



Population Structure and Genetic Connectivity of Squat Lobsters (*Munida* Leach, 1820) Associated With Vulnerable Marine Ecosystems in the Southwest Pacific Ocean

Ruo-Jin Yan^{1*}, Kareen E. Schnabel², Ashley A. Rowden^{1,2}, Xiang-Zhao Guo¹ and Jonathan P. A. Gardner¹

¹ School of Biological Sciences, Victoria University of Wellington, Wellington, New Zealand, ² National Institute of Water and Atmospheric Research, Wellington, New Zealand

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*Correspondence:

Ruo-Jin Yan
rjyan@suda.edu.cn

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Studies of genetic diversity and population genetic structure in deep-sea fauna mainly focus on vulnerable marine ecosystem (VME) indicator taxa, whilst relatively few studies have focussed on VME-associated taxa whose distributions are not exclusively limited to VMEs. Knowledge of genetic connectivity (gene flow) amongst populations of VME-associated taxa, such as squat lobsters, will contribute to ongoing management decision-making related to the protection of VMEs. To better understand the genetic diversity and genetic structure of squat lobster populations (*Munida isos*, *Munida endeavourae* and *Munida gracilis*) at different spatial scales (biogeographic provinces, regions and geomorphic features) in the southwest Pacific Ocean, mitochondrial cytochrome *c* oxidase subunit I (COI) region and nuclear microsatellite markers were employed. Overall, the levels of genetic diversity were high for the COI region and moderate for the microsatellite loci across the three *Munida* species. Analysis of molecular variance (AMOVA) of COI variation revealed no significant genetic differentiation, whereas AMOVA of microsatellite variation revealed significant genetic differentiation amongst the three species, but at different spatial scales. Based on microsatellite variation, a range of analyses [Structure, principal coordinate analysis (PCoA), discriminant analysis of principal components (DAPC)] provided some evidence of limited genetic differentiation at different spatial scales across the three species. Low to moderate levels of assignment success (~40–60%) based on microsatellite variation were achieved for the three *Munida* species, suggesting high levels of gene flow and possible panmixia. Nonetheless, for *M. isos*, populations from the Tasmanian slope were genetically differentiated from all other populations and may act as source populations, whereas populations from the Kermadec Ridge region may be sink populations for all three *Munida* species. Our results highlight the need to consider gene flow at trans-national scales when managing anthropogenic impacts on VMEs. The results are discussed in the context of existing marine protected areas (MPAs), which can contribute new information useful to the management of VMEs within the southwest Pacific Ocean.

Keywords: genetic diversity, genetic connectivity, management, conservation, southwest Pacific Ocean, vulnerable marine ecosystems

INTRODUCTION

The deep sea is the largest ecosystem on earth, encompassing more than 90% of the global ocean area (Ramirez-Llodra et al., 2010; Taylor and Roterman, 2017). It harbours a wide range of marine ecosystem services with unique abiotic and biological characteristics that support a rich diversity of life (Ramirez-Llodra et al., 2010). For several decades, considerable attention has been paid to the ongoing declines in marine biodiversity caused by pressures including environmental change, pollution and human exploitation (Pandolfi et al., 2003; Ramirez-Llodra et al., 2011; Cordes et al., 2016). Consequently, international agreement on the need for marine protected areas (MPAs) has been reached (Food and Agricultural Organisation of the United Nations, 2009) that is widely acknowledged to be an important step to help reduce anthropogenic impacts on biodiversity (Leathwick et al., 2008; Gjerde et al., 2016).

Vulnerable marine ecosystems (VMEs), including seamounts, canyons, hydrothermal vent and cold seep habitats, as well as cold-water coral reefs and sponge beds, are vulnerable to the impact of intense or long-term anthropogenic activities, in particular to bottom trawling (Food and Agricultural Organisation of the United Nations, 2009). There is now increasing awareness that VMEs need protection from anthropogenic activities such as trawling that have been carried out for decades, but also from future activities such as mining which may commence in the near future (e.g. Beger et al., 2014; Boschen et al., 2016; van Dover et al., 2018). MPAs provide a means to restrict damaging human activities and thereby allow for the conservation of critical natural habitats such as VMEs, which can help facilitate the maintenance and restoration of biodiversity and ecosystem structure and function (Agardy, 1994; Clark et al., 2011). Given that population declines can lead to reduced individual fitness, reduced population resilience and possible eventual extinction, an understanding of the population connectivity of VME indicator taxa is useful for informing the design and implementation of MPAs, for assessing the effectiveness of existing MPAs and for improving the management and mitigation of impacts on VMEs (Palumbi, 2003; Miller and Gunasekera, 2017).

The presence of a VME can be indicated by taxa that form a VME, e.g. coral species that form complex reef habitats (refer to Parker et al., 2009 for the South Pacific Ocean and Parker and Bowden, 2010 for the Southern Ocean and Antarctica). Whilst there are numerous studies examining the population structure of VME indicator taxa (e.g. in the southwest Pacific Ocean—Miller et al., 2010, 2011; Herrera et al., 2012; Miller and Gunasekera, 2017; Zeng et al., 2017; Holland et al., 2019; Zeng et al., 2019), relatively few studies have focussed on VME-associated taxa whose distributions are not exclusively limited to VMEs but are often found together with VMEs (e.g. crinoids, brisingid seastars *sensu*; Parker et al., 2009). Squat lobsters are highly diverse anomuran crustaceans that are sometimes, but not always, closely associated with VMEs (e.g. coral thickets and sponge gardens) (Lumsden et al., 2007; Baba et al., 2008; Baeza, 2011). As such, squat lobsters are an abundant and ecologically important group

that may provide new insights into the patterns of genetic connectivity amongst VMEs.

Using a range of different genetic markers, previous studies of population genetic structure for deep-sea squat lobsters across all or parts of their ranges have, perhaps not surprisingly, failed to reveal a consistent pattern of results. Whilst several different studies have reported no genetic population differentiation (e.g. Samadi et al., 2006; Haye et al., 2010; Bors et al., 2012; Cabezas et al., 2012; Roterman et al., 2016; Wang et al., 2016), others have reported evidence of limited to moderate genetic differences amongst populations (e.g. Pérez-Barros et al., 2014; Yang et al., 2016) and still others have reported no population genetic differentiation based on one marker type but population genetic differentiation based on a different marker type (e.g. Thaler et al., 2014). As VME-associated taxa, knowledge of connectivity amongst deep-sea squat lobster populations can be used to contribute to ongoing management decision-making about protection by assessing the effectiveness of existing MPAs and informing the placement of new MPAs. However, in the absence of consistent patterns of population genetic differentiation amongst squat lobster species reported in the literature, it is clear that new and region-specific assessments of genetic connectivity need to be carried out.

In the present study, the population genetic variation of three locally abundant *Munida* species [*Munida isos* (Ahyong and Poore, 2004), *Munida endeavourae* (Ahyong and Poore, 2004) and *Munida gracilis* (Henderson, 1885)] from the southwest Pacific Ocean region are investigated to better understand genetic diversity and genetic connectivity amongst VMEs. *M. isos* and *M. endeavourae* are widespread around New Zealand and south-eastern Australia where they occur from the edge of the continental shelves (~400 m) to bathyal depths of 1800–2700 m, respectively. *M. gracilis* is a widespread New Zealand endemic species with a slightly shallower depth distribution of about 100–1200 m (Baba et al., 2008; Schnabel, unpublished). *Munida* species have free-swimming planktonic larval phases that may drift with ocean currents, but the larval dispersal strategies of these species are unknown in detail. However, it is presumed that they have similar development to other munidids which include between four and six planktotrophic zoal larval stages and 15–83 days of larval development (Baba et al., 2011), which in turn suggests that larval dispersal over distances of 10s, if not 100s of km is likely. *Munida* species are associated with VMEs rather than being a functional component of VMEs as are many habitat-forming corals and sponges (Zeng et al., 2017, 2019). Squat lobsters therefore are a good choice of group to help investigate patterns of gene flow and population genetic differentiation amongst VMEs, and provide a valuable contrast to the more widely studied VME-forming groups. In conjunction, information about genetic connectivity and barriers to gene flow that may result in geographic genetic differentiation and/or genetic hotspots can be used in the planning of the placement and size of new MPAs (e.g. Miller et al., 2010, 2011; Bors et al., 2012; Miller and Gunasekera, 2017; Zeng et al., 2017, 2019; Holland et al., 2019). Based on the presumptive dispersal strategies and the

known habitat preferences of these species, it is hypothesised that *M. isos* and *M. endeavourae*, which are typically found on physically isolated seamounts, will exhibit population genetic heterogeneity within the study area. In contrast, it is hypothesised that *M. gracilis*, which is also found on soft sediments that are generally contiguous over large areas of the deep sea, allowing greater physical movement of adults and larvae, will exhibit higher levels of population genetic homogeneity. If the connectivity of species inhabiting only seamounts differs from the connectivity of species inhabiting seamounts and non-seamount habitats, then this has implications for spatial management measures of these species across their respective ranges. We used a spatially explicit hierarchical framework to test these hypotheses, and to explore the drivers of the observed genetic connectivity patterns, to provide information that will contribute to the management of VMEs within the southwest Pacific Ocean.

