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Population Genetic Differentiation on the Hydrothermal Vent Crabs *Xenograpsus testudinatus* along Depth and Geographical Gradients in the Western Pacific

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Abstract: Connectivity in deep-sea organisms must be considered across both depth gradient and horizontal geographical scales. The depth-differentiation hypothesis suggests that strong environmental gradients (e.g., light, temperature, pressure) and habitat heterogeneity in the deep-sea can create selection pressure, and this can result in genetic population divergence. The hydrothermal vent crab *Xenograpsus testudinatus* (Xenograpsidae) is common in vents at Kueishan Island, Taiwan, ranging from 10 to about 300 m depths. *Xenograpsus testudinatus* has also been found in shallow water vents (3–20 m) at Kagoshima and the Izu archipelago of Japan. We examine the sequence divergences in the mitochondrial COI, 16S rRNA and D-loop genes, to test the hypothesis that there is significant genetic differentiation among populations of *X. testudinatus* along the depth gradient at Kueishan Island (30, 200, 209–224 and 250–275 m), and among different geographical regions (Kueishan, Kagoshima and the Izu archipelago) in the West Pacific. There is neither significant population differentiation among shallow or deep-sea vents, nor between geographical locations. Vertical migration of zoea, upwelling on the eastern coast of Taiwan and the strong effect of the Kuroshio Currents has probably resulted in a high level of planktonic larval dispersal of *X. testudinatus* along the depth and geographical gradients in the Western Pacific.

Keywords: population connectivity; deep-sea; shallow waters

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1. Introduction

The life cycles of marine invertebrates typically include planktonic larval and sedentary adult phases. The duration of planktonic larval development (PLD), larval development modes (planktotrophic or lecithrotrophic) and larval behavior can affect the larval dispersal and connectivity from shallow water [1–3] to even bathyal and abyssal depths [4–6]. Population genetics of intertidal or shallow water species are often focused on connectivity along the horizontal geographical scales [7–11]. Compared to shallow water species, deep-sea (>200 m deep) organisms often inhabit a relatively wider depth gradient [12]. Connectivity of deep-sea populations occurs across two dimensions—depth gradient and horizontal geographical scales [12]. Strong depth (400 m) and geographical scale population differentiation, for example, were reported in the deep-sea coral *Callogorgia delta* on the Atlantic coast of the USA [13].

The exclusive hydrothermal vent crab *Xenograpsus testudinatus* (Xenograpsidae) was first discovered in Yilan, Taiwan [14]. There is a large population living in the hydrothermal vents at Kueishan Island, from shallow (depth 10–30 m) to deep-sea at about 300 m depth

(horizontal distance between shallow and deep-sea vents is 4–5 km) (Figure 1) [14–16]. The composition of gases released from shallow and deep-sea hydrothermal vents in Kueishan Island is similar, with a high concentration of carbon dioxide (90–99%) and hydrogen sulphide (0.8–8.4%) but low levels of sulphur dioxide (<0.03%) and hydrogen chloride (<50 ppm) [17]. In summer, the surface seawater temperature is about 25 °C and drops to 10 °C in depths around 300 m. *Xenograpsus testudinatus* is the only hydrothermal vent species known to be present in both shallow and deep-sea vents [16,18]. The species is an opportunist, feeding on plankton killed by the vent plumes as well as other dead organisms in the vent region [16,19]. Recently, *X. testudinatus* populations were identified from Japanese shallow water hydrothermal vents at Showa Iojima Island off Kagoshima [20,21] (3–5 m depth) and Shikine-jima Island (10–14 m depth) at the Izu archipelago [22]. Planktonic early zoeal stages and benthic megalopas of *X. testudinatus* were found at the shallow water vents at Kueishan Island, Taiwan, suggesting that the larvae were recruiting around the vents [23,24]. It is possible that the depth-environmental gradient, including light, temperature, pressure and dissolved oxygen levels, can drive selection to occur in vents along the depth gradient and result in depth-driven population genetic differentiation. In contrast, larvae may actively migrate vertically, resulting in substantial gene flow across depths. Shallow water vents at Kueishan Island, Taiwan, Kagoshima and the Izu archipelago in southern Japan are influenced by the strong Kuroshio Current, which flows from the east coast of Taiwan and to the Pacific coast of Japan and can facilitate larval dispersal of larvae among these vents. During the East Asian winter monsoon (February to April), the northward flow of Kuroshio Current almost stops, and currents can be reversed by strong monsoon winds [25]. The larvae of vent crab can thus be transported between the vent sites at Japan and Taiwan by the Kuroshio Current and winter monsoon winds.

The present study uses the molecular divergence of the mitochondrial (mtDNA) COI, 16S rRNA and D-loop genetic markers to test the hypothesis if *X. testudinatus* exhibits genetic differentiation along the depth gradient and geographical regions in the West Pacific.

