is known to increase phytodetrital deposition to crestal and slope sediments (Nodder et al., 2007), influencing meio- and macrofaunal biomass (Nodder et al., 2003; Berkenbusch et al., 2011). Consequently, distinct communities have previously been recorded within the wider benthic invertebrate community on Chatham Rise crest (e.g., McKnight and Probert, 1997; Probert et al., 1996, 2009). Similarly, we detected several stations with closely-related amphipod communities on the southern crest of the Chatham Rise (group I), in close proximity to the mean location of the strongest

temperature gradients in the Subtropical Front (44°S) (Uddstrom and Oien, 1999; Sutton, 2001).

Amphipods are an abundant and diverse group in bathyal habitats. The similarity between the results of the present study and those of earlier investigations focussing on other bathyal benthic taxa from the same region suggests that they may provide a proxy for overall benthic community diversity. For example, a previous study on polychaete worms also noted that communities of shallow-water genera on the Challenger Plateau were more like those of the Chatham Rise, than samples taken from the deeper waters of the Challenger Plateau (Probert and Grove, 1998). Similar changes in wider macrobenthic assemblages relative to pelagic productivity have been previously recorded on New Zealand continental margins (Probert and Grove, 1998; Probert et al., 2001), but comparisons over spatial scales comparable to those of our study are rare.

Our findings may have been influenced by the slight mismatch in the timing of sampling between study regions. Chatham Rise sampling took place during late April, whereas the Challenger Plateau was sampled 3 – 4 weeks later, during late May/early June. While this was unavoidable, the lower average abundance on the Challenger Plateau stations may reflect this temporal shift. Indeed, the differences we observed may have been even greater had we sampled during the spring blooms. Close coupling between epibenthic crustacean biomass and pelagic productivity has been demonstrated previously (Brandt, 1995), and can increase over a period of weeks in response to an increase in food supply (Brandt, 1996).

CONCLUSIONS

In contrast to our initial expectations, benthic community composition for three western Challenger Plateau stations was highly spatially variable. We suggest that this is due to decreased amphipod abundance on the western Challenger Plateau and subsequent reductions in consistency of family catch rates for patchily distributed fauna. The low abundance is likely caused by the reduced pelagic productivity on the western Challenger Plateau, leading to decreased food supply to the benthos. Disparate amphipod communities were also located on the Chatham Rise, which also had similarly low amphipod abundance. Overall, surface chlorophyll *a* correlated most strongly with amphipod community composition, suggesting strong benthic-pelagic links. The high abundances and diversities of amphipod crustaceans on both the Chatham Rise and Challenger Plateau emphasises their importance in New Zealand bathyal habitats.

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Appendix 1.1

Amphipod family abundance (ind. 1000 m^{-2}) in Brenke sledge supranets from 20 stations on Chatham Rise and Challenger Plateau (see Fig. 1.1 and Table 1.1 for station locations).

	CR024	CR041	CR049	CR065	CR083	CR099	CR127	CR136	CR141	CR160
Phoxocephalidae	427	103	199	130	190	310	22	72	19	90
Urothoidae	356	58	238	35	160	114	4	29	125	39
Eusiridae	82	42	24	51	81	27	1	15	102	46
Exoedicerotidae	150	97	65	43	68	39	4	32	2	26
Ischyroceridae	12	30	41	40	22	109	9	11	1	69
Aoridae	21	32	158	24	38	82	7	62	8	90
Lysianassidae	244	29	35	105	44	22	0	8	13	34
Oedicerotidae	44	3	69	0	17	20	5	62	13	68
Liljeborgiidae	38	10	104	8	0	41	5	10	19	99
Synopiidae	18	15	45	5	4	20	2	8	0	94
Unidentified family	0	0	179	19	2	36	2	5	0	5
Gammaridae	0	0	50	5	13	56	29	0	1	0
Stenothoidae	9	2	17	5	2	17	1	14	0	14
Pardaliscidae	3	1	0	0	0	0	13	25	0	58
Caprellidae	6	5	13	3	2	2	2	5	3	5
Ampeliscidae	0	1	15	0	3	24	3	14	0	11
Stegocephalidae	0	2	6	8	0	0	2	1	0	12
Melitidae	0	0	0	0	0	0	0	4	0	0
Cyphocarididae	0	0	0	0	0	0	0	0	0	0
Dexaminidae	0	0	4	0	2	0	1	1	0	8
Pachynidae	6	0	0	0	1	2	0	1	0	1
Amarillidae	0	0	2	0	1	2	0	0	0	0
Epimeridae	0	0	0	0	0	0	0	1	0	0
Cyproideidae	0	0	0	0	0	0	0	0	0	1
Amphilochidae	0	0	0	0	0	0	0	0	2	0
Ochlesidae	0	0	0	0	0	0	0	0	0	0
Corophiidae	0	0	0	0	0	0	0	0	0	0
Unidentified material	59	15	56	24	44	49	11	10	6	27

Appendix 1.1 *cont.*

Amphipod family abundance (ind. 1000 m^{-2}) in Brenke sledge supranets from 20 stations on Chatham Rise and Challenger Plateau (see Fig. 1.1 and Table 1.1 for station locations).

	CR178	CR251	CR255	CR276	CR285	CP029	CP051	CP105	CP119	CP139
Phoxocephalidae	163	779	464	238	100	164	1	28	152	62
Urothoidae	112	189	413	121	57	36	0	2	25	30
Eusiridae	59	97	311	131	54	7	4	19	199	134
Exoedicerotidae	64	220	100	20	16	33	1	23	65	45
Ischyroceridae	54	180	100	96	12	24	9	2	69	16
Aoridae	8	56	146	26	15	48	6	13	3	40
Lysianassidae	8	32	37	20	7	65	0	1	12	15
Oedicerotidae	16	83	142	55	5	23	4	31	34	15
Liljeborgiidae	4	19	121	53	8	20	0	24	34	27
Synopiidae	18	36	22	83	28	3	2	6	34	44
Unidentified family	1	100	1	29	0	0	2	24	6	0
Gammaridae	0	33	40	20	5	0	2	26	2	2
Stenothoidae	4	32	10	2	2	0	0	6	2	10
Pardaliscidae	0	2	13	6	16	0	0	1	3	8
Caprellidae	5	4	6	75	5	0	0	2	0	1
Ampeliscidae	0	1	2	12	2	2	2	1	6	1
Stegocephalidae	0	1	0	5	0	2	0	2	6	3
Melitidae	2	1	2	19	10	0	0	2	1	8
Cyphocarididae	0	9	0	0	2	22	1	6	3	0
Dexaminidae	7	4	5	0	1	0	5	1	0	0
Pachynidae	0	0	2	0	1	2	0	0	0	0
Amarillidae	0	0	0	1	0	0	0	0	0	0
Epimeridae	0	2	0	0	0	0	0	0	1	1
Cyproideidae	0	1	0	0	0	0	0	0	0	1
Amphilochidae	0	0	0	0	0	0	0	0	0	0
Ochlesidae	0	2	0	0	0	0	0	0	0	0
Corophiidae	0	0	0	0	0	0	0	1	0	0
Unidentified material	25	35	137	43	21	19	3	12	59	14

CHAPTER 2

GREATER AMPHIPOD DIVERSITY ASSOCIATED WITH ENVIRONMENTAL HETEROGENEITY IN DEEP-SEA HABITATS[†]

Keywords: New Zealand – Phoxocephalidae – Continental shelf – DNA Barcoding – biodiversity

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ABSTRACT

Cytochrome oxidase *c* subunit I (COI) DNA sequences were used to estimate species diversity and to compare phoxocephalid amphipod assemblages among 20 sites encompassing a range of environmental conditions. Two regions, east (Chatham Rise) and west (Challenger Plateau) of New Zealand were sampled with an epibenthic sled. Using a comparison among identified morphospecies, we found a clear gap in sequence divergences between 6 and 13% and used a 6% threshold to designate molecular operational taxonomic units (MOTUs), as a surrogate to putative species. DNA sequences (n=297) revealed high total diversity (n=49 MOTUs), as well as high beta diversity (28 MOTUs found at single location only). Analysis of COI sequences also allowed detection of morphologically conserved taxa which might otherwise have been overlooked. Despite the relatively limited sampling, novel phoxocephalid MOTUs were found at most stations, especially on the Challenger Plateau and the flanks of the Chatham Rise. Analyses of inter-station assemblages revealed a major split between regions, indicating minimal overlap in taxon distributions on eastern and western continental margins. A cluster of highly similar stations was identified, broadly distributed over the crest of the Chatham Rise, in association with elevated food availability, likely caused by higher surface productivity and relatively shallow depth. A multivariate analysis of phoxocephalid assemblages with environmental parameters confirmed these findings and revealed a strong correlation with measures of food supply. This study highlights the value of molecular approaches, and in particular COI sequences, for quantifying and comparing diversity in taxonomically under-studied or under-sampled taxa.

INTRODUCTION

Determining and understanding changes in community structure and composition across environmental gradients is an essential element of community ecology (Levin, 1992). However, with an estimated 10^7 species in the deep-sea (Grassle and Maciolek, 1992), the vast majority of which await discovery and/or formal descriptions (e.g. Brandt and Berge 2007, Lörz and Brandt 2003), comparisons of deep-sea communities are frequently limited to a relatively small group of easily identified megafaunal taxa or to the analysis of higher taxonomic levels. Due to a lack of accurate taxonomic information, macrofauna are often overlooked despite their domination of biomass, especially at relatively shallow (200 – 4000 m) depths (Rex et al., 2006). To overcome this lack of taxonomic data, we utilise a molecular-based approach to assess diversity and community composition of phoxocephalid amphipods from a series of stations along the deep-sea continental margins of New Zealand and contrast diversity within and among stations with environmental variability.

Many factors may contribute to the distribution of biota in deep-sea ecosystems (Snelgrove and Butman, 1994; Ellingsen, 2002). Spatially variable inputs of organic matter attract specialised fauna, shaping local and regional biodiversity (Snelgrove et al., 1992; 1996). Sediment type also alters benthic community composition by favouring different habitat and feeding preferences among species (Stransky and Brandt, 2010). Bathymetric variation in the form of geomorphic features, such as submarine canyons, gullies, terraces and hills, are also influential in structuring the composition and functioning of deep-sea biota (Levin and Dayton, 2009; Rathburn et al., 2009). Over large spatial scales, depth and latitude act as indirect surrogates for temperature and pressure, which control species

distributions by means of physiological constraints (Gappa et al., 2006; Brandt et al., 2007, McArthur et al., 2010).

The dynamic topography and spatially variable food supply of New Zealand's continental margins provide an ideal setting for examining the links between diversity and deep-sea environmental parameters. Chatham Rise and Challenger Plateau are prominent marine geomorphic structures, connected by Cook Strait (Fig. 2.1) and both regions present a contrasting range of environmental parameters (Table 2.1). Chatham Rise, an extensive submarine ridge, east of New Zealand is characterised by high productivity in surface waters, associated with the Subtropical Front (STF) (Uddstrom and Oien, 1999; Murphy et al., 2001; Sutton, 2001). As a result, benthic communities beneath the STF are subject to elevated inputs of organic matter (Nodder et al., 2003). Challenger Plateau lies west of New Zealand and is a region of similar depth range but with less topographic relief and lower pelagic productivity relative to the Chatham Rise.

In order to examine the influence of environmental parameters on understudied components of the deep-sea macrofauna, we focussed on phoxocephalid amphipods as they are among the most abundant and diverse amphipod families in southern hemisphere deep-sea habitats (Senna, 2010, Knox et al., 2012). Amphipods provide a significant food source to higher trophic levels (Dauby et al., 2003), and the behaviour of burrowing groups such as the Phoxocephalidae may influence benthic carbon cycling in upper sediment layers (Brandt, 1995). Despite their ecological significance, New Zealand phoxocephalid taxonomy remains poorly studied, with only 17 formally described species (mostly from relatively accessible coastal habitats), and at least a further 15 housed in collections awaiting descriptions (Webber et al., 2010). The Phoxocephalidae of New Zealand's deep-sea regions are almost completely unstudied. However, with increased access to DNA sequencing

facilities, it is now possible to generate and analyse species-level diversity data for this ecologically important taxon.

The use of molecular markers, in particular the cytochrome oxidase *c* subunit I (COI) gene locus, in combination with traditional morphological methods has added to the accuracy with which many taxa can be identified and reliably compared to one another both within and between studies (Hebert et al., 2003). Genetic data are unaffected by the common pitfalls of morphological identifications such as phenotypic plasticity, sexual dimorphism and life stage morphology differences. Moreover, DNA sequences have also revealed previously unrecognised, potentially cryptic species in several animal groups (Hebert et al., 2004, Witt et al., 2006, Zemlak et al., 2009). In habitats such as the deep-sea, where the majority of amphipod species are undescribed, molecular data can provide a taxonomic framework which can also benefit future species descriptions (e.g. Lörz et al., 2011; 2012).

The ability to delineate species in undescribed and/or taxonomically challenging taxa hinges on the degree of intraspecific versus interspecific COI sequence variation and the distinctness of the so-called "barcoding gap" (Meyer and Paulay, 2005). In cases where an extensive taxonomic framework exists, studies have found that COI sequences form discrete clusters corresponding to morphological species (Witt et al., 2006; Costa et al., 2009; Hou et al., 2009; Radulovici et al., 2009) with interspecific variation on average 25 times greater than intraspecific. Consequently, COI divergences can be used to demarcate species identities in difficult taxonomic taxa. For example, Plaisance et al. (2009) used COI sequences to identify the crustacean cryptofauna of *Pocillopora* coral heads and compare biodiversity among stations using a 5% sequence similarity threshold. Similarly, Radulovici et

al. (2009) used a threshold of 3% sequence divergence to classify sequenced individuals as different marine crustacean species.

Here, we used levels of genetic divergence within and among taxa to estimate species-level diversity in New Zealand's deep-sea Phoxocephalidae and to relate this diversity with environmental parameters. Based on previous studies on similarly underexplored habitats (e.g. Brandt and Berge, 2007; Williams et al., 2010), we anticipated finding high diversity. We then tested the hypothesis that the diversity of deep-sea macrofauna is correlated with increased food supply associated with higher surface productivity.

Table 2.1.

Epibenthic sled trawl distance, depth range and environmental variables of study stations on the Chatham Rise and Challenger Plateau. Average autumn surface chlorophyl a concentration derived from SeaWiFS data (1998 – 2007) (range of autumn values in brackets) and environmental variables used in statistical analyses. Organic sediment measurements and mud and clay content are derived from multicore samples (Nodder et al., 2011).

Station	Trawl dist (m)	Depth range (m)	Surface chl *a* (mg m^{-3})	TOM (%)	$CaCO_3$ (%)	Clay (% <4 µm)	Mud content (% <63 µm)
Chatham Rise (Voyage TAN0705)							
CR024	340	512 – 513	0.55 (0.40 – 0.68)	3.5	9.7	8.2	89.8
CR041	926	478 – 479	0.49 (0.27 – 0.72)	4.0	14.7	10.2	85.8
CR049	463	1235 – 1239	0.49 (0.27 – 0.72)	1.8	37.6	8.5	82.9
CR065	370	769 – 771	0.45 (0.26 – 0.62)	2.8	29.5	16.5	45.8
CR083	1204	529 – 530	0.42 (0.24 – 0.59)	2.5	19.5	10.6	87.3
CR099	586	1076 – 1103	0.31 (0.21 – 0.39)				
CR127	923	933 – 940	0.48 (0.39 – 0.61)				
CR136	926	638 – 644	0.41 (0.33 – 0.54)	2.5	23.0	15.1	63.8
CR141	889	196 – 218	0.41 (0.33 – 0.54)				
CR160	1037	1023 – 1026	0.36 (0.29 – 0.41)	5.0	61.6	14.6	37.5
CR178	963	424 – 425	0.40 (0.28 – 0.51)	3.9	60.9	31.1	44.9
CR251	1111	520 – 530	0.37 (0.29 – 0.48)	3.1	49.8	27.9	44.2
CR255	945	346 – 346	0.40 (0.28 – 0.51)				
CR276	945	1194 – 1199	0.40 (0.32 – 0.48)	3.3	13.1	4.2	94.1
CR285	923	418 – 422	0.55 (0.40 – 0.68)				
Challenger Plateau (Voyage TAN0707)							
CP029	1074	480 – 480	0.18 (0.15 – 0.22)	1.9	89.5	18.4	35.5
CP051	963	1207 – 1213	0.16 (0.13 – 0.18)	1.8	86.1	18.7	41.8
CP105	926	803 – 805	0.22 (0.18 – 0.27)	5.7	68.6	6.4	83.5
CP119	883	529 – 534	0.27 (0.22 – 0.37)	2.0	56.9	10.5	67.6
CP139	926	264 – 266	0.31 (0.25 – 0.41)	1.9	27.4	4.5	90.1

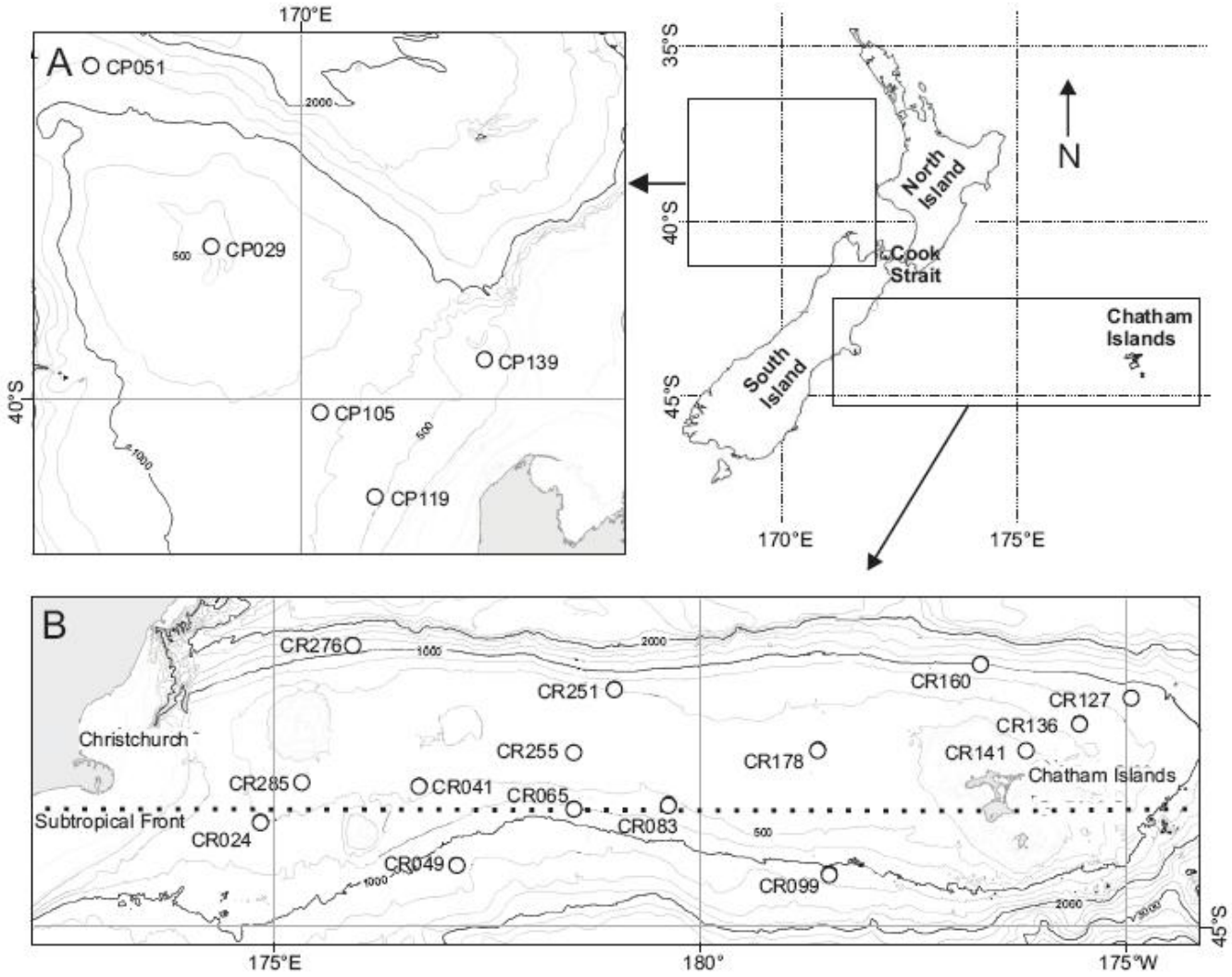

FIGURE 2.1. Bathymetry and Brenke sledge sampling locations on (A) Challenger Plateau (TAN0707) and (B) Chatham Rise (TAN0705). The approximate location of the Subtropical Front (44° S) is shown as a dotted line on the Chatham Rise map.

