UTTAR PRADESH JOURNAL OF ZOOLOGY

41(1): 42-52, 2020
ISSN: 0256-971X (P)

RAPD-PCR ANALYSIS OF FRESH WATER FISH SPECIES IN THREE RESERVOIRS OF NORTH MAHARASHTRA, INDIA

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Received: 04 January 2020
Accepted: 10 March 2020
Published: 14 March 2020

Original Research Article

ABSTRACT

Assessment of genetic variation among the carp fishes captured from rivers of north Maharashtra is prime objective of the present study. The genetic variability among fresh water carp fish belonging to family Cyprinidae inhabiting three reservoirs of Maharashtra was examined in department of Zoology using random amplified polymorphic DNA (RAPD) during February 2018 to January 2019. Scale samples of each test fish were processed for extraction of genomic DNA. Ten Oligonucleotide primers were screened and three primers were selected to amplify DNA of test fish. Three out of 10 primers were good candidate for developing molecular markers. Using pattern analysis of polymorphic and monomorphic bands generated by three primers in 70 carp fish belonging to 24 genera of Cyprinidae family phylogenetic tree constructed. This study represents a first step towards the generation of DNA markers for species diagnosis and assessment of genetic diversity of economically important carp fish species.

Keywords: Fish; RAPD; PCR; molecular markers.

1. INTRODUCTION

Explaining the disparity in species richness among clades and areas is a major goal of evolutionary biology. One of the more intriguing patterns of biodiversity is the extraordinary number of species on continents compared to oceans [1]. In fact, it has been estimated that species diversity on continents is 25 times more than that found in oceans [2], despite the fact that oceans are vastly greater in size. For aquatic organisms, freshwater habitats make up only ~0.01% of available aquatic habitat and yet harbor 40% of all fish species. Actinopterygii is the class of freshwater ray-finned fishes that comprises ~30,000 species and Cyprinidae is one of the most diverse families of Actinopterygii having 220 genera including 2420 fish species worldwide.

Modern taxonomic work regularly employs internal anatomy, physiology, behavior, genes, isozymes, geography, and morphological characters, which remain the cornerstone of taxonomic treatments [3]. However, there are difficulties in relying primarily on morphology when attempting to identify fish diversity during various stages of their development for example larvae or when examining fragmentary, partial, or processed remains. Even when intact adult specimens are available, the morphological characters used to discern species can be so subtle that identification is difficult, even for trained taxonomists [4].

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Species is the fundamental unit of comparison in biology, from anatomy to behavior, development, ecology, evolution, genetics, molecular biology, paleontology, physiology, systematic, and so forth [5]. Thus, the capability to identify species correctly is crucial in order to minimize "error cascades" resulting from the use of bad taxonomy in science [6]. Specifically, such errors are known to have a significant impact on the population assessment of over fished species [7].

Traditionally, for the identification of species, morphological characters are used. However, the development of molecular biology created a new set of useful tools to identifying species. Protein electrophoresis on starch gel was first used more than 45 years ago to identify species. Since then, many studies have been published using a diverse assemblage of molecular approaches and markers to identify species, such as allozymes [8].

While humanity is facing increasing evidence of the erosion of Earth's biodiversity, the approach of identifying the fish species by performing RAPD analysis is proving its effectiveness in characterizing the complexity of the biodiversity realm at a pace unequalled by other characters [9]. Several researchers signified the importance of RAPD analysis in identification of freshwater fish species. Pertaining to available literature it was noted that there is no previous record of RAPD-PCR profiling of members of family Cyprinidae found in the three reservoirs located in three districts of North Maharashtra region.

Since, the RAPD-PCR analysis plays instrumental role in understanding the genetic variability of fish populations, the present work was undertaken to amplify genomic DNA of seventy freshwater fish belonging to family Cyprinidae collected from three freshwater resources using short primer of arbitrary sequence so as to detect high levels of polymorphism and produce genetic markers. The results described here could be used for stocks maintenance of the studied species in hatchery programs.

