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ANALYSES OF GENETIC DIVERSITY OF *BADIS BADIS* (HAMILTON-BUCHANAN 1822) FROM THREE RIVERINE SYSTEMS IN SUB-HIMALAYAN BIODIVERSITY HOTSPOT OF WEST BENGAL, INDIA USING RAPD AND ISSR FINGERPRINTING

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Badis badis is a threatened freshwater fish in the Indian scenario, and the population genetic architecture of this fish is largely unexplored in the eastern sub-Himalayan biodiversity hotspot of West Bengal, India, also known as the Terai and the Dooars. Total seventeen populations from three major river systems viz. Mahananda (Terai), Teesta and Jaldhaka (Dooars) have been studied through RAPD and ISSR fingerprinting. The polymorphism, genetic diversity and Shannon's Information index were calculated for each population. The highest values for Nei's genetic diversity (0.1436± 0.1963 and 0.1409 ±0.1954 after RAPD and ISSR analyses) and Shannon's information index (0.2150±0.2794 and 0.2109 ±0.2785 after RAPD and ISSR analyses) were found in Jaldhaka river system. The UPGMA dendrogram revealed that the Mahananda and Teesta populations form a single group and the Jaldhaka population forms a separate group. Comparing with other related studies the genetic diversity *Badis badis* was found to be low in the three main riverine systems of the Terai and Dooars region of West Bengal, although the Jaldhaka population showed a comparatively higher level of genetic diversity. Therefore, Jaldhaka population should be managed and, conserved to preserve the available gene pool of this threatened species in this region. Low levels of genetic diversity were found in the present study among the seventeen populations validating the recent assignment of its threatened status.

Key words: Badis badis, RAPD, ISSR, genetic diversity, sub-Himalayan hotspot region.

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INTRODUCTION

Badis badis (Hamilton-Buchanan, 1822) (Actinopterygii, Perciformes, Badidae) is a tropical, freshwater species that has ornamental importance and thus possesses commercial value. The species has been categorized as vulnerably susceptible in the list of threatened freshwater fishes of India by NBFGR, Lucknow (LAKRA, 2010). However, studies with regard to genetic diversity of fish species especially in the eastern sub-Himalayan region of West Bengal, India have been found to be very inadequate. From the standpoint of validating its threatened status, assessment of its genetic diversity from this particular region with an aim towards conserving its wild population thus seem to be of great significance.

Genetic variability of an organism is of paramount importance for the sustenance of the whole population; therefore, the erosion of genetic diversity of a population curtails its adaptability and increases the risk of its extinction. The vulnerable organisms having small population size may experience continuous erosion in the genetic variation (LANDWEBER and DOBSON, 1999). However, maintaining the genetic variation in a threatened species is essential to ascertain its adaptation, expansion and reestablishment in natural populations. The study of intra and inter-population genetic variability is profoundly utilizable to gain information on individual identity, genetic relatedness and genetic diversity within as well as between them.

Molecular markers are realistic and useful tool to investigate and monitor the genetic structure of populations both in native and captive condition (ALAM *et al.,* 2010). RAPD and ISSR techniques have been widely used for genetic diversity studies because they are easy, cost effective and fast methods, especially when other sophisticated methods are not yet developed. Moreover, the RAPD and ISSR amplifications do not require prior knowledge of flanking sequence of the genome of the concerned species (ZIETKIEWICZ *et al.*, 1994). RAPD-PCR technique has been extensively used to characterize genetic structure as well as to study genetic diversity of different fishes such as *Epinephelus* sp. (ROY *et al.*, 2014), *Oreochromis* sp. (KADER *et al.*, 2013), *Onchorhynchus* sp. and *Schizothorax* sp. (VASAVE *et al.*, 2014), *Mystus* sp. (HASAN and GOSWAMI, 2015a, b; *Badis* sp. (MUKHOPADHYAY and BHATTACHARJEE, 2014b), *Tenualosa* sp. (BRAHMANE *et al.*, 2006), *Monopterus* sp. (ALAM *et al.*, 2010), *Barilius bendelisis* and *B. Barna* (MISHRA *et al.*, 2012). ISSR primers usually amplify DNA segments present at an amplifiable distance between two oppositely oriented identical microsatellite repeat regions (REDDY *et al.*, 2002). ISSR markers are generated via PCR amplification with a single microsatellite primer anchored (either 5′ or 3′) with one to three nucleotides that aim to eliminate slippage-related artefacts. The amplified regions represent the flanking sequence between two microsatellite sites and the absence of bands is interpreted as a loss of a locus by the deletion of the microsatellite site or chromosomal rearrangement (WOLFE and LISTON, 1998). Some advantages related to these markers are rapidness, small quantities of template DNA, less number of PCR reactions and high annealing temperatures that reduce the quantity of artefacts (PAZZA *et al.*, 2007). ISSR is a multi-locus marker and highly polymorphic in nature (MALTAGLIATI *et al.*, 2006; LI *et al.*, 2013). Thus, ISSR-PCR technique was preferred over to other dominant markers by several groups of researchers in studying genetic diversity at the interspecific and intraspecific levels (CHEN *et al.*, 2009; PANARARI-ANTUNES *et al.*, 2011; SAAD *et al.*, 2012; HANIFFA *et al.*, 2014; LABASTIDA *et al.*, 2015). Therefore, due to the unavailability of microsatellite or SNP markers in *Badis badis* till date, we resorted to the time-tested RAPD and ISSR primers to ascertain and compare the available genetic variations in this ichthyofauna.

We have previously estimated the genetic diversity of *Badis badis* in the Mahananda river system through RAPD marker analyses (MUKHOPADHYAY and BHATTACHARJEE, 2014a). The objectives of the present study was to (1) estimate the intra-population genetic diversity of *Badis badis* of the three major riverine systems (Mahananda, Teesta and Jaldhaka) of the sub-Himalayan Dooars region of West Bengal, India through different diversity indices by RAPD and ISSR fingerprinting, (2) compare the genetic diversity between the three riverine populations and (3) ascertain the genetic distance and genetic relatedness of the populations from the major river streams of this region.

MATERIALS AND METHODS

Survey and Sample Collection

An extensive survey has been carried out in different spots of the major streams of eastern sub-Himalayan region of West Bengal, India. We have collected the total 170 fish samples from Mahananda-Balasan, Teesta River and Jaldhaka river system of the Terai and Dooars Region of eastern sub-Himalayan West Bengal, India. Total seventeen spots were selected for collection (ten samples from each collection site) of the fish samples (six spots from Mahananda-Balasan river system, seven spots from Teesta river system and four spots from Jaldhaka river system). The geographic co-ordinates were recorded with the help of GPS (eTrex Vista HCx, Garmin, USA). We have previously reported that the genetic diversity of this fish population has dwindled in the nearby riverine system (MUKHOPADHYAY and BHATTACHARJEE, 2014a). Therefore a limited number of individuals (ten individuals from each collection site) were involved for further population genetic analyses because of the waning population structure of this species in the study region. The collection spots were as follows: TR-1, TR-2, TR-3, TR-4, TR-5 and TR-6 (Mahananda river system from Terai region); and DR-1, DR-2, DR-3, DR-4, DR-5, DR-6, DR-7, DR-8, DR-9, DR-10 and DR-11 (Teesta and Jaldhaka river system from Dooars region). The local names and geographical co-ordinates of the collection spots are mentioned in Figure 1 and Table 1. Fishes were identified according to SHAW and SHEBBEARE (1937) and TALWAR and JHINGRAN (1991).