METHODS

Study sites and sampling methods

Sampling of the Chatham Rise was undertaken on a voyage from 29 March to 30 April 2007, and on the Challenger Plateau from 26 May to 7 June 2007 (National Institute of Water and Atmospheric Research [NIWA] voyages TAN0705 and TAN0707 respectively). In total, 20 stations were sampled (15 from the Chatham Rise and five from the Challenger Plateau) spanning a similar depth range (200 – 1200 m) at both locations (Table 2.1). An epibenthic sledge, built after Brenke (2005) and hereafter referred to as a "Brenke sledge", was deployed once at each station. The Brenke sledge carries two stacked sampling boxes: lower (epi) and upper (supra), each with an opening of 100 cm width by 35 cm height and a 500 µm mesh capture net. Closing doors prevented pelagic by-catch during Brenke sledge descent and retrieval. Upon retrieval, net contents were immediately rinsed with surface seawater into containers. To avoid damaging delicate specimens, samples were then elutriated, a process that allowed lighter organic matter to pass onto 500 µm sieves using gentle water agitation. Following elutriation, remaining material was also sieved. All material was immediately preserved in 95% ethanol for later analyses and is stored at the NIWA invertebrate collection in Wellington, New Zealand.

Sample sorting and taxonomic identification

In order to minimise future damage to specimens, epi- and supra Brenke sledge nets were subsampled while onboard *RV Tangaroa* by hand picking amphipod specimens from total contents immediately after initial processing. Supranet contents were completely sorted, allowing semi-quantitative inter-station abundance analyses. To account for variable trawl distances (see Table 2.1), numbers of individuals within supranet samples are presented as

densities per 1000 m^2. To maximise the number of specimens available for diversity analyses, the partially sorted epinet subsamples were combined with full supranet data.

We identified Phoxocephalidae to the family-level using Lowry and Springthorpe (2001). Individuals were then sorted into putative morpho-species based on morphological similarity for subsequent molecular work. We obtained COI sequences from representatives of all morpho-species present at each station to determine the consistency of taxon identity between geographically disparate locations. Within stations, potential sorting errors and cryptic species were screened out by taking a subsample that was proportional in size to the total number of morpho-species representatives present at each station (see Appendix 2.1). We aimed to generate COI sequences for at least 10% of the total number of individuals up to a maximum of 10 sequences per station. Of the 4099 available phoxocephalid specimens, we selected 360 specimens, representing all putative taxa and all twenty stations for DNA sequencing (Table 2.2). The first uropod (a taxonomically uninformative appendage) was dissected from the left side of each specimen and used for DNA analyses, thus providing intact voucher specimens to match each sequence.

Genetic analyses

Laboratory work was jointly carried out at the Biodiversity Institute of Ontario (BIO) and at the University of Waikato. DNA extractions at BIO used a Glass Fiber Plate DNA Extraction (AcroPrep) method (Ivanova et al., 2006) whereas Waikato DNA extractions were carried out using REDExtract-N-Amp Tissue Kits (Sigma) following manufacturer's instructions. The primer pair LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al*.,* 1994) was used to amplify a 654 bp fragment of the COI gene. Samples which did not amplify successfully were re-run using

CrustDF1 (5'-GGTCWACAAAYCATAAAGAYATTGG-3') and CrustDR1 (5'-TAAACYTCAGGRTGACCRAARAAYCA-3') (Steinke, 2007 unpublished). The PCR thermal regime for both sets of COI primers involved initial denaturing at 94 °C for 1 min; five cycles at 94 °C for 1 min, 45 °C for 1.5 min and 72 °C for 1.5 min; 35 cycles of 94 °C for 1 min, 50 °C for 1.5 min and 72 °C for 1 min followed by a final cycle at 72 °C for 5 min. The PCR product was cleaned by Sephadex (BIO) or by the addition of 0.1µL EXO I enzyme (10 U/µL), 0.2 µL shrimp alkaline phosphate (SAP) (1 U/µL) and 2.7 µL sterile H_20 incubated at 37 °C for 30 min and 80 °C for 15 min (Waikato). Prior to sequencing, the clean PCR product was diluted 1:10 with sterile water and 2 – 5 µL of it was sequenced in both directions using ABI 3730xl (BIO) and 3130xl (Waikato) automated DNA sequencers. All sequences and supporting information have been deposited in BOLD (project ANZCM) and GenBank® (accession numbers *TBA*).

The 360 target specimens yielded 297 usable COI sequences, sequenced in both directions, for an overall success rate of 82%. The specimens which were not sequenced successfully either did not amplify (suggesting sample degradation or primer incompatibility) or produced short or unreadable sequences (e.g. mixed signals, pseudogenes or contamination problems) and were evenly spread across stations / regions. DNA sequences were edited in Sequencher or Geneious and aligned using the Clustal W algorithm in MEGA 5.0 (Tamura et al., 2011), where they were checked for stop codons (none were detected, indicating that only mitochondrial DNA was amplified). Sequence length averaged 623 base pairs (bp) and ranged from 351 to 658 bp.

Statistical analyses

The Kimura 2-parameter (K2P) distance metric was used to assess the level of inter and intraspecific genetic divergence by plotting pairwise sequence comparisons at K2P intervals

of 1% revealing a clear gap between intra and interspecific genetic variation between 6 and 13% (Fig. 2.2). Sequence divergences have been previously used to designate molecular operational taxonomic units (MOTUs) as a surrogate for species-level diversity (e.g. Floyd et al., 2002). To choose appropriate sequence dissimilarity threshold(s), we tested the number of MOTUs found as a function of the value of K2P dissimilarity (Fig. 2.3). We observed a steep decrease in the number of MOTUs from 0 to 2% representing the coalescent. At 6% sequence dissimilarity, an inflexion point leads to a plateau that lasts until a threshold value of 10% within which no additional MOTUs are added. This inflexion point represents the switch from intraspecific sequence variability to interspecific sequence variability. On the basis of this analysis, we employed a 6% threshold for MOTU discrimination.

Based on taxon presence / absence at each station, taxon accumulation curves were computed for all 20 stations as well as by individual region in PRIMER 6 (Clarke, 1993) using the number of observed MOTUs. We also used presence / absence data to create cluster diagrams and MDS plots of inter-station assemblage similarity in PRIMER 6. This was compared with environmental parameters (depth, average autumn surface chlorophyll *a*, TOM, $CaCO_3$, clay and mud content) (Table 2.1) from fifteen stations taken concurrently with our research cruises. The collection and analysis of environmental data are fully described in Nodder et al. (2011) and Knox et al. (2012). All variables were normally distributed with the exception of depth, which was corrected by a $\log^{10}$ transformation. Correlations between community structure (presence-absence) and environmental parameters were examined using the BIOENV procedure in PRIMER 6 (Clarke and Ainsworth, 1993). The contribution of individual MOTUs to the results of cluster and MDS analyses was examined using the similarity percentages (SIMPER) procedure in PRIMER 6 (Clarke, 1993).

Table 2.2.
Abundance and diversity of Phoxocephalid amphipods on Chatham Rise and Challenger Plateau. Singleton molecular taxonomic units (MOTUs) are those which occur at a single station only.

Station	Supranet (ind. 1000 m^{-2})	Combined nets raw abundance	Number of COI sequences	Number of MOTUs		
				Total	Singletons	%
CR024	389	207	32	8	1	13
CR041	103	216	27	8	0	0
CR049	190	126	10	4	1	25
CR065	130	88	12	6	2	33
CR083	170	375	29	10	1	10
CR099	310	189	11	5	0	0
CR127	22	24	3	3	1	33
CR136	72	82	12	4	0	0
CR141	19	26	6	3	1	33
CR160	90	99	11	2	0	0
CR178	160	230	18	10	2	20
CR251	780	966	17	8	0	0
CR255	470	544	23	8	1	13
CR276	238	245	12	5	0	0
CR285	100	168	14	8	0	0
CP029	164	231	22	6	3	50
CP051	1	11	5	5	5	100
CP105	28	73	14	6	5	83
CP119	153	138	9	4	2	50
CP139	59	61	10	5	3	60

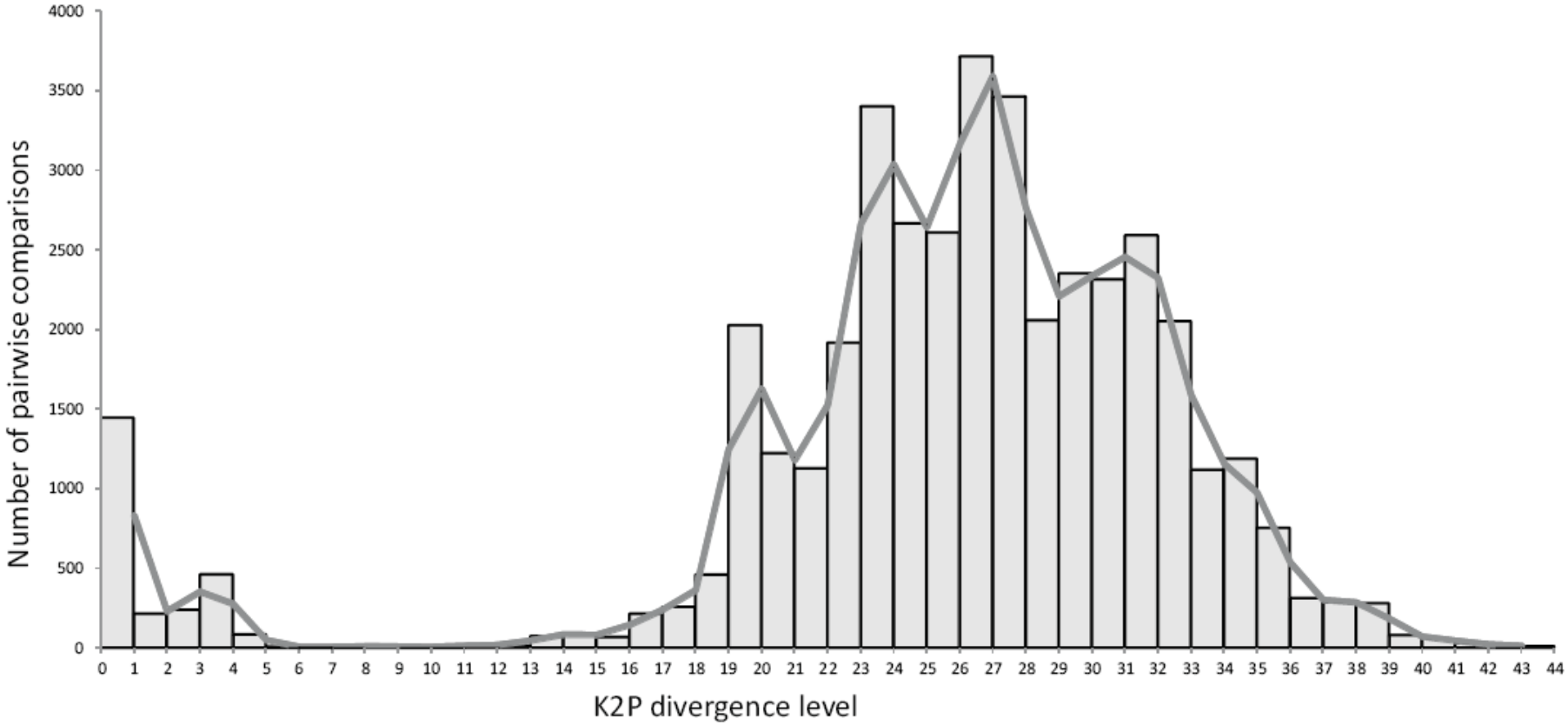

FIGURE 2.2. Pairwise K2P divergence levels of 297 phoxocephalid COI sequences. Bars represent actual counts between intervals, line is the moving average (n=2).

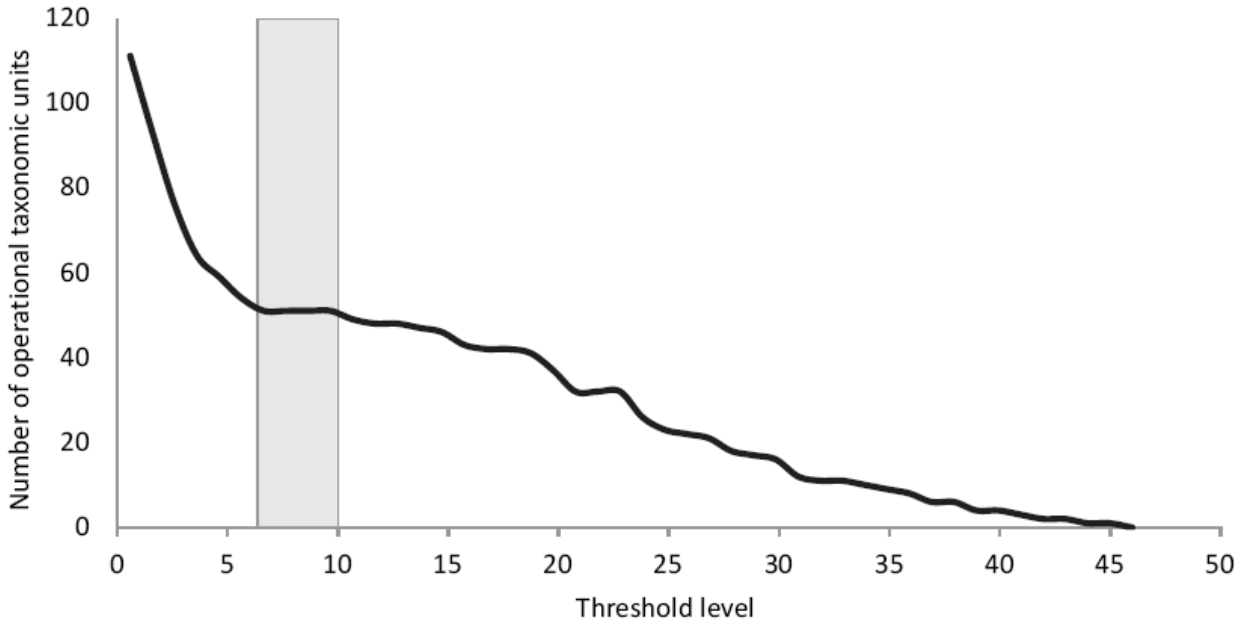

FIGURE 2.3. Number of molecular operational taxonomic units (MOTUs) as a function of threshold limits based on K2P sequence divergence. Grey shading highlights the 'barcoding gap' region, between 6 and 10%, where no MOTUs are added.

RESULTS

Phoxocephalid diversity

Inter-station phoxocephalid abundances from the supranets ranged from 1 – 780 individuals per 1000 m^{-2}, revealing great variability among stations (Table 2.2). Despite differences in abundance, the number of MOTUs was relatively consistent among stations, ranging from two to ten (Table 2.2). Using a 6% threshold, 49 phoxocephalid MOTUs were identified in total. Most of these agreed with initial morpho-species designations. In some cases however, DNA data revealed deep divergences among morphologically conserved MOTUs, initially assigned to the same morpho-species. Such discrepancies occurred both within stations and among populations from different stations.

A total of 29 MOTUs were found on the 15 Chatham Rise stations while the five Challenger Plateau stations housed 21 MOTUs. Only one taxon was found on both regions. Most MOTUs (28/49) only occurred at a single station only, especially on the Challenger Plateau, where such singleton taxa made up >50% of all MOTUs present at each station (Table 2.2). The most distal station on the Challenger Plateau housed only five MOTUs, none of which were collected elsewhere. Deep water stations (>750 m) on the south Chatham Rise (CR049, CR065 and CR099) were also relatively high in singleton taxa (Table 2.2). Shallow stations on the Chatham Rise, especially those with the highest concentration of surface chlorophyll *a* (Table 2.1), tended to have more MOTUs than deeper locations (Table 2.2). Accumulation curves (Fig. 2.4) show a steady rise with increasing sampling over all 20 stations. Taken alone, regions appeared to accumulate MOTUs at different rates (Fig. 2.4). The Chatham Rise (n=15 stations) accumulated MOTUs less rapidly than the Challenger Plateau (n=5 stations) though this is possibly due to different sampling intensity.

Community analyses

Cluster and MDS analyses revealed inter-regional differences, with virtually no similarity in assemblages on the Chatham Rise and Challenger Plateau (Fig.2. 5). Within regions, the Chatham Rise crest stations were closely clustered, indicating similar phoxocephalid assemblages. Deeper (>1000 m) regions of northern and southern Chatham Rise (CR049, CR099 and CR276) harboured separate, closely clustered assemblages (Fig. 2.5). The remaining Chatham Rise stations (CR065, CR127, and CR141) were less closely clustered. Of these, one (CR141) was from our shallowest station (207 m), while the other two were both from intermediate depths (750 – 1000 m). Of the four Challenger Plateau stations, CP029, CP119 and CP139 were all from shallow depths (<550 m) and clustered closely, relative to the remaining station (CP105) which was taken at 800 m depth. Station CP051, omitted from multivariate analyses due to its total dissimilarity with all other stations, was from the deepest and most distal region sampled on the Challenger Plateau.

Using the BIOENV procedure, the best explanatory variables of phoxocephalid assemblages were surface chlorophyll a (46% variability), depth (31%) and TOM (31%). The best correlation (63%) was achieved with a combination of these three variables. All other environmental parameters were only weakly correlated with phoxocephalid assemblages (<6%). Due to the lack of MOTUs with inter-regional distributions, SIMPER analyses were conducted within regional sets only. Three MOTUs (SP02, 03 and 04), all with wide distributions (see Appendix 2.1), contributed most to average station similarity on the Chatham Rise. On the Challenger Plateau, SP30 and 31 (also widely distributed) contributed most to average assemblage similarity.