2. MATERIALS AND METHODS

2.1 Study Area with Sampling Sites for Collection of Fishes

Specimens of carp fish belonging to family Cyprinidae were obtained from commercial artisanal catches made at following sampling sites:

- Hatnur dam (21°4'27.79"N 75°57'7.81"E) on river Tapi behind Varangaon Ordnance factory near Hatnur village, Tal Bhusawal in Jalgaon district,
- Gangapur dam (20°1'24.39"N 73°39'21.02"E) on river Godavari river in near Nasik city in Nasik district, and
- Lower Panzara/Akkalpada dam (20°56'47.92"N 74°26'12.27"E) on river Panzara near Akkalpada village in Dhule district (Fig. 1).

Local fishermen helped in identifying the fish species which were tagged with site and species specific markers. Morphological features of each tagged carp fish were noted for further investigation. Specimens were preserved in cool box filled with ice and transferred to the post graduate research center in the department of Zoology of Arts, Science and Chopda in Jalgaon district of Maharashtra for estimating genetic variations.

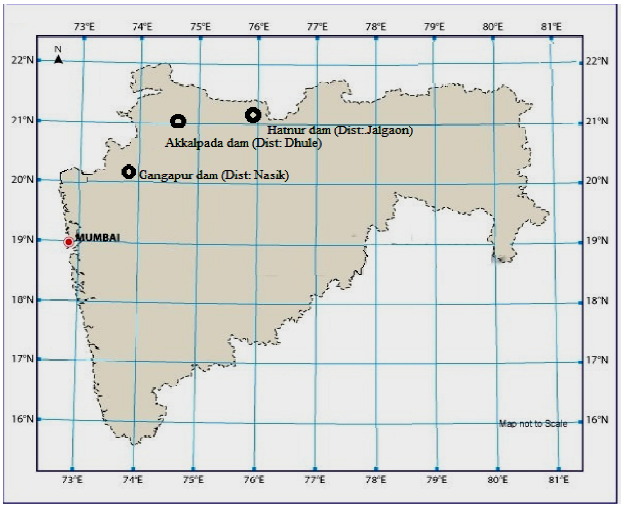

Fig. 1. Location of three sampling sites in the region of North Maharashtra for collection of carp fishes

2.2 Isolation of Genomic DNA from Carp Fish

For the isolation of total genomic DNA, a short procedure was applied according to a modified protocol reported by Wu et al. [10]. Approximately, 50 mg of fish scales were taken from each species and dried on a filter paper. The scales were then cut into small pieces and placed in a 2 ml-Eppendorf tube containing 940 µl lysis buffer (200 mM Tris-HCl, pH 8.0; 100 mM EDTA, pH 8.0; 250 mM NaCl), 30 µl proteinase K (10 mg/ml) and 30 µl 20 percent SDS. The content in the tubes were incubated at 48°C for 45-50 min in a water bath. After incubation, 500 µl volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added to the tube containing lysed scales cells. The contents were then mixed properly by gently inverting the tube for 10 min to precipitate the proteins and other part of the nucleic acids. The tube was then rotated for 10 min at 9,200 g. The top aqueous layer was transferred to a new 1.5 ml-Eppendorf tube, leaving interface and lower phase.

The DNA was precipitated by adding equal volume of isopropanol and 0.2 volumes of 10 M ammonium acetate and inverting the tubes gently several times. The precipitated DNA was then pelleted by centrifugation at 13,200 g for 10 min. The supernatant was removed by pouring out gently, taking care to avoid loss of DNA pellet. The pellet was then washed briefly in 500 µl chilled 70 percent ethanol, air-dried and resuspended in 200 µl sterile water/ TE buffer. The presence of DNA was confirmed with diphenylamine test.

After ensuring complete solubility of DNA, the purity factor (A_{260}/A_{280} nm) was measured by UV spectrophotometer (BioEra, Pune) and its integrity was checked by loading 10 µl DNA preparation (2 µl extracted DNA, 2 µl dye and 6 µl sterile water) on 0.8 percent agarose gel and stained with ethidium bromide. The extracted DNA samples were then stored at -20°C till their further use. These DNAs were used as templates in a PCR based search for producing RAPD markers.

