

RESEARCH ARTICLE

Population genetic differentiation of the hydrothermal vent crab *Austinograea alayseae* (Crustacea: Bythograeidae) in the Southwest Pacific Ocean

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Abstract

To understand the origin, migration, and distribution of organisms across disjunct deep-sea vent habitats, previous studies have documented the population genetic structures of widely distributed fauna, such as gastropods, bivalves, barnacles, and squat lobsters. However, a limited number of investigations has been conducted in the Southwest Pacific Ocean, and many questions remain. In this study, we determined the population structure of the bythograeid crab *Austinograea alayseae* from three adjacent vent systems (Manus Basin, North Fiji Basin, and Tonga Arc) in the Southwest Pacific Ocean using the sequences of two mitochondrial genes (*COI* and *16S rDNA*) and one nuclear gene (*28S rDNA*). Populations were divided into a Manus clade and a North Fiji–Tonga clade, with sequence divergence values in the middle of the barcoding gap for bythograeids. We inferred that hydrographic and/or physical barriers act on the gene flow of *A. alayseae* between the Manus and North Fiji basins. *Austinograea alayseae* individuals interact freely between the North Fiji Basin and the Lau Basin (Tonga Arc). Although further studies of genetic differentiation over a geological time scale, life-history attributes, and genome-based population genetics are needed to improve our understanding of the evolutionary history of *A. alayseae*, our results contribute to elucidating the phylogeny, evolution, and biogeography of bythograeids.

Introduction

Hydrothermal vent environments are characterized by a lack of light (aside from that generated by high-temperature fluids [1]), lack of photosynthesis, high pressure, steep temperature gradients, and high levels of metals and dissolved gases [2–4]. Since the discovery of hydrothermal vents along the Galapagos Rift in 1977, the description of new species has progressed

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steadily, and new hydrothermal vent sites have been discovered in deep areas of the seafloor. Seven hundred valid species had been described by 2011, and description of additional species continues [5]. Phylogenetic evidence based on molecular markers generally supports traditional taxonomy, and sometimes reveals the existence of sibling (or cryptic) species [6–8]. However, the origin, migration, and distribution of vent fauna across disjunct vent habitats that may undergo repetitive creation and extinction cycles at a time scale similar to the generation times of vent species are not yet fully understood.

Hydrological and physical processes are the most important factors leading to allopatric speciation in the deep-sea [9–11]. Additionally, in hydrothermal vent ecosystems, the continuity or discontinuity of ocean circulation, tectonic history, seawater temperature, and hydrothermal fluid components strongly influence the biogeographic distribution of vent fauna [12]. Based on biogeographic relationships, genetic pools, phylogenetic analyses of vent species, and larval dispersal modeling, approximately 700 known volcanic areas on the deep-sea floor can be clustered into 11 biogeographic provinces [13–15]. Previous genetic studies have noted that the populations of species in vent fields within back-arc basins are well connected, although basin-to-basin transport is impeded by dispersal barriers and directionality [7, 15, 16].

Large hydrothermal vent areas in the Southwest Pacific Ocean are defined as belonging to the single “South-West Pacific Area” biogeographic province, which consists primarily of the Manus Basin, the North Fiji Basin, and the Lau/Tonga region [13, 14]. Although the vent communities in the three regions are visually similar, biogeographic relations indicate that the North Fiji Basin and Lau/Tonga regions are most closely related [17]. Additionally, based on previous geological studies, volcanic areas spanning from North Fiji to Lau/Tonga have been proposed to originate from the disruption of a single arc [11, 18, 19]. To elucidate the genetic flows and distribution patterns of hydrothermal vent fauna among basins, the biodiversity and population genetic structures must be understood at a large spatial scale. To date, investigations in these areas are less well documented and many questions remain [15, 20, 21] (Table 1).

The distribution of the decapod family Bythograeidae Williams, 1980, is restricted to hydrothermal vents (or in close proximity to vents), and these species are typically considered to be omnivorous predators in back-arc basin vent communities [17, 26, 27]. Based on morphological taxonomy and molecular phylogenetics, this family is recognized as a sister taxon of the superfamily Xanthoidea, containing 16 species in six genera, including two recently described *Austinograea* species [28, 29–33]. Most bythograeid species occur in the Pacific Ocean; only two species, *Austinograea rodriguezensis* (Indian Ocean) and *Segonzacia mesatlantica* (Atlantic Ocean), are known outside of the Pacific. In particular, all species in the genus *Austinograea* except *A. rodriguezensis* colonize hydrothermal vents in the western Pacific Ocean [17, 33, 34]. The factors that drove the accelerated adaptive radiation and speciation of *Austinograea* in the Pacific Ocean remain unknown. The examination of phylogenetic relationships among *Austinograea* species could help to clarify the interaction between biological evolution and geographical processes at deep-sea hydrothermal vents. Population genetic analyses could contribute to our understanding of the larval dispersal capacity [35–37], but genetic analysis of bythograeids at the population level has been performed in only one species, *A. rodriguezensis*.

