Research Article

Study of the Genetic Diversity of the Ornamental Fish *Badis badis* (Hamilton-Buchanan, 1822) in the Terai Region of Sub-Himalayan West Bengal, India

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Dwarf chameleon fish or *Badis badis*, a lesser known ornamental freshwater fish, has recently been included in the Indian threatened category of fish list. There are insufficient studies with regard to the assessment of genetic background of this ichthyofauna, especially in the western sub-Himalayan region of West Bengal, India, popularly known as the Terai. The present study is the first attempt to investigate the present status of the genetic background of this species in the Mahananda and Balason rivers, major streams of this region. Twenty-one selective RAPD primers generated 53 and 60 polymorphic fragments in the Mahananda and Balason populations, respectively. The proportion of polymorphic loci, Nei's genetic diversity (H), and Shannon's index (H') were 0.4416 , 0.1654 ± 0.023 , and 0.2450 ± 0.2907 , respectively, in Mahananda river population and were 0.5041 , 0.1983 ± 0.2126 , and 0.2901 ± 0.3037 , respectively, in Balason river population. Inbreeding coefficient and degree of gene differentiation were also calculated. The H and H' were found to be 0.1601 ± 0.1944 and 0.2363 ± 0.2782 , respectively, in overall Mahananda-Balason river system. Our study revealed considerable lack of genetic variation among the individuals of *Badis badis*. The genetic data obtained from the present study lend support to the view that there is a scope of stock improvement for this ichthyofauna.

1. Introduction

Badis badis (Hamilton-Buchanan, 1822) (Actinopterygii, Perciformes, Badidae) or dwarf chameleon fish is a tropical, benthopelagic freshwater species that attains up to 5 cm in total length. It has noteworthy ornamental and thus commercial value and has recently been included within the vulnerable category in the list of threatened freshwater fishes of India by National Bureau of Fish Genetic Resources (NBFGR, Lucknow, India) [1]. However, there have been insufficient studies with regard to the estimation of genetic diversity of this fish species, especially in the eastern sub-Himalayan region of West Bengal, India, popularly known as Terai. The region being included within the Eastern Himalayan hotspot, our results will be important from the standpoint of validation of its threatened status and its conservation/sustainability of wild population, if required.

Genetic variability or diversity is an essential characteristic of any population for the fitness of individuals as well as survival of the whole population, permitting adaptation to the changing environmental conditions and stress. Therefore the degradation of genetic diversity of a species reduces its capability for adaptation and increases the risk of its extinction [2–5]. Inbreeding is implicated in the erosion of genetic variation within natural populations, especially in small populations, by reducing the number of heterozygotes and hence reducing the mean phenotypic values of useful traits [6]. The endangered/vulnerable organisms having small population size may experience a continuous reduction in the level of genetic variation. Therefore, the study of genetic variability is of major importance to conservation, sustainability, and management of wild population which depends on the firsthand knowledge of the amount of variation existing in a local reproductive population [7].

PCR-based random amplified polymorphic DNA (RAPD-PCR) is a widely used molecular tool in detection and characterization of genetic polymorphisms in natural populations with higher speed and efficiency [8, 9]. RAPD-PCR amplifies DNA segments of variable lengths and such length polymorphisms are inherited in a Mendelian fashion and thus can be used as genetic markers [10]. RAPD analysis has been extensively used to evaluate genetic diversity in *Brycon lundii*, *Astyanax altiparanae* and *Prochilodus marginatus* [11–13], flounder [14], *Labeo rohita* [15], catfish [16, 17], mud eel [18], ornamental reef fishes [19], crocodile [20], black rat snake [21], and hilsa [22]; and in subspecies identification of tilapia [23], *Badis badis*, *Dario dario* [24], and lamprey [25]. A very limited genomic research has been carried out in *Badis badis* species till date and insufficient genomic information is available to perform other sophisticated fingerprinting techniques where whole genomic sequence is necessary; therefore, RAPD fingerprinting is the best choice for genetic diversity analysis in this species.

