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Genetic diversity of Tripureswari duck using microsatellite markers

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ABSTRACT

Tripureswari duck, a recently recognized indigenous duck breed of Tripura, represents a valuable genetic resource for rural livelihoods and sustainable poultry production. However, information on its molecular genetic diversity is limited, restricting the development of effective conservation and breeding strategies. Therefore, the present study was undertaken to characterize the genetic diversity and population structure of Tripureswari ducks using microsatellite markers. Genomic DNA from 54 birds sampled across different districts of Tripura was analyzed using 12 duck-specific microsatellite loci. All loci were polymorphic and collectively resolved 48 alleles, with the number of alleles per locus ranging from 2 to 7 (mean: 4.00 ± 0.48). The allele sizes spanned from 110 bp (CAUD008) to 307 bp (CAUD025). Average heterozygosity, polymorphic information content (PIC), effective number of alleles, and Shannon's information index were 0.62 ± 0.04, 0.55 ± 0.04, 2.93 ± 0.31, and 1.11 ± 0.10, respectively, indicating moderate to high genetic variability within the population. The overall within-population inbreeding coefficient (F_{IS} = 0.82 ± 0.07) and significant deviations from Hardy–Weinberg equilibrium across loci suggested heterozygote deficiency, likely resulting from non-random mating, selection, and inbreeding. CAUD014 had the lowest PIC value (0.366), whereas APL036 recorded the highest (0.779). Most markers exhibited moderate to high PIC values, demonstrating their effectiveness for genetic characterization studies. These findings provide the first comprehensive microsatellite-based assessment of genetic diversity in Tripureswari ducks and establish baseline information for designing conservation, genetic improvement, and sustainable utilization programmes for this indigenous duck breed.

Keywords: Heterozygosity, Hardy-Weinberg equilibrium, Microsatellites, Tripureswari duck

Ducks hold a significant position in rural India, ranking just after chickens as a source of animal protein. They constitute approximately 10% of the total poultry population in the country and contribute about 7–8% to the national egg production (Gupta 2014). According to Padhi and Giri (2024), India ranked fifth in the global duck population in 2020, with 35.51 million ducks while China led the world with 682.94 million ducks, followed by Vietnam (86.56 million), Bangladesh (59.72 million), and Indonesia (58.24 million). Within India, ducks are predominantly found in the states of Assam, West Bengal, Andhra Pradesh, Odisha, Bihar, Tamil Nadu, and Kerala (Debnath *et al.* 2023). In the North-East India, Tripura ranked third in duck population with 8.5 lakh birds, out of which 0.78 million are native (*desi*) ducks (20th Livestock Census 2019). Padhi and Giri (2024) highlighted the considerable potential for genetic improvement of *desi* ducks, as their egg production varies widely under different rearing systems. They also reported per capita availability of duck meat at 89.13 gram per annum in India. Currently, India has only nine recognized duck breeds, four of which are native to the North-Eastern region, as per ICAR–National Bureau of Animal Genetic Resources (NBAGR), Karnal.

The native duck of Tripura, officially registered as the Tripureswari breed by ICAR–NBAGR, has an average body weight of approximately 1.199 kg at 12 months of age and an annual egg production ranging from 70 to 101 eggs (ICAR-NBAGR 2025). The breed exhibits dark brown or mixed plumage. The head color of drakes and ducks ranges from green to black, white, brown, grey, and yellowish brown, while their bills, shanks, and feet are typically orange or yellow (Debnath *et al.* 2023). The Tripureswari ducks represent a valuable genetic resource for rural livelihoods and sustainable poultry production. However, information on their molecular genetic diversity is limited, restricting the development of effective conservation and breeding strategies. Genetic analysis using microsatellite markers is a prerequisite for unlocking their genetic potential. According to the FAO (2007), microsatellites are the preferred molecular markers for genetic studies of native breeds. Microsatellites are short, tandem repeated DNA sequences (1–6 base pairs) that are

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highly variable, polymorphic, widely distributed across the genome, and suitable for genome analysis (Alyethodi *et al.* 2010, Huang *et al.* 2005). These markers are mainly located in centromeric regions (Hans *et al.* 2004) and are extensively used in genetic profiling due to their high level of polymorphism (Hans 2004, Su and Chen 2009, Jain *et al.* 2014). They serve as powerful tools for genome analysis, genetic and linkage mapping, parentage verification, and quantitative trait loci (QTL) analysis (Das *et al.* 2019).

