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Assessing genetic diversity of tiger shrimp *Penaeus monodon* Fabricius, 1798 utilising microsatellite DNA markers

P. V. Mini[1,2], K. S. Prithvisagar[3], Iddya Karunasagar[4] and Indrani Karunasagar[3]*

[1]Kannur University, Government College Kasargod, Vidyanagar, Kasaragod - 671 123, Kerala, India
[2]Department of Fishery Microbiology, College of Fisheries, Mangaluru - 575 002, Karnataka, India
[3]NITTE (Deemed to be University), Technology Enabling Centre, NITTE University Centre for Science Education and Research, Deralakatte, Mangaluru - 575 018, Karnataka, India
[4]NITTE (Deemed to be University), University Enclave, Medical Sciences Complex, Deralakatte, Mangaluru - 575 018, Karnataka, India

Abstract

The giant tiger shrimp, *Penaeus monodon* Fabricius, 1798 is a significant commercial shrimp species, yet limited information is available on the genetic makeup and diversity of its natural populations. As aquaculture industry relies on wild populations for breeding, understanding their genetic makeup is vital for resource management and diversity preservation. While selective breeding typically targets observable traits, incorporating genetic markers for traits like growth and disease resistance can improve breeding programs. In this study, highly variable microsatellite DNA markers, were used to analyse the genetic variation among *P. monodon* populations in India. The study found these markers to be highly polymorphic, enabling clear population differentiation and revealing significant genetic sub-division, including a marked distinction between east and west coast populations. Such polymorphic microsatellite loci are valuable tools for gene mapping and pedigree analysis in *P. monodon*.

*Correspondence e-mail:
indrani.karunasagar@nitte.edu.in

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Introduction

In the Asia-Pacific region, aquaculture has expanded both in scale and species diversity, with crustaceans contributing a significant share of production. In India, penaeid shrimps are the predominant group among cultured edible crustaceans (Thornber *et al.,* 2020), India exported 17,81,602 t of seafood valued at ₹60,523.89 crore (US$7.38 billion) during 2023-24. Frozen shrimp remained the dominant commodity in the export basket, contributing ₹40,013.54 crore (US$4.88 billion). It accounted for 40.19% of the total export volume and 66.12% of the overall dollar earnings, with export quantities reaching 7.16 lakh t during the year (https://mpeda.gov.in/?page_id=3447). The tiger shrimp *Penaeus monodon* Fabricius, 1798 (Decapoda: Penaeidae), is the second largest species used for shrimp farming in India and has been cultured in other tropical and subtropical regions of the world. With the availability of specific pathogen-free (SPF) broodstock, many Asian countries have switched to culture of *Penaeus vannamei*. Other significant commercial species include *P. chinensis, P. merguiensis* and *P. indicus*. Aquaculture often relies on wild populations of broodstock, to maintain and restore genetic diversity and therefore understanding natural populations is crucial for effective resource management and conservation (Kobayashi *et al.,* 2025). Genetic diversity in natural populations helps identify suitable stocks for selective breeding and must be well understood before initiating cultivation. According to Erckmane and Sugunan (2003), genetic diversity is a key component of populations that contribute to both short-term fitness of individuals and long-term survival of populations by facilitating adaptation to shifting environmental conditions. Species with considerable genetic diversity are more likely to exhibit rapid growth, stable development, high viability and fecundity as well as greater resistance to disease and environmental stress. Therefore, for any breeding management and conservation program to be successful, understanding the genetic origin and population structure of the species is crucial (Robledo *et al.,* 2024). However, aquaculture production of *P. monodon* proved to be unsustainable due to a lack of biological and genetic knowledge needed to achieve desirable traits such as fast growth, disease resistance and captive reproductive maturation without reliance on wild brooders (Guppy *et al.,* 2018). Basic knowledge of the genetic loci responsible for viral resistance, immunological responses, and rapid growth in shrimp is missing. Identifying loci linked to key traits through linkage and quantitative trait locus (QTL) mappingis essential to speed up genetic improvement in shrimp breeding programs (Sui *et al.,* 2024). Assessing genetic variations within and across populations is a critical first step towards the genetic improvement of this species. Knowledge about the genetic diversity and population differentiation is vital for designing effective genetics-based stock enhancement programs, which require reliable population markers to accurately determine the origin of each shrimp (Mandal *et al.,* 2012).

