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Genetic variation in endangered butter catfish, *Ompok bimaculatus* (bloch) populations revealed by random amplified polymorphic DNA (RAPD) fingerprinting

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Abstract

This study was conducted to provide baseline information on the genetic population structure of *Ompok bimaculatus*, an endangered catfish of Bangladesh. Random amplified polymorphic DNA (RAPD) fingerprinting analysis was performed to assess the genetic variation in two wild (Chalan beel and Tola haor) and one hatchery (Brahmmaputra Fish Seed Complex-BFSC) populations of *O. bimaculatus*,. Five selected decamer random primers amplified a total of 34 RAPD bands among which 24 were found to be polymorphic. The percentage of polymorphic loci, intra-population similarity indices, gene diversity and Shannon's information indidex values were 64.71%, 77.57% , 0.249±0.216 and 0.365±0.303 for Chalan *beel*, 58.82%, 75.45%, 0.219±0.215 and 0.322±0.304 for Tola *haor* population and 52.94%, 86.49%, 0.214±0.219 and 0.311±0.312 for the hatchery, BFSC, respectively. The coefficient of population differentiation (PhiPT) between the Chalan *beel* - BFSC and Tola *haor* - BFSC pairs were found to be significant. The gene flow (N_m) between the population pairs ranged from 1.899 to 5.052. The highest inter-population similarity (S_{ij}) was found between Chalan *beel*-BFSC populations. Among the three populations, the highest genetic distance (0.157) was found between Tola *haor* and the BFSC population. The results of the present study indicated a substantial level of genetic variation in the enangerd *O. bimaculatus* populations in Bangladesh and significant differentiation among the populations..

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Introduction

The extent of genetic variation in a population indicates what sorts of events it might have experienced in the past, what about the current situation and what is the probability of sustenance in future. Low level of genetic diversity, having a negative correlation with the potential for adaptation to changing environmental conditions, may cause threat to the survival of endangered species (Lynch et al. 1995; Loew, 2000). Therefore, baseline data on the fine-scale genetic population structure of endangered species are prerequisite for identifying and protecting populations that can be used as a potential source for future restocking programs in nature and for saving genetically weaker populations from extinction (Kimberling et al., 1996; Crozier, 1997). The objective of the study was to provide baseline information on the genetic population structure of endangered *Ompok bimaculatus* (Bloch) popularly known as "butter catfish" and locally called 'kani pabda'. *O. bimaculatus* is an indigenous medium size food fish found in natural water bodies i.e. *haors (*large natural depressions*)*, *baors* (ox-bow lakes), rivers, beels (low-lying seasonal water bodies) and floodplains of Bangladesh (Rahman, 1989). It has a wide geographical distribution covering India, Pakistan, Afganistan, Myanmar, Thailand, Java, Sumatra, Borneo and China (Talwar and Jhingran, 1991). Its natural population has declined in the last decade of the 20th century in Bangladesh due to overexploitation and serious destruction and fragmentation of most aquatic ecosystems and injudicious application of insecticides in the paddy fields and the species is classified as endangered (IUCN 2000). Less availability of *O. bimaculatus* has also been reported in the biggest freshwater tectonic lake in Assam, a northeastern province of India neighbouring Bangladesh (Kar et al., 2006). A species is termed as endangered when its population size is declined to a certain level which may lead to a reduction in the level of genetic variation (Alam et al., 2010). In an attempt to conserve the valuable fish species from further decline, induced breeding and fry rearing techniques of this species have been developed. Considering high market price (one of the highest valued fish species in Bangladesh) and consumer demand fish farmers are showing interest in its culture and it is being cultured to a very limited scale in Bangladesh (Parween, 2007).

To study the population genetic structure of the important catfish, we selected Chalan beel and Tola haor because these are very large natural waterbodies and considered important reservoir of freshwater fish species of Bangladesh. We, examining the random amplified polymorphic DNA (RAPD), report for the first time the genetic variation for two wild and one hatchery populations of *O. bimaculatus*.

