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Short Communication

Population Genetic Analysis of Striped Dwarf Catfish Golsha, *Mystus cavasius* (Hamilton 1822) Using Allozyme Markers in Bangladesh

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Abstract

Population genetic variation, in four populations of golsha, *Mystus cavasius* (Hamilton 1822) from Bangladesh, namely the Bulla River, the Dhonu River, the Mithamoin *haor* (dead basin of oxbow lakes) and the Bonhi *baor* (closed waterlogged areas), were analysed using allozyme markers. Seven loci encoded in muscle and liver tissues by four enzymes were examined using starch gel electrophoresis. Five loci were polymorphic (P_{99}). The mean proportion of polymorphic loci was highest (71.43%) in the Mithamoin *haor* population whereas the highest mean number of alleles per locus (2.143) was observed in both the Mithamoin and Bonhi *baor* populations. The observed and the expected heterozygosity (0.176 and 0.193 respectively) were highest in the Bonhi *baor* population. None of the pairwise F_{ST} values was statistically significant. The highest (0.109-0.125) F_{ST} and genetic distance (D) values were found between the Bonhi *baor* and all other populations reflecting the more distant location, in a separate catchment, of the Bonhi population. The genetic distance also showed the Bonhi as an outlier separated from other three populations. Although the Dhonu and to a lesser degree, the Bonhi populations showed some evidence of deviation from Hardy-Weinberg equilibrium, all populations had sufficient variation for use in selective breeding programmes and for the commercial propagation of golsha as future broodstocks.

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Introduction

The striped dwarf catfish golsha, *Mystus cavasius* (Hamilton 1822) belongs to one of the richest and most important family of teleosts, Bagridae (210 valid species), in the order Siluriformes whose members are distributed all over the world (Day 1878). There are 79 species and subspecies of *Mystus* worldwide (Nelson 1994). The golsha is widely distributed in Bangladesh, India, Nepal, Pakistan, Sri Lanka, Myanmar, Thailand, Indo-China, Malaysia, East Indies, Syria and West Africa (Yadav 1999). Known locally as golsha or kabashi-tengra, it is considered one of the most important small indigenous species (SIS) in Bangladesh and grows to a length of 20 to 23 cm (IUCN 2000). It is easily recognized by its elongated and compressed body, obtuse snout and, slightly longer upper jaw. In addition, the dorsal, adipose and caudal fins are shaded with melanophores and a black spot is present behind the operculum.

Golsha is one of the catfishes which has a high market value (USD $10 \cdot 1^{-1}$). Populations have declined considerably due to increased fishing pressure and various anthropogenic activities leading to siltation, aquatic pollution, and loss of natural habitat for spawning and growth. Although endangered in Bangladesh, a few endemic golsha populations are still found in tidal rivers and lakes and in beels (a natural depression of soil which contain water usually surrounded by landmasses but smaller than haor), canals, ditches, haors (dead basin of oxbow lakes) , baors (closed waterlogged areas) and inundated fields (DoF 2010). Attempts are needed to boost golsha production through effective management in natural water bodies. However, better information on taxonomy and genetic variation of wild golsha populations is needed to inform management for improved stock sustainability.

Some remarkable changes in body colour and morphology has been reported in *M. cavasius* in different closed water bodies (Rasul 2010) suggesting genetic variation among these populations. Genetic variation in a species enhances the capability of organism to adapt to changing environment and is necessary for the survival and biological potential of fish stocks (Tave 1999). Allozyme electrophoresis has been one of the most popular approaches used to examine population genetic and stock structure in fishes (Allendorf 1983). Allozyme data are available for related catfishes including the yellow catfish *Mystus nemurus* (Valenciennes 1840) in Thailand (Leesa-Nga et al. 2000) and Malaysia (Usmani et al. 2003). Information on *Mystus vittatus* (Bloch 1794) in India has been obtained using the RAPD-PCR technique (Garg et al. 2009). However, there is no information to date on *M. cavasius*. The present study was undertaken to provide some basic information on the genetic variation of wild populations of *M. cavasius* using allozyme markers.

