ICES Journal of Marine Science, 2022, **0**, 1–11
DOI: 10.1093/icesjms/fsac130
Original Article

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Genome-wide SNPs reveal fine-scale genetic structure in ornate spiny lobster *Panulirus ornatus* throughout Indo-West Pacific Ocean

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Genome-wide, single nucleotide polymorphism (SNP) typing can improve the management of valuable marine species by delineating finer scale population structure compared with traditional markers. This approach was applied to the spiny lobster, *Panulirus ornatus* distributed in the Indo-West Pacific and is one of the most highly valuable seafood products in the world. A total of 3008 SNPs was generated from DArTseq sequencing of 224 lobsters sampled at 13 locations across the Indo-Pacific. SNPs revealed a highly significant genetic structure among samples (analysis of molecular variance $F_{ST} = 0.046$). Pairwise genetic comparison showed significant differences among the majority of sampling locations. Outlier loci (including an outlier SNP mapped to the CASc gene with different allele frequencies among sampling locations) revealed highly significant pairwise differentiation, especially a genetic break between regional populations in northern Australia and South East Asia. Significant pairwise differences in outliers among sampling locations, even over small geographic scales, suggest a possible role of local adaptation on the population structure. Genetic differences identified among samples from northern Australia and South East Asia are sufficient to refute the single-stock hypothesis proposed using conventional genetic markers. The results of genome-level SNPs identify five management units across the species' range, with significant implications for the future fisheries management and aquaculture development of this species.

Keywords: Australia, ornate spiny lobster, population genomics, population structure, SNP, South East Asia.

Introduction

Understanding population structure is important in the management of commercial marine species undergoing intensive harvesting, especially where the natural range of the species crosses multiple fisheries jurisdictions (Kough *et al.*, 2013; Garavelli *et al.*, 2018). Studies with mitochondrial DNA (mtDNA) sequence and microsatellite markers have not been able to reveal fine-scale population genetic or stock structure in many marine taxa (e.g. Dao *et al.*, 2015). These genetic markers may have lower power in detecting cryptic population structure, resolving complex connectivity patterns, and detecting differences in genetic diversity (Jeffries *et al.*, 2016). Microsatellite markers have practical limitations for some analyses of population genetic structure (Putman and Carbone, 2014), and mtDNA markers may overestimate (in diverged populations) or underestimate (fine-scale genetic differences) the overall genetic differences among populations (Hurst and Jiggins, 2005). Although resolving the genetic structure in populations of marine organisms is often challenging, genome-wide single nucleotide polymorphisms (SNPs) have successfully been employed with sufficient resolution to detect even weak genetic structure in populations of marine organisms, whereas other markers have not (Woodings *et al.*, 2018). Genome-wide SNP markers discovered by reduced representation sequencing (RADseq, DArTseq, GBS) have resolved fine-scale genetic structure in migratory pelagic fishes (Grewe *et al.*, 2015; Mamoozadeh *et al.*, 2020), bivalves (Lal *et al.*, 2017), American lobster (Benestan *et al.*, 2015), and spiny lobsters with long pelagic larval durations (PLD) (Woodings *et al.*, 2018).

Due to the sizes and complexities of marine invertebrate genomes (Tan *et al.*, 2020), population genomic studies have so far been somewhat limited in this taxonomic group. *Panulirus* spiny lobsters generally have a wide distribution (>5000 km), long PLDs (>4 months), and are presumed to have a high level of connectivity among populations, especially throughout the Indo-Pacific (Dao *et al.*, 2015). Spiny lobsters attract research interest because of their ecological importance, high commercial value and population declines, most often due to overfishing and climate change (Phillips *et al.*, 2013). Although genome-wide SNP studies have been applied to identify genetic stocks in some spiny lobsters (e.g. Woodings *et al.*, 2018), no such studies have yet examined the entire distribution of an Indo-Pacific species. For example, a SNP study of *P. homarus* examined only the periphery of the species' distribution along the coast of Oman, but still showed the existence of population structure (Al-Breiki *et al.*, 2018).

The ornate spiny lobster *P. ornatus* (Fabricius, 1798) occurs in coastal shallow waters and coral reefs ranging from the eastern coast of Africa across the Indian Ocean to the western Pacific Ocean, including northeastern Australia (NEA), and as far north as southern Japan (Dao *et al.*, 2015). Considerable genetic connectivity among populations of this species is believed to be maintained by its 4–6 month pelagic larval stage, which mostly occurs in the open ocean. The semi-enclosed region within South East Asia (SEA) that forms the South China

Received: March 10, 2022. **Revised:** June 20, 2022. **Accepted:** June 20, 2022
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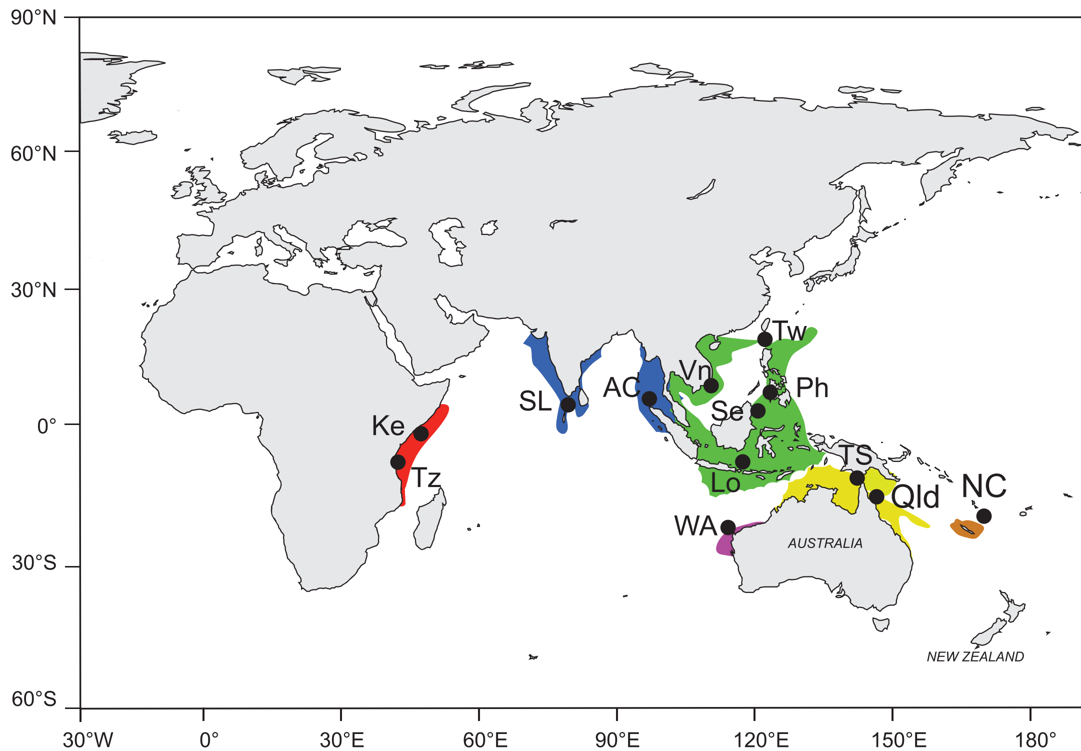

