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Genetic Variation among Dwarf Gourami (*Trichogaster lalia*, Osphronemidae) populations using Random Amplified Polymorphic DNA(RAPD) markers

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ABSTRACT:

Random amplified polymorphic DNA (RAPD) was applied to generate species-specific diagnostic fragment patterns for the molecular identification of the ornamental aquarium fish species *Trichogaster lalia,* more commonly known as dwarf gourami. The species were collected from various geographically distant locations of Assam. After initial screening, four primers having a length of 10 arbitary nucleotide sequence were used which generated the RAPD profile for *Trichogaster* species. The primers produced 39 bands in total. In the experiment 22 polymorphic bands and 7 monomorphic bands were produced. The genetic distance of an individual ranged from 0.03 to 0.38. The average genetic distance among the individuals showed that more than 0.03 species are genetically more similar

Keywords:
Genetic diversity, RAPD, Dwarf gourami.

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INTRODUCTION

Osphronemidae is the most diverse family of fish with 11 genera and more than 30 species distributed throughout the Indian Sub-Continent, South East Asia and China, of which only 7 genera are reported from India (Jayaram,1999);and *Trichogaster lalia (*commonly known as dwarf gourami) is a popular species of this family among aquarist for their blue colour. The fish species is distinct from its other ornate in having a translucent blue colour accented with fine stripes. This fish is beautifully appointed and makes a brilliant display in the aquarium. The fish can be diagnosed with head blunt and unarmed, moderate sized eyes, lateral line absent, caudal fin fan-shaped, pectoral fin low and ventral fin extending to end of anal fin. Dwarf gourami is distributed over Asian subcontinent (Pakistan, Nepal, Bangladesh, Myanmar, Borneo). In India it is naturally found in Tripura, (Ganga and Yamuna river system) and in Assam it is found in Brahmaputra river and in various beels of Assam.(**IUCN status:** Least concern LC). However, there is insufficient studies in regard to the estimation of genetic diversity of this fish species.

A vital attribute of a population which signifies the fitness of the individuals as well as survival of the entire population is its genetic variability. Genetic variation refers to the adaptive potentiality of individuals to the changing environmental conditions and stress. Hence the squalor of genetic diversity of a species reduces its capability for adaptation and increases the risk of its extinction. In a small population inbreeding is concerned with the wearing down of genetic variation within the populations, which is responsible for lowering down of heterozygosity and subsequently causes diminution of mean phenotypic values of useful traits. Therefore, the study of genetic variability is of foremost importance to conservation, sustainability, and management of wild population which depends on the first-hand knowledge of the amount of variation existing in a local reproductive population (Frankham, 1995).

Table 1 sequence of primer

PRIMER	PRIMER SEQUENCE	Tm
R8	GACAGGGACC	34
R13	GTGTCTCAGG	34
R18	TCCCGCCTCA	34
R19	AACGCGTCGG	34

PCR-based Random Amplified Polymorphic DNA (RAPD-PCR) is an extensively used molecular tool in detection and characterization of genetic polymorphisms in natural populations (Welsh and McClelland 1990). RAPD-PCR amplifies DNA segments of variable lengths and such length polymorphisms can be used as genetic markers (Williams *et al.*, 1990). RAPD analysis has been extensively used to evaluate genetic diversity in various species. However, a very little genetic information of *Trichogaster lalia* is available. Hence RAPD-PCR was carried out for the particular species so that it provides avenues and information for further sophisticated fingerprinting techniques.

Morphometry refers to the study of the body design of an individual and it is considered as an useful method for identification of an individual. It has also proved to be a beneficial tool for the taxonomist for measuring discreteness and relationships among various taxonomic categories (Ihssen *et al*., 1981; Melvin *et al*., 1992;Quilang *et al*., 2007; Nowak *et al*.,). It is only through the study of morphometric characters that helps us to mark out the origin of a particular fish species. . The interactive effect of environment, selection and heredity on the body shapes and sizes within a species, can be understood using morphometric characters (Cadrin, 2000).The present study was undertaken to find the genetic variation of different species of *Trichogaster lalia* using RAPD-PCR besides their morphometric identification.