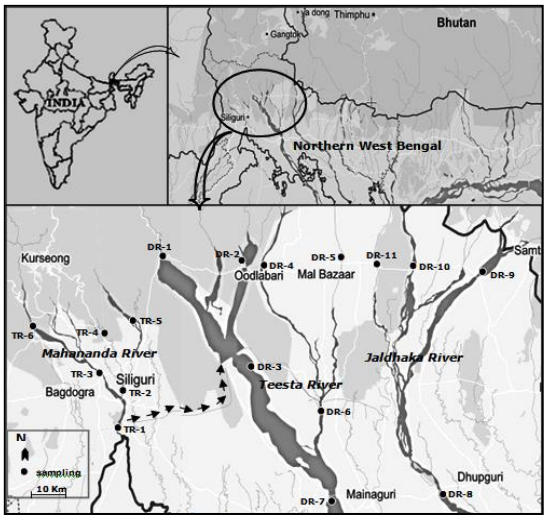

Figure 1. Map showing collection spots of *Badis badis* from the three major river streams of the Terai (TR) and Dooars (DR) region in the northern part of West Bengal, India. Geographical locations and altitudes were recorded by hand-held GPS. The alphabets in capital bold case indicate the collection spots. The arrow indicates the narrow water channel that carries water Mahananda Barrage, Fulbari to Teesta River Barrage, Gajoldoba. See Table 1 for details.

Table 1. Sample (Badis badis) collection spots with their local names and the geographic co-ordinates

Sl/No.	Population Code	Name of the River and adjacent place	Geographic position and GPS Record
1	TR-1	Mahananda Barrage, Fulbari.	26°38.884´N; 88°24.125´E; Elevation 319 AMSL
2	TR-2	Mahananda-Panchanoi River Junction, Siliguri.	26°42.125´N; 88°24.717´E; Elevation 620 AMSL
3	TR-3	Balason River, Palpara, Matigara.	26°43.177´N; 88°22.825´E; Elevation 646 AMSL
4	TR-4	Panchanoi River, Siliguri.	26°43.007´N; 88°24.396´E; Elevation 666 AMSL
5	TR-5	Mahananda River, Champasari, Siliguri.	26°44.452´N; 88°25.497´E; Elevation 717 AMSL
6	TR-6	Balason River, Tarabari.	26°45.632´N; 88°18.912´E; Elevation 731 AMSL
7	DR-1	Sevok (Teesta River)	N 26°53´043, E 88°28´367 Elevation 480 AMSL
8	DR-2	Ghish River	N 26°52´327, E 88°36´355 Elevation 536 AMSL
9	DR-3	Gajoldoba (Teesta River Barrage)	N 26°44´584, E 88°35´314 Elevation 354 AMSL
10	DR-4	Chel River	N 26°51´499, E 88°38´048 Elevation 522 AMSL
11	DR-5	Neora River	N 26°52´486, E 88°46´205 Elevation 527 AMSL
12	DR-6	Dharla River	N 26°40´496, E 88°44´126 Elevation 299 AMSL
13	DR-7	Jalpaiguri (Teesta River)	N 26°33´499, E 88°45´369 Elevation 274 AMSL
14	DR-8	Jaldhaka River	26°34´13.17 N, 88°56´14.26 E Elevation 267 AMSL
15	DR-9	Murti River	26°52´57.73 N, 88°49´44.98 E Elevation 578 AMSL
16	DR-10	Ghotia River	26°52´14.89 N, 88°53´37.98 E Elevation 540 AMSL
17	DR-11	Diana River	26°51´37.96 N, 89°00´07.40 E Elevation 647 AMSL

*AMSL= Above Mean Sea Level; Terai (TR) and Dooars (DR) region

Isolation of high molecular weight genomic DNA and quantification

Genomic DNA (gDNA) was extracted from a tiny amount of tissue samples (10-15 mg of fin clips from the caudal and ventral portions) from the *Badis badis* using commercial DNA isolation Kit (DNeasy Blood and Tissue Kit, Qiagen) following a standardized method

(MUKHOPADHYAY and BHATTACHARJEE, 2014b).The extracted gDNA samples were stored in 1.5 ml microcentrifuge tube at -20ºC till further analysis. The gDNA samples were subjected to spectrophotometric quantification (Rayleigh UV-2601 Spectrophotometer, Beijing, China). The concentration of the extracted gDNA was adjusted to 50 ng/µl for each PCR amplification.

Primer selection

Forty arbitrary decamer RAPD primers of random sequences (Kit-A and Kit-B, twenty primers from each kit) were purchased from Imperial Life Science Pvt. Ltd., India. Firstly, all the populations were screened with the forty primers and finally twenty (ten primers from Kit-A and ten primers from Kit-B) were selected for further analyses on the basis of the variability and reproducibility of the bands obtained (Table 2). The GC content of the primers was between 60-70%. Twenty-one ISSR primers, purchased from Xcelris Genomics, India, were used to screen two populations; and finally, fifteen ISSR primers (3´-anchored) were selected for further analyses on the basis of the variability and reproducibility (Table 2). The annealing temperatures of the ISSR primer were optimized for each amplification and are depicted in Table 2.

Table 2. Primer names, Sequence, GC content, annealing temperature, number of fragments and number of polymorphic fragments amplified by different RAPD and ISSR primers.

RAPD						
Sl/No.	Primer	Sequence (5´➔3´)	G+ C Content (%)	Annealing temperature	Total number of fragments scored	Number of polymorphic bands
1	OPA-01	CAGGCCCTTC	70	36ºC	8	8
2	OPA-02	TGCCGAGCTG	70		5	3
3	OPA-04	AATCGGGCTG	60		7	6
4	OPA-07	GAAACGGGTG	60		7	7
5	OPA-09	GGGTAACGCC	70		7	7
6	OPA-10	GTGATCGCAG	60		5	5
7	OPA-13	CAGCACCCAC	70		6	4
8	OPA-16	AGCCAGCGAA	60		8	8
9	OPA-19	CAAACGTCGG	60		8	8
10	OPA-20	GTTGCGATCC	60		7	5
11	OPB-01	GTTTCGCTCC	60		6	6
12	OPB-03	CATCCCCCTG	70		5	1
13	OPB-04	GGACTGGAGT	60		6	4
14	OPB-06	TGCTCTGCCC	70		6	3
15	OPB-07	GGTGACGCAG	70		9	9
16	OPB-11	GTAGACCCGT	60		8	8
17	OPB-12	CCTTGACGCA	60		9	8
18	OPB-15	GGAGGGTGTT	70		8	2
19	OPB-17	AGGGAACGAG	60		8	1
20	OPB-18	CCACAGCAGT	60		8	2
Total = 141						**Total = 105**