Figure 1. Sampling locations for ornate spiny lobsters in its distribution range in the Indo-Pacific Ocean. The colour shadings represent the managing units proposed for the species in this study. Samples were available from Tanzania (Tz), Kenya (Ke), Sri Lanka (SL), Aceh (AC), Vietnam (Vn), Taiwan (Tw), the Philippines (Ph), Semporna (Se), Lombok (Lo), New Caledonia (NC), Torres Strait (TS), Queensland coast (Qld), and Exmouth Gulf in Western Australia (WA).

Sea basin, is an important area for the fisheries and aquaculture of this lobster species, and has the potential to limit genetic connectivity of populations (Dao *et al.*, 2015). *Panulirus ornatus* is one of the most valuable seafood species in the world per unit, typically fetching over US$150 kg^{-1} in live seafood markets in Asia, with most landings coming from SEA and NEA. The large value of the species has resulted in overharvesting and has encouraged the development of aquaculture production using wild juveniles harvested from coastal areas as seed for ongrowing (Jeffs, 2010; Radhakrishnan *et al.*, 2019). The ongrowing of wild-caught juvenile *P. ornatus* in sea-cages started in the 1990s in Vietnam in response to market value, especially in Chinese live seafood markets (Jones, 2010; Jeffs *et al.*, 2020; Priyambodo *et al.*, 2020). Since that time, production has grown to over 1600 t a year, valued at over US$120 M with millions of seed lobsters harvested and translocated into Vietnam from distant fisheries. These translocations have created concerns about potential population-wide impacts from overharvesting, spread of disease, genetic mixing, and local extinction (Hughes *et al.*, 2003; Dao *et al.*, 2015).

More recently, the development of large-scale hatchery rearing of *P. ornatus* eggs to juveniles has emerged in Australia using local broodstock as an alternative supply of seed lobsters for aquaculture that can be exported into growing nations from Australia (Smith, 2017; Radhakrishnan *et al.*, 2019). Rapidly advancing aquaculture requires careful selection of wild broodstock with sufficient genetic diversity to initiate the breeding programme. Therefore, understanding the degree of divergence among populations can be useful for informing both fisheries management and aquaculture development.

Previous mtDNA and microsatellite studies of *P. ornatus* have revealed genetic structure only over large geographic distances, such as between the western Indian Ocean (WIO) and SEA (Dao *et al.*, 2015; Yellapu *et al.*, 2017). The analysis of outlier SNPs has shown strength in detecting genetic differences among spiny lobster populations previously interpreted as single populations based on microsatellite or mtDNA markers. For example, SNPs revealed distinct genetic differences between New Zealand and Australian populations of *Jasus edwardsii* (Villacorta-Rath *et al.*, 2016) that were previously not readily apparent using microsatellite markers (Morgan *et al.*, 2013; Thomas and Bell, 2013).

This study aimed to determine if the application of high-resolution genome-wide markers would reveal further genetic diversity, genetic structure, and local adaptation, facilitating better management of the species for commercial harvest and aquaculture development. Here, a genome-wide SNP dataset was used to determine population genetic structure of *P. ornatus* in the broader Indo-Pacific, and at a fine scale in the most intensely fished regions of SEA and NEA.

Material and methods

Muscle tissue from the pleopod of ornate spiny lobster specimens was sampled at 12 locations in the Indo-West Pacific, at the western-most extent of the range of *P. ornatus* in Tanzania across to New Caledonia (NC) at the eastern-most extent of the distribution (Figure 1, Table 1). Muscle tissue samples were purchased from local fisheries or donated by research organizations and preserved in 95% ethanol until DNA extraction. The samples (~40 mg) were submitted to Diversity Array

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Table 1. Number of samples sequenced using DArTseq for each location and respective genetic diversity indices.

Location (code)	N	$sMLH$	SD	H_e	SD	$H_{n.b}$	SD	H_o	SD	AR	F_{IS}	g^2	p
Tanzania (Tz)	10	0.990	0.729	0.157	0.156	0.166	0.165	0.118	0.137	1.16	0.300	0.056	1.0
Kenya (Ke)	15	1.120	0.088	0.158	0.149	0.163	0.154	0.139	0.147	1.16	0.151	0.029	1.0
Sri Lanka (SL)	23	0.999	0.036	0.161	0.141	0.165	0.144	0.148	0.140	1.16	0.103	0.004	0.96
Aceh (AC)	4	0.983	0.103	0.134	0.178	0.156	0.209	0.099	0.172	1.14	0.411	0.076	0.96
Vietnam (Vn)*	22	0.998	0.043	0.167	0.139	0.171	0.143	0.156	0.139	1.17	0.091	0.007	0.70
Philippines (Ph)	5	1.000	0.027	0.149	0.170	0.167	0.192	0.136	0.181	1.15	0.208	0.114	0.10
Semporna (Se)	49	1.000	0.027	0.168	0.130	0.171	0.132	0.156	0.127	1.17	0.086	0.001	0.10
Lombok (Lo)	29	0.977	0.162	0.161	0.133	0.164	0.136	0.125	0.111	1.16	0.243	0.070	0.017
New Caledonia (NC)	5	0.9998	0.019	0.150	0.165	0.167	0.184	0.154	0.197	1.16	0.089	0.001	0.16
Torres Strait (TS)	31	0.994	0.083	0.168	0.138	0.170	0.140	0.151	0.133	1.17	0.114	0.015	1.0
Queensland (Qld)	20	0.992	0.094	0.164	0.140	0.168	0.144	0.145	0.136	1.17	0.140	0.020	1.0
Western Australia (WA**)	11	0.998	0.053	0.144	0.164	0.151	0.172	0.145	0.193	1.15	0.04	0.007	1.0
Overall	224	1.000	0.023			0.165	0.044	-		1.16	0.18	0.026	0.97