Effective resource utilisation in fisheries requires management at the population level, making identification and characterisation of population units essential. However, limited techniques for determining genetic variation, have often led fisheries management to rely on physical traits (Wong *et al.,* 2021). Developing efficient molecular methods is therefore crucial for evaluating genetic diversity among stocks. Molecular genetic markers enable direct assessment of genetic variation and distribution, serving as a powerful tools to define the genotypes and predict performance in animal breeding. DNA markers allow direct assessment and utilisation of genetic variation across the entire genome. Key advantage of DNA-based research is that genetic diversity can be directly evaluated at the most fundamental level, before intracellular processes such as gene transcription, RNA splicing, protein translation and post-translational modifications alter expression patterns. Another benefit is the ability to access nearly all of an organism's genetic material for analysis. Common genetic markers used in the aquaculture include, allozymes, mitochondrial DNA (mtDNA), restriction fragment length polymorphism (RFLP), single nucleotide polymorphism (SNP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellites and expressed sequence tag (EST) markers.

Simple sequence repeats (SSR), often known as microsatellites, are a special class of tandemly repeated polymorphic sequences that are widely distributed throughout genomes and exhibit significant levels of allelic polymorphism. These repeats which can occur in tens to hundreds of copies show extreme variability in both plants and animals (Sigang *et al.,* 2021). Microsatellites are classified as mono, di, tri, tetra, penta and hexa nucleotide repeats, with minimum repeat thresholds of >10 for mononucleotides, 6 for dinucleotides, 4 for trinucleotides, and 3 for tetra- to hexa-nucleotides (Estoup *et al.,* 1993), They occur as perfect (continuous repeat units), imperfect (interrupted by 1–3 bases), or compound (multiple repeat types separated by <3 bases) forms. (Maneeruttanarungroj *et al.,* 2006). Informativeness of microsatellite markers is known to rise with the number of repeats (Bull *et al.,* 1999). Because of their high variability, SSRs are valuable molecular markers for diverse genetic studies, including population genetics, pedigree verification, and stock management (Maneeruttanarungroj *et al.,* 2006). In shrimps, microsatellite markers have proven particularly effective for pedigrees confirmation and for assessing genetic diversity in hatchery populations (Wong *et al.,* 2021). Microsatellite DNA markers have successfully distinguished *P. monodon* broodstocks from various Indian coastal regions with varying sensitivity to WSSV (Chakrabarty *et al.,* 2015).

Microsatellites are valuable tools for forensic and ancient DNA investigations in population genetics and conservation, but a key drawback is that most species require *de novo* isolation of microsatellites for initial analysis. The two main techniques for isolating microsatellites are genomic library-based methods and mining microsatellites from nucleotide sequencing data, with the latter being more effective (Moniruzzaman *et al.,* 2015). In the coming decades, quantitative and molecular genetics are expected to dominate animal breeding, where genetic evaluation traditionally relies on phenotypic analysis, while molecular genetics begins with examining known alleles or DNA sequences and their effects on phenotypes.

