Aquat. Living Resour. 2023, 36, 5

https://doi.org/10.1051/alr/2023002

Aquatic Living Resources
Available online at:
www.alr-journal.org

RESEARCH ARTICLE OPEN ACCESS

Genetic diversity and structure assessment of *Macrobrachium nipponense* populations: implications for the protection and management of genetic resources

Yiwei Xiong[1,a], Sufei Jiang[1,a], Lijuan Zhang[2], Jisheng Wang[2], Wenyi Zhang[1], Shubo Jin[1], Yongsheng Gong[1], Yan Wu[1], Hui Qiao[1,2] and Hongtuo Fu[1,2,*]

[1] Key Laboratory of Freshwater Fisheries and Germplasm Resources Utilization, Ministry of Agriculture, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Wuxi, Jiangsu, PR China
[2]Wuxi Fisheries College, Nanjing Agricultural University, Wuxi, Jiangsu, PR China

Received 1 September 2022 / Accepted 18 January 2023

Handling Editor: Carlos Saavedra

Abstract – This article presents a study of D-loop sequences to characterize the genetic diversity of wild *Macrobrachium nipponense* populations in Yixing natural waters including two reservoirs (Hengshan reservoir, HS; Youche reservoir, YC), 3 brooks (Linjin Dang, LJD; Magong Dushan Dang, MDD; Yangshan Dang, YSD) and 3 shallow lakes (Dongjiu lake, DJ; Xijiu lake, XJ; Tuanjiu lake, XJ), and compared the genetic differentiation and population structure with wild populations of Taihu Lake (TH), Yangtze River (YZ), and the main local artificially bred varieties "Taihu No. 2" (TH-2). A 747 bp D-loop sequence fragment was amplified in 321 individuals and the results exhibited a higher content of A+T (80.03%) than C+G (19.97%). A total of 110 haplotypes were identified. The *h* and π value proved the diversity of these populations was at the same level with high genetic diversity. TH-2 and YZ showed remarkable diversity, and XJ is even better. *Fst* estimates suggested that YZ and TH-2 were significant differentiation with other Yixing populations ($P < 0.05$). Three populations from shallow lake (DJ, XJ and TJ) displayed significant differentiated with the left Yixing ones ($P < 0.05$). The pairwise genetic distance, as well as haplotype network results, also suggested that all these 11 populations did not diverge at the species level (<15%). The *P* values of Tajima's *D* and Fu *Fs* were relatively greater than 0.1 ($P > 0.1$) and the nucleotide mismatch distribution analysis showed multiple peaks, giving a conclusion that the populations did not exhibited expansion. All these results suggested that TH-2 and YZ have remarkable diversity, and the germplasm resources and genetic diversity of *M. nipponense* in Yixing are very good and are suitable for original materials of breeding.

Keywords: *Macrobrachium nipponense* / mitochondrial DNA D-Loop / genetic diversity / genetic structure

1 Introduction

Macrobrachium nipponense, commonly known as oriental river prawn or river shrimp, is an economically important indigenous prawn species in China, widely distributed in lakes, rivers, reservoirs, and other freshwater water areas throughout the country (Fu et al., 2004). Because of its fast growth, high adaptability, and strong reproductive capacity, this species has been continuously bred since the late 1950s, especially in the lower reaches of the Yangtze River and Taihu Lake basin. However, due to natural and anthropogenic factors, the wild *M. nipponense* populations are currently surviving in smaller habitat areas; they are reducing and catches are declining (Fu et al., 2018). In 2001, the Freshwater Fisheries Research Center of the Chinese Academy of Fishery Sciences set up a scientific research team to carry out research on the improvement of prawn varieties. After years of efforts, two new prawn varieties were successfully cultivated: the hybrid prawn 'Taihu No. 1' and prawn 'Taihu No. 2'. Compared to the local wild prawn populations, individual specifications of both strains had been significantly improved. Its growth rate increased by more than 30% and the average yield per mu increased by 25% (mu, Chinese unit of landmeasurement that is commonly 666.7 square meters). the 'Taihu No. 2' showed

*Corresponding author: fuht@ffrc.cn
[a] These authors contributed equally to this work and should be considered co-first authors.

Table 1. Information of populations of *M. nipponense* included in the present research.

