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Genetic Variation among Cat Fish (*Mystus vittatus*) Population Assessed by Randomly Amplified Polymorphic (RAPD) Markers from Assam, India

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Abstract

Mystus vittatus is a small indigenous fish species having higher nutritional value in terms of protein, micronutrients, vitamins and minerals. But the catfish aquaculture including Mystus sp has not been developed extensively for its aquaculture potential even though the demand of catfishes in the Indian domestic markets are very high. Therefore for good aquacultural practices and to maintain a healthy gene pool, detailed knowledge on the population structure of Mystus sp. is needed.

In the present study molecular and morphological analysis of a population of *Mystus vittatus* caught from four different freshwater bodies of Assam about 100-400 km away from each other was done using RAPD markers. Total 412 RAPD fragments were generated using nine decamer primers of arbitrary nucleotide sequences. In the experiment 322 polymorphic bands and 90 monomorphic bands were produced which shows 78.15% of polymorphism and 21.84% of monomorphism. UPGMA dendrogram constructed on the basis of genetic distance formed three distinct clusters indicating comparatively higher level of genetic variations in the studied M. vittatus populations in Assam. 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 for future examinations of genetic variations within the populations of fishes which are commercially important and the possible use of DNA markers in future may create new avenues for cat fish molecular biological research in this part of world.

Keywords: Genetic; Diversity; RAPD; Markers; Cat fish

Introduction

The striped dwarf catfish *Mystus vittatus* is an economically important and favourite food fish in the South East Asian countries. This important species for aquaculture is naturally distributed in India, Bangladesh, Pakistan, Sri Lanka, Nepal, Myanmar and Thailand [1]. 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 [2]. 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. For the development of effective management strategies information on population structure is essential to conserve the biodiversity associated with different species, sub-species, stocks and races [3]. Therefore for good aquacultural practices and to maintain a healthy gene pool, detailed knowledge on the population structure of *Mystus sp.* is needed.

All organisms are subject to mutations because of normal cellular operations or interactions with the environment, leading to genetic variation (polymorphism). Genetic variation in a species enhances the capability of organism to adapt to changing environment and is necessary for survival of the species [4]. Genetic variation arises between individuals leading to differentiation at the level of population, species and higher order taxonomic groups apart from other evolutionary forces like selection and genetic drift. Molecular markers along with the development of new statistical tools has revolutionized the analytical power necessary to explore the genetic diversity, both in native populations and in captive lots [5]. Nowadays, a wide range of new molecular techniques have been explored and reported for fishes [6,7]. Williams *et al.* first introduced the Random amplified polymorphic DNA (RAPD) technique [8]. 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 [9]. RAPD has also been used to estimate genetic diversity and variations needed to study fish management and conservation practices, even with endangered species [10,11]

It is based on the PCR amplification of discrete regions of genome with short oligonucleotide primers of arbitrary sequence [12]. 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 [13].

The information on morphometric measurements of fishes and the study of statistical relationship among them are essential for taxonomic work [14]. Moreover, to know the origin of stock, separation of stocks or identification of commercially important species of fishes, morphometric characters are frequently used [15,16]. These type of study are important for understanding the interactive effect of environment, selection and heredity on the body shapes and sizes within a species [17]. Several studies on the comparative morphometrics of different fish populations have been conducted [18]. 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 vittatus* populations. The objectives of this study are focused on morphometric identification and

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detection of RAPD pattern for determination of the genetic variation of a population of *Mystus vittatus* from Assam, India.

Materials and Methods

Fish sampling sites and morphometric measurements of fishes

Geographically, populations of *Mystus vittatus* 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 June, 2013. 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 [19,20]. The muscle tissues were isolated from freshly caught fishes and preserved at -20°C for further use.

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 [21]. 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 280nm and the concentration of extracted DNA was adjusted to 50 ng/µl for PCR amplification.

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.

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 $MgCl_2$ and 1 µl template DNA. A negative control, without template DNA was also included in each round of reactions. After preheating for 5 mins at 94°C, PCR was run for 35 cycles. It consisted of a 94°C denaturation step (1min), 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.

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).

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. vittatus* 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 or UPGMA [22] 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,

N_{AB}=total number of RAPD bands shared in common between individuals A and B

N_A=total number of bands scored for individual A

N_B=total number of bands scored for individual B [23],

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 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* vittatus [24].

