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Population Genetic Diversity and Differentiation of Mitten Crab, Genus *Eriocheir*, Based on Microsatellite Markers

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Abstract: Mitten crab, *Eriocheir sensu stricto*, is an important indigenous aquatic species and food source in Eastern Asia. Genetic diversity is an important prerequisite for the conservation and utilization of germplasm resources. However, the genetic diversity and differentiation of *Eriocheir s. s.* remains unclear. This study evaluated population genetic diversity and genetic differentiation of three *Eriocheir s. s.* populations from Suifenhe (SFH), Liaohe (LH), and Nanliujiang (NLJ), China, based on 19 microsatellite markers. The mean observed number of alleles (N_a) was 22.84 alleles, and the mean polymorphism information content (*PIC*) was 0.86, which demonstrated high genetic diversity. The allele frequency distribution showed an "L" shape, ranging from 0.01 to 0.74. The genetic diversity parameter values of the LH population were higher than those of the other two populations. All pairwise F_{ST} values showed significant differences among the three *Eriocheir s. s.* populations ($p < 0.01$). The value of Nei's genetic distance (D_S) varied from 0.31 (between SFH and NLJ populations) to 0.33 (between SFH and LH populations). Overall, The results illustrate that the three wild *Eriocheir s. s.* populations have high genetic diversity and high genetic differentiation. This study provides the basis for *Eriocheir s. s.* utilization in China.

Keywords: *Eriocheir sensu stricto*; genetic diversity; genetic differentiation; microsatellite

1. Introduction

The mitten crab, *Eriocheir sensu stricto*, belongs to the Class Crustacea, Order Decapoda, and Family Syringidae, and inhabits the coastal rivers and estuaries from Heilongjiang Province to Guangxi Zhuang Autonomous Region along the coast of China [1,2]. The Chinese mitten crab (*Eriocheir sinensis*), as one of the most widely developed and utilized mitten crabs, is an economically important species for freshwater aquaculture in China because of its delicate flavor and high nutritional value [3,4]. The aquaculture yield of *E. sinensis* has increased steadily during the past two decades and stabilized at an annual yield between 750,000 and 800,000 tons in the past four years [5]. In the wild, the major natural habits for *E. sinensis* are in the Liaohe (LH) and Yangtze (YZ) rivers in China [6], forming the LH and YZ populations of *E. sinensis* [7]. The LH population of *E. sinensis* is mainly cultured in paddies, lakes, and a few ponds in northern China. However, YZ populations of *E. sinensis* are mainly cultured in earth ponds, lakes, and a few paddies in southern China [8]. Of these two populations, the YZ population of *E. sinensis* is considered more popular for aquaculture practitioners because of larger adult size and good flavor [9]. However, the shortage of wild juveniles from the YZ river basin caused by over-exploitation drove frequent stock transfer among the different geographical populations (mainly transport from the Rivers LH and Oujiang (OJ) to the YZ river basin) in the 1990s [6]. This transport has caused the YZ population of *E. sinensis* to become a

mixed gene pool of LH and OJ *E. sinensis*. Little is known about transport from the YZ or OJ populations to the LH delta for culture. Meanwhile, combined with the previous studies [10], the LH population of *E. sinensis* is a relatively pure gene pool of *Eriocheir s. s.* compared with the YZ population in the Yangtze delta.

Currently, the majority of *Eriocheir s. s.* derived from the respective river basins have definite species names. The *Eriocheir s. s.* derived from river basin Tumenjiang (TMJ) in the northeast of China comprises overlapping distributions or a genetically mixed population of *E. sinensis* and *E. japonicus* based on morphometrics analysis [11]. *Eriocheir s. s.* derived from the river basins LH, Yellow (YL), and YZ are treated as *E. sinensis* based on mitochondrial and microsatellite markers [12,13], because only slight differences in morphological and molecular characteristics were previously observed [14,15]. *Eriocheir s. s.* derived from the river basins OJ and Minjiang (MJ) are classified into a mixed population of *E. sinensis* and *E. hepuensis* based on mitochondrial and microsatellite markers [12,16]. The *Eriocheir s. s.* derived from the river basin Nanliujiang (NLJ) is classified as *E. hepuensis* based on morphometrics and microsatellite marker analysis [12,17].

The *Eriocheir s. s.* derived from river basin SFH is indigenous and distributed in the southeast of Heilongjiang Province, China. According to previous reports [18], the SFH population of *Eriocheir s. s.* is considered as *E. japonicus* because *Eriocheir s. s.* is a migratory aquatic animal, and the megalopa migrate upstream to the river basin SFH from the Sea of Japan. However, based on landmark-based morphometric analysis, the *Eriocheir s. s.* in the SFH river basin, has closer morphological characteristics that more closely resemble *E. sinensis* [19]. Currently, there are no studies in the literature that report the genetic diversity and differentiation of *Eriocheir s. s.* in the SFH river basin from the perspective of genetics. Hence, we used microsatellite loci to explore the genetic diversity and differentiation of *Eriocheir s. s.* in the SFH river basin.

