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POPULATION GENETIC STRUCTURE OF AN ENDANGERED KALIBAUS, *Labeo calbasu* (HAMILTON, 1822) REVEALED BY MICROSATELLITE DNA MARKERS

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ABSTRACT

The population genetic structure of kalibaus *Labeo calbasu* collected from four wild and a hatchery population was studied using microsatellite DNA marker analysis. Five heterologous microsatellite markers (*Lr10, Lr21, Lr24, Lr26* and *CcatG1*) developed from rohu (*Labeo rohita)* and catla (*Gibelion catla*) were analyzed to test the genetic variability of kalibaus stocks. The number of alleles observed in the loci ranged from 2-10. The loci were found to be polymorphic ($<P_{95}$) in all the populations. The average numbers of possessed alleles were higher by the four wild stocks than the hatchery stock. The average number of allele was the highest in the Jamuna population (5.8) and the least in the Hatchery population (4.8). The observed average heterozygosity (H_o) in the Jamuna population (0.776) was the highest followed by the Halda (0.667), the Haor (0.661) and the Padma (0.642) populations. Except loci *Lr10* and *Lr24* in the Halda and locus *Lr10* in the Padma and Hatchery populations, significant deviations from Hardy-Weinberg Equilibrium (HWE) were detected in all cases. The F_{ST} values and the N_m values indicated high level of differentiation and a low level of gene flow between the populations. The largest genetic distance value (D = 0.543) was measured between the Jamuna and the Hatchery populations while the least value (D =0.124) was observed between the Padma and the Halda populations. The estimated genetic population structure and potential applications of microsatellite markers may assist the proper management of kalibaus populations in the wild.

INTRODUCTION

Kalibaus *Labeo calbasu* (Hamilton, 1822), is one of the four Indian major carps available in Bangladesh (family: Cyprinidae, order: Cypriniformes), possessing a karyotype with 25 pairs of diploid (2n) chromosomes (Reddy, 1990). *L. calbasu* is distributed in many countries namely Bangladesh - the Padma–Brahmaputra, i.e. Padma, Jamuna, Arial Khan, Kumar and Old Brahmaputra and the Halda River and Pakistan, India, Myanmar, Thailand and China (Reddy, 1990).

L. calbasu has great commercial importance due to its adaptability to a wide range of environments, palatability and consumer preference. At the beginning of 1980s, when carp polyculture was started in Bangladesh owing to the success of artificial propagation, all four Indian major carps (*Labeo ro-*

hita, *Gibelion catla*, *Cirrhinus mrigala* and *L. calbasu*) were brought under aquaculture. However, due to slower growth (compared to other three) and unavailability of the fingerlings, farmers lost their interest in *L. calbasu*. Over the last few decades the abundance of this species has seriously been decreased in nature due to overfishing, habitat degradation, aquatic pollution and other anthropogenic and natural causes. *L. calbasu* is now enlisted as an endangered species from the biodiversity point of view (IUCN, 2000). Moreover, there has been a long-term decline in genetic quality of *L. calbasu* due to inbreeding, inter-species hybridization and improper brood management practice by the hatchery owners. A little amount of *L. calbasu* seed is presently being produced in a few hatcheries of Bangladesh without considering the genetic quality.

Many hatcheries of Bangladesh practice hybridization of kalibaus with rohu (*Labeo rohita)* or catla (*Gibelion catla*). The sustainable utilization of genetic resources, including fish, is a vital part in improving the standard of living in a developing country like Bangladesh. Therefore, it is high time to save *L. calbasu* from extinction and bring it back in natural environments and culture systems. A number of studies reported that cultured fish stocks showed lower genetic diversity than wild populations (Frost et al., 2006). Both landmark-based morphometric analysis and microsatellite markers are recognized methods for stock selection and identification (Turan et al., 2006; Hossain et al., 2010). There have been few attempts to evaluate the population structure of *L. calbasu* using different methods based on phenotypic and genetic aspects.

Genetic variation is used as a useful tool for characterization of different species or strains, comparison of farmed with wild populations. Maintenance of genetic variation is the most important concern for the management of hatchery stocks of any species. Molecular marker is a powerful tool which has been extensively used to evaluate genetic diversity and structure of farmed food fish species, such as Asian seabass (Zhu et al., 2006), oyster (Carlsson et al., 2006) and tilapia (Rutten et al., 2004), and used for identification of Quantitative Trait Loci (QTLs) and applied to assist in the breeding program and broodstock management (Jackson et al., 2003). In Bangladesh, microsatellite DNA marker has been used for some fish species including catla (Alam and Islam, 2005), silver carp and bighead carp (Mia et al., 2005), common carp (Mondol et al., 2006), walking catfish (Islam et al., 2007), mrigal (Hasnat, 2007) and rohu (Alam et al., 2009).