N: number of individuals sampled in each population, H_E: expected heterozygosity, $H_{n.b}$: sample size corrected expected heterozygosity, H_O: observed heterozygosity, $sMLH$: multilocus heterozygosity averaged on samples and standardized for sample size, AR: allelic richness, F_{IS} inbreeding coefficient, g^2 identity disequilibrium parameter, and SD: standard deviation.
*Two individuals from south of Taiwan pooled with adjacent Vn samples, as there was no genetic difference between these two locations. **Results from the genetic cluster of a subset of seven samples from WA (identified using DAPC): $g^2 = 0.0015$; $p = 0.001$.

Technology (DArT) Pty Ltd Canberra, Australia for DArTseq genotyping. The genomic DNA was extracted from tissue and double digested by using PtsI and HpaII restriction enzymes (RE) at DArT Pty Ltd, Canberra. RE-specific adapter ligated fragments were size selected and pooled for library preparation (each library of 94 samples and two negative controls) and sequenced on Illumina HiSeq2500 platform for 77 cycles (see the technical details in https://www.diversityarrays.com/papers_category/research/).

Raw sequence quality control of reads and SNP calling was carried out using the DArT proprietary pipeline (Lind *et al.*, 2017), and a final genotype matrix was supplied. Further quality control of the raw SNP data was performed in R 3.6.2 (R Core Team, 2020) using the dartR 1.1 (Gruber *et al.*, 2018) to create a final dataset (referred to below as "all SNPs") for population genetics analyses. For this purpose, SNP loci were filtered out stringently using the following criteria: monomorphic, read depth below 5, technical repeatability (consistency of marker in technical replicates) <95%, more than one SNP per tag, non-missing call rates below 90%, or Hamming distance threshold of 0.2. Loci were assessed for deviation from Hardy-Weinberg (HWE, $p < 0.00001$) and linkage disequilibrium (LD, $r^2 = 0.80$) in Plink 1.9 (Chang *et al.*, 2015).

Population genetic diversity indices, including expected (H_E) and observed (H_O) heterozygosity, average sample size corrected expected heterozygosity ($H_{n.b}$), and inbreeding coefficient (F_{IS}) were calculated in Genetix 4.05 (Belkhir *et al.*, 2015). The standard multi-locus heterozygosity ($sMLH$) and identity disequilibrium parameter (g^2) and allelic richness (AR) were estimated using "inbreedR" (Stoffel *et al.*, 2016) with the final filtered set of 3008 SNPs.

The presence of outlier loci was assessed using "OutFLANK" (Whitlock and Lotterhos, 2015) with a q-value of 0.01 and trimming of 5% loci from both ends. An FDIST-like approach (Excoffier *et al.*, 2009) for identifying loci under selection based on F-statistics was implemented in Arlequin 3.5 (Excoffier and Lischer, 2010) with default settings and 100 000 simulations ($p = 0.05$). Furthermore, detection of outlier SNPs using principal component analysis was carried out with the pcadapt package (Luu *et al.*, 2017). The false discovery rate (FDR) correction was used to identify outlier loci. Outlier loci identified by all three methods (shared outliers) were used for genetic structure analysis and clustering. There were thus three final data sets: "all SNPs", "shared outliers", and "putatively neutral SNPs" (with all outliers removed). The shared outlier loci were blasted against the annotated transcriptome of *P. ornatus* (E-value threshold of 1×10^{-6}, Accession number: PRJNA664650, CrustyBase, Hyde *et al.*, 2020). The allele frequency of outlier loci with matches in CrustyBase was visualized for each sampling location in R. All SNPs were used in a redundancy analysis (RDA) to identify putative adaptive loci in association with maximum and minimum sea surface temperatures (SST_max or SST_min) at sampling locations in "vegan" (Oksanen *et al.*, 2020).

Analysis of molecular variance (AMOVA) among all sampling locations and Weir and Cockerham (1984) unbiased pairwise F_{ST} and associated p-values were estimated in StAMPP package (Pembleton *et al.*, 2013) for the all SNPs, putative neutral loci and outlier loci using 10 000 bootstraps, with FDR adjustment of the p-values. A Mantel test was carried out with the dartR gl.ibd function and 10 000 permutations on a comparison of the pairwise genetic distance matrix between locations of the whole SNPs dataset and the geographic distance matrix (converted from latitude and longitude of sampling locations by "geodist") (Karney, 2013).

The genetic structure of populations was inferred using discriminant analysis of principal components (DAPC) and admixture analyses. DAPC was performed in adegenet package (Jombart and Ahmed, 2011) to visualize relationships in multivariate space, either with or without estimating the optimal number of clusters and with the number of principal components retained using a-score approach. Population structure analysis was undertaken with the program ADMIXTURE (Alexander *et al.*, 2009) with cross validation (CV) enabled for $K = 1$ to $K = 15$ populations, with five iterations each, to find the optimal K. A second run with 10 replicates was used to narrow find the optimal number of clusters with the smallest CV errors. Clusters were visualized as bar-plots in R. Population clustering was also carried out in the "LEA" (Frichot and François, 2015). Phylogeographic relationships among all individuals were illustrated as a minimum spanning network (MSN) with "poppr" (Kamvar *et al.*, 2014) using bitwise distance estimated form allele frequency of the 3008 SNPs with 10 000 bootstrap replicates. The relative asymmetric

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Table 2. Pairwise F_{ST} values among populations of ornate spiny lobster.

