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Genetic variation among cat fish (*Mystus cavasius)* population assessed by randomly amplified polymorphic (RAPD) markers from Assam, India

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Abstract
In the present study molecular and morphological analysis of a population of Mystus cavasius caught from four different freshwater bodies of Assam, India about 100-400 km away from each other was done using RAPD markers. Total 578 RAPD fragments were generated using nine decamer primers of arbitrary nucleotide sequences. In the experiment 448 polymorphic bands and 130 monomorphic bands were produced which shows 77.50% of polymorphism and 22.49% of monomorphism. UPGMA dendrogram constructed on the basis of genetic distance formed four distinct clusters, indicating comparatively higher level of genetic variations in the studied M. cavasius populations in Assam. The genetic diversity data can be applied in the areas of research on evolution, conservation and management of cat fish resources and genetic improvement programmes. RAPD analysis for genetic diversity study provides a basis to obtain genetic variation within and among populations.

Keywords: Genetic, diversity, RAPD, markers, cat fish

1. Introduction
Bagridae family, the richest and the most important of the teleostei class comprising of 27 genera (six in Indian region) is widely distributed in Asia and Africa (Talwar & Jhingran, 1991). Even though the catfishes are in great demand in the Indian domestic markets, the catfish aquaculture, including *Mystus* sp has not yet been developed for its aquaculture potential. (Tripathi 1996). The entire demand for this fish in the domestic market is met through capture from river bodies and hence the effective management of wild stocks is critical. Information on population structure is useful for the development of management strategies that will conserve the biodiversity associated with different species, sub-species, stocks and races (Turan *et al.* 2005). Thus, detailed knowledge on the population structure of *Mystus sp.* is needed for sound management and successful commercial fishing of this species.
Genetic diversity and gene pools which helps in adaptation and survival is considered to be a key component for conservation and management of populations (Andayani, *et al*., 2001) [1]. Molecular markers along with the development of new statistical tools has indeed revolutionized the analytical power necessary to explore the genetic diversity, both in native populations and in captive lots (Tamanna *et al*., 2012) [25]. In recent years, a wide range of new molecular techniques have been explored and reported for fishes (Lehmann *et al*., 2000; Jayasankar, 2004). Random amplified polymorphic DNA (RAPD) one such technique which was first introduced by Williams *et al.* (1990). RAPD technique is the one of the most frequently used molecular methods for taxonomic and systematic analyses of various organisms and has provided important applications in catfish (Bartish et al., 2000). DNA fingerprinting has tremendous potential in aquaculture and in fisheries as a tool for identification of individuals (Jong-Man, 2001) and population genetics studies. (Bielawski and Pumo, 1997; Smith et al., 1997; Mamuris et al., 1998). RAPD is a technique based on the PCR amplification of discrete regions of genome with short oligonucleotide primers of arbitrary sequence (Welsh and McClelland, 1990; Williams *et al.*, 1990). It utilizes single, arbitrary, decamer DNA oligonucleotide primers to amplify regions of the genome based on the polymerase chain reaction (Hadrys *et al*., 1992; Williams *et al*., 1993). The characters assessed through RAPD are useful for genetic studies because they provide various types of

data-taxonomic population, inheritance pattern of various organisms including fishes (David and Pandian, 2006).

Morphometric studies of fish populations are very important for understanding the interactive effect of environment, selection and heredity on the body shapes and sizes within a species (Cadrin 2000). Several studies on the comparative morphometrics of different fish populations have been conducted (Ibanez- Aguirre *et al.* 2006). Animals with the same morphometric characteristics are often assumed to constitute a stock; this idea has been used widely in fishery stock differentiation studies (Avsar 1994).

The study of genetic diversity of catfishes of Assam is very much limited, so in the present study, this technique was applied to analyze the genetic relationship among *Mystus cavasius* populations. The objectives of this study are focused on morphometric identification and detection of RAPD pattern for determination of the genetic variation of a population of *Mystus cavasius* from Assam.

