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GENETIC DIVERSITY AND POPULATION STRUCTURE ANALYSES OF THREATENED *Amblyceps mangois* FROM SUB-HIMALAYAN WEST BENGAL, INDIA THROUGH RAPD AND ISSR FINGERPRINTING

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ABSTRACT

Amblyceps mangois or the "Indian torrent catfish" is a tropical, freshwater, hill-stream species that has ornamental-commercial value and has been included within the "Endangered" category in the list of threatened freshwater fishes of India. A total fourteen populations from the Terai and Dooars region of northern West Bengal, India were analyzed to study the genetic architecture of this species with the help of RAPD and ISSR markers. The observed number of alleles (S), Nei's gene diversity (H) and Shannon's information index (H´ or I) showed the highest values in the Teesta river system and the lowest values in the Mahananda river system. The UPGMA-based dendrogram and PCoA, based on RAPD and ISSR fingerprints, showed that the Mahananda and the Teesta river populations formed a group distinct from the remaining Jaldhaka river population. We further considered the fourteen riverine populations into nine groups according to the continuity of the water flow for *SHE* analysis. It was found that the three components, i.e. the pattern of diversity (H´), richness (S) and evenness (E), have varied and fluctuated across all fourteen populations from higher to lower altitude as the river flows downstream. AMOVA, PhiPT and genetic hierarchical analyses showed that a distinct hierarchical structure is present in *Amblyceps* populations in the study region. Low levels of genetic diversity/variation and genetic hierarchical structure with high genetic divergence were found in the present study as an indicator of the recent picture of threatened status of this species. This study is the initial attempt to characterize and evaluate the genetic architecture of the species from this region and there is a scope to manage the evolutionary significant units (ESU) for conservation purpose.

How to Cite

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INTRODUCTION

Amblyceps mangois (Hamilton-Buchanan, 1822) (Actinopterygii, Siluriformes, Amblycipidae) or the "Indian torrent catfish" is a tropical, freshwater, hill-stream species that has ornamental and commercial value. This species has been included within the "Endangered" category in the list of threatened freshwater fishes of India by the National Bureau of Fish Genetic Resources (NBFGR, Lucknow, India) (Lakra et al., 2010). However, there has been lack of studies with regard to the estimation of genetic diversity of this fish species, especially in the eastern sub-Himalayan region of West Bengal, India. The region being included within the Eastern Himalayan biodiversity hotspot, these results will be necessary from the standpoint of validation of its threatened status in this region and its conservation/sustainability of wild population. Moreover this species has an ornamental value thus management and proper rehabilitation of this ichthyofauna in the wild is essential from the standpoint of livelihood of rural fishermen and their economic upliftment (Das, 2015). Therefore, breeding and proper farming of this indigenous ornamental fish species can help in the restoration and conservation of available biodiversity of the study region and thus the ornamental fish trade will go a long way to provide employment in the region (Sharma and Dhanze, 2018).

Genetic variation is essential for the survivability and reproductive fitness of any organism and the whole population relies on this for its sustenance. Therefore, the corrosion of genetic diversity/variability within a population restrains its capacity for adaptation and augments the risk of its extinction. Moreover, the vulnerable and small population experiences a continual reduction in the genetic variation leading to its extinction (Landweber and Dobson, 1999). However, detecting the available genetic variation and its conservation in an endangered species is essential to determine its adaptation, expansion and its opportune reestablishment in a natural habitat. Therefore the study of genetic variability within and between local populations helps to acquire information on individual identity, breeding patterns, degree of relatedness and genetic variability within as well as between them. Genetic variations can be assessed by the means of DNA polymorphisms.

RAPD and ISSR analysis can be carried out on organisms to gain a first-hand data about the available genetic variation and utilize arbitrary primer to develop specific banding pattern to detect polymorphisms for virtually any organism whose genomic sequences are unavailable (Zietkiewicz et al., 1994). RAPD-PCR technique has been extensively used to characterize genetic structure as well as to study genetic diversity of many fish species, such as *Horabagrus brachysoma* (Muneer et al., 2009), *Heteropneustes fossilis* (Sultana et al., 2010), *Clarias batrachus* (Garg et al., 2010), *Badis* sp. (Mukhopadhyay and Bhattacharjee, 2014a), *Mystus* sp. (Hasan and Goswami, 2015) and *Barilius barna* (Paul et al., 2016). ISSR-PCR technique was used in studying genetic diversity at the interspecific and intraspecific levels of species (Panarari-Antunes et al., 2011; Saad et al., 2012; Haniffa et al., 2014; Labastida et al., 2015; Paul et al., 2016). Therefore, due to the unavailability of microsatellite markers in *Amblyceps mangois* till date, we resorted to the time-tested RAPD and ISSR primers to ascertain and compare the available genetic variations in this ichthyofauna.

The objective of the present study was to (1) estimate the intra-population genetic diversity of *Amblyceps mangois* from the three major riverine systems (Mahananda, Teesta and Jaldhaka) of the sub-Himalayan Terai and 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, (4) determine the transmutation in the diversity pattern through SHE analysis among different populations of *Badis badis* from the streams of the region and (5) comprehend the hierarchical genetic diversity analyses among different Badis populations to define the evolutionary significant unit (ESU).

MATERIAL AND METHODS

Survey and sample collection

An extensive survey was carried out (about 5000 km^2) in different spots of the major streams of eastern sub-Himalayan region of West Bengal, India. Total of 140 fish samples were collected from the Mahananda-Balasan, Teesta River and Jaldhaka river systems of the Terai and Dooars region of eastern sub-Himalayan West Bengal, India. Total of fourteen spots were selected for collection (ten samples from each collection site) of the fish samples (three spots from the Mahananda-Balasan river system, seven spots from the Teesta river system and four spots from the Jaldhaka river system). The geographic co-ordinates were recorded with the help of GPS (eTrex Vista HCx, Garmin, USA). A limited number of individuals (ten from each collection site) were collected for the study of population genetic analyses because they are included in the list of endangered category by NBFGR (Lakra et al., 2010). The collection spots were as follows: ATR-1, ATR-2 and ATR-3 (Mahananda river system from Terai region), and ADR-1, ADR-2, ADR-3, ADR-4, ADR-5, ADR-6, ADR-7 (Teesta river system from Dooars region) and ADR-8, ADR-9, ADR-10 and ADR-11 (Jaldhaka river system from Dooars region) (Fig. 1). Fishes were identified according to Talwar and Jhingran (1991).

Isolation of genomic DNA and quantification

Genomic DNA (gDNA) was extracted from tissue samples (10-15 mg of clips from the caudal and ventral fins) from *Amblyceps mangois* using commercial DNA

