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Genetic variation among three wild populations of stinging catfish (*Heteropneustes fossilis*) by allozyme electrophoresis

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Abstract

Stinging catfish (*Heteropneustes fossilis*) is one of the most popular indigenous catfish having considerable potential for aquaculture and commercial importance in Bangladesh. With a view to assessing the genetic status of *H. fossilis*, three samples Chalan Beel (Pabna), Burungi Beel (Jamalpur) and Bagapura Beel (Mymensingh) were analyzed. For genetic variation study, five enzymes (LDH, EST, MDH, PGM and GPI) were used encoded by eight loci of which three were polymorphic (*Mdh-1**, *Est-1** and *Gpi-1**). The highest mean proportion of polymorphic loci, mean number of allele and the mean proportion of heterozygous loci per individual of the Chalan Beel population were observed (25.00%, 1.250 and 6.250%, respectively). The highest gene flow (33.5) and lowest population differentiation (0.0074) found in Burungi Beel-Bagapura Beel indicated the close relationship among them. In the Nei's UPGMA dendrogram, the Chalan Beel population formed one cluster by the genetic distance of 0.0371 and the other cluster was formed by Burungi Beel and Bagapura Beel populations (*D*=0.003). The results suggested that a considerable genetic variation is maintained among the natural *H. fossilis* populations.

Keywords: Allozyme electrophoresis, stinging catfish, *Heteropneustes fossilis*, genetic variation, dendrogram

INTRODUCTION

The stinging catfish, *Heteropneustes fossilis* (Bloch) is locally known as "shing". It is important catfish indigenous to many Asian countries (Akand *et al.* 1991). The indigenous catfish, *H. fossilis*, is a popular and valuable fish in Bangladesh. It is popular not only for its good taste but also highly esteemed from nutritional and medical point of view. Popularity of this species for cultivation is also high due to extreme hardiness, fast growth, high fecundity, efficient feed utilization and ability to survive in poorly oxygenated water.

Habitat degradation, disease, over fishing, injudicious application of pesticides in agricultural fields and release of industrial effluent have recently become great constraints for fish biodiversity in most aquatic ecosystems in Bangladesh, and thus remarkable changes have already been observed in natural *H. fossilis* populations such as either in erosion or in genetic diversity. There might be several strains or sub-population of *H. fossilis* in different closed water bodies. In order to understand those variations, intensive study on existing genetic diversity of natural populations of *H. fossilis* in Bangladesh is urgently needed.

Genetic variation serves as a useful tool for characterization of different species or strains within a species and evaluation of changes in genetic structure of species over time. To manage any biological resources

effectively, researchers must identify the level of genetic variation within and among populations. Allozyme electrophoresis is a well-established technique for revealing genetic variation. This molecular marker has been used as an effective tool for fish population studies and fishery management (Utter 1991). The genetic diversity of various catfishes was studied by this technique; Senanan *et al.* (2004) studied hybrid catfish (female Thai walking catfish, *Clarias macrocephalus* and male African catfish, *C. gariepinus*) through allozyme electrophrosis. Hardjamulia *et al.* (2001) worked on inter-specific characterization of *Clarias batrachus* strains. Zawadzki *et al.* (2000) investigated three species of armored catfish of the genus *Loricariichthys* by starch gel isozyme electrophoresis. Allozyme studies have not been undertaken to identify genetic variation of wild *H. fossilis* in Bangladesh. The objective of the present study is to assess the population structure of stinging catfish which may serve as an important part of the effort in the measures for the conservation and production of highly diversified stock of this species.

METHODOLOGY

Research Laboratory: The experiment was accomplished in the Fisheries Biology and Genetics Laboratory, Bangladesh Agricultural University, Mymensingh. The equipment and other facilities available at the Fisheries Faculty were also used whenever required. The research study was conducted from November 2007 to May 2008.

Sample collection: The experimental fish were collected from three different *beels* (wetlands) in Bangladesh (Figure 1). Details of sources, number of specimens and date of collection are shown in Table 1.