2. Materials and Methods

2.1 Fish sampling sites and morphometric measurements of fishes

Geographically, populations of *Mystus cavasius* were caught from freshwater bodies of Assam about 100-400 km away from each other, that is, Kolong river at Morigaon (Morigaon District) ; Deepor beel at Guwahati (Kamrup District) ; Kani beel(Dibrugarh District); Dhemaji local fish market (Dhemaji District) in the month of December, 2012. A total of 60 fish specimens were collected from all the locations with the help of local fishermen and 20 fish specimens were randomly selected for morphometric measurements and estimating genetic variations. All the fish specimens were kept in the iceboxes and brought to the laboratory for further study. For the morphometric measurements, total 24 parameters were considered. Fish specimens were morphologically identified with taxonomic keys (Srivastava, 2000; Jayaram, 1999). The muscle tissues were isolated from freshly caught fishes and preserved at -20 °C for further use.

2.2 Isolation of Genomic DNA from Fish Tissue

For the isolation of total genomic DNA, a modified protocol was followed using Sambrook and Russel (Molecular Cloning-A Lab. Manual). UV-VIS spectrophotometer was used to check quality as well as quantity of isolated DNA. Optical densities of the DNA samples were measured at 260 nm and 280 nm and the concentration of extracted DNA was adjusted to 50 ng/ µl for PCR amplification.

2.3 PCR primers

In the present study, 30 commercially available RAPD primers (10 to 20 base long) made by Xcelris Genomics, India were used to initiate PCR amplifications. Primers were randomly selected on the basis of GC content and annealing temperature for RAPD-PCR amplifications.

2.4 PCR amplification

The reaction mixture (10 µl) for PCR was composed of 1 µl of 10 X Taq polymerase buffer, 1 µl of 2.5 mM dNTPs, 1 µl of RAPD primer, 0.15 µl Taq DNA polymerase (2 U/ µl), 5.55 µl PCR grade water, 0.3 µl of 50 mM MgCl2 and 1 µl template DNA. A negative control, without template DNA was also included in each round of reactions. After preheating for 5 min*s* at 94 °C, PCR was run for 35 cycles. It consisted of a 94 °C denaturation step (1 min), 37 °C annealing step (1 min) and 72 °C elongation step (2 min) in a thermal cycler (Biorad). At the end of the run, a final extension period was appended (72 °C, 10 min) and then stored at 4 °C until the PCR products were analyzed.

2.5 Agarose gel electrophoresis

The amplified DNA fragments were separated on 1.8% agarose gel and stained with Ethidium bromide. A low range DNA marker of 100 bp from Bangalore Genei, Bangalore, India was run with each gel. The amplified pattern was visualized on an UV transilluminator and photographed by gel documentation system (BIORAD, USA).

2.6 Statistical analysis

The RAPD fragments were scored for the presence and absence of fragments on the gel photographs and RAPD fragments were compared among the *M. cavasius* populations. RAPD banding patterns were recorded on spreadsheets, which were used to determine gene diversity, gene flow, number of polymorphic loci and genetic distance through a construct by an un-weighted pair group method of arithmetic mean of UPGMA (Nei, 1978) using GGT 2.0 software (http://www.dpw.wau.nl/pv/pub/ggt/)

The similarity index (SI) values between the RAPD profiles of any 2 individuals on the same gel were calculated using following formula:

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

Where,

NAB = total number of RAPD bands shared in common between individuals A and B

NA = total number of bands scored for individual A

NB = total number of bands scored for individual B
(Lynch, 1990).

Cluster analysis was carried out using GGT 2.0 version software. Dendograms were constructed by employing UPGMA (Unweighted Pair Group Method with Arithmetic Mean) based on Sneath and Sokal (1973) to study the genetic variability within the species.Similarly the same method was followed to construct the dendogram to study the phylogenetic relationship among the genotypes of Mystus cavasius.

3. Results and Discussion

The morphometric variation among the different individuals of *Mystus cavasius* was found to be very low (Table-1).

Table 1: Morphometric measurements of *Mystus cavasius* (in proportion to Total length)

Characters	Minimum	Maximum	Mean±SD
Forked Furcal length***:TL***	83.16	96.25	7.16±***0***.644
Eye Diameter***:TL***	2.78	5.26	0.35±***0***.102
Caudal peduncle***:TL***	10	12.66	0.93±0.11
Dorsal fin Height***:TL***	10.53	14.29	1.04±.066
Dorsal fin Length***:TL***	10	12.79	***0***.91±0.94
Pectoral fin length***:TL***	11.11	18.95	1.24±***0***.335
Ventral fin height***:TL***	7.14	12.5	0.8±***0***.19
Ventral fin length***:TL***	1.39	3.75	***0***.21±***0***.083
Anal fin height***:TL***	8.57	12.66	***0***.92±***0***.178
Caudal fin length***:TL***	16.67	21.28	1.6±***0***.293
2nd Dorsal fin length***:TL***	10.47	12.66	0.96±***0***.128
Body width***:TL***	6.67	17.72	1.08±.346

