



Research article

Analysis of the population structure and genetic diversity of the red swamp crayfish (*Procambarus clarkii*) in China using SSR markers

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ABSTRACT

Background: *Procambarus clarkii* produces high-quality, delicious meat that is high in protein, low in fat, and rich in calcium and phosphorus. It has become an important aquatic resource in China. Our objectives are (i) to analyze the level of genetic diversity of *P. clarkii* populations; (ii) to explore the genetic differentiation (Gst); and (iii) to propose appropriate strategies for the conservation.

Results: In this study, Shannon's index (I) and Nei's gene diversity index (H) for *P. clarkii* were high (I = 0.3462 and H = 0.2325 on average and I = 0.6264, H = 0.4377 at the species level) based on the SSR markers. The expected heterozygosity value of 17 microsatellite loci in 25 crayfish populations was 0.9317, the observed heterozygosity value was 0.9121, and the observed number of alleles per locus was 2.000; and the effective number of alleles per locus was 1.8075. Among the *P. clarkii* populations, the inbreeding coefficient within populations (Fis) was 0.2315, overall inbreeding coefficient (Fit) was 0.4438, genetic differentiation coefficient among populations (Fst) was 0.3145 and gene differentiation (Gst) was 0.4785 based on SSR analyses. The cluster analysis results obtained by unweighted pair-group method with arithmetic mean (UPGMA) analysis, principal coordinate analysis (PCoA) and STRUCTURE analysis were similar. A mantel test showed that the isolation-by-distance pattern was not significant.

Conclusions: The high Gst among *P. clarkii* populations is attributed to genetic drift and geographic isolation. The results indicated that more *P. clarkii* populations should be collected when formulating conservation and aquaculture strategies.

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1. Introduction

Procambarus clarkii is commonly known as the freshwater crayfish. Originating in North America, it has been introduced to Southeast Asia and other regions since the 1930s. It is widely distributed in China and has established natural populations. *P. clarkii* is an important freshwater crayfish species in China's natural waters. It is found in Jiangsu, Zhejiang, Anhui, Shanghai, and other provinces and cities. *P.*

clarkii has recently become a popular freshwater aquaculture species due to its high market value and consumption demand in inland China [1]. Because of its fast growth, convenient management and high yield, it has good aquaculture potential. However, with the continuous increase in fishing, the spread of disease and the pollution of its living environment, its biological resources are constantly threatened. Therefore, to promote the sustainable development of the *P. clarkii* industry, studying its genetic diversity has become an important task.

Genetic diversity means the product of species' genetic variation and adaptation ability to the environment in the long-term evolutionary process. It is an important basis for species' sustainable survival and development in the long-term complex process of habitat change.

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Accurate evaluation of genetic diversity of germplasm resources can provide predictive guidance for parent selection, progeny genetic variation and heterosis prediction, and improve breeding efficiency. Therefore, understanding genetic structure and genetic differentiation of germplasm resources can help to protect and utilize germplasm resources more effectively [2]. Molecular marker technology is widely used in the field of biological resource protection [2,3]. Many techniques can be used to detect genetic diversity based on polymerase chain reaction (PCR). Simple sequence repeats (SSRs) are among the most effective markers for studying genetic diversity and differentiation among populations [4,5] and can be more informative than other types of markers because they are codominant. In addition, the heterozygosity of polymorphic loci detected by SSRs was higher than that detected by some other markers [6]. In fact, SSRs are a DNA fingerprinting technique, which is close to an ideal marker system for genetic diversity analysis and population genetics research [7,8,9,10]. SSR markers have been applied in *Meretrix petechialis* [11], *Prunus sibirica* L. [12], *Ruditapes philippinarum* [13], *Juniperus thurifera* L. [14], *Crassostrea gigas* [15], *Mytilus coruscus* [16], and *Ligumia nasuta* [8].

Some microsatellite loci are available in *P. clarkii* in some local areas [17], and in recent years, molecular research using microsatellite biology has been carried out in *P. clarkii*. Wang et al. [18] used microsatellite primers to study the genetic diversity of 4 geographical populations of *P. clarkii*, Peng et al. [19] used 7 pairs of microsatellite primers to analyze the genetic diversity of 3 geographical populations, and Xing et al. [20] used 8 microsatellite markers to analyze the genetic diversity of *P. clarkii* in 8 areas. Therefore, SSRs are considered appropriate molecular tools for analyzing population structure and genetic diversity between and within *P. clarkii* populations. These studies on genetic diversity using microsatellite sequences were mostly only concentrated in Hubei, Jiangsu and other major *P. clarkii*-breeding provinces; thus, more polymorphic microsatellites are still required in this species to obtain a better understanding of the *P. clarkii* genetics in China.

To the best of our knowledge, there are no reports on genetic diversity analysis of *P. clarkii* populations in most areas of China. In the present study, SSR markers were employed (i) to assess the genetic diversity level within/among *P. clarkii* populations; (ii) to explore the genetic differentiation (Gst) among these populations; and (iii) to discuss the appropriate strategies for conservation of *P. clarkii* germplasm resources.

2. Materials and methods

2.1. Animals of *P. clarkii*

A total of 375 *P. clarkii* individuals were collected from 25 populations, representing the main distribution areas of *P. clarkii* in China. Table 1 and Fig. 1 show the detailed locations of the research populations based on GPS positioning. All samples were from wild populations. All animals of *P. clarkii* came from five regions: Northwest China (WW, ZW, BT, LF and LY); Qinling and the Huaihe River (HZ and JN); North China and Northeast China (HS, NH, MY and PJ); the Yangtze River basin (YZ, CM, HF, JX, NC, YY, QJ, ZX and CD); and South and Southwest China (QN, DL, GL, ZQ and QZ) (Table 1 and Fig. 1). Each *P. clarkii* was ice compressed for 10 min to maintain hypothermic anesthesia and then used MS-222 for next anesthesia. Fresh tail muscle was collected, dried immediately in silica gel, and then stored at -70°C for later processing.

2.2. DNA extraction and PCR amplification

Total *P. clarkii* genomic DNA was extracted according to the protocol of Liu et al. [21] and Sambrook and Russell [22]. DNA quality and quantity were determined using 1.5% agarose gel electrophoresis and

Table 1
The *P. clarkii* populations used in the present study.

Population code	Sample size	Location	Longitude (E)	Latitude (N)
WW	15	Wuwei, Gansu Province, China	102.85	38.38
ZW	15	Zhongwei, Ningxia Hui Autonomous Region, China	105.91	36.81
BT	15	Baotou, Inner Mongolia Autonomous region, China	109.80	40.76
LF	15	Linfen, Shanxi Province, China	110.69	36.45
HZ	15	Hanzhong, Shaanxi Province, China	107.12	33.05
LY	15	Luoyang, Henan Province, China	112.75	34.63
HS	15	Hengshui, Hebei Province, China	115.63	37.65
NH	15	Ninghe, Tianjin City, China	117.58	39.32
MY	15	Miyun, Beijing City, China	116.99	40.53
PJ	15	Panjin, Liaoning Province, China	122.13	40.98
JN	15	Jining, Shandong Province, China	117.19	34.77
YZ	15	Yangzhou, Jiangsu Province, China	119.42	32.74
CM	15	Chongming, Shanghai City, China	121.57	31.71
HF	15	Hefei, Anhui Province, China	117.33	31.57
JX	15	Jiaxing, Zhejiang Province, China	120.32	30.34
NC	15	Nanchang, Jiangxi Province, China	116.35	28.39
YY	15	Yiyang, Hunan Province, China	112.38	28.86
QJ	15	Qianjiang, Hubei Province, China	112.81	30.50
ZX	15	Zhongxian, Chongqing City, China	108.07	30.33
CD	15	Chengdu, Sichuan Province, China	103.74	30.73
QN	15	Qiannan, Guizhou Province, China	106.69	25.40
DL	15	Dali, Yunnan Province, China	100.18	25.38
GL	15	Guilin, Guangxi Province, China	110.38	25.18
ZQ	15	Zhaoqing, Guangdong Province, China	112.35	23.14
QZ	15	Quanzhou, Fujian Province, China	118.51	24.96

a spectrophotometer. The samples of *P. clarkii* DNA were stored at -20°C for later analysis.

