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Research Article

GENETIC STRUCTURE OF ENDANGERED BATA (*LABEO BATA*, HAMILTON) INFERRED FROM LANDMARK-BASED MORPHOMETRIC AND MERISTIC MEASUREMENTS AND ALLOZYME MARKERS

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Abstract: Landmark-based morphometric and meristic counts were conducted to evaluate the population status of *L. bata* collected from three rivers namely the Tista, the Padma and the Brahmaputra and one hatchery sources. Seven of 13 morphometric characters, four of 8 meristic counts and eleven of 25 truss measurements revealed a significant inter-stock variation ($P<0.01$) among all samples. Plotting discriminant function DF1 and DF2 showed differentiation among stocks for both measurements. The first and second DF accounted 67.9% and 22.3% group variability respectively, explaining 90.2% of total group variability. A dendrogram based on morphometric and landmark distances placed the Tista and the Brahmaputra populations in one cluster with one sub-cluster with the Padma population while the hatchery population formed separate cluster. Allozyme electrophoresis was also carried out to assess the population genetic structure of *L. bata* with five enzymes (*EST*, *GPI*, *LDH*, *MDH* and *PGM*). The enzymes were controlled by eight presumptive loci (*Est-1**, *Gpi-1**, *Gpi-2**, *Ldh-1**, *Ldh-2**, *Mdh-1**, *Mdh-2** and *Pgm**) where highest (six) polymorphic loci were found in Brahmaputra population. The mean proportion of polymorphic loci (%) observed as 62.50, 50, 75 and 62.50 in the Tista, the Padma, the Brahmaputra and the hatchery populations respectively. The highest (1.875) mean number of allele per locus was observed in the Brahmaputra river population. The mean proportion of heterozygous loci per individual was 19.48% and the average observed heterozygosity (H_o) and expected heterozygosity (H_e) were 0.195 and 0.176 respectively for all populations. The lowest (0.032) pair-wise population differentiation (F_{ST}) and highest (7.649) gene flow (*Nm*) were found in the Padma and the Brahmaputra river population indicating close relationship among them. In the Nei's UPGMA dendrogram, the Tista population separated from other cluster by the highest genetic distance, D=0.171. The Padma and the Brahmaputra populations of *L. bata* made one cluster (D=0.009) and separated from the hatchery population by the genetic distance of 0.048. This study revealed considerable but inequitable variations among populations in wild and hatchery populations of *L. bata* using two methods at a time.

Keywords: Electrophoresis, heterozygosity, Stock identification, Genetic differentiation

Introduction

Labeo bata is a benthopelagic, potamodromous minor carp in South and South-East Asia with great demand as table fish due to its deliciousness, nutrient contents and less spine[1,2]. The bata are normally captured from the natural sources i.e., *haors*, *baors*, *beels*, and rivers in Bangladesh. However, the natural production has been deteriorated by more fishing pressure, dam construction and other anthropological effects. *L. bata* is now one of the 56 freshwater fish species denoted as endangered in Bangladesh[3]. It is therefore important to know the genetic structure of existing population for adopting appropriate conservation approaches. Landmarks and allozyme markers can be used to help know the population structure of any species. Landmarks refer to some arbitrarily selected points on a fish's body and with the help of these points the individual fish shape can be analyzed[4,5]. The landmark based differences among stocks of a species are recognized as important for evaluating the population structure and as a basis for identifying stocks[6,7]. Genetic analysis using allozyme markers is also an effective tool for population studies[8] where the electrophoretic data are used to define population structure and estimate intra and/or inter population gene flow through the analysis of genotype frequencies at multiple and independent loci. Since the late 1960s, the electrophoretic analysis of enzymatic proteins has become one of the vital sources of information about genetic structuring among fish populations. Genetic structuring using biochemical markers alongwith the collected information from landmark based experiments could help endangered bata to sustain in natural environment following proper conservation approach. Therefore the objective of the present work was to investigate the genetic variations of wild and hatchery populations of *L. bata* to identify the genetically diversified populations so that recommendations can be given to minimize the level of threat on this endangered species.

Materials and methods

Research station

The experiments were conducted in the Fish Genetics and Biotechnology Laboratory, Department of Fisheries Biology and Genetics and Field Laboratory Complex, Faculty of Fisheries, Bangladesh Agricultural University. The equipment and other facilities available at the faculty were used whenever required.

Sample collection
The experimental fish were collected from three rivers viz. the Tista, the Brahmaputra, the Padma (Fig. 1) and one hatchery in Bangladesh. Details of sources, number of specimens and date of collection are shown in Table 1.

Experiment 1: Characterizing *L. bata* by landmark-based analyses
Measurement of morphometric and meristic characters
Thirteen morphometric characters and body weights (Fig. 2) were measured following the conventional method described by Hubbs and Lagler (1958) to the nearest 0.1 cm using a slide calipers. Description of morphometric characters used for analysis of *L. bata* population variations is given in Table 2. Eight meristic characters, viz., dorsal fin rays (DFR); pectoral fin rays (PcFR); pelvic fin rays (PvFR); anal fin rays (AFR); caudal fin rays (CFR); branchiostegal rays (BR); scales above the lateral line and scales below the lateral line were studied following the methods of Hubbs and Lagler (1958).

Measurement of Landmark distances of the species
For measurement of Landmark distances of the species the truss network system described for fish body morphometrics[9] was used to construct a network on fish body. Twelve landmarks determining 25 distances were produced and measured as illustrated in Fig. 3. Data points were arranged in "trusses" around the fish, a layout which maximises the number of measurements and increases the sensitivity of the analysis[10]. Fish were laid out on a piece of graph paper on wax coated tray and fixed into position by the insertion of pins along the body. Finally each landmark was obtained from the distances on the graph paper which were measured using vernier calipers.

Statistical analysis
A multivariate discriminant analysis was used for morphometric data to identify the combination of variables that best separate *L. bata* species. Size-dependent variation for all the characters was removed by an allometric formula given by Elliott et al. (1995): $M_{adj} = M (L_s / L_o)^{b}$, where M: Original measurement, M_{adj}: Size adjusted measurement, L_o: Total length of fish, and L_s: Overall mean of total length for all fish from all samples. Parameter b was estimated for each character from the observed data as the slope of the regression of log M on log L_o, using all fish in all groups. The efficiency of size adjustment transformations was assessed by testing the significance of the correlation between transformed variable and TL. The degree of similarity among samples in the overall analysis and relative importance of each measurement for group separation were assessed by discriminant function analysis (DFA). Univariate analysis of variance (ANOVA) was carried out to test the significance of morphological differences. Comparison of meristic characters was done using non parametric Kruskal-Wallis test.

Experiment 2: Studying genetic variation of *L. bata* by allozyme markers
Allozyme electrophoresis is a biochemical technique referring to the migration of protein particles under the influence of an electric field. With the allozyme electrophoretic separation technique, the protein product of a given gene is assayed and genetic polymorphism is studied by comparing protein mobilities in an electrical field. In the present study, horizontal starch gel electrophoresis method was used.

Collection and storage of tissues
The muscle tissues were collected from each sample (N=30), kept in marked airtight small size plastic bags and stored in a freezer (-18 ºC).