2.3 Screening and Selection of PCR Primers

In the present study, 10 commercially available RAPD primers (10 bases long) made by Chromus Biotech, Bangalore, India were used to initiate PCR amplifications.

2.4 PCR Amplification

For standardizing $MgCl_2$ concentration in PCR reactions for getting consistency in amplification products, a concentration gradient from 1.0 to 3.0 mM was applied, and DNA template concentration was optimized for PCR reaction using initially five concentrations of DNA 5, 10, 20, 40 and 80 ng per PCR reaction. The reaction mixture (10 µl) for PCR was composed of 1 µl of 10X Taq polymerase buffer [100 mM Tris, pH 9.0; 500 mM KCl; 0.1% gelatin, 2.0 mM $MgCl_2$], 1 µl of 2.5mM dNTPs, 1 µl of RAPD primer, 0.15 µl Taq DNA polymerase (2 U/ µl), 5.55µl 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 1 µl of the sample (80 ng/µl) added to the reaction mixture from different fish samples which would act as template. The ingredients for above reactions were purchased from Chromus Biotech Pvt. Ltd Bangalore, India.

After preheating the reaction mixture for 5 minutes at 94°C, PCR was run for 35 cycles. It consisted of a 94°C denaturation step (for 1 min), 37°C annealing step (for 1 min) and 72°C elongation step (for 2 min) in a thermal cycler (BioEra, Pune, India). 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.

Table 1. Code and sequence of decamer primers used in the RAPD analysis of 24 genera of carp fish

Serial. number	Code of primer	Sequence of the primer
1	RACB-08	5'–GTGACGTAGG-3'
2	RACB-15	5'–TTCCGAACCC-3'
3	RACB-07	5'–GACCGCTTGT-3'
4	RACB-02	5'–GGACCCAACC-3
5	RACB-01	5'–CAGGCCCTTC-3'
6	RACB-05	5'–CGGCCCCGGC-3'
7	RACB-18	5'–GAAGAACGCT-3'
8	RACB-34	5'–GACGGATCAG-3'
9	RACB-14	5'–CGGAGAGCCC-3'
10	RACB-31	5'–GGGTAACGCC-3'

2.5 Agarose Gel Electrophoresis

The amplified DNA fragments were separated on 1.8% agarose gel and stained with ethidium bromide. Standard marker DNA (M) of ø X 174 DNA marker digested with Hae III that produce 100, 200, 300, 400, 500, 600, 800 and 1000 bp DNA ladder (Chromus Biotech, Bangalore, India) was run with each gel. The amplified pattern was visualized on an UV transilluminator and photographed by gel documentation system (BioEra, Pune, India).

2.6 Statistical Analysis

The morphometric features of 24 genera and 70 fish species were statistically analyzed using GraphPad Prism 6 software. For the analysis and comparison of the patterns, a set of distinct, well-separated bands were selected. The genotypes were analyzed in the form of binary variables by recording the presence (one) or absence (zero) of these bands only, neglecting other (weak and unresolved groups of) bands. Each locus can be treated as a two-allele system, with only one of the allele per locus being amplifiable by the PCR. We also assumed that marker alleles from different loci do not co-migrate to the same position on a gel, and that populations are under the Hardy-Weinberg equilibrium [11]. Genetic similarity between 24 genera of carp fish was estimated according to Nei and Li [12]. Nei's unbiased genetic distance was calculated among different genera with all markers, including monomorphic markers. 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 of UPGMA [13].

Cluster analysis was carried out using GGT 2.0 version software. Dendrograms were constructed by employing online D-UPGMA (Unweighted Pair Group Method with Arithmetic Mean) to study the genetic variability within the species [14]. Similarly, the same method was followed to construct the dendrogram to study the phylogenetic relationship among different genera of carp fishes.

3. RESULTS AND DISCUSSION

Total 568 fish specimen collected from three different dams constructed on Tapi, Godavari and Panzara rivers in northern Maharashtra. Based on standard identification keys proposed by Talwar and Jhingran [15], local fishermen and extended cooperation from Zoological survey of India (Pune), fish were categorized into 24 genera of family Cyprinidae (Table 2).