2.3. SSR-PCR amplification

The SSR primers (Table 2) used in this study were the same as those in Li et al. [17]. The final reaction mixture containing 0.1 μM forward and

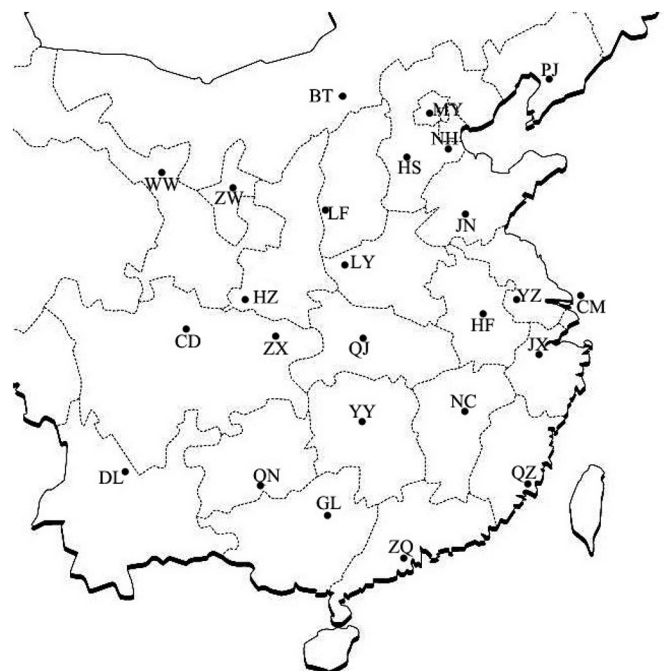


Fig. 1. The locations of *P. clarkii* populations sampled in this study were assigned to three geographical regions, as described in Table 1.

Table 2
Primers used for the molecular analyses.

SSR Locus	Primer	Ta (°C)	SSR motif	Allele size (bp)	No. of alleles	Accession number
PclG02	F:CTCCCCATGCACTCTGGCTCTGT R:TGGCGAATTTTGCTGTTCTGTC	66	(GATA)3GAGAA(GATA) ₅	216–224	3	AF290919
PclG03	F:CTCTCCACCAGTCATTTCTT R:AAGCTTACAATAATATAGATAGAC	52	(TCTA) ₂₀	216–420	4	AF290920
PclG04	F:TATATCAGTCAATCTGTCCAG R:TCAGTAAGTAGATTGATAGAAGG	54	(TCTA) ₃ ...(TCTA) ₂ ...(TCTA) ₂₉ ...(TCTA) ₂	170–290	4	AF290921
PclG07	F:CCTCCACCAGGTTATCTATTCA R:GTGGGTGTGGCGCTCTGTGT	63	(TCTA) ₈	100–160	4	AF290922
PclG08	F:ACGATAAATGATAGATGGATGAA R:CCGGTCTGTCTGTCTGTCA	62	(GATA) ₁₆	148–220	5	AF290923
PclG09	F:TATGCACCTTTACCTGAAT R:TGTTGGTGTGGTCAATCA	60	(TCTA) ₁₄	80–160	4	AF290924
PclG10	F:TGCTCAGCAAACTGTATTCACT R:CAATGGTCTGTGATTTGGTGTCT	54	(TAGA) ₂ TA(TAGA) ₁₆	90–176	3	AF290925
PclG13	F:CTCTCTGGCGCTGTATTATAGC R:TGAAGAGCAGAGTGAGGATCTC	62	(TCTA) ₁₂	130–150	2	AF290926
PclG15	F:GGCGTGACCCAACGTGTCTT R:GGCTGGCCACTTTGTAGCTGAG	70	(TATC)2TGTC(TATC) ₁₇ TA TT(TATC) ₃	150–185	3	AF290927
PclG16	F:CTCGAATGTCCACCTGAGA R:TCATTATGGATTTTGTCAATCTAT	54	(TCTA) ₁₈ TCTC(TATC) ₃	80–160	4	AF290928
PclG17	F:GTCGGGAACCTATTACAGTGTAT R:AAGAGCGAAGAAAGAGATAAAGAT	57	(TCTA) ₁₄	156–190	4	AF290929
PclG27	F:AATCTTAAGATCATGAAAAAGGTA R:TTTAAGGAACGTATAAGAAAAGAC	57	(TATC) ₄ CATC(TATC) ₈	80–150	6	AF290932
PclG28	F:CTCGCGGATTTACTGAAAT R:AGAAGAAAAGGGATATAAGTAAAG	60	(GATA) ₂₂ (GA) ₅	210–270	3	AF290933
PclG29	F:GAAAGTCAATGGGTGATAGGTAAC R:TTTTTGGGCTATGTGACGAG	65	(TATC) ₉	95–165	3	AF290934
PclG33	F:TTCGAGGCGTTGCTGATTGTAAGT R:CAAGGAAGCGTATAGCCGGAGTCT	68	(GT) ₂₁	120–180	5	AF290936
PclG37	F:TAAATAAGTGGCGTGTAGACGAG R:TAACTAAGCCAGGTTGCTCCAG	66	(CA) ₄ CG(CA) ₁₅ CG (CA) ₂₃	80–180	7	AF290939
PclG48	F:CTGTTGGTGAATTTCCGCAATTT R:AGATTCAACGCTGTCTCTGATC	66	(CA) ₁₂	146–190	2	AF290941

reverse primers, 0.15 mM MgCl₂, template DNA (approximately 10 ng), 0.05 mM dNTPs, 0.8 U of Taq DNA polymerase, and 10× PCR Buffer (Takara Biomedical Technology (Beijing) Co., Ltd., Beijing, China) was used for amplification. The amplifications were performed by a Thermocycler PTC 200™ Programmable Thermal Controller (Bio-Rad, USA) as follows: first, one cycle of 5 min at 95°C; second, 36 cycles of 50 s at 95°C, 40 s of annealing at the respective primer annealing temperatures, and then 50 s of elongation at 72°C; and third, a final extension of 4 min at 72°C. The PCR products were analyzed by 1.5% agarose gel electrophoresis. A DNA labeling (DL2000; Takara Biomedical Technology (Beijing) Co., Ltd., Beijing, China) marker was used as a molecular size ladder. The experiments were repeated three times, and the data were analyzed using only clear and consistent bands.

2.4. Statistical analysis

The SSR primer banding patterns were scored as absent (0) or present (1). POPGENE version 1.32 [23] was used to calculate the genetic diversity and distance matrix, Shannon's information index (I) [24], Nei's genetic diversity (H); observed heterozygosity (Ho); expected heterozygosity (He); Hardy-Weimberg equilibrium (HWE) [25], gene differentiation (Gst) and gene flow (Nm). NTSYS-pc version 2.10 [26] was used to perform a Mantel test between Nei's genetic distance and geographic distance. The inbreeding coefficient (Fis), the total inbreeding coefficient (Fit) and the genetic differentiation coefficient (Gst) among populations were calculated using arlequin 3.5.2.2 and the frequency of null alleles (P_N) was calculated using GenePop 4.7. Numerical Taxonomy Multivariate Analysis System (NTSYS-pc) software (version 2.10) [27] was used to construct a two-dimensional array by principal coordinate analysis (PCoA) and a

UPGMA tree based on the genetic distance matrices generated. Finally, the STRUCTURE (version 2.3.4) program was used to test for genetic admixture across species boundaries. The population structure of SSRs was analyzed by the Bayesian analysis method. The number of populations was estimated as described by Evanno et al. [28] using the STRUCTURE HARVESTER software [29].

3. Results

3.1. Genetic relationships and genetic diversity in *P. clarkii*

Seventeen pairs of SSR primers were used to assess the genetic relationships and genetic diversity of 25 *P. clarkii* populations (Table 2). In total, 60 bands were observed in the SSR products, and all 60 (100.00%) were polymorphic among the *P. clarkii* populations. The highest and the lowest genetic diversities of *P. clarkii* were observed in populations NC (H = 0.3191, I = 0.4708) and JN (H = 0.1367, I = 0.2003), respectively (Table 3). The mean within-population diversity of *P. clarkii* was low (H = 0.2325, I = 0.3462), but *P. clarkii* exhibited high diversity at the species level (H = 0.4377, I = 0.6264) based on SSRs. The expected heterozygosity (He) value of 17 microsatellite loci in 25 *P. clarkii* populations was 0.9317, the observed heterozygosity (Ho) value was 0.9121, and the observed number of alleles per locus was 2.000; and the effective number of alleles per locus was 1.8075 (Table 3). Hardy Weinberg equilibrium deviation index (HWE) test showed that 25 populations did not show heterozygous deletion sites (Table 4). Table 4 shows that pclg09 is monomorphic in the ZW population, pclg10, pclg17 and pclg28 are monomorphic in the HF population, pclg13 is monomorphic in the WW and HF population, and the rest of the loci are polymorphic in each

Table 3
Genetic diversity within *P. clarkii* populations based on SSR markers.

