Pertanika J. Trop. Agric. Sci. 49 (2): 453 - 469 (2026)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Phylogeography and Genetic Structure of the Giant Freshwater Prawn (Decapoda: Palaemonidae) in Peninsular Malaysia using Mitochondrial DNA Analysis

Dania Aziz[1]*, Afrina Irdina Roszizul Kahairi[1], Athirah Mohd Bakri[1], Nur Syuhada Mohd Fuad[1], Izzah 'Atirah Mohd Hir Tarmidi[1], Yuzine Esa[1,2], Md. Lifat Rahi[3], and Muhammad Dailami[4]

[1]Microalgae - Biota Technology and Innovation Group (ALBIC), Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Selangor, Malaysia
[2]International Institute of Aquaculture and Aquatic Sciences, Universiti Putra Malaysia, Lot 960 Jalan Kemang 6, 71050 Port Dickson, Negeri Sembilan, Malaysia
[3]Fisheries and Marine Resource Technology Discipline, Life Science School, Khulna University, 9208 Khulna, Bangladesh
[4]Faculty of Fisheries and Marine Science, Universitas Brawijaya, 65145 Malang, Indonesia

ABSTRACT

The Giant Freshwater Prawn (GFP), *Macrobrachium rosenbergii,* is a cornerstone of global freshwater aquaculture, especially in Southeast Asia, due to its increasing demand for high-quality seed stock and market value, yet the genetic structure of its natural populations in Malaysia remains poorly understood. This study evaluated the population structure and genetic diversity in the wild GFP across Peninsular Malaysia to identify superior strains for sustainable breeding programmes. This study analysed a 572 base pair segment of the mitochondrial cytochrome *c* oxidase 1 (COX1) gene from 149 GFP individuals collected from six river systems across Peninsular Malaysia. Twelve distinct haplotypes were identified, with phylogenetic analysis showing polyphyletic relationships among populations. The Kelantan river population exhibited the highest level of genetic diversity (π = 0.0024) and haplotype diversity (h = 0.5885), whereas the Perak river population showed no polymorphism. These results suggest a historical population bottleneck that drastically reduces the effective population size, in turn leading to loss of rare alleles and thus the increase in genetic homogeneity. As indicated by negative Tajima's D and Fu's FS values, significant deviations from neutrality, together with unimodal mismatch distributions,

ARTICLE INFO

Article history:
Received: 04 July 2025
Accepted: 15 April 2026
Published: 28 April 2026

DOI: https://doi.org/10.47836/pjtas.49.2.22

E-mail addresses:
dania@upm.edu.my (Dania Aziz)
afrinairdina@gmail.com (Afrina Irdina Roszizul Kahairi)
athirahbakri17@gmail.com (Athirah Mohd Bakri)
202086@student.upm.edu.my (Nur Syuhada Mohd Fuad)
206339@student.upm.edu.my (Izzah 'Atirah Mohd Hir Tarmidi)
yuzine@upm.edu.my (Yuzine Esa)
lifatrahi@gmail.com (Md. Lifat Rahi)
muhdailami@ub.ac.id (Muhammad Dailami)
* Corresponding author

e-ISSN: 2231-8542

suggest a historical bottleneck followed by rapid population growth and the subsequent accumulation of rare mutations. These findings provide a critical genetic baseline for the management of wild stocks and the selection of high-diversity founder populations for the GFP aquaculture industry in Malaysia.

Keywords: COX1 gene, genetic diversity, genetic variation, *Macrobrachium rosenbergii,* population structure

INTRODUCTION

The Giant Freshwater Prawn (GFP), locally known as "Udang Galah" in Malaysia or scientifically known as *Macrobrachium rosenbergii*, is a highly sought-after, commercially valuable species globally (Yu et al., 2019). In 2022, global production reached 294 thousand tons, making it one of the most extensively cultured decapod species, and its production is essential to the aquaculture industries globally (Zheng et al., 2024). The prawns are distributed from Northwest India down to Southeast Asia, Papua New Guinea, the Philippines, and Northern Australia (Banu & Christianus, 2016). The GFP has four distinct stages in its life cycle, which are the egg, larva, juvenile and adult. While mating occurs throughout the year, peak breeding periods are observed during the monsoon seasons (October to January, and March to May), when increased rainfall and optimal water temperatures trigger downstream migration and mass spawning (Mitra et al., 2005). Notably, the successful development of GFP larvae requires brackish water, as survival rates are drastically reduced if they are not transferred to such an environment within a few days of hatching. In their natural habitat, GFP larvae primarily feed on zooplankton, small insects and the larvae of other aquatic invertebrates. After mating, gravid females migrate towards brackish water where the eggs hatch as free-swimming larvae before transitioning to post-larvae. These post-larvae then migrate upstream into freshwater systems, where they continue their life cycle into adulthood, a critical aspect of their survival and reproductive success. Moreover, this migration also serves to reduce predation risk for the prawns (Li et al., 2010).

Understanding the biodiversity and population structure in aquatic ecosystems requires genetic analysis of aquatic species. The Cytochrome c oxidase subunit 1 (COX1) gene is a mitochondrial marker widely adopted in DNA barcoding for species identification and classification (Hebert et al., 2003). The COX1 gene has proven particularly effective in distinguishing closely related species, identifying cryptic taxa and investigating intraspecific diversity (Trivedi et al., 2016). This gene has been successfully utilised in a variety of organisms, including both vertebrates and invertebrates (Oba et al., 2015; Tavares & Baker, 2008; Trivedi et al., 2016), yet vice versa for plants and some species of fungi (Ward et al., 2005). Compared to other mitochondrial genes, COX1 gave the signal for a better

phylogenetic analysis (Strüder-Kypke & Lynn, 2010). Even though still in the preliminary level, application of COX1 already showed positive results for barcode dinoflagellates, *Paramecium* sp., and *Nemertea* (Stern et al., 2010; Sundberg et al., 2016; Zhao et al., 2013). So, mixed analyses of nuclear and mitochondrial markers might assist in narrowing the knowledge gap (Blaxter, 2016). In this study, we aim to analyse the genetic structure of the wild GFP populations in Peninsular Malaysia using the mitochondrial cytochrome c oxidase 1 (COX1) gene. This approach enables the assessment of genetic diversity and population differentiation, which is important for identifying genetically diverse and potentially superior strains for aquaculture. The findings can support the selection of suitable broodstock, improve selective breeding strategies, reduce the risk of inbreeding, and contribute to sustainable aquaculture management and conservation of valuable wild genetic resources.