Materials and Methods

Golsha were collected from four wild populations; one *haor*, one *baor* and two rivers in Bangladesh during October to November, 2010 (Table 1, Fig. 1.). The live golsha fish were brought to the Laboratory of Fisheries Biology and Genetics, Bangladesh Agricultural University,

Mymensingh where tissue samples (muscle and liver) were taken from each individual and stored at -18 ^{o}C until electrophoretic analysis. Horizontal starch gel electrophoresis with amine-citrate buffers (CA 6.1) and histochemical-staining techniques were used according to Shaw and Prasad (1970) to analyse four enzymes [glucose-6-phosphate isomerase (GPI, 5.3.1.9), lactate dehydrogenase (LDH, 1.1.1.27), malate dehydrogenase (MDH, 1.1.1.37) and phosphoglucomutase (PGM, 5.4.2.2)].

Table 1. Population of golsha (*M. cavasius*) analysed in the study.

Population no.	Localities River/*Haor*/*Baor**ψ	No. of individuals	Date of collection
1	Bulla river (Subornogram, Noakhali) **[Bulla]**	30	25 October 2010
2	Dhonu river (Mohonganj, Netrokona) **[Dhonu]**	30	21 October 2010
3	Mithamoin *haor* (Mithamoin, Kishorganj) **[Mithamoin]**	30	28 October 2010
4	Bonhi *baor* (Gopalganj, Khulna) **[Bonhi]**	30	10 November 2010

***** Collection of samples with the sadar area and district; items in bold show the code name of each population.

ψ Distance between the Dhonu river and the Mithamoin *haor* populations is about 40 km; others distance are about 250-300 km