	Tz	Ke	SL	AC	Vn	Ph	Se	Lo	NC	TS	Qld	WA
Tz	0	0.009	**0.126**	0.041	**0.182**	**0.113**	**0.195**	**0.178**	**0.177**	**0.227**	**0.200**	**0.283**
Ke	**0.012**	0	**0.133**	**0.094**	**0.235**	**0.149**	**0.256**	**0.234**	**0.238**	**0.287**	**0.255**	**0.325**
SL	**0.017**	**0.016**	0	0.001	**0.072**	−0.019	**0.067**	**0.054**	**0.155**	**0.247**	**0.203**	**0.296**
AC	0.002	**0.028**	**0.028**	0	0.0128	−0.016	**0.047**	−0.000	**0.091**	**0.214**	**0.166**	**0.172**
Vn*	**0.018**	**0.023**	**0.008**	**0.022**	0	0.0313	**0.016**	**0.005**	**0.103**	**0.216**	**0.179**	**0.300**
Ph	**0.011**	**0.027**	**0.012**	0.004	0.0029	0	0.015	−0.021	**0.115**	**0.209**	**0158**	**0.176**
Se	**0.021**	**0.025**	**0.0089**	**0.025**	0.0004	**0.004**	0	0.010	**0.120**	**0.234**	**0.194**	**0.343**
Lo	**0.019**	**0.024**	**0.0086**	**0.017**	0.0006	0.003	0.002	0	**0.086**	**0.219**	**0.179**	**0.297**
NC	**0.013**	**0.030**	**0.014**	0.015	**0.004**	0.005	**0.005**	−0.0005	0	**0.038**	**0.034**	**0.219**
TS	**0.028**	**0.033**	**0.018**	**0.030**	**0.007**	**0.012**	**0.007**	**0.008**	0.003	0	**0.011**	**0.370**
Qld	**0.026**	**0.032**	**0.015**	**0.025**	**0.006**	**0.008**	**0.006**	**0.004**	0.003	**0.003**	0	**0.315**
WA	**0.070**	**0.077**	**0.061**	**0.082**	**0.053**	**0.067**	**0.050**	**0.050**	**0.069**	**0.058**	**0.060**	0

*Two individuals from south of Taiwan pooled with adjacent Vn samples, as there was no genetic difference between these two locations. Above diagonal: from 51 shared outlier loci. Below diagonal: from the whole 3008 SNPs dataset. Significant values ($p < 0.01$) are shown in bold.

migration rates among populations were examined by the divMigrate function of "diveRsity" (Sundqvist *et al.*, 2016) in R from allele frequency data with 10 000 bootstraps.

Results

After filtering, 3008 SNPs were retained for 224 lobsters (Supplementary Table S1). A total of 22 individuals were removed during genotyping and filtering due to large proportions of missing genotypes. The H_e of populations ranged from 0.134 in Aceh (AC) to 0.168 in Semporna (Se), which had the smallest ($n = 4$) and largest ($n = 49$) samples size, respectively (Table 1). Hence, to improve comparison of the data, we also present $H_{n.b}$ index as an average expected heterozygosity corrected for sample size, which shows a similar but less divergent pattern. The H_o values ranged from 0.09 in AC to 0.156 in Se, and was greater than $H_{n.b}$, except for samples from Western Australia (WA) and NC. *sMLH* had the smallest values for AC (0.98) and largest value for Kenya (1.20). None of these H measures revealed a significant difference between sample locations. The largest and smallest values of F_{IS} were observed in AC (0.41) and Semporna (0.086), respectively, all significantly greater than zero. This dataset showed significant deviation of g^2 from zero for seven distinct samples from WA as a separate cluster ($g^2 = 0.001$; $p = 0.001$).

The AMOVA test on the whole SNP dataset showed significant genetic structure ($F_{ST} = 0.046$, $p < 0.0001$) among sampling locations. Pairwise F_{ST} varied from −0.0005 between NC and Lo to 0.082 between AC and WA (Table 2). WA showed a pronounced pairwise significant difference from all other locations. The second largest regional differentiation based on pairwise F_{ST} was observed between the WIO locations (Tz and Ke) and other locations. There were lower levels of divergence among the SEA locations (Vn, Ph, Se, and Lo) and among the NEA locations (TS and Qld) and NC. The pattern of population difference, especially at the regional scale, was observable in the MSN network constructed using all SNP allele frequencies (Supplementary Figure S1). In total, 408 Outlier loci were detected, including 258 loci by Arlequin, 221 loci with pcadapt, and 51 loci with OutFLANK (Supplementary Table S3). There were 51 loci shared between the Arlequin and OutFLANK methods, with 17 loci shared among all three methods, including PcAdapt.

AMOVA of neutral loci (all outlier loci excluded) showed weaker, but still significant genetic structure ($F_{ST} = 0.019$, $p < 0.0001$). Although pairwise F_{ST} values with neutral loci were still largely significant among the three major regions (WIO, SEA, and NEA), in comparisons to between locations within regions, which had much smaller, frequently non-significant, values, especially in the central SEA distribution range (Vn, Ph, Se, and Lo) (Supplementary Table S2). The Mantel test comparing genetic and geographic distances did not show a significant pattern of isolation-by-distance among sampling locations for the whole SNP dataset ($r = 0.145$, $p = 0.236$).

The global F_{ST} from our AMOVA analysis on 51 shared outlier loci showed a more pronounced overall differentiation ($F_{ST} = 0.331$, $p < 0.0001$) and pairwise differences between sampled locations. The outliers differed significantly among almost all sampling locations except largely for locations with low sample size (AC and Ph) (Table 2). The significant pairwise difference ($F_{ST} = 0.012$) between Tz and Ke within the all SNPs was not observed with outlier SNPs.

The DAPC plot based on all SNPs (Figure 2a) showed five main population clusters including WIO (Tz and Ke), Central Indian Ocean (CIO: SL and AC), SEA (Vn, Ph, Se, and Lo), WA, and NEA (TS and Qld) plus NC (colour shades in Figure 1). The NC cluster was somewhat intermediate to both SEA and NEA locations, whereas the CIO samples were intermediate to both WIO and SEA. The DAPC based on shared outlier loci showed a similar pattern of genetic relationships among locations, except there was a comparatively greater differentiation of the NEA samples (shown in dimension 1) and less differentiation among the other regions (Figure 2b). When optimizing genetic groupings regardless of sampling location, DAPC showed four optimal clusters for all SNPs and six clusters for the outlier SNPs (Supplementary Figure S2). Seven of the eleven samples from WA formed a distinct cluster for both all SNPs and outlier analyses. The majority of Tz and Ke samples in WIO also grouped together in both datasets.