Population	PPB (%)	Ao	Ae	H	Ho	He	I
WW	68.33	1.6833 ± 0.4691	1.3417 ± 0.3263	0.2129 ± 0.1780	0.3268 ± 0.0872	0.5612 ± 0.1231	0.3287 ± 0.2568
ZW	55.00	1.5500 ± 0.5017	1.2779 ± 0.3206	0.1738 ± 0.1803	0.3319 ± 0.1127	0.5287 ± 0.1345	0.2681 ± 0.2651
BT	60.00	1.6000 ± 0.4940	1.2801 ± 0.3194	0.1758 ± 0.1782	0.3628 ± 0.1285	0.5629 ± 0.1827	0.2737 ± 0.2595
LF	56.67	1.5667 ± 0.4997	1.2914 ± 0.3340	0.1793 ± 0.1849	0.3927 ± 0.1842	0.5928 ± 0.1672	0.2754 ± 0.2689
HZ	48.33	1.4833 ± 0.5039	1.2730 ± 0.3412	0.1642 ± 0.1928	0.3877 ± 0.1926	0.5671 ± 0.1874	0.2474 ± 0.2908
LY	65.00	1.6500 ± 0.4810	1.3510 ± 0.3510	0.2123 ± 0.1892	0.3987 ± 0.1134	0.5921 ± 0.1763	0.3231 ± 0.2712
HS	41.67	1.4167 ± 0.4972	1.2580 ± 0.3645	0.1490 ± 0.1986	0.4125 ± 0.1827	0.6213 ± 0.2124	0.2215 ± 0.2852
NH	61.67	1.6167 ± 0.4903	1.3918 ± 0.4044	0.2218 ± 0.2112	0.4200 ± 0.1978	0.6161 ± 0.2316	0.3278 ± 0.2970
MY	63.33	1.6333 ± 0.4860	1.4246 ± 0.3947	0.2421 ± 0.2079	0.3987 ± 0.1276	0.6078 ± 0.2012	0.3564 ± 0.2948
PJ	58.33	1.5833 ± 0.4972	1.3773 ± 0.3938	0.2164 ± 0.2072	0.4102 ± 0.1276	0.5873 ± 0.2145	0.3207 ± 0.2945
JN	35.00	1.3500 ± 0.4810	1.2419 ± 0.3698	0.1367 ± 0.1999	0.4148 ± 0.7568	0.6305 ± 0.2124	0.2003 ± 0.2865
YZ	53.33	1.5333 ± 0.5031	1.3770 ± 0.4171	0.2090 ± 0.2190	0.4756 ± 0.2317	0.6254 ± 0.2136	0.3045 ± 0.3093
CM	63.33	1.6333 ± 0.4860	1.4127 ± 0.4078	0.2331 ± 0.2105	0.6728 ± 0.2126	0.6896 ± 0.1728	0.3442 ± 0.2953
HF	75.00	1.7500 ± 0.4367	1.4842 ± 0.3959	0.2734 ± 0.2038	0.4512 ± 0.1768	0.4132 ± 0.1345	0.4028 ± 0.2823
JX	81.67	1.8167 ± 0.3902	1.5222 ± 0.3680	0.2993 ± 0.1871	0.6517 ± 0.1681	0.6023 ± 0.1872	0.4425 ± 0.2579
NC	83.33	1.8333 ± 0.3758	1.5509 ± 0.3369	0.3191 ± 0.1725	0.4409 ± 0.1128	0.5419 ± 0.1827	0.4708 ± 0.2403
YY	81.67	1.8167 ± 0.3902	1.5437 ± 0.3660	0.3097 ± 0.1854	0.7512 ± 0.1687	0.7219 ± 0.1565	0.4558 ± 0.2555
QJ	80.00	1.8000 ± 0.4034	1.4993 ± 0.3782	0.2869 ± 0.1891	0.6814 ± 0.1126	0.6432 ± 0.1138	0.4266 ± 0.2598
ZX	78.33	1.7833 ± 0.4155	1.4327 ± 0.3649	0.2564 ± 0.1854	0.6812 ± 0.1187	0.7218 ± 0.1988	0.3887 ± 0.2559
CD	81.67	1.8167 ± 1.4183	1.4183 ± 0.3599	0.2502 ± 0.1822	0.6545 ± 0.2300	0.7432 ± 0.1987	0.3830 ± 0.2491
QN	71.67	1.7167 ± 0.4544	1.4599 ± 0.4024	0.2598 ± 0.2061	0.6578 ± 0.1989	0.6829 ± 0.1126	0.3837 ± 0.2860
DL	63.33	1.6333 ± 0.4860	1.4365 ± 0.4018	0.2463 ± 0.2116	0.5825 ± 0.1632	0.5923 ± 0.1985	0.3606 ± 0.2989
GL	66.67	1.6667 ± 0.4754	1.4482 ± 0.4100	0.2509 ± 0.2125	0.5521 ± 0.1782	0.5988 ± 0.1892	0.3680 ± 0.2969
ZQ	66.67	1.6667 ± 0.4754	1.4295 ± 0.4073	0.2414 ± 0.2127	0.7266 ± 0.2219	0.7328 ± 0.1825	0.3553 ± 0.2970
QZ	73.33	1.7333 ± 0.4459	1.5212 ± 0.3937	0.2916 ± 0.2028	0.7718 ± 0.2892	0.7819 ± 0.2436	0.4253 ± 0.2832
Mean	65.33	1.6533	1.4018	0.2325	0.5203	0.6224	0.3462
Species level	100%	2.0000 ± 0.0000	1.8075 ± 0.2086	0.4377 ± 0.0808	0.9121 ± 0.2976	0.9317 ± 0.3415	0.6264 ± 0.0918

Note: PPB: Percentage of polymorphic bands; Ao: Observed number of alleles per locus; Ae: Effective number of alleles per locus; H: Nei's gene diversity (1979); Ho: observed heterozygosity; He: expected heterozygosity; I: Shannon's information index.

population. HWE deviation index (*d*) test showed that there were more heterozygous deletion sites in WW, ZW and HF3 populations, 15, 11 and 13, respectively, and heterozygous deletion sites in other populations. The frequency of invalid alleles was between 0.053 and 0.276 (Table 5).

Among the *P. clarkii* populations, the inbreeding coefficient within populations (*F_{is}*) was 0.2315, overall inbreeding coefficient (*F_{it}*) was 0.4438, genetic differentiation coefficient among populations (*F_{st}*) was 0.3145 and *G_{st}* was 0.4785 based on SSR analyses. The *N_m* was 0.5450 in *P. clarkii* populations based on SSR analysis. These data indicate limited gene exchange among populations of *P. clarkii* (Table 6). The *G_{st}* and *N_m* among pairs of populations were shown in Table 7. Nei's genetic distance in *P. clarkii* ranged from 0.0491 (CM vs. HF) to 0.5655 (ZW vs. QZ) based on POPGENE analysis of SSRs (Table 8).

3.2. Phylogenetic analysis

The UPGMA tree clustered all populations into five groups based on Nei's genetic distance calculated with SSRs (Fig. 2). Populations WW, ZW, BT, LF and LY formed the first group, which could be divided into two subgroups: population WW in Gansu Province formed its own subgroup, and the other subgroup included populations ZW, BT, LF and LY in Ningxia, Shanxi, and Henan Provinces and the Inner Mongolia Autonomous Region. Populations HZ and JN from Qinling and the Huaihe River in Shaanxi and Shandong Provinces formed a second group. Populations PJ, HS, NH, MY and JN in Liaoning and Hebei Provinces, Tianjin and Beijing formed a third group. Populations YZ in Jiangsu Province, CM in Shanghai city, HF in Anhui Province, QJ in Hubei Province, ZX in Chongqing city, CD in Sichuan Province, NC in Jiangxi Province, and YY in Hunan Province in the Yangtze River basin formed a fourth group. The remaining populations, QN in Guizhou Province, GL and QZ in Guangxi Zhuang Autonomous Region, ZQ in Guangdong Province, and DL in Yunnan Province, formed a fifth group.

The SSR markers of the 25 populations were subjected to PCoA (Fig. 3). The results shown in the scatter plot and clustering plot were consistent and could be divided into five categories. The PCoA

of all 375 individuals in the 25 populations accounted for 44.67% (axis 1) and 27.15% (axis 2) of the total variance based on the SSR analyses, respectively. In the PCoA plot, populations (QN in Guizhou Province, GL and QZ in Guangxi, DL in Yunnan, and ZQ in Guangdong) occupied similar positions along axis 1. Seven *P. clarkii* populations, namely, YZ in Jiangsu Province, CM in Shanghai city, HF in Anhui Province, QJ in Hubei Province, ZX in Chongqing city, CD in Sichuan Province, and NC in Jiangxi Province, also showed genetic similarity to a similar degree; however, as shown in Fig. 2, YY in Hunan Province belonged to the Yangtze River basin, but it was opposite from other regions in the same group. Five populations (PJ, HS, NH, MY and JN in Hebei and Liaoning Provinces, Tianjin and Beijing) also showed genetic similarity to a similar degree. The other populations, HZ and JN in Qinling and the Huaihe River region in Shaanxi and Shandong Provinces, occupied a wide range of positions along axes 1 and 2 and showed genetic similarity to a similar degree.

The STRUCTURE (version 2.3.4) program was used to calculate the genetic structure of the *P. clarkii* samples. Since the species was initially considered an individual population, individual genotypes were allocated to the species using STRUCTURE, but the population size was uncertain. With *K* = 2, *P. clarkii* formed two groups. With *K* = 4, the populations from North China and Northeast China formed one group, those from Qinling and the Huaihe River region formed a second group, the Yangtze River basin population formed a third group, and populations from South and Southwest China formed a fourth group (Fig. 4). With *K* = 5, the populations from five regions clustered with each other. With *K* = 6, the same clustering was observed as with *K* = 5, but with the population from the Yangtze River basin divided into two subgroups. When using the recently developed Evanno method, the *K* value supported *k* = 7–25. Each region showed population subdivision. The structure simulation through STRUCTURE HARVESTER confirmed that the highest peak was at *K* = 3, inferring that 25 populations can incorporate all individuals with the maximum likelihood (Fig. S1, Table S1). The Mantel test results revealed no

Table 4
Analysis of Hardy–Weinberg equilibrium (HWE) at 17 SSR loci among different populations of *P. clarkii*.

