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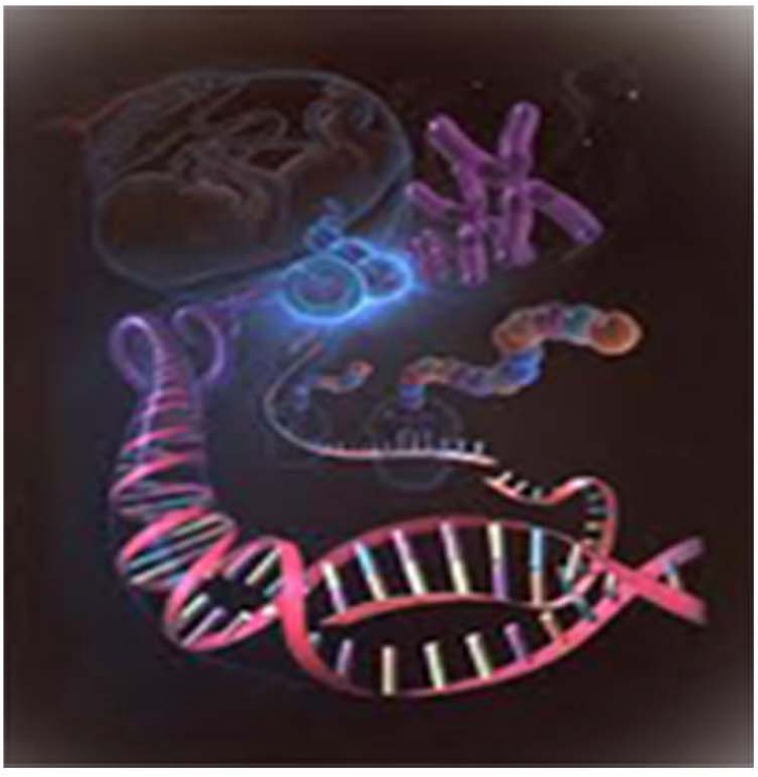

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Research Paper

MORPHOLOGICAL AND GENETIC VARIATIONS IN WILD AND HATCHERY POPULATIONS OF GONIA (*LABEO GONIUS*, HAMILTON) USING TRUSS MEASUREMENT AND ALLOZYME MARKERS

Afroza Begum[1], Md. Mukhlesur Rahman Khan[1], Kamrun Nahar[1], Maruf Hossain Minar[1], Nargis Sultana[1] and Mohd. Golam Quader Khan[1*]

Corresponding Author: **Mohd. Golam Quader Khan, ✉ khanmgq@yahoo.com*

The morphological and genetic variations of wild and hatchery stocks of Gonia *Labeo gonius* (Hamilton, 1822) were studied based on morphometric measurements, meristic counts and allozyme analyses. Samples were collected from two rivers (the Brahamaputra and the Bulla), two *haors* (the Mithamoin and the Kotiadi) and one Hatchery of Bangladesh. Significant differences were observed in nine (L_F, L_S, L_H, D_E, L_{Po}, D_{HB}, D_{LB}, L_{Pc} and L_A) out of 13 transformed morphometric measurements and 13 (1-2, 1-11, 3-10, 3-8, 4-5, 4-10, 4-7, 5-7, 7-8, 8-9, 9-10, 10-11 and 11-12) of 25 truss network measurements and five (pectoral fin rays, anal fin rays, caudal fin rays, scales above lateral line and scales below lateral line) of eight meristic counts among the samples (P<0.05). Discriminant Function Analysis (DFA) of morphometric and landmark measurements showed that the first DF accounted for 43.1% and the second DF accounted for 27.1% of among-group variability and together they explained 70.2% of the total among-group variability. Allozyme markers analysis of the same populations showed that the lowest pair-wise population differentiation (F_{ST}) (0.026) and highest gene flow (*Nm*) (9.323) were found between the Kotiadi and the Hatchery populations indicating close relationship among them. The UPGMA dendrogram (Nei, 1987) constructed from Nei's (1972) genetic distance showed that five populations formed separate sets of clusters. The present study revealed that the lower level of morphological differences was existed among the five populations of *L. gonius* but the genetic analyses indicated considerable variability among the stocks. Nonetheless, genetic analysis showed that these levels were significant and the population structure should be analyzed using markers able to detect a greater degree of population differentiation.

Keywords: Landmark, Allozyme electrophoresis, Stock identification, Genetic differentiation, Genetic conservation, Gene flow

INTRODUCTION

Gonia, *Labeo gonius* (Hamilton, 1822) is a common species of minor carps under Cyprinidae family distributed in natural waters of Pakistan, India, Nepal and Myanmar (Talwar and Jhingran, 1991). In Bangladesh this fish is

[1] Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

normally captured from the natural sources belonging to *haors, baors, beels* and rivers of Kishoreganj, Narsingdi and Noakhali districts and now being cultured in captive condition (DoF, 2011). It attains a maximum length of about 150 cm (Talwar and Jhingran, 1991) and weight of 1.36 kg (Rahman, 1989) and has characteristic shiny color with small scales that draw consumers' attention.

However, the natural production of gonia is being deteriorated gradually by more fishing pressure, dam construction, environmental pollutions and other anthropological effects such as pesticides, herbicides and other agro-chemicals. It is now one of the 56 freshwater species that has been enlisted as critical or somewhat endangered in Bangladesh (IUCN, 2000). Besides, the genetic impurity of gonia is being observed because of introgressed hybridization between gonia and bata, gonia and rohu, gonia and mrigal, etc. It is the high time to identify the purity of gonia and to conserve the original stock for consumption as well as for sustainable production. Therefore identification of 'pure' gonia, reliable scientific approach is necessary for conserving the original stock for mass scale propagation.