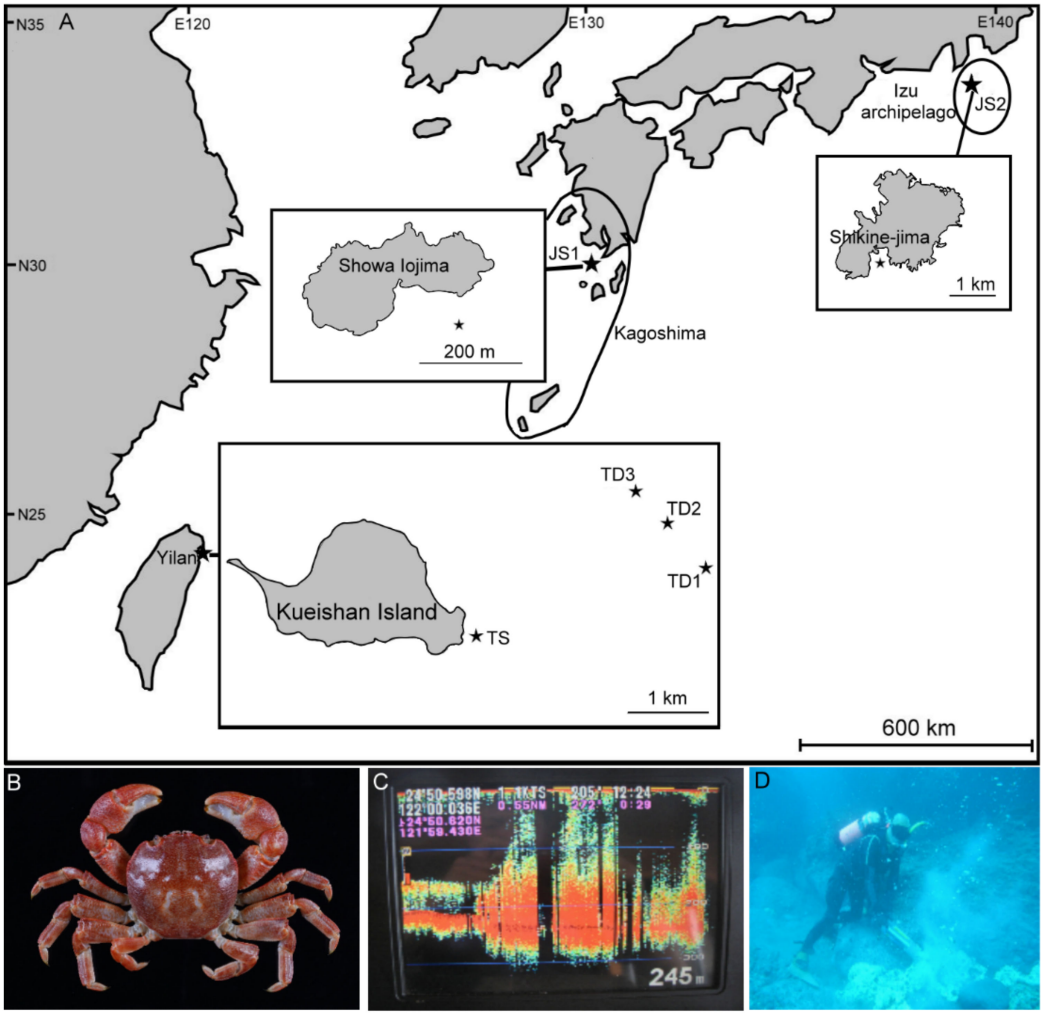

Figure 1. (**A**) The six populations of *Xenograpsus testudinatus* analyzed in this study and indicated by TS, TD1–3 and JS1–2. TS: Shallow water population at Kueishan Island, Taiwan; TD: Deep-sea population at Kueishan Island, Taiwan; JS: Shallow water population at Japan. "★" means population sampling locality. (**B**) The hydrothermal vent crab *Xenograpsus testudinatus*. (**C**) Depth sonar in sampling vessels, showing numerous gas productions from hydrothermal vents at Kueishan Island, Taiwan at a depth of 245 m. (**D**) SCUBA sampling on shallow water vents at Kueishan Island, Taiwan.

2. Materials and Methods

2.1. Sampling Sites and Sample Collection

Five populations of vent crab *X. testudinatus* in the Western Pacific were collected, including one shallow (15–30 m, 51 individuals, abbreviated as TS) and three deep-sea populations ranging from 16 to 31 individuals (TD1: 200, TD2: 209–224, TD3: 250–275 m) off Kueishan Island at Taiwan and a shallow water population off Showa Iojima Island at Kagoshima (JS2) (Figure 1). COI sequences of the *X. testudinatus* population (JS1) at Shikine-Jima Island at the Izu archipelago of Japan were available in the Genbank (Figure 1; Table 1). Deep-sea *Xenograpsus* populations (TD1, 2 and 3) were collected by trawls (voucher specimens deposited in National Taiwan Ocean University) (Table 1; Figure 1C shows bubbling from a deep-sea vent using depth sonar investigation in the sampling vessels). For the Kagoshima population (JS2), 30 individuals were collected from the shallow water vent by diving in 2011 [20] (voucher specimens deposited in Lee Kong Chian Natural History Museum, Singapore). The Izu archipelago population was based on the sequences data of three individuals (JS1) available in the GenBank [22]. Crude genomic DNA of 151 collected individuals (Table 1) from this study were extracted from the cheliped muscles

using the QIAGEN® DNeasy Blood and Tissue Kit (Cat. No. 69504, Valencia, CA, USA) following the protocol of the manufacturer.

