Genetic Diversity Among *Puntius Sophore* Complex Using Restriction Fragment Length Polymorphism

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Abstract: *Restriction Fragment Length Polymorphism (RFLP) analysis of genomic DNA was employed to characterize species of Puntius sophore complex, a benthopelagic freshwater fish species. Samples of five species of Puntius viz, Puntius sophore, Puntius amphibius, Puntius chola, Puntius bimaculatus and Puntius dorsalis from the east flowing Tamiraparani and Kuzhithurai and west flowing Tamiraparani river basins (Western Ghats) were analyzed. The restriction enzyme employed (Hind III) generated species-specific DNA patterns for each of the five species. DNA exhibited considerable polymorphism among species. All samples showed relatively high values of diversity. The relation between species is discussed.*

Key Words: *RFLP, Hind III, Genetic diversity, Puntius sophore complex.*

1. Introduction

Genetic diversity is the sum of total information available in a genome of particular species or races[1]. Genetic diversity occurs at intra-population level within a species. Meiotic recombination gives rise to innumerable combinations of alleles, which generate intra-population genetic diversity. In natural population mutations, recombination`s, genetic drift and gene flow reshuffle it, which is either preserved (or) rejected by the selection.

According to Gopal[2], more than 20% of the fauna in India is aquatic and majority of them are fresh water. Among the fresh water organisms fishes are the best group that exists at or near the top of the food chain and can serve as the indicators of a balanced ecosystem[3].

Current nomenclature and systematic status of many species is solely based on morphometric and meristic characters often that lead to taxonomic uncertainties at various taxonomic levels. About 950 primary freshwater fishes are reported from Indian region. Among them more than 100 species are synonymics with other closely related species[4,5]. Uncertain systemic position and taxonomic ambiguity of organisms can play havoc in conservation of our resources. Genetic tools are being used significantly to resolve these taxonomic ambiguities and to increase production in the world aquaculture as well as to conserve and protect fish biodiversity.

Puntius sophore complex consist of five species. These species are small barbs belonging to the genera *Puntius*, lives in freshwater environment (bentholopelagic) and commonly inhabitats aquatic resources of tropical climate. Most of them are endemic to streams and rivers of Western Ghats and Eastern Himalayas. Thesse species are more popular among ornamental keepers[6]. They have similarity in external / phenotypic characters (occurrence of two black spots on the dorsal as well as caudal peduncle, expect *Puntius amphibius*) that lead to taxonomic uncertainty and amphiguity in species determination. An attempt was made in the present study to access the genetic diversity and to further characterize species of *Puntius sophore* complex from the east flowing Tamiraparani and Kuzhithurai and west flowing Tamiraparani river basins of Western Ghats by Restriction Fragment Length Polymorphism (RFLP).

2. Materials and Methods

2.1 Sample Collection

Samples were collected from different locations in east flowing rivers Tamiraparani and Kuzhithurai and west flowing Tamiraparani river basins of Western Ghats (Table 1). Sampling was performed by using cast and dragnets. After collection, portions of muscle tissues were fixed in formaldehyde for further analysis.

2.2 Morphological characters

The morphometric and meristic measurements were done by Hubbes and Laggler (1956)[7] method. The morphometric measurements were taken at the accuracy of 0.01mm using digital verneir.

DNA Isolation:

DNA was isolated from the muscle tissues by slight variations of standard phenol:chloroform protocol (Sambrook et al., 1989)[8]. The isolated DNA was quantified by using UV spectrophotometer.

RFLP-Analysis:

For restriction digestion, 20 µl of the sample DNA was digested in a total of 50 µl of the reaction mixture, containing 10U of *Hind III* restriction enzyme, which was incubated at 37^0C for 2hrs, digestion was carried out as specified by the supplier (New England Biolabs). Restriction fragments were separated by elecxtrophoresis in 1% agarose gel. Molecular weights of the fragments were estimated by comparison to *Hind III* λ DNA digests (New England Biolabs). The electrophoresed gel was documented with gel documentation unit (FOTO / UV21). The intensity and volume of each separated fragment was documented by using total LAB gel analyzing software. Electrophorogram was prepared for each lane and the band volumes were used for construction of cluster using Bioinformatics software STATISTICA (Version 6.0).