Truss measurements constructed with the help of landmark points are powerful tools (Hossain *et al*., 2010) which can be used for the stock identification of gonia as well as other species. Landmark is a point of correspondence on an object that matches between and within populations (Barlow, 1961; Swain and Foote, 1999) and often subject to strong natural and sexual selection that may vary across a species range (Arnold, 1983; Bels *et al*., 2003). Recently landmark morphology data for kalibaus (Hossain *et al*., 2010), Chub mackerel (Erguden *et al*., 2009), rohu (Hasan *et al*., 2007) and Thai pangas (Khan *et al*., 2004) have been developed home and abroad. In addition to the stock identification technique with truss measurements, allozyme electrophoresis, a molecular technique, can be applied for quantifying genetic variation at the level of populations, species and higher taxonomic designations (Chauhan *et al*., 2010). Allozyme electrophoresis provides an extensive morphological quantitative survey (Menezes *et al*., 1993) and has become an effective tool for fish population studies and fishery management. The genetic variation of a number of species of Bangladesh has been studied by several authors, for example, Bata, *Labeo bata* (Suraiya *et al*., 2009), local sharpunti, *Puntius sarana* (Pervej, 2005), Catla, *Catla catla* (Alam *et al*., 2004), Rohu, *Labeo rohita* (Alam *et al*., 2002), Silver carp, *Hypophthalmichthys molitrix* and Bighead carp, *Aristichthys nobilis* (Alam and Khan, 2004). However, there is no known information on stock identification and genetic variation of *L. gonius* in Bangladesh.

The identification of the gene pool variation in gonia stocks could sustain their purity to develop breeding program for sustainable production. The present work was concentrated with the landmark-based morphometric and meristic studies and investigated the genetic variation of the wild and hatchery populations of *L. gonius* to identify diversified populations through allozyme markers.

MATERIALS AND METHODS

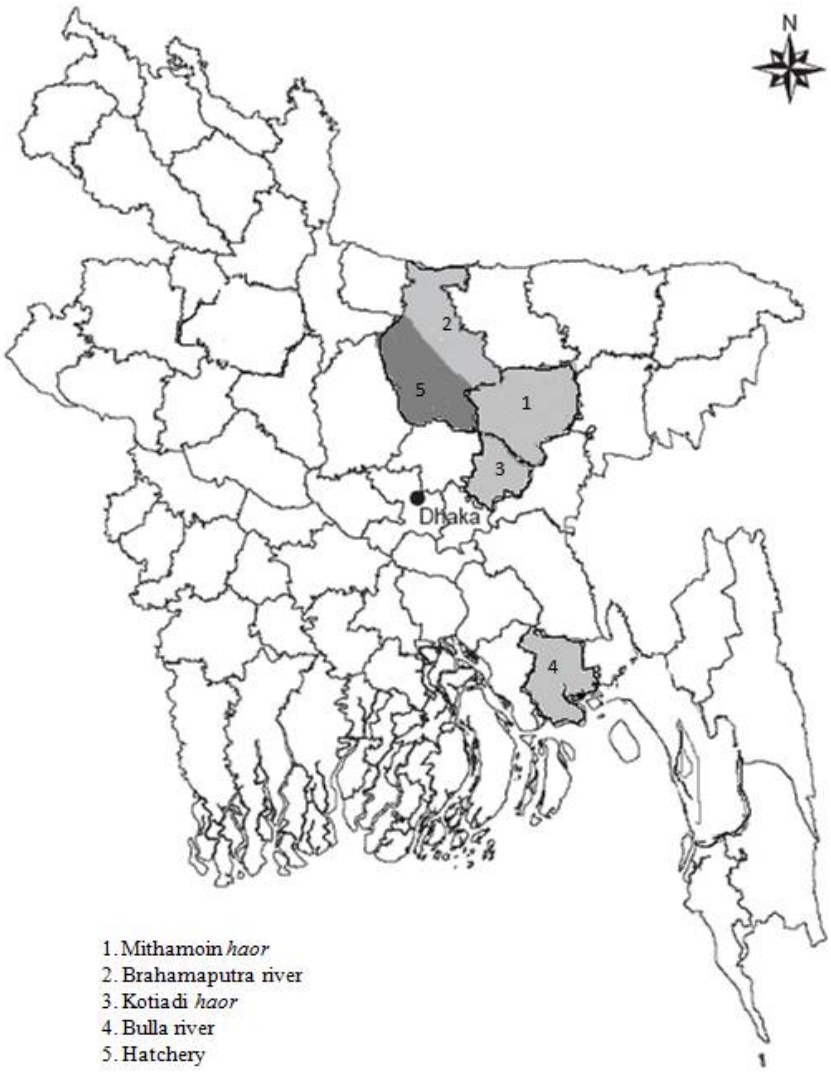
Collection of Samples

A total of 150 *L. gonius* was collected from five different locations in Bangladesh during July 25, 2011 to March 1, 2012 (Table 1 and Figure 1). The samples were then brought to the laboratory

Table 1: Sampling Details of *L. gonius* Collected from Different Locations in Bangladesh

Population No.	Sources of collection River/*Haor*	Site Co-ordinates	No. of Individuals	Length Mean±SE	Date of Collection
1	Mithamoin *haor* (Karimganj, Kishorganj)	24° 25′ N 90° 46′ E	30	19.31 ± 0.77	September 8, 2011
2	Brahmaputra river (Sadar, Mymensingh)	24° 38′ N 90° 16′ E	30	22.44 ± 0.36	July 25, 2011
3	Kotiadi *haor* (Kotiadi, Narsingdi)	23° 92′ N 90° 73′ E	30	24.11 ± 0.18	January 12, 2012
4	Bulla river (Subornogram, Noakhali)	22° 83′ N 91° 10′ E	30	25.35 ± 0.55	March 1, 2012
5	Hatchery (Trishal, Mymensingh	24° 38′ N 90° 16′ E	30	27.48 ± 1.06	February 15, 2012

Figure 1: Sample Collection Sites of Gonia (*L. gonius*) in the Present Study



of fish genetics and Biotechnology, Bangladesh Agricultural University, Mymensingh for morphometric, meristic and landmark studies. Then muscles and liver samples were stored at –18°C for allozyme electrophoresis.