Microsatellite marker is able to detect lower level of genetic variation than isozyme and mtDNA RFLP (Desvignes et al., 2001). Microsatellite markers have added new dimension in the field of fisheries and aquaculture as many populations are subjected to bottleneck, inbreeding, genetic drift and as a consequence exhibit low variation that cannot be detected by other markers. As kalibaus is an endangered fish, it may be subjected to bottleneck, inbreeding, genetic drift, gene introgression etc. and exhibit low production performance in the aquaculture practices. Therefore, it is necessary to study genetic status of this endangered fish. The present study evaluated the genetic diversity and population structure of endangered carp, kalbaus *L. calbasu* from five different stocks – three major rivers, a *haor* (wetlands in the northeastern part of Bangladesh - bowl or saucer shaped shallow depressions) and a hatchery using microsatellite markers.

MATERIALS AND METHODS

A total of 165 adult *L. calbasu* samples, 33 each from five different locations were collected during August 2007- October 2008 from five different locations: the Jamuna, the Padma, the Halda, the Tola Haor and a Hatchery (Fig. 1). The sampling sites were selected to cover genetic variation on a wide geographical distribution range of the species.

Briefly, approximately 50 mg of caudal fin tissue was clipped from each individual in separate eppendorf tubes and stored at -18 °C.. The fin clips were cut into small pieces with scissors and ground with a tissue grinder in a 1.5 ml microcentrifuge tube. The tissue was digested overnight at 37°C in a 1.5 ml microcentrifuge tube containing 500 µl of extraction buffer (100 mM M Tris-HCl pH 8; 10 mM EDTA; 250 mM NaCl and 1% SDS) and 30 µl of Proteinase K (10 mg/ml). Total DNA was extracted with two washes of phenol: chloroforms: isoamyl alcohol (25:24:1) and one of chloroform: isoamyl (24:1) followed by ethanol precipitation. Finally, the DNA samples were stored in freezer at -18 °C.

After completion of PCR, 2.5 µl loading dye was added to each PCR tube and mixed well and centrifuged briefly containing ethidium bromide in 1× TBE buffer. After confirmation on agarose gel, the gel containing 19:1 acrylamide:bis-acrylamide and 7 M urea. Electrophoresis was conducted using a SequiGen GT sequencing gel electrophoresis system (Bio-Rad Laboratories, Hercules, CA). The PCR products and 100 bp DNA ladder (GENEI, Bangalore, India) were preheated at 95 °C for 5 minutes and cooled immediately on ice before loading. The DNA ladder was loaded on either side of the gel and was run at 60 W for required length of time (1 hr 50).