MATERIALS AND METHODS:

Fish sampling sites and morphometric measurements of fishes:

Geographically, populations of *Trichogaster lalia* were procured from freshwater bodies of Assam about 100 - 400 km away from each other, that is, Deepor Beel (Brahmaputra river, Kamrup District) ; Kolong river at Morigaon (Morigaon District) ; Katli Beel, Mankachar(Dhrubri District) in the month of August 2013 as this species are more available during the rainy season.. 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, a total of 16 parameters were considered. Fish specimens were morphologically identified with taxonomic keys (Srivastava, 2000; Jayaram, 2002). The muscle tissues were isolated from freshly caught fishes and preserved at -20°C for further use.

Isolation of genomic DNA from fish tissue:

The genomic DNA was isolated using the protocol as described by Sambrook and Russel,(2001) with slight modifications **.**The quality as well as quantity of isolated DNA was checked with the help of a UV-VIS Spectrophotometer. Optical densities of the DNA samples were measured at 260 nm and 280nm**.**The genomic DNA that showed the ratio of absorption at 260nm to 280nm in between 1.6-2.0 were selected for RAPD-PCR .

PCR amplification:

A total of 25 commercially available RAPD primers,10 mers each, were procured from Genei (Merck Bioscience), India, and used for RAPD- PCR amplifications (Table 1:)The primers that showed the maximum number of bands were selected for the study. The RAPD-PCR reaction was carried out as per William's et.al 1990 protocol with slight modification. The PCR master mix constituted of 3 µl of dNTP mix (2.5mM each),100ng of Random primer, 20ng of DNA template, 3U Taq DNA polymerase(3U/ µl), 1X Taq polymerase buffer(Supplied as 10X concentration) containing 15mM $MgCl_2$, and PCR grade water to make the volume to 25 µl. The PCR amplification protocol comprised of an initial denaturation for 5 min at 94° C,followed by 35cycles of denaturation at 94 °C (1 min), annealing at 37°C (1 min) and an elongation step of 72°C (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).

Agarose gel electrophoresis:

The amplified DNA products were mixed with gel loading buffer and run on 1.8% agarose gel containing Ethidium bromide (10mg/ml) at 60 V for 2 hours. A 100bp DNA ladder from Bangalore Genei (Merck Bioscience) was run with each gel. The banding pattern was visualized on an UV transilluminator and photographed by gel documentation system (BIORAD, USA).

Data analysis:

The amplified DNA fragments were scored for the presence (1) and absence (0) of fragments on the gel photographs and RAPD fragments were compared among the *T. lalia* 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 (Nei, 1978) using 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} = 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 (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 *Trichogaster lalia.*

RESULTS AND DISCUSSION:

To determine the morphometric variation among the different individuals the following attributes were taken into consideration-in relation to Standard length (SL%) are Body depth, Head length, Caudal peduncle length, Caudal peduncle depth, Dorsal fin base length, Dorsal fin length, Pectoral fin length, Pelvic fin length, Anal fin length, Pre dorsal length and the Post dorsal length, and in relation to Head length (HL%) are Head depth, Head width, Snout length, Eye diameter and Inter orbital distance. The result among the 20 individuals varied as per the table 2

Table:2 Morphometric Variation among Different species of *Trichogaster lalia*

PARAMETERS	Range (mm)	Mean± SD
% SL		
Body depth	41.6 - 44.3	43.2±0.6
Head length	36.0 - 37.0	36.6±0.3
Caudal peduncle length	01.5 -02.0	01.7±0.1
Caudal peduncle depth	17.6 -18.3	17.9±0.2
Dorsal fin base length	57.0 -59.6	58.8±0.6
Dorsal fin length	10.0 -11.5	10.9±0.4
Pectoral fin length	30.5 -31.4	30.9±0.3
Pelvic fin length	85.9 -88.2	87.3±0.6
Anal fin length	64.0 -66.3	65.3±0.7
Pre dorsal length	43.5 -46.1	45.3±0.7
Post dorsal length	57.8-60.0	58.9±0.6
%HL		
Head depth	95.0 -97.0	96.3±0.5
Head width	48.3 -49.5	48.9±0.3
Snout length	29.8 -31.0	30.2±0.3
Eye diameter	29.0 -30.5	29.5±0.4
Inter orbital distance	35.3 -36.5	35.9±0.3

