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Genetic structure of *Macrobrachium nipponense*, an important farmed freshwater shrimp in China, in the Three Gorges Reservoir

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Abstract – *Macrobrachium nipponense* is a major farmed species freshwater shrimp in China and its genetic diversity is of high value in aquaculture. The construction of the Three Gorges Project has slowed down the water velocity in the Three Gorges Reservoir (TGR) and made the water more clear, which is more favourable for the survival and reproduction of *M. nipponense*. However, there are lack of studies on the genetic diversity of *M. nipponense* populations in the TGR. In this study, mitochondrial COI gene sequences were used as molecular marker to analyze the genetic diversity, genetic differentiation and historical demography of eight *M. nipponense* populations in the TGR. The results showed that the *M. nipponense* populations in TGR have high genetic diversity. There was some genetic differentiation between the four populations in lower reaches of the TGR and four populations in the upper reaches of the TGR. We also found that there was a significant correlation between genetic distance and geographical distance among the *M. nipponense* populations in the TGR. The *M. nipponense* population in the TGR has experienced population reduction in the recent year, which might be related to the ice age movement in the Little Ice Age and human activities. This study provides a theoretical basis for the rational development and protection of *M. nipponense* resources in the TGR.

Keywords: Three Gorges Reservoir / *Macrobrachium nipponense* / genetic diversity / population genetic structure

1 Introduction

Shrimp and crab are important aquaculture species in China, whose annual yield is second only to fish (Bureau of Fishery, Ministry of Agriculture and Affairs of the P.R.C, 2020). *M. nipponense* belongs to *Macrobrachium* in Palaemonidae, which is one of the most widely cultured freshwater shrimps in China.In 2019, the cultured yield of *M. nipponense* in China was up to 225,300 tons, second only to that of *Litopenaeus Vannamei* and *Procambarus clarkii* (Bureau of Fishery, Ministry of Agriculture and Affairs of the P.R.C, 2020). Improved variety play an important role in aquaculture (Sae-Lim et al., 2017). Continuous cultivation of new variety is an important guarantee for the healthy and sustainable development of aquaculture (Janssen et al., 2017). Wild germplasm resources carrying genes of potential value in improving yield, quality, and adaptation to the environment provide a wide range of raw materials for improved farmed varieties (Pathirana and Carimi, 2022). Therefore, protecting wild germplasm resources plays an important role in aquaculture (Hill et al., 2021; Janssen et al., 2018; Wang et al., 2021).

A comprehensive survey of the genetic diversity of *M. nipponense* can provide guidance for better utilization of germplasm resources for genetic improvement (Xiong et al., 2023). As an important farmed shrimp in China, there have been a large number of studies on the genetic diversity of *M. nipponense* populations in major producing areas or important water areas. For example, Feng et al. (2008) used mitochondrial COI gene sequence as a molecular marker study the genetic diversity of *M. nipponense* populations in the five freshwater lakes of China (i.e. Poyang Lake, Dongting Lake, Taihu Lake, Chaohu Lake, and Hongze Lake), they found that each populations has good genetic diversity, and there was a large genetic differentiation among each populations. And

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Feng et al. (2021) also used mitochondrial COI gene sequence as a molecular marker to investigate the genetic diversity of *M. nipponense* from four water systems in Henan Province, China. High haplotype diversity and low nucleotide diversity were observed, and there was no significant genetic differentiation for *M. nipponense* from the four water systems. The demographic history analysis showed that *M. nipponense* of Henan province did not experience recent population expansion. There are also many studies using microsatellite markers (SSR), for example, Ma et al. (2012) used nine polymorphic microsatellite markers to analyze the genetic diversity and structure of four *M. nipponense* populations in Qiandao Lake, China, and found the four populations displayed high genetic diversity. Mutation-drift equilibrium analysis showed no significant bottleneck effect. A moderate level of differentiation among these populations. However, most studies on the genetic diversity of *M. nipponense* were conducted in natural water areas, and few studies were conducted in areas that has been subjected to strong transformations by human activities, such as reservoirs.

The Yangtze River is the longest river in Asia and has abundant hydroenergy. The Three Gorges Dam was built in Sandouping, Yichang, in 1993 in order to solve the flood problem in the middle reaches of the Yangtze River and develop the hydroenergy resources of the Yangtze River (New and Xie, 2008). The Three Gorges Dam is 181 meters high. After the construction of the Three Gorges Dam, a 663 km long reservoir has been formed from Zigui to Jiangjin in the Yangtze River, with a water area of about 1,084 km^2 (Xiang et al., 2021). After the impounding of the TGR, water flow velocity slowed down obviously, water surface area increased, providing more habitat for *M. nipponense* (Xiang et al., 2021b; Yang et al., 2018). At the same time, the slower flow rate of water makes sediment deposition, water clearing and algae productivity increased, which providing more food sources for *M. nipponense* and was conducive to the proliferation of *M. nipponense* (Long et al., 2011; Mirzajani et al., 2020; Xu et al., 2011). As we thought the change of hydrological environment had increased the population number of *M. nipponense* in the TGR (Yang et al., 2018). A comprehensive understanding of the genetic structure of *M. nipponense* populations in the TGR is conducive to the conservation and rational development of *M. nipponense* in the TGR (Alal et al., 2021).

At present, there are a few studies on *M. nipponense* in the TGR. Chen Sibao et al. (2020) studied the reproductive biology of *M. nipponense* population in the TGR and found that the spawning time of *M. nipponense* in the TGR was from late April to early October (Chen et al., 2020). In terms of genetic diversity, only Fu et al. (2010) used microsatellite (SSR) markers to study the genetic diversity of *M. nipponense* populations in the Yangtze River Basin in 2007, and designed two sample sites (Wanzhou and Chongqing) in the TGR. Fu et al. (2010) found that the genetic diversity of Wanzhou and Chongqing populations were at high level, and the degree of genetic differentiation between Chongqing and Wanzhou populations was low. But there are only two sample sites involved in Fu et al. (2010), which failed to fully reveal the genetic diversity pattern of *M. nipponense* populations in the TGR.

Due to the advantages of strict maternal inheritance and high mutation rate, mitochondrial DNA have been widely used in population genetics research. Therefore, in this study, partial sequences of mitochondrial cytochrome oxidase subunit I (COI) genes were used as molecular markers to analyze the genetic diversity, inter-population genetic differentiation, and historical demography of eight *M. nipponense* populations in the head, middle and tail of the TGR. In order to comprehensively understand the genetic diversity and genetic structure of *M. nipponense* populations in the TGR, and to provide theoretical basis for the protection and rational development of wild germplasm resources of *M. nipponense* populations in the TGR.