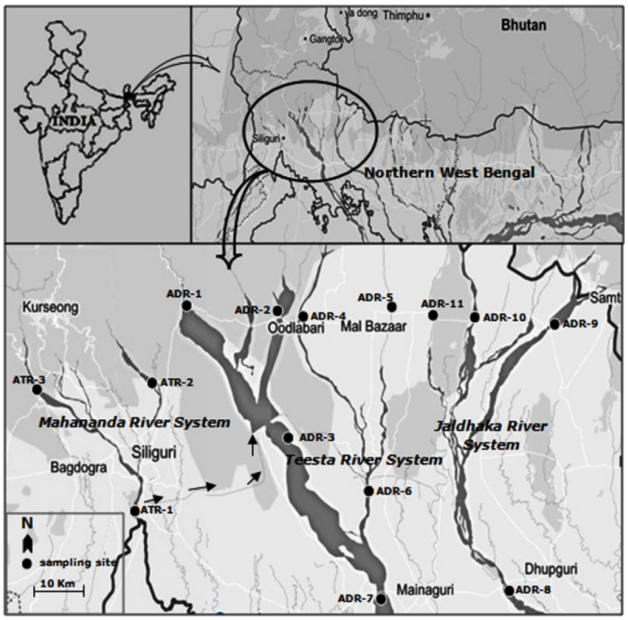


Fig 1. Collection spots of *Amblyceps mangois* from the three major river streams of the Terai (TR) and Dooars (DR) region in the northern part of West Bengal, India. Geographical locations and altitudes were recorded by hand-held GPS. The alphabets in capital bold case indicate the collection spots. ATR-1= Mahananda Barrage, Fulbari [26°38.884´N; 88°24.125´E; 319 AMSL], ATR-2= Mahananda River, Champasari [26°44.452´N; 88°25.497´E; 717 AMSL] and ATR-3= Balason River, Tarabari [26°45.632´N; 88°18.912´E; 731 AMSL]. ADR-1=Sevok (Teesta River) [N 26°53´043, E 88°28´367 Elev 480 AMSL], ADR-2=Ghish River [N 26°52´327, E 88°36´355 Elev 536 AMSL], ADR-3= Gajoldoba (Teesta River Barrage) [N 26°44´584, E 88°35´314 Elev 354 AMSL], ADR-4=Chel River [N 26°51´499, E 88°38´048 Elev 522 AMSL], ADR-5= Neora River [N 26°52´486, E 88°46´205 Elev 527 AMSL], ADR-6= Dharla River [N 26°40´496, E 88°44´126 Elev 299 AMSL], ADR-7=Jalpaiguri (Teesta River) [N 26°33´499, E 88°45´369 Elev 274 AMSL], ADR-8=Jaldhaka River [26°34´13.17 N, 88°56´14.26 E Elev 267 AMSL], ADR-9= Murti River [26°52´57.73 N, 88°49´44.98 E Elev 578 AMSL], ADR-10= Ghotia River [26°52´14.89 N, 88°53´37.98 E Elev 540 AMSL] and ADR-11= Diana River [26°51´37.96 N, 89°00´07.40 E Elev 647 AMSL]. The arrow indicates the narrow water channel that carries water Mahananda Barrage, Fulbari to the Teesta River Barrage, Gajoldoba.

isolation Kit (DNeasy Blood and Tissue Kit, Qiagen), following a standardized method (Mukhopadhyay and Bhattacharjee, 2014b). The gDNA samples were subjected to spectrophotometric quantification (Rayleigh UV-2601 Spectrophotometer, Beijing, China). The concentration of the extracted gDNA was adjusted to 50-100 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 primers (ten primers from Kit-A, i.e. OPA-01 to OPA-16 and ten primers from Kit-B, i.e. OPB-01 to OPB-17) were selected for further analyses on the basis of the variability and reproducibility of the bands obtained (Table 1). The GC content of the primers was between 60-70%. Twenty-one ISSR primers, purchased from Xcelris Genomics, India were used to screen all populations, and finally, twelve ISSR primers (ISSR-01 to ISSR-21, all 3´-anchored) were selected for further analyses on the basis of the variability and reproducibility. The annealing temperatures of the ISSR primer were optimized for each amplification and are depicted in Table 1.

RAPD and ISSR-PCR amplifications were performed in a 96 well Eppendorf® thermal cycler (Eppendorf, Germany) 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 (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 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 94°C for 5 min followed by 50 cycles (RAPD) or 40 cycles (ISSR) of 94°C, 1 min for denaturation; 35°C (RAPD) and 38°C - 47°C (ISSR) (specific and optimal annealing temperature for each primer, see Table 1), 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 an ethidium bromide (0.5 µg/ml) pre-stained 1.4% (for RAPD) / 1.6% (for ISSR) (w/v) agarose gel (Lonza, Basel, Switzerland) at a constant voltage 100 V and current 100 mA in TAE buffer (40 mM Tris-HCl, pH 8.0; 20 mM Acetic acid; 1 mM EDTA, pH 8.0) using BenchTop Labsystems 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 (Spectroline BI-O-Vision®NY, USA) and photographed using a Nikon D3100 camera.

RAPD and ISSR data analyses

RAPD and ISSR data from *Amblyceps mangois* populations were analyzed for assessing intra-population genetic variability within each of the fourteen 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 (William et al., 1990, Lynch and

Table 1. Sequence, GC content, annealing temperature of RAPD and ISSR primers

Sl/No.	Primer	Sequence (5´ 3´)	G+ C Content (%)	Annealing temperature
		RAPD		
1	OPA-01	CAGGCCCTTC	70	
2	OPA-02	TGCCGAGCTG	70	
3	OPA-04	AATCGGGCTG	60	
4	OPA-06	GGTCCCTGAC	60	
5	OPA-08	GTGACGTAGG	70	
6	OPA-10	GTGATCGCAG	60	
7	OPA-12	TCGGCGATAG	70	
8	OPA-13	CAGCACCCAC	60	
9	OPA-14	TCTGTGCTGG	60	
10	OPA-16	AGCCAGCGAA	60	36°C
11	OPB-01	GTTTCGCTCC	60	
12	OPB-04	GGACTGGAGT	70	
13	OPB-06	TGCTCTGCCC	60	
14	OPB-07	GGTGACGCAG	70	
15	OPB-08	GTCCACACGG	70	
16	OPB-09	TGGGGGACTC	60	
17	OPB-10	CTGCTGGGAC	60	
18	OPB-11	GTAGACCCGT	70	
19	OPB-12	CCTTGACGCA	60	
20	OPB-17	AGGGAACGAG	60	
		ISSR		
1	ISSR-1	$(CT)_8TG$	50	
2	ISSR-2	$(CT)_8AC$	50	44°C
3	ISSR-3	$(CT)_8GC$	55.6	
4	ISSR-4	$(CA)_6AG$	50	
5	ISSR-5	$(CA)_6AC$	50	
6	ISSR-7	$(CAC)_3GC$	72.7	31°C
7	ISSR-8	$(GAG)_3GC$	72.7	
8	ISSR-9	$(GTG)_3GC$	72.7	
9	ISSR-13	$(GT)_6CC$	57.1	36°C
11	ISSR-19	$(GGAC)_3A$	69.2	42°C
12	ISSR-20	$(GGAC)_3C$	76.9	
13	ISSR-21	$(GGAC)_4$	75	44°C

PCR amplification and documentation of amplified products

Milligan, 1994). The RAPD and ISSR profile generated was compared within and between populations in a pair-wise manner.

The RAPD and ISSR data was analysed using three software packages viz. Popgene ver. 1.32 (Yeh et al., 1999), TFPGA (Tools for Population Genetic Analysis) ver.1.3 (Miller, 1997) and GenAlEx 6.5 (Peakall and Smouse, 2006; Peakall and Smouse, 2012). Different indices of diversity measurement were used for the assessment of genetic background of *Amblyceps mangois* species. The data matrix was used to estimate the observed number of alleles [$(1/K)\Sigma\ n_i$, where K= number of loci and n_i= the number of alleles detected per locus], effective number of alleles ($1/\Sigma p_i^2$, 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 $I= -\Sigma\ p_i log2\ p_i$, 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 was found where all RAPD and ISSR bands have equal abundance. For a 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 was 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 software TFPGA ver. 1.3 and Arlequin ver. 3.1 (Excoffier and Schneider, 2005). The Nei's genetic distance matrix was subjected to generate unweighted pair-group method using arithmetical averages (UPGMA) based dendrogram through linkage procedure, using the software Popgene ver. 1.32 (Yeh et al., 1999), and the tree was verified by Phylip ver. 3.69 (Felsenstein, 2005), and finally the tree was modified and prepared by FigTree ver.1.3.1 (Rambaut and Drummond, 2010) using in-built default parameter settings. Principal Component Analyses were carried out using the distance matrix to observe the clustering of all fourteen populations more sophistically using GenAlEx 6.5 software (Peakall and Smouse, 2006; Peakall and Smouse, 2012).

To analyze the inter-population genetic differentiation and hierarchical genetic structure between seven different populations of *Amblyceps mangois*, pair-wise 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 as estimated from the pooled allele frequencies) (Wright, 1969). The F_{ST} is equivalent to the coefficient of gene differentiation G_{ST} ($G_{ST}=D_{ST}/H_T$). The estimated gene flow (Nm) between the pairwise populations was also calculated by the formula $Nm=0.5(1 - G_{ST})/G_{ST}$ (McDonald and McDermott, 1993). The Analysis of Molecular Variance (AMOVA) and PhiPT [Estimated variance among population / (estimated variance within population + estimated variance among population)] analyses were performed to determine the hierarchical genetic structure of the populations using GenAlEx 6.5 software (Peakall and Smouse, 2006; Peakall and Smouse, 2012). The Shannon's information index (H´), measure of evenness (E) and observed number of alleles, i.e. richness (S), sum up to *SHE* analysis and this analysis was carried out manually (H´=lnE+ lnS) in MS-Excel 2007 software (Hayek and Buzas, 1997; Magurran, 2004). SHE analyses were carried out to describe the change in the diversity pattern though different subpopulations in a spatial scale across the river streams.