MATERIALS AND METHODS

Sample Collection and DNA Extraction

Specimens were obtained from the National Institute of Water and Atmospheric (NIWA) Research Invertebrate Collection (Wellington, New Zealand) and Museum Victoria (Melbourne, Australia). These were archived individuals collected mainly by scientific expeditions in the southwest Pacific Ocean since the 2000s. Most specimens were collected within the New Zealand's Exclusive Economic Zone (EEZ), with additional samples from the south Tasman Sea within the Australian EEZ, and from the Louisville Seamount Chain, an Area Beyond National Jurisdiction, to the northeast of New Zealand's EEZ (Figure 1). All specimens were preserved in > 80% ethanol after collection. The majority of specimens of *M. isos* and *M. endeavourae* were from seamount habitats, whilst the majority of specimens of *M. gracilis* were from soft sediment habitats at relatively shallower depths. Sample availability depends on collection activity at any given site with the result that our site-specific sample sizes are highly variable, but not low given these constraints. Sample details are summarised in Table 1; full details are presented in Supplementary Tables S1–S3.

Abdominal muscle or pereopod tissue samples were collected and stored individually in 95% ethanol before being transferred to the laboratory, for further analysis. Whole genomic DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. DNA integrity and molecular weight were assessed using electrophoresis in 3% agarose gels and with a NanoPhotometer (Implen, Munich, Germany). Samples exhibiting poor quality DNA were discarded.

Squat lobster samples had previously been identified using the methods and keys described in Ahyong and Poore (2004) and Baba (2005). Species identification was further confirmed by genetic screening with GenBank accession numbers MF457406 (*M. isos*) and KJ544249 (*M. gracilis*) using the mitochondrial universal DNA barcoding primers LCO1490/HCO2198 (Folmer et al., 1994).

Data Testing Framework

For *M. isos*, for which there were large sample sizes and good spatial coverage, a spatially explicit hierarchical testing framework was employed to evaluate genetic differentiation amongst populations. This approach follows Zeng et al. (2017, 2019) and Holland et al. (2019).

First, samples of *M. isos* were assigned to the two deep, bathyal (800–3500 m), ocean floor biogeographic provinces of Watling et al. (2013); BY6, referred to here as the Northern province, and BY10 or the Southern province, with a boundary between them at approximately 45°S (Figure 1). The differential water mass characteristics (e.g. salinity, temperature and particulate organic carbon flux) of the two provinces may affect the Northern–Southern population distribution, resulting in a province-scale pattern of genetic differentiation across the study area.

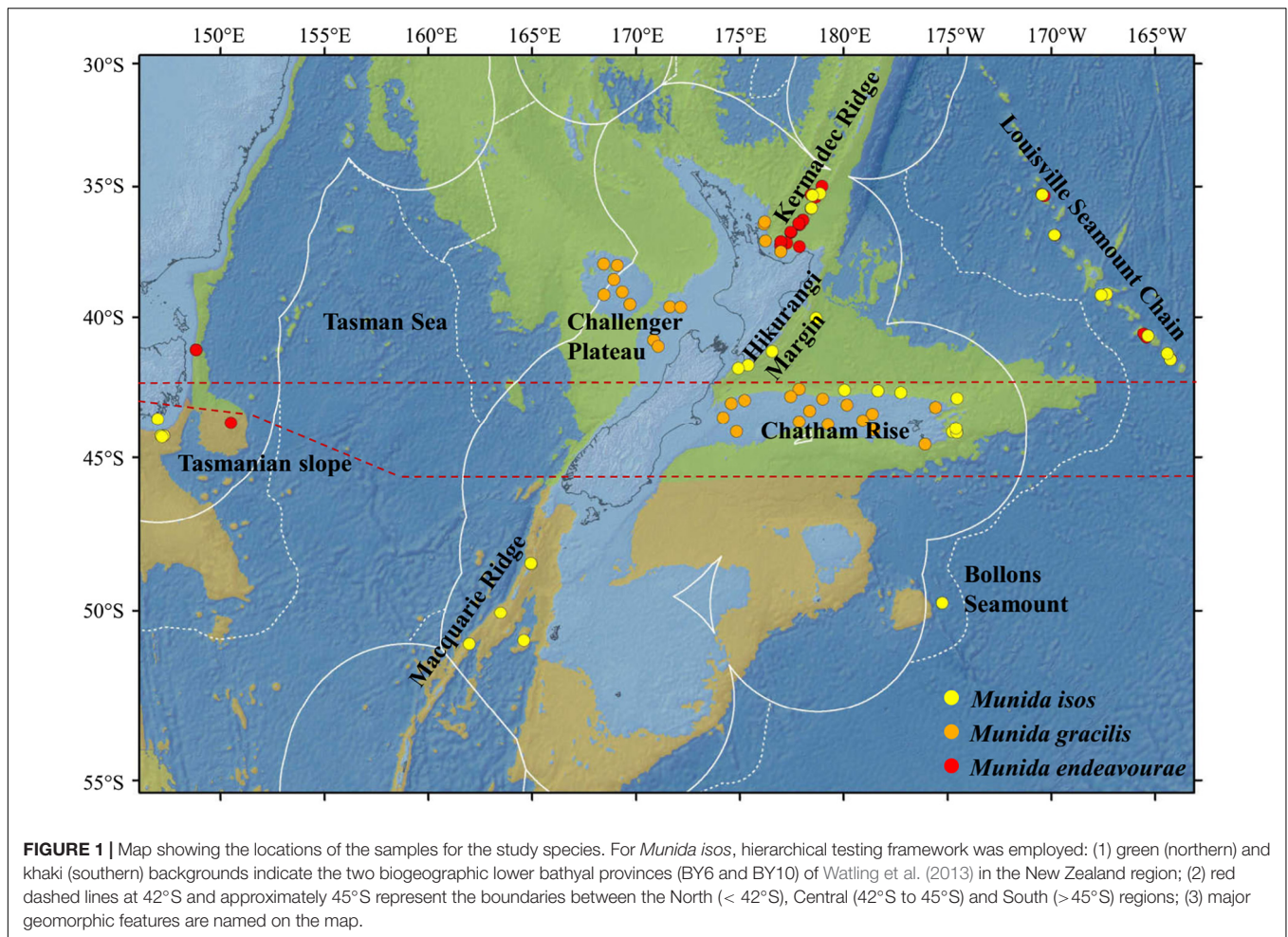
Second, samples were assigned to three regions; North (north of Chatham Rise), Central (Chatham Rise) and South (south of Chatham Rise) regions (Figure 1). Subtropical Water (STW) that reaches New Zealand region via the East Australian Current and the South Pacific subtropical gyre, and Subantarctic Water (SAW) that is driven north by Ekman transport and westerly winds, meet along the Chatham Rise forming the Subtropical Front (Chiswell et al., 2015). This front may act as a barrier to pelagic larval dispersal, leading to a North–Central–South region scale pattern of genetic subdivision of populations, and a high level of genetic diversity may be expected in the Central region on the Chatham Rise where North–South population mixing may occur.

Third, samples were assigned to populations according to major geomorphic features of the seafloor in the study area: Kermadec Ridge, Louisville Seamount Chain, Hikurangi Margin, Chatham Rise, Macquarie Ridge and Tasmanian slope (Figure 1). Given the species-specific distribution and habitat requirement, the distinctive topographic and local hydrodynamic characteristics may act as barriers to gene flow (Rowden et al., 2010a,b; Zeng et al., 2017). Samples from Bollons Seamount were included in the province and region spatial scale analyses, but not in geomorphic feature scale analyses, due to the limited sample size.

For *M. endeavourae* and *M. gracilis*, due to patchy sampling site coverage, samples were only assigned to populations according to major geomorphic features for the assessment of genetic connectivity. For *M. endeavourae*, populations were included from the Kermadec Ridge, Louisville Seamount Chain and the Tasmanian slope. For *M. gracilis*, populations included those from the Kermadec Ridge, Chatham Rise and Challenger Plateau (Figure 1).

COI Sequencing and Data Analyses

The mitochondrial DNA protein encoding cytochrome *c* oxidase subunit I (COI) region was amplified for the three *Munida* species using universal invertebrate primers LCO1490 and HCO2198 (Folmer et al., 1994), yielding fragments of 649 bp in length. Reactions were performed in a 20 μL total volume containing approximately 1 μL of genomic DNA (~30 ng/μL), 10 μL MyTaq Red Mix (Total Lab Systems Ltd., Auckland, New Zealand), 1 μL of both primers (forward and reverse) at a concentration



of 10 μM and with the addition of 7 μL ddH₂O. PCR amplifications for COI sequencing were carried out in a 96-well PCR Thermal Cycler (Applied Biosystems) programmed as follows: denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 30 s, 48°C for 30 s, extension at 72°C for 45 s and a final extension at 72°C for 10 min. PCR products were visualised on a 1.5% agarose gel and then sequenced on an ABI 3730xl DNA analyser. Despite multiple attempts, some COI fragments could not be amplified for several samples. Sequences have been deposited in GenBank (MK155662–MK156049).

Cytochrome *c* oxidase subunit I sequences (forward strand only) were aligned in MEGA v7 (Kumar et al., 2016) following manual checking and editing using DNASTar software (Burland, 2000). Species-specific genetic diversity statistics including number of haplotypes (*h*), average number of nucleotide differences (*K*), number of segregating sites (*S*), haplotype diversity (*H_d*) and nucleotide diversity (π) were calculated using DNASP v6.11.01 (Rozas et al., 2017) and Arlequin v.3.5 (Excoffier and Lischer, 2010).

To assess hierarchical population genetic differentiation amongst squat lobster populations, the uncorrected *p* distances within/between regions were calculated in MEGA v7 (Kumar et al., 2016). Arlequin v.3.5 (Excoffier and Lischer, 2010)

was used to perform a spatially explicit analysis of molecular variance (AMOVA) and to compute pairwise Φ_{ST} values. All groupings were evaluated with 10,000 permutations. Haplotype networks were constructed using a median-joining method in Popart (Leigh and Bryant, 2015). For each species, a maximum likelihood (ML) tree was drawn under the best-fit evolutionary model with the lowest Bayesian information criterion (BIC) score and 500 bootstraps in MEGA v7 (Kumar et al., 2016). Two species, *Munida thoe* (AY351013) and a yeti crab, *Kiwa tyleri* (KP763652), were designated as outgroups, the former being congeneric and the latter a more distantly related squat lobster. Nodes with bootstrap values > 90% were included on the final ML trees.

