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Characterization of Geographically Isolated Stocks of *Lamellidens marginalis* (Lamarck, 1819) Using Microsatellite Markers

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Abstract

The Indian freshwater mussel, *Lamellidens marginalis*, is one of the key candidate species for pearl production in India. The conservation status of *L. marginalis* has been assessed as 'least concern', and the population trend of the species is not known. However, the increased anthropogenic activities such as overexploitation, release of industrial or agricultural waste, and construction of dams are posing threats to the natural stocks. For sustainable management of resources, knowledge of genetic stock structure is essential. Further, the genetic variation between the genetic stocks could manifest in the form of pearl quality. We analyzed the genetic structures of five populations collected from Tripura (2 locations), Maharashtra, Gujarat, and Odisha using three microsatellite (di-nucleotide SSR: 2; trinucleotide: 1) markers. Locus-wise, the number of alleles varied from 2 to 4, with an average of 2 alleles per locus. The mean observed and expected heterozygosity values varied from 0.253 to 0.345 and 0.459 to 0.638, respectively. All the stocks were in Hardy–Weinberg equilibrium, and no null alleles were observed. Pairwise F*ST* analyses showed moderate genetic differentiation among the stocks of Maharashtra, Odisha, and Gujarat. The loci showed moderate diversity within the stocks and revealed moderate to high genetic differentiation among the stocks.

Keywords:
Lamellidens marginalis; Genetic structure; Microsatellite loci; Genetic variation

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Introduction

The Indian freshwater mussel *Lamellidens marginalis* (Lamarck, 1819) is a key candidate species for freshwater pearl production in India (Janaki Ram, 2003). Taxonomically, it belongs to the family Unionidae under the order Unionida and is native to South Asia, including India, Bangladesh, Nepal, Myanmar, and Sri Lanka (Nesemann *et al*., 2007). This species thrives in a range of freshwater habitats, from stagnant ponds and lakes to slow-moving streams and rivulets. Unlike its congener *L. corrianus*, *L. marginalis* exhibits separate sexes and reaches first maturity at a length of 6.5 to 8 cm (Ghosh & Ghose, 1972). *L. marginalis* is known to spawn throughout the year (Misra *et al*., 2010; Behera *et al*., 2014), with optimal spawning occurring at water temperatures between 20 and 25°C (Gaikwad & Kamble, 2014).

L. marginalis primarily consumes algae, protozoa, bacteria, and suspended river sediments. Using its gills and labial palps to filter food, it also accumulates environmental contaminants such as heavy metals (Waldichuk, 1974) and pesticides (Minakshi & Mahajan, 2013), particularly in the gills, digestive glands, and labial palps (Ramesh *et al*., 2011; Kumar *et al*., 2012; Das *et al*., 2014). This characteristic makes it an effective bio-indicator for monitoring water quality (Ramesha *et al*., 2013). Owing to its economic, ecological, and medicinal significance, *L. marginalis* is considered an important mollusc in India (Prabhakar & Roy, 2009; Baby *et al*., 2010). Its soft tissues are valued as a protein

source for humans, fish, and poultry (Haldar *et al*., 2014), while different body parts are traditionally used to treat ailments such as rheumatism, asthma, anemia, tuberculosis, muscle dystrophy, menstrual disorders, and paralysis (Prabhakar & Roy, 2009). Its sedentary nature, wide distribution, and filter-feeding behavior make it a promising species for pollution toxicity assessment and biomonitoring (Rittschof & McClellan, 2005).

The natural and xenogeneic pearl formation capability further enhances its commercial value (Ram, 1989). In India, *L. marginalis* exhibits a broad geographical distribution across various freshwater ecosystems, with the northeastern region in particular showing high intraspecific diversity, likely due to its unique geological characteristics. Although it is currently listed as 'Least Concern' on the IUCN Red List, its population trends remain unclear (Madhyastha *et al*., 2010). Human activities such as mining, pollution, and habitat degradation pose potential threats to its survival and could lead to population declines.

Understanding the genetic stock structure of *L. marginalis* is crucial for developing sustainable management and conservation strategies. Moreover, variations in genetic makeup among populations may influence pearl quality, further underscoring the need for genetic characterization. Despite its significance, no comprehensive study has been conducted to assess the genetic diversity and population structure of this species. Among various molecular tools, microsatellite markers are particularly effective for population genetic studies due to their locus-specificity, high polymorphism, co-dominant inheritance, reproducibility, cost-effectiveness, and suitability for automation (Saiki *et al*., 1988; Tautz, 1989). They are widely used in population genetics, conservation biology, and evolutionary studies (Mojekwu & Anumudu, 2013). In the present study, we employed three microsatellite markers to investigate the genetic diversity and population structure of *L. marginalis* across five populations sampled from Tripura (two locations), Maharashtra, Gujarat, and Odisha.