Population	Locality	Tissue samples collected	Number of samples sequenced
HS	Hengshan reservoir, Yixing, 119°33' 36.94"E, 31°13' 55.18"N	35	29
YC	Youche reservoir, Yixing, 119°44' 54.55"E, 31°13' 5.11"N	35	30
LJD	Linjin brook, Yixing, 119°39' 11.88"E, 31°27' 8.60"N	35	28
MDD	Magong and Dushan brook, Yixing, 119°43' 9.19"E, 31°27' 10.18"N	35	26
YSD	Yangshan brook, Yixing, 119°55' 43.43"E, 31°24' 30.94"N	35	31
DJ	Dongjiu lake, Yixing, 119°51' 48.52"E, 31°21' 27.61"N	35	32
XJ	Xijiu lake, Yixing, 119°44' 16.61"E, 31°23' 57.52"N	35	30
TJ	Tuanjiu lake, Yixing, 119°47' 37.43"E, 31°22' 40.99"N	35	32
TH	Taihu lake, Wuxi, 120°03' 26.06"E, 31°19' 16.32"N	35	28
YZ	Yangtze River, Zhenjiang, 119°39' 41.60"E, 32°12' 24.01"N	35	27
TH-2	"Taihu No. 2", Dapu scientific research test base of Freshwater fisheries research center of the Chinese academy of fishery sciences,Yixing, 119°55' 47.14"E, 31°18' 53.08"N	35	28

Notes: HS: Hengshan reservoir population; YC: Youche reservoir population; LJD: Linjin brook population; MDD: Magong and Dushan brook population; YSD: Yangshan brook population; DJ: Dongjiu lake population; XJ: Xijiu lake population; TJ: Tuanjiu lake population; TH: Taihu lake population; YZ: Yangtze River population; TH-2: "Taihu No. 2" population.

better specifications than 'Taihu No.1'and has been cultured on a large scale and promoted in Jiangsu, Anhui, Zhejiang, Sichuan, Tianjin, Hubei, and other provinces (cities). However, the market is still short of supply. In 2019, the Chinese government implemented a comprehensive strategy to step up the conservation of the Yangtze River, and subsequently a fishing ban was imposed in the Taihu Lake basin. These measures are of great significance for the conservation of the wild *M. nipponense* germplasm resources and sustainable development of aquaculture.

In recent studies, a number of molecular markers, such as SSR (simple sequence repeats) and COI (cytochrome c oxidase subunit I), were applied to analyze wild *M. nipponense* populations in different areas of China (Chen et al., 2015, 2017; Ma et al., 2012; Qiao et al., 2013). Yixing city is located in the lower reaches of the Yangtze River in Jiangsu Province, on the west bank of Taihu Lake. The city's natural water system is very developed and diverse, and it includes a number of reservoirs, brooks, and shallow lakes that are connected with Taihu Lake. The excellent geographical environment is perfect for the survival of prawns, but to date no studies have been conducted on the genetic characteristics of the local wild prawn population in Yixing.

The mitochondrial DNA (mtDNA) D-loop sequence is widely used in population genetic analysis because it does not encode proteins and is not affected by selection (Liao et al., 2016; Maltsev et al., 2015). In the present study, wild *M. nipponense* populations were sampled from eight different natural water bodies in Yixing city, including reservoirs, brooks, and shallow lakes, and D-loop sequences were employed to examine their genetic diversity. Then, the genetic differentiation and structure of these populations were compared with those of wild populations from Taihu Lake, Yangtze River, and the main local artificial strain 'Taihu No. 2'. The results of this study may contribute to improving the germplasm resources of wild *M. nipponense* populations in China and provide reference data to assist in the formulation of policies for the species' breeding program.

2 Materials and methods

2.1 Ethics statement

The study was approved by the Animal Care and Use Ethics Committee in the Freshwater Fisheries Research Center (Wuxi, China).

2.2 Sample collection

Wild samples of *M. nipponense* were collected from eight different natural water bodies in Yixing city, consisting of two reservoirs (Hengshan reservoir, Youche reservoir), three brooks (Linjin Dang, Magong Dushan Dang, Yangshan Dang), and three shallow lakes (Dongjiu lake, Xijiu lake, Tuanjiu lake) from February to March 2021. Wild prawns from the Yangtze River in Zhenjiang city and Taihu Lake in Wuxi city were also sampled. Individuals from the main local artificially bred strain 'Taihu No. 2' were obtained from the Dapu scientific research test base of the Freshwater Fisheries Research Center at the Chinese Academy of Fishery Sciences in Yixing. The detailed information on the samples is included in Table 1 and the locations are shown in Figure S1. In total, 35 individual prawns were sampled for each population. Muscle tissue samples were stored in 95% ethanol.

2.3 Total DNA extraction, PCR amplification, and sequencing

About 50 mg of muscle was sampled from each individual and was used to extract total DNA following the protocol

Table 2. Genetic diversity parameters of 11 *M. nipponense* populations.

