Zoological Studies **62:**24 (2023)
doi:10.6620/ZS.2023.62-24

Zoological Studies

Open Access

Genetic Structure of the Endemic Fiddler Crab *Uca* (*Xeruca*) *formosensis* on the West Coast of Taiwan

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Received 20 January 2022 / Accepted 17 February 2023 / Published 1 June 2023
Communicated by His-Te Shih

Xeruca formosensis is the only endemic species of fiddler crab on the west coast of Taiwan. However, its natural habitats and populations have been compromised by excessive anthropogenic activities and improper land use over the past four decades. In light of these changes, we sought to evaluate the genetic diversity and gene flow of the species by examining the genetic variation of *X. formosensis* at different sampling locations. To this end, we performed molecular analyses of three endonuclease-amplified fragment length polymorphisms (TE-AFLP) and the cytochrome oxidase subunit I (*COI*) marker from leg muscle samples. We found that the genetic variation within sampling locations was higher than that among sampling locations, and the expected heterozygosity of genetic diversity (H_j) was 0.152 for TE-AFLP data. Meanwhile, the *COI* marker showed high haplotype diversity (h = 0.976 ± 0.008) and a low genetic differentiation level (F_{ST} = 0.021) in *X. formosensis* populations. Importantly, the genetic connectivity of *X. formosensis* may be influenced by larval-stage crabs drifting between coastal and marine habitats. As such, crab gene flow is promoted among populations by larval exchange via nearshore currents. Although *X. formosensis* has high gene flow, the species could undergo an extinction crisis if the population sizes continue to decline, as with most endangered species. In order to maintain the natural habitats and population size of *X. formosensis*, long-term monitoring and investigation will be necessary.

Key words: *Xeruca formosensis*, Genetic variation, Larvae drifting, Genetic diversity.

BACKGROUND

In Taiwan, there are 15 known species of fiddler crabs, with *Xeruca formosensis* being the only endemic species on the west coast. The first record of this species was made at Lugang in Changhua County in 1918 (Shih et al. 1999), and it was announced as a new species (originally *Uca formosensis*) in 1921 (Shih et al. 1999). In 2016, the genus was changed to *Xeruca* based on morphological and molecular evidence, including mitochondrial 16S rDNA, cytochrome oxidase subunit I (*COI*), and nuclear 28S rDNA (Shih 2015; Shih et al. 2016), which has been further supported by the mitogenome analysis (MY Liu and Shih 2022). Previous studies indicated that *X. formosensis* inhabits tidal areas where there are wide flats with very clayey-muddy substrate and no mangroves on the supratidal zone (Shih et al. 1999; Liou 2012). In the past, large populations of this species existed at several locations, including Zengwen estuary, Dadu estuary, Xiangshan area, and Shengang area (Fig. 1). In particular, the Shengang area of Changhua County was once known as the "hometown of *X. formosensis*" (Shih 1997; Liou 2012). However, recent anthropogenic activities and improper land use

Citation: Hung KC, Liou CY, Wen CC, Lin HC. 2023. Genetic structure of the endemic fiddler crab *Uca* (*Xeruca*) *formosensis* on the west coast of Taiwan. Zool Stud **62:**24. doi:10.6620/ZS.2023.62-24.

have led to marked declines in the natural habitats and populations of *X. formosensis*. Human endeavors such as landfills, fish farms and artificial mangrove planting have nearly eliminated *X. formosensis* from many areas (Shih et al. 1999; Chen 2008; Liao et al. 2008; Liou 2012). For example, inappropriate dike construction along the coastline of Shengang area has displaced *X. formosensis* and allowed both *Austruca lactea* and *Tubuca arcuata* to become two of the most dominant species in the wetland (Liou 2010). In addition, expansion of aquaculture facilities near the Zengwen estuary in the Tainan City greatly damaged the habitats of *X. formosensis* such that only three stable populations remain in this location (Liou 2012). Meanwhile, construction of a water recycling center and anthropogenic planting of a mangrove forest in the Xiangshan area has led to a gradual decrease of the *X. formosensis* population in the past decade (Wu and Chang 2008).

According to the International Union for Conservation of Nature and Natural Resources (IUCN), regulations for protecting focal species should make use of gene flow, genetic variation and genetic differentiation as key indexes (Frankel 1974; Araujo