			ISSR			
1	ISSR-1	$(CT)_8TG$	50		5	4
2	ISSR-2	$(CT)_8AC$	50	45ºC	7	5
3	ISSR-3	$(CT)_8GC$	55.6		7	6
4	ISSR-4	$(CA)_6AG$	50		7	7
5	ISSR-5	$(CA)_6AC$	50		6	5
6	ISSR-7	$(CAC)_3GC$	72.7	33ºC	8	7
7	ISSR-8	$(GAG)_3GC$	72.7		8	6
8	ISSR-9	$(GTG)_3GC$	72.7		7	6
9	ISSR-10	$(GGAC)_3T$	69.2	42ºC	11	8
10	ISSR-11	$(GA)_6CC$	57.1	37ºC	9	7
11	ISSR-13	$(GT)_6CC$	57.1		8	5
12	ISSR-15	$(ACC)_6$	66.66	33ºC	8	6
13	ISSR-19	$(GGAC)_3A$	69.2	42ºC	9	7
14	ISSR-20	$(GGAC)_3C$	76.9		8	6
15	ISSR-21	$(GGAC)_4$	75	45ºC	8	6
			Total = 116			**Total = 91**

PCR amplification and documentation of amplified products

RAPD and ISSR-PCR amplifications were performed in a 96 well Eppendorf® thermal cycler in a final reaction volume of 25 µl, each containing a final concentrations of ~100-150 ng of isolated gDNA, 1.6 pM of oligonucleotide primers (both for RAPD and ISSR), standard Taq polymerase buffer (10mMTris-HCl, pH 8.3, 50 mMKCl, 1.5 mM $MgCl_2$) (NEB, USA), 200 µM of each dNTPs (dATP, dTTP, dCTP, dGTP) (NEB, USA), and one unit of Taq DNA Polymerase (NEB, USA). After the standardization of each reaction regime, all PCR amplifications were replicated to verify reproducibility and authenticity of the DNA bands. PCR cycling programs were as follows: initial denaturation at 94º C for 5 min followed by 50 cycles (RAPD) or 40 cycles (ISSR) of 94ºC, 1 min for denaturation; 35ºC (RAPD) and 38ºC - 47ºC (ISSR) (specific and optimal annealing temperature for each primer, see Table 1), 1 min for annealing; 72 ºC, 2 min for elongation and finally an extension at 72 ºC for 10 min. The amplified products were electrophoresed in an ethidium bromide (0.5µg/ml)-pre-stained 1.4% (for RAPD) / 1.6% (for ISSR) (w/v) agarose gels (Lonza, Basel, Switzerland) at a constant voltage 100 V and current 100 mA in TAE buffer (40 mMTris-HCl, pH 8.0; 20 mM Acetic acid; 1 mM EDTA, pH 8.0) using BenchTopLabsystems BT-MS-300, Taiwan electrophoretic apparatus. The molecular weight of each band was estimated using a standard 100 base pair ladder (NEB, USA). The gels were visualized on the UV-transilluminator (SpectrolineBI-O-Vision®NY, USA) and photographed using a Nikon D3100 camera.

RAPD and ISSR data analyses

RAPD and ISSR data from seventeen *Badis badis* populations were analyzed for assessing intra-population genetic variability within each of the seventeen collection sites. The RAPD and ISSR 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 visible 'dominant' allele was in Hardy-Weinberg equilibrium with the corresponding 'recessive' null allele or the absent fragment (WILLIAMS *et al.*, 1990; LYNCH and MILLIGAN, 1994). The binary scores obtained from all the twenty primers in the RAPD analyses and fifteen primers in the ISSR analyses were then individually pooled for constructing a single data matrix for further analysis. The RAPD and ISSR profile generated was compared within and between populations in a pair-wise manner.

The RAPD data was analysed using two softwares viz., Popgene ver. 1.32 (YEH *et al.*, 1999), TFPGA (Tools for Population Genetic Analysis) ver.1.3 (MILLER, 1997). Different indices of diversity measurement were used for the assessment of 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= number of loci and n_i= 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) (KIMURA and CROW, 1964), number of polymorphic loci, proportion of polymorphic loci, Nei's genetic diversity (H) (NEI, 1973), Shannon's Information Index (H' or $I= -\sum p_i log2\ p_i$, where H' or I is diversity and $p_i$is the frequency of a particular RAPD or ISSR band) (LEWONTIN, 1972). 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 and ISSR bands have equal abundance. For better interpretation of Shannon's Information index we have used the exponential function of Shannon's Index i.e, $e^{H'}$ and subsequently calculated the measures of evenness ($E= e^{H'}/S$, where S is the observed number of alleles) and Heip's index of evenness using the formula $E_{Heip}= e^{H'}-1/S-1$ (HEIP, 1974).

The binary matrix prepared from all scored fragments were used to generate Nei's unbiased measures of genetic identity and genetic distance matrix (NEI, 1978) using the software Popgene ver. 1.32 and the output data matrix was also verified separately using the softwares TFPGA ver. 1.3 and Arlequin ver. 3.1 (EXCOFFIER and SCHNEIDER, 2005). The Nei's genetic distance matrix was subjected to unweighted pair-group method using arithmetical averages (UPGMA) to generate a dendrogram through linkage procedure using the software Phylip ver. 3.69 (FELSENSTEIN, 2005) and FigTree ver.1.3.1 (RAMBAUT, 2010).

RESULTS

RAPD and ISSR data were analyzed to ascertain and compare the genetic variability and differences within and between populations as well as genetic distances among the *Badis badis* populations of three riverine system of the Terai and Dooars region of West Bengal India. The DNA band fingerprinting generated through RAPD and ISSR assay in the present study was used to evaluate the genetic variation within and between the populations of *B. Badis* from the two major rivers of the Terai and Dooars region.

RAPD and ISSR band profiles

Twenty RAPD primers generated total 141 amplified fragments, out of which 105 fragments were polymorphic in nature (Table 2). The percentage of polymorphism was 74.16. The number of amplified fragments generated by OPA primers were 68 (48.22%) and OPB were 73 (51.77%). The number of amplified polymorphic fragments generated by OPA primers were 61 (58.10%) and OPB were 44 (41.09%). The number of amplified fragments ranged from 5 (OPA02, OPA10 and OPB03) to 9 (OPB 07 and OPB 12) (Table 2). The number of polymorphic fragments ranged from 1 (OPB 03 and OPB 17) to 9 (OPB 07) (Table 2). The highest numbers of amplified fragments (nine) and polymorphic fragments (nine) were noticeable in primers OPB 07 and OPB 12; and OPB 07 respectively (Table 2). Fifteen ISSR primers generated a total of 116 amplified fragments, out of which 91 fragments were polymorphic in nature (Table 2). The percentage of polymorphism was 78.44%. The number of amplified fragments ranges from 5 (ISSR-1) to 11(ISSR-10) (Table 2). The number of polymorphic fragments ranges from 4 (ISSR-1) to 8 (ISSR-10) (Table 2). The highest number of amplified fragments (eleven) and polymorphic fragments (eight) was noticeable in primer ISSR-10 (Table 2).

