

# Morpho-genetic analysis of three river populations of bhagna, labeoariza (hamilton 1807) in Bangladesh

## Abstract

Morphological and genetical analysis of Bhagna *Labeoariza H.* populations was investigated from three river populations (the Atrai, the Jamuna and the Brahmaputra) of Bangladesh to identify its diversified populations. Therefore, about 30 similar size of fish samples were collected from each of those three river populations and analyzed nine morphometric and meristic characters and their genetic variation by allozyme markers. All of the morphometric characters were significantly higher in ( $P < 0.05$ ) in Atrai population compare to other two river populations. However, among nine meristic characters, five were showed significant differences ( $P < 0.05$ ) among each other. The genetic variations were analyzed with five enzymes (EST, PGM, GPI, LDH and MDH) in CA 6.1 buffer system. Two polymorphic loci *Est-1\** and *Pgm\** were interpretable in muscle with starch gel electrophoresis. The highest mean number of allele per locus was observed in the Atrai population (1.375). The mean proportion of heterozygous loci per individual was 4.305% for all populations. The average observed heterozygosity ( $H_o$ ) and expected heterozygosity ( $H_e$ ) were 0.042 and 0.058, respectively for all the populations. The lowest pair-wise population differentiation ( $F_{ST}$ ) (0.039) and the highest gene flow ( $N_m$ ) (6.148) were found in the Jamuna–Brahmaputra indicating close relationship among them. In the Nei's (1972) UPGMA dendrogram, the Jamuna and the Brahmaputra populations of *L. ariza* made one cluster ( $D=0.004$ ) and separated from the Atrai population by the genetic distance of 0.066. The results suggest that a considerable genetic variation is existed among the natural *L. ariza* populations in Bangladesh.

**Keywords:** *Labeoariza L.*, Morphology, Genetic variation, Dendrogram, River population

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**Abbreviations:** ANOVA, Analysis Of Variances; AFR, Anal Fin Rays; BR, Branchiostegal Rays; CFR, Caudal Fin Rays; DFR, Dorsal Fin Rays; ED, Eye Diameter; FL, Fork Length; GPI, Glucose-6-Phosphate Isomerase; HL, Head Length; HBD, Highest Body Depth; LBD, Low Body Depth, LDH, Lactate Dehydrogenase; MDH, Malate Dehydrogenase; Pr-OL, Pre-Orbital Length; PDL, Pre-Dorsal Length; P<sub>C</sub>FR, Pectoral Fin Rays; P<sub>P</sub>FR, Pelvic Fin Rays; PGM, Phosphoglucumutase; SaLL, Scale Along the Lateral Line; SL, Standard Length; SabLL, Scale Above the Lateral Line; SbLL, Scale Below the Lateral Line; SPSS, Statistical Social Package Program ; TL, Total Length

## Introduction

*Labeoariza* (Hamilton 1807) is commonly identified as Bhagna, Reba carp, Bhagna bata, Raik or Tatkini and locally it is regarded as one of the most desired indigenous minor carp species in Bangladesh. Bhagna is distributed over Indo-Pacific region.<sup>1-4</sup> Bhagna additionally encompasses a wide range of geographical distribution. Primarily, this fish is found in Asia: Indus plain and adjoining hilly areas (Pakistan); Ganges-Brahmaputra basin (India, Nepal and Bangladesh); Mahanadi, Krishna and some more diminutive basins in southern India; Karnafuly and adjacent more diminutive basins in Chittagong Hill Tracts (Bangladesh).<sup>4-6</sup> As well, Bhagna is widely distributed in most of the small creeks, rivers, floodplains and natural depressions of Bangladesh.<sup>7,8</sup>

Bhagna can be facily apperceived by its consequential characteristics like the lower body is shiny and silvery; the upper body is deep bluish or darkish and medium-sized silvery scales. The lateral line consists of 38 or 39 scales and most of them appeared as

elongated ebony marks from tail to the base margin of the operculum during the early stage of life. During the adult stage, the ebony mark becomes shorter and remains in between the terminus of the belly and the operculum.<sup>2,3,7-9</sup> The thin stripes or a black mark is varying from subdued to bold. The dorsal profile is relatively more convex than the abdomen and, margin of snout is projecting, ciliated and covering upper lip completely and snout obtusely rounded. On the other hand, lower lip is very deficient and thin and is connected with skin of isthmus by a bridge and the upper lip is entire. The post labial groove is absent. The lower jaw is provided with a tubercle at symphysis. A pair of short rostral barbels present and mouth is inferior. Scales are hexagonal. Dorsal fin originates in advance of pelvics, midway between snout tip and posterior end of anal base. Pelvic fin origin nearer to snout tip than to caudal base. Pectoral fin shorter than head by less than eye-diameter. Longest dorsal ray may be slightly longer than head.<sup>3,9</sup>