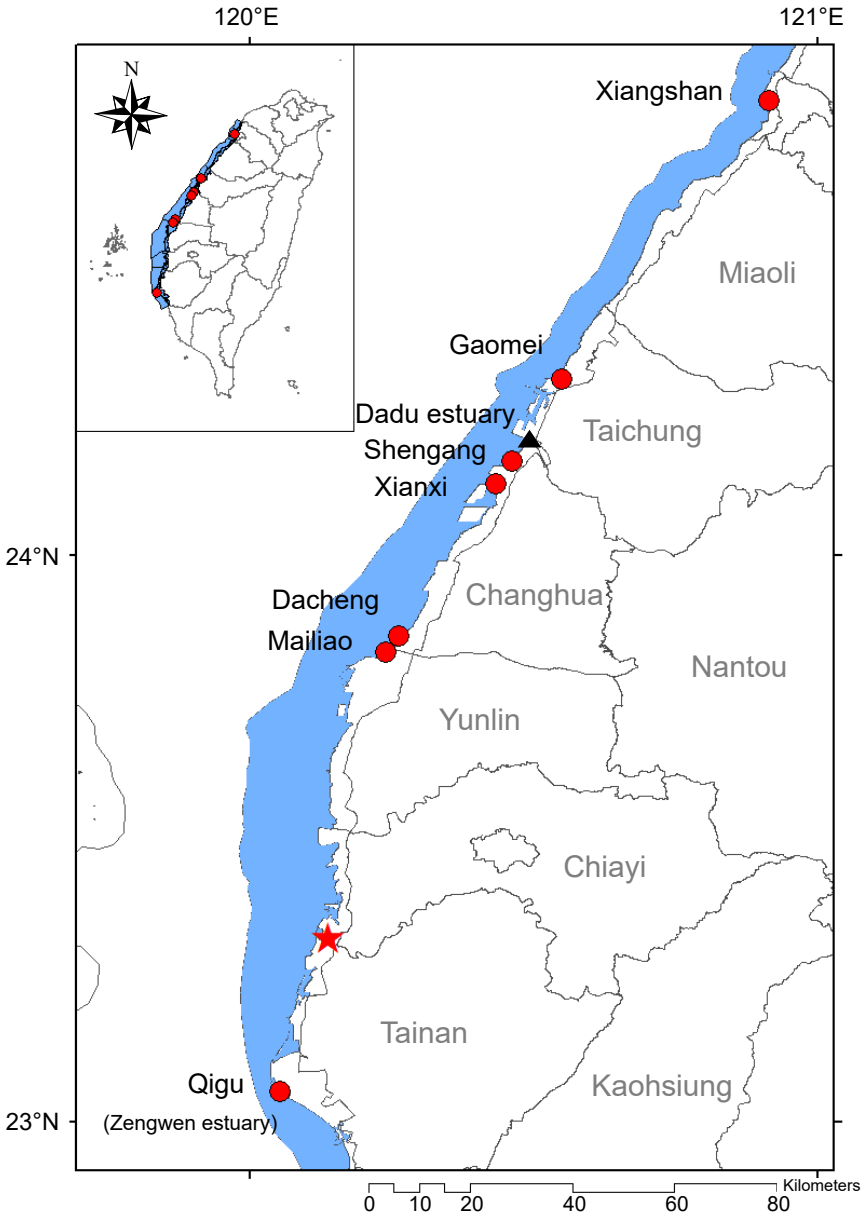

Fig. 1. Sampling sites for *Xeruca formosensis* in the west coast of Taiwan. Red circles represent the sampled populations, the red star represents the proposed population in Bazhang River at Jiayi County (this population was not yet sampled), and the black triangle represents the Dadu estuary.

and Ramos 2000; Geist 2010). Moreover, excessive anthropogenic development and inappropriate land use not only impact the natural habitats of organisms but also lead to decreased genetic diversity and increased risk of population extinction (Dixo et al. 2009; Haag et al. 2010). As such, research on the genetic structure and diversity among geographically distinct populations can be highly useful for identifying management units for vulnerable species (Baeza et al. 2019; Mendiola and Ravago-Gotanco 2021).

DNA fingerprinting and *COI* markers are robust established methods that can be used to efficiently produce large amounts of accurate genetic data for genetic diversity analyses and further conservation purposes. Many DNA fingerprinting methods have been implemented, including amplified fragment length polymorphisms (AFLP), and three endonuclease-amplified fragment length polymorphisms (TE-AFLP). These fingerprinting methods have been widely applied in different areas, including genetic structure research on crustaceans (Fratini and Vannini 2002; Herborg et al. 2007; Wieman et al. 2014; Seeb et al. 2002). Similarly, the *COI* marker of crustacea is useful for detecting and evaluating intraspecific diversity (Tang et al. 2003; Aoki et al. 2008). Since knowledge of genetic diversity and structure is important for species conservation, detailed studies to gather genetic structural information on the endemic fiddler crab *X. formosensis* are warranted. However, up to now, beside the molecular studies mentioned above (Shih 2015; Shih et al. 2016; MY Liu and Shih 2022), most studies on this species have focused on population size, behavior, habitat selection, adult and larval morphology (Takahasi 1935; Shih et al. 1999; Shih et al. 2005; Liao et al. 2008; Chen and Lee 2008; YC Zhang and Shih 2022). Only two studies have reported allozyme analyses, and these were conducted in 1984 and 1999 (Zhang 1984; Shih 1999). Thus, it is important to identify the current state of genetic variation at high-resolution using modern methods in order to better understand the genetic structure of *X. formosensis* among populations.

The habitats of *X. formosensis* are mostly found within the wetlands of the eastern Taiwan Strait, which is located between the South China Sea and the East China Sea on the western coast of Taiwan. The Taiwan Strait contains several major currents (Hsiao et al. 2011), including the China Coastal Current, the extension of the South China Sea Warm Current, and the intrusion from the Kuroshio Current (Hsieh et al. 2004; Lan et al. 2004; Guan and Fang 2006; Dur et al. 2007). These ocean currents are subject to strong seasonal influence by monsoons (Wyrtki 1961; Liang et al. 2003). In the summer, a northward intrusion of the current from the South China Sea and branch of the Kuroshio arises as the southwest monsoon appears from May to November. In winter months, the intrusion from the Kuroshio is blocked by the northeasterly monsoon, which lasts from approximately December to February depending on climatological conditions (Hu et al. 2010). Based on these environmental conditions and its life history, the breeding season of *X. formosensis* occurs in the summer, from April to September.

In light of recent habitat destruction and population declines, we sought to evaluate the species genetic diversity and structure for *X. formosensis*. To this end, we investigated the genetic variation across seven sampling locations that were geographically divided into three groups (north, central and south). We analyzed the expected heterozygosity of genetic diversity and population structure (including genetic clustering among locations) based on TE-AFLP DNA fingerprinting (Pritchard et al. 2000; Wieman et al. 2014). Furthermore, we determined the nucleotide diversity (π) and haplotype diversity (h) from mitochondrial DNA sequencing data. Multiple statistical methods, such as analysis of molecular variance (AMOVA), mismatch distribution, principal coordinate analysis (PCoA) and historical effective population estimation, were used to explore the endemic genetic status of *X. formosensis*. Overall, our findings contribute to understanding the current status of the endemic species, *X. formosensis*, in Taiwan.

MATERIALS AND METHODS

Sample collection

In total, 280 samples were collected from seven different locations in mudflat intertidal zones on the west coast of Taiwan with natural distributions of *X. formosensis* (Table 1). Adult *X. formosensis* individuals were collected from the following seven different coastal locations: Xiangshan area of Hsinchu City, Gaomei area in Taichung City, Shengang area, Xianxi area, and Dacheng in Changhua County, Mailiao area in Yunlin County, and Qigu area in the Tainan City; hereafter, these areas are respectively referred to as Xiangshan, Gaomei, Shengang, Xianxi, Dacheng, Mailiao, and Qigu. The *X. formosensis* sampling locations were further grouped according to geographic area: north (Xiangshan); central, (Gaomei, Shengang, Xianxi, Dacheng, and Mailiao); and south (Qigu) (Table 1). Total genomic DNA was extracted from adult individuals using the MasterPureTM DNA Purification Kit (Epicentre, United States) and stored in Tris-EDTA at -20°C for later use.