The main objectives of our study was to (1) ascertain the present status of the genetic background of the *Badis badis* species in the Terai region (especially in the Mahananda and Balason river system) of sub-Himalayan Terai region of West Bengal, India, (2) determine the genetic diversity within and between the populations of *Badis badis*, (3) determine the total genetic diversity of Mahananda-Balason river system of Terai region of sub-Himalayan West Bengal, India. Our results provide firsthand information that may be used in the proper maintenance and conservation of this fish fauna as well as in other dwindled populations.

2. Materials and Methods

2.1. Survey and Sample Collection. A detailed survey has been carried out in different spots of the major streams of the Terai region of sub-Himalayan West Bengal, India. A total of twenty samples of *Badis badis* were collected mainly during the monsoon and after monsoon period from Mahananda and Balason rivers (ten samples from each river system) (Hamilton-Buchanan 1822) during 2012–2013 and geographic coordinates of the collection spots were recorded with the help of handheld GPS (eTrex Vista HCx, Garmin, USA). Individuals mostly measuring 4 cm were obtained with the help of a “scoop net” from the submerged vegetation/gravels along the sides of the streams. The collection spots were as follows: in Mahananda River [M1 = 26°42.125'N; 88°24.717'E, Elevation-620 ft AMSL and M2 = 26°44.452'N; 88°25.497'E, Elevation-717 ft AMSL]; in Balason River [B1 = 26°43.177'N; 88°22.825'E, Elevation-646 ft AMSL and B2 = 26°45.632'N; 88°18.912'E, Elevation-731 ft AMSL]; P = 26°43.007'N; 88°24.396'E, Elevation-666 ft AMSL and F = 26°38.884'N; 88°24.125'E, Elevation-319 ft AMSL (Figure 1). The M1-M2, B1-B2, P, and F constitute together the whole Mahananda-Balason river system of the Terai region of West Bengal, India. Fishes were identified according to Talwar and Jhingran [26] and were photographed.

2.2. Isolation of High Molecular Weight DNA and Quantification. Genomic DNA (gDNA) extraction was done from tiny amount of tissue samples (10–15 mg of fin clips from the caudal and ventral portions and/or 25–30 pieces of scales from the dorsal portion were collected noninvasively) from each fish following Mukhopadhyay and Bhattacharjee [27]. The DNA samples were resuspended in 100 μ L sterile Type-1 Milli-Q water and stored at -20°C till further analysis. The extracted DNA samples were subjected to spectrophotometric analysis for quantification in Rayleigh UV2601 Spectrophotometer, Beijing, China, and the concentration of extracted DNA was adjusted to 50 ng/ μ L after suitable dilution of the extracted DNA samples for each PCR amplification.

2.3. Primer Selection. Forty arbitrary decamer primers of random sequences (Kit-A and Kit-B, twenty primers from each kit) were purchased from Imperial Life Science Pvt. Ltd., India. Twenty-one primers (10 from Kit-A and 11 from Kit-B) were selected for further analyses on the basis of the numbers, variability, and reproducibility of the bands obtained (Table 1).

2.4. RAPD-PCR and Documentation of Amplified Products. RAPD analyses were performed in a 96-well Peltier Thermal Cycler (Applied Biosystems 2720, Life Technologies, USA) in a final reaction volume of 25 μ L, each containing final concentrations of $\sim$ 100–150 ng of isolated gDNA, 1.6 pM of OPA or OPB primer, 1X Standard Taq Polymerase buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, and 1.5 mM MgCl_2) (NEB, USA), 200 μ M of each of dNTPs (dATP, dTTP, dCTP, and dGTP) (NEB, USA), and one unit of Taq DNA Polymerase (NEB, USA). PCR cycling programs were as follows: initial denaturation at 94°C for 5 min followed by 40 cycles of 94°C , 1 min for denaturation; 35°C , 1 min for annealing; 72°C , 2 min for elongation; and finally an extension at 72°C for 10 min. The amplified products were electrophoresed in 1.4% (w/v) agarose gel (Lonza, Basel, Switzerland) pre-stained with ethidium bromide (0.5 $\mu\text{g}/\text{mL}$) at a constant voltage 100 V and current 100 mA in TAE buffer (40 mM Tris-HCl, pH 8.0; 20 mM acetic acid; and 1 mM EDTA, pH 8.0) using BenchTop LabSystems BT-MS-300, Taiwan electrophoretic apparatus. Molecular weight of each band was estimated using a standard 100 base pair ladder (NEB, USA) and/or Lambda EcoRI/HindIII double digest DNA size marker (NEB, USA). The gels were visualized on the UV-transilluminator (Spectroline BI-O-Vision NY, USA) and photographed using a digital camera.

