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Research Paper

# Intraspecific Genetic Variation of Trichogaster Fasciatus Bloch & Schneider, 1801 (Osphronemidae) Assessed By Random Amplified Polymorphic Dna (Rapd) Analysis

Zoology

KEYWORDS : Trichogaster fasciatus, Intraspecific genetic diversity, RAPD

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## ABSTRACT

*The egg shaped with orange and bluish bars freshwater fish Trichogaster fasciatus is a member of family Osphronemidae and is commonly known as striped or banded gourami. It is one of the popular fish among aquaculturist due to its ornamental value and has great utility to relieve pain from spider bite. An unique feature of this fish is it can spit on insect to bring them to surface level. However to enhance its aquaculture potentiality and to maintain a vigorous gene pool, detailed knowledge is required on the population structure of Trichogaster. This study was carried out by using RAPD markers to investigate morphological and molecular aspect of a population of Trichogaster fasciatus collected from different freshwater bodies of Assam about 100-450 km away from each other. A total of 36 amplified bands were produced using five decamer primers having arbitrary nucleotide sequence. The results reveal that 22 polymorphic bands and 14 monomorphic bands were produced which shows 65.32 % of polymorphism and 34.64% of monomorphism. Based on the genetic distance UPGMA dendogram was constructed which revealed the formation of three main clusters signifying comparatively higher level of genetic variations in the studied T. fasciatus populations in Assam. Once the population structure is known, restoration of the ornamental fish population can be done. Thus the present study may serve as a prospect for examinations of genetic variations within the populations of fishes which are ornamentally significant and the possible use of DNA markers in future may pave path for ornamental fish molecular biological research.*

## 1. Introduction:

The egg shaped with orange and bluish bars freshwater fish *Trichogaster fasciatus* is a member of family *Osphronemidae* and is mainly distributed in Southern Asia (Froese, Rainer and Daniel Pauly 2014). It is generally known as Banded or Striped Gourami (Biswas and Lepcha, 2004). It is well known as ornamental fish in countries like the USA, Singapore, Japan, China etc, though it was considered as a weed fish in India (Dwivedi, 2004). For its ornamental value it has a great demand in these foreign countries, to satisfy which the species has been pushed to a threatened condition (Mukherjee, 2004). Moreover the application of pesticides and insecticides in the agricultural fields, chemicals leach out and pollutes the natural environment affecting the breeding and feeding ground of the fish. This has lead the scientists to work for the refurbishment of the fish population and save it from being vulnerable (Behera *et al*., 2015). Though India has a vast fish biological resource comprising of more than 10% of world fish diversity (Das and Pandey 1998) but most of the indigenous aquarium fish have not been characterized and documented genetically. The identification and characterisation based on morphological characters are sometimes insufficient to identify a species especially in the early stages of development (Ayoma *et al*., 2000). The fishes *Badis badis* ( Hamilton 1822) and *Dario dario* (Kullander and Britz 2002) (Actinopterygii, Perciformes, Badidae) can be distinguished morphologiocally and Hamilton (1822) placed the fishes in genus *Labrus* and named them *Labrus badis* and *Labrus dario* respectively, but much later Talwar and Jhingran (1991) exhibited that both *B.badis* and *D.dario* to be *B.badis* (*B.badis bengalensis*) and recently Kullander and Britz (2002) placed *Badis badis bengalensis* in the new genus *Dario* after revising the family *Badidae*.

According to Andayani *et al.,* (2001) genetic diversity and gene pools are considered to be a key component in population conservation and management. With the advent of molecular markers and new statistical tools the genetic diversity can be assessed easily both in native populations and in captive lots (Tamanna *et al.,* 2012). A wide range of molecular techniques has been reported in recent years (Lehmann *et al.,* 2000; Jayashankar, 2004). Williams *et al*., (1990) introduced one such technique called Random amplified Polymorphic DNA (RAPD). It is frequently used molecular technique for taxonomic and systematic analyses of various organisms and Bartish *et al*., (2000) had reported its applications in catfish. RAPD has been used in identification of individuals in aquaculture and fisheries (Jong-Man, 2001) and population genetics studies ( Bielawski and Pumo, 1997; Smith *et al*., 1997; Mamuris *et al.,* 1998). In RAPD discrete regions of the genome is amplified with short oligonucleotide sequences (Welsh and McClelland, 1990; Williams *et al.,* 1990). Various types of data relating to taxonomic positions and inheritance patterns of various organisms including fishes can be assessed through RAPD (David and Pandian, 2006). The study of genetic diversity of *Trichogaster* species is very much limited and so in the present study RAPD techniques was applied to analyse genetic relationship among the different species. The objective of this study is to identify the intraspecific genetic variation of *Trichogaster fasciatus* Bloch & Schneider, 1801 (Osphronemidae) using random amplified polymorphic DNA (RAPD) analysis