Materials and Methods

Sample Collection and DNA Extraction

A total of 175 individuals of *Lamellidens marginalis* (Lamarck, 1819) were collected from five locations: Raigad, Maharashtra (n = 35), Bhubaneswar, Odisha (n = 35), Gandhinagar, Gujarat (n = 35), and two locations (Maharanipura and Hawaibara) along the Khowai River, Tripura (n = 70), at different time points. The individuals were transported to the lab for further analysis. About 100 mg of mantle tissue was collected aseptically and washed with distilled water. Total DNA was extracted using a genomic DNA purification kit. Extracted genomic DNA was stored at −20 °C for further molecular analysis.

Microsatellite Genotyping

A total of 175 individuals of *Lamellidens marginalis* collected from five different populations were genotyped using three species-specific microsatellite loci: *Lm171* (Di-nucleotide), *Lm3982* (Di-nucleotide), and *Lm251* (tri-nucleotides), developed by Varshney *et al*. (2020). PCR amplification was performed in 25μL reaction volumes, each containing 1 Unit of Taq DNA polymerase, 1X of 10X Taq buffer, 200 μM of each dNTP, 10 picomoles of forward and reverse primers, and approximately 100 ng of genomic DNA, with nuclease-free water added to reach the final volume. Thermal cycling was carried out in a thermal cycler with the following conditions: an initial denaturation at 94 °C for 5 minutes; 30 cycles of denaturation at 94°C for 30 seconds, annealing for 30 seconds at primer-specific temperatures (57°C for *Lm171*, and 55°C for both *Lm3982* and *Lm125*), and extension at 72°C for 1 minute; followed by a final extension at 72°C for 10 minutes and a hold at 4°C. The amplified products were separated using polyacrylamide gel electrophoresis (PAGE) alongside a pBR322 DNA-MspI digest ladder to estimate fragment sizes. Gels were stained with silver nitrate for visualization. Amplicons within the expected size range were considered valid alleles. Individuals displaying two distinct amplicons of similar intensity within the expected range were classified as heterozygous, while those showing a single amplicon were considered homozygous for the respective locus. Genotyping was performed using MyImageAnalysis software (Thermo Fisher Scientific, USA).

Data Analysis

The microsatellite genotype data were examined for potential PCR-related errors, including null alleles, stuttering, and allele dropout, using MICRO-CHECKER (van Oosterhout *et al*., 2004). Polymorphic Information Content (PIC) values for each locus were calculated using Cervus v3.0 (Marshall *et al*., 1998) to evaluate the informativeness of the markers. Key genetic parameters, including allele frequencies, observed and expected heterozygosity, deviations from Hardy-Weinberg equilibrium, and genetic differentiation among populations, were estimated using GenAlEx 6.4 software (Peakall and Smouse, 2006). Further, Principal Coordinate Analysis (PCoA), Analysis of Molecular Variance (AMOVA), and identification of private alleles were also conducted in GenAlEx to explore population structure and assess within- and between-population genetic variation.

Results and discussion

Frequency of Null Alleles

Null alleles were detected at loci *Lm251* and *Lm3892* with a frequency value of more than 0.25 in Maharanipura and Hawaibari stocks (Table 1). The genotypes of these loci were adjusted using Micro-checker software for further genetic stock characterization. Null alleles are undetected alleles that result from the preferential amplification of shorter alleles during PCR. Their occurrence can be attributed to several factors, including poor DNA template quality or quantity, mutations at the primer

Table 1. Summary of Null allele frequency in *Lamellidens marginalis*

Population	Locus	Oosterhout	Chakraborty	Brookfield 1
Gujarat	*Lm 171*	0.034	0.036	0.023
	Lm 3892	0.060	0.066	0.041
	Lm 251	0.069	0.769	0.047
Odisha	*Lm 171*	0.013	0.013	0.011
	Lm3892	0.172	0.201	0.013
	Lm 251	0.120	0.148	0.101
Maharashtra	*Lm 171*	0.148	0.191	0.128
	Lm 3891	0.132	0.165	0.093
	Lm 251	0.047	0.051	0.032
Tripura (Maharanipura)	*Lm171*	0.047	0.051	0.032
	Lm3892	0.180	0.245	0.130
	Lm 251*	**0.264**	**0.428**	**0.20**
Tripura (Hawaibari)	*Lm 171*	0.232	0.350	0.172
	Lm3892*	**0.250**	**0.528**	**0.162**
	Lm251	0.142	0.182	0.100

*Values in Bold letters indicate null alleles

binding sites, PCR slippage, or small sample sizes (Gagneux *et al*., 1997). The presence of these null alleles aligns with previous reports of high null allele frequencies in molluscs and bivalves (Li *et al*., 2003; Chiesa *et al*., 2016).