Materials and methods

Collection of fish sample and extraction of DNA

In order to study genetic variations within and among different populations of *O. bimaculatus*, samples were collected from three different sources: Chalan *beel* under Natore district and the Tola *haor* under Netrokona district during July-August, 2009. The hatchery samples were collected from Brahmmaputra Fish Seed Complex (BFSC), Mymensingh in September, 2009. The hatchery operator collected *O. bimaculatus* broods from a renowned natural water body of Bangladesh named as "Tanguar *haor*" in Sunamgonj district. In order to perform RAPD fingerprinting, a total 60 fish, 20 from each population were taken randomly. The fish were anesthetized with MS222 and the tissue samples were clipped from the caudal fin of each fish and immediately preserved in individual microfuge tube containing 95% ethanol and stored at -20°C. For isolation of genomic DNA, approximately 40 mg of fin tissues from each sample was cut into small pieces and ground with a tissue grinder in 1.5 ml microfuge tube. The genomic DNA was isolated following cell rupture and proteinase-K digestion, phenol: chloroform: isoamyl alcohol (25:24:1; v/v/v) extraction, and ethanol precipitation method as described by Islam and Alam (2004). The quality of all DNA samples were tested (degradation of DNA) by electrophoresis on 1% agarose gel and the

quantity was estimated using a photometer (Biophotometer plus, Eppendorf, Germany).

Polymerase chain reaction (PCR) and electrophoresis

We screened fifteen decamer primers from three kits (six from kit A, seven from kit B and two from kit C) of random sequence (Operon technologies, Inc., Alameda, CA, USA) on random samples from each population. A final subset of five primers which displayed reproducible, scorable and clear bands was considered for analysis of the entire samples of 60 fish.

The amplification conditions originally recommended by Williams *et al.* (1990) were applied and optimized for *O. bimaculatus* samples. PCR reactions were performed on each DNA in a 10 µl reaction mix containing 1 µl of 10× *Taq* polymerase buffer, 0.2µl of primer, 1 µl of dNTPs and 1 unit of *Taq* DNA polymerase (GENEI, Bangalore, India) and 150 ng µl of genomic DNA. The PCR was conducted using a gradient thermal cycler (Mastercycler Gradient Eppendorf, Germany). The reaction mix was preheated at 94°C for 3 min followed by 40 cycles of 30 sec denaturation at 94°C, 1 min annealing at 38°C and 2 min DNA chain elongation or extension at 72°C. After the last cycle, a final step of 7 min at 72°C was added to allow complete extension of all amplified fragments followed by holding at 4°C. The amplified products from each sample were separated electrophoretically on 1.4% ethidium bromide containing agarose gel (GENEI, India) and the image was captured using a Gel Documentation system.

Collection and analysis of RAPD data

The image profiles of banding patterns were recorded and molecular weight of each band was determined by AlphaEaseFC (Version 4.0) software. The banding pattern was scored on the basis of presence or absence of clear, visible and reproducible bands. The RAPD fragments were compared among the samples collected from different populations. The data were analyzed based on the principle that a band is considered to be polymorphic if it is present in some individuals and absent in others. A single data matrix was constructed pooling the scores obtained for all primers in all individuals under the three populations and used to estimate gene frequency, polymorphic loci, Nei's (1973) gene diversity, observed number of alleles (N_a), effective number of alleles (N_e), Shannon's Information Index (I), gene flow (N_m), genetic distance (Nei, 1972) and homogeneity test (χ^2 test) between the population pairs using the POPGENE (Version 1.31) (Yeh *et al.*, 1999) computer program. We examined the hierarchy of molecular variance and differentiation (Phi-PT) between the population pairs by AMOVA using the software GenAlex 6.4 (Peakall and Smouse, 2006). The similarity index values (*SI*) between the RAPD profiles of any two individuals on the gels were calculated from RAPD markers of the same molecular weight on the data matrix according to the following formula:

Similarity index (*SI*) = $2N_{AB}/ (N_A + N_B)$

Where, N_{AB} is the total number of RAPD bands shared by individuals A and B, and N_A and N_B are the total number of bands produced by individual A and B, respectively, with regard to all assay units (Lynch, 1990). Within population similarity (S_i) was calculated as the average of SI across all possible comparisons between individuals within a population. Between population similarity (S_{ij}), was calculated as the average similarity between randomly paired individuals from population i and j (Lynch and Milligan, 1994).