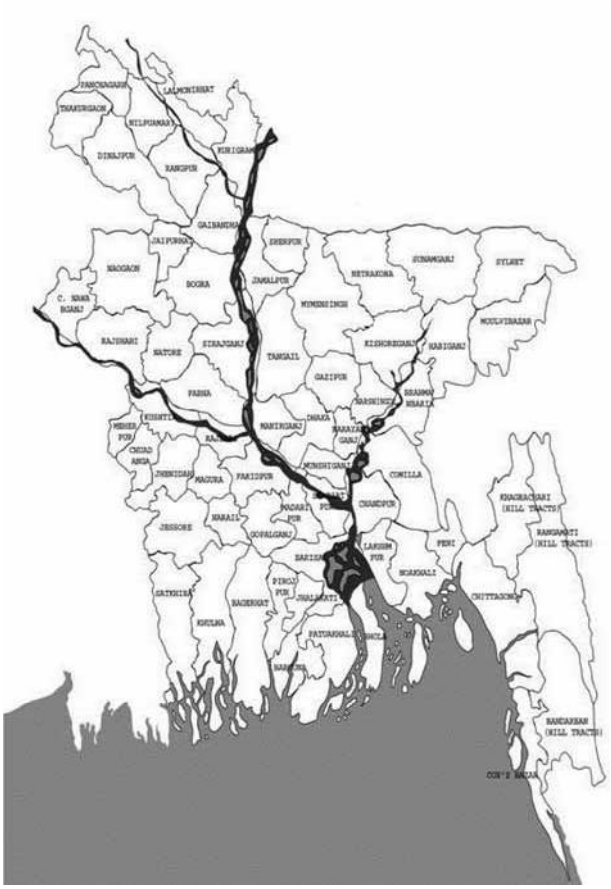

Fig. 1. Sampling sites of *L. calbasu* in Bangaldesh

No microsatellite marker has been developed from *L. calbasu.* Twenty five (25) primers developed from other related carp species were tested of which nine were amplified. The experiment was conducted by five among nine amplified primers. Four microsatellite loci (*Lr10, Lr21, Lr24* and *Lr26*) developed from rohu by Das et al. (2005) and one microsatellite locus (*CcatG1*) developed from catla by Naish and Skibinski (1998) were used in this experiment.

RESULTS

Cross species amplification

Developed from rohu, catla and common carp (*Cyprinus carpio*) were tested in this experiment for PCR amplification using kalibaus DNA as template of which nine primers were amplified successfully (Table 1). Out of nine loci, four microsatellite markers (*Lr10, Lr21, Lr24* and *Lr26*) developed from rohu (Das et al., 2005) and one microsatellite marker (*CcatG1*) developed from catla (Naish and Skibinski, 1998), yielded consistently scorable bands. A total of thirty alleles were detected in the five loci examined.

Genetic variation

The microsatellite *CcatG1* was most polymorphic with ten alleles while the locus *Lr24* was least polymorphic with two alleles (Table 2). The average number of allele was highest in the Jamuna population (5.8), followed by the Padma (5.2), the Halda (5) and the *Haor* (5) populations. The Hatchery populations possessed the least number (4.8) of average alleles (Table 2). Therefore, the wild populations possessed higher average allele number than the cultured stock. The highest *Ne* (7.383) was found in locus *CcatG1,* while the locus *Lr24* had the least *Ne* (1.832) (Table 2). The Jamuna stock possessed the highest average *Ne* (3.805) followed by the *Haor* (3.797), the Padma (3.785), the Halda (3.661) and the Hatchery (3.054) populations (Table 2).

The observed average heterozygosity (H_o) in the Jamuna population (0.776) was the highest which was followed by the Halda (0.667), the *Haor* (0.661) and the Padma (0.642) populations. The hatchery stock had the lowest observed average heterozygosity (H_o) (0.370) (Table 2). The 1-*Ho*/*He* values

Table 1. Primers of microsatellite loci which developed from *Labeo rohita*, *Gibelion catla* and *Cyprinus carpio* tested for cross-species amplification in *L. calbasu*

Resource Species	Primer pairs tested (no.)	Locus	Reference	Successful amplified result in *L. calbasu* (%)
Labeo rohita	12	*Lr1, Lr3, Lr6, Lr10, Lr12, Lr14a, Lr14b, Lr20, Lr21, Lr23, Lr24, Lr26*	Das et al., 2005	5 (45)
Gibelion catla	9	*CcatG1 CcatG2, C catG3*	Naish and Skibinski, 1998	3 (33)
		Cc6, Cc 7, Cc 8, Cc 9, Cc10, Cc 12	McConnell et al., (2001)	
Cyprinus carpio	4	*MFW 7, MFW 19, MFW 24, MFW 26*	Crooijmans et al., 1997	1 (25)
Total tested	25			9 (36)

Table 2. Allelic variations in five microsatellite loci in a sample of five populations of *L. calbasu* (*N*= Number of alleles, *Ne*= Effective number of alleles, H_o= Heterozygosity observed, H_e= Heterozygosity expected, inbreeding index ($f = 1- H_o / H_e$)