Gel preparation
The gels were prepared using 40.8 g (12%) of hydrolyzed potato starch (STARCH-SIGMA-ALDRICH CHEME, Steinheim, Germany) in 340 ml of distilled water and appropriate buffer. At first the starch powder was weighed by an electronic balance (METTLER TOLEDO, PG503-SDR, Switzerland), transferred into a 1 L Erlenmeyer flask containing 17 ml (1/20 of total liquid volume) of electrophoresis gel buffer (CA 6.1). Then 323 ml of distilled water (19/20 of total liquid volume) was added to the mixture and immediately swirled to generate a uniform suspension and finally was heated to boil for 8-10 min using a bunsen burner until transparent solution of the gel was observed. Then the boiled gel solution was degassed for approximately one minute to remove air foam by gentle shaking of the flask. The boiled starch was then poured onto the glass (18 cm x 22 cm x 0.5 cm) attached with plastic frame (16 cm x 20 cm x 0.5 cm). The hot gel was allowed to cool at room temperature for about 2 hrs. The gel was covered with OHP sheet to prevent desiccation after 30 min. Finally, after cooling the gel was preserved overnight into a refrigerator at 4 ^{o}C to increase hardness for slicing of the gel easily.

Sucking of muscle tissues by paper wick
The muscle tissues were taken out of the freezer for thawing. The inoculation of protein extract from muscle was sucked using paper wicks (Advantec-Toyo). Thus, muscle tissue from each individual of a population was sucked by paper wicks and dried with soak paper for removing of extra-absorbed enzyme.

Loading of sucked wick and running of electrophoresis
After overnight solidification, the gel was taken out from the refrigerator and cut through approximately 6 cm from the cathodal end to make two pieces. Then the sucked paper wicks were loaded between the two pieces of gel from left to right direction and two wicks containing a dye marker (ethylene blue) were placed at both end of the gel as an indicator of determining the rate of protein migration. The electrophoresis was performed in a buffer system after Clayton and Tretiak (1972).

Gel slicing and staining
The gel was sliced horizontally into five or more sections after completion of electrophoresis, depending on the thickness of the gel. This was done sequentially by placing 1 mm glass pieces one after another on the lower side of the gel and drawing monofilament strings through the gel over which glass-sheets were placed. The sliced gels were placed into individual staining trays with the cut-side of the slice

facing upward. The enzymes analyzed, E.C. numbers and enzyme patterns for horizontal starch-gel electrophoresis are presented in Table 3. The components of staining solution with some co-factors that were used in this experiment are listed in Table 4. All staining were routinely accomplished in an oven set at 45-55 ^{o}C.

Gel preservation

The staining gel was washed with 10% acetic acid and kept in 10% glycerin for 10-15 min. Finally, the gel was kept on and covered by cellophane paper. Then the gel was kept in oven at 55 ^{0}C for overnight for drying.

Genetic data analysis

The allele frequencies were calculated by direct count of the proportion of different alleles. The distributions of observed genotypes were compared with the expected ones, calculated from the Hardy-Weinberg (H-W) equilibrium using a Chi-square (χ^2) test. The allele frequency and the analyses of Chi-square (χ^2) test were performed using Gene Alex (Peakall and Smouse 2005) computer program. It estimates allele frequency by using the following formula:

Allele frequency = (2Nxx + Nxy) / 2N

Where, Nxx = the number of homozygous individuals

Nxy = the number of heterozygous individuals and

N = the number of samples

The mean proportions of polymorphic loci per population, the mean number of alleles per loci and the mean proportion of heterozygous loci per individuals were determined to observe the extent of genetic variability for each population. Mean proportion of polymorphic loci was analyzed with the help of POPGENE (version 1.31) (Yeh et al. 1999) computer package program. The mean number of allele per locus was calculated directly from the observed number of alleles by dividing with the observed number of locus. The mean proportions of heterozygous loci per individual were calculated directly from observed total number of heterozygote loci by dividing with the total number of individual in a population. Expected heterozygosity (H_e) and observed heterozygosity (H_o) were also calculated after Nei (1972) using the following formula and with the help of POPGENE (version 1.31) (Yeh et al. 1999) computer package program.

$h_e = 2n\,(1 - \Sigma\, x_i^2/N)/\,(2n - 1)$

Where, n is the number of sampled individuals and x_i is the frequency of i-th allele at each locus. N is the number of loci examined. Thus, average heterozygosity (H_e) was calculated as $He = \Sigma\, h_e$ / r, Where, r is the number of loci examined (Nei and Roychoudhury 1973). Observed average heterozygosity phenotypes (H_o) was calculated as $H_o = \Sigma\, h_o$ / r , Where h_o is the total heterozygous phenotypes in each locus and r is the number of loci examined.

F_{ST} is the inbreeding coefficient within subpopulations relative to the total. It is more or less equivalent to G_{ST} which provides the measure of genetic differentiation among populations i.e., the proportion of total genetic diversity (equivalent to heterozygosity) that is distributed among the populations. If all the subpopulations are in Hardy-Weinberg equilibrium with the same allele frequencies then the F_{ST} = 0. The F_{ST} represent the degree of population genetic differentiation. The number of individuals that migrate from one subdivided population to another is revealed as gene flow (N_m). F_{ST} and N_m were measured by using the following formula with the help of GeneAlex (version 6) (Peakall and Smouse 2005) computer program by using the following formula:

$$F_{ST} = \frac{H_T - \text{Average } H_e}{H_T}$$

where, H_T is the gene diversity in the total population, measured by total allelic frequency of the examined population.

Gene flow (N_m) for four populations was measured by using following formula with the help of POPGENE (version 1.31) (Yeh et al. 1999) computer package program.

N_m = Gene flow estimated from F_{ST}, $N_m = [(1/ F_{ST}) - 1]/4$

Genetic distance values (D) (Nei, 1972) were calculated as following formula:

$D = -\ln J_{XY}/\sqrt{\sum J_X J_Y}$

Where, $J_X = \sum X_i^2/$ r in population X, $J_Y = \sum Y_i^2/$ r in population Y, and $J_{XY} = \sum X_i Y_i$. X_i and Y_i are the frequency of the i-th allele of a given locus in the two populations of fishes compared and r is the number of loci frequencies for all possible pairs of populations. The preceding analysis of allozyme data were performed using POPGENE (version 1.32) and G-Stat (version 3.1)[11] and TREEVIEW[12] package computer program. Based on the *D*-values, dendrogram and radial tree were made by the UPGMA (unweighted pair-group method using arithmetic average) method[13].

Results

Morphometric and Landmark distances

Univariate statistics showed that seven morphometric (HL, P<0.001; ED P<0.001; PROL, P<0.001; POOL, P<0.001; HBD, P<0.001; DFL, P<0.01 and PCFL, F=5.116; P<0.01) and eleven truss measurements (1-2, 2-3, 2-12, 2-11, 3-4, 4-5, 4-10, 4-9, 5-6, 5-7 and 7-8) revealed a significant inter stock variation between all samples in varying degrees (Table 5.)

Pooled within group correlation between discriminant variables and discriminant functions (DFs) revealed that seven morphometric (ED, POOL, HBD, PROL, PCFL, FL, AFL) and eight landmark measurements (4-10, 2-12, 11-12, 4-9, 5-7, 2-9, 9-10, 3-10) contributed to the first DF and five morphometric characters (HL, DFL, MG, PVFL, SL) and fourteen landmark measurements (1-2, 2-3, 10-11, 5-6, 3-4, 4-5, 4-7, 7-8, 3-8, 3-11, 6-7, 8-9, 1-12, 3-9) contributed second DF (Table 6). Plotting discriminant function DF1 and DF2 showed differentiation among populations for both morphometric and landmark measurement. For both morphometric and landmark measurements the first and second DF accounted 67.9% and 22.3% group variability respectively, explaining 90.2% of total group variability. The Brahmaputra and the hatchery populations were clearly separated from each other and from the Padma and the Tista populations but the Padma and the Tista populations scattered and overlapped each other in the discriminant space (Fig. 4).The results suggested that there was limited

intermingling among populations and the populations were separated.