2.5. RAPD Data Analyses. RAPD data were analyzed for assessing within and between populations genetic variability of *Badis badis* in the two major rivers. The RAPD marker profiles were determined by direct comparison of the amplified profiles and the data obtained were computed and analyzed in the form of binary variables (1 = band present or 0 = band absent). Each locus was treated as a two-allele system, where only one of the alleles per locus was amplifiable by PCR and each fragment represented a Mendelian locus in which the

FIGURE 1: Map showing collection spots of *Badis badis* from the major streams of the Terai region of West Bengal, India. Geographical coordinates and altitudes, recorded by hand-held Garmin eTrex Vista HCx GPS, are as follows: M1 = 26°42.125'N; 88°24.717'E; 620 ft AMSL; M2 = 26°44.452'N; 88°25.497'E; 717 ft AMSL; B1 = 26°43.177'N; 88°22.825'E; 646 ft AMSL and B2 = 26°45.632'N; 88°18.912'E; 731 ft AMSL, P = 26°43.007'N; 88°24.396'E; 666 ft AMSL and F = 26°38.884'N; 88°24.125'E; 319 ft AMSL.

visible “dominant” allele was in Hardy-Weinberg equilibrium (HWE) with the corresponding “recessive” null allele or the absent fragment [9, 28]. The binary scores obtained from all the twenty-one primers in the RAPD analyses were then pooled for constructing a single data matrix for further analysis. Each set of PCR was repeated for at least three times to check the reproducibility of the banding pattern generated.

The RAPD data was analysed using three software applications, namely, Popgene ver. 1.32 [29], TFGPA (Tools for Population Genetic Analysis) ver. 1.3 [30], and GenAlEx 6.5 [31, 32]. Total number of RAPD bands, monomorphic bands, and polymorphic bands within and between populations were calculated manually by directly scoring the RAPD amplified banding profiles from gel photographs. Different indices of diversity measurement were used for assessing the genetic background of *Badis badis* species. The data matrix was used to estimate the observed number of alleles $[(1/K) \sum n_i]$, where K is the number of loci and n_i is the number of alleles detected per locus, effective number of alleles $(1/\sum p_i^2)$, where p_i is frequency of particular RAPD band [33], number of polymorphic loci, proportion of polymorphic loci, Nei's genetic diversity (H) [34], and Shannon's information index (H' or $I = -\sum p_i \log 2p_i$, where H' or I is diversity

and p_i is the frequency of a particular RAPD band) [35]. The rates of polymorphism were calculated using the criterion for polymorphism in which the frequency of the most common allele was ≤ 0.95 or ≤ 0.99 . The maximum diversity has been found where all RAPD bands had equal abundance. For better interpretation of Shannon's information index we have used the exponential function of Shannon's index, that is, $e^{H'}$, and subsequently calculated the measures of evenness ($E = e^{H'}/S$, where S is the observed number of alleles). The proportion of total inbreeding within the population was assessed by the formula $F_{IS} = F_{IT} - F_{ST}/1 - F_{ST}$ (where F_{IS} is the measure of inbreeding or departure from Hardy-Weinberg (HW) proportions within local demes or subpopulations, F_{ST} is the measure of allele frequency divergence among subpopulations relative to metapopulation, and F_{IT} is the measure of the overall departure from HW proportions for individuals with the metapopulation) [36].

To analyze the population genetic differentiation of *Badis badis*, F_{ST} values were calculated using the formula $F_{ST} = 1 - H_S/H_T$ (where H_S is the average expected heterozygosity estimated from each subpopulation and H_T is the total gene diversity or expected heterozygosity in the total population

TABLE 1: Code and sequence of random primers used for detection of polymorphism in *B. badis* populations.