Previous studies have demonstrated the genetic diversity and differentiation of *E. sinensis* and *E. hepuensis* [6,10,12,17,20]. Due to the relatively pure germplasm of *Eriocheir s. s.* in the LH river basin, we selected the LH population of *Eriocheir s. s.* as the representative *E. sinensis* in this study. Likewise, the NLJ population is a representative population of *E. hepuensis*. This study was conducted using classical population genetics to assess the genetic diversity and differentiation of these three wild *Eriocheir s. s.* populations (especially the SFH population) and to provide appropriate guidelines for the conservation and utilization of germplasm resources.

2. Materials and Methods

2.1. Sample Collection

Wild *Eriocheir s. s.* samples were captured from September to November 2021 in the SFH (131.32° E, 44.05° N, Mudanjing City, Heilongjiang Province, China), LH (121.81° E, 40.95° N, Panjin City, Liaoning Province, China), and NLJ (109.04° E, 21.39° N, Beihai City, Guangxi Province, China) river basins. The geographic locations of *Eriocheir s. s.* populations are shown in Figure 1. The sample size of each basin was 60 adult individuals, including 30 females and 30 males with a weight range of 80–100 g. Subsequently, all live individuals were transported to the Key Open Laboratory of Cold Water Fish Germplasm Resources and Breeding of Heilongjiang Province, Heilongjiang Fisheries Research Institute (CAFS), Harbin, China. According to the guidelines for the care and use of laboratory animals of Heilongjiang River Fisheries Research Institute (CAFS), *Eriocheir s. s.* were anesthetized, and the sampled pleopod muscles from the legs of each individual were stored at −80 °C before DNA extraction.

2.2. DNA Extraction

Total genomic DNA was extracted from leg muscle tissue using the Magnetic Animal Tissue Genomic DNA Kit produced by Tiangen Biotech (Beijing, China) Co., Ltd. (DP341-2). After extraction, the gel was dyed with ethidium bromide replacement, and captured by GeneSnap with the Syngene System (Bio-Rad, Hercules, CA, USA). Subsequently, DNA

concentration was measured using a spectrophotometer (Quawell, San Jose, CA, USA). The genomic DNA samples were diluted to 50 ng/μL and stored at −20 °C before use.
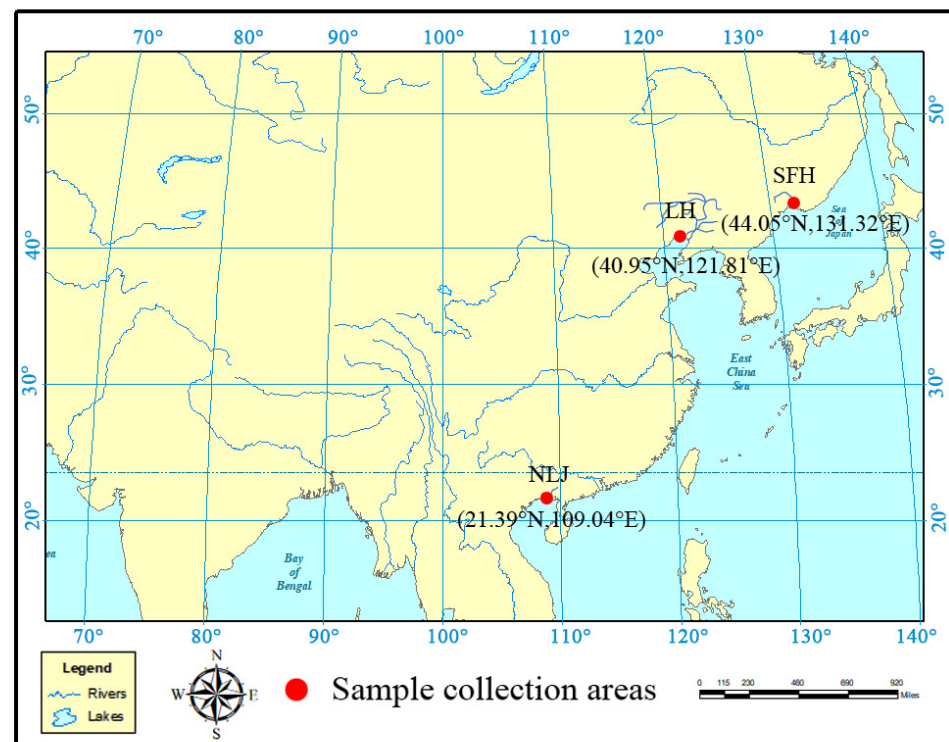
Figure 1. Sampling locations of *Eriocheir s. s.* populations. The sampling locations, from north to south, were Suifenhe River (SFH), Liaohe River (LH), and Nanliujiang River (NLJ), respectively.