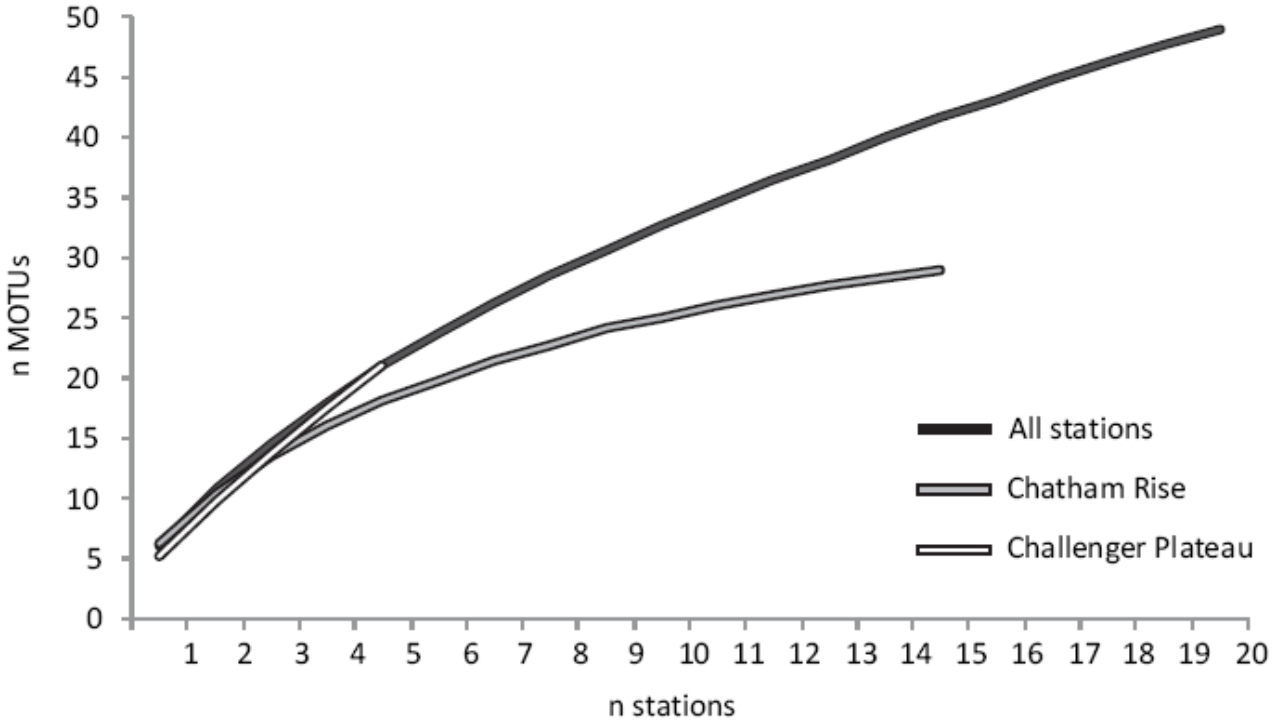

FIGURE 2.4. Molecular operational unit (MOTU) accumulation curves based on observed MOTUs from combined data (all stations) and individual regions (Chatham Rise and Challenger Plateau).

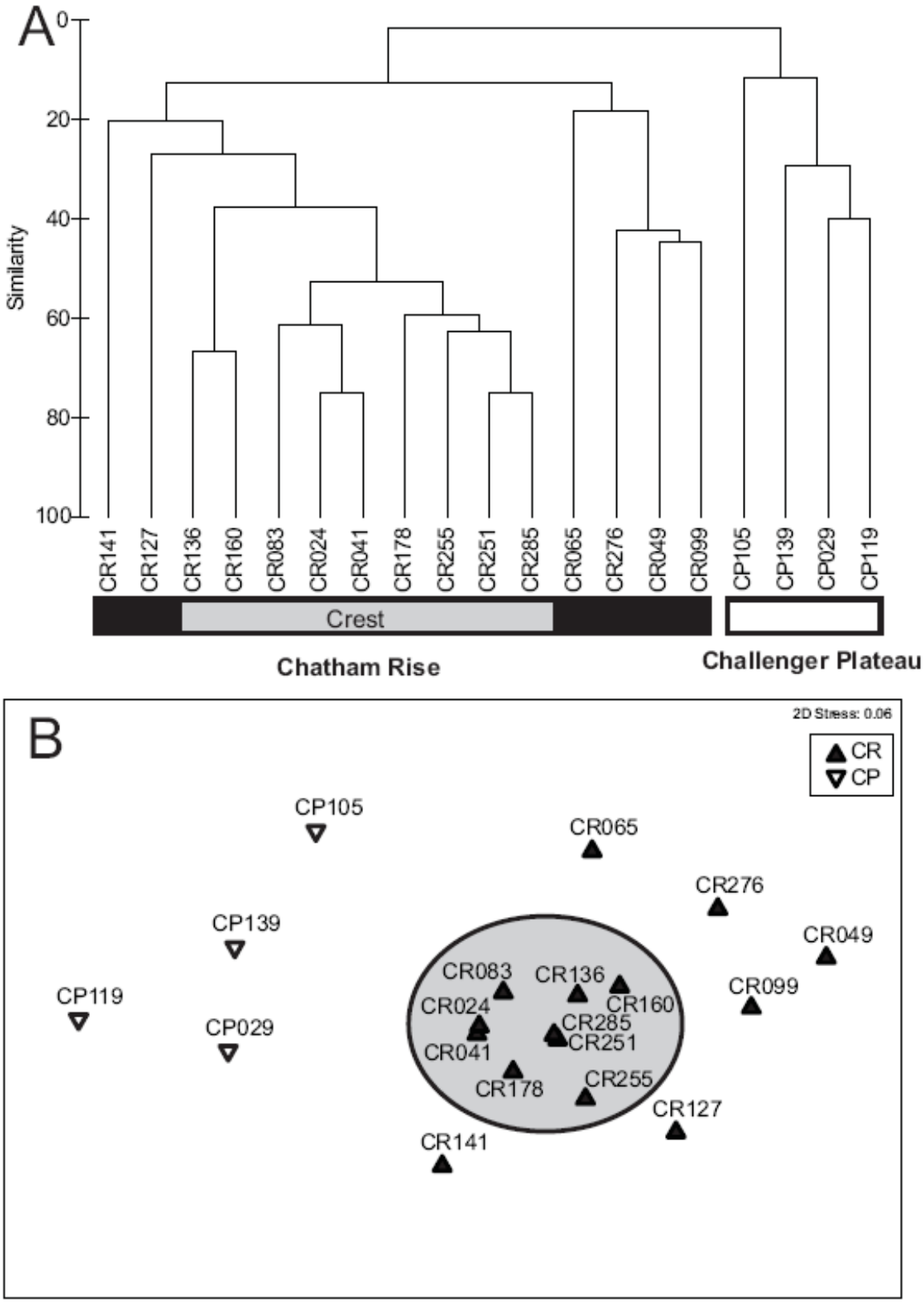

FIGURE 2.5. (A) Dendrogram based on presence / absence station data of phoxocephalid MOTUs. Bars at the bottom indicate groupings based on Bray– Curtis similarity. (B) MDS representation of phoxocephalid composition data. Shape colours and shaded area correspond with groupings identified in A.

DISCUSSION

Phoxocephalid diversity

This study identified 49 MOTU's from 4100 individuals sampled from 20 sites on continental margins of New Zealand. While our MOTUs require detailed taxonomic analysis before gaining formal species recognition, our results suggest the presence of a diverse phoxocephalid fauna on New Zealand's continental margins. The global diversity for Phoxocephalidae currently stands at 222 species, most described from Australian shallow waters (n=89), which is regarded as the epicentre for their evolution (Barnard and Karaman, 1983). Previous work has also shown that the Phoxocephalidae are the most abundant family on New Zealand's continental margins, accounting for approximately 25% of total amphipod abundance (Knox et al., 2012). Collectively, these findings suggest that Phoxocephalidae are a numerically dominant and diverse component of these deep-sea ecosystems.

Our genetic data revealed a number of cases in which deep divergences (>10%) occurred among organisms with high morphological similarity. Most of these lineages were allopatric and would have been overlooked without the sequence data. Prior studies have revealed similar cases of deep genetic divergence among amphipod populations in both marine and freshwater environments (Stevens and Hogg, 2004; Witt et al., 2006) linked to both weak dispersal capabilities and allopatry. We found only one MOTU that occurred in both study regions, suggesting that immigration between eastern and western deep-sea regions of New Zealand is rare for phoxocephalids. Changing landmass and sea levels may have isolated once contiguous populations in New Zealand's deep-sea regions. For example, Cook Strait, which currently separates the North and South Islands of New Zealand and links

eastern and western deep-sea regions, only formed during the Pleistocene (Fleming, 1979; Stevens et al., 1995). Prior to this, Chatham Rise and Challenger Plateau populations' would have been isolated by the New Zealand landmass with connectivity only possible via northern or southern routes. Connectivity over such large distances would be difficult due to shifting environmental conditions associated with latitudinal changes and may explain the lack of present-day overlap in phoxocephalid MOTU distributions.

Nearly 60% (29/49) of phoxocephalid MOTUs were only found at a single location, often represented by a single specimen. The Challenger Plateau, and the deeper, slope regions (>750 m) north and south of Chatham Rise had the highest numbers of these singleton taxa, whereas stations on the crest of Chatham Rise (<750 m) generally harboured the same group of MOTUs. The high incidence of rare taxa has been found in other poorly explored deep sea regions (e.g. Schlacher et al., 2007, Williams et al., 2010), and may be due to the comparatively small number of samples taken and the distances between stations. This highlights the benefits of undertaking further, sampling of deep-sea habitats in order to clarify taxon distribution and diversity. The high beta diversity was somewhat surprising because of the wide coverage of the sampling apparatus. Each of our tows sampled an area of roughly 1000 m^{-2}, which exceeds the spatial coverage of other sampling gear capable of retaining small macrofauna, such as box- or multi-cores. Replicate Brenke sled tows are rare, due to the costs of sampling and sample processing, although they have been found to contain quite different species compositions (Kaiser et al., 2008), suggesting high spatial variability in macrofaunal species distributions at the scale of hundreds to thousands of metres. While acknowledging the logistic constraints inherent in deep-sea research, future studies could benefit from additional data on variability among tows.

The fact that most phoxocephalid MOTUs in our study could not be linked to any described species, emphasises the benefits of surrogate measures for quantifying species-level diversity in unexplored and/or under-sampled habitats. This is particularly relevant for morphologically conservative taxa such as amphipods. The COI sequences we used were effective at discriminating among recognisable morpho-species and also for identifying cryptic taxa which would otherwise have been overlooked. SIMPER analyses also identified several taxa which were numerically dominant in phoxocephalid assemblages. Based on these data, taxonomic attention can then be focussed on identifying morphological correlates, within and among taxa, as well as the adaptive advantages that these confer on the animals (Packer et al., 2009; Smith et al., 2009).

Community analyses and relationships with environmental parameters

Due to the high number of MOTUs restricted to a single station, our community analyses were mostly characterised by highly dissimilar stations. Stations on the Chatham Rise and the Challenger Plateau clustered separately, reflecting virtually no overlap in assemblages between regions. Macrofaunal comparisons between the two regions are rare. However, a previous study on polychaete worms found that communities of shallow water genera on the Challenger Plateau were more like those of the Chatham Rise, than samples taken from the deeper waters of the Challenger Plateau (Probert and Grove, 1998). The greater differentiation detected in our study likely reflects its increased taxonomic resolution and the low vagility of amphipods, linked to their lack a dispersive larval life stage.

Cluster analyses revealed a set of relatively similar stations located on the crest of Chatham Rise and more disparate stations in deeper waters to the north and south (Fig. 2.5). Previous work on Chatham Rise macroinvertebrates has shown that communities show

increasing divergence with depth separation (e.g. Probert et al., 2009; Knox et al., 2012). The crest of the Chatham Rise is characterised by persistent strong easterly currents with near-bed speeds of 20 – 35cm s^{-1} (Heath, 1983; Chiswell, 1994). Connectivity among populations on the crest may be higher than those on the slope because current speed on the Chatham Rise declines with increasing depth (Nodder and Northcote, 2001). Closely related crest communities may also reflect relatively stable environmental conditions along the crest of the Chatham Rise, where food supply is elevated by increased phytodetritus deposition from the STF (Nodder et al., 2003; 2007). In shallow regions of the Chatham Rise crest, patches of organic enrichment caused by episodic surface blooms may increase the number of available niches and thus increase diversity. BIOENV analyses confirm this view, showing a strong correlation between phoxocephalid communities and surface chlorophyll *a*. Dissimilar communities at deep stations could relate to the decline in food availability with depth (Rex, 1981) and the degree to which this permits populations to develop. Highly specialised species with large ranges and low populations are likely to characterise such environments (Sanders, 1968; Snelgrove et al., 1992). The Challenger Plateau, with its relative lack of organic input, also has reduced phoxocephalid MOTU overlap among stations, although this may also reflect the smaller number of samples taken.

In summary, we have highlighted the benefits of molecular techniques to improve both taxonomic resolution and the estimation of faunal diversity in an understudied taxonomic group. Further, we combined these data with information on environmental parameters to determine factors influencing the distribution and diversity of macrofaunal taxa in one section of the deep sea, the world's largest ecosystem. Future studies may benefit from the use of next generation sequencing platforms to more rapidly assess levels

of diversity within and among habitats. However, their application first requires the development of appropriate barcode reference libraries as that developed in this study.

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Appendix 2.1.

COI sample counts for all species and stations.

Species	CR024	CR041	CR049	CR065	CR083	CR099	CR127	CR136	CR141	CR160
01		4			2					
02	2	5		1	10			5		7
03					4	1	1	4		4
04	4	1			1			2	3	
05	10	6			3					
06	4	3								
07	8	6			2		1			
08			1			2				
09	1	1							2	
10				1						
11	2			2	1					
12										
13		1			4			1		
14				1		1				
15					1					
16						4				
17			4							
18										
19			4			3				
20										
21										
22				5						
23				2						
24					1					
25										
26	1									
27			1							
28									1	
29							1			
30										
31										
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49										

Appendix 2.1 *cont.*

COI sample counts for all species and stations.

Species	CR178	CR251	CR255	CR276	CR285	CP029	CP051	CP105	CP119	CP139
01						1		1		4
02	2	5		6	5					
03	2	2	4		1					
04	1	1	3		2					
05	1	1	2		1					
06	3	1	7		2					
07		4			1					
08			1	2						
09	4									
10				2	1					
11										
12	1		1		1					
13										
14		2								
15	2									
16				1						
17				1						
18		1	3							
19										
20			2							
21	1									
22										
23										
24										
25	1									
26										
27										
28										
29										
30						1			2	1
31						1			1	
32								2		
33								4		
34									4	
35						6				
36								2		
37							1			
38									2	
39								1		
40								4		
41										1
42						1				
43						12				
44							1			
45							1			
46							1			
47							1			
48										3
49										1

CHAPTER 3

GENETIC DIVERGENCE AMONG DEEP-SEA AMPHIPODS (CRUSTACEA)[†]

Keywords: Allopatry – Continental shelf – DNA Barcoding – Genetic connectivity – Pleistocene

[†]submitted under the same title as: Matthew A. Knox, Ian D. Hogg, Conrad A. Pilditch, Paul D.N. Hebert and Dirk Steinke.

ABSTRACT

To examine genetic divergence among populations in deep-sea habitats, we analysed mitochondrial DNA (COI) sequences from amphipod crustacean taxa inhabiting New Zealand's continental margins. Sampling was undertaken on two prominent geomorphic features east and west of New Zealand, the Chatham Rise (n=15) and Challenger Plateau (n=5) and encompassed putative barriers to gene flow including undersea ridges, oceanic convergences and historical land barriers. We used a phylogenetic analyses of three relatively abundant and widespread taxa; and combined available genetic data from a further 59 taxa collected from the same stations, to examine mean levels of inter-station genetic divergences. Similar levels of genetic divergence were found between eastern and western regions for all three taxa. Within regions, populations were generally genetically homogenous, often over great distances. Our inter-station analysis of the wider amphipod community revealed similar patterns of genetic structure for most taxa. Based on molecular clock estimates, divergences may correspond to isolation following landmass changes during the Pleistocene. However, levels of genetic divergence between regions were lower than those previously found for nearby coastal taxa, suggesting slower rates of molecular evolution in deep-sea organisms. We conclude that, similar to New Zealand coastal taxa, deep-sea Amphipoda also exhibit clear genetic separation of eastern and western regions, possibly reflecting past landmass changes.

INTRODUCTION

The deep-sea (>200 m) is a vast and essentially continuous environment with few obvious barriers to gene flow (Gage and Tyler, 1991). Consequently, deep-sea organisms are generally thought to have broad distributions and to exhibit little genetic structure due to high levels of gene flow (Etter et al., 1999; Zardus et al., 2006). In other marine habitats, vicariant events such as uplift and landmass changes can disrupt gene flow and isolate formerly interbreeding populations, leading to deeply divergent, geographically sorted lineages (Heads, 2005; Kelly et al., 2006; Stevens and Hogg, 2004). Little is currently known about the effects of these processes on deep-sea taxa. Obtaining adequate sample sizes for studies of population genetic structure means that target taxa need to be abundant and widespread. However, obtaining large samples of such species in the deep-sea across a wide geographic range is problematic due to logistical challenges and sampling costs. The lack of taxonomic information for many deep-sea taxa (Brandt and Berge, 2007; Lörz and Brandt, 2003) also limits potential study taxa. Our study examines levels and patterns of sequence divergence in three relatively abundant, widespread amphipod taxa (*Ampelisca* cf. *chiltoni, Oediceroides* cf. *apicalis* and Phoxocephalidae sp).We supplemented these data with an analysis of a further 59 taxa to examine mean levels of genetic divergence among locations.

In the few prior studies of deep-sea taxa, genetic divergences among populations have been associated with a range of factors including topography (Le Goff-Vitry et al., 2004; Weinberg et al., 2003), currents (Aboim et al., 2005), depth (Etter et al., 2005; Zardus et al., 2006) and oxygen levels (Creasey et al., 1997; Rogers, 2000). Patterns of genetic variation suggest that some cases of divergence have arisen from vicariance (Howell et al., 2004; Roques et al., 2002). For example, mid-ocean ridges may impede gene flow and

isolate previously contiguous populations at abyssal depths (Etter et al., 2011), while changing sea levels on continental margins during the Pleistocene apparently isolated mollusc populations in the Sea of Japan, leading to regional divergence (Iguchi et al., 2007).

Because of the high environmental heterogeneity on continental margins (Danovaro et al., 2008; Gooday et al., 2010), organisms in these habitats appear to exhibit greater population structure than those from abyssal depths, suggesting that continental margins may be primary sites for evolutionary radiations (Etter et al., 2005). The continental margins of New Zealand are greatly extended by the presence of several large geomorphic features including the Challenger Plateau and the Chatham Rise. These regions feature variable topography (Fig. 3.1A, B) as well as a complex geological history, providing an ideal setting for examining putative barriers to dispersal. Chatham Rise is a broad submarine ridge, stretching eastwards for over 800 km from east of New Zealand's South Island near Christchurch to the Chatham Islands and beyond (Fig. 3.1B). The rise is generally flat-topped at 300 – 400 m depth and descends into deeper waters (>2500 m) to the north and south. The Subtropical Front (STF), which marks the mixing of subtropical and subantarctic surface waters, appears to be bathymetrically locked to the southern flank of the rise near 44°S (Sutton, 2001; Uddstrom and Oien, 1999) and represents a zone of elevated primary production (Murphy et al., 2001) and benthic biomass (Nodder et al., 2003). Due to the STF, the crest of the Chatham Rise is characterised by persistent, strong, east-flowing currents (Chiswell, 1994; Heath, 1983). In contrast to Chatham Rise, Challenger Plateau is a flat-lying continental block, submerged beneath the Tasman Sea (Fig. 3.1A) with few obvious barriers to gene flow among populations. The two regions are currently connected by Cook Strait. However, uplift during Pleistocene caused this inter-regional dispersal pathway to completely close (Fig. 3.2), potentially isolating eastern and western populations.