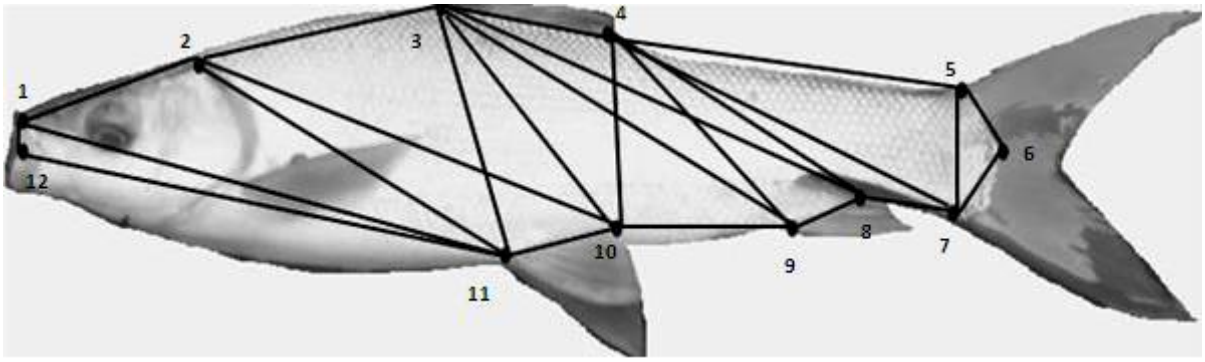
Morphometric Measurements

Fourteen morphometric characters were measured along the body of fish to the nearest 0.05 mm with digital callipers and metallic ruler, following the conventional method described by Hubbs and Lagler (1958). The total length (L_T), head length (L_H), standard length (L_S), fork length (L_F) and pre-orbital length (L_{Pr}) were measured from the tip of the snout, to the longest caudal fin ray, to the posterior margin of the opercular, to the end of the vertebral column, to the middle part of the fork of the tail and to the anterior margin of the eye, respectively. Eye diameter (D_E) was taken from the external border of the eyes. Post-orbital length (L_{Po}) was the distance from the posterior margin of the eye to the end of the operculum. Highest body depth (D_{HB}) and lowest body depth (D_{LB}) were taken as the diameter of the highest and lowest part of body. The dorsal-fin length (L_D), anal-fin length (L_A), pelvic-fin length (L_{Pe}) and pectoral fin length (L_P) were measured as the length of the base of dorsal fin, pectoral fin, pelvic fin and anal fin, respectively. Mouth gap (G_M) was the distance between upper and lower lip. The methodology applied to analyze the morphometric characters was the same as those applied for landmark-based morphometric analysis.

Landmark-Based Morphometric Analysis

Twenty-five morphometric variables were taken as interlandmark distances over the body of individuals using a digital calliper (0·05 mm precision). These variables were based on 12 landmarks (Figure 2) obtained by truss network following Strauss and Bookstein (1982). A multivariate discriminant analysis was used for morphometric data to identify the combinations of variables that separate *L. gonius* samples best. Prior to the analysis, size effects from the

Figure 2: Locations of the 12 landmarks for constructing the truss network on fish (filled circle), Landmarks refer to: (1) anterior tip of snout at upper jaw, (2) most posterior aspect of neurocranium (beginning of scaled nape), (3) origin of dorsal fin, (4) insertion of dorsal fin, (5) anterior attachment of dorsal membrane from caudal fin, (6) posterior end of vertebrae column, (7) anterior attachment of ventral membrane from caudal fin, (8) insertion of anal fin, (9) origin of anal fin, (10) insertion of pelvic fin, (11) origin of pelvic fin and (12) corner of the jaws



data set were eliminated. Variations were attributed to body shape differences, and not to the relative sizes of the fish. In the present study, there were significant linear correlations among all measured characters and the L_T of the fish. Therefore, it was necessary to remove size-dependent variations from all of the characters. An allometric formula given by Elliott *et al*. (1995) was used to remove the size effect from the dataset:

$$M_{adj} = M (L_s / L_o)^{b}$$

where, M was the original measurement, M_{adj} was the size-adjusted measurement, L_o was the L_T of the fish, and L_s was the overall mean of the L_T 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 the size adjustment transformations was assessed by testing the significance of the correlation between transformed variable and the L_T. A univariate analysis of variance (ANOVA) was carried out to test the significance of morphological differences. In addition, size-adjusted data were standardized and submitted to a Discriminant Function Analysis (DFA). All statistical analyses were done using SPSS v.17 (SPSS, Chicago, IL, USA).

Meristics Analysis

Eight meristic (dorsal, pectoral, pelvic, anal, and caudal fin rays, branchiostegal rays, scales above and below the lateral line) counts were taken from the left side of each specimen. Kruskal-Wallis non-parametric ANOVA (Hoaglin *et al.*, 1991) was used to test for significant differences in meristic counts of the samples.

Allozyme Electrophoresis Study

Horizontal starch gel electrophoresis and histochemical-staining techniques were used according to Shaw and Prasad (1970). The CA 6.1 buffer was used to analyze the allelic variations among all *L. gonius* populations. The enzymes studied, loci, enzyme patterns, Enzyme Commission (EC) numbers and tissue of samples are shown in Table 2. Amine-citrate buffers (CA 6.1) (Clayton and Tretaik, 1972) were used in allozyme electrophoresis. Gel slices

Table 2: Ten Enzymes Analyzed in *L. gonius* Populations

Enzymes (Abbreviation)	Loci	Enzyme Patterns	E.C. Number	Tissue
Aspartate aminotranferase (AAT)	-	Dimer	2.6.1.1	M/L
Alcohol dehydrogenase (ADH)	-	Dimer	1.1.1.1	M/L
Esterase (EST)	*Est-1*Est-2**	Monomer	3.1.1.1	M
Glyceroldehyde-3-phosphate dehydrogenase (G3PDH)	*G3pdh-1* G3pdh-2**	Dimer	1.1.1.8	M
Glucose-6-phosphate isomerase (GPI)	*Gpi-1*Gpi-2**	Dimer	5.3.1.9	M
Glucose-6-phosphate dehydrogenase (G6PDH)	*G6pdh-1*G6pdh-2**	Dimer	1.1.1.49	M
Isocitrate dehydrogenase (IDHP)	*Idhp-1*Idhp-2**	Dimer	1.1.1.42	M
Lactate dehydrogenase (LDH)	*Ldh-1*Ldh-2**	Tetramer	1.1.1.27	M
Malate dehydrogenase (MDH)	*Mdh-1* Mdh-2**	Dimer	1.1.1.37	M
Phosphoglucomutase (PGM)	*Pgm**	Monomer	5.4.2.2	M

Note: M=Muscle, L=Liver.