Results

Polymorphism of RAPD markers

The five primers: OPA04, OPB08, OPB13, OPC04 and OPC06 used in the analysis produced different RAPD patterns. A total of 34 reproducible and consistently scorable bands were obtained through PCR of which 24 (70.59%) were found to be polymorphic (P_{95}) (Table 1). The number of bands per primers ranged from 6-8. The proportion of polymorphic bands for each primer ranged from 50.00% to 83.33%. The overall polymorphism

produced by primer OPA04 was the highest and that produced by primer OPB08 was the lowest. The sizes of the RAPD bands resulted from the four primers ranged from 183 – 1627 bp. (Table 1). A representative type of RAPD profiles of three different populations of *O. bimaculatus* for the primers OPB08 and OPB13 are shown in Fig. 1.

Table 1. Random Amplified Polymorphic DNA primers with corresponding bands scored, their size ranges and polymorphic bands amplified from each of the samples of the three populations of *O. bimaculatus*.

Primers	Sequence (5′-3′)	No. of scorable bands	Size range (bp)	Polymorphic bands	Polymorphism (%)
OPA04	AATCGGGCTG	6	332-1617	5	83.33
OPB08	GGACTGGAGT	8	243-1559	4	50.00
OPB13	GTCCACACGG	6	401-1627	4	66.66
OPC04	TTCCCCCGCT	8	314-1574	6	75.00
OPC06	GAACGGACTC	6	183-1451	5	83.33
Total		**34**		**24**	**71.66**

Table 2. Estimates of genetic variation: number and proportion of polymorphic loci, gene diversity, no. of alleles (na), effective no. of alleles (ne) and intra- and inter-population similarity indices for the studied *O. bimaculatus* populations.

Parameters	Population		
	Chalan *beel*	Tola *haor*	BFSC
No. of polymorphic loci	22	20	18
Percentage of polymorphic loci	64.71	58.82	52.94
Gene diversity (Mean±SD)	0.249±0.216	0.219±0.215	0.214±0.219
Observed no. of alleles (n_a)	1.647	1.588	1.384
Effective no. of alleles (n_e)	1.447	1.388	1.384
No. of private bands	1	0	0
Shannon's Information Index (I)	0.365±0.303	0.322±0.303	0.311±0.312
Intra-population similarity index (S_i)	77.57	75.65	86.49

Genetic variability parameters

The proportion of polymorphic loci, gene diversity and Shannon's Information Index were higher in Chalan *beel* population followed by those of the Tola *haor* and BFSC population respectively. The effective number of alleles was also higher in the Chalan beel population compared to the other two populations (Table 2). The Chalan *beel* population had one

private band however the band was not observed in all the samples of the population. So, it cannot be termed as population diagnostic band.

Intra- and inter-population similarity indices

The intra-population similarity index (S_i) values ranged from 75.65 to 86.49% (Table 2) while the inter-population similarity index (S_{ij}) values ranged from 75.02 to 80.63% (Table 3). Intra-population similarity index (S_i) for the BFSC population was found to be higher than those for the Chalan *beel* and Tola *haor* population. On the other hand, inter-population similarity or between populations similarity index (S_{ij}) for Chalan *beel* vs BFSC samples was found to be the highest while that for the Tola *haor* vs BFSC population was found to be the lowest (Table 3).

Table 3. Inter-population similarity indices, population differentiation (Phi-PT), percent of genetic variation within and between populations, gene flow (Nm), genetic distance, Genetic identity and loci causing significant departure from homogeneity between the population pairs of *O. bimaculatus*.