Table 1. *Xenograpsus testudinatus* material used in this study. The references provided refer to sequences available in the GenBank. "n/a" means the detailed information not provided.

Locality	Habitat & Population Name Abbreviation	Latitude & Longitude	Depth (m)	Sampling Date	Sample Sizes	Source
Kueishan Is., Yilan, Taiwan	Shallow waters (TS) ★	24°50.112′ N 121°57.741′ E	10–15	9 August 2013	51	This study
		n/a	n/a	n/a	1	EU727203 [26]
		n/a	15	11 August 2008	4 #	AB933570-AB933573 [20]
	Deep sea (TD1)	24°50.662′ N 121°59.697′ E	200	13 July 2005	16	This study
	Deep sea (TD2) ★	24°50.994′ N 121°59.431′ E	209–224	12 August 2010	31	This study
	Deep sea (TD3)	24°51.231′ N 121°59.204′ E	252–275	4 September 2008	23	This study
Showa Iojima Is., Kagoshima, Japan	Shallow waters (JS1) ★	30°48.16′ N 130°20.5′ E	3–5	14 May 2011	30	This study
		30°48.16′ N 130°20.5′ E	3–5	14 May 2011	5 #	AB933569, AB933574-AB933577 [20]
Shikine-Jima Is., Izu archipelago, Japan	Shallow waters (JS2)	n/a	10–14	17 July 2018	3 #	LC490686-LC490688 [22]

★—the population also contributed to D-loop sequences; #—the individuals provided to the COI sequence only from the GenBank.

2.2. Molecular Analyses

Universal primer set, LCO1490/HCO2198 [27] and 16Sar/16S1472 [28,29] were employed for getting the COI and 16S rDNA datasets, respectively. PCR reactions were performed in 25 µL with 20–100 ng of the DNA extractions, 2.5 µL of 10× polymerase buffer, 15 mM of magnesium chloride ($MgCl_2$), 2.5 mM of deoxyribonucleotide triphosphate mix (dNTPs), 5 µM of each primer, and 1 unit of *Taq* polymerase (5 units μL^{-1}; Super-Therm, Takara Bio, Kusatsu, Japan). The PCR cycling profiles were as follows: 5 min at 95 °C for initial denaturation, then 40 cycles of 30 s at 94 °C, 40 s at 47.8 °C for COI and 46.5 °C for 16S rRNA genes, respectively, 40 s at 72 °C, and the final extension for 5 min at 72 °C. The PCR products were sent to a commercial bio-company (Mission Biotech, Taipei, Taiwan) after checking for the correct size and good quality by 1% agarose gel. The same PCR primer set was used for sequencing on an ABI 3730 genetic analyzer (Applied Biosystems, Center for Integrated BioSystems, Logan, UT, USA). The output sequences were edited for contig assembly by SeqManProTM (LASERGENE®; DNASTAR, Madison, WI, USA).

All of 151 individuals were successful in obtaining the sequences from both genes in this study (COI-657 bp: OM764349–OM764499, 16S rDNA-543 bp: OM777525–OM777675) and are deposited in GenBank. An additional 13 sequences of mitochondrial COI gene from shallow water vent were obtained from GenBank and added to this newly generated dataset, including five individuals from Kueishan Island [20,26] at Taiwan, five individuals from Showa Iojima Island [20] and three from Shikine-Jima Island, Japan [22]. Meanwhile, the specific primer set was designed by referring to the complete mtDNA of *X. testudinatus*

(EU727203) [26] to obtain the sequences of the D-loop region. A forward primer Xeno_12SF: 5′-ATATTACAATAACACTATACTATA-3′ was modified from the conserved sequence of a small subunit ribosomal RNA (12S rRNA) to flank with the reverse primer Xeno_GlnR: 5′-AATGGTGTAATTCCATTACGTATAA-3′ on the tRNA glutamine gene. The process of PCR and dataset acquisition was the same as the above-mentioned, except for the annealing temperature, which was changed to 49.5 °C. However, sequences were obtained only from 68 specimens (441–573 bp) of 3 populations (TS, TD2 and JS1, Table 1) due to the poor DNA qualities of the 2 deep-sea populations (TD1 and TD3) samples. All D-loop sequences generated from this study are also deposited in GenBank (OM778196–OM778263), and the same region from the complete mtDNA (EU727203) was added for the subsequent analyses.