Materials and methods

Wild shrimp (*P. monodon*) samples were collected from trawling grounds off Karnataka (Mangalore) and Kerala (Calicut and Kannur) on the west coast and from Andhra Pradesh (Kakinada) and Tamil Nadu (Nagapattinam) on the east coast of India. DNA was isolated using phenol chloroform method as described by Tassanakajon *et al*. (1997). The stability and integrity of DNA was established by resolving the extracted DNA on a 0.8% gel. The concentration of DNA was adjusted to 25 ng μl^{-1} and stored at 4°C to be used as template for PCR. Extracted DNA was subjected to microsatellite PCR using 8 primers namely Pm2.41, Pm4.1, Pm 4.38, Pm4.45, Pm4.53, Pm4.7, Pm4.82 and Pm4.85 (Table 1). The products were resolved in 2% agarose gels. The total number of alleles was counted from the total number of individuals studied for a particular locus. Genetic distance, observed and expected heterozygosities were calculated with software POPGENE version 1.31 (Yeh *et al*., 1999). Departures from Hardy-Weinberg equilibrium (HWE) at each population were tested and a global test of heterozygote deficit across loci and across populations was conducted by GENEPOP Version 3.3 (Raymond and Rousset, 1995). The Markov chain method was employed to estimate the probability of significant deviation from HWE using GENEPOP with the following parameters: Dememorisation=1000, Batches=100 and Iterations = 1000. Critically significant levels for the test were adjusted using a sequential Bonferroni approach. The Wright's F statistics, Pairwise F_{ST} and R_{ST} values between all populations were also calculated with GENEPOP. Using the PHYLIP Version 3.5 Fitch Programme, a phylogenetic tree of the populations was created based on genetic distance (Felsenstein, 1993).

Results

All eight of the microsatellite primers exhibited a substantial degree of polymorphism. Fig. 1 and 2 show the gel patterns produced by these primers and Table 2 lists the total number of alleles detected at each locus. Among the loci, Pm4.85 displayed the highest allelic diversity with 18 observed alleles, while Pm4.45 had the lowest with 7 alleles. Allelic diversity reflects the genetic variability within a population. One key aspect of this variability is allelic richness,

Table 1. Sequence and annealing temperatures of microsatellite primers

Sl. No.	Primer code	Primer sequence	Annealing temperature (°C)	References
1	Pm 4.82F Pm 4.82R	5'-ATTCATCAGCTAGCCTTG 5'-CGTTTACTGCATTCACTACC	52	Xu *et al*. (1999)
2	Pm 2.41F Pm 2.41R	5'-AAGGCAGATTTTCTAGCC 5'-ATCAAGGGAGACATTCAG	52	Xu *et al*. (1999)
3	Pm 4.85F Pm 4.85R	5'-CTTCGGCGGAAATATGTG 5'-TTGTGTTTGTGCGAGTGC	56	Xu *et al*. (1999)
4	Pm 4.45F Pm 4.45R	5'-ATCTCTACCAACCTGTCAGC 5'-TTAGTGAACCCCCTTCGTG	48	Xu *et al*. (1999)
5	Pm 4.1F Pm 4.1R	5'-AAAACGGCGGTTGCTTCTC 5'-CCTCGGTATGGTGATGACATG	52	Xu *et al*. (1999)
6	Pm 4.7F Pm 4.7R	5'-TCACTCTTTCTGTCGCTGTC 5'-TACGCTCGTGCAATGTTTG	52	Xu *et al*. (1999)
7	Pm 4.38F Pm 4.38R	5'-CTCATATTCAGAGCAACGAC 5'-CCCGTGTGCATCATTATC	52	Xu *et al*. (1999)
8	Pm 4.53 F Pm 4.53R	5'-GTTAAAGGTCAGAGGACACAAG 5'-TCAAACATGAGCACACCG	52	Xu *et al*. (1999)

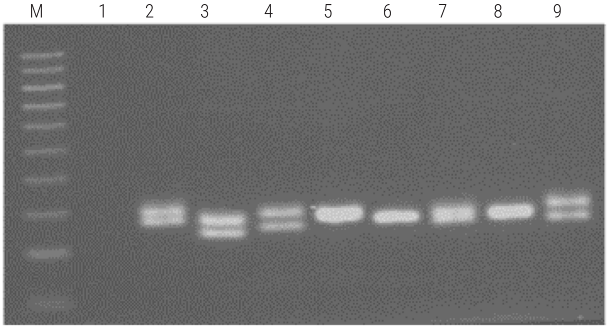

Fig. 1. Microsatellite pattern generated by Pm 2.41 with populations of *P. monodon.* Lane M: Molecular weight marker; Lane 1: Negative control; Lanes 2-9: *P. monodon* from Kakinada