Population	Variable sites	Number of haplotypes	Haplotypes diversity (h)	Nucleotide diversity (π)
HS	29	29	0.7438 ± 0.0753	0.01169 ± 0.00618
YC	32	19	0.8519 ± 0.0349	0.01512 ± 0.00787
LJD	36	26	0.9262 ± 0.0319	0.00962 ± 0.00518
MDD	67	26	0.9508 ± 0.0208	0.02135 ± 0.01095
YSD	66	40	0.9505 ± 0.0171	0.01822 ± 0.00935
DJ	38	24	0.9275 ± 0.0388	0.01321 ± 0.00698
XJ	49	22	0.9881 ± 0.0163	0.01211 ± 0.00645
TJ	35	27	0.9180 ± 0.0436	0.00991 ± 0.00531
TH	67	28	0.8836 ± 0.0496	0.01535 ± 0.00798
YZ	100	27	0.9601 ± 0.0259	0.02885 ± 0.01462
TH-2	88	28	0.9656 ± 0.0182	0.03571 ± 0.01796

described in Jiang et al. (2019). The D-loop amplification primers were designed based on the D-loop gene sequence fragment of *M. nipponense* available in Genbank (accession number: MZ614458). The primer sequences were F: 5'-TTTACTCCCAGTCTAACC-3', R: 5'-TTCAT-TATTCGCCCTATC-3'. The PCR procedure was conducted in 50-μL reaction mixtures following the steps reported in Jiang et al. (2019). PCR products were detected by electrophoresis and fragments were sequenced with an ABI3730 automated sequencer (Invitrogen Biotechnology Co., Ltd, USA).

2.4 Data analysis

BioEdit version 7 (Hall, 1999) was used to edit and align the D-loop sequences. Nucleotide diversity (π) and haplotype diversity (h) were estimated using ARLEQUIN 3.5 (Excoffier and Lischer, 2010), and genetic distances were estimated in MEGA 7 (Kimura, 1980). The analysis of molecular variance model (AMOVA) was used to estimate genetic variation following 1000 permutations in ARLEQUIN 3.5. Pairwise genetic differentiations (F_{ST}) were calculated with 10,000 permutations in ARLEQUIN 3.5 and the false discovery rate (FDR) was implemented using the method of Benjamini and Hochberg (1995). The best model to approximate sequence evolution was estimated in MEGA 7 (Kumar et al., 2016; Tamura et al., 2011). Evolutionary analyses were conducted using the maximum likelihood method in MEGA 7 based on the Tamura-Nei model (Kumar et al., 2016). Bootstrap values were based on 1000 rapid bootstrap replicates. The median-joining method in POPART was used to build a haplotype network (Leigh and Bryant, 2015). The neutrality test values were calculated in ARLEQUIN 3.5 (Excoffier and Lischer, 2010; Fu, 1997; Tajima, 1989). The mismatch-distribution analysis was carried out in DnaSP v5 (Librado and Rozas, 2009).

3 Results

3.1 Base composition and variation analysis of the D-Loop region sequences

The D-Loop region sequences of *M. nipponense* were amplified in 385 individuals from 11 populations. Through alignment analysis, incomplete and invalid sequences were removed and 720-bp-long sequences obtained from 321 individuals were retained for further analysis. The YZ and TH-2 populations had the most variable sites, while the HS and YC populations had the least variable ones (Tab. 2). The mean basic nucleotide composition in the obtained sequences was as follows: T = 36.28%, T, A = 43.75%, C = 10.75%, and G = 9.22%. The A + T and C + G contents were estimated at 80.03% and 19.97%, respectively (Tab. S1).

A total of 110 haplotypes were observed from the 321 individuals examined (Table S2, accession number: OP972608-OP972717). We found no insertion/deletion (indel) polymorphisms. Among these 110 haplotypes, the least unique were detected in the HS population (Hap17, Hap19, and Hap21). The YZ population had the highest number (20) of unique haplotypes (Hap91–Hap110). The remaining haplotypes were shared by two or more populations. The additional details about distribution of haplotypes were listed in Table S2.

3.2 Population genetic structure analysis

The genetic diversity parameters obtained are presented in Table 2. XJ population showed the highest h value (0.9881), while HS population had the lowest h (0.7438). Eight populations (XJ, TH-2, YZ, MDD, YSD, DJ, LJD, TJ), their h value exceed more than 0.9. The h of TH and YC populations were between 0.8 and 0.9. For π values, TH-2 population had the highest value (0.035714) while LJD population had the lowest value (0.00962). The π values of three populations (TH-2, YZ and MDD) were greater 0.02. The π values of six populations (YSD, TH, YC, DJ, XJ and HS) were between 0.01 and 0.02. The π values of TJ and LJD populations were no more than0.01. The XJ and HS populations displayed the highest and lowest h values, respectively, while the highest π value was detected in TH-2 and the lowest in LJD.