RAPD polymorphisms:

Among the 25 primers screened initially ,only 4 primers R-8,R-13,R-18,R-19 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 four primers produced 39 well amplified bands, with reproducible banding patterns of which 22 were polymorphic and 7 were monomorphic. The RAPD profiles obtained in the representative sample of *Trichogaster lalia* is shown in Fig 1.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.

RESULTS AND DISCUSSION

The study revealed little morphological differences among the *T.lalia* populations by morphometric studies that were sampled from different rivers and localities in Assam. Most of the morphometric characteristics of the fishes in the present study were similar and there is overlap in the range of each of traditional morphometric measurements taken (Table 2) 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 (Ponniah and Gopalkrishnan, 2000; Garg *et al.*, 2009b).

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 (Barman *et al*., 2003; Jayasankar *et al.,* 2004; Lopera-Barrero *et al*., 2006; Shair *et al*., 2011 In the present study conducted using decamer random primers, four primers generated a

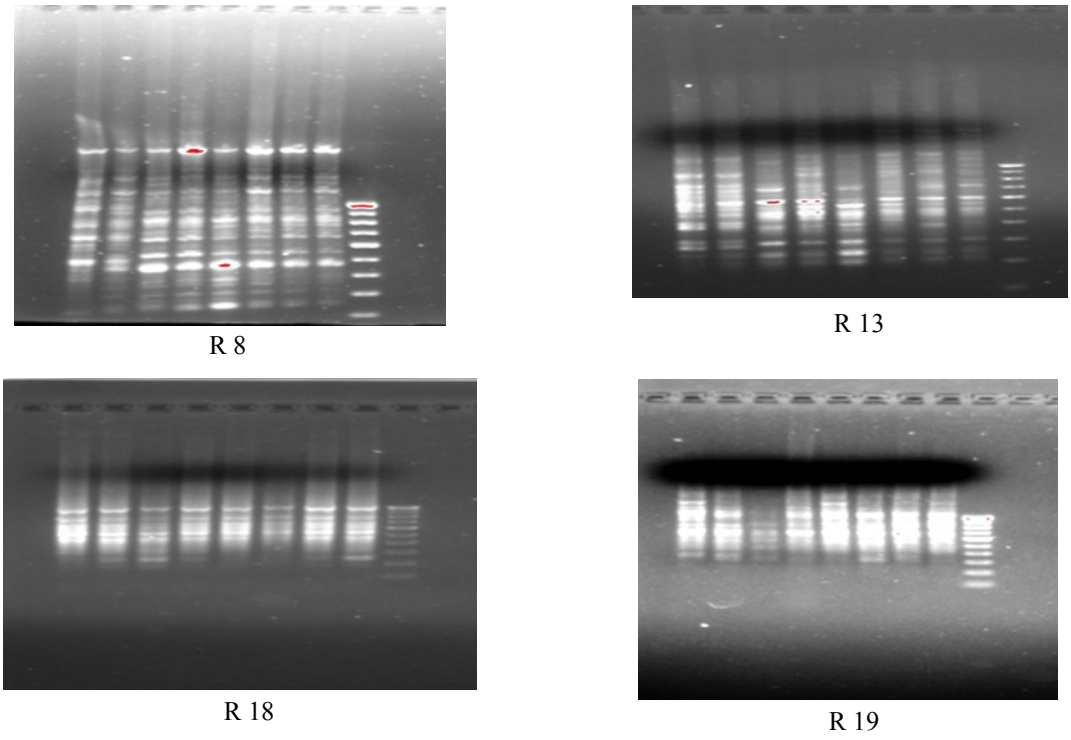

Fig1: RAPD profiles obtained with different primers (R 8, R13, R18 and R19)