2.3. Data Analyses

A total of 164 sequences from the mitochondrial COI gene, 152 sequences from the 16S rRNA gene and 69 D-loop sequences were used for the subsequent analyses (Table 1). Among the 13 mtCOI gene reference sequences already available in the GenBank, the nucleotide length on the Shikine-jima Islands population (LC490686–LC490688) is shorter than 657 bp—we filled the missing nucleotides with the letter "N" instead of "-" for performing the analyses. Meanwhile, those individuals only provided the COI sequences and without any 16S rDNA information except for one of complete mitogenome [26] (Table 1). The COI dataset was translated into the corresponding amino acids by MEGA v.7.0.14 [30] for checking whether the nuclear mitochondrial pseudogenes (numts) were included [31]. All sequences alignments were performed by MAFFT v.7 [32] using the "auto" strategy and we adjusted some nucleotide sites or gaps by eyes using the BioEdit v.7.2.5 [33]. An optimal model of DNA substitution was determined for pairwise distance calculation using jModelTest v.2.1.3 [34] and corrected pairwise distance was estimated by MEGA v.7.0.14 for comparing the genetic divergence on six populations. The sequence polymorphism information of three mitochondrial genes was estimated using DnaSP v.6.12.03 [35], including the number of haplotypes (H), number of polymorphic sites (S), haplotype diversity (h), nucleotide diversity (π), and Tajima's D [36] for each population.

Due to the different sample sizes of each genetic dataset and variable nucleotide divergent information, the sequences were separated into (I) COI only (164 individuals, 657 bp), (II) COI + 16S rRNA (152 individuals, 1201 bp), and (III) COI + 16S rRNA + D-loop (69 individuals, 1753 bp) for the subsequent analyses. F_{ST} for pairwise genetic differentiation between each population (TS, TD1, 2, 3 and JS1, 2) was calculated using Arlequin v.3.5 [37]. One thousand permutations were implemented to assess the significance of the statistics. Network v. 10.2.0.0. (https://www.fluxus-engineering.com/network_terms.htm, accessed on 30 December 2020) was used to construct the haplotype networks by using the median-joining algorithm [38] for examining the genealogy of the haplotypes on these *X. testudinatus* populations.

As a result of the larger sample sizes and with only COI markers available, studying the substitution rate calibration, the inference of past population dynamics were evaluated only on the mtCOI dataset by using the coalescent Bayesian skyline plot [39] in BEAST v.2.6.2 [40]. The analyses on all three deep-sea and shallow water vents at Kueishan Island, and shallow water vents between Kueishan Island and Kagoshima, Kagoshima with the Izu archipelagos and all combined populations were run using a strict clock model with the mutation rates on 1.66×10^{-8} substitutions/site/year, which were suggested from the non-Jamaican land crabs on mtCOI [41]. All HKY + I models of nucleotide substitution were evaluated as the optimal model from the AIC criterion in jModelTest on four groups then used to set up the site model. A total of 2.5×10^7 generations sampled every 1000th generation were analyzed and the first 10% discarded as burn-in. Tracer v1.7 [42] was used to check the convergence from the effective sample sizes (ESS) of all parameters (ESS > 200 for each group) and to calculate the mean value, the upper and lower bounds of the 95% highest posterior density interval of effective population sizes, and to draw skyline plots.

3. Results

A total of 164 COI gene sequences were analyzed and represented 78 haplotypes without any stop codons, and 152 sequences on the 16S rRNA gene revealed 14 haplotypes were successfully sequenced from *X. testudinatus* from shallow water vents at Taiwan (TS) and Kagoshima (JS2) and three deep-sea vents at Taiwan (TD1–TD3). Sixty-nine sequences of the D-loop gene were successfully sequenced from the shallow water vents at Taiwan (TS) and Kagoshima (JS2) and one deep-sea vent (TD2, 209–224 m) at Kueishan Island.

Ranges of sequence divergences within and among depth populations in Taiwan were similar. The population divergence in the COI and 16S rRNA genes for the shallow water (TS; 15 m), and three deep-sea populations (TD1–TD3; 200, 209–224 and 250–279 m), ranged from 0% to 1.4% and 0% to 0.6%, respectively. Among three deep-sea populations (TD1–TD3), the divergences were close to the comparison of shallow waters (TS) (COI: 0–1.2%; 16S rRNA: 0–0.4%). In the D-loop gene, sequence divergence for the shallow water (TS; 15 m) and deep-sea (TD2; 209–224 m) populations were 0.4–6.2% and 0.6–6.0%, respectively. The population divergence in the D-loop gene between shallow water and deep-sea at Kueishan Island (TS vs. TD2) was 0.2–6.3%.

Studying sequences divergences along the horizontal geographical gradient, sequence divergences within and among populations in Kueishan Island, Kagoshima and Izu archipelago were similar. With regards to population sequence divergences in shallow water populations at Kueishan Island (TS) (COI: 0–1.4%; 16S rRNA: 0–0.4%; D-loop: 0.4–6.2%), Kagoshima (JS1) (COI: 0–1.6%; 16S rRNA: 0–0.4%; D-loop: 0.4–5.7%), and Izu (JS2) (COI: 0–0.6%), the overall differences were less than 1.6% for COI, 0.4% for 16S rRNA and 6.2% for D-loop. From pairwise comparisons, the divergence in the COI gene was 0–1.6% between Kagoshima (JS1) and Izu (JS2). The COI ranges between the Kueishan Island shallow vents (TS) and Kagoshima (JS1) ones were similar to the pairwise Kagoshima-Izu (JS1 vs. JS2) comparison (COI: 0–1.7%; 16S rRNA: 0–0.4%; D-loop: 0.2–6.4%). Pairwise comparison in sequence divergences between Kueishan Island shallow water vents (TS) and Izu (JS2) was 0.0–1.4% for the COI gene.