In the admixture plot from the all SNPs dataset (optimal number of K was 4), the same regional groupings were largely evident (Figure 3a). It was also apparent that the CIO sample from SL comprised individuals that appeared to be an admixture of the WIO and SEA clusters. The major difference in the admixture plot from outlier loci was that the NEA samples formed a largely distinct cluster (Figure 3b, $K = 3$). This

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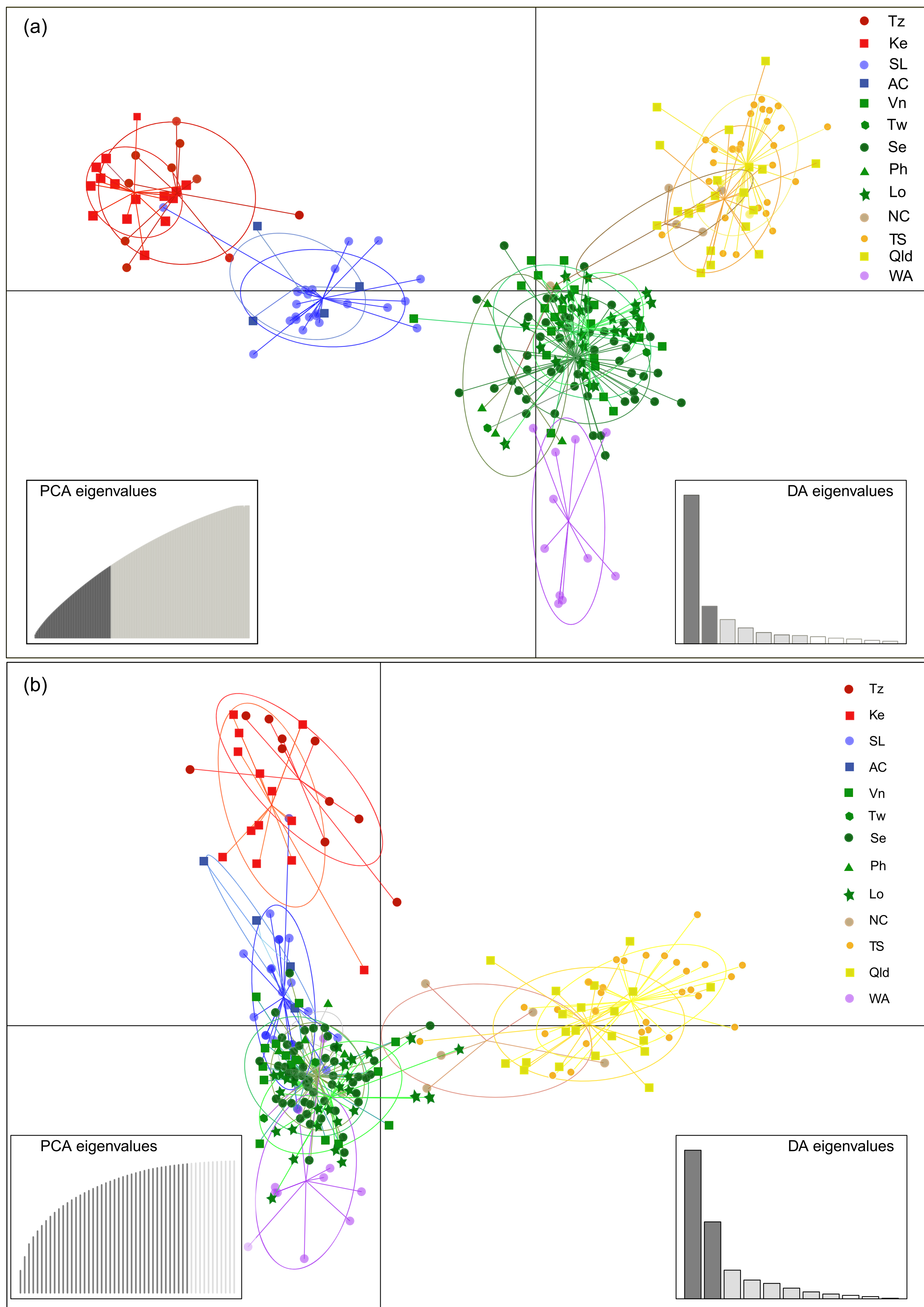

Figure 2. DAPC scatter plot of *P. ornatus* genetic structure using (a) the all SNPs dataset and (b) with 51 outlier SNPs. The samples have been grouped based on sampling locations. Abbreviations; Tz: Tanzania, Ke: Kenya, SL: Sri Lanka, AC: Aceh, Vn: Vietnam, Ph: Philippines, Se: Semporna, Lo: Lombok, NC: New Caledonia, TS: Torres Strait, Qld: Queensland, and WA: Western Australia.