Molecular methods

DNA fingerprinting TE-AFLP genotypes data

Based on AFLP, the TE-AFLP method consists of three parts, as described by van der Wurff et al. (2000). First, total genomic DNA (~100 ng) was digested by *Xba*I, *Bam*HI, and RsaI, and two sets of adapters (*Xba*I adapter sequences were 5'-ACGTTGTGGCGGCGTCGGACTAGA-3' and 3'-CCGCCGCAGCTCTGATCT-5'; *Bam*HI adapter sequences were 5'-ACGAAGTCCCGCGCCAGCAA GATCC-3' and 3'-GGGCGCGGTCGTTCTAG-5') were selectively ligated using an enzyme-specific sequence to restriction fragments in a single reaction volume. Each reaction contained 2 µl 10X Ligase buffer, 2.0 µl 500 mM NaCl, 7.5 U ligase (NEB, USA), 1.25 U *Xba*I (Promega, USA), 4.0 µl *Bam*HI adapter (1 pmol/µl and 4.0 µl *Xba*I adapter (1 pmol/µl). Second, a labelled *Xba*I primer (5'-GGCGTCGAGACTAGACC-3' *, * represents the fluorescein-labeled site) and unlabeled *Bam*HI-CC primer (5'-GTTTCGCGCCAGCAAGAT CC-3') were used for selective amplification. Each reaction included 0.5 µl digestion/ligation DNA sample, 2.5 µl 5× PCR buffer, 0.25 µl *Bam*HI-C primer (10pmol/ µl), 0.25 µl *Xba*I-CC primer (10 pmol/µl), 0.125 µl Taq polymerase (Go-Taq, Promega, USA), 0.25 µl 10 mM dNTPs and 11 µl distilled water (van der Wurff et al. 2000). Third, polymerase chain reaction (PCR) was performed on a thermal cycler (Eppendorf Master cycler gradient 5331, Eppendorf AG, Germany) using an initial denaturation step at 95°C for 3 min, followed by 10 cycles (denaturation at 95°C for 30 s, annealing at 70°C for 30 s, elongation at 72°C for 1 min), 40 cycles (denaturation at 95°C for 30 s, annealing at 60°C for 30 s, elongation at 72°C for 1 min), and final elongation at 72°C for 20 min. After PCR, the samples were stored at -20°C for later experimentation. PCR products were used for SNP genotyping (130 loci for each sample). Gene-Mapper software was used to transfer SNP data to a 1 and 0 matrix format by detecting the presence of each band in the gel (Mission biotech Ltd, Taipei, Taiwan).

Mitochondrial DNA *COI* sequencing

A 648 base-pair region of the *COI* gene was amplified using the following universal primers: HCO-2198: 5'-TAAACTTCAGGGTGACCAAAAAAT CA-3' and LCO1490: 5'-GGTCAACAAATCATAAA GATATTGG-3' (Folmer et al. 1994; Shih 2015). PCR amplification conditions consisted of an initial denaturation step at 95°C for 5 min, followed by 35 cycles of 30 s at 95°C, 30 s for primer annealing at 51°C, and extension at 72°C for 15 min; after the reaction, samples were held at 4°C (Shih 2015). PCR products were separated by electrophoresis on a 2% agarose gel to check quality.

Molecular data analyses

TE-AFLP genotypes

Genetic clusters were assessed based on TE-AFLP genotype data of *X. formosensis* by Bayesian assignment test using STRUCTURE 2.3.4 (Pritchard et al. 2000). The program operation (number of subpopulations) was set as follows: 3,000 lengths burn-in period; 30,000 MCMC reps after burn-in; admixture model of ancestry info; and range of genetic demes (K) from 1 to 7, with three repetitions per genetic cluster (K) for allele frequencies correlated with the population of the frequency model. The best value of K was detected by calculating ΔK (Evanno et al. 2005). The genotype data were also used to estimate the molecular variance among/within sampling location (Excoffier et al. 1992),

Table 1. Sampling information on the fiddler crab *Xeruca formosensis*, including sampling location, group assignment (according to geographic distance), county, geographic coordinates, number of DNA samples for mtDNA (*COI*) and TE-AFLP markers analyses

Sampling location	Group	County	Geographic coordinates	Number of DNA samples	
				mtDNA (*COI*)	TE-AFLP analyses
Xiangshan	North group	Hsinchu	24°48'02.9"N 120°54'57.5"E	29	40
Gaomei	Central group	Taichung	24°18'39.7"N 120°32'59.7"E	21	40
Shengang	Central group	North Changhua	24°09'54.3"N 120°27'54.6"E	21	40
Xianxi	Central group	North Changhua	24°07'31.0"N 120°25'59.2"E	11	40
Dacheng	Central group	South Changhua	23°51'27.7"N 120°15'43.3"E	20	40
Mailiao	Central group	Yunlin	23°49'44.0"N 120°14'10.2"E	22	40
Qigu	South group	Tainan	23°03'27.9"N 120°03'36.0"E	28	40

and PCoA was performed using GeneAlex 6.501 (Peakall and Smouse 2006) based on an Euclidean distance matrix. Nei's heterozygosity (H_j) (Nei 1987) and the percentage of polymorphic loci (%P) for each location were calculated using AFLP-SURV version 1.0 (Lynch and Milligan 1994; Vekemans et al. 2002). A consensus tree of *X. formosensis* was constructed by the Phylip 3.6 software (Felsenstein 1993) based on the unweighted pair group method with arithmetic mean (UPGMA).

Mitochondrial DNA *COI* sequencing data

The analysis of molecular variance (AMOVA) was performed on data from 152 individuals using Arlequin 3.5.2.2. Molecular diversity indices, including the number of haplotypes (Nh), haplotype diversity (h), nucleotide diversity (π), pairwise fixation index (F_{ST}) estimates, and the average number of nucleotide differences (k), were estimated using DnaSP, version 6.0. Two neutral hypothesis tests, the Tajima D test (Tajima 1983) and Fu's Fs test (Fu 1997) were performed to detect the expansion of the whole population of *X. formosensis*. The mismatch distribution (Lavery et al. 1996) was determined using Arlequin with a spatial expansion model, and the sum of square deviation (SSD) of observed frequencies was greater than the expected frequencies ($P > 0.05$), indicating that this population conforms to an expansion model (Harpending 1994; Deli et al. 2016). In addition, the unit of mutational time (τ) and absolute time since expansion (T) at the same time were assessed according to mismatch distribution, following analyses described in previous studies (Rogers and Harpending 1992; Harpending 1994; Tokuyama et al. 2020). The effective population size was estimated based on formulae from a previous study (Watterson 1975), and the mutation rate ($\mu = 2.1 \times 10^{-8}$) was adopted from a previous study on the terrestrial crab genus *Sesarma* spp. (Wares and Cunningham 2007; Wieman et al. 2014). Furthermore, a haplotype network was constructed using PopART 1.7 by the minimum spanning network (MSN) method (Leigh and Bryant 2015; Hardianto et al. 2022). The nucleotide sequences of *X. formosensis* from this study were deposited into GenBank (National Center for Biotechnology Information, NCBI) under accession numbers ON692540-ON692691.

RESULTS

TE-AFLP data

Total TE-AFLP data were obtained from 280 individuals and used for AMOVA. The results showed 93% genetic variation within location and 98% genetic variation within groups (Table 2). The Bayesian assignment test revealed two genetic clusters for *X. formosensis* ($\Delta K = 2$) (Fig. 2). Comparing the results of the three groups of *X. formosensis* in this study (north, central and south groups) indicated that both genetic diversity and polymorphic loci in the central group were lower than those of the north and south groups (Table 3); those for Shengang ($H_j = 0.033$; %P = 16.92%) were the lowest. The highest genetic diversity and polymorphic loci were seen in the south group, especially those in Mailiao. For the whole population, the percentage of polymorphic loci was 86.48%, and the expected heterozygosity of genetic diversity index was 0.152 (Table 3). PCoA analysis did not show obvious clustering of the seven *X. formosensis* sampling locations (Fig. 3). Moreover, the UPGMA tree only indicated that the distance of the north group was relatively farther than others; the analysis did not clearly distinguish the southern and central groups too (Fig. 4).