Table 2: Morphometric measurements of *Mystus cavasius* (in proportion to standard length)

Characters	Minimum	Maximum	Mean±SD
Body Weight, gm	3.4	5.6	4.565±0.621
Total length, cm	7.6	9.3	8.56±0.526
Standard length, cm	5.9	7	6.58±0.399
Head length***:SL***	16.95	24.24	1.43±0.179
Pre Dorsal length***:SL***	32.86	38.46	4.24±5.755
Post Dorsal length***:SL***	64.29	70	4.39±0.239
Pre Orbital length***:SL***	7.14	8.47	0.5±0
Post Orbital length***:SL***	5.71	9.09	0.5±.063
Head Width***:SL***	8.47	15.15	0.88±0.16
Body Depth***:SL***	17.14	29.23	1.51±0.158
Head length excluding snout***:SL***	14.28	16.95	1±0
Snout length***:SL***	6.35	7.69	0.47±.046
Anal Fin length***:SL***	7.14	12.31	0.59±.094

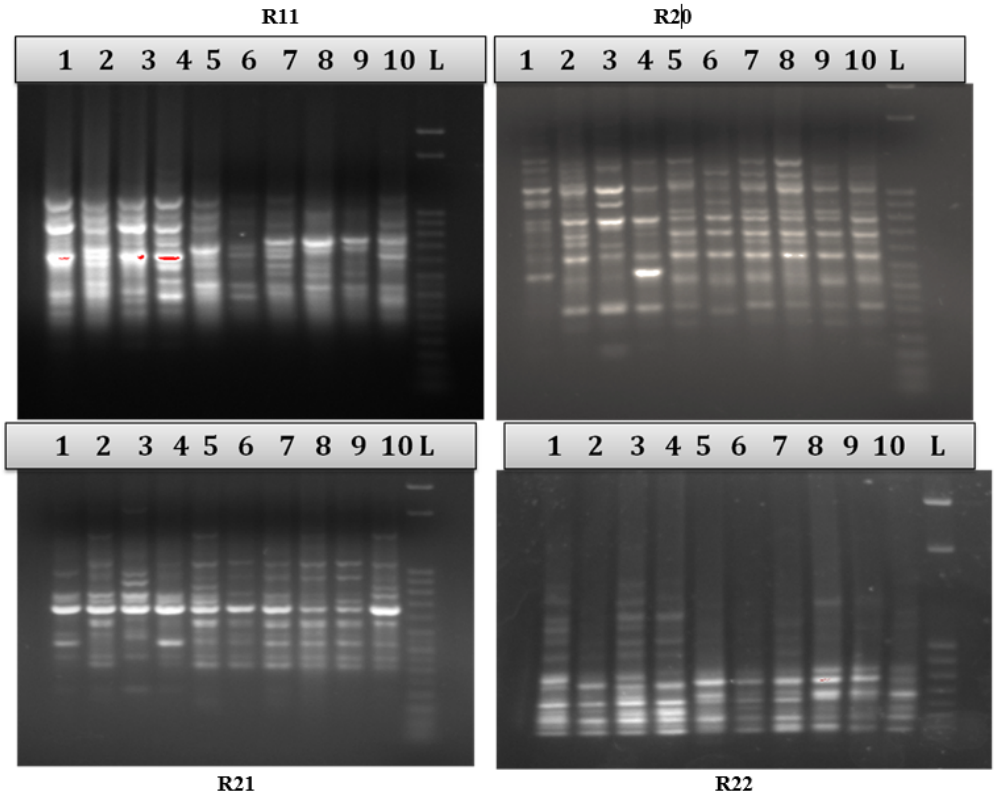

Fig 1: RAPD profile of *Mystus cavasius* with primers R11, R20, R21 and R22

3.1 RAPD polymorphisms

Among the 30 primers initially tested, only nine R-4, R-5,R-6, R-11, R-12, R-13, R-20, R-21 and R-2***2*** were selected that yielded relatively large number of good quality bands. All the