Austinograea alayseae Guinot, 1990, is distributed widely in hydrothermal vent fields from Manus to Lau/Tonga in the Southwest Pacific Ocean [17, 34, 38]. The origin, migration, adaptation, and genetic structure of this species in the Southwest Pacific Ocean are unclear, despite previous studies of its phylogeny using DNA barcoding and mitochondrial genomics [17, 34, 39]. In this study, we obtained two mitochondrial sequences and one nuclear gene sequence of *A. alayseae* collected from hydrothermal vents in the Manus and North Fiji basins and the Tonga Arc, and confirmed the phylogenetic position of this species within the Bythograeidae.

Table 1. Summary of the population structure of hydrothermal vent organisms living in the Southwest Pacific Ocean. Different shade indicates population differentiation based on *cytochrome oxidase subunit I (COI)* sequences of individuals from distinct geographical regions.

Phylum	Species	Region			References
		Manus	N. Fiji	Tonga/Lau	
Arthropoda	<i>Munidopsis lauensis</i>		-		[7]
	<i>Chorocaris</i> sp. 2			-	[7]
	<i>Eochionelasmus ohtai</i>		-	-	[22]
Mollusca	<i>Vulcanolepas</i> cf. <i>parensis</i>		-	-	[22]
	<i>Olgasolaris tollmanni</i>		-		[23]
	<i>Bathymodiolus manuensis</i>		-	-	[24]
	<i>Ifremeria nautilei</i>				[16, 25]

-, indicates unavailable or undiscovered samples or no data.

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We also identified population genetic divergence and migration events of *A. alayseae*, and discuss the biogeographic connections among these three vent regions in the Southwest Pacific Ocean.

Materials and methods

Ethics statement

Permission for sampling in Fiji's Exclusive Economic Zone (EEZ) was granted by the Ministry of Land and Natural Resources, Republic of Fiji, to KIOST Minerals Limited, Korea Institute of Ocean Science and Technology. The permit for the collection of biological samples in Tonga's EEZ was issued by the Minister for Lands and Natural Resources, Kingdom of Tonga, to KIOST Minerals Limited, Korea Institute of Ocean Science and Technology. The license for exclusive exploration in the Manus Basin was granted by the government of Papua New Guinea to Nautilus Minerals, and biological samples were loaned to Duke University for scientific research.

Vent crab sampling and identification

Specimens of bythograeid crabs were collected using suction samplers mounted on remotely operated vehicles from twelve vent sites in the Manus Basin, the North Fiji Basin, and the Tonga Arc of the Southwest Pacific Ocean (Fig 1; Table 2). On board, all specimens were immediately preserved in 95% ethanol or stored at -80°C until genetic analysis. The specimens were identified as *A. alayseae*, *A. hourdezi*, and *Gandalfus puia* on the basis of the morphological characteristics and *cytochrome oxidase subunit I (COI)* DNA barcodes, following the methods used in previous studies [33, 39, 40]. Detailed information about the specimens is provided in S1 Table.

DNA extraction, PCR amplification, and sequencing

A microscopic section of muscle tissue was dissected from a pereopod of each specimen for DNA extraction. Total genomic DNA was extracted using the RED Extract-N-Amp PCR Kit (Sigma-Aldrich Co., Brooklyn, NY, USA) [41]. The partial sequences of two mitochondrial genes (*COI* and *16S rDNA*) and one nuclear gene (*28S rDNA*) were determined using previously published primers (Table 3). PCR amplification was performed in a total volume of 50 μL containing 1 μL genomic DNA, 4 μL dNTP mixture (2.5 mM each), 1 μL (10 pmol) of each primer, 5 μL 10X Ex Taq Buffer (Mg²⁺ plus), and 1.25 U Takara Ex Taq DNA Polymerase