ID	WW	ZW	BT	LF	HZ	LY	HS	NH	MY	PJ	JN	YZ	CM	HF	JX	NC	YY	QJ	ZX	CD	QN	DL	GL	ZQ	OZ
PclG02	0.0428	0.3013	0.0238	0.1192	0.0256	0.2987	0.2156	0.0269	0.0892	0.2528	0.0199	0.0344	0.1118	0.3378	0.0230	0.0221	0.0291	0.0391	0.2717	0.3982	0.0695	0.3982	0.0892	0.1922	0.1830
PclG03	0.4125	0.0218	0.4230	0.0682	0.2126	0.2487	0.3023	0.5821	0.3588	0.0934	0.3720	0.3221	0.0416	0.0294	0.0519	0.5502	0.4819	0.2981	0.0396	0.0319	0.5942	0.3892	0.5529	0.4319	0.0892
PclG04	0.5318	0.0636	0.3891	0.3928	0.6342	0.0178	0.0560	0.6029	0.5098	0.392	0.5572	0.5132	0.3521	0.0578	0.4782	0.6720	0.3982	0.2987	0.4297	0.0427	0.6173	0.3832	0.4892	0.4329	0.4382
PclG07	0.1673	0.4137	0.1928	0.3097	0.3189	0.0192	0.2583	0.1432	0.1782	0.3139	0.3071	0.1168	0.3270	0.2461	0.3319	0.0960	0.3891	0.1182	0.3947	0.2827	0.1982	0.3971	0.0392	0.1492	0.3763
PclG08	0.4632	0.3028	0.2423	0.3928	0.3237	0.4866	0.0608	0.2976	0.5690	0.2790	0.3320	0.5472	0.2839	0.0509	0.2320	0.2500	0.3982	0.2718	0.3082	0.0728	0.2991	0.4872	0.3091	0.2985	0.2901
PclG09	0.6156	0.0000	0.7718	0.0894	0.0462	0.1947	0.0724	0.7823	0.032	0.6618	0.0499	0.6437	0.6012	0.0664	0.6710	0.7412	0.0529	0.4981	0.5589	0.0460	0.6432	0.0782	0.7829	0.7289	0.5892
PclG10	0.3132	0.6230	0.2091	0.7075	0.0462	0.1426	0.0136	0.2090	0.2682	0.8290	0.388	0.2873	0.8199	0.0000	0.6728	0.2290	0.0757	0.3199	0.7812	0.0152	0.3197	0.0794	0.0892	0.1872	0.6028
PclG13	0.0000	0.2416	0.2832	0.0724	0.6992	0.1872	0.0011	0.2019	0.2893	0.0574	0.0891	0.2735	0.0314	0.0000	0.0917	0.2012	0.0720	0.2012	0.0982	0.0693	0.2129	0.0727	0.0921	0.0932	0.7191
PclG15	0.1672	0.0832	0.0419	0.3641	0.2619	0.1705	0.0763	0.0505	0.2217	0.2587	0.2240	0.1266	0.3641	0.0754	0.2450	0.0309	0.2079	0.0207	0.4872	0.0694	0.1983	0.3329	0.3981	0.4092	0.2219
PclG16	0.1254	0.1398	0.1928	0.1119	0.1427	0.1804	0.1389	0.1420	0.1817	0.1297	0.1981	0.1628	0.1119	0.1228	0.2521	0.1420	0.2196	0.1382	0.1274	0.0981	0.1981	0.1984	0.3982	0.4192	0.2319
PclG17	0.3312	0.0142	0.000	0.3058	0.1982	0.1905	0.0125	0.0153	0.3155	0.3146	0.1978	0.3068	0.3058	0.0000	0.3129	0.0011	0.1828	0.0092	0.4217	0.0078	0.0296	0.2285	0.2218	0.2018	0.3320
PclG27	0.2489	0.1891	0.0732	0.048	0.4891	0.4075	0.1642	0.0685	0.0982	0.0540	0.4096	0.2070	0.0481	0.1592	0.0527	0.0775	0.4194	0.0791	0.0985	0.1682	0.0984	0.3887	0.4287	0.3389	0.0389
PclG28	0.5612	0.0254	0.2874	0.7788	0.1019	0.3130	0.021	0.3189	0.6578	0.6283	0.2642	0.6782	0.7788	0.0000	0.6329	0.2529	0.3129	0.2985	0.6751	0.0341	0.4199	0.2764	0.4187	0.4007	0.6180
PclG29	0.1279	0.1892	0.4178	0.2638	0.1998	0.1805	0.0873	0.5051	0.3145	0.2590	0.1764	0.3319	0.2638	0.0778	0.2519	0.4052	0.1891	0.2957	0.2985	0.1206	0.2874	0.1095	0.2273	0.2765	0.3326
PclG33	0.4318	0.4678	0.4686	0.3248	0.3056	0.2129	0.5128	0.2892	0.5519	0.3319	0.3152	0.5316	0.3248	0.5205	0.3129	0.2913	0.3025	0.3319	0.3189	0.6891	0.3129	0.3189	0.1971	0.1683	0.3315
PclG37	0.2819	0.4072	0.3897	0.3545	0.3132	0.3020	0.4298	0.5380	0.3984	0.2891	0.3169	0.3012	0.3545	0.4138	0.3619	0.5540	0.2974	0.5018	0.3782	0.3092	0.6983	0.3192	0.2254	0.2194	0.3294
PclG48	0.3132	0.1599	0.3139	0.0615	0.3895	0.3120	0.1829	0.4982	0.1365	0.0921	0.3819	0.1247	0.0615	0.1508	0.0319	0.2880	0.4194	0.2581	0.0615	0.1932	0.4552	0.2984	0.4007	0.3882	0.0497

Table 5
Frequency of null alleles at 17 SSR loci among populations of *P. clarkii*.

ID	P _N	ID	P _N
PclG02	0.173	PclG16	0.195
PclG03	0.196	PclG17	0.145
PclG04	0.055	PclG27	0.164
PclG07	0.078	PclG28	0.079
PclG08	0.062	PclG29	0.276
PclG09	0.053	PclG33	0.194
PclG10	0.188	PclG37	0.181
PclG13	0.089	PclG48	0.077
PclG15	0.176	Mean	0.140

Note: P_N: frequency of null alleles.

Table 6
Nei's analysis of gene differentiation among *P. clarkii* populations.

	Fit	Fis	Fst	Gst	Nm
<i>P. clarkii</i>	0.4438 ± 0.0061	0.2315 ± 0.0050	0.3145	0.4785	0.5450

Note: Fis: Inbreeding coefficient within populations; Fit: Overall inbreeding coefficient; Fst: Genetic differentiation coefficient among populations; Gst: Nei's genetic differentiation; Nm: Gene flow

significant positive correlation between the geographical distance and genetic distance of *P. clarkii* populations ($r = 0.39694$, $p = 4.9406$).

4. Discussion

4.1. Genetic diversity

Genetic diversity is the product of the long-term evolution of species or populations [30,31]. The extent of genetic diversity of *P. clarkii* determined in the present study was consistent with the previously reported genetic diversity of *P. clarkii* in other local areas, such as $H = 0.35$, as determined by SSRs in Anhui [32]; $H = 0.334$, as determined by SSRs in Anhui [20]; and $H = 0.2959$, as determined by AFLP markers in 6 populations of the Middle and Lower Reaches of the Yangtze River [20]. However, lower genetic diversity was detected elsewhere, such as $H = 0.667$, as determined by SSRs in 8 populations of East China and Guangxi [17], and $H = 0.5208$, as determined by SSRs in 4 populations of the Lower Reaches of the Yangtze River [18]. At the population and species levels, *P. clarkii* showed high genetic diversity, while some shrimps showed a different level of genetic diversity, for example, *Penaeus monodon* ($H = 0.0917$ to 0.1271 , $I = 0.1484$ to 0.2032) [33], *Macrobrachium rosenbergii* ($PPB = 0.853$ – 0.941 , $He = 0.848$ – 0.896) [34], *Litopenaeus vannamei* ($PPB = 0.88$ to 0.92) [35], *Panulirus polyphagus* ($H = 0.1135$, $I = 0.1680$), *P. ornatus* ($H = 0.1262$, $I = 0.1884$), *P. penicillatus* ($H = 0.1185$, $I = 0.1731$), *P. versicolor* ($H = 0.1735$, $I = 0.1990$), *P. homorus* ($H = 0.1264$, $I = 0.1877$), *P. stipsoni* ($H = 0.0834$, $I = 0.1232$), *P. japonicus* ($H = 0.0589$, $I = 0.0864$) [36]. It also showed lower genetic diversity than other aquatic animal *Macraa veneriformis* ($H = 0.307$, $I = 0.476$) [37], *Crassostrea gigas* ($H = 0.755/0.882/0.448$) [15], *Saccharina japonica* ($I = 0.617$, $H = 0.416$) [38], and *Perinereis aihuhitensis* ($H = 0.6889/0.6587$) [39]. This consistency in species genetic diversity may be due to the species distribution. Generally, species that are geographically widespread have a higher level of genetic variability than those with a narrow geographic distribution [40]. *P. clarkii* is widely distributed throughout China. Conversely, the genetic diversity of *P. clarkii* was high because of the wide species distribution.