Intra-population genetic diversity:

Mahananda river system

Based on the RAPD profile the number of polymorphic loci and the percentage of polymorphic loci varied across six populations (TR-1 to TR-6) (Table 3). The highest number of polymorphic loci was observed in TR-6 population (44 numbers) and the percentage of polymorphism was 31.21. The lowest number of polymorphic loci was observed in TR-3 population (34 numbers) and the percentage of polymorphism was 24.11. The observed number of alleles or allelic richness (S) varied from 1.2411± 0.4293in TR-3 population to 1.3121± 0.4650 in TR-6 population (Table 3). The Nei's genetic diversity (H) was highest (0.1166± 0.1890) in TR-6 population and lowest (0.0865± 0.1690) in TR-3 population (Table 3). The Shannon's information index (H′ or I) was highest (0.1717±0.2709) in TR-6 population and lowest (0.1283±0.2433) in TR-3 population (Table 3). However, the measure of evenness (E) was highest (0.916037) in TR-3 population and lowest (0.904902) in TR-6 population (Table 3). The Heip's measure of evenness was highest (0.600197) in TR-6 population and lowest (0.567789) in TR-3 population (Table 3). The diversity indices based on the ISSR analyses were also in accordance with the RAPD data. The highest number of polymorphic loci was observed in TR-6 population (38 numbers) and the percentage of polymorphism was 32.76. The lowest number of polymorphic loci was observed in TR-3 population (26 numbers) and the percentage of polymorphism was 22.41. The observed number of alleles or allelic richness (S) varies from 1.2241± 0.4188 in TR-3 population to 1.3276± 0.4714 in TR-6 population (Table 3). The Nei's genetic diversity (H) was highest (0.1136± 0.1806) in TR-6 population and lowest (0.0767± 0.1593) in TR-3 population (Table 3). The Shannon's information index (H′ or I) was highest (0.1704±0.2619) in TR-6 population and lowest (0.1149±0.230) in TR-3 population (Table 3). However, the measure of evenness (E) was highest (0.916037) in TR-3 population and lowest (0.904902) in TR-6 population (Table 3). The Heip's measure of evenness was highest (0.600197) in TR-6 population and lowest (0.567789) in TR-3 population (Table 3).

Table 3. Intra-population genetic diversity indices based on RAPD and ISSR analyses of Mahananda-Balason (TR) river system.

Populations	Molecular Markers						
	RAPD						
	N_p	N_{per}	S	H	H´ or I	E= $e^{H'}/S$	$E_{Heip}=(e^{H'}-1/S-1)$
Mahananda Barrage, Fulbari **(TR-1)**	40	28.37%	1.2837± 0.4524	0.1061± 0.1841	0.1562± 0.2637	0.910696	0.595911
Mahananda-Panchanoi River Junction**(TR-2)**	35	24.82%	1.2482± 0.4335	0.0898± 0.1718	0.1330± 0.2471	0.915118	0.573127
Balason River, Palpara, Matigara **(TR-3)**	34	24.11%	1.2411± 0.4293	0.0865± 0.1690	0.1283± 0.2433	0.916037	0.567789
Panchanoi River **(TR-4)**	36	25.53%	1.2553± 0.4376	0.0946± 0.1766	0.1395± 0.2533	0.915876	0.586364
Mahananda River, Champasari**(TR-5)**	37	26.24%	1.2624± 0.4415	0.0953± 0.1764	0.1409± 0.2531	0.912001	0.576637
Balason River, Tarabari**(TR-6)**	44	31.21%	1.3121± 0.4650	0.1166± 0.1890	0.1717± 0.2709	0.904902	0.600197

ISSR						
N_p	N_{per}	S	H	H´ or I	E= $e^{H'}/S$	$E_{Heip}=(e^{H'}-1/S-1)$
34	29.31%	1.2931± 0.4572	0.1024± 0.1751	0.1534± 0.2540	0.910696	0.595911
27	23.28%	1.2328± 0.4244	0.0806± 0.1629	0.1204± 0.2354	0.915118	0.573127
26	22.41%	1.2241± 0.4188	0.0767± 0.1593	0.1149± 0.2305	0.916037	0.567789
30	25.86%	1.2586± 0.4398	0.0924± 0.1733	0.1372± 0.2493	0.915876	0.586364
32	27.59%	1.259± 0.4489	0.0959± 0.1749	0.1428± 0.2513	0.912001	0.576637
38	32.76%	1.3276± 0.4714	0.1136± 0.1806	0.1704± 0.2619	0.904902	0.600197

Note:N_p=number of polymorphic loci, N_{per}=percentage of polymorphic loci, S=observed number of alleles, H=Nei's gene diversity, H´ or I= Shannon's Information index, E= measure of evenness, E_{Heip}= Heip's evenness index.

Teesta river System

Both RAPD and ISSR analyses showed that the diversity indices varied across populations from DR-1 to DR-7 (Table 4). The highest number of polymorphic loci was observed in DR-4 population and the percentages of polymorphism were found to be 34.75 in RAPD and 34.48 in ISSR analyses. The lowest number of polymorphic loci was observed in DR-3 population and the percentages of polymorphism were found to be 21.28 in RAPD and 21.55 in ISSR analyses. The observed number of alleles or allelic richness (S) varied from 1.2128±0.4107 and 1.2155±0.4130 in DR-3 population to 1.3475±0.4779 and 1.3448±0.4774 in DR-4 population by RAPD and ISSR analyses respectively (Table 4). The Nei's genetic diversity (H) was highest (0.1291± 0.1964 and 0.1242 ±0.1917) in DR-4 population and lowest (0.0762± 0.1611 and 0.0726 ± 0.1549) in DR-3 population in RAPD and ISSR analyses respectively (Table 4). The Shannon's information index (H´ or I) was highest (0.1897±0.2802 and 0.1838 ±0.2746) in DR-4 population and lowest (0.1130±0.2322 and 0.1071±0.2248) in DR-3 population in RAPD and ISSR analyses respectively (Table 4). The measure of evenness (E) was highest (0.923179 and 0.917543) in DR-3 population in RAPD and ISSR respectively; and lowest (0.897133) in DR-4 population in RAPD and 0.888922 in DR-1 population in ISSR analyses (Table 4). The Heip's measure of evenness was highest (0.607581 and 0.593509) in DR-2 population in RAPD and ISSR analyses and lowest (0.539581) in DR-5 population in RAPD and 0.501088 in DR-6 population in ISSR analyses (Table 4).