The morphometric measurements later confirmed 70 species of carp fish belonging to 24 genera of carp fish inhabited three freshwater reservoirs namely, Hatnur, Gangapur and Akkalpada formed in three rivers of the study area. Recorded observations related to occurrence in abundance, moderate or absence of fish indicated that the river Tapi and Godavari harbor most of the carp fish species than the river Panzara, which may be due to the size, source, geographical location of the dam and interference of anthropogenic activities.

3.1 RAPD-PCR Analysis of 24 Genera of Carp Fish

The genomic DNA isolated from representatives of 24 genera (G1 to G24) of carp fish were processed for PCR amplification using 10 primers following the same protocol. The PCR products run on agarose gel were scored manually. Among ten primers, the promising results for generating stable and easy to score and reproducible bands were selected for genotyping of 24 genera by three primers namely, RACB-01, RACB-08 and RACB-14. For primer RACB-01, a total number of 59 bands of amplicons were visualized. Their size ranged from 200 to 1000 bp (Fig. 2 a & b). Within the PCR products of size of the bands having 700 bp were monomorphic, and the primer produced 59.39% polymorphic loci. For Primer RCAB-08, the number of amplified products scored was 48 (Fig. 3 a & b). These products ranged in size from 100 to 1000 bp. Twenty-three of PCR products with 650 bp were monomorphic and the primer produced 52.08% polymorphic loci. Primer RACB-14 produced 37 PCR products (Fig. 4 a & b). Their size ranged between 200 to 1000 bp. Twenty-three amplicons of size 800 bp were seen to be specific, hence monomorphic and 37.84% of loci which are polymorphic were obtained by this primer.

A total of 144 amplified bands produced by three selected primers within 24 genera of Cyprinid fish, on average 51.39% bands were polymorphic (Table 3). Average number of polymorphic bands varied from 1.5 to 6.5. In particular, primer RACB-01 produced highest number of amplified fragments with an average of 6.5 followed by RACB-08 and RACB-14 produced the lowest number of fragments with an average of 1.5.

Table 2. Number of carp fish species reported in 24 genera of family Cyprinidae captured from freshwater habitats in North Maharashtra region

Sr. no.	Genera of fish	N	Sr. no.	Genera of fish	N
G1	*Labeo*	10	G13	*Tor*	01
G2	*Catla*	01	G14	*Salmostoma*	03
G3	*Cyprinus*	01	G15	*Hypophthalmichthys*	02
G4	*Cirrhinus*	04	G16	*Barilius*	03
G5	*Ctenopharyngodon*	01	G17	*Parluciosoma*	01
G6	*Gonoproktopterus*	03	G18	*Danio*	05
G7	*Neolissochilus*	02	G19	*Esomus*	03
G8	*Oreichthys*	01	G20	*Amblypharyngodon*	01
G9	*Osteobrama*	05	G21	*Aspidoparia*	02
G10	*Osteochilus*	03	G22	*Crossocheilus*	01
G11	*Puntius*	10	G23	*Garra*	03
G12	*Rasbora*	03	G24	*Barbodes*	01

Sr. No = Serial number \\\ N = Number of species reported

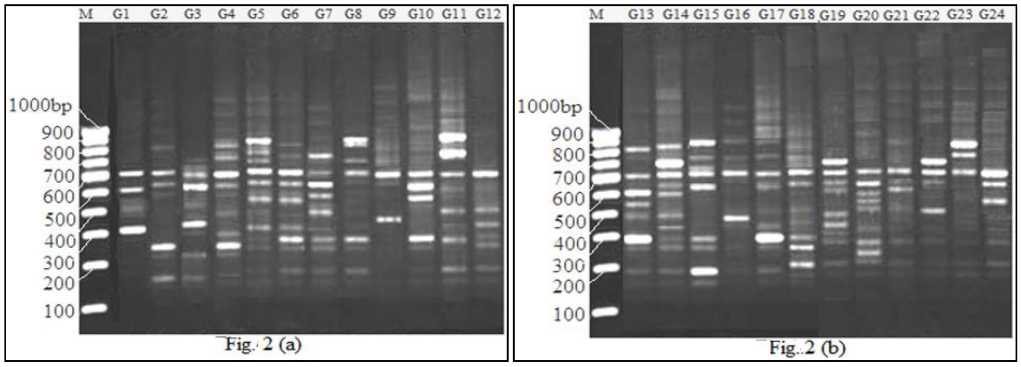

Fig. 2(a & b). RAPD patterns generated in 24 genera of carp fish species using primer RACB-01. M, ø X 174 DNA marker digested with *Hae*III that produced 1000 to100 bp DNA ladder