Bhagna feeds on plankton and detritus and is an omnivore and column feeder in nature and grows to a largest size of 40 to 50 cm in length.<sup>8,10,11</sup> This fish additionally grows up to 30.0 cm in standard length and weight of 1,360 g. Females and males procure sexual maturity within one year and customarily by the terminus of first year. This fish breeds in flooded shallow water from June to September.<sup>5</sup> The spawning season of Bhagna ranges from April to the August with a peak spawning time during the rainy time in flowing flood waters of these months.<sup>7,10</sup> It is also a prolific breeder, laying about 3 million ova per female.<sup>5</sup> Absolute fecundity of Bhagna was recorded between 2, 00,000 and 2, 50,000 per kg body weight of female.<sup>7</sup>

It is an eco-friendly fish and an important source of carbohydrates, proteins, fats, vitamins, minerals, iron and calcium.<sup>12</sup> Its flesh is

oily, tasteful and people would like to eat due to its lucrative size, high nutrition, attractive flavor and less spines.<sup>5,9</sup> It is a congruous species for aquaculture.<sup>5,9</sup> but the wild population of Bhagna is rapidly declining due to sundry causes. Over the last few years, the population of Bhagna has been declining considerably due to incremented and indiscriminant fishing pressure and sundry anthropogenic activities leading to aquatic pollution, siltation and loss of natural habitat for the magnification and spawning.<sup>7,9,10</sup> These activities are perpetually eradicating the spawning and breeding grounds and causing havoc to the availability of fry, fingerlings and brood fish.<sup>7</sup> As a result, the fish is recently listed as one of the most paramount vulnerably susceptible and threatened species in Bangladesh.<sup>13-15</sup> Although they are listed as threatened and vulnerable, a small number of the fish is still available in different rivers, beels, haors and baors.<sup>15</sup> For these reasons, it is necessary to conserve and rehabilitable through breeding and culture practices. Correspondingly, with the development of breeding and culture practices, information will be generated for promoting effective management and conservation practices and that will be helpful to protect the fish from extinction. So, immediate attempts should be taken to boost up Bhagna production through effective management in natural water bodies and a proper knowledge about their morpho-genetic variation is necessary.

During 1960s, molecular genetics studies on proteins such as hemoglobin and transferrin but attentions quickly turned to enzymatic proteins (allozyme/isozyme variation) on which most subsequent studies have been based.<sup>16</sup> Since, fish geneticists have been utilizing protein electrophoresis as their primary implement to characterize population-level genetic variation in a variety of fish species.<sup>17</sup> Isozymes the elements of different alleles at the same locus are named as allozymes. Allozyme electrophoresis represents the process for identifying genetic variation at the level of enzymes, which are directly encoded by DNA. The allelic variants give elevate to protein variants called allozymes that differ marginally in electric charges. Allozymes are co-dominant Mendelian characters (both alleles are individually expressed in a heterozygous individual) and characters are passed from parent to offspring in a prognosticable manner.<sup>18</sup> Though co-dominant marker, they are functional for analyzing genetic variability and genetic identification of species.<sup>19,20</sup> Fish population structure identification of several species has been carried out using allozyme technique.<sup>21-25</sup> Allozymes were also found to be supportive in generating species-specific profiles and resolving taxonomic ambiguities in several species.<sup>26-28</sup> In this regard, Allozyme electrophoresis an effective tool for fish population studies and fishery management.<sup>29</sup> However, there is no known information on stock identification and genetic variation of *L. ariza* in Bangladesh. Therefore, the present research was concentrated to investigate the morpho-genetic variation of the three major river populations of *L. ariza* regarding their present status to identify the diversified *L. ariza* populations through using allozyme markers.

## Materials and methods

### Fish specimens

About 30 experimental fish were collected from each of three rivers viz. the Atrai (Dinajpur district), the

Brahmaputra (Mymensingh district) and the Jamuna (Sirajganj district) River in Bangladesh (Table 1).

### Morphological variation study

The conventional method was used to measure the nine morphometric characters as noted: Total Length (TL); Fork Length

(FL); Standard Length (SL); Head Length (HL); Eye Diameter (ED); Pre-Orbital Length (Pr-OL); Pre-Dorsal Length (PDL); The Highest Body Depth (HBD); Low Body Depth (LBD) To The Nearest 0.1 Cm Using A Slide Calipers. Nine Meristic Characters Were Also Studied By Following The Conventional Methods: Branchiostegal Rays (BR); Dorsal Fin Rays (DFR); Pectoral Fin Rays (P<sub>c</sub>FR); Pelvic Fin Rays (P<sub>e</sub>FR); Anal Fin Rays (AFR), Caudal Fin Rays (CFR), Scale Above The Lateral Line (SabLL), Scale Below The Lateral Line (SbLL) And Scale Along The Lateral Line (SaLL). A magnifying glass was used to count the fin rays and only the principal rays were counted as separate ray.