Based on the AMOVA, 16.46% of the total genetic variation was attributed to genetic differences among populations and 83.54% to variation within populations (Tab. 3). The F_{ST} value was high ($F_{ST} = 0.165$) and significant (P-value < 0.000). The population pairwise F_{ST} results after FDR testing are shown in Table 4. The F_{ST} values between populations varied from −0.0111 (between the HS and TH populations) to 0.469 (between the YZ and TJ

Table 3. Analysis of molecular variance (AMOVA) results for 11 *M. nipponense* populations.

Source of variation	*d.f*	Sum of squares	Variance components	Percentage of variation
Among populations	10	416.140	1.26580	16.46
Within populations	311	1895.546	6.42558	83.54
Total	321	2311.686	7.69138	100
F_{ST}	0.16457 (P-value = 0.00000)			

Table 4. Pairwise F_{ST} values of 11 *M. nipponense* populations.

	HS	YC	LJD	MDD	YSD	DJ	XJ	TJ	TH	YZ	TH-2
HS		0.283	0.1825	0.293	0.174	0.000*	0.000*	0.000*	0.000*	0.000*	0.445
YC	0.019		0.341	0.457	0.211	0.046*	0.000*	0.000*	0.000*	0.000*	0.551
LJD	0.082	0.064		0.178	0.345	0.048	0.045*	0.000*	0.000*	0.000*	0.466
MDD	0.010	0.006	0.038		0.379	0.058	0.000*	0.000*	0.071	0.000*	0.847
YSD	0.037	0.022	0.0311	–0.001		0.217	0.151	0.017*	0.000*	0.000*	0.467
DJ	0.158	0.126	0.126	0.081	0.028		0.457	0.433	0.000*	0.000*	0.000*
XJ	0.146	0.134	0.073	0.070	0.022	–0.009		0.273	0.000*	0.000*	0.033*
TJ	0.269	0.224	0.188	0.156	0.088	–0.001	0.008		0.000*	0.000*	0.000*
TH	–0.011	0.004	0.014	–0.018	0.009	0.114	0.091	0.201		0.000*	0.000*
YZ	0.366	0.331	0.425	0.294	0.329	0.399	0.415	0.469	0.341		0.000*
TH-2	0.151	0.139	0.173	0.048	0.112	0.163	0.154	0.214	0.109	0.252	

Notes: Pairwise F_{ST}, below the diagonal; significance of corresponding P-values (above the diagonal) based on pairwise differences in the concatenated mtDNA sequences.
*: $P < 0.05$ as per Benjamini & Hochberg (1995) false discovery rate correction.

Table 5. Genetic distance of 11 *M. nipponense* populations.

	HS	YC	LJD	MDD	YSD	DJ	XJ	TJ	TH	YZ	TH-2
HS											
YC	0.014										
LJD	0.012	0.013									
MDD	0.017	0.019	0.016								
YSD	0.016	0.017	0.015	0.020							
DJ	0.015	0.016	0.013	0.019	0.016						
XJ	0.014	0.016	0.012	0.018	0.016	0.013					
TJ	0.015	0.016	0.012	0.019	0.016	0.012	0.011				
TH	0.014	0.016	0.013	0.018	0.017	0.016	0.015	0.016			
YZ	0.032	0.033	0.034	0.036	0.036	0.036	0.036	0.037	0.034		
TH-2	0.029	0.030	0.028	0.031	0.031	0.030	0.029	0.030	0.029	0.044	

populations). The results indicated that the genetic differentiation between YZ and other populations was statistically highly significant ($P < 0.05$). The TH population was significantly differentiated from all the other populations ($P < 0.05$) except for MDD ($P > 0.05$). TH-2 was significantly differentiated from the XJ, TJ, TH, and YZ populations ($P < 0.05$). Among the Yixing populations, TJ was genetically differentiated from HS, YC, LJD, MDD, and YSD ($P < 0.05$); XJ was genetically differentiated from HS, YC, LJD, and MDD ($P < 0.05$); and DJ was genetically differentiated from HS and YC ($P < 0.05$). No obvious differentiation was detected in the remaining pairwise combinations of populations ($P > 0.05$).