Mitochondrial DNA *COI* sequencing data

A 648 base-pair coding region of the *COI* gene was sequenced from 152 adult individuals collected from seven locations of *X. formosensis*. According to *COI* sequencing results, we found nucleotide frequencies of A = 27.2%, T = 36.5%, C = 20.1%, and G = 16.1%. The results of AMOVA showed 96–98% genetic variation within sampling locations (Table 2), and mean F_{ST} value obtained from the AMOVA was 0.021 (Table 4), with a significant P-value ($P < 0.05$). Pairwise comparisons showed negative F_{ST} values between Xiangshan and Mailiao and between Shengang and Mailiao (Table 5); negative values indicate essential genetic homogeneity. The pairwise F_{ST} values comparing between the north, central and south groups were positive (Table 6). Notably, when comparing between the north and central groups, and between the north and south groups, the pairwise F_{ST} values were significant ($P < 0.05$). However, comparing between the south and central groups yielded a non-significant pairwise F_{ST} value (P value = 0.23).

According to our molecular diversity analysis of the *COI* marker, haplotype diversity (h) was high in all locations, with Xiangshan having the highest value ($h = 0.993 \pm 0.014$). In addition, the nucleotide diversity values (π) for Xiangshan, Xianxi, Qigu and Mailiao populations were higher than those for Gaomei, Shengang and Dacheng. As shown in table 3, 118 haplotypes were detected among all samples. The overall haplotype diversity index was 0.976 ± 0.008 (range, 0.924–0.993), while the nucleotide diversity

index was 0.010 ± 0.004 (range, 0.007–0.016), and the average number of nucleotide differences (*k*) was 5.95 (range, 4.65–7.37). Further, the nucleotide diversity indexes for Gaomei and Shengang were lower than those for the rest of the examined locations. Haplotype network analysis revealed no obvious patterns (Fig. 5).

The results of Tajima's *D* test ($P < 0.05$) and Fu's *F*s test ($P < 0.02$) showed negative values for our *X. formosensis* samples (Table 4). Mismatch distribution plots exhibited a single peak for *X. formosensis*, which indicates a past expansion (Fig. 6). Furthermore, the units of mutational rate prior to the present (τ) was 4.684, and the absolute time (T years) since the expansion of the *X. formosensis* was 108,861 years ago (Table 4). We also determined that the population size post-expansion ($\theta_1 = 28.44$) was higher than before the expansion ($\theta_0 = 2.02$), and the mode value of theta in the MCMC parameters was 0.09823. The effective population size (N_e) was 4.68×10^6 for *X. formosensis* (Table 4). Furthermore, according to the mutation rate we adopted, the *X. formosensis* population is predicted to have expanded in the past 100,000 years.

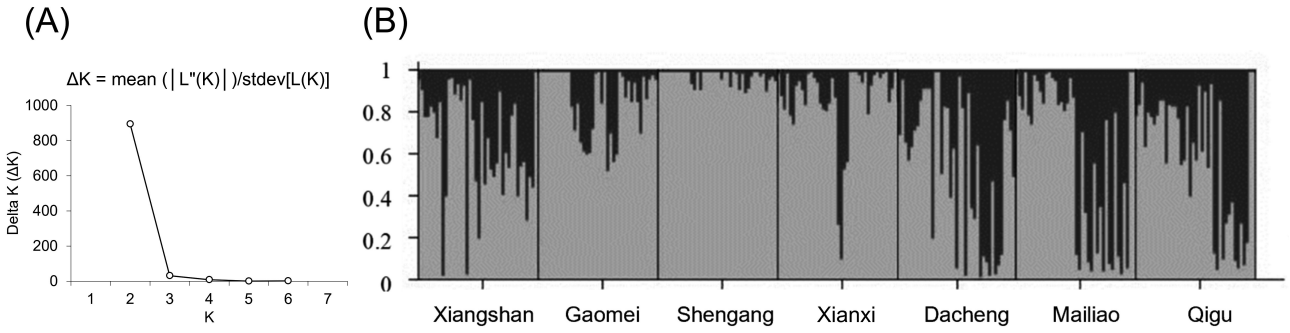

Fig. 2. Genetic analysis as inferred by the assignment test and using a Bayesian approach in STRUCTURE 2.3.4. A) Estimation of populations using a Δ*K* value of 2 based on TE-AFLP markers. B) The result of Bayesian assignment test indicted two genetic clusters for seven locations of *X. formosensis*.

Table 2. Analysis of molecular variance for seven sampling locations, three groups (north, central and south) and two subpopulations (north and others) of *Xeruca formosensis* based on TE-AFLP and mitochondrial DNA cytochrome oxidase subunit I (*COI*) data

DNA data type	Source	*d.f.*	Percentage of variation	Sum of squares	Variance components
TE-AFLP	Among 7 sampling locations	6	7.00	236.22	0.73
	Within sampling location	273	93.00	2771.10	10.15
	Total	279	100	3007.32	10.88
	Among 3 groups	2	2.86	71.17	0.31
	Within group	277	97.14	2936.15	10.60
	Total	279	100	3007.32	10.91
	Among 2 populations	1	1.51	22.06	0.17
	Within populations	278	98.49	2985.26	10.74
	Total	279	100	3007.32	10.90
mtDNA *COI*	Among 7 sampling locations	6	2.07	25.55	0.06
	Within sampling location	146	97.93	426.94	2.92
	Total	152	100	452.48	2.99
	Among 3 groups	2	2.06	1.45	0.08
	Within group	149	97.94	588.10	3.95
	Total	151	100	602.79	4.03
	Among 2 populations	1	3.39	10.45	0.14
	Within populations	150	96.61	592.34	3.95
	Total	151	100	602.79	4.09

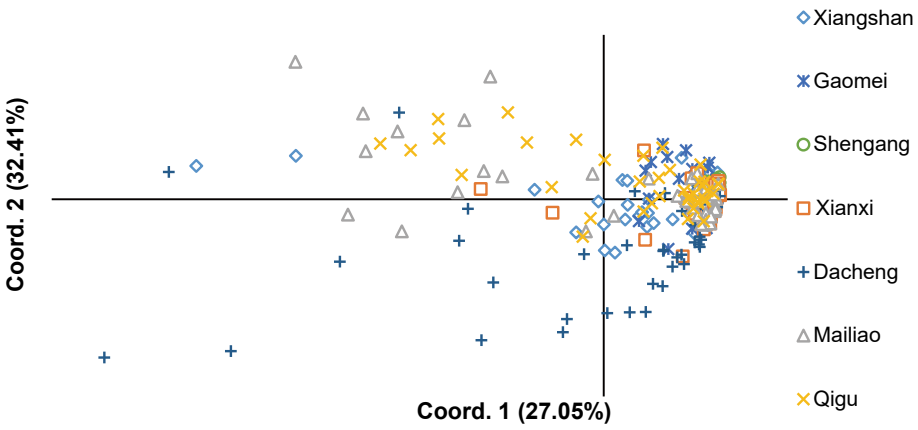

Fig. 3. Results of principal coordinate analysis (PCoA) of TE-AFLP markers.