2 Materials and methods

2.1 Sample collection

M. nipponense samples were collected from August 2020 to June 2022 at eight sites in the TGR of the Yangtze River, including Gudongkou (GDK), Xiakou (XK), Shuitianba (STB), Wushan (WS), Wanzhou (WZ), Fengdu (FD), Mudong (MD) and Jiangjin (JJ). GDK, XK and STB are sample points in the tributary of the head area of the reservoir; WS is the sample point in the main stream of the head area of the reservoir; WZ and FD are the sample points in the main stream of the middle area of the reservoir; MD and JJ are sample points in the tributary of the tail area of the reservoir (Fig. 1 and Tab. 1). The experimental samples were collected using cage net, and species identification was carried out with reference to *Fauna Sinica* (Li et al., 2007). A total of 229 *M. nipponense* samples were collected from the eight sampling sites in the TGR. A small amount of abdominal muscle was cutted from *M. nipponense* and stored in 95% alcohol at −20°C in School of Life Sciences, Jianghan University.

2.2 DNA extraction, amplification and sequencing

Total DNA was extracted using the Animal Tissues DNA Isolation Kit (Forge Biotechnology Co., Ltd, Chengdu China). The amplification primers for the mitochondrial gene COI fragment sequence were the LCO1490 5'-GGT CAA ATC ATA AAG ATA TTG G-3' and HCO2198 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3' (Cui et al., 2018). The total reaction volume for the polymerase chain reaction (PCR) was 30 μL, containing PrimeSTAR Max Premix (2×) 15 μL (containing dNTPs, $MgCl_2$, Taq DNA polymerase, and reaction buffer; Takara Bio., Ltd), 2.5 μL each of forward and reverse primers (10 mmol/L), 3 μL template DNA, and 7 μL ddH_2O. The PCR amplification procedure was as follows: pre-denaturation at 97 °C for 3 min; followed by 35 cycles of denaturation at 98 °C for 15 s, annealing at 46 °C for 15 s, extension at 72 °C for 20 s; final extension at 72 °C for 5 min and storage at 4 °C. After the PCR products were subjected to agarose gel (1%) electrophoresis, the samples with accurate and clear target bands were sent to Wuhan Tianyi-Huayu Gen Sci Tech Co., Ltd. for purification, recovery, and sequence determination.

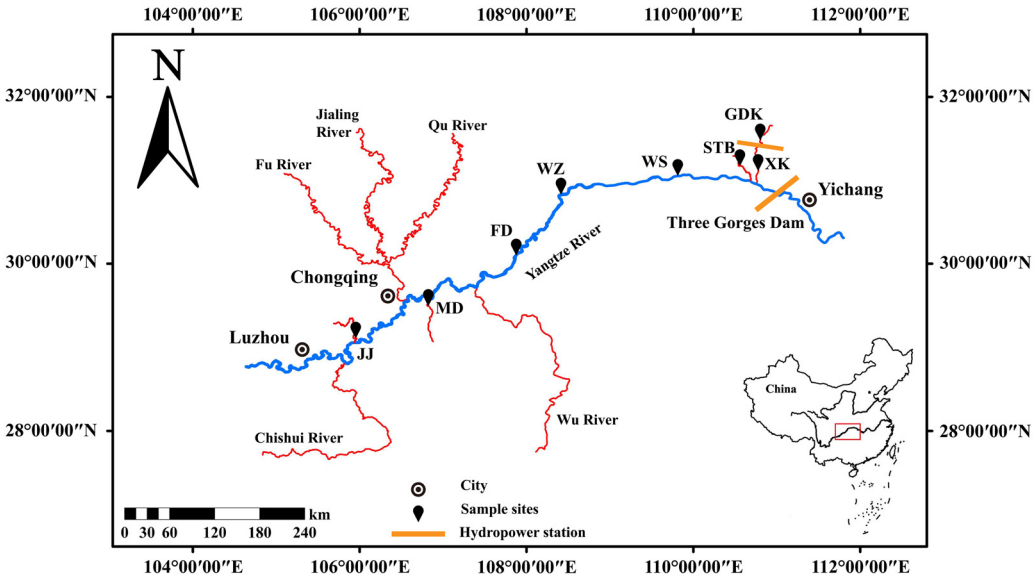

Fig. 1. Sampling sites of *M. nipponense* in the TGR.

Table 1. Sampling sites and number of individuals of *M. nipponense* in the TGR.

Population code	Tissue samples collected	Number of samples sequenced	Locality	Sample site
GDK	30	29	110°44′58.64″E, 31°22′11.38″N.	Xiangxi River, Gudongong Village, Yichang
XK	30	30	110°46′09.78″E, 31°09′48.81″N.	Xiangxi River, Xiakou Town, Yichang City
STB	30	29	110°40′55.89″E, 31°03′58.98″N.	Yuanshui River, Shuitianba Town, Yichang
WS	30	27	109°53′03.91″E, 31°04′01.38″N.	Yangtze River, Wushan county, Chongqing
WZ	30	28	108°23′37.25″E, 30°48′57.81″N.	Yangtze River, Wanzhou District, Chongqing
FD	30	28	107°46′01.59″E, 29°55′44.33″N.	Yangtze River, Fengdu County, Chongqing
MD	30	29	106°50′17.03″E, 29°33′57.11″N.	Mudong River, Mudong Town, Chongqing
JJ	30	29	105°56′20.73″E, 29°03′43.46″N.	Zhuyangxi River, Jiangjin District, Chongqing
Total	240	229		Three Gorges Reservoir Hubei and Chongqing, China

2.3 Sequence analysis

The raw sequences were checked and edited with reference to the .ab1 chromatogram files, and the sequences were aligned using MEGA 7 software (Kumar et al., 2016). The population genetic diversity indices calculated included the number of segregating sites (s), haplotype number (h), haplotype diversity (H_d), and nucleotide diversity (P_i) were calculated using DnaSP 5.10 software (Librado and Rozas, 2009).

