

# Mitochondrial DNA (COI) analyses reveal that amphipod diversity is associated with environmental heterogeneity in deep-sea habitats

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## Abstract

The relationship between species diversity and environmental parameters is poorly understood for the mobile macrofauna of deep-sea habitats due to under-sampling and subsequent lack of accurate taxonomic information. To redress this, cytochrome oxidase *c* subunit I (COI) DNA sequences were used to estimate species diversity and to compare phoxocephalid amphipod assemblages among 20 stations encompassing a range of environmental conditions. Two regions, east (Chatham Rise) and west (Challenger Plateau) of New Zealand were sampled to depths of 200–1200 m 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). Novel phoxocephalid MOTUs were found at most stations, especially on Challenger Plateau and the flanks of Chatham Rise. Analyses of interstation assemblages revealed a major split between regions, indicating minimal overlap in taxon distributions. A cluster of highly similar stations was identified, broadly distributed over the crest of Chatham Rise, in association with elevated food availability, probably resulting from higher surface productivity and relatively shallow depth. Accordingly, multivariate analysis revealed a strong correlation between phoxocephalid assemblages and food supply. This study highlights the value of molecular approaches, in particular COI sequences, for quantifying and comparing diversity in under-sampled and/or under-studied taxa.

*Keywords:* biodiversity, continental shelf, DNA barcoding, New Zealand, Phoxocephalidae

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## 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 & Maciolek 1992), the vast majority of which await discovery and/or formal descriptions

(Lörz & Brandt 2003; Brandt & Berge 2007), 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 utilize a molecular-based approach to assess diversity and community composition of phoxocephalid amphipods from a series of stations along the

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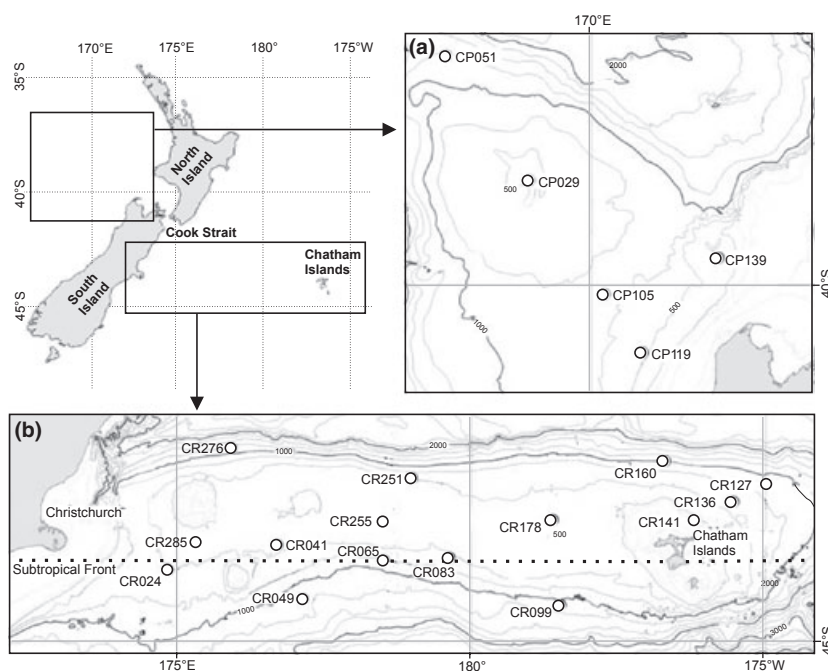
deep-sea continental margins of New Zealand and contrast diversity within and among stations with environmental variability.

Many factors may contribute to the distribution of biota in deep-sea ecosystems (Snelgrove & Butman 1994; Ellingsen 2002). Spatially variable inputs of organic matter attract specialized 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 & Brandt 2010). Bathymetric variation in the form of geomorphic features, such as submarine canyons, gullies, terraces and hills create habitat heterogeneity influential in structuring community composition (Levin & 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, with possible migratory connections for inhabitants via Cook Strait (Fig. 1). Both regions present a contrasting range of environmental parameters such as surface productivity, benthic organic content and sediment grain sizes (Table 1). Chatham Rise is a broad submarine ridge stretching eastwards

for over 800 km from east of New Zealand's South Island to the Chatham Islands and beyond. The rise is generally flat-topped at 300–400 m depth and descends into deeper waters (>2500 m) to the north and south (Fig. 1). Chatham Rise is characterized by high productivity in surface waters, associated with the Subtropical Front (STF), which marks the mixing of subtropical and subantarctic surface waters and appears to be bathymetrically locked to the southern flank of the rise near 44°S (Uddstrom & 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 (Fig. 1) and lower pelagic productivity relative to Chatham Rise.