The genetic distance among the 11 populations ranged from 0.011 to 0.044 (Tab. 5). The largest value was observed between YZ and TH-2 (0.044), which was higher than the values among the other nine populations (all below 0.20). Moreover, the genetic distance between YZ and the other nine populations was above 0.032, and that between TH-2 and the other nine populations was between 0.028 and 0.031. The genetic distance between the TJ and XJ populations was the smallest (0.011). The clustering analysis results (Fig. 1) showed the eight Yixing populations were grouped with TH and TH-2 in a main branch, while the YZ population clustered separately into a single branch. The haplotype network obtained from the comparison of all sequences displayed no

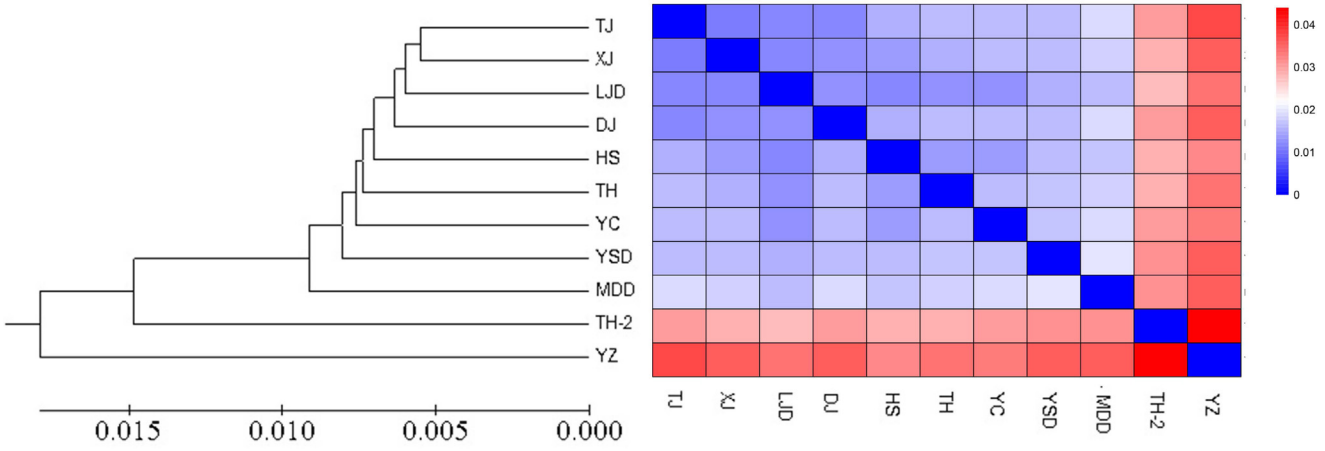

Fig. 1. The phylogenetic tree with the heatmap of 11 *M. nipponense* populations. Notes: left: the phylogenetic tree based on genetic distances; right: the genetic distance between each pair of populations is plotted in each cell. Colors are described in the palette on the right referred to distance values.

significant differences in haplotypes and geography among the *M. nipponense* populations (Fig. 2). Haplotypes did not cluster according to the classification of populations, which showed that individuals of different groups interlaced with each other to form complex clusters.

3.3 Population dynamics analysis

The neutrality tests showed that most Tajima's *D* values were negative except in HS, YC, and TH-2, while Fu's *Fs* values were negative in LJD, DJ, XJ, TJ, and YZ. Overall, the *P* values were relatively greater than 0.1 ($P > 0.1$), which indicated that the neutrality tests values were not significant (Tab. S3). The mismatch distribution analysis showed multiple peaks, suggesting that the population size remained relatively stable and there was a demographic equilibrium in all populations (Fig. 3).

4 Discussion

Within the context of the Yangtze River conservation and Taihu Lake fishing ban, the protection and appropriate use of the elite local germplasm resources are of great significance for the sustainable and efficient development of the prawn aquaculture industry. *M. nipponense* is an excellent local prawn species and its farming has become an important way to increase agricultural efficiency and the income of farmers in China (Fu et al., 2018). Therefore, it is very important to identify local populations with superior qualities and protect them to ensure the sustainable development of the *M. nipponense* seed industry, breeding, and aquaculture.

Some reports have been published on the genetic diversity of *M. nipponense* in China, mainly based on SSR and COI markers. SSR was employed in the genetic diversity analysis of populations in the Yellow River and Qiandao Lake, and showed that the wild populations had a high genetic diversity (Ma et al., 2012; Qiao et al., 2013). Mitochondrial DNA COI and 16SrRNA fragments were used in a study on the genetic structure of a wild population from Taiwan and showed that population expansion occurred recently (Chen et al., 2015, 2017). In the present study, the abundant variable sites and haplotypes detected suggested that the D-loop was an effective molecular marker to detect genetic differences in *M. nipponense* populations. The mean basic nucleotide configurations obtained indicated that the content of A+T was higher than that of C+G. This was similarly observed in other shrimp and crab species, and is consistent with the fact that commonly the GC combination is relatively scarce while AT content is high in the mtDNA base composition of arthropods (Beati et al., 2013).