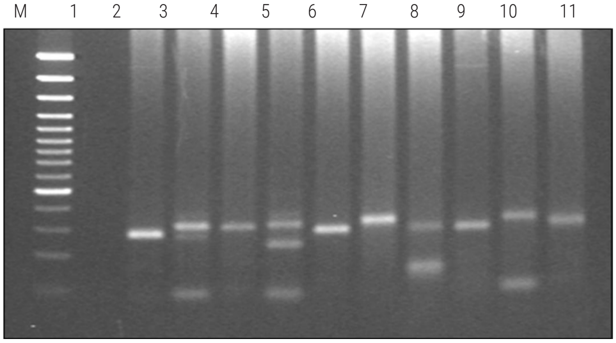

Fig. 2. Microsatellite pattern generated by Pm 4.85 with populations of *P. monodon.* Lane M: Molecular weight marker; Lane1: Negative control; Lanes 2-11: *P. monodon* from Calicut

Table 2. Total number of observed alleles for each locus

Sl. No	Loci	Observed number of alleles	Mol. wt (bp)
1	2.41	13	214-310
2	4.1	12	128-210
3	4.38	12	138-195
4	4.45	7	250-392
5	4.53	13	244-400
6	4.7	10	184-245
7	4.82	10	133-196
8	4.85	18	220-382

defined as the average number of alleles per locus. In this study, the average number of detected alleles per locus ranged from 5.0 (Pm4.45) to 8.6 (Pm2.41). The mean number of alleles per locus was 7.75 in Calicut and 5.5 in Kannur and across population with an overall average of 6.65 alleles per population and locus. The highest number of genotypes was observed in the Calicut population and at locus Pm4.82, while the lowest values were recorded in the Kannur population and at locus Pm 4.45. Among the markers Pm4.82 produced the greatest number of alleles. Gene diversity, also known as population heterozygosity (H_0), a widely used indicator of genetic variation was also assessed in this study. The observed heterozygosity (H_0) measures the proportion of heterozygous individuals at a given locus. Among the populations, Nagapattinam exhibited the highest average H_0 (0.4070), while Mangalore showed the lowest (0.1252). Among the loci, the highest average H_0 was recorded at Pm4.53 (0.5827) and the lowest at Pm4.7 (0.0405) (Table 3).

Expected heterozygosity (H_E) calculated from the estimated allele frequencies of each population, ranged from the lowest of 0.7555 in Kannur to the highest of 0.7933 in Calicut. Across loci, the average H_E values varied from 0.7768 to 0.8815, with pm4.53 showing the

Table 3. Average observed (H_0) and expected (H_E) heterozygosity values for the populations analysed

Population	Average H_0	Average H_E
Calicut	0.2922	0.7933
Kannur	0.3284	0.7555
Mangalore	0.1252	0.7708
Kakinada	0.2607	0.7590
Nagapattinam	0.4070	0.7727

highest locus-specific value. When comparing the observed (H_O) and expected (H_E) heterozygosities. Nagapattinam exhibited the highest average H_O, while Mangalore recorded the lowest, while H_E was lowest in Kannur and highest in Calicut (Table 3). Tests for Hardy-Weinberg equilibrium (HWE), conducted using the sequential Bonferroni method, revealed significant departures in 37 of the 40 comparisons. With the exception of Kannur and Kakinada at locus Pm 4.53 and Nagapattinam at Pm 4.45, all populations at each locus exhibited a heterozygote deficit. Global tests, both among populations within a locus and between populations within a locus, also revealed significant deviations from HWE ($p<0.05$) (Table 4).

F_{ST} values were calculated to evaluate genetic differentiation, reflecting differences in allele frequencies among populations. At all eight loci, the F_{ST} data revealed significant genetic differentiation among the five *P. monodon* populations, with an overall F_{ST} value of 0.0857. Except for loci Pm4.45 and Pm4.7, which are associated with inbreeding at the subpopulation level, all loci showed strong population-level genetic divergence, due to heterozygous deficiency in one or more populations, The positive F_{IS} value (0.6635) further indicated a marked deficit of heterozygotes within populations across all loci, consistent with heterozygous deficiency in one or more populations (Table 5).