RESULTS

Intra-population genetic diversity study

Mahananda river system

Based on the RAPD profile, the number of polymorphic loci and the percentage of polymorphic loci vary across three populations (ATR-1 to ATR-3). The highest percentage of polymorphism was observed in ATR-3 population (34.04) and the lowest percentage of polymorphism was observed in ATR-2 population (28.37). The Nei's genetic diversity (H) was highest (0.1290±0.1954) in ATR-3 population and lowest (0.1052±0.1837) in ATR-2 population. The Shannon's information index (H´ or I) was highest (0.1896±0.2798) in ATR-3 population and lowest (0.1549±0.2629) in ATR-2 population. The Heip's measure of evenness was highest (0.613296) in ATR-3 population and lowest (0.590558) in ATR-2 population. The diversity indices based on the ISSR analyses were also in accordance with the RAPD data. The highest percentage of polymorphism was observed in ATR-3 population (33.70) and the lowest percentage of polymorphism was observed in ATR-2 population (28.26). The Nei's genetic diversity (H) was highest (0.1186± 0.1844) in ATR-3 population and lowest (0.0992± 0.1783) in ATR-2 population. The Shannon's information index (H´ or I) was highest (0.1773± 0.2667) in ATR-3 population and lowest (0.1474± 0.2554) in ATR-2 population. However, the Heip's measure of evenness was highest (0.575636) in ATR-3 population and lowest (0.561987) in ATR-2 population (Table 2).

Teesta river system

Based on the RAPD profile, the number of polymorphic loci and the percentage of polymorphic loci vary across seven populations (ADR-1 to ADR-7). The highest percentage of polymorphism was observed in ADR-6 population (35.46) and the lowest percentage of polymorphism was observed in ADR-3 population (23.40). The Nei's genetic diversity (H) was highest (0.1326±0.1984) in

ADR-6 population and lowest (0.0861±0.1704) in ADR-3 population. The Shannon's information index (H´ or I) was highest (0.1946±0.2829) in ADR-6 population and lowest (0.1271±0.2447) in ADR-3 population. However, the Heip's measure of evenness was highest (0.615613) in ADR-2 population and lowest (0.579190) in ADR-3 population. The diversity indices based on the ISSR analyses were also in accordance with the RAPD data. The highest percentage of polymorphism was observed in ADR-6 population (41.30) the lowest percentage of polymorphism was observed in ADR-3 population (20.65). The Nei's genetic diversity (H) was highest (0.1551±0.2082) in ADR-6 population and lowest (0.0699±0.1533) in ADR-3 population. The Shannon's information index (H´ or I) was highest (0.2271±0.2954) in ADR-6 population and lowest (0.1048±0.2221) in ADR-3 population. The Heip's measure of evenness was highest (0.617325) in ADR-6 population and lowest (0.516291) in ADR-5 and ADR-7 populations (Table 3).

Jaldhaka river system

Based on the RAPD profile, the number of polymorphic loci and the percentage of polymorphic loci vary across four populations (ADR-8 to ADR-11). The highest percentage of polymorphism was observed in ADR-11 population (43.26), the lowest percentage of polymorphism was observed in ADR-8 population (39.72). The Nei's genetic diversity (H) was highest (0.1436±0.1963) in ADR-11 population and lowest (0.1378±0.1955) in ADR-8 population. The Shannon's information index (H´ or I) was highest (0.2150±0.2794) in ADR-11 population and lowest (0.2052±0.2791) in ADR-8 population. However, the Heip's measure of evenness was highest (0.593598) in ADR-9 population and lowest (0.554466) in ADR-11 population. The diversity indices based on the ISSR analyses were also in accordance with the RAPD data. The highest percentage of polymorphism was observed in ADR-11 population (41.30) and the lowest percentage of polymorphism was observed in ADR-8 population (39.13). The Nei's genetic diversity (H) was highest (0.1320±0.1910) in ADR-11 population and lowest (0.1290±0.1913) in ADR-8 population. The Shannon's information index (H´ or I) was highest (0.2102±0.2716) in ADR-11 population and lowest (0.1935±0.2725) in ADR-8 population. However, the Heip's measure of evenness was highest (0.568144) in ADR-9 population and lowest (0.52752) in ADR-11 population (Table 4).

Inter-population genetic diversity study

The RAPD and ISSR based analyses showed that the Teesta river system has the highest detectable polymorphic loci, i.e. 118 and 66 in number, respectively (Table 3). In contrast, the Mahananda river system showed lower number of polymorphic loci, i.e. 57 and 36 based on RAPD and ISSR analyses, respectively (Table 2, 3, 4). The observed number of alleles (S), Nei's gene diversity (H) and Shannon's information index (H´ or I) showed highest values in the Teesta river system, i.e. 1.8369±0.3708, 0.2991±0.1780 and 0.4457± 0.2441 by RAPD analysis, and 1.7174±0.4527, 0.2295±0.1965 and 0.3461±0.2759 by ISSR analysis, respectively (Table 3 and Fig. 2), and lowest in the Mahananda river system, i.e. 1.4043±0.4925, 0.1485±0.1988 and 0.2200±0.2853 by RAPD analysis, and 1.3913±0.4907, 0.1239±0.1794 and 0.1891±0.2609 by ISSR analysis, respectively (Table 2, 3, 4 and Fig. 2). Heip's measure of evenness was used for better interpretation of the measure of evenness. It was found that the Teesta river system was more *even* in genetic diversity distribution than other populations (Fig. 2).

Genetic relationship

Based on the RAPD and ISSR analyses, the Nei's genetic distance was highest between ADR-3 and ADR-8 populations (0.4979) and lowest between ADR-8 and ADR-9 population (0.0041) (Table 5).

The genetic identity was highest between ADR-8 and ADR-9 (0.9960) and lowest between ADR-3 and ADR-8 populations (0.6078) (Table 5).

The UPGMA based dendrogram based on the Nei's unbiased genetic distance and identity matrix after RAPD and ISSR analyses showed clear representation of genetic relationship of fourteen populations of *Amblyceps mangois* of the three major riverine systems (Mahananda, Teesta and Jaldhaka) of the sub Himalayan West Bengal. The dendrogram based on RAPD and ISSR analyses showed that the Mahananda and Teesta river populations (ATR-1 to ATR-3 and ADR-1 to ADR-7) formed a distinct group from the remaining Jaldhaka river population (ADR-8 to ADR-11) (Fig. 3). The principal component analyses clearly showed the clustering of fourteen populations into distinct three groups, two groups for the Mahananda and Teesta river populations and a separate group for the Jaldhaka river population (Fig. 4).

SHE analyses

SHE analyses revealed the distribution of three biodiversity components - richness (S), diversity (H´) and evenness (E) - of *Amblyceps mangois* gene pool into fourteen different riverine populations of the Terai and Dooars regions. It was found that the lnS and H´ components were highest in ADR-9 population (0.335829173 and 0.2094, respectively) and lowest in ADR-3 population (0.201470376 and 0.1183, respectively) (Fig. 5, lower panel). The lnE value was highest in ADR-11 population (-0.146201636) and lowest in ADR-2 population (-0.083039273) (Fig. 5, lower panel). The *SHE* analysis plot revealed the observed pattern for distribution of three components viz. S (richness), H´ (Shannon's information index) and E (evenness) in relation to altitudinal gradient of the river streams across fourteen different populations. The fourteen riverine populations were divided into nine groups according to the continuity of the water flow through the river from upstream to downstream viz. ATR-3 and ATR-1 constituting the first group (Plot A), ATR-2 and ATR-1 constituting the second

Table 2. Intra-population genetic diversity indices based on RAPD and ISSR analyses of the Mahananda-Balason river systems