Macrofauna inhabiting New Zealand's continental margins are highly diverse (McKnight and Probert, 1997; Probert and Grove 1998), with benthic Amphipoda especially well represented (Knox et al., 2012; Lörz, 2010). Amphipods were chosen as our target taxon in part because of their ecological importance in continental shelf systems (Brandt, 1995; Dauby et al., 2003). However, amphipod species are also good candidates for vicariance-induced diversification because of their lack of larval dispersal stages (Bilton et al., 2002). Prior studies have established their propensity to form genetically discrete populations (Cristescu et al., 2003).

This study examines genetic divergence among amphipod taxa found on New Zealand's continental shelf (200 – 1200 m). This setting includes undersea ridges, oceanic convergences and historical land barriers; all possible barriers to gene flow. We obtained individuals of several amphipod taxa from 20 stations separated by up to 1600 km and determined levels of sequence variation at the mitochondrial cytochrome oxidase *c* subunit I (COI) gene. Our aim was to assess levels of genetic divergence within and between the two major geographic features (Chatham Rise and Challenger Plateau) and to test the hypothesis that genetically divergent populations have arisen as a consequence of isolation caused by hydrogeographic barriers.

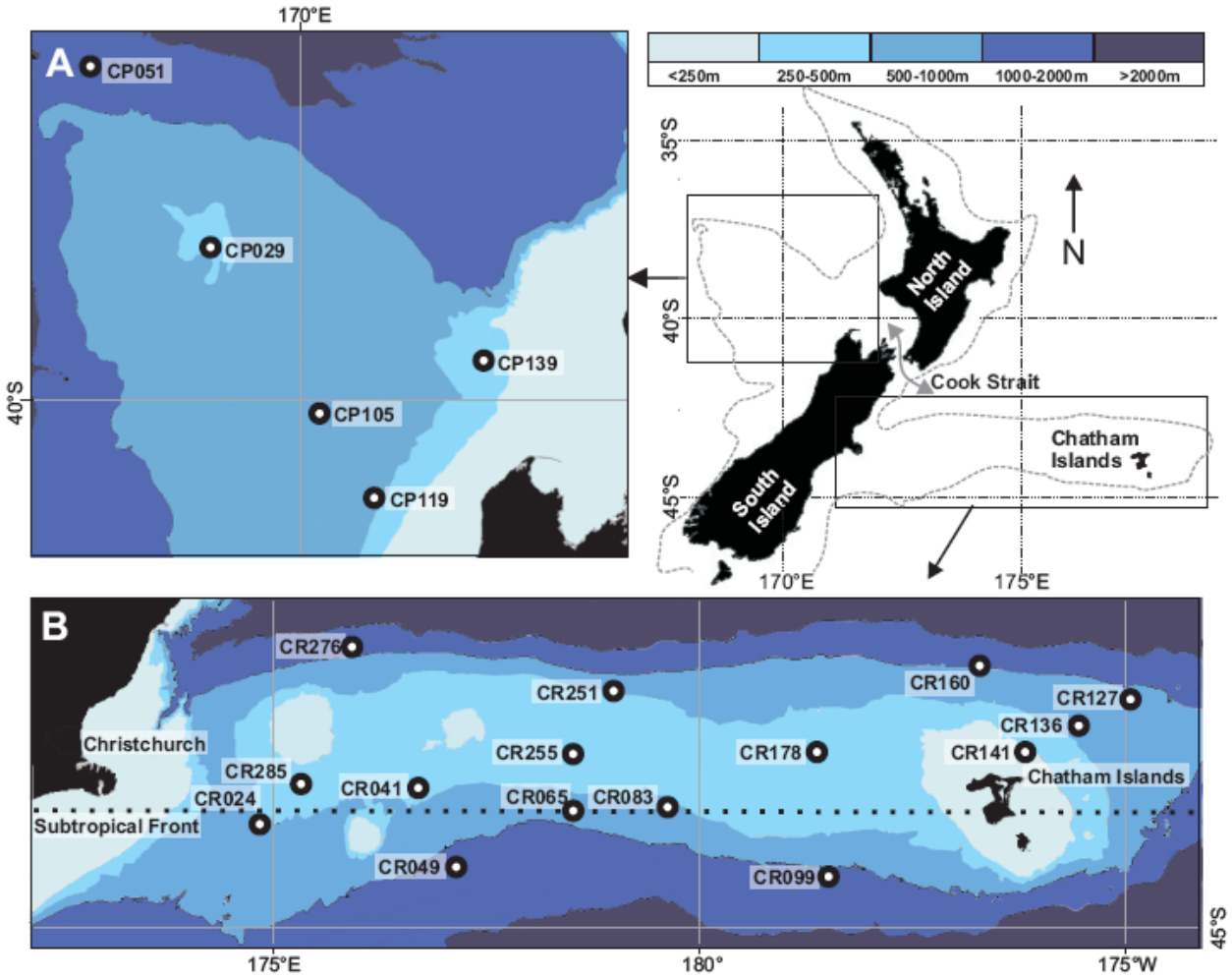


FIGURE 3.1. Bathymetry and Brenke sledge sampling locations on (A) Challenger Plateau (TAN0707) and (B) Chatham Rise (TAN0705). The approximate location of the Subtropical Front (44° S) (black dotted line) is shown on the Chatham Rise map. The location of Cook Strait (grey arrow) and the 1000 m isobath (dotted grey line) are shown on the New Zealand map.

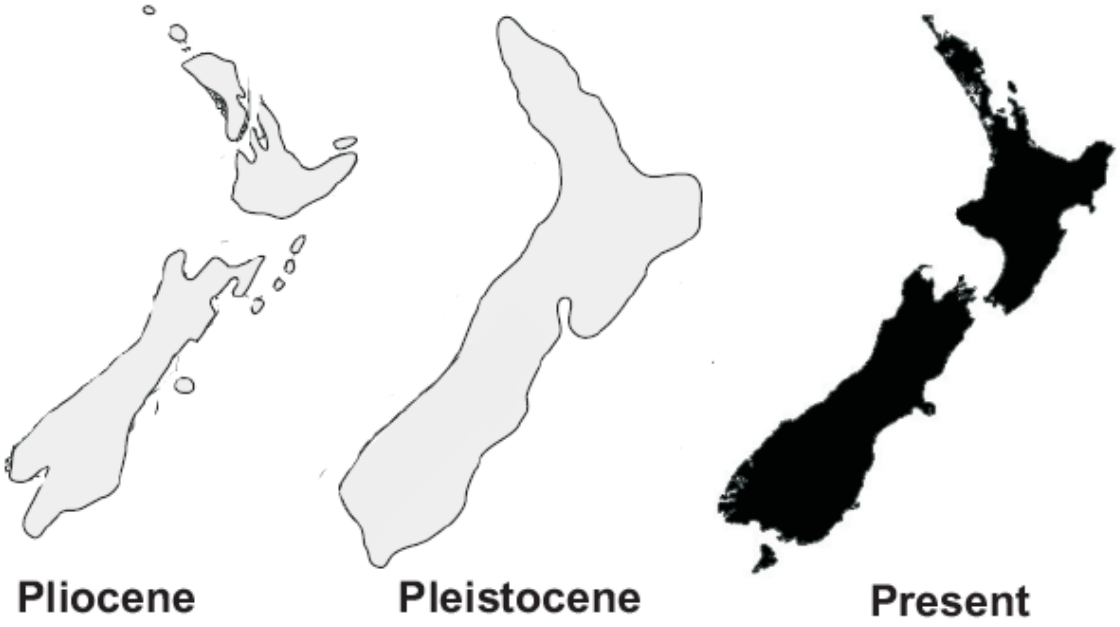


FIGURE 3.2. The changing landmass of New Zealand. The Pliocene (6 – 2 Mya) landmass provided few barriers for genetic exchange between the east and west coasts in contrast to the Pleistocene (~2 Mya). Figures adapted from Fleming (1979) and Stevens et al. (1995).

METHODS

Study sites and sampling methodology

Sampling of Chatham Rise was undertaken on a voyage from 29 March to 30 April 2007, and Challenger Plateau was sampled from 26 May to 7 June 2007 (National Institute of Water and Atmospheric Research [NIWA] voyages TAN0705 and TAN0707 respectively). In total, 20 stations were sampled (15 from Chatham Rise and 5 from Challenger Plateau) spanning a similar depth range (200 – 1200 m) at both locations (Fig. 3.1). An epibenthic sledge (Brenke, 2005), hereafter referred to as a "Brenke sledge", was deployed once at each station. The Brenke sledge carries two stacked sampling boxes: lower (epi) and upper (supra), each with an opening of 100 cm width by 35 cm height and a 500 µm mesh capture net. Closing doors prevented pelagic by-catch during Brenke sledge descent and retrieval. Upon retrieval, net contents were immediately rinsed with surface seawater into containers. To avoid damaging delicate specimens, samples were then elutriated, a process that allowed lighter organic matter to pass onto 500 µm sieves using gentle water agitation. Following elutriation, remaining material was also sieved. All material was immediately preserved in 95% ethanol for later analyses and is stored at the NIWA invertebrate collection in Wellington, New Zealand.

Amphipoda were identified to a family-level using Lowry and Springthorpe (2001) and specimens from each station were sorted to putative morphospecies. Three taxa, *Ampelisca* cf. *chiltoni, Oediceroides* cf. *apicalis* and Phoxocephalidae sp. (identified using Barnard, 1961), occurred at several stations and were targeted for phylogenetic analyses. A further 59 taxa with more limited distributions (occurring at 2 or more stations) were selected for a second analysis to determine mean levels of genetic divergence among all

available amphipod taxa and stations. The first uropod (a taxonomically uninformative appendage) was dissected from the left side of each specimen and used for DNA analyses, thus providing intact voucher specimens for future reference.

Genetic protocols

We targeted the mitochondrial cytochrome *c* oxidase subunit I (COI) gene, to determine patterns of genetic divergence and to apply evolutionary models. The COI gene is a common marker for studies of intraspecific variation (Hebert et al., 2003), and has previously provided insights into evolutionary processes for amphipod taxa (Costa et al., 2009; Kelly et al., 2006; Witt et al., 2006). Furthermore, the timing of population divergences can be estimated using sequence divergences based on molecular clock calibrations (Knowlton and Weigt, 1998). In total, 494 COI sequences were obtained from the 62 taxa. Of these, 65 sequences belonged to the three taxa targeted for phylogenetic analyses; *Ampelisca* cf. *chiltoni* (n=28)*, Oediceroides* cf. *apicalis* (n=25) and Phoxocephalidae sp. (n=12). A caprellid amphipod was also sequenced for use as outgroup in subsequent analyses.

Laboratory work was jointly carried out at the Canadian Centre for DNA Barcoding (CCDB) and University of Waikato. DNA extractions at the CCDB used Glass Fiber Plate DNA Extraction method (Ivanova et al., 2006), whereas Waikato DNA extractions were carried out using REDExtract-N-Amp Tissue Kit (Sigma) following manufacturer's instructions. The primer pair LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al., 1994) was used to amplify a 658 bp fragment of the COI gene. Samples which did not amplify successfully were re-run using CrustDF1 (5'-GGTCWACAAAYCATAAAGAYATTGG-3') and CrustDR1 (5'-TAAACYTCAGGRTGACCRAARAAYCA-3') (D. Steinke unpublished data). The PCR thermal regime for both primer sets was: initial denaturing at 94 °C for 1 min; five cycles at 94 °C for

1 min, 45 °C for 1.5 min and 72 °C for 1.5 min; 35 cycles of 94 °C for 1 min, 50 °C for 1.5 min and 72 °C for 1 min followed by a final cycle at 72 °C for 5 min. Each PCR product was cleaned by Sephadex (CCDB) or by the addition of 0.1 µL EXO I enzyme (10 U/µL), 0.2 µL shrimp alkaline phosphate (SAP) (1 U/µL) and 2.7 µL sterile H_20 incubated at 37 °C for 30 min and 80 °C for 15 min (Waikato). Prior to sequencing, clean PCR product was diluted 1:10 with sterile water and 2 – 5 µL of product was sequenced in both directions using ABI 3730xl (CCDB) and 3130xl (Waikato) automated DNA sequencers. All sequences and supporting information have been deposited in the Barcode of Life Datasystems (BOLD) database (Ratnasingham and Hebert, 2007) in project *ANZCM*, and cross-referenced to GenBank® (Accession numbers *TBA*). DNA sequences were edited in Sequencher or Geneious and aligned using the Clustal W algorithm in MEGA 5.0 (Tamura et al., 2011), where they were checked for the presence of stop codons.

Statistical analyses

Phylogenetic trees consisting of sequences from *Ampelisca* cf. *chiltoni, Oediceroides* cf. *apicalis,* Phoxocephalidae sp. and Caprellidae sp. (outgroup) were constructed in MEGA 5.0 (Tamura et al., 2011) using maximum likelihood and a GTR + I model selected using JModeltest (Posada, 2008). Confidence levels for cladistic analysis were estimated using 1000 bootstrap replicates. Using the same model parameters, sequence differences and mean distances within and between Chatham Rise and Challenger Plateau stations were calculated in MEGA. To further assess genetic structure, we estimated a haplotype joining network for each taxon using the algorithm of (Templeton et al., 1992) in TCS ver. 1.21 (Clement et al., 2000) with a 95% connection limit. Since this level failed to allow the connection of all groups, we allowed a maximum number of mutational steps of 15, and used this information only to suggest possible points where distinct haplotypes may

connect. The degree of genetic variation within and among populations was tested using AMOVA (Analyses of Molecular Variance; (Excoffier et al., 1992)) carried out in Arlequin ver. 3.11, using 10,000 permutations of haplotypes among populations and an alpha value of 0.05 (Excoffier et al., 2005). Approximate divergence times between eastern and western regions were calculated using the molecular clock estimation for COI of 1.4 – 2.3% nucleotide sequence divergence per million years (Knowlton and Weigt, 1998; Schubart et al., 1998), rates previously applied to phylogeographic studies on amphipods (Krebes et al., 2010; Pilgrim et al., 2009). Clock-like behaviour was confirmed within each taxon, using a relative rate test (Tajima, 1993) conducted in MEGA prior to the analyses.

To examine mean levels of inter-station genetic divergences, genetic data from 59 benthic amphipod taxa (n=434 COI sequences) belonging to 15 families were imported into Arlequin (see Appendix 3.1 for full taxonomic and geographic data). We then calculated the numbers of pairwise differences between populations (PiXY) for each of the 59 taxa. Due to inconsistencies in sequence length between taxon groups, PiXY was standardised to pairwise differences per 100 base pairs. Data for all taxa were then combined into a pairwise data matrix (Appendix 3.2) so that all available comparisons could be analysed simultaneously. To determine the average genetic relatedness of amphipod taxa across stations and regions, single linkage cluster analysis was carried out in PRIMER (Clarke, 1993). To investigate inter-regional genetic divergences along with taxon-specific depth preferences, we compared data from 11 taxa (including *Ampelisca* cf. *chiltoni, Oediceroides* cf. *apicalis* and Phoxocephalidae sp.) with distributions on both Chatham Rise and Challenger Plateau. Standardised PiXY values were used to compare average intra (where possible) and inter-regional levels of genetic divergence. Based on distributional data we

classified each of the 11 taxa (see Appendix 3.1) as inhabiting either “shallow” (200 – 1000 m) or “deep” water (>1000 m).

RESULTS

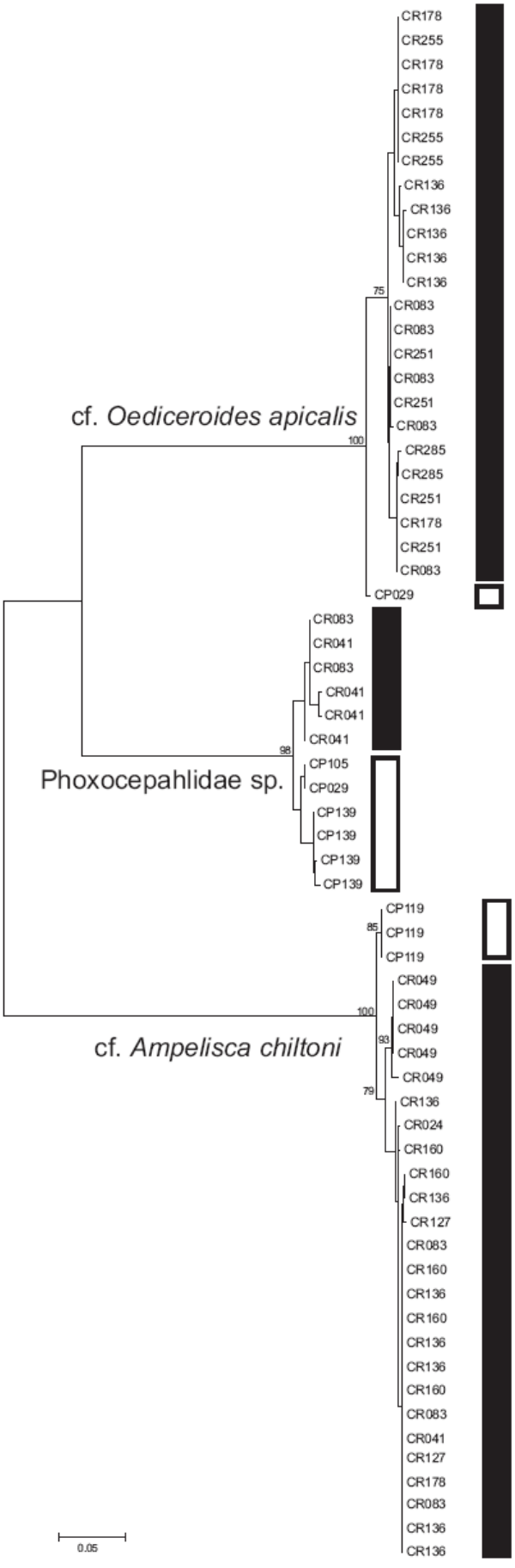
Each of the three target taxa were clearly delineated from one another by COI sequence data (Fig. 3.3). Taxa also showed within-species genetic structure with Chatham Rise populations clearly distinct from those on Challenger Plateau (Fig. 3.3). Within-species divergences between Chatham Rise and Challenger Plateau ranged from 2.2 – 2.8%, while divergences within Chatham Rise were <1% and <1.6% for Challenger Plateau. Haplotype joining networks, estimated for each taxon, show either nine or ten missing haplotypes between Challenger Plateau and Chatham Rise populations (Fig. 3.4A, B, C). There was also evidence for genetic differentiation within regions. Near-shore populations (CP139) of Phoxocephalidae sp. on Challenger Plateau were separated by seven missing haplotypes from offshore populations (CP029, CP105) (Fig. 3.4B). Populations of *Ampelisca* cf. *chiltoni* on the southern slopes (CR049) of Chatham Rise were separated from other rise populations by eight missing haplotypes (Fig. 3.4C). We also found haplotype diversity within populations. For example, several different haplotypes were found among *Oediceroides* cf. *apicalis* at CR083 and CR178 (Fig. 3.4A).

We conducted AMOVA tests for each taxon to quantify the level of variability attributable to inter-regional differences. Variation among regions accounted for 68%, 62% and 62% of the total COI genetic variation in *Ampelisca* cf. *chiltoni, Oediceroides* cf. *apicalis* and Phoxocephalidae sp. respectively. The remaining variation was mainly explained by genetic diversity among stations within regions (21%, 23% and 25%) while the genetic variation within stations accounted for only 8%, 15% and 13% of the total genetic variation in *Ampelisca* cf. *chiltoni, Oediceroides* cf. *apicalis* and Phoxocephalidae sp., respectively.