MATERIALS AND METHODS

Sampling Locations

The GFP samples for this study were collected from various rivers across Peninsular Malaysia (Table 1), including Sungai Linggi (Negeri Sembilan), Sungai Perak (Perak), Sungai Pahang (Pahang), Kuala Selangor (Selangor), Sungai Selehong (Kelantan), and Sungai Kerian (Pulau Pinang). These samples were obtained from wild populations and subsequently stored at -20 ˚C in the Genetics Laboratory of Universiti Putra Malaysia prior to DNA extraction.

Table 1
Sample collection from six different populations in Peninsular Malaysia

Location	Sample Size
Sungai Linggi, Negeri Sembilan	20
Sungai Perak, Perak	29
Sungai Pahang, Pahang	23
Kuala Selangor, Selangor	24
Sungai Selehong, Kelantan	30
Sungai Kerian, Pulau Pinang	23

DNA Extraction and Polymerase Chain Reaction (PCR)

Genomic DNA was extracted from the muscle tissue of GFP samples using the ReliaPrep gDNA Tissue Miniprep System (Promega Corp, Madison, USA) according to the manufacturer's instructions. The extracted DNA was kept at 4 °C prior to the PCR protocol. Approximately 25 mg of muscle tissue was used for each specimen. The mitochondrial cytochrome c oxidase subunit I (COX1) gene was amplified using the universal primers FishF1 and FishR1 (Table 2). Each PCR reaction was performed in a total volume of 25 µl containing 14.3 µl sterile distilled water, 5 µl Taq buffer 5×, 2.0 µl of 25 mM MgCl, 0.5 µl of 10 mM dNTP, 0.5 µl of 10 µM of each primer, 0.2 µl of 5 µ µ-1 of Taq DNA polymerase

Table 2
Primer used in this study and the sequences

Primer	Sequences
FishF1	5'-TCAACCAACCACAAAGACATTGGCAC-3'
FishR1	5'-TAGACTTCTGGGTGGCCAAAGAATCA-3'

*Source: Ward et al. (2005)

and 2 µl of DNA template. Amplification was carried out using a Mastercycler Gradient PCR system (Eppendorf, Hamburg, Germany) under the following cycling conditions: initial denaturation at 94 °C for 3 minutes, followed by 32 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 1 minute, and extension at 72 °C for 1 minute 30 seconds, with a final extension at 72 °C for 2 minutes. The amplified PCR products were separated on a 1% agarose gel stained with ethidium bromide and visualised under UV illumination using an AlphaImager HP system (BioTechne, USA). A BenchTop 1kb DNA Ladder (Promega Corp, Madison, USA) was used as the molecular size marker. PCR products that yielded clear bands of the expected size were purified prior to sequencing and subsequently submitted to First BASE Laboratories Sdn. Bhd. for forward-direction sequencing using an ABI 377 automated sequencer (Applied Biosystems).

Data Analyses

The phylogenetic analysis was constructed with the inclusion of two sequences from GenBank (MF622020 and MW381290). The Kimura 2-parameter evolution model was used to calculate sequence divergence, sequences were grouped by using the Neighbour-Joining method (Figure 1) and bootstrapped with 1000 replications using MEGA version 7 (Kumar et al., 2016). Genetic structure of the GFP was obtained from the summary statistics of Tajima's D and Fu's Fs using ARLEQUIN version 3.0 (Excoffier et al., 2005). The mismatch distribution analysis was performed to detect recent population expansion by comparing the distribution of the number of pairwise differences between population and their theoretical distribution expected under a model of sudden (stepwise) demographic expansion. In the mismatch distribution analysis, SSD (Sum of Squared Deviations) and r statistics are used to assess the goodness-of-fit of the observed mismatch distribution to the expected distribution under specific demographic models. The SSD is a measure that quantifies the difference between the observed mismatch distribution and the expected distribution under a particular demographic model. The r statistic, also known as Harpending's raggedness index, is another measure used to assess the smoothness of the observed mismatch distribution.

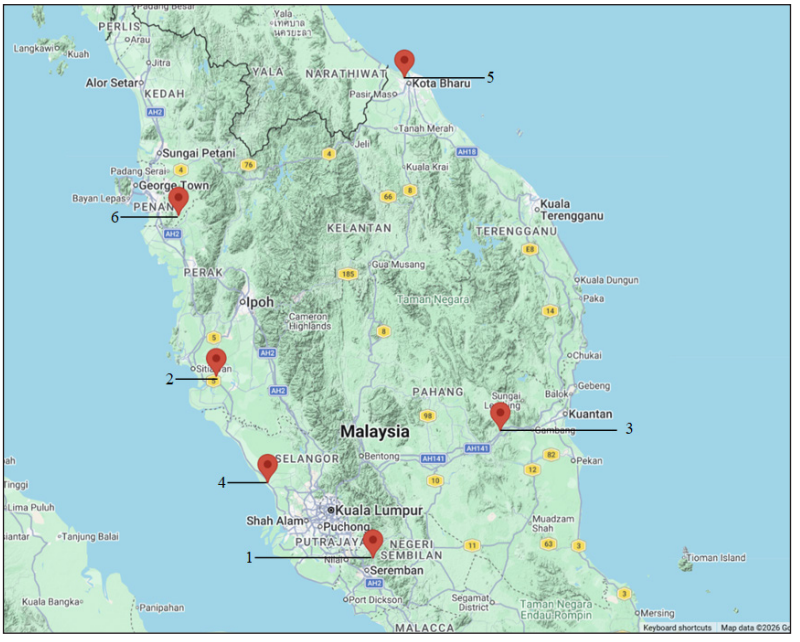

Figure 1. Sampling sites in Peninsular Malaysia; 1. Sungai Linggi, Negeri Sembilan; 2. Sungai Perak, Perak; 3. Sungai Pahang, Pahang; 4. Kuala Selangor, Selangor; 5. Sungai Selehong, Kelantan; 6. Sungai Kerian, Pulau Pinang