Analysis of molecular variance (AMOVA) was performed on the *M. nipponense* population in the TGR to quantify the sources of genetic variation in Arlequin 3.0 software, 229 *M. nipponense* were divided into eight groups according to geographical location (Excoffier et al., 2005). Pairwise genetic differentiation index (F_{ST} values) among 8 *M. nipponense* populations with the significance level examined by 1000 permutations were calculated in Arlequin 3.0 (Excoffier et al., 2005). STRUCTURE analysis was applied to infer the number of genetically differentiated clusters (K) using STRUCTURE v2.3.4 (Pritchard et al., 2000). The parameters were set as follows: a burn-in period of 100,000 iterations followed by 500,000 recorded iterations for $K = 1$ to $K = 8$ clusters and 15 iterations per K values (Pritchard et al., 2000). The most probable number of clusters present in this dataset was determined using the Evanno's ΔK approach using Structure Harvester online (Earl and Vonholdt, 2012; Evanno et al., 2005) (available at http://taylor0.biology.ucla.edu/structureHarvester/).

Table 2. Genetic diversity of eight *M. nipponense* populations in TGR.

Population	Number of individuals	Number of polymorphic sites	Number of haplotypes	Haplotype diversity	Nucleotide diversity
GDK	29	22	8	0.525 ± 0.110	0.00932 ± 0.00221
XK	30	33	12	0.745 ± 0.082	0.01260 ± 0.00231
STB	29	30	10	0.687 ± 0.091	0.00995 ± 0.00238
WS	27	35	13	0.801 ± 0.077	0.01411 ± 0.00191
WZ	28	38	16	0.894 ± 0.050	0.01790 ± 0.00100
FD	28	39	14	0.926 ± 0.029	0.01829 ± 0.00108
MD	29	35	12	0.862 ± 0.052	0.01770 ± 0.00104
JJ	29	35	12	0.857 ± 0.051	0.01594 ± 0.00133
Total	229	51	46	0.801 ± 0.026	0.01540 ± 0.00060

Additionally, the genetic distance based on Kimura 2-parameter (K_2P) model was calculated using MEGA 7 software to construct a genetic distance matrix between *M. nipponense* populations (Kumar et al., 2016). The distance along the river between each sampling point was measured using the LocaSpace Viewer 4.33 to construct a geographic distance matrix between *M. nipponense* populations in the TGR. The Mantel test was performed using the "vegan" package in R software in order to identify the relationship between the genetic distance matrix and geographical distance matrix of *M. nipponense* populations in the TGR.

To detect whether there was distinct lineage differentiation among haplotypes or populations, a phylogenetic tree of haplotypes was reconstructed using the Bayesian (BI) approaches based on Hasegawa-Kishino-Yano (HKY) model plus gamma distribution rate (+G) plus evolutionarily invariable (+I) model (The best-fit model was calculated using the Bayesian Information Criterion (BIC) in jModeltest 2.1.7) in MrBayes 3.2.7 (Darriba et al., 2012; Ronquist et al., 2012), with *Macrobrachium tratense* (MW845636), *Macrobrachium villosimanus* (MW845638) and *Macrobrachium yui* (MW845641) as the outgroup. Four chains (three hot, one cold) were run for 10^8 generations, the last 75% generations were used do phylogenetic analyses. A haplotype network was constructed using Median-joining in Popart 1.7 software (Leigh and Bryant, 2015).

Mismatch distribution analyses using DnaSP 5.10 software and neutrality tests (Tajima's *D* test and Fu' *FS* test) using Arlequin 3.0 software were conducted to test for historical population expansion of the *M. nipponense* populations in the TGR (Excoffier et al., 2005; Librado and Rozas, 2009). The Bayesian skyline plot (BSP) was constructed using BEAST v2.7.0 software to estimate the historical demography of the *M. nipponense* population in the TGR (Suchard et al., 2018). The best-fit model for each population was calculated using the BIC in jModeltest 2.1.7 software (Darriba et al., 2012). Referring to the study by Feng et al. (2021), the base evolution rate of the COI gene use 1.4% per million years, which was estimated based on the divergence of sister species of the Palaemonidae caused by the Isthmus of Panama (Knowlton and Weigt 1998). And using a strict molecular clock model with a Markov chain Monte Carlo (MCMC) length of 1.5×10^9 generations, and finally, Tracer 1.5 software was used to detect the results of an effective sample size greater than 200 (ESS > 200), which were output as Bayesian skyline plots (Rambaut and Drummond, 2007).

3 Results

3.1 Sequence characterization and genetic diversity

A total of 229 mitochondrial COI gene sequences of *M. nipponense* from the TGR were obtained in this study, and the clearly sequenced 635-bp fragments were selected for analysis. There were no base insertions or deletions were observed. There were 51 variable sites in the 229 sequences, accounting for 8.03% of the total number of sites, including 10 singleton variable sites, and 41 parsimony informative sites. The mean total nucleotide composition of 229 sequences was A = 29.8%, T = 29.3%, G = 18.4%, C = 22.5%, that showed an AT bias, which was consistent with the base composition of other crustaceans mitochondrial genome (Wei, 2022).

A total of 46 haplotypes (GenBank accession numbers: OQ978506-OQ978551) were identified in this study, and each sampled population has 8–16 haplotypes. The overall haplotype diversity was 0.801, with the lowest haplotype diversity of 0.525 found in the GDK population and the highest haplotype diversity of 0.926 found in the FD population. The overall nucleotide diversity was 0.01540, with the lowest nucleotide diversity of 0.00932 found in the GDK population and the highest nucleotide diversity of 0.01829 found in the FD population (Tab. 2).

3.2 Population structure

Results of AMOVA demonstrated that genetic variations mainly originated from within populations (92.96%), while variation among populations was relatively low (7.04%) (Tab. 3). The fixation index F_{st} was 0.07 ($0.01 < P < 0.05$), indicating that there might be a slight degree of genetic differentiation among the eight *M nipponensis* populations in the TGR.

The pairwise F_{ST} values ranged from 0.0667 to 0.2529, among which the F_{ST} values between the GDK and FD populations were the largest, and the F_{ST} values between FD and JJ populations were the smallest (Sewall 1978) (Fig. 2). The F_{ST} values between populations showed that the four populations in the lower reaches of the TGR (GDK, XK, STB and WS) has widespread genetic differentiation among the four populations in the upper reaches of the TGR (WZ, FD, MD and JJ), and this suggests that the F_{ST} values may be related to the geographical distance between *M. nipponense* populations.

Table 3. AMOVA of eight *M. nipponense* populations based on mitochondrial COI gene.