3. Results

Morphological characters

Five different species of *Puntius sophore* complex collected from southern parts of Western Ghats and 14 morphological characters were studied in the present study (Table 2). There was a great variation among the species of *Puntius sophore* complex and the variations were significant. The major variations in morphometric characters is head length, body depth, predorsal length, snout length and the ratio of eye diameter and inter orbit width.

Genomic DNA Analysis:

The total genomic DNA quantified by using spectrophotometer at 260nm showed variation among the species, and it ranged from 0.24 µg/ml to 0.36 µg/ml (Table 3). *Puntius sophore* had highest (0.72 µg/ml) and *Puntius dorsalis* the lowest (0.48 µg/ml).

RFLP- Analysis:

The restriction analysis showed that there was a clear banding pattern in each DNA sample (Fig:1). Digestion with *Hind III* produced species-specific DNA fragment patterns in all five species of *Puntius sophore* complex studied. Using electrophorogram DNA fragments were identified. Band volume and absorption intensity of each bands in corresponding lanes were analyzed. The band volume ranged between 0.4 to 4.0 n.moles (Table 4).

Volume of restriction fragment in each lane was subjected to phylogenetic tree construction using STATISTICA software. The results showed that four species viz, *Puntius sophore, Puntius amphibius*, *Puntius chola* and *Puntius dorsalis* were clustered together, based on genetic distance. Whereas *Puntius bimaculatus* showed distinct genetic make up (Fig:2).

4. Discussion

Due to uncertainty and amphiguity in *Puntius sophore* complex species determination[9], the present study on classical morphological characters was done. The result showed some variation in characters like head length, body depth, predorsal length, snout length and the ratio of eye diameter and inter orbit width. It is not enough to address the genetic structure of individual species. Since, species are often arranged in to hierarchies of meta population, population and sub population with varied distribution of genetic variation within and among these levels of organization[10]. so molecular techniques such as estimation of genomic DNA and restriction Fragment Length Polymorphism were performed to

assess the genetic diversity. There is a considerable variation in genomic size (varying from 0.7 µg/ml to 0.11 µg/ml) among the species. In cyprinids, the genomic size varies from 1.6 to 4.4 µg/ml [11,12]. In general interpopulation genome size varies in closely related species[13]. The genomic size is essential for atleast three reasons[14,15]. Firstly, it provides some valuable clue regarding genome evolution. Secondly, genome size can be correlated to some qualitative characteristics such as cell volume. Thirdly, during molecular genetic study it is used to calculate the number of copies of genes present in the genome of the species.

Electrophoresis of the restricted fragments showed significant variation in *Puntius sophore* complex. Among the species *Puntius sophore* has high genetic diversity (Table 3). This variation in genome size may occur due to mutation. The existence of many closely related halotypes that are partially geographically localized has been associated with species or subset of species with historically intermediate levels of gene flow between geographic species[16]. In this scenario ancestral halotypes may be dispersed over a wide area where as more recent mutations are conformed to specific areas[17]. Differentiation among samples from *Puntius sophore* complex is consistent with previous findings for fish species using protein electrophoresis[18,19] and mitochondrial DNA polymorphism[20].

Present study infers the genetic variation as marked distinction and the genetic structure of the species of *Puntius sophore* complex appears to be unique among species. These unique genetic resources should be preserved. Degree of habitat specialization, population tolerance and water quality can affect the absolute and relative population size leading to changes in genetic structure. Water quality directly erodes variation through directional selection[21]. For conservation such genetic make up combining our understanding of how ecology and habitat specialization relates to genetic variation should be of value in designing, management strategies even when direct genetic information is not available.