A dendrogram based on morphometric and land-mark distances data was shown for the population of the Tista, the Padma, the Brahmaputra and the hatchery. Two main clusters were formed among four populations. The Tista and the Brahmaputra populations formed one cluster with one sub-cluster with the Padma population. On the other hand hatchery population formed separated cluster based on the distance of squared Euclidean dissimilarity (Fig. 5).

Meristic characters

Meristic counts for all samples ranged between 10-12 for dorsal fin rays, 15-17 for pectoral fin rays, 8-10 for pelvic fin rays, 7-8 for anal fin rays, 20-23 for caudal fin rays, 3 for branchiostegal rays, 6.5-8.5 for scales above lateral line and 5.5- 6.5 for scales below lateral line. The mean number of dorsal, anal, caudal fin rays and branchiostegal rays were not significantly different among fish from the four stocks (Kruskal-Wallis test (H), $P>0.05$) and difference were found in other characters ($P<0.001$) (Table 7).

Experiment 2. Study on genetic variation of wild and hatchery populations of *L. bata* using allozyme markers

Alleles and genotypes

The electrophoretic patterns of muscle tissue showed that the enzymes were controlled by the genes at 8 presumptive loci. Two alleles (**a* and **b*) were found in five loci (*Est-1*, Mdh1*, Mdh2*, Gpi-1** and *Gpi-2*)* where two to three genotypes (**aa, *ab* and **bb*) were produced. Five genotypes (**aa, *ab, *bb, *bc* and **cc*) were found in one locus (*Pgm**) by three alleles (**a, *b* and **c*) and only one genotype (**aa)* was observed for *Ldh-1** and *Ldh-2**. On the average 2.5 genotypes were produced by 1.875 alleles at the 8 loci (Table 8).

Polymorphic loci and allele frequencies

Among the eight loci, the Tista river and the hatchery populations showed five polymorphic loci (*Mdh-2*, Pgm*, Gpi-1*, Gpi-2** and *Est-1**) and the Padma population showed four polymorphic loci (*Mdh-1*, Mdh-2*, Pgm** and *Est-1**). On the other hand, the Brahmaputra population showed six polymorphic loci (*Mdh-1*, Mdh-2*, Pgm*, Gpi-1*, Gpi-2** and *Est-1**). The chi-square (χ^2) test was done in all the cases of polymorphic loci between observed and expected genotypes based on Hardy-Weinberg equilibrium. The probability of all polymorphic loci has been showed in Table 9. The Tista, the Padma and the Brahmaputra populations showed significant variation in allele frequencies of *Gpi-2*, Pgm** and *Mdh-2** loci respectively.

With monomeric enzyme esterase (EST), allele **a* was dominant in the Padma and the Brahmaputra populations but present in all populations and the frequency ranged from 0.417 to 0.833. Allele **b* was dominant in the Tista and hatchery populations and also present in all populations with frequencies ranged from 0.167 to 0.617 (Table 9). The monomeric enzyme phosphoglucomutase (PGM) was found to be controlled by single *Pgm** locus (Fig. 6). The *Pgm** was polymorphic in all the four populations. With tetrameric enzyme lactate dehydrogenase (LDH), two Monomorphic loci *Ldh-1** and *Ldh-2** were identified. The dimeric enzyme malate dehydrogenase (MDH) presumably controlled by two different loci, *Mdh-1** and *Mdh-2**. The *Mdh-1** was monomorphic with the allelic frequency of **a* = 1.000 in the Tista river and hatchery but was polymorphic in the Padma river and the Brahmaputra river populations with the allelic frequency of **a* ranged from 0.983 to 0.100, and **b* with 0.017. The *Mdh-2** was polymorphic in all the populations with the allelic frequency of **a* ranged from 0.133 to 0.350 and **b* ranged from 0.650 to 0.867. The dimeric enzyme glucose-6-phosphate isomerase (GPI) exhibited three banding patterns presumably controlled by at least two different loci, *Gpi-1** and *Gpi-2**. Both the *Gpi-1** and *Gpi-2** loci were polymorphic in the three populations (the Tista, the Brahmaputra and hatchery). The *Gpi-1** was polymorphic with the allelic frequency of **a* ranged from 0.75 to 0.95 and **b* ranged from 0.05 to 0.25, (Table 9). The *Gpi-2** was polymorphic with the allelic frequency of **a* ranged from 0.167 to 0.683 and **b* ranged from 0.317 to 0.833 respectively. On the other hand both the *Gpi-1** and *Gpi-2** loci were monomorphic with the allelic frequency of **a*=1.000 and **b*=1.000 respectively in the Padma population.

Chi-square (χ^2) test

The Tista, the Padma and the Brahmaputra populations showed significant variation in allele frequency of *Gpi-2**, *Pgm** and *Mdh-2** loci respectively ($P<0.05$). On the other hand, no significant variation occurred in allele frequencies in hatchery population.

Genetic variability

The mean proportions of polymorphic loci in the Tista, Padma, Brahmaputra and the hatchery populations were 62.5, 50, 75 and 62.5% respectively. The mean number of alleles per locus (*Na*) for all populations was 1.688 and ranged from 1.625 to 1.875 (Table 10). The mean proportion of heterozygous loci per individual for all populations was 19.84% and ranged from 10% (Padma) to 22.92% (Tista). The observed heterozygosity (H_o) was 0.195 in average and ranged from 0.1 (Padma) to 0.229 (Tista). The average expected heterozygosity (H_e) was 0.176 and ranged from 0.094 (Padma) to 0.217 (hatchery). The summarized genetic variations are shown in Table 10.

Population differentiation (F_{ST}) and Gene flow (N_m)

The genetic differentiation (F_{ST}) and the gene flow (N_m) over all four populations were 0.226 and 0.857 respectively. In pair-wise analysis, comparatively higher N_m value (7.649) was estimated between the Padma and the Brahmaputra populations corresponding lower level of F_{ST} value (0.032) (Table 11).

Genetic distance

A matrix of genetic distance (D)[14] was constructed based on allelic frequencies of all loci among four populations ranged from 0.009 to 0.171. The minimum genetic distance (D=0.009) was observed between the Padma and the Brahmaputra river populations, while the maximum value (D=0.171) was found between the Padma and the Tista river populations (Table 12). The UPGMA dendrogram (Nei, 1987) constructed from Nei's (1972) genetic distance resulted in two major clusters among the four populations

(Fig. 7). Cluster-1 consisted of the Tista river only and separated from other cluster by the highest genetic distance, D = 0.171. The cluster-2 consisted of three populations and divided into two sub-clusters and separated from each other by the genetic distance, D = 0.048. The sub-cluster-1 was made by the hatchery population and separated from Brahmaputra river population by the 0.042. The sub-cluster-2 consisted of the Padma river and the Brahmaputra river populations and separated from each other by the smallest *D*-value (0.009).