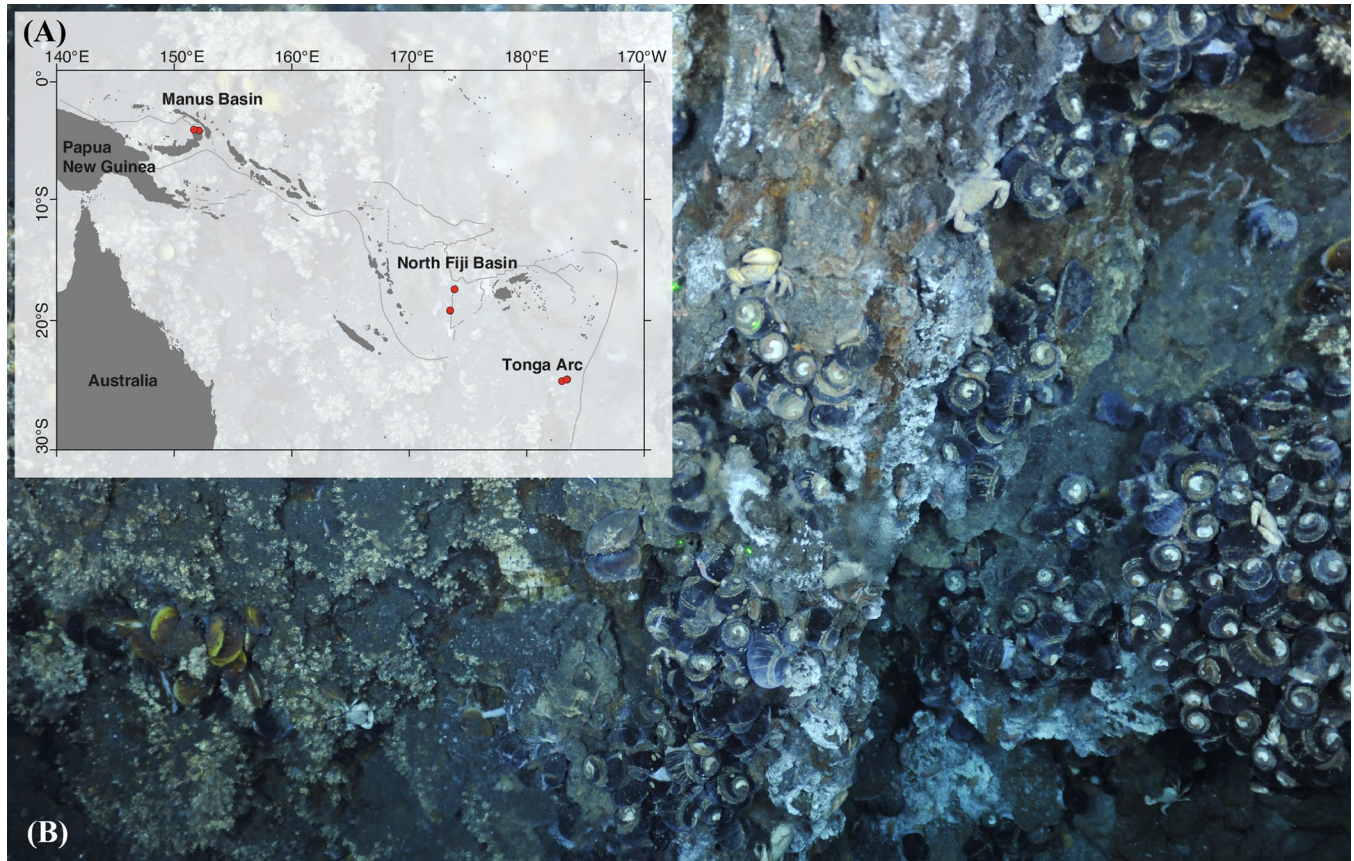


Fig 1. Map of *A. alayseae* sampling locations in the Southwest Pacific Ocean and a photograph of a hydrothermal vent in the North Fiji Basin and its associated biological communities. (A) Sampling locations of *A. alayseae*. Lines represent subduction zones. Red circles indicate sampling areas. Some sampling sites located short distances (<5 km) from each other (R1968-C6 and R1970-C3, R1964-C1 and 1966-C11, TA25A and TA25D) are marked with single circle. (B) An active vent at the 1970-C3 sampling site in the North Fiji Basin. The dominant visible taxa in the image are the provannid snail *Ifremeria nautiliei* (center bottom and right) and the balanomorph barnacle *Eochionelasmus ohtai* (left). A small cluster of mytilid mussels, *Bathymodiolus* sp., and alvinocaridid shrimp species are visible. Scattered individuals of the bythograeid crab *A. alayseae* are generally seen in association with *I. nautiliei* and *E. ohtai*.

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Table 2. Sampling information for bythograeid crabs collected from hydrothermal vent regions in the Southwest Pacific Ocean.

Region	Field ID	Number of specimens			Latitude	Longitude
		<i>A. alayseae</i>	<i>A. hourdezi</i>	<i>G. puia</i>		
Manus Basin	Solwara 1	9	-	-	3.79° S	152.09° E
	Solwara 8	1	-	-	3.73° S	151.67° E
Fiji Basin	R1970-C3	8	-	-	17.11° S	173.87° E
	R1959-C2	-	2	-	19.05° S	173.48° E
	R1964-C1	1	5	-	18.85° S	173.50° E
	R1966-C11	1	-	-	18.82° S	173.50° E
	R1966-C12	-	1	-	18.82° S	173.50° E
	R1968-C6	1	-	-	17.12° S	173.87° E
Tonga Arc	TA26A	1	-	2	24.48° S	177.00° W
	TA25A	4	-	-	24.35° S	176.57° W
	TA25C	-	-	1	24.35° S	176.54° W
	TA25D	12	-	2	24.35° S	176.57° W

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Table 3. Primers used for PCR amplification.