Sl/No.	Primer	Sequence (5' → 3')	G + C Content (%)
1	OPA-01	CAGGCCCTTC	70
2	OPA-02	TGCCGAGCTG	70
3	OPA-04	AATCGGGCTG	60
4	OPA-07	GAAACGGGTG	60
5	OPA-09	GGGTAACGCC	70
6	OPA-10	GTGATCGCAG	60
7	OPA-13	CAGCACCCAC	70
8	OPA-16	AGCCAGCGAA	60
9	OPA-19	CAAACGTCCG	60
10	OPA-20	GTTGCGATCC	60
11	OPB-01	GTTTCGCTCC	60
12	OPB-03	CATCCCCCTG	70
13	OPB-04	GGACTGGAGT	60
14	OPB-05	TGCGCCCTTC	70
15	OPB-06	TGCTCTGCCC	70
16	OPB-07	GGTGACGCAG	70
17	OPB-11	GTAGACCCGT	60
18	OPB-12	CCTTGACGCA	60
19	OPB-13	TTCCCCCGCT	70
20	OPB-17	AGGGAACGAG	60
21	OPB-18	CCACAGCAGT	60

as estimated from the pooled allele frequencies) [36]. The diversity among populations ($D_{ST} = H_T - H_S$) was also calculated to ascertain the interpopulation diversity between different pairwise populations.

3. Results

RAPD data were analyzed to ascertain within and between populations genetic variability as well as genetic differences within and between the *Badis badis* populations of two major river streams of the Terai region of West Bengal India. The banding patterns generated through RAPD assay in the present study were used to evaluate the genetic variation within and between the populations of *B. badis* from the two major rivers of Terai region.

3.1. RAPD Profile. Twenty-one RAPD primers generated in total 120 (average 5.71) bands in M1-M2 population and 121 (average 5.76) bands in B1-B2 population (Figure 2). The number of bands ranged from 9 (OPA16, OPB04, and OPB17) to 3 (OPB11 and OPB18) in M1-M2 population and from 9 (OPB04) to 3 (OPB18) in B1-B2 population, respectively, (Table 2). The number of monomorphic bands ranged from 5 (OPA01, OPB04, and OPB06) to 1 (OPB03) in M1-M2 population and from 5 (OPA13 and OPB01) to 0 (OPB03 and OPB13) in B1-B2 population, respectively, (Table 2). The number of polymorphic bands ranged from 6 (OPB17) to 0 (OPB18) in M1-M2 population and from 5 (OPB17) to 0 (OPA13 and OPB18) in B1-B2 population, respectively, (Table 2). The total number of bands, monomorphic bands, and polymorphic

bands obtained between the M1-M2 population and the B1-B2 population ranged from 10 (OPB13) to 3 (OPB18), from 6 (OPA01) to 0 (OPB03 and OPB13), and from 9 (OPB12) to 1 (OPA10, OPA13, and OPB18), respectively, (Table 2).

3.2. Within Population Diversity. The total number of monomorphic and polymorphic bands generated after amplification with twenty-one RAPD primers in M1-M2 population was 67 (average 3.19) and 53 (average 2.52), respectively, (Table 2). The total number of monomorphic and polymorphic bands generated in B1-B2 population was 61 (average 2.90) and 60 (average 2.86), respectively, (Table 2). Diversity and evenness indices were estimated in the two *Badis badis* populations separately (Table 3). The richness or observed number of alleles and effective number of alleles of M1-M2 population were 1.4500 ± 0.4996 and 1.2863 ± 0.3797 , respectively. The proportion of polymorphic loci was 0.4416 (44.16%). The Nei's genetic diversity (H) and Shannon's information index (H' or I) were 0.1654 ± 0.2023 and 0.2450 ± 0.2907 , respectively. The richness or observed number of alleles and effective number of alleles of B1-B2 population were 1.5041 ± 0.5021 and 1.3501 ± 0.3946 , respectively. The proportion of polymorphic loci was 0.5041 (50.41%). The Nei's genetic diversity (H) and Shannon's information index (H' or I) were 0.1983 ± 0.2126 and 0.2901 ± 0.3037 , respectively. The measure of evenness (E) was 0.8811 and 0.8886 in M1-M2 and B1-B2 populations, respectively. The inbreeding coefficient (F_{IS}) of M1-M2 and B1-B2 populations was 0.1387 and 0.1316, respectively (Table 3).