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



**Fig. 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.

**Table 1** Epibenthic sled trawl distance, depth range and environmental variables of study stations on the Chatham Rise and Challenger Plateau

| Station                             | Trawl dist (m) | Depth range (m) | Surface chl <i>a</i> (mg/m <sup>3</sup> ) | Total organic matter (%) | CaCO <sub>3</sub> (%) | Clay content (% <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                   |

Average autumn surface chlorophyll *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).

(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 has added to the accuracy with which taxa can be identified and reliably compared with one another both within and among 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 morphological differences. Moreover, DNA sequences have also revealed previously unrecognized, 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 that can also benefit future species descriptions (Lörz *et al.* 2011, 2012).

The ability to delineate species in undescribed and/or taxonomically challenging taxa hinges on the degree of intraspecific vs. interspecific COI sequence variation and

the distinctness of this so-called 'barcoding gap' (Meyer & 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 morphologically similar 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 similar southern hemisphere deep-sea habitats (Williams *et al.* 2010), we anticipated finding high diversity. We then tested the hypothesis that the community composition of deep-sea macrofauna is correlated with increased food supply associated with higher surface productivity.

## Methods

### Sampling methods

Sampling of Chatham Rise was undertaken on a voyage 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) voyages TAN0705 and TAN0707, respectively]. In total, 20 stations were sampled (15 from Chatham Rise and five from Challenger Plateau) spanning a similar depth range (200–1200 m) at both locations (Table 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- $\mu$ m 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- $\mu$ m 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

To minimize 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 interstation abundance analyses. To account for variable trawl distances (see Table 1), numbers of individuals within supranet samples are presented as densities per 1000 m<sup>2</sup>. To maximize 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 & Springthorpe (2001). Individuals were then sorted into putative morphospecies based on morphological similarity for subsequent molecular work. We obtained COI sequences from representatives of all morphospecies 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 morphospecies' representatives present at

each station (see Appendix). 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 20 stations for DNA sequencing (Table 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 Fibre 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'-TAAACTTCAGGGTGACCAAAAAA TCA-3') (Folmer *et al.* 1994) was used to amplify a 658 bp fragment of the COI gene. Samples which did not amplify successfully were rerun 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 0.1  $\mu$ L EXO I enzyme (10 U/ $\mu$ L), 0.2  $\mu$ L shrimp alkaline phosphate (1 U/ $\mu$ L) and 2.7  $\mu$ L sterile H<sub>2</sub>O 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  $\mu$ L 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® (see data accessibility section for Accession numbers).

The 360 target specimens yielded 297 usable COI sequences, sequenced in both directions, for an overall success rate of 82%. The specimens that 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

| Station | Supranet abundance<br>(ind./1000m <sup>2</sup> ) | Combined nets<br>raw abundance | Number of COI<br>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  |

**Table 2** Abundance and diversity of phoxocephalid amphipods on Chatham Rise and Challenger Plateau

Singleton molecular operational taxonomic units (MOTUs) are those which occur at a single station only.

COI, cytochrome oxidase *c* subunit I.

(Tamura *et al.* 2011), where they were checked for stop codons (none was detected, indicating that only mitochondrial DNA was amplified). Sequence length averaged 623 base pairs (bp) and ranged from 351 to 658 bp.