Allelic Richness and Diversity in Populations

The level of polymorphism at a locus is typically expressed in terms of the number of alleles and gene diversity. A higher number of alleles indicates greater marker power for analyzing stock structure (Estoup *et al*., 1998). In the present study, each population, comprising 35 individuals, was screened using three microsatellite loci, resulting in a total of 105 PCR reactions (35 individuals × 3 loci) to assess allele richness within each group. The Gujarat stock exhibited a total of six alleles across the three loci, with an average of two alleles per locus and an effective allele number of 1.98. The Odisha population showed the highest allelic diversity, with nine alleles and an average of three alleles per locus. In the Maharashtra stock, a total of eight alleles were observed across all three loci. Both Maharanipura and Hawaibari stocks displayed a total of six alleles. Altogether, across all populations and loci, a cumulative total of 35 alleles were detected, yielding an average of approximately 11 alleles per locus. Among the five stocks, the Odisha population exhibited relatively greater allelic richness compared to the others (Table 2). The allelic frequencies of the three polymorphic microsatellite loci across the populations are presented in Table 1.Comparable findings have been reported in other molluscan species: *Margaritifera margaritifera* (freshwater pearl mussel) showed 2 to 12 alleles across 13 microsatellite loci with an average of 6.8 alleles per locus (Geist *et al*., 2003); *Mytilus trossulus* (Baltic blue mussel) exhibited 3 to 13 alleles across 6 loci (Gardeström *et al*., 2008); *Mytilus galloprovincialis* (Mediterranean blue mussel) showed 2 to 8 alleles with an average of 5.2 per locus (Li *et al*., 2011); *Haliotis discus hannai* (Pacific abalone) exhibited a much broader range of 15 to 64 alleles per locus, with an average of 23.5 (An *et al*., 2011); and *Pteria penguin* (pearl oyster) showed 5 to 26 alleles per locus (Zhang *et al*., 2016).

Table 2. Observed (N_a) and Effective (N_e) of alleles in *Lamellidens marginalis*

Locus	Gujarat		Odisha		Maharashtra		Maharanipura		Hawaibari		Overall population	
	N_a	N_e	N_a	N_e	N_a	N_e	N_a	N_e	N_a	N_e	N_a	N_e
Lm 171	2	1.997	4	3.834	3	2.974	2	1.995	2	1.995	13	10.8
Lm3982	2	1.960	2	1.972	3	1.956	2	1.980	2	1.471	11	9.339
Lm251	2	2	3	2.834	2	1.995	2	2.0	2	1.956	11	10.785
Total	6	5.957	9	8.64	8	6.925	6	5.975	6	5.422	35	32.92
Mean	2	1.98	3	2.88	2.6	2.30	2	1.99	2	1.80	11.6	10.95

Table 3. Distribution of alleles at several lociLocus Allele/n Gujarat Odisha Maharashtra

Locus	Allele/n	Gujarat	Odisha	Maharashtra	Tripura1 (Maharanipura)	Tripura2 (Hawaibari))
Lm171	**N**	28	25	20	20	20
	160	0.000	0.000	0.000	0.000	0.525
	170	0.000	0.200	0.000	0.000	0.000
	178	0.000	0.000	0.000	0.000	0.475
	180	0.518	0.000	0.000	0.475	0.000
	190	0.482	0.200	0.000	0.525	0.000
	192	0.000	0.000	0.375	0.000	0.000
	200	0.000	0.000	0.325	0.000	0.000
	202	0.000	0.320	0.000	0.000	0.000
	216	0.000	0.280	0.300	0.000	0.000
Lm3892	**N**	28	25	20	20	20
	142	0.571	0.000	0.000	0.000	0.000
	147	0.000	0.440	0.425	0.000	0.000
	150	0.429	0.560	0.000	0.550	0.800
	160	0.000	0.000	0.575	0.450	0.200
Lm251	**N**	28	25	20	20	20
	120	0.500	0.380	0.525	0.000	0.000
	126	0.500	0.220	0.000	0.000	0.000
	128	0.000	0.000	0.475	0.500	0.575
	130	0.000	0.400	0.000	0.000	0.000
	132	0.000	0.000	0.000	0.500	0.425

Allele Distribution Pattern across Microsatellite Loci

The microsatellite locus *Lm171* revealed a total of nine alleles, with fragment sizes ranging from 160 to 216 base pairs (bp). An allele of 190 bp was commonly observed in the populations of Gujarat, Odisha, and Maharanipura, while the 180 bp allele was shared between the Gujarat and Maharanipura stocks. A unique 170 bp allele was found exclusively in the Odisha population. Two alleles, 192 bp and 200 bp were specific to the Maharashtra population. Additionally, alleles of 160 bp and 178 bp were restricted to the Hawaibari population, Tripura (Table 3).

At the *Lm3982* locus, four alleles were identified, with sizes ranging from 142 to 160 bp. The 142 bp allele was specific to the Gujarat population, whereas the 150 bp allele was widespread, present in the Gujarat, Odisha, Maharanipura, and Hawaibari populations. The 160 bp allele was detected in Maharashtra, Maharanipura, and Hawaibari stocks.

The *Lm 251* locus exhibited five alleles, ranging from 120 to 132 bp. The 120 bp allele was identified in populations from Gujarat, Odisha, and Maharashtra. The 126 bp allele was detected in both Gujarat and Odisha. An allele of 128 bp was observed in the populations of Maharashtra, Maharanipura, and Hawaibari. These allele distribution patterns across the loci are shown in Figures 1 to 12.