All analyses described above were carried out at three spatial scales (Northern–Southern provinces, North–Central–South regions and geomorphic features) for *M. isos*, and at the geomorphic level only for *M. endeavourae* and *M. gracilis*.

Microsatellite Genotyping and Data Analyses

From the 20 microsatellite markers described in Yan et al. (2019), 17, 11 and 9 microsatellite primer pairs were assayed for variation

TABLE 1 | Summary of sample sizes (*N*, number of specimens) for *Munida isos* at three spatial scales, and for *M. endeavourae* and *M. gracilis* at the geomorphic feature scale.

	Spatial class	Code	COI	Microsatellite
<i>Munida isos</i>	Northern province	Northern	214	219
	Southern province	Southern	134	136
	Total (provinces)		348	355
	North region	North	106	109
	Central region	Central	108	110
	South region	South	134	136
	Total (regions)		348	355
	Kermadec Ridge	KR	13	13
	Louisville Seamount Chain	LS	82	85
	Hikurangi Margin	HM	11	11
Chatham Rise	CR	108	110	
Macquarie Ridge	MR	59	60	
Tasmanian slope	TA	72	73	
Total (geomorphic features)		345	352	
<i>M. endeavourae</i>	Kermadec Ridge	KR	38	42
	Louisville Seamount Chain	LS	28	28
	Tasmanian slope	TS	23	26
	Total (geomorphic features)		89	96
<i>M. gracilis</i>	Kermadec Ridge	KR	19	20
	Chatham Rise	CR	154	155
	Challenger Plateau	CP	159	164
	Total (geomorphic features)		332	339

for *M. isos*, *M. endeavourae* and *M. gracilis*, respectively, based on their scoring reliability (**Supplementary Table S4**). Locus-specific PCR amplification conditions followed Yan et al. (2019). PCR products were run alongside a LIZ500 internal size standard on an ABI 3730xl DNA analyser (Sangon Inc., Shanghai, China). Alleles were scored automatically and reviewed manually using PeakScanner v2.0 (Applied Biosystems).

The genotype data were initially assessed in Lositan (Antao et al., 2008) for the neutrality test in an island model under the infinite alleles model (IAM) with 50,000 simulations. Microchecker v2.2.3 (van Oosterhout et al., 2004) was subsequently employed to detect the presence of null alleles, large allele dropout and scoring errors caused by stuttering, as well as to calculate null allele frequency (r) using the Brookfield (1996) method for each locus.

To quantify population genetic diversity, summary statistics including the actual number of alleles (N_A), effective number of alleles (N_E), observed (H_O) and expected (H_E) heterozygosity, polymorphism information content (PIC) values, allelic richness (A_R), number of private alleles (N_{AP}) and inbreeding coefficient (F_{IS}) were calculated for each locus using GenAEx v6.5 (Peakall and Smouse, 2012), Cervus v3.03 (Kalinowski et al., 2007) and Fstat v2.9.3.2 (Goudet, 2002). Exact tests of deviation from Hardy–Weinberg equilibrium (HWE) (Guo and Thompson, 1992) for each locus \times population combination and tests for genotypic linkage disequilibrium (LD) between pairs of loci were performed in Arlequin v3.5 (Excoffier and Lischer, 2010) and Genepop v4.2 (Raymond and Rousset, 1995; Rousset, 2008). Significance levels ($p < 0.05$) for multiple pair-wise tests

were adjusted using the false discovery rate (FDR) correction (Benjamini and Hochberg, 1995).

To quantify population genetic differentiation, Nei's genetic distance (D_A) (Nei et al., 1983) and pairwise F_{ST} values were computed using Populations v1.2.31¹ and Arlequin v3.5 (Excoffier and Lischer, 2010). The latter was used to perform spatially explicit AMOVA, with all groupings tested with 10,000 permutations, for the different spatial scales.

General relationships amongst individuals were depicted through a principal coordinate analysis (PCoA) on the basis of a covariance pairwise genetic distance matrix in GenAEx v6.5 (Peakall and Smouse, 2012). Discriminant analysis of principal components (DAPC) (Jombart et al., 2010) was applied to test for genetic structure amongst populations with the 'adegenet' package v2.0.1 (Jombart and Ahmed, 2011) in the program R v3.4.1 (R Development Core Team, 2017). For *M. isos*, DAPC was conducted at region and population scales but not at the province level due to the limited number of groups. To determine the best K , denoting the most likely number of putative genetic clusters, a Bayesian clustering approach was implemented in Structure v2.3.4 (Pritchard et al., 2000) using an admixture ancestry model with corrected initial ALPHA values ($\sim 1/K$), uncorrelated allele frequencies and prior location information (Hubisz et al., 2009; Wang, 2017). Preliminary testing revealed that choice of model made no substantive difference to analysis outcomes. Each Markov chain Monte Carlo (MCMC) simulation was run for 500,000 iterations, with an initial burn-in period of

¹<http://bioinformatics.org/~tryphon/populations/>

50,000 iterations. Fifty independent runs were performed for each cluster set. The ΔK metric (Evanno et al., 2005) was employed to calculate the best K (average number of nucleotide differences) in Structure Harvester². Post-processing of Structure results was carried out using CLUMPAK (Kopelman et al., 2015). A second clustering approach was carried out in BAPS v6 (Corander et al., 2008) as a complement to the Structure analyses. Calculations were performed under the mixture model, with the maximum number of populations set as the number of regions, i.e. six, three and three for *M. isos*, *M. endeavourae* and *M. gracilis*, respectively.

Individual assignment tests and first generation migrant detection with 'L_home' likelihood computation were implemented in GeneClass v2 (Piry et al., 2004). The contemporary migration rates amongst populations were calculated in BayesAss v3 (Wilson and Rannala, 2003), which can produce accurate estimates of migration and self-replenishment when F_{ST} is ≥ 0.05 (Faubet et al., 2007). Calculation parameters were set as follows: 10,000,000 iterations, of which 3,000,000 were burn-in; sampling frequency 1000; migration rates, allele frequencies and inbreeding coefficients set at 1, 1 and 1, respectively, to make the acceptance rates between 20 and 60%.

All analyses described above were carried out at three spatial scales (Northern–Southern provinces, North–Central–South regions and geomorphic features) for *M. isos*, and at geomorphic feature level for *M. endeavourae* and *M. gracilis*.

RESULTS

Population Genetic Structure Based on COI Region Genetic Diversity

Haplotypic diversity was very high across populations for all provinces, regions and geomorphic features of the three *Munida* species. For *M. isos*, 111 haplotypes, based on a 649-bp fragment of the gene, were obtained from 348 individuals at both the province and region spatial scales. The haplotype diversity and nucleotide diversity values of populations were slightly greater in populations of the Northern province than in the Southern province, and both estimates in the North region were greater than the other two regions (detailed results are reported in **Supplementary Table S5**). At the geomorphic feature scale, a total of 110, 67 and 200 COI haplotypes were obtained from 345, 89 and 332 individuals of *M. isos*, *M. endeavourae* and *M. gracilis*, respectively. Notably, the mean population haplotype diversity and nucleotide diversity values for *M. isos* (0.764; 0.26%) were lower than for *M. endeavourae* (0.980; 0.59%) and *M. gracilis* (0.973; 0.56%), respectively.

Genetic Differentiation

As revealed by uncorrected p distances and Φ_{ST} values, genetic differentiation amongst populations of provinces, regions and geomorphic features was low in the three *Munida* species (uncorrected p distances in the range of 0.002–0.006; Φ_{ST} values in the range of 0.000–0.035). Specifically, all of the

uncorrected p distances were relatively low, but within the Decapoda intraspecific barcode threshold (0.285–1.375%) (da Silva et al., 2011). Significance ($p < 0.05$) was detected for three Φ_{ST} values, all from population pairs at geographical feature scale of *M. isos*, yet none was observed amongst regions or province population pairs of *M. isos*, nor population pairs of geomorphic features for *M. endeavourae* or *M. gracilis* (**Supplementary Table S6**). This lack of differentiation was supported AMOVA results, with no significant genetic differentiation observed for all Φ -statistic values and over 99% of the variance being attributed to differences within populations (**Supplementary Table S7**).