While global efforts in molecular characterization using microsatellites are increasing, there remains a scarcity of data on India's native duck breeds. Therefore, the present study was undertaken to characterize the genetic diversity and population structure of Tripureswari ducks using microsatellite markers.

MATERIALS AND METHODS

Experimental birds: Tripureswari ducks, primarily reared by small and marginal farmers and distributed across various regions of Tripura, were selected for the present study. Venous blood samples (~0.5 ml) were randomly collected from 54 ducks across different locations within the core breeding tract. The samples were preserved at –20°C for subsequent molecular analysis.

Some samples used in the present study originated from the same geographical breeding tract investigated by Debnath *et al.* (2023); however, the objectives and marker panels differed substantially. The previous study focused on the general genetic characterization of indigenous ducks of Tripura, whereas the present study specifically evaluated the Tripureswari duck population using a separate panel of twelve duck-specific microsatellite markers. The inclusion of additional loci enabled a more detailed assessment of allelic diversity, polymorphic information content, heterozygosity, and inbreeding parameters relevant to the recently recognized breed.

DNA isolation, quality assessment and quantification: Genomic DNA was extracted using the standard phenol-chloroform method as described by Kagami *et al.* (1990). The quality of extracted DNA was assessed via 0.8% horizontal submarine agarose gel electrophoresis (AGE), and the quantity was measured using an Eppendorf BioSpectrometer. DNA samples exhibiting intact bands and an optical density (OD) ratio of 260/280 nm between 1.7 and 1.9 were selected for further analysis. The final concentration of PCR-grade DNA was standardized to 50 ng/µl.

Microsatellite primers: A panel of twelve duck-specific microsatellite markers, as reported by Huang *et al.* (2005) and Veneranda *et al.* (2019), was utilized in this study. Forward and reverse primers were synthesized by GCC Biotech, Joychandipur, Bakrahat, West Bengal, India. PCR conditions for each primer pair were optimized according

Table 1. A panel of duck microsatellite loci with their primers and optimized annealing temperatures (T_a)

Sl. No.	Primer Name	Nucleotide Sequence of left (L) and right (R) primers	T_a (ºC)	
			Optimized T_a	Under Reference*
1	CAUD008	L- GTTAAGAAAATCAGAAGAGCG R- CTTGAGTTAGGCTAAGTGTG	63.5	63.5
2	CAUD012	L- ATTGCCTTTCAGTGGAGTTTC R- CGGCTCTAAACACATGAATG	63.5	63.5
3	CAUD014	L- CACAACTGACGGCACAAAGT R- CTGAGTTTTTCCCGCCTCTA	58.1	58.1
4	CAUD021	L- TGCAGGTTCCATGTGTTAGA R- TAGCAACAAATGAGAAATGAGT	60.8	60.8
5	CAUD022	L- CATGCTGAGTGTCCTATCCT R- CCAGGTCAGGCGTGTGCT	55.5	55.5
6	CAUD025	L- AGTTCATCCCGATTTGTAGC R- AAATGCAGTGAGGTAAACCC	63.5	63.5
7	CAUD031	L- AGCATCTGGACTTTTTCTGGA R- CACCCCAGGCTCTGAGATAA	51.4	51.4
8	CAUD033	L- ACCCAGAAGAGTCAAGAATAG R- GAGTATTCCTGGTCTGTGCT	58.1	58.1
9	CAUD034	L- TACTGCATATCACTAGAGGA R- TAGGCATACTCGGGTTTAG	55.5	55.5
10	APL023	L- GAAGAGGCAGTGGCAACG R- GCTGAGATGCTCCCAGGAC	60.0	65.0
11	APL026	L- AACAGGGATAACATGAGAAGTGG R- TGAGCAGCTGTCTGGTATCTATTC	55.0	63.0
12	APL036	L- ATGCTTTGCTGTTGGAGAGC R- TCCACTGGGTGCAAACAAG	60.0	64.0

T_a (°C) denotes optimized annealing temperatures in degree centigrade.
* Huang *et al.* (2005) and Magpantay *et. al.* (2019)

to protocols established by Huang *et al.* (2005) and Magpantay *et al.* (2019). Details of the primer sequences and their corresponding annealing temperatures (T_a) are listed in Table 1.