**Table 1** Sources and number of *L. ariza* samples collected from three river systems of Bangladesh

Sample No.	Populations	District (Collection Site)	No. of specimens
1	Atrai River	Dinajpur (Chirirbandar)	30
2	Brahmaputra River	Mymensingh (Sadar)	30
3	Jamuna River	Sirajganj (Kajipur)	30

### Morphological data analyses

Due to limited number of fish in each group, non-parametric statistical analysis was used in all the comparisons for morphological data analysis.<sup>30</sup> Kruskal-Wallis non-parametric analysis of variance (ANOVA) was used to analyze the differences in morphometric characters and meristic counts of fish.<sup>31</sup> In this instance a non-parametric post hoc test.<sup>30</sup> was conducted where significant differences between groups were detected. SPSS (version 12) program software was used to analyze all of the data.

### Genetic variation study

About 30 fish were used to analyze the genetic variations for each river populations. Allozyme markers through horizontal starch gel electrophoresis were used to perform this study.<sup>32</sup> The enzymes analyzed and enzyme commission (EC) numbers are shown in Table 2. Allozyme electrophoresis was performed by using amine-citrate buffers (CA 6.1).<sup>33</sup> (Table 3). The components of staining solution with some co-factors that were used in this experiment are listed in Table 4. Gel slices (1 mm) were stained histochemically for different enzymes.<sup>34</sup>

**Table 2** Examined enzymes used for allozyme electrophoresis

Enzymes	Enzyme Patterns	E.C. Number
Esterase (EST)	Monomer	3.1.1.1
Glucose-6-Phosphate Isomerase (GPI)	Dimer	5.3.1.9
Lactate Dehydrogenase (LDH)	Tetramer	1.1.1.27
Malate Dehydrogenase (MDH)	Dimer	1.1.1.37
Phosphoglucumutase (PGM)	Monomer	5.4.2.2

### Genetic variation data analyses

The allele frequency and the analyses of Chi-square ( $\chi^2$ ) test were performed using Gene Alex.<sup>35</sup> computer program (version 6). The POPGENE (version 1.31) computer package program was used to determine the mean proportion of polymorphic loci.<sup>36</sup> The mean number of allele per locus was determined and calculated directly from the number of observed alleles by dividing with the number of observed locus. The mean proportions of heterozygous loci per individual were calculated directly from total number of observed heterozygote loci by dividing with the total number of individual in a population. POPGENE (version 1.31) computer package program was also used to calculate the expected heterozygosity ( $H_e$ ), observed heterozygosity ( $H_o$ ), population differentiation ( $F_{ST}$ ) and gene flow

( $N_m$ ). Genetic distance values ( $D$ ) was estimated through.<sup>37</sup> Based on the  $D$ -values, dendrogram was made by the UPGMA (unweighted pair group method using arithmetic average) method.<sup>37</sup>

## Results

### Morphological variation observations

The recorded nine morphometric characters from the samples of three river populations of *L. ariza* shown in the Table 5. The observed all nine morphometric characters of the Atrai population demonstrated significantly higher average values compared to the other two populations (the Jamuna & Brahmaputra). The proportion

of five morphometric characters (TL: HL, SL: HL, ED: HL, SL: HBD and TL: HBD) of all river populations were significantly different ( $P < 0.05$ ) from each other. The recorded nine meristic characters from the three river populations of *L. ariza* are shown in Table 6. Of the nine meristic characters, four characters (BR, VFR, SabLL and SbLL) of all river populations showed no significant difference ( $P > 0.05$ ) from each other. The number of DFR, PFR and SaLL of the Atrai and the Jamuna populations were significantly higher ( $P < 0.05$ ) than the Brahmaputra population. On the other hand, the number of CFR of the Brahmaputra population was significantly different from other two populations (Table 6).

**Table 3** Composition of buffer system and allozyme electrophoretic process

Buffer system	Gel buffer	Running time	Voltage	References
CA 6.1 Electrode Buffer 0.04 M Citric acid adjust pH to 6.1 with NaOH	1:20 dilution of electrode buffer	Thick gel (0.5 cm) Initial: 30 min Final: 5-6 hrs	100 V 150 V	(Clayton & Tretiak, 1995)

**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* <sup>1</sup> (60 ml) 25 mg a-naphthyl acetate 70 mg Fast blue BB salt
GPI	*	-	*	NADP	0.05 M Tris-HCl* <sup>2</sup> (80 ml) 100 mg Fructose-6-phosphate (Na) 110 $\mu$ l Glucose-6-phosphate dehydrogenase 1 ml 1 M Magnesium chloride
LDH	*	*	-	NAD	0.05 M Tris-HCl* <sup>2</sup> (80 ml) 6 ml Sodium lactate
MDH	*	*	-	NAD	0.05 M Tris-HCl* <sup>2</sup> (80 ml) 6 ml Sodium malate
PGM	*	-	*	NADP	0.05 M Tris-HCl* <sup>2</sup> (80 ml) 150 mg Glucose-1-phosphate (Na) 70 $\mu$ l Glucose-6-phosphate dehydrogenase 1 ml 1 M Magnesium chloride