(1 mm) were histochemically stained for different enzyme activities as described by Aebersold *et al.* (1987). Allele frequencies were calculated directly from observed genotypes. The distribution of observed genotypes was compared with that of expected, calculated from the Hardy-Weinberg equilibrium using a χ^2 test. The most common allele existed in a frequency less than 0.95 at a given locus; this locus was regarded as polymorphic. The allozyme data were analyzed using POPGENE v.1.32) (Yeh *et al.*, 1999) and TREEVIEW (Roderick, 2000) computer program packages. Using POPGENE program, the mean proportion of polymorphic loci and the average number of alleles per locus were calculated to quantify genetic variability for each population (Lewontin and Hubby, 1966; Lewontin, 1974). The observed heterozygosity (H_o) and expected heterozygosity (H_e) were examined according to Nei and Roychoudhury (1973). Based on Nei's (1972) genetic distance (D), a dendrogram was constructed using the Unweighted Pair-Group Method of Arithmetic averages (UPGMA) for the analysis of divergence and relationships among populations. Genetic differentiation between pairs of populations was analyzed by calculating pair-wise F_{ST} values (Goudet, 1995) and testing their significance by permuting individuals between populations using the program FSTAT (Weir and Cockerham, 1984).

RESULTS

Morphometric, Landmark and Meristic Analyses

Univariate statistics (ANOVA) showed that among the 13 transformed morphometric characters, nine characters (L_F, L_S, L_H, D_E, L_{Po}, D_{HB}, D_{LB}, L_{Pc} and L_A) and from the 25 truss measurements, 13 measurements (1-2, 1-11, 3-10, 3-8, 4-5, 4-10, 4-7, 5-7, 7-8, 8-9, 9-10, 10-11 and 11-12) were found to be significant (*P<0.05 , **P<0.01 and ***P<0.001) among the population (Table 3).

Pooled within-group correlations between discriminant variables and Discriminant Functions (DFs) revealed that among the 13 morphometric and the 25 truss measurements, one morphometric measurement (L_F) and one truss measurement (8-9) contributed to the first DF, four morphometric measurements (L_{Po}, L_S, L_H and L_D) and eight truss measurements (11-12, 1-11, 4-7, 4-5, 5-7, 2-12, 3-11 and 3-9) contributed to the second DF. The third DF was contributed by three morphometric measurements (D_E, D_{LB} and L_A) and six truss measurements (1-2, 3-10, 3-4, 1-12, 5-6 and 2-11). The remaining 5 morphometric measurements (L_{Pc}, D_{HB}, G_M, L_{Pr} and L_{Pe}) and 10 truss measurements (9-10, 7-8, 4-10, 3-8, 2-9, 10-11, 4-8, 4-9, 6-7 and 2-3) contributed to the forth DF and implied that those characters were the most important in the description of population characteristics (Table 4).

Plotting discriminant function DF1 and DF2 showed a clear differentiation between the species as well as among the stocks for both morphometric and landmark measurements. The DFA produced four types of DFs (the first, second, third and forth DF) for both morphometric and landmark measurements. For both measurements, the first DF accounted for 80.06% and the second DF accounted for 13.2% of among group variability and together they explained 93.26% of the total among-group variability (Table 4). It showed that all the populations were clearly separated from each other in the discriminant plot (Figure 3). A correct classification of individuals by discriminant analysis showed that 100% of

Table 3: Univariate Statistics (ANOVA) Testing Differences Among Samples from Morphometric and 25 Truss Measurements

Characters	Wilks' Lambda	F	Significance
L_F	0.107	301.438	0.000***
L_S	0.666	18.202	0.000***
L_H	0.723	13.913	0.000***
D_E	0.917	3.267	0.013*
L_{Pr}	0.973	1.000	0.410
L_{Po}	0.471	40.720	0.000***
D_{HB}	0.893	4.333	0.002**
D_{LB}	0.855	6.125	0.000***
L_D	0.973	1.000	0.410
L_P	0.568	27.575	0.000***
L_{Pe}	0.990	0.382	0.821
L_A	0.934	2.544	0.042*
G_M	0.973	1.004	0.407
1-2	0.733	13.203	0.000***
1-12	0.973	1.016	0.401
1-11	0.852	6.309	0.000***
2-3	0.982	0.668	0.615
2-12	0.950	1.927	0.109
2-11	0.985	0.540	0.706
2-9	0.960	1.499	0.205
3-4	0.965	1.311	0.269
3-11	0.969	1.151	0.335
3-10	0.773	10.620	0.000***
3-9	0.973	0.992	0.414
3-8	0.916	3.319	0.012*
4-5	0.877	5.079	0.001**
4-10	0.901	4.005	0.004**
4-9	0.971	1.074	0.372
4-8	0.967	1.243	0.295

Table 3 (Cont.)

Characters	Wilks' Lambda	F	Significance
4-7	0.843	6.738	0.000***
5-6	0.978	0.829	0.509
5-7	0.919	3.198	0.015*
6-7	0.962	1.437	0.225
7-8	0.919	3.207	0.015*
8-9	0.924	2.966	0.022*
9-10	0.805	8.754	0.000***
10-11	0.860	5.881	0.000***
11-12	0.655	19.052	0.000***

Note: *P<0.05, **P<0.01, ***P<0.001.

Table 4: Pooled Within-Groups Correlations Between Discriminating Variables and Standardized Canonical Discriminant Functions (Variables Ordered by Absolute Size of Correlation Within Function

Characters	Function			
	First DF	Second DF	Third DF	Forth DF
L_F	0.820*	–0.053	0.025	–0.069
8-9	0.075*	0.059	–0.016	0.047
L_{PoO}	0.220	0.340*	–0.335	0.207
11-12	0.120	0.306*	0.207	-0.096
L_S	0.127	–0.292*	0.014	0.239
1-11	0.033	–0.214*	-0.117	0.058
L_H	0.152	0.175*	0.044	0.039
4-7	0.081	0.168*	–0.056	0.133
4-5	0.075	0.140*	–0.090	0.000
5-7	0.053	0.122*	0.077	–0.014
2-12	0.015	0.112*	0.068	–0.089
3-11	0.019	0.082*	0.070	0.022
L_D	0.017	0.079*	0.060	0.014
3-9	0.018	0.078*	0.061	0.012
1-2	-0.070	0.026	–0.467*	-0.218

Table 4 (Cont.)