PCR reaction mix and amplification protocol: The PCR amplification was performed in a 25 µl reaction volume, containing 50 ng of template DNA (1 µl), 10 pM of each primer (0.5 µl each with final concentration of 0.2 µM each), 10 mM deoxyribonucleotide triphosphate (dNTPs) (0.5 µl with final concentration of 200 µM each), 0.5 µl Taq DNA polymerase (1.25U), optimized concentration of magnesium chloride with 10X Reaction Buffer (2.5 µl) and nuclease free water to make up the final volume. The PCR reactions were conducted in 0.2 ml nuclease-free Maxiamp flat-cap PCR tubes using an Eppendorf Nexus Gradient Master Cycler. The thermal cycling conditions were initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, optimized annealing temperature (T_a°C) for each primer pair (Table 1) for 1 minute and extension at 72°C for 1 minute followed by final extension at 72°C for 5 minutes and holding at 4°C indefinitely.

To ensure PCR reliability, each amplification run included a no-template negative control containing all reaction components except genomic DNA to detect possible contamination. In addition, a previously validated DNA sample exhibiting consistent amplification of the target microsatellite loci was included as a positive/reference control during PCR optimization and standardization.

Gel electrophoresis: PCR products were first visualized by electrophoresis on a 2% horizontal submarine agarose gel for 60 minutes at 2–5 V/cm. Bands were visualized under UV light using the Gel Doc™ EZ System (Bio-Rad Laboratories, USA). Amplification products were accepted for analysis only when the controls performed as expected and clear, reproducible bands were observed. The 2% agarose gel was used for initial verification of PCR amplification and product specificity, while the 3.4% Metaphor agarose gel, which provides substantially higher resolution, was used for microsatellite allele separation and accurate genotyping. Approximately 10 µl of PCR product and 3 µl of 50 bp DNA ladder were loaded to assess molecular sizes (Debnath *et al.* 2017, Debnath *et al.* 2023). For precise allele identification, confirmed amplicons were electrophoresed on a 3.4% Metaphor™ agarose gel (MAGE) at 6–8 V/cm for 2 hours and 30 minutes. Allelic bands were documented using the Gel Doc™ EZ System, and allele sizes were estimated by comparison with the 50 bp ladder using Image Lab 6 software (Bio-Rad Laboratories, USA). The number of observed alleles and probable genotypes at each microsatellite locus for all samples were recorded for genotyping and subsequent analysis.

The present study employed stringent DNA quality checks, optimized PCR conditions, and scoring of only clear, reproducible bands were employed to minimize genotyping errors. The selected loci produced clearly distinguishable allele classes, and allele sizing was standardized using molecular size markers (50 bp DNA ladder) and Image Lab software. Ambiguous or weak amplification products were excluded from allele scoring, and only clearly resolved bands were considered for subsequent population genetic analyses. Where applicable, selected samples were re-amplified to verify the consistency of allele profiles. Since the objective of the study was to estimate population genetic diversity parameters rather than perform fine-scale allele discrimination, the resolution obtained was considered adequate.

Statistical analysis: Population genetic parameters including allele frequencies, observed and effective number of alleles, Shannon's information index, Wright's fixation index (F-statistics), observed and expected heterozygosity, chi-square for goodness of fit test, and likelihood ratio test (G-square) were analyzed using POPGENE® version 1.31 software, following the methodology described by Yeh *et al.* (1999). These analyses were conducted based on the compiled genotypic data from the twelve microsatellite loci across the sampled Tripureswari ducks. Although POPGENE 1.31 is an older software package, it remains a validated and widely accepted tool for estimating allele frequencies, heterozygosity, Hardy–Weinberg equilibrium, Shannon's information index, and F-statistics from microsatellite data. Its use also facilitates comparison with previous studies on indigenous duck populations. Its limitations were additionally acknowledged, including the absence of advanced population structure analyses and reduced functionality compared with newer software platforms.

In addition, average heterozygosity and polymorphic information content (PIC) for each microsatellite locus were calculated using the formulas of Nei (1978) and Botstein *et al.* (1980), as also applied by Debnath *et al.* (2023).