## 2. Materials and Methods:

### 2.1 Sample collection and Morphometric measurements:

A total of 80 samples of *Trichogaster fasciatus* were collected from the Brahmaputra River (Maligaon,Guwahati), Nangal Bhanga Stream (Chirang District), Bogibeel Dibrugarh, Deepor Beel,Guwahati ,Patiasala beel (Jorhat), Kolong River and Jalugoti beel (Morigaon District), Katli Beel (Dhubri District), from the state of Assam, India in the month of August 2013.. Their weight ranged from 15-80mg.The fishes were maintained temporarily in an aquarium maintaining the required condition of water parameters and temperature at laboratory of UGC SAP (DRS) Project, Department of Zoology, G.U. 20 fish specimens were randomly selected for morphometric measurements and to assess genetic variation. The fish samples were identified with the aid of taxonomic keys (Vishwanath *et al*., 2014).The muscle tissues were isolated from freshly caught fishes and preserved at -20°C for further use.

### 2.2 Genomic DNA extraction

For the isolation of total genomic DNA standard phenol chloroform isoamyl alcohol was followed as described by Sambrook & Russel (2001) with minor modification. Tissues (25 - 30 mg) were placed in a 1.5 ml microcentrifuge tube and homogenized by using Eppendorf micro-pestle, with the help of liquid nitrogen. In the homogenized tissue, 700µl of lysis buffer (10 mM NaCl, 0.25 mM EDTA, 0.1% SDS and 0.25 M Tris HCl) and 20µl proteinase

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K were added, mixed gently and incubated overnight at 55°C in a waterbath (Yorco Serological) for complete lysis of cells. After incubation, 500 µl phenol : chloroform: isoamyl alcohol (25:24:1) were added, mixed gently and centrifuged at 12,000 rpm at room temperature (High speed Eppendorf centrifuge,5424R) for 5 min. The supernatant was then transferred to a new micro-centrifuge tube and half volume of 7.5 M sodium acetate and 2 volume of 100% chilled ethanol was added and mixed well and incubated at -20°C for 30 min. The mixture was centrifuged at 12,000 rpm for 15 min at room temperature. The supernatant was discarded and a comma shaped milky white pellet visible. 500µl of 70% chilled ethanol was added and centrifuged at 12000 rpm for 10 min. Again, 70% ethanol was added and centrifuged at 12000 rpm for 10 min at room temperature. The supernatant was carefully discarded and allowed to air dry. The air dried DNA was dissolved in 30µl PCR grade water. UV-VIS spectrophotometer (Nano-Drop ND-1000, USA) was used to check quality as well as quantity of isolated DNA. DNA quality and concentration were determined by agarose gel electrophoresis.

**2.3 RAPD reactions**

RAPD-PCR reaction was performed as per the protocol described by Williams *et al.,* (1990) and Welsh and McClelland (1990) with slight modifications. A master mix was prepared, containing 1.5mM MgCl2, 2.5mM dNTP's, 10mM primer, 25ng of DNA template 1U/µl Taq DNA polymerase, 10 X Taq polymerase buffer and PCR grade water to make the volume to 25µl. A total of 20 commercially available RAPD primers (10 to 20 base long) (make Bangalore Genei, India) were used to initiate PCR amplifications, of which only five yielded reproducible bands **(table 1).** Primers were randomly selected on the basis of GC content and annealing temperature for RAPD-PCR amplification. After preheating for 5 min at 94°C, PCR was run for 35cycles. 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 (Bio-Rad). At the end of the run, a final extension period was appended (72°C for 2 min) and then it was subjected to a hold temperature of 10°C. The PCR products thus obtained was run in a 2% Agarose Gel electrophoresis.