Invasive populations often suffer from a decrease in genetic diversity due to bottleneck effects or genetic drift [41]. However, the genetic diversity of some species does not decrease significantly after invasion [42]. For example, the populations of

Table 7
 Matrix of genetic differentiation G_{st} (below diagonal) and gene flow Nm (above diagonal) among populations of 25 *P. clarkii*.

ID	WW	ZW	BT	LF	HZ	LY	HS	NH	MY	PJ	JN	YZ	CM	HF	JX	NC	YY	QJ	ZX	CD	QN	DL	GL	ZQ	QZ
WW	1.3022	1.6014	1.7865	0.9566	1.7178	0.7503	0.9904	1.0645	0.8583	0.6130	0.8698	1.2213	1.2807	1.5588	2.1443	1.3422	1.2413	1.1425	1.3004	0.9282	0.7993	0.9293	0.8381	0.9864	
ZW	0.2774	2.9340	3.5461	0.9685	2.5388	0.5881	0.7104	0.8522	0.6741	0.5328	0.6977	0.8397	0.9676	1.1073	1.3187	1.2768	0.9050	0.8500	1.0273	0.9585	0.7879	0.6276	0.7729	0.7783	
BT	0.2379	0.1456	3.4619	0.9113	3.3978	0.5599	0.7500	0.8944	0.6944	0.4795	0.6456	0.8557	0.9802	1.0505	1.3658	1.1833	0.8760	0.7981	1.0000	0.8376	0.8575	0.6787	0.6901	0.8605	
LF	0.2187	0.1236	1.0573	3.8407	1.0209	0.5895	0.7536	0.7726	0.6578	0.6024	0.7124	0.9160	1.0593	1.1566	1.4785	1.4175	0.9371	0.9073	0.9837	1.0337	0.9793	0.7137	0.8411	0.8689	
HZ	0.3433	0.3405	0.3543	0.3211	1.0209	0.4524	0.5764	0.5581	0.5557	0.6057	0.6070	0.7072	0.7671	0.8542	0.9811	1.0710	0.7401	0.7232	0.7971	0.8759	0.6665	0.6852	0.7721	0.7739	
LY	0.2225	0.1645	0.1283	0.1152	0.3288	0.5739	0.6715	0.8665	0.6923	0.5296	0.4362	0.3600	1.0242	1.1644	1.3714	1.5687	0.9758	0.8830	1.0814	0.9488	0.9928	0.9527	0.7561	0.8172	
HS	0.3999	0.4595	0.4718	0.4589	0.5250	0.4656	0.8801	1.0740	0.7946	0.3441	0.8739	0.8272	0.8616	0.9118	0.8932	0.8010	1.0018	1.0236	1.0262	0.6780	0.5320	0.5685	0.5398	0.6857	
NH	0.3355	0.4131	0.4000	0.3988	0.4645	0.4268	0.3623	1.1036	1.6170	0.5213	1.0535	0.9083	0.9141	1.4644	1.3463	0.9704	1.0809	1.2588	1.2957	0.7204	0.6730	0.7155	0.6403	0.8210	
MY	0.3196	0.3698	0.3586	0.3929	0.4725	0.3659	0.3177	0.3118	1.1361	0.4183	1.1369	1.1516	1.1792	1.3572	1.2842	1.1021	1.5082	1.2691	1.5502	1.7204	0.8854	0.8706	0.8702	1.1286	
PJ	0.3681	0.4259	0.4186	0.4318	0.4736	0.4194	0.3862	0.2362	1.1361	0.4143	0.8294	0.7996	0.8973	1.3352	1.0654	0.8478	0.9944	1.0427	1.2700	0.7164	0.7062	0.8058	0.6670	0.8953	
JN	0.4492	0.4841	0.5105	0.4535	0.4522	0.4856	0.5924	0.4896	0.5445	0.5469	0.5149	0.5682	0.6644	0.7700	0.6764	0.5834	0.6589	0.7099	0.7088	0.4772	0.4772	0.6490	0.6251	0.9930	
YZ	0.3650	0.4175	0.4365	0.4124	0.4517	0.6642	0.3639	0.3219	0.3055	0.3761	0.5167	2.3327	2.4239	2.4239	2.4297	1.7575	1.1768	2.4460	2.7351	2.5424	1.0287	0.7439	0.7701	0.7375	
CM	0.2905	0.3732	0.3688	0.3531	0.4142	0.8889	0.3767	0.3550	0.3027	0.3847	0.4927	0.1765	4.1510	3.2264	2.1930	1.6690	3.1205	3.8111	1.0932	0.8055	0.8175	0.7842	1.0078	1.4064	
HF	0.2808	0.3407	0.3378	0.3207	0.3946	0.3280	0.3672	0.3536	0.2978	0.3578	0.4681	0.1710	0.1075	3.7269	2.5885	1.6670	3.5269	3.2682	3.2893	1.4098	1.0394	1.1502	1.1281	1.4064	
JX	0.2429	0.3111	0.3225	0.3018	0.3692	0.3004	0.3542	0.2545	0.2699	0.2725	0.4294	0.1707	0.1342	0.1183	0.1354	2.1458	3.9662	4.0499	5.0807	1.6048	1.2359	1.2845	1.1376	1.5145	
NC	0.1891	0.2749	0.2680	0.2527	0.3376	0.2672	0.3589	0.2708	0.2802	0.3194	0.3937	0.2215	0.1857	0.1619	0.1354	3.8111	3.6246	2.8671	2.8514	1.8062	1.4208	1.4290	1.4126	1.8591	
YY	0.2714	0.2814	0.2970	0.2608	0.3183	0.2417	0.3843	0.3400	0.3121	0.3710	0.4250	0.2982	0.2305	0.2307	0.1890	0.1160	0.1212	2.8186	2.1360	2.2032	1.8395	1.5610	1.3453	1.2382	
QJ	0.2871	0.3559	0.3634	0.3479	0.4042	0.3388	0.3329	0.3163	0.2492	0.3346	0.4615	0.1697	0.1387	0.1242	0.1120	0.1212	0.1507	3.8111	3.6246	2.8671	2.8514	1.8062	1.4208	1.4126	
ZX	0.3044	0.3704	0.3852	0.3553	0.4088	0.3615	0.3282	0.2843	0.2826	0.3241	0.4314	0.1546	0.1381	0.1327	0.1099	0.1485	0.1897	0.0784	5.8796	5.4029	1.7154	1.2561	1.3316	1.3017	
CD	0.2777	0.3274	0.3333	0.3370	0.3855	0.3233	0.3276	0.2784	0.2439	0.2825	0.4133	0.1643	0.1160	0.1320	0.0896	0.1492	0.1850	0.0847	0.0900	5.0535	1.5579	1.1300	1.2589	1.1595	
QN	0.3501	0.3428	0.3738	0.3260	0.3634	1.0464	0.4244	0.4087	0.2990	0.4110	0.4136	0.3271	0.3138	0.2618	0.2375	0.2168	0.2137	0.2257	0.2430	0.2643	1.3982	1.0741	1.3982	1.0741	
DL	0.3848	0.3882	0.3683	0.3380	0.4286	0.3162	0.4845	0.4263	0.3609	0.4145	0.5117	0.4020	0.3830	0.3248	0.2880	0.2603	0.2426	0.2847	0.3068	0.3176	0.2445	1.5452	1.4163	1.7677	
GL	0.3761	0.4434	0.4242	0.4120	0.4219	0.3451	0.4679	0.4113	0.3648	0.3829	0.5117	0.3937	0.3795	0.3030	0.2802	0.2592	0.2710	0.2730	0.2843	0.2634	0.2609	0.2522	1.4822	1.0949	
ZQ	0.3737	0.3928	0.4201	0.3728	0.3930	0.3981	0.4808	0.4385	0.3650	0.4285	0.4352	0.4040	0.3894	0.3071	0.3053	0.2614	0.2877	0.2775	0.3013	0.3176	0.2205	0.3135	0.1832	1.6672	
QZ	0.3364	0.3911	0.3675	0.3653	0.3925	0.3796	0.4217	0.3785	0.3070	0.3583	0.4444	0.3349	0.3316	0.2623	0.2482	0.2119	0.2117	0.2333	0.2584	0.2751	0.2183	0.1917	0.0630	0.2307	

Table 8
Genetic identities and distances of the 25 *P. clarkii* populations based on SSR data.