RESULTS AND DISCUSSION

Microsatellite allelic profile: The allelic profile of Tripureswari ducks at twelve duck-specific microsatellite loci is summarized in Table 2, with a total of 48 distinct alleles identified. The molecular sizes of these alleles ranged from 110 bp at locus CAUD008 to 307 bp at locus CAUD025, as illustrated by representative samples in Fig. 1. The number of alleles per locus varied between 2 and 7, with the lowest allele count observed at CAUD012 and CAUD014, while APL036 exhibited the highest number of alleles.

The observed allele numbers and sizes were generally consistent with earlier studies. For instance, Huang *et al.* (2005) analyzed 25 microsatellite loci, including CAUD008, CAUD012, CAUD014, CAUD021, CAUD022, CAUD025, CAUD031, CAUD033, and CAUD034 in Pekin ducks using a 3100 Genetic Analyzer (ABI), and reported similar allele numbers at CAUD012, CAUD014, and CAUD025, with variation at CAUD008, CAUD021, CAUD022, CAUD031, CAUD033, and CAUD034. Gaur *et al.* (2010) studied 24 microsatellite

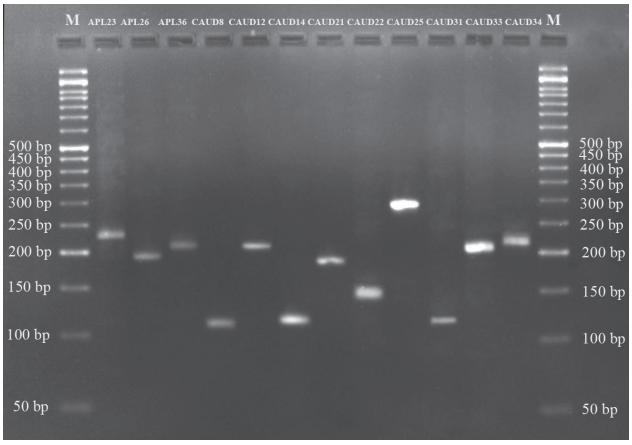

Fig.1. Representative samples of duck-specific microsatellite loci for Tripureswari duck

loci including CAUD022, CAUD025, CAUD031, and CAUD033 across five unrelated duck populations from West Bengal, Odisha, Jharkhand, Tamil Nadu, and Khaki Campbell ducks of Ranchi using the 3100 Avant Automated DNA Analyzer and reported variation in the number of alleles at CAUD022, CAUD025, CAUD031, and CAUD033, with some similarities in allele counts at CAUD022 (Odisha) and CAUD025 (Khaki Campbell, Ranchi). Goel and Goel (2016) analyzed Muscovy ducks from Manipur, Assam, and West Bengal using 2% agarose gel and observed allele numbers similar to our findings at CAUD031 and CAUD033, though allele sizes varied. In contrast, both the number and sizes of alleles at CAUD025 differed. Agatep *et al.* (2016) assessed 28 microsatellite loci including APL023, APL026, and APL036 in Philippine Mallard, Khaki Campbell, and Pekin ducks using 8% polyacrylamide gel electrophoresis (PAGE), and reported different allele counts and sizes at those loci. Similarly, Magpantay *et al.* (2019) examined 22 microsatellite (SSR) markers in Philippine Mallard populations using a Fragment Analyzer (Advanced Analytical Technologies Inc., USA), noting variability in the number and size of alleles at loci APL023, APL026, and APL036. The observed variation in allele numbers and sizes across studies is likely due to in genetic differences in the duck populations, as well as methodological differences in electrophoresis platforms and allele size determination techniques.

In the present study, allele frequencies across the analyzed microsatellite loci ranged from 0.019 at locus APL023 to 0.75 at APL026. Among the 48 resolved alleles, 36 (75%) exhibited frequencies exceeding 10%, indicating their potential significance within the population. As no prior data on allele frequencies in ducks was available for direct comparison, these findings offer valuable baseline information. Notably, Debnath *et al.* (2023) and Susanti *et al.* (2021) suggested that alleles with frequencies around 10% may serve as effective markers for population-specific tagging.