2.3. Microsatellite Markers and PCR Amplification

A total of 20 loci (Table 1) was screened with information regarding microsatellite primers obtained from the previous literature [21–25]. The primer sequences and allele size ranges are listed in Table 2. All forward primers (5′ end) were fluorescently labeled with either an FAM or HEX tag, and all reverse primers were ordinary primers. These primers were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China).

Table 1. Characterization of *E. sinensis* microsatellites.

Locus Name	Primer Sequences (5′–3′)	Core Sequence	Temperature	Allele Size Range
A9	F: GCAATGAGACTCAACAGGAGA R: AATGTGGCTCACCTGACGT	$(GA)_7(GT)_7$	60	200–282
Eril1	F: GATAGACCGTAAATGAGACGGCTG R: GGACGGAGAAAACTAGAGACCACA	$(GGA)_9$	65	151–174
Eril2	F: GGATTTACTTAAGTTGGGGCTCGT R: CGACGCAGTTTTGTCTAGAGACCT	$(GAG)_8$	65	119–161
Eril3	F: CAGCGAAAAACAGGAAGCATTTAG R: GGAAAGGGAAAGTGAAGGATGAAT	$(AC)_{11}$	65	140–212
Eril4	F: TTCTTTGAGCGACATGCAAAAGT R: AGACAGACAGACAAAAACGCTCCT	$(TG)_{29}$	65	132–192
Eril5	F: TAGGGGGTTTTAGGTGTGGTGATA R: ATTTATGTGGAGGGAATGGGAGAT	$(TGA)_9$	65	125–167
Eril8	F: TGTTGAGTGTGATGTTTGTGATGC R: TAATAGCGGCCAAACTTTGTTGAT	$(GT)_{12}$	65	160–226

Table 1. *Cont.*

Locus Name	Primer Sequences (5′–3′)	Core Sequence	Temperature	Allele Size Range
Eril10	F: TACCTTTTTCAGGGTGAGTGAAGG R: AAGGACAGGAGGGAAAATGAGAGT	$(GT)_{23}$	65	138–208
Eril12	F: ACCCATCTCAAGTCCAGACTCATC R: AGAGGATGCAAGGGAAATAAGGAG	$(CCT)_8$	65	152–170
Eril13	F: AAGGAAGGCAGTTAGGAGGGTATG R: TTATTATTGTGGCGACGAAGGGT	$(AC)_{11}$	65	189–259
Eril16	F: TCCTCCCTATGCTCTTTGTAGGTG R: AAGGCCCAGGAGTATGGTGAAC	$(AC)_{11}$	65	150–192
Esin18	F: CACCGTAAGGTTCCGTAA R: AAGCACCCATAAGTCAATGTA	$(GT)_{26}$	58	170–225
Esin42	F: GCACCGCAGTGATAATGTAGTGG R: GATCCTCGTGTGGGCGTGCTTAC	$(AC)_{19}$	53	235–275
Esin67	F: TTTGGGATTCACCTTGTCAACTT R: CGACGCACGACAGAGGAGAGG	$(GT)_{11}$	53	105–170
Scaffold256287_157596	F: CACGAAAGGGTAAAAGGGGTACAT R: TCTTCTGTTTCATCCTCGTCTTCC	$(AGG)_9$	63	102–126
Scaffold16450_13154	F: TATTTTCTCCCCCTCCATCCTTTA R: GCATCGCCTACACCGTTTATTATC	$(GGA)_8$	55	147–189
Scaffold387247_202848	F: TCCTCCTCCTCATCCTCATCA R: CTCGTGACTGGAAAATGCGAC	$(AG)_{40}$	55	134–173
Scaffold101834_74240	F: TGTTCCTCTTGTTCTGTTTTGTTCC R: GGTTGCTTAATGGTGGCTTATGAA	$(CTA)_{10}$	53	112–235
Scaffold242247_151216	F: TCCATAACAGTCCTCTGAGTTCCC R: AGGAGAGAGTGTTGGCATGGTAAG	$(CTC)_8$	56	133–175
Scaffold306931_218734	F: CAATTTGCGCTAAATAGATGTCCC R: CCTCGAAGTTTGGGGTTAAAGGTA	$(AGT)_9$	63	235–301

Table 2. Genetic diversity of 19 microsatellite loci in three *Eriocheir s. s.* populations.