Source of variation	d.f.	Sum of squares	Variance component	Percentage of variation	F_{st} and P value of F_{st}
Among groups	7	101.638	0.3472Va	7.04	0.07040(0.00000)
Among populations within groups	221	1013.029	4.5839Vb	92.96	
Total	228	1114.677			

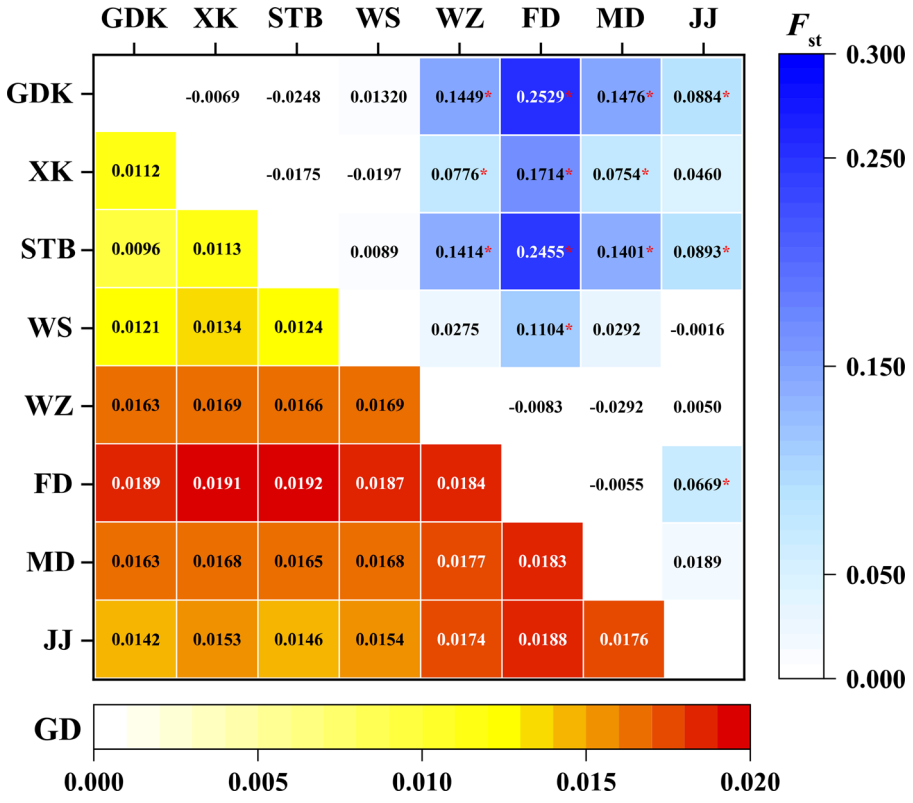

Fig. 2. Pairwise F_{st} values (above the diagonal) and genetic distance (GD, below the diagonal) based on mitochondrial COI gene among eight *M. nipponense* populations in the TGR. All results were tested for significance 1000 times, and the value marked by a red asterisk (*) represents a passing significance test.

The results of STRUCTURE analysis of 229 *M. nipponense* in the TGR showed that ΔK reached a maximum value when $K = 6$. Figure 3 shows the distribution of genetic clusters of each *M. nipponense* population in the TGR when $K = 2$–8. No matter what the value of K is, we can clearly see that the proportion of genetic clusters between the four populations in lower reaches of the TGR and the four populations in the upper reaches of the TGR is different, but the number of genetic clusters in each population is the same. This is consistent with the results of pairwise F_{ST} values.

The genetic distance (GD) among the eight populations ranged from 0.96% to 1.92%, with the biggest genetic distances was observed between FD and XK (Fig. 2) and the result of the Mantel test was 0.6078 with P value of 0.014. This indicates that there is a significantly correlation between genetic distance and geographical distance of *M. nipponense* populations in the TGR.

3.3 Haplotype phylogenetic relationship

The haplotype BI phylogenetic tree of *M. nipponense* in the TGR did not show obvious clustering trend with high support rate, and the private haplotypes of each population did not cluster according to geographical distribution (Fig. 4).

The haplotype network of *M. nipponense* was roughly overlaps with the BI phylogenetic tree, and the distribution of haplotypes did not show obvious phylogeographic pattern. The haplotypes were closely related to each other, and the mutation steps between all haplotypes are very few (generally only 1–2 step). The network shows that Hap4, Hap10 and Hap17 are abundant and located at the base of the BI phylogenetic tree. It is implied that these haplotypes may be ancestral haplotypes of *M. nipponense* population in the TGR (Fig. 5).