Table 1: Study material and location

S.No.	Species	Location
1	*Puntius amphibius*	Tamiraparani, Kuzhithurai
2	*Puntius bimaculatus*	Tamiraparani, Kuzhithurai
3	*Puntius sophore*	Tamiraparani, Kuzhithurai
4	*Puntius chola*	Tamiraparani, Kuzhithurai
5	*Puntius dorsalis*	Tamiraparani, Kuzhithurai

Table 2: Morphological characters of *Puntius sophore* complex

S.No.	Character	Mean				
		Puntius amphibius	*Puntius bimaculatus*	*Puntius sophore*	*Puntius chola*	*Puntius dorsalis*
	% of standard length					
1	Body width	16.0	15.6	15.3	14.8	17.6
2	Body depth	25.9	26.9	35.3	26.2	32.0
3	Head length	26.1	28.4	27.0	29.2	28.6
4	Predorsal length	49.2	05.1	49.1	53.2	59.3
5	Length of caudal peduncle	13.0	14.2	11.2	12.2	13.4
6	Length of anal fin	15.7	19.7	16.5	18.1	20.3
7	Length of pelvic fin	17.9	19.1	15.6	19.0	20.2
8	Length of pectoral fin	19.1	18.9	20.4	19.6	19.1
9	Snout length	08.4	09.5	08.1	09.0	10.4
10	Eye diameter	09.7	08.1	08.8	09.2	10.8
	% of head length					
11	Eye diameter	37.2	28.4	28.9	29.4	34.9
12	Snout length	32.1	33.7	30.1	30.9	36.6
13	Length of pectoral fin	73.0	66.9	75.7	67.3	49.8
14	Eye diameter / inter orbit width	03.5	05.2	04.6	03.5	03.8

Table 3: Level of genomic DNA of *Puntius sophore* complex

Species	OD value (260nm)	DNA content (µg/ml)
Puntius amphibius	0.54	0.27
Puntius bimaculatus	0.60	0.30
Puntius sophore	0.72	0.36
Puntius chola	0.64	0.32
Puntius dorsalis	0.48	0.24

Table 4: Number of bands in each lane, band volume and Rf value of electrophorogram of *Puntius sophore* complex

Species	Band	Volume	Rf
Puntius amphibius	1	85,969.00	0.155
	2	86,727.00	0.245
	3	86,171.00	0.424
Puntius bimaculatus	4	102,407.00	0.661
Puntius sophore	1	127,951.00	0.232
	2	200,066.00	0.700
Puntius chola	1	137,078.00	0.215
	2	16,984.00	0.456
Puntius dorsalis	3	11,005.00	0.755
	1	128,613.00	0.232
	2	22,496.00	0.540
	3	18,173.00	0.785
	1	99,411.00	0.211
	2	43,333.00	0.295
	3	45,204.00	0.363
	4	11,461.00	0.654