Populations	Molecular Markers													
	RAPD							ISSR						
	N_p	N_{per}	S	H	H´ or I	E= $e^{H'}/S$	E_{Heip}= ($e^{H'}$-1/ S-1)	N_p	N_{per}	S	H	H´ or I	E= $e^{H'}/S$	E_{Heip}= ($e^{H'}$-1/ S-1)
Mahananda Barrage, Fulbari **(ATR-1)**	43	30.50%	1.3050± 0.4620	0.1159± 0.1899	0.1701± 0.2718	0.90837	0.607946	28	30.43%	1.3043± 0.4627	0.1055± 0.1775	0.1580± 0.2568	0.897927	0.562492
Mahananda River, Champasari **(ATR-2)**	40	28.37%	1.2837± 0.4524	0.1052± 0.1837	0.1549± 0.2629	0.909513	0.590558	26	28.26%	1.2826± 0 .4527	0.0992± 0.1783	0.1474± 0.2554	0.903491	0.561987
Balason River, Tarabari **(ATR-3)**	48	34.04%	1.3404± 0.4755	0.1290± 0.1954	0.1896± 0.2798	0.901795	0.613296	31	33.70%	1.3370± 0 .4753	0.1186± 0.1844	0.1773± 0.2667	0.893036	0.575636
Mahananda River System	57	40.43%	1.4043± 0.4925	0.1485± 0.1988	0.2200± 0.2853	0.887329	0.608649	36	39.13%	1.3913± 0.4907	0.1239± 0.1794	0.1891± 0.2609	0.868369	0.531975

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.

Table 3. Intra-population genetic diversity indices based on RAPD and ISSR analyses of the Teesta river system

Populations	Molecular Markers													
	RAPD							ISSR						
	N_p	N_{per}	S	H	H´ or I	E= $e^{H'}/S$	E_{Heip}= ($e^{H'}$-1/ S-1)	N_p	N_{per}	S	H	H´ or I	E= $e^{H'}/S$	E_{Heip}= ($e^{H'}$-1/ S-1)
Sevok (Teesta River) **(ADR-1)**	48	34.04%	1.3404± 0.4755	0.1224± 0.1944	0.1802± 0.2761	0.893358	0.580073	35	38.08%	1.3804± 0.4882	0.1382± 0.2009	0.2035± 0.2855	0.88792	0.593284
Ghish River **(ADR-2)**	38	26.95%	1.2695± 0.4453	1.1050± 0.1848	0.1535± 0.2648	0.918399	0.615613	23	25%	1.2500± 0.4354	0.0978± 0.1795	0.1433± 0.2583	0.923261	0.616304
Gajoldoba (Teesta River Barrage) **(ADR-3)**	33	23.40%	1.2340± 0.4249	0.0861± 0.1704	0.1271± 0.2447	0.920203	0.579190	19	20.65%	1.2065± 0.4070	0.0699± 0.1533	0.1048± 0.2221	0.920421	0.535053
Chel River **(ADR-4)**	34	24.11%	1.2411± 0.4293	0.0884± 0.1710	0.1308± 0.2460	0.91833	0.579593	30	32.61%	1.3261± 0.4713	0.1148± 0.1880	0.1700± 0.2688	0.893828	0.568245
Neora River **(ADR-5)**	36	25.53%	1.2553± 0.4376	0.08070± 0.1685	0.1299± 0.2425	0.907125	0.543339	22	23.91%	1.2391± 0.4289	0.0774± 0.1602	0.1164± 0.2307	0.906662	0.516291
Dharla River **(ADR-6)**	50	35.46%	1.3546± 0.4801	0.1326± 0.1984	0.1946± 0.2829	0.896815	0.605823	38	41.30%	1.4130± 0.4951	0.1551± 0.2082	0.2271± 0.2954	0.88815	0.617325
Jalpaiguri (Teesta River) **(ADR-7)**	36	25.53%	1.2553± 0.4376	0.0998± 0.1855	0.1448± 0.2634	0.920743	0.610295	22	23.91%	1.2391± 0.4289	0.0774± 0.1602	0.1164± 0.2307	0.906662	0.516291
Teesta River System	118	83.69%	1.8369± 0.3708	0.2991± 0.1780	0.4457± 0.2441	0.850119	0.671028	66	71.74%	1.7174± 0.4527	0.2295± 0.1965	0.3461± 0.2759	0.823072	0.576448

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.

Table 4. Intra-population genetic diversity indices based on RAPD and ISSR analyses of the Jaldhaka river system.

Populations	Molecular Markers													
	RAPD							ISSR						
	N_p	N_{per}	S	H	H´ or I	E= $e^{H'}$/S	E_{Heip}= ($e^{H'}$-1/ S-1)	N_p	N_{per}	S	H	H´ or I	E= $e^{H'}$/S	E_{Heip}= ($e^{H'}$-1/ S-1)
Jaldhaka River **(ADR-8)**	56	39.72%	1.3972± 0.4911	0.1378± 0.1955	0.2052± 0.2791	0.878736	0.573441	36	39.13%	1.3913± 0.4907	0.1290± 0.1913	0.1935± 0.2725	0.872198	0.54559
Murti River **(ADR-9)**	57	40.72%	1.3992± 0.4927	0.1426± 0.1972	0.2117± 0.2822	0.884467	0.593598	37	40.22%	1.4022± 0.4930	0.1314± 0.1940	0.2058± 0.2772	0.876129	0.568144
Ghotia River **(ADR-10)**	58	40.79%	1.4032± 0.4952	0.1430± 0.1987	0.2075± 0.2856	0.88076	0.580558	37	40.28%	1.4022± 0.4930	0.1314± 0.1944	0.2058± 0.2772	0.868105	0.540172
Diana River **(ADR-11)**	61	43.26%	1.4326± 0.4972	0.1436± 0.1963	0.2150± 0.2794	0.865463	0.554466	38	41.30%	1.4130± 0.4951	0.1320± 0.1910	0.2102± 0.2716	0.861901	0.52752
Jaldhaka River System	70	49.65%	1.4965± 0.5018	0.1471± 0.1891	0.2247± 0.2701	0.836583	0.507446	44	47.83%	1.4783± 0.5023	0.1383± 0.1875	0.2117± 0.2674	0.835945	0.492948

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.

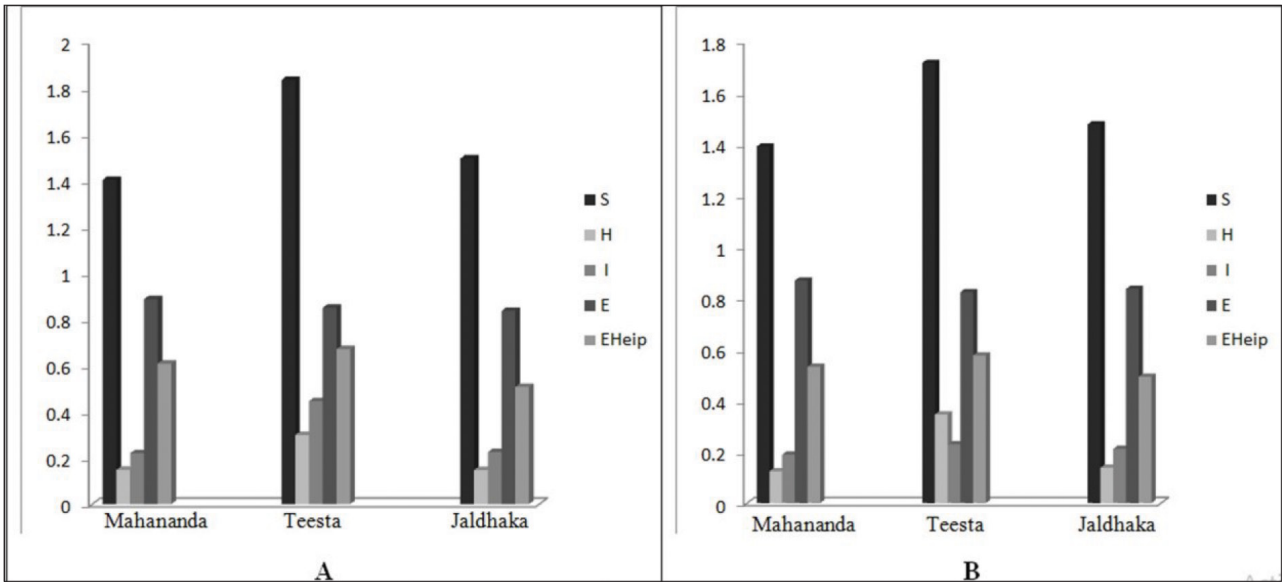


Fig 2. Comparison of genetic diversity between three river system populations by A. RAPD and B. ISSR analyses. S=observed number of alleles, H= Nei's gene diversity, H´ or I= Shannon's information index, E= measure of evenness, E_{Heip}= Heip's evenness index.