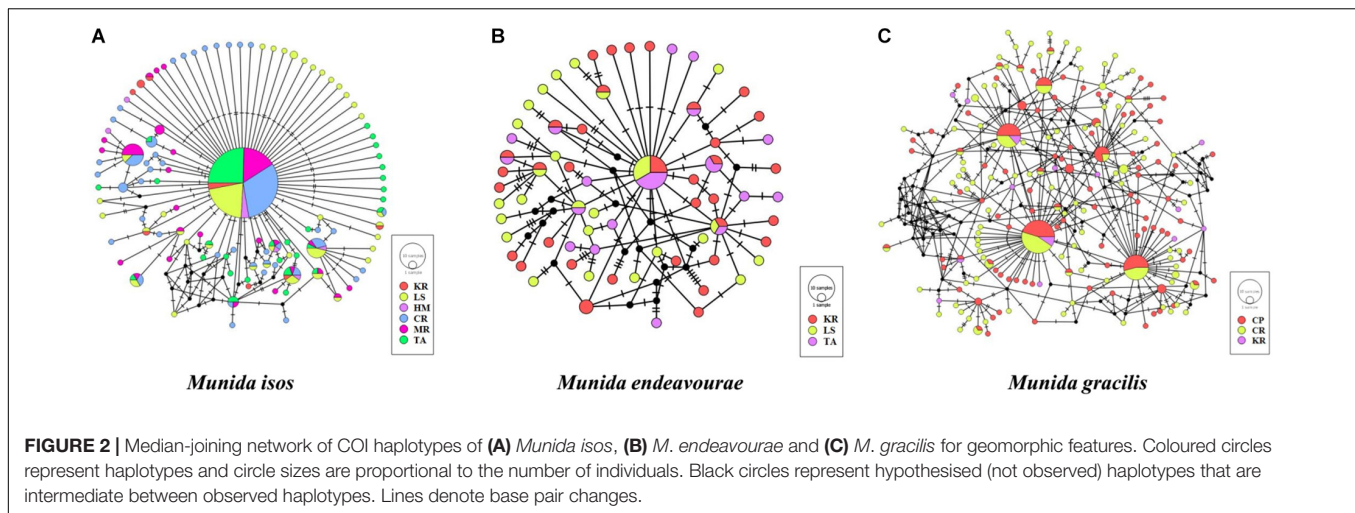
The median-joining networks, representing the most parsimonious relationship amongst haplotypes, were plotted for the three *Munida* species. For *M. isos*, a starburst-like pattern was observed from the 111 haplotypes, but no pattern of genetic structure was detected in populations at either the province (**Supplementary Figure S1**) or region scales (**Supplementary Figure S2**). At the geomorphic feature scale, haplotypes from both *M. isos* and *M. endeavourae* populations exhibited a starburst-like pattern, consistent with the mutational evolution of new haplotypes over time (**Figure 2**). However, the number of equally parsimonious haplotype connections was too great in *M. gracilis* for the most likely network to be determined. Similarly, there was no evidence of a pattern of genetic structure amongst populations of the three *Munida* species across their distributional ranges (**Supplementary Figure S3**). The ML phylogenetic trees were similar for the three *Munida* species, with all haplotypes occurring in a major non-differentiated cluster with strong support, and clearly differentiated from the two outgroup taxa (**Supplementary Figure S4**). These analyses indicate that there is no clear evidence of genetic subdivision amongst populations at the geomorphic feature, region or province scale for the three *Munida* species.

Population Genetic Structure Based on Microsatellite Variation Genetic Diversity

For all three species Lositan analyses indicated that no microsatellite locus was likely to be experiencing selection. Null alleles ($r > 0.2$) were detected at loci MI_06 (*M. isos*) and MI_02 (*M. gracilis*), but not observed at these loci in the two other species. Evidence of homozygote excess and stuttering was observed, but these were randomly distributed across loci in the three *Munida* species. No large allele dropout was indicated at any locus.

In total, 167, 63 and 74 alleles with size ranges of 78–134, 83–172 and 82–200 bp were amplified at 17, 11 and nine microsatellite loci in *M. isos*, *M. endeavourae* and *M. gracilis*, respectively. In general, allelic variation (e.g. H_E and PIC) was only moderately high. Four loci (MI_21, MI_29, MI_39 and MI_40) in *M. isos*, three (MI_06, MI_10 and MI_39) in *M. endeavourae* and one (MI_14) in *M. gracilis* exhibited a relatively low level of polymorphism compared to other loci (details are summarised in **Supplementary Table S8**). The *M. isos* of the Northern province exhibited a higher level of genetic diversity than those of the Southern province. The average number of effective alleles

²<http://taylor0.biology.ucla.edu/structureHarvester/>



and expected heterozygosity were greatest in populations of the Central region, but the largest number of private alleles was found in the South region. At the geomorphic feature level, the observed and expected heterozygosities in the populations were reasonably consistent in all features for the three species (detailed results are reported in **Supplementary Table S5**). At the geomorphic feature scale, populations of *M. isos* and *M. gracilis* exhibited moderate levels of genetic diversity whilst *M. endeavourae* showed slightly lower genetic diversity. Private alleles were identified in nearly all populations of geomorphic features across the three *Munida* species, with the highest numbers being observed for the Chatham Rise (*M. isos* and *M. gracilis*) and Kermadec Ridge (*M. endeavourae*) populations, whilst none were detected for the Kermadec Ridge population of *M. gracilis*. Average F_{IS} values were positive across populations of geomorphic features, with the only exception being the population of *M. endeavourae* from the Tasmanian slope, which exhibited a deficit of heterozygotes.

For *M. isos*, after FDR correction for multiple comparisons, 28 (82.35%) and 37 (72.55%) tests were significantly different from HWE ($p < 0.05$) at pairwise combinations of loci \times populations at the province and region spatial scales, respectively. For geomorphic features, 38 (37.25%), 5 (15.15%) and 14 (51.85%) tests were significantly different from HWE ($p < 0.05$) in *M. isos*, *M. endeavourae* and *M. gracilis*, respectively, with the majority exhibiting heterozygote deficiencies. In particular, loci MI_25 and MI_02 demonstrated significant departures from HWE within all populations at the geomorphic feature scale of *M. isos* and *M. gracilis*, respectively.

For *M. isos*, only four of 136 locus-pairs showed significant LD after FDR correction ($p < 0.05$). No evidence of LD was found in *M. endeavourae* and *M. gracilis*.

Genetic Differentiation

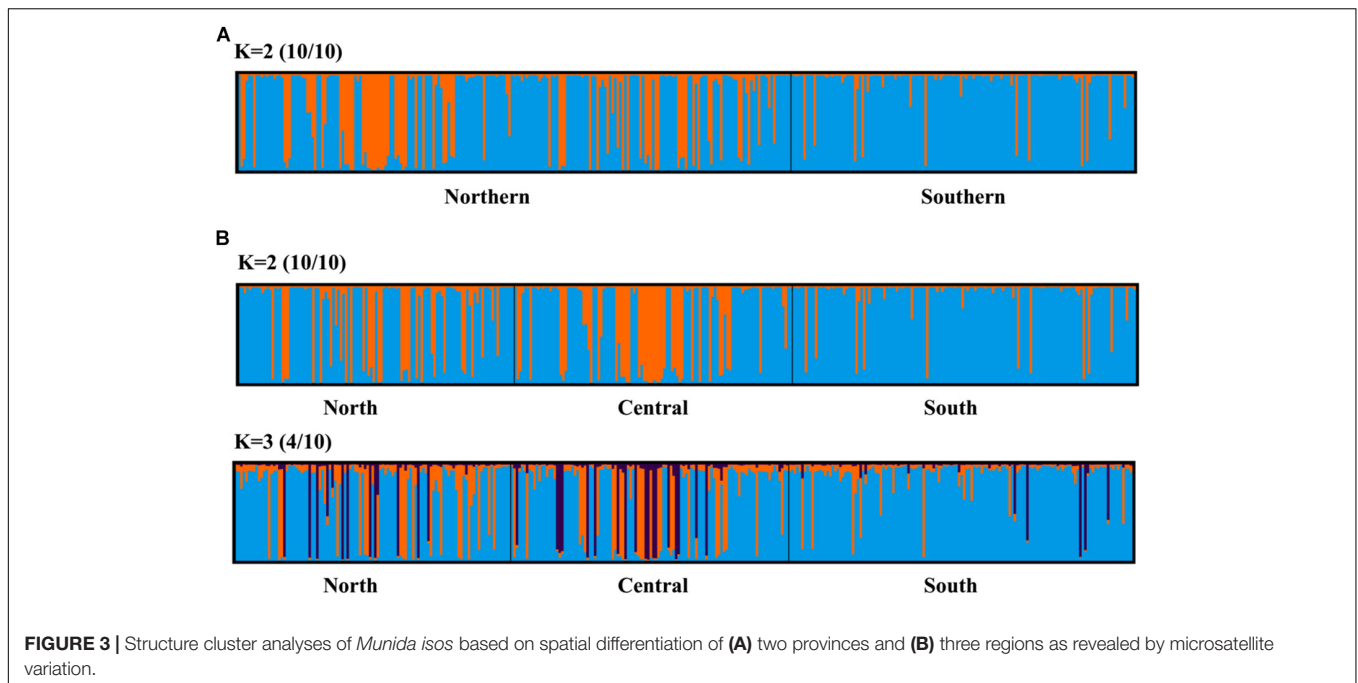
Genetic population differentiation was low for the three species, as revealed by Nei's genetic distance (D_A) and F_{ST} values (D_A in the range of 0.015–0.107; F_{ST} in the range of 0.001–0.051). For *M. isos*, F_{ST} values amongst populations of the two provinces and three regions remained low but significant ($p < 0.05$). For *M. isos* at the geomorphic feature scale, only the pairwise comparison

between populations of the Hikurangi Margin and Tasmanian slope ($F_{ST} = 0.051$) exhibited a moderate level of differentiation (**Supplementary Table S6**). For population pairs of the other two species, significant difference from zero ($p < 0.05$) was detected for thirteen F_{ST} values after correction, of which 11 and two were from *M. isos* and *M. endeavourae*, respectively.

Analysis of molecular variance revealed significant genetic differentiation at different spatial levels: within all samples of *M. isos* and *M. gracilis*; amongst the populations of the two provinces, three regions, and six geomorphic features for *M. isos* and amongst populations of the three geomorphic features for *M. endeavourae* and *M. gracilis* (**Supplementary Table S7**). Nonetheless, AMOVA partitioned more than 96% of the genetic variation within samples for the three *Munida* species, leaving less than 4% variance attributable to the differences amongst populations and amongst populations within groups.