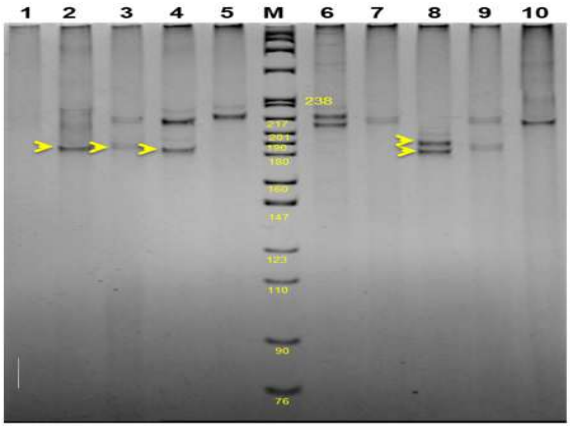

Fig.1. Allele distribution at locus Lm 171 of *Lamellidens marginalis*. Lane M: DNA ladder (pBR322-Msp1 digest); Lane $^{1-10}$: *L. marginalis* individuals from Gujarat

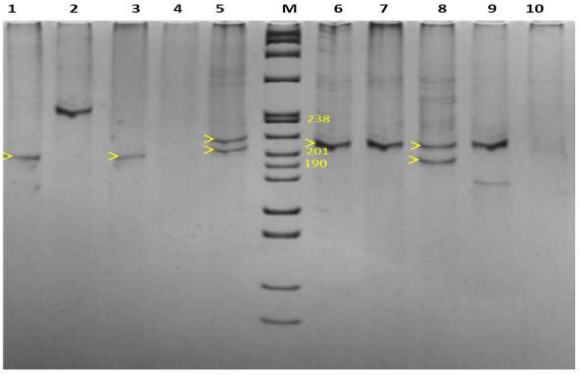

Fig.2. Allele distribution at locus 171 of nargtoa!ts, Lane M: DNA ladder(pBR322-Msp^{1}digest); Lane 1-10: Lane 1-10: *L. marginalis* individuals from Maharashtra.

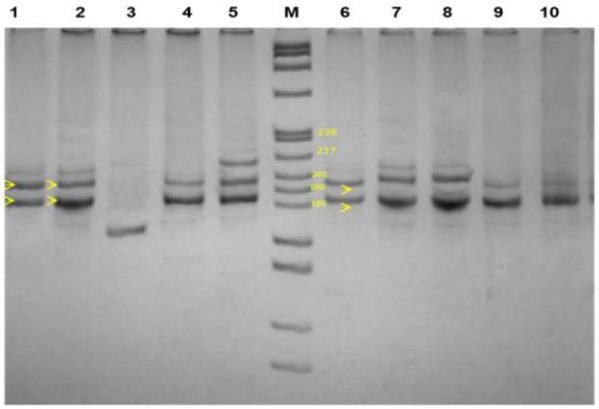

Fig.3. Allele distribution at locus *Lm 171* of *Lamellidens marginalis*. Lane M: DNA ladder (pBR322-Msp1 digest); Lane 1-10: *L. marginalis* individuals from Odisha.

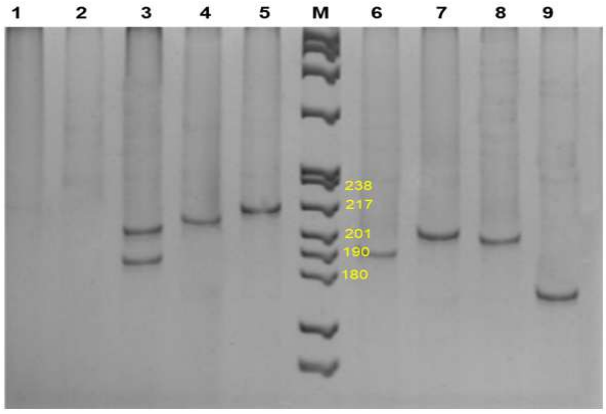

Fig.4. Allele distribution at locus *Lm 171* of *Lamellidens marginalis*. Lane M: DNA ladder (pBR322-Msp1 digest); Lane 1-10: *L. marginalis* individuals from Tripura (Maharanipura).

