RESEARCH ARTICLE
eISSN 2234-1757
Fish Aquat Sci. 2026;29(9):622-633
https://doi.org/10.47853/FAS.2026.e53
FAS Fisheries and Aquatic Sciences

Genetic diversity in farmed and wild populations of the giant freshwater prawn (*Macrobrachium rosenbergii*) based on microsatellite DNA marker analysis

Md. Jubayer Alam[#], Md. Belal Hossen[#], Md. Samsul Alam[*]

Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

Abstract

Macrobrachium rosenbergii is one of the most important aquaculture species in Bangladesh. Understanding a population's genetic structure is vital for developing effective conservation strategies, allowing population monitoring, and identifying vulnerable groups. This study assessed genetic diversity in two hatchery populations (Fish-Tech prawn hatchery and CP prawn hatchery) and two wild populations (Indurkani point of Kocha River-2022 and Zianagar point of Kocha River-2023) of *M. rosenbergii* using microsatellite DNA markers. Nine microsatellite loci were analyzed, and all were found to be polymorphic across the populations. The number of alleles (N_a) per locus ranged from 3 to 11. The mean N_a in the Fish-Tech, CP, and Kocha River samples was 6.000, 6.778, and 7.055, respectively. The observed heterozygosity (H_o) was lower than the expected heterozygosity (He) in all four populations. Polymorphism information content (PIC) values ranged from 0.335 to 0.887 across four populations, indicating moderate to high marker effectiveness. Significant deviations from Hardy–Weinberg expectations were observed in 35 of 36 tests. The population differentiation (F_{ST}) values indicate a moderate genetic differentiation among the populations. The analysis of molecular variance (AMOVA) revealed that only 10% of the variance exists among populations. Complying with the neighbor-joining tree and principal coordinate analysis (PCoA), and STRUCTURE analysis revealed two distinct clusters; two samples of the Kocha River comprise one cluster, and the two hatchery samples comprise another cluster. These findings provide a baseline for future breeding program development and genetic monitoring.

Keywords: Genetic diversity, *Macrobrachium rosenbergii*, Microsatellite marker

Introduction

The giant freshwater prawn, *Macrobrachium rosenbergii,* is one of the most cultivable crustaceans and the largest *Macrobrachium* species with commercial significance. This species is naturally distributed in South and Southeast Asia, parts of Oceania, and some Pacific islands, and has been introduced in more than 40 countries (Bala et al., 2017). Bangladesh is well-suited for giant

Received: Oct 21, 2025 **Revised:** Jan 30, 2026 **Accepted:** Apr 24, 2026
[#]These authors contributed equally to this work.
[*]**Corresponding author:** Md. Samsul Alam
Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
Tel: +880-91-67401-6, **Fax:** +880-91-61510, **E-mail:** samsul.alam@bau.edu.bd

freshwater prawn farming due to its favorable agroclimatic conditions and extensive productive inland and brackish water bodies (Ahmed et al., 2008; Wahab et al., 2012). Commercial prawn farming began in Bangladesh in the early 1990s. A high demand for *M. rosenbergii* in local and international markets presents a significant opportunity to enhance its farming in Bangladesh (Ahmed et al., 2008; Alam & Alam, 2014). Farming *M. rosenbergii* represents a crucial segment of the aquaculture sector and is a vital source of income for Bangladesh. Captive breeding and the production of postlarvae (PL) play significant roles in the commercial aquaculture of *M. rosenbergii* in many countries, including China, India, Thailand, Vietnam, and Bangladesh.

Challenges such as slow growth rates, size variations at harvest, diseases, and deterioration of culture environments hinder the growth of the *M. rosenbergii* farming sector (Chareontawee et al., 2007). In addition to several other factors, poor genetic diversity is believed to be a primary cause of PL's low productivity. A broodstock with a greater genetic diversity is essential for a sustainable breeding program and efficient management strategy. The genetic diversity of a population promotes the expression of diverse physical traits in individuals, enabling them to adapt to stress, disease, and unfavorable environmental conditions, thereby ensuring long-term survival. Inbreeding has led to genetic deterioration in most hatchery populations, potentially negatively impacting the culture period, growth, economic returns, and natural ecosystems (Nguyen Thanh et al., 2015). Genetic degradation impedes the sustainable development of this species. Genetic diversity is crucial for stock improvement, enabling the prioritization of populations for selection based on genetic criteria (Petit et al., 1998). Genetic diversity is recognized to facilitate better breeding plans by selecting genetically diverse broodstocks and ensuring the maintenance of genetic variation (Charoentawee et al., 2006). The evolutionary potential and fitness of a population are positively correlated with genetic diversity (Vandewoestijne et al., 2008). Therefore, understanding genetic diversity is essential for developing effective conservation strategies. The assessment of genetic variation, determination of inbreeding, and identification of species have entered a new era with the application of genetic markers. Numerous applications of genetic markers include differentiating natural populations for better fishery management, analyzing population genetic structures, conserving, identifying selected lines, identifying quantitative trait loci in aquaculture, and identifying taxa, food products, and forensic samples (Layton et al., 2020; Wenne et al., 2007).

Through molecular techniques, pure stocks with greater genetic diversity can be identified and preserved to produce high-quality broodstock. Microsatellite DNA markers are frequently used to characterize the genetic makeup of both wild and cultured populations for management and breeding purposes. For example, a breeding program can use microsatellite DNA markers to build pedigrees and estimate heritability (Vandeputte et al., 2004). Microsatellites are highly polymorphic, neutral, and codominant DNA markers based on the variable number of short, usually 2–4 bp nucleotide repeats (Yu et al., 2019), that can resolve low genetic differentiation effectively, and play a pivotal role in describing the genetic structure, conserving genetic resources, and enhancing aquaculture by identifying potential sources for broodstock development. Specific microsatellite primers for *M. rosenbergii* have been developed in Australia (Chand et al., 2005), India (Bhat et al., 2009; Divu et al., 2008), Malaysia (Bhassu et al., 2008), and in Thailand (Charoentawee et al., 2006). Different genetic parameters of *M. rosenbergii* have been studied in North America (Schneider et al., 2013), China (Nguyen Thanh et al., 2015; Yu et al., 2019), and Thailand (Chareontawee et al., 2007).