Table 4. Intra-population genetic diversity indices based on RAPD and ISSR analyses of Teesta (DR) river system

Populations	**Molecular Markers**						
	RAPD						
	N_p	**N_{per}**	**S**	**H**	**H´ or I**	**E= $e^{H'}$/S**	**E_{Heip}=($e^{H'}$-1/S-1)**
Sevok (Teesta River) **(DR-1)**	47	33.33%	1.3333± 0.4731	0.1191± 0.1925	0.1756± 0.2735	0.893993	0.575941
Ghish River **(DR-2)**	36	25.53%	1.2553± 0.4376	0.0984± 0.1800	0.1442± 0.2584	0.92019	0.60758
Gajoldoba (Teesta River Barrage) **(DR-3)**	30	21.28%	1.2128± 0.4107	0.0762± 0.1611	0.1130± 0.2322	0.923179	0.562181
Chel River **(DR-4)**	49	34.75%	1.3475± 0.4779	0.1291± 0.1964	0.1897± 0.2802	0.897133	0.601113
Neora River **(DR-5)**	34	24.11%	1.2411± 0.4293	0.0820± 0.1655	0.1223± 0.2378	0.910558	0.539581
Dharla River **(DR-6)**	31	21.99%	1.2199± 0.4156	0.0801± 0.1641	0.1187± 0.2366	0.923053	0.573134
Jalpaiguri (Teesta River) **(DR-7)**	34	24.11%	1.2411± 0.4293	0.0932± 0.1805	0.1355± 0.2566	0.922657	0.601863

ISSR						
N_p	**N_{per}**	**S**	**H**	**H´ or I**	**E= $e^{H'}/S$**	**$E_{Heip}=(e^{H'}-1/S-1)$**
40	34.48%	1.3448 ± 0.4774	0.1207 ± 0.1924	0.1785 ± 0.2734	0.888922	0.566772
31	26.72 %	1.2672± 0.4444	0.0999 ± 0.1793	0.1472 ± 0.2579	0.914288	0.593509
25	21.55%	1.2155 ± 0.4130	0.0726 ± 0.1549	0.1071 ± 0.2248	0.917543	0.534914
40	34.48%	1.3448 ± 0.4774	0.1242 ± 0.1917	0.1838 ± 0.2746	0.893646	0.585196
29	25.00%	1.2500 ± 0.4349	0.0807 ± 0.1629	0.1214 ± 0.2342	0.903261	0.516306
26	22.41%	1.2256 ± 0.4211	0.0746 ± 0.1649	0.1091 ± 0.2028	0.908164	0.501088
27	23.27%	1.2376 ± 0.4111	0.0786 ± 0.1675	0.1151 ± 0.2228	0.906582	0.513408

Note: N_p=number of polymorphic loci, N_{per}=percentage of polymorphic loci, S=observed number of alleles, H=Nei's gene diversity, H´ or I= Shannon's Information index, E= measure of evenness, E_{Heip}= Heip's evenness index.

Jaldhaka river system

The RAPD and ISSR analyses showed that the polymorphism varied across four populations of Jaldhaka river system. The highest number of polymorphic loci was observed in DR-11 population and the percentages of polymorphism were found to be 43.26 in RAPD and 42.24 in ISSR analyses. The lowest number of polymorphic loci was observed in DR-8 population and the percentages of polymorphism were found to be 36.17 in RAPD and 39.66 in ISSR analyses. The observed number of alleles or allelic richness (S) varied from 1.3171± 0.4811 and 1.3966 ±0.4913in DR-8 population to 1.4326± 0.4972 and 1.4224 ±0.4961 in DR-11 population by RAPD and ISSR analyses respectively (Table 5). The Nei's genetic diversity (H) was highest (0.1436± 0.1963 and 0.1409 ±0.1954) in DR-11 population and lowest (0.1048± 0.1855 and 0.1348 ± 0.1927) in DR-8 population in RAPD and ISSR analyses respectively (Table 5). The Shannon's information index (H´ or I) was highest (0.2150±0.2794 and 0.2109 ±0.2785) in DR-11 population and lowest (0.1652±0.2788 and 0.2017 ±0.2756) in DR-8 population in RAPD and ISSR analyses respectively (Table 5). Whereas, the measure of evenness (E) was highest (0.895626) in DR-8 population in RAPD analyses and (0.878639) in DR-9 population in ISSR respectively; and lowest (0.865463 and 868102) in DR-11 population in RAPD and ISSR analyses respectively (Table 5). The Heip's measure of evenness was highest (0.573441 and 0.585355) in DR-9 population in RAPD and ISSR analyses respectively and lowest (0.553043) in DR-10 population in RAPD analyses and (0.555845) in DR-11 population in ISSR analyses (Table 5).

Table 5. Intra-population genetic diversity indices based on RAPD and ISSR analyses of Jaldhaka river system.

Populations	Molecular Markers						
	RAPD						
	N_p	N_{per}	S	H	H´ or I	E= $e^{H'}/S$	$E_{Heip}=(e^{H'}-1/S-1)$
Jaldhaka River **(DR-8)**	51	36.17 %	1.3171 ± 0.4811	0.1048 ± 0.1855	0.1652 ± 0.2788	0.895626	0.566474
Murti River **(DR-9)**	56	39.72 %	1.3972 ± 0.4911	0.1378 ± 0.1955	0.2052 ± 0.2791	0.878736	0.573441
Ghotia River **(DR-10)**	52	36.88 %	1.3471 ± 0.4656	0.1068 ± 0.1756	0.1756 ± 0.2588	0.884835	0.553043
Diana River **(DR-11)**	61	43.26 %	1.4326 ± 0.4972	0.1436 ± 0.1963	0.2150± 0.2794	0.865463	0.554466

ISSR						
N_p	N_{per}	S	H	H´ or I	E= $e^{H'}/S$	$E_{Heip}=(e^{H'}-1/S-1)$
46	39.66%	1.3966 ± 0.4913	0.1348 ± 0.1927	0.2017± 0.2756	0.876042	0.563492
48	41.38%	1.4138 ± 0.4946	0.1403 ± 0.1960	0.2103 ± 0.2810	0.878639	0.585355
47	40.52%	1.4052 ± 0.4931	0.1374 ± 0.1962	0.2049 ± 0.2789	0.873472	0.561212
49	42.24%	1.4224 ± 0.4961	0.1409 ± 0.1954	0.2109 ± 0.2785	0.868102	0.555845

Note: N_p=number of polymorphic loci, N_{per}=percentage of polymorphic loci, S=observed number of alleles, H=Nei's gene diversity, H´ or I= Shannon's Information index, E= measure of evenness, E_{Heip}= Heip's evenness index.

Genetic distance and dendrogram

Based on the RAPD analyses, the Nei's genetic distance was highest between DR-7 and DR-8 populations (0.5956) and lowest between TR-4 and TR-5 population (0.0001) (Table 6A). Based on the ISSR analyses, the Nei's genetic distance was highest between DR-3 and DR-8 populations (0.5370) and lowest between TR-8 and TR-9 population (0.0040) (Table 6B). The UPGMA dendrogram based on the Nei's unbiased genetic distance and identity matrix after RAPD and ISSR analyses showed clear representation of genetic relationship of seventeen populations of *Badis badis* of the three major riverine systems (Mahananda, Teesta and Jaldhaka of the sub Himalayan West Bengal. Both the RAPD and ISSR-based dendrograms showed that

the Mahananda and Teesta river populations (TR-1 to TR-6 and DR-1 to DR-7) formed a distinct group from the remaining Jaldhaka river population (DR-8-DR-11) (Figure 2A and 2B).