ID	VW	ZW	BT	LF	HZ	LY	HS	NH	MV	PJ	JN	YZ	CM	HF	JX	NC	YY	QJ	ZX	CD	QN	DL	GL	ZQ	OZ
VW	0	228	688	808	606	948	1138	1324	1300	1698	1273	1639	1870	1490	1838	1596	1369	1252	950	824	1383	1393	1587	1900	2096
ZW	0.2031	0	593	582	515	723	920	1106	1099	1502	1043	1401	1638	1268	1600	1393	1193	1058	840	778	1269	1398	1440	1746	1891
BT	0.1630	0.0920	0	528	878	708	600	693	649	1030	832	1256	1458	1180	1472	1440	1364	1172	1164	1238	1624	1891	1720	1971	1938
LF	0.2185	0.0932	0.0945	0	531	181	409	638	684	1072	464	835	1054	709	1042	920	842	646	721	920	1164	1585	1212	1454	1423
HZ	0.2866	0.2694	0.2762	0.2628	0	530	942	1180	1214	1605	918	1161	1376	968	1320	970	712	626	320	398	762	1060	916	1230	1450
LY	0.2344	0.1312	0.0963	0.0889	0.2688	0	451	693	749	1111	388	693	909	542	891	734	674	467	633	903	1044	1541	1067	1287	1240
HS	0.3868	0.4533	0.4680	0.4753	0.4695	0.4937	0	244	298	664	272	686	861	672	906	1008	1068	855	1086	1329	1494	1892	1486	1660	1460
NH	0.3508	0.4706	0.4349	0.4861	0.4464	0.5509	0.2661	0	99	430	428	767	901	818	978	1176	1280	1065	1320	1566	1720	2216	1702	1860	1596
MV	0.3293	0.3891	0.3596	0.4596	0.4599	0.4029	0.2174	0.2146	0	408	522	870	1004	916	1077	1274	1362	1146	1378	1605	1790	2264	1781	1951	1692
PJ	0.3938	0.4849	0.4424	0.5237	0.4767	0.5218	0.2435	0.1388	0.2098	0	793	1003	1058	1122	1161	1495	1655	1448	1742	1996	2127	2643	2082	2202	1835
JN	0.4010	0.4199	0.4531	0.3729	0.2616	0.4429	0.4042	0.3673	0.4445	0.4432	0	424	620	404	645	750	863	655	980	1286	1337	1907	1287	1431	1187
YZ	0.3835	0.4426	0.4534	0.4893	0.3814	0.5337	0.2285	0.2328	0.2230	0.3018	0.3460	0	216	214	223	534	799	658	1104	1468	1336	2002	1199	1244	836
CM	0.2713	0.3904	0.3432	0.3598	0.3306	0.3792	0.2858	0.3214	0.2289	0.3743	0.3354	0.1209	0	411	127	644	952	841	1299	1668	1491	2182	1322	1310	806
HF	0.2961	0.3863	0.3420	0.3939	0.3548	0.3909	0.3047	0.3292	0.2433	0.3771	0.3561	0.1189	0.0491	0	355	369	593	442	890	1261	1125	1790	1005	1081	783
JX	0.3323	0.4172	0.4193	0.4533	0.4139	0.4359	0.3784	0.2119	0.2673	0.2777	0.3813	0.1372	0.1144	0.1101	0	524	846	755	1219	1595	1386	2091	1203	1187	687
NC	0.2206	0.3611	0.3147	0.3382	0.3396	0.3747	0.3500	0.2653	0.2802	0.3999	0.3453	0.1622	0.1310	0.1185	0.1410	0	344	350	777	1156	860	1574	673	708	502
YY	0.3566	0.3795	0.3567	0.3267	0.2876	0.2759	0.4022	0.3706	0.3443	0.4873	0.3584	0.3032	0.2110	0.2228	0.2191	0.1397	0	215	460	833	540	1241	424	611	750
QJ	0.3571	0.4476	0.4440	0.4775	0.4017	0.4594	0.2817	0.2725	0.2228	0.3596	0.3777	0.1246	0.1010	0.1055	0.0963	0.1037	0.1417	0	464	843	701	1348	636	823	840
ZX	0.3878	0.5014	0.5078	0.5069	0.4416	0.5216	0.2832	0.2346	0.2478	0.3206	0.3224	0.1061	0.1059	0.1100	0.1092	0.1348	0.2185	0.0636	0	380	450	923	600	917	1207
CD	0.3392	0.4273	0.4190	0.5047	0.3976	0.4552	0.2734	0.2285	0.2109	0.2839	0.4098	0.1203	0.1012	0.1317	0.0922	0.1515	0.1982	0.0664	0.0806	0	588	667	847	1182	1572
QN	0.4051	0.4368	0.4767	0.4182	0.3180	0.3960	0.4502	0.4655	0.3131	0.4750	0.2876	0.3340	0.2959	0.2841	0.2990	0.2536	0.2297	0.2256	0.2710	0.3096	0	727	291	615	1133
DL	0.4662	0.5470	0.4679	0.4384	0.4517	0.4620	0.5268	0.4457	0.3859	0.4386	0.4632	0.4207	0.4011	0.3720	0.3444	0.3363	0.2845	0.3210	0.3527	0.4079	0.2389	0	992	1265	1852
GL	0.5285	0.6295	0.5206	0.5476	0.4044	0.5606	0.5130	0.4156	0.3924	0.3685	0.3938	0.4120	0.3915	0.3119	0.3313	0.3149	0.3096	0.2843	0.3249	0.3667	0.2641	0.2238	0	338	857
ZQ	0.4464	0.4412	0.4803	0.4066	0.3489	0.4798	0.4484	0.4021	0.3809	0.3786	0.2774	0.3547	0.3307	0.2572	0.2881	0.2620	0.2787	0.2334	0.2814	0.3295	0.1909	0.3070	0.1583	0	662
OZ	0.4975	0.5655	0.4562	0.5132	0.3982	0.5108	0.4772	0.4114	0.3353	0.3630	0.3622	0.3498	0.3346	0.2787	0.3001	0.2668	0.2500	0.2561	0.2998	0.3376	0.2509	0.2009	0.0447	0.2061	0

Note: Nei's genetic distances are given below the diagonal, and geographical distances (km) are given above the diagonal.

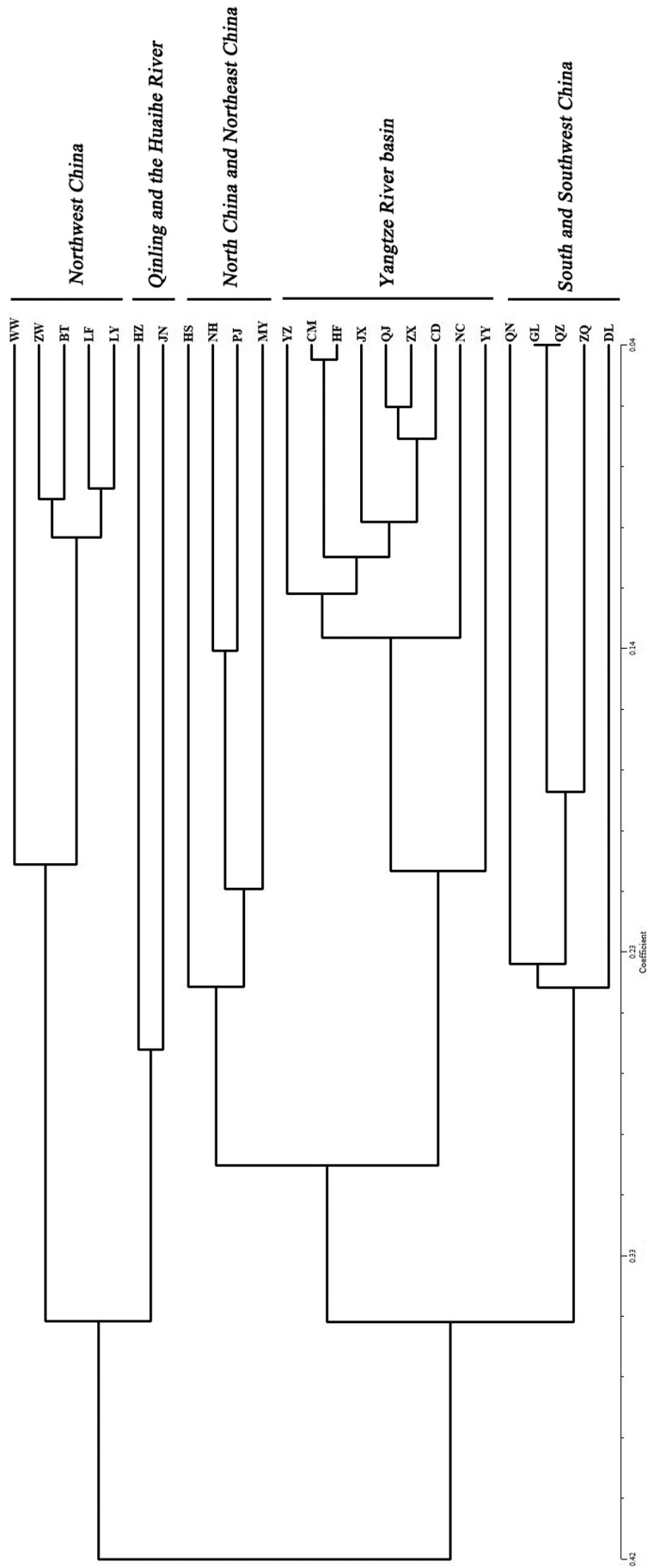


Fig. 2. The phylogenetic tree for the 25 populations of *P. clarkei* based on SSR markers using the UPGMA method based on Nei's (1979) genetic distances. The numbers in branches are percentage values over 1000 bootstrap replicates. Only bootstrap values over 50% are shown.

