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Full Length Research Paper

Comparative analysis of inter population genetic diversity in *Puntius filametosus* using restriction fragment length polymorphism (RFLP) analysis

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The genetic variation in different population of the freshwater cyprinid *Puntius filamentosus* was studied using restriction fragment length polymorphism (RFLP) analysis. Samples were collected from five different locations of southern Western Ghats, India. The morphometric characters of population from Alancholai showed little variation when compared with other population. The genomic size of the different population of *P. filamentosus* found between 3.45 and 3.80 ng/mg. The result of RFLP analysis showed that the population from Alancholai had distinct fragment length and scored high band volume (12.430 nmoles). The result of cluster analysis showed that Alancholai population had distinct genetic structure and it did not cluster with other population. The study inferred that the population from Alancholai appeared to be unique among the other population of *P. filamentosus.*

Key words: *Puntius filamentosus,* genetic diversity, RFLP, Western Ghats, India.

INTRODUCTION

Conservation of genetic diversity is one of the important components for bioresource management. Species are often arranged into hierarchies of metapopulation, population and subpopulation with varied distribution of genetic variation within and among these levels of organization (Selander and Johnson, 1973; Baumgartner, 1985). The genetic diversity in a species provides an inherent ability to adapt and evolve in a changing environment. A species possessing higher amount of genetic diversity is more capable than those with less diversity to evolve in response to environmental stresses (Frankel and Soule, 1981). India is one of the `megadiversity' countries with richest storehouse for genetic resources from all organisms. Among aquatic organism, fishes are the best known group that exist at or near the top of the food chain and can serve as indicator of a balanced ecosystem (Karr et al., 1986).

The knowledge on aquatic diversity and conservation of genetic resources has intensified during last few decades. In the advent of recent molecular techniques like DNA fingerprinting, Restriction Fragment Length Polymorphism and Random Amplified Polymorphic DNA, which provides the means for quantitative screening of genetic variation (DeLong et al., 1989; Giovannoni and Cary, 1993). The information on genetic resource of Indian fish fauna is very limited, especially native cyprinids.

The present study attempts to identify and estimate genetic variation within different population of *Puntius filamentosus* from southern Western Ghats. *P. filamentosus* is a small cyprinid, commonly known as black-spot barb and it has wide geographical distribution in Asian countries (Talwar and Jhingran, 1991). It is a highly adopted species commonly inhibitat in lower stretches of streams, rivers and river associated wetlands and swamps (Arunachalam et al., 2000).

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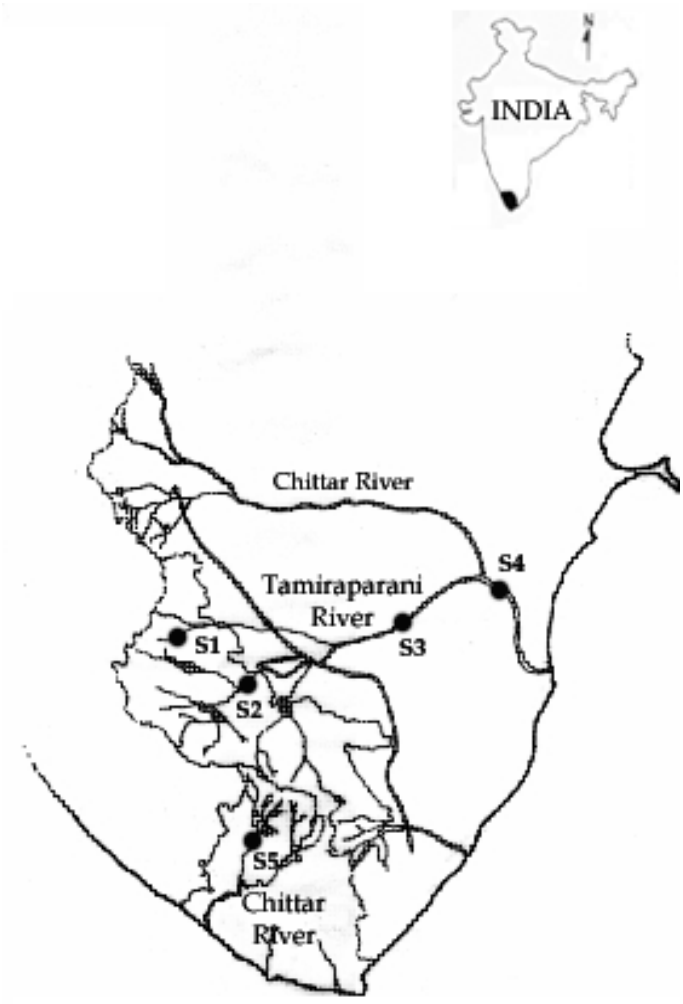

Figure 1. Location of the sampling sites at southern Western Ghats (S1- Gadana; S2- Papanasam; S3- Tirunelveli; S4- Vallanadu; S5- Alancholai).