In Bangladesh, the genetic diversity of the wild population of *M. rosenbergii* was also studied a decade ago (Khan et al., 2014), and a declining trend in the effective population size was reported. In 2014, only 349 MT (metric ton) of *M. rosenbergii* were caught from the major rivers of Bangladesh, which has increased to 4,084 MT in 2024 (DOF, 2024) due to increased fishing pressure, which may reduce the genetic diversity of *M. rosenbergii* in the wild population. However, the present status of the genetic parameters of the Bangladeshi wild population of *M. rosenbergii* is not well known. The Kocha River in the district of Pirojpur is one of the natural sources of berried (egg-bearing) and PL of prawn (Khan et al., 2014; Shofiquzzoha et al., 2016) in Bangladesh. The study of genetic diversity and population structure of this river would provide a better understanding of the genetic structure of the prawn used in the hatchery to produce post-larvae and cultured in the farms. Moreover, the genetic parameters of the hatchery-produced population of *M. rosenbergii* in Bangladesh have yet to be explored. Therefore, the present study aimed to examine the genetic diversity of two farmed (hatchery-produced) populations and two wild populations of *M. rosenbergii* using nine microsatellite markers. Understanding a population's genetic structure is vital for developing effective conservation strategies, for monitoring

population and identifying vulnerable groups.

Materials and Methods

Sample collection

A total of 80 samples (20 from each of the populations) were collected from one wild and two hatchery sources. The samples of the wild population were collected from two different locations (Indurkani point and Zianagar point, as labelled 2 and 3 respectively in Fig. 1) of the Kocha River in Pirojpur district in two consecutive years, 2022 and 2023, while the hatchery-produced samples were collected from the CP hatchery (C.P. Bangladesh Bagerhat, Bangladesh) in Bagerhat district (as labelled 4 in Fig. 1) and the Fish-Tech hatchery (Fishtech BD Limited, Patuakhali, Bangladesh) in Patuakhali district (as labelled 6 in Fig. 1). Between the two hatcheries, the CP prawn hatchery imported the berried (egg-bearing mother prawn) from Thailand, and the Fish-Tech prawn hatchery used their pond-developed berried for PL production. We collected the PLs from hatchery sources and reared them separately until the juvenile stage. Pleopods were collected from 20 individuals in each population, preserved in 95% ethanol, and transported to the Fish Genetics and Biotechnology Laboratory at Bangladesh Agricultural University, where they were stored at –20℃.

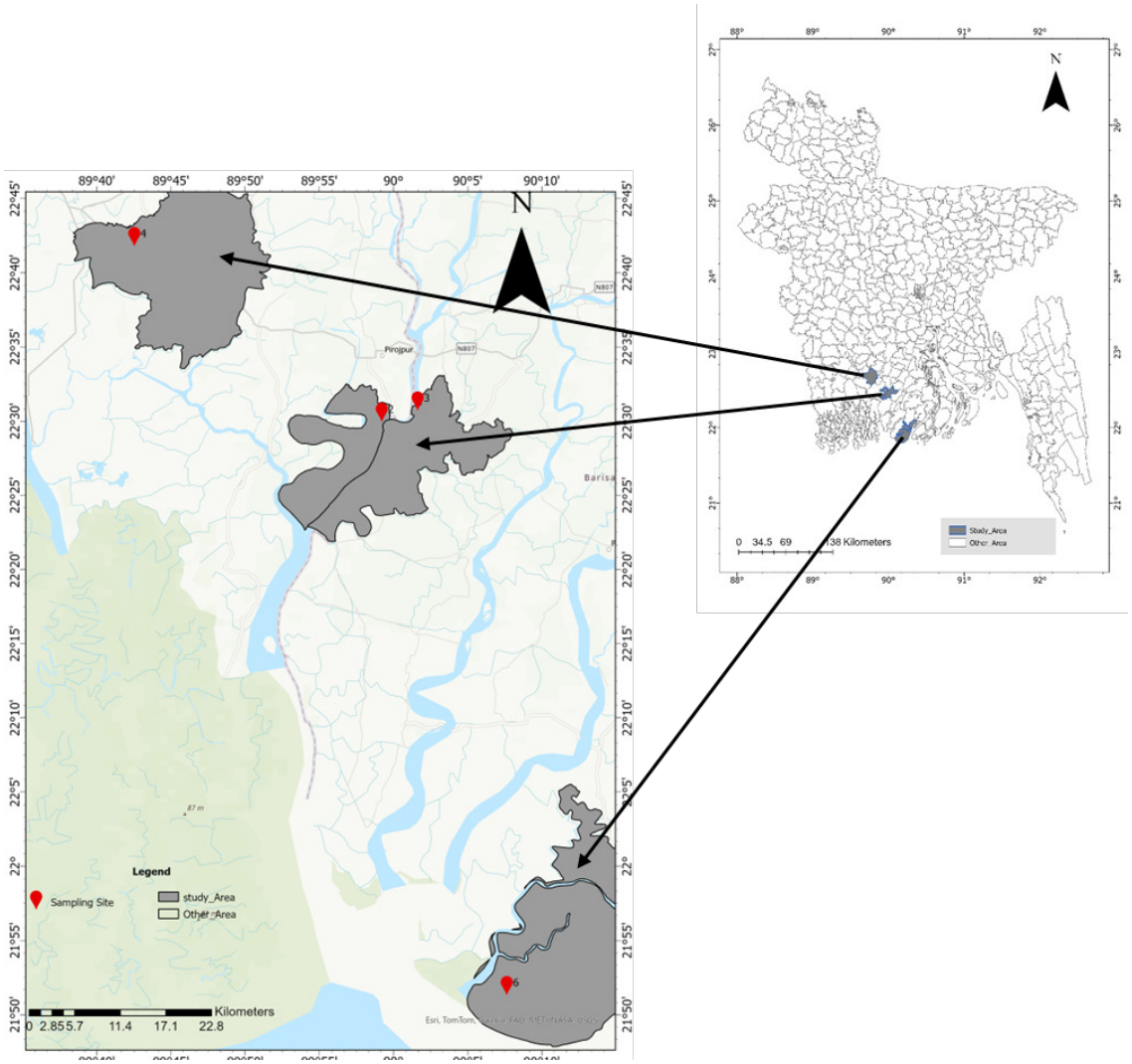

Fig. 1. Study area map showing the sampling sites for different populations of *Macrobrachium rosenbergii*. The sampling sites are labelled with different numeric values. The value 2 indicates the Indurkani point of Kocha River; 3 indicates the Zianagar point of Kocha River; 4 indicates the CP prawn hatchery and 6 indicates the Fish-Tech prawn hatchery.

https://doi.org/10.47853/FAS.2026.e53

Genomic DNA extraction

Approximately 30 mg of pleopod was used for genomic DNA extraction using phenol-chloroform-isoamyl alcohol (PCI) and the ethanol precipitation method (Ayub et al., 2008). The quantity of DNA was measured by a spectrophotometer (Genova Nano spectrophotometer, Staffordshire, UK).