RESULTS

Sequence Characteristics and Haplotype Composition

A total of 572 base pairs (bp) of the COX1 gene were successfully retrieved after sequence alignment. 24 sites (4.19%) were identified as variable, including 15 parsimoniously informative sites, while the remaining 548 sites (95.8%) were conserved. The detected haplotypes contained eight substitutions, consisting of seven transitions and one transversion. The mean nucleotide composition was as follows: A = 27.3%, T = 27.6%, C = 26.9% and G = 18.2%. From the 149 GFP samples analysed, 12 distinct haplotypes were identified. All identified haplotypes were deposited in GenBank under accession numbers (PP337789- PP337800).

Genetic Diversity and Population Variation

Genetic diversity analysis revealed that GFP from Sungai Selehong (Kelantan) exhibited the highest genetic richness, with a haplotype diversity (h) of 0.5885 along with a relatively low nucleotide diversity (π = 0.0024). In contrast, the GFP from Sungai Perak (Perak) population displayed no genetic variation (h = 0.0000; π = 0.0000), with both haplotype and nucleotide diversity values equal to zero (Table 3), indicating a state of genetic homogeneity in that river system.

Table 3
Distribution of 12 observed haplotypes, nucleotide diversity, number of haplotypes, haplotype diversity and number of polymorphic sites among populations of GFP

Haplotypes	GenBank Accession Numbers	Populations					
		N. Sembilan (20)	Perak (29)	Pahang (23)	Selangor (24)	Kelantan (30)	Penang (23)
Hap 1	PP337789	0.45	-	-	-	-	-
Hap 2	PP337790	0.50	1.00	-	0.83	0.63	-
Hap 3	PP337791	0.05	-	0.04	0.13	0.10	-
Hap 4	PP337792	-	-	0.83	-	-	-
Hap 5	PP337793	-	-	0.04	-	-	-
Hap 6	PP337794	-	-	0.04	-	-	-
Hap 7	PP337795	-	-	0.04	-	-	-
Hap 8	PP337796	-	-	-	-	0.10	-
Hap 9	PP337797	-	-	-	-	0.07	-
Hap 10	PP337798	-	-	-	-	0.07	-
Hap 11	PP337799	-	-	-	-	0.03	-
Hap 12	PP337800	-	-	-	-	-	0.04
Nucleotide Diversity (PiJC)		0.0011	0	0.002	0.0004	0.0024	0.0007
Number of Haplotypes		3	1	5	2	6	3
Haplotype Diversity (Hd)		0.57368	0	0.3241	0.22826	0.58851	0.4229
Number of Polymorphic Sites		2	0	13	1	13	2

Phylogenetic Relationships among Populations

Phylogenetic analysis based on the Neighbour-Joining (NJ) tree revealed two distinct clades with high bootstrap values (Figure 2). The first clade (L1) contains 11 haplotypes, representing 148 samples, along with GFP sequences from Thailand available in GenBank. The second clade (L2) consisted of a single haplotype, Haplotype 11, which was exclusive to a single sample from Kelantan. Among the 12 identified haplotypes, five were unique to specific populations: Hap 5, Hap 6 and Hap 7 from Pahang, Hap 11 from Kelantan and Hap 12 from Pulau Pinang. The greatest genetic distance (2.9%) was observed between Hap 11 (Kelantan) and Hap 5/Hap 7 (Pahang), while the smallest distance was recorded between Hap 6 and Hap 4 (Table 4).

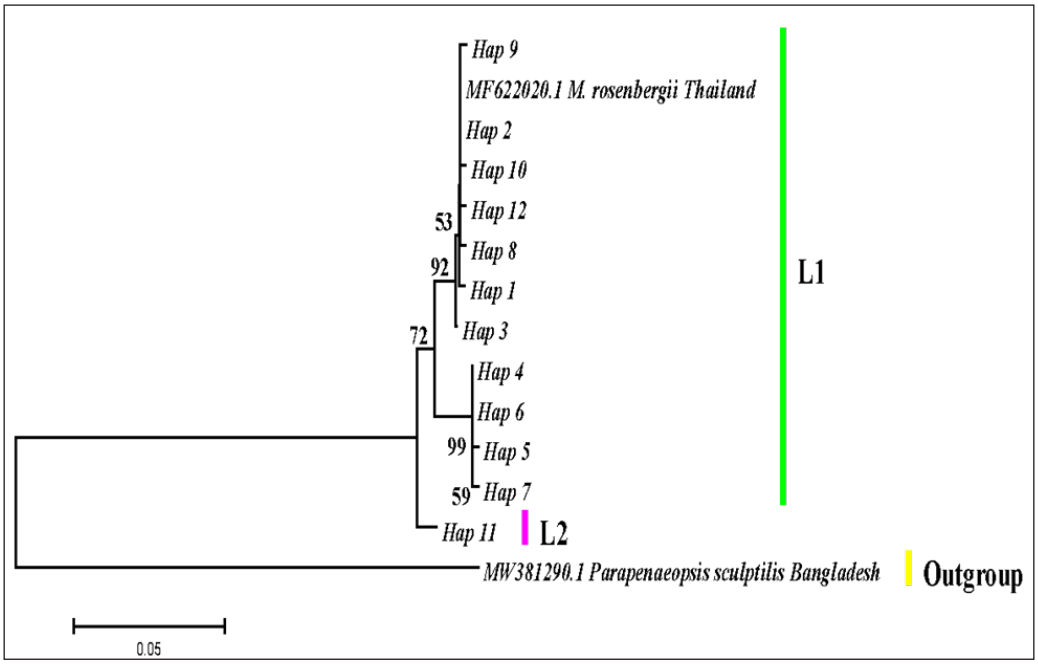

Figure 2. Neighbour-joining (NJ) tree showing relationships among the seabasses. The number at each node represents the bootstrap value (%) based on the 1000 pseudoreplications of the dataset