Cluster analysis using combined data from the 59 taxa (Fig. 3.5) revealed a clear genetic difference between amphipod populations on Chatham Rise and Challenger Plateau. Within both regions, populations were relatively homogenous and genetic differences between stations were low (Fig. 3.5). The only exception was station CP051, which grouped separately from other stations and CP105, which appears to be more closely grouped with the Chatham Rise stations. In both cases, low amphipod abundances and a high proportion of unique taxa led to a relatively low number of available comparisons with other populations at different stations (Appendix 3.2). To examine taxon-specific patterns, we also recorded the genetic differences (Standardised PiXY values) within and between regions for individual species with distributions spanning both regions (n=11 taxa). Of these taxa, three were found predominantly at stations >1000 m depth and had the lowest levels of genetic divergence (<1.3 changes per 100bp). With the exception of one taxon (sp. 59 Synopiidae), the other, relatively shallow taxa (n=8) all exhibited greater levels (>1.8 changes per 100bp) of genetic divergence between regions (Table 3.1).

Table 3.1.
Intra and inter-regional genetic differences (number of changes per 100 base pairs) and average depth for populations of 11 amphipod taxa with distributions on both Chatham Rise and Challenger Plateau.

Identification	intra CR	intra CP	Inter-region	Average depth (m)
sp01 *Ampelisca* cf. *chiltoni*	0.6		2.2	744
sp02 *Oediceroides* cf. *apicalis*	0.7		2.1	481
sp03 Phoxocephalidae sp.	0.3	1.1	2.5	511
sp11 Eusiridae sp.	0.1		3.6	436
sp13 Eusiridae sp.	0.2		1.8	634
sp15 Eusiridae sp.			2.1	306
sp29 Lysianassidae sp.			3.0	413
sp59 Synopiidae sp.	0.0		0.9	508
sp18 Gammaridae sp.	0.3		0.8	1001
sp39 Pardaliscidae sp.		1.5	1.3	984
sp60 Unknown family			1.3	1179


CR178
CR255
CR178
CR178
CR178
CR255
CR255
CR136
CR136
CR136
CR136
CR136
75
CR083
CR083
CR251
CR083
CR251
CR083
cf. Oediceroides apicalis
100
CR285
CR285
CR251
CR178
CR251
CR083
CP029
CR083
CR041
CR083
CR041
CR041
CR041
98
CP105
Phoxocepahlidae sp.
CP029
CP139
CP139
CP139
CP139
CP119
85
CP119
CP119
CR049
CR049
100
CR049
93
CR049
cf. Ampelisca chiltoni
CR049
79
CR136
CR024
CR160
CR160
CR136
CR127
CR083
CR160
CR136
CR160
CR136
CR136
CR160
CR083
CR041
CR127
CR178
CR083
CR136
CR136
0.05

Previous page: FIGURE 3.3. Maximum likelihood tree of COI sequences belonging to *Oediceroides* cf. *apicalis*, Phoxocephalidae sp. and *Ampelisca* cf. *chiltoni* from populations located on the continental margins of New Zealand. Numbers above bars denote bootstrap confidence derived from 1000 psuedoreplicates. Black bars indicate Chatham Rise locations, white Challenger Plateau.

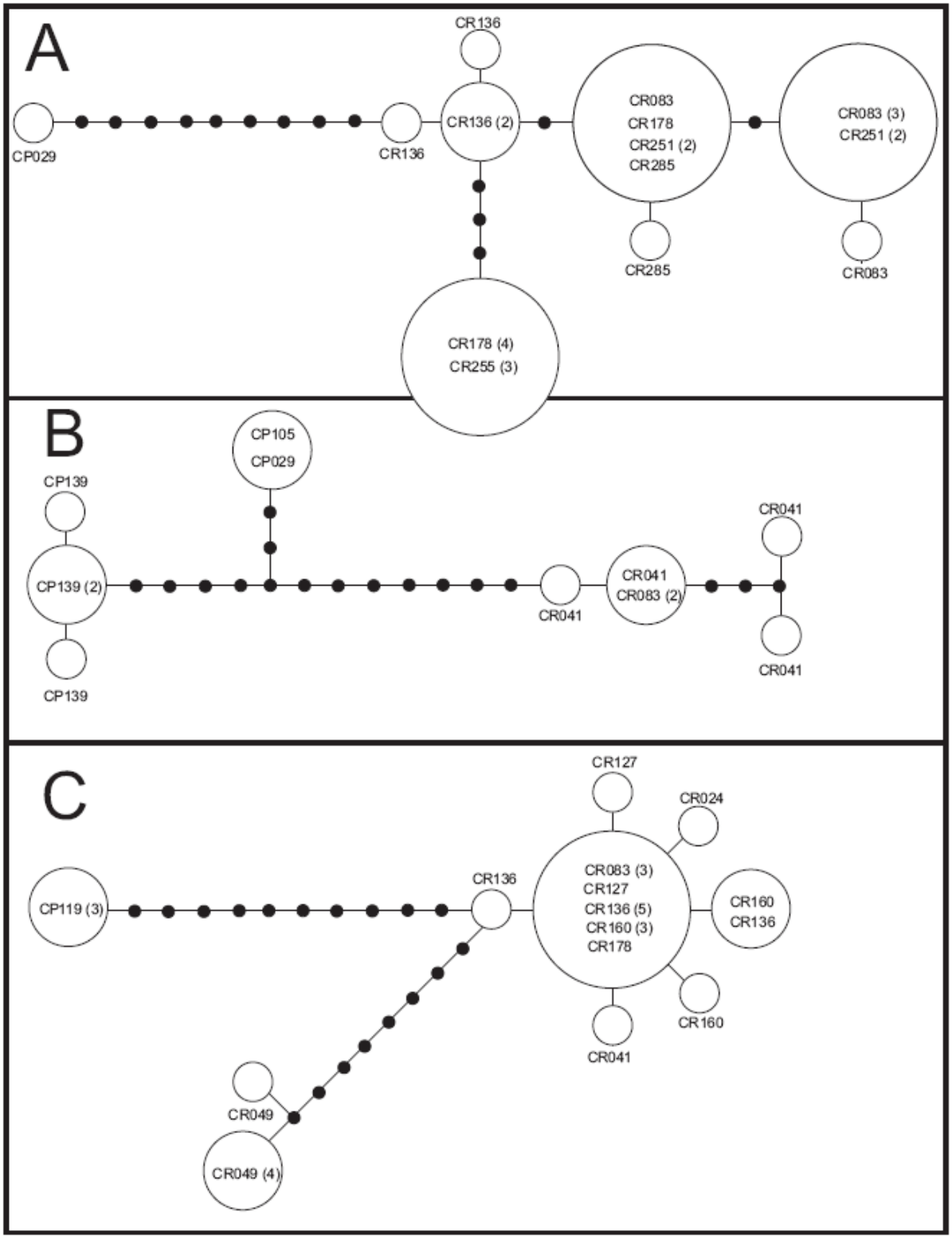


FIGURE 3.4. Haplotype joining network for (A) *Oediceroides* cf. *apicalis*, (B) Phoxocephalidae sp. and (C) *Ampelisca* cf. *chiltoni*. Missing haplotypes/mutational steps are indicated by small solid circles. The size of each white circle is proportional to the number of haplotypes (small=1; medium=2 – 4; large=5+).

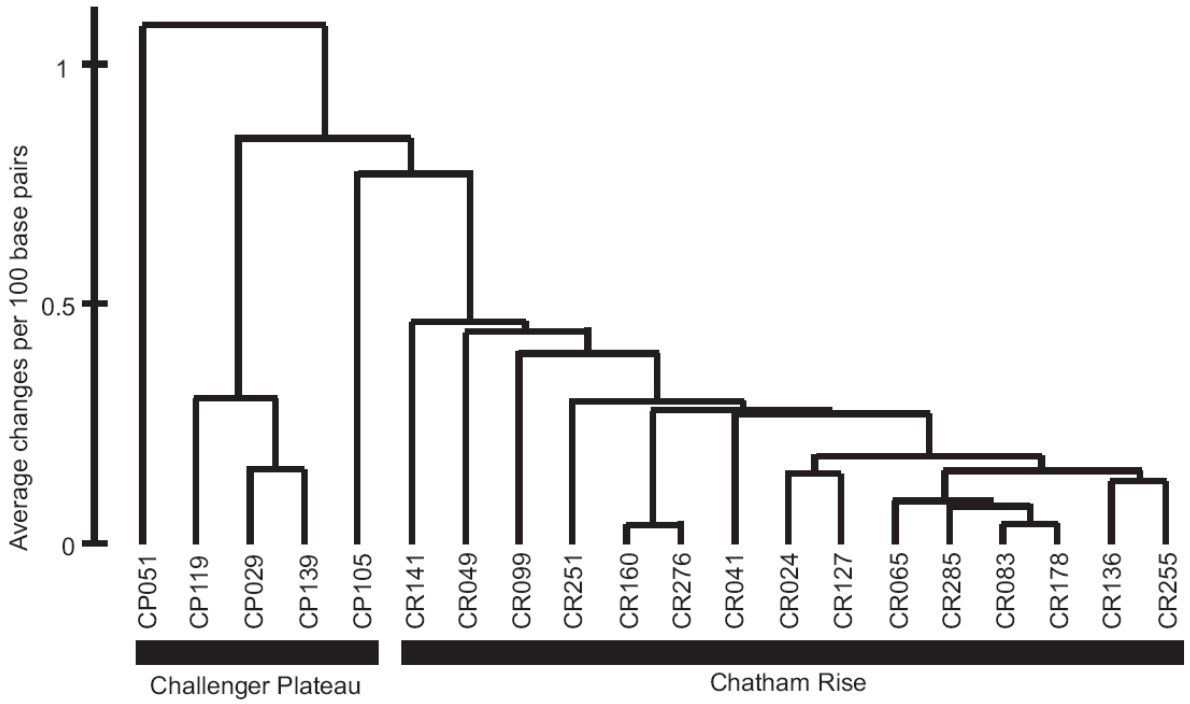


FIGURE 3.5. Single linkage cluster diagram of inter-population genetic differences averaged per 100 base pairs. Data derived from averaged inter-population comparisons of 59 amphipod taxa from 20 stations on the continental margins of New Zealand.

DISCUSSION

Similar levels of COI sequence divergence between eastern and western sampling sites for each of our three study taxa suggest similar evolutionary histories. The closure of Cook Strait is likely to have isolated populations on Chatham Rise and Challenger Plateau and similar patterns of vicariance following the Pleistocene uplift have been observed for many of New Zealand's coastal taxa (Ross et al., 2009). Population differentiation in association with Pleistocene isolation has been previously observed among deep-sea benthic invertebrates (Iguchi et al., 2007) and may be an important mechanism underlying the high biodiversity of deep-sea ecosystems.

Because of the broad geographic ranges and genetic homogeneity of many species (Etter et al., 2011; Zardus et al., 2006), deep-sea organisms are thought to have large, continuous populations with high levels of gene flow. Our finding of limited genetic divergence within regions (e.g. *Ampelisca* cf. *chiltoni* from stations CR024 and CR127 which are 830 km apart) suggests that gene flow is often considerable. Genetic homogeneity over great distances is a characteristic of other deep-sea organisms and possibly reflects environmental stability and lack of dispersal barriers (France and Kocher, 1996; Zardus et al., 2006).

Bathymetric gradients are often associated with genetic structure, suggesting that they impose greater constraints on species dispersal than geographic distance (McClain and Hardy, 2010). Previous research has found intraspecific genetic divergences among populations inhabiting different depth strata, with a distinct boundary at 3300 – 3500 m (France and Kocher, 1996; Zardus et al., 2006). Our study involved the analysis of sites with a 1000 m depth range on both sides of Chatham Rise. The focussing of phytodetritus on

southern Chatham Rise slopes (Nodder et al., 2003; 2007) and the differential properties of overlying waters (subtropical vs. subantarctic) present an opportunity to examine the influence of elevated food supply with concurrent depth changes on the population genetics of deep-sea amphipods. Our results indicate genetically divergent populations on the southern slopes of Chatham Rise (*Ampelisca* cf. *chiltoni* at station CR049), while northern populations (CR127, CR160) inhabiting similar depths were homogenous with crest populations. This suggests that factors other than depth, such as food supply and temperature influence genetic structure in deep-sea amphipod taxa.

Similar levels of sequence divergence within and between regions were revealed by our analysis of the additional 59 amphipod taxa. Many of the intraspecific comparisons within regions involved just a few stations, but they often spanned large distances. Genetic homogeneity among such widely separated populations implies recent gene flow among stations within regions and suggests the potential for long distance dispersal or the presence of unsampled, intermediate populations (Schlacher et al., 2007; Williams et al., 2010).

We found consistent levels of genetic divergence between eastern and western populations in each of our study three taxa (2 – 3%), suggesting that eastern and western populations last shared a common ancestor between 0.96 and 2 million years ago based on standard molecular clocks. However, these divergences are lower than those in nearby coastal amphipod taxa. For example, the estuarine amphipods *Paracorophium excavatum* and *P. lucasi*, which were both also isolated by Pleistocene landmass changes (Fig. 3.2), exhibit higher levels of genetic divergence (6 – 7%) (Knox et al., 2011) and more closely match the approximate timing (~2 Mya) of the Cook Strait closure (Fleming, 1979; Stevens

et al., 1995). The lower levels of divergence in our deep-sea taxa suggest a slower evolutionary rate, possibly reflecting the impacts of deep-sea environmental conditions on generation length or metabolic rate. Certainly, the molecular clock calibrations of (Knowlton and Weigt, 1998) were developed using coastal taxa exposed to very different environmental conditions than those in the deep-sea. The low food supply and low temperatures of deep-sea ecosystems mean that most organisms are slow growing and produce fewer offspring compared with their shallow-water counterparts (Gage and Tyler, 1991). Furthermore, the deep sea fauna may experience more stable selective pressures than taxa in other habitats as a consequence of the relatively stable environmental conditions. Specifically, genetic variants are less likely to accumulate and persist given the temporally and spatially stable environmental conditions, resulting in relative genetic homogeneity. This is supported by the observation that within the deep-sea, genetic homogeneity is highest at abyssal depths (Etter et al., 2005), where environmental stability would be greatest.

Founder effects, which follow genetic bottlenecks caused by disturbance, can cause populations to diverge genetically (Grant et al., 2001; Jakobsson and Rosenberg, 2007). A relative lack of catastrophic disturbance events in the deep-sea might also result in reduced rates of mitochondrial evolution. Other studies however, suggest that elevated environmental stability is a mechanism for increasing genetic diversity, arguing that deep-sea taxa retain a higher proportion of ancestral polymorphisms due to the decreased likelihood of population bottlenecks (Etter et al., 1999; Zardus et al., 2006).

Species specific depth preference may contribute to contrasting phylogeographic patterns observed among some taxa. Those taxa which occurred at the relatively shallow

stations (<1000 m) were found to have increased inter-regional genetic divergences, suggesting that these taxa are restricted to the band of continental shelf <1000 m that surrounds New Zealand (Fig. 3.1). Accordingly, isolation following Pleistocene landmass changes (e.g. closing of Cook Strait; Fig. 3.2) may be responsible for the observed genetic structure within these taxa. In contrast, taxa collected from deeper stations exhibit reduced levels genetic divergence between regions, suggesting that these populations may be connected via un-sampled populations inhabiting northern and/or southern continental margins.

Overall, our findings suggest that, similar to coastal fauna, vicariance in response to Pleistocene landmass changes appears to concur with present day patterns of genetic structure in amphipod taxa inhabiting New Zealand's continental margins. Eastern and western populations showed genetic divergence at similar levels in a range of amphipod taxa. However, divergence levels were lower than those found for coastal amphipod taxa, which suggests that evolutionary rates may be slower in deep-sea amphipods.

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Appendix 3.1.

Distribution and sequence count data for 62 amphipod taxa on Chatham Rise and Challenger Plateau sampling stations.

Identification	CR024	CR041	CR049	CR065	CR083	CR099	CR127	CR136	CR141	CR160	CR178	CR251	CR255	CR276	CR285	CP029	CP051	CP105	CP119	CP139
sp. 01 *Ampelisca* cf. *chiltoni*	1	1	5		3		2	7		5	1								3	
sp. 02 *Oediceroides* cf. *apicalis*					5			5			5	4	2		3	1				
sp. 03 Phoxocephalidae sp.		4			2											1		1		4
sp. 04 Ampeliscidae						6							2		3					
sp. 05 Aoridae		3									2	4								
sp. 06 Aoridae	1				2						3	3								
sp. 07 Aoridae					2			1												
sp. 08 Cyphocarididae																8			3	
sp. 09 Dexaminidae		1									3	1	2							
sp. 10 Eusiridae											1	3								
sp. 11 Eusiridae	2				1							2	2							3
sp. 12 Eusiridae		3											4							
sp. 13 Eusiridae										1			2						1	
sp. 14 Eusiridae								2		1										
sp. 15 Eusiridae													1							6
sp. 16 Eusiridae	1	1											1							
sp. 17 Gammaridae	1				1						1	1								
sp. 18 Gammaridae			10				5			3								6		
sp. 19 Gammaridae		1										1								
sp. 20 Ischyroceridae					1							4								
sp. 21 Ischyroceridae						1				2										
sp. 22 Ischyroceridae		1								1										
sp. 23 Ischyroceridae	1										1									
sp. 24 Ischyroceridae		1										3								
sp. 25 Ischyroceridae		1				1														
sp. 26 Liljeborgiidae	7	6	3										1							
sp. 27 Liljeborgiidae																	1	1		
sp. 28 Lysianassidae												2	5							
sp. 29 Lysianassidae													2			3				
sp. 30 Lysianassidae		4		2	6															
sp. 31 Lysianassidae									1				1							

Appendix 3.1. *cont.*

Distribution and sequence count data for 62 amphipod taxa on Chatham Rise and Challenger Plateau sampling stations.

Identification	CR024	CR041	CR049	CR065	CR083	CR099	CR127	CR136	CR141	CR160	CR178	CR251	CR255	CR276	CR285	CP029	CP051	CP105	CP119	CP139
sp. 32 Lysianassidae	2				2							1	2							
sp. 33 Lysianassidae	1				3															
sp. 34 Oedicerotidae		1										1								
sp. 35 Oedicerotidae	4				1		1	5		1										
sp. 36 Oedicerotidae			2							3										
sp. 37 Oedicerotidae										1		2		1						
sp. 38 Oedicerotidae	1	1																		
sp. 39 Pardaliscidae							1										1	1		
sp. 40 Phoxocephalidae		1											2							
sp. 41 Phoxocephalidae												3	1							
sp. 42 Phoxocephalidae	7	5			1								1		1					
sp. 43 Phoxocephalidae	1	4		1	8			2				5		4	5					
sp. 44 Phoxocephalidae										7				1						
sp. 45 Phoxocephalidae				1		2						2								
sp. 46 Phoxocephalidae		1			4			1												
sp. 47 Phoxocephalidae					4	3	1	4		4	3	2	4		1					
sp. 48 Phoxocephalidae																1			1	1
sp. 49 Phoxocephalidae			1			2								3						
sp. 50 Phoxocephalidae	6	3			1							2			1					
sp. 51 Phoxocephalidae														2	1					
sp. 52 Phoxocephalidae	2			1	1															
sp. 53 Phoxocephalidae	4	1			1			2	3		2	2								
sp. 54 Phoxocephalidae					1						2									
sp. 55 Phoxocephalidae			2											1						
sp. 56 Phoxocephalidae									2		3									
sp. 57 Phoxocephalidae	1	3									3	1	7		2					
sp. 58 Phoxocephalidae						4								1						
sp. 59 Synopiidae	2	12																	8	
sp. 60 Unknown Family			5			1											1			
sp. 61 Unknown Family			2	1																
sp. 62 Urothoidae					2	1														

Appendix 3.2

Pairwise comparisons of genetic variation within 59 amphipod taxa over twenty populations. Data corresponds to average numbers of pairwise differences between populations per 100 bp (below line) and number of inter-station comparisons analysed (above line). Multiple data values between the same stations were averaged and unavailable pariwise comparisons were treated as missing data.