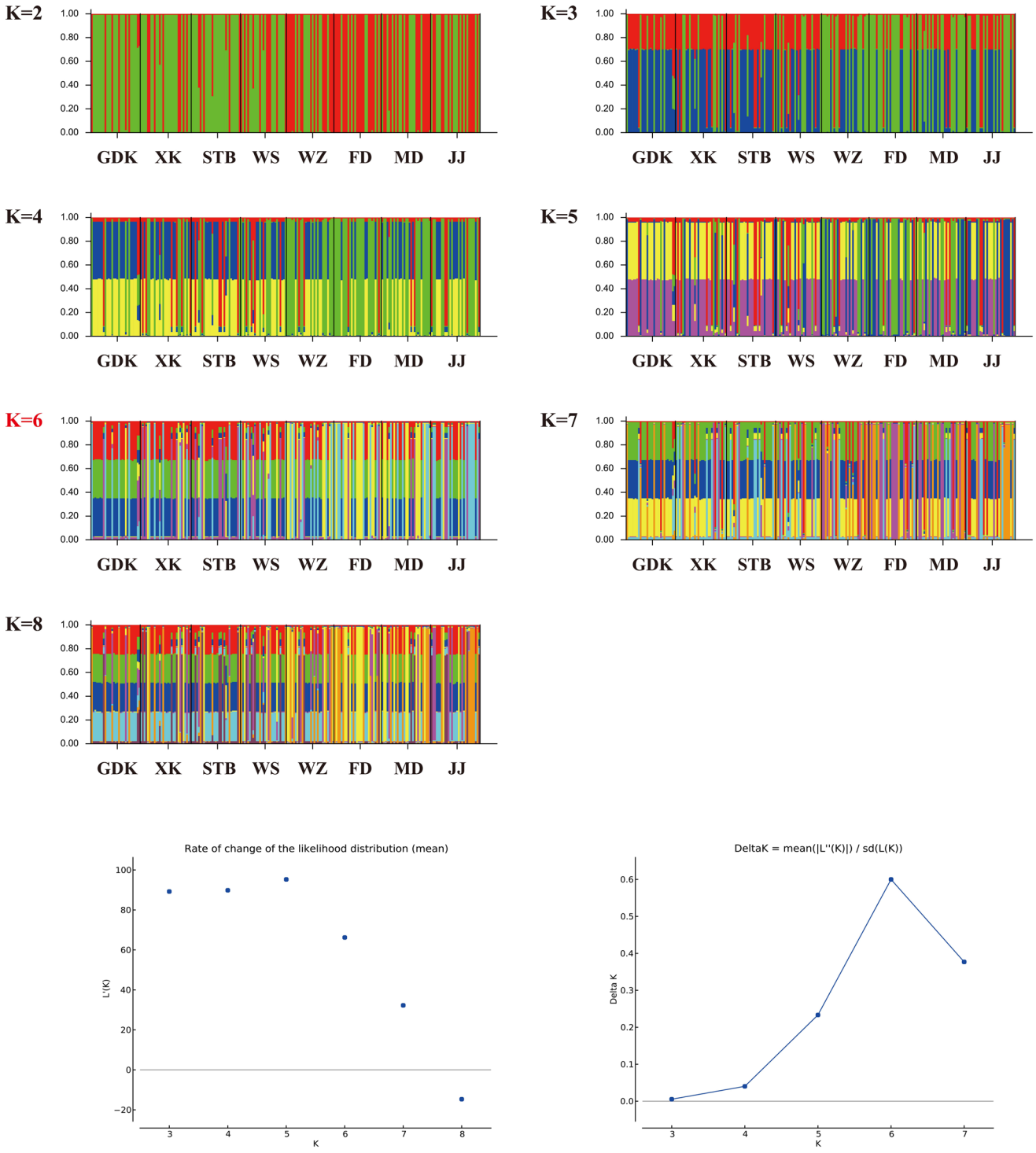

Fig. 3. STRUCTURE analysis of eight *M. nipponense* populations in the TGR based on COI gene.

3.4 Historical demography

The *P* values of Tajima's *D*-test and Fu's *FS*-test for the eight *M. nipponense* populations in the TGR were not significantly ($P < 0.05$), indicating that the variation in all *M. nipponense* populations did not significantly deviate from neutral mutation (Tab. 4). The mismatch distribution analyse plots for each *M. nipponense* population and for the total population were all significantly multipeaked, again suggesting that no population expansion occurred historically in the *M. nipponense* population from the TGR (Fig. 6). The Bayesian skyline plot indicated that all populations have experienced population contraction in the recent years. Effective population size of all *M. nipponense* populations

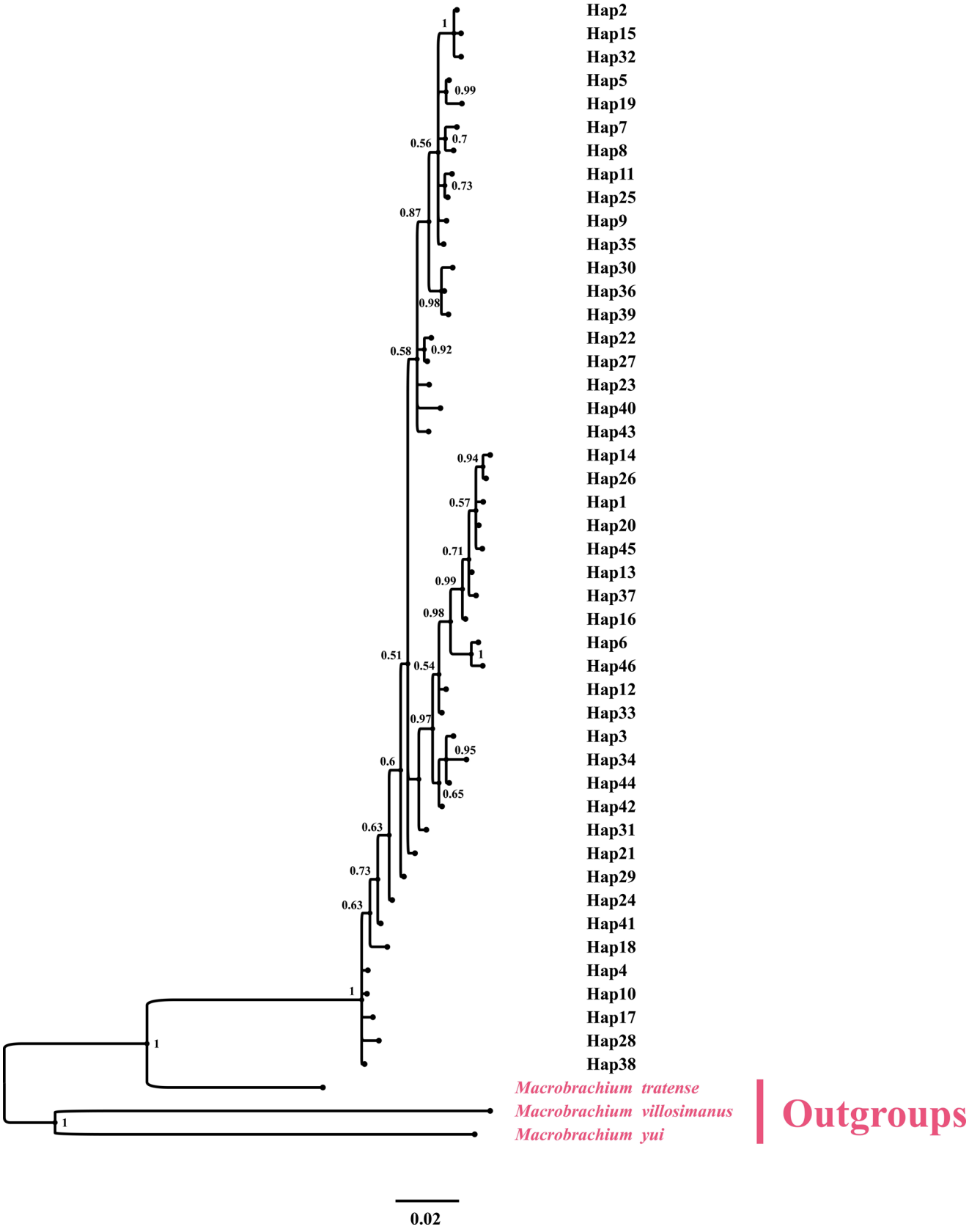

Fig. 4. BI phylogenetic tree of *M. nipponense* haplotypes constructed based on COI gene.