Genetic Differentiation Among Populations

Pairwise F_{st} values and results of the Chi-square tests for genetic differentiation among the GFP populations were presented in Table 5. However, there were no significant genetic differences among Peninsular Malaysia populations except samples from Sungai Linggi (Negeri Sembilan) and Sungai Pahang (Pahang) with the other populations in Peninsular Malaysia. AMOVA results (Table 6) revealed that the majority of the variance was from inter-population (83.34%), and only 16.66% of the total resulted from intra-population differentiation. The neutrality test of Tajima's D and Fu's Fs analysis was represented in Table 7. Negative Tajima's D was observed in all populations except Sungai Linggi (Negeri Sembilan) and Sungai Perak (Perak). However, only Sungai Pahang (Pahang), Sungai Selehong (Kelantan) and Sungai Kerian (Pulau Pinang) depicted a negative value for Fu's Fs test. The mismatch distribution was generally displayed as a multimodal pattern for all populations (Figure 3).

Table 4
Pairwise Tamura-Nei genetic distance among 12 haplotypes of GFP, one haplotype of Thailand GFP and one haplotype of Parapenaeopsis sculptilis

Haplotypes	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Hap 1	-													
Hap 2	0.002	-												
Hap 3	0.004	0.002	-											
Hap 4	0.023	0.021	0.019	-										
Hap 5	0.025	0.023	0.021	0.002	-									
Hap 6	0.023	0.021	0.019	0.000	0.002	-								
Hap 7	0.025	0.023	0.021	0.002	0.004	0.002	-							
Hap 8	0.004	0.002	0.004	0.023	0.025	0.023	0.025	-						
Hap 9	0.004	0.002	0.004	0.023	0.025	0.023	0.025	0.004	-					
Hap 10	0.004	0.002	0.004	0.023	0.025	0.023	0.025	0.004	0.004	-				
Hap 11	0.019	0.021	0.019	0.027	0.029	0.027	0.029	0.023	0.023	0.021	-			
Hap 12	0.004	0.002	0.004	0.023	0.025	0.023	0.025	0.004	0.004	0.004	0.023	-		
Macrobrachium rosenbergii	0.002	0.000	0.002	0.021	0.023	0.021	0.023	0.002	0.002	0.002	0.021	0.002	-	
Parapenaeopsis sculptilis	0.308	0.308	0.308	0.308	0.311	0.308	0.312	0.306	0.311	0.311	0.301	0.308	0.308	-

Table 5
Population subdivision (F_{st}) values and Chi-square probability test for population differentiation based on 1000 permutations of the sequence dataset among six populations of GFP

	1. N. Sembilan	2. Perak	3. Pahang	4. Selangor	5. Kelantan	6. P. Pinang
1	-					
2	0.436*	-				
3	0.920*	0.953*	-			
4	0.318*	0.102ns	0.937*	-		
5	0.137*	0.038*	0.886*	0.000ns	-	
6	0.283*	0.176*	0.925*	-0.011ns	0.00ns	-

Note. *$p < 0.001$; ns, not significant

Table 6
Hierarchical analysis of molecular variance (AMOVA) among populations GFP

Source of Variation	Sum of Squares	Variance Components	Percentage of Variation
Among Populations	197.718	1.58601	83.34
Within Populations	45.329	0.31699	16.66

Table 7
Mismatch distribution parameters, Tajima's D and Fu's FS neutrality tests of GFP from six different populations

	Tajima's D Test		Fu's FS Test		Mismatch Distribution (95% Confidence Intervals)	
Populations	Tajima's D	Tajima's D p-value	Fu's FS	Fu's FS p-value	r	SSD
N. Sembilan	0.2384	0.6970	0.2037	0.4560	0.2416	0.0376
Perak	0.0000	1.0000	0.0000	N. A.	0.0000	0.0000
Pahang	-2.3796	0.0010*	-0.6216	0.3230	0.2585	0.0095
Selangor	-0.2484	0.3090	0.2301	0.3060	0.3475	0.3005
Kelantan	-1.9489	0.0080*	-0.7737	0.3190	0.1348	0.0148
P. Pinang	-0.4084	0.3690	-0.3448	0.2600	0.1776	0.0118
Mean	-0.7912	0.3973	-0.2177	N. A.	0.1934	0.0624
Standard Deviation	0.9992	0.3583	0.3903	N. A.	0.1091	0.1071

Note. *Indicates significant level $p < 0.05$. Tajima's D and Fu's Fs are both neutrality test values