\*: After use showed activity; \*<sup>1</sup>: Phosphate Buffer (Ph 7.0): Mixture of 0.2 M Sodium Dihydrogen Phosphate and 0.2 M Disodium Hydrogen Phosphate; \*<sup>2</sup>Trishcl Buffer (pH 8.7): Tris (Hydroxymethyl) Aminomethane, pH Adjusted with 1N HCl; NAD: B Nicotinamide Adenine Dinucleotide; NADP: B-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** Morphometric characters (average  $\pm$ SE) as recorded from *L. ariza* sample of three river populations in Bangladesh (n=100)

Morphometric characters	Atrai population	Jamuna population	Brahmaputra population
Total Length (TL)	16.21 $\pm$ 0.42 <sup>a</sup>	14.20 $\pm$ 0.33 <sup>b</sup>	13.87 $\pm$ 0.47 <sup>c</sup>
Fork Length (FL)	14.00 $\pm$ 0.39 <sup>a</sup>	11.99 $\pm$ 0.25 <sup>c</sup>	12.16 $\pm$ 0.26 <sup>b</sup>
Standard Length (SL)	13.03 $\pm$ 0.39 <sup>a</sup>	11.19 $\pm$ 0.59 <sup>c</sup>	11.22 $\pm$ 0.41 <sup>b</sup>
Head Length (HL)	2.85 $\pm$ 0.14 <sup>a</sup>	2.84 $\pm$ 0.11 <sup>b</sup>	2.35 $\pm$ 0.16 <sup>c</sup>
Eye Diameter (ED)	0.76 $\pm$ 0.07 <sup>a</sup>	0.64 $\pm$ 0.06 <sup>b</sup>	0.64 $\pm$ 0.06 <sup>b</sup>
Pre Orbital Length (Pr-OL)	0.82 $\pm$ 0.12 <sup>a</sup>	0.82 $\pm$ 0.06 <sup>a</sup>	0.64 $\pm$ 0.16 <sup>b</sup>
Pre- Dorsal Length (PDL)	5.56 $\pm$ 0.18 <sup>a</sup>	4.97 $\pm$ 0.17 <sup>b</sup>	4.71 $\pm$ 0.23 <sup>c</sup>
Highest Body Depth (HBD)	3.53 $\pm$ 0.17 <sup>a</sup>	3.36 $\pm$ 0.13 <sup>b</sup>	3.11 $\pm$ 0.07 <sup>c</sup>
Low Body Depth (LBD)	1.79 $\pm$ 0.08 <sup>a</sup>	1.42 $\pm$ 0.06 <sup>b</sup>	1.31 $\pm$ 0.08 <sup>c</sup>
TL : HL	6.00 $\pm$ 0.01 <sup>a</sup>	5.00 $\pm$ 0.10 <sup>c</sup>	5.90 $\pm$ 0.10 <sup>b</sup>
SL: HL	4.57 $\pm$ 0.03 <sup>b</sup>	3.94 $\pm$ 0.01 <sup>c</sup>	4.77 $\pm$ 0.02 <sup>a</sup>
TL:HBD	4.82 $\pm$ 0.03 <sup>a</sup>	4.02 $\pm$ 0.01 <sup>c</sup>	4.45 $\pm$ 0.04 <sup>b</sup>
SL: HBD	3.87 $\pm$ 0.03 <sup>a</sup>	3.16 $\pm$ 0.02 <sup>c</sup>	3.60 $\pm$ 0.01 <sup>b</sup>
EL:HL	4.50 $\pm$ 0.05 <sup>a</sup>	4.43 $\pm$ 0.03 <sup>b</sup>	3.67 $\pm$ 0.04 <sup>c</sup>

**Table 6** Meristic characters (average  $\pm$ SE) as recorded from *L. ariza* sample of three river populations in Bangladesh (n=100)

Meristic characters	Atrai population	Jamuna population	Brahmaputra population
Branchiostegal Rays (BR)	3.00 $\pm$ 0.00 <sup>a</sup>	3.00 $\pm$ 0.00 <sup>a</sup>	3.00 $\pm$ 0.00 <sup>a</sup>
Dorsal Fin Ray (DFR)	11.03 $\pm$ 0.18 <sup>a</sup>	10.86 $\pm$ 0.57 <sup>b</sup>	10.26 $\pm$ 0.52 <sup>c</sup>
Pectoral Fin Rays (PFR)	16.23 $\pm$ 0.43 <sup>b</sup>	16.26 $\pm$ 0.44 <sup>a</sup>	16.00 $\pm$ 0.00 <sup>c</sup>
Ventral Fin Ray (VFR)	9.00 $\pm$ 0.00 <sup>a</sup>	9.00 $\pm$ 0.00 <sup>a</sup>	9.00 $\pm$ 0.00 <sup>a</sup>
Anal Fin Ray (AFR)	7.03 $\pm$ 0.18 <sup>a</sup>	6.23 $\pm$ 0.43 <sup>c</sup>	6.76 $\pm$ 0.43 <sup>b</sup>
Caudal Fin Ray (CFR)	18.60 $\pm$ 0.72 <sup>b</sup>	(6.23 $\pm$ 0.43 <sup>c</sup> ) 18.26 $\pm$ 0.44 <sup>c</sup>	18.70 $\pm$ 0.46 <sup>a</sup>
Scale Along Lateral Line (SaLL)	37.53 $\pm$ 0.81 <sup>a</sup>	35.63 $\pm$ 1.27 <sup>b</sup>	35.53 $\pm$ 1.35 <sup>c</sup>
Scale Above Lateral Line (SaLL)	7.50 $\pm$ 0.00 <sup>a</sup>	7.50 $\pm$ 0.00 <sup>a</sup>	7.50 $\pm$ 0.00 <sup>a</sup>
Scale Below Lateral Line (SbLL)	6.50 $\pm$ 0.00 <sup>a</sup>	6.50 $\pm$ 0.00 <sup>a</sup>	6.50 $\pm$ 0.00 <sup>a</sup>