Microsatellite loci	Parameters	Jamuna	Padma	Halda	*Haor*	Hatchery
Lr10	N	4	4	4	4	3
	Ne	3.170	3.227	3.419	3.212	2.450
	H_o	0.788	0.667	0.818	0.485	0.606
	H_e	0.685	0.690	0.708	0.689	0.592
	1- H_o / H_e	-0.151	0.034	-0.156	0.296	-0.024
	H-W test	22.41**	7.77 NS	9.87 NS	37.56***	2.22NS
		(6)	(6)	(6)	(6)	(3)
Lr21	N	6	6	6	6	4
	Ne	4.279	3.636	4.436	4.287	2.401
	H_o	0.697	0.697	0.636	0.879	0.273
	He	0.766	0.725	0.775	0.767	0.584
	1- H_o / H_e	0.090	0.039	0.178	-0.146	0.533
	H-W test	97.62***	82.26***	45.92***	36.83**	34.95***
		(15)	(15)	(15)	(15)	(6)
Lr24	N	5	2	2	2	3
	Ne	2.789	1.832	1.936	1.832	1.919
	H_o	0.788	0.697	0.515	0.636	0.182
	H_e	0.641	0.454	0.483	0.454	0.479
	1- H_o / H_e	-0.228	-0.535	-0.066	-0.401	0.620
	H-W test	53.34***	9.44**	0.14 NS	5.31*	24.30***
		(10)	(1)	(1)	(1)	(3)
Lr26	N	4	5	5	4	4
	Ne	3.463	3.588	3.125	2.271	1.561
	H_o	0.818	0.758	0.879	0.697	0.182
	H_e	0.711	0.721	0.680	0.560	0.360
	1- H_o / H_e	-0.150	-0.050	-0.292	-0.245	0.494
	H-W test	44.79***	93.78***	56.36***	78.75***	22.01**
		(6)	(10)	(10)	(6)	(6)
CcatG1	N	10	9	8	9	10
	Ne	5.325	6.640	5.391	7.383	6.936
	H_o	0.788	0.394	0.485	0.606	0.606
	H_e	0.812	0.849	0.815	0.865	0.856
	1- H_o / H_e	0.030	0.536	0.405	0.299	0.292
	H-W test	175.93***	105.48***	85.95***	91.55***	105.05***
		(45)	(36)	(28)	(36)	(45)
Average number of alleles		5.8	5.2	5	5	4.8
Average Effective no. of alleles		3.805	3.785	3.661	3.797	3.054
Average H_o over loci		0.776	0.642	0.667	0.661	0.370
Average H_e over loci		0.723	0.688	0.692	0.667	0.574
Polymorphism (P_{95})		100	100	100	100	100

Statistically significant values are marked with asterisks.
* P<0.05, ** P<0.01, *** P<0.001, NS=Not Significant

were negative in both the Jamuna and Halda populations at *Lr10*, *Lr24* and *Lr26*; in the Padma at *Lr24* and *Lr26;* in the *Haor* at *Lr21*, *Lr24* and *Lr26;* and in the Hatchery population at *Lr10* (Table 2). The 1-*Ho*/*He* values were negative which means that all the sampled populations were excess of heterozygosity.

Deviation from Hardy-Weinberg proportion

The deviations from Hardy-Weinberg Equilibrium at *Lr21*, *Lr24*, *Lr26* and *CcatG1* in the Jamuna; *Lr21, Lr26* and *CcatG1* in the Padma and Halda; *Lr10*, *Lr26* and *CcatG1* in the *Haor*; *Lr21 Lr24* and *CcatG1* in the Hatchery and at *Lr21* in the Jamuna were

very high (P<0.001). The deviations from Hardy-Weinberg Equilibrium at *Lr10* in the Jamuna, *Lr24* in the Padma, *Lr21* in the *Haor*, *Lr26* in the Hatchery populations were high (P<0.01). The deviations from Hardy-Weinberg Equilibrium at *Lr24* in the *Haor* population was relatively low (P<0.05) (Table 2).

Bottleneck test

Since the H_0 values were higher than H_e in *L. calbasu* populations, this shows that there might be bottleneck in some populations except the Hatchery population. The ratio (5:0) was significantly different from the expected ratio (1:1) for nonbottlenecked, equilibrium populations (Sign test: P = 0.064; Wilcoxon test: one tail for $H_{ex,}$ P = 0.015). On the other hand, the data sets from the Jamuna, the Padma and the *Haor* population that did not have a significant heterozygosity excess (Sign test: P = 0.678, 0.299 and 0.295 and Wilcoxon test: one tail for $H_{ex,}$ P = 0.406, 0.031 and 0.031 respectively), deviated toward an excess of heterozygosity as expected for bottleneck populations (nearly significant). Only the Hatchery population had significant excess of heterozygosity (H_{ex}/H_d = 2/3) (Table 3).