Gene	Primer	Sequence	Application	Reference
COI	AAnd2+300	5' -TCC ACA TCA TTA ATT CTT ATA GCC CTC C-3'	Long PCR primers for COI of <i>A. alayseae</i>	[34]
	AAatp6-450	5' -AGC AAG TGT TCC TGG ACG AAT AAT G-3'		
	AACO1+sF	5' -TTT CTA CAA ATC ATA AAG ACA TTG G-3'	Sequencing primers for the COI barcoding region of <i>A. alayseae</i>	
	AACO1-sR	5' -AGC ATG GCG TAG ATC ATA CCT AGA G-3'		
	LCO1490	5' -GGT CAA CAA ATC ATA AAG ATA TTG G-3'		
HCO2198	5' -TAA ACT TCA GGG TGA CCA AAA AAT CA-3'	Universal primers for the COI barcoding region of <i>A. hourdezi</i> and <i>G. puia</i>	[42]	
16S rDNA	16Sa	5' -CGC CTG TTT ATC AAA AAC AT-3'	Universal primers for mitochondrial 16S rDNA	[43]
	16Sb	5' -CTC CGG TTT GAA CTC AGA TCA-3'		
28S rDNA	28Sa	5' -GAC CCG TCT TGA AAC ACG GA-3'	Universal primers for nuclear 28S rDNA	[44]
	28Sb	5' -TCG GAA GGA ACC AGC TAC TA-3'		

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(Takara Biotechnology Co., Tokyo, Japan) under the following conditions: initial denaturation at 94°C for 2 min, followed by 40 cycles of denaturation (10 s at 95°C), primer annealing (30 s at 42°C in the first 5 cycles and 30 s at 48°C in the last 35 cycles for 16S rDNA; 30 s at 53°C for 28S rDNA), and extension (2 min at 72°C), and then a final extension (2 min at 72°C). To avoid interference from nuclear mitochondrial pseudogenes, PCR amplification for COI of *A. alayseae* was carried out according to the method of Kim et al. (2013) [34]. Finally, Sanger sequencing was conducted at Macrogen Service (Seoul, Korea) using the ABI PRISM 3730XL Analyzer (Applied Biosystems) with BigDye (R) Terminator v3.1 Cycle Sequencing kits (Applied Biosystems). Sequences obtained in this study were trimmed and annotated using Geneious Prime (Biomatters, Auckland, New Zealand), then adjusted through visual inspection. The newly obtained sequences of *A. alayseae*, *A. hourdezi*, and *G. puia* (S1–S3 Tables) were registered in GenBank.

Phylogenetic analysis

The new sequences obtained in this study were aligned with those of other bythograeids and the Atlantic blue crab *Callinectes sapidus*, used as an outgroup (S2 Table), which were retrieved from GenBank using the Geneious alignment method implemented in Geneious Prime, and adjusted through visual inspection. Intra- and interspecific variations in individual gene alignment were calculated using MEGA X [45] based on the p-distance value.

To construct a phylogenetic tree of bythograeid crabs, individual COI, 16S rRNA, and 28S rRNA gene alignments were concatenated to form a single multiple-sequence alignment using Geneious Prime. The best-fitting model of nucleotide substitution was then determined using the Akaike information criterion (AIC) in JModelTest 2.1.7 [46], and the model GTR + I + G was selected as the best evolution model. Next, maximum likelihood (ML) and Bayesian inference (BI) tests were performed using RAxML version 8.2.11 [47] and MrBayes 3.2.6 [48], respectively, implemented in Geneious Prime with the gene partition option. Confidence in the resulting bythograeid relationships was assessed based on the bootstrap proportion (BP) with 100 replications for the ML model. For the BI analysis, four Markov chain Monte Carlo chains were run for 1,000,000 generations and sampled every 200 generations. Bayesian posterior probability (BPP) values were estimated after the initial 500 (10%) trees were discarded as burn-in.

Nucleotide diversity and haplotype network

The number of polymorphic sites, nucleotide diversity, number of haplotypes, haplotype diversity, Tajima's *D*, Fu's *F_S*, and pairwise differences (*F_{ST}*) were estimated using DnaSP v5.0

[49] and Arlequin v. 3.5 [50]. Significance levels for Tajima's D , Fu's F_S , and pairwise F_{ST} were corrected using the Bonferroni method [51]. Analysis of molecular variance (AMOVA) was performed in Arlequin v. 3.5 [50] to detect the population differentiation of *COI* haplotypes. To determine the genetic relationships among hydrothermal vent field populations and *COI* haplotypes, median-joining networks [52] were created using Arlequin v. 3.5 [50] and graphed with PopArt v. 1.7 [53]. Migration rates, population sizes, and relative numbers of migrants were estimated using MIGRATE v. 4.4.0 [54].

Results

Phylogenetic position of *A. alayseae* within the bythograeid lineage

Austinograea alayseae is distributed widely across hydrothermal vent areas in the Southwest Pacific Ocean. In this study, we obtained the sequences of three genes, two mitochondrial genes (*COI* and *16S rDNA*) and one nuclear gene (*28S rDNA*), from 38 specimens of *A. alayseae*, which included 10 individuals from the Manus Basin (Manus population), 11 individuals from the North Fiji Basin (North Fiji population), and 17 individuals from the Tonga Arc (Tonga population). Then, phylogenetic trees of bythograeids were constructed using the concatenated sequences of the three genes (Fig 2; S1 Table). We could not include sequences of *A. jolliveti* and *B. intermedia* because they were not available from open-access sequence databases. The tree topologies were consistent with those obtained in previous studies [17, 34, 55]. Three genera, *Austinograea*, *Bythograea*, and *Gandalfus*, were each supported strongly as monophyletic taxa, with high support values of 99% BP and 1.00 BPP, 98% BP and 1.00 BPP, and 90% BP and 1.00 BPP, respectively. The newly added individuals of *A. alayseae* formed a monophyletic assemblage with 100% BP and 1.00 BPP, representing one of the most recently derived species in the bythograeid lineage. *Austinograea alayseae* individuals were separated into two clades, the Manus clade and the North Fiji–Tonga clade, which had very short branch lengths with respect to the collection region.