Discussion

Characterizing *L. bata* using Landmark-based morphometric and meristic variations analysis

Morphological measurements have widely been used to differentiate various fish populations[15,16]. The present results demonstrated significant differences in morphometric characters among the populations of the Tista, the Padma, the Brahmaputra and the hatchery. The morphometric differences among the stocks could be expected because of geographical separation and origin from different ancestors. Random genetic drift may also result in variation in different geographically separated groups, thus causing differentiation in phenotypes. Such differentiation was observed by morphometric and landmark distance in European anchovy *Engraulis encrasicolus* in the Black, the Marmara and the Aegean Seas[17]. For both morphometric and landmark measurement, plotting discriminant function DF1 and DF2 showed a clear differentiation among stocks and the first and second DFs accounted 67.9% and 22.3% group variability respectively, explaining 90.2% of total group variability. The Brahmaputra and the hatchery populations were clearly separated from each other whereas the Padma and the Tista populations scattered and overlapped each other in the discriminant space. The results suggested that there was limited intermingling among populations and the populations were separated. Plotting discriminant function explained 100% of total between group variability and clearly discriminated the Eastern Mediterranean sea sample from the Baltic and the Aegean sea samples in an analysis of morphometric and landmark distance of twaite shad (*Alosa fallax*) among three areas in turkish seas. In the present study, the mean number of pectoral fin rays, pelvic fin rays, scale above lateral line and scales below lateral line differed significantly (P<0.001) among the four stocks. Many authors reported that the final number of structures achieved by meristic attribute is determined by the environmental characteristics prevailing during a critical stage in the development of the individuals, during which they are more phenotypically influenced by the environment[18]. Differences in meristic counts in European anchovy *Engraulis encrasicolus*) in the Black, the Marmara and the Aegean Seas samples and in Japanese charr (*Salvelinus leucomaenis*) among the tributaries of the Hohki River and the Naka River systems were considered to be affected by environmental factors by Erdogan et al. (2009) and Nakamura (2003)[19] respectively.

Studying genetic variation of *L. bata* using allozyme markers

In the present study genetic variations were analyzed with five enzymes (LDH, MDH, PGM, GPI and EST) in CA 6.1 buffer system and they produced clear resolution in the muscle tissue of the four populations of *L. bata*. According to Khan the allelic enzyme activity varies from buffer to buffer, species to species and also tissue specific. Alam et al reported that two buffer systems (CA 6.1 and CA 7.0) showed clear resolution for at least four enzymes GPI, LDH, MDH and PGM for muscle tissue.

In the present study, all populations showed two common alleles **a* and **b* except locus *Pgm** in the Padma population where a rare allele **c* (0.05) was observed. Such allelic mobility has also been observed by some authors. For example, by Alam et al in the natural population of an Indian major carp, *Labeo rohita*, by Pervej in the locus *Est-1** of *Puntius sarana* population of SK (Shukair and Kangsha). However different species and buffer systems can have different allelic mobility. The presence of such rare allele in the Padma population could be an evidence of superiority of that very stock. However, the polymorphism in Brahmaputra population revealed higher (6 loci) than those observed in Padma (4) and other two (5) indicating the increase of gene pool diversity in the Brahmaputra. The presence of mean proportion polymorphic loci of Brahmaputra (75%) is comparable with the work of Garg et al. who studied genetic diversity of two populations of catfish *Mystus vittatus* using RAPD markers and obtained high polymorphism (64.98%). 50% polymorphic loci for natural population of common carp was reported by Kohlmann and Petra as well as in wild and hatchery populations of *L. rohita* by [20]Alam *et al*. whereas 57.14% in another Indian major carp, *Catla catla*. The average heterozygous loci for all population of *L. bata* in this study was 19.48%, higher than the average heterozygous loci obtained by Pervej (13.33%) for the three populations of sharpunti (*P. sarana*) and than that obtained by Alam et al[21]. for both hatchery and natural populations of rohu, *L. rohita* (15%).

The average heterozygosity (*Ho* or *He*) is considered as a good indicator of the genetic variability throughout the genome of the population. Nevo (1978) reported that an average observed heterozygosity (H_o) value for bony fishes was 0.051. The average observed heterozygosity (H_o) obtained in the present study (0.195) was higher than those obtained by Pouyaud et al. (0.091) and Na-Nakorn et al. (0.038-0.080) in case of *Clarias macrocephalus.* Nasren et al. found the average observed heterozygosity ranged from 0.64 to 0.75 in *H. fossilis*. Islam et al. (2007) found average heterozygosity 0.67 to 0.83 in *C. batrachus.* The highest observed and expected heterozygosity (H_o=0.229 and H_e=0.212) exhibited by the Tista populations in the current study indicated that the gene pool was maintained effectively.

The co-efficient of gene differentiation (F_{ST}) in all four *L. bata* populations examined for all loci was 0.226, indicated the presence of population with a significant genetic differentiation. The F_{ST} values for some freshwater fishes obtained by other workers were rather high, for example 0.774 in loach (Khan and Arai, 0.698 in freshwater Gobi. However, the number of individuals that migrate from one population to another was the high as revealed as N_m=0.857 in this study. The genetic distance between the population

pairs ranged from 0.009 to 0.171. The highest genetic distance was found between the Padma river and the Tista river population (0.171) which might be due to distant origin of broods. The observed genetic distances among the four populations of *L. bata* `in the present study are much more lower than the findings of Khan et al. who found the higher genetic distance between the Padma and the Halda (*D*=0.263) or between the Halda and other six populations (*D* =0.112) for *L. rohita*. Leesa-Nga et al. mentioned that the *D*-values of yellow catfish *Mystus nemurus* ranged from 0.005 to 0.164 and suggested that the highest genetic distance among them was the subspecies level. Nei found that in a variety of animals, *D* is approximately 1.0 for inter species comparisons, around 0.1 for subspecies, and 0.01 for local races. Ayala reported that the *D*-value between subspecies is approximately 0.20. Considering from the above-mentioned criteria, the studied *L. bata* may be categorized as subspecies or local population.

The higher genetic variation in the distant river population, however, is not justified by in the present study. Although it seems Tista is geographically distant, it is actually the Brahmaputra which exhibited highest genetic variability (Table 10). Therefore geographic distance is a very unreliable means to infer the relatedness. However, use of only seven allozyme loci might not infer the correnct relationship among populations. The result of the present study could be useful as the baseline information of *L. bata* populations for further study regarding conservation genetics and population management program.