FIGURE 2: Representative 1.4% agarose gel showing RAPD fragment patterns generated using OPA09, 10 and OPB01, 03 primers from Mahananda (M1 and M2) (a) to (d) and Balason river populations (B1 and B2) (e) to (h). m1: 100 base pair DNA ladder; m2: Lambda DNA EcoRI/HindIII double digest. Lanes 1 to 10 indicate 10 individuals from each river populations

3.3. Between Population Diversity and Genetic Differentiation. The total number of RAPD fragments scored between the Mahananda (M1-M2) and the Balason (B1-B2) populations was 150 (average 7.14) and the number of monomorphic and polymorphic bands scored was 49 (average 2.33) and 101 (average 4.81), respectively, (Table 2). The richness or

observed number of alleles and effective number of alleles of among the Mahananda and Balason populations were 1.6733 ± 0.4706 and 1.3752 ± 0.3642 , respectively, (Table 3). The proportion of polymorphic loci was 0.6733 (67.33%) (Table 3). The Nei's genetic diversity (H) and Shannon's information index (H' or I) were 0.2228 ± 0.1934 and 0.3364

TABLE 2: RAPD band profiles for the twenty-one primers in *Badis badis* population from two different locations of the Terai region of West Bengal.

Primers	Populations								
		“M1-M2”			“B1-B2”		“Between M1-M2 and B1-B2”		
	Total number of bands	Total number of monomorphic bands	Total number of polymorphic bands	Total number of bands	Total number of monomorphic bands	Total number of polymorphic bands	Total number of bands	Total number of monomorphic bands	Total number of polymorphic bands
OPA-01	8	5	3	8	4	4	9	6	3
OPA-02	5	3	2	6	2	4	7	3	4
OPA-04	5	3	2	5	2	3	7	3	4
OPA-07	7	3	4	5	3	2	7	2	5
OPA-09	5	3	2	6	4	2	6	3	3
OPA-10	4	3	1	4	1	3	5	4	1
OPA-13	6	4	2	5	5	0	6	5	1
OPA-16	9	4	5	5	4	1	9	2	7
OPA-19	5	2	3	5	2	3	8	2	6
OPA-20	7	3	4	6	2	4	8	3	5
OPB-01	4	1	3	7	5	2	8	1	7
OPB-03	4	1	3	4	0	4	5	0	5
OPB-04	5	5	0	9	5	4	9	2	7
OPB-05	6	4	2	8	4	4	8	3	5
OPB-06	5	5	0	5	3	2	6	3	3
OPB-07	9	4	5	7	3	4	9	1	8
OPB-11	3	2	1	5	2	3	6	1	5
OPB-12	7	4	3	6	4	2	10	1	9
OPB-13	4	2	2	4	0	4	5	0	5
OPB-17	9	3	6	8	3	5	9	2	7
OPB-18	3	3	0	3	3	0	3	2	1
Total	120	67	53	121	61	60	150	49	101
Average	5.71	3.19	2.52	5.76	2.90	2.86	7.14	2.33	4.81

M1 and M2 sites are from Mahananda river; B1 and B2 sites are from Balason river.

TABLE 3: Within and between populations genetic diversity, evenness, and inbreeding coefficient of *B. badis* based on RAPD analyses.

Population name	S ($\pm$ SD)	Effective number of alleles ($\pm$ SD)	Proportion of polymorphic loci	H ($\pm$ SD)	H' or I ($\pm$ SD)	$E = e^{H'}/S$	F_{IS}
M1/M2	1.4500 $\pm$ 0.4996	1.2863 $\pm$ 0.3697	0.4416 (44.16%)	0.1654 $\pm$ 0.2023	0.2450 $\pm$ 0.2907	0.8811	0.1387
B1/B2	1.5041 $\pm$ 0.5021	1.3502 $\pm$ 0.3946	0.5041 (50.41%)	0.1983 $\pm$ 0.2126	0.2901 $\pm$ 0.3037	0.8886	0.1316
M1/M2 and B1/B2	1.6733 $\pm$ 0.4706	1.3752 $\pm$ 0.3642	0.6733 (67.33%)	0.2228 $\pm$ 0.1934	0.3364 $\pm$ 0.2747	0.8360	0.1290
Mahananda-Balason river system	1.4372 $\pm$ 0.4680	1.2894 $\pm$ 0.3724	0.4371 (43.71%)	0.1601 $\pm$ 0.1944	0.2363 $\pm$ 0.2782	0.8876	0.0884

S : Observed number of alleles or richness; H: Nei's gene diversity; H' or I: Shannon's information index; E: measure of evenness; $F_{IS} = F_{IT} - F_{ST}/1 - F_{ST}$ = Inbreeding coefficient.