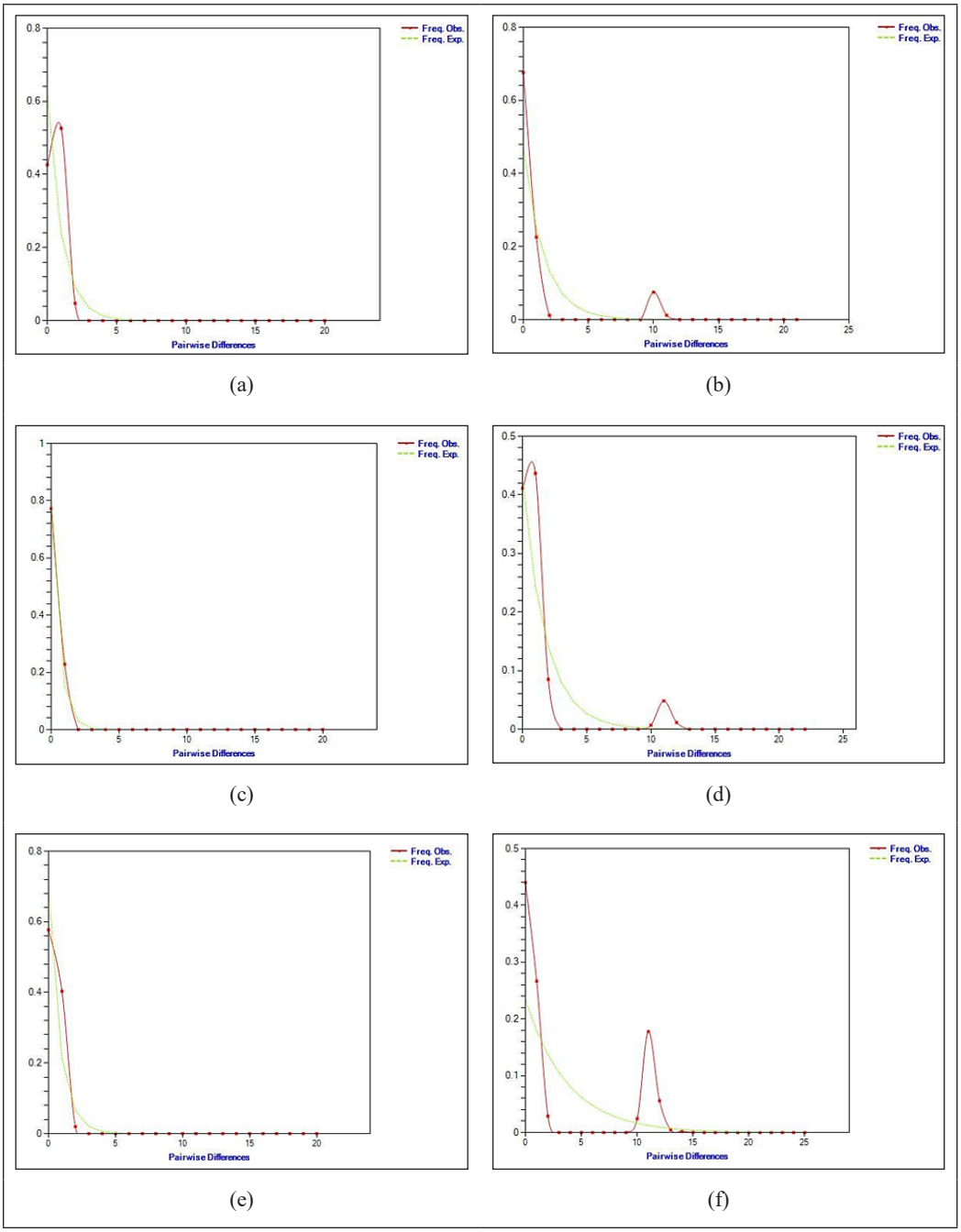

Figure 3. Mismatch distribution graphs of GFP for the: (a) N. Sembilan; (b) Pahang; (c) Selangor; (d) Kelantan; (e) P. Pinang; and (f) Total Populations

DISCUSSION

Phylogeography and Genetic Structure

The cytochrome c oxidase 1 (COX1) gene of the Giant Freshwater Prawn (*Macrobrachium rosenbergii*) was successfully sequenced from all samples collected from six distinct rivers across Malaysia. Among the populations examined, Sungai Selehong (Kelantan) exhibited the highest genetic variation. This variation was assessed through two primary genetic markers: nucleotide diversity (π) and haplotype diversity (h) (Senanan et al., 2015). Despite Sungai Selehong (Kelantan) population showing higher values of both π and h compared to the other populations, it was classified into the second category of population history, which is characterised by high haplotype diversity and low nucleotide diversity, suggesting a recent population expansion following a historical bottleneck (Grant & Bowen, 1998). Furthermore, the Java Island cluster, encompassing the Tabuk River and Peureulak populations, was assorted as a distinct group in genetics and became an isolated group, notably due to the significant genetic distance between populations. This fact is strengthened by the complete absence of shared haplotypes among the other four studied populations, which shows evidence of prolonged isolation and restricted gene flow. These are likely influenced by geographical separation and also the demographic history of the populations. These results emphasise the necessity for region-specific conservation and broodstock management to maintain the genetic integrity and adaptive potential of *M. rosenbergii* populations (Aliah et al., 2022).

A period of low effective population size, followed by a rapid expansion phase, is probably the cause of the separation. This result is consistent with the findings by Rogers and Herpending (1992), who found that accelerated population growth facilitated the retention of new mutations. This demographic signature generally features a dominant ancestral haplotype surrounded by peripheral haplotypes and often described as "tip" haplotypes in a star-like network. These peripheral haplotypes (Hap 11) represent recent mutational derivatives that differ by one or two nucleotide substitutions from the central, high-frequency haplotype (e.g. Hap 3). Given this, a phylogenetic analysis was constructed using a Neighbour-Joining (NJ) tree with high bootstrap support, and the results revealed that the new mutation, L2, was entirely composed of the unique haplotype Hap 11. This aligns with the previous assumption, thus within the L1 population, further examination of subclusters identified population-specific haplotypes, such as Hap 5, Hap 6 and Hap 7. Das et al. (2018) suggested that the emergence of these unique haplotypes is a result of the independent mutation events that occur within populations, increasing their genetic diversity.