Locus	N_a	N_e	H_o	H_e	I	*PIC*	F_{IS}	F_{IT}	F_{ST}	N_m	*EWTN*
A9	34.00	20.34	0.85	0.95	3.20	0.95	0.08	0.10	0.02	12.01	0.09
Eril1	12.00	3.98	0.74 **	0.75	1.71	0.71	−0.01	0.02	0.03	7.97	0.28
Eril2	17.00	6.98	0.54 **	0.86	2.32	0.85	0.34	0.37	0.04	6.38	0.20
Eril3	46.00	26.62	0.99 **	0.97	3.49	0.96	−0.04	−0.02	0.02	14.30	0.06
Eril4	39.00	23.52	0.69 **	0.96	3.35	0.96	0.26	0.27	0.02	12.27	0.08
Eril5	12.00	3.15	0.69 **	0.68	1.63	0.66	−0.05	−0.01	0.04	5.98	0.29
Eril8	28.00	17.82	0.84 **	0.95	3.09	0.94	0.08	0.11	0.03	7.23	0.12
Eril10	26.00	15.38	0.91	0.94	2.91	0.93	0.01	0.03	0.02	10.05	0.13
Eril12	10.00	5.20	0.73 **	0.81	1.86	0.78	0.07	0.09	0.02	10.97	0.34
Eril13	29.00	17.53	0.94	0.95	3.04	0.94	−0.02	0.01	0.03	9.61	0.11
Eril16	24.00	7.17	0.78 **	0.86	2.44	0.85	0.06	0.09	0.03	9.18	0.14
Esin18	29.00	18.56	0.68 **	0.95	3.07	0.94	0.26	0.28	0.02	16.15	0.11
Esin42	18.00	10.34	0.86	0.91	2.52	0.90	0.02	0.05	0.03	8.16	0.19
Esin67	25.00	12.98	0.83 **	0.93	2.79	0.92	0.06	0.10	0.04	6.42	0.13
Scaffold256287_157596	9.00	4.61	0.63 **	0.79	1.75	0.76	0.19	0.20	0.01	20.80	0.36
Scaffold16450_13154	12.00	6.78	0.80	0.86	2.07	0.84	0.04	0.06	0.02	10.97	0.28
Scaffold101834_74240	34.00	19.67	0.84 **	0.95	3.20	0.95	0.09	0.12	0.03	7.83	0.09
Scaffold242247_151216	12.00	3.73	0.46 **	0.73	1.62	0.69	0.23	0.37	0.19	1.08	0.28
Scaffold306931_218734	18.00	7.70	0.75	0.87	2.31	0.86	0.11	0.13	0.03	9.73	0.19
Average	22.84	12.21	0.77	0.88	2.55	0.86	0.09	0.12	0.03	7.31	0.18

Note: ** means extremely significant deviation from Hardy–Weinberg equilibrium ($p < 0.01$); *EWTN*: Ewens–Watterson test for neutrality.

A total volume of 20 μL was used for each reaction, including 1 μL genomic DNA (50 ng/μL), 0.5 μL forward primer (10 mmol/L), 0.5 μL reverse primer (10 mmol/L), $2 \times$ Es Taq Master Mix (CW Biotech Co., Ltd., Beijing, China), and 8 μL ddH$_2$O. PCR products were obtained with a PCR instrument (Bio-Rad, Hercules, CA, USA). The PCR amplification conditions were as follows: firstly, pre-denaturation, 94 °C for 2 min; secondly, a total of 35 cycles including denaturation at 94 °C for 30 s, optimal annealing temperature (listed in Table 1) for 30 s, and 72 °C for 30 s; and finally, an extension step at 72 °C for 10 min. After all the PCR products were detected by agarose gel electrophoresis, the PCR products of each FAM and HEX primer were mixed together, and then sent to Sangon Biotech Co., Ltd. (Shanghai, China) for short tandem repeat (STR) sequencing. Formamide denaturation was used, ROX-500 was used as the internal standard, and an ABI-3730XL automatic DNA sequencer (ABI, Waltham, MA, USA) was used for detection and analysis. The peak map was scanned by Genemapper version 3.5 software (Thermo, MA, USA) and the length of allele fragment represented by the target peak was read. All data were imported into Microsoft Excel 2017 for further analysis [13,26].

2.4. Statistical Analysis

According to the size and quantity of each locus gene fragment, the genotype of each sample was determined. The measured genetic diversity, genetic differentiation, and genetic structure parameters were according to the method of Wang et al. [16], which included observed number of alleles (N_a), expected number of alleles (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), Shannon Wiener index (I), polymorphic information content (PIC), inbreeding coefficient (F_{IS}), total departure of genotype frequencies from HWE (F_{IT}), genetic differentiation coefficient (F_{ST}), gene flow (N_m), Ewens–Watterson test for neutrality ($EWTN$), test of departure for Hardy–Weinberg equilibrium (HWE, $p < 0.05$), Nei's genetic distance (D_S), neighbor-joining phylogenetic tree, population pairwise F_{ST}, and analysis of molecular variance (AMOVA) of *Eriocheir s. s.* species were calculated by POPGEN version 3.2 (San Jose, CA, USA), PIC_CALC version 0.6, MEGA version 5.0 (Mega Limited, Auckland, New Zealand), and ARLEQUIN version 3.5 (University of Berne, Swiss).