Genetic Structure

Principal coordinate analysis, with relatively low cumulative variation represented by the first two factors, revealed no evidence of genetic differentiation across populations of provinces or regions for *M. isos*, and amongst populations of geomorphic features for the three *Munida* species (**Supplementary Figures S5, S6**), whilst Structure showed the highest support for two clusters ($\Delta K = 2$) for all datasets. However, given that the ΔK approach is unable to detect the best K when $K = 1$, post-processing of Structure bar plots was conducted in CLUMPAK. As a result, $K = 1$ and $K = 2$ could not be successfully differentiated between for *M. isos* (10/10; 10/10) at three spatial scales (provinces, regions and geomorphic features) and *M. gracilis* (10/10; 10/10) at geomorphic feature level, whereas $K = 2$ (5/10) was rejected in favour of $K = 1$ for *M. endeavourae* (**Figures 3, 4**). For *M. isos*, when $K = 2$, individuals from the Southern province and the South region were mostly assigned to the blue cluster based on provinces and region scales. At the geomorphic feature scale, the majority of individuals from the Hikurangi Margin, Macquarie Ridge and Tasmanian slope populations were assigned to the blue cluster, whilst populations from other features showed a more complex genetic structure



with a shared membership of both the blue and orange clusters. This finding was supported by the fact that 10 out of 11 significant pairwise F_{ST} values were detected in populations from these three geomorphic features (Hikurangi Margin, Macquarie Ridge and Tasmanian slope). For *M. endeavourae*, no pattern was revealed, with all individuals assigned equally to both clusters, indicating no structure. For *M. gracilis*, individuals from populations of all three geomorphic features were randomly assigned to two clusters, echoing the AMOVA results as major variance was attributed to the differences within geomorphic features. In the BAPS analyses, the inferred number of populations was one ($K = 1$) for all datasets of three *Munida* species, indicating no genetic structure.

Results from DAPC, an analysis that can maximise genetic variation amongst groups whilst minimising separation within groups, indicated three genetic clusters corresponding to the three regions as defined by the first two principal components (PCs) for *M. isos* (Figure 5). At the geomorphic feature level, two, three and three major genetic clusters were calculated for *M. isos*, *M. endeavourae* and *M. gracilis*, respectively (Figure 6). For *M. isos*, PC1 separated the Tasmanian slope population from the populations of all other geomorphic features. The major cluster was further divided by PC2 into individuals from populations of the Kermadec Ridge and Louisville Seamount Chain, Chatham Rise and Macquarie Ridge and the Hikurangi Margin, which was congruent with the F_{ST} and Structure results. For *M. endeavourae* and *M. gracilis*, three distinct clusters corresponding to the populations from the three geomorphic features were revealed, with clusters overlapping to different degrees for the two species.

Gene Flow and Connectivity

Assignment-based tests performed in GeneClass had a low to moderate level of classification success. For *M. isos*, 231

(65.1%) and 179 (50.4%) individuals were correctly assigned to their province and region populations, respectively. At the geomorphic feature level, a total of 143 (40.6%), 53 (55.2%) and 156 (40.0%) individuals were correctly assigned to their own geomorphic feature populations for *M. isos*, *M. endeavourae* and *M. gracilis*, respectively. The 'L_home' statistics demonstrated some evidence of first-generation migrants amongst sampled province/region/geomorphic feature populations. In total, 19, two and eight individuals were below the threshold of the assignment tests ($\alpha = 0.01$) for *M. isos*, *M. endeavourae* and *M. gracilis*, respectively, increasing the possibility that these individuals may be first generation immigrants that have been introduced from an unknown source.

For *M. isos*, BayesAss analyses were unable to reach the standard of 20–60% in the province and region spatial scale datasets: only results for geomorphic features are presented. At the geomorphic feature level, estimates of contemporary migrants between populations were low, and the proportions of self-recruitment were high to very high (Table 2). For *M. isos*, the Macquarie Ridge (81.22%) and Tasmanian slope (96.43%) populations exhibited evidence of genetic isolation with high proportions of contribution from within each population (self-recruitment). Similar patterns were observed for individuals from the Louisville Seamount Chain for *M. endeavourae* (95.47%) and from the Challenger Plateau for *M. gracilis* (88.26%). Notably, the Kermadec Ridge region was identified as a sink population (only receiving migrants from other populations) for all *Munida* species. For *M. isos*, the Tasmanian slope population was identified as a source population (providing migrants to other populations while receiving no or few migrants). This was incongruent with findings from *M. endeavourae*, for which the Tasmanian slope population (68.01%) acted as a sink population, receiving migrants (30.27%) from the Louisville Seamount

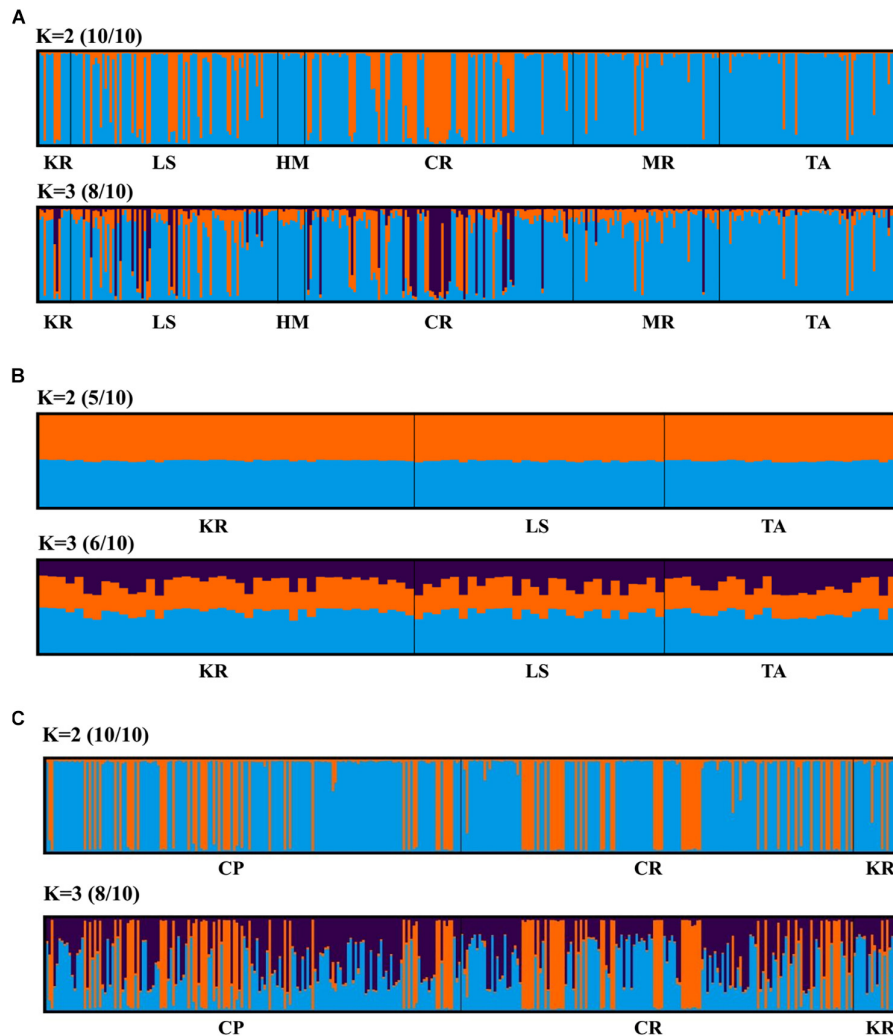


FIGURE 4 | Structure cluster analyses of (A) *Munida isos*, (B) *M. endeavourae* and (C) *M. gracilis* based on microsatellite variation among populations on geomorphic features.

Chain population. For *M. gracilis*, populations from both the Chatham Rise and Challenger Plateau were both source and sink populations.