Significant levels of both intra- and inter-population genetic variation were observed across the other five Malaysian river systems (Terengganu, Kelantan, Pahang, Selangor, and Negeri Sembilan), especially the Sungai Selehong (Kelantan) population, which

results demonstrate a high intra-population diversity and are also characterised by elevated nucleotide diversity (π) and haplotype diversity (h). This is in contrast to the genetic homogeneity observed in the Perak population, which shows otherwise. Moreover, this demographic signature aligns with a star-like phylogeny, where a high frequency of unique peripheral (tip) haplotypes (Hap 11) cluster around a central, ancestral haplotype (Hap 3). Thus, following a historical bottleneck, this pattern suggests a rapid population expansion, where accelerated growth facilitated the retention of new mutational derivatives. Contrary to previous findings, this study showed that the Perak population appears to be constrained by purifying selection and limited effective population size (Khan et al., 2021; Zhang et al., 2021), whereas the diversity in the other five rivers suggests a higher adaptive potential and a vigorous response to environmental changes. This result is consistent with that of a study by Whiteley et al. (2015), who reported that the gene flow through mechanisms such as genetic rescue initiatives and evolutionary rescue has been proposed as a strategy to maintain the viability of small, isolated populations. Despite this limitation, genetic rescue can help mitigate the effects of deleterious alleles that contribute to the genetic load in the gene pool, thereby promoting population growth, while evolutionary rescue enhances genetic variation, supporting long-term viability of *M. rosenbergii* in rivers across Peninsular Malaysia (Hoffmann et al., 2021).

In addition, genetic differences between haplotypes were found to be minimal, ranging from 0% to 0.2%, which suggests this may arise from mutations among closely related species. While the observed trend showed minimal genetic divergence between haplotypes, it was suggested that shallow evolutionary history, indicating that these lineages could possibly have only recently diverged from a common ancestor. Consequently, *M. rosenbergii* likely has a high degree of sequence similarity across different geographical locations in Peninsular Malaysia, and this reflects recent common ancestry or ongoing gene flow that has prevented significant genetic drift. Although significant genetic differentiation was observed between populations ($p<0.05$), the consistent variations in haplotypes, coupled with elevated Fst values, suggest minimal or no gene flow between geographically distant populations or those separated by extensive river systems. The lack of migration between these populations is further evidenced by the substantial population subdivision, which is consistent with the idea that migration plays a critical role in shaping genetic structure (Natasha et al., 2022).

Neutrality and Mismatch Distribution Tests

Neutrality tests and mismatch distribution are two effective tools used to study the demographic events in populations (Mousset et al., 2004). A stable population is typically indicated when the value of the neutrality test approaches zero. In addition, populations with negative and statistically significant neutrality test values are often considered to

have undergone historical expansion (Guo et al., 2023). Tajima's D test emphasises old mutations, while Fu's Fs test is more sensitive to recent mutation (Gunyakti Kilinc et al., 2020). The negative Tajima's D values observed in Pahang, Selangor, Kelantan, and Pulau Pinang suggest a history of population expansion, with an excess of rare nucleotide variants. The negative value observed in Fu's Fs test further supports this claim, except for the Selangor population, which displayed a positive value. These genetic patterns may be attributed to the founder effect (Dutta et al., 2020).

Significant negative Tajima's D values for Kelantan and Pahang populations were supported by the stable multimodal mismatch distribution graphs as shown in Figure 3, which suggest strong genetic subdivision and a stable population size, indicating demographic equilibrium (Gupta et al., 2018). While genetic hitchhiking could contribute to these patterns, the negative values in the neutrality tests seem indicative of purifying selection counterbalancing the genetic variation (Hills et al., 2008; Ruiz-Pesini et al., 2004). In contrast, unimodal mismatch distributions observed in the Selangor and Pulau Pinang populations rejected the neutrality test results. Therefore, this deviation may be due to recent population expansion events that may occur in these regions (Yan et al., 2023).

In Peninsular Malaysia, the characterisation of the phylogeography and genetic structure of *M. rosenbergii* offers a critical framework for transitioning from the traditional backyard aquaculture to a knowledgeable, genetically informed aquaculture. Therefore, by identifying the two distinct genetic clusters (L1 and L2) in this study, the results offer a baseline biological roadmap for the development of superior domestic broodstock, which farmers and industries could use as a guide. Consequently, breeders can leverage this spatial genetic information to implement strategic hybridisation of the species for production and potentially utilise heterosis to enhance commercially desirable traits such as growth rate, feed conversion efficiency, and disease resistance. Furthermore, the identification of unique haplotypes provides a molecular identification that allows for traceability and standardisation of specific lineages within closed-cycle hatchery systems, in turn reducing the industry's chronic reliance on unverified wild-caught postlarvae. This approach will assist in maintaining the genetic variations in the gene pool.

However, the findings still serve as an essential diagnostic tool beyond productivity for industry sustainability and risk mitigation. The observation from the Perak population highlights a significant vulnerability to inbreeding depression, possibly due to notably low genetic diversity in specific populations, which typically manifests as increased larval mortality, stunted and deformed growth and reduced environmental tolerance. In a world where climate change occurs annually, aquaculture management must prioritise the maintenance of genetic variance by avoiding the over-utilisation of broodstocks from bottlenecked populations and instead incorporating diverse genetic material from identified high-diversity population clusters to increase the heterozygosity and ensure long-term

viability. Future research should focus more on the development of climate-resilient strains that are capable of withstanding fluctuating water temperatures and emerging pathogens. This essential approach called genetic "insurance" is vital to realise the effort from shifting traditional aquaculture to develop a national breeding programme, which will harmonise conservation efforts by integrating phylogeographic insights with commercial objectives. This will ensure that the GFP will remain a robust and reliable food source despite the ever-changing ecological landscape.