The values (mean $\pm$ SE) of N_a, N_e, H_o, H_e, I, PIC, and N_m of each population were calculated using Microsoft Excel 2017 based on values of per locus.

## 3. Results

3.1. Genetic Diversity of Microsatellite Loci

The genetic diversity parameters of 19 microsatellite loci in three *Eriocheir s. s.* populations are shown in Table 2. A total number of 434 alleles was observed in 180 individuals of three wild *Eriocheir s. s.* populations, with an overall mean of 22.84 alleles per locus and a range from 9 (*Scaffold242247_157596*) to 46 (*Eril13*) (Figure 2). The N_e values ranged from 3.73 (*Scaffold242247_151216*) to 26.62 (*Eril3*), with a mean of 12.21 alleles per locus. The H_o values varied from 0.46 (*Scaffold242247_151216*) to 0.99 (*Eril3*) with a mean of 0.77, while H_e values ranged from 0.73 (*Scaffold242247_151216*) to 0.97 (*Eril3*) with a mean of 0.88. The I values lay in the range from 1.62 (*Scaffold242247_151216*) to 3.49 (*Eril3*) with a mean of 2.55 per locus, respectively. The PIC values were all more than 0.5, ranging from 0.66 (*Eril5*) to 0.96 (*Eril3*), with an overall mean of 0.86, and indicated a high degree of polymorphism at each locus. The F_{IS} values ranged from −0.04 (*Eril3*) to 0.34 (*Eril2*), with an average of 0.09, while the F_{IT} values ranged from −0.02 (*Eril3*) to 0.37 (*Eril2*), with a mean of 0.12. The F_{ST} values lay in the range from 0.01 (*Scaffold256287_157596*) to 0.04 (*Eril5*) with a mean of 0.03. Except for the F_{ST} value of the locus (*Scaffold242247_151216*) of 0.19, which exceeded the threshold of 0.05, the F_{ST} values of the remaining loci were all less than 0.05. The N_m values ranged from 1.08 (*Scaffold242247_151216*) to 20.80 (*Scaffold242247_157596*), with an average of 7.31. The $EWTN$ values per locus were 0.18 on average, ranging from 0.06 (*Eril3*) to 0.36 (S*caffold242247_157596*). Heterozygosity excess or deficiency was detected by multi-locus tests for *HWE*; in total, 13 out of 19 loci revealed significant deviation

from *HWE* ($p < 0.01$), including 11 loci with a heterozygosity deficiency, and 2 loci with a heterozygosity excess.
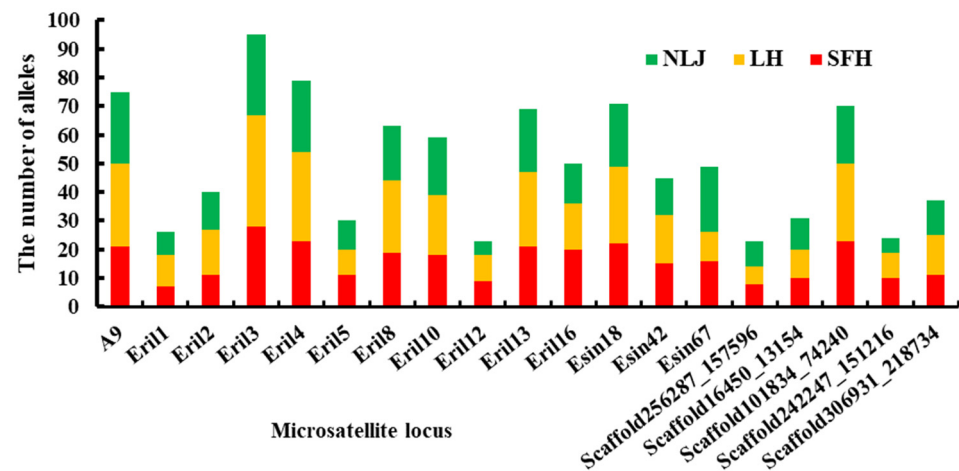
Figure 2. Number of alleles for each microsatellite locus in *Eriocheir s. s.* populations. Green, yellow, and red colors represent the Nanliujiang River (NLJ), Liaohe River (LH), and Suifenhe River (SFH) populations, respectively.