Polymerase chain reaction (PCR) amplification

Nine pairs of primers for *M. rosenbergii* (Mbr-l, Mbr-2, Mbr-3, Mbr-5, Mbr-7, Mbr-8, Mbr-9, Mbr-10, and Mbr-11 developed by Charoentawee et al. (2006) were used in this study. Polymerase chain reactions (PCRs) were performed in 20 µl containing 10 µl of PCR Master Mix, 2 µl of DNA sample, 1 µl of forward primer, 1 µl of reverse primer, and autoclaved double-distilled water to a final volume of 6 µl. PCR was performed by a gradient thermocycler (Eppendorf Mastercycler® nexus X2, Hamburg, Germany) with the following cycling conditions: initial denaturation at 95℃ for 3 min; 35 cycles of denaturation at 95℃ for 30 s, annealing at 56℃ for 30s, and extension at 72℃ for 1 min; followed by a final extension at 72℃ for 7 min. The PCR products were electrophoresed on a 6% polyacrylamide gel, using a 100 bp ladder and a Mini Gel Tank, to separate the microsatellite alleles. The gel was then stained in a 0.5% ethidium bromide solution for 15 min and washed with water to enhance visualization under an ultraviolet (UV) illuminator.

Statistical analysis of microsatellite data

The software AlphaEaseFC version 4.0 (Khan et al., 2014) was used for genotyping. Population genetic parameters such as the number of alleles (N_a), effective number of alleles (N_e), frequency of alleles, observed heterozygosity (H_o), expected heterozygosity (H_e), genetic distance (D_A) between the population pairs, and chi-square test for the goodness of fit to Hardy–Weinberg expectation (HWE), principal coordinate analysis (PCoA), genetic differentiation coefficient (F_{ST}), and analysis of molecular variance (AMOVA) were performed using the software GenAlEx version 6.5 (Peakall & Smouse, 2012) with 1,000 permutations. Fixation index within a specific subpopulation (F_{IS}) was calculated in PopGene (Yeh et al., 1997). Pairwise fixation index for population differentiation (F_{ST}) values were corrected for the presence of null alleles using the excluding null allele (ENA) method using FreeNA (Chapuis & Estoup, 2007). The statistical significance of F_{ST} values between population pairs was assessed using the Bonferroni test in ARLEQUIN 3.0 (Excoffier et al., 2005) with 1,000 genotype permutations. Polymorphism information content (PIC) values were calculated using the formula PIC = 1 – ΣPi2, where pi is the frequency of the ith allele (Smith et al., 1997). The model-based approach for determining the genetic structure of four different populations of *M. rosenbergii* was analyzed using STRUCTURE 2.3.4 (Pritchard et al., 2000) to assess genomic clustering (k). In the assumption population menu, the assumed K value was set from 1 to 6, with 100,000 repetitions for the burn-in period length and the number of Markov chain Monte Carlo (MCMC) replications after burn-in. The ΔK is the most likely number of K that represents the population structure (Evanno et al., 2005). To obtain a representative K value for data modelling, 10 independent runs were performed for each value from 1 to 6. The software MEGA version 11 (Tamura et al., 2021) was used to construct the neighbor-joining dendrogram. Before statistical analysis, genotype data were checked for data integrity using Micro Checker version 2.2.3 (Van Oosterhout et al., 2004) to identify null alleles, allelic dropout, and stuttering-related scoring errors. STRUCTURE Harvester (Earl & vonHoldt, 2012) was used to visualize STRUCTURE output, including likelihood values and ΔK, to determine the most probable number of genetic clusters.

Results

Allele size and frequency

We genotyped nine microsatellite loci in four *M. rosenbergii* populations, all of which were polymorphic. A total of 88 alleles ranging from 220 to 382 bp were detected at nine microsatellite loci with an average of 9.777 alleles per locus. The detailed allele frequencies are shown in Supplementary Table S1. A total of 11 private alleles were detected (Table 1). The CP hatchery population had the highest number of private alleles, with seven, among all other populations. The Kocha river-2022 population had three, and the Kocha river-2023 had one private allele, whereas no private allele was identified in the Fish-Tech hatchery population.

Intra-population genetic variation

All loci were polymorphic (P_{95}) across the four populations studied. The N_a varies from 3 (Mbr-7, Mbr-10) to 11(Mbr-1). The average N_a varied from 6 ± 0.799 (Fish-Tech) to 7.111 ± 0.735 (Kocha River population of both years). The N_e ranged from 1.504 for Mbr-7 (CP hatchery) to 7.921 for Mbr-1 (Kocha

Table 1. Frequencies of private alleles found at different microsatellite loci in four populations of *Macrobrachium rosenbergii*

Locus	Allele	Fish-Tech hatchery	CP hatchery	Kocha River	
				2022	2023
Mbr-1	259	0.000	0.025	0.000	0.000
	269	0.000	0.075	0.000	0.000
	329	0.000	0.000	0.150	0.000
	335	0.000	0.000	0.100	0.000
Mbr-7	247	0.000	0.050	0.000	0.000
Mbr-8	231	0.000	0.100	0.000	0.000
Mbr-9	262	0.000	0.100	0.000	0.000
	270	0.000	0.000	0.000	0.025
Mbr-10	220	0.000	0.125	0.000	0.000
	255	0.000	0.000	0.025	0.000
	266	0.000	0.075	0.000	0.000

River-2022). The mean effective allele number varied from (3.865 ± 0.591) for the Fish-Tech hatchery population to (5.741 ± 0.673) for the Kocha River population sampled in 2022. The average observed heterozygosity across loci ranged from 0.183 ± 0.095 (Fish-Tech hatchery) to 0.444 ± 0.089 (CP hatchery). On the other hand, the average H_e was greater than the observed heterozygosity (H_o) in all the populations, ranging from 0.681 ± 0.052 (Fish-Tech hatchery) to 0.807 ± 0.021 (Kocha River-2022). A significant departure from the HWE was observed in 35 of the 36 tests ($p < 0.05$) (Table 2). Most of the PIC values were greater than 0.5, indicating their high effectiveness in genetic population analysis. The PIC values at Mbr-7 (CP hatchery) population and Mbr-10 (Fish-Tech hatchery) were 0.335 and 0.386, respectively, indicating moderate effectiveness. The results from Micro-Checker indicated no scoring errors or allele dropouts; however, null alleles were observed at 8 of 9 loci across all four populations (Table 3). Among the four experimental populations of the *M. rosenbergii*, the average null allele frequency of the CP hatchery population was lowest (0.1577) compared with the other three populations. However, all the experimental populations showed a higher average value of null allele frequency (Table 3) than the recommended threshold value of 0.02 (Dakin & Avise 2004).

Hierarchical partitioning of genetic diversity

AMOVA revealed that the most significant proportion of total variation, 61%, was due to variation between individuals, and 29% was attributed to within-individual variation within a population. In contrast, only 10% of the total variation was attributed to differences among the populations (Table 4).