MATERIALS AND METHODS

Fish samples were collected from five different location viz., Gadana, Papanasam, Tirunelveli, Vallanadu in east flowing river Tamiraparani and Alancholai in west flowing Chittar river basin of southern Western Ghats (Figure 1). Sampling was performed by using cast net and drag net. A portion of gill and muscle tissues were fixed in isopropyl alcohol and they were kept in the ice cubes for further laboratory analysis. Few individuals were also fixed in formaldehyde for further morphometric analysis. The morphometric measurements were followed by Hubbes and Laggler (1958).

The genomic DNA was isolated by phenol-chloroform method based on Sambrook et al. (1989). Amount of DNA present in each sample were determined using UV-spectrophotometer. Isolated DNA samples were subjected to Restriction enzyme digestion at 37°C for 2 h using *Hind III* enzyme. After incubation each samples were loaded into 1% agarose gel and electrophoresed at 50 - 100 V for one and half hours. After electrophoresis gel was placed in the gel document unit (FOTODYNE) and bands were visualized and they were photographed using NIKON digital camera. The DNA fragment in each lane was viewed and number of bands and band volume were documented using TOTAL LAB gel analyzing software. The band volume data were used for construction of similarity cluster using STATISTICA software (version 6.5).

RESULTS

In the present study fourteen classical morphometric characters were studied in different population of *P. filamentosus* (Table 1). The morphometric characters did not vary much among the population from Tamiraparani River basin; however, the population from Alancholai had significant difference in some morphometric characters. It distinguished from Tirunelveli and Gadana population in body width (25.0 vs. 32.2 and 38.7 in % of standard length), body depth (40.2 vs. 30.7 and 22.9 in % of standard length), head length (19.7 vs. 25.3 and 28.8 in % of standard length), eye diameter (39.7 vs. 35.0 and 31.3 in % of head length), inter orbital width (49.3 vs. 53.3 and 58.8 in % of eye diameter), pectoral fin length (13.5 vs. 17.8 and 20.4 in % of standard length) and pelvic fin (15.5 vs. 19.5 and 19.2 in % of standard length). It also differed from Papanasam population in predorsal length (47.2 vs. 65.8 in % of standard length), length of caudal peduncle (12.6 vs. 25.2 in % of standard length) and length of anal fin (13.1 vs. 20.5 in % of standard length).

Phenotypically it exhibited variation in body colour patterns (caudal and ventral fin are deeply red whereas sample from east flowing river were red tinted with black edge). Moreover, it had variation in shape and size of body blotch [a block oval solid blotch on the entire caudal peduncle and extended up to caudal fin whereas the oval shaped blotch did not extend in the base of caudal peduncle of other population (Figure 2)].

The DNA content of each population and the corresponding OD values were given in Table 2. The genomic size of *P. filamentosus* ranged from 3.45 to 3.80 ng/mg. The result of restriction analysis showed that there was clear separate DNA banding patterns in different population and the fragment migration were ranged from 5000 to 2500 bp. Based on electophorogram, different bands of fragments in each lane and band volume were analyzed (Table 3). Electrophorogram analysis revealed that the maximum fragment length polymorphism in population from Alancholai which had five fragments and the total volume of bands in the entire lane was 12.430 nmoles. The DNA samples from Gadana and Tirunelveli had 3 distinct bands and the total volume of bands in the entire lane was 9.605 and 6.622 nmoles, respectively. Whereas the population from Papanasam and Vallanadu had only two fragments and the total volume of bands in the entire lane was 7.442 and 4.506 nmoles, correspondingly. The cluster analysis showed the population from Papanasam, Tirunelveli, Vallanadu and Gadana were grouped together. Where as the population form Alancholai had distinct genetic distance and it did not cluster with other population (Figure 3).

Table 1. Morphometric measurement of *Puntius filamentosus* from different locations.