Characters	Function			
	First DF	Second DF	Third DF	Forth DF
3-10	0.039	0.235	0.265*	0.154
D_E	0.029	0.022	0.228*	0.139
D_{LB}	0.041	0.172	0.198*	–0.115
L_{AF}	0.034	–0.091	0.154*	–0.034
3-4	–0.017	–0.050	0.142*	–0.007
1–12	–0.011	–0.009	0.138*	–0.063
5-6	0.002	0.013	0.135*	0.009
2-11	0.025	–0.004	0.072*	0.034
L_P	0.139	–0.352	0.151	–0.386*
9-10	0.020	0.185	–0.208	0.325*
7-8	-0.003	–0.009	0.100	0.321*
4-10	-0.017	–0.041	0.184	0.286*
3-8	0.046	–0.061	0.124	–0.216*
D_{HB}	-0.003	0.133	-0.167	0.205*
2-9	0.001	–0.043	0.063	–0.204*
10-11	0.023	–0.179	0.153	–0.196*
G_M	-0.001	–0.055	–0.036	0.151*
L_{Pr}	-0.015	–0.038	0.058	0.147*
4-8	0.036	0.039	0.005	–0.138*
4-9	–0.007	0.054	0.075	–0.134*
6-7	–0.002	0.075	0.105	–0.110*
2-3	0.008	0.054	0.002	–0.107*
L_P	-0.005	–0.008	–0.046	0.101*
% variance	80.6	13.2	4.0	2.1

Note: * Largest absolute correlation between each variable and discriminant function.

individuals could be classified in their correct priori grouping (Table 5).

Among the 8 meristic counts of *L. gonius* three characters, i.e., pelvic fin, anal fin and branchiostegal rays (9-9, 7-8 and 3-3 respectively) were more similar among all the populations. Other five characters, i.e., dorsal fin rays, pectoral fin rays, caudal fin rays, scales above lateral line and scales below lateral line were found in variable ranges among the populations. Kruskal-Wallis test (*H*) showed significant (P<0.05) *H*-value at *df* = 4, 55.873 for pectoral fin rays, 12.347 for anal fin rays, 29.878 for caudal fin rays, 29.315 for scales above lateral line and 17.795 for scales below lateral line.

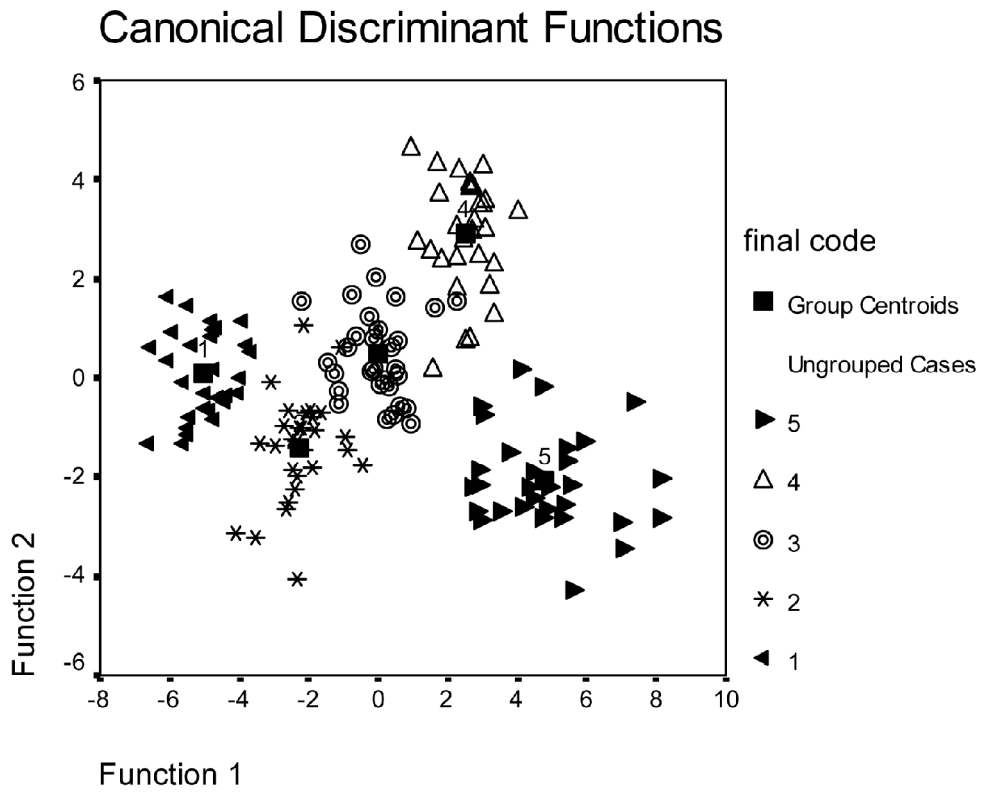
Genetic Variation

Among the 10 enzymes studied, seven presumptive loci were identified where five loci were (*Gpi-1*, Gpi-2*, Mdh-1*, Mdh-2** and *Pgm**) were polymorphic (P<0.95). The LDH showed two loci (*Ldh-1** and *Ldh-2**) which were monomorphic. Two enzymes (AAT and ADH) did not produce any scorable bands. Because of complex banding pattern, enzymatic bands with EST, G3PDH, G6PDH and IDH were not interpretable. The highest number (5) of polymorphic five was found in the Brahmaputra and Hatchery population followed by Kotiadi and Bulla population (4). Only three polymorphic was present loci in the Mithamoin (Table 6).

Deviation from Hardy-Weinberg Expectations and Genetic Variation

The χ^2 test was performed in all the cases of polymorphic loci between observed and expected genotypes, based on Hardy-Weinberg equilibrium (HWE). The test showed that among the five loci, the Mithamoin *haor* in *Gpi-2**, the Brahmaputra river in *Mdh-1**, the Kotiadi *haor* in *Pgm** and Bulla river populations *Mdh-2** were found to be deviated (P<0.05) from Hardy-Weinberg proportions (Table 6). The mean proportion of polymorphic loci (71.3%), the mean number of alleles per locus (*Na*) (1.857) and mean

Figure 3: Sample Centroids of the Discriminant Function Scores Based on Morphometric and Truss Measurements (1. Mithamoin *haor*, 2. Brahamaputra river 3. Kotiadi *haor* 4. Bulla river and 5. Hatchery)



proportion of heterozygous loci per individual (18.095%) in the Brahmaputra river population were higher than those other in four populations. Again the highest observed (0.181) and the expected (0.210) heterozygosity (H_e) was found in the Brahmaputra. The excess of homozygosity was found only in hatchery population (–0.056) (Table 7). The 1-*Ho/He* values were positive for all populations except the hatchery which meant that the respective populations were good in heterozygosity level (Table 7).