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REFERENCES

1. Riede K. Global register of migratory species from global to regional scales. Final report of the R&D-Projekt 808 05 081. Federal Agency for Nature Conservation, Bonn, Germany, **2004**; 329.
2. Tripathi SH, N Ross, N Hasan. The role of small indigenous fish species in food and nutritional security in Bangladesh. NAGA News Letter, July-December, **1997**; 13.
3. IUCN-Bangladesh, Red book of Threatened Fishes of Bangladesh, **2000**; 31.
4. Barlow W. Causes and significance of morphological variation in the fishes. *Syst Zool*, **1961**; 10: 105-117.
5. Swain DP, CJ Foote. Stocks and chameleons: the use of phenotypic variation in stock identification. *Fish. Res***, 1999**; 43: 113-128.
6. Turan C. Stock identification of Mediterranean horse mackerel (*Trachurus mediterraneus*) using morphometric and meristic characters. *J. Mar. Sci***, 2004**; 61: 774-781.
7. Randall JE, RL Pyle. *Synodus orientalis*, a new lizardfish (Aulopiformes: Synodontidae) from Taiwan and Japan, with correction of the Asian records of *S. lobelia. Zool. Stud*, **2008**; 47: 657-662.
8. Ryman N, F Utter. Population genetics and fishery management. University of Washington Press, Seattle, **1987**; 420.
9. Hossain MAR, M Nahiduzzaman, D Saha, MUH Khanam, MS Alam. Landmark-based morphometric and meristic variations of the endangered carp, Kalibaus, *Labeo calbasu*, from stocks of two isolated rivers, the Jamuna and Halda and a hatchery. *Zool. Stud*. **2010**, 49: 556-563.
10. Strauss RE, FL Bookstein. The Truss: body form reconstruction in morphometrics. *Syst. Zool*, **1982**; 31: 113-135.
11. Siegismund HR. Genetical statistical programs for the analysis of population data, **1995**; 180.
12. Roderick DMP. TREEVIEW. Tree drawing software for Apple Macintosh and Microsoft Windows. Tree View manual, **2000**; 27.
13. Nei M. Genetic distance between populations. *Am. Natl*. **1972**; 106: 283-292.
14. Nei M. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **1987**; 89: 583-590.
15. Uiblein F. Morphological variability between populations of *Neobythites stefanovi* (Pisces: Ophidiidae) from deep red sea and Gulf of Aden. *Marine Ecol. Prog. Ser.* **1995**; 124: 23-29.
16. Hurlbut T, D Clay. Morphometric and meristic differences between shallow and deepwater populations of whitehake (*Urophyeis tenuis*) in the Southern Gulf of St. Lawrence. Can. J. Fish. *Aquat. Sci*. **1998**; 55: 2274-2282.
17. Erguden D, O Bayram, AE Zeliha, T Cemal. Morphologic structuring between populations of chub mackerel *Scomber japonicus* in the Black, Marmara, Aegean and northeastern Mediterranean Seas. *Fish Sci*, 2009; 75: 129-135.
18. Tudeala S. Morphological variability in a Mediterranean, genetically homogeneous population of the European anchovy, *Engraulis encrasicolus*. *Fish. Res*, **1999**; 42: 229-243.
19. Nakamura T. Meristic and morphometric variations in fluvial Japanese charr between river systems and among tributaries of a river system. Environ. *Biol. Fish*, **2003**; 66: 133-141.
20. Alam MA, MSH Akanda, MMR Khan, MS Alam. Comparison of genetic variability between a hatchery and a river population of rohu (*Labeo rohita*) by allozyme electrophoresis. *Pakistan J. Biol. Sci*. **2002**; 5(9): 959-961.
21. Alam MA, MSH Akanda, MMR Khan, MA Rahman, MS Alam. Comparison of allozyme variation between hatchery and wild population of catla (*Catla catla*: Cyprinidae). *Bull. Fac. Sci. University of the Ryukyus*, **2004**; 78: 357-364

Table 1. Sources, number of fish and date of collection of *L. bata* samples

Sample No.	Populations	District (Collection site)	No. of specimens	Date of collection
1	Brahmaputra river	Mymensingh	30	04.02.2012
2	Tista river	Rangpur	30	10.10.2011
3	Padma river	Rajshahi	30	20.01.2012
4	Hatchery	Mymensingh	30	15.09.2011

Table 2. Morphometric characters used for analysis of *L. bata* population

SL No.	Character	Description
1	Total length (TL)	Distance from the tip of the snout to the longest caudal fin ray
2	Fork length (FL)	Distance from the tip of the snout to the middle part of the fork of the tail
3	Standard length (SL)	Distance from the tip of the snout to the end of the vertebral column
4	Head length (HL)	Distance from the tip of the snout to the posterior margin of the opercula
5	Eye length (EL)	Diameter of the eye
6	Pre-orbital length (PROL)	Distance from the tip of the snout to the anterior margin of the eye
7	Post-orbital length (POOL)	Distance from the posterior margin of the eye to the end of the operculum
8	Highest body depth (HBD)	Diameter of the highest body part
9	Lowest body depth (LBD)	Diameter of the lowest body part
10	Dorsal fin length (DFL)	Length of the base of dorsal fin
11	Pectoral fin length (PCFL)	Length of the base of pectoral fin
12	Pelvic fin length (PVFL)	Length of the base of pelvic fin
13	Anal fin length (AFL)	Length of the base of anal fin

Table 3. Enzymes used for allozyme electrophoresis

Enzymes	Enzyme patterns	E.C. Number	Tissue*
Lactate dehydrogenase (LDH)	Tetramer	5.3.1.9	M
Malate dehydrogenase (MDH)	Dimer	1.1.1.27	M
Phosphoglucomutase (PGM)	Monomer	1.1.1.37	M
Glucose-6-phosphate isomerase (GPI)	Dimer	5.4.2.2	M
Esterase (EST)	Monomer	3.1.1.1	M

*M: Muscle

Table 4. Components of staining buffer used for allozyme electrophoresis

Enzyme name	PMS (1mg)	NBT (10 mg)	MTT (10 mg)	Cofactor (20 mg)	Other components
EST	-	-	-	-	0.1M Phosphate buffer*[1] (60 ml) 25 mg α-napthyl acetate 70 mg Fast blue BB salt
GPI	*	-	*	NADP	0.05 M Tris-HCl*[2] (80 ml) 100 mg Fructose-6-phosphate (Na) 110 µl Glucose-6-phosphate dehydrogenase 1 ml 1 M Magnesium chloride
LDH	*	*	-	NAD	0.05 M Tris-HCl*[2] (80 ml) 6 ml Sodium lactate
MDH	*	*	-	NAD	0.05 M Tris-HCl*[2] (80 ml) 6 ml Sodium malate
PGM	*	-	*	NADP	0.05 M Tris-HCl*[2](80 ml) 150 mg Glucose-1-phosphate (Na) 70 µl Glucose-6-phosphate dehydrogenase 1 ml 1 M Magnesium chloride

* After use showed activity; *[1] Phosphate buffer (pH 7.0): mixture of 0.2 M sodium dihydrogen phosphate and 0.2 M disodium hydrogen phosphate; *[2] Tris HCl buffer (pH 8.7): Tris (hydroxymethyl) aminomethane, pH adjusted with 1N HCl NAD: β-nicotinamide adenine dinucleotide, NADP: β-nicotinamide adenine, dinucleotide phosphate, sodium salt; NBT: nitro blue tetrazolium; MTT: (3-[4, 5 dimethyl-2-thiazolyl]-2, 5 diphenyl tetrazolium bromide); PMS: phenazine methosulfate

Table 5. Univariate statistics (ANOVA) testing differences among samples from thirteen (13) morphometric and twenty five (25) Landmark measurements

Characters	Wilks' Lambda	F
FL	0.953	1.898
SL	0.989	0.414
HL	0.809	9.133***
ED	0.562	30.081***
PROL	0.856	6.494***
POOL	0.746	13.200***
HBD	0.834	7.719***
LBD	0.960	1.607
DFL	0.902	4.208**
PCFL	0.883	5.116**
PVFL	0.952	1.952
AFL	0.980	0.774
MG	0.939	2.506
1-2	0.880	5.267**
1-12	0.989	0.429
1-11	0.990	0.409
2-3	0.876	5.453**
2-12	0.911	3.791*
2-11	0.811	9.002***
2-9	0.938	2.545
3-4	0.925	3.123*
3-11	0.941	2.442
3-10	0.981	0.754
3-9	0.992	0.302
3-8	0.951	2.010
4-5	0.919	3.424*
4-10	0.892	4.678**
4-9	0.931	2.864*
4-8	0.977	0.921
4-7	0.948	2.137
5-6	0.886	4.975**
5-7	0.924	3.200*
6-7	0.987	0.493
7-8	0.922	3.284*
8-9	0.989	0.422
9-10	0.954	1.865
10-11	0.786	10.549***
11-12	0.911	3.768*