Table 6. Nei's original measures of genetic identity and genetic distance based on A. RAPD and B. ISSR analysis of seventeen populations of Badis badis. Nei's genetic identity (above diagonal) and genetic distance (below diagonal). The shaded box shows highest and lowest level of genetic distances.

	DR-1	DR-2	DR- 3	DR-4	DR-5	DR-6	DR-7	DR-8	DR-9	DR-10	DR-11	TR-1	TR-2	TR-3	TR-4	TR-5	TR-6
DR-1	****	0.7782	0.7766	0.8439	0.8536	0.6740	0.7235	0.6728	0.6781	0.6766	0.6782	0.7330	0.7856	0.7891	0.7857	0.7865	0.7679
DR-2	0.2508	****	0.9896	0.7845	0.7421	0.6688	0.6554	0.6108	0.6115	0.6202	0.6265	0.9305	0.9813	0.9845	0.9802	0.9809	0.9458
DR-3	0.2529	0.0104	****	0.7869	0.7442	0.6553	0.6500	0.6009	0.6015	0.6102	0.6162	0.9371	0.9920	0.9951	0.9909	0.9917	0.9571
DR-4	0.1697	0.2427	0.2397	****	0.8551	0.7020	0.7346	0.7600	0.7601	0.7671	0.7621	0.7695	0.7785	0.7822	0.7787	0.7794	0.7691
DR-5	0.1583	0.2983	0.2954	0.1565	****	0.7521	0.6706	0.7174	0.7175	0.7232	0.7182	0.7324	0.7529	0.7564	0.7530	0.7537	0.7222
DR-6	0.3946	0.4023	0.4227	0.3538	0.2849	****	0.6511	0.6119	0.6161	0.6138	0.6126	0.6715	0.6616	0.6653	0.6666	0.6672	0.6722
DR-7	0.3236	0.4226	0.4308	0.3085	0.3996	0.4291	****	0.5513	0.5586	0.5540	0.5554	0.6643	0.6494	0.6532	0.6493	0.6499	0.6539
DR-8	0.3963	0.4930	0.5093	0.2744	0.3321	0.4912	0.5956	****	0.9958	0.9909	0.9872	0.6166	0.6202	0.6141	0.6225	0.6216	0.6207
DR-9	0.3884	0.4918	0.5082	0.2743	0.3320	0.4843	0.5824	0.0043	****	0.9897	0.9866	0.6167	0.6206	0.6144	0.6222	0.6213	0.6194
DR-10	0.3906	0.4776	0.4940	0.2651	0.3240	0.4881	0.5906	0.0092	0.0104	****	0.9929	0.6294	0.6282	0.6231	0.6300	0.6292	0.6285
DR-11	0.3884	0.4676	0.4841	0.2717	0.3310	0.4901	0.5881	0.0129	0.0135	0.0071	****	0.6238	0.6355	0.6298	0.6340	0.6331	0.6305
TR-1	0.3105	0.0720	0.0650	0.2621	0.3114	0.3983	0.4091	0.4836	0.4833	0.4630	0.4719	****	0.9439	0.9409	0.9421	0.9426	0.9378
TR-2	0.2414	0.0188	0.0080	0.2503	0.2838	0.4130	0.4317	0.4777	0.4770	0.4648	0.4533	0.0577	****	0.9969	0.9983	0.9980	0.9631
TR-3	0.2368	0.0156	0.0049	0.2457	0.2791	0.4075	0.4259	0.4877	0.4871	0.4730	0.4623	0.0609	0.0031	****	0.9960	0.9968	0.9619
TR-4	0.2411	0.0200	0.0091	0.2502	0.2837	0.4055	0.4319	0.4740	0.4745	0.4621	0.4557	0.0597	0.0017	0.0040	****	0.9999	0.9654
TR-5	0.2402	0.0192	0.0084	0.2492	0.2828	0.4046	0.4309	0.4754	0.4760	0.4633	0.4571	0.0591	0.0020	0.0032	0.0001	****	0.9655
TR-6	0.2641	0.0558	0.0439	0.2626	0.3255	0.3972	0.4248	0.4769	0.4790	0.4644	0.4612	0.0642	0.0376	0.0389	0.0352	0.0351	****

A

	DR-1	DR-2	DR- 3	DR-4	DR-5	DR-6	DR-7	DR-8	DR-9	DR-10	DR-11	TR-1	TR-2	TR-3	TR-4	TR-5	TR-6
DR-1	****	0.7734	0.7715	0.8324	0.8426	0.8354	0.8469	0.6708	0.6774	0.6764	0.6793	0.7841	0.7607	0.7642	0.7715	0.7682	0.7745
DR-2	0.2570	****	0.9875	0.7765	0.7312	0.7625	0.7344	0.6024	0.6027	0.6097	0.6144	0.9601	0.9729	0.9828	0.9539	0.9775	0.9654
DR-3	0.2595	0.0126	****	0.7795	0.7359	0.7652	0.7395	0.5845	0.5847	0.5919	0.5963	0.9749	0.9850	0.9948	0.9648	0.9905	0.9789
DR-4	0.1834	0.2529	0.2491	****	0.8683	0.9841	0.8609	0.7568	0.7564	0.7663	0.7604	0.7655	0.7696	0.7722	0.7507	0.7771	0.7682
DR-5	0.1712	0.3130	0.3066	0.1412	****	0.8742	0.9944	0.7240	0.7247	0.7281	0.7215	0.7549	0.7294	0.7288	0.7382	0.7333	0.7428
DR-6	0.1798	0.2711	0.2677	0.0160	0.1344	****	0.8665	0.7884	0.7869	0.7978	0.7936	0.7708	0.7575	0.7591	0.7478	0.7652	0.7565
DR-7	0.1662	0.3087	0.3018	0.1498	0.0056	0.1433	****	0.7292	0.7300	0.7334	0.7267	0.7586	0.7329	0.7323	0.7417	0.7368	0.7463
DR-8	0.3992	0.5068	0.5370	0.2787	0.3230	0.2377	0.3158	****	0.9960	0.9922	0.9874	0.6309	0.5984	0.5887	0.6105	0.6050	0.5930
DR-9	0.3895	0.5063	0.5367	0.2792	0.3220	0.2396	0.3147	0.0040	****	0.9900	0.9856	0.6291	0.5967	0.5875	0.6074	0.6043	0.5920
DR-10	0.3909	0.4948	0.5245	0.2662	0.3173	0.2259	0.3101	0.0079	0.0100	****	0.9937	0.6364	0.6036	0.5945	0.6172	0.6122	0.5991
DR-11	0.3867	0.4871	0.5170	0.2739	0.3264	0.2311	0.3192	0.0127	0.0145	0.0063	****	0.6346	0.5997	0.5930	0.6144	0.6122	0.5959
TR-1	0.2432	0.0407	0.0255	0.2672	0.2812	0.2604	0.2763	0.4607	0.4635	0.4519	0.4548	****	0.9772	0.9755	0.9749	0.9841	0.9684
TR-2	0.2735	0.0275	0.0151	0.2619	0.3155	0.2777	0.3108	0.5135	0.5164	0.5049	0.5113	0.0231	****	0.9944	0.9752	0.9929	0.9788
TR-3	0.2689	0.0174	0.0052	0.2585	0.3164	0.2756	0.3116	0.5298	0.5319	0.5201	0.5225	0.0248	0.0057	****	0.9689	0.9912	0.9831
TR-4	0.2594	0.0472	0.0358	0.2867	0.3036	0.2906	0.2987	0.4936	0.4986	0.4825	0.4872	0.0254	0.0251	0.0316	****	0.9760	0.9665
TR-5	0.2637	0.0227	0.0095	0.2522	0.3101	0.2677	0.3054	0.5026	0.5037	0.4907	0.4908	0.0160	0.0071	0.0088	0.0242	****	0.9826
TR-6	0.2555	0.0352	0.0213	0.2637	0.2973	0.2791	0.2926	0.5226	0.5242	0.5124	0.5176	0.0321	0.0215	0.0171	0.0341	0.0175	****