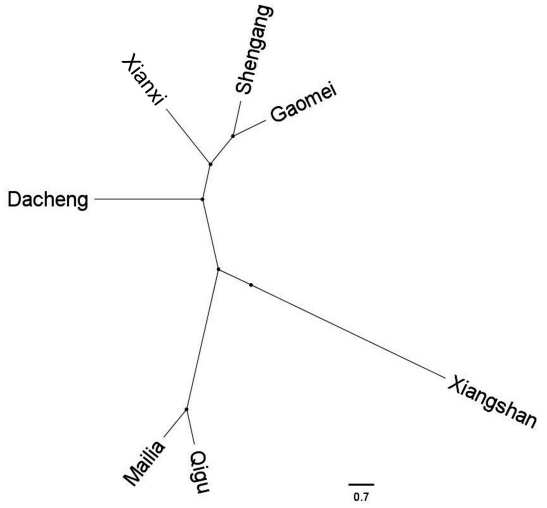

Fig. 4. *X. formosensis* consensus tree constructed by the UPGMA method based on TE-AFLP markers.

Table 3. Nuclear and mitochondrial COI DNA diversity indexes for the fiddler crab *Xeruca formosensis*

Genetic marker	TE-AFLP		mtDNA (*COI*)			
Sampling location and groups	H_j	%P	N_h	$h \pm SD$	$\pi \pm SD$	k
Xiangshan	0.197	82.31 %	25	0.993 ± 0.014	0.016 ± 0.008	7.12
Gaomei	0.085	43.08 %	11	0.924 ± 0.033	0.007 ± 0.004	4.65
Shengang	0.033	16.92 %	18	0.976 ± 0.023	0.009 ± 0.005	5.23
Xianxi	0.106	46.92 %	10	0.946 ± 0.066	0.012 ± 0.007	6.98
Dacheng	0.221	80.00 %	18	0.984 ± 0.024	0.010 ± 0.005	5.36
Mailiao	0.221	96.92 %	14	0.939 ± 0.035	0.010 ± 0.005	5.91
Qigu	0.206	88.46 %	22	0.980 ± 0.014	0.010 ± 0.006	6.22
North group	0.197	82.31 %	25	0.993 ± 0.012	0.016 ± 0.008	7.12
Central group	0.147	59.23 %	71	0.972 ± 0.011	0.012 ± 0.006	5.41
South group	0.206	88.46 %	22	0.982 ± 0.015	0.012 ± 0.006	5.75
North populations	0.197	82.31 %	25	0.993 ± 0.012	0.016 ± 0.008	7.12
Other populations	0.160	67.69 %	85	0.980 ± 0.007	0.011 ± 0.006	7.37
Whole population	0.152	86.48 %	118	0.976 ± 0.008	0.010 ± 0.004	5.95

H_j: expected heterozygosity of genetic diversity. %P: percentage of polymorphic loci. N_h: number of haplotypes. h: haplotype diversity. π: nucleotide diversity. k: average number of nucleotide differences.

DISCUSSION

Genetic variation and structure

Our analysis of TE-AFLP data and *COI* marker from *X. formosensis* showed that the genetic variation within the sampling location was higher than it was among sampling locations. These results indicate that *X. formosensis* possesses characteristics of genetic structure that are typical of decapods (Tracey et al. 1975; Brian et al. 2006). Importantly, *X. formosensis* currently has greater than 90% genetic variation within location and group, and the mean F_{ST} value (0.021, with significant *P*-value) suggests the possibility of an *X. formosensis* metapopulation (Wang et al. 2019; Tokuyama et al. 2020). In addition, this result indicates that *X. formosensis* still has high gene flow among sampling locations, even though it faces survival pressures and habitat destruction. Notably, the pairwise F_{ST} among the three groups showed that the northern group was significantly different from the other two ($P < 0.05$), but there was no significant difference between the southern and central groups. In line with this result, the UPGMA tree only indicated a grouping of the northern group and others (Fig 4). Thus, we concluded that *X. formosensis* should be divided into two populations (north and others) rather than three (north, central and south). This conclusion led us to further conduct the AMOVA analysis and calculate diversity indexes for the two populations (north and others); the results are shown in tables 2 and 3.

The genetic diversity value ($H_j = 0.152$) we observed for *X. formosensis* is higher than that of another fiddler crab species, *Uca maracoani* (0.062), reported in Wieman et al. 2014. However, it is lower than the reported values for other brachyurans, such as swimming crab (*Portunus trituberculatus*) and *Cancer setosus* (Gomez-Uchida et al. 2003; Liu et al.

Table 4. Neutrality test and mismatch distribution values for *Xeruca formosensis*

	N_e	T_D	F_{us}	τ	T	θ_0	θ_1	SSD	F_{ST}
Whole population	4,680,000	-2.109 **	-3.332 **	4.684	108,861.37	2.02	28.44	0.0013	0.021 **

T_D: Tajima D test. F_{us}: Fu's *F*s test. τ: time since expansion in units of mutational time. T: absolute time since expansion in years. θ_0: estimated value of population size before expansion. θ_1: estimated value of post-expansion population size. SSD: sum of squared deviation value. F_{ST}: fixation index. ** mean significant value ($P < 0.05$).

Table 5. The pairwise F_{ST} value (below the diagonal) and pairwise F_{ST} *P*-value (above the diagonal) for the *COI* marker among 7 sampling location of *Xeruca formosensis*

	Xiangshan	Gaomei	Shengang	Xianxi	Dacheng	Mailiao	Qigu
Xiangshan	-	0.0371 ± 0.006**	0.2168 ± 0.010	0.0156 ± 0.004**	0.0537 ± 0.006	0.9043 ± 0.013	0.0156 ± 0.004**
Gaomei	0.0362	-	0.1602 ± 0.012	0.0664 ± 0.007	0.0898 ± 0.010	0.1641 ± 0.014	0.1426 ± 0.011
Shengang	0.0090	0.0165	-	0.1113 ± 0.011	0.3291 ± 0.018	0.5889 ± 0.014	0.2940 ± 0.019
Xianxi	0.0547	0.0344	0.0263	-	0.2832 ± 0.015	0.0566 ± 0.008	0.1660 ± 0.012
Dacheng	0.0322	0.0257	0.0053	0.0063	-	0.1953 ± 0.012	0.2539 ± 0.014
Mailiao	-0.0291	0.0250	-0.0156	0.0674	0.0177	-	0.1055 ± 0.010
Qigu	0.0435	0.0133	0.0055	0.0130	0.0053	0.0341	-