*P<0.05, **P<0.01, ***P<0.001

Table 6. Pooled within-groups correlations between discriminating variables and discriminant functions (DFs; variables ordered by size of correlation within function, * denotes largest absolute correlation between each variable and DFs)

Characters	Function		
	DF1	DF2	DF3
ED	-0.488(*)	0.293	-0.039
POOL	0.334(*)	0.119	0.074
HBD	0.245(*)	0.154	0.075
PROL	0.224(*)	0.138	0.085
PCFL	-0.206(*)	-0.010	0.144
4-10	0.202(*)	-0.040	-0.003
2-12	-0.181(*)	-0.042	-0.051
11-12	0.167(*)	0.119	-0.073
4-9	0.155(*)	-0.042	0.070
5-7	0.151(*)	-0.101	0.123
2-9	0.150(*)	-0.006	-0.004
FL	0.124(*)	0.004	-0.101
9-10	0.121(*)	-0.058	0.070
AFL	0.080(*)	0.029	-0.028
3-10	0.080(*)	0.020	0.024
HL	0.196	0.360(*)	-0.010
1-2	-0.094	0.339(*)	-0.007
2-3	-0.092	0.339(*)	0.000
DFL	-0.052	0.317(*)	-0.107
10-11	-0.249	0.304(*)	0.076
5-6	-0.135	0.276(*)	0.081
3-4	-0.069	0.261(*)	-0.058
4-5	0.075	-0.261(*)	0.128
MG	0.014	0.259(*)	0.002
4-7	-0.011	-0.224(*)	0.127
7-8	-0.117	-0.217(*)	0.022
PVFL	0.003	0.215(*)	0.121
3-8	-0.018	0.213(*)	0.133
3-11	0.125	0.130(*)	-0.058
6-7	-0.007	0.111(*)	0.043
8-9	0.010	0.105(*)	0.009
SL	-0.015	0.101(*)	0.029
1-12	-0.050	0.064(*)	-0.001
3-9	0.037	0.061(*)	0.018
2-11	0.222	0.073	0.446(*)
4-8	0.046	-0.062	0.183(*)
LBD	0.110	0.035	0.112(*)
1-11	0.019	-0.075	0.098(*)

Table 7. Comparison of the meristic characters of *L. bata*

Meristic characters	Population (Range)				H-value
	Tista	Padma	Brahmaputra	Hatchery	
Dorsal fin ray (DFR)	12 (10-12)	12 (11-12)	12 (11-12)	11 (10-12)	5.658
Pectoral fin ray (PcFR)	16 (15-17)	16 (15-17)	17 (16-17)	16 (16-17)	28.60***
Pelvic fin ray (PeFR)	9 (9-10)	9	9 (9-10)	9 (8-9)	17.31***
Anal fin ray (AFR)	7 (7-8)	7 (7-8)	7 (7-8)	7 (7-8)	7.498
Caudal fin ray (CFR)	21(20-23)	22 (20-23)	22 (20-23)	22 (21-23)	6.067
Branchiostegal ray (BR)	3	3	3	3	0.000
Scales above lateral line (ScALL)	7.5 (7.5-8.5)	8 (7.5-8.5)	7.5 (7.5-8.5)	7.5 (6.5-8.5)	27.40***
Scales below lateral line (ScBLL)	5.5 (5.5-6.5)	6.5 (5.5-7.5)	5.5 (5.5-6.5)	5.5 (5.5-6.5)	66.31***

***$P < 0.001$

Table 8. List of the alleles and genotypes examined in *L. bata* populations

Locus	Alleles		Genotypes	
	No.	Type	No.	Type
*Est-1**	2	**a*, **b*	3	**aa*, **ab*, **bb*
*Pgm**	3	**a*, **b*, **c*	5	**aa*, **ab*, **bb*, **bc*, **cc*
*Ldh-1**	1	**a*	1	**aa*
*Ldh-2**	1	**a*	1	**aa*
*Mdh-1**	2	**a*, **b*	2	**aa*, **ab*
*Mdh2**	2	**a*, **b*	2	**ab*, **bb*
*Gpi-1**	2	**a*, **b*	3	**aa ab*, **bb*
*Gpi-2**	2	**a*, **b*	3	**aa*, **ab*, **bb*
Average	**1.875**		**2.5**	

Table 9. Allele frequency at 8 presumptive loci of *L. bata* populations

	Allele	Tista (N=30)	Padhma (N=30)	Brahmaputra (N=30)	Hatchery (N=30)
*Est-1**	**a*	0.417	0.833	0.833	0.383
	**b*	0.583	0.167	0.167	0.617
	P	0.491NS	0.302NS	0.302NS	0.582NS
	χ^2	0.474	1.065	1.065	0.304
	d.f	1	1	1	1
*Pgm**	**a*	0.700	0.017	0.050	0.117
	**b*	0.300	0.933	0.917	0.883
	**c*	-	0.050	0.033	-
	P	0.722NS	0.000***	0.978NS	0.506NS
	χ^2	0.127	19.000	0.195	0.441
	d.f	1	3	1	1
*Ldh-1**	**a*	1.000	1.000	1.000	1.000
	P	-	-	-	-
	χ^2				
	d.f				
*Ldh-2**	**a*	1.000	1.000	1.000	1.000
	P	-	-	-	-
	χ^2				
	d.f				
*Mdh-1**	**a*	1.000	0.983	0.983	1.000
	**b*	-	0.017	0.017	-
	P		1.000NS	1.000NS	
	χ^2		000	000	
	d.f		1	1	
*Mdh-2**	**a*	0.133	0.183	0.350	0.167
	**b*	0.867	0.817	0.650	0.833
	P	0.434NS	0.244NS	0.004***	0.302NS
	χ^2	0.612	1.356	8.218	1.065
	d.f	1	1	1	1
*Gpi-1**	**a*	0.950	1.000	0.883	0.750
	**b*	0.050	-	0.117	0.250
	P	0.815NS		00.442NS	0.229NS
	χ^2	0.055		0.506	1.446NS
	d.f	1		1	1
*Gpi-2**	**a*	0.683	-	0.167	0.250
	**b*	0.317	1.000	0.833	0.750
	P	0.014***		0.302NS	0.079NS
	χ^2	6.048		1.065	3.075
	d.f	1		1	1

Statistically significant values are marked with asterisks.
P: Probability of chi-square value, significant level: ****P*<0.05, NS: Non-significant

Table 10. Genetic variabilities at 8 presumptive loci of *L. bata* populations

Population	The mean proportion of polymorphic loci* (%)	The mean number of alleles per locus (*Na*)	The mean proportion of heterozygous loci per individual (%)	Heterozygosity			
				H_o	H_e	H_o/H_e	$1-H_o/H_e$
Tista	62.5	1.625	22.92	0.229	0.212	1.08	-0.08
Padma	50	1.625	10.0	0.1	0.094	1.06	-0.06
Brahmaputra	75	1.875	22.5	0.225	0.179	1.26	-0.26
Hatchery	62.5	1.625	22.5	0.225	0.217	1.03	-0.03
Average	**62.50**	**1.688**	**19.48**	**0.195**	**0.176**	**1.11**	**-0.43**

* $P \leq 0.95$

Table 11. Pair-wise and overall population differentiations (F_{ST}) and gene flow (N_m) in four *L. bata* populations