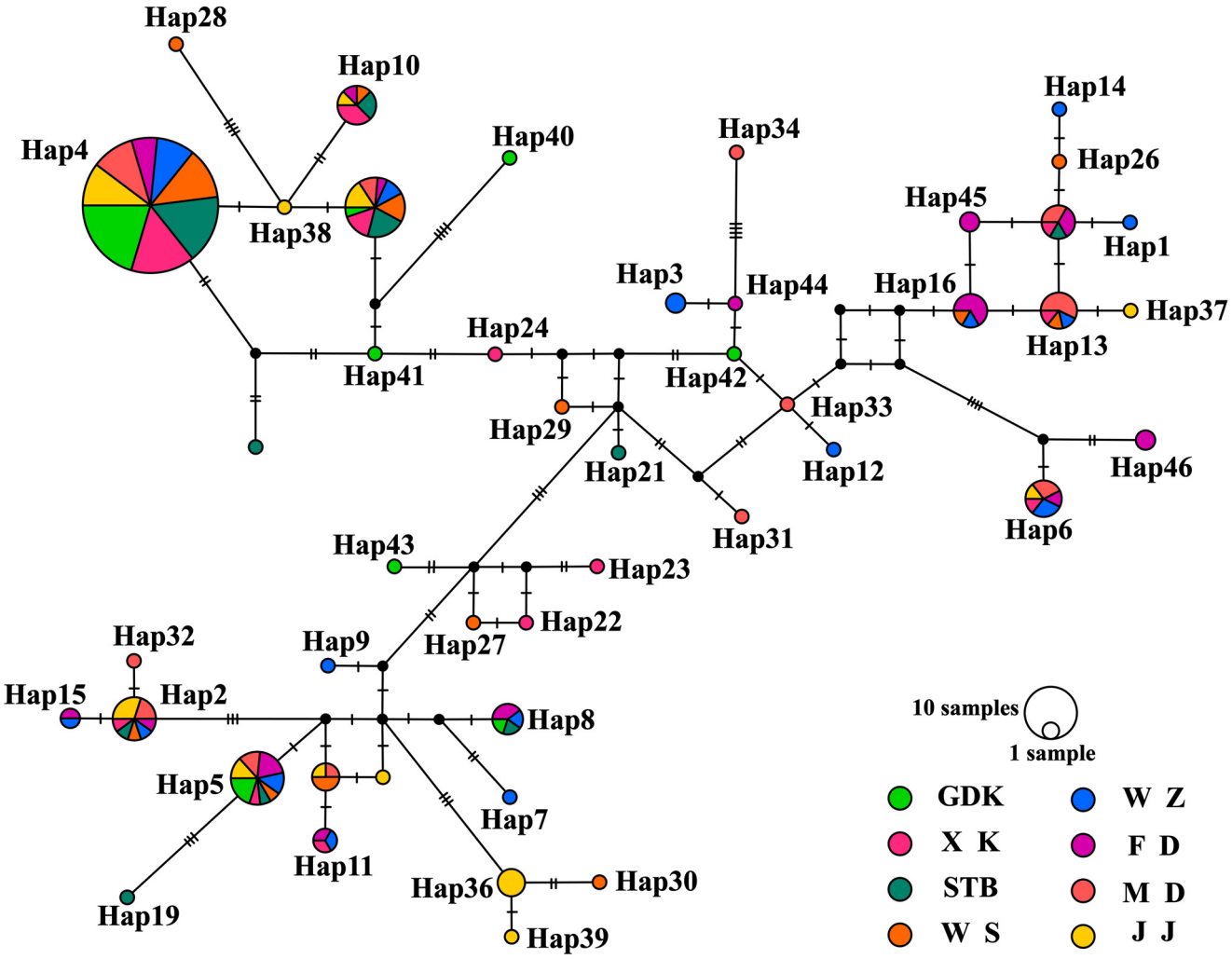

Fig. 5. The network diagram of *M. nipponense* haplotypes constructed based on COI gene. Each circle represents a haplotype, and the size of the circle is proportional to the number of individuals in the population.

Table 4. Neutrality tests for *M. nipponense* population based on mitochondrial COI gene.

Population	GDK	XK	STB	WS	WZ	FD	MD	JJ	Total
number of sequences	29	30	29	27	28	28	29	29	229
Tajima's *D*	0.202	–0.144	–0.630	–0.051	0.610	0.591	0.958	0.499	0.447
P value	0.615	0.483	0.285	0.550	0.810	0.781	0.868	0.765	0.742
Fu' *FS*	2.632	0.887	1.231	0.212	–0.676	0.731	2.234	1.746	–6.008
P value	0.869	0.684	0.740	0.575	0.399	0.665	0.806	0.776	0.145

in GTR began to decrease by about 700 years ago, and it was reducing faster and faster, and before that, the effective population size of all populations was largely stable (Fig. 7).

4 Discussion

4.1 Genetic diversity and historical demography

In broad terms, germplasm is the diversity of a farmed species and its wild relatives that can hybridise and produce fertile progeny (Pathirana and Carimi, 2022). And a comprehensive survey of genetic diversity can provide guidance for better utilization of germplasm resources for genetic improvement (Fu, 2015). Haplotype diversity and nucleotide diversity are important indicators that are often used to evaluate biological genetic diversity. The haplotype diversity of the *M. nipponense* population in the TGR was 0.801, and the nucleotide diversity was 0.01540, indicating that the genetic diversity is high (Grant and Bowen, 1998). The genetic diversity of the *M. nipponense* population in the TGR was not only higher than that of the *M. nipponense* population