In terms of nucleotide sequence divergence, all *28S rDNA* sequences were identical among all three populations of *A. alayseae* (Table 4). The proportions of intraspecific variation in *16S rDNA* and *COI* were 0.00–1.69% and 0.00–4.08%, respectively. We observed the maximum variation between the Manus and North Fiji–Tonga clades (Table 5). In particular, the maximum value of *COI* variation was found in the middle of the barcoding gap, which is the gap between inter- and intraspecific variation, for bythograeids [34, 40].

Population genetic divergence of *A. alayseae*

We examined whether the differences between these two *A. alayseae* clades represented genetic divergence at the population level. Alignment of the *COI* sequences of *A. alayseae* allowed detection of 32 variable nucleotide sites at the third position and two sites at the first position in the codons. The degree of intrapopulation variation ranged from 0.00% to 1.19%, and the maximum divergence was found in the North Fiji population (Table 5). The degree of interpopulation variation between the Manus population and the other two regions was 3.23–4.08%, and the maximum value for divergence between the North Fiji and Tonga populations was only 1.02%, within the range of intrapopulation variation among the three populations. In addition, based on pairwise comparison of F_{ST} , the Manus population differed significantly from the North Fiji and Tonga populations, whereas no difference was found between the North Fiji and Tonga populations.

Based on the variable sites in the *COI* sequences of *A. alayseae*, we examined 20 haplotypes, which showed an overall haplotype diversity of 0.94 (± 0.022). The Manus, North Fiji, and Tonga populations consisted of seven, eight, and nine haplotypes, respectively. All three

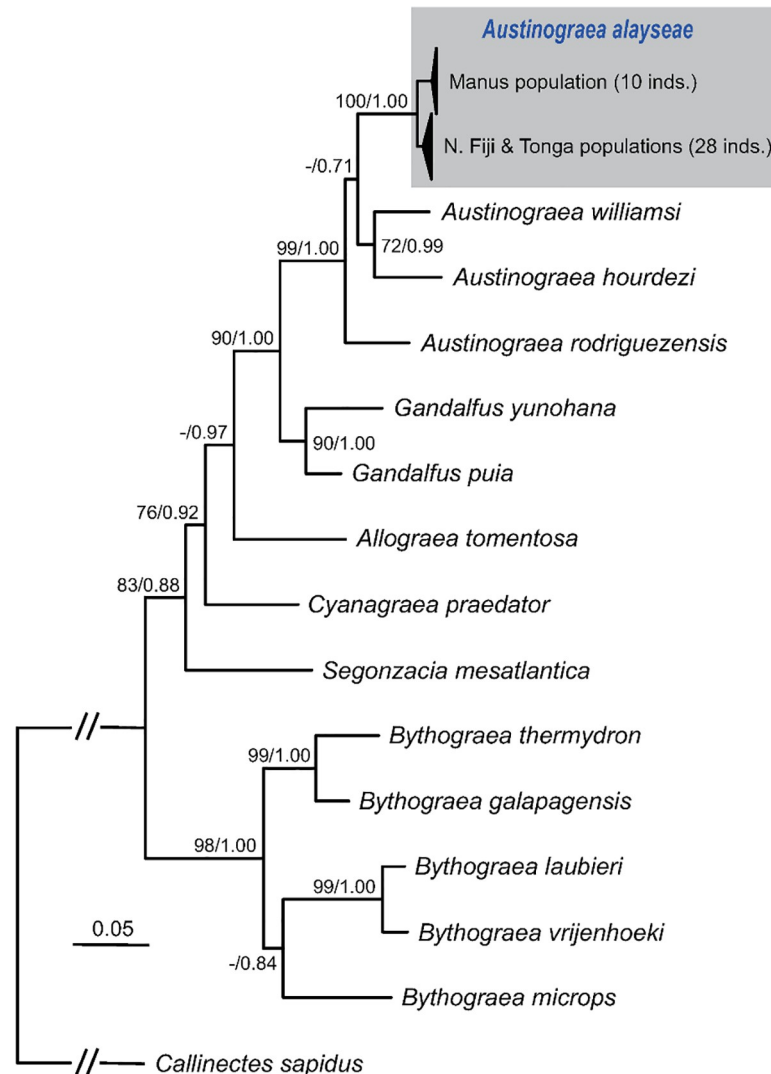


Fig 2. Phylogenetic tree of bythograeid crabs, constructed using a concatenated dataset of *COI*, *16S rRNA*, and *28S rRNA* genes. The gray-shaded box highlights three *Austinograea alayseae* populations. Numbers at internodes are the maximum likelihood bootstrap proportions (left) and Bayesian posterior probabilities (right). Asterisks indicate bootstrap values <60%.