group (Plot B), ADR-1, ADR-3 and ADR-7 constituting the third group (Plot-C), ADR-2, ADR-3 and ADR-7 constituting the fourth group (Plot-D), ADR-4, ADR-6 and ADR-7 constituting the fifth group (Plot-E), ADR-5, ADR-6 and ADR-7 constituting the sixth group (Plot-F), ADR-11 and ADR-8 constituting the seventh group (Plot-G), ADR-10 and ADR-8 constituting the eighth group (Plot-H), and ADR-9 and ADR-8 constituting the ninth group (Plot-I) (Fig. 5, upper panal). It was found that as the river flows downward, in most of the cases the diversity decreased but evenness increased (Plot- A, C, E, I), diversity and evenness both increased (Plot-F), both decreased (Plot-D, G), diversity increased and evenness decreased (Plot-B), or remained the same (Plot-H) (Fig. 5, upper panel).

Genetic hierarchical analyses

The three river systems have different natural hierarchical structure. Therefore, the three river systems were divided into 1st and 2nd order (for the Mahananda river system), 1st to 5th order (for the Teesta river system) and 1st to 3rd order

Table 1. Matrix showing values of Nei's (1978) unbiased measures of genetic similarity (above diagonal) and genetic distances (below diagonal). The square boxes indicate the highest and lowest genetic similarity and genetic distance between two pair of population.

pop ID	ADR-1	ADR-2	ADR-3	ADR-4	ADR-5	ADR-6	ADR-7	ADR-8	ADR-9	ADR-10	ADR-11	ATR-1	ATR-2	ATR-3
ADR-1	****	0.7524	0.7524	0.7530	0.8622	0.8601	0.7923	0.6897	0.6971	0.6949	0.6941	0.7325	0.7543	0.7536
ADR-2	0.2845	****	0.9874	0.7281	0.7490	0.7884	0.6906	0.6196	0.6212	0.6293	0.6323	0.9371	0.9768	0.9504
ADR-3	0.2845	0.0126	****	0.7210	0.7539	0.7929	0.6906	0.6078	0.6090	0.6170	0.6197	0.9483	0.9890	0.9634
ADR-4	0.2837	0.3173	0,3271	****	0.7755	0.8062	0.7238	0.6526	0.6556	0.6600	0.6575	0.7304	0.7252	0.7324
ADR-5	0.1483	0.2891	0.2825	0.2542	****	0.8533	0.8044	0.7115	0.7126	0.7163	0.7116	0.7553	0.7565	0.7386
ADR-6	0.1507	0.2377	0.2321	0.2155	0.1586	****	0.7779	0.7660	0.7660	0.7743	0.7708	0.7820	0.7855	0.7744
ADR-7	0.2328	0.3702	0.3702	0.3232	0.2176	0.2512	****	0.6151	0.6202	0.6178	0.6170	0.7092	0.6897	0.6957
ADR-8	0.3715	0.4786	0.4979	0.4268	0.3404	0.2666	0.4861	****	0.9960	0.9907	0.9871	0.6290	0.6200	0.6173
ADR-9	0.3608	0.4762	0.4960	0.4222	0.3388	0.2666	0.4778	0.0041	****	0.9894	0.9863	0.6295	0.6208	0.6173
ADR-10	0.3641	0.4631	0.4828	0.4154	0.3337	0.2558	0.4816	0.0093	0.0106	****	0.9936	0.6401	0.6289	0.6258
ADR-11	0.3651	0.4584	0.4785	0.4193	0.3403	0.2604	0.4829	0.0130	0.0138	0.0064	****	0.6334	0.6281	0.6230
ATR-1	0.3112	0.0650	0.0531	0.3142	0.2807	0.2459	0.3436	0.4637	0.4629	0.4461	0.4566	****	0.9571	0.9441
ATR-2	0.2820	0.0235	0.0111	0.3213	0.2790	0.2415	0.3715	0.4780	0.4768	0.4639	0.4650	0.0439	****	0.9688
ATR-3	0.2829	0.0509	0.0372	0.3114	0.3029	0.2557	0.3629	0.4824	0.4824	0.4688	0.4733	0.0576	0.0317	****

(for the Jaldhaka river system) hierarchy. This division helped us to compare the riverine populations constituting the different order of hierarchy sophisticatedly (Fig. 6). In the Mahananda river system the first order hierarchical group (between population ATR-3 and ATR-2) showed a lower value of genetic differentiation (F_{ST} = 0.1084) and a higher value of gene flow (N_m = 4.1117) than the 2nd order hierarchical population (Fig. 6). The variance among population and PhiPT value of the first order hierarchical group were also lower, i.e. 0.983 (7%) and 0.069 (*p* value = 0.006) respectively, than the 2nd order hierarchical population (Fig. 6). In the Teesta river system, the first order hierarchical group (between population ADR-1 and ADR-2) and the second order hierarchical group (between population ADR-1, ADR-2 and ADR-3) showed a lower value of genetic differentiation (F_{ST} = 0.4870 and 0.4928, respectively) and a higher value of gene flow (N_m = 0.5268 and 0.5145) than other hierarchical orders of population (Fig. 6). The variance among population and PhiPT value of the first order hierarchical group (between population ADR-1 and ADR-2) and the second order hierarchical group (between population ADR-1, ADR-2 and ADR-3) were also lower, i.e. 18.36 (63%) and 15.82 (58%), and 0.595 (*p* value = 0.001) and 0.580 (*p* value = 0.001) respectively, than the other hierarchical orders of populations (Fig. 6). In the Jaldhaka river system all three hierarchical groups, i.e. the first order, second order and third order showed very low genetic differentiation (F_{ST} = 0.0196, 0.0318, 0.0387, 0.0125, 0.0427), and high amount of gene flow (N_m = 25.0247, 15.2365, 12.4330, 39.3447 and 11.2136) (Fig. 6). Although the second order populations (ADR-9 and ADR-8) showed low F_{ST} and high gene flow among other hierarchical orders, there was no variance among populations in any hierarchical orders and there were significant negative PhiPT values in all hierarchical orders of the Jaldhaka populations (Fig. 6).

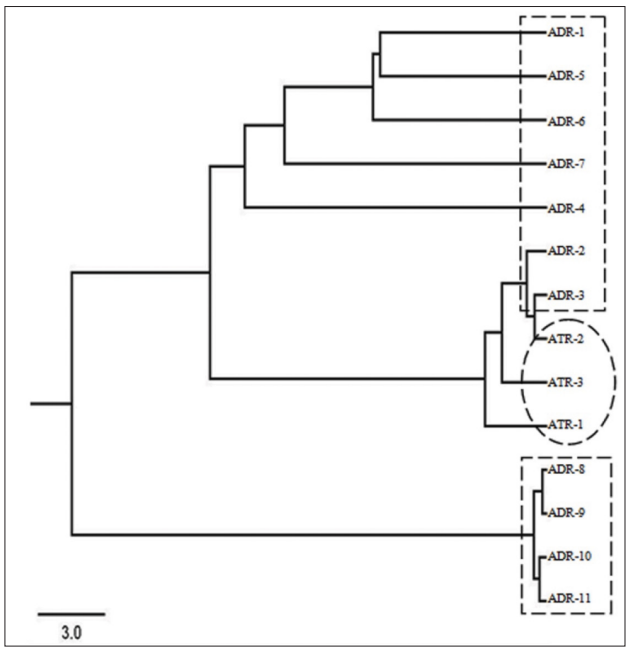


Fig 3. UPGMA dendrogram based on Nei's (1978) unbiased genetic distance matrix. The dotted square box and circle indicates the clustering of Dooars and Terai populations.