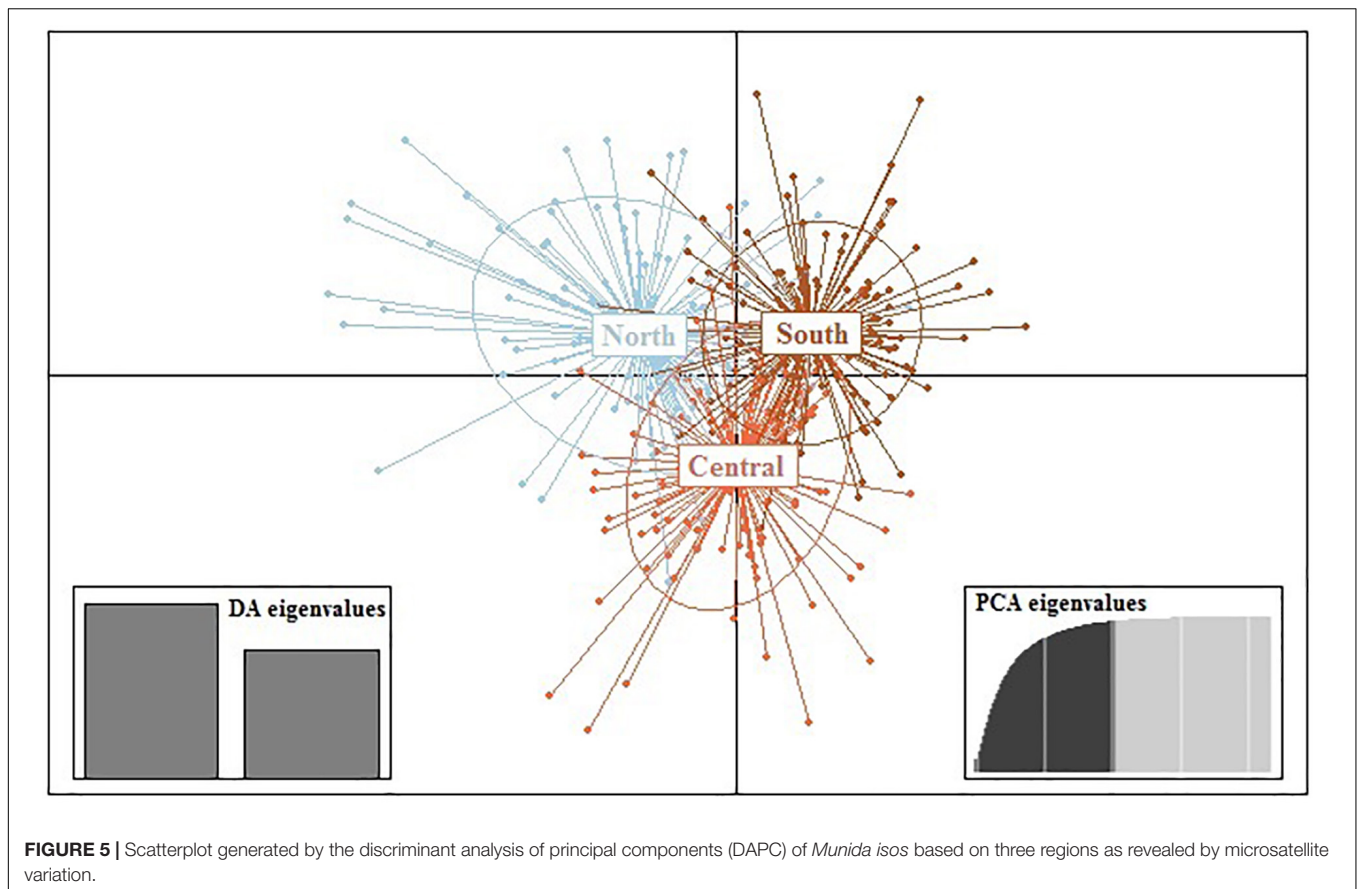
DISCUSSION

Three munidid squat lobsters, *M. isos*, *M. endeavourae* and *M. gracilis*, have been tested for levels of genetic diversity and patterns of genetic connectivity in the southwest Pacific Ocean based on mitochondrial COI region and nuclear microsatellite variation. Overall, the level of genetic diversity was high as revealed by the COI region, and moderate as revealed by microsatellite markers, across all three *Munida* species. There was no evidence of genetic subdivision amongst populations of the three *Munida* species based on the COI sequence data, and only limited genetic differentiation was observed in *M. isos* and *M. gracilis* based on microsatellite variation (none was detected for

M. endeavourae using microsatellites). These results provide new insights into the genetic connectivity of VME-associated taxa in the southwest Pacific Ocean.

Genetic Diversity

Genetic diversity of the three *Munida* species was randomly distributed across the study area, as indicated by both the COI region and microsatellite markers. The mitochondrial COI region, in particular, exhibited a high level of genetic diversity for haplotypes across the three *Munida* species. Notably, within the genus *Munida*, high levels of polymorphisms in COI can be indicative of a relatively fast mutation rate, or because population sizes are large, or because the taxa have an ancient origin, allowing for mutations to accumulate over time (Coykendall et al., 2017). The observation of high haplotype diversity was consistent with findings from other deep-sea squat lobsters, such as the chirostyloid *K. tyleri* (Roterman et al., 2016) and the munidid *Pleuroncodes monodon* (Haye et al., 2010). Nonetheless, *M. isos*



exhibited lower genetic diversity compared to *M. endeavourae* and *M. gracilis*, as revealed by other genetic diversity estimates based on COI region, whilst *M. endeavourae* showed the lowest genetic diversity on the basis of microsatellite markers with respect to expected heterozygosity and effective number of alleles that could reflect genetic drift (Hedrick, 1999). Given that sample size is uneven when comparing populations of *M. isos* and *M. gracilis*, allelic richness, which is independent on sample size, is included as an index to evaluate the level of genetic diversity for the newly developed microsatellite markers. Consequently, these microsatellite markers performed reasonably well, exhibited a moderate level of genetic diversity and provided valuable genetic information for the three *Munida* species.

Significant deviations from HWE were observed in the three *Munida* species, which is common in squat lobster species (Molecular Ecology Resources Primer Development Consortium et al., 2010; Boyle et al., 2013; Nakajima et al., 2018). Deviations from HWE may be attributed to causes such as null alleles and the Wahlund effect (Johnson and Black, 1984). The presence of null alleles may result in mistyping of homozygotes and heterozygotes, leading to an over-estimation of genetic differentiation (DeWoody et al., 2006). However, null alleles are commonly observed in a variety of marine invertebrate species (e.g. Dailianis et al., 2011; Thaler et al., 2014; Roterman et al., 2016) and in population genetic studies

null allele frequencies below 0.2 are commonly accepted when using microsatellite markers (Dakin and Avise, 2004). In this study, although low-to-moderate frequencies of null alleles were detected at a number of loci across the three *Munida* species, only two loci exhibited null allele frequencies above 0.2. Given that little genetic differentiation was observed amongst populations of *M. isos* and *M. gracilis*, the null alleles at these two loci were not expected to influence the overall interpretation of the results. The Wahlund effect is caused by individuals from different localities that are characterised by different genotype proportions, being mixed during sampling or during data analysis, resulting in excess homozygosity and significant F_{IS} estimates (Freeland, 2005; Waples, 2014). The highly significant deficit of heterozygote genotypes, as revealed by positive inbreeding coefficients, was obtained across all populations in the three *Munida* species with only one exception (i.e. the Tasmanian slope population of *M. endeavourae*), indicating the presence of inbreeding. Moreover, HWE deviations occurred in the form of homozygote excesses, which might also result from small sample size (e.g. populations from Kermadec Ridge and Hikurangi Margin in *M. isos*), given the potential for extensive gene flow amongst populations and population demographic history (e.g. expansions or selective sweeps). However, small sample size and limited spatial scale sampling are unavoidable constraints in population genetic studies of deep-sea organisms (Baco et al., 2016). For microsatellite-based population genetic

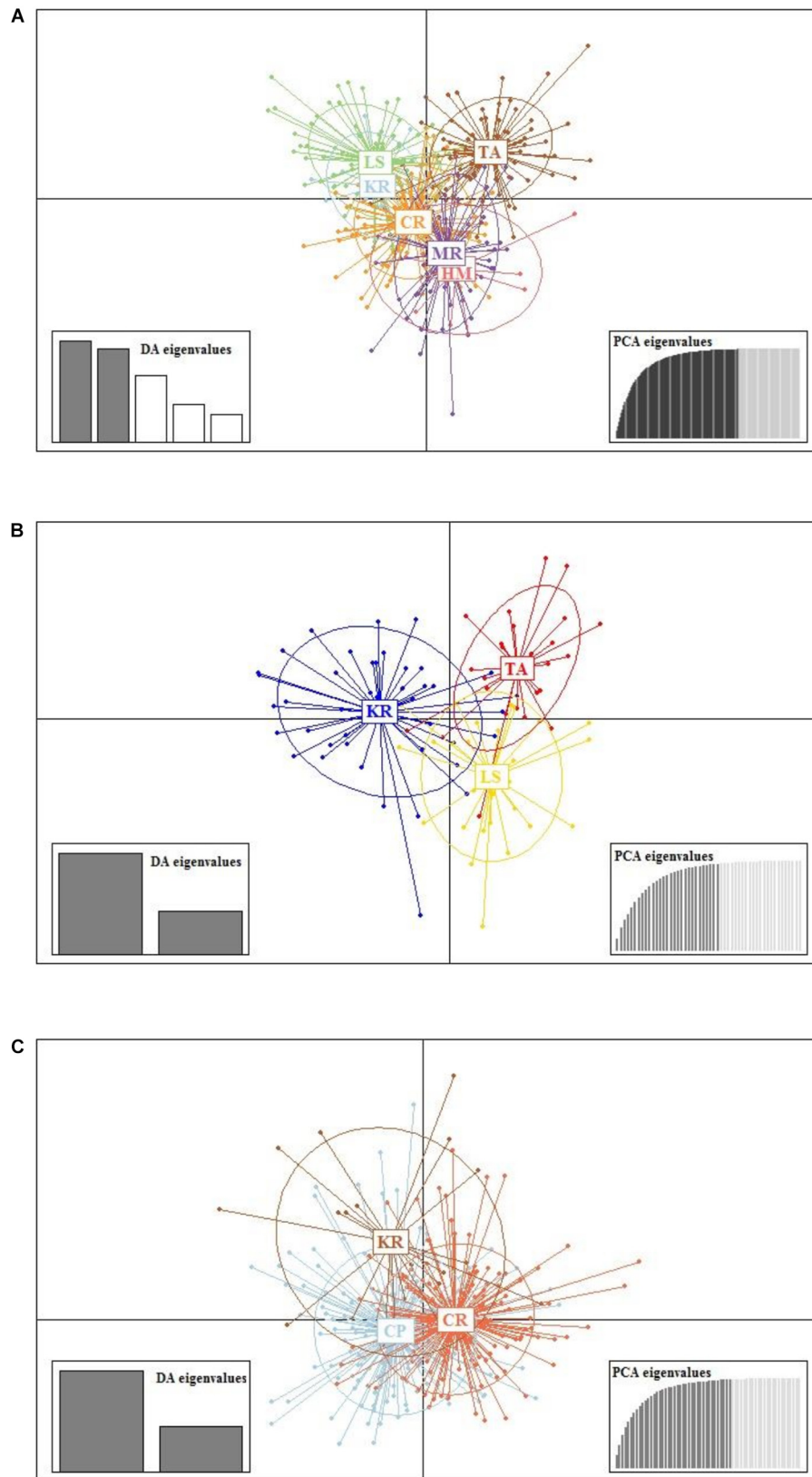


FIGURE 6 | Scatterplot generated by the discriminant analysis of principal components (DAPC) of **(A)** *Munida isos*, **(B)** *M. endeavourae* and **(C)** *M. gracilis* based on microsatellite variation among populations of geomorphic features.