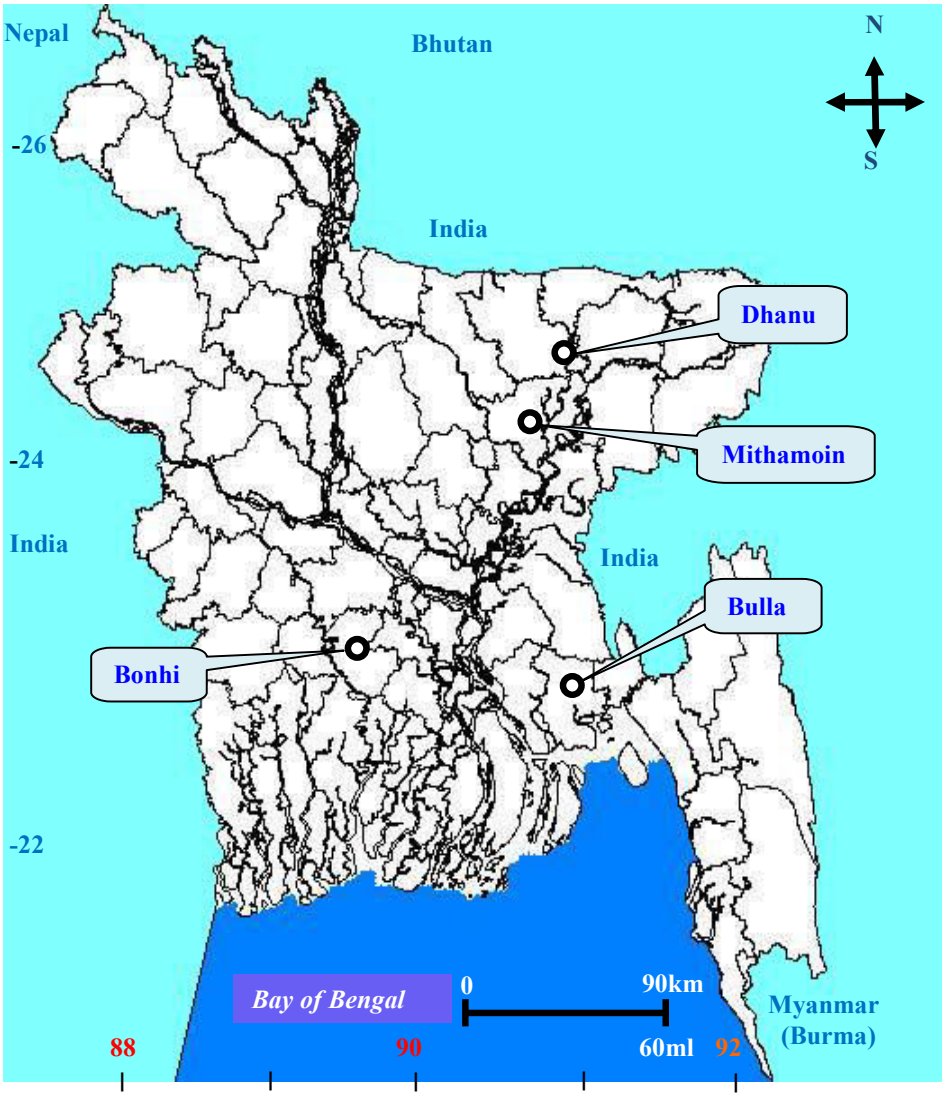

Fig. 1. Map showing sample collection sites of golsha (*M. cavasius*) populations.

Allele frequencies were calculated directly from observed genotypes. When the most common allele existed in a frequency less than 0.99 at a given locus, this locus was regarded as polymorphic. The distribution of observed genotypes was compared with that expected from the Hardy-Weinberg Equilibrium (HWE) using a χ^2 test. The analysis of allozyme data, calculation of population genetic parameters and tree building were performed using POPGENE (version 1.32) (Yeh et al. 1999), G–STAT (version 3.1) (Siegismund 1995) and TREEVIEW (Roderick 2000) computer programme packages. The tree (dendrogram) was constructed using the unweighted pair-group method of arithmetic averages (UPGMA) for the analysis of divergence and relationships among populations using Nei's (1972) genetic distance (*D*). F_{ST} values between pairs of populations were analysed and their significance tested using the programme ARLEQUIN 2.0 (Schneider et al. 2000).

Results

The electrophoretic patterns of muscle and liver samples showed that the enzymes were controlled by seven presumptive loci, two of which (*Ldh-1** and *Ldh-2**) were monomorphic and five were polymorphic (*Gpi-1*, Gpi-2*, Mdh-1*, Mdh-2** and *Pgm**). The allele frequencies are shown in Table 2. The χ^2 test showed significant ($P < 0.05$) deviations from the allele frequencies expected under conditions of HWE at *Gpi-1** and *Pgm** in the Dhonu population and at *Gpi-2** in the Bonhi population (Table 2). The lower observed values of *Ho* suggest deficits of heterozygotes. The mean proportion of polymorphic loci was highest (71.43%) in the Mithamoin *haor* whereas the highest mean number of allele per locus (2.143) was observed in both the Mithamoin and the Bonhi populations. The observed and the expected heterozygosities (0.176 and 0.193 respectively) were highest in the Bonhi *baor* population (Table 3).

Table 2. Allele frequency at 5 polymorphic loci of four *M. cavasius* populations.

	Allele	Bulla	Dhonu	Mithamoin	Bonhi
Gpi-1*	***a**	0.017	-	-	0.017
	***b**	0.933	0.967	0.017	0.966
	***c**	0.050	0.033	0.983	0.017
	P	0.990NS	0.000*	1.000NS	0.999NS
	χ^2 (d.f)	0.113 (3)	59.018 (1)	0.000 (1)	0.018 (3)
Gpi-2*	***a**	-	0.050	-	-
	***b**	-	0.233	0.167	0.050
	***c**	1.000	0.700	0.816	0.550
	***d**	-	0.017	0.017	0.350
	***e**	-	-	-	0.050
	P	-	0.989NS	0.716NS	0.003*
	χ^2 (d.f)	-	0.903 (6)	1.356 (3)	19.891 (6)
Mdh-1*	***a**	0.150	-	0 .017	0.417
	***b**	0.833	1.000	0.983	0.583
	***c**	0.017	-	-	-
	P	0.785NS	-	1.000NS	0.491NS