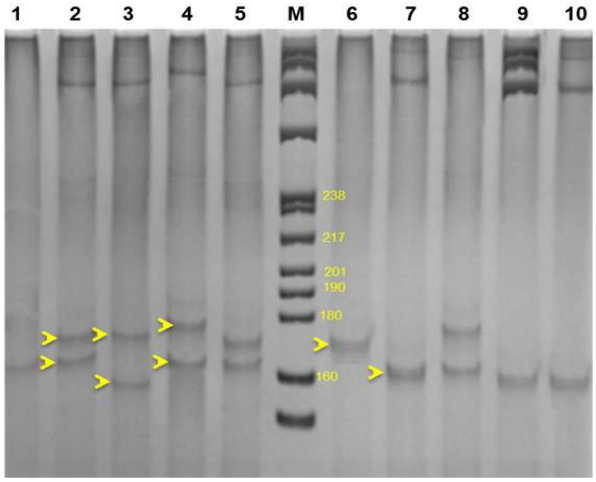

Fig.5. Allele distribution at locus *Lm 171* of *Lamellidens marginalis*. Lane M: DNA ladder (pBR322-Msp1 digest); Lane 1-10: *L. marginalis* individuals from Tripura (Hawaibara).

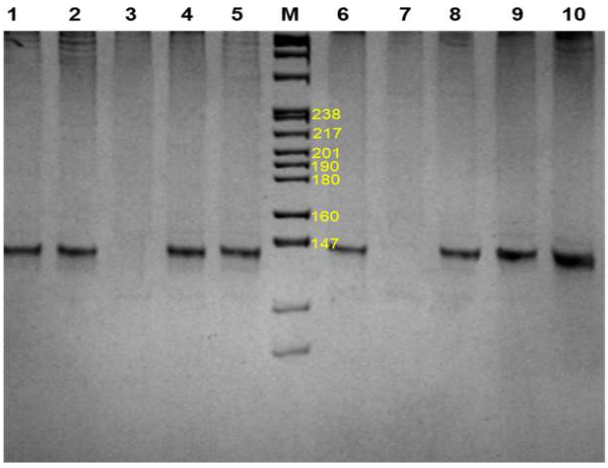

Fig.6. Allele distribution at locus *Lm 3982* of *Lamellidens marginalis*. Lane M: DNA ladder (pBR322-Msp1 digest); Lane 1-10: *L. marginalis* individuals from Gujarat.

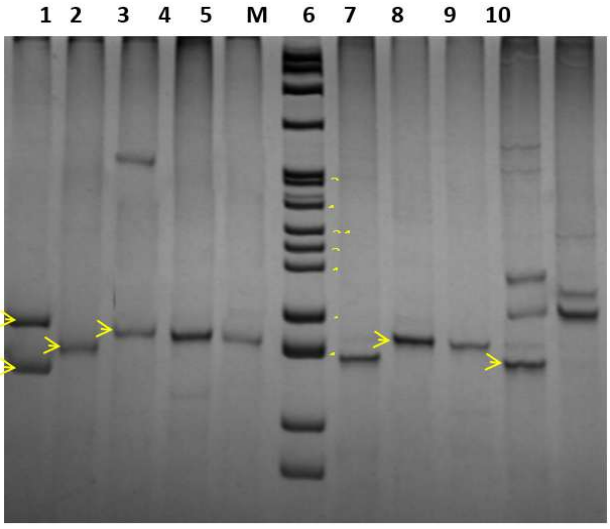

Fig.7. Allele distribution at locus *Lm 3982* of *Lamellidens marginalis*. Lane M: DNA ladder (pBR322-Msp1 digest); Lane 1-10: *L. marginalis* individuals from Odisha.

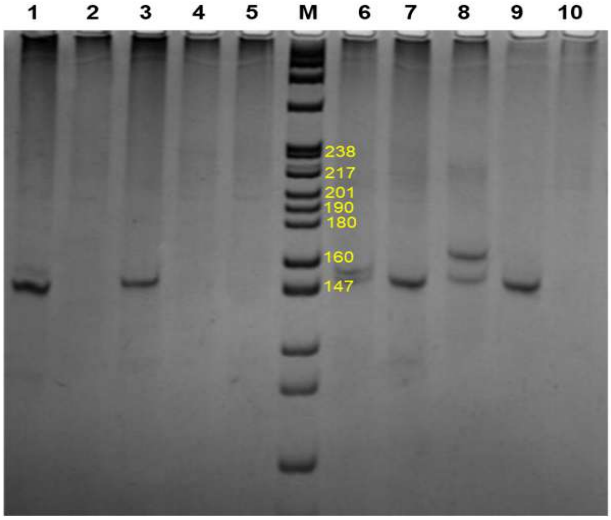

Fig.8. Allele distribution at locus *Lm 3982* of *Lamellidens marginalis*. Lane M: DNA ladder (pBR322-Msp1 digest); Lane 1-10: *L. marginalis* individuals from Odisha.