DISCUSSION

RAPD and ISSR-PCR can be utilized as an efficient molecular implement to differentiate spatially and/or genetically isolated populations and have been widely

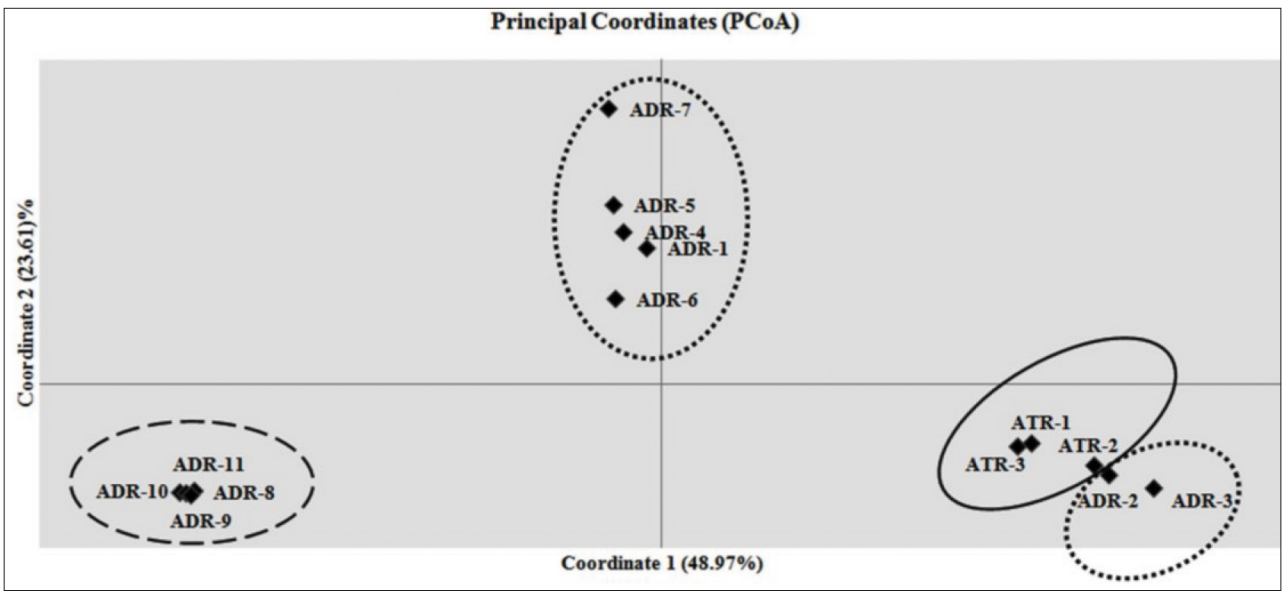
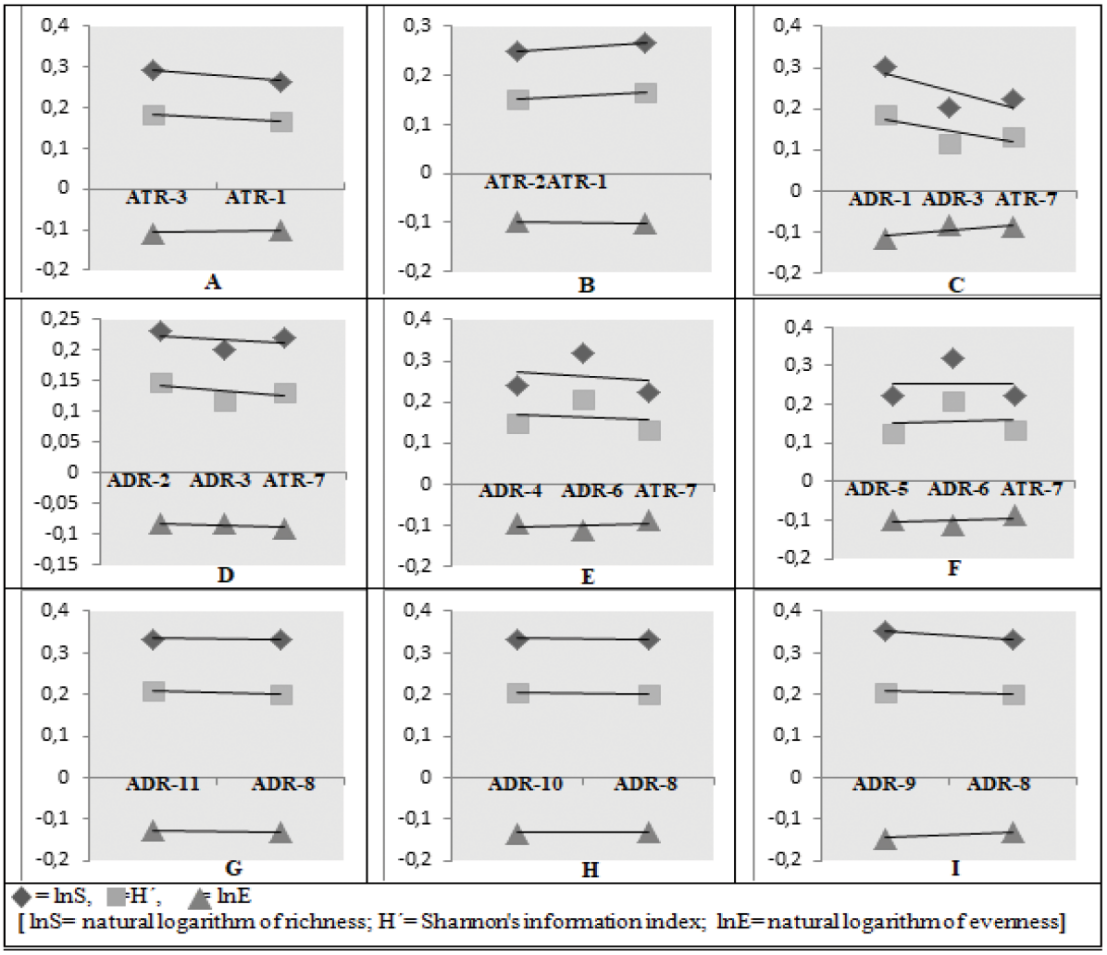


Fig 4. Principal Component Analysis based on covariance matrix without data standardization of *Amblyceps mangois* populations of three river systems. Continuous, dotted and dashed line circles represent clustering of the Mahananda, Teesta and Jaldhaka river populations. Coordinates 1 and 2 explain 48.97% and 23.61% of the variations, respectively.



Population	lnS	H´	lnE
Mahananda Barrage, Fulbari, (ATR-1)	0.265973129	0.1653	-0.100673129
Mahananda River, Champasari, (ATR-2)	0.249434885	0.1519	-0.097534885
Balason River, Tarabari, (ATR-3)	0.291997747	0.1847	-0.107297747
Sevok(Teesta River), (ADR-1)	0.304686671	0.1894	-0.115286671
Ghish River, (ADR-2)	0.232539273	0.1495	-0.083039273
Gajoldoba(Teesta River Barrage), (ADR-3)	0.201470376	0.1183	-0.083170376
Chel River, (ADR-4)	0.242710857	0.1463	-0.096410857
Neora River, (ADR-5)	0.222263164	0.1246	-0.097663164
Dharla River, (ADR-6)	0.320415442	0.2074	-0.113015442
Jalpaiguri (Teesta River), (ADR-7)	0.222263164	0.1336	-0.088663164
Jaldhaka River, (ADR-8)	0.332751036	0.2006	-0.132151036
Murti River, (ADR-9)	0.335829173	0.2094	-0.126429173
Ghotia River, (ADR-10)	0.335829173	0.2032	-0.132629173
Diana River, (ADR-11)	0.354101636	0.2079	-0.146201636

Fig 5. SHE analyses showing observed patterns of diversity changes of *Amblyceps mangois* in three river system populations.