Results and Discussion

The morphometric variation among the different individuals of *Mystus vittatus* was found to be very low (Table 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-22 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 412 reproducible and consistently scorable RAPD bands of which 322 were found to be polymorphic and 90 were monomorphic. The number of bands per primer ranged from 5 to 13. Among the primers, R-4 and R-21 gave DNA profile with highest number of bands while R-13 gave the least (Table 2). The RAPD profile of the bands obtained in the population of *M.vittatus* with primer R-4,R-5,R-6 and R-11 is shown in the Figure 1 as representative photographs. The UPGMA dendrogam was prepared based on genetic distance by the GGT 2.0 software. The unweighted dendrogam divided all the genotypes in three clusters.

Little m orphological differences were revealed among the *M.vittatus* populations by morphometric and meristic studies that were sampled from different rivers and localities in Assam. Most of the morphometric

Characters	Minimum	Maximum	Mean ± SD
Forked Furcal length:TL	6.2	7.9	7.16 ± 0.644
Eye Diameter:TL	0.2	0.5	0.35 ± 0.102
Caudal peduncle:TL	0.7	1.0	0.93 ± 0.11
Dorsal fin Height:TL	1.0	1.2	1.04 ± 0.066
Dorsal fin Length:TL	0.8	1.1	0.91 ± 0.094
Pectoral fin length:TL	0.8	1.8	1.24 ± 0.335
Ventral fin height:TL	0.1	0.3	0.8 ± 0.19
Ventral fin length:TL	0.1	0.3	0.21 ± 0.083
Anal fin height:TL	0.7	1.2	0.92 ± 0.178
Caudal fin length:TL	1.2	2.0	1.6 ± 0.293
2nd Dorsal fin length:TL	0.8	1.2	0.96 ± 0.128
Body width:TL	0.6	1.5	1.08 ± 0.346

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

Characters	Minimum	Maximum	Mean ± SD
Body Weight,gm	2.35	5.15	3.59 ± 1.087
Total length,cm	7.1	9.5	8.17 ± 0.88
Standard length,cm	5.3	7.5	6.74 ± 0.9
Head length:SL	26.09	31.08	1.89 ± 0.281
Pre Dorsal length:SL	35.71	40.74	2.6 ± 0.335
Post Dorsal length:SL	55.71	64.81	3.96 ± 0.4
Pre Orbital length:SL	6.67	9.26	0.55 ± 0.081
Post Orbital length:SL	12	16.36	0.95 ± 0.067
Head Width:SL	7.55	13.51	0.77 ± 0.205
Body Depth:SL	6.67	20.29	0.91 ± 0.394
Head length excluding snout:SL	13.33	20.27	1.15 ± 0.216
Snout length:SL	3.77	6.76	0.36 ± 0.88
Anal Fin length:SL	7.55	11.59	0.64 ± 0.128

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

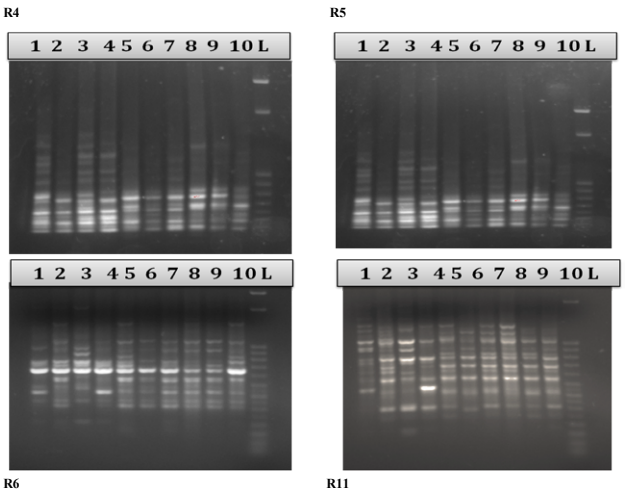

Figure 1: RAPD profile of *Mystus vittatus* with primers R4, R5, R6 and R11.

Sl no	Polymorphism	*Mystus vittatus*								
		R-4	R-5	R-6	R-11	R-12	R-13	R20	R21	R22
1	Total no of bands	58	36	38	48	37	35	53	58	49
2	Total no of polymorphic bands	48	26	28	38	27	25	43	48	39
3	Total no of monomorphic bands	10	10	10	10	10	10	10	10	10
5	Polymorphism (%)	82.75	78.8	73.6	79.16	72.9	71.4	81.1	82.7	79.5
6	Monomorphism (%)	18.18	27.7	26.3	27.7	27.1	28.5	18.8	17.2	20.5

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

characteristics of the fishes in the present study were similar and there is overlap in the range of each of traditional morphometric measurements taken (Tables 2 and 3). The morphometric data may not be enough to support the established genetic structure of the population that often leads to taxonomic uncertainity in many occasions because of the considerable geographical and ecological variability in form [25,26]. In *Hilsa* sp., significant differences in allele frequencies and morphological variations were observed from nine different sites within Bangladesh by which may be due to the local environmental conditions [27].