High genetic diversity means high adaptive survival potential and high evolutionary potential, which are advantageous for conservation and the utilization of germplasm resources. The π and h values are good indicators of genetic variation in a population (Jiang et al., 2019). All the11 populations examined in this study showed $h > 0.7$ and $\pi > 0.009$ proving that they are highly genetically diverse. The TH-2, YZ, and XJ populations showed remarkable diversity, especially the latter. The diversity of reservoir populations was slightly lower than that of other populations, the h of YC and HS populations were the lowest (ranged from 0.7438 to 0.8519). All the results suggested that the germplasm resources and genetic diversity of *M. nipponense* in Yixing are of excellent quality and the species is suitable for breeding.

The pairwise F_{ST} value was considerably lower than the genetic differentiation index of the eight populations in China (Zhang et al., 2022). This might be due to frequent interaction between these populations given the relatively short geographical distance separating them. The genetic distance ranged from 0.011 to 0.044, indicating that the differences among the 11 populations were considerably smaller than those at the species level (<15%) for *Macrobrachium* spp. (Zhang et al., 2009). The YZ and TH-2 populations showed a high genetic differentiation from the other Yixing populations due to geographical isolation and parental origin differences. According to the distribution analysis of sampling sites, the brooks, lakes, and reservoirs of Yixing are connected with