	CR 024	CR 041	CR 049	CR 065	CR 083	CR 099	CR 127	CR 136	CR 141	CR 160	CR 178	CR 251	CR 255	CR 276	CR 285	CP 029	CP 051	CP 105	CP 119	CP 139
CR024		9	1	3	11	1	1	3	1	1	5	8	6	1	4	0	0	0	1	1
CR041	0.31		1	2	6	1	0	3	1	1	4	9	7	1	4	0	0	0	1	0
CR049	2.16	2.38		1	0	2	1	0	0	2	0	0	1	2	0	0	1	1	0	0
CR065	0.19	0.28	0.44		3	1	0	1	0	0	0	1	1	1	1	0	0	0	0	0
CR083	0.21	0.31	1.74	0.2		3	2	6	1	2	5	9	4	1	4	0	0	0	0	1
CR099	1.97	1.02	0.56	1.65	0.96		1	1	0	2	2	2	3	2	2	0	1	0	0	0
CR127	0.15		0.62		1.53	2.76		2	0	3	1	1	1	0	1	0	1	2	0	0
CR136	0.58	0.69	1.74	0.31	0.36	0.43	1.62		1	3	2	3	1	1	2	0	0	0	1	0
CR141	0.85	0.79			1.15			1.51		0	2	1	1	0	0	0	0	0	0	0
CR160	0.35	0.39	0.68		0.55	1.07	0.95	0.43			1	2	2	2	1	0	0	1	1	0
CR178	0.32	0.47	1.91		0.05	1.35	2.52	0.29	0.66	0.96		8	3	0	2	0	0	0	0	0
CR251	0.44	0.44		0.63	0.3	1.2	3.06	0.38	1.15	0.59	0.31		8	2	4	0	0	0	0	1
CR255	0.37	0.81	2.4	0.21	0.16	0.77	3.06	0.13	0.46	0.53	0.28	0.32		0	3	1	0	0	1	2
CR276	0.63	0.43	1.08	0.47	0.51	1.41		0.78		0.04		0.58			2	0	0	0	0	0
CR285	0.29	0.36		0.09	0.08	0.4	3.06	0.27		0.9	0.61	0.59	0.26	0.28		0	0	0	0	0
CP029		2.35			2.18			2.11			2.11	2.11	2.99		2.17		0	0	2	1
CP051			1.29			1.25	1.84											2	0	0
CP105		2.53	0.81		2.39		0.77			0.83						0.18	1.08		0	0
CP119	0.85	0.89	2.41		2.14	2.23		2.28		1.89	2.32		1.73			0.3				1
CP139	3.69	2.81			3.69							3.52	2.85			0.16		1.75	0.32	

THESIS CONCLUSION

Accurate assessments of biodiversity are essential to monitor anthropogenic activities such as land-use changes, over-harvesting and global warming, as well as to understand ecosystem function and evolutionary processes. This thesis focused on the diversity of deep sea amphipods from New Zealand's Chatham Rise and Challenger Plateau and their relationship with environmental variables. In the past, such studies would have relied primarily on morphological data. However, ready access to molecular data is now available through access to high throughput DNA sequencing and has provided an additional capacity to assess the diversity of under-explored ecosystems such as that examined here. This thesis has expanded knowledge of New Zealand deep-sea amphipod diversity at the family, species and genetic levels and provided insight into the ecological and evolutionary processes which influence these taxa.

High amphipod densities (up to 2074 individuals 1000 m^{-2}) were recovered and identified from Brenke sledge supranets (Chapter 1). This value is similar to those found using suprabenthic sampling equipment elsewhere (e.g. Linse et al., 2002; Lörz and Brandt, 2003). The strong influence of elevated food supply (in association with the subtropical front) on Chatham Rise amphipod communities was detected at both family (Chapter 1) and species-level (Chapter 2), suggesting strong benthic-pelagic links. In addition, Cook Strait was identified as an important biogoegraphic barrier for amphipods inhabiting New Zealand continental margins both in terms of restricting species ranges (Chapter 2) and gene-flow (Chapter 3).

Species-level diversity was found to be very high in the amphipod family Phoxocephalidae. Based on divergence thresholds among COI DNA sequences I estimated

species-level diversity using molecular taxonomic units (MOTUs). This method revealed unexpectedly high total diversity (n=49 MOTUs), far exceeding the currently recognised New Zealand species (n=17; Webber et al., 2010). Global diversity for Phoxocephalidae currently stands at 222 species, most described from Australian shallow waters (n=89), which is regarded as the epicentre for their evolution (Barnard and Karaman, 1983). The high beta diversity revealed in this chapter also implies that the full extent of diversity on the deep-sea floor may be grossly underestimated. By extrapolating this information to the other 26 families (identified in Chapter 1), the number of amphipod species inhabiting the continental margins of New Zealand, many of which will be new to science, is likely to number in the hundreds.

The data generated in this thesis also confirm the value of molecular based approaches for the assessment of biodiversity. Using multivariate analyses of MOTU presence / absence, a cluster of highly similar stations was identified, broadly distributed over the crest of the Chatham Rise in association with elevated food availability. While the family-level analyses of Chapter 1 suggested that land proximal Challenger Plateau stations were similar to those of Chatham Rise, species-level analyses in Chapter 2 revealed only one of 49 MOTUs was distributed on both Chatham Rise and Challenger Plateau, suggesting limited species exchange between regions. Increased resolution of DNA barcodes highlights the usefulness of this approach to assess biodiversity.

High biodiversity in the deep-sea seems intrinsically unlikely due to the apparent lack of disturbance and barriers to gene-flow, which lead to genetically divergent lineages elsewhere. The final chapter of my thesis was focussed at the genetic level and sought to examine the influence of putative barriers to gene-flow in deep-sea habitats. In addition to a

detailed analysis focussed on a few taxa, I sought to compare genetic connectivity among stations using as many taxa as possible. This comparative approach focussed the analysis on geographic barriers and selective processes that influence genetic connectivity at an ecosystem scale, the understanding of which is imperative for large-scale management efforts (Kelly and Palumbi, 2010). Overall, populations inhabiting Chatham Rise and Challenger Plateau were found to be genetically distinct in several amphipod taxa. The east–west pattern of genetic structure was similar to that seen in nearby coastal amphipod taxa (*Paracorophium excavatum* and *P. lucasi*; Knox et al., 2011; Appendix I) and suggests vicariance following Pleistocene landmass changes, namely the closure of Cook Strait (Fleming, 1979; Stevens et al., 1995). However, the actual levels of divergence (2 – 3%) were lower than those seen in *Paracorophium* (6 – 7%). On the basis of these results, I concluded that vicariance has played an important role in the evolutionary radiation of the deep-sea fauna and that evolutionary rates may need to be revised for deep-sea taxa.

FUTURE DIRECTIONS

This research on New Zealand's continental margin amphipod fauna and has also identified several areas which would benefit from further study. Specifically, the extension of the taxonomic scope, horizontal and vertical sampling coverage and utilisation of recent advances in DNA sequencing technology would compliment and expand upon the findings of this thesis.

Taxonomic knowledge and expertise below the level of family was lacking for the great majority of the amphipod taxa collected on the two ocean voyages. The results of Chapter 2 suggest that there may be hundreds of undescribed amphipod species in the material collected at the 20 stations analysed in this thesis. Further sampling, especially at

deeper sites (>1000 m) would undoubtedly provide even more novel specimens. A greater future emphasis on taxonomic research would expand the taxonomic breadth available for analysis and allow testing of the generality of the findings of this thesis.

One of the values of this thesis research was the ability to link amphipod community groupings (Chapters 1 and 2), to environmental variables collected at the same stations. However, some of the environmental variables which were compared (e.g. sediment variables collected with multi-corer), can vary widely over relatively small scales due to their patchy spatial distribution on bathyal seafloor habitats and did not offer ideal comparisons with sledge data. Brenke sledge trawl lengths were up to 1 km long, sweeping an area at least 100 cm wide, whereas a multi-corer sample covers only a fraction of this area (0.008 m^{-2}). Future studies attempting to match environmental variables from multi-cores to towed sledges should take this limitation into account by increasing the number of multi-core deployments to expand spatial coverage.

The continent of Zealandia is nearly 4 million square kilometres, 93% of which is submerged. However, the study of large scale biogeographic patterns in this region has been largely restricted to terrestrial flora and fauna. Sampling macrofauna from other nearby undersea features such as Campbell Plateau, Norfolk Ridge or Lord Howe Rise and comparing species assemblages with those of Chatham Rise and Challenger Plateau would improve understanding of biogeography on the continental shelf of Zealandia.

My research focussed on the shallowest section of the deep-sea, the continental margins. Future research could test the finding of increased beta diversity with increasing depth, revealed in Chapter 2, over a more extensive depth range. Furthermore, expanding the sampling range would also help to elucidate potential depth-specific patterns of genetic

divergence. The results of Chapter 3 suggested that taxa which occurred at the relatively shallow stations (<1000 m) had increased inter-regional genetic divergences in accordance with isolation following Pleistocene landmass changes (closing of Cook Strait). In contrast, taxa collected at deeper stations tended to exhibit reduced levels genetic divergence between regions, suggesting that these populations are connected, not by Cook Strait, but perhaps via un-sampled populations inhabiting northern and/or southern continental margins. Unfortunately, there were insufficient data to test this hypothesis, but future sampling, especially at deeper stations would allow more detailed examination of changing patterns of genetic variation with depth. Examining intraspecific genetic structure within and among other nearby regions such as Campbell Plateau, Norfolk Ridge or Lord Howe Rise would also provide further insight into evolutionary processes in deep-sea habitats.

One of the major bottlenecks of biodiversity studies is the time-consuming nature of sorting and identifying samples. This has led to developments in molecular estimates of biodiversity, similar to Chapter 2 of this thesis. Future DNA based studies on New Zealand's continental margin fauna may extend the taxonomic breadth and detail of the present work, thus contributing to a database of COI sequences to match current taxonomic information. Such databases will be extremely valuable as molecular approaches to bio-monitoring are adopted. Next generation sequencing (NGS) approaches can rapidly generate considerable sequence data (Schuster, 2008), with exciting applications for improving the accuracy and efficiency of routine bio-monitoring for many ecosystems (e.g. Hajibabaei, 2011), including the deep-sea. NGS of entire or partial sledge tows would eliminate the time-consuming sorting phase, allowing rapid assessment of biodiversity in deep-sea habitats, which are being increasingly affected by human influence.

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APPENDIX 1

THE ROLE OF VICARIANCE AND DISPERSAL ON NEW ZEALAND'S ESTUARINE BIODIVERSITY: THE CASE OF *PARACOROPHIUM* (CRUSTACEA: AMPHIPODA) †

Keywords: allopatric isolation – Arthropoda – biodiversity – cryptic – dispersal – Pleistocene – vicariance

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ABSTRACT

To investigate the role of vicariance and dispersal on New Zealand's estuarine biodiversity, we examined variability in mitochondrial cytochrome *c* oxidase subunit I (COI) gene sequences for the amphipod genus *Paracorophium.* Individuals from the two nominate endemic species (*Paracorophium excavatum* and *Paracorophium lucasi*) were collected from sites throughout the North and South Islands. Sequence divergences of 12.8% were detected among the species. However, divergences of up to 11.7% were also observed between well supported clades, suggesting the possibility of cryptic species. Nested clade analyses identified four distinct lineages from within both *P. excavatum* and *P. lucasi*, with boundaries between clades corresponding to topographical features (e.g. Cook Strait, North and East Cape). Sequence divergences of 3.7 – 4.9% were also observed within geographic regions (e.g. east and west coasts of the upper North Island). Genetic structure in *Paracorophium* appears to represent prolonged isolation and allopatric evolutionary processes dating back to the Upper Miocene and continuing through the Pliocene and early Pleistocene. On the basis of molecular clock estimates from sequence divergences and reconstructions of New Zealand's geological past, we suggest that sea level and landmass changes during the early Pleistocene (2 Mya) resulted in the isolation of previously contiguous populations leading to the present-day patterns. COI genetic structure was largely congruent with previously observed allozyme patterns and highlights the utility of COI as an appropriate marker for phylogeographic studies of the New Zealand estuarine fauna.

INTRODUCTION

Present day patterns of intra- and interspecific genetic variation are often the result of past vicariant events (Avise, 1992). Landmass and sea level changes can disrupt gene flow, isolate formerly interbreeding populations and, through mutation, genetic drift and natural selection, result in the formation of distinct evolutionary lineages (Walker and Avise, 1998; Heads, 2005). The use of molecular techniques (e.g. allozyme electrophoresis, DNA sequencing), has allowed for a finer level of taxonomic resolution and demonstrated that many taxa consist of highly genetically diverged, species complexes (Bickford et al., 2007; Costa et al., 2009), with genetic structure often suggesting vicariant speciation processes (Kelly et al., 2006).

Over the past 12 Myr, the New Zealand landmass has undergone significant changes. At the beginning of the Pleistocene (approximately 2 Mya) the North and South Islands formed a single landmass when uplift separated the east and west coasts of what is now the North Island. Prior to this, marine intrusions during the Pliocene (6 – 2 Mya) and upper Miocene (12 – 6 Mya) may have turned northern New Zealand into an archipelago of smaller ephemeral islands (Fleming, 1979; Stevens et al., 1995). These geological processes resulted in changes in population connectivity for New Zealand coastal fauna by altering available dispersal pathways.

New Zealand's geological history is reflected in the population genetics of many of its extant coastal species. For example, an east–west pattern of genetic structure has been detected for several taxa (Schnabel et al., 2000; Jones et al., 2008). However, this pattern is not ubiquitous (Ross et al., 2009). In addition to landmass changes, species-specific differences in abilities to disperse across hydrodynamic features (Bilton et al., 2002;

Valanko, et al., 2010), and/or the requirement for specific settlement habitats (e.g. estuaries; Stevens and Hogg, 2004; Jones et al., 2008), may also contribute to present-day phylogeographic patterns for New Zealand coastal species.

In New Zealand, the amphipod genus *Paracorophium* occurs in estuarine habitats (Marsden, 2002), and may be ideal for studying vicariant processes. The direct development of *Paracorophium* should reduce migration and, thus, gene flow between neighbouring populations compared to planktonic larval dispersers (Bilton et al., 2002; Cristescu et al., 2003). The genus occurs throughout New Zealand (except the west coast of the South Island), and occupies a latitudinal range from the subtropical north (35.0°S) to the temperate south of New Zealand (46.4°S) (Stevens and Hogg, 2004). Throughout this range, connectivity among *Paracorophium* populations may be limited by dispersal abilities and the availability of suitable habitat. Estuaries are spatially discrete habitats, often separated by ecophysiological boundaries, as well as geographical distance, which may limit gene flow (Cognetti and Maltagliati, 2000; Dawson et al., 2001). Currently, three recognized *Paracorophium* species (*Paracorophium excavatum*, *Paracorophium lucasi*, and *Paracorophium brisbanensis*) occur in New Zealand (Chapman et al., 2002). Of these, *P. brisbanensis* is a recent introduction (originating from Australia) and, to date, has only been found in Tauranga Harbour (Stevens et al., 2002). Both endemic species are euryhaline, occurring in fine organic sediments in the mid to upper tidal areas of sand or mudflats of harbours and inlets, as well as in regions of rivers and freshwater lakes (Chapman et al., 2002).

Previous allozyme analyses have shown that *P. excavatum* and *P. lucasi* populations sampled from around New Zealand are highly structured genetically (Stevens and Hogg,

2004). Populations sharing a coastline had low levels of genetic differentiation that did not correlate with geographical distance. By contrast, populations not sharing a coastline (i.e. isolated by land or oceanographic barriers) showed higher levels of differentiation indicating restricted gene flow. The levels of divergence were similar to interspecific levels found in other amphipod species and it was proposed that at least two groups from the *P. lucasi* complex and three from the *P. excavatum* complex corresponded to sibling species.

To further investigate patterns of genetic divergence for *Paracorophium* spp. in New Zealand and to apply evolutionary models, we used the mitochondrial cytochrome *c* oxidase subunit I (COI) gene. The COI gene has become an increasingly common marker for studies of intra- and interspecific variation (Hebert et al., 2003), and has provided insights into ongoing evolutionary processes within taxa (Witt et al., 2008). Furthermore, population divergence times may be estimated from mitochondrial sequence divergence based on molecular clock calculations calibrated with known geological events (Knowlton and Weigt, 1998). The geological history of New Zealand during the Pliocene and Pleistocene was used to identify processes that may be responsible for the present-day genetic structure observed in *Paracorophium*.

METHODS

Study Sites and Sample Collection

Collections of endemic New Zealand *Paracorophium* (*P. excavatum* and *P. lucasi*) were made between September 1998 and August 2000 from 37 sites throughout New Zealand (Stevens and Hogg, 2004). Samples were initially stored at -75 °C and then later transferred to 95% ethanol for longer-term storage. Individuals were morphologically identified *sensu* Chapman et al. (2002). On the basis of the patterns of variation reported by Stevens and Hogg (2004), we selected 93 individuals (51 *P. lucasi* and 42 *P. excavatum*) from 27 of these sites for COI analyses (Fig. 4.1, Table 4.1). We also collected individuals of the introduced *P. brisbanensis*, from Tauranga Harbour in October 2009. Two adult male specimens were recovered and identified using morphological characters described in Chapman (2002). Owing to their close phylogenetic links to the New Zealand *Paracorophium* fauna, these specimens were used as an outgroup taxon for subsequent genetic analyses.

Genetic Protocols

DNA extractions for *P. excavatum* and *P. lucasi* were undertaken using a 'salting out' protocol (Sunnucks and Hales, 1996; Aljanabi and Martinez, 1997). The primer pair LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al., 1994) was used to amplify a 658-bp fragment of the gene for COI. Ten microlitre polymerase chain reaction (PCR) reactions contained 1 µL of 10x PCR buffer plus $MgCl_2$ (Roche), 2 µL of 10 mM dNTP (Boehringer Mannheim), 0.5 µL of each primer (10 pmol µL-1), 0.5 mL of bovine serum albumin (0.0005%), 0.5 U of *Taq* polymerase (Roche) and 1 µL of unquantified DNA. The PCR thermal regime involved initial denaturing at 94 °C for 1 min; five cycles at 94 °C for 1 min, 45 °C for

1.5 min, and 72 °C for 1.5 min; 35 cycles of 94 °C for 1 min, 50 °C for 1.5 min, and 72 °C for 1 min; followed by a final cycle at 72 °C for 5 min. The PCR product was cleaned by the addition of 0.1 mL EXO I enzyme (10 U μL^{-1}), 0.2 mL of shrimp alkaline phosphate (SAP) (1 U μL^{-1}) and 2.7 mL of sterile H2O incubated at 37 °C for 30 min and 80 °C for 15 min. DNA extractions and PCR for *P. brisbanensis* were carried out using Sigma REDExtract-n-Amp kits in accordance with the manufacturer's instructions. Clean up and sequencing occurred as for *P. excavatum* and *P. lucasi*.

Before sequencing, clean PCR product was diluted 1 : 10 with sterile water and 2 – 5 µL of product was sequenced in both directions using a MegaBACE DNA Analysis System (Amersham Biosciences) or an ABI 3130 automated DNA sequencer (Applied Biosystems Inc.), both at the University of Waikato DNA Sequencing facility. Sequences were aligned using SEQUENCHER, version 4.1.2 (Gene Codes) sequence editor and verified as being derived from amphipod DNA using the GenBank® BLAST algorithm. A 530-bp fragment was used for all subsequent analyses. All sequences have been deposited in GenBank® under accession numbers HQ857914–HQ858008).