Pairwise F_{ST} and R_{ST} analyses were used to estimate the levels of population differentiation. While Wright's F_{ST} measures allele frequency differences, R_{ST} incorporates allelic size variance into its computations. Both indices revealed significant genetic differentiation among all population pairs, with the strongest separation observed between Nagapattinam and the Kannur (Table 6). Estimated average genetic distances between population pairs ranged from 0.2247 (between Mangalore and Kakinada) to 0.6360 (between Mangalore and Nagapattinam) (Table 7). The genetic distance-based UPGMA dendrogram clearly separated east and west coast populations. Nagapattinam and Kakinada formed a distinct east coast cluster, whereas on the west coast, Calicut and Mangalore clustered first and then joined by Kannur (Fig. 3).

Table 4. The p values for estimating the probability of significant deviation from HWE

HWE	Calicut	Kannur	Mangalore	Kakinada	Nagapattinam
4.85	**0.0000** **(0.0000)**	**0.0184** **(0.0004)**	**0.0000** **(0.0000)**	**0.0000** **(0.0000)**	**0.0003** (0.2503)
4.82	**0.0000** **(0.0000)**	**0.0004** **(0.0000)**	**0.0000** **(0.0000)**	**0.0000** **(0.0000)**	**0.0000** **(0.0000)**
2.41	**0.0000** **(0.0000)**	**0.0000** **(0.0000)**	**0.0000** **(0.0000)**	**0.0000** **(0.0000)**	**0.0002** **(0.0076)**
4.70	**0.0000** **(0.0000)**	**0.0000** **(0.0000)**	**0.0000** **(0.0000)**	**0.0000** **(0.0000)**	**0.0000** **(0.0000)**
4.53	**0.0001** **(0.0000)**	0.8842 (0.8010)	**0.0000** **(0.0000)**	0.0807 (0.0277)	**0.0007** **(0.0008)**
4.38	**0.0000** **(0.0000)**	**0.0000** **(0.0039)**	**0.0000** **(0.0000)**	**0.0000** **(0.0000)**	**0.0000** **(0.0000)**
4.10	**0.0000** **(0.0000)**	**0.0000** **(0.0014)**	**0.0000** **(0.0000)**	**0.0000** **(0.0000)**	**0.0000** **(0.0000)**
4.45	**0.0000** **(0.0042)**	**0.0014** **(0.0089)**	**0.0000** **(0.0000)**	**0.0000** **(0.0000)**	0.6677 (0.8587)

Number in parentheses indicates the probability (p) of significant heterozygosity deficiency. Significant p ($p<0.05$) values are in boldface.

Table 5. F Statistics of all the populations in each locus

Locus	F_{IS}	F_{ST}
4.85	0.5058	0.0817
4.82	0.7351	0.0857
2.41	0.6219	0.0492
4.70	0.9114	0.0400
4.53	0.3104	0.0673
4.38	0.9115	0.1880
4.10	0.6860	0.1400
4.45	0.6214	0.0372
All:	0.6635	0.0857

Discussion

The geographic distribution of genotypes that define subspecies, races, or stocks is closely related to the genetic variation within a species. This distribution helps distinguish DNA-based variation from phenotypic differences that may arise as a result of population-level adaptation and can be effectively detected using molecular markers. Such markers may offer valuable tools to aid in breeding programs, as they can assist in selection of individuals with desirable traits such as enhanced growth rates or greater disease resistance for use as bloodstock or seed stock.