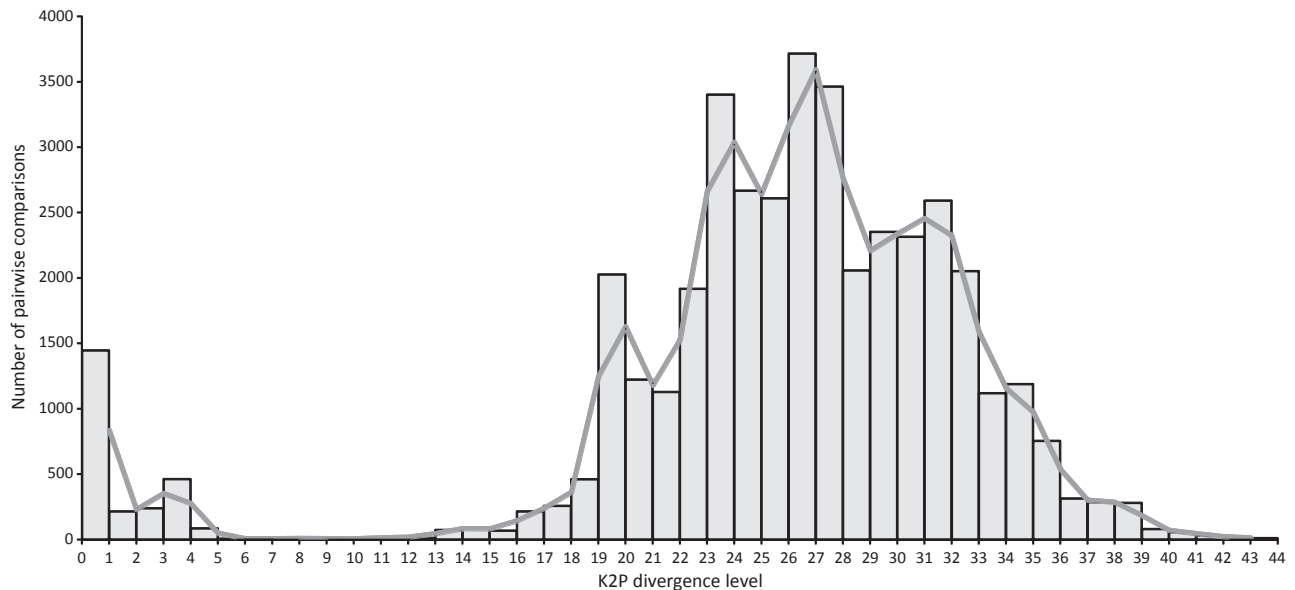
### Statistical analyses

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). 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. 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

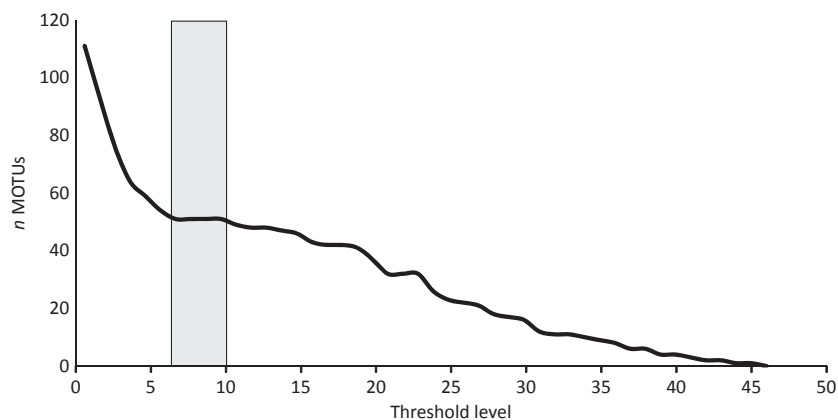
interspecific sequence variability. On the basis of this analysis, we employed a 6% threshold for MOTU discrimination.

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 multidimensional scaling (MDS) plots of interstation assemblage similarity in PRIMER 6. This was compared with environmental parameters (depth, average autumn surface chlorophyll *a*, total organic matter (TOM), CaCO<sub>3</sub>, clay and mud content) (Table 1) from 15 stations taken concurrently with our research cruises. The collection and analysis of environmental data are fully described by 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<sup>10</sup> transformation. Correlations between community structure (presence/absence) and environmental parameters were examined using the BIOENV procedure in PRIMER 6 (Clarke & 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).





**Fig. 2** Pairwise K2P divergence levels of 297 phoxocephalid cytochrome oxidase *c* subunit I sequences. Bars represent actual counts between intervals, line is the moving average ( $n = 2$ ).