Table 2. Allelic and genetic variations at nine microsatellite loci in four populations of *Macrobrachium rosenbergii*

Microsatellite loci	Parameters	Fish-Tech hatchery	CP hatchery	Kocha River	
				2022	2023
Mbr-1	N_a	8	9	10	11
	N_e	4.762	4.790	7.921	8.696
	H_o	0.200	0.450	0.050	0.200
	H_e	0.790	0.791	0.874	0.885
	F_{IS}	0.747	0.431	0.943	0.774
	H–W test	90.97 (28)***	56.23 (36)*	164.35 (45)***	151.91(55)***
	PIC	0.790	0.791	0.874	0.885
Mbr-2	N_a	10	8	10	8
	N_e	5.755	5.970	8.081	5.063
	H_o	0.900	0.800	0.600	0.900
	H_e	0.826	0.833	0.876	0.803
	F_{IS}	–0.089	0.039	0.315	–0.121
	H–W test	68.05 (45)*	49.76 (28)**	93.88 (45)***	44.69 (28)*
	PIC	0.826	0.833	0.880	0.802
Mbr-3	N_a	7	8	10	10
	N_e	5.229	5.263	8.889	7.407
	H_o	0.050	0.700	0.300	0.150
	H_e	0.809	0.810	0.888	0.865
	F_{IS}	0.938	0.136	0.662	0.827
	HW–test	100.16 (21)***	45.06 (28)*	120.46(45)***	134.40 (45)***
	PIC	0.848	0.810	0.887	0.865
Mbr-5	N_a	8	8	6	6
	N_e	6.557	4.278	4.103	3.883
	H_o	0.300	0.550	0.050	0.350
	H_e	0.848	0.766	0.756	0.743
	F_{IS}	0.646	0.282	0.934	0.529
	HW–test	69.30(28)***	34.55(28)ns	80.80 (15)***	33.14 (15)**
	PIC	0.848	0.766	0.756	0.742
Mbr-7	N_a	3	3	6	5
	N_e	2.410	1.504	5.128	2.941
	H_o	0.000	0.000	0.000	0.000
	H_e	0.585	0.335	0.805	0.660
	F_{IS}	1.000	1.000	1.000	1.000
	H–W test	40.00 (3)***	40.00 (3)***	100.00 (15)***	80.00 (10)***
	PIC	0.585	0.335	0.805	0.660
Mbr-8	N_a	5	6	6	7
	N_e	2.332	2.454	5.263	5.369
	H_o	0.100	0.300	0.200	0.050
	H_e	0.571	0.593	0.810	0.814
	F_{IS}	0.825	0.494	0.795	0.939
	H–W test	40.16 (10)***	66.04(15)***	73.60 (15)***	100.40 (20)***
	PIC	0.571	0.593	0.810	0.810

https://doi.org/10.47853/FAS.2026.e53

Table 2. Continued

Microsatellite loci	Parameters	Fish-Tech hatchery	CP hatchery	Kocha River	
				2022	2023
Mbr-9	N_a	5	6	6	7
	N_e	2.332	2.454	5.263	5.369
	H_o	0.100	0.300	0.200	0.050
	H_e	0.571	0.593	0.810	0.814
	F_{IS}	0.825	0.494	0.795	0.939
	H–W test	40.16 (10)***	66.04(15)***	73.60 (15)***	100.40 (20)***
	PIC	0.571	0.593	0.810	0.810
Mbr-10	N_a	3	5	5	5
	N_e	1.629	3.053	3.265	4.167
	H_o	0.050	0.300	0.150	0.100
	H_e	0.386	0.673	0.694	0.760
	F_{IS}	0.871	0.554	0.748	0.868
	H–W test	20.24 (3)***	43.35 (10)***	40.80 (10)***	60.55 (10)***
	PIC	0.386	0.672	0.694	0.760
Mbr-11	N_a	5	9	6	6
	N_e	3.704	6.299	4.571	5.442
	H_o	0.000	0.700	0.050	0.050
	H_e	0.730	0.841	0.781	0.816
	F_{IS}	1.000	0.168	0.936	0.939
	H–W test	80.00 (10)***	61.88 (36)**	90.45 (15)***	100.80 (21)***
	PIC	0.730	0.841	0.781	0.816
Mean ± SE of alleles (N_a)		6.000 ± 2.397	6.778 ± 2.108	7.111 ± 2.204	7.000 ± 2.236
Mean ± SE of effective alleles (N_e)		4.046 ± 1.802	4.081 ± 1.630	5.741 ± 2.018	5.300 ± 1.775
Mean ± SE of H_o over loci		0.183 ± 0.095	0.444 ± 0.089	0.156 ± 0.065	0.206 ± 0.094
Mean ± SE of H_e over loci		0.681 ± 0.052	0.703 ± 0.054	0.807 ± 0.021	0.793 ± 0.022
Mean ± SE of F_{IS} over loci		0.761 ± 0.113	0.424 ± 0.102	0.814 ± 0.074	0.743 ± 0.118
Polymorphism (P_{95})		1.0	1.0	1.0	1.0

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
N_a, number of alleles; N_e, number of effective alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; F_{IS}, fixation index; H–W test, Hardy–Weinberg test; ns, not significant; PIC, polymorphic information content.

Table 3. Frequency of null alleles for each locus across populations (brookfield 2 method)

Locus	Null	Fish-Tech hatchery	CP hatchery	Kocha River	
				2022	2023
Mbr-1	Yes	0.3296	0.1905	0.4396	0.3634
Mbr-2	No	0	0.0177	0.0147	0
Mbr-3	Yes	0.4195	0.06608	0.3113	0.3834
Mbr-5	Yes	0.2963	0.1224	0.4021	0.2253
Mbr-7	Yes	0.3691	0.2509	0.446	0.3976
Mbr-8	Yes	0.337	0.2884	0.4366	0.413
Mbr-9	Yes	0.2999	0.1837	0.337	0.4211
Mbr-10	Yes	0.2426	0.2224	0.321	0.375
Mbr-11	Yes	0.4220	0.0767	0.4105	0.4219
Range		0–0.4220	0.0177–0.2884	0.0147–0.4460	0–0.4219
Mean		0.3018	0.1577	0.3465	0.3334

Table 4. AMOVA of the four populations of *Macrobrachium rosenbergii*

Source of variation	*df*	Sum square	Mean square	Estimated variation	Percentage variation (%)	*p* value
Among populations	3	66.481	22.160	0.407	10	< 0.001
Among individuals	76	447.975	5.894	2.391	61	
Within individuals	80	89.000	1.113	1.113	29	
Total	159	603.456		3.910	100	

AMOVA, analysis of molecular variance.