The allele frequency distribution is shown in Figure 3. The allele frequency distribution showed an "L" shape, ranging from 0.01 to 0.74. The proportion between 0.0 and 0.1 was the highest, reaching more than 80%, followed by 0.1~0.2, accounting for approximately 10% of the total. The remaining allele frequencies accounted for lower proportions.
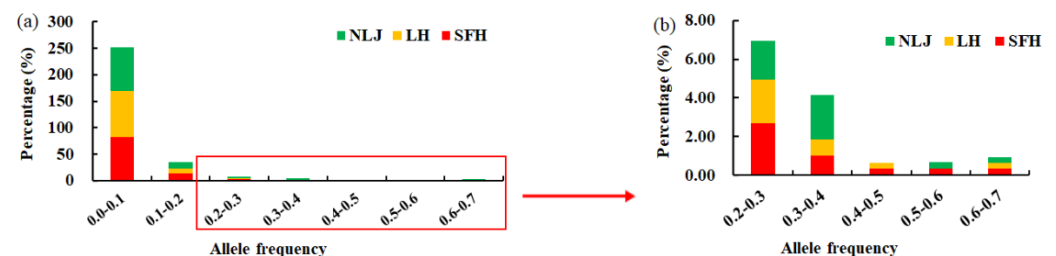
Figure 3. Allele frequency distribution of *Eriocheir s. s.* populations. (**a**) The percentage contents of 0.0–0.7 allele frequency distribution; (**b**) the percentage contents of 0.2–0.7 allele frequency distribution. Green, yellow, and red colors represent the Nanliujiang River (NLJ), Liaohe River (LH), and Suifenhe River (SFH) populations, respectively.

3.2. Genetic Diversity of Eriocheir s. s. Populations

The genetic diversity parameters for *Eriocheir s. s.* populations are shown in Figure 4. The mean N_a values varied from 15.95 ± 1.43 (SFH) to 18.53 ± 2.16 (LH) (Figure 4a). Likewise, the mean N_e values ranged from 8.43 ± 0.88 (SFH) to 11.79 ± 1.72 (LH) (Figure 4b). The mean *PIC* values varied from 0.81 ± 0.03 (NLJ) to 0.85 ± 0.03 (LH) (Figure 4c), while the average *I* values ranged from 2.22 ± 0.15 (NLJ) to 2.46 ± 0.15 (LH) (Figure 4d). The average H_o values ranged from 0.73 ± 0.04 (SFH) to 0.79 ± 0.04 (LH) (Figure 4e), while the average H_e values varied from 0.84 ± 0.03 (NLJ) to 0.88 ± 0.02 (LH) (Figure 4f). The mean N_m values varied from 7.56 ± 1.54 (SFH) to 11.09 ± 1.11 (LH) (Figure 4g). Overall, the parameter values for the LH population were higher than those of the remaining two populations. However, no significant differences were observed for these populations among these three wild populations ($p > 0.05$).
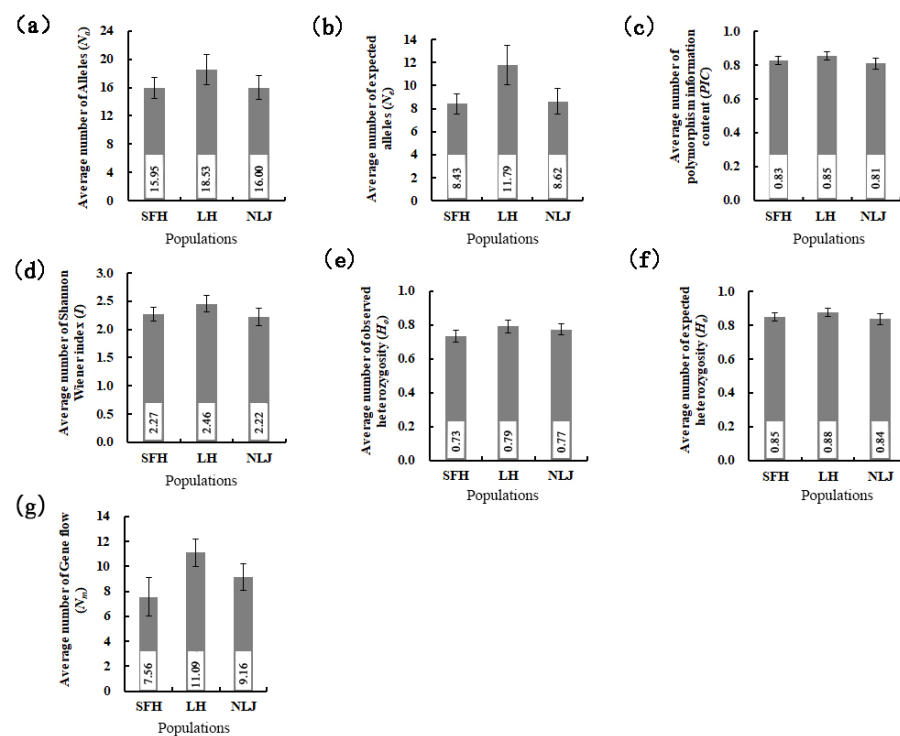
Figure 4. Genetic diversity parameters for three *Eriocheir s. s.* populations. (**a**) Average number of observed alleles (N_a); (**b**) average number of expected alleles (N_e); (**c**) average number of polymorphic information content (*PIC*); (**d**) average number of Shannon Wiener index (*I*); (**e**) average number of observed heterozygosity (H_o); (**f**) average number of expected heterozygosity (H_e); and (**g**) average number of gene flow (N_m) estimated after screening 19 microsatellite loci in three wild *Eriocheir s. s.* populations.