**Fig. 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*

Interstation phoxocephalid abundances from the supranets ranged from 1 to 780 individuals per 1000 m<sup>2</sup>, revealing great variability among stations (Table 2). Despite differences in abundance, the number of MOTUs was relatively consistent among stations, ranging from two to ten (Table 2). Using a 6% threshold, a total of 49 phoxocephalid MOTUs were identified. Most of these agreed with initial morphospecies designations. In some cases, however, DNA data revealed deep divergences among morphologically conserved MOTUs, initially assigned to the same morphospecies. 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 supported 21 MOTUs. Only one taxon was found in both regions. Most MOTUs (28/49) occurred at a single station only, especially on Challenger Plateau, where such singleton taxa made up >50% of all MOTUs present at each station (Table 2). The most distal station on Challenger Plateau supported 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). Shallow stations on Chatham Rise, especially those with the highest concentration of surface chlorophyll *a* (Table 1), tended to have more MOTUs than deeper locations (Table 2). Accumulation curves (Fig. 4) show a steady rise with increasing sampling over all 20 stations. Taken alone, regions appeared to accumulate

MOTUs at different rates (Fig. 4). Chatham Rise ( $n = 15$  stations) accumulated MOTUs less rapidly than 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 Chatham Rise and Challenger Plateau (Fig. 5). Within regions, 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. 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 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

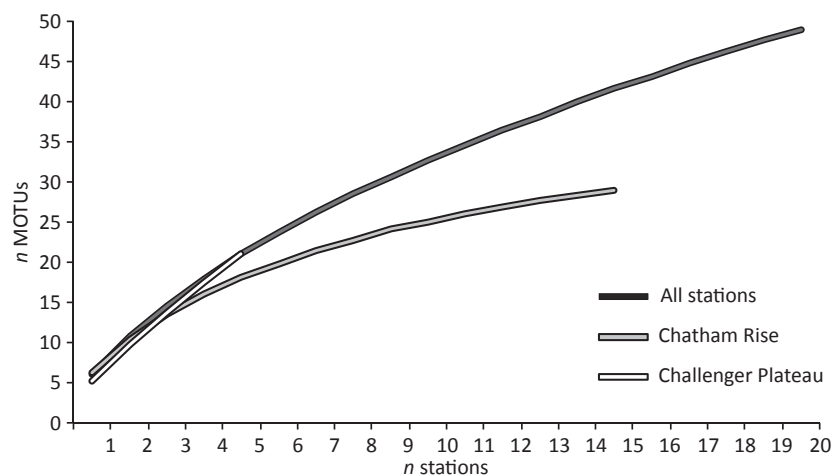
(see Appendix), contributed most to average station similarity on Chatham Rise. On Challenger Plateau, SP30 and 31 (also widely distributed) contributed most to average assemblage similarity.

### Discussion

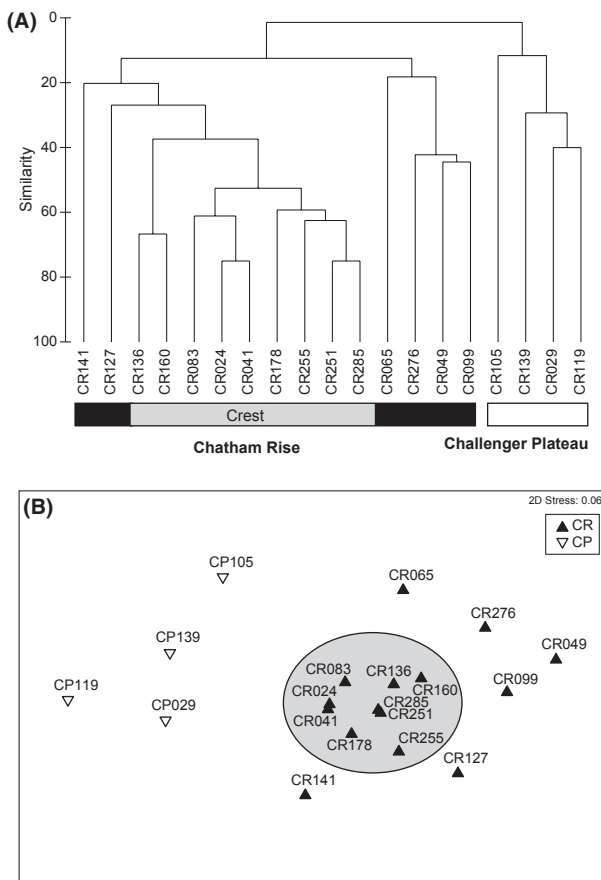
#### *Phoxocephalid diversity*

We identified 49 MOTUs from 4100 individuals sampled from 20 stations on the continental margins of New Zealand. While our MOTUs require detailed taxonomic analysis before gaining formal species recognition, our results suggest that the presence of a diverse phoxocephalid fauna on New Zealand's continental margins. In contrast, similar regional studies from continental margins elsewhere identified relatively few phoxocephalid species ( $n = 7$ , Brandt 1997;  $n = 1$ , Lörz & Brandt 2003). 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 & 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