primers produced different RAPD patterns, and the number of fragments amplified per primer varied. The nine primers yielded a total of 578 reproducible and consistently scorable RAPD bands of which 448 were found to be polymorphic and 130 were monomorphic. The number of bands per primer ranged from 5 to 13 (Table 3). Among the primers, R-6 gave DNA profile with highest number of bands while R-20 gave the least (Table 3) (Fig) The RAPD profile of the bands obtained in the population of *M. cavasius* with primer R-11,R-20,R-21 and R-22 is shown in the Figure 2 as representative photographs. The UPGA dendrogram was prepared based on genetic distance by the GGT 2.0 software. The unweighted dendrogram divided all the genotypes in four clusters.

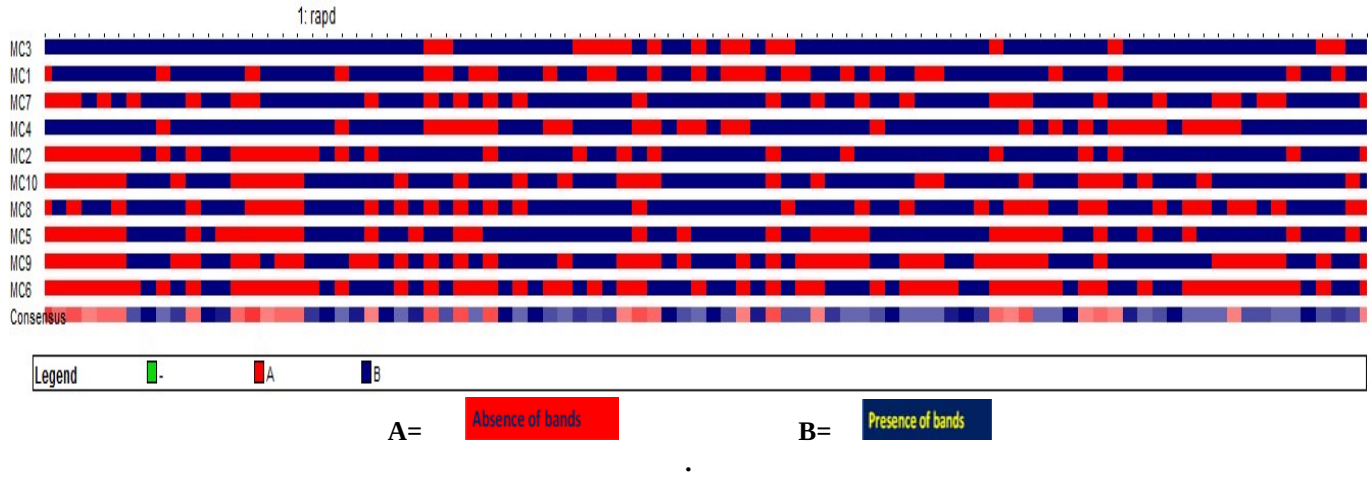

Fig 2: Genetic polymorphisms in 10 genotypes of *Mystus cavasius*

Table 3: Pattern of polymorphism (primer wise) in 10 individuals of *Mystus cavasius.*

S. No	Polymorphism	*Mystus cavasius*								
		R-4	R-5	R-6	R-11	R-12	R-13	R20	R21	R22
1	Total no of bands	76	82	84	83	60	36	34	60	63
2	Total no of polymorphic bands	56	62	64	63	50	26	24	50	53
3	Total no of monomorphic bands	20	20	20	20	10	10	10	10	10
5	Polymorphism(%)	73.6	75.6	76.1	75.9	83.3	72.2	70.5	83.3	84.1
6	Monomorphism(%)	26.3	24.9	23.8	24.1	16.6	27.7	29.4	16.6	15.8