The haplotype and nucleotide diversities in Taiwan (one shallow water and three deep-sea populations; TS vs. TD1–TD3), Kagoshima (JS1) and Izu (JS2) in the COI gene ranged from 0.667 to 0.969 and 0.004 to 0.006, respectively, while the D-loop gene range was from 1 and 0.032 to 0.041. Haplotype and nucleotide diversity is lowest in the 16S rRNA gene when compared to COI and D-loop, ranging from 0.303 to 0.632 and 0.0006 to 0.002, respectively. Tajima's Test (*T's D*) evaluation showed that all values are negative (<0), and significant *p*-values indicated that the populations experienced the recent expansions (Table 2).

The F_{ST} index showed (Table 3) that there was no significant genetic differentiation among deep-sea (TD1, 2, 3)/shallow waters (TS) at Kueishan Island on COI, COI + 16S rRNA, and COI + 16S rRNA + D-loop sequence analyses. The COI + 16S rRNA genes revealed significant genetic differentiation between TD2 and TS at Kueishan Island (*p*-values = 0.036) (Table 3). There were no significant differences in shallow water samples along the geographical gradient (TS, JS1, JS2) (*p*-values > 0.05) (Table 3). However, of the 78 haplotypes on the mtDNA COI dataset, haplotypes 1 and 15 were the dominant (n = 21) and the relatively ancestral type and were present in all six sampling populations. Haplotype 50 was the second commonest type (n = 13) (Figure 2A). The number of haplotypes increases to 99 on the combined dataset of COI + 16S rRNA genes and a very similar topology is shown for the COI + 16S rRNA gene (haplotype 15 is the dominant, n = 16) when compared to the COI gene only (Figure 2B). As each sequence of the D-loop gene represents a unique haplotype (Table 2), the topology of the haplotype network did not show any ancestor or dominant haplotype (Figure 2C). Overall, there was no discrete grouping of haplotypes among populations of *X. testudinatus*.

Table 2. Sequence polymorphisms in the COI, 16S rRNA and D-loop genes of *Xenograpsus testudinatus*. *N* = No. of sequences; *H* = No. of haplotypes; *S* = No. of polymorphic sites; *h* = haplotype diversity (standard deviation); π = nucleotide diversity (standard deviation); *T's D* = Tajima's Test.

Population	*N*	*H*	*S*	*h*	π	*T's D*
COI gene						
Kueishan Is., Shallow waters (TS)	56	38	40	0.969 (0.013)	0.006 (0.0004)	−1.95 *
Kueishan Is., Deep-sea (TD1)	16	13	18	0.967 (0.036)	0.006 (0.0007)	−1.16
Kueishan Is., Deep-sea (TD2)	31	20	20	0.955 (0.022)	0.005 (0.0004)	−1.39
Kueishan Is., Deep-sea (TD3)	23	16	16	0.964 (0.022)	0.005 (0.0005)	−0.95
Showa Iojima Is., Shallow waters (JS1)	35	17	20	0.921 (0.026)	0.005 (0.0005)	−1.28
Shikine-Jima Is., Shallow waters (JS2)	3	2	2	0.667 (0.314)	0.004 (0.0019)	None
Total	164	78	54	0.898 (0.018)	0.006 (0.0003)	−2.19 **
16S rRNA gene						
Kueishan Is., Shallow waters (TS)	52	5	5	0.370 (0.081)	0.0009 (0.0002)	−1.45
Kueishan Is., Deep-sea (TD1)	16	4	3	0.592 (0.122)	0.001 (0.0004)	−0.47
Kueishan Is., Deep-sea (TD2)	31	4	3	0.553 (0.060)	0.001 (0.001)	−0.41
Kueishan Is., Deep-sea (TD3)	23	6	4	0.632 (0.089)	0.002 (0.0003)	−0.69
Showa Iojima Is., Shallow waters (JS1)	30	4	3	0.303 (0.104)	0.0006 (0.0002)	−1.36
Total	152	14	12	0.435 (0.0001)	0.001 (0.0001)	−1.92 *
D-loop gene						
Kueishan Is., Shallow waters (TS)	27	27	81	1 (0.011)	0.037 (0.002)	−1.28
Kueishan Is., Deep-sea (TD2)	18	18	73	1 (0.019)	0.041 (0.002)	−1.00
Showa Iojima Is., Shallow waters (JS1)	24	24	68	1 (0.012)	0.032 (0.002)	−1.38
Total	69	60	111	1 (0.003)	0.034 (0.001)	−1.68

* Significant $p < 0.05$; ** $p < 0.01$.

Table 3. Pairwise comparison of F_{ST} index among the six populations based on COI, COI + 16S rRNA and COI + 16S rRNA +D-loop genes sequences of *Xenograpsus testudinatus*. Population abbreviations are listed in Table 1. * Indicates p value < 0.05; n/s = no dataset available; – The pairwise valeus should not be shown in the same locality.