Co-dominant markers such as microsatellites are highly informative in determining genetic relatedness among germplasm pools. This approach offers a powerful framework for examining the substructure of closely related populations, particularly those collected from small geographic areas with limited isolation. Because microsatellites follow Mendelian inheritance, their polymorphism can be used to estimate key population genetic

Table 6. Pair-wise F_{ST} and R_{ST} among populations of *P. monodon*

	Calicut	Kannur	Mangalore	Kakinada	Nagapattinam
Calicut		0.1088	0.0831	0.0772	0.3192
Kannur	0.0955		0.0559	0.0087	0.3480
Mangalore	0.0799	0.0694		0.0765	0.3385
Kakinada	0.0922	0.0434	0.0405		0.3048
Nagapattinam	0.1024	0.1141	0.1057	0.0960	

R_{ST} (Slatkin, 1995) values are above the diagonal and F_{ST} (Ø) (Weir and Cockerham, 1984) values are below the diagonal

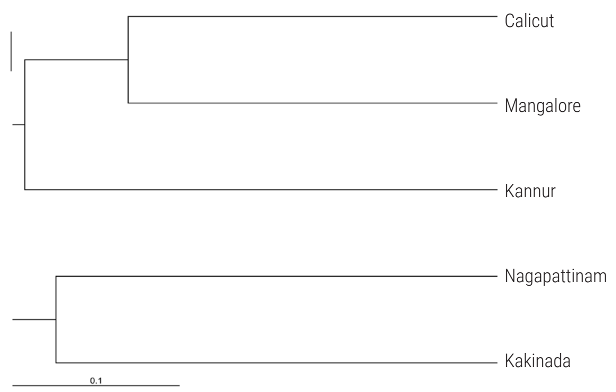

Fig. 3. Dendrogram derived from Nei's genetic distance depicting the genetic distance among west coast and east coast populations

Table 7. Genetic distance among populations of *P. monodon*

Pop ID	1	2	3	4	5
1	****				
2	0.6013	****			
3	0.3716	0.5492	****		
4	0.4345	0.3068	0.2247	****	
5	0.6151	0.5507	0.6360	0.5303	****

*Pop1-Calicut, Pop2-Kannur, Pop3-Mangalore, Pop4-Kakinada, Pop5-Nagapattinam

parameters, like allele frequency, heterozygosity, Hardy-Weinberg equilibrium, genetic distance, inbreeding coefficients and the extent of subpopulation mixing in natural waters. In genetic surveys, the number of alleles per locus, the range of allele sizes and the degree of population differentiation at each locus are especially important. In this study, all eight selected primer sets successfully amplified microsatellite loci and revealed polymorphism across all *P. monodon* populations examined.

Allele frequency and the mean number of alleles per locus (allelic richness) are key indicators of genetic variation. In this study, the number of alleles per locus ranged from 7 to 18, which is lower than values reported for *P. monodon* from other regions (Benzie, 2000; Xu *et al.*, 2001). Previous microsatellite surveys revealed 14 to 28 alleles at 2 loci (Supungul *et al*., 2000) and 19 to 30 alleles at 5 loci (Tassanakajon, *et al*., 1998b) in *P. monodon* from Thailand and 5 to 12 alleles per locus for *Metapenaeus affinis* from Persian Gulf (Shokoohmand, *et al.,* 2018). Despite this comparatively moderate allele variation, the microsatellite loci observed in this study showed enough resolution to clearly differentiate populations. This is an advantage over highly polymorphic loci that require larger sample sizes for accurate analyses. Among the loci, Pm2.41 exhibited the highest mean number of observed alleles per locus (8.6), while among populations Calicut showed the highest mean allelic richness (7.75). Overall, the mean number of alleles per locus per population was 6.65 and all the eight loci showed significant difference in allele frequency distributions across populations.