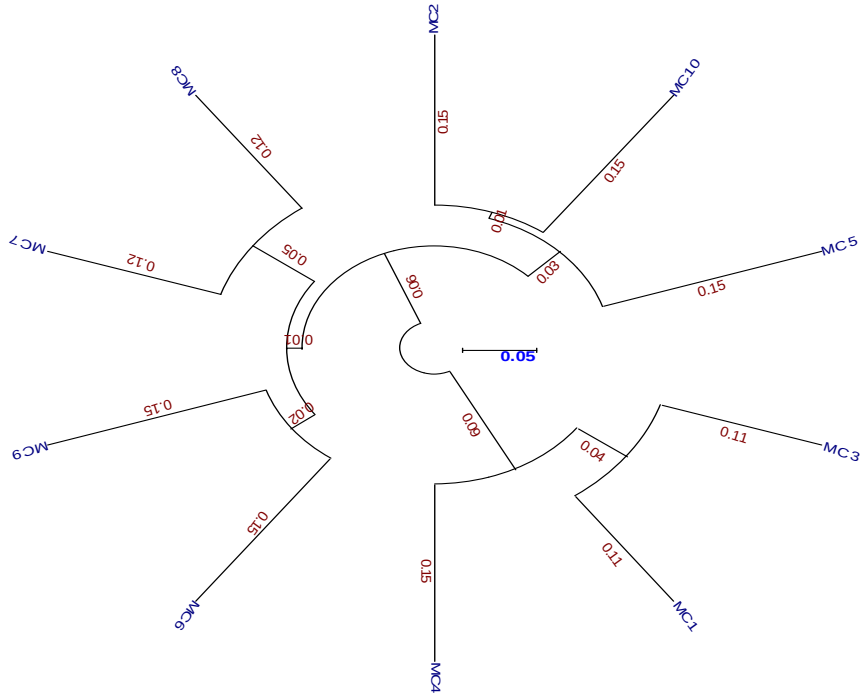

Fig 3: Genetic similarity in 10 genotypes of *Mystus cavasius*

Genetic diversity patterns among populations can provide clues to the populations' life histories and degree of evolutionary isolation because genetic diversity or variation represents the existence of variants (alleles) of individual genes resulting from alterations of the DNA sequence (Williamson, 2001; Çiftci and Okumus, 2002). The alleles of

a particular gene may occur in different frequencies in different groups of interbreeding individuals (populations) and the genetic variation of a particular species is therefore distributed both within populations (expressed as different allele combinations between individuals) and between populations (differences in occurrence and frequency between populations).

Most of the morphometric characteristics of the fishes in the present study were similar and often overlap. These morphometric data may not be enough to support the established genetic structure of the population that often leads to taxonomic uncertainty in many occasions because of the considerable geographical and ecological variability in form (Ponniah and Gopalkrishnan, 2000; Gargetal., 2009). Significant differences in allele frequencies and morphological variations in *Hilsa sp* were observed from nine different sites within Bangladesh as reported by Salini et al., which may be due to the local environmental conditions.

In the present study among the 30 single decamer random primers, nine primers generated a total of 578 bands in the population which were found to be both polymorphic and monomorphic. In the experiment 448 polymorphic bands and 130 monomorphic bands were produced which shows 77.50 % of polymorphism and 22.49% of monomorphism (Fig-1). Polymorphism for genetic similarity among the different individuals of *M. cavasius* which was analyzed using GGT software is expressed in Fig-3. Yellow colour shows a significant relationship between the genotypes. It remarks that all are significantly related genotypes. The cluster analysis and dendogram showing genetic relationship between 10 genotypes of *M.cavasius* showed formation of 4 clusters (Fig-3). Cluster I include genotype 7 and 8; Cluster II include genotype 2,10 and 5;Cluster III include genotype 3,1 and 4 ;Cluster IV include genotype 6 and 9.

The present study indicates that comparatively higher level of genetic variation exists in the studied *M. cavasius* populations in Assam. Garg et al., 2009 could discriminate between the different populations of *M. vittatus* in reservoirs of Madhya Pradesh by RAPD analysis. According to them the intraspecific genetic similarity between the individuals of the population was due to geological variations or changes in the aquatic environment. Our statistical analysis showed considerable genetic variation among the genotypes of *M.cavasius* collected from different locations of Assam. This population genetic differentiation may be due to ecological, geographical and evolutionary factors. The genetic diversity data has varied applications in research on evolution, conservation and management of natural resources and genetic improvement programmes, etc. RAPD analysis for genetic diversity study provides a basis to obtain genetic variation within and among populations. Once the population structure is known, scientific management for optimal harvest and conservation of the catfish fishery resource can be undertaken. Therefore, the present study may serve as a reference point for future examinations of genetic variations within the populations of fishes which are commercially important and the possible use of DNA markers in the future may create new avenues for fish molecular biological research.

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