Table 3. Heterozygosity excess under two-phase mutation model at five microsatellite loci from each of five populations of *L. calbasu*

Population	Sign Test		Wilcoxon Test
	H_{ex}/H_d	P	P (one tail for H_{ex})
Jamuna	3/2	0.678	0.406
Padma	4/1	0.299	0.031
Halda	5/0	0.064	0.015
Haor	4/1	0.295	0.031
Hatchery	2/3	0.328	0.593

P = probability, *Hex* = heterozygosity excess, *Hd* = heterozygosity deficiency

Inter-population genetic structure

The F_{ST} between the population pairs were compared and it was found that the population differentiation values between all the population pairs are significant. This result indicates that the populations are not homogeneous. The F_{ST} (population differentiation) value between the Jamuna population and the Hatchery population was the highest (0.109), while the F_{ST} value between the Padma population and the Halda population was the lowest (0.024). The N_m (gene flow) value between the Padma and the Halda across all loci was the highest (10.306) and the N_m value between the Jamuna and the Hatchery was the lowest (2.045) (Table 4). Analysis of molecular variance (AMOVA) revealed that 18% of the molecular variance existed among the populations and 82% existed within populations (Table 5).

Genetic distance

A matrix of genetic distance (Nei, 1972) was built based on allelic frequencies of all loci (Table 6). The largest genetic distance value (D = 0.543) was measured between the Jamuna and the Hatchery population while the smallest value (D =0.124) was observed between the Padma and the Halda populations.

Table 4. Multilocus F_{ST} (below diagonal) and N_m (above diagonal) values between pairs of five populations of *L. calbasu* across all loci

Populations	Jamuna	Padma	Halda	*Haor*	Hatchery
Jamuna	*	4.949	4.479	2.959	2.045
Padma	0.048	*	10.306	5.381	2.716
Halda	0.053	0.024	*	5.102	2.686
Haor	0.078	0.044	0.047	*	3.460
Hatchery	0.109	0.084	0.085	0.067	*

$N_m = [(1/F_{ST})-1]/4$

Table 5. Summary of analysis of molecular variance (AMOVA) among and within the populations

Source	df	SS	MS	Est. Var.	%
Among Pops	4	119.079	29.770	0.790	18%
Within Pops	160	589.455	3.684	3.684	82%
Total	164	708.533		4.475	100%

Table 6. Nei's (1972) genetic distance in five populations of *L. calbasu*

Populations	Jamuna	Padma	Halda	*Haor*	Hatchery
Jamuna	****				
Padma	0.251	****			
Halda	0.295	0.124	****		
Haor	0.455	0.235	0.247	****	
Hatchery	0.543	0.381	0.379	0.242	****

Dendrogram

The UPGMA dendrogram based on Nei's (1972) genetic distance resulted in two major clusters. The first cluster was separated into two sub-clusters: Jamuna stock alone in one cluster and remaining three stocks (the Padma, the Halda and the *Haor*) in the other cluster. Hatchery stock was in the second cluster (Fig. 2).

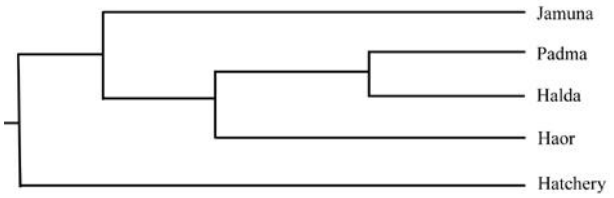

Fig. 2. UPGMA dendrogram based on Nei's (1972) genetic distance between five populations of *L. calbasu*, according to the microsatellite DNA analysis

DISCUSSION

This is the first extensive study of genetic diversity and population structure of endangered *L. calbasu* using microsatellite DNA markers in Bangladesh.

Cross species amplification

Cross species amplification is dependent on the phylogenetic relationship of species for which microsatellites were originally developed (Hulak et al., 2010). Catla and rohu are closely related species to kalibaus under the same family and it was expected that the markers developed from rohu and catla should also work in kalibaus. These results suggest that time and money invested in evaluating the cross-species amplification of microsatellite loci in target species may be worth it. The cross-species amplification data indicated that allelic diversity was low in *L. calbasu* compared with the original species. Therefore, greater attention should be been given to primer optimization or redesign.