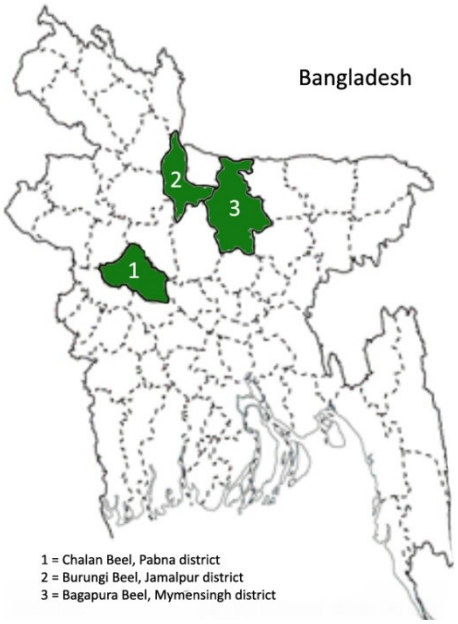

Figure 1: Map of Bangladesh indicating sampling points

Table 1: Sources, number of specimens and date of collection of *Heteropneustes fossilis* populations

Sample No.	*Waterbody /Beel*	*Collection site (District)*	*No. of fish*	*Date of collection*
1	Chalan	Bhangura (Pabna)	30	Nov 27, 2007
2	Burungi	Melandaha (Jamalpur)	30	Nov 29, 2007
3	Bagapura	Mymensingh Sadar (Mymensingh)	30	Dec 04, 2007

Allozyme study: Muscle samples were taken from each individual and stored at –18^{o}C until electrophoretic analysis. 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. In this way, at first the starch powder was weighed by electronic balance (METTLER TOLEDO, PG503-SDR, Switzerland) and was transferred into a 1L Erlenmeyer flask containing 17ml (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 until transparent solution of the gel was observed using a Bunsen burner. 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 x 22 x 0.5 cm^3) attached with plastic frame (16 x 20 x 0.5 cm^3). The hot gel was allowed to cool at room temperature for about 2 hours. The gel was covered with OHP sheet to prevent desiccation of gel after 30 min. Finally, after cooling the gel was preserved overnight into a refrigerator at 5-80C to increase hardness for easily slicing of the gel. Enzyme were assessed using horizontal starch gel electrophoresis in one buffer systems (CA 6.1) and gel staining procedures were based on the methods of (Shaw and Prasad, 1970). The enzymes examined were as follows: Esterase (EST; EC 3.1.1.1), Glucose-6- phosphate isomerase (GPI; EC 5.3.1.9), Lactate dehydrogenase (LDH; EC 1.1.1.27), Malate dehydrogenase (MDH; EC 1.1.1.37) and Phosphoglucomutase (PGM; EC 5.4.2.2). After staining, the gel slice were preserved using cellophane paper, 10% acetic acid and 10% glycerin. At first, the staining gel washed with 10% acetic acid kept in 10% glycerin for 10-15 min. Finally, the gel was kept on the cellophane paper and covered by other cellophane paper. Then the gel was kept in oven at 50 to 55^{o}C since over night for drying the gel.

Genetic data analysis: Allelic frequencies were inferred directly from observed genotypes. Hardy-Weinberg equilibrium of genotype frequencies was tested using a

chi-square (χ^2) test. When the most common allele existed in a frequency less than 0.95 at a given locus, this locus was regarded as polymorphic. The preceding analysis of allozyme data was performed using POPGENE, (version 1.32) (Yeh *et al.* 1999). Using this program, the mean proportion of heterozygous loci per individual, mean proportion of polymorphic loci per population, and average number of alleles per locus were calculated to quantify genetic variability for each population (Lewontin and Hubby 1966, Lewontin 1974). Expected (He) and observed heterozygosity (Ho) were also calculated. The preceding analysis of allozyme data was performed using POPGENE, (version 1.32) (Yeh *et al.* 1999). The inbreeding co-efficient (Fis) was measured to estimate the deviation of random mating (heterozygote deficiency or excess) (Wright 1978). To examine the genetic divergence and relationships among populations, Nei's (1972) standard genetic distance was calculated and a UPGMA tree based on the genetic distance matrix was constructed according to Nei (1972).