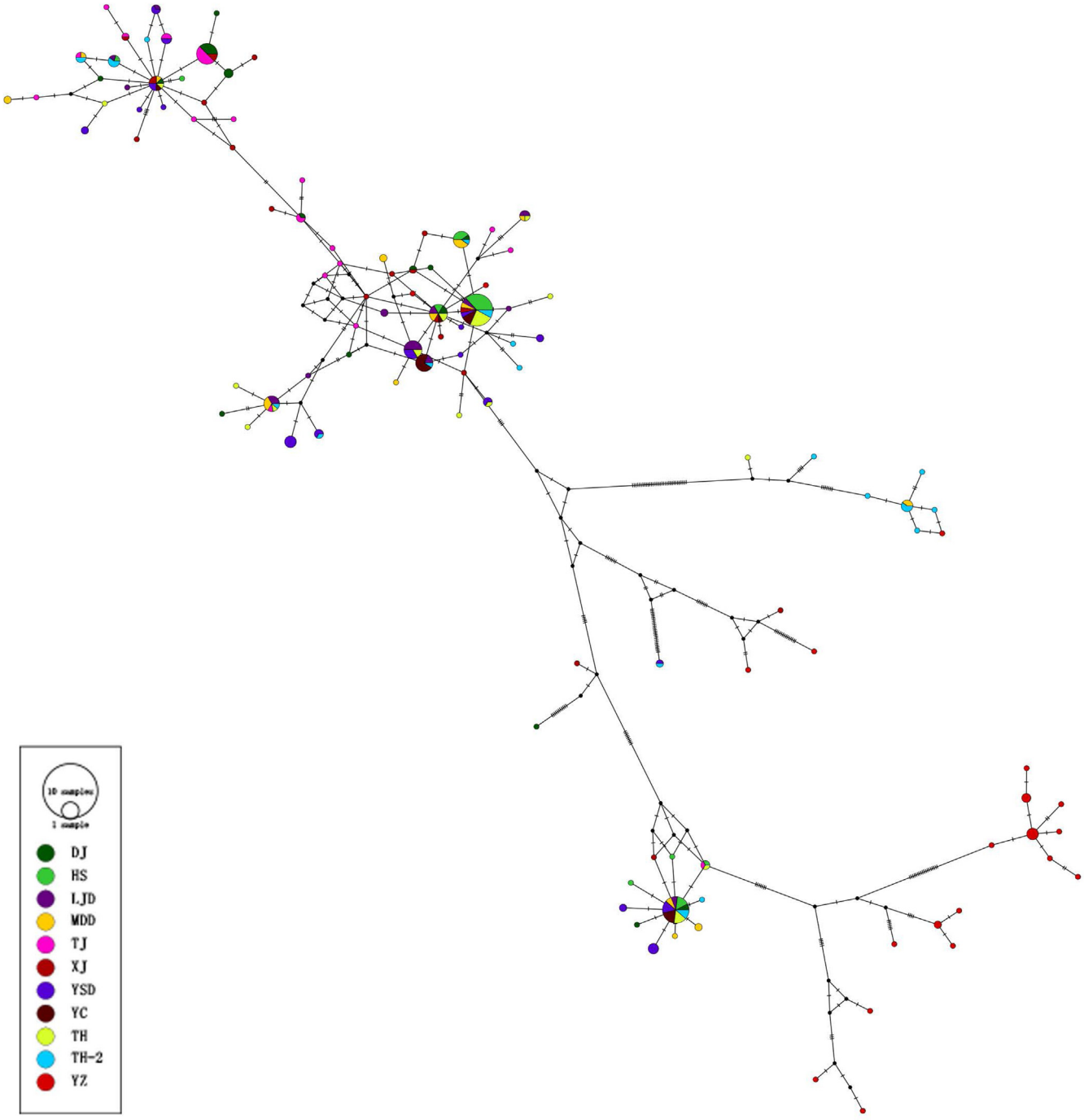

Fig. 2. The median-joining network based on haplotype frequencies of *M. nipponense*. The circle represents haplotype; circle size is proportional to the number of individuals in the haplotype. Each line in the network represents a single mutational change, and the branches are scaled to the number of polymorphic sites between each haplotype. Each black circle represents one missing haplotype.

Taihu lake by trenches, which results in frequent water exchange. The haplotype network results also revealed the same inconspicuous differences in populations.

Population genetics studies have suggested that the main causes of population differentiation are genetic drift and natural selection, and that the process is also influenced by population history dynamics (Chen et al., 2005). The neutrality and mismatch distribution tests have been used to measure the historical evolution of populations. The populations have undergone expansion in the past, as shown by the Tajima's *D* value deviating significantly from the neutral test and the nucleoside acid mismatch curve presenting a single-peak distribution (Fratini et al., 2005). In this study, the *P* values (Tajima's *D* and Fu *Fs*) were relatively greater than 0.1

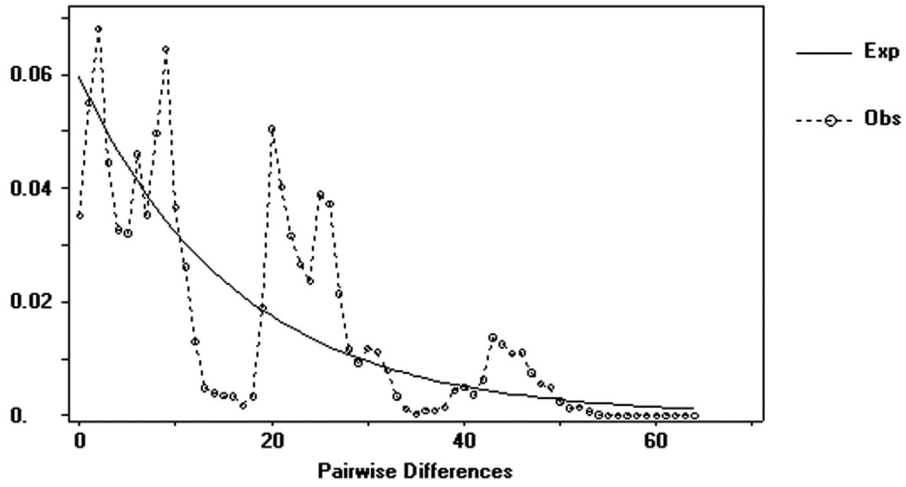

Fig. 3. Mismatch distribution for the 11 inferred populations of *M. nipponense.*

($P > 0.1$) and the nucleotide mismatch distribution analysis showed multiple peaks, indicating that the populations did not undergo expansion.

5 Conclusions

In this study, D-loop sequences were employed to examine the genetic diversity of eight wild *M. nipponense* populations in Yixing's natural waters, and their genetic differentiation and population structure were compared with those of wild populations of TH, YZ, and the main local strain TH-2. Our results showed that the TH-2 and YZ populations have a remarkable genetic diversity; the germplasm resources of *M. nipponense* in Yixing are in excellent condition and populations show a high genetic diversity, suggesting this species is suitable for breeding.

Competing interests

The authors declare no conflicts of interest.

Acknowledgments. This research was supported by grants from Central Public-interest Scientific Institution Basal Research Fund CAFS (2020TD36); the seed industry revitalization project of Jiangsu province (JBGS [2021] 118); Jiangsu Agricultural Industry Technology System; the National Key R&D Program of China (2018YFD0901303); earmarked fund for CARS (CARS-48); the New cultivar breeding Major Project of Jiangsu province (PZCZ201745). Thanks for the Jiangsu Province Platform for the Conservation and Utilization of Agricultural Germplasm.

Author contributions

Hongtuo Fu and Hui Qiao designed the research; Yiwei, Xiong, Sufei Jiang collect samples and performed the study; Lijuan Zhang and Wenyi Zhang analyzed the data;, Jisheng Wang, Shubo Jin, Yongsheng Gong and Yan Wu contributed reagents, materials and tools; Sufei Jiang and Hui Qiao drafted the manuscript; Hongtuo Fu revised the manuscript; all authors approved the final version.