Inter Population Genetic Structure and Genetic Differentiation

The pair-wise genetic differentiation (F_{ST}) in above diagonal and genetic distance (D) in below diagonal were estimated in five populations of *L. gonius* based on five polymorphic loci (Table 8). The highest F_{ST} value (0.402) was observed between the pair of Mithamoin *haor* and Hatchery populations and the lowest (0.026) was between the pair of Kotiadi *haor* and the Hatchery populations. The observed F_{ST} values between all the pairs of populations were found to be significant (P<0.05) (Table 8). The minimum genetic distance (D=0.006) was observed between the Kotiadi *haor* and the Hatchery populations, while the maximum (D=0.211) was found between the Mithamoin *haor* and the Bulla river populations. The UPGMA dendrogram resulted in two major clusters among the five populations. Cluster-1 consisted of the Mithamoin

Table 5: Correct Classification of Individuals (*L. gonius*) Collected from Five Different Stocks into Their Original Population

Stock	Predicted Group Membership					Total
	Mithamoin	Brahmaputra	Kotiadi	Bulla	Hatchery	
OriginalCount	30	0	0	0	0	30
	0	30	0	0	0	30
	0	0	30	0	0	30
	0	0	0	30	0	30
	0	0	0	0	30	30
%	100	0	0	0	0	100
	0	100	0	0	0	100
	0	0	100	0	0	100
	0	0	0	100	0	100
	0	0	0	0	100	100

Note: 100% of original grouped cases correctly classified.

Table 6: Allele Frequencies at 5 Polymorphic Loci of *L. gonius* Population

Locus	Allele Frequency					
	Allele	Mithamoin (N = 30)	Brahmaputra (N = 30)	Kotiadi (N = 30)	Bulla (N = 30)	Hatchery (N = 30)
*Gpi-1**	**a*	1	0.15	0.15	0.067	0.05
	**b*	-	0.833	0.85	0.917	0.95
	**c*	-	0.017	-	0.016	-
	P	-	0.785NS	0.365NS	0.978NS	0.815 NS
	χ^2	-	1.065	0.819	0.195	0.055
	d.f	-	3	1	3	1
	Ho	0	0.333	0.3	0.167	0.18
	He	0	0.287	0.259	0.157	0.417
*Gpi-2**	**a*	0.1	-	0.15	-	-
	**b*	0.8	0.917	0.85	0.917	0.917
	**c*	0.1	0.083	0.017	0.083	0.083
	P	0.002*	0.658NS	0.365NS	0.658NS	0.658NS
	χ^2	14.85	0.195	0.819	0.195	0.195
	d.f	3	1	1	1	1
	Ho	0.267	0.167	0.3	0.167	0.213
	He	0.346	0.155	0.259	0.155	0.219

Table 6 (Cont.)

Locus	Allele Frequency					
	Allele	Mithamoin (N = 30)	Brahmaputra (N = 30)	Kotiadi (N = 30)	Bulla (N = 30)	Hatchery (N = 30)
*Mdh-1**	**a*	0.333	0.367	-	-	-
	**b*	0.65	0.633	0.933	1	0.95
	**c*	0.017	-	0.067	-	0.05
	P	0.142NS	0.001*	0.737NS	-	0. 815NS
	Ç^2	5.448	10.372	0.113	-	0.054
	d.f	3	1	1	-	1
	Ho	0.3	0.2	0.133	0	0.147
	He	0.47	0.472	0.126	0	0.286
*Mdh-2**	**a*	-	0.083	-	0.383	0.067
	**b*	1	0.917	1	0.617	0.933
	P	-	0.658NS	-	0.004*	0.737NS
	Ç2	-	0.195	-	8.154	0.113
	d.f	-	1	-	1	1
	Ho	0	0.166	0	0.233	0.107
	He	0	0.155	0	0.481	0.191
*Pgm**	**a*	0.95	0.733	0.817	0.833	0.917
	**b*	0.05	0.267	0.183	0.167	0.083
	P	0.815NS	0.974NS	0.010*	0.094NS	0.658NS
	Ç2	0.054	0.001	6.673	2.803	0.195
	d.f	1	1	1	1	1
	Ho	0.1	0.4	0.167	0.2	0.207
	He	0.097	0.398	0.304	0.282	0.256

Note: *$P<0.05$, NS: Non-significant.

haor only and separated from the other cluster by the highest genetic distance of *D*=0.211. The cluster-2 consisted of four populations and divided into two subclusters. The subcluster-1 consisted of the Brahmaputra populations only and separated from other subcluster by the genetic distance of *D*=0.143. The subcluster-2 again was divided into two groups, the group-1 consisted of only the Bulla population and was separated from other group by the genetic distance of *D*=0.042. The group-2 was made by the Kotiadi and the Hatchery populations and was separated from each other by the smallest *D*-value 0.006 (Figure 4).

DISCUSSION

Morphometric, landmark and meristic characters have been used here as a first step to analyze the potential differentiation of *L. gonius* populations. Morphometric and meristic studies

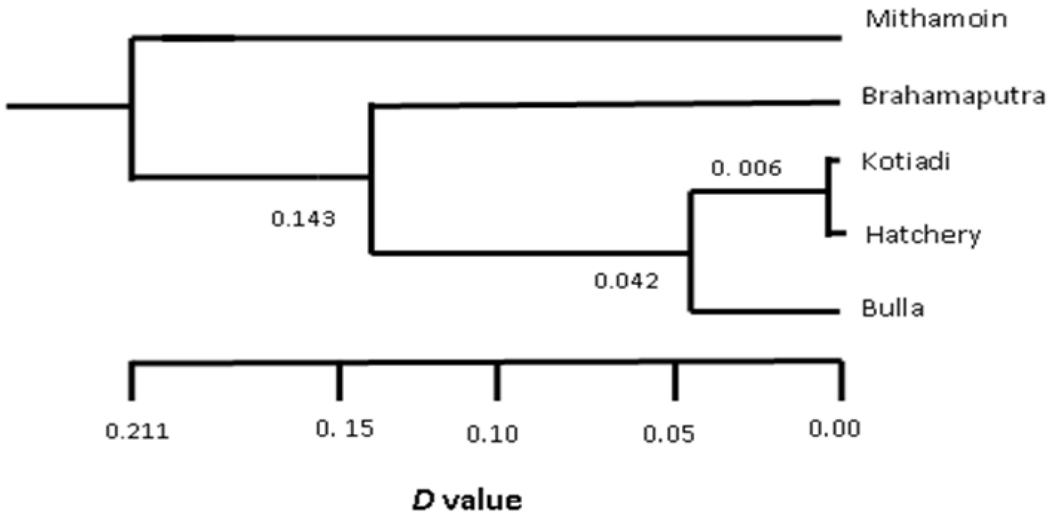
Table 7: Genetic Variabilities at 5 Polymorphic Loci of *L. gonius* Populations