Population genetic analysis of microsatellite data: The population genetic analysis revealed that the mean ± SE of

Table 2. Number of alleles, their molecular sizes and frequencies at various duck-specific microsatellite loci in Tripureswari duck

Microsatellite loci	Number of alleles	Alleles	Size (bp)	Allele frequency
CAUD008	3	A	110	0.167
		B	114	0.463
		C	118	0.370
CAUD012	2	A	213	0.519
		B	219	0.482
CAUD014	2	A	121	0.407
		B	126	0.593
CAUD021	3	A	190	0.463
		B	197	0.389
		C	202	0.148
CAUD022	3	A	138	0.037
		B	147	0.556
		C	152	0.407
CAUD025	3	A	298	0.352
		B	303	0.407
		C	307	0.241
CAUD031	6	A	115	0.130
		B	121	0.037
		C	129	0.259
		D	133	0.361
		E	138	0.157
		F	143	0.056
CAUD033	5	A	203	0.296
		B	209	0.278
		C	213	0.111
		D	218	0.241
		E	223	0.074
CAUD034	4	A	224	0.074
		B	230	0.546
		C	236	0.352
		D	258	0.028
APL023	6	A	162	0.315
		B	188	0.019
		C	214	0.380
		D	233	0.139
		E	241	0.111
		F	278	0.037
APL026	4	A	154	0.750
		B	163	0.157
		C	173	0.037
		D	181	0.056
APL036	7	A	156	0.019
		B	175	0.120
		C	184	0.056
		D	194	0.157
		E	206	0.204
		F	215	0.296
		G	225	0.148
Mean ± SE	4 ± 0.48			

Table 3. The measures of heterozygosity and diversity statistics, chi-square (χ^2) and likelihood ratio test for Hardy-Weinberg equilibrium at various duck specific microsatellite loci in Tripureswari duck

Microsatellite loci	df	PIC	I	N_a	N_e	F_{IS}	Nei's H	Chi square	G square
CAUD008	3	0.54	1.02	3	2.64	1.00	0.62	112.11***	112.53***
CAUD012	1	0.38	0.69	2	2.00	1.00	0.50	55.022***	75.80***
CAUD014	1	0.37	0.68	2	1.93	1.00	0.48	55.09***	74.01***
CAUD021	3	0.53	1.01	3	2.58	1.00	0.61	112.46***	110.78***
CAUD022	3	0.42	0.81	3	2.10	0.93	0.52	71.20***	77.98***
CAUD025	3	0.58	1.08	3	2.88	1.00	0.65	111.33***	118.27***
CAUD031	15	0.72	1.56	6	4.10	0.76	0.76	227.91***	131.71***
CAUD033	10	0.72	1.50	5	4.15	1.00	0.76	231.72***	165.73***
CAUD034	6	0.49	0.99	4	2.33	0.90	0.57	116.56***	97.11***
APL023	15	0.68	1.45	6	3.62	0.36	0.72	88.94***	91.25***
APL026	6	0.38	0.79	4	1.69	0.50	0.41	42.56***	38.76***
APL036	21	0.78	1.75	7	5.16	0.43	0.81	74.62***	73.38***
Mean ±SE		0.55 ± 0.04	1.11 ± 0.10	4 ± 0.48	2.93 ± 0.31	0.82 ± 0.07	0.62 ± 0.04		

df, degree of freedom; H, heterozygosity; PIC, polymorphic information content; N_a, observed number of alleles; N_e, effective number of alleles; I, Shannon's information, F_{IS}, Wright's fixation index (1978), ***P≤0.001

the observed number of alleles (N_a), effective number of alleles (N_e), Shannon's Information Index (I), and Wright's fixation index (F_{IS}) were 4.00 ± 0.48, 2.93 ± 0.31, 1.11 ± 0.10, and 0.82 ± 0.07, respectively. The effective number of alleles ranged from 1.69 at locus APL026 to 5.16 at APL036 (Table 3). There are no earlier reports available on these genetic parameters (N_a, N_e, I, and F_{IS}) for the same set of microsatellite loci in the Tripureswari duck, making the present findings a baseline reference. However, Debnath *et al.* (2023) used Shannon's index to indicate gene diversity in native ducks of Tripura, supporting the genetic diversity observed.

According to Das *et al.* (2015), F_{IS} is an indicator of inbreeding, where a positive F_{IS} denotes a deficiency in heterozygotes (*i.e.*, more inbreeding than expected), and a negative F_{IS} indicates an excess of heterozygotes (less inbreeding). Consistently positive F_{IS} values in the present study suggested heterozygote deficiency, plausibly due to a small, unselected population. The F_{IS} = 1.00 value appeared solely as an evidence of extreme inbreeding, but likely due to the allele spacing and genotyping platform employed in this study. However, lower effective allele numbers than observed allele counts still indicated the presence of genetic variation. Comparative studies by Magpantay *et al.* (2019) reported different F_{IS} values for APL023 (0.23), APL026 (0.38), and APL036 (0.70), while Goel and Goel (2016) found variable F_{IS} values for CAUD025 (0.46), CAUD031 (0.45), and CAUD033 (0.47). The differences in F_{IS} values across studies may be attributed to population structure variations.