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populations showed relatively high (>0.8) degrees of haplotype diversity. The highest diversity value (0.93 ± 0.067) was obtained for the North Fiji population. Four haplotypes were identified in both the North Fiji and Tonga populations, whereas none were shared between the Manus and North Fiji–Tonga populations. According to the AMOVA, haplotype variation between the Manus and North Fiji–Tonga populations was greater than that between North Fiji and Tonga populations and those among individuals within each population (90.88% vs. 0.00% and 9.12%, respectively), suggesting genetic isolation of *A. alayseae* in the Manus and North Fiji–Tonga hydrothermal vent field regions. On the other hand, based on migration rates and population sizes, the estimated number of migrants between North Fiji and Tonga indicated bidirectional flow of *A. alayseae* from North Fiji to Tonga (4289 migrants/generation) and from Tonga to North Fiji (4467).

Table 4. Intra- and interspecies variations in COI, 16S rDNA, and 28S rDNA sequences of Bythograeidae. Sequence variations were calculated from the nucleotide sequences using the p-distance method in MEGA X.

Group	Variation (%)					
	COI		16S		28S	
	Min.	Max.	Min.	Max.	Min.	Max.
Bythograeidae						
Intraspecies						
<i>Austinograea alayseae</i>	0	4.08	0	1.69	0	0
<i>A. hourdezi</i>	0	0	0	0.22	0	0
<i>A. rodriguezensis</i>	0	0.68	0	0.38	-	-
<i>Gandalfus puia</i>	0	0.51	0	0.38	0	0
<i>G. yunohana</i>	-	-	0	0	-	-
<i>Segonzacia mesatlantica</i>	-	-	0	0	-	-
<i>Cyanagraea praedator</i>	-	-	0.81	0.81	-	-
Interspecies						
<i>Austinograea</i> (4 [†])	10.93	12.08	4.73	7.54	0.42	1.69
<i>Bythograea</i> (5 [†])	6.62	16.13	0.62	7.48	0.31	3.94
<i>Gandalfus</i> (2 [†])	10.50	10.50	5.38	5.38	-	-
Among bythograeid genera (6 [‡])	12.22	17.17	8.71	14.85	0.66	7.45

†Number of species

‡Number of genera

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In the haplotype network, the COI haplotypes of *A. alayseae* were divided distinctly into two clades, the Manus clade and the North Fiji–Tonga clade, with 14 nucleotide substitutions (Fig 3), which well reflects the phylogenetic relationship inferred using three genes (Fig 2). This result indicates that at some time in the past, *A. alayseae* living in the Southwest Pacific Ocean might have experienced a strong population bottleneck, which influenced separation of the two clades (Fig 2; Table 5). Since then, both clades have undergone independent population expansion (Tajima’s *D* and Fu’s *F_S* < 0).

Population genetic structure of invertebrates in the Southwest Pacific Ocean

Among vent organisms distributed evenly around hydrothermal vent fields in the Southwest Pacific Ocean, the vent crab *A. alayseae*, vent shrimp *Chorocaris* sp. 2, and black snail *Ifremeria*

Table 5. Variation among COI nucleotide sequences of the bythograeid crab *Austinograea alayseae* collected from three back-arc basins in the Southwest Pacific Ocean.

Population		N	S	H	h	D	F _S	Nucleotide diversity (%)			Pairwise F _{ST}
								Min	Max	Mean	
Intra-population	Manus	10	8	7	0.87 ± 0.107	-1.87 ^a	-4.04 ^a	0.00	0.68	0.27	-
	N. Fiji	11	9	8	0.93 ± 0.067	-0.62	-3.46 ^a	0.00	1.19	0.44	-
	Tonga	17	9	9	0.87 ± 0.068	-1.18	-4.41 ^a	0.00	0.68	0.30	-
	N. Fiji–Tonga	28	13	13	0.90 ± 0.036	-1.24	-6.91 ^a	0.00	0.47	0.27	-
	Overall	38	34	20	0.94 ± 0.022	0.76	-2.18	0.80	2.54	1.67	-
Inter-population	Manus and N. Fiji	-	-	-	-	-	-	3.23	4.08	3.70	0.90*
	Manus and Tonga	-	-	-	-	-	-	3.23	4.08	3.65	0.92*
	N. Fiji and Tonga	-	-	-	-	-	-	0.00	1.02	0.37	0.00

N, sample size; S, polymorphic sites; H, total number of haplotypes; h, haplotype diversity; D, Tajima’s D; F, Fu’s F_S; F_{ST}, F-statistic.

^a indicates statistically significant values at α = 0.017 after Bonferroni correction.