3.3. Genetic Differentiation of Eriocheir s. s. Populations

All pairwise F_{ST} values between populations, i.e., indices of population differentiation, were significantly different among these three *Eriocheir s. s.* populations ($p < 0.01$; Table 3 above the diagonal). The pairwise F_{ST} value ranged from 0.0352 (between SFH and LH) to 0.0409 (between SFH and NLJ). A significant pattern of differentiation was observed over all three *Eriocheir s. s.* populations ($F_{ST} = 0.03822$, $p < 0.01$; Table 4). Results of AMOVA showed significant differences both among and within populations ($p < 0.01$; Table 4), and 3.82% of the genetic variation was among populations and 96.18% within populations (Table 4). Unbiased genetic distances (Table 3 below the diagonal) over 19 microsatellite loci were estimated. The D_S value varied from 0.3144 (between SFH and NLJ populations) to 0.3310 (between SFH and LH populations). The genetic relationships among *Eriocheir s. s.* populations were further assessed based on D_S values by the reconstruction of phylogenetic trees. The neighbor-joining phylogenetic tree result is shown in Figure 5. SFH and NLJ populations were grouped together, and they then clustered together with the LH population.

Table 3. Nei's genetic distance (D_S, below the diagonal) and genetic differentiation coefficient (pairwise F_{ST} values, above the diagonal) for *Eriocheir s. s.* populations.

Populations	SFH	LH	NLJ
SFH	——	0.0352 **	0.0409 **
LH	0.3310	——	0.0385 **
NLJ	0.3144	0.3292	——

Note: ** denotes significant difference ($p < 0.01$). SFH, LH, and NLJ represent the *Eriocheir s. s.* from Suifenhe, Liaohe, and Nanliujiang river basins, respectively.

Table 4. AMOVA results for *Eriocheir s. s.* populations.

Source of Variation	d. f.	Sum of Squares	Variance Components	Percentage of Variation (%)	F_{ST}	p
Among populations	2	77.080	0.31807 Va	3.82	0.03822	0.00000
Within populations	285	2281.438	8.00504 Vb	96.18		
Total	287	2358.517	8.32312	100.00		
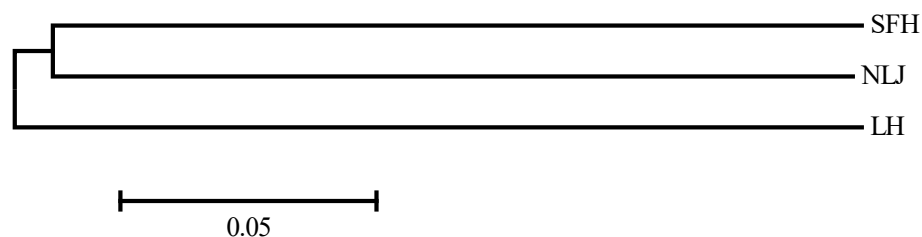
Figure 5. Neighbor-joining phylogenetic tree of *Eriocheir s. s.* based on Nei's genetic distance. SFH, LH, and NLJ represent the *Eriocheir s. s.* from Suifenhe, Liaohe, and Nanliujiang river basins, respectively.

4. Discussion

4.1. Genetic Diversity

The higher the genetic diversity, the greater the potential response to natural selection in the population, and the stronger the ability to adapt to the environment, which is conducive to the survival and evolution of the population [27]. In this study, 19 out of 20 highly polymorphic loci were screened to assess the genetic diversity of 180 wild *Eriocheir s. s.* individuals from three river basins, SFH, LH, and NLJ. The numbers of samples and loci in this study are sufficient to evaluate the genetic diversity parameters, because 30 samples and 10 loci are usually enough for genetic diversity analysis, while larger numbers of samples and loci have a great impact in terms of improving the accuracy of the genetic diversity parameters N_a, N_e, and H_o [28]. In this study, the N_a value was relatively high at *Esin18*, *Esin42*, and *Esin67* loci within individuals compared with earlier studies [6,13,16,29]; this value represented the high number of alleles in our sampled individuals. These N_a values, either low or high, may be determined by the numbers of samples or electrophoretic typing techniques [16]. All the average values of the N_e and I parameters at 19 loci showed high genetic diversity within individuals, and the results are similar to those of previous studies [13,30,31]. Polymorphism information content (*PIC*), i.e., a metric of genetic diversity, was not lower than 0.5, which illustrated high genetic diversity per locus. Through comparison with the literature, both wild or cultured *Eriocheir s. s.* were found to have high genetic diversity, unlike wild *Cyprinus carpio* [32], *Ctenopharyngodon idellus* [33], *Pelteobagrus fulvidraco* [34], *Portunus trituberculatus* [35], and *Litopenaeus vannamei* [36]. The allele frequencies of the three wild *Eriocheir s. s.* populations in this study displayed an "L" shape, indicating that the wild populations did not experience a recent bottleneck effect in their allele frequency distribution [37]. The heterozygosity in a population can reflect the level of genetic variation of that population. The higher the proportion of heterozygotes in the population, the greater the genetic variation [38]. The H_o and H_e values in this study illustrated high genetic variation, suggesting that the population can easily adapt to the changing environment. If the individuals are in *HWE,* the H_o and H_e values are close to each other at the locus. Otherwise, the individuals exhibit an excess or deficiency of heterozygotes [39]. In this study, 13 out of 19 microsatellite loci deviated from *HWE*, and 11 out of 13 deviated microsatellite loci showed heterozygote deficiency.