Characters	Gadana (n = 10)		Papanasam (n = 10)		Tirunelveli (n = 8)		Vallanadu (n = 8)		Alancholai (n = 12)	
	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD
% of standard length										
Body width	36.2 - 41.0	38.7±0.02	24.9 - 29. 2	26.0±0.02	29.6 - 35.2	32.2±0.03	17.9 - 29.9	25.3±0.06	19.0 - 30.1	25.0±0.01
Body depth	21.1 - 24.4	22.9±0.02	28.6 - 35. 3	31.3±0.04	27.0.34.0	30.7±0.04	31.8 - 34.3	33.3±0.01	39.3 - 41.7	40.2±0.01
Head length	28.2 - 29.3	28.8±0.01	36.3 - 40. 1	39.3±0.01	24.8 - 25.6	25.3±0.01	22.1 - 24.9	23.9±0.16	19.3 - 20.3	19.7±0.01
Predorsal length	41.0 - 46.6	42.6±0.29	59.9 - 69. 3	65.8±0.52	48.4 - 49.6	49.1±0.01	44.0 - 48.3	46.5±0.02	45.0 - 49.3	47.2±0.04
Length of caudal peduncle	12.7 - 13.2	12.9±0.01	22.1 - 28. 3	25.2±0.03	11.6 - 12.6	12.1±0.01	11.5 - 12.9	12.2±0.01	11.6 - 13.3	12.6±0.01
Length of anal fin	14.6 - 16.5	15.4±0.01	19.9 - 20. 9	20.5±0.01	11.4 - 15.4	14.8±0.01	11.3 - 14.0	13.6±0.01	11.9 - 13.8	13.1±0.01
Length of pelvic fin	18.9 - 19.5	19.2±0.01	21.3 - 21. 8	21.4±0.02	19.2 - 20.1	19.5±0.01	14.3 - 15.9	15.0±0.01	14.9 - 15.9	15.5±0.01
Length of pectoral fin	19.9 - 21.0	20.4±0.01	22.1 - 23. 8	23.1±0.01	17.1 - 18.3	17.8±0.01	13.3 - 14.0	13.8±0.01	13.9 - 14.9	13.5±0.02
Snout length	08.9 - 09.5	09.2±0.01	08.9 - 09. 6	07.8±0.05	07.6 - 08.1	07.8±0.01	07.1 - 07.6	07.4±0.01	21.8 - 24.1	23.3±0.01
Eye diameter	09.1 - 09.9	09.5±0.01	09.3 - 10. 7	10.1±0.01	08.0 - 09.1	08.4±0.01	06.9 - 08.2	07.9±0.01	07.1 - 08.2	07.7±0.01
% of head length										
Eye diameter	29.9 - 32.9	31.3±0.02	27.0 - 30. 1	28.8±0.02	34.1 - 35.9	35.0±0.01	32.4 - 34.3	33.2±0.01	38.0 - 40.8	39.7±0.01
Snout length	30.3 - 32.6	31.9±0.01	18.3 - 19. 4	18.8±0.01	24.9 - 28.1	26.5±0.02	28.1 - 29.4	28.7±0.01	28.1 - 29.4	28.7±0.01
Length of pectoral fin	68.9 - 72.2	70.9±0.02	71.1 - 74. 6	73.0±0.02	67.1 - 69.3	68.3±0.01	79.3 - 89.3	82.9±0.06	78.0 - 89.9	83.6±0.03
Eye diameter / inter orbit width	58.1 - 59.2	58.8±0.01	52.2 - 54. 1	53.1±0.01	51.8 - 55.2	53.3±0.02	43.3 - 59.8	50.8±0.08	39.3 - 50.6	49.3±0.03

DISCUSSION

Most of the morphometric characters of fishes are similar and often overlap within the population. This morphometric data are not enough to support the established genetic structure of the population often that leads to taxonomic uncertainty (Daniel, 1997; Ponniah and Gopalakrishnan, 2000). The genomic size of *P. filamentosus* is ranged from 3.45 to 3.9 ng/mg and no much variation among population. In cyprinids, the genomic size varies between 1.6 and 4.4 ng/mg (Buth et al., 1991; Gold et al., 1992). In general, intra-population genome size is very small and it is also not much in related species (Fontana, 1976). The genomic size is essential for at least three reasons. First, it provides some valuable clue regarding genome evolution. Secondly, genome size can be correlated to some quantitative characteristics such as cell volume. Thirdly, during molecular genetic study it is used in calculation of number of copies of gene present in genome of species (Dolittle and Sapienzi, 1980; Orgel and Crick, 1980). Electrophorogram analy-sis has showed significant variation in fragment length within the population of *P. filamentosus*.