Populations	F_{ST}		N_m*	
	Pair-wise	Overall	Pair-wise	Overall
Tista-Padma	0.311	0.226	0.555	0.857
Tista- Brahmaputra	0.226		0.856	
Tista-Hatchery	0.145		1.478	
Padma- Brahmaputra	0.032		7.649	
Padma- Hatchery	0.121		1.820	
Brahmaputra- Hatchery	0.078		2.942	

* N_m = Gene flow estimated from $F_{ST} = 0.25(1- F_{ST}) / F_{ST}$

Table 12. Nei's (1972) original measures of genetic identity (above diagonal) and genetic distance (below diagonal) estimated among 4 populations of *L. bata* based on 8 loci

Populations	Padma	Tista	Brahmaputra	Hatchery
Padma	****	0.843	0.991	0.953
Tista	0.171	****	0.862	0.910
Brahmaputra	0.009	0.149	****	0.960
Hatchery	0.048	0.095	0.042	****

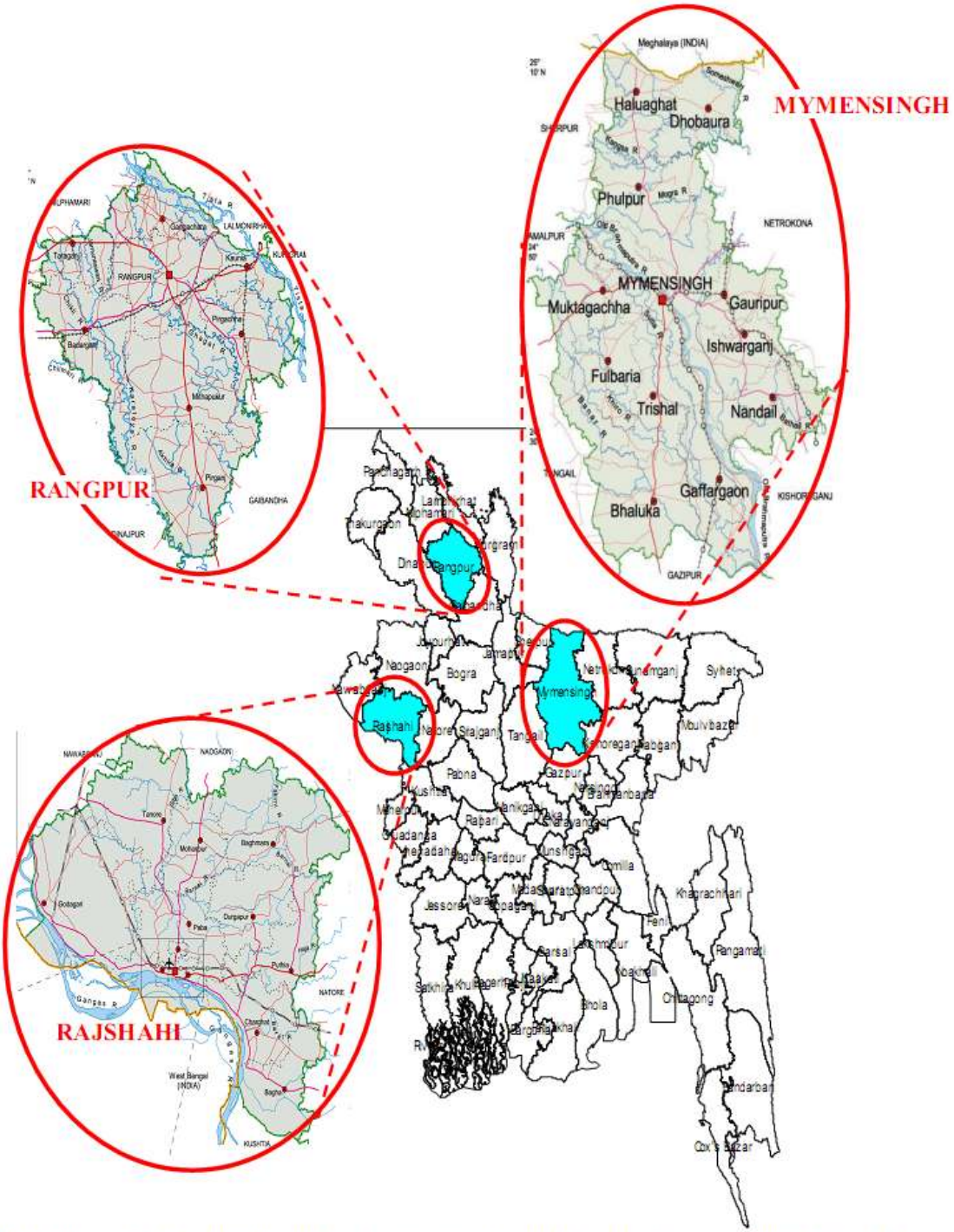

Fig. 1. Map of Bangladesh showing collection sites of wild and hatchery populations of *L. bata.*

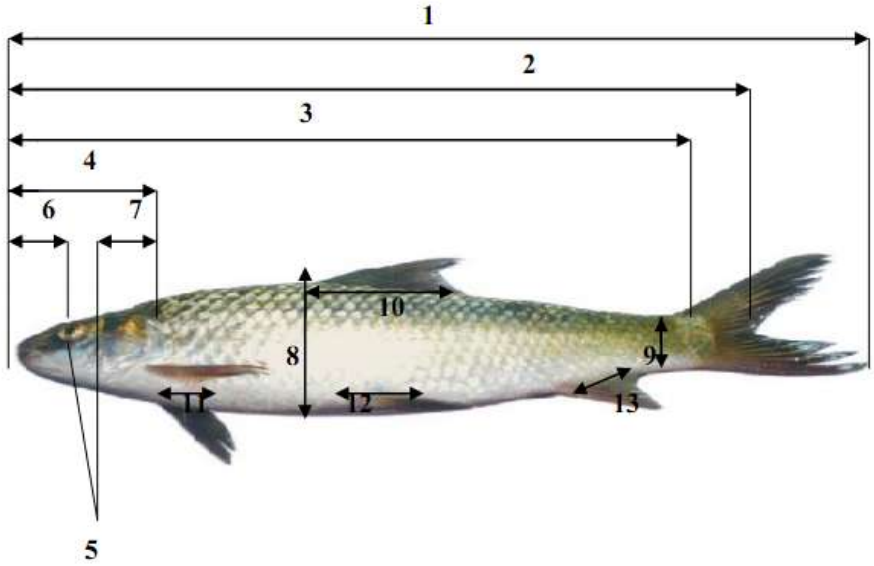

Fig. 2. Morphometric characters of *L. bata*. 1-total length (TL); 2-fork length (FL); 3-standered length; 4-head length (HL); 5-eye diameter (ED); 6-pre-orbital length (PROL); 7-post-orbital length (POOL); 8-highest body depth (HBD); 9-lowest body depth (LBD); 10-dorsal fin length (DFL); 11-pectoral fin length (PCFL); 12-pelvic fin length (PVFL) and 13-anal fin length (AFL).