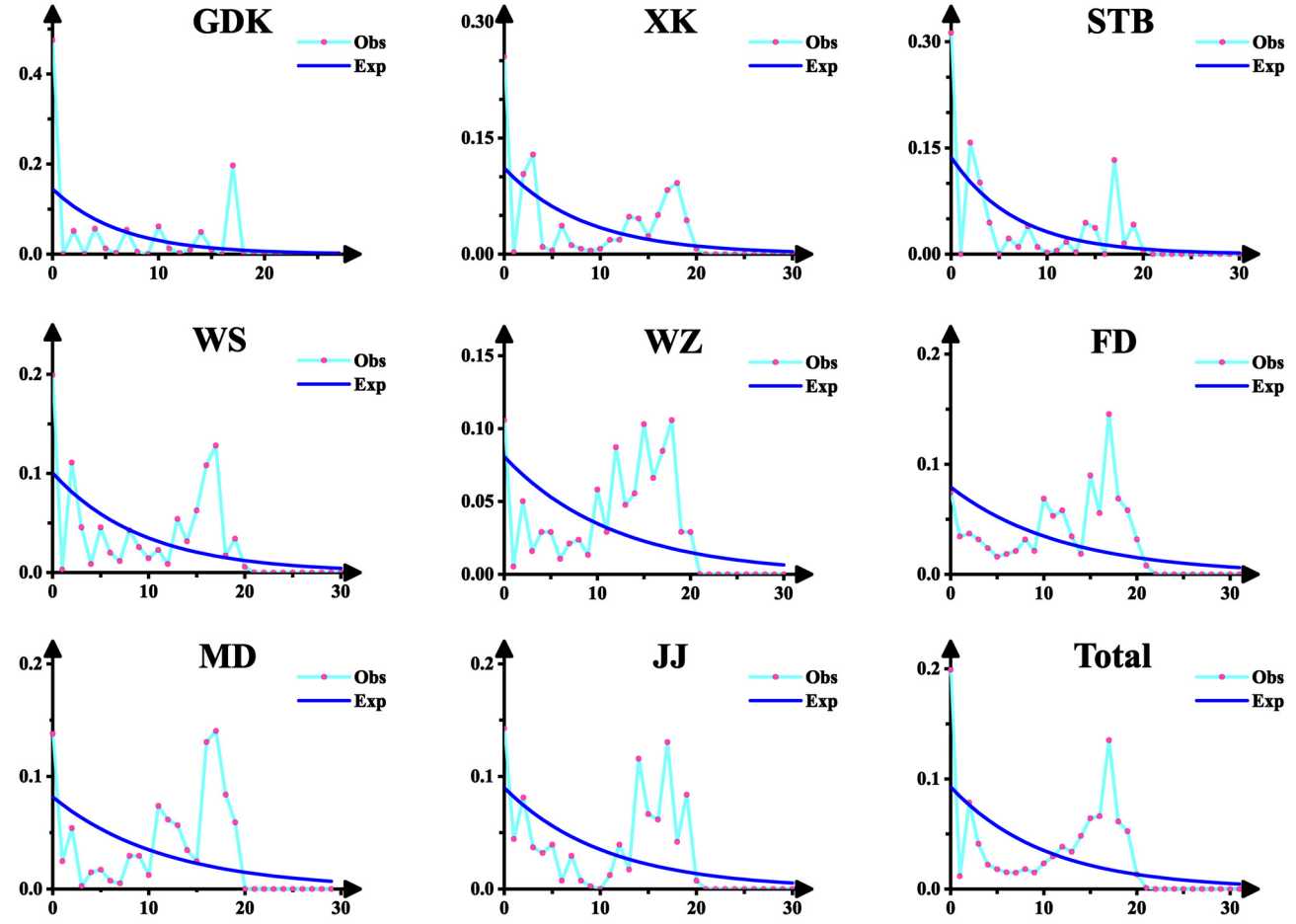

Fig. 6. Mismatch distribution analyse for each *M. nipponense* population from different localities.

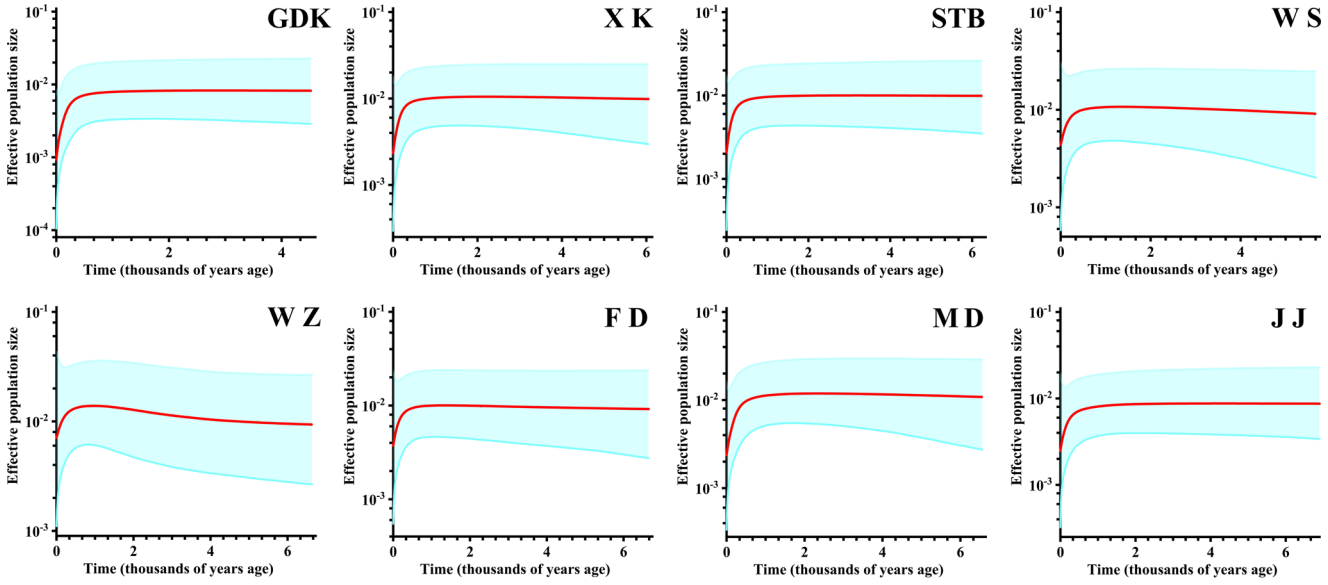

Fig. 7. Bayesian skyline plot of the effective population sizes through time for *M. nipponense* population. The X axis is the time scale in units of thousands of years, and the Y axis is the estimated effective population size.

in the Henan Province ($H_d = 0.78574$, $P_i = 0.01059$) but also higher than that of the *M. nipponense* populations in freshwater lakes in China such as Dongting Lake ($H_d = 0.716$, $P_i = 0.01151$), Chaohu Lake ($H_d = 0.795$, $P_i = 0.00494$) and so on (Feng et al., 2008, 2021). The high genetic diversity of *M. nipponense* suggesting that there may be a large number of germplasm resources with excellent quality in the TGR, especially the WZ and FD populations, which are suitable for breeding work.