$\pm$ 0.2747, respectively. The measure of evenness (E) was 0.8360 (Table 3). The expected (H_T) and mean heterozygosity (H_S), diversity among populations (D_{ST}), and degree of gene differentiation (F_{ST}) were estimated between Mahananda and Balason populations. The expected and mean heterozygosity were found to be 0.2228 ± 0.0374 and 0.1758 ± 0.0289 ,

respectively, (Table 4). The diversity among the Mahananda and Balason populations was 0.0470 (Table 4). The degree of gene differentiation between the Mahananda and Balason populations was calculated to be 0.2109 (Table 4). The inbreeding coefficient (F_{IS}) between populations was found to be 0.1290 (Table 3).

TABLE 4: Between populations diversity and gene differentiation in two different populations of *B. badis* based on RAPD analyses.

Populations	H_T	H_S	D_{ST}	F_{ST}
Mahananda (M1/M2) and Balason (B1/B2)	0.2228 ± 0.0374	0.1758 ± 0.0289	0.0470	0.2109

H_T : expected heterozygosity in random mating total population; H_S : mean expected heterozygosity within random mating subpopulations; D_{ST} : diversity among populations; F_{ST} : degree of gene differentiation among populations in terms of allele frequencies.

3.4. Total Genetic Diversity of Mahananda-Balason River System. The observed number of alleles (richness) and effective number of alleles of the Mahananda-Balason river system (combining all the sites, namely, M1-M2, B1-B2, P, and F) were found to be 1.4372 ± 0.4680 and 1.2894 ± 0.3724 , respectively, (Table 3). The proportion of polymorphic loci was 0.4371 (43.71%) (Table 3). The Nei's genetic diversity (H) and Shannon's information index (H' or I) were 0.1601 ± 0.1944 and 0.2363 ± 0.2782 , respectively. The measure of evenness (E) was 0.8876. The inbreeding coefficient (F_{IS}) of the whole river system population was calculated to be 0.0884 (Table 3).

4. Discussion

RAPD-PCR can be used as an efficient molecular tool to differentiate allopatrically and/or sympatrically isolated populations and has been widely used to delineate the available gene pool of locally adapted populations of a species that may have arisen either by means of genetic selection under different environmental pressure or as a result of genetic drift [37]. To our knowledge, the present study is the first attempt to explore the present status of population specific genetic background of this fish fauna in the major river streams in the Terai region of this sub-Himalayan hotspot of North Eastern India. Our results showed that there is considerable lack of genetic variation among populations of *Badis badis* collected from the Mahananda-Balason river system.

Forty decamer primers were initially used to screen two different populations, and twenty-one primers were finally chosen for the study based on reproducibility of banding patterns (Table 1). The obtained results showed that the analyzed decamer primers permitted the detection of polymorphic fragments in *Badis badis*, revealing lower levels of genetic variability within and between two populations of major river streams of the study region. In the present study the number of amplified fragments generated by primer OPB12 was 6–10, 8–9 by primer OPB17, and 3 by primer OPB18, which were comparable with the amplified fragments observed by Brahmane et al. [24] (OPB12 = 4–6 bands, OPB17 = 3–6 bands, and OPB18 = 3–4 bands) in *Badis badis* populations collected from three districts of West Bengal namely, Nadia, North 24 Parganas, and Coochbehar. While there is an absence of comparable data on *Badis badis* and other related species from other Indian rivers, we have compared our