**Table 1. Sequence of primers**

| PRIMER | 5'-3' SEQUENCE | Tm Value |
|---|---|---|
| R11 | CTTCACCCGA | 30 |
| R12 | CCCGCTACAC | 34 |
| R13 | GTGTCTCAGG | 32 |
| R14 | GTGGGCTGAC | 34 |
| R15 | GTCCATGCCA | 32 |

**2.4 RAPD data analysis**

The amplified DNA fragments were scored for the presence (1) and absence (0) of bands on the gel photographs and RAPD fragments of *T.fasciatus* population were compared. RAPD banding patterns were recorded on spreadsheets, which were used to determine gene diversity, number of polymorphic loci and genetic distance through a construct by an un-weighted pair group method of arithmetic mean of UPGMA (Nei, 1978). UPGMA clustering method was used to generate a dendogram by computing genetic similarity values in GGT 2.0 software. 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, $N_{AB}$ is the total number of RAPD bands shared by individuals A and B.

$N_A$ & $N_B$ = total bands scored for individual A & B, respectively.

**3. Results & Discussions:**

**3.1 Morphometric Analysis**

The morphometric variation among the different individuals of *Trichogaster fasciatus* was found to be very low (Table 2). The coefficient of variation (CV) is calculated and the estimated values ranged from0.78(head depth ) to 10.96 (snout length)

**TABLE 2: Morphometric data of *T. fasciatus***

| Parameters | Range | Mean ± SD | $C_V$ |
|---|---|---|---|
| SL % | | | |
| Body depth | 41.8-44.3 | 43.2±0.8 | 1.85 |
| Head length | 30.9-32.9 | 31.9±0.6 | 1.88 |
| Caudal peduncle length | 1.7-2.1 | 1.9±0.1 | 5.26 |
| Caudal peduncle depth | 14.3-16.7 | 15.6±0.8 | 5.13 |
| Dorsal fin base length | 58.2-62.2 | 60.6±1.0 | 1.65 |
| Dorsal fin length | 5.5-8.5 | 7.8±0.8 | 10.26 |
| Pectoral fin length | 29.3-32.2 | 30.7±0.8 | 2.61 |
| Pelvic fin length | 65.4-68.3 | 67.4±0.8 | 1.19 |
| Anal fin length | 65.1-69.0 | 67.5±0.3 | 0.44 |
| Pre dorsal length | 30.9-34.1 | 32.5±0.9 | 2.77 |
| Post dorsal length | 63.8-66.0 | 65.0±0.7 | 1.08 |
| HL % | | | |
| Head depth | 114.1-117.2 | 115.7±0.9 | 0.78 |
| Head width | 59.9-63.3 | 62.7±1.0 | 1.59 |
| Snout length | 18.5-25.9 | 21.9±2.4 | 10.96 |
| Eye diameter | 14.8-19.2 | 18.0±1.2 | 6.67 |
| Inter orbital distance | 47.7-49.0 | 48.2±0.4 | 0.83 |

**3.2 RAPD polymorphisms**

Among the 20 primers initially tested, only five, R-11, R-12, R-13, R-14 and R-15 were selected that yielded relatively large number of good quality bands. The number of fragments amplified per primer was variable. The five primers yielded a total of 36 reproducible and consistently scorable RAPD bands of which 22 were found to be polymorphic and 14 were monomorphic. RAPD-PCR showed bands in the range of 300-1200 bp. Figure 1, represents the RAPD profile of the bands obtained in the population of *T.fasciatus* with primer R-11 and R-12 and R15 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. The phylogenetic relationship between the members of *T.fasciatus* species from different geographical location was illustrated by UPGMA dendogram based on genetic distances calculated on the basis of allele sharing similarity co-efficient. The dendogram with RAPD markers grouped the population into three main clusters *i.e* TF1(Maligaon,Kamrup district ) and TF4 (Deepor beel, Kamrup district) formed one cluster and TF2( Chirang district) alone was included in one cluster and the third cluster was formed of TF8(Katli beel,Dhubri) which was closely related to TF3 (Bogibeel,Dibrugarh), TF5(Patiachala beel,

Jorhat),TF 6(Kalang, Morigaon)and TF7 (Jalugati beel, Jorhat ) formed the other subcluster (Fig. 2).