	χ^2 (d.f)	1.065 (3)	-	0.000 (1)	0.474 (1)
Mdh-2*	***a**	0.033	-	-	-
	***b**	0.967	1.000	0.966	1.000
	***c**	-	-	0.017	-
	***d**	-	-	0.017	-
	P	0.895NS	-	0.999NS	-
	χ^2 (d.f)	0.018 (1)	-	0.018 (3)	-
Pgm*	***a**	-	0.067	0.033	0.017
	***b**	0.967	0.833	0.950	0.883
	***c**	0.033	0.100	0.017	0.100
	P	0.895NS	0.009*	0.997NS	0.444NS
	χ^2 (d.f)	0.018 (1)	11.404 (3)	0.055 (3)	2.677 (3)

P: Probability of chi-square value, significant level: $*P < 0.05$, NS: Not-significant.

Table 3. Genetic variabilities at 5 polymorphic loci of *M. cavasius* populations.

Population	**The mean proportion of polymorphic loci (%)**	**The mean number of alleles per locus (*Na*)**	**The mean proportion of heterozygous loci per individual (%)**	**Heterozygosity**				**Shannon's information index**
				H_o	H_e	H_o/H_e	1-H_o/H_e	
Bulla	57.14	1.86	15	0.086	0.078	1.103	-0.103	0.154
Dhonu	42.86	1.86	35	0.100	0.117	0.893	0.107	0.217
Mithamoin	71.43	2.14	24	0.086	0.077	1.116	-0.116	0.157
Bonhi	57.14	2.14	31	0.176	0.193	0.912	0.088	0.322
Mean±SE	**57.14±5.22**	**2.00±0.07**	**26.2±3.9**	**0.112± 0.019**	**0.116± 0.024**	**1.006± 0.054**	**- 0.006± 0.054**	**0.213± 0.035**

No significant difference was found among the pair-wise F_{ST} values (Table 4). However, there was an indication that the measures of genetic differentiation were related to the degree of geographical separation of golsha populations. The pair-wise population differentiation (F_{ST}) value between the Bonhi *baor* and the Mithamoin *haor* was the highest (0.125) while the genetic distant value (*D*) was the lowest (0.003) between the two closest populations Dhonu River and Mithamoin *haor* among all the populations. The highest F_{ST} value represents a high level of population differentiation (Table 4).

Table 4. Pair-wise population differentiations (F_{ST}) (above diagonal) and genetic distance (below diagonal) estimated among four populations of *M. cavasius* based on 5 loci.

Populations	Bulla	Dhonu	Mithamoin	Bonhi
Bulla	****	0.078	0.049	0.114
Dhonu	0.018	****	0.017	0.109
Mithamoin	0.009	0.003	****	0.125
Bonhi	0.038	0.044	0.042	****

The highest genetic distance (0.044) showed the Bonhi river as an outlier separated from other three populations. Three other populations, the Mithamoin haor first grouped with the Bulla river population by D = 0.038 and then with the Dhonu river population by D = 0.042 due to their geographic separation (Table 4).

Discussion

The lowest F_{ST} value (0.017) of *M. cavasius* populations as obtained in the present study is lower than that obtained for other freshwater fishes such as loach (0.774) (Khan and Arai 2000) and freshwater golsha (0.612) (Garg et al. 2009). However, the lack of statistical significance of all F_{ST} values suggests little genetic differentiation among the populations. Nevertheless, there does appear to be an increase in both measures of genetic differentiation of the populations (D and F_{ST}) with increasing geographical separation. The Bonhi *baor* is a dead basin of oxbow lakes far removed from the other populations. It is the largest *baor* (about 282 ha) in Bangladesh which is used for nursing of many wild fish species, and is the largest breeding ground of *M. cavasius* population having water depth 180-210 cm (sometimes 300 cm) and good vegetation to assist growth and survival.

Although there were indications of heterozygote deficits at two sites these were minor and affected different loci, suggesting no major effects of inbreeding at any of the populations. The general fit otherwise to HWE allele frequencies and the occurrence of reasonable levels of variation suggests any of these populations would be acceptable sources for broodstock. Although F_{ST} values were not significant, the hints of increasing genetic differentiation with increasing geographical separation suggests young fish should not be used to stock sites great distances from the origin of their parents until further work establish the nature of spatial variation in genetic variation in this species more clearly.

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