Genetic variability

In the present study, all the five loci were found to be polymorphic in five populations of *L. calbasu*. Average effective number of alleles and gene diversity were different in the wild and hatchery stocks, suggesting significant difference of genetic variability between wild and hatchery stocks. Similar results were also reported from microsatellite and RAPD markers analyses (Bartfai et al., 2003 and Lal et al., 2004). Das et al. (2005) developed these microsatellite markers and Alam et al. (2009) obtained similar genetic polymorphism in an Indian farmed population of rohu.

The Hatchery populations possessed the least number (4.8) of average alleles and thus they have the highest number of null alleles (6 each). Loss of allelic variation has also been reported for hatchery populations of catla by Alam and Islam (2005) and Hansen et al. (2006), and for *Clarias batrachus* hatchery populations by Islam et al. (2007). The higher proportion of polymorphic loci and gene diversity in the individuals of the Jamuna River are usually expected, because it is well known that the Jamuna is a large river. Therefore, in Bangladesh, genetically more diversified *L. calbasu* individuals can only be found in the Jamuna River.

The hatchery population might have been founded with a small effective number of parents (Ne). However, it was not possible to confirm from the hatchery owners which Ne was maintained in the hatchery under study, as no record was maintained in the hatchery. It is, however, a common practice to maintain only a small number of broodfish in Bangladeshi hatcheries. The losses of alleles and heterozygosity may increase with bottlenecking and inbreeding through time in the hatchery stocks (Alam and Islam, 2005). Therefore, measures should be taken to prevent inbreeding in the commercial hatcheries through proper broodstock management practices.

Population bottleneck

Since the H_0 values were higher than H_e in *L. calbasu* populations, this shows the possibility of bot-

tleneck in some populations. The tests of mutation drift equilibrium for detecting genetic bottlenecks revealed signs of population decline of the species. In the present study, the Hatchery population with a significant heterozygosity excess reveals risk of sever population bottlenecks. According to the statements of the hatchery owner, the hatchery was established seven years ago with brood fish collected from a hatchery. The samples of *L. calbasu* used in the present study were progeny of the fourth generation. At present, population is declining in the Halda River for many reasons such as overexploitation, habitat destruction, water pollution, siltation etc.

Population structure

Except loci *Lr10* and *Lr24* in the Halda and locus *Lr10* in the Padma population, deviation from Hardy-Weinberg were found at all loci. Deviation of all populations at *Lr21* and *CcatG1* loci from Hardy-Weinberg expectations were found to be relatively larger due to deficiency in heterozygosity and loss of alleles in all samples. Alam and Islam (2005) found that the hatchery population of catla deviated from Hardy-Weinberg equilibrium at a number of loci. Deviations from Hardy-Weinberg expectation might be due to inbreeding.

Pair-wise F_{ST} estimates between the Jamuna and hatchery populations were higher than all other population pairs and significant population differentiation was observed between the Jamuna and the hatchery population of *L. calbasu*. Geological structures separate the Jamuna River from the hatchery populations and may limit the gene flow between the Jamuna and hatchery population. The effect of geographical distance on F_{ST} and gene flow (N_m) values has been reported in stream-living brown trout collected from different sections of the main stream in Jamtland, central Sweden (Carlsson et al., 1999).

The genetic differentiation in the populations of *L. calbasu* may be caused by artificial selection or random genetic drift or founder effect in the hatchery stocks.

Genetic distance

The UPGMA dendrogram based on Nei's (1972) genetic distance resulted in two major clusters. Hatchery stock was in the second cluster. The clustering suggests that the status of hatchery population were unique where the origin of broods might be geographically distant from wild stocks. However, it is imperative that effective coordination of ecological and genetic approaches is helpful to conserve this endangered species.

Conclusion

The result of the present study has proven to understand the intra and inter-population genetic variation and population status. For proper conservation of a species and effective management strategy, genetic monitoring is essential because a population may suffer the risk of genetic drift, bottleneck, inbreeding, founder effect etc. Hatchery owner should maintain good brood management system through collecting brood of different sources. It may be an important way to maintain diverse gene pool. However, further study with large number of populations, including all segments of the country with microsatellite DNA markers, is recommended in order to have more information about population genetic structure of this endangered fish species. Therefore, it is very essential to develop specific primer for *L. calbasu.*

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Sažetak

GENETSKA STRUKTURA POPULACIJA UGROŽENOG CIPRINIDA *Labeo calbasu* (HAMILTON, 1822) ISTRAŽENA POMOĆU MIKROSATELITSKIH MARKERA