results with some evolutionary related species as well as some vulnerable species found in geographically related areas. In a study carried out by Kader et al. [38] on three *Tilapia* species (*Oreochromis niloticus*, *O. aureus*, and *Tilapia zilli*) in Egypt and by Chandra et al. [39] on vulnerable *Eutropiichthys vacha* in India, 15 RAPD primers generated 201 and 45 polymorphic amplified fragments, respectively. In a different study carried out by Alam et al. [18] and Miah et al. [40] on another locally vulnerable species of Mud eel (*Monopterus albus*) in Bangladesh, the number of polymorphic bands generated were 30 out of 39 bands and 24 out of 73 bands, respectively. Another study on endemic catfish (*Horabagrus brachysoma*) in the Western Ghat region of South India and the *Clarias batrachus* in Indian riverine system reported the number of polymorphic bands to be 75 and 358, respectively [41, 42]. The difference in the number and sizes of the amplified fragments can be ascribed to the sequence of the primers used and the source of the template DNA when compared to comparable studies. We have chosen a large number of decamer primers to provide robustness to our results [43].

The possible reason behind the observed high between populations allelic richness may be ascribed to the spatial distribution of the two populations (Figure 1). In a study on three *Tilapia* species in Egypt, the proportion of polymorphic loci was 51.8%, 58.6%, and 60.3%, respectively [38] and the proportion of polymorphic loci of *Tilapia zilli* and *Oreochromis niloticus* populations in Lake Victoria of Africa was 65.0% and 59.0%, respectively [44]. In the studies on vulnerable Mud eel of Bangladesh, the proportion of polymorphic loci was 76.92% [18] and 32.87% [40], while in the studies on endemic yellow catfish *Horabagrus brachysoma* and *Clarias batrachus* the proportion of polymorphic loci was 60.48% [41] and 77.49% [42], respectively. In the present study the proportion of polymorphic loci varies from 0.4371 (43.71%) in overall population to 0.6733 (67.33%) in between populations (Table 3). The overall proportion of polymorphic loci (43.71%) is lower than that found in phylogenetically related and vulnerable fish fauna indicating a substantial decline of genetic variability within *Badis badis* population of the sub-Himalayan Terai region.

Our study revealed that the Nei's genetic diversity (H) within the Mahananda (M1-M2) and Balason (B1-B2) populations is 0.1654 and 0.1983, respectively, whereas genetic diversity between populations is 0.2228 (Table 3). The overall genetic diversity of the river system was 0.1601 (Table 3). An earlier study carried out by Mwanja et al. [44] on *Oreochromis niloticus* populations in eastern Africa revealed that the genetic diversity ranged from highest (Lake Kachira = 0.27 and Lake Nabugabo = 0.29) to intermediate (Lake Albert = 0.17 and Lake Victoria = 0.20) and to lowest (Lake Edward = 0.14) values. The studies carried out by Appleyard and Mather [45] in *Oreochromis mossambicus* populations in Fiji islands displayed a lower (0.091 to 0.111) to highest (0.165 to 0.187) levels of observed heterozygosity. Moreover, Kader et al. [38] found that the genetic diversity was higher in *Tilapia zilli* (0.249) than in the *O. niloticus* and *O. aureus* populations (0.219 and 0.238, resp.). In the study carried out on vulnerable *Monopterus albus* in Bangladesh and on endemic species *Horabagrus brachysoma* in India [41] Nei's genetic diversity

was 0.285 and 0.222, respectively. Therefore in comparison it can be said that genetic diversity of *Badis badis* population in the Mahananda-Balason river system ranges from lower to intermediate values.

In our study the Shannon's information index (H') was calculated to be 0.240 ± 0.2907 in Mahananda (M1-M2), 0.2901 ± 0.3037 in Balason (B1-B2), and 0.3364 ± 0.2747 in Mahananda-Balason taken together, respectively, whereas the H' of overall river system was 0.2363 ± 0.2782 (Table 3). These results are similar to the findings of Chandra et al. [39] on *Eutropiichthys vacha*, where the Shannon's index was 0.280 and 0.300 in Patna and Madhepura populations, respectively. Moreover, Kader et al. [38] reported that *Tilapia zilli* population had a higher proportion of heterozygous genotypes ($H' = 0.363$) than *O. niloticus* ($H' = 0.318$) and *O. aureus* populations ($H' = 0.347$). In two different studies carried out by Alam et al. [18] and Miah et al. [40] on mud eel *Monopterus albus* in Bangladesh, the Shannon's information index was 0.423 and 0.213, respectively. Comparing our data with that of *Oreochromis sp.* and *Eutropiichthys vacha* it was found that the genetic diversity of *Badis badis* in the major river streams (Mahananda and Balason) of Terai region is lower.