** mean significant value ($P < 0.05$).

Table 6. The pairwise F_{ST} value (below the diagonal) and pairwise F_{ST} *P*-value (above the diagonal) for the *COI* marker among 3 groups (north group, central group, and south group) of *Xeruca formosensis*

	North group	Central group	South group
North group	-	0.01367 ± 0.0037 **	0.01758 ± 0.0039 **
Central group	0.028	-	0.22852 ± 0.0131
South group	0.043	0.0035	-

** mean significant value ($P < 0.05$), and for details of groups, see Table 1.

2012). According to mitochondrial *COI* DNA diversity analyses, *X. formosensis* has higher levels of haplotype diversity and nucleotide diversity (Table 3) than fiddler crabs and other crabs from mangrove, coastal and marine habitats (Azuma et al. 2007; Aoki et al. 2008; Darling et al. 2008; Wieman et al. 2014). Nevertheless, the diversity of *X. formosensis* is lower than that of its sympatric species, *A. lactea* (Tokuyama et al. 2020). In addition, the highest value of genetic diversity (H_j) was found in the Mailiao and Dacheng sites (Table 5). The Mailiao sampling site is a large, abandoned fish farm near the Sixth Naphtha Cracking Complex, south of the Zhuoshui River. According to our personal observations, the distribution area was approximately

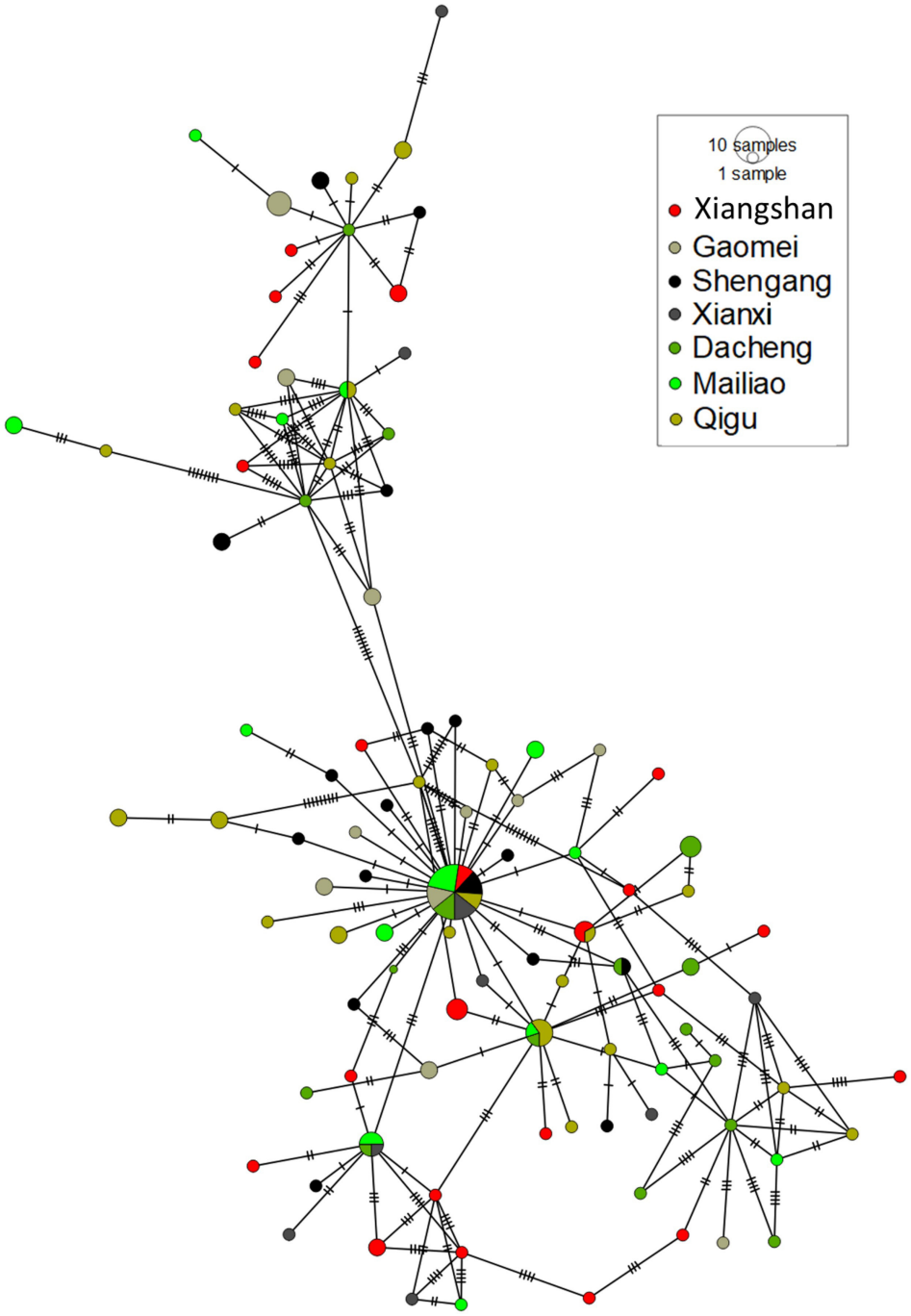

Fig. 5. TCS network showing the relationships among the recorded haplotypes of *X. formosensis* based on the analysis of a 648 base pair region of the mitochondrial gene *COI* using PopART.

41 hectares [about 4 individuals (ind)/m^2]. Relative to other locations, the number of individuals, distribution area, genetic diversity (0.221), and percentage of polymorphic loci (97%) were all higher for the Mailiao site than the other six sampling locations (Table 3). Of note, the Dacheng site is located just north of the Zhuoshui River, only about 4 km from the Mailiao location.