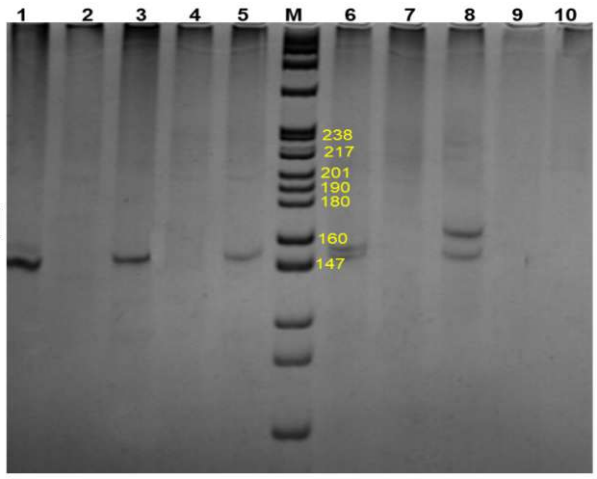

Fig.9. Allele distribution at locus *Lm 3982* of *Lamellidens marginalis*. Lane M: DNA ladder (pBR322-Msp1 digest); Lane 1-10: *L. marginalis* individuals from Tripura (Maharanipura).

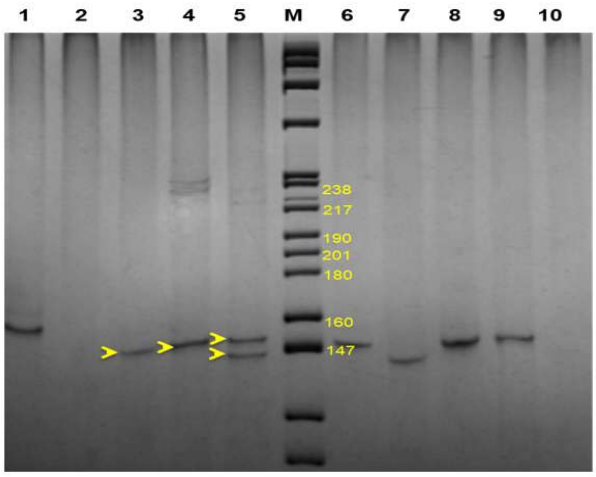

Fig.10. Allele distribution at locus *Lm 3982* of *Lamellidens marginalis*. Lane M: DNA ladder (pBR322-Msp1 digest); Lane 1-10: *L. marginalis* individuals from Tripura (Hawaibara).

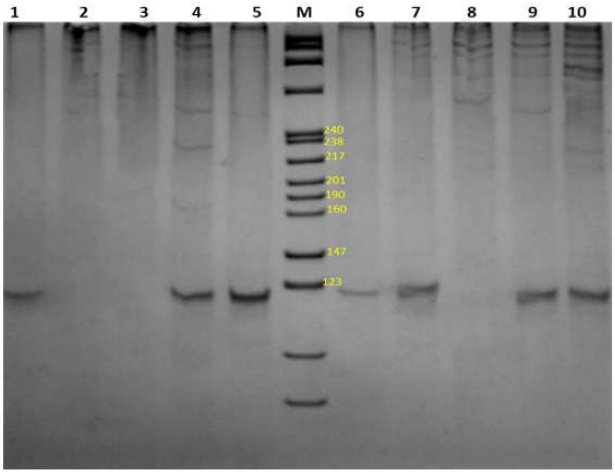

Fig.11. Allele distribution at locus *Lm 251* of *Lamellidens marginalis*. Lane M: DNA ladder (pBR322-Msp1 digest); Lane 1-10: *L. marginalis* individuals from Gujarat.

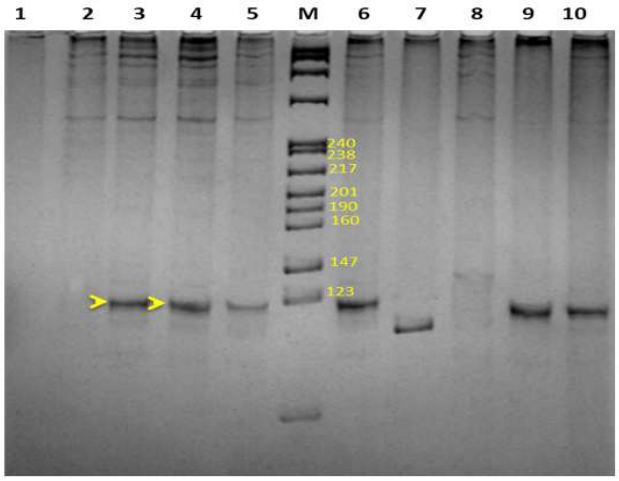

Fig.12. Allele distribution at locus *Lm 251* of *Lamellidens marginalis*. Lane M: DNA ladder (pBR322-Msp1 digest); Lane 1-10: *L. marginalis* individuals from Odisha.