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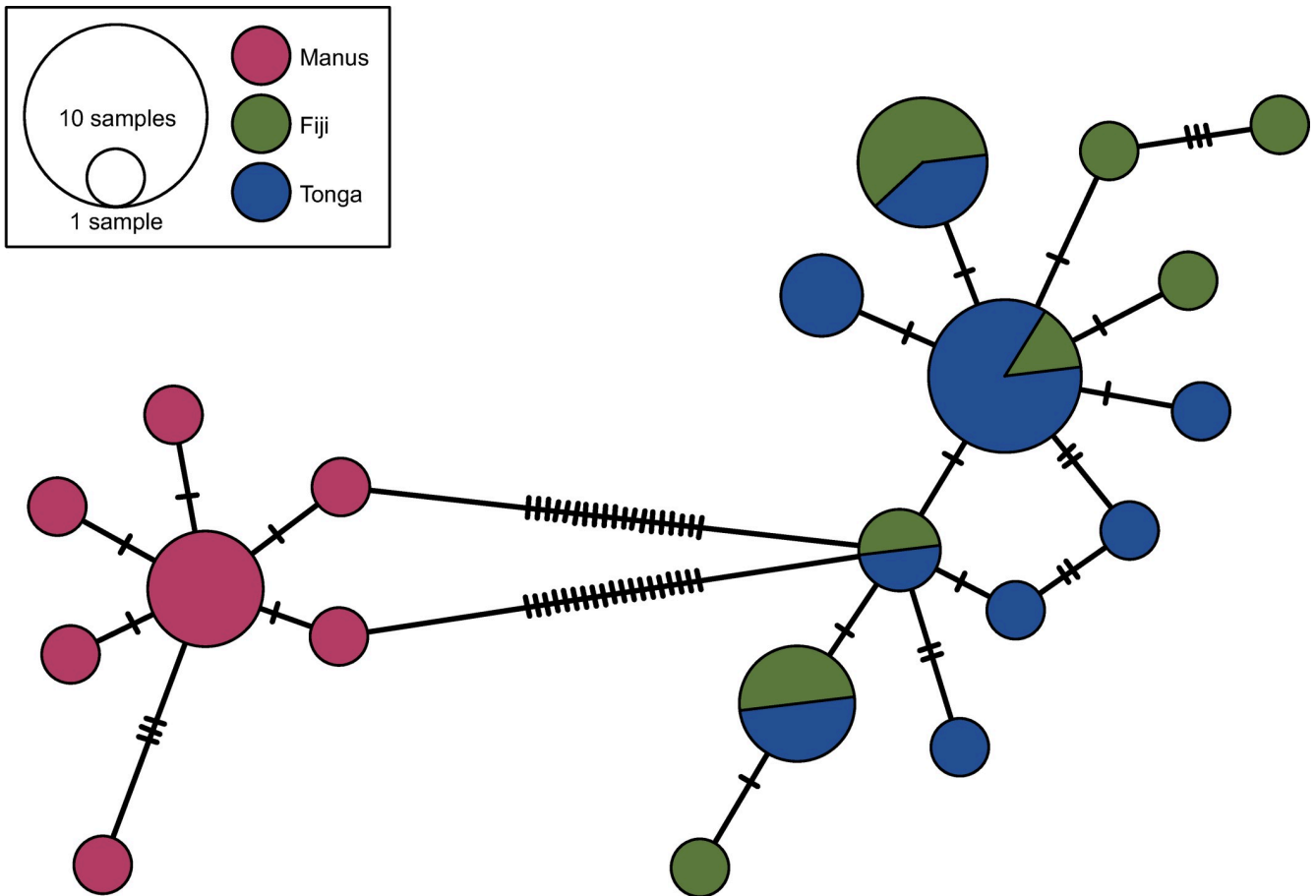


Fig 3. Median-joining network based on *COI* haplotypes from the bythograeid crab *Austinograea alayseae* collected from hydrothermal vent fields in the Southwest Pacific Ocean. Circle size and color reflect haplotype frequency and vent field, respectively. The number of short bars on each branch indicates the number of nucleotide substitutions between haplotypes.

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nautili exhibit distinctive population genetic structures over a certain spatial scale or distance (Table 1) [7, 25]. The population genetic structure of *A. alayseae* recorded in this study demonstrated such distinct distribution patterns, with two segregated populations formed in the Manus and North Fiji–Tonga regions. This distribution is similar to those of *Chorocaris* sp. 2 and *I. nautili*. However, whereas the populations of *Chorocaris* sp. 2 and *I. nautili* share intermediate haplotypes, *A. alayseae* does not share any intermediate haplotype between clades (Fig 3). Based on this difference among the three species, we inferred that the *A. alayseae* clades were generated through a stepwise mutation process over a long period of time, with the possibility that intermediate haplotypes became extinct.

Discussion

Previous studies have confirmed the monophyly of bythograeid crabs [17], which are distinguished from other Brachyura by reduction of the eyes at the adult stage and complete adaptation to hydrothermal vent environments [56]. Based on phylogenetic analysis, bythograeid crabs have been divided into two main groups, the *Bythograea* clade and a clade composed of *Austinograea*, *Gandalfus*, *Allograea*, *Cyanagraea*, and *Segonzacia* [17]. However, the established phylogenetic trees were constructed using specimens from a single vent area for each

species, although some species (*A. alayseae*, *A. hourdezi*, *A. rodriguezensis*, *S. mesatlantica*, *B. laubieri*, and *B. thermydron*) are known to have wide distributions among hydrothermal vent fields. Genetic divergence analysis of bythograeids at the population level has been performed for only one species, *A. rodriguezensis*, and revealed no genetic differentiation between populations at two vent fields separated by 700 km (Central Indian Ridge) [55, 57].