RESULTS AND DISCUSSION

Alleles and genotypes: The electrophoretic patterns of muscle tissue showed that the enzymes were controlled by the genes at 8 presumptive loci where two genotypes (**aa* and **ab*) were found for the three loci (*Est-1**, *Mdh-1** and *Gpi-1**) by two alleles (**a* and **b*), and only one genotype (**aa*) was observed for *Ldh-1**, *Ldh-2**, *Mdh-2**, *Pgm** and *Gpi-2**. On the average 1.50 genotypes were produced by 1.357 alleles at the 8 loci (Table 2).

Table 2: List of alleles and genotypes examined in *Heteropneustes fossilis*

Locus	Alleles		Genotypes	
	No.	Types	No.	Types
*Ldh-1**	1	**a*	1	**aa*
*Ldh-2**	1	**a*	1	**aa*
*Est-1**	2	**a,*b*	2	**aa, *ab*
*Mdh-1**	2	**a,*b*	2	**aa, *ab*
*Mdh-2**	1	**a*	1	**aa*
*Pgm**	1	**a*	1	**aa*
*Gpi-1**	2	**a,*b*	3	**aa, *ab, *bb*
*Gpi-2**	1	**a*	1	**aa*
Average	1.375		1.50	

Allele frequencies: Allele frequencies were calculated directly from observed genotypes at eight loci in 90 specimens of three natural populations of *H. fossilis*. The allele frequencies for the eight loci in each population analyzed are shown in Table 3. Among the eight loci, the Chalan Beel population showed two (*Est-1** and *Gpi-1**, Figure2 and 3), the Burungi Beel population showed two (*Mdh-1** and *Est-1**) and the Bagapura Beel population showed one (Mdh-1*) polymorphic loci. 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 Chalan Beel population showed significant variation only in allele frequency of *Gip-1** locus whereas no significant variation occur in allelic frequencies in other populations.

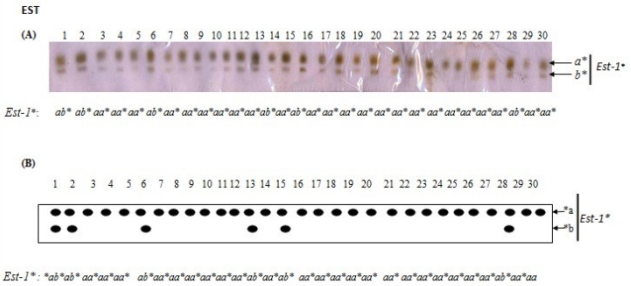

Figure 2: (A) Electrophoregram of enzyme esterase (EST) and (B) Schematic representation of *Est-1** loci in the Chalan Beel population of *Heteropneustes fossilis*

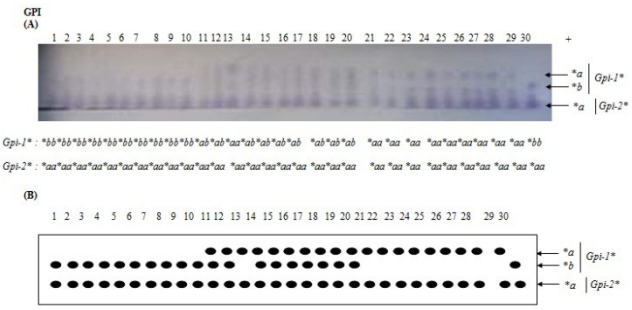

Figure 3: (A) Electrophoregram of enzyme glucose-6- phosphate isomerase (GPI) and (B) Schematic representation of *Gpi-1** and *Gpi-2** loci in the Chalan Beel population

In the present study five enzymes LDH, EST, MDH, PGM and GPI were used in CA 6.1 buffer system and found clear resolution for muscle tissue among the three populations of stinging catfish. According to Khan (1999) the allelic enzyme activity varies from buffer to buffer, species to species and also tissue specific. Alam *et al.* (2002) 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.