CONCLUSION

The present study underscores the critical importance of understanding the genetic diversity and population structure of *Macrobrachium rosenbergii* populations for effective breeding programmes, aquaculture management and conservation. Generally, the findings revealed a significant genetic differentiation between two clusters (L1 and L2), likely driven by historical events. Notably, a major concern arising from this study is the low genetic diversity in populations such as Perak, which could pose a significant threat to their long-term viability. The identification of unique haplotypes and the observation of mutation accumulation provide valuable insights into the species' evolutionary processes. Concurrently, it should be noted that future research needs to prioritise phylogeographical studies and conservation strategies supporting the long-term resilience of *M. rosenbergii* populations to ensure the sustainability of this important species and enhance aquaculture breeding programmes.

ACKNOWLEDGEMENT

The completion of this research paper would not have been possible without the support and guidance from all the co-authors and Laboratory Staff of Genetics Lab, Faculty of Agriculture, Universiti Putra Malaysia and The International Institute of Aquaculture and Aquatic Sciences (I-AQUAS) at UPM, Port Dickson, Malaysia, providing the laboratory facilities and support, which the authors gratefully acknowledge.

FUNDING

This work is funded by Universiti Putra Malaysia (UPM) through the GP-IPB: Geran Putra Inisiatif Putra Bekumpulan with grant number GP-IPB/2020/9687901 (Pusat Kos: 9687901).

REFERENCES

Aliah, R. S., Sutanti, & Fetrilisa, S. (2022). Mitochondrial DNA diversity in four populations of Indonesian freshwater giant prawn (*Macrobrachium rosenbergii*). *Jurnal Bioteknologi & Biosains Indonesia (JBBI)*, *9*(1), 46-56. https://doi.org/10.55981/jbbi.2022.1785

Banu, R., & Christianus, A. (2016). Giant freshwater prawn *Macrobrachium rosenbergii* farming: A review on its current status and prospective in Malaysia. *Journal of Aquaculture Research & Development*, *7*(4), Article 1000423. https://doi.org/10.4172/2155-9546.1000423

Blaxter, M. (2016). Imagining Sisyphus happy: DNA barcoding and the unnamed majority. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1702). https://doi.org/10.1098/rstb.2015.0329

Das, S. P., Swain, S., Jena, J., & Das, P. (2018). Genetic diversity and population structure of *Cirrhinus mrigala* revealed by mitochondrial ATPase 6 gene. *Mitochondrial DNA Part A: DNA Mapping, Sequencing, and Analysis*, *29*(4), 495-500. https://doi.org/10.1080/24701394.2017.1310852

Dutta, N., Kumar, R., Abhinav, S., Vindhya, P., & Sangeeta, M. (2020). Mitochondrial DNA markers reveal genetic connectivity among populations of osteoglossiform fish *Chitala chitala*. *Molecular Biology Reports*, *47*(11), 8579-8592. https://doi.org/10.1007/s11033-020-05901-w

Excoffier, L., Laval, G., & Schneider, S. (2005). Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics*, *1*, 47-50. https://doi.org/10.1177/117693430500100003

Grant, W. S., & Bowen, B. W. (1998). Obituary: Professor Michael Laskowski Sr. *Acta Biochimica Polonica*, *30*(2), 113-114.

Gunyakti Kilinc, S., Celik, F., Kesik, H. K., & Simsek, S. (2020). In silico analysis of the biodiversity and conservation status of mitochondrial cytochrome C oxidase subunit 1 (CO1) gene of *Taenia multiceps*. *Acta Parasitologica*, *65*(4), 852-858. https://doi.org/10.1007/s11686-020-00236-9

Guo, J., Li, J., Massart, S., He, K., Francis, F., & Wang, Z. (2023). Analysis of the genetic diversity of two *Rhopalosiphum* species from China and Europe based on nuclear and mitochondrial genes. *Insects*, *14*(1), Article 57. https://doi.org/10.3390/insects14010057

Gupta, S. K., Kumar, A., Angom, S., Singh, B., Ghazi, M. G. U., Tuboi, C., & Hussain, S. A. (2018). Genetic analysis of endangered hog deer (*Axis porcinus*) reveals two distinct lineages from the Indian subcontinent. *Scientific Reports*, *8*(1), 1-12. https://doi.org/10.1038/s41598-018-34482-9

Hebert, P. D. N., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, *270*(1512), 313-321. https://doi.org/10.1098/rspb.2002.2218

Hills, T., Nyakaana, S., Tumusiime, C., Oguge, N., Siegismund, H. R., Arctander, P., & Muwanika, V. (2008). Mitochondrial DNA diversity and population structure of *Praomys taitae* (Rodentia: Muridae) Heller 1911, in the fragmented forest patches of Taita Hills, Kenya. *Journal of Zoology*, *276*(4), 499-504. https://doi.org/10.1590/S0038-23532008000600027

Hoffmann, A. A., Miller, A. D., & Weeks, A. R. (2021). Genetic mixing for population management: From genetic rescue to provenancing. *Evolutionary Applications*, *14*(3), 634-652. https://doi.org/10.1111/eva.13154

Khan, A., Patel, K., Shukla, H., Viswanathan, A., van der Valk, T., Borthakur, U., Nigam, P., Zachariah, A., Jhala, Y. V., Kardos, M., & Ramakrishnan, U. (2021). Genomic evidence for inbreeding depression and

purging of deleterious genetic variation in Indian tigers. *Proceedings of the National Academy of Sciences*, *118*(49), Article e2023018118. https://doi.org/10.1073/pnas.2023018118

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, *33*(7), 1870-1874. https://doi.org/10.1093/molbev/msw054

Li, L., Zheng, B., & Liu, L. (2010). Biomonitoring and bioindicators used for river ecosystems: Definitions, approaches and trends. *Procedia Environmental Sciences*, *2*, 1510-1524. https://doi.org/10.1016/j.proenv.2010.10.164

Mitra, G., Chattopadhay, D. N., & Mukhopadhay, P. K. (2005). Nutrition and feeding in freshwater prawn (*Macrobrachium rosenbergii*) farming. *Aqua Feeds: Formulation & Beyond*, *2*(1), 7-9.