In the *Bythograea* clade, *B. laubieri* and *B. vrijenhoeki*, which co-occur on the Southeast Pacific Rise, are the most recently diverged species [31]. These sympatric species have relatively little genetic difference (6.6%) in their *COI* sequences, which is the minimum interspecific variation observed in the family Bythograeidae (Table 4) [40]. In this study, populations of *A. alayseae* showed relatively large degrees of intraspecific variation (3.23–4.08%) between the Manus and North Fiji–Tonga clades. An analysis of bythograeids excluding *A. alayseae* revealed <1% intraspecific variation and 6.62–16.13% interspecific variation (Table 4). Although the proportion of variation between the *A. alayseae* clades is closer to the interspecific variation patterns of other bythograeids, we could not identify any morphological difference based on the original descriptions of *Austinograea* [29, 33]. In many cases, genetic differentiation among related species in recently diverged lineages is not reflected in morphological traits, which occasionally leads to taxonomic ambiguity [58–60]. After experiencing a population bottleneck, the original *A. alayseae* population in the Southwest Pacific Ocean could have experienced independent population expansion and genetic differentiation of the two *A. alayseae* clades. These findings for *A. alayseae* raise the possibility of incipient speciation, despite the current absence of morphological traits distinguishing the clades. In addition, Guinot and Segonzac (2018) [33] noted the presence of the invalid *Austinograea* species, *Austinograea* sp. aff. *A. alayseae*, in the Manus Basin. Thus, we cannot rule out the possibility that our specimens from the Manus Basin may be *Austinograea* sp. aff. *A. alayseae* which currently does not have confirmed taxonomic status (a new species or subspecies) due to insufficient morphological information and unavailable DNA sequences in public databases.

Larval dispersal is a main driver of gene flow and distribution of vent invertebrates, and it is related to local- and regional-scale geological and hydrological barriers, species-specific development processes, dispersal depth, and water temperature [9, 11, 12]. According to the modeling of potential larval dispersal distances with a planktonic larval duration of 83 days in the Southwest Pacific Ocean, a bidirectional connection was suggested only between North Fiji and New Hebrides (500 km apart), with an event of dispersal between these regions occurring once every 5,000–12,000 years [15]. Even with an increased planktonic larval duration of 170 days, the model did not assure a bidirectional connection between the Manus and North Fiji basins. Moreover, based on previous studies of the bythograeid crab *B. thermydron* and other species [61–63], the estimated duration of *A. alayseae* larval development does not exceed 3 months, which is too short for dispersal over a sufficient distance.

To elucidate the genetically differentiated clades of *A. alayseae*, geological and hydrographic barriers in the Southwest Pacific Ocean were also considered. Previous studies of geological features have led researchers to propose that the Vanuatu and New Guinea archipelagos, Solomon Islands, and New Hebrides, which are relatively young and not fully developed back-arc basins, acted as barriers or stepping stones, forming a genetic connection between the Manus and North Fiji basins [7, 15, 16]. Although we did not observe intermediate haplotypes between the Manus and North Fiji–Tonga clades, considering the recent differentiation of these clades, such intermediates may exist in newly formed vent regions. On the other hand, to explain the connection between the North Fiji and Tonga populations, we considered the theory that these volcanic areas originated from the disruption of a single arc <10 Mya [18]. Based on the estimated divergence times of decapod species [64] and our results, the divergence of the North Fiji–Tonga clade correlates with this geological event. Thus, the wide area

of vent fields ranging from North Fiji to the Tonga/Lau region functioned as a geographical boundary, leading to reproductive isolation within the region and restricted gene flow from outside. In addition, the water masses in the North Fiji and Lau/Tonga regions are well mixed by the South Equatorial Current system [15, 65]. This mixing might have enabled bidirectional migration of *A. alayseae* between the North Fiji Basin and Tonga Arc. After larval dispersal, *A. alayseae* could have settled quickly and reliably in these regions, which have similar environmental features [12, 18].

Many questions remain unanswered concerning the origin and evolution of vent fauna in short-lived patchy habitats that last no more than a few decades. To improve our understanding of evolutionary processes, further research should be considered, including biogeographic analysis of additional taxa, investigation of mutation rates and average generation times of species over a geological time scale, and examination of the roles of other environmental features and life-history attributes, as well as genome-based population studies. Furthermore, to elucidate the gene flow of hydrothermal vent fauna between basins, we should investigate additional specimens of known species from unstudied regions throughout the Southwest Pacific Ocean.

Supporting information

S1 Table. Information about *A. alayseae* specimens used in this study. Classification, sampling locations, sample IDs, and DNA sample IDs of the species and GenBank accession numbers of genetic sequences used in this study.

(XLSX)

S2 Table. Information about Bythograeidae specimens used for phylogenetic analysis.

Classification and sampling locations of the species and GenBank accession numbers of genetic sequences used.

(XLSX)

S3 Table. Information about Bythograeidae specimens used for distance calculation. Classification and sampling locations of the species and GenBank accession numbers of the *COI* gene sequences used.

(XLSX)

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