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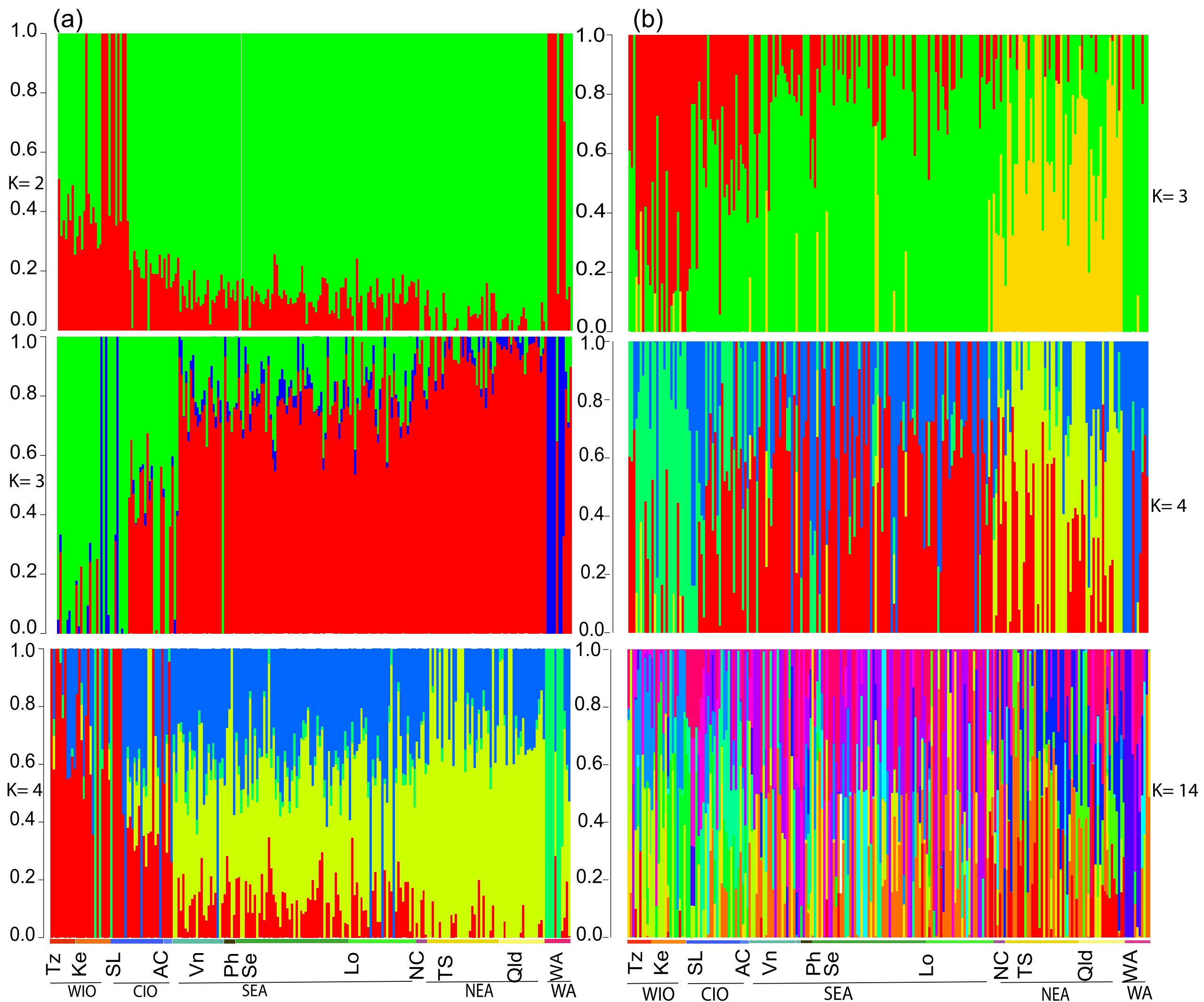

Figure 3. Barplot of the admixture analysis for (a) the all SNPs dataset with results for $K = 2$, $K = 3$, and $K = 4$ (optimal K) and (b) the outlier loci dataset with $K = 3$, $K = 4$, and $K = 14$ (optimal K).

clustering pattern was far more evident in the outlier loci than all SNPs, revealing three clusters, WIO, NEA, and the remainder of sampling locations. The WA cluster appeared at $K = 4$ with outlier loci. Although the optimal number of clusters with outlier loci was $K = 14$, the pattern of these 14 clusters was not related to sampling location. Similar patterns were observed with LEA sNMF clustering (Supplementary Figure S3). Migration analysis (Supplementary Figure S4) showed similar patterns, in particular a strong limitation in gene flow with the WA population. There were also relatively low levels of gene flow with the Tz, NC, AC, and Ph populations.

The RDA analysis did not show any significant correlation between SST (SST_max, SST_min) and the outlier SNPs. The BLAST analysis of the 17 shared outlier loci from *P. ornatus* gave two hits: pyruvate: ferredoxin (Flavodoxin) oxidoreductase family gene and CASc gene with possible roles in metabolism and the immune system, respectively. The allele frequencies of the SNP mapping to the CASc gene were highly associated with the two NEA locations (Supplementary Figure S5), which had previously been highlighted above in the outlier admixture analysis.

Discussion

This study was the first to use a genome-wide SNPs dataset for samples obtained across the entire distribution of the economically important spiny lobster *P. ornatus*. The genetic diversity, broad- and fine-scale genetic structure were investigated using DArTseq™ SNP genotyping. In addition to genetic differences at a regional broad-scale, fine-scale genetic structure was also observed between SEA and NEA, contrary to previous studies undertaken using microsatellite and mtDNA markers (Dao *et al.*, 2015; Yellapu *et al.*, 2017). Furthermore, the potential outlier loci suggested local adaptation may be contributing to the observed population structure.

Small overall observed heterozygosity and large F_{IS} values observed in some locations may indicate inbreeding. The distinct genetic cluster of seven individuals from WA identified by DAPC displayed a significant deviation of g^2 values from zero, supporting possible inbreeding in the isolated WA population. F_{IS} values observed for *P. ornatus* were similar to those observed in populations of other crustaceans, such as *P. homarus* (Al-Breiki *et al.*, 2018), *P. cygnus* (Kennington *et al.*, 2013), and *Portunus pelagicus* (Dang *et al.*, 2019). Methodological

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aspects such as null alleles, relatively small sample size (in the case of AC, Ph, and Ke) and possible genotyping error can also result in large F_{IS} values. The genome-wide heterozygosity (*sMLH*, as a better measure of genome-wide diversity) in *P. ornatus* was greater than those observed in the congener *P. homarus* based on a dataset covering a much smaller geographic range on the coast of Oman (Al-Breiki *et al.*, 2018). The observed levels of genetic diversity should be sufficient for selection of wild broodstock for aquaculture locally, but large F_{IS} values and low levels of genetic diversity in Aceh and WA, may indicate small local population sizes, potentially a result of fisheries pressure. Further investigation with larger sample sizes for each location is warranted.