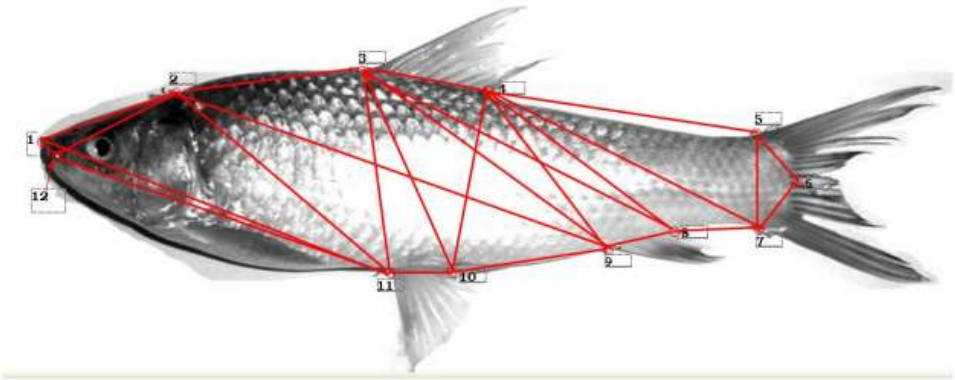

Fig. 3. Locations of 12 landmarks used for the shape analysis of *L. bata*.

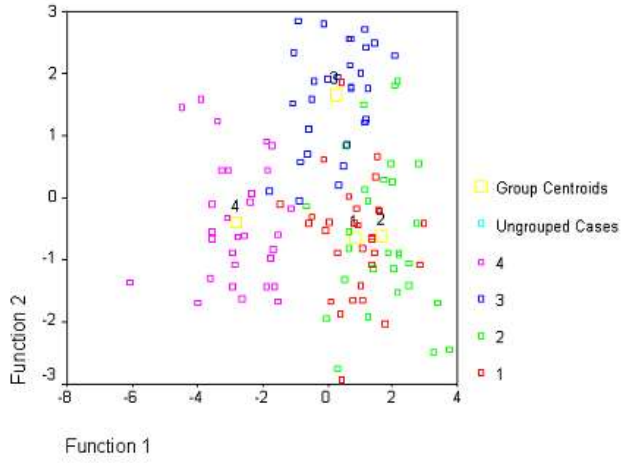

Fig. 4. Sampled centroids of discriminant function scores based on morphometric and truss measurements (1. the Tista, 2. the Padma, 3. the Brahmaputra, and 4. Hatchery).

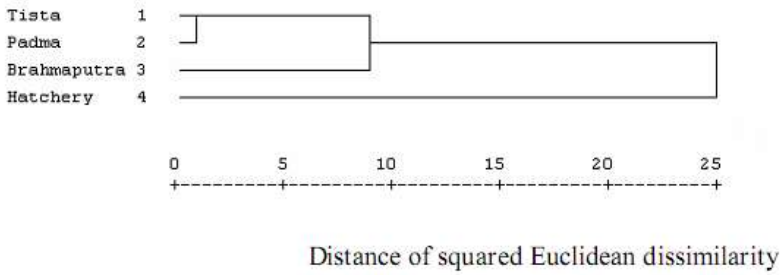

Fig. 5. Dendrogram based on morphometric characters and Landmark distances of the Tista, the Padma, the Brahmaputra and the hatchery populations of *L. bata.*

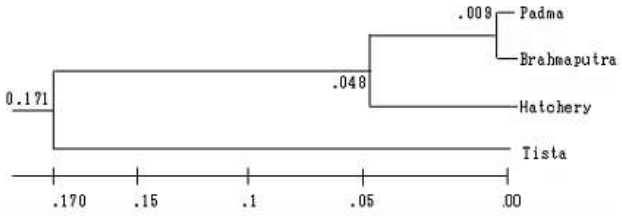

Fig. 7. UPGMA dendrogram based on Nei's (1972) genetic distance, summarizing the data on differentiation among four populations of *L. bata* according to the allozyme analysis.

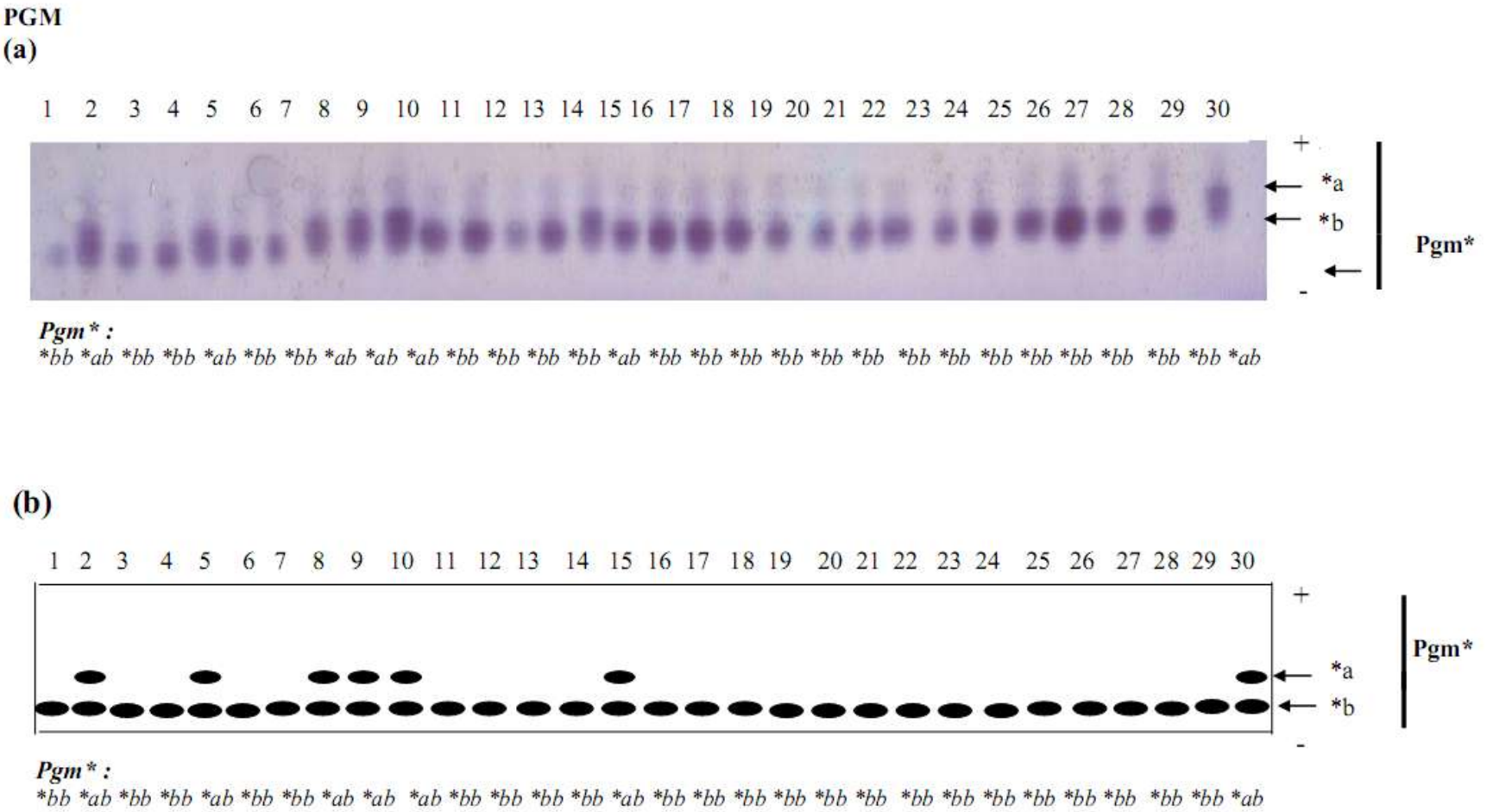

Fig. 6. (a) Electrophoregram of phosphoglucomutase (PGM) and (b) schematic representation of electrophoretic pattern of *Pgm locus in *L. bata* (Hatchery population, 1-30).**