### Genetic variation observations

The enzymes were controlled and regulated by the genes at 8 presumptive loci as indicated by the electrophoretic patterns of muscle tissue. One of the 8 loci, the *Est-1*\* formed two genotypes (\*aa and \*ab) by two alleles (\*a and \*b), whereas only one locus *Pgm*\* formed four genotypes (\*aa, \*ab, \*bb and \*bc) by three alleles (\*a, \*b and \*c) (Table 7). Six loci viz. *Mdh-1*\*, *Mdh-2*\*, *Ldh-1*\*, *Ldh-2*\*, *Gpi-1*\* and *Gpi-2*\* formed only homozygous genotype (\*aa) with fixed allele \*a. On the average 2 genotypes were produced by 1.6 alleles at the 5 loci are shown in (Table 7). Allele frequencies were measured from observed genotypes at eight loci in 90 samples of three river populations (Table 8). Among the eight loci, two polymorphic loci (*Est-1*\* and *Pgm*\*) was identified from the Atrai and the Jamuna populations. On the other hand, only one polymorphic locus (*Est-1*\*) was identified from the Brahmaputra population. The chi-square ( $\chi^2$ ) test was done in all the cases of polymorphic loci between observed and expected genotypes, based on Hardy-Weinberg equilibrium. The probability of two polymorphic loci is also showed in Table 8. The significant

variations in allele frequency of *Pgm*\* and *Est-1*\* loci were observed in The Atrai and the Jamuna populations, whereas other population did not show any significant variation in allele frequencies (Table 8). The monomeric enzyme esterase (EST) presumably controlled by at least 2 loci, *Est-1*\* and *Est-2*\* and exhibited two banding patterns consisting of two heterodimers and one homodimer (Figure 1). Allele \*a frequency ranged from 0.850 to 0.950 and was dominant in all three populations. Allele \*b frequencies ranged from 0.050 to 0.183 and was present in all three populations (Table 8). The *Est-2*\* was not readable due to complex banding pattern. The single *Pgm*\* locus controls the monomeric enzyme phosphoglucosmutase (PGM) (Figure 2). The *Pgm*\* was monomorphic in the Brahmaputra population and polymorphic in the Atrai and the Jamuna populations with the allelic frequency of \*a was ranged from 0.300 to 1.000. Allele \*b was also present in the Atrai and the Jamuna populations with frequencies ranged from 0.183 to 0.683 but it was absent in the Brahmaputra population (Table 8) where the rare allele \*c (0.016) is present in the Atrai population (Figure 2).

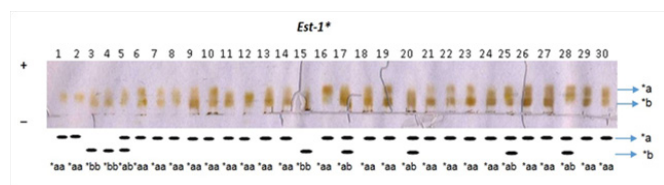
**Table 7** Meristic characters (average  $\pm$ SE) as recorded from *L. ariza* sample of three river populations in Bangladesh (n=100)

Locus	Alleles		Genotypes	
	No.	Type	No.	Type
<i>Est-1</i> *	2.0	*a, *b	3.0	*aa, *ab, *bb
<i>Pgm</i> *	3.0	*a, *b, *c	4.0	*aa, *ab, *bb, *bc
<i>Ldh-1</i> *	1.0	*a	1.0	*aa
<i>Mdh-1</i> *	1.0	*a	1.0	*aa
<i>Gpi-1</i> *	1.0	*a	1.0	*aa
Average	1.6		2.0	

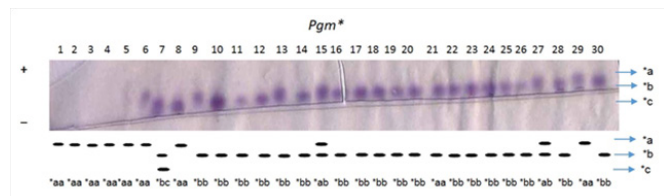
**Table 8** P: Probability of Chi-Square Value; \*\*\* Significant Level:  $P < 0.01$ ; \*\* Significant Level:  $P < 0.05$ ; \*Significant Level:  $P < 0.10$ ; NS: Non-Significant