According to Lewontin (1974), 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 is a commonly used measure of electrophoretically detectable variation in a population. Another commonly used measure of genetic variation is the average frequency of heterozygous loci per individual (*H*).

Table 3: Allelic frequencies at 8 presumptive loci among *Heteropneustes fossilis* populations

Locus	Allele frequency			
	Allele	Chalan Beel	Burungi Beel	Bagapura Beel
*Ldh-1**	*a	1.0000	1.0000	1.0000
*Ldh-2**	*a	1.0000	1.0000	1.0000
*Est-1**	*a	0.9000	0.9500	1.0000
	*b	0.1000	0.0500	-
*Mdh-1**	*a	1.0000	0.9167	0.9333
	*b	-	0.0833	0.0667
*Mdh-2**	*a	1.0000	1.0000	1.0000
*Pgm**	*a	1.0000	1.0000	1.0000
*Gpi-1**	*a	0.4833	1.0000	1.0000
	*b	0.5167	-	-
*Gpi-2**	*a	1.0000	1.0000	1.0000

Genetic variability: The mean proportions of polymorphic loci in Chalan Beel, Burungi Beel and Bagapura Beel populations were 25.00, 25.00 and 12.50% respectively and average polymorphic loci was 20.83%. The mean number of alleles per locus (N_a) for all populations were 1.125 in average and ranged from 1.125 (Bagapura Beel) to 1.250 (Chalan Beel and Burungi Beel) (Table 4). The mean number of effective alleles (*Ne*) per locus for all populations were 1.0686 in average and ranged from 1.0178 (Bagapura Beel) to 1.1522 (Chalan Beel) (Table 4). The mean proportion of heterozygous loci per individuals for all populations was 3.75% in average and ranged from 1.67% (Bagapura Beel) to 6.25% (Chalan Beel). The observed heterozygosity (H_o) was 0.0375 in average and ranged from 0.0167 (Bagapura Beel) to 0.0625 (ChalanBeel). The average expected heterozygosity (H_e) was 0.0445 and ranged from 0.0158 (Bagapura Beel) to 0.0864 (Chalan Beel). The genetic variation summaries are shown in Table 4.

In this study, the observed proportion of polymorphic loci per population ranged from 12.50% to 25.00% (average 20.83%) which is relatively similar (average 22%) to that reported by Hanzawa *et al.* (1988) for freshwater dace, 18% of 20 species for pangasid catfish by Pouyaud *et al.* (1998), 19.42% for four hatchery population of Thai pangas, *P. hypophthanmus* reported by Shahid (2004) but much lower (65.22%) than that for yellow catfish *Mystus nemurus* reported by Leesa-Nga *et al.* (2000), 50% for Indian *C. gariepinus* reported by Lal *et al.* (2003) and 36.36% for four populations of Thai pangas, *P. hypophthalmus* reported by Eunus (2004). Nevo *et al.* (1984) estimated polymorphic loci (*P*) as 15.2% ($P \leq 0.95$) for polymorphism in fish in general. Therefore, the studied *H. fossilis* populations showed a lower level of polymorphism in comparison with the above mentioned catfishes.

Table 4: Genetic variability at 8 loci in *Heteropneustes fossilis* populations

Populations	Mean proportion of polymorphic loci* (%)	Mean number of alleles (Na) per locus	Mean number of effective alleles (N_e) per locus	Mean proportion of heterozygous loci per individual (%)	Heterozygosity		
					H_o	H_e	H_o/H_e
ChalanBeel	25.00	1.250	1.1522	6.25	0.0625	0.0864	0.7234
BurungiBeel	25.00	1.250	1.0357	3.33	0.0333	0.0315	1.0571
BagapuraBeel	12.50	1.125	1.0178	1.67	0.0167	0.0158	1.0560
Average	20.83	1.125	1.0686	3.75	0.0375	0.0445	0.9455