Heterozygosity (H_O), is widely used by ecologists and aquaculturists as a general indicator of genetic diversity. However, this metric has several shortcomings, particularly in fully capturing the spectrum of genetic variation (Kanaka *et al.,* 2023). In the present study, Nagapattinam showed the highest observed heterozygosity, whereas Mangalore had the lowest. Among the loci, Pm4.53 exhibited the greatest average H_O (0.5827), whicle Pm4.7 had the lowest (0.0405). Because populations with many alleles at low frequencies, typically exhibit high genetic diversity, the expected heterozygocity (H_E) for unrelated individuals approaches 1. Here, the average H_E across the eight loci was 0.8356, indicating a high proportion of genetically diverse and unrelated individuals. This contrast sharply with the much lower average heterozygosity (7.5%) reported in crustaceans using allozyme markers (Ali *et al.,* 2024), highlighting the greater sensitivity of microsatellite loci in detecting genetic variability. Despite this high $H_{E.}$ a heterozygosity deficit, the difference between the expected and observed heterozygosity, was evident across populations, a pattern frequently reported in aquatic species using microsatellite markers (Gonzalez-Castellano *et al.,* 2018). Importantly, high genetic diversity should not be interpreted as a direct indicator of high genetic diversity. In this study. Statistically a reduced number of alleles and genotypes, both critical measure of genetic variation, signaled a decline in overall genetic diversity (Xu *et al.,* 2001). Notably the Nagapattinam population, although exhibiting the highest $H_{O,}$ also showed the fewest alleles and genotypes per locus.

When there is no discernible difference between H_O and H_E, a population is considered to follow the Hardy-Weinberg rule. In the present study, however nearly all tests (37 of 40) showed high significant departures from HWE, primarily due to an excess of homozygotes, at most loci and across populations. Similar deviations from HWE have been reported for *P. monodon* populations from Thailand (Supungul *et al.,* 2000) and the Philippines (Xu *et al.,* 2001), as well as for several loci in other penaeid shrimps such as *Farfantepenaeus californiensis* and *Litopenaeus stylirostris,* which also exhibited allozyme based heterozygote deficits (de la Rosa *et al.,* 2000). Several factors have been proposed to explain such heterozygote deficits, including null alleles, technical artefacts, population mixing (Wahlund effect), mixing of cohorts (the "family" Wahlund effect) among others (Castric *et al.,* 2002). Inbreeding is an unlikely explanation in this case, because true inbreeding would be expected to produce linkage disequilibrium (non-random association of alleles across loci), which was not detected (Castric *et al.,* 2002). Null alleles, or the inability of an individual to amplify one of the alleles, or mis-scoring as a result of stutter bands, could also account for heterozygote deficits (Rico *et al.,* 2017). Although null alleles are typically expected at a single location in all populations, this study detected evidence for their presence practically at all loci. The possibility of null alleles cannot be excluded, especially since the analysis of a null-allele corrected data set in *P. monodon* (Xu *et al.,* 2001) was shown to reduce the number of departures from HWE. Technical artefacts such as shadow or stutter bands may also result in the incorrect classification of heterozygotes, particularly those with closely spaced alleles as homozygotes, thereby inflating homozygote counts and producing deviations from Hardy-Weinberg expectations (Morin *et al.,* 2009). However, in the present study, the allele scoring was based on the most intense and clearly resolved bands, which should minimise errors in genotype assignment despite the potential influence of stutter bands. Each geographic sample may contain adults originating from multiple breeding stocks, due to the extensive transplantation of *P. monodon* larvae that occurs every year. Wild *P. monodon* females are capable of spawning multiple times per year, producing 0.2 to 8 million eggs per spawning, which may be sufficient to replenish substantial portions of the adult population. According to Hedgecock (1994), such large reproductive output can lead to "sweepstakes recruitment" where only a few highly successful breeders contribute disproportionately to the next generation, creating family effects that disrupt Hardy-Weinberg equilibrium in marine organisms. While this mechanism has been proposed to explain homozygote excess in the shrimp *P. monodon* (Supungul *et al.,* 2000) and in the fish *Clupea pallasi* (O'Connell *et al.,* 1998), the present data do not allow a definitive conclusion about which hypothesis best accounts for the observed Hardy-Weinberg disequilibrium in *P. monodon*.