Broad-scale genetic structure

Population genetic analyses of genome-wide SNPs revealed five main groupings for ornate spiny lobster composed of WIO, CIO, WA, SEA, and NEA. Population structure throughout the Indo-West Pacific has been identified in both the spiny lobster *P. penicillatus* (Abdullah *et al.*, 2014) using mtDNA markers, and in the giant black tiger shrimp (*Penaeus monodon*) using genome-wide SNPs with both neutral and outlier loci (Vu *et al.*, 2021). In the current study, SNPs identified distinct genetic clusters for WIO, CIO, SEA, and the Coral Sea. The WIO genetic break in *P. ornatus* is not surprising since it has been observed in several studies across different taxa (spiny lobster: Truelove *et al.*, 2015; Farhadi *et al.*, 2017; striped marlin: Mamoozadeh *et al.*, 2020; shrimp: Vu *et al.*, 2021) even at subspecies level (Lavery *et al.*, 2014). Populations in the centre of the species distribution (SEA) showed some genetic divergence but did not form distinct genetic clusters. With neutral loci, these populations showed weak or no genetic structure (Supplementary Table S2). Genetic divergences of peripheral Indo-West Pacific populations have been observed in the congener species *P. homarus* (Farhadi *et al.*, 2017) and *P. penicillatus* (Iacchei *et al.*, 2016). This peripheral divergence was not as pronounced in *P. ornatus*, although this species does not have as wide a distribution as the other two. The genetic divergence of the samples from WA and WIO (Tanzania and Kenya) in the peripheries of the *P. ornatus* distribution was previously supported by mtDNA data (Yellapu *et al.*, 2017) and confirmed with the genome-wide SNP data, but with the smaller pairwise F_{ST} values here. It is of relevance that the CIO population of SL appears to contain an admixture of genetic material from both its western (WIO) and eastern (SEA) neighbouring regions. This is shown both in the admixture analyses (Figure 3) and in the network analysis (Supplementary Figure S1).

Discordance in the magnitude of regional genetic divergence as measured by mtDNA compared to the nuclear genome has been well documented in the congener species *P. homarus* (Farhadi *et al.*, 2017). A larger degree of genetic isolation in mtDNA can be related to its larger mutation rate, smaller effective population size (Hurst and Jiggins, 2005), or a recent change in gene flow pattern (Toews and Brelsford, 2012) with secondary contact between differentiated populations. As mtDNA sequences may retain a much greater amount of phylogenetic signal than SNP allele frequencies, the mtDNA data may reflect a historical pattern of relationships among populations, whereas the SNP data may better reflect contemporary patterns of gene flow (Lavery *et al.*, 2014). Recent gene flow change due to demographic expansion or a reduction in a dispersal barrier (e.g. due to a change in ocean currents), resulting in secondary contact, selective sweeps (Toews and Brelsford, 2012), or perhaps a larger nuclear effective population size slowing down the population divergence (Yellapu *et al.*, 2017) are possible explanations of such a discord.

Local adaptation may also contribute to genetic differences among populations of *P. ornatus*. The most distinctive result from the analysis of outlier loci was the much more prominent genetic clustering of NEA locations (TS and Qld) as separate from the adjacent SEA populations. Although this distinct regional grouping was suggested by the all SNPs, the outlier loci revealed a much stronger pattern, particularly in the admixture analyses. This may perhaps be driven via some adaptive processes, suggested by the strong correlation with allele frequencies in at least one locus. The much larger pairwise differentiation values from the outlier SNPs compared to all SNPs and neutral SNPs may indicate the potential existence of selection and local adaptation, despite relatively large gene-flow at the smaller scale. Although the presence of genetic differences at such small scales seems paradoxical for a species with a 4–6 month larval period (Smith *et al.*, 2009), the outlier SNPs have shown differences within a population of *P. homarus* with a similar larval period when sampled over only a 100 km distance off the Omani coast (Al-Breiki *et al.*, 2018).

This suggests that local adaptation may play an important role for this species as has been observed in other crustacean populations with large distribution ranges. A population genetic study on black tiger shrimp using genome wide SNPs found the genetic structure of the population throughout the Indo-West Pacific was related to both geographic isolation and localized environmental factors (Vu *et al.*, 2020). Outlier loci have also detected significant genetic structure in other spiny lobsters species, such as *J. edwardsii* among locations in New Zealand and Australia (Villacorta-Rath *et al.*, 2016) and in *Sagmariasus verreauxi* (Woodings *et al.*, 2018), with both lobster species having much longer larval periods than *P. ornatus* (Phillips *et al.*, 2006; Jeffs *et al.*, 2013).

Fine-scale genetic structure

Our data revealed significant genetic differences between sampling locations of *P. ornatus* in the eastern Indian Ocean and West Pacific, and we observed fine-scale genetic differences between locations in NEA (Torres Strait and Queensland) and the adjacent four locations sampled in SEA. Outlier loci also showed genetic differences between Queensland and Torres Strait, as well as among some sampling locations within SEA (Table 2), but these were not supported by admixture analysis. Previous research using mtDNA and microsatellites markers indicate a single genetic stock in this entire sampling area (Dao *et al.*, 2015; Yellapu *et al.*, 2017).

A pattern of genetic divergence between NEA and SEA has not previously been reported in species with a long larval period. However, the pattern is similar to that observed in two grouper species by mtDNA and microsatellite data, which showed significant differences between samples taken from Australian waters in Queensland and/or Torres Strait and those from Bali and the Philippines (Ma *et al.*, 2018). An east–west division has been reported for migratory Spanish mackerel (*Scomberomorus commerson*) along the Wallace Line (SEA and Northern Australia), consistent with our findings for *P. ornatus* (Sulaiman and Ovenden, 2010). Small but

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significant genetic differences have also been observed in shark species between Java and NEA (Ovenden *et al*., 2009). Other examples include the gastropod, *Haliotis asinine* (Jeffrey *et al*., 2006), and the seagrass, *Thalassia hemprichii* (Hernawan *et al*., 2017) with similar patterns, supporting the idea of historical demographic processes explaining the present genetic pattern. An SNP study of the pearl oyster, *Pinctada margaritifera*, showed significant genetic differences between samples from the Great Barrier Reef of Australia and Manado in North Sulawesi, Indonesia (Lal *et al*., 2017). A possible reason for the presence of a genetic break between NEA and SEA may be a strong retention of *P. ornatus* phyllosoma larvae within the strong Coral Sea Gyre, and the limited outflow of water from the South China Sea into the Pacific and Indian Oceans (Dennis *et al*., 2001; Dao *et al*., 2015).