However, a notable feature of the present study was the high inbreeding coefficient (F_{IS} = 0.82 ± 0.07), which appears to be greater than those reported in many indigenous duck populations. While positive F_{IS} values have been documented in native ducks from North-East India and other Asian populations, the magnitude observed in Tripureswari ducks suggests a greater degree of heterozygote deficiency. This may reflect the relatively small effective population size of the breed, geographical concentration within Tripura, repeated use of a limited number of breeding males, and restricted exchange of breeding stock among village flocks. Such a pattern emphasized the need for immediate genetic management despite the presence of appreciable overall diversity.

Compared with other Indian indigenous ducks, Tripureswari ducks, therefore, present an interesting genetic profile characterized by the coexistence of substantial allelic diversity and evidence of inbreeding. This indicated that although valuable genetic variation was still present within the breed, proactive conservation measures were required to prevent future erosion of genetic resources. The findings also reinforced the importance of recognizing and genetically characterizing region-specific duck breeds, as each population might possess unique adaptive traits and genetic attributes that contribute to India's poultry biodiversity. From a conservation perspective, continued inbreeding may lead to a reduction in genetic variability, increased homozygosity, and a greater risk of inbreeding depression, which can adversely affect fitness-related traits such as reproductive performance, disease resistance, adaptability, and long-term population viability. Therefore, conservation efforts should focus on maintaining genetic diversity through systematic selection of breeding stock, exchange of breeding males among flocks, avoidance of close-relative mating, and establishment of nucleus breeding and conservation programmes.

Average heterozygosity and polymorphic information content (PIC): Nei's heterozygosity across loci ranged from 0.41 at APL026 to 0.81 at APL036. Ten loci including CAUD008 (0.62), CAUD012 (0.50), CAUD021 (0.61), CAUD022 (0.52), CAUD025 (0.65), CAUD031 (0.76), CAUD033 (0.76), CAUD034 (0.57), APL023 (0.72), and

APL036 (0.81) had heterozygosity values greater than 0.50. Only CAUD014 (0.48) and APL026 (0.41) had values below 0.50. The overall average heterozygosity was 0.62 ± 0.04 (Table 3), indicating substantial genetic diversity. The genetic diversity estimates obtained in the present study indicated that Tripureswari ducks possess moderate to high levels of genetic variation. The average heterozygosity (0.62 ± 0.04) observed in this population is comparable to or higher than values reported for several indigenous duck populations of India. Das *et al.* (2019) had also reported substantial levels of heterozygosity, highlighting the rich genetic diversity maintained in traditionally managed duck populations while studying four indigenous duck populations of North-East India. Similarly, Debnath *et al.* (2023) reported considerable genetic variability in indigenous ducks of Tripura using microsatellite markers, suggesting that native duck populations of the region continue to retain valuable genetic resources despite the absence of organized breeding programmes.

Polymorphic Information Content (PIC), a measure of marker informativeness and genetic diversity, ranged from 0.37 (CAUD014) to 0.78 (APL036), with an average PIC of 0.55 ± 0.04. All twelve loci were polymorphic. According to classification by Botstein *et al.* (1980), a PIC > 0.50 indicates high diversity, 0.25–0.50 indicates moderate diversity, and <0.25 reflects low diversity. Therefore, most loci showed high to moderate polymorphism. The average PIC value (0.55 ± 0.04) observed in the present study further supported the existence of substantial genetic variation in Tripureswari ducks. Comparable levels of marker informativeness have been reported in indigenous ducks from other parts of India, including populations studied by Gaur *et al.* (2010) and Goel and Goel (2016). Such findings collectively indicated further emphasized appreciable levels of genetic diversity, likely due to adaptation to diverse agro-climatic conditions and relatively low selection intensity under traditional production systems. Comparative values from Huang *et al.* (2005) and Magpantay *et al.* (2019) revealed variations in PIC for several loci, such as high PIC at CAUD022 (0.62) and low values at CAUD012 (0.04) and CAUD014 (0.32). Differences might have resulted from variations in duck populations, methodological differences, or allelic fixation or loss.