**Fig. 4** Molecular operational unit (MOTU) accumulation curves based on observed MOTUs from combined data (all stations) and individual regions (Chatham Rise and Challenger Plateau).



**Fig. 5** (A) Dendrogram based on presence/absence station data of phoxocephalid molecular operational taxonomic units. Bars at the bottom indicate groupings based on Bray–Curtis similarity. (B) Multidimensional scaling representation of phoxocephalid composition data. Shape colours and shaded area correspond with groupings identified in A.

environments (Stevens & 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 migration 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 the physiological constraints imposed by changing environmental conditions associated with

latitude 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. 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 (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 approximately 1000 m<sup>2</sup>, which exceeds the spatial coverage of other sampling gear capable of retaining small macrofauna, such as box- or multicores. 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 local- and temporal-scale variability among tows.

The fact that most phoxocephalid MOTUs in our study could not be linked to any described species emphasizes 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 recognizable morphospecies and also for identifying cryptic taxa which would otherwise have been overlooked. SIMPER analyses also identified several common taxa. Given the limited knowledge of New Zealand phoxocephalids, these common taxa would be an appropriate focus for future morphological or taxonomic study (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 characterized by highly dissimilar stations. Stations on Chatham Rise and 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 Challenger Plateau were more like those of Chatham Rise than samples taken from the deeper waters of Challenger Plateau (Probert & Grove 1998). The greater differentiation detected in our study likely reflects our higher taxonomic resolution.

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. 5). Previous work on Chatham Rise macroinvertebrates has shown increasing community divergence with depth separation (Probert *et al.* 2009; Knox *et al.* 2012). The crest of Chatham Rise is characterized by persistent strong easterly currents with near-bed speeds of 20–35 cm/s (Heath 1983; Chiswell 1994). Connectivity among populations on the crest may be higher than those on the slope because current speed on Chatham Rise declines with increasing depth (Nodder & Northcote 2001). Closely related crest communities may also reflect relatively stable environmental conditions along the crest of Chatham Rise, where food supply is elevated by increased phytodetritus deposition from the STF (Nodder *et al.* 2003, 2007). 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 large populations to develop. Highly specialized species with large ranges and low populations are likely to characterize such environments due to niche partitioning (Sanders 1968; Snelgrove *et al.* 1992). 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 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, the construction of DNA barcode reference libraries such as that developed here, linked with appropriately archived voucher specimens, will be essential.

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All authors are interested in the use of molecular data for enhancing our understanding of ecological and evolutionary processes. Using a range of genetic markers, they seek to identify patterns of biodiversity and biogeographic variation in relation to environmental factors in both aquatic and terrestrial ecosystems.

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### Data accessibility

DNA sequences: GenBank® Accessions; GU689627, GU689628, GU689632, GU689633, GU689635–GU689637, GU689641, GU689647, GU689652, GU689653, GU689668, GU689670, GU689691, GU689693–GU689695, GU689722, GU689727, GU689741–GU689743, GU689745–GU689754, GU689757, GU689758, HM373019, HM373030, HM373038, HM434047, HM434048, HM880293–HM880307, HM880310–HM880327, HM880329–HM880359, JX216841–JX217034.

BoLD project: Amphipods of New Zealand's continental margins (ANZCM).