Populations	The mean proportion of polymorphic loci (%)	The mean number of alleles (*Na*) per locus	The mean proportion of heterozygous loci per individual (%)	Heterozygosity			
				Ho	*He*	*Ho/He*	1- *Ho/He*
Mithamoin	42.86	1.714	9.524	0.095	0.131	0.725	0.275
Brahmaputra	71.43	1.857	18.095	0.181	0.210	0.862	0.138
Kotiadi	57.14	1.571	12.857	0.129	0.136	0.948	0.052
Bulla	57.14	1.714	10.952	0.109	0.154	0.708	0.292
Hatchery	71.43	1.714	8.095	0.095	0.090	1.056	-0.056
Average	60	1.714	11.905	0.122	0.144	0.847	0.140

Table 8: Nei's (1972) Pair-wise Genetic Differentiation (F_{ST}) Value (Above Diagonal) and Genetic Distance (Below Diagonal) Estimated Among 5 Populations of *L. gonius*

Populations	Mithamoin	Brahmaputra	Kotiadi	Bulla	Hatchery
Mithamoin	-	0.248	0.315	0.369	0.402
Brahmaputra	0.142	-	0.058	0.088	0.074
Kotiadi	0.149	0.024	-	0.080	0.026
Bulla	0.211	0.042	0.029	-	0.062
Hatchery	0.178	0.026	0.006	0.017	-

Figure 4: UPGMA Dendrogram Based on Nei's (1972) Genetic Distance, Summarizing the data on Differentiation Among Five Populations of *L. gonius*, According to the Allozyme Analysis



provide useful results for identifying fish stocks (Ihssen *et al*., 1981). The results indicated the existence of morphologically differentiated groups of *L. gonius* in Bangladesh. Both DFAs and ANOVA suggested five phenotypically distinct local populations with varying degrees of differentiation.

Morphometric differences among stocks are expected, because they are geographically separated and may have originated from different ancestors (Hossain, *et al.* 2010). Therefore, it is not unlikely that obvious environmental variations exist in *L. gonius* collected from five different stocks. Fishes are very sensitive to environmental changes and quickly adapt themselves by changing some of morphometric characteristics. It is well-known that morphological characters can show high plasticity in response to differences in environmental conditions, such as food abundance and temperature (Allendorf and Phelps, 1988; Swain, *et al.* 1991; Wimberger, 1992). In this study, the fish demonstrated greater variances in morphological traits both within and between populations and could be susceptible to environmentally induced morphological variations. Among the 13 transformed morphometric characters, 9 characters (L_F, L_S, L_H, D_E, L_{Po}, D_{HB}, D_{LB}, L_{Pc} and L_A) and from the 25 truss measurements, 13 measurements (1-2, 1-11, 3-10, 3-8, 4-5, 4-10, 4-7, 5-7, 7-8, 8-9, 9-10, 10-11 and 11-12) were found to be significant at variable degrees (*P<0.05 , **P<0.01 and ***P<0.001). Therefore, stock identification based on morphological characters must be confirmed by genetic evidence to verify that the phenotypic differences reflect some degree of reproductive isolation rather than simply environmental differences. On the other hand, stock discrimination by morphological markers might be appropriate for fisheries management even this phenotypic divergence is not reflected by genetic differentiation.

To our knowledge, there are no reports on the morphological characterization among different populations of *L. gonius* till now. To elucidate the facts, truss measurements were employed in this experiment. Truss network systems are a powerful tool for identifying stocks of fish species (Turan *et al.*, 2004). An unbiased network of morphometric measurements over a two dimensional outline of a fish removes the need to find the types of characters and optimal number of characters for stock separation, and provides information over the entire fish form (Turan *et al.*, 2004). The truss network system can effectively be used to distinguish among the stocks. In the present study, discriminant analysis was also performed. It is a statistical method used in identifying fish populations. In this study, morphometric character and truss parameters were combined and used to differentiate the populations of *L. gonius*. For both morphometric and landmark measurements, the 1st DF accounted for 80.06 % and the 2nd DF accounted for 13.2 % of among group variability and together they explained 93.8 % of the total among-group variability. It suggested that there was no intermingling among the five studied populations of *L. gonius*. It also explains that first DF was more informative than the second DF in explaining differences among the stocks. Plotting DFs revealed moderate isolation in morphometrics among the stocks. It showed that the discriminant analysis was applicable as effective method in identifying populations, strains, and subspecies which have nearer relations. A correct classification of individuals by discriminant analysis showed that 100% of individuals could be classified in their correct priori grouping (Table 5).

The morphometric differentiation was supported by the meristic traits. Meristic characteristics commonly used to distinguish species were analyzed using the numbering approach. The numbers of scales, both of pectoral and pelvic fins, the eye size and the body height have been used as criteria for separating species (Norman, 1937). Features of this kind are easy to examine, require no subjective numerical conversation in analysis and are important in fish identification and species separation. Three characters, i.e., pelvic fin, anal fin and branchiostegal rays (9-9, 7-8 and 3-3 respectively) were similar among all the population and 5 characters, i.e., dorsal fin rays, pectoral fin rays, caudal fin rays, scales above lateral line and scales below lateral line found in variable ranges among the populations. Kruskal-Wallis test (*H*) showed significant ($P<0.05$) H-value at $df = 4$, for pectoral fin rays, anal fin rays, caudal fin rays, scales above lateral line and for scales below lateral line.