Statistical Analyses

Chi-squared tests implemented in PAUP* 4.0b10 (Swofford, 2002) were used to determine whether the assumption of equal base frequencies among sequences was violated, first on all sites, then for parsimony informative sites only, and, finally, for the third codon position only. COI sequences were then used to construct a maximum likelihood (ML) phylogeny using PHYML (Guindon and Gascuel, 2003) and the generalized time reversible (GTR) evolutionary model selected by jMODELTEST, version 0.1.1 (Posada, 2008). Confidence in the cladistic analyses was assessed by bootstrap analysis with 1000 pseudoreplicates for the

ML tree. Uncorrected and GTR corrected percent sequence divergence was calculated for each sample in PAUP*. Sequence differences and net distances between haplogroups were calculated in MEGA, version 4 (Tamura et al., 2007) using uncorrected *p* distances. Pairwise data were then imported into PRIMER, version 6.1.5 (Clarke and Gorley, 2006) to create a cluster diagram showing percentage divergence.

To further assess genetic structure, we estimated a haplotype network for both *P. excavatum* and *P. lucasi* using the algorithm of Templeton et al.(1992) in TCS, version 1.21 (Clement et al., 2000). A 95% connection limit was used for both species. Because this level failed to allow connection of all groups, we allowed a maximum number of mutational steps of 60, and used this information only to suggest possible points where the distinct groups may connect. Populations were specified by their Global Positioning System coordinates and sample sizes, and then defined into a nested structure including outgroup probabilities (Castelloe and Templeton, 1994), following nested clade analysis (NCA) guidelines *sensu* Crandall (1996). To quantify the degree of genetic variation within and among populations, phylogroups highlighted by haplotype networks were also tested by means of analyses of molecular variance (AMOVA) (Excoffier et al., 1992). AMOVA analyses were carried out using ARLEQUIN, version 3.11, using 10 000 permutations of haplotypes among populations and an a value of 0.05 (Excoffier et al., 2005).

Divergence times were estimated by using a molecular clock approximation for CO1 of 1.4 – 2.3% nucleotide sequence divergence per Myr. This rate is derived from comparisons between geological evidence and invertebrate mitochondrial data from several studies (Knowlton and Weigt, 1998; Schubart et al., 1998) and has been applied previously to phylogeographic studies of amphipods (Pilgrim et al., 2009; Krebes et al., 2010). Clock-like

behaviour was confirmed within each species, using a relative rates test (Tajima, 1993) conducted in MEGA before the analyses.

Table 4.1.

Sampling codes and locations. N=Northland, NE=North Island east coast, NW=North Island west coast, L= North Island Lake, CS= Cook Straight, SE= South Island east coast and SW=South Island west coast. Number of seqeunces and haplotypes from each location are presented.

Paracorophium excavatum		lat (S)	long (E)	n seq	n haplo
Rangauna Harbour	N2	35°01'	173°15'	2	2
Omaha Bay	N6	36°35'	174°76'	1	1
Tauranga Harbour	N8	37°40'	176°10'	7	7
Foxton	CS2	40°18'	175°15'	8	7
Nelson	CS5	41°17'	173°14'	7	3
Collingwood	CS6	40°41'	172°40'	1	1
Lake Waikare	L1	37°26	175°13'	1	1
Whanganui Inlet	SW1	40°34'	172°38'	1	1
Little Wanganui	SW2	41°23'	172°03'	1	1
Christchurch	SE1	43°32'	172°43'	1	1
Shag River	SE2	45°29'	170°47'	1	1
Karitane	SE3	45°38'	170°38'	2	2
Tomahawk Lagoon	SE4	45°51'	170°32'	3	2
Brighton River	SE5	45°57'	170°20'	1	1
Lake Waihola	SE6	46°01'	170°05'	1	1
Waikawa Harbour	SE7	46°38'	169°07'	2	2
Fortrose	SE9	46°34'	168°47'	2	2

Paracorophium lucasi		lat (S)	long (E)	n seq	n haplo
Houhora Harbour	N1	34°48'	173°06'	2	1
Awanui River	N3	35°01'	173°17'	4	2
Taumarere	N4	35°20'	174°06'	3	2
Tauranga Harbour	N8	37°40'	176°10'	19	9
Rawene	NW1	35°26'	173°31'	3	2
Raglan Harbour	NW3	37°48'	174°57'	2	1
Lake Waikare	L1	37°26	175°13'	2	1
Lake Rotoiti	L3	38°01'	176°21'	2	1
Foxton	CS2	40°18'	175°15'	2	2
Wairau River	CS4	41°29'	174°02'	1	1
Collingwood	CS6	40°41'	172°40'	1	1
Gisborne	NE1	38°34'	177°56'	2	2
Napier	NE2	39°30'	176°48'	5	2
Porangahau	NE3	40°38'	176°22'	3	2

Paracorophium brisbanensis		lat (S)	long (E)		
Tauranga Harbour	N8	37°40'	176°10'	2	2

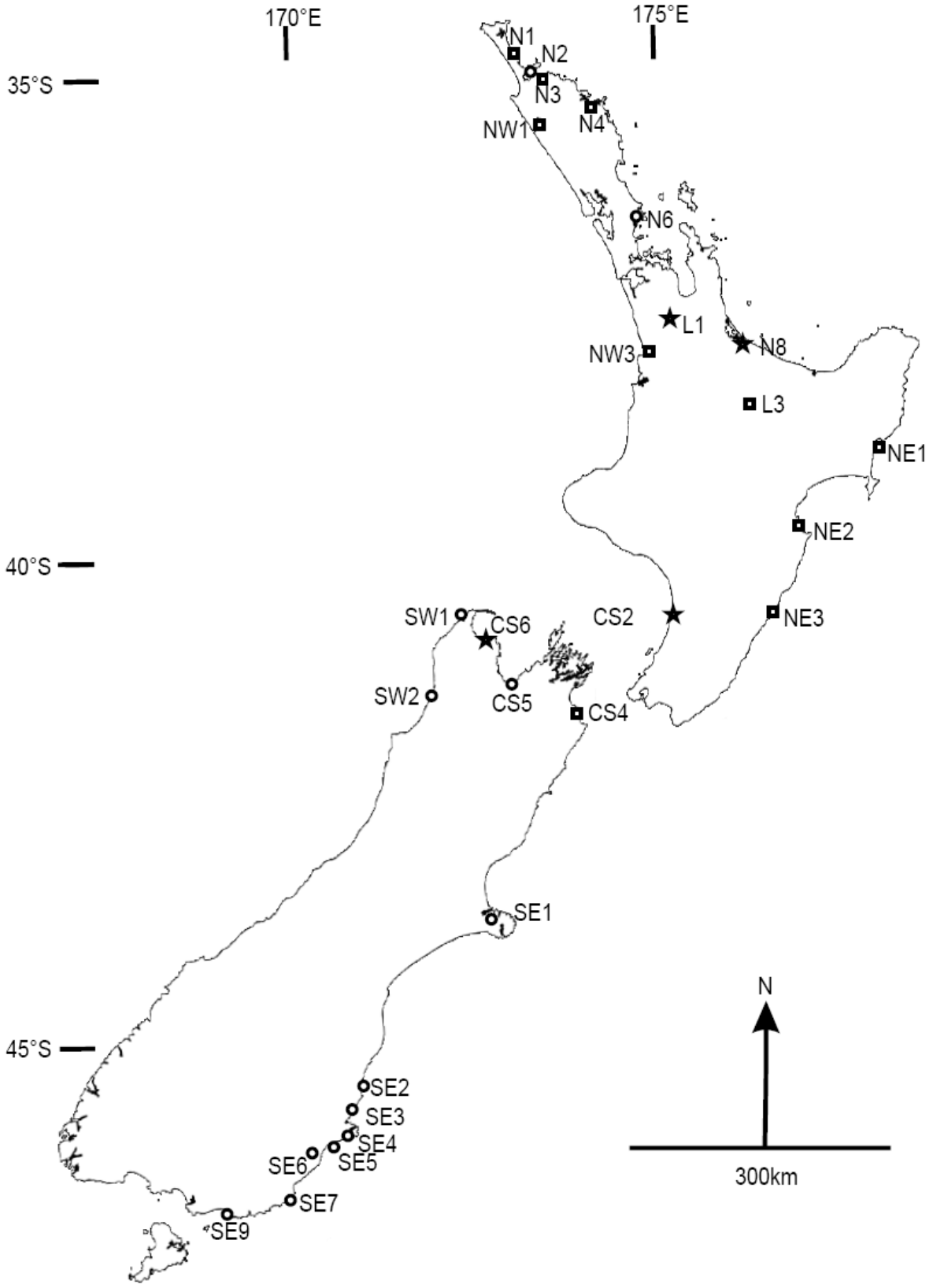


FIGURE 4.1. Sampling locations of *Paracorophium excavatum* (circles) and *Paracorophium lucasi* (squares) in New Zealand. Stars show sympatric occurrences. *Paracorophium brisbanensis* was found at N8.

RESULTS

A total of 73 different haplotypes (27 *P. lucasi*, 36 *P. excavatum*, and two *P. brisbanensis*) were detected with pairwise sequence differences in the range 0 – 15% in a trimmed 530-bp fragment of COI. Of the 530 bp, 163 were parsimony informative and no insertion or deletion was detected. The nucleotide composition across all sequences was: A=26.7%, T=38.5%, C=15.7%, and G=19.1%. The assumption of homogeneity of base frequencies was supported when considering all codon positions, as well as parsimony informative and third codon sites.

Each of the three recognized species (*P. brisbanensis*, *P. excavatum*, and *P. lucasi*) could be clearly delineated on the basis of the COI data and all species-level divisions were well supported by bootstrap replicates (Fig. 4.2). Uncorrected distances between individuals and groups were used because the difference between this and GTR corrected data was slight. The two New Zealand endemic species were the most closely related with 12.8% sequence divergence, whereas the non-indigenous *P. brisbanensis* differed from *P. excavatum* and *P. lucasi* by 16.1% and 14.3%, respectively.

COI sequences revealed considerable genetic substructure within both New Zealand endemic *Paracorophium* species. For *P. excavatum* populations, ML analysis revealed a highly structured pattern of genetic subdivision, split into four major clades (PE1 – 4), each with high bootstrap support (Fig. 4.2). The three most widely distributed clades (PE2, 3 and 4), occupy separate coastlines and discrete latitudinal ranges. PE2 is confined to northern east coast locations, PE3 to western Cook Strait, and PE4 to the southern east coast of the South Island. A fourth clade (PE1) was found only at Nelson and was grouped most closely with PE2, rather than the more geographically proximate PE3 populations (Fig. 4.2). Similar

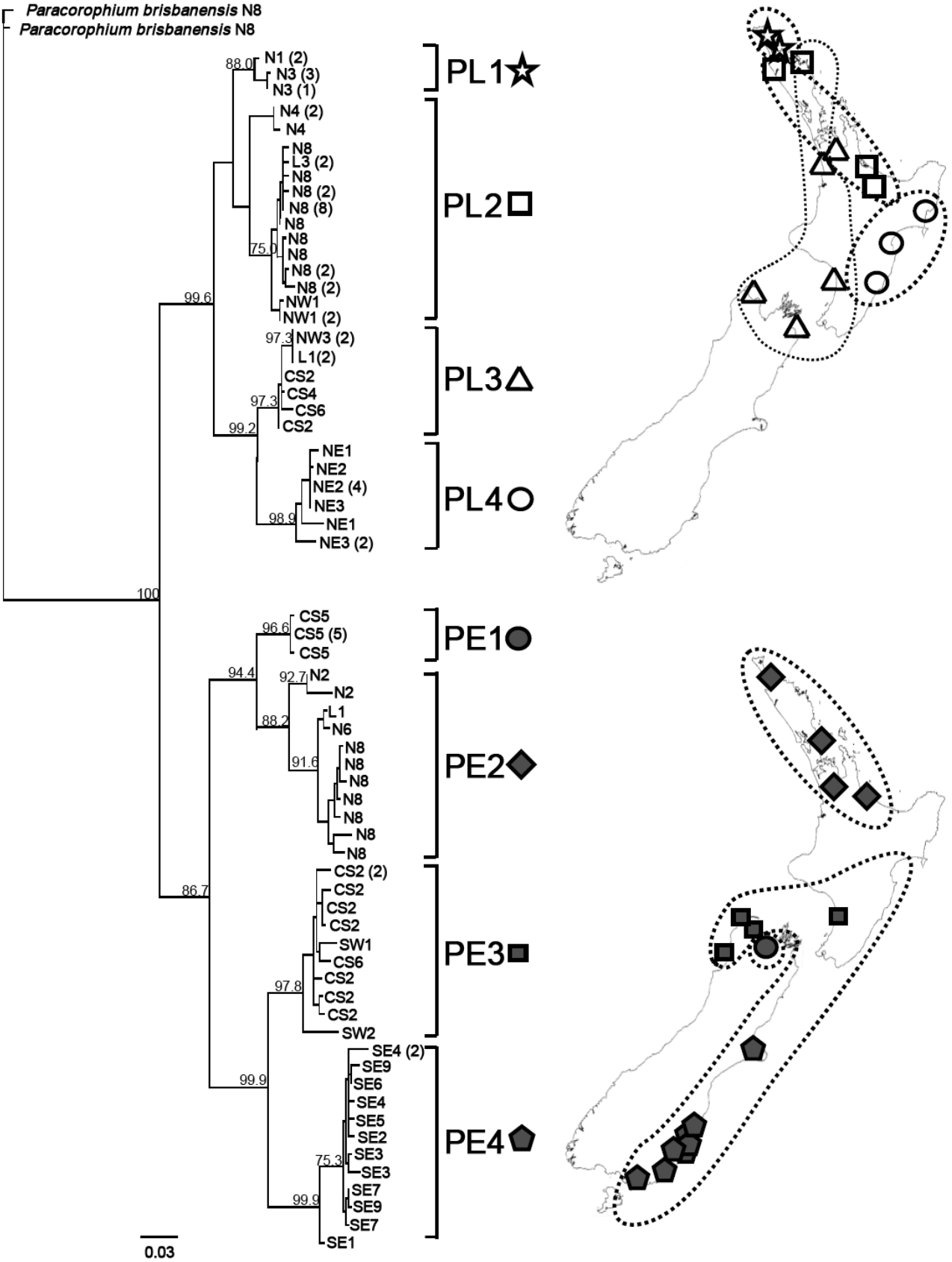
relationships were evident in the percentage divergence cluster diagram (Fig. 4.3). The combined PE1/2 and PE3/4 clades were clearly the most divergent populations of either species at 11.7% (Fig. 4.3). Because divergence levels between *P. excavatum* and *P. lucasi* were only marginally higher than this (12.8%), the combined PE1/2 and PE3/4 lineages are tentatively labelled *P. excavatum* A and B, respectively. Within *P. excavatum* A, groupings PE1 and 2 corresponded to divergences of 6.5% and, in *P. excavatum* B, PE3 and 4 groupings diverged by 7.7%. Further genetic substructure was identified within *P. excavatum* clades (PE1 – 4), most notably the divergent northern population at N2 within PE2, which was separated from the other PE2 populations by 4.9% sequence divergence.

For *P. lucasi* populations, ML analysis also identified four clades (PL1 – 4) with distinct geographic limits, each receiving high bootstrap support (Fig. 4.2). PL1 consisted of two northernmost populations: N1 and N3. PL2 contained the remaining northern east coast populations, as well as a northern west coast population at NW1. PL3 was represented by the remaining western populations, as well as those in the vicinity of Cook Strait, whereas PL4 contained only east coast populations, south of East Cape (Fig. 4.2). Divergences between clades ranged from 4.6% (PL1 and 2) to 8.3% (PL1 and 4) (Fig. 4.3). As was the case for *P. excavatum*, northern populations of *P. lucasi* were highly divergent, with pairwise differences of up to 4.6%.

Haplotype networks were generated separately for both *P. excavatum* and *P. lucasi* (Fig. 4.4A, B). The NCA resulted in two six-step and four five-step clades in both species. In *P. excavatum*, structure at the five step level mostly agreed with groupings identified with ML analyses. The only exception was the placement of N2 population with CS5 (PE1), rather than with populations from PE2. Similarly, in *P. lucasi*, NCA was in agreement with ML

analyses, with the only difference being the suggested placement of the N4 population within PL1 rather than PL2.

On the basis of the five-step groupings found with NCA (Fig. 4.4A, B), we conducted AMOVA tests for each species. This demonstrated that 76.3% (*P. excavatum*) and 72.8% (*P. lucasi*) of the variation is observed among the five-step groups identified by NCA, which are confined to specific areas. The remaining variation is mainly explained by the genetic diversity among populations within groups (13.8% and 18.6%), whereas the genetic variation within populations' accounts for only 9.9% and 8.6% of the total variation in *P. excavatum* and *P. lucasi* respectively.


Paracorophium brisbanensis N8
Paracorophium brisbanensis N8
N1 (2)
N3 (3)
N3 (1)
N4 (2)
N4
N8
L3 (2)
N8
N8 (2)
N8 (8)
N8
N8
N8
N8 (2)
N8 (2)
NW1
NW1 (2)
NW3 (2)
L1(2)
CS2
CS4
CS6
CS2
NE1
NE2
NE2 (4)
NE3
NE1
NE3 (2)
CS5
CS5 (5)
CS5
N2
N2
L1
N6
N8
N8
N8
N8
N8
N8
N8
CS2 (2)
CS2
CS2
CS2
SW1
CS6
CS2
CS2
CS2
SW2
SE4 (2)
SE9
SE6
SE4
SE5
SE2
SE3
SE3
SE7
SE9
SE7
SE1
88.0
75.0
99.6
97.3
97.3
99.2
98.9
100
96.6
94.4
92.7
88.2
91.6
86.7
97.8
99.9
75.3
99.9
0.03
PL1
PL2
PL3
PL4
PE1
PE2
PE3
PE4

Previous page: FIGURE 4.2. Maximum likelihood phylogram showing relationships among *Paracorophium lucasi* (clusters PL1–4) and *Paracorophium excavatum* (clusters PE1–4) cytochrome *c* oxidase subunit I (COI) haplotypes using a generalized time reversible model of sequence evolution selected by jMODELTEST. The haplotype number is one unless specified in parenthesis. Nodal support is indicated by bootstrap values (> 75) estimated from 1000 replications. The tree was rooted with *P. brisbanensis* sequences from Tauranga Harbour. COI sequence groupings for each species are indicated on the adjacent maps. Dotted lines represent groupings previously found with allozyme markers (Stevens and Hogg, 2004).