The overall population genetic structure and migration pattern analysis revealed by SNPs suggest that recruitment for all populations of *P. ornatus* involves a greater degree of self-seeding at the regional scale and a lesser degree of broad connectivity than might be expected given the lengthy pelagic period (4–6 months) and the oceanic larval development in this species.

In *P. ornatus*, population differentiation tests using outlier loci and pairwise F_{ST} showed weak but significant differences among locations in SEA, including the island of Lombok in Indonesia, Vietnam, and Philippines. Moreover, these pairwise genetic distances were not aligned with Euclidean geographic distances, suggesting it was not simply geographic isolation driving these differences. Genetic differences within SEA have been observed in other marine organisms such as the giant clam, *Tridacna crocea* (Kochzius and Nuryanto, 2008), clownfish (Timm and Kochzius, 2008), and the seagrass, *Enhalus acoroides* (Putra *et al*., 2018). The small genetic divergence observed here between NEA vs. NC with outlier SNPs is in line with the results from microsatellite loci in a previous study (Yellapu, 2016). However, these were based on small sample sizes, and further sampling is required to test whether *P. ornatus* in NC is significantly different from populations in NEA (Qld and TS).

The 14 optimal genetic clusters ($K = 14$) identified in the outlier SNPs highlights the patchy genetic heterogeneity of ornate spiny lobster. It appears that there are currently two genetic clusters within the WA sample: a genetically unique cluster of seven individuals, and four individuals genetically similar to nearby SEA individuals, which could be due to recent dispersal. These same four individuals were also genetically discriminated from a distinct WA cluster using mtDNA data (Yellapu *et al*., 2017). Divergence of a peripheral local population, despite some migration, has also been observed in *P. homarus*. Possible causes include a difference in spawning time through local adaptation, or simply patchiness (as observed in south Australia for the *J. edwardsii* by Villacorta-Rath *et al*., 2016). This small population also deserves further investigation.

Fisheries and aquaculture implications

The use of genome-wide SNPs provided greater statistical power for revealing patterns of genetic variation across the distribution of *P. ornatus*. The results confirm the existence of multiple genetic stocks of the species throughout its range, including a break in genetic connectivity of the stocks between NEA and SEA. Genetic differences are sufficient to consider the implementation of management units for the WIO, CIO, WA, SEA, and NEA (including Torres Strait and Queensland, also likely the Gulf of Papua). Moreover, the observed small genetic differences of the Sumatra region (AC) and the most eastern stock in NC should be clarified by accessing further samples. Additionally, strong limitation in gene flow observed from and towards the WA population can be an indication of the long-distance translocation of individuals for aquaculture practice.

A nucleotide BLAST search for the outlier SNPs in this study returned few matches due to the lack of a well-annotated genomic resource. However, the SNP identified in an immune response-related gene may reveal an adaptive response of the population to pathogens that may play a role in the survival of ornate spiny lobsters at a regional scale. The identified SNP in the CASc (Caspase) gene has a role in modulating the immune system. For example, it provides antiviral activity against white spot syndrome virus infection in crayfish, *Cherax quadricarinatus* (Li *et al*., 2019). Viral disease has also been reported in *Panulirus,* such as the deadly *P. argus* Virus 1 (PaV1), which infects *P. argus* in the Caribbean and appears to have spread through the population via larval transfer (Kough *et al*., 2015). Likewise, large losses of aquaculture lobsters due to disease in Vietnam (Petersen and Phuong, 2010; Nur, 2018; Jones *et al*., 2019) may be exacerbated by long distance importation of wild seed with insufficient genetic resistance to pathogens encountered in Vietnamese waters. Therefore, the selection of wild broodstock for breeding programmes for aquaculture should carefully consider the genetic source, to take advantage of both local adaptation, and the potential benefits of cross-breeding.

Conclusions

Our study showed the broadscale genetic structure throughout the distribution of the ornate spiny lobster, and also revealed fine-scale genetic structure among SEA sampling locations, NEA and NC. Based on this population genetic structure, at least five fisheries management units are suggested. We also propose potential management units in NC and even within SEA, which require confirmation by further sampling in the region. There is some evidence that the local adaptation has contributed to shaping the population structure of *P. ornatus*. Selective breeding programmes for aquaculture stocks should consider the outlier loci for candidate gene discovery, as they may yield distinct phenotypic advantages, such as improved resistance to pathogens.

Data availability

The filtered SNP dataset is available in STRUCTURE file format as Supplementary file S2.

Ethics Statement

All samples were collected in line with local and international fisheries management, marine protected area controls, and corresponding regulations. *Panulirus ornatus* is not an endangered or protected species. This research was fully compliant with the European Union's regulation under Directive 2010/63/EU on the protection of animals used for scientific purposes.

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Supplementary Data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

Conflict of interest statement

The authors declare no potential competing interest of interest.

Funding

This work was supported by the Australian Research Council's Research Hub for Sustainable Onshore Lobster Aquaculture [grant number IH190100014].

Author contributions

AF: conceptualization, methodology, data analysis, and writing–draft preparation, reviewing, and editing. FP: data analysis and writing–reviewing and editing. BY: sample collection and methodology. SL: writing–reviewing and editing. AJ: conceptualization, funding acquisition, sampling collection, and writing–reviewing and editing.

Acknowledgements

The authors would like thank Victor Estilo (University of the Philippines), Julius Sarria (Darden Aquasciences), Matt Slater (Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research), Nguyen Thi Bich Ngoc (Research Institute for Aquaculture, Vietnam), Matthew Johnston (University of Western Australia), Tin-Yam Chan (National Taiwan Ocean University), Stephen Battaglene (University of Tasmania), Sirajudeen Mohammed Raffi (Annamalai University), and Sharizal Shaarani (UTAS Nexus Aquasciences Pty Ltd) for their assistance in obtaining samples. They express their gratitude towards Stewart Grant for the constructive revision and comments.

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Handling Editor: William Grant

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