Dryad entry (alignment file): doi: 10.5061/dryad.5h6n5.x

Appendix

COI sample counts for all species and stations

| Species | CR024 | CR041 | CR049 | CR065 | CR083 | CR099 | CR127 | CR136 | CR141 | CR160 | CR178 | CR251 | CR255 | CR276 | CR285 | CP029 | CP051 | CP105 | CP119 | CP139 |
|---------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 01      | 4     |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 1     |       |       |       | 4     |
| 02      | 2     | 5     | 1     | 10    | 2     |       | 5     |       | 7     | 2     | 2     | 5     |       | 6     | 5     |       |       |       |       |       |
| 03      |       |       |       | 4     | 4     | 1     | 4     |       | 4     | 2     | 2     | 2     | 4     |       | 1     |       |       |       |       |       |
| 04      | 4     | 1     |       | 1     | 1     | 1     | 2     | 3     |       | 1     | 1     | 1     | 3     |       | 2     |       |       |       |       |       |
| 05      | 10    | 6     |       | 3     | 3     |       |       |       |       | 1     | 1     | 1     | 2     |       | 1     |       |       |       |       |       |
| 06      | 4     | 3     |       |       |       |       |       |       | 3     | 3     | 1     | 1     | 7     |       | 2     |       |       |       |       |       |
| 07      | 8     | 6     |       | 2     | 2     | 1     |       |       |       |       | 4     |       |       | 1     |       |       |       |       |       |       |
| 08      |       |       | 1     |       |       |       |       |       |       |       |       |       | 1     | 2     |       |       |       |       |       |       |
| 09      | 1     | 1     |       |       |       | 2     |       |       | 2     | 4     |       |       |       | 2     | 1     |       |       |       |       |       |
| 10      |       |       | 1     |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
| 11      | 2     |       | 2     | 1     |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
| 12      |       |       |       |       |       |       |       |       |       | 1     | 1     | 1     |       |       | 1     |       |       |       |       |       |
| 13      |       | 1     |       | 4     | 4     | 1     | 1     |       |       |       | 2     |       |       |       |       |       |       |       |       |       |
| 14      |       |       | 1     | 1     |       |       |       |       |       | 2     |       |       |       |       |       |       |       |       |       |       |
| 15      |       |       |       | 1     |       | 4     |       |       |       |       |       |       |       | 1     |       |       |       |       |       |       |
| 16      |       |       |       |       |       |       |       |       |       |       |       |       |       | 1     |       |       |       |       |       |       |
| 17      |       |       | 4     |       |       |       |       |       |       |       |       |       |       | 1     |       |       |       |       |       |       |
| 18      |       |       | 4     |       |       | 3     |       |       |       |       |       | 1     | 3     |       |       |       |       |       |       |       |
| 19      |       |       | 4     |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
| 20      |       |       |       |       |       |       |       |       |       |       |       |       | 2     |       |       |       |       |       |       |       |
| 21      |       |       |       |       |       |       |       |       |       | 1     |       |       |       |       |       |       |       |       |       |       |
| 22      |       |       | 5     |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
| 23      |       |       | 2     |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
| 24      |       |       |       | 1     |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
| 25      |       |       |       |       |       |       |       |       |       | 1     |       |       |       |       |       |       |       |       |       |       |
| 26      | 1     |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
| 27      |       |       | 1     |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
| 28      |       |       |       |       |       |       |       |       | 1     |       |       |       |       |       |       |       |       |       |       |       |
| 29      |       |       |       |       |       | 1     |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
| 30      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 1     |       |       | 2     | 1     |
| 31      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 1     |       |       | 1     |       |
| 32      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 2     |       |       |       |
| 33      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 4     |       |       |       |       |
| 34      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 4     |       |
| 35      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 6     |       |       |       |       |

(continued)

(continued)

| Species | CR024 | CR041 | CR049 | CR065 | CR083 | CR099 | CR127 | CR136 | CR141 | CR160 | CR178 | CR251 | CR255 | CR276 | CR285 | CP029 | CP051 | CP105 | CP119 | CP139 |
|---------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 36      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 2     |       |       |       |
| 37      |       |       |       |       |       |       |       |       |       |       |       | 1     |       |       |       |       |       |       |       |       |
| 38      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 2     |       |
| 39      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 1     |       |       |       |
| 40      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 4     |       |       |       |
| 41      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 1     |
| 42      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 1     |       |       |       |       |
| 43      |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 12    |       |       |       |       |       |
| 44      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
| 45      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 1     |       |       |       |       |
| 46      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 1     |       |       |       |       |
| 47      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 1     |       |       |       |       |
| 48      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 3     |
| 49      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       | 1     |