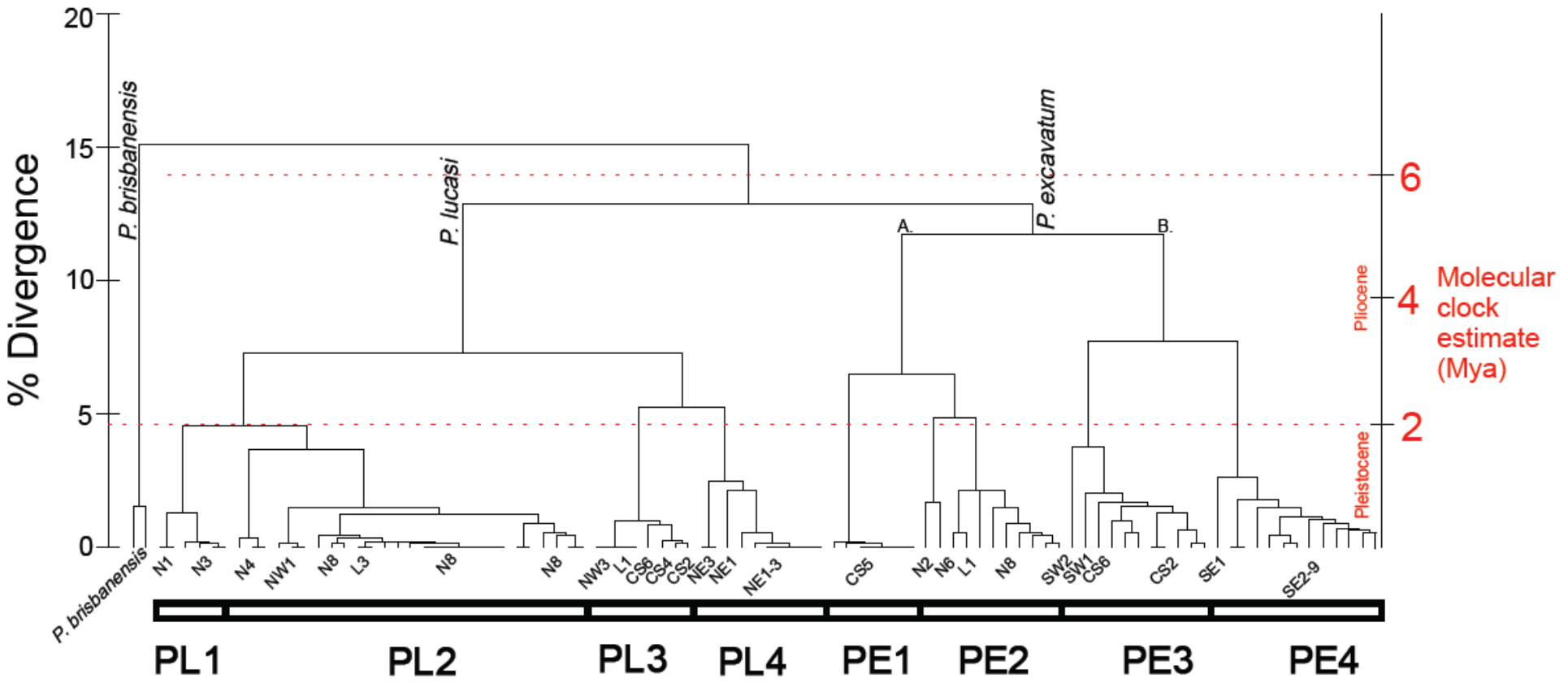
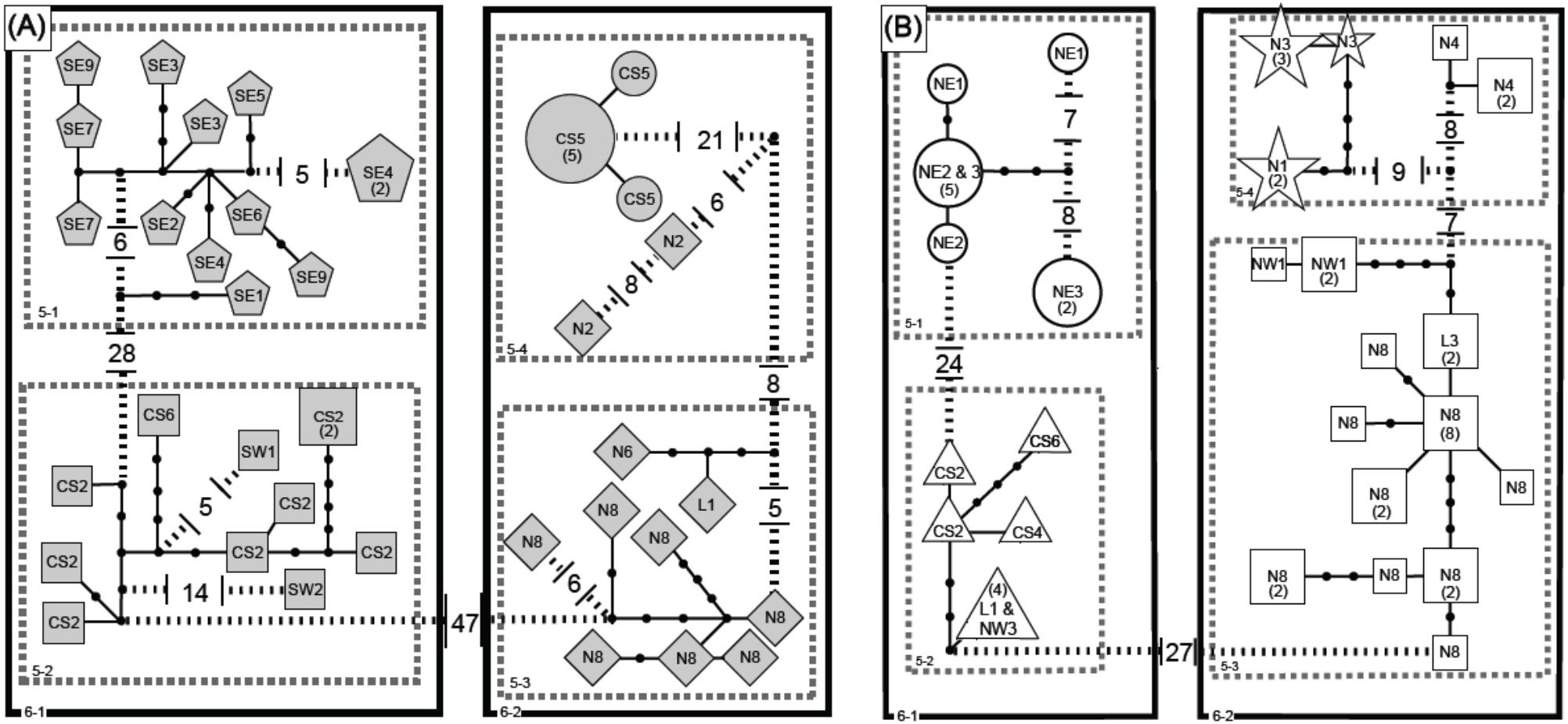


FIGURE 4.3. Percentage difference of *Paracorophium* cytochrome *c* oxidase subunit I sequences based on uncorrected pairwise sequence differences. Population substructure (PL1 – 4 and PE1 – 4) from a maximum likelihood phylogram correspond to groupings with > 5% divergence. On the basis of the relatively high sequence divergence approaching that between *Paracorophium excavatum* and *Paracorophium lucasi*, *Paracorophium excavatum* is tentatively split into two lineages: A and B. Molecular clock estimates shown on the right *y*-axis are based on 2.3% sequence divergence per million years.


(A)
SE9
SE3
SE5
SE7
SE3
5
SE4 (2)
SE7
SE2
SE6
6
SE4
SE9
SE1
5-1
28
CS6
CS2 (2)
SW1
CS2
5
CS2
CS2
CS2
CS2
CS2
14
SW2
CS2
5-2
6-1
47
CS5
CS5 (5)
21
CS5
6
N2
8
N2
5-4
8
N6
N8
L1
5
N8
N8
6
N8
N8
N8
N8
N8
5-3
6-2
(B)
NE1
NE1
7
NE2 & 3 (5)
8
NE2
NE3 (2)
5-1
24
CS6
CS2
CS2
CS4
(4) L1 & NW3
5-2
6-1
27
N3 (3)
N3
N4
N4 (2)
8
N1 (2)
9
5-4
7
NW1
NW1 (2)
L3 (2)
N8
N8
N8 (8)
N8
N8 (2)
N8
N8 (2)
N8
N8 (2)
N8
5-3
6-2

Previous page: FIGURE 4.4. Nested clade design for (A) the 36 unique mitochondrial (mt)DNA [cytochrome *c* oxidase subunit I (COI)] haplotypes from 42 individuals of *Paracorophium excavatum* and (B) the 27 unique mtDNA (COI) haplotypes from 51 individuals of *Paracorophium lucasi.* Groupings (PE1 – 4 and PL1 – 4) identified by previous maximum likelihood analyses are also shown using the same symbols as in Fig. 4.2. The number of individuals within each haplotype is identified in parentheses when *N* > 1. Missing haplotypes/mutational steps are indicated by a '●'. In cases where the number of missing haplotypes exceeds 5, the proposed link between distinct groups is indicated by a '- - -| # |- - -', where '#' is the number of mutational steps between these groups. The clades are identified using a two number system, where the first number refers to the nesting hierarchy and the second is an arbitrary, individual clade identifier. Thick-lined rectangles enclose six-step clades; broken lined filled rectangles enclose five-step clades.

DISCUSSION

We observed deeply divergent lineages within each nominate species of New Zealand *Paracorophium*, with levels of genetic divergence among populations approaching those commonly seen between species. For example, of the 1781 crustacean species analysed by Hebert et al. (2003), the mean level of COI divergence was 15.4% (SD 6.6%) and 18% of species were found to be differentiated by < 8%. Although genetic structure is commonly observed among populations of New Zealand coastal taxa (see Ross et al., 2009), the divergences we observed between *Paracorophium* populations are in the upper range of those previously reported in estuarine amphipods (Kelly et al., 2006; Rock et al., 2007). Furthermore, because we detected COI sequence divergences of only 12.8% between *P. excavatum* and *P. lucasi*, and up to 11.7% among *Paracorophium* populations, our results suggest the strong possibility of cryptic species complexes within both *P. excavatum* and *P. lucasi.* On the basis of a previous allozyme analysis of *Paracorophium*, Stevens and Hogg (2004) suggested that *P. excavatum* was likely to consist of a complex of at least four morphologically cryptic species and that *P. lucasi* consisted of at least three species. Collectively, this provides strong evidence for multiple evolutionary units with both *Paracorophium* species. However, further analyses based on morphological, behavioural or physiological features would need to be undertaken before any definitive conclusions on specific status could be made.

Each of our analyses (ML, NCA, and AMOVA) revealed a similar phylogeographic pattern of deeply divergent, geographically sorted, lineages. Groupings within both species corresponded with shared coastlines and topographical features, such as Cook Strait and East Cape. In both species, populations diverged by 7.7% (PE3/4) and 5.3% (PL3/4) were

identified on east and west coasts in the Cook Strait region. This break was previously detected using allozyme data for *P. lucasi* but not for *P. excavatum* (Stevens and Hogg, 2004). For *P. lucasi*, East Cape appeared to mark the divergence between PL2/4 groups, and this break was also detected using allozyme data. However, using COI data, *P. lucasi* L1 populations were grouped more closely with PL3. This contrasted with the allozyme data (Stevens and Hogg, 2004), where L1 was most closely related to populations at N8, NW1, and L3. Such minor differences in phylogeographic patterns for allozyme and COI analyses are not uncommon (Muller, 2000; Hurwood et al., 2003), and may be attributable to differential rates of evolution and/or marker-specific selection criteria (e.g. sex biased dispersal; Stevens et al., 2006).

Significant genetic structure was detected within both species' northernmost population groupings. COI samples from within the Northland region showed a relatively high degree of sequence divergence (> 4%). The observed genetic patterns in northern populations of *Paracorophium* are consistent with previous research that has demonstrated Northland to be a hotspot of genetic diversity in terrestrial and freshwater taxa (Morgan-Richards, 1997; Gleeson et al, 1999; Armstrong and De Lange, 2005; Chapple et al., 2008). During the Pliocene, the northern tip of New Zealand formed an archipelago of islands (Fig. 4.5), which may have been sufficient to isolate ancestral populations of Northland *Paracorophium*. In general, other marine species do not appear to exhibit significant genetic substructure in the Northland region (Sponer and Roy, 2002; Goldstien et al., 2006). The propensity of *Paracorophium* to form genetically distinct subpopulations may reflect its limited dispersal abilities and the spatially discrete nature of estuarine habitats.

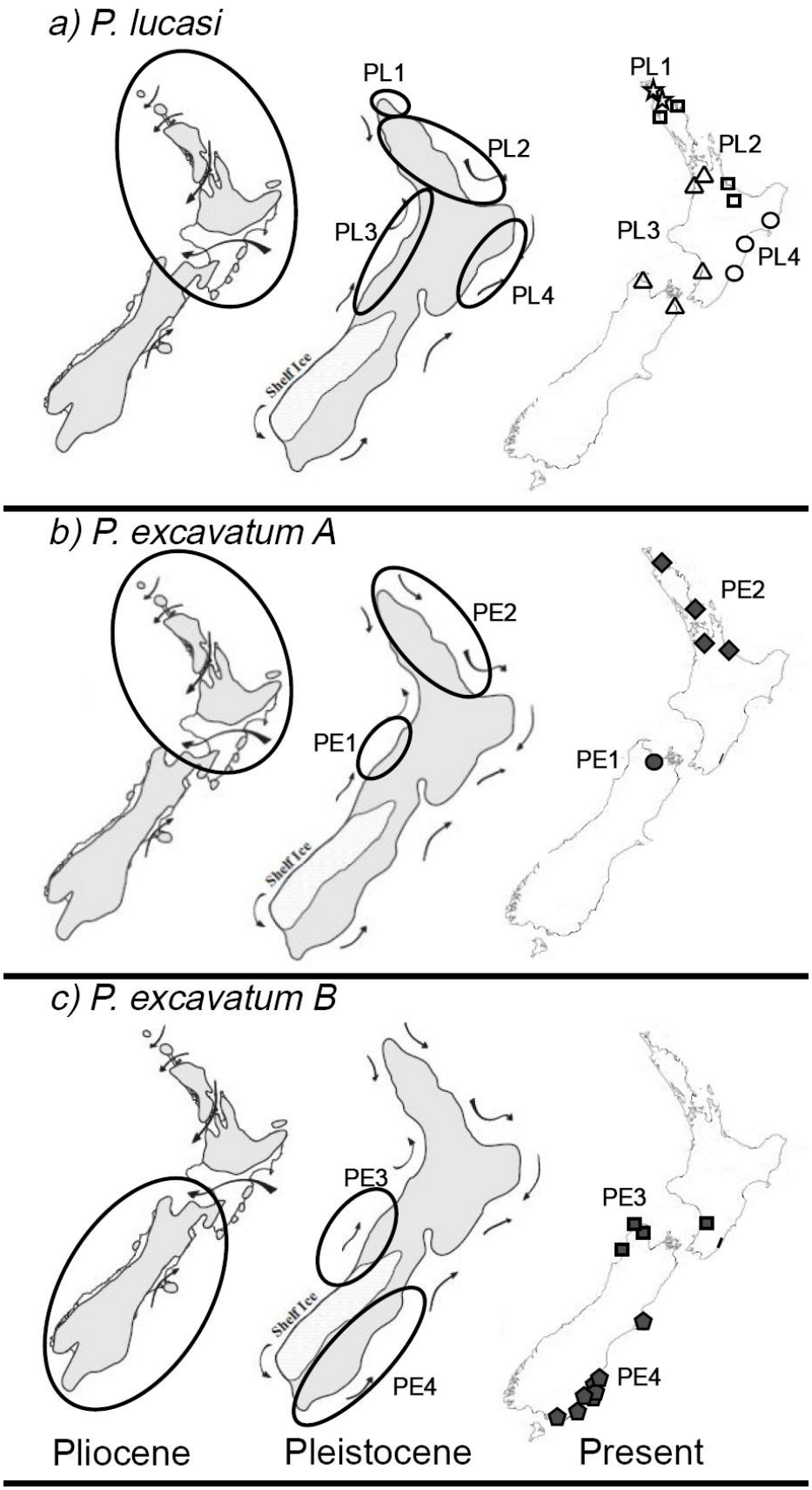
Applying a conservative molecular clock rate of 1.4 – 2.3% nucleotide sequence divergence per Myr to interspecific COI divergences (Knowlton and Weigt, 1998; Schubart et al., 1998), it appears that *P. lucasi* and *P. excavatum* last shared a common ancestor 5.6 – 9.2 Mya. Within *P. excavatum*, the divergent lineages A and B appear to have been separated for almost as long as *P. excavatum* and *P. lucasi* (5.1 – 8.4 Mya). COI sequence divergence places the isolation of *P. lucasi* and *P. excavatum* A and B during the upper Miocene period (12 – 6 Mya). At this time, the landmass of New Zealand formed a single island lacking internal sea ways, which may have isolated east and west coast populations of ancestral *Paracorophium*, allowing the speciation of *P. excavatum* and *P. lucasi*, as well as *P. excavatum* A and B.

Several genetically distinct clades appear to have resulted from the splitting of *Paracorophium* lineages into eastern and western populations (i.e. PE1 and 2, PE3 and 4, and PL2 and 3). On the basis of percentage sequence variation, all lineages appear to have diverged at approximately the same time. COI sequence divergence places the splitting of these groupings somewhere in the range 2.3 – 5.6 Mya. This range drops to 2.3 and 3.4 Mya when using the 2.3% per million years calibration and implies frequent gene flow during the Pliocene (6 – 2 Mya), followed by isolation of eastern and western populations during the Late Pliocene/Pleistocene (approximately 2 – 3 Mya).

These findings are consistent with allopatric evolution as a result of rising sea levels and uplift of the North Island during the early Pleistocene (approximately 2 Mya). Landmass changes during the Pliocene period resulted in New Zealand forming an archipelago-like chain of islands (Fig. 4.5), resulting in improved dispersal potential between the east and west coasts. However, during the Pleistocene, New Zealand was once again a single island,

which may have led to east–west splitting of *P. lucasi* and *P. excavatum* lineages A and B (Fig. 4.5). At this time, the East Cape appears to have presented and maintained a barrier to mixing of northern and southern east coast *P. lucasi* (PL2 and PL4) populations (Fig. 4.5A). Similarly, east coast and central west coast *P. lucasi* populations (PL2 and 3) may have diverged from one another because uplift prevented exchange between eastern and western coastlines (Fig. 4.5A). The *P. excavatum* population at CS5 (PE1) may be the only surviving west coast population of a similarly split northern *P. excavatum* lineage, which would explain its relatedness to northern *P. excavatum* (PE2), rather than more proximate populations comprising PE3 (Fig. 4.5B). COI divergences between PE3 and PE4 populations suggests that southern *P. excavatum* (B) diverged with the closure of Cook Strait (Fig. 4.5C).

The levels of genetic divergences we found (up to 11.7%) suggest multiple lineages within both currently recognised New Zealand *Paracorophium* species. On the basis of these divergences, populations formed east and west coast groupings consistent with cessation of gene flow following uplift and a drop in sea level during the early Pleistocene (approximately 2 Mya). The overall genetic structure in *Paracorophium* species appears to have resulted from periods of vicariance dating back to the Upper Miocene and continuing through the Pliocene and early Pleistocene.


a) P. lucasi
PL1
PL2
PL3
PL4
Shelf Ice
PL1
PL2
PL3
PL4
b) P. excavatum A
PE2
PE1
Shelf Ice
PE2
PE1
c) P. excavatum B
PE3
PE4
Shelf Ice
PE3
PE4
Pliocene
Pleistocene
Present

Previous page: FIGURE 4.5. The changing landmass of New Zealand and its proposed effect on gene flow in three *Paracorophium* lineages; A) *P. lucasi*, B) *P. excavatum* A, C) *P. excavatum* B. On the basis of present-day cytochrome *c* oxidase subunit I (COI) phylogeographic patterns, theorized species distributions during the Pliocene and Pleistocene are represented by ovals. The COI population groupings of the present study are represented in the present-day maps. Arrows represent prevailing ocean circulation. The Pliocene (6 – 2 Mya) landmass provided few barriers for aquatic dispersal between the east and west coasts, in contrast to the Pleistocene. Adapted from Fleming (1979) and Stevens et al. (1995).

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