Locus	Allele Frequency			
	Allele	Atrai (N=30)	Jamuna (N=30)	Brahmaputra (N=30)
<i>Est-1</i> *	*a	0.950	0.816	0.850
	*b	0.050	0.183	0.150
P		0.815 NS	0.009***	0.365 NS
$\chi^2$ d.f.		0.054*	6.673*	0.818*
<i>Gpi-1</i> *	*a	1.000	1.000	1.000
<i>Gpi-2</i> *	*a	1.000	1.000	1.000
$\chi^2$ d.f.		0	0	0
<i>Ldh-1</i> *	*a	1.000	1.000	1.000
<i>Ldh-2</i> *	*a	1.000	1.000	1.000
$\chi^2$ d.f.		0	0	0
<i>Mdh-1</i> *	*a	1.000	1.000	1.000
<i>Mdh-2</i> *	*a	1.000	1.000	1.000
$\chi^2$ d.f.		0	0	0
<i>Pgm</i> *	*a	0.300	0.816	1.000
	*b	0.683	0.183	-
	*c	0.016	-	-
P		0.001***	0.932 NS	NS
$\chi^2$ d.f.		14.925**	0.007	0
		3	1	-





**Figure 1** Electrophoregram of esterase (EST) and the schematic representation of electrophoretic patterns of *Est-1\** locus in the Jamuna population of Baghna.



**Figure 2** Electrophoregram of phosphoglucumutase (PGM) and the schematic representation of electrophoretic patterns of *Pgm\** locus in the Atrai population of Baghna.

The tetrameric enzyme lactate dehydrogenase (LDH) presumably controlled by two loci *Ldh-1\** and *Ldh-2\** and exhibited five banding pattern. The result indicated that the *Ldh-1\** and *Ldh-2\** loci were monomorphic and in all the three river population the allelic frequency of *\*a* was found to be 1.00 (Table 8). The dimeric enzyme malate dehydrogenase (MDH) presumably controlled by at least two different loci, *Mdh-1\** and *Mdh-2\** and exhibited three banding patterns consisting of two heterodimers and one homodimer. The result also indicated that both the *Mdh-1\** and *Mdh-2\** were monomorphic and in all the three river population the allelic frequency of *\*a* was found to be 1.00 (Table 8). The dimeric enzyme glucose-6-phosphate isomerase (GPI) presumably controlled by at least two

different loci, *Gpi-1\** and *Gpi-2\** and exhibited three banding patterns consisting of one homodimer and two heterodimers. Both the *Gpi-1\** and *Gpi-2\** were also monomorphic and in all the three river population the allelic frequency of *\*a* was found to be 1.00 (Table 8). In all the cases of polymorphic loci, the chi-square test based on Hardy-Weinberg equilibrium was carried out between observed and expected genotypes. Although the test was not effective in most of the cases in which the expected values were less than five, there were generally good agreements between observation and expectation (Table 8). However, significant variation in allele frequency of locus *Est-1\** was observed in the Atrai and Jamuna river populations. On the other hand, significant variation in allele frequency of locus *Pgm\** was observed in the Atrai population.

The genetic variability at 8 presumptive loci of *L. ariza* populations collected from 3 river population of Bangladesh is shown in Table 9. The mean fraction of polymorphic loci in the Atrai, Jamuna and Brahmaputra populations were 25, 25 and 12.5% respectively. The number of alleles per locus ( $N_a$ ) ranged from 1.125 (Brahmaputra) to 1.375 (Atrai) with a mean value of 1.250 for all populations. The mean fraction of heterozygous loci per individual was found to be 4.305% for all population. The observed heterozygosity ( $H_o$ ) ranged from 0.033 (Atrai) to 0.058 (Jamuna) with a mean value of 0.042. The expected heterozygosity ( $H_e$ ) ranged from 0.032 (Brahmaputra) to 0.076 (Jamuna) with a mean value of 0.058 for all population. The summary of the gene flow ( $N_m$ ) and population differentiation ( $F_{ST}$ ) are given in Table 10. The analysis of gene diversity within populations measured the gene flow ( $N_m$ ) and the genetic differentiation ( $F_{ST}$ ) over all the three river populations Bhagna are 0.652 and 0.277, respectively. In pair-wise analysis, relatively higher  $N_m$  value (6.148) was measured between the Jamuna and the Brahmaputra populations corresponding the lower level of  $F_{ST}$  value (0.039).