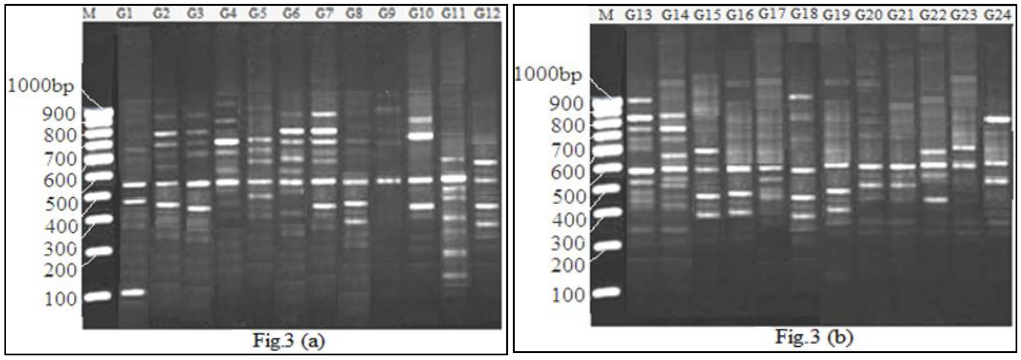

Fig. 3(a & b). RAPD patterns generated in 24 genera of carp fish species using primer RACB-08. M, ø X 174 DNA marker digested with *Hae*III that produced 1000 to100 bp DNA ladder

Depending on the data produced from RAPD-PCR amplification of representative carp fish from 24 genera of family Cyprinidae, Genetic distance and similarity coefficient were obtained from statistical analysis that provided information about the genetic similarity between carp fish collected from three different freshwater resources located in north Maharashtra.

RAPD bands in this study were always variant (i.e., strong, faint, fuzzy and sharp bands) generated with each primer because one or more copies of DNA may exist per genome or may be attributed to the varying degree of the annealing process between the primer and the DNA. This problem of mixed bands shows the well-known sensitivity of PCRs [16]. RAPD fragments generated by third primer (RACB-14)

produced low polymorphism among fishes studied. This primer sequences may have annealed to variable sequences, which might be of great utility at lower taxonomic levels, e.g. for the differentiation of closely related species. However, in RAPD fragments generated by RACB-01 and RACB-08 primers, there were high degree of polymorphism; their sequences may be considered as more conserved sequences, which are most useful in higher taxonomic levels and evolutionary relationships. These results are in agreement with Bardkci and Skibinski [17] who stated that, patterns of similarities and differences between populations showed broad agreement across primers and the overall similarity level varied between primers. Thus, primer choosing is a very important for this technique.

RAPD fingerprinting has been used to construct a genetic linkage map [18]. In the present part of investigation, representatives of 24 genera of carp fish were studied genetically through the RAPD technique to put a species fingerprint and to identify the similarity coefficient among the fishes under study. This coefficient represents a measure of the shared bands two or more different species within the same, and different, primers. These are important measurements that help to quantify the degree of relationships between different species.

The description of this similarity coefficient is not simple, especially when more than one character is involved in the same cluster. The dendrogram constructed by online D-UPGMA (Fig. 5) showed the genetic similarity and diversity among the representatives of 24 genera of family Cyprinidae. The present study concluded that there were 14 clusters in which there are high similarity between 20 different genera such as *Osteobrama* and *Labeo, Parapsilorhynchus* and *Garra, Ctenopharyngodon* and *Hypophthalamichthys, Salmostoma* and *Aspidoparia, Rasbora* and *Amblypharyngodon, Barilus* and *Esomus, Barbodes* and *Catla, Cyprinus* and *Cirrhinus, Neolissochilus* and *Tor, Osteochilus* and *Punctius*. These observations may indicate that there is high probability of hybridization between the members of these genera, but not between the members of genera *Oreichthys, Danio, Gonoproktopterus* and *Crossocheilus*. These findings are an indication of the presence of distinct character in these four genera of family Cyprinidae territoriality, in spite of the close morphology of body shape they all share. The UPGMA dendrogram constructed on the basis of similarity matrix indicated the segregation of the 24 genera of carp fish collected from three dams in North Maharashtra, India.