Population pairs	S_{ij}	Phi-PT value	Percent of molecular variance BP(WP)	Gene flow (N_m)	Genetic Distance (GI)	Loci causing significant departure from homogeneity (χ^2 values)
Chalan *beel* - Tola *haor*	75.62	0.043^{NS}	4% (96%)	5.05	0.062 (0.939)	RB08_05 (7.543)*** RB13_01 (3.756)* RB13_03 (5.760)* RC06_03 (9.249)**
Chalan *beel* - BFSC	80.63	0.089*	9% (91%)	4.16	0.075 (0.928)	RB04_07 (9.249)** RB04_08 (9.249)*** RB08_05 (5.844)* RC06_05** (7.543)
Tola haor - *BFSC*	75.02	0.257**	26% (74%)	1.89	0.157 (0.854)	RB04_07 (7.542)** RB04_08 (9.249)** RB08_05 (20.000)*** RB13_01 (5.760)* RB13_03 (5.760)* RB13_05 (4.236)* RC06_05 (16.180)***

NS: Statistically not significant; * P<0.05; ** P<0.01; ***P<0.001

BP: Between populations; WP: Within population; S_{ij}: Inter-population similarity indices

GI: Genetic Identity

Gene flow, genetic distance and population differentiation

The gene flow between the Chalan *beel* and Tola *haor* population was the highest while that between the Tola *haor* and BFSC population was the lowest. The highest (0.157) and lowest (0.062) genetic distances were found between the Tola *haor* and BFSC populations and between the Chalan beel and Tola *haor* populations respectively (Table 3). The between-population molecular variance ranged from 4% to 26%. The population differentiation (Phi-PT) values between the Chalan *beel* and BFSC populations as well as between the Tola *haor* and BFSC populations were found to be significant while the PhiPT value between the Chalan *beel* and Tola *haor* population was found to be insignificant (P=0.17). Significant departure from homogeneity between the population pairs were observed in eight (OPB04_07, OPB04_08, OPB08_05, OPB13_01, OPB13_03. OPB13_05, OPC06_03, and OPC06_05) of the 24 polymorphic loci (Table 3).