Supplementary Material

Table S1. The nucleotide composition from 11 *M. nipponense* populations.
Table S2. Distribution of haplotypes of 11 *M. nipponense* populations.
Table S3. Neutrality tests for11 *M. nipponense* populations
Figure S1. A location map for the 11 inferred populations of *M. nipponense*.

The Supplementary Material is available at https://www.alr-journal.org/10.1051/alr/2023002/olm.

References

Beati L, Nava S, Burkman EJ, Barros-Battesti DM, Labruna MB, Guglielmone AA, Caceres AG, Guzman-Cornejo CM, Leon R, Durden LA, Faccini JL. 2013. *Amblyomma cajennense* (Fabricius, 1787) (Acari: Ixodidae), the Cayenne tick: phylogeography and evidence for allopatric speciation. *BMC Evolut Biol* 13: 1–20.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc: Ser B* 57: 289–300.

Chen PC, Shih CH, Chu TJ, Lee YC, Tzeng TD. 2017. Phylogeography and genetic structure of the oriental river prawn *Macrobrachium nipponense* (Crustacea: Decapoda: Palaemonidae) in East Asia. *PLOS ONE* 12.

Chen PC, Shih CH, Chu TJ, Wang D, Lee YC, Tzeng TD. 2015. Population structure and historical demography of the oriental river prawn (*Macrobrachium nipponense*) in Taiwan. *PLoS ONE* 10.

Chen SY, Su YH, Wu SF, Sha T, Zhang YP. 2005. Mitochondrial diversity and phylogeographic structure of Chinese domestic goats. *Mol Phylogenetics Evol* 37: 804–814.

Excoffier L, Lischer HE. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour* 10: 564–567.

Fratini S, Zaccara S, Barbaresi S, Grandjean F, Souty-Grosset C, Crosa G, Gherardi F. 2005. Phylogeography of the threatened crayfish (genus Austropotamobius) in Italy: implications for its taxonomy and conservation. *Heredity* 94: 108–118.

Fu HT, Gong YS, Wu Y, Xu P, Wu CJ. 2004. Artificial interspecific hybridization between Macrobrachium species. *Aquaculture* 232: 215–223.

Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* 147: 915–925.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nuclc Acids Symp Ser* 41: 95–98.

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16: 111–120.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33: 1870–1874.

Leigh JW, Bryant D. 2015. POPART: full-feature software for haplotype network construction. *Methods Ecol Evol* 6: 1110–1116.

Liao Y, Mo G, Sun J, Wei F, Liao DJ. 2016. Genetic diversity of Guangxi chicken breeds assessed with microsatellites and the mitochondrial DNA D-loop region. *Mol Biol Rep* 43: 415–425.

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25: 1451–1452.

Ma KY, Feng JB, Li JL. 2012. Genetic variation based on microsatellite analysis of the oriental river prawn, *Macrobrachium nipponense* from Qiandao Lake in China. *Genet Mol Res* 11: 4235–4244.

Maltsev AN, Stakheev VV, Bogdanov AS, Fomina ES, Kotenkova EV. 2015. Phylogenetic relationships of intraspecific forms of the house mouse *Mus musculus*: analysis of variability of the control region (D-loop) of mitochondrial DNA. *Doklady Biol Sci* 465: 285–288.

Qiao H, Lv D, Jiang SF, Sun SM Gong YS, Xiong YW, Jin SB, Fu HT. 2013. Genetic diversity analysis of oriental river prawn, *Macrobrachium nipponense*, in Yellow River using microsatellite marker. *Genet Mol Res* 12: 5694–5703.

Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123: 585–595.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEG A5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28: 2731–2739.

Zhang QY, Cheng QQ, Guan WB. 2009. Mitochondrial COI gene sequence variation and taxonomic status of three *Macrobrachium* species. *Zool Res* 30: 613–619.

Zhang WY, Jiang SF, Salumy KR, Xuan ZY, Xiong YW, Jin SB, Gong YS, Wu Y, Fu HT. 2022. Comparison of genetic diversity and population structure of eight *Macrobrachium nipponense* populations in China based on D-loop sequences. *Aquac Rep* 23: 101086.

Cite this article as: Xiong Y, Jiang S, Zhang L, Wang J, Zhang W, Jin S, Gong Y, Wu Y, Qiao H, Fu H. 2023. Genetic diversity and structure assessment of *Macrobrachium nipponense* populations: implications for the protection and management of genetic resources. *Aquat. Living Resour.* 36: 5