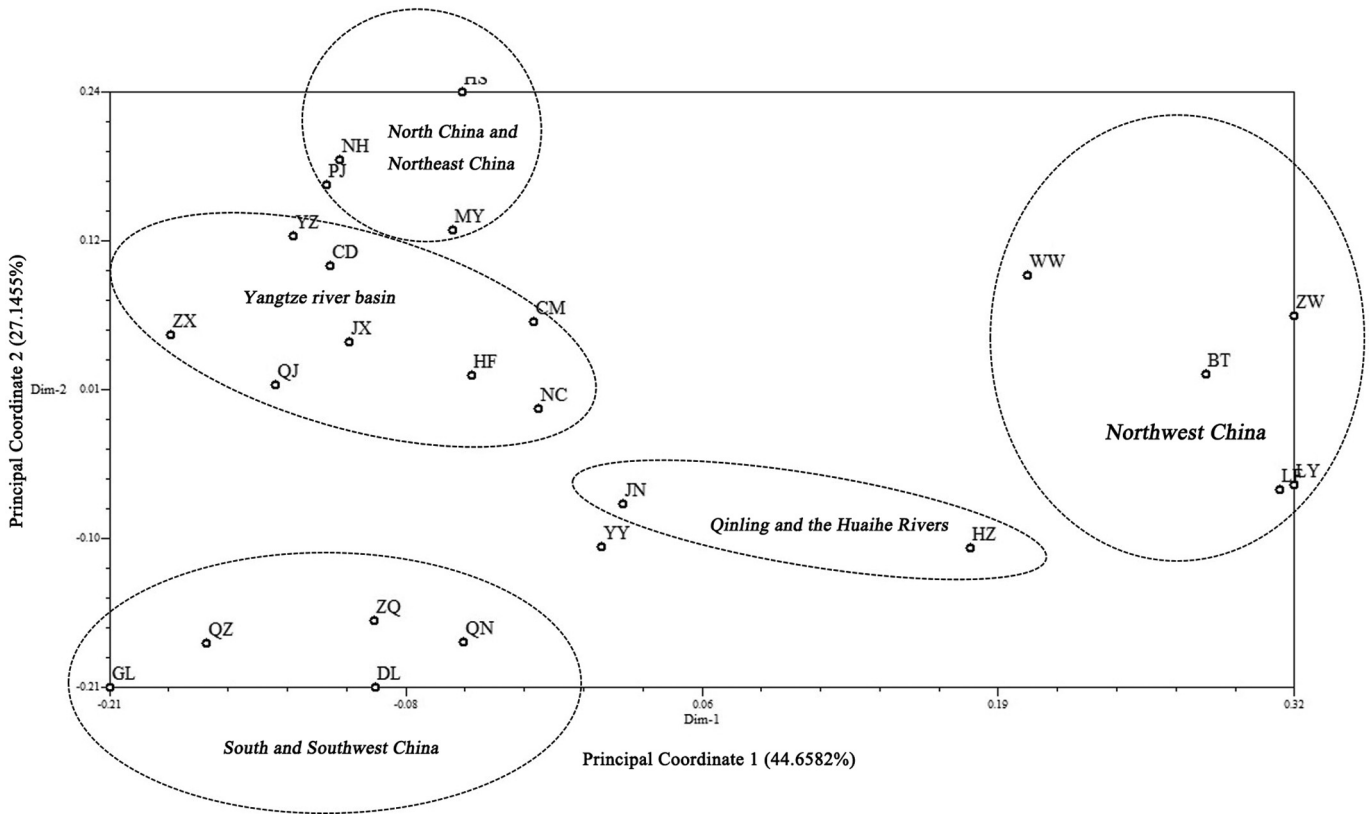


Fig. 3. A two-dimensional plot of the principal coordinates analysis (PCoA) based on SSR data for *P. clarkii*.

Dreissena bugensis [43] and *Alternanthera philoxeroides* invading Nanjing and other places in China have high genetic diversity [44]. This phenomenon may be related to the following factors. First, multiple introductions or single introductions of large numbers of individuals from the same or different sources may lead to higher genetic diversity of the population [43,45,46,47,48]. Second, the genetic diversity of invasive species increases by crossbreeding or mutation after they successfully enter new environments [44,49]. Furthermore, *P. clarkii* is one kind of freshwater crayfish with poor migration ability, but the populations of *P. clarkii* in this study showed a high diversity level. The following reasons might be taken account: multiple introductions from the same source or different sources or a single introduction of a large number of individuals may lead to high genetic diversity in a population. Besides, after successful entry of invasive species into the new environment, genetic variation increases through crossbreeding or mutation. Huner et al. [50] supported that the successful invasion of *P. clarkii* is mainly due to its ecological plasticity, which enables it to survive stably in a variety of environments. Barbaresi and Gherardi [51] pointed out that some biological characteristics of alien invasive species made them gain competitive advantage over indigenous species in some environments. *P. clarkii* is a fast-growing species with R-type propagation strategy [52], the embryo development of *P. clarkii* only lasts 14–21 d. It can lay eggs more than 2–3 times a year, as a result one parent *P. clarkii* can hold 50–200 larvae at last. It is generally believed that successful alien species often have a wide range of adaptation and strong tolerance to various environmental factors [53]. Species with wide ecological range can effectively utilize various resources to achieve higher density and establish wild population [54]. Correia [55] showed that *P. clarkii* could successfully establish a population in the place of introduction was the result of its wide ecological niche and diversified food. The *P. clarkii* has a high adaptability to the

hydrological and temperature conditions of the new habitat [56]. In most water bodies, such as small water bodies, short-term ponding ditches, and water bodies disturbed by human beings, the *P. clarkii* can reproduce [57], and adapt well to the strong seasonal fluctuation of water level in its living area [51]. *P. clarkii* can endure extreme environment and survive in polluted water [51], low concentration of dissolved oxygen, high salinity and acidity [50]. Because of its strong tolerance to adverse conditions, it can even spread across land, so it can cross geographical barriers and establish populations in isolated water bodies [58]. These factors contribute to the genetic variation and distribution pattern of *P. clarkii*. Therefore, the diversity of *P. clarkii* populations was increased because the connections between rivers were the main route of dispersal. Artificial introduction promoted gene exchange among the *P. clarkii* populations in different areas, which had some impact on the genetic diversity of the *P. clarkii* populations. Seasonal fluctuations of water levels in lakes and wetlands in the middle and lower reaches of the Yangtze River affected the genetic diversity of *P. clarkii*.

According to the difference in PPB, the genetic diversity of the Southern provinces was higher than that of the Northern provinces. These results might be due to the factor of the water system in the southern provinces that is more developed than that in the northern provinces, which facilitates the gene exchange of *P. clarkii*. For example, in the southern provinces, where populations QN, GZ, GL and ZQ reside, the water system density is much higher than in the northern provinces, where the BT population lives; thus, the distribution of *P. clarkii* in the southern provinces is consequently longer. In addition, the different populations show the same levels of diversity and the same patterns of bands in each locus, for example, HF, JX, NC, YY, QJ, ZX and CD all in Yangtze River basin. In general, the genetic structure of *P. clarkii* populations in China has not had a significant impact and still has a relatively rich