Genetska struktura populacija ciprinida *Labeo calbasu,* prikupljena od četiri divlje i jedne uzgajane populacije, proučavana je koristeći mikrosatelitske markere. Pet heterogenih mikrosatelitskih markera (*Lr10, Lr21, Lr24, Lr26* i *CcatG1*) sastavljenih za indijske ciprinide su proučavani da bi se istražila genetska varijabilnost *L. calbasu* populacija. Broj uočenih alela u lokusu se kretao od 2 do 10. U proučavanim populacijama lokusi su bili polimorfni ($<P_{95}$). Prosječan broj alela je bio veći kod divljih nego kod uzgajanih populacija. Prosječan broj alela je bio najveći u Jamuna populaciji (5.8), te najmanji u uzgajanoj populaciji (4.8). Uočena prosječna heterozigotnost (H_o) u Jamuna populaciji (0.776) bila je najveća, slijedile su je populacije Halda (0.667), Haor (0.661) i Padma (0.642). Izuzev lokusa *Lr10* i *Lr24* u

populaciji Halda i lokusa *Lr10* u Padma populaciji i uzgajanoj populaciji, zabilježeno je značajno odstupanje od Hardy-Weinbergovog zakona ravnoteže (HWE) u svim slučajevima. Vrijednosti F_{ST} i N_m ukazuju na visok stupanj diferencijacije i nizak stupanj protoka gena između populacija. Najveća genetska udaljenost (D = 0.543) je izmjerena između populacije Jamuna i uzgajane populacije, dok je najmanja vrijednost (D = 0.124) uočena između populacija Padma i Halda. Procijenjena genetska struktura populacije i moguća upotreba mikrosatelitskih markera mogu pomoći pri upravljanju populacija *L. calbasu* u otvorenim vodama.

Ključne riječi: međuvrsna amplifikacija, *Labeo calbasu,* ugrožene vrste, mikrosatelitski DNA markeri

REFERENCES

Alam, M. S., Islam, M. S. (2005): Population genetic structure of *Catla catla* (Hamilton) revealed by microsatellite DNA markers. Aquaculture, 246, 151-160.

Alam, M. S., Jahan, M., Hossain, M. M., Islam, M. S. (2009): Population genetic structure of three major river populations of rohu, *Labeo rohita* (Cyprinidae: Cypriniformes) using microsatellite DNA markers. Gen. Genom., 31, 1, 43-51.

Bartfai, R., Egedi, S., Yue, G. H., Kovacs, B., Urbanyi, B., Tama, G., Horvath, L., Orban, L. (2003): Genetic analysis of two carp broodstocks by RAPD and microsatellite markers. Aquaculture, 219, 157-167.

Carlsson, J., Morrison, C. L., Reece, K. S. (2006): Wild and aquaculture populations of the eastern oyster compared using microsatellites. Heredity, 97, 595-598.

Carlsson, J., Olsen, K. H., Nilsson, J., Averli, A., Stabell, O. B. (1999): Microsatellites reveal fine-scale genetic structure in stream-living brown trout. Journal of Fish Biology, 55, 1290-1303.

Crooijmans, R. P. M. A., Vander, P. J. J., Groenen, M. A. M., Bierbooms, V. A. F., Komen, J. (1997): Microsatellite markers in common carp (*Cyprinus carpio L.*). Anim. Genet., 28, 129-134.

Das, P., Barat, A., Meher, P. K., Ray, P. P., Majumdar, D. (2005): Isolation and characterization of polymorphic microsatellites in *Labeo rohita* and their cross species amplification in related species. Molecular Ecology Notes, 5, 231.

Desvignes, J. F., Laroche, J., Durand, J. D., Bouvet, Y. (2001): Genetic variability in reared stocks of common carp (*Cyprinus carpio* L.) based on allozymes and microsatellites. Aquaculture, 194, 291-301.

Excoffier L., Laval, G. and Schneider, S. 2005. ARELEQUIN ver.3.0: An integrated software package for population genetics data analysis. Evol. Bioinformatics Online, 1, 47-50.

Frost, L. A., Evans, B. S., Jerry, D. R. (2006): Loss of genetic diversity due to hatchery culture practices in barramundi (*Lates calcarifer*). Aquaculture, 261, 1056-1064.

Hansen, M. M., Simonsen, V., Mensberg, K. L. D., Sarder, M. R. I., Alam, M. S. (2006): Loss of genetic variation in hatchery-reared Indian major carp, *Catla catla*. Fish Biol., 69, 229-241.