The findings of the study indicated that all twelve duck-specific microsatellite loci examined are polymorphic in the Tripureswari duck population, with most showing high PIC values (>0.50), underscoring their suitability as informative genetic markers. These loci can be effectively used for assessing genetic diversity and may also be applied for the genetic characterization of other indigenous duck populations in India.

*Hardy-Weinberg equilibrium (HWE):*Hardy-Weinberg equilibrium was assessed using chi-square and G-square tests. Deviation from Hardy–Weinberg equilibrium at each microsatellite locus was evaluated using chi-square (χ^2) and likelihood ratio (G^2) tests implemented in POPGENE version 1.31. The significance of the test statistics was determined by comparing the calculated χ^2 and G^2 values with the corresponding chi-square distribution based on the appropriate degrees of freedom. Probability values generated by the software were used to assess statistical significance, and loci with $P \leq 0.001$ were considered significantly deviated from Hardy–Weinberg equilibrium. All microsatellite loci exhibited highly significant deviations from Hardy–Weinberg equilibrium ($P < 0.001$), supporting the inference that the population was influenced by factors such as non-random mating, inbreeding, selection, or genetic drift and/or methodological limitations associated with allele resolution.

Although the Tripureswari duck population exhibited moderate to high genetic diversity, the high inbreeding coefficient and significant deviation from Hardy–Weinberg equilibrium highlighting the need for immediate conservation and genetic management interventions. The present study was designed primarily to assess within-population genetic diversity of Tripureswari ducks using microsatellite markers. Since samples were analyzed as a single population and no distinct geographical or genetic subgroups were defined, Analysis of Molecular Variance (AMOVA) and phylogenetic/tree-based approaches were beyond the scope of the current investigation and would provide limited additional biological interpretation.

The present findings have important implications for the conservation and genetic improvement of the Tripureswari duck. The moderate to high levels of genetic diversity detected across the studied microsatellite loci indicate the existence of a valuable genetic reservoir that can be utilized in future breeding programmes. At the same time, the high inbreeding coefficient and significant departure from Hardy–Weinberg equilibrium suggest the need for immediate genetic management to prevent further loss of diversity. The identified polymorphic markers may serve as useful tools for monitoring genetic variability, assessing population structure, identifying genetically diverse breeding stocks, and minimizing inbreeding in organized breeding programmes. These results can support the development of scientifically managed nucleus breeding flocks and community-based conservation programmes aimed at maintaining the breed's genetic integrity while improving productive performance. Furthermore, the baseline genetic information generated in this study may assist policymakers and breed conservation agencies in prioritizing the Tripureswari duck for conservation initiatives, germplasm preservation, and sustainable utilization under state and national livestock biodiversity programmes.

Based on the findings of the present study, several management interventions may be recommended for the sustainable conservation and improvement of the Tripureswari duck population. First, the exchange of breeding males among villages and flocks should be encouraged to reduce inbreeding and increase gene flow. Second, mating among close relatives should be avoided through proper identification and record-keeping of

breeding birds. Third, a nucleus breeding flock may be established under institutional supervision to maintain genetic diversity while implementing selective breeding for economically important traits such as egg production, growth, and survivability. Fourth, periodic genetic monitoring using microsatellite or other molecular markers should be undertaken to assess changes in genetic variability and inbreeding levels over time. Fifth, community-based conservation programmes involving local farmers should be promoted to preserve the breed in its native production environment. Finally, cryopreservation of germplasm and maintenance of *ex situ* conservation populations may be considered as safeguards against future genetic erosion. Management strategies such as controlled mating, exchange of breeding males among flocks, establishment of nucleus breeding units, periodic genetic monitoring, community-based conservation, and germplasm preservation are recommended to reduce inbreeding and maintain the genetic diversity of the Tripureswari duck population.

A limitation of the present study is that microsatellite alleles were scored using high-resolution Metaphor™ agarose gel electrophoresis rather than capillary electrophoresis. Although this approach was adequate for detecting polymorphism and estimating population-level diversity parameters, closely spaced alleles differing by only a few base pairs may not have been resolved with complete accuracy. As a result, heterozygosity may have been underestimated at some loci. Therefore, estimates of F_{IS} and Hardy–Weinberg disequilibrium should be interpreted with appropriate caution.

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