TABLE 2 | Estimated migration rates from source populations (top) to recipient populations (side) for three *Munida* species based on microsatellite variation.

	Code	Source					
<i>Munida isos</i>		KR	LS	HM	CR	MR	TA
	KR	68.41%	6.59%				16.35%
	LS		72.23%				20.31%
	HM			68.59%		19.99%	5.02%
	CR				78.74%	6.22%	14.02%
	MR					81.22%	14.87%
	TA						96.43%
<i>M. endeavourae</i>		KR	LS	TA			
	KR	79.65%	19.45%				
	LS		95.47%				
	TA		30.27%	68.01%			
<i>M. gracilis</i>		KR	CR	CP			
	KR	68.13%	17.86%	14.00%			
	CR		76.81%	22.98%			
	CP		11.54%	88.26%			

Empty cells denote migration rates <0.05 , estimates of self-recruitment in bold (on the diagonal).

studies, 25–30 individuals per population is considered sufficient to provide accurate estimations of genetic diversity and gene flow (Hale et al., 2012), and the majority of the populations tested in the three *Munida* species meet this criterion.

Genetic Differentiation and Connectivity

Genetic differentiation at different spatial scales was evaluated based on mitochondrial COI region and nuclear microsatellite marker variation in the three *Munida* species. Levels of site-specific genetic diversity are a function, at least in part, of sample size and we recognise that our sample sizes are very variable amongst our sites. This variability reflects the differential availability of archived material in the museum collections, which is itself a function of the very different sampling efforts that our sites have been subject to over the last several decades and is a known and appreciated constraint for deep-sea population genetics research (e.g. Boschen et al., 2015; Baco et al., 2016; Zeng et al., 2017, 2019). For the COI region, according to the Decapoda intraspecific barcode threshold (0.285–1.375%) (da Silva et al., 2011), the maximum pairwise uncorrected p distances amongst the three *Munida* populations did not suggest the presence of cryptic species. These results support previous findings for *M. gracilis* in the New Zealand region (0–1.9%) (Bors et al., 2012) and align with observations in other squat lobsters, including *Fennerogalatea chacei* (0.13–0.61%) (Rodríguez-Flores et al., 2017). Despite the large geographic coverage of sampling sites, low and statistically non-significant global Φ_{ST} values were observed for the three *Munida* species, suggesting genetic similarity in the squat lobster populations across the southwest Pacific Ocean. In contrast, microsatellite markers revealed some limited evidence of genetic differentiation amongst populations of *M. isos* and *M. gracilis*, but not *M. endeavourae*. Although nuclear microsatellite markers showed signs of low levels of genetic differentiation for the three *Munida* species, more than 96% of the variance was attributed to differences within populations, again suggesting high levels of

gene flow amongst populations leading to panmixia. Collectively, little genetic differentiation was observed amongst populations of three *Munida* species in the southwest Pacific Ocean, either from seamounts or soft sediments. Therefore, the hypothesis that *M. isos* and *M. endeavourae* (typically found on isolated seamounts) exhibit population genetic differentiation was rejected, whilst the hypothesis that gene flow amongst populations of *M. gracilis*, (typically found on soft sediments) results in high levels of population genetic homogeneity was accepted. The absence of genetic structure is usually interpreted as being consistent with either panmixia or recent demographic expansion following a genetic bottleneck event can leave little or no signature in the genome. Additionally, a recent colonisation from another area of the species' distribution is possible, although we see no evidence of this. This lack of population genetic differentiation has been observed in a number of squat lobster species (e.g. Samadi et al., 2006; Haye et al., 2010; Bors et al., 2012; Cabezas et al., 2012; Wang et al., 2016), including for *M. gracilis* in the New Zealand region (Bors et al., 2012). Similarly, no significant population genetic structure was observed across the same province, region and geomorphic feature scales for a deep-sea sponge species in the study area (Zeng et al., 2019). However, this pattern of genetic similarity is not consistent across all deep-sea marine species, including corals and sponges (e.g. Miller et al., 2011; Herrera et al., 2012; Miller and Gunasekera, 2017). For example, within the Southwest Pacific region, a North–Central–South region pattern of structure has been detected in a cup coral based on ITS sequence variation (Miller et al., 2010), province-scale differentiation has been reported in branching stony corals using a combination of D-loop, ITS and microsatellite markers (Zeng et al., 2017), and genetic differentiation has been detected in a deep-sea sponge species based on province, region and geomorphic feature spatial scales as revealed by COI, Cytb and microsatellite variation (Zeng et al., 2019).

Based on mitochondrial COI sequence data, an overall lack of population genetic structure was detected for the three *Munida*

species accordingly to ML tree and median-joining network. Similar haplotype networks have been reported for other squat lobster taxa, such as *K. tyleri* (Roterman et al., 2016), *Munida intermedia* (García-Merchán et al., 2012), *M. gracilis* (Bors et al., 2012), *Munida gregaria* (Pérez-Barros et al., 2014) and *P. monodon* (Haye et al., 2010). For nuclear microsatellite data, given that the differentiation amongst populations was weak for the three *Munida* species, it was difficult to infer population genetic structure. The Bayesian clustering software programmes Structure and BAPS can both perform reasonably well at low levels of genetic differentiation and are able to correctly identify the maximum (upper limit) number of genetically distinct groups (Latch et al., 2006). In this study, considering the low F_{ST} values for all three *Munida* species, Structure and BAPS were employed to calculate the best K (average number of nucleotide differences). Little or no structure was found for the three *Munida* species when using both approaches. Nonetheless, populations from the Tasmanian slope are potentially genetically differentiated, but not isolated, from the other *M. isos* populations as revealed by results from the F_{ST} , Structure and DAPC analyses. Considering the difficulty in detecting any structure, we refrain from speculating why *M. isos* populations are potentially more structured compared to those of the other two *Munida* species.

The three *Munida* species exhibited low to moderate levels of assignment success, indicative of panmixia across the study area, and which is most probably driven by high levels of gene flow in the region. Interestingly, estimations of migration rate were not always consistent for populations from the Tasmanian slope, which acted as a source population for *M. isos* and as a sink population for *M. endeavourae*. Moreover, populations from the Kermadec Ridge acted as sink populations in the three *Munida* species, which was inconsistent with a previous study of two stony corals where the Kermadec Ridge is a migrant source (Zeng et al., 2017). Given the fact that BayesAss can only produce accurate estimates of migration and self-replenishment when F_{ST} is ≥ 0.05 (Faubet et al., 2007), all estimates of migration rates as revealed by BayesAss should be cautiously interpreted and are best viewed in combination with other analyses.

Overall, we have evidence of moderate to high levels of gene flow, of low levels of genetic differentiation and of low levels of assignment success, all of which point to population genetic homogeneity or perhaps even panmixia at large spatial scales. But we also have evidence of limited differentiation of the Tasmanian slope populations from all other populations to the east, in the New Zealand EEZ and beyond (i.e. the Louisville Seamount Chain) based on F_{ST} values and the DAPC plots. We interpret these results as indicating that the Tasmanian slope populations are differentiated to a small extent, perhaps at the limits of detection by some of the analyses, from all other populations. Our data suggest that there is likely to be asymmetrical gene flow from the Tasmanian slope populations to the east (i.e. the Tasmanian slope populations are source populations), and this is supported by, and consistent with, the regional physical oceanography. For example, the major surface current circulation, the STW and SAW, both flow eastwards (from Australia towards New Zealand) before meeting and mixing along the Chatham Rise to the east of New Zealand (Chiswell et al., 2015). Our data point

to gene flow from the Australian EEZ to the New Zealand EEZ, but not vice versa, a finding that is consistent with results for other species such as shallow water rock lobsters and the intertidal black nerite that have a long pelagic larval duration (e.g. Chiswell et al., 2003; Bruce et al., 2007; Morgan et al., 2013; Gardner et al., submitted).

Larval dispersal is crucial for connectivity and colonisation through migration in deep-sea environments. Specific larval development characteristics are unknown for *M. isos*, *M. endeavourae* and *M. gracilis* but based on egg size, a 'regular' (not abbreviated) planktotrophic larva is inferred as in other *Munida* species (Baba et al., 2011). The only complete larval development study was conducted for a congeneric species, *M. gregaria*, with a PLD that may reach 83 days at a rearing temperature of 8°C in the laboratory (Pérez-Barros et al., 2007). The potentially long PLD of the three *Munida* species that lasts for perhaps several months is but one of various possible explanations for the panmixia observed in the study region. The larval lifespans may be extended by low temperature-induced metabolic rate reduction in a low-temperature environment, lessening the impact of dispersal strategy on patterns of genetic differentiation (Roterman et al., 2016). In addition, the density of seamounts, as potential stepping stones, may be higher than is presently thought, facilitating dispersal, and deep water currents may act as a driving force for the enhancement of connectivity as well (Handayani et al., 2014). Interestingly, the estimates of genetic homogeneity in marine organisms are often found to be inconsistent with expectations from inferred PLD, which may be due to oversimplification of PLD estimation (Weersing and Toonen, 2009; Selkoe and Toonen, 2011; Faurby and Barber, 2012). Developmental biology studies of the three *Munida* species in the future should test the potential relationship between PLD and connectivity in squat lobster populations across their distributional range. Understanding this relationship will help inform the construction of biophysical models that are also used to inform the design of MPA networks (Hilário et al., 2015).