Hossain, M. A. R., Nahiduzzaman, M., Saha, D., Khanam, M. U. H., Alam, M. S. (2010): Landmark based morphometrics of an endangered carp kalibaus, *Labeo calbasu* stocks. Zoological Studies, 49, 556-563.

Hulak, M., Kaspar, V., Kozak, P., Burice, M., Filipova, L., Petrusek, A. (2010) Cross-species amplification of microsatellite markers in the invasive spiny-cheek crayfish (*Orconectes limosus*): assessment and application. Appl. Genet., 51, 1, 73–78.

Islam, M. N., Islam, M. S., Alam, M. S. (2007): Genetic structure of different populations of walking catfish (*Clarias batrachus* L.) in Bangladesh. Biochem. Genet., 45, 647-662.

Islam, M.S., Alam, M. S. (2004): Randomly amplified polymorphic DNA analysis of four different populations of the Indian major carp, *Labeo rohita* (Hamilton). J. Appl. Ichthyol., 20, 407-412.

IUCN (2000): Red Book of Threatened Fishes of Bangladesh. Islam, M.A., M. Ameen and Nishat, A. (Eds.). The World Conservation Union, Dhaka, Bangladesh, pp. 116.

Jackson, T. R., Martin-Robichu, D.J., Reith, M. E. (2003): Application of DNA markers to the management of Atlantic halibut (*Hippoglossus hippoglossus*) broodstock. Aquaculture, 220, 245-259.

Lal, K. K., Chauhan, T., Mandal, A., Singh, R. K., Khulbe, L., Ponniah, A. G. and Mohindra, V. (2004): Identification of microsatellite DNA markers for population structure analysis of Indian major carp (*Cirrhinus mrigala*) (Hamilton-Buchanan, 1882). J. Appl. Ichthyol., 20, 87-91.

Mia, M. Y., Taggart, J. B., Gimour, A. E., Gheyas, A. A., Das, T. K., Kohinoor, A. H. M., Rahman, M. A., Sattar, M. A., Hussain, M. G., Mazid, M. A., Penman, D. J., McAndrew, B. J. (2005): Detection of hybridization between Chinese carp species

(*Hypophthalmichthys molitrix* and *Aristichthys nobilis*) in hatchery broodstock in Bangladesh, using DNA microsatellite loci. Aquaculture, 247, 167-273.

McConnell, S. K. J., Leamon, J., Skibinski, D. O. F., Mair, G. C. (2001): Microsatellite markers from the Indian major carp species, *Catla catla*. Molecular Ecology Notes., 1, 115.

Mondol, M. R. K., Islam, M. S., Alam, M. S. (2006): Characterization of different strains of common carp (*Cyprinus carpio* L.) (Cyprinidae, Cypriniformes) in Bangladesh using microsatellite DNA markers. Gen. Mol. Biol., 29, 4, 626-633.

Naish, K. A., Skibinski, D. O. F. (1998): Tetranucleotide microsatellite loci for Indian major carp. Journal of Fish Biology, 53, 886-889.

Nei, M. (1972): Genetic distance between populations. Am. Nat., 106, 283-292.

Peakall, R., Smouse, P. E. (2005): GenAlEx V6.1: Genetic Analysis in Excel. Population genetic software for teaching and research. Australian National University, Canberra, Australia. http://www.anu.edu.au/ BoZo/GenAlEx.

Reddy, P. V. G. K. (1990): Genetic resources of Indian major carps. FAO Fisheries Technical Papers - T387, pp. 76.

Rutten, M. J. M., Komen, H., Deerenberg, R. M., Siwek, M., Bovenhuis H. (2004): Genetic characterization of four strains of Nile tilapia (*Oreochromis niloticus* L.) using microsatellite markers. Anim. Genet., 35, 93-97.

Turan, C., Oral, M., Ozturk, B., Duzgunes, E. (2006): Morphometric and meristic variation between stocks of bluefish (*Pomatomus saltatrix*) in the Black, Marmara, Aegean and northeastern Mediterranean Seas. Fish. Res., 79, 139-147.

Zhu, Z. Y., Lin, G., Lo, L. C., Xu, Y. X., Renee, C., Yue, G. H. (2006): Genetic analyses of Asian sea bass stocks using novel polymorphic microsatellites. Aquaculture, 256, 167-173.