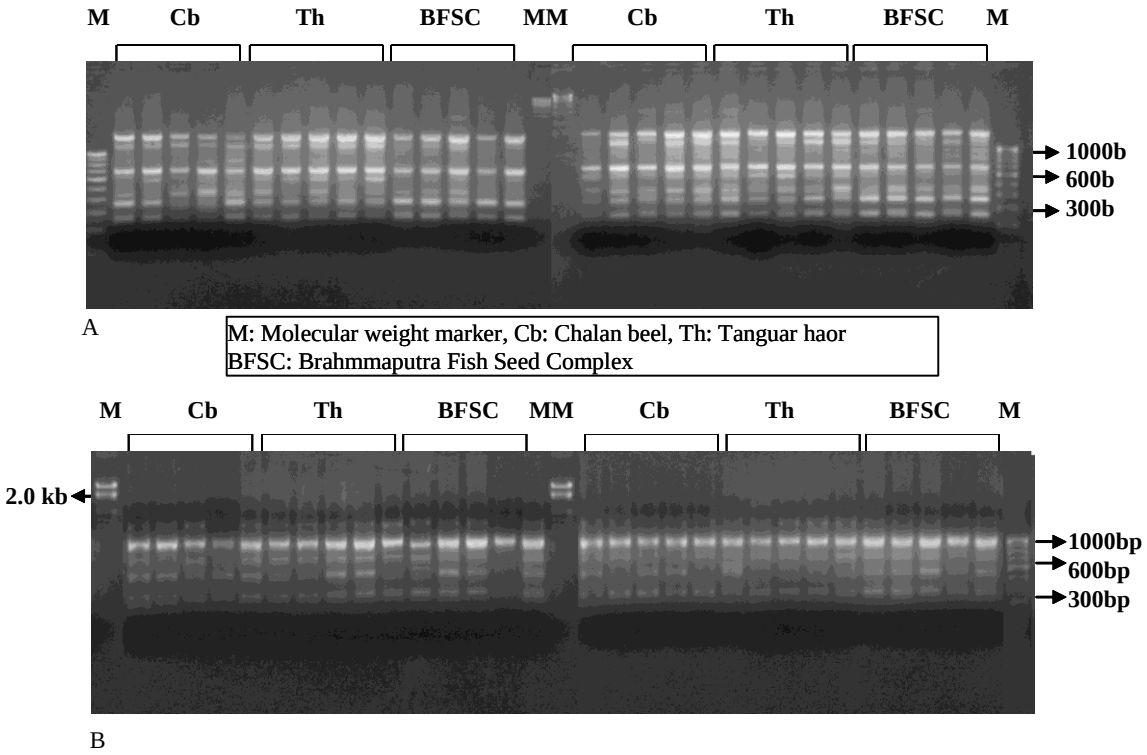

Fig. 1. Random Amplified Polymorphic DNA profiles of *O. bimaculatus* for the primer OPB08 (A) and OPB13 (B); M: Molecular weight markers (Lambda DNA/EcoRI/HindIII digest and 100bp DNA ladder; (Cb: Chalan *beel*, Th: Tola *haor* and Bh: Brahmmaputra hatchery).

Discussion

Molecular markers are realistic and useful tools for the investigation and monitoring of genetic conditions both in natural populations and in captive stocks. RAPD fingerprinting technique is simple, fast, sensitive and allows the examination of genomic variation without prior knowledge of DNA sequences (Williams *et al.*, 1990; Welsh and McClelland, 1990). We have used five random primers for DNA fingerprinting of *O. bimaculatus* that generated a total of 34 bands from the 60 individuals in the three populations of which 70.59% were polymorphic. The percentage of polymorphic loci in the three different populations of *O. bimaculatus* was found to be similar to that observed in the African catfish *Clarias gariepinus* 69.5% (Saad *et al.*, 2009) but slightly lower than those reported in the walking catfish, *Clarias batrachus* 86.66% (Garg *et al.*, 2010) and in the Asian stinging catfish, *Heteropneustes fossilis* 83.87% (Sultana *et al.*, 2010). However, the value was higher those in two populations of *Heteropneustes fossilis* (18.75%) reported by Garg *et al.* (2009) and three populations of *Clarias batrachus* (25-35.5%) reported by Khedkar et al. (2010) in India. Hassanien et al. (2008) reported polymorphic in five populations of European Seabass in the range of 44% to 64%. The percentage of polymorphic loci obtained in the Chalan *beel* population of *O. bimaculatus* (64.71%) is indicative of a relatively higher level of genetic variation. On the other hand, the lowest percentage of polymorphic loci (52.94%) found in the BFSC population of *O. bimaculatus* is indicative of inbreeding in the hatchery population. Compared with the respective natural populations, lowest percentage of polymorphic loci (64.52%) were also reported in the hatchery population of *Heteropneustes fossilis* (Sultana *et al.*, 2010), *Catla catla* (Rahman *et al.*, 2009) and *Labeo rohita* (Islam and Alam 2004) by RAPD marker analysis.

In the present study, high quality bands with good reproducibility were achieved in the size range of 183-1627bp. The sizes of highly reproducible RAPD

markers ranged from 200 to 1500bp in channel catfish (*Ictalurus punctatus*) (Liu *et al.* 1999), 200 to 1500 in snakehead (Channa striata) (Ambak et al., 2006) and from 172 to 1677bp in *Clarias batrachus* (Garg *et al.*, 2010). Khedkar *et al.* (2010) found good quality bands in the range of 100 to 1200bp in *Clarias batrachus*. The mean number of alleles observed across all primers for all the loci was 1.7059±0.4625. Like percentage of polymorphic loci, the number of alleles is also higher in the Chalan *beel* population. The values for intra- population similarity indices (S_i) were slightly higher, ranging from 75.65 to 86.49% than the inter-population similarity indices (S_{ij}) which ranged from 75.02 to 80.63%. The band-sharing based intra-population similarity index (S_i) for BFSC population (86.49%) was found to be higher than those for two other populations. On the other hand, inter-population similarity similarity index (S_{ij}) for Chalan *beel* vs BFSC populations (80.63%) was found to be higher than those for the other population pairs. The S_{ij} value for Tola *haor* vs BFSC populations (75.02%) was found to be the lowest. The average value of similarity indices was found to be 79.57% for the three studied populations. Almost similar level of intra-population similarity indices in all the population implies that individuals within each population were genetically close to each other. The similar results were also observed in different Indian major carps and other fishes like *Labeo rohita* (94.88%) (Islam and Alam, 2004); *Catla catla* (87.89) (Rahman *et al.*, 2009); *Barbodes gonionotus* (94.26%) (Akter *et al.*, 2010); freshwater mud eel, *Monopterus cuchia* (88.33%) (Alam *et al.*, 2010).