3.2 RAPD-PCR Profiling of 70 Species of Carp Fish Belonging to Family Cyprinidae

In the present study, the members of each genus of family Cyprinidae were processed for RAPD-PCR profiling to have the phylogenetic tree. In the group of 70 carp fish species selected for above-mentioned objectives, 10 species included in each of the genus *Labeo* and *Punctius*, followed by 5 species in each of the genus *Danio* and *Osteobrama*, 4 species of the genus *Cirrhinus*, 3 species of each genus *Gonoproktopterus, Osteochilus, Rasbora, Salmostoma, Barilius, Esomus,* and *Garra,* followed by 2 species of the genus *Neolissochilus, Hypophthalmichthys* and *Aspidoparia.* All these members were processed to evaluate the percentage of their polymorphism and monomorphism based on their RAPD-PCR banding patterns. The rest of the genera of carp fish namely, *Catla, Cyprinus, Ctenopharyngodon, Oreichthys, Tor, Parluciosoma, Amblypharyngodon, Crossocheilus* and *Barbodes* were represented by single species, which were already shown their phylogenetic status in the dendrogram.

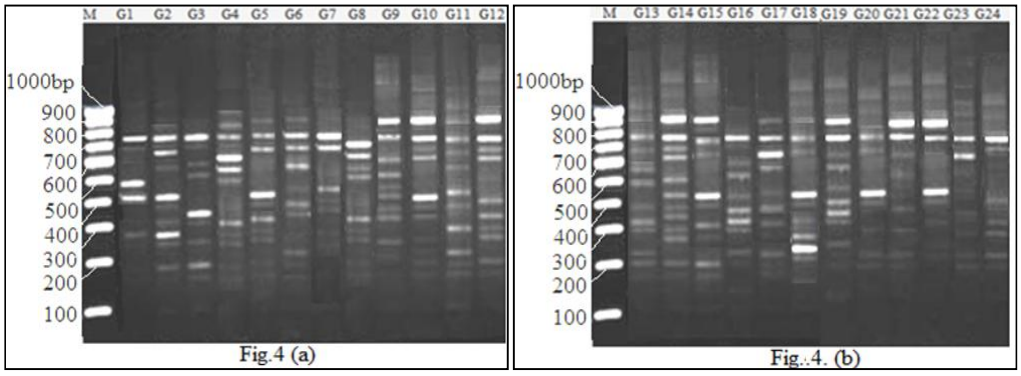

Fig. 4(a & b). RAPD patterns generated in 24 genera of carp fish species using primer RACB-14. M, ø X 174 DNA marker digested with *Hae*III that produced 1000 to 100 bp DNA ladder

Table 3. Pattern of polymorphic and monomorphic bands generated using three primers in 24 genera of Cyprinidae fish

Parameters	RACB-01	RACB-08	RACB-14	Total
Total No. of bands	59	48	37	144
Number of polymorphic bands	35	25	14	74
Number of monomorphic bands	24	23	23	70
Percentage of Polymorphism	59.39	52.08	37.84	51.39
Percentage of Monomorphism	40.61	47.92	61.16	48.61