B

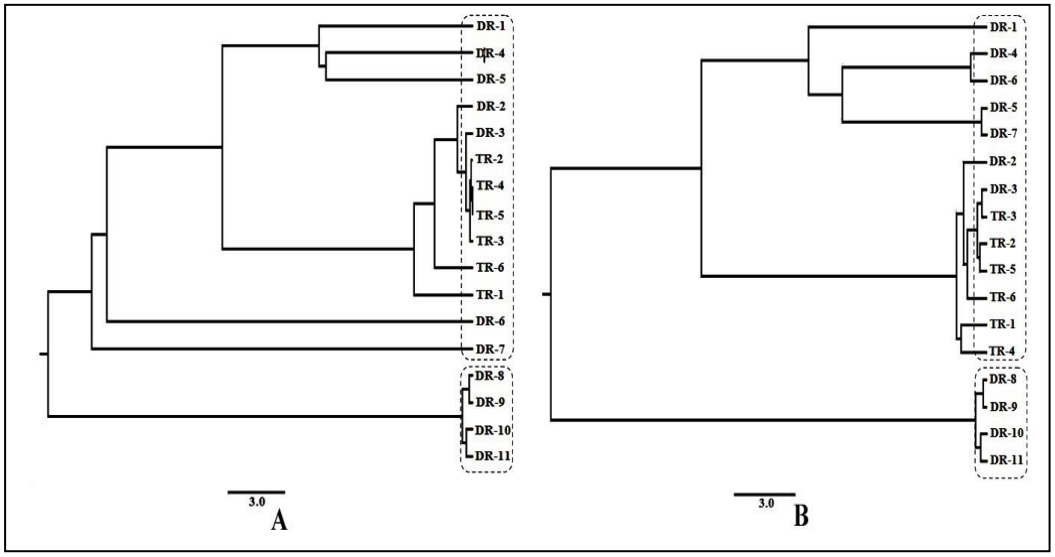

Figure 2. UPGMA dendrogram based on A. RAPD and B. ISSR marker of the seventeen populations of *Badis badis* from the Terai (TR) and Dooars (DR) region of West Bengal, India. Dotted line indicates the groups.

DISCUSSIONS

RAPD and ISSR techniques can be utilized as efficient molecular tools to differentiate spatially and/or genetically isolated populations and to characterize the available gene pool of locally adapted populations that may have arisen either through genetic selection under different environmental conditions or as a result of genetic drift (FUCHS *et al.*, 1998). To our knowledge, the present study is the first endeavour to explore and compare the present status of the genetic background of this fish fauna in the major river streams of the Terai and Dooars region of this sub-Himalayan hotspot of North Eastern India. A total of twenty decamer RAPD primers and fifteen ISSR primers were used to characterize total *seventeen* different populations of *Badis badis* species for the study, based on the reproducibility of banding patterns (Table 2). Since other popular sophisticated markers are not available in this fish species, we have used both RAPD and ISSR techniques for the estimation of genetic diversity of *Badis badis* species to increase the robustness of the study protocol. In the present study, the number of amplified fragments generated by RAPD primer OPB12 was 9 and that by primer OPB18 was 8 (Table 2), which were comparable with the amplified fragments observed by our previous studies on *Badis badis* (OPB12=6–10 and OPB18=3) in the Terai region of West Bengal India (MUKHOPADHYAY and BHATTACHARJEE, 2014b) and BRAHMANE *et al.* (2008) (OPB12 = 4–6 bands, and OPB18 = 3-4 bands) in *Badis badis* populations collected from West Bengal. In a different study on *Barilius barna* in the Teesta river of Dooars region of West Bengal we have found the number of amplified fragment generated by OPB12 was 15 and OPB18 was 17 (PAUL *et al.*, 2016). Another study carried out by MISHRA *et al.* (2012) on *Barilius barna* species in Uttarakhand, India, eight RAPD primers generated total 35 bands of which 14 bands were polymorphic in nature.

Maximum polymorphic bands (50.00%) were produced by primer OPB08 and OPB12 whereas minimum polymorphic bands (33.33%) were produced by primer OPA18, OPB15, OPB18, and OPH03. The study carried out by KADER *et al.* (2013) on three vulnerable Tilapia species (*Oreochromis niloticus, O. aureus,* and *Tilapia zilli*) in Egypt and by CHANDRA *et al.* (2010) on vulnerable *Eutropiichthys vacha* in India, 15 RAPD primers generated 201 and 45 polymorphic amplified fragments, respectively. In our another study we have found total 124 fragments after amplification with ten RAPD decamer primers, out of which 111 fragments were polymorphic (PAUL *et al.*, 2016). In our present study we have found total 105 and 91 polymorphic fragments out of total 141 and 116 amplified fragments in RAPD and ISSR respectively (Table 2). The lower number of polymorphic fragments generated by ISSR amplification than the RAPD (Table 2) might be ascribed to lower number of priming sites of the ISSR primers or owing to the fact that ISSR primers bind to the genomic DNA comparatively specific site than that by the RAPD primers.