In the present study, the nucleotide diversity of the central group was the lowest (0.012 ± 0.006), but this population showed relatively high haplotype diversity values. This pattern is consistent with a rapidly growing population derived from a sporadic or small effective population (Hardianto et al. 2022). However, Xianxi showed both high haplotype and nucleotide diversity (h = 0.946 ± 0.066; π = 0.012 ± 0.007) based on the *COI* data, suggesting that the source of larvae at Xianxi might be a different location or that it was large and stable (Hardianto et al. 2022). Of note, the number of individuals found at Xianxi is typically much smaller than at other sites (according to our field observations over the past few years), and both the percentage of polymorphic loci and the genetic diversity were relatively low (H_j = 0.106; %P = 46.92%) based on TE-AFLP data. Furthermore, a previous study reported low values of polymorphic loci and the genetic diversity based on allozyme analysis if the habitat was not suitable for *X. formosensis* (Shih 1999). Together, these results suggest that the population at Xianxi might be undergoing survival pressure. The Xianxi site is located on the coastal wetland of the Changhua coastal industrial park, which was developed in 1979 (https://www.rtaiwanr.com/lukang/changhua-coastal-industrial-park). In a field survey conducted in 2014, we found that the number of *X. formosensis* individuals was relatively smaller than at other locations. In 2019, floating solar panels were installed at the nearby mudflat. It is uncertain what influence this development may have on the *X. formosensis* in the future. Since our *COI* and TE-AFLP results showed different patterns at Xianxi, further study with more sampling will be needed to more accurately assess the genetic structure, only if the habitat does not disappear due to anthropogenic activities. Similar to the situation at Xianxi, the Shengang population had similarly high haplotype and nucleotide diversity based on the *COI* data, but *X. formosensis* in Shengang had the lowest genetic diversity and polymorphic loci values. In 1999, the population size in Shengang abruptly decreased due to preparatory construction for a controversial landfill project (Shih 1997; Liou 2012) that was ultimately shelved. Between 2003 and 2006, the trail and embankment were constructed again (Chen 2008); however, this construction project did not improve the habitat. In fact, the conditions became worse, and the number of *X. formosensis* individuals at this site has declined in the past 15 years. Recently, the species has been replaced by other species of fiddler crabs, namely *A. lactea*, *T. arcuata* and *Gelasimus borealis*. The population density of *X. formosensis* dropped from 0.59 ind/m^2 in 1991 (Lee 1991) to only 0.022 ind/m^2, according to the results of our field survey in 2019 (Fig. 7).

Genetic cluster analysis using TE-AFLP marker data revealed that there were two genetic clusters for each sampling location and also that the genetic structures of populations at Gaomei, Shengang and Xianxi showed little difference from the other locations. Nevertheless, the nucleotide diversity value from *COI* marker data was lower for the central group than it

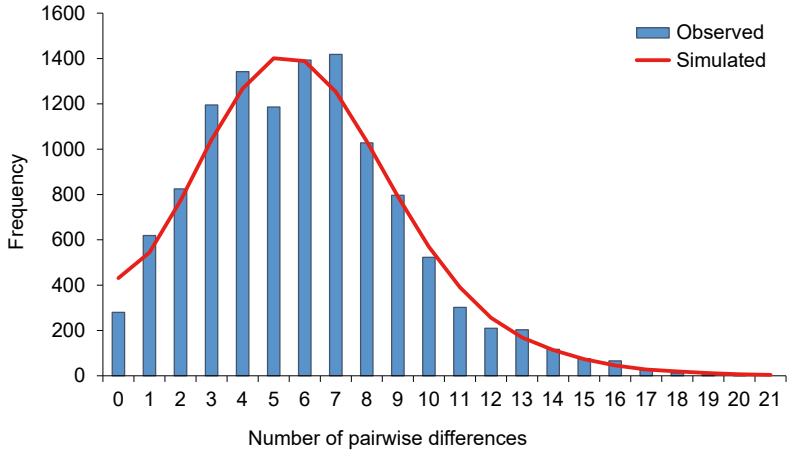

Fig. 6. Mismatch distribution for *X. formosensis* determined using Arlequin 3.5.2.2 and based on mitochondrial DNA cytochrome oxidase subunit I (*COI*) data. Blue-colored bars represent the observed frequency and the red-colored curve represents the expected frequencies under a sudden expansion model.

was for the other sites. Likewise, the genetic diversity, percentage of polymorphic loci, haplotype diversity and nucleotide diversity of the central group were all relatively low as well (Table 3). According to previous reports, several populations of *X. formosensis* have been abruptly reduced by different developmental pressures (Shih 1997; Shih et al. 1999). For example, the *X. formosensis* population in the Gaomei area disappeared due to the construction of a breakwater and seawall at natural shorelines about 20 years ago. Thereafter, only a few isolated individuals have been sporadically seen near the mudflat. Larger numbers of individuals did not appear next to the seawall until the summer (approximately June to September) of 2017. The PCoA of TE-AFLP data indicated that there was no obvious grouping of individuals by sampling location. Additionally, the haplotype network analysis of *COI* data indicated widespread haplotypes in each location. The *COI* haplotype network analysis also revealed a "star-like" tree topology for *X. formosensis*, which is an indication that the population has recently undergone a founder effect or population bottleneck (Slatkin and Hudson 1991). Under such circumstances, a sudden population expansion would increase the probability of retaining new mutations (Fu 1997).

Population historical expansion and dynamics

The values from Tajima's *D* and Fu's *F*s test were significantly negative (Table 4), and mismatch distribution indicated a low sum square deviation (SDD) value of 0.0013, which was not significant (> 0.05). These results suggest that the *X. formosensis* are currently expanding. When we apply a mutation rate adopted from *Sesarma* spp., the *X. formosensis* population appears to have expanded since 100,000 years ago (about 130,000 to 12,000 years ago in the Late Pleistocene) (Walker et al. 2012). At that time in Taiwan, the sea level was lower and the land area was larger than now (Oshir and Noihra 2000; Tokuyama et al. 2020). To be more precise, we calculated an expansion time for *X. formosensis* of 108,861 years ago. In contrast, the *A. lactea* population in Taiwan expanded 30,000 years ago (Tokuyama et al. 2020), when the local ocean environment was relatively stable.

The genetic connectivity among sampling locations may be affected by inshore water circulation patterns along the coastal area (Gordon 2007; Silva et al. 2010; Hardianto et al. 2022). Several studies also show that coastal environmental factors and the life cycles of marine crustaceans (inshore crab, *Carcinus maenas*; fiddler crabs, *Austruca lactea*, *Austruca perplexa* and *T. arcuata*) greatly affect the population structure (Peliz et al. 2007; Moksnes et al. 2014; Aoki et al. 2008; Bashevkin et al. 2020; Tokuyama et al. 2020; Hardianto et al. 2022). Other species in the ocean (*e.g.*, *Acanthochromis polyacanthus* and tropical reef fishes) are also subject to similar influences (Planes et al. 2001; Rocha et al. 2005). Both connectivity and population dynamics are driven by interactions between hydrology and life history (Cowen and Sponaugle 2009; Mendiola and Ravago-Gotanco 2021). Our molecular data indicated *X. formosensis* populations at the western coast in Taiwan should be considered as a metapopulation, although the gene flow in the central group might be somehow restricted. There may also be a correlation between the hydrology of the western inshore of Taiwan and the genetic connectivity of *X. formosensis*, since many previous studies have shown that larval-stage crabs play an important role in reducing geographical impacts on population dynamics (Peliz et al. 2007; Moksnes et al. 2014; Bashevkin et al. 2020). As previously mentioned, the dispersal mechanisms of *X. formosensis* larvae may be affected by many coastal