Hierarchical Structure	Source of variation between population	F_{ST}	N_m	AMOVA		PhiPT (p value)
				Among population (%)	Within population (%)	
	Mahananda River System					
1st Order	ATR-3 # ATR-2	0.1084	4.1117	0.983 (7)	13.217 (93)	0.069 (0.006)
2nd Order	ATR-3, ATR-2 # ATR-1	0.1847	2.2068	2.181 (14)	13.259 (86)	0.141 (0.001)
	Teesta River System					
1st Order	ADR-1 # ADR-2	0.4870	0.5268	18.36 (63)	12.65 (37)	0.595 (0.001)
1st Order	ADR-2 # ADR-4	0.6187	0.4109	28.70 (72)	11.31 (28)	0.717 (0.001)
1st Order	ADR-4 # ADR-5	0.5285	0.4461	22.17 (67)	11.02 (33)	0.668 (0.001)
2nd Order	ADR-1, ADR-2 # ADR-3	0.4928	0.5145	15.82 (58)	11.47 (42)	0.580 (0.001)
2nd Order	ADR-4, ADR-5 # ADR-6	0.5102	0.4801	24.30 (66)	12.48 (40)	0.657 (0.001)
3rd Order	ADR-1, ADR-2, ADR-3 # ADR-4, ADR-5, ADR-6	0.5907	0.3465	20.20 (63)	11.98 (38)	0.628 (0.001)
4th Order	ADR-1, ADR-2, ADR-3 # ADR-7	0.5992	0.3345	22.65 (67)	11.20 (33)	0.669 (0.001)
4th Order	ADR-4, ADR-5, ADR-6 # ADR-7	0.5770	0.3666	21.02 (64)	11.96 (36)	0.637 (0.001)
5th Order	ADR-1, ADR-2, ADR-3, ADR-4, ADR-5, ADR-6 # ADR-7	0.5489	0.3081	22.02 (65)	11.74 (35)	0.652 (0.001)
	Jaldhaka River System					
1st Order	ADR-11 # ADR-10	0.0196	25.0247	0.0 (0)	16.35 (100)	-0.088 (1.000)
1st Order	ADR-10 # ADR-9	0.0318	15.2365	0.0 (0)	16.59 (100)	-0.062 (0.991)
2nd Order	ADR-11, ADR-10 # ADR-8	0.0387	12.4330	0.0 (0)	16.37 (100)	-0.079 (1.000)
2nd Order	ADR-9 # ADR-8	0.0125	39.3447	0.0 (0)	16.67 (100)	-0.090 (0.999)
3rd Order	ADR-11, ADR-10, ADR-9 # ADR-8	0.0427	11.2136	0.0 (0)	16.51 (100)	-0.077 (1.000)

F_{ST} = Population genetic differentiation, N_m = Estimated gene flow, AMOVA= Analysis of molecular variance, PhiPT= Estimated variance among population/(Estimated variance within population + Estimated variance among population), probability values based on 999 permutations. # indicates the comparison between populations or groups of populations.

Fig 6. Genetic hierarchial model of fourteen different populations of *Amblyceps mangois*. The populations are arranged in hierarchical orders as first, second, third, fourth, fifth order populations. The dots represent collection spots and arrow indicates the direction of water flow.

used to characterize the available gene pool of locally habituated populations. The present study is the first endeavour to explore the present status of population categorical genetic background of this threatened fish fauna in the major river streams in the Terai and Dooars region of this sub-Himalayan hotspot of North Eastern India. RAPD and ISSR technique-based estimations of genetic diversity may be suitable for assessing the impact of different stresses upon a population as well as wide range of ecosystems. Since homozygotes cannot be distinguished from heterozygotes by the RAPD and ISSR techniques, the absence of amplification of a band in two genotypes does not necessarily represent genetic similarity between them.

In the study carried out in Bangladesh with two species of vulnerable Mud eel (Alam et al., 2010; Miah et al., 2013), endemic yellow catfish *Horabagrus brachysoma* (Muneer et al., 2009) and *Clarias batrachus* (Khedkar et al. 2010), the proportion of polymorphism was 76.92%, 32.87%, 60.48% and 77.49%, respectively. In another study carried out in Bangladesh on catfish *Mystus vittatus* the polymorphisms observed were 88.64% (Chalan beel), 84.09% (Mohanganj haor) and 90.91% (Kangsha river) (Tamanna et al., 2012). In a study carried out in Brazil on *Pimelodus maculatus*, the proportions of polymorphic loci estimated for the lower, middle and the upper Tietê river were 60.19%, 51.94%, and 52.43%, respectively, and 56.49%, 54.81% and 61.51% for the lower, middle and upper Paranapanema, respectively (Almeida et al., 2003). In our earlier study with *Badis badis* the proportion of polymorphic loci varies from 0.4371 (43.71%) in overall population to 0.6733 (67.33%) in between populations (Mukhopadhyay and Bhattacharjee, 2014a). In the present study, it was found that the individual population polymorphism was between lower and moderate in the Teesta river system (23.40% in ADR-3 to 35.46% in ADR-6), moderate in the Mahananda river system (28.37% in ATR-2 to 34.04% in ATR-3 population) and moderate in the Jaldhaka river system (39.72% in ADR-8 to 43.26% in ADR-11 population) (Tables 2, 3, 4). Therefore, our present study revealed that a substantial decline of genetic variability within *Amblyceps mangois* population of the sub-Himalayan Terai and Dooars region.

The Nei's genetic diversity, Shannon's information index varied across three river systems (Table 2, 3, 4 and Fig. 2). Although considering the intra-population genetic variation, the Jaldhaka river system population showed a higher level of genetic variation than the Mahananda and Teesta river system populations (Table 2, 3, 4). In studies carried out in vulnerable *Monopterus cuchia* in Bangladesh (Alam et al., 2010) and on endemic species *Horabagrus brachysoma* in India (Muneer et al., 2009), the Nei's genetic diversity was 0.285 and 0.222, respectively. These results are in accordance with the findings of Chandra et al. (2010) on *Eutropiichthys vacha* where the Shannon's information Index was 0.280 and 0.300 in two different geographical populations. In two different studies carried out by Alam et al. (2010) and Miah et al. (2013) on mud eel *Monopterus cuchia* in Bangladesh, the Shannon's indices were 0.423 and 0.213, respectively. In another study carried out in Bangladesh on catfish *Mystus vittatus*, the genetic diversity was found to be 0.259±0.163, 0.198±0.136 and 0.216±0.138, and Shannon's information indices were 0.403±0.03, 0.327±0.03 and 0.354±0.02 in Chalan beel, Mohanganj haor and Kangsha river, respectively (Tamanna et al., 2012). Another separate study of ours revealed that the Nei's genetic diversity (*H*) of *Badis badis* Mahananda and Balason river population was 0.1654 and 0.1983, respectively, and the Shannon's information index (*H´*) was calculated to be 0.2450 ± 0.2907 in the Mahananda river and 0.2901 ± 0.3037 in the Balason river (Mukhopadhyay and Bhattacharjee, 2014a), and the Shannon's information index ranged from 0.1648 ± 0.2691 to 0.2205 ± 0.2950 in the Terai region of West Bengal India (Mukhopadhyay and Bhattacharjee, 2015). In a different study on *Barilius barna* isolated from the Teesta river, it was found that the Nei's genetic diversity ranged from 0.172 ± 0.189 to 0.293 ± 0.164 and the Shannon's information index (I) ranged from 0.265 ± 0.268 to 0.445 ± 0.220 (Paul et al., 2016). 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, in comparison with other studies, it was found that the genetic diversity was comparatively lower in the three river systems viz. the Mahananda, Teesta and Jaldhaka of the study region. The UPGMA-based dendrogram showed two distinct clusters of Terai and Dooars *Amblyceps mangois* population, i.e. one cluster consisting of ten populations of the Mahananda river system and Teesta river system, and a separate cluster with four populations of the Jaldhaka river system (Fig. 3). The ADR-2 and ADR-3 population of the Teesta river system form a cluster with the Mahananda river populations, i.e. ATR-1, ATR-2 and ATR-3. This clustering is obvious because there is a man-made water-canal that links two (Mahanada and Teesta) river systems (Fig. 1). This canal was mainly constructed for irrigation purpose of nearby agricultural fields and to channelize the extra water when the flood occurs in the rivers during monsoon season. This canal causes the admixture of *Amblyceps* gene pool of these two river systems, i.e. the Mahananda and Teesta, and grouping of the populations into a single cluster (Fig. 3). Whereas the Jaldhaka river system is totally allopatrically isolated from the other two neighbouring river systems and therefore the Jaldhaka river populations formed a distinct cluster separate from the Mahananda and Teesta river system cluster (Fig. 1 and Fig. 3).