The proportion of total genetic variation that exists within populations and areas among geographic regions can be quantified using genetic diversity metrics such as Wright's F statistics or Nei's genetic diversity statistics (Nei, 1987). When compared to allozymes, microsatellites reveal a finer level of population substructure in *P. monodon* (Wong *et al.,* 2021). Among these measures, F_{ST} method is widely used to assess the degree of genetic differentiation between populations. An F_{ST} value of 1 suggests complete separation, where no alleles are shared at polymorphic loci, whereas an F_{ST} estimate of 0 indicates no detectable geographic structure, implying that the sampled individuals may represent a single panmictic population. The *P. monodon* populations analysed showed significant genetic differentiation, with an overall F_{ST} value of 0.0857. The F_{IS} statistic, which measures inbreeding within subpopulations, was strongly positive (0.6635), indicating a pronounced heterozygote deficit and substantial reduction in the average proportion of heterozygous genotypes across all loci. The pair wise F_{ST} and R_{ST} comparisons further confirmed that this genetic structure is present within each geographic zone, supporting the view that each area represents a distinct population unit. Among all regions, the Nagapattinam population displayed the highest level of genetic differentiation, reflected in its high F_{ST} and R_{ST} values.

The estimated average genetic distances between population pairs ranged from 0.2247 (between Mangalore and Kakinada) to 0.6360 (between Mangalore and Nagapattinam). A genetic-distance based dendrogram clearly separated populations from the east and west coasts, reflecting distinct regional groupings. These patterns imply that long-term gene flow even over thousands of years, has not been sufficient to eliminate the genetic variations that has accumulated through geographic separation.

Factors such as allelic size range, the number of alleles and the likelihood of population differentiation at a locus are important when selecting markers for microsatellite surveys. Beacham *et al.* (2002) reported that loci with 10 or fewer alleles achieved only about 30% of the identification accuracy obtained with loci containing more than 10 alleles. Generally, the ability of individual identification increases with the number of alleles present at a locus. In the present study, all loci, with the exception of one, produced 10 or more alleles and showed clear polymorphism, with each primer set generating a large number of genotypes. This high level of polymorphism provided the flexibility to choose the best locus suited for a given application. For example, highly polymorphic loci with lots of alleles are perfect for pedigree analysis, gene mapping and other fine-scale genetic studies. Overall, the findings of this study demonstrate that the black tiger shrimp, *P. monodon,* contains highly polymorphic microsatellite markers suitable for population genetic analysis and selective breeding programs. A consistently high F_{ST} value across loci highlights the strong genetic differentiation observed among *P. monodon* populations. Although microsatellites with relatively low within-population variation can still be useful for analysing genetic diversity between populations, such loci are less effective for parentage analysis or relatedness estimation due to their limited discriminatory power. Microsatellites are the preferred markers for population studies because of their high sensitivity, reproducibility, capacity to identify all alleles within a population, and inherently high variability at each locus. These features provide substantially greater information per unit test, compared with other marker systems, making them valuable for both fundamental and applied research in population genetics. Such data can guide strategies for the sustainable exploitation and management of *P. monodon* genetic resources (Orosco and Lluisma, 2018). Among the markers examined, locus Pm 2.41 stood first among the numerous microsatellite markers utilised in the current investigation, owing to its high and stable allele numbers, broad range of genotypes and balanced estimates of observed and anticipated heterozygosity, along with high F_{ST} and a low F_{IS}. These characteristics give Pm2.41 a strong discriminatory power to distinguish between populations.

To conclude, microsatellite markers are powerful and reliable tools for assessing the genetic diversity of tiger shrimp, *P. monodon*. Research using these markers has revealed substantial genetic variation within wild populations, often attributable to historical colonisation events and the presence of multiple geographic lineages (Aguirre-Pabon *et al.,* 2023). In contrast, hatchery-reared stocks generally exhibit reduced genetic diversity (Wong *et al.,* 2021), raising concerns about their long-term sustainability and resilience. The development and use of genome-wide SSR panels has further advanced our understanding of genome adaptation and plasticity (Vu *et al.,* 2021; Huerlimann *et al.,* 2022) and has proven valuable in selective breeding and disease resistance programs (Chakrabarty *et al.,* 2015). Overall, these findings highlight the crucial role of SSR markers in the management, conservation and genetic improvement of tiger shrimp stocks, supporting both sustainable aquaculture practices and preservation of natural genetic resources.

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