*$P \leq 0.95$

The mean number of alleles per locus (1.125) as obtained in the present study was lower than that obtained by Na-Nakorn *et al.* (1998) (1.275) for *Clarias macrocephalus* and that obtained by Barua *et al.* (2004) (1.3) for *P. hypophthalmus*. Sumantadinata and Taniguchi (1990) found number of alleles per locus in the range from 1.087 to 1.217 (mean 1.159) for Indonesian stocks of common carp and that for Japanese stocks was found to range from 1.174 to 1.391 (mean 1.296). Shahid (2004) found the mean number of alleles per locus were 1.3 for the Thai pangas. The value obtained in this study was found to be lower.

The average heterozygous loci per individual obtained was 3.75% (range from 1.67 to 6.25) which was much lower than the results of 11.25% obtained by Barua *et al.* (2004), 13.33% obtained by Eunus (2004) for four hatchery populations of Thai pangas, *P. hypophthalmus* and 6.3% obtained by Grobler *et al.* (1997) for African catfishes, *Clarias gariepinus*. This indicates the low level of heterozygous loci per individual in the present samples.

The average observed heterozygosity (H_o) obtained in the present study (0.0375) is lower than that (0.178) reported by Muneer *et al.* (2007) for yellow catfish, *H. brachysoma* and that (0.091) reported by Pouyaud *et al.* (1998) but similar (0.038) to that obtained by Hardjamulia *et al.* (2001) for *C. batrachus* strains from Java and Sumatra. The average observed heterozygosity (H_o) obtained in the present study (0.0375) is also similar to that reported by Triantafyllidis *et al.* (1999) for European catfish species, *S.*

glanis and *S. aristotelis*. The higher observed and expected heterozygosity (H_o=0.0625 and H_e=0.0864) exhibited by the Chalan Beel population, which is closer to the average values of H_o=0.055 obtained for teleosts (Kirpichnikov 1992), indicated that the gene pool of the Chalan Beel population was maintained effectively. Nevo (1978) reported that an average observed heterozygosity (H_o) value for bony fishes was 0.051. However, the He values obtained in the present study do not fall in the range of values (H_e = 0.02 to 0.03) which are generally considered the lower margins of genetic variability for fishes (Nevo *et al.* 1984, Kirpichnikov 1992). The level of heterozygosity is related with the size of populations within a species. The species with small populations might lose 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 programmes. The average heterozygosity (H_o or H_e) is considered a good indicator of the genetic variability throughout the genome of the population (Leary and Booke 1990, Allendorf and Ryman 1986).

Population differentiation (F_{ST}) and Gene flow (N_m): The summary of the genetic differentiation (F_{ST}) and gene flow (N_m) are given in Table 5. The Nei's (1972) analysis of gene diversity within populations estimated the genetic differentiation (F_{ST}) and the gene flow (N_m) over all three populations were 0.2622 and 0.7035 respectively. In pair-wise analysis, comparatively higher N_m value (33.50) was estimated between the Burungi Beel and the Bagapura Beel populations corresponding lower level of F_{ST} value (0.0074) (Table 5).

Table 5: Pair-wise and overall population differentiations (F_{ST}) and Gene flow (N_m) in three *Heteropneustes fossilis* populations

Population	F_{ST}		N_m*	
	Pair-wise	Overall	Pair-wise	Overall
Chalan-Burungi	0.2296		0.8387	
Burungi-Bagapura	0.0074	0.2622	33.5000	0.7035
Bagapura-Chalan	0.2593		0.7142	

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

The co-efficient of gene differentiation (F_{ST}) in all three natural populations of stinging catfish examined (Nei 1975) for all loci was 0.2622, which is larger (0.0396) than that reported by Nasren (2007) for four population of shing while analysis with microsatellite marker, but lower than that obtained (0.792) for Thai pangas, *P. hypophthalmus* (Barua *et al.* 2004) and that obtained for other freshwater fishes such as loach (0.774) reported by Khan and Arai (2000).