Nakamura (2003) found differences in meristic counts in Japanese charr, *Salvelinus leucomaenis* among the river systems (Naka and Tone rivers, central Japan) and among the tributaries of the Naka River (Ashinagasawa, Akasawa, Ushirosawa and Moto-okashirasawa streams). Hossain *et al.* (2010) also found variable meristic counts in Kalibaus, *Labeo calbasu* among the stocks of two isolated rivers, the Jamuna and Halda, and a hatchery. Meristic characters have genetic basis but the environment may modify the expression of their characters as morphology is especially dependent on environmental conditions during early life-history stages (Ryman *et al*., 1984; Lindsey, 1988; Cheverud, 1988).

The allozyme variation of *L. gonius* populations revealed by five polymorphic loci and two monomorphic loci (*Ldh-1** and *Ldh-2**) and others did not show clear resolution in both the muscle and liver tissues. This could be due to buffer system, species and tissue specificity in the populations. Variations of five enzymatic loci, i.e., *Gpi-1**, *Gpi-2**, *Mdh-1*, Mdh-2** and *Pgm** were observed among all populations. Three alleles ***a*, ***b* and ***c* were found common among all the populations in the present study. Lewontin (1974) reported that the amount of genetic variation in a population can be estimated only if one has information about the number of loci at which variation occurs (polymorphic loci). Electrophoretic data provide such information and thus can be used to monitor levels of genetic variation in populations (Leary and Booke, 1990). The proportion of polymorphic loci (*P*) is a commonly used measure of electrophoretically detectable variation in a population.

In this study, the observed proportion of polymorphic loci per population ranged from 42.86% to 71.43% (average 60%). Nevo (1978) estimated polymorphic loci (*P*) as 15.2% ($P<0.95$) for polymorphism in fish in general. Umma Salma Tonny *et al*. (2012) studied genetic diversity between GIFT and GIFU using allozyme markers and estimated polymorphic loci per population was 50%. Suraiya *et al*. (2009) recorded 16.67% polymorphic loci in *L. bata*. Therefore, concerning the above mentioned range the studied *L. gonius* population showed a high level of polymorphism.

The mean number of heterozygous loci per individual in the present study was 11.905%. The average heterozygous loci of 13.33% per individual observed by Pervej (2005) for the three populations of sharpunti (*P. sarana*) which was lower than the value (15%) reported by Alam *et al.* (2002) for both hatchery and natural

populations of rohu. Our results indicated the status of heterozygous loci notably nearer to the above mentioned range by Pervej (2005).

The average observed heterozygosity (*Ho*) obtained in the present study (0.122) was higher than that those reported by Na-Nakorn *et al*. (1998) and by Pouyaud *et al*. (1998) in case of *Clarias macrocephalus* (0.038-0.080, and 0.091 respectively). The higher observed and expected heterozygosity (H_o = 0.181 and H_e = 0.210) exhibited by the Brahmaputra population indicated that the gene pool might be maintained effectively. However, the average observed heterozygosity was much higher in the study by Nasren *et al*. (2009) (0.64-0.75) in *H. fossilis* and by Islam *et al*. (2007) (0.67-0.83) in *C. batrachus*. Nevo (1978) reported that an average observed heterozygosity (*Ho*) value for bony fish was 0.051. The H_e values (0.090-0.210) exceeded the range of values obtained by Kirpichnikov (1992) (H_e = 0.02 to 0.03) as well as of Pervej (2005) (H_e = 0.062-0.118) indicating higher margins of genetic variability. The level of heterozygosity is often related with the size of the populations within a species. It is often assumed that the species with small populations might lost variation due to genetic drift (Reina *et al.,* 1994). The practical interest of higher heterozygosity (H_o) value of a population can be aimed at genetic breeding programs. The average heterozygosity (H_o or H_e) is considered as a good indicator of the genetic variability throughout the genome of the population (Leary and Booke, 1990; Allendorf and Ryman, 1986).

Based on the Nei's (1972) genetic distance (*D*-value), the UPGMA dendrogram showed that the five populations can be grouped into two major clusters. Cluster-1 with Mithamoin *haor* separated from other cluster by the highest genetic distance of *D*=0.211. The observed genetic distances among the two clusters consisting of three populations of *P. sarana* in the study by Imran *et al.* (2010) (*D*=0.0183) and the average distances within the species of pangasid catfish (*D*=0.106) by Pouyaud *et al*. (1998) are lower than that observed in the present study. Leesa-Nga-SN *et al*. (2000) 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. Similar results were observed by Shimizu *et al*. (1993) and also suggested that the highest genetic differentiation among the five groups of *Rhinogobius* was the species or subspecies level. Nei (1972) 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 (1975) reported that the *D*-value between subspecies is approximately 0.20. The higher genetic distance obtained in the present study for the *L. gonius* therefore strongly reflect a sub-species diversity in the said species.

CONCLUSION

Morphometric characters were found to be more suitable than meristic characters for a good discrimination among *L. gonius* populations. The differences among the stocks may have been due to environmental as well as genetic variations. The results of the study are useful as baseline information of *L. gonius* populations for further studies. In both aquaculture and open water management, it is essential to select the genetically superior stock with better features. More research, especially on genetic studies and investigation of environmental factors should be carried out for mass seed production and conservation of selected superior stocks through proper management to save the endangered species from extinction.

Finally, the morphological and allozyme results suggested that the considerable environmental and genetic variation existed among the populations of *L. gonius*. This has major implications in understanding morphological and genetic diversity among populations and this can be used as baseline information for further study viz. DNA level work (RAPD, RFLP, microsatellite, etc.) should be undertaken with increased number of samples from different locations to find out more informative results for better clarification and confirmation of genetic variation. So, this study will not only help to know the genetic structure of the species, but will open the window of conserving this endangered species from extinction in near future. Populations with high level of genetic diversity will give high conservation status. Mass seed production and conservation of the genetically superior stock through proper management is highly recommended to save this species from being extinction. So, selective breeding program can be performed using genetically superior stock to increase the gene pool variation to conserve.

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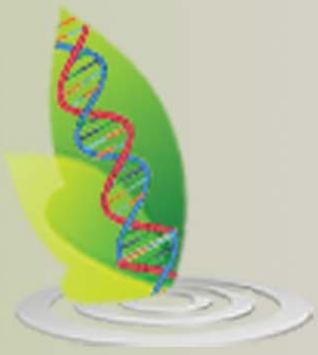

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