Observed (Ho) and Expected (He) Heterozygosities

In the Gujarat stock, the observed heterozygosity (Ho) values ranged from 0.429 at loci *Lm3982* and *Lm251* to 0.464 at *Lm171*, with a mean Ho of 0.440. The expected heterozygosity (He) values in this stock ranged from 0.490 (*Lm3982*) to 0.500 (*Lm125*), with an average He of 0.496. The Odisha stock exhibited a higher level of genetic diversity, with an average observed heterozygosity of 0.507, ranging from 0.320 (*Lm3982*) to 0.720 (*Lm171*). The expected heterozygosity in this stock ranged from 0.493 to 0.739, with a mean value of 0.626. In the Maharashtra stock, observed heterozygosity values ranged from 0.350 (*Lm3982*) to 0.450 (*Lm171* and *Lm251*), with an average of 0.417. The expected heterozygosity values in this population ranged from 0.489 (*Lm3982*) to 0.664 (*Lm171*), with an average of 0.550. The Maharanipura stock showed lower observed heterozygosity, with values ranging from 0.300 (*Lm3982*) to 0.450 (*Lm171*) and a mean of 0.314. Expected heterozygosity values in this stock ranged from 0.495 (*Lm3982*) to 0.500 (*Lm251*), with an average of 0.498. In the Hawaibari stock, the average observed and expected heterozygosity values were 0.436 and 0.447, respectively (Table 4). Among the five populations, the Odisha stock exhibited relatively higher genetic diversity. This could be attributed to the fact that individuals from this stock were collected from CIFA, where mussels from different sources were pooled and reared together, potentially increasing genetic variation. In contrast, the relatively low genetic diversity observed in other stocks may be due to smaller sample sizes and the use of a limited number of microsatellite loci.

Table 4. Summary statistics of microsatellite loci of *L. marginalis*

Locus	Parameter	Gujarat	Odisha	Maharashtra	Maharanipura	Hawaibari
Lm171	Na	2	4	3	2	2
	H_0	0.464	0.720	0.450	0.450	0.250
	H_e	0.499	0.739	0.664	0.499	0.499
	PIC	0.488	0.698	0.598	0.480	0.475
	P_{HWE}	0.710	1.000	0.039	0.662	0.026*
Lm3892	Na	2	2	2	2	2
	H_0	0.429	0.320	0.350	0.300	0.100
	H_e	0.490	0.493	0.489	0.495	0.320
	PIC	0.485	0.488	0.465	0.482	0.315
	P_{HWE}	0.508	0.080	0.204	0.078	0.002*
Lm251	Na	2	3	2	2	2
	H_0	0.429	0.480	0.450	0.200	0.100
	H_e	0.500	0.647	0.499	0.500	0.320
	PIC	0.495	0.595	0.485	0.490	0.275
	P_{HWE}	0.450	0.167	0.662	0.007*	0.204

In the present study, the Polymorphic Information Content (PIC) values ranged from 0.275 at locus *Lm251* to 0.698 at *Lm171*. According to the classification by Botstein *et al*. (1980), co-dominant markers are considered highly informative when PIC > 0.5, reasonably informative when PIC falls between 0.25 and 0.5, and slightly informative when PIC < 0.25. Loci with many alleles and a PIC value close to 1 are considered most desirable. In this study, all loci fell within the slightly to reasonably informative range. The highest PIC value, 0.698, was observed in the Odisha population at locus *Lm171*, which corresponds to its higher allelic richness. In contrast, the lowest PIC value of 0.275 was recorded for the Hawaibari population from Tripura. Comparatively high PIC values have also been reported in other molluscan species, such as *Lamprotula leai* (0.374 to 0.927) (Jin-Jin *et al*., 2015) and *Pteria penguin* (0.656 to 0.929) (Zhang *et al*., 2016).

Genetic Diversity Analysis

Most of the populations did not show significant deviations from Hardy-Weinberg Equilibrium (HWE), as indicated by high p-values (P > 0.05). However, deviations from HWE were observed in specific cases. The Maharanipura stock showed significant deviation at locus *Lm251*, while the Hawaibari stock deviated from HWE at loci *Lm171* and *Lm3892* (Table 4). Such deviations can arise due to technical factors, such as a small sample size, the presence of null alleles, or the Wahlund effect (Gagneux *et al*., 1997), as well as biological factors including limited population size, non-random mating, natural selection, migration, or mutation. Van Oosterhout *et al*. (2004) proposed that overall homozygosity in populations may result from deviations from panmixia, inbreeding, short allele dominance, or large allele dropout. In the case of the Maharanipura and Hawaibari populations, the presence of null alleles and small sample sizes are likely contributors to the observed deviations from HWE.

Pairwise F_{ST} values between the stocks ranged from 0.137 (Maharanipura-Hawaibari) to 0.303 (Gujarat-Hawaibari) (Table 5). In the present study, around 46% of the total variation was attributed to differences among the stocks, while within-stock variation accounted for 54% of the total variation (Table 6). The Principal Component Analysis (PCoA) revealed separate clusters of individuals corresponding to different stocks. Nevertheless, few samples from

Table 5. Pairwise genetic differentiation values (F_{ST}) among the stocks of *Lamellidens marginalis*

Population	Gujarat	Odisha	Maharashtra	Tripura1 (Maharanipura)	Tripura2 (Hawaibari)
Gujarat	0.000				
Odisha	0.267	0.000			
Maharashtra	0.167	0.182	0.000		
Tripura (Maharanipur)	0.184	0.207	0.225	0.000	
Tripura2 (Hawaibari)	0.303	0.221	0.276	0.137	0.000