We have used this exponential function of Shannon's index (i.e., $e^{H'}$) to calculate the measure of evenness [46]. The measure of evenness was more or less similar in these two populations: 0.8811 in Mahananda population and 0.8886 in Balason population (Table 3). Therefore, this indicates that the gene pool of these two populations is genetically quite even in distribution. This might happen as an outcome of addition of juveniles due to the joining of the major streams with small tributaries, especially during the monsoonal breeding season, which may result in substantial amount of gene flow between these two major river stream populations.

While the value of F_{IS} ranged from -1.0 (all the individuals within a subpopulation being heterozygotes) to $+1.0$ (no observed heterozygotes are present within a subpopulation), we have found that the Mahananda and Balason river system showed positive values of the inbreeding coefficient [Mahananda population (M1-M2) = 0.1387, Balason population (B1-B2) = 0.1316, between Mahananda and Balason population (M1-M2 and B1-B2) = 0.1290; and 0.0884 in the overall Mahananda-Balason river system] indicated a lower number of heterozygotes within these two populations. Abdul Muneer et al. [41] also reported that the F_{IS} value of *Horabagrus brachysoma* was 0.504 indicating a deficiency of heterozygotes. Nonrandom mating or inbreeding, spatial Wahlund effect, and expression of null alleles are the leading causes of heterozygotes deficiency in small populations. However, in small populations sexual differences in allele frequencies are likely to cause an increase in heterozygotes relative to Hardy-Weinberg proportions and therefore there is a tendency for the excess of heterozygotes [47].

Fixation index or F_{ST} is a measure of genetic divergence among subpopulations that ranges from 0 (when all subpopulations have equal allele frequencies) to 1 (when all the subpopulations are fixed for different alleles) [47]. We have found that between the Mahananda and Balason populations

the D_{ST} value was 0.047 and the F_{ST} value was 0.2109 (Table 4). In a different study carried out by Abdul Muneer et al. [41] on *Horabagrus brachysoma* the F_{ST} value ranged from the maximum (0.219) between (0.045) Meenachil and Nethravathi populations to the minimum between (0.045) Meenachil and Chalakkudi populations. The lower value of D_{ST} in the present study indicates that the diversity between these two populations was low and a higher F_{ST} value indicates that gene differentiation was high. The observed D_{ST} and F_{ST} values may be attributed to several factors, namely, lack of migration, lower level of gene flow, water flow pattern, water volume, fishing extent, and other possible anthropogenic and/or geological activities. This decrease in genetic diversity and variability within and between the gene pools of the *Badis* population in the study area may result due to flow pattern disturbances owing to sand/stone excavation, indiscriminate fishing and pesticide run-offs from adjacent tea gardens in the hilly areas of lower Himalayas, as the river streams flow from higher to lower altitudes. All of these causes can culminate into the observed lower level of the genetic diversity pattern and genetic erosion in *Badis badis* populations across the Mahananda and Balason river system.

However there was a weak point in this study, as a limited number of (ten samples from each river stream) individuals were involved for genetic analyses, primarily because of the dwindling population structure of the species and also to have ease in data handling and analyses. However a large number of decamer primers were used to provide robustness to the study. The present study using twenty-one RAPD primers revealed that a lower level of genetic diversity exists in the *Badis badis* population of the Mahananda-Balason river system in the Terai region. Several anthropogenic interferences like indiscriminate fishing, effluents from adjacent households and factories, heavy use of fertilizers and pesticides in the nearby tea gardens and their subsequent run-offs into the hilly parts of the streams, or excavation of river bed along the banks for sand and gravel, dam building activities at the upper reaches of the rivers all may be responsible for the lack of the overall diversity of this threatened and ornamental fish species in this region. Therefore, conservational interventions may be directed towards the Terai population. Proper steps should be taken to improve the environment to avoid further losses. The genetic data obtained from the present study lend support to the view that there is a scope of stock improvement for this threatened ornamental fish.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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