Genetic identity measures in the pair-wise of populations show the proportion of individuals of the two populations that are genetically identical. A higher level of genetic identity (0.939) found between the Chalan *beel* and Tola *haor* populations of *O. bimaculatus* indicate that genetically they are more similar than the others. The same genetic identity (0.939) was obtained between two wild populations of *Heteropneustes fossilis* (Sultana *et al.*, 2010). Among the three populations, the highest genetic distance was found between the Tola *haor* and BFSC compared with Chalan *beel* and Tola *Haor* and Chalan *beel* and BFSC populations. The coefficient of gene differentiation (Phi-PT) value indicated significant differentiation between the hatchery (NFSC) and the two wild populations. However, no significant differentiation was observed between the two wild populations. The results of the present study demonstrated that there is a close genetically similar relationship between Chalan *beel* and Tola *haor* populations than to the BFSC population.

RAPD marker has proven to be useful tools to investigate the population genetic status. Despite the fact that no specific markers were found to discriminate *O. bimaculatus* populations, the RAPD fingerprinting revealed some degree of divergence between them. The result of the present study can be used as baseline information regarding the genetic variation and population structure before undertaking any breeding program and for future study. Further studies involving large number of samples and loci are recommended to have a precise knowledge about the population status of *O. bimaculatus*. Mass seed production and conservation of the available populations through proper management of the populations in order to maintain their genetic quality is highly recommended to save this endangered species from extinction. The fish has already been started to culture, though in a very limited scale, in Bangladesh. It is expected that this effort may help restore the natural population though restocking programs in near future.

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References

Akter S, Sultana S, Khan MSR, Nahiduzzaman M, Hossain MAR, Alam MS. 2010. Genetic characterization of critically endangered *Puntius sarana* (Hamilton) and the exotic *Barbonymus gonionotus* (Bleeker) (Cyprinidae: Cypriniformes) by DNA Fingerprinting. International Journal of BioScience and Agriculture Technology **2**, 21-27.

Alam MS, Islam MS, Alam MS. 2010. DNA fingerprinting of the freshwater mud eel, *Monopterus cuchia* (Hamilton) by Randomly Amplified Polymorphic DNA (RAPD) Marker. International Journal of Biotechnology and Biochemistry **6,** 27-278.

Ambak M A, Bolong AM A, Ismail P, Tam BM. 2006. Genetic variation of snakehead fish (Channa striata) populations using random amplified polymorphic DNA. Biotechnology **5(1),** 104-110.

Crozier RH. 1997. Preserving the information content of species: genetic diversity, phylogeny and conservation worth Annual Review of Ecology and Systematics **28,** 243–268.

Garg RK, Sairkar P, Silawat N, Vijay N, Batav N, Mehrotra NN. 2009. Genetic diversity between two populations of *Heteropneustes fossilis* (Bloch) using RAPD profile. International Journal of Zoological Research **5,** 171-177.

Garg RK, Sairkar P, Silawat N, Vijay N, Batav N, Mehrotra NN. 2010. Assessment of genetic diversity of *Clarias batrachus* using RAPD markers in three water bodies of Bhopal. Journal of Environmental Biology **31,** 749-753.

Hassanien HA. 2008. Use of randomly amplified polymorphic DNA (RAPD) analysis to detect genetic variation in Seabass *Dicentrarchus labrax*. Journal of Fisheries and Aquatic Sciences **3**, 39-46.