Populations	TD1	TD2	TD3	TS	JS1	JS2
			COI gene			
TD1	–					
TD2	−0.0193	–				
TD3	−0.0293	−0.0129	–			
TS	−0.0188	0.0143	−0.0054	–		
JS1	−0.0189	0.0029	−0.0031	0.0100	–	
JS2	−0.2990	−0.1308	−0.1720	−0.2825	−0.1709	–
			COI + 16S rRNA genes			
TD1	–					n/s
TD2	−0.0138	–				n/s
TD3	−0.0241	0.0136	–			n/s
TS	−0.0105	0.0328 *	0.0108	–		n/s
JS1	−0.0123	0.0249	0.0076	−0.0023	–	n/s
			COI + 16S rRNA + D-loop genes			
TD2	n/s	–	n/s			n/s
TS	n/s	−0.0048	n/s	–		n/s
JS1	n/s	0.0109	n/s	−0.0039	–	n/s

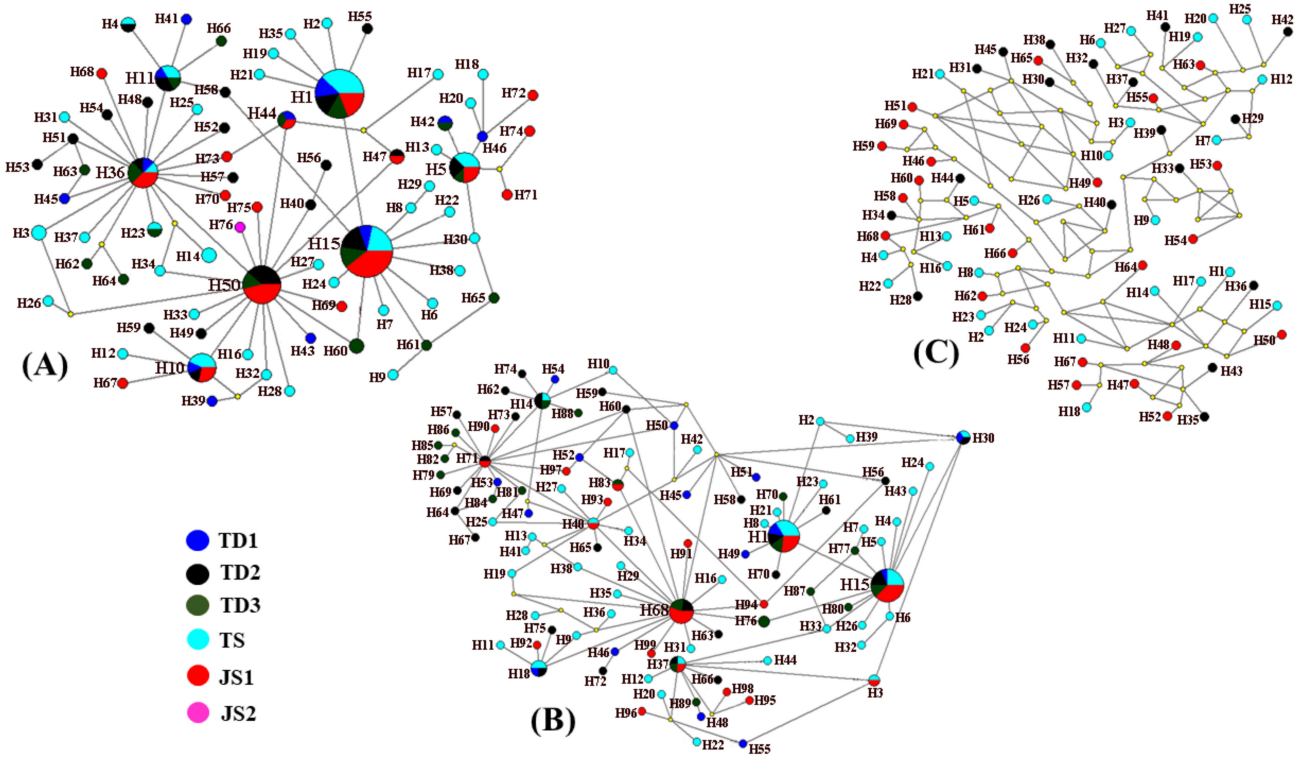

Figure 2. Median-joining networks constructed from (**A**) 78 haplotypes on COI gene, (**B**) 99 haplotypes on COI + 16S rRNA genes, and (**C**) 69 unique haplotypes on COI + 16S rRNA+ D-loop dataset of *Xenograpsus testudinatus* among populations in Taiwan and Japan. Size of circle corresponding to relative numbers for each haplotype. TS: Shallow water population in Taiwan; TD: Deep-sea population in Taiwan; JS: Shallow water population in Japan.

The neutral test (Tajima's D statistic) indicates that the populations of *X. testudinatus* have experienced a history of expansion (negative values) (Table 2); the construction of Bayesian skyline plots showed that it occurred weekly and continued until around 33,000 years before the present (Figure 3A). Comparing different populations separately, we find that the deep and shallow waters vent area at Kueishan Island was the earliest to stabilize (around 45,000 years ago, Figure 3C). Populations in the deep-water vent of Kueishan Island (Figure 3B) and shallow waters vents at Kueishan Island and Kagoshima (Figure 3D) stabilized slightly later (around 42,000 years ago). The Kagoshima shallow water vents were the most recent phase of the expansion at around 15,000 years ago (Figure 3E).