Inter-population genetic divergence

The genetic differentiation (F_{ST}) values (both uncorrected and ENA corrected) are shown in Table 5. The uncorrected and ENA corrected F_{ST} values ranged from 0.027 to 0.105 and 0.010 to 0.108, respectively, indicating very low to moderate differentiation among the populations. The highest value of F_{ST} with ENA correction was 0.108 between the Kocha River 2023 population and the CP hatchery population, and the lowest F_{ST} value (0.010) was observed between the Kocha River 2022 population and the Kocha River 2023 population. All pairwise F_{ST} (both uncorrected and ENA corrected) comparisons were significant ($p < 0.05$), except for the Kocha River populations collected in 2022 and 2023 ($F_{ST} = 0.027$, $p = 0.315$). The highest D_A (0.925) was detected between the CP hatchery population and the Kocha River-2023 population, whereas the lowest distance (0.589) was detected between the Kocha River-2022 and the Fish-Tech hatchery (Table 5).

The Neighbour-joining dendrogram based on Nei's D_A data grouped the two hatchery populations (Fish-Tech and CP hatchery) in the same cluster, and the two wild populations (Kocha River-2022 and Kocha River-2023) in another cluster (Fig. 2). Principal coordinate analysis (PCoA) of the four populations revealed relationships among them. The first and second coordinates represent 9.33% and 7.97% of the variation, respectively (Fig. 3).

STRUCTURE software was used to elucidate population structure under an admixture model. The optimal number of clusters (K) was determined by the peak ΔK in the

Table 5. Uncorrected F_{ST} (above diagonal). ENA corrected FST (above diagonal within parentheses) and genetic distance (below diagonal) between pairs of four populations of *Macrobrachium rosenbergii* across nine microsatellite loci

Fish-Tech hatchery			CP hatchery	Kocha River	
				2022	2023
Fish-Tech hatchery		0	0.105 (0.105)	0.076 (0.069)	0.097 (0.101)
CP hatchery		0.700*	0	0.072 (0.075)	0.097 (0.108)
Kocha River	2022	0.589*	0.598*	0	0.027 (0.010)
	2023	0.869*	0.925*	0.239ns	0

* Indicates significant genetic distance between pair ($p < 0.05$).
ns, non-significant.

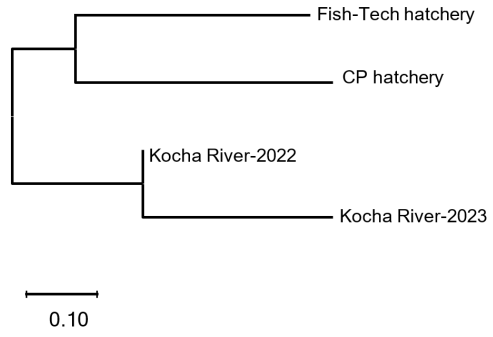

Fig. 2. Dendrogram based on pairwise Nei's genetic distances (neighbor-joining method).

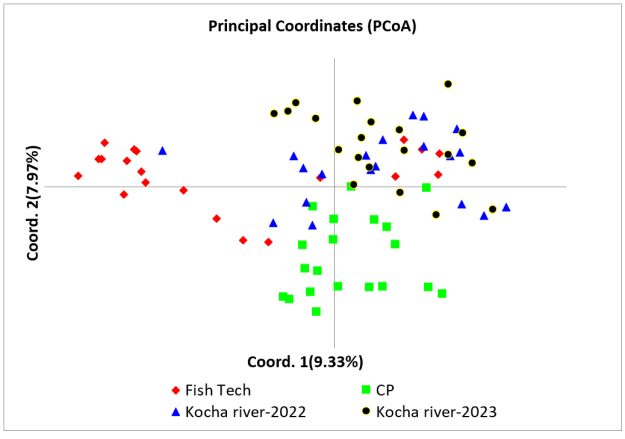

Fig. 3. Principal coordinate analysis (PCoA) of four populations of *Macrobrachium rosenbergii* based on nine microsatellite loci.

STRUCTURE analysis. The highest ΔK value was observed at K = 2, dividing the four populations into two groups (Fig. 4). One group includes two cultured populations, while the other consists of two wild populations.

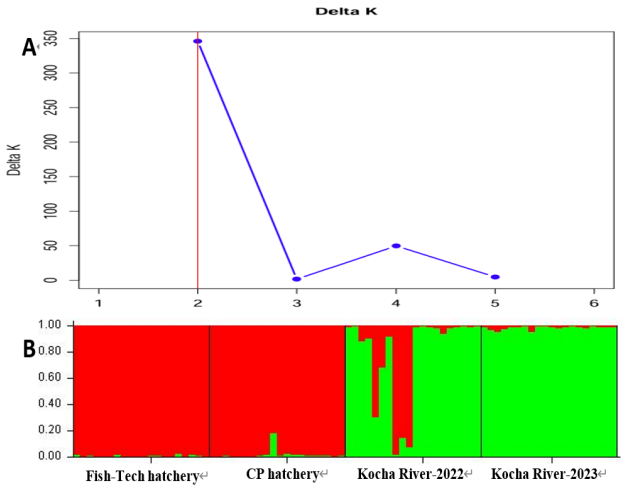

Fig. 4. Clustering analysis of four *Macrobrachium rosenbergii* populations using Structure software. (A) The highest ΔK value = 2, and (B) clustering of four populations. Red color indicates the cluster of hatchery population, while green color indicates the cluster of wild population.

Discussion

Genetic diversity is fundamental for evolutionary potential and population fitness (Ibrahim et al., 2025). Higher genetic diversity increases the capability for adaptation and breeding potential. In contrast, reduced genetic diversity jeopardizes survival and increases the risk of extinction, and it is also crucial for the sustainable aquaculture of *M. rosenbergii*. We employed nine microsatellite markers to assess the genetic diversity of four *M. rosenbergii* populations from four distinct locations in Bangladesh. The N_a, H_o, H_e, and PIC are typically considered standard metrics to quantify population genetic diversity (Shete et al., 2000). In the present study, we detected 88 alleles at nine loci, ranging from 3 to 11, which is very similar to the number reported by Khan et al. (2014) for three river populations of *M. rosenbergii* in Bangladesh.