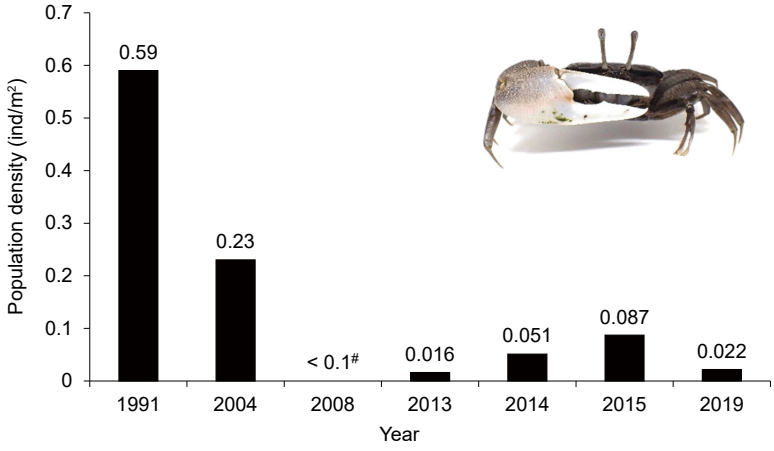

Fig. 7. Timeline of the population density change of *X. formosensis* at the Shengang area from 1991 to 2019. #No population density data available.

environmental factors, but the larval drifting dynamics and specific influences remain to be clarified. Therefore, it would be highly valuable to construct a drifting simulation of *X. formosensis* larvae in order to better understand the population genetics of the species.

Drift dynamics of crab larvae are known to play an important role in the settlement of fiddler crabs (Silva et al. 2010; Ituarte et al. 2012; Tokuyama et al. 2020). It is also thought that the population genetic connectivity of benthic species can be maintained by very few larvae drift processes (Bashevkin et al. 2020). While crab larvae can swim among intertidal zones, this behavior is coordinately affected by many marine environmental factors (Ituarte et al. 2012; Hardianto et al. 2022). Thus, inshore currents and hydrology appear to be the major determinants of most migration and gene flow (Peliz et al. 2007). For example, the different vertical positions of inshore crab (*Carcinus maenas*) larvae may critically affect the direction of dispersal by different current systems or tidal cycles (Moksnes et al. 2014). Furthermore, green crab (*Carcinus maenas*) larvae were shown to spread along a coastal area by surface ocean currents, along with diel vertical migration (DVM) behaviors of zooplankton and fish dispersal in intertidal or coastal regions (Queiroga et al. 1994; Peliz et al. 2007). Similarly, the larvae of *X. formosensis* are thought to spread and migrate with the help of the oceanographic conditions, which is expected to drive gene flow among subpopulations along the coast.

Management and conservation

A previous study reported that some populations of *X. formosensis* have gradually disappeared, including those in Keelung, Yongan (Kaohsiung) and Yilan County (Shih et al. 1999). In 1992, the *X. formosensis* populations in the Qigu area of Tainan City were huge, relatively stable, and completely distributed (Liou 2012). However over the next 20 years, much of the habitat in the original distribution area was destroyed, causing the crabs to become displaced or disappear and leaving a patchy distribution (Liou 2012). Therefore, a conservation strategy to prevent reductions in gene flow is urgently needed in order to protect the remaining small populations (Aoki and Wada 2013). A parallel example is *T. arcuate*, a common fiddler crab with a high genetic diversity in its Taiwan population (Aoki et al. 2008; Shih et al. 2022). Based on the analysis of restriction fragment length polymorphism (RFLP), Aoki et al. (2008) reported the population of *T. arcuata* in Nakagusuku Bay of Okinawa, Japan is not only small, but its genetic and nucleotide diversities are lower than those in other populations. Thus, they suggested that destruction of the nearby *T. arcuata* habitat on the coast of Okinawa may hinder gene flow. The *X. formosensis* in Xiangshan faces a similar situation, as the density dropped from 1.58 ind/m^2 in 2005 to only 0.25 ind/m^2 in 2006 (Wang et al. 2012). This population was negatively affected by the improper transplantation of mangroves (*e.g.*, *Kandelia candel*) and a water recycling center built in April 2006 (Shih et al. 1999; Liao et al. 2008). Along with the overall decrease in numbers, the high-density distribution area of *X. formosensis* declined from 8,000 m^2 to 2,500 m^2 (Wang et al. 2012). Until recently, *X. formosensis* could survive in the area due to some protective measures (Liao et al. 2008). However, it has been suggested that if wild populations continue to decline due to environmental pressures or some other factors, eventually the genetic diversity and gene flow of the population will decrease, and the population's extinction risk will increase (Hoffmann and Willi 2008; Aoki and Wada 2013). Therefore, it should be a priority to protect small populations with higher extinction risks than larger populations, so that gene flow among populations can continue.

Although the two molecular methods used in this study, TE-AFLP and *COI* marker, are modern analyses that revealed the population genetics of *X. formosensis*, there are limitations to our study. For example, future efforts should include the use of high-throughput molecular and powerful genetic marker methods such as RAD sequencing to increase the resolution of genetic structure and to more precisely detect intensity and directionality of gene flow (Davey and Blaxter 2011; Moksnes et al. 2014; Jeffery et al. 2017; Baeza et al. 2019).

CONCLUSIONS

Both TE-AFLP and *COI* marker results indicated that the *X. formosensis* on the western coast of Taiwan can be considered a metapopulation that is divided into two subpopulations (north and others). Despite the high gene flow in this metapopulation, if the local populations of *X. formosensis* along the western coast continue to decline, the species may face a crisis similar to those of other endangered species. Maintenance of suitable habitats will be crucial future work for restoring the populations of *X. formosensis*. We plan to continue our surveys and record the population genetics at several locations to gain a more comprehensive understanding of how the *X. formosensis* population is changing over time. Based on our genetic findings so far, environmental factors may allow for robust gene flow among locations and reduce the degree of differentiation between location. Therefore, future studies on the scale of spreading and patterns of drifting

behavior for *X. formosensis* larvae will be necessary to guide appropriate conservation efforts in the future.

Acknowledgments: Fiddler crab sampling in Qigu of Tainan City was performed with the approval of the Endemic Species Research Institute, Taiwan. Dr. Yong-Chao Su of Kaohsiung Medical University provided many suggestions on genetic analyses. The first author also thanks members of the Department of Life Science, Tunghai University for their help during the sampling. Furthermore, we thank Hsi-Te Shih and the two anonymous reviewers for helpful comments.

Authors' contributions: HKC, LHC collected the specimens and designed the research of genetic analysis; LCY provided detailed information on specimens and collection sites; WCC provided hydrodynamic information about patterns of larval dispersal. All authors contributed to the study conception and interpreted the results. All authors read and approved the final manuscript.

Competing interests: The authors declare that they have no conflicts of interests.

Availability of data and materials: The datasets generated or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication: Not applicable.

Ethics approval consent to participate: Not applicable.

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