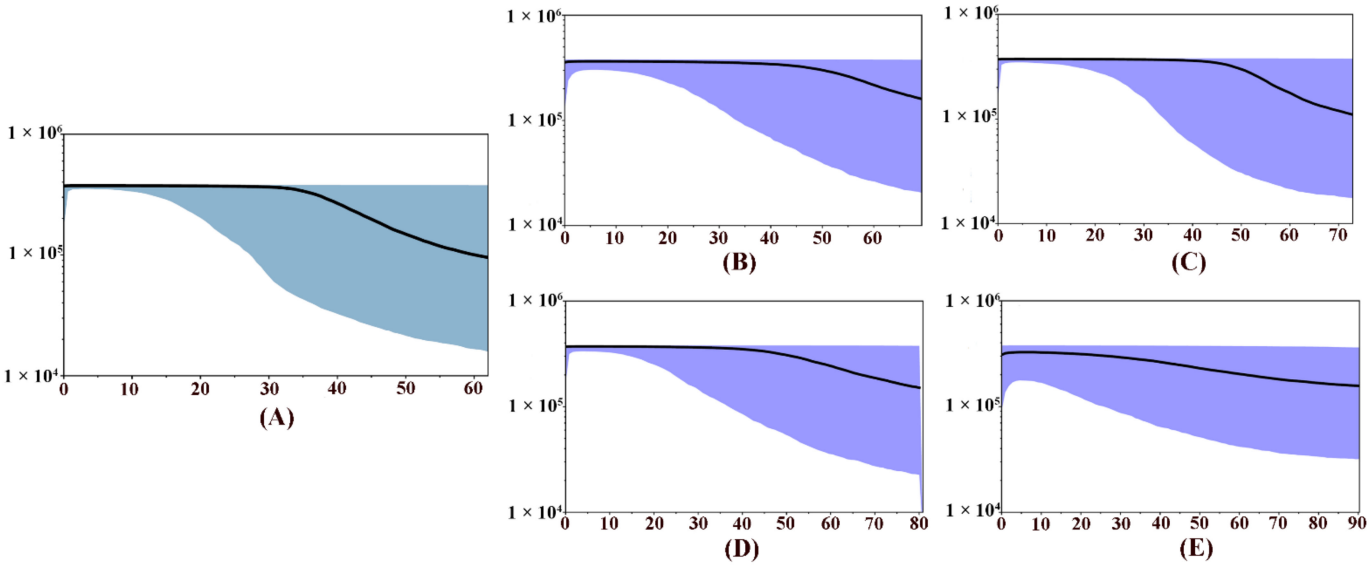

Figure 3. Bayesian skyline plots construction based on mitochondrial COI gene dataset from the different sampling populations of (**A**) all sequences used in this study, (**B**) only deep-sea vent at Kueishan Island of Taiwan, (**C**) deep-sea and shallow water vents at Kueishan Island of Taiwan, (**D**) shallow water vents at Kueishan Island and Kagoshima, and (**E**) shallow water vents at Kagoshima and the Izu archipelagos of Japan. Estimations were according to a mutation rate of 1.66×10^{-8} substitutions per site per year. The *X*-axis indicates the thousands of years before present, the *Y*-axis indicates the effective population size (*Ne*) estimates by generation time (*T*). The bold line means the median estimate of the calculated effective population size, and the shaded area means the intervals of 95% confidence.

4. Discussion

The present study revealed neither depths nor geographical distances affect the population differentiation of the vent crab *Xenograpsus testudinatus* in the West Pacific. The complete larval development of *X. testudinatus* has not been studied and the length of PLD remains unknown. The reproductive season of *X. testudinatus* is in summer when larvae are released, with settlement occurring in November [24]. The Kuroshio is a strong current affecting the north-south gene flow along the Pacific coast of Honshu, Kyushu, Okinawa, Taiwan and the Philippines, and its velocity can be up to 1 m s^{-1} and composed of complex gyres [43]. We assumed the larval development of *X. testudinatus* ranged from 15 to 25 days based on the development model of other species within Grapsoidea [44,45] and the current speed of Kuroshio is 1 ms^{-1}, larvae of *X. testudinatus* can be dispersed from Taiwan to the Pacific coast of Japan within the PLD (15 days: 1296 km, 25 days: 2160 km). During the period of the strong East Asian winter monsoon (February to April), the current flow can be reversed by monsoon winds [25], and the mixing of Japanese and Taiwan larval populations can therefore occur. It is known that intertidal and bathy-pelagic communities have a large degree of gene flow along the Kuroshio region, resulting in a lack of population

genetic differentiation by distance [46–49], and the data shows that *X. testudinatus* has the same pattern. There is substantial population connectivity among deep-sea vents assemblages in the West Pacific (including vent associated *Neoverruca* barnacles, *Bathymodiolus* mussels, *Shinkailepas* limpets and *Alvinocaris* shrimps) which can be caused by the strong mixing of the deep waters due to Kuroshio Current [42] interplaying with complex eddy movements [50–54].