Different authors have reported variations in the N_a in *M. rosenbergii*, such as 12–18 (Chand et al., 2005); 3–5 (Bhat et al., 2009); 4–20 (Chareontawee et al., 2007); 3–16 (Divu et al., 2008); 3–25 (Schneider et al., 2013); and 3–12 (Bhassu et al., 2008). The N_a in a population generally increases with the population size, meaning larger populations tend to have more genetic diversity. This relationship occurs because larger populations are more likely to retain and accumulate rare alleles through mutation and genetic drift. The N_e represents the theoretical number of equally frequent alleles that would be required to produce the same level of H_e as observed in a real population with its diverse allele frequencies.

https://doi.org/10.47853/FAS.2026.e53

Usually, N_e is always less than or equal to the actual N_a. In the present study, we found a lower number of effective alleles than the actual number across the loci in all experimental populations, which is consistent with the findings of Khan et al. (2014), Nguyen Thanh et al. (2015), and Luan et al. (2006). We also found lower H_o than the H_e, indicating heterozygote deficiency across the loci in all studied populations. Similar observations have also been reported by Nguyen Thanh et al. (2015), Sánchez-Velásquez et al. (2022), and Ma et al. (2012). Several factors, e.g. founder effects, random genetic drift (Lacy, 1987), reduced effective breeding number, selection pressure at a particular locus, and variation in mating success, may contribute to low heterozygosity in the hatchery population (Wahidah et al., 2023). However, the reason behind the lower heterozygosity in natural populations is difficult to explain because various factors, such as sample bottlenecks (Allendorf, 1986), continuous environmental changes resulting from both anthropogenic and ecological activities (Mimura et al., 2017; Schaberg et al., 2008), may cause the loss of alleles and reduced genetic diversity. Additionally, scoring errors, allele dropout, and the presence of null alleles (Nguyen Thanh et al., 2015; Van Oosterhout et al., 2004) may also contribute to heterozygote deficiency. Heterozygote deficiency in the hatchery and wild populations found in the present study could be due to a small effective population and the presence of null alleles (Table 3). In any population, null alleles may arise from primer length (Wattier et al., 1998), poor primer annealing (Di Nunzio et al., 2022; Jahnke et al., 2022; Kwok et al., 1990), or insufficient amount of template DNA in the sample (Gagneux et al., 1997; Jahnke et al., 2022). To evaluate the potential impact of null alleles on downstream analyses, we applied the commonly used 20% threshold recommended by Dakin & Avise (2004). Their simulations demonstrate that null alleles with frequencies below $p = 0.20$ introduce only minor downward bias in exclusion probabilities and generally do not compromise population genetic inference. In contrast, null alleles exceeding this threshold can inflate allele frequency estimates and substantially increase false parentage exclusions. In our dataset, several loci (e.g., Mbr-1, Mbr-3, Mbr-7, Mbr-8, Mbr-11) exceeded the 0.30 level in one or more populations, indicating moderate to high null allele presence. However, these loci were retained because downstream analyses (ENA corrected FST, STRUCTURE clustering) are robust to null alleles, ensuring that the observed genetic patterns were not artefacts of amplification failure.

The PIC value is used to measure the informativeness of the markers and is typically categorized as high (PIC > 0.5), moderate (0.5 > PIC > 0.25), or low (PIC < 0.25) (Botstein et al., 1980). It also measures genetic variability within and between populations; higher PIC values indicate greater genetic richness and environmental adaptability (Avval, 2017). In this study, most loci exhibited high effectiveness (PIC > 0.5), except for Mbr-7 (CP) and Mbr-10 (Fish-Tech), which showed moderate effectiveness (0.25 < PIC < 0.5) (Table 2).

In this study, we found evidence of departures from the HWE in all the populations. Among the four populations, all loci of the river and the Fish-Tech hatchery populations were significantly deviated from the HWE (35 out of the 36 tests), indicating heterozygote deficiency ($F_{IS} > 0$) relative to Hardy–Weinberg expectation, which may be attributed to inbreeding, nonrandom mating, variations in mating success, small sample sizes (Chareontawee et al., 2007), and reduced effective breeding numbers (Innes & Elliott, 2006; Schneider et al., 2013) in case of the hatchery population and Wahlund effects in case of the wild population. Although sampling in the Kocha River was conducted at two locations in two consecutive years, there was no physical barrier to prevent gene flow between the two subpopulations. So, mixing of individuals between these two sub-populations may cause a reduction in heterozygosity due to the Wahlund effect, and thus contribute to the deviation from HWE (Chareontawee et al. (2007). The presence of null alleles in the observed loci may also have contributed to the deviation from HWE (Gopalakrishnan et al., 2009; Nguyen Thanh et al., 2015) in the present study.

Private alleles are genetic variants unique to a specific population or individual and play a vital role in understanding a population's genetic diversity and history. In the present study, we found the highest number (7) of private alleles in the CP hatchery population, indicating greater genetic divergence from the Bangladesh-origin populations. Khan et al. (2014), Chareontawee et al. (2007), and Schneider et al. (2013) also detected private alleles in different populations of *M. rosenbergii*.

AMOVA revealed significant variation within populations (90%) and limited differentiation among populations (10%) (Table 4), indicating high genetic diversity within each population and significant gene flow or recent shared ancestry among populations. This suggests that populations are relatively well connected and the differences between them are minor. The population pairwise F_{ST} is a key measure of genetic diversity among populations (Ibrahim et al., 2025). The F_{ST} values represent degrees of genetic differentiation and are categorized as follows: moderate, $0.05 < F_{ST} < 0.15$; high, $0.15 < F_{ST} < 0.25$; and very high, $F_{ST} > 0.25$ (Wright, 1965). The presence of null alleles can lead to overestimation of both F_{ST} and D_A (Chapuis & Estoup, 2007), which can make the

populations more distinct. In the present study, we calculated ENA corrected F_{ST} along with the normal F_{ST} to avoid the bias of the null allele. The uncorrected and ENA corrected F_{ST} values were found very close to each other, indicating the null allele present in the dataset contributed an insignificant impact on the pairwise F_{ST} value. Similar to Ding et al. (2024), we found very low to moderate genetic differentiation between the population pair (Table 5). We observed the highest ENA corrected F_{ST} (F_{ST} = 0.108) between the Kocha River 2023 and the CP hatchery population. This might have occurred due to differences in their origin as the CP hatchery collected berried from Thailand for PL production. Conversely, a very low F_{ST} (0.010) between two wild populations collected from two different locations of the Kocha River (Fig. 1) in 2022 and 2023 indicates a close relationship, as they belong to the same river. The neighbour-joining dendrogram (Fig. 2) also supports a close relationship between the two Kocha River populations, as they are grouped in the same cluster, with the lowest D_A of 0.239. The other cluster comprises two cultured populations. The STRUCTURE analysis grouped four *M. rosenbergii* populations into two clusters (K = 2) (Fig. 4), one comprising the Fish-Tech and CP hatchery populations, and the other consisting of two wild populations from the Kocha River. A very low F_{ST} value (0.010) and a low D_A (0.239) between the two Kocha River populations support their association within the same group. Furthermore, similar results were obtained using the PCoA (Fig. 3).