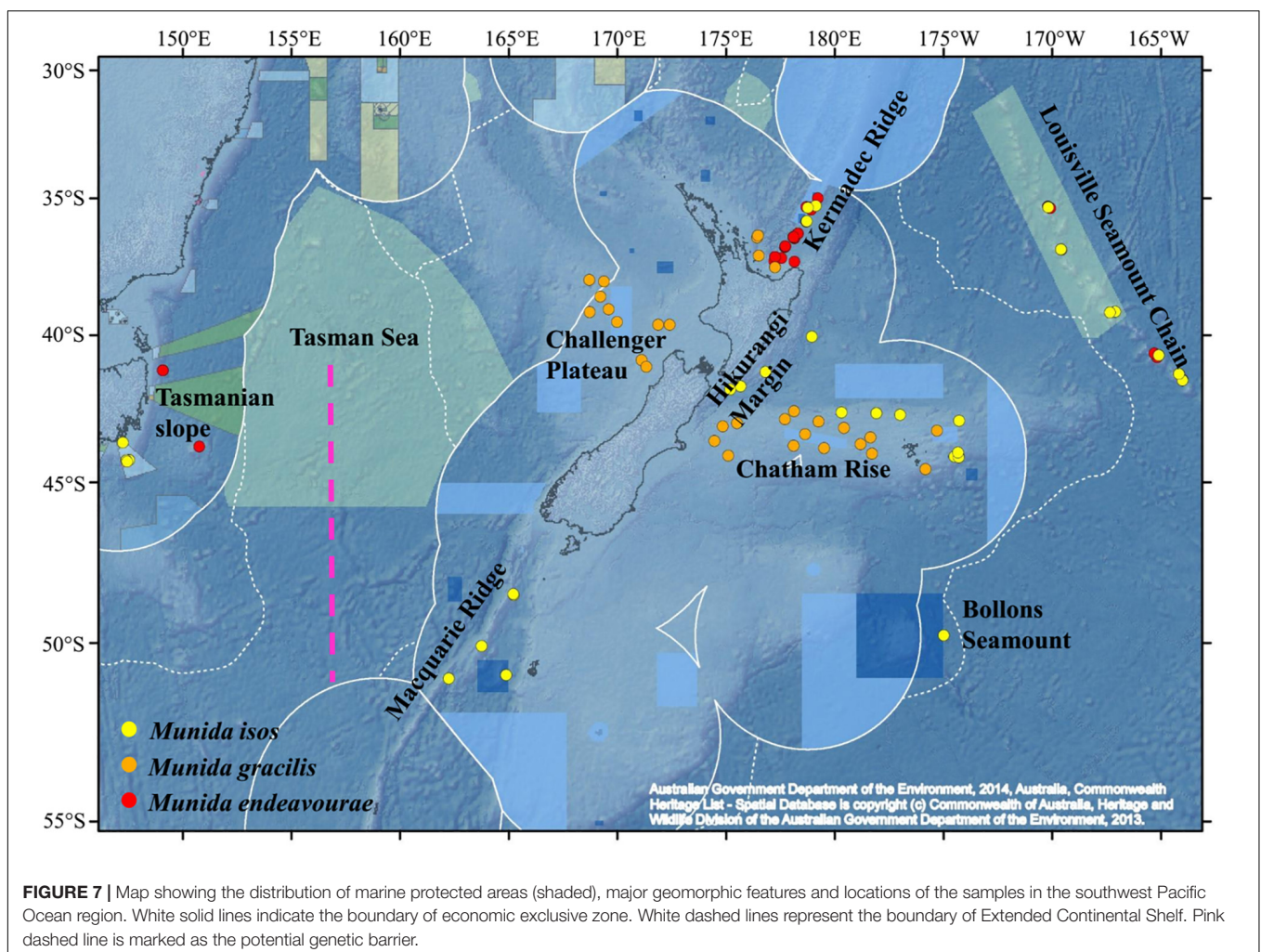
Implications for Conservation and Management

In recent years, VMEs have suffered severe damage from anthropogenic activities at a regional or local scale, as well as from physical environmental changes such as increasing ocean temperature and acidification (Thresher et al., 2015; Gollner et al., 2017). To date, some efforts have been made to lessen human impacts on the VMEs by establishing MPAs that provide a means to restrict human activity for a conservation purpose to protect critical natural habitats, which can help facilitate the protection and restoration of biodiversity and ecosystem function of VMEs (Agardy, 1994; Clark et al., 2011). An understanding of spatially explicit genetic diversity as well as patterns of connectivity within and amongst populations of deep-sea organisms that characterise or are associated with VMEs, is fundamental to inform the management of impacts on VMEs, through assessing the effectiveness of current MPAs and informing the design of the spatial arrangement of new MPAs (Palumbi, 2003; Coleman et al., 2011; Pujolar et al., 2013; Santini et al., 2016).

In 2001, New Zealand implemented a seamount management strategy, with 17 areas (containing 19 seamounts) within New Zealand's EEZ closed to all bottom trawling in order to protect the benthic habitat and communities on seamounts, including VMEs (Brodie and Clark, 2003). Following these seamount closures, in 2007 the New Zealand Government closed 17 benthic protected areas (BPAs) within the EEZ to dredging and bottom trawling (and prohibiting mid-water trawling within 50 m of the seafloor) to protect benthic biodiversity, including VMEs (Helson et al., 2010). As a consequence, 32% of New Zealand's seafloor is now protected from dredging and bottom trawling (but no other form of anthropogenic disturbance), a total area which comprises roughly four times the landmass of New Zealand (Figure 7). In 2007, the United Nations General Assembly Resolution 61/105 on sustainable fisheries (United Nations General Assembly, 2007) appealed to States and regional fisheries management organisations to protect VMEs from significant adverse impacts caused by destructive fishing activities in international waters. Consequently, in the South Pacific Ocean, interim measures to protect VMEs have been adopted by the South Pacific Regional Fisheries Management

Organization (SPRFMO). These measures include 20 min longitude \times 20 min latitude areas closed to bottom trawling, as well as areas open to trawling, some of which include the provision of a 'move-on rule' to avoid damage to VMEs (Penney et al., 2009). To date, the suitability and effectiveness of the BPAs and seamount closures within New Zealand's EEZ, and the SPRFMO interim measures, have been assessed using cost-benefit analyses (Leathwick et al., 2008; Penney and Guinotte, 2013), as well as some limited evaluation using genetic tools and connectivity patterns (e.g. Boschen et al., 2016; Holland et al., 2019).

A key finding of this study is that the relatively high levels of genetic connectivity and the low levels of genetic differentiation reported amongst geographically isolated populations of *Munida* spp. suggests that any damage to a localised site squat lobster population (e.g. natural disturbance such as slumps or man-made disturbance such as fishing or mining) may be counteracted by the high levels of larval connectivity from other sites. Whilst this appears to be true for *Munida* spp. that are associated with the VMEs, the same cannot be said of VME habitat-forming taxa such as corals and sponges (Zeng et al., 2017, 2019).



Squat lobster populations on the Tasmanian slope are located in the 9991 km² Huon Commonwealth Marine Reserve (part of the Southeast Commonwealth Marine Reserve Network) within the Australian EEZ. Three of the sample sites are within the Habitat Protection Zone (IUCN IV), with mining prohibition and commercial fishing needing to be authorised, whilst the fourth site for this population is situated in the Huon-Multiple Use Zone (IUCN VI), where activities such as mining and fishing can occur if authorised (Iftekhar et al., 2014). Thus, the conservation of populations of a VME-associated taxon on the Tasmanian slope is managed. Moreover, the population of *M. isos* in the Huon Marine Park is a potential source population for populations within New Zealand's EEZ, and therefore its protection is helpful for the conservation of the genetic diversity of this species in the New Zealand region.

Squat lobsters from within New Zealand's EEZ and beyond (i.e. at the Louisville Seamount Chain) exhibited high levels of gene flow across New Zealand's EEZ and beyond. Thus, it is essential to have MPAs distributed throughout these regions to reflect and to protect this wide-ranging genetic connectivity. Significantly, it is necessary to separately protect VMEs on the Louisville Seamount Chain, because the impacts of bottom trawling on VMEs in the SPRFMO Convention Area are managed separately from the management of similar impacts in New Zealand's EEZ. Furthermore, the currently closed areas on the Louisville Seamount Chain are part of a broader protection measure that not only distributes VME protection elsewhere in the SPRFMO Convention Area, but also includes limits on VME indicator taxa bycatch (including VME habitat associated taxa).

Ongoing monitoring of genetic diversity and patterns of connectivity within and amongst populations of deep-sea fauna before and after bottom trawling will allow researchers to evaluate the impact of anthropogenic activity on population differentiation and connectivity, and will provide valuable information to inform best practices in management and conservation. Collectively, the results of this study illustrate the importance of considering the international cross-boundary implications of conservation measures when trying to design effective MPA networks (e.g. Miller et al., 2018; van Dover et al., 2018).

DATA AVAILABILITY STATEMENT

The datasets for this study can be found in the NCBI GenBank (<https://www.ncbi.nlm.nih.gov/nucleotide/>).

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AUTHOR CONTRIBUTIONS

AR and JG designed the research project. KS and AR contributed to the collection of the analysed samples. KS and R-JY provided the identifications of the squat lobster species. R-JY and X-ZG conducted the laboratory and statistical analyses. The first draft of the manuscript was written by R-JY. R-JY, KS, AR, and JG contributed to the reworking and editing of the draft manuscript towards the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2019.00791/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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