This study showed that the genetic diversity parameters of N_a, N_e, *PIC*, I, and N_m values in the LH *Eriocheir s. s.* population were slightly higher than the other two *Eriocheir s. s.* populations, SFH and NLJ, indicating that the wild LH *Eriocheir s. s.* population had higher genetic diversity. Two reasons may explain this result. First, the LH *Eriocheir s. s.* population is an important species cultured in paddies and lakes in northeastern China,

with a large population size, accompanied by proliferation and release. On the other hand, wild germplasm of the SFH and NLJ rivers has not been developed and utilized, and there are few river branches in its basin, which inhibits inter-population migration. Second, SFH and NLJ *Eriocheir s. s.* may not be the same species as LH *E. sinensis*. The microsatellite markers were specific primers for *E. sinensis*, but not for other *Eriocheir s. s.*; therefore, the microsatellite markers may not be absolutely precise for SFH and NLJ *Eriocheir s. s.* population analysis [16]. This may explain why genetic diversity parameters of the SFH and NLJ *Eriocheir s. s.* populations are lower than those of the LH *Eriocheir s. s.* population. To analyze the population genetic diversity of the SFH and NLJ *Eriocheir s. s.* populations, it is urgent to conduct screenings using genetic markers appropriate for evolutionary studies of this lineage.

4.2. Genetic Differentiation

Gene flow (N_m) and the genetic differentiation coefficient (F_{ST}) are two important parameters used to analyze the degree of genetic differentiation [32]. This study demonstrated a relatively high N_m value (7.31) and low F_{ST} value (0.0330) among populations at all loci, which implied low genetic differentiation. Pairwise F_{ST} values indicated genetic differentiation among the SFH, LH, and NLJ *Eriocheir s. s.* populations. Previous studies also demonstrated genetic differentiation, although the pairwise F_{ST} value between populations LH and NLJ was 0.0324 [6], which is very close to the pairwise F_{ST} value (0.0385) in this study. Likewise, the pairwise F_{ST} values among SFH, LH, and NLJ in this study were higher than those in earlier studies [13,16], e.g., 0.0050 (between LH and YZ) and 0.0105 (between YZ and MJ). Although the pairwise F_{ST} values (0.0352, 0.0385, and 0.0409) in this study were lower than 0.05, the values still suggest considerable genetic differentiation for *Eriocheir s. s.* Pairwise estimations of Nei's D_S exhibited a similar result. The D_S values (0.3144, 0.3292, and 0.3310) were higher than in previous studies [13,16,23,29,40], which showed a relatively great genetic distance among SFH, LH, and NLJ *Eriocheir s. s.* populations. Based on Nei's D_S, the neighbor-joining phylogenetic tree was constructed among these three wild *Eriocheir s. s.* populations. This study illustrated that populations SFH and NLJ were closer to each other than to the LH population, which was consistent with results from a previous study [41] but contradicted those from Jiang et al.'s study [19]. In Jiang et al.'s study, the SFH population was clustered with the YZ population (*E. sinensis*) rather than the NLJ population. However, the NLJ population clustered with *E. japonicus* was sampled in Hyogo, Japan. Two reasons may explain the difference. First, microsatellite analysis is more accurate from the perspective of genetics, while a morphological method was used in Jiang et al.'s work. Second, *E. japonicus* was sampled in Hyogo, Japan (35.62° N, 134.48° E), which is far from the SFH population. Therefore, the authors maintained that *E. japonicus* should have many different geographical populations, just like *E. sinensis*, including the LH, YL, and YZ populations. The Hyogo population of *E. japonicus* is located in the south of Japan's islands.

Results of AMOVA analysis revealed that total variation among populations was 3.82%, whereas 96.18% of variation was observed within populations. Low variation among populations may be caused by the *Eriocheir s. s.* lifecycle. *Eriocheir s. s.* has a catadromous lifecycle and travels through many different habitats during migration. Therefore, it was interpreted that *Eriocheir s. s.* probably loses local environmental adaptability [16].

5. Conclusions

Three wild *Eriocheir s. s.* populations (SFH, LH, and NLJ) had high genetic diversity and significant genetic differentiation. The study will provide important support for the conversation and management of *Eriocheir s. s.* populations.

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