Among the population, sample from Alancholai River (west flowing river) was genetically different among the other population and stands apart largely owing to high genetic diversity (total band volume 12.430 nmoles). Moreover, it also exhibits phenotypic characters such as body colour pattern and shape of blotch at the caudal peduncle (Figure 2). These variations in fragment length may be due to mutation. The existence of many closely related haplotypes that are only partially and geographically localized has been associated with species or subset of species with historically intermediate levels of gene flow between geographic populations (Avise et al., 1987). In this scenario, ancestral haplotypes may be dispersed over a wide area whereas more recent mutation are conformed to specific areas (Bermingham and Avise, 1986). The differentiation among sample from separate region is consistent with previous findings for fish species using protein electrophoresis (Gyllelnsten, 1985; Shakleen and Kannon, 1986; Comparani and Rodino, 1980) and

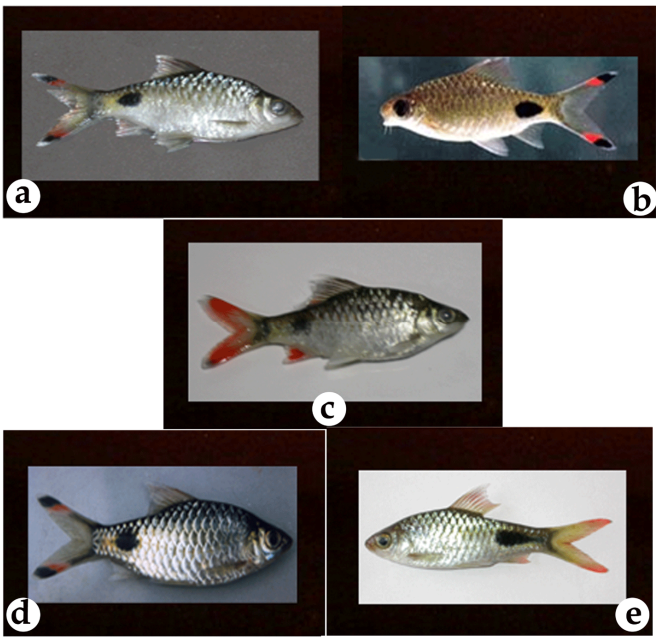

Figure 2. *Puntius filamentosus* from different location. (a) Gadana; (b) Papanasam; (c) Tirunelveli; (d) Vallanadu; (e) Alancholai.

Table 2. Genomic size of *Puntius filementosus* from different locations.

Population	OD value	DNA content (ng/mg)
Gadana	0.072	3.60
Papanasam	0.078	3.85
Tirunelveli	0.069	3.45
Vallanadu	0.076	3.80
Alancholai	0.070	3.69

DNA fingerprinting (DeLong et al., 1989; Giovannoni and Cary, 1993). In this present study, it was inferred that genetic variation has marked distinction and the genetic structure of the population of *P. filamentosus* from Alancholai River appears to be unique among population of other region. For conserving such unique genetic makeup, the combination of our understanding of how ecology and habitat specialization relates to genetic variation should be of value in designing and management of rare germplasm.

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Table 3. Number of fragments and Band volume of electrophorogram of *Punitius filementosus*.

Population	Number of fragments	Band volume (nmoles)	Total Band volume (nmoles)
Gadana	1	2.823	9.605
	2	5.826	
	3	0.956	
Alancholai	1	4.826	12.430
	2	3.268	
	3	2.058	
	4	1.562	
	5	0.716	
Papanasam	1	2.616	7.442
	2	4.826	
Tirunelveli	1	3.052	6.622
	2	2.106	
	3	0.570	
Vallanadu	1	1.893	4.506
	2	2.613	

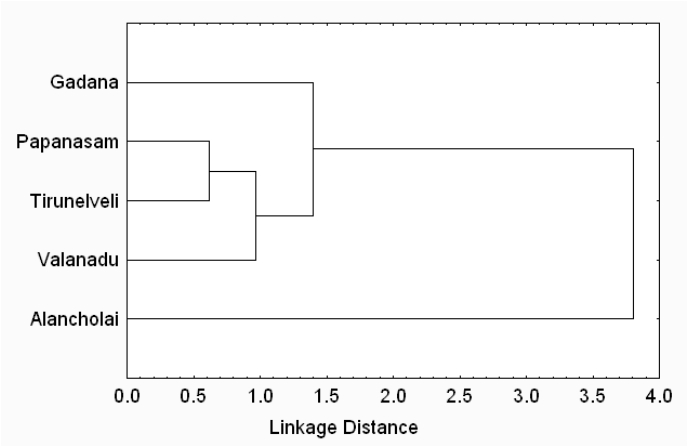

Figure 3. Genetic distance between different populations of *Puntius filamentosus*.

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