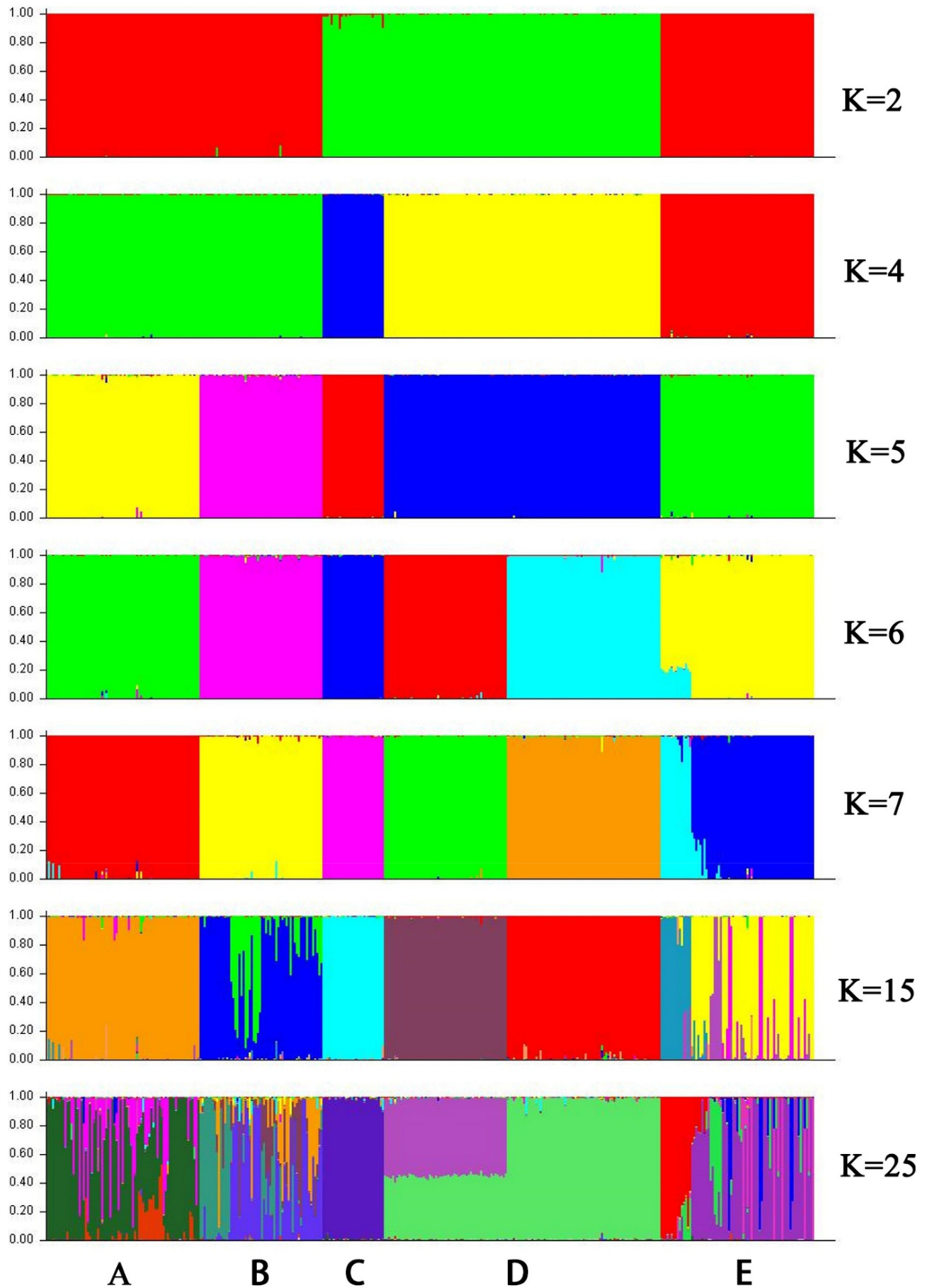


Fig. 4. Genetic relationships of *P. clarkii* (375 individuals) among the 25 populations estimated using STRUCTURE (version 2.3.4) based on SSR data. Each thin vertical colored line represents an individual. Each color represents an accession, and the color of an individual represents its proportional membership in the different populations. Notes: Northwest China (A), Qinling and the Huaihe Rivers (B), North China and Northeast China (C), Yangtze River basin (D), South and Southwest China (E).

genetic diversity. Since no significant differences in the genetic parameters of *P. clarkii* populations in the similar areas were observed. Therefore, distant hybridization should be carried out among individuals to ensure the relative stability of genetic diversity and improve the nuclear genetic diversity of these populations.

4.2. Gene differentiation

Gene differentiation and gene flow are two important indicators for evaluating the genetic structure of a population. According to Buso et al. [59] a G_{st} value of more than 0.25 indicates a very high degree of genetic differentiation, a G_{st} ranging from 0.15 to 0.25 suggests a high degree of genetic differentiation, a G_{st} ranging from 0.05 to 0.15 represents moderate genetic differentiation, and a G_{st} less than 0.05 represents a small amount of genetic differentiation. In this study, the genetic differentiation among populations of *P. clarkii* was very high ($G_{st} = 0.4785$) (Table 4). The results are similar to those of Liu et al. [21].

Population differentiation is expected to occur to varying degrees due to differences in the regional distribution, life cycle and reproductive pattern among species. Compared with the gene flow in *Pelodiscus sinensis* ($N_m = 5.9993$) [60], that in *P. clarkii* ($N_m = 0.5450$) is limited, which enhances the genetic differentiation in this species. Generally, a degree of gene flow ($N_m = 1$) indicates gene flow sufficient to prevent population genetic differentiation due to genetic drift [61]. Studies also show that gene flow between populations is relatively low. Various factors can explain the degree of G_{st} among species, including the geographical distribution, the breeding system, population genetic drift and genetic isolation [62]. The relatively low gene flow among the *P. clarkii* populations is explained by two processes: reproductive isolation and fluctuations in the water level, which are important factors affecting population structure. The 25 groups involved in this study belong to different water systems, such as lakes and wetlands in the middle and lower reaches of the Yangtze River basin, which are subject to water level fluctuations. Their water levels fluctuate seasonally and are related to the water level of the Yangtze River, while many groups in the north belong to arid areas and lack reproductive communication.

4.3. Genetic relationships

All populations have some variable morphological characteristics, including stripes, color, body width and body length, but it is difficult to recognize them. They are usually used to identify differences in populations of *P. clarkii*, depending on the observer's ability. In fact, similar forms are found among populations [63]. Therefore, molecular markers have been used to distinguish these species and populations in recent years [17,18,19,32]. In this study, SSR markers were used to explore variation in DNA among *P. clarkii* populations in most provinces of China. The samples used were from the whole distribution and were more representative than those collected from local areas. According to UPGMA cluster analysis, the 25 populations were divided into 5 groups (Fig. 2). Similarly, according to the results of structural analysis, when $k = 5$, all *P. clarkii* populations from closely related regions were clustered in the same group (Fig. 4). The SSR markers revealed that the Northwest China, Qinling and the Huaihe River, North China and Northeast China, Yangtze River basin, and South and Southwest China populations clustered with each other, although the Northwest China, Qinling and the Huaihe River, North China and Northeast China, Yangtze River basin, and South and Southwest China populations appeared to be distinct based on PCoA. The results of this study are consistent with the distribution of rivers and showed that the polymorphism of the 25 populations was relatively moderate, indicating that SSR-based marker analysis was effective and that the population differences were highly consistent [27].

4.4. Strategies for conservation and sustainable utilization of *P. clarkii*

Because of the important market value of *P. clarkii*, the scale of *P. clarkii* farming has been expanding in recent years. Although, the genetic diversity of wild *P. clarkii* germplasm resources keeps at a high level, it is necessary to take some protective measures. In recent years, the natural habitat of *P. clarkii* has been seriously damaged due to urban activities, industrial development, increased market demand and pollution. The ultimate goals are to ensure population protection and sustainable use and to maintain evolutionary potential [40]. Some other aquatic species face a similar problem: limited harvesting for use and resources, such as in *Penaeus monodon* [33], *Macrobrachium rosenbergii* [34], *Litopenaeus vannamei* [35], *Panulirus polyphagus*, *P. ornatus*, *P. penicillatus*, *P. versicolor*, *P. homorus*, *P. stipsoni*, *P. japonicus* [36], *C. gigas* [15], *Macra veneriformis* [37], medical leech [21] and *S. japonica* [38]. Because of founder effects or bottleneck effects, the genetic diversity of migrant populations is generally lower than that of their original population [43]. Compared with the results of Barbaresi et al. [47], the genetic diversity of *P. clarkii* in China measured here is lower than that in Western Europe and North America. Population differentiation occurs, and some loci are lost. It can be concluded that the genetic diversity of *P. clarkia* was maintained at a high level due to its strong adaptability and fast reproduction after it migrated to China. Therefore, breeding during the process of artificial reproduction or breeding of *P. clarkii* can combine morphological characteristics, select parents from different populations as the source group, and introduce improved varieties from abroad to promote the healthy and sustainable development of the *P. clarkii* farming industry.

Therefore, artificial breeding programs seem to be a feasible and necessary method with which to create new high-quality *P. clarkii* varieties. Determining the genetic structure of *P. clarkii* is helpful for artificially cultivating high-quality germplasm. The resources of crayfish should be collected as much as possible and kept in different places. In conclusion, adequate artificial *P. clarkii* farms should be established to meet the strong market demand. Only in this way can wild crayfish resources be protected.

5. Conclusions

In conclusion, the present study found that genetic diversity determined by SSR marker analysis of *P. clarkii* was high at the species level because of the fact that this species is widely distributed. The high G_{st} among *P. clarkii* populations is attributed to genetic drift and geographic isolation. In this study, the genetic structure of *P. clarkii* populations was studied. The results indicated that more *P. clarkii* populations should be collected when formulating conservation and aquaculture strategies.

Conflict of interest

All authors declare no conflict of interest.

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Supplementary material

<https://doi.org/10.1016/j.ejbt.2020.06.007>

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