Islam MS, Alam MS. 2004. Randomly amplified polymorphic DNA analysis of four different populations of the Indian major carp, *Labeo rohita* (Hamilton). Journal of Applied Ichthyology **20**, 407-412.

IUCN (International Union for Conservation of Nature). 2000. *Red Book of Threatened Fishes of Bangladesh*, IUCN- The World Conservation Union.

Kar D, Nagarathna AV, Ramachandra TV, Dey SC. 2006. Fish diversity and conservation aspects in an aquatic ecosystem in northeastern India. Zoo's Print Journal **21**, 2308-2315.

Khedkar GD, Reddy ACS, Persis M, Ravinder K, Muzumdar K. 2010. *Clarias batrachus* (Linn.1758) population is lacking genetic diversity in India. Molecular Biology Reports **37**, 1355-1362.

Kimberling DN, Ferreira AR, Shuster SM, Keim P. 1996. RAPD marker estimation of genetic structure among isolated northern leopard frog populations in the south-western USA. Molecular Ecology **5**, 521-529.

Liu ZJ, Li P, Argue BJ, Dunham RA. 1999. Random amplified polymorphic DNA markers: usefulness for gene mapping and analysis of genetic variation of catfish. Aquaculture **174**, 59–68.

Loew SS. 2000. The role of genetics in conservation biology In: Quantitative Methods for Conservation Biology (eds. Ferson S, Burgman M), Springer Verlag, NY. p. 226–258.

Lynch M. 1990. The similarity index and DNA fingerprinting. Molecular Biology and Evolution **7**, 478-484.

Lynch M, Milligan BG. 1994. Analysis of population genetic structure with RAPD markers. Molecular Ecology **3**, 91-99.

Lynch M, Conery J, Burger R. 1995. Mutation accumulation and the extinction of small populations. American Naturalist **146**, 489– 518.

Nei M. 1972. Genetic distance between populations. American Naturalist **106**, 283-292.

Nei M. 1973. Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences USA **70**, 3321-3323.

Parween S. 2007. *Ompok bimaculatus* (Bloch, 1797). In: Siddiqui, K.U., Islam, M.A., Kabir, S.M.H., Ahmed, M., Ahmed, A.T.A., Rahman, A.K.A., Haque, E.U., Ahmed, Z.U., Begum, Z.N.T., Hasan, M.A., Khondker, M., and Rahman, M.M. (eds.). *Encyclopedia of Flora and Fauna of Bangladesh*, Vol. 23. *Freshwater Fishes*. Asiatic Society of Bangladesh, Dhaka. p.127.

Peakall R, Smouse PE. 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology. Notes **6**, 288-295.

Rahman AKA. 1989. Freshwater fishes of Bangladesh. The Zoological Society of Bangladesh. 168-169pp.

Rahman SMZ, Khan MR, Islam S, Alam MS. 2009. Genetic variation of wild and hatchery populations of the catla Indian major carp (*Catla catla* Hamilton 1822: Cypriniformes, Cyprinidae) revealed by RAPD markers. Genetics and Molecular Biology, **32,** 197-201.

Saad YM, Hanafi MS, Essa MA, Guerges AA, Ali SF. 2009. Genetic signatures of some Egyptian *Clarias gariepinus* populations. Global Veterinarian **3**, 503-508.

Sultana S, Akter S, Hossain MAR, Alam MS. 2010. DNA fingerprinting of the Asian stinging catfish (*Heteropneustes fossilis,* Bloch) by Random Amplified Polymorphic DNA markers. International Journal of Biotechnology Applications **2,** 0975-9123.

Talwar PK, Jhingran AG. 1991. *Inland fishes of India and adjacent countries*. Oxford & IBH Publishing Co. PVT. Ltd., New Delhi, Vol. 2: 584pp.

Welsh J, McClelland M. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research **18**, 7213-7218.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research **18**, 6531-6535.

Yeh FC, Yang RC, Boyle T. 1999. POPGENE version 1.31: Microsoft Window-based freeware for population genetic analysis. ftp://ftp.microsoft.com/Softlib/HPGI.EXE