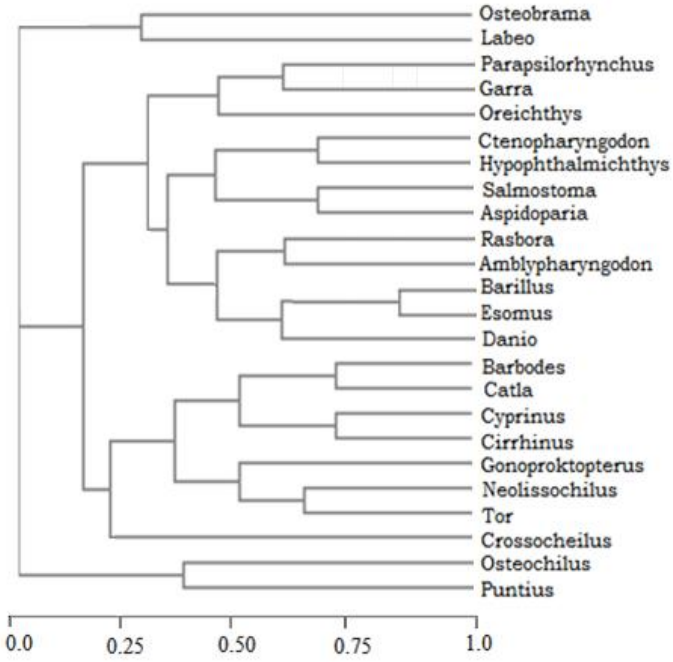

Fig. 5. UPGMA dendrogram showing the relationship and diversity among representatives of 24 genera of family Cyprinidae

Based on the RAPD-PCR profiling of all the 70 carp fish species collected from three different freshwater resources in North Maharashtra region, the consolidated dendrogram was constructed using online D-UPGMA (Fig. 6). RAPD fragments observed in the 70 carp fish species of Cyprinidae family, showed a reasonable degree of genetic diversity within and between the populations. Our results illustrate that RAPD analysis is a rapid and convenient technique to generate useful genetic markers in carp fishes. The low levels of within-species genetic variation exhibited in are due to their limited migration and pair fidelity mode of reproduction. Similar observations were reported by Berman et al. [19] in carp species. RAPD technique can be used as a rapid method for developing genetic molecular markers for gold fishes. Comparatively, the random primer RACB-01 and RACB-08 seems to be good candidate for developing genetic markers in carp fish. Species diagnostic RAPD markers developed in this manner can be utilized to determine from generation to generation a comparison of growth performance of each carp fish species under commercial growing conditions.

The advances in DNA techniques have had a great impact in addressing problems in many aspects of biology including population genetic studies. RAPD analysis has been used to discriminate between the different populations of variety of fish. The effectiveness of RAPD in detecting polymorphism among different carp fish varieties, their applicability in population studies, and the establishment of genetic relationships among various carp fish populations has been investigated this study. The result obtained from this study has proven to be useful in discrimination, characterization and differentiation of the 70 fish belonging to family Cyprinidae and clustering them according to their origin.