Bayesian skyline plot analyses showed that all the studied populations of *X. testudinatus* had a similar demographic history, with population expansion taking place from 85,000~70,000 until 42,000~15,000 years before the present (Figure 3), which was in the period of the last glacial maxima (LGM). During the LGM, the sea level was lower by 120 m [55] compared to present-day levels. We hypothesize that when sea levels rose after the LGM, vent crab populations in northern Taiwan expanded and were dispersed by the Kuroshio Current to vent sites to the north.

The lack of genetic differentiation among the shallow and deep-water populations in Taiwan suggests the larvae of *X. testudinatus* actively disperses along the depth gradient. There are various factors that can affect the vertical migration behavior of marine invertebrate larvae. Daigle and Metaxas [56] revealed that larvae from different taxa have different responses towards the depth thermocline under laboratory conditions. Echinoderm larvae can move vertically across the thermoclines. Scallop larvae, however, always stay below the thermocline and maintain at specific depths. Brachyuran larvae are strong swimmers and have cyclic migrations (period of a tide cycle or a day) along the depth gradient and are not affected much by the presence of thermocline (see review by Anger et al. [57]). Zoea I and megalopa of *X. testudinatus* have well-developed eyes and display positive phototaxis [23]. Zoea and megalopa of *X. testudinatus* can actively swim to the shallow depths during the day and be transported by the Kuroshio Current. Larvae of *X. testudinatus* may be able to control their vertical position and be dispersed horizontally to larger geographical distances [58]. On the east coast of Taiwan, there are also seasonal upwellings (May to September) which can facilitate the vertical migration of vent crab larvae from the deep-sea to the surface [59]. Vertical movement pattern was observed in the deep-sea hydrothermal vent limpet *Shinkailepas myojinensis*, the larvae migrating from the deep-sea vents to the ocean surface from 4 to 43 days. Surface waters provide larvae with a greater supply of phytoplankton and opportunities to be dispersed by strong currents to greater geographical scales [53]. This indicates that larval behavior explains the observed vertical connectivity of shallow and deep-sea populations.

Brachyuran larvae are negatively buoyant and can naturally sink to benthic habitats for settlement. During the late larval development of *X. testudinatus*, the megalopa probably needs physical and/or chemical cues in hydrothermal vents for locating the appropriate habitats for settlement. For example, the settlement of larvae of the deep-sea hydrothermal vent barnacle *Neoverruca* responds to higher temperature stimulation in vent mouths [59,60]. When late stages of naupliar larvae of *Neoverruca* experience an increase in water temperature (indication on arrivals in the vent region), it develops rapidly into cyprids for settlement. Since *X. testudinatus* is exclusively found in vents, its larvae must respond to environmental cues associated with the habitat. Whether the settlement cues are temperature or chemical will still need to be determined. Dahms et al. [24] observed megalopa of *X. testudinatus* preferring to stay near the substrate, while Hwang et al. [61] reported the settlement of megalopa and juvenile crabs on sulphur aggregates at Kueishan Island. The sulphur aggregates provide important settlement sites and nursery grounds for the first crabs.

The high level of larval dispersal of *X. testudinatus* along depth and geographical gradients is probably a pattern shared among some deep-sea organisms in the West Pacific—a consequence of their behavior and biology as well as the strong effects of the Kuroshio Current. Other hydrothermal systems and species assemblages show different patterns. In the Fiji and Lau Basins, for example, the vent neolepadid barnacles [62,63] and vent crabs (*Austinograea*) [64] exhibit a strong genetic differentiation between sites. The Xenograpsidae

is a relatively old thoracotreme family and on the basis of a recent molecular study, dates from the late Cretaceous [65]. The present findings, that it has a relatively slow molecular differentiation rate with low genetic divergences across depth and geography, may be associated with its highly specialized habitat requirements. It also explains why the family is so small, with only one genus and three known species. The strong dispersal of *X. testudinatus* in the West Pacific also casts doubt on its taxonomic validity, where it is likely to be conspecific with the Japanese vent crab *X. novaeinsularis*. *Xenograpsus novaeinsularis* was originally described from the Ogasawara Islands and subsequently reported from the Mariana Arc [66,67]. No molecular sequences are available, as they all had been preserved in formalin. *Xenograpsus novaeinsularis* and *X. testudinatus* are morphologically very close and there is enough variation to suggest they may be the same [20]. Certainly, the specimens from Shikine-jima Island in the Izu archipelago are in the same volcanic arc as the Ogasawara islands, and their genetic sequences [22] are almost identical to those from Taiwan and southern Japan. Future research is needed to investigate the genetic identity and population ecology of *X. testudinatus* and *X. novaeinsularis* in the West Pacific. The third species, the New Zealand vent crab, *X. ngatama*, was described and is only known from the depths of Macauley Cauldron [68] and is genetically close but distinct from *X. testudinatus* (unpublished data). It is possible that *X. ngatama* also occurs in shallow water vents when more surveys are done.

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