Grant and Bowen (1998) showed that if a population has high genetic diversity ($H_d > 0.5$, $P_i > 0.005$), then it may be due to a long evolutionary history in a large stable population. The haplotype network of *M. nipponense* in the TGR does not show a simple star-shaped structure. The neutrality tests and mismatch distribution analyses showed that the population size of *M. nipponense* in the TGR may remained stable in history, indicating that *M. nipponense* in the TGR may not have experienced severe bottleneck effect or population expansion (Wang et al., 2023). A large number of genetic mutations had been accumulated in *M. nipponense* population in the TGR, resulting in high genetic diversity in the long evolutionary history (Chen et al., 2017; Chiang and Schaal, 1999; Wang et al., 2000). Secondly, larger population size also favored the maintenance of high genetic diversity in *M. nipponense* population. Genetic drift can reduce population genetic diversity, and this effect is more significant in small populations. *M. nipponense* is the dominant species of Crustacea in the TGR with a large population, and little influence from genetic drift. The large population size of *M. nipponense* in the TGR is conducive to keep its high genetic diversity.

Bayesian skyline plot indicated that *M. nipponense* populations in the TGR began to shrink 700 years ago, which may be related to the Little Ice Age that began in the 13th century and ended in the early 20th century (Zhu, 1973). The Earth's climate alternates between ice ages and warm periods, with aquatic organisms generally experiencing population contraction or bottlenecking during colder ice ages and population expansion during warmer interglacial periods (Hewitt, 2000, 1996; Li 2015). The Little Ice Age is the nearest ice age, and the average temperature during it was approximately 1–2 °C lower than present temperature, even in some places the minimum temperature may be 5–7 °C lower than the modern, which may have contributed to the contraction of *M. nipponense* populations in the TGR (Chang et al., 2020; Zhou et al., 2019; Zhu, 1973). However, in the 20th century, with the end of the Little Ice Age and the gradual warming of the global climate. The results of BSP show that effective population size of *M. nipponense* in the TGR was still shrinking. Fishing intensity increased may have been a major factor in the decline of effective populations of *M. nipponense* in the TGR after the 20th century (Jackson et al., 2001; Limburg and Waldman, 2009). Wild fishing was the main source of *M. nipponense* in the market before large-scale farming, and the fishing intensity of *M. nipponense* gradually increased with the improvement of fishing technology in the middle of the last century (Chen et al., 2011; Li, 2020). However, the genetic diversity of all *M. nipponense* populations in the TGR is still at high level, suggesting that population contraction at the Little Ice Age had a limited impact on the genetic diversity of *M. nipponense* in the TGR.

4.2 Population structure

Results of AMOVA suggest that genetic differentiation may exist among the eight *M. nipponense* populations in the TGR. The Mantel test indicated that there was a significantly positive correlation between genetic distance and geographical distance, and the pairwise F_{ST} values also indicate widespread genetic differentiation between the head area and tail area populations of the TGR. In addition, it is clearly show from the results of STRUCTURE analysis, that the proportion of genetic clusters between the four populations in lower reaches of the TGR and the four populations in the upper reaches of the TGR is a little bit different. This is similar to the study of Feng et al. (2008) on genetic diversity of *M. nipponense* in five major freshwater lakes in China and Cui et al. (2018) on genetic diversity of *M. nipponense* in the Huaihe River Basin in China. Genetic differentiation is common among *M. nipponense* populations whose distance are far, and the degree of genetic differentiation is related to geographical distance to a certain extent.

The weak mobility of *M. nipponense* may be the cause of genetic differentiation between the four populations in lower reaches of the TGR and four populations in the upper reaches of the TGR. Firstly, *M. nipponense* is small in size and poor in locomotion ability, which makes it difficult to carry out long-distance migration. Second, the female will stick the fertilized eggs to her abdomen after spawning (Ma et al., 2012). This behavior can improve the survival rate of fertilized eggs, but it prevents the *M. nipponense* from expanding gene exchange among different populations like species that produces drifting eggs such as *Hypophthalmichthys molitrix* and *Aristichthys nobilis* (Wang et al., 2003). In conclusion, because of the long geographical distance between head area populations and tail area populations in the TGR, there is little gene flow between them, so resulting in genetic differentiation (Joanna et al., 2015; Wang et al., 2003). Therefore, *M nipponensis* in the TGR cannot be simply regarded as a conservation unit, and the genetic structure of *M nipponensis* in the TGR needs to be continuously investigated.

The absence of genetic differentiation between the GDK population and other populations, despite the barrier imposed by the Gudongkou Hydropower Station, may be related to the relatively short period of time since the Gudongkou Hydropower Station was built. A sufficient number of isolated generations is an important factor in generating genetic differentiation, Ruzich et al. (2019) have found that the effects of dam barrier on the genetic diversity and differentiation of fish populations may not be reflected in a short period of time, but 40–60 generations are sufficient to show. The Gudongkou Hydropower Station cut off links between GDK groups and others in 1995, less than 30 years, and thus the GDK population may not have accumulated sufficient genetic differences from other populations in the head area of the TGR (Cui et al., 2018; Gong 2006).

5 Conclusions

In this study, COI gene sequences were employed to examine the genetic diversity, genetic differentiation and historical demography of eight *M. nipponense* populations in

the TGR. The results showed that *M. nipponense* population in the TGR had high genetic diversity, so germplasm resources are abundant, and the screening work of excellent germplasm should be carried out to make sure the germplasm resources in the TGR can be used to breed, especially the FD and WZ populations because of their high genetic diversity. In addition, there was some genetic differentiation between the four populations in lower reaches of the TGR and the four populations in the upper reaches of the TGR. Therefore, we advise to segment two management units of *M. nipponense* in the TGR when we management or develop them. It should be noted that studies based only on mitochondrial genes as markers may have some limitations. Markers such as SSR and SNP should be used to analysis the genetic diversity and genetic structure of *M. nipponense* populations in the TGR in the future.

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Conflicts of interest

All the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability statement

The original data of COI marker have been deposited in Figshare: Wang, Dong (2023). ALL COI sequence.fas. figshare. Dataset. https://doi.org/10.6084/m9.figshare.22817753.v1.

Author contribution statement

Dong Wang performed the laboratory work, and wrote the manuscript. Le Hu, Fubin Zhang and Hongyan Liu helped in data analysis. Fengqun Zheng and Mengyu Gong helped in laboratory work. Fei Xiong designed the study and revised the manuscript. Dongdong Zhai helped in revised the manuscript. All authors read and approved the final version of the manuscript. Dongdong Zhai also contributed to sampling. All authors read and approved the final manuscript.

Supplementary material

Table 1. Pairwise F_{st} values (above the diagonal) and corrected P values (below the diagonal) based on mitochondrial COI gene among eight *M. nipponense* populations in the TGR.

Table 2. Genetic distance (GD) based on mitochondrial COI gene among eight *M. nipponense* populations in the TGR.

Table 3. Geographical distance (GD, above the diagonal) among eight *M. nipponense* populations in the TGR.

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