DNA fingerprinting method has tremendous potential in aquaculture and in fisheries as a tool for identification of individuals [28] and population genetics studies [29-31]. RAPD-PCR is a useful tool for estimating the genetic variability and degree of similarity among fish species as has been reported by other workers [32]. Using a RAPD analysis, the intrapopulation variation was detected with different primers in tilapia [33]. Chong et.al could identify and characterize *Mystus nemurus* populations by RAPD analysis in Malaysia [34]. RAPD technique has also been used to determine genetic variation within and among three populations of *Mystus vittatus* by Tamanna et.al in Bangladesh in the year 2012 [5].

In the present study among the 30 single decamer random primers, nine primers generated a total of 412 bands in the population which were found to be both polymorphic and monomorphic. In the experiment 322 polymorphic bands and 90 monomorphic bands were produced which shows 78.15% of polymorphism and 21.84% of monomorphism (Figure 1). Polymorphism for genetic similarity among the different individuals of *M. vittatus* which was analyzed using GGT software is expressed in Figure 2. The cluster analysis and dendogram showing genetic relationship between 10 genotypes of *M.vittatus* showed formation of 3 clusters (Figure 3). Cluster I include genotype 4, 7, 9 and 8; Cluster II include genotype 10,3 and 1; Cluster III include genotype 6, 5 and 2.

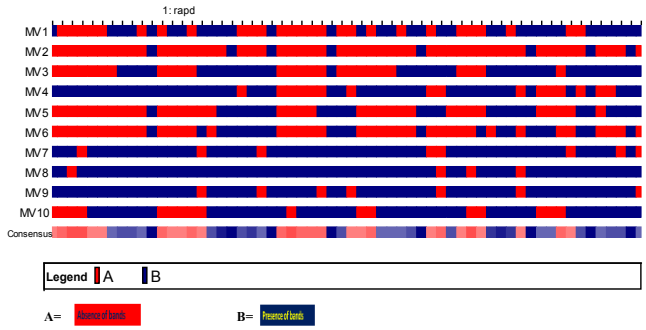

Figure 2: Genetic polymorphisms in 10 genotypes of *Mystusvittatus*.

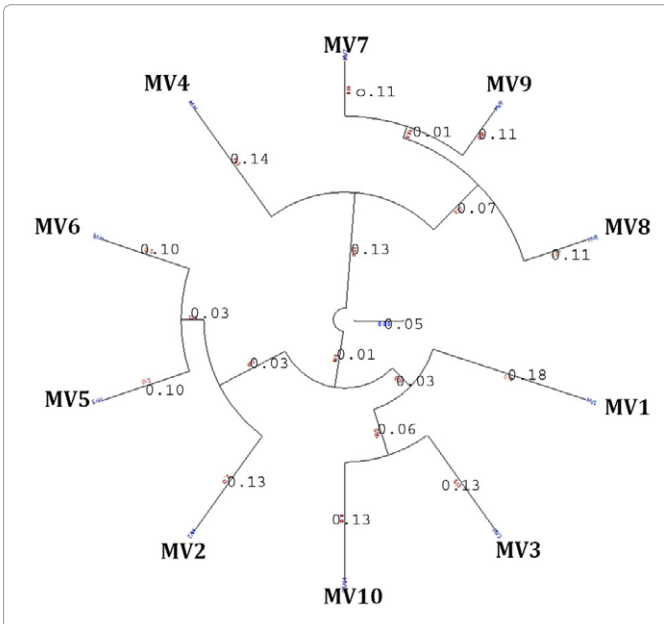

Figure 3: Genetic similarity in 10 genotypes of *Mystusvittatus*.

The present study indicates that comparatively higher level of genetic variation exists in the studied *M. vittatus* populations in Assam. Garg et al. could discriminate between the different populations of *M.vittatus* in reservoirs of Madhya Pradesh by RAPD analysis [35]. According to them the intraspecific genetic similarity between the individuals of the population was due to geological variations or changes in aquatic environment. Our statistical analysis showed considerable genetic variation among the genotypes of *M.vittatus* 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 future may create new avenues for fish molecular biological research.

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