(In the figure lane 1,2,3,4,5,6,7,8, represents the representative individual. And lane 9 represents the 100bp ladder)

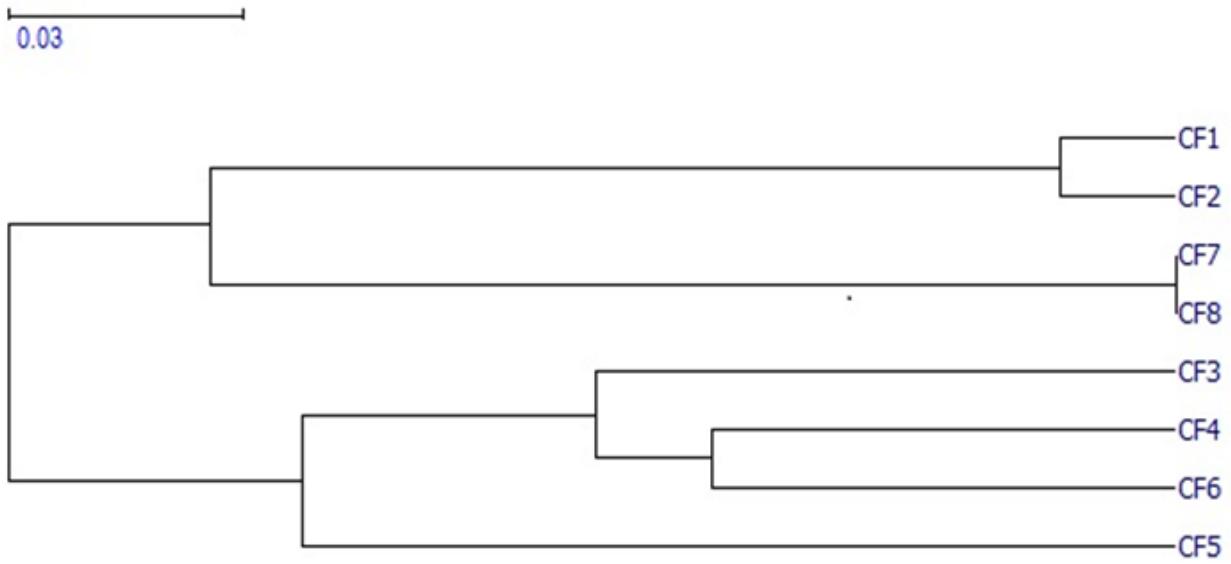

FIG2 :Phylogenetic Tree Constructed By Similarity Co-Efficient (Jaccards) CF represents the representative samples of *Trichogaster lalia*

total of 39 bands in the population which were found to be both polymorphic and monomorphic. In the experiment 22 polymorphic bands and 7 monomorphic bands were produced which shows 0.56% of polymorphism and 0.18 % of monomorphism. The cluster analysis and dendogram showing genetic relationship between 8 genotypes of *T.lalia* showed formation of 2 clusters (Fig-2). Cluster I include genotype1,2,7and 8(individuals 7 and 8 were closely related); Cluster II include genotype

	CF1	CF2	CF3	CF4	CF5	CF6	CF7	CF8
CF1								
CF2	0.03							
CF3	**0.38**	**0.35**						
CF4	0.29	0.26	0.15					
CF5	**0.35**	**0.38**	**0.15**	**0.29**				
CF6	0.35	0.32	0.15	0.12	0.24			
CF7	**0.26**	**0.24**	**0.29**	**0.21**	**0.32**	**0.26**		
CF8	0.26	0.24	0.29	0.21	0.32	0.26	0.00	

Fig 3:Genetic distance among the individuals (CF)

3,4,5and 6.The genetic distance obtained using GGT software shows that the species are genetically similar (Fig-3).

CONCLUSION

Once the population structure is known, various avenues including conservation of ornamental fish can be undertaken. Thus, the present study may help researchers 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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