Table 6. Analysis of Molecular Variance of microsatellite in five stocks of the *Lamellidens marginalis*

Source	df	SS	MS	Est. Var.	%
Among Pops	4	163.981	40.995	1.732	46%
Within Pops	108	222.453	2.060	2.060	54%
Total	112	386.434		3.792	100%

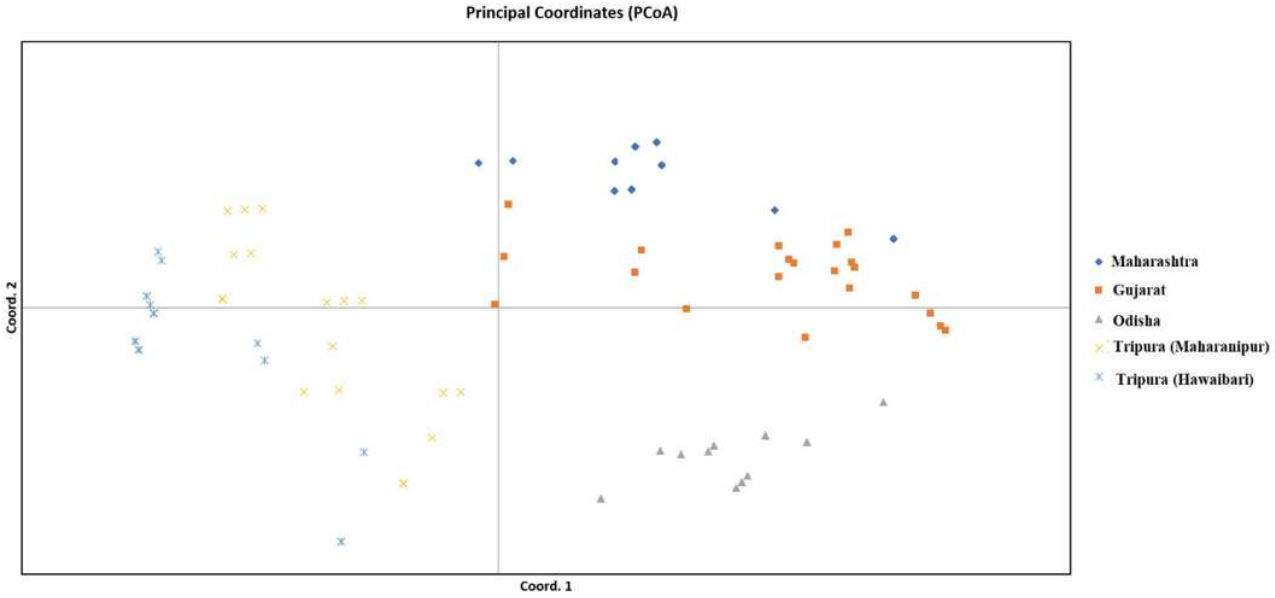

Fig.13. Principal coordinate analysis (PCoA) of *Lamellidens marginalis*

Hawaibari and Maharanipura were clustered relatively close compared to other stocks (Fig.13)

The values indicate the existence of sufficient genetic differentiation between the stocks. Jin-Jin *et al*. (2015) reported F_{ST} values ranging from 0.073 to 0.146 in the freshwater mussel *Lamprotula leai*. According to the classification by Wright (1978) and Hartl & Clark (1997), F_{ST} values between 0 and 0.05 indicate little genetic differentiation; values between 0.05 and 0.15 suggest moderate differentiation; values from 0.15 to 0.25 reflect great differentiation; and values above 0.25 indicate very great genetic differentiation. Based on these criteria, the Maharanipura and Hawaibari populations showed moderate genetic differentiation (F_{ST} = 0.137), suggesting some level of gene flow between these two geographically closer stocks. In contrast, very high genetic differentiation was observed among the populations of Gujarat, Maharashtra, Odisha, and Tripura, particularly between Gujarat and Hawaibari. This strong differentiation can be attributed to geographic isolation, which limits gene flow and leads to distinct genetic structuring among populations.

Conclusion

The present study highlights the moderate to high levels of genetic diversity across populations of *Lamellidens marginalis* using microsatellite markers, with Odisha showing the highest allelic richness and heterozygosity, likely due to stock mixing at the hatchery level. While most populations conformed to Hardy-Weinberg Equilibrium, deviations observed in the Maharanipura and Hawaibari stocks were attributed to the presence of null alleles and smaller sample sizes.

In light of these findings, it is essential to adopt location-specific management strategies to conserve the genetic diversity of *L. marginalis*. For hatchery operations, care should be taken to avoid mixing genetically distinct stocks without proper genetic evaluation, as it may disrupt local adaptations. Conservation measures should prioritize the protection of natural habitats, especially in regions like Tripura and Gujarat, where isolated stocks show significant genetic differentiation. Establishing genetic baselines and monitoring programs will support sustainable pearl culture, minimize inbreeding risks, and help maintain the evolutionary potential of this economically and ecologically important freshwater mussel.

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