Genetic differentiation:The genetic distance (*D*) values among three populations ranged from 0.003 to 0.0371. The minimum genetic distance (*D*=0.003) was observed between the Burungi Beel and Bagapura Beel populations, while the maximum value (*D*=0.0371) was found between the Chalan Beel and Bagapura Beel populations (Table 6).

The UPGMA dendrogram (Nei 1978) constructed from Nei's (1972) genetic distances is shown in Figure 4. The UPGMA dendrogram showed two clusters among the three natural populations. The Burungi Beel and the Bagapura Beel populations formed one cluster while the Chalan Beel population formed another cluster. The dendrogram showed that the Chalan Beel population separated from the Burungi Beel and the Bagapura Beel populations by a genetic distance of 0.0371 where the Burungi Beel population is different from the Bagapura Beel population by the genetic distance of 0.003 (Table 6).

Table 6: Nei's original measures of genetic identity (above diagonal) and genetic distance (below diagonal) estimated among 3 populations of *Heteropneustes fossilis*

Population	Chalan Beel	Burungi Beel	Bagapura Beel
Chalan Beel	****	0.9637	0.9636
Burungi Beel	0.0370	****	0.9997
Bagapura Beel	0.0371	0.0003	****

The observed genetic distances among the three populations of stinging catfish in the present study were much more lower than the findings of Pouyaud *et al.* (1998) who found the average distances within the species *P. polyuranodon* (*D*=0.106) between population of Kalimantan and the population of Chao Phraya or *P. micronema* (*D*=0.145) between population of TelukKuantan in Sumatra and population of sole in Japan. Leesa-Nga *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. On the other hand, the genetic distance (*D*=0.109) between interspecies of *P. nasutus* and *P. conchophilus* and also similar value (*D*=0.158) shown in the distance between *P. bocourti* and *P. djambal* (Pouyaud *et al.* 1998). 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. Considering from the above-mentioned criteria, the studied *H. fossilis* may be categorized as local race or population.

The observed genetic distance differs between the Chalan Beel and other populations might be due to geographical isolation because the Chalan Beel population is geographically isolated from the Burungi Beel and the Bagapura Beel populations. On the other hand, there is no geographical barrier between the Burungi Beel and the Bagapura Beel populations. So there might be a possibility of mixing of the Bagapura Beel and the Burungi Beel populations.

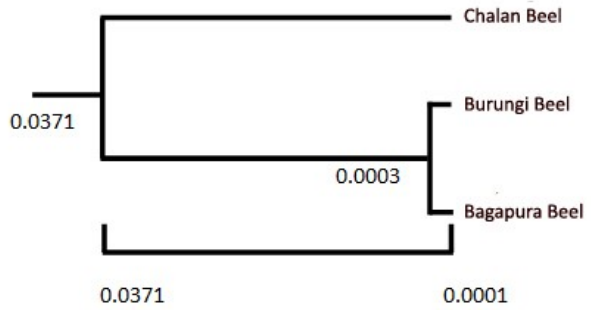

Figure 4: UPGMA dendrogram showing the genetic distance (*D*) among three natural populations (Nei 1972)

CONCLUSION

The result of the present study would be useful to know genetic variation and structure of different populations of the studied species before undertaking any stock improvement and conservation program. To discuss the detailed population structures over time, further studies are required dealing with a large number of populations intensively sampled from different parts of the country. Moreover, large sample size and more enzymes are required. This information would help to identify distinct population groups existing in Bangladesh and consequently enables to formulate genetic conservation plan. Moreover, it may show the impacts of brood stocks management practices. However, there are few draw backs in the present study. Due to limitation of highly sophisticated equipment and reagents, it was not possible to analyze huge number of individuals per stocks. We screened 30 individuals from each of the stocks and numbers of enzyme were only five, which is not sufficient to draw a conclusion about the genetic structure of the three stocks of stinging catfish in Bangladesh. Further studies involving large number of samples and more loci are needed to have a precise knowledge about the genetic structure of this valuable fish species.

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