Buzas and Hayek (1996) reported that the Shannon's index of diversity can be decomposed into two metric components, namely species richness (E) and evenness (S) (H´= lnE + lnS), but sometimes it becomes difficult to ascribe whether the diversity component is influenced

by greater/lower richness or greater/lower evenness of values or both. However, this decomposition also allows investigators to describe the change in the diversity pattern through different subpopulations in a spatial and temporal scale. In our study, it was found that the three components, i.e. the pattern of diversity (H´), richness (S) and evenness (E), have varied across all fourteen populations in a hierarchical manner from higher to lower altitude as the river flows downstream (Fig. 5), which is most obvious in naturally subdivided populations. Therefore, the *SHE* analysis can deduce the change or break in the diversity pattern of the populations along a distinct gradient. Hayek and Buzas (1997) pointed out that often the diversity (H´) changes because the differences between richness (S) and evenness (E) do not offset each other (i.e. $H'_1 \neq H'_2$, $S_1 \neq S_2$, $E_1 \neq E_2$, where 1 and 2 in suffix are any two populations) and such *SHE* plot is log normal one. *SHE* analysis appears to be a useful approach for defining the diversity. Our data revealed that as the river streams converged from higher to lower altitude, there was a fluctuation in the diversity patterns. In most of the cases the genetic diversity and richness of *Amblyceps mangois* populations decreased and evenness increased but in some cases the diversity and evenness both increased or decreased, or remained the same (Fig. 5, upper panel). The flow pattern disturbance and human interferences (such as fishing and pesticide run-offs) as the river streams flow from higher to lower altitudes may cause the overall fluctuation and break in diversity and richness pattern within the gene pool of *Amblyceps* population. All of these causes can culminate into the observed decline, modification and change in diversity pattern and richness in *Amblyceps mangois* populations across the river stream along the altitudinal gradient.

In this study, a low to high (0.0125 to 0.6187) levels of genetic differentiation (F_{ST}) across different populations of *Amblyceps mangois* in three river systems were detected in a hierarchical manner and the gene flow was low to high between different populations. A residual level of genetic admixture was carried out through narrow channels between different populations because of the submergence of the channels during monsoon season. A direct evidence of population differentiation was revealed by the AMOVA. A high level of variance among populations and genetic differentiation (F_{ST}) was detected in the second order hierarchical population (consisting of populations ATR-3, ATR-2 and ATR-1) of the Mahananda river system and first order hierarchical population (consisting of populations ADR-2 and ADR-4) of the Teesta river system, which indicates that these hierarchical groups are genetically and reproductively isolated and a sporadic gene flow occurred (Fig. 6). Moreover in the Jaldhaka river system the observed variance among populations was nil and very low amount of genetic differentiation with a high amount of gene flow indicates that this river system is made up of genetically similar sub populations (Fig. 6). Results of PhiPT were also congruent with the results of F_{ST}. In a study carried out in Bangladesh on catfish *Mystus vittatus*, the differentiation (PhiPT) values were found to be insignificant, indicating that there was no significant differentiation among the three studied populations (Tamanna et al., 2012). The gene flow estimated for Brazilian *Pimelodus maculatus* in the Paranapanema river was 4.4646, 2.1732 and 1.8776 between the lower and middle, lower and upper, and middle and upper parts, respectively, which showed significant genetic differentiation (Almeida et al., 2003). According to Wright (1978), genetic differentiation between 0.05 and 0.15 is considered as moderate population structuring, and with probabilities between 0.15 and 0.25 of high population structuring. The study carried out in Bangladesh on endemic yellow catfish *Horabagrus brachysoma*, the F_{ST} value ranged from minimum 0.045 to maximum 0.219 (Muneer et al., 2009). In our previous study on *Badis badis* in the Terai region of West Bengal, India, it was found that the degree of gene differentiation ranged from 0.5996 to 0.2560, and gene flow ranged from 0.3339 to 1.4534. In the same study with the same species, it was found that between the Mahananda and Balason populations the F_{ST} value was 0.2109 (Mukhopadhyay and Bhattacharjee, 2015).

Compared to other related studies, the present study revealed a significant level of genetic divergence, and population differentiation occurred in the Mahananda and Teesta river systems, and therefore populations are highly structured. That is, a population having local breeding units in each stream has high genetic divergence from similar breeding units in other streams and needs to be managed as evolutionary significant units (ESU) for conservation purpose. Therefore in the Mahananda river system, there is a need to manage the whole river system as evident from the hierarchy analyses and in the Teesta river system it is necessary to manage the first order streams to conserve the gene pool of *Amblyceps* of the whole river system. This study is the initial attempt to characterize and evaluate the genetic architecture of *Amblyceps mangois* from the three major river systems of sub-Himalayan Terai and Dooars region of West Bengal, India. Low levels of genetic diversity/variation and genetic hierarchical structure with high genetic divergence were found in the present study among the fourteen populations as an indicative of the recent picture of threatened status of this species. In addition to over-fishing, presence of barrage/dam at the upper reaches of the river system, 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, Teesta and Jaldhaka river populations. A correlative study based on these anthropogenic factors with that of the available genetic diversity in this species can add a newer element to find out the cause of this low level of genetic variability as well as a possible conservational strategy.

CONCLUSION

This study is the initial attempt to characterize and evaluate the genetic architecture of *Amblyceps mangois* from the three major river systems of sub-Himalayan Terai and Dooars region of West Bengal, India. Low levels of genetic diversity/variation and genetic hierarchical structure with high genetic divergence were found in the present study among the fourteen populations as an indicative of the recent picture of threatened status of this species. In addition to over-fishing, presence of barrage/dam at the upper reaches of the river system, 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, Teesta and Jaldhaka river populations. A correlative study based on these anthropogenic factors with that of the available genetic diversity in this species can add a newer element to find out the cause of this low level of genetic variability as well as a possible conservational strategy.

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SAŽETAK

RAPD I ISSR ANALIZA GENETIČKE RAZNOLIKOSTI I STRUKTURE POPULACIJE UGROŽENE VRSTE *Amblyceps mangois* IZ POD-HIMALAJSKOG ZAPADNOG BENGALA, INDIJA

Amblyceps mangois ili "indijski bujični som" je tropska, slatkovodna, vrsta brdskih potoka koja u Indiji ima ukrasno-komercijalnu vrijednost a uključena je u kategoriju "ugrožene" na popisu ugroženih slatkovodnih riba Indije. Kako bi se, uz pomoć markera RAPD i ISSR, istražila genetička arhitektura ove vrste, analizirano je ukupno četrnaest populacija iz regije Terai i Dooars na sjeveru Zapadnog Bengala u Indiji. Opaženi broj alela (S), Nei-ova genska raznolikost (H) i Shannonov indeks podataka (H´ ili I) ukazali su na najviše vrijednosti u riječnom slivu Teesta i najniže vrijednosti u slivu rijeke Mahananda. Dendrogram na bazi UPGMA i PCoA, temeljeni na RAPD i ISSR otiscima, ukazale su na formiranje skupine populacije rijeka Mahananda i Teesta koja se razlikuje od preostale populacije rijeke Jaldhaka. Četrnaest riječnih populacija su dalje razmatrane u devet skupina prema kontinuitetu protoka vode za SHE analizu. Utvrđeno je da su tri komponente tj. uzorak raznolikosti (H'), bogatstva (S) i jednolikosti (E), varirale i fluktuirale u svih četrnaest populacija s više na nižu nadmorsku visinu, kako rijeka teče nizvodno. AMOVA, PhiPT i genetske hijerarhijske analize ukazale su na izrazitu hijerarhijsku strukturu prisutnu u populaciji Ambliceps-a na istraživanom području. U istraživanju su nađene niske razine genetske raznolikosti/varijacija i genetska hijerarhijska struktura s visokom genetskom divergencijom, kao pokazatelj nedavne slike ugroženog statusa ove vrste. Ova studija je početni pokušaj karakterizacije i procjene genetske arhitekture ribljih vrsta iz ove regije i postoji prostor za upravljanje značajnim jedinicama evolucije (ESU) u svrhu njihovog očuvanja.

Ključne riječi: Indijski bujični som, genetska hijerarhija, SHE analiza, sub-himalajski *hotspot*

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