Genetic variability is vital for survival and is a crucial factor for growth and interaction with the environment. The genetic structure of a population can be altered through interventions, potentially leading to a loss of genetic potential and hindering its ability to adapt to environmental changes, ultimately resulting in a decline in genetic diversity. Along with various factors, e.g. garbage dumping, agricultural pesticide and industrial waste runoff, the overexploitation of commercially important species is a principal cause of population decline (Schneider et al., 2013). Therefore, understanding the genetic structure of natural populations is crucial for the sustainable management of population diversity. Similar to Nguyen Thanh et al. (2015), the present study revealed relatively low levels of genetic diversity in the cultured and wild population of *M. rosenbergii*.

Conclusion

The findings of the present study revealed that among the four studied *M. rosenbergii* populations, the Thai-originated CP hatchery population exhibited genetic superiority (in terms of the mean H_o and the highest number of private alleles) relative to those of the other three populations. However, similar to many baseline microsatellite studies, the present study employed a limited sample size of 80 individuals (20 per population) and a modest number of loci (nine microsatellite loci). Consequently, the present study had limited statistical power to detect subtle structural or temporal changes. So, the findings of the study can serve as a baseline and be useful in future studies on the genetic diversity of different populations of *M. rosenbergii*, using additional loci (e.g., single nucleotide polymorphisms [SNPs] or additional simple sequence repeats [SSRs]) and larger sample sizes from multiple hatchery and wild sources. Besides, we recommend regular sourcing of berried from the wild population and periodic exchange of berried among local hatcheries and a marker-assisted selection (MAS) to select broodstock with the lowest kinship and high heterozygosity for maintaining the long-term sustainability and productivity of the hatchery stocks. Simultaneously, strict local government policies should be imposed for the sustainable exploitation of the river population of *M. rosenbergii* in Bangladesh.

Supplementary Materials

Supplementary materials are only available online from: https://doi.org/10.47853/FAS.2026.e53

Competing interests

No potential conflict of interest relevant to this article was reported.

Funding sources

Krishi Gobeshona Foundation (KGF), Bangladesh (Grant No. TF-85/F-20).

Acknowledgements

Fishtech BD Limited (Patuakhali, Bangladesh) in Patuakhali district, for generously providing *M. rosenbergii* samples.

Availability of data and materials

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Ethics approval and consent to participate

This research has been approved by the Animal Welfare and Ethics Committee of the Bangladesh Agricultural University, with permit No.: ESRC/FISH/41.

https://doi.org/10.47853/FAS.2026.e53

ORCID

Md. Jubayer Alam https://orcid.org/0000-0003-1913-1062
Md. Belal Hossen https://orcid.org/0009-0006-4799-6775
Md. Samsul Alam https://orcid.org/0000-0003-0476-1766

References

Ahmed N, Demaine H, Muir JF. Freshwater prawn farming in Bangladesh: history, present status and future prospects. Aquac Res. 2008;39:806-19.

Alam MS, Alam MN. Development of the giant freshwater prawn *Macrobrachium rosenbergii* (De Man 1779) broodstock in culture ponds of South-Western Bangladesh: a case study. J Entomol Zool Stud. 2014;2:108-13.

Allendorf FW. Genetic drift and the loss of alleles versus heterozygosity. Zoo Biol. 1986;5:181-90.

Avval SE. Assessing polymorphism information content (PIC) using SSR molecular markers on local species of *Citrullus colocynthis*. Case study: Iran, Sistan-Balouchestan Province. J Mol Biol Res. 2017;7:42-9.

Ayub F, Sarker MY, Alam MS. Prevalence of white spot syndrome virus infection detected by one-step and nested PCR in selected tiger shrimp (*Penaeus monodon*) hatcheries. Aquac Int. 2008;16:405-15.

Bala B, Mallik M, Saclain S, Islam MS. Genetic variation in wild and hatchery populations of giant freshwater prawn (*Macrobrachium rosenbergii*) revealed by randomly amplified polymorphic DNA markers. J Genet Eng Biotechnol. 2017;15:23-30.

Bhassu S, See LM, Hassan R, Siraj SS, Tan SG. Isolation and characterization of microsatellite loci in the Malaysian giant freshwater prawn, *Macrobrachium rosenbergii*. Mol Ecol Resour. 2008;8:983-5.

Bhat S, Patel A, Das P, Meher PK, Pillai BR, Jayasankar P. Isolation and characterization of microsatellite loci in giant freshwater prawn, *Macrobrachium rosenbergii*. Conserv Genet. 2009;10:1473-5.

Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet. 1980;32:314-31.

Chand V, De Bruyn M, Mather PB. Microsatellite loci in the eastern form of the giant freshwater prawn (*Macrobrachium rosenbergii*). Mol Ecol Notes. 2005;5:308-10.

Chapuis MP, Estoup A. Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol. 2007;24:621-31.

Chareontawee K, Poompuang S, Na-Nakorn U, Kamonrat W. Genetic diversity of hatchery stocks of giant freshwater prawn (*Macrobrachium rosenbergii*) in Thailand. Aquaculture. 2007;271:121-9.

Charoentawee K, Poompuang S, Na-Nakorn U. Isolation and characterization of microsatellites in giant freshwater prawn *Macrobrachium rosenbergii*. Mol Ecol Notes. 2006;6:823-5.

Dakin EE, Avise JC. Microsatellite null alleles in parentage analysis. Heredity. 2004;93:504-9.

Department of Fisheries (DOF). Yearbook of fisheries statistics of Bangladesh, 2023-24. Dhaka: Department of Fisheries; Ministry of Fisheries and Livestock; 2024.

Ding Q, Shi M, Ji P, Qin L, Gao X, Zhang X, et al. Genetic diversity and differentiation of cultured *Macrobrachium rosenbergii* in China using newly developed microsatellite multiplex PCR panels. Aquac Int. 2024;32:7895-910.

Di Nunzio A, Tinto N, Iafusco F, Zibetti A, Ricci P, Di Nunzio C. Null allele can bring to interpretative problems in a deficitary paternity case. Forensic Sci Int Genet Suppl Ser. 2022;8:115-6.

Divu D, Khushiramani R, Malathi S, Karunasagar I, Karunasagar I. Isolation, characterization and evaluation of microsatellite DNA markers in giant freshwater prawn *Macrobrachium rosenbergii*, from South India. Aquaculture. 2008;284:281-4.

Earl DA, vonHoldt BM. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour. 2012;4:359-61.

Evanno G, Regnaut S, Goudet J. Detecting the number of clusters of individuals using the software structure: a simulation study. Mol Ecol. 2005;14:2611-20.

Excoffier L, Laval G, Schneider S. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform. 2005;1:47-50.

Gagneux P, Boesch C, Woodruff DS. Microsatellite scoring errors associated with noninvasive genotyping based on nuclear DNA amplified from shed hair. Mol Ecol. 1997;6:861-8.

Gopalakrishnan A, Musammilu KK, Basheer VS, Lijo J, Padmakumar KG, Lal KK, et al. Low genetic differentiation in the populations of the Malabar carp *Labeo dussumieri* as revealed by allozymes, microsatellites and RAPD. Asian Fish Sci. 2009;22:359-91.