Mousset, S., Derome, N., & Veuille, M. (2004). A test of neutrality and constant population size based on the mismatch distribution. *Molecular Biology and Evolution*, *21*(4), 724-731. https://doi.org/10.1093/molbev/msh066

Natasha, J., Stockwell, B. L., Marie, A. D., Hampton, J., Smith, N., Nicol, S., & Rico, C. (2022). No population structure of bigeye tunas (*Thunnus obesus*) in the Western and Central Pacific Ocean indicated by single nucleotide polymorphisms. *Frontiers in Marine Science*, *9*, 1-11, Article 799684. https://doi.org/10.3389/fmars.2022.799684

Oba, Y., Ôhira, H., Murase, Y., Moriyama, A., & Kumazawa, Y. (2015). DNA barcoding of Japanese click beetles (Coleoptera, Elateridae). *PLoS ONE*, *10*(1), 1-16, Article e0116612. https://doi.org/10.1371/journal.pone.0116612

Rogers, A. R., & Harpending, H. (1992). Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, *9*(3), 552-569.

Ruiz-Pesini, E., Mishmar, D., Brandon, M., Procaccio, V., & Wallace, D. C. (2004). Effects of purifying and adaptive selection on regional variation in human mtDNA. *Science*, *303*(2004), 223-226. https://doi.org/10.1126/science.1088434

Senanan, W., Pechsiri, J., Sonkaew, S., Na-Nakorn, U., Sean-in, N., & Yashiro, R. (2015). Genetic relatedness and differentiation of hatchery populations of Asian seabass (*Lates calcarifer*) (Bloch, 1790) broodstock in Thailand inferred from microsatellite genetic markers. *Aquaculture Research*, *46*(12), 2897-2912. https://doi.org/10.1111/are.12442

Stern, R. F., Horak, A., Andrew, R. L., Coffroth, M. A., Andersen, R. A., Küpper, F. C., Jameson, I., Hoppenrath, M., Véron, B., Kasai, F., Brand, J., James, E. R., & Keeling, P. J. (2010). Environmental barcoding reveals massive dinoflagellate diversity in marine environments. *PLoS ONE*, *5*(11), Article e13991. https://doi.org/10.1371/journal.pone.0013991

Strüder-Kypke, M. C., & Lynn, D. H. (2010). Comparative analysis of the mitochondrial cytochrome c oxidase subunit 1 (CO1) gene in ciliates (Alveolata, Ciliophora) and evaluation of its suitability as a biodiversity marker. *Systematics and Biodiversity*, *8*(1), 131-148. https://doi.org/10.1080/14772000903507744

Sundberg, P., Kvist, S., & Strand, M. (2016). Evaluating the utility of single-locus DNA barcoding for the identification of ribbon worms (Phylum Nemertea). *PLoS ONE*, *11*(5), 1-17, Article e0155541. https://doi.org/10.1371/journal.pone.0155541

Tavares, E. S., & Baker, A. J. (2008). Single mitochondrial gene barcodes reliably identify sister-species in diverse clades of birds. *BMC Evolutionary Biology*, *8*(1), 1-14, Article 81. https://doi.org/10.1186/1471-2148-8-81

Trivedi, S., Aloufi, A. A., Ansari, A. A., & Ghosh, S. K. (2016). Role of DNA barcoding in marine biodiversity assessment and conservation: An update. *Saudi Journal of Biological Sciences*, *23*(2), 161-171. https://doi.org/10.1016/j.sjbs.2015.01.001

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. N. (2005). DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *360*(1462), 1847-1857. https://doi.org/10.1098/rstb.2005.1716

Whiteley, A. R., Fitzpatrick, S. W., Funk, W. C., & Tallmon, D. A. (2015). Genetic rescue to the rescue. *Trends in Ecology & Evolution*, *30*(1), 42-49. https://doi.org/10.1016/j.tree.2014.10.009

Yan, S., Ma, P., Zuo, C., Zhu, Y., Ma, X., & Zhang, Z. (2023). Genetic analysis based on mitochondrial *nad2* gene reveals a recent population expansion of the invasive mussel, *Mytella strigata*, in China. *Genes*, *14*(11), Article 2038. https://doi.org/10.3390/genes14112038

Yu, L., Zhu, X., Liang, J., Fan, J., & Chen, C. (2019). Analysis of genetic structure of wild and cultured giant freshwater prawn (*Macrobrachium rosenbergii*) using newly developed microsatellite. *Frontiers in Marine Science*, *6*, 1-9, Article 323. https://doi.org/10.3389/fmars.2019.00323

Zheng, Y., Guo, G., Lv, Y., Gao, Q., Zhou, D., Zhang, L., Tu, G., Weng, S., Li, C., He, J., & Wang, M. (2024). A chromosome-level genome assembly of giant river prawn (*Macrobrachium rosenbergii*). *Scientific Data*, *11*(1). https://doi.org/10.1038/s41597-024-03804-0

Zhang, L., Sun, K., Csorba, G., Hughes, A. C., Jin, L., Xiao, Y., & Feng, J. (2021). Complete mitochondrial genomes reveal robust phylogenetic signals and evidence of positive selection in horseshoe bats. *BMC Ecology and Evolution*, *2*, 1-15 Article 199. https://doi.org/10.1186/s12862-021-01926-2

Zhao, Y., Gentekaki, E., Yi, Z., & Lin, X. (2013). Genetic differentiation of the mitochondrial cytochrome oxidase c subunit I gene in genus *Paramecium* (Protista, Ciliophora). *PLoS ONE*, *8*(10), Article e77044. https://doi.org/10.1371/journal.pone.0077044