**Table 9** Genetic variabilities at 8 presumptive loci of *L. ariza* populations collected from 3 river population of Bangladesh

Population	Mean Polymorphic Loci* (%)	Mean Number of Alleles Per Locus ( $N_a$ )	The Mean Proportion of Heterozygous Loci Per Individual (%)	Heterozygosity		
				$H_o$	$H_e$	$H_o / H_e$
Atrai	25.000	1.375	3.333	0.033	0.068	0.485
Jamuna	25.000	1.250	5.833	0.058	0.076	0.763
Brahmaputra	12.500	1.125	3.750	0.037	0.032	1.156
Average	20.830	1.250	4.305	0.042	0.058	0.801

\*  $P \leq 0.95$

**Table 10** Pair-wise and overall population differentiations ( $F_{ST}$ ) and Gene flow ( $N_m$ ) in three *L. ariza* populations.

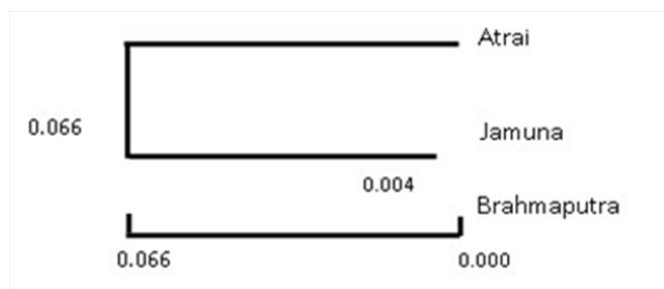
Populations	$F_{ST}$		$N_m^*$	
	Pair-Wise	Overall	Pair-Wise	Overall
Atrai-Jamuna	0.207		0.956	
Jamuna-Brahmaputra	0.039	0.277	6.148	0.652
Atrai-Brahmaputra	0.396		0.380	

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

**Table 11** Nei's (1972) original measures of genetic identity (above diagonal) and genetic distance (below diagonal) estimated among 3 populations of *L. ariza* based on 8 loci

Populations	Atrai	Jamuna	Brahmaputra
Atrai	***	0.962	0.935
Jamuna	0.037	***	0.995
Brahmaputra	0.066	0.004	***

The genetic distance ( $D$ ) values ranged from 0.004 to 0.037 among the three river populations (Table 11). The lowest genetic distance ( $D=0.004$ ) was detected between the Brahmaputra and the Jamuna populations, while the highest value ( $D=0.066$ ) was found in between the Atrai and the Brahmaputra populations. The UPGMA dendrogram constructed from Nei's (1972) to show the genetic distances is shown in Figure 3. The UPGMA dendrogram displayed two clusters among the three river populations. The Jamuna and Brahmaputra population formed one cluster while the Atrai population formed another cluster. The dendrogram showed that the Atrai population separated from the Jamuna and the Brahmaputra populations by a genetic distance of 0.066 where the Jamuna population is different from the Brahmaputra population by the genetic distance of 0.004 (Table 11).



**Figure 3** Nei's (1972) UPGMA dendrogram showing the genetic distance ( $D$ ) among three river populations.

## Discussion

### Morphological variation study

In this study, we collected all the samples at the similar time but higher total length of the Atrai population compared to that of the other populations might be due to relatively early natural breeding in that area due to natural climatic conditions. The offspring grow faster in early developing stage in natural habitat which might be the cause of growth variation in the Atrai population. In natural conditions, the growth of a particular species could be influenced by the different environmental factors which in turn, reflect the reproductive activity of that species.<sup>38</sup> The proportion of total length and standard length of *L. ariza* with head length as obtained in the present study (5.00-6.00 and 3.00-4.77 times, respectively) is slightly lower than that reported in the previous study (5.8-6.3 and 4.5-4.8 times, respectively).<sup>3</sup> In the present study, of the nine meristic characters of *L. ariza* of the Atrai, Jamuna and the Brahmaputra populations, the dorsal fin rays (11.03, 10.86 and 10.26), pectoral fin rays (16.23, 16.26 and 16.00), anal fin rays (7.03, 6.23 and 6.76), caudal fin rays (18.60, 18.26 and 18.70) and scale along lateral line (37.53, 35.63 and 35.53) were found to vary. These variations may be due to different environmental factors, geographical location of experimental population.

The present findings agreed with previous study by<sup>39</sup> who reported that the meristic counts of fishes are considered to be affected by environmental factors such as water temperature, pH etc in fresh water. The previous study reported 36-38 scales in lateral line of *L. ariza* populations and this meristic character strongly supported the present study.<sup>3</sup> In the above mentioned observations, the morphological characteristic of all *L. ariza* populations seems to be somewhat similar. Although this species is already going to be threatened but their morphological characters are not destroyed. The observed morphological characters of *L. ariza* populations in Bangladesh showed minor variation from others and it is possibly due to the fact that their original sources might be from different

locations. The mean values of each character diverse considerably by population, representing its exclusive characteristics.<sup>3</sup>

### Genetic variation study

Five enzymes viz. GPI, LDH, EST, MDH and PGM were used in CA 6.1 buffer system and they produced clear resolution in the muscle tissue of the three populations of *L. ariza*. The enzyme GPI did not display clear resolution which might be due to the tissue and species specificity and utilized buffer system. If one has the information about the number of loci at which variation occurs (polymorphic loci), then only it would be possible to estimate the amount of genetic variation in a population.<sup>40</sup> In order to monitor the levels of genetic variation in population, we can use the electrophoretic data because it provides such information of existence of polymorphic loci.<sup>41</sup> The fraction of polymorphic loci ( $P$ ) can be commonly used to estimate the electrophoretically noticeable variation in a population. Other commonly used measures of genetic variations are allele frequency in loci, mean number of allele per locus, average frequency of heterozygous loci per individual ( $H$ ), observed heterozygosity ( $H_o$ ) and expected heterozygosity ( $H_e$ ).