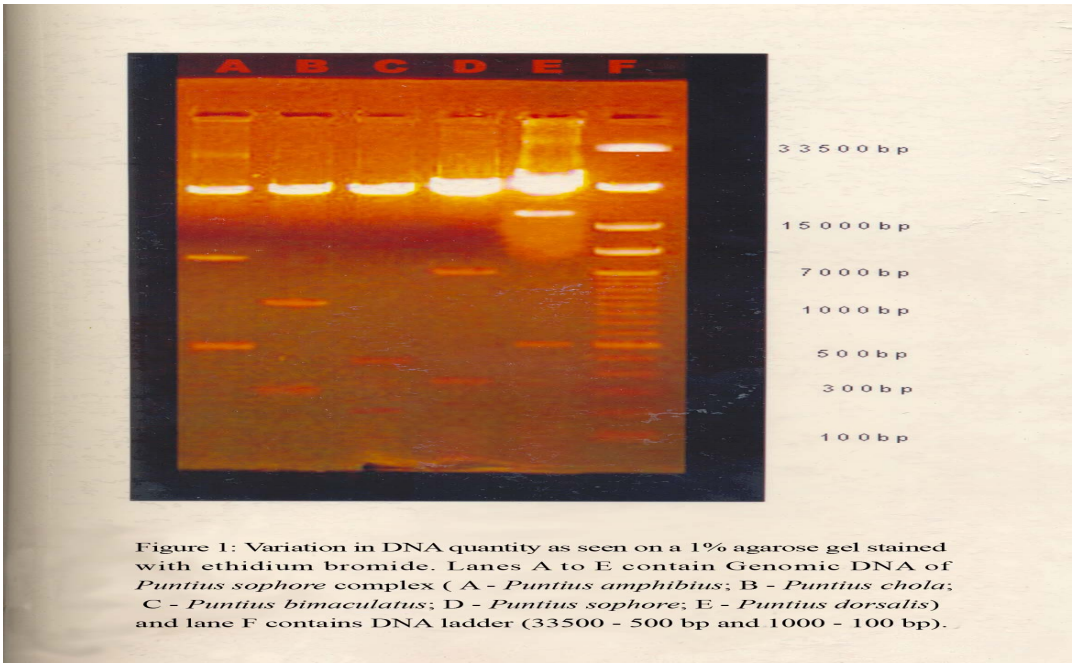

Figure 1: Variation in DNA quantity as seen on a 1% agarose gel stained with ethidium bromide. Lanes A to E contain Genomic DNA of *Puntius sophore* complex (A - *Puntius amphibius*; B - *Puntius chola*; C - *Puntius bimaculatus*; D - *Puntius sophore*; E - *Puntius dorsalis*) and lane F contains DNA ladder (33500 - 500 bp and 1000 - 100 bp).

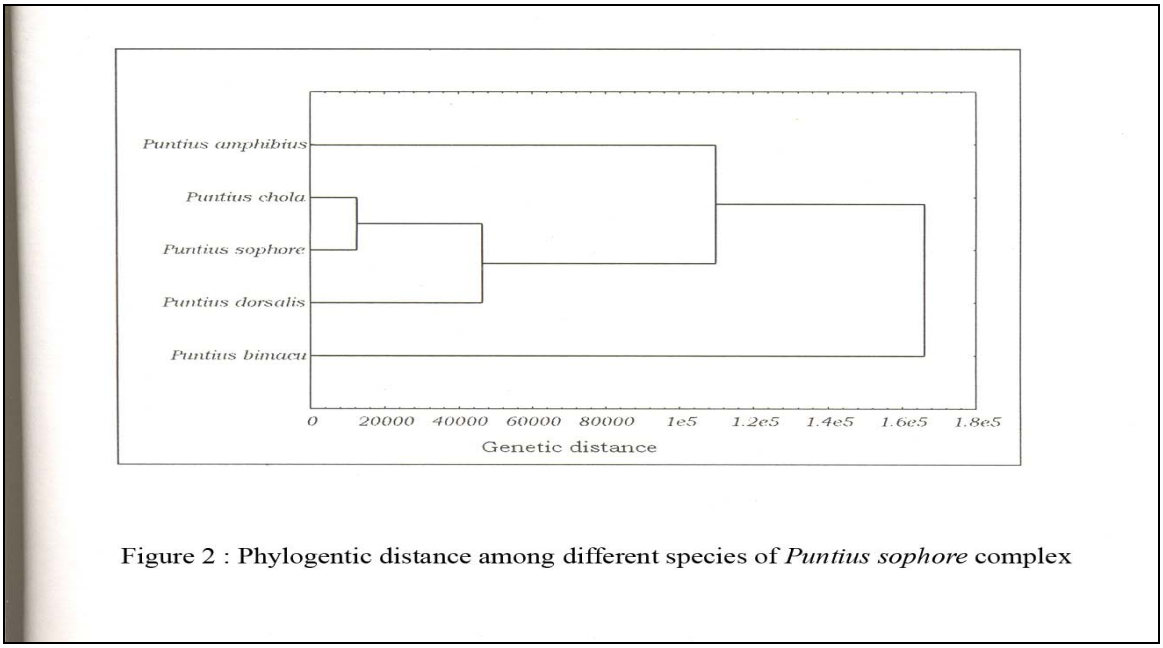

Figure 2 : Phylogentic distance among different species of *Puntius sophore* complex

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