The genetic diversity that exists in a population can give indications of the population's life histories and the degree of evolutionary isolation as genetic diversity signifies the existence of variants (alleles) of individual genes resulting from alterations of the DNA sequence (Çiftci and Okumus, 2002).The morphometric characters in the present study were found to be similar and often overlap. However morphometric data not be adequate to support the well-known 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; Garg *et al*., 2009).

Homozygosity and heterozygosity in a population is exhibited by monomorphic and polymorphic loci respectively. Several hypothesis were put forward to explain homozygote in fish population including inbreeding population or the presence of a non expressed allele (Appleyard *et al*., 2001). In the present study among the 20 single decamer random primers, five primer generated a total of 36 bands in the population which were found to be both polymorphic and monomorphic. In the experiment 22 polymorphic bands and 14 monomorphic bands were produced which shows 65.32% of polymorphism and 22.49% of monomorphism

The present study reveals comparatively higher level of genetic variation in the population of *T.fasciatus* studied from different geographical locations of Assam. Awasthi, 2014 conducted similar intraspecific variation studies in five populations of *C.lalia* in Kolkatta and found highly deviated from each other, this might be due to geographic isolation and difference in environmental condition, and moreover this could have been hindering the movement of fishes to intermingle with populations of other. Our statistical analysis highlighted genetic variation among the genotypes of *T.fasciatus* of different geographical locations of Assam. Ecological and geographical and evolutionary factors might be attributed to the observed genetic variation in the population of the fish species. The result of RAPD can further be extended using other molecular methodologies. Today aquaculture genetics at the zenith of genetic engineering leading to better opportunities in the study of fish molecular biological research. Thus the present study may dole out as an allusion point for intraspecific genetic variation of fishes in future.

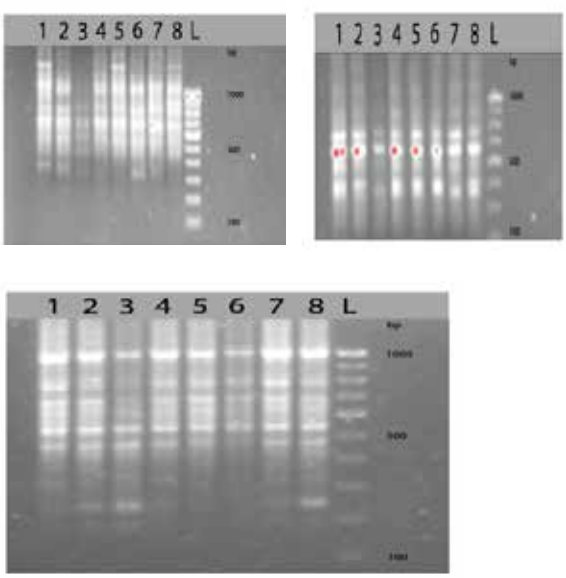


**FIG 1: RAPD profile of *T.fasciatus* with primer R11, R12 and R15respectively.**

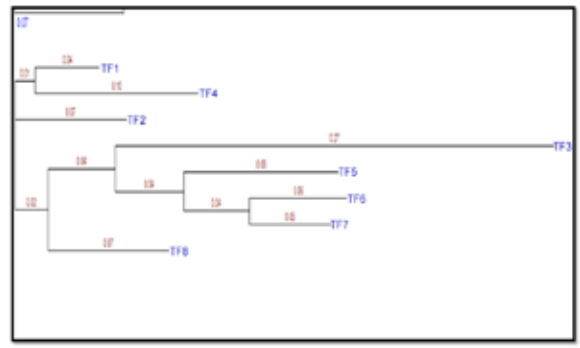


**FIG 2: Dendrogram of eight individuals of *Trichogaster fasciata* (TF)**

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