In the present study, all populations showed two common alleles  $*a$  and  $*b$  except locus  $Pgm^*$  in the Atrai population where a rare allele  $*c$  (0.033) was observed. Similarly a rare allele  $c^*$  (0.05) in  $Pgm^*$  was also reported by.<sup>42</sup> in the natural population of rohu. The rare allele ( $*c=0.033$ ) was also presented in the locus  $Est-1^*$  of *Puntius sarana* population Shukair and Kangsha, Bangladesh.<sup>43</sup> However, the presences of this rare allele in the Atrai population were the evidence of superiority of the Atrai population and absence of allele  $*c$  in other populations provided the evidence of the loss of genetic variability.

The average fraction of polymorphic loci per population of the Atrai and the Jamunariver were higher (25%) than the Brahmaputra (12.5) population which indicates the increase of gene pool diversity in the Baghna population of the Atrai and Jamuna River than the Brahmaputra population. It might be due to the geographical barrier between different stocks. The previous study estimated polymorphic loci ( $P$ ) as 15.2% ( $P \leq 0.95$ ) for polymorphism in fish in general.<sup>44</sup> Therefore, the studied *L. ariza* population of the Atrai showed a decent level of polymorphism which close to the above mentioned range. The average heterozygous loci of 13.33% per individual within the range (6.67-13.33%) was obtained for the three populations of sharpunti (*P. sarana*).<sup>43</sup> and for both hatchery and natural populations of rohu the value was reported to be lower than 15%.<sup>42</sup> In our study, the mean number of heterozygous loci per individual (4.305%) indicated that the status of heterozygous loci remarkably remains in all the three river populations. The average observed heterozygosity ( $H_o$ ) obtained in the present study (0.042) is lower than that (0.091).<sup>45</sup> but similar to that (0.038-0.080) [46] in case of *Clarias macrocephalus*. The higher observed and expected heterozygosity ( $H_o = 0.058$  and  $H_e = 0.076$ ) exhibited by the Jamuna population of Bhagna indicated that the gene pool was preserved effectually. The  $H_o$  values indicated that those corresponding to the Jamuna of *L. ariza* populations are closer to the average values of  $H_o = 0.055$  obtained for teleosts.<sup>47</sup> It was stated that an average observed heterozygosity ( $H_o$ ) value for teleost fishes was found to be 0.051.<sup>48</sup> However, the  $H_e$  values (0.032-0.076) obtained in the present study do not exceed the range of values ( $H_e = 0.062$  to 0.118) which are generally considered as the higher margins of genetic variability.<sup>44</sup>

The co-efficient of gene differentiation ( $F_{ST}$ ) in all three *L. ariza* populations examined for all loci was 0.277. This result indicated

the presence of population with a trivial genetic differentiation and the number of individuals that migrate from one population to another is high as revealed as  $N_m = 0.652$ . Between the Jamuna-Brahmaputra populations, the pair-wise population gene flow was higher (6.148) than all other between population comparisons with corresponding the lowest  $F_{ST}$  value of 0.039. The  $F_{ST}$  value (0.277) of *L. ariza* populations as obtained in the present study is lower than that obtained for other freshwater fishes such as loach (0.774) .<sup>49</sup> and freshwater Gobi (0.698).<sup>50</sup> However, the present  $F_{ST}$  value indicates that there are little genetic differentiation exists among the populations.

The UPGMA dendrogram showed that the three populations can be congregated into two as revealed in the dendrogram (Figure 3). First group is consists of only a single Atrai population and separated by the  $D=0.066$  from second one whereas second group consists of the Jamuna and Brahmaputra populations. In second group, it was observed that the Jamuna population was separated from the Brahmaputra population at  $D=0.004$ . It 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. The  $D$ -value between subspecies is approximately 0.20.<sup>51</sup> Considering from the above-mentioned criteria, the studied *L. ariza* may be categorized as local race or population.

## Conclusion

Following above discussion of allozyme variabilities it is observed that the Atrai population showed higher genetic variation and followed by the Jamuna and the Brahmaputra populations. The higher genetic variation in the Atrai population is due to geographical distance because the Atrai River is geographically far from the Jamuna and the Brahmaputra rivers. On the other hand, the Jamuna River is geographically nearer to the Brahmaputra River. But in the present study, the Atrai sample was collected from the Chirrirbandar Upazilla (Dinajpur) where there is less possibility of mixing with the Jamuna and the Brahmaputra populations.

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