Ibrahim S, Ruhul A, Li J, Yang G, Yi S, Xia Z, et al. Genetic diversity of selective breeding populations of giant freshwater prawn (*Macrobrachium rosenbergii*) based on SSR and

mitochondrial D-loop gene. Diversity. 2025;17:437.
Innes BH, Elliott NG. Genetic diversity in a Tasmanian hatchery population of Atlantic salmon (*Salmo salar* L.) compared with its Canadian progenitor population. Aquac Res. 2006;37:563-9.
Jahnke G, Smidla J, Deák T, Oláh R, Szőke BÁ, Nyitrainé Sárdy DÁ. The SSR null allele problem, and its consequences in pedigree reconstruction and population genetic studies in viticulture. Horticulturae. 2022;8:658.
Khan SR, Akter H, Sultana N, Khan MGQ, Wahab MA, Alam MS. Genetic diversity in three river populations of the giant freshwater prawn (*Macrobrachium rosenbergii*) in Bangladesh assessed by microsatellite DNA markers. Int J Agric Biol. 2014;16:195-200.
Kwok S, Kellogg DE, McKinney N, Spasic D, Goda L, Levenson C, et al. Effects of primer-template mismatches on the polymerase chain reaction: human immunodeficiency virus type 1 model studies. Nucleic Acids Res. 1990;18:999-1005.
Lacy RC. Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection, and population subdivision. Conserv Biol. 1987;1:143-58.
Layton KKS, Dempson B, Snelgrove PVR, Duffy SJ, Messmer AM, Paterson IG, et al. Resolving fine-scale population structure and fishery exploitation using sequenced microsatellites in a northern fish. Evol Appl. 2020;13:1055-68.
Luan S, Kong J, Wang QY. Genetic variation of wild and cultured populations of the Kuruma prawn *Marsupenaeus japonicus* (Bate 1888) using microsatellites. Aquac Res. 2006;37:785-92.
Ma KY, Feng JB, Li JL. Genetic variation based on microsatellite analysis of the oriental river prawn, *Macrobrachium nipponense* from Qiandao Lake in China. Genet Mol Res. 2012;11:4235-44.
Mimura M, Yahara T, Faith DP, Vázquez-Domínguez E, Colautti RI, Araki H, et al. Understanding and monitoring the consequences of human impacts on intraspecific variation. Evol Appl. 2017;10:121-39.
Nguyen Thanh H, Liu Q, Zhao L, Zhang H, Liu J. Genetic diversity of the cultured giant freshwater prawn (*Macrobrachium rosenbergii*) in China based on microsatellite markers. Biochem Syst Ecol. 2015;59:144-54.
Peakall R, Smouse PE. GenALEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics. 2012;28:2537-9.
Petit RJ, El Mousadik A, Pons O. Identifying populations for conservation on the basis of genetic markers. Conserv Biol. 1998;12:844-55.
Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000;155:945-59.
Sánchez-Velásquez JJ, Pinedo-Bernal PN, Reyes-Flores LE, Yzásiga-Barrera C, Zelada-Mázmela E. Genetic diversity and relatedness inferred from microsatellite loci as a tool for broodstock management of fine flounder *Paralichthys adspersus*. Aquac Fish. 2022;7:664-74.
Schaberg PG, DeHayes DH, Hawley GJ, Nijensohn SE. Anthropogenic alterations of genetic diversity within tree populations: implications for forest ecosystem resilience. For Ecol Manag. 2008;256:855-62.
Schneider KJ, Tidwell JH, Gomelsky B, Pomper KW, Waldbieser GC, Saillant E, et al. Genetic diversity of cultured and wild populations of the giant freshwater prawn *Macrobrachium rosenbergii* (de Man, 1879) based on microsatellite analysis. Aquac Res. 2013;44:1425-37.
Shete S, Tiwari H, Elston RC. On estimating the heterozygosity and polymorphism information content value. Theor Popul Biol. 2000;57:265-71.
Shofiquzzoha AFM, Haque SM, Wahab MA. Reproductive performance of freshwater prawn *Macrobrachium rosenbergii* (De Man 1879) broodstocks grown on different diets. J Bangladesh Agric Univ. 2016;14:229-234.
Smith JSC, Chin ECL, Shu H, Smith OS, Wall SJ, Senior ML, et al. An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisons with data from RFLPs and pedigree. Theor Appl Genet. 1997;95:163-73.
Tamura K, Stecher G, Kumar S. MEGA11: molecular evolutionary genetics analysis version 11. Mol Biol Evol. 2021;38:3022-7.
Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes. 2004;4:535-8.
Vandeputte M, Kocour M, Mauger S, Dupont-Nivet M, De Guerry D, Rodina M, et al. Heritability estimates for growth-related traits using microsatellite parentage assignment in juvenile common carp (*Cyprinus carpio* L.). Aquaculture. 2004;235:223-36.
Vandewoestijne S, Schtickzelle N, Baguette M. Positive correlation between genetic diversity and fitness in a large, well-connected metapopulation. BMC Biol. 2008;6:46.
Wahab MA, Ahmad-Al-Nahid S, Ahmed N, Haque MM, Karim M. Current status and prospects of farming the giant river

https://doi.org/10.47853/FAS.2026.e53

prawn *Macrobrachium rosenbergii* (De Man) in Bangladesh. Aquac Res. 2012;43:970-83.

Wahidah, Andy Omar SB, Trijuno DD, Amrullah, Khatimah K, Nugroho E. Genetic variation of giant freshwater prawns *Macrobrachium rosenbergii* wild population of South Sulawesi, Indonesia. Biodiversitas. 2023;24:3081-90.

Wattier R, Engel CR, Saumitou-Laprade P, Valero M. Short allele dominance as a source of heterozygote deficiency at microsatellite loci: experimental evidence at the dinucleotide locus Gv1CT in *Gracilaria gracilis* (Rhodophyta). Mol Ecol. 1998;7:1569-73.

Wenne R, Boudry P, Hemmer-Hansen J, Lubieniecki KP, Was A, Kause A. What role for genomics in fisheries management and aquaculture? Aquat Living Resour. 2007;20:241-55.

Wright S. The interpretation of population structure by *F*-statistics with special regard to systems of mating. Evolution. 1965;19:395-420.

Yeh FC, Yang RC, Boyle TBJ, Ye ZH, Mao JX. POPGENE, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, Edmonton, AB: University of Alberta; 1997.

Yu L, Zhu X, Liang J, Fan J, Chen C. Analysis of genetic structure of wild and cultured giant freshwater prawn (*Macrobrachium rosenbergii*) using newly developed microsatellite. Front Mar Sci. 2019;6:323.