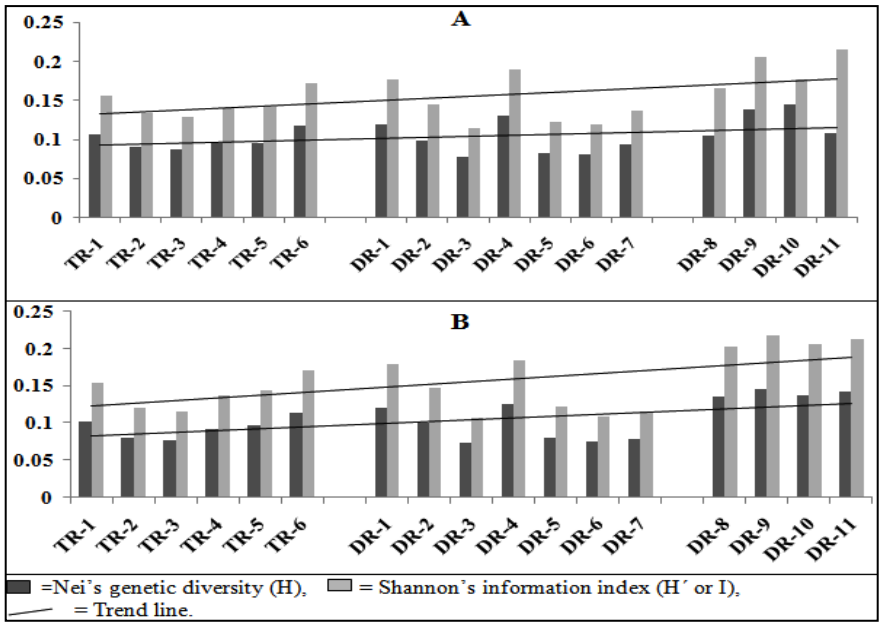

Figure 3: The graphical representation of Nei's genetic diversity (H) and Shannon's information index (H´ or I) of total seventeen riverine populations (TR-1 to TR-6 and DR-1 to DR11) of *Badis badis* from sub-Himalayan Terai and Dooars region of West Bengal, India. A. RAPD analyses. B. ISSR analyses.

In our present study the highest values for Nei's genetic diversity and Shannon's information index were found in DR11 population of Jaldhaka river system (H= 0.1436± 0.1963 and 0.1409 ±0.1954; H´ or I= 0.2150±0.2794 and 0.2109 ±0.2785 after RAPD and ISSR analyses respectively) (Table 5 and Figure 3). Our another study on *Barilius barna* isolated from Teesta river revealed that the Nei's genetic diversity ranged from 0.172 ± 0.189 to 0.293 ± 0.164 and the Shannon's information index ranged from 0.265 ± 0.268 to 0.445 ± 0.220 (PAUL *et al.*,

2016). In the study carried out on *B. Barna* in Uttarakhand, India, MISHRA *et al.* (2012) reported that the Nei's genetic diversity of *B. barna* species was highest (0.4972) at the locus OPB18 while the lowest (0.1339) at the loci OPB12 and the mean value of gene diversity was found to be 0.1606. MWANJA *et al.* (2008) reported that the genetic diversity of *Oreochromis niloticus* in Africa ranges from lowest (0.14) to highest (0.27) values. MISHRA *et al.,* (2012) found that Shannon's information index in *B. barna* populations ranged from 0.2592 to 0.6904 with a mean value of 0.2331. Moreover, KADER *et al.*, (2013) reported that the Shannon's index was higher in *Tilapia* (0.363) population compared to two species of *Oreochromis* populations (0.318 and 0.347). The range of Nei's genetic diversity ranges from 0 to 1 (NEI, 1973) and Shannon's Information index ranges from 1.5 to 3.5 (LEWONTIN, 1972). Therefore, comparitive discussion with other related studies revealed that the genetic diversity was reasonably lower in three river system viz. Mahananda, Teesta and Jaldhaka of the study region (Table 3, 4 and 5; Figure 3). Contrastingly the Jaldhaka population showed a high level of genetic diversity compared to Mahananda and Teesta river system (Figure 3). The UPGMA based dendrogram and Nei's measure of genetic distance clearly revealed the genetic relationship among three river system, where the Mahananda and Teesta population forms a group and Jaldhaka population forms a separate group. The close association of Mahananda river population with the Teesta river population especially with the DR-3 population was mainly because of the connection of Mahananda riverine system with the Teesta river system by a water channel. The Mahananda river system has converged to the Fulbari Barrage (TR-1) and this Barrage connects to the Teesta river system at the Teesta Barrage (DR-3) via narrow channel (Figure 1). This channel causes admixture of Mahananda river population with the Teesta river population.

Different anthropogenic pressures (e.g. to over-fishing, presence of barrages/dams pesticide run offs from the nearby tea gardens, and urban effluents) could be the possible reasons behind the lower catch frequency and low level of genetic diversity of the studied fish in the Mahananda and Teesta river population. Whereas the Jaldhaka population experiences less anthropogenic pressure leads to comparatively high level of diversity than other two river systems. Therefore, the Jaldhaka population should be managed and conserved to preserve the available gene pool of this threatened species. A correlative study based on these anthropogenic factors with that of the available genetic diversity in this species can add a newer dimension to find out the cause of this low level of genetic variability as well as a possible conservational strategy. Information on intra and interspecific genetic variation from the present study should form a baseline on which further studies can be undertaken.

CONCLUSIONS

This study is the first attempt to characterize and compare the genetic architecture of *Badis badis* from the three major river system of sub-Himalayan biodiversity hotpost region of West Bengal, India. A low level of genetic diversity was found in the present study among the seventeen populations as an indicative of the recent threatened status of this species. As the species is commercially important and has an ornamental value; and the region being located in the sub-Himalayan hotspot region, the management and proper rehabilitation of this ichthyofauna in the wild is essential from the standpoint of livelihood of rural fishermen and their economic upliftment.

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ANALIZA GENETIČKOG DIVERZITETA *Badis badis* (HAMILTON-BUCHANAN 1822) U TRI REČNA SLIVA POD-HIMALAJSKOG *HOTSPOT*-A ZA BIODIVERZITETA ZAPADNOG BENGALA, INDIJA UPOTREBOM RAPD I ISSR FINGERPRINTINGA

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Izvod

Badis badis je ugrožena slatkovodna riba u Indiji, a njena populaciona genetička arhiktetura je u velikoj meri neistražena u istočnom pod-himalajskom hotspo-u za biodiverzitet u zapadnom Bengalu, Indija poznatim i kao terai i Dooars. Ukupno sedamnest populacija iz tri glavna rečna sliva Mahanadi (Terai), Teesta i Jaldhaka (Dooars) su proučavani pomoću RAPD i ISSR fingerpritinga. Polimorfizam, genetički diverzitet i Shann-ov informacioni indeks su izračunati za svaku populaciju. najviša vrednost Nei genetičkog diverziteta (0.1436± 0.1963 i 0.1409 ±0.1954 za RAPD i ISSR analizu) i Shannon-og informacionog indeksa (0.2150±0.2794 i 0.2109 ±0.2785 za RAPD i ISSR analizu) utvrđeni su u Jaldhaka rečnom sistemu. UPGMA dendrogram je pokazao da su Mahananda i Teesta populacije iz jedne grupe, a da je Jaldhaka u odvojenoj grupi. U odnosu na ostala proučavanja genetičkog diverziteta *Badis badis*, u ovom istraživanju je utvrđen mali diverzitet, s tim što je populacija imala nešto viši diveruitet u rečnom slivu Jaldhaka. Zbog toga Jaldhaka populacija treba da bude konzervirana da bi se zaštitio genetski *pool* ove ugrožene vrste. Nizak nivo genetičke raznovrsnosti pronađen je u ovoj studiji među sedamaest populacija opravdavaju nedavno dodeljivanje ugroženog statusa ovoj vrsti.

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