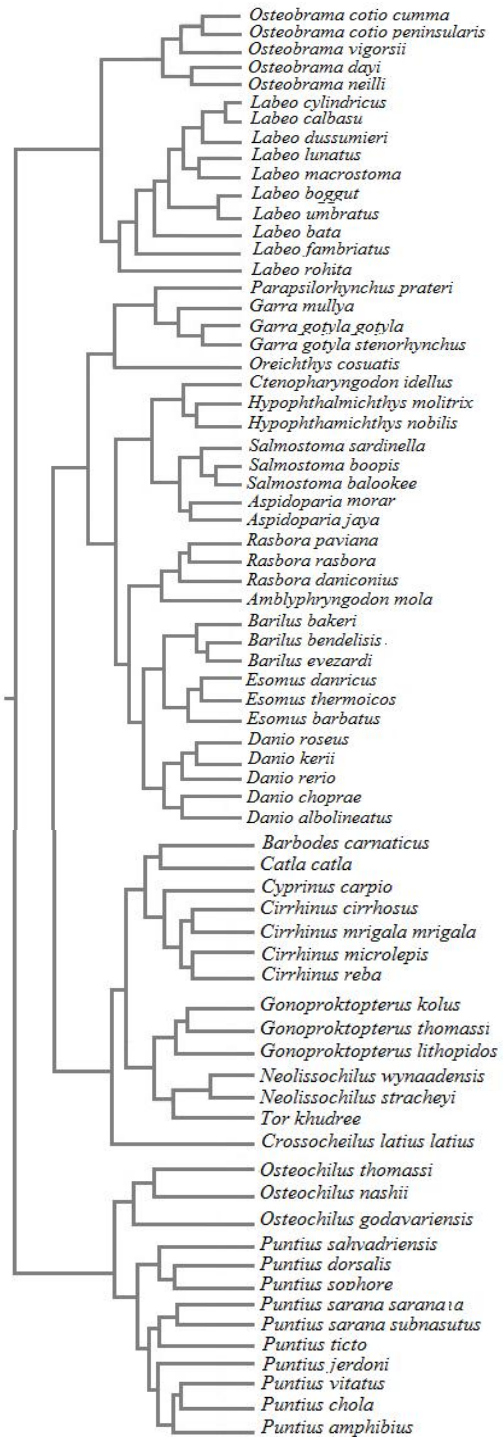

Fig. 6. UPGMA dendrogram showing the relationship and diversity among seventy carp fish species of family Cyprinidae

However, it is essential to optimize RAPD amplification condition and ascertain the reproducibility of RAPD markers for individual taxa prior to apply RAPD fingerprinting to any genetic analysis. By using three different primers, RAPD fragments showed a reasonable degree of genetic similarity as well as variation within and between the species. This observation clearly depicted the genetic similarity and genetic variation within the 70 carp fish species. The genetic data collected during this work will guide the choice of genotypes to cross according to their lineage belonging or their level of diversity.

The RAPD-PCR amplification with a single decamer primer to produce a DNA fingerprint of DNA fragments was affecting essentially with the type of primer, DNA template and reaction conditions. So that these advantages in comparison to other DNA fingerprinting method, such as restriction fragment length polymorphism (RFLP) [20] and Single Strand conformation polymorphism (SSCP) [21] that need larger quantity of pure DNA, target gene or few loci of the gene and some information of specific primers or restriction enzymes. In spite of the morphological similarity among different species of Cyprinidae, molecular profiles showed that there was apparent variation in bands amplified by using the same three RAPD primers. The number of bands on the agarose gel depends on the number of appropriately oriented and target sites present in DNA in that species or individual. This result is compatible with the result of Stacey et al. [22].

The results of RAPD profiles showed strongly differentiated fingerprints of the seventy carp fish species, so discrimination among the tested species was easy. This result coincided with the result of Welsh and McClelland [23], Hadreys et al. [24] and Mustufa et al. [25] which established a method to differentiate between cyprinids and other fish families in Iraq. The present study revealed that the seventy carp species were accurately distinctive species as shown in the RAPD molecular profiles. Although, Clark and Lanigan [26] highlighted that the RAPD technique is less laborious compared with other fingerprinting techniques, producing results were having low statistical error. In the other hand this method needs accurate work, and multiple decamere primers should be used to generate a number of molecular markers to establish fingerprints.

Therefore DNA fingerprint similarity is being used to infer the level of genetic variation within and between natural populations [27]. RAPD variation differed among primers and species of carp fish since the amplified bands per primer were different. Many studies indicated to those differences [28]. RAPD protocol was used to differentiate the cyprinids. Barman et al. [19] previously used RAPD-PCR protocol to investigate the genetic diversity between Indian major Carps, *Labeo rohita, L. calbasu, Catla*

catla and *Cirrhinus mrigala*. For many groups, even qualified taxonomist can't find characters for reliable identification for early life stages of fish species particularly in cyprinid species due to rapid growth and similar features among them, while using RAPD protocol would solve this problem. Thus, RAPD technique is a very useful tool for studying the genetic structure and phylogenetic of the species of Cyprinidae. Iberian *Barbus* species of Cyprinidae family were investigated by RAPD to resolve the phylogenetic relationships [29,30]. The fact that RAPD-PCR technique surveys numerous loci in the genome makes the method particularly attractive for analysis of genetic distance and phylogeny reconstruction. RAPD method exhibits more pronounced effect of isolation by distance among populations of *Mullus surmuletus* in comparison with allozyms [31]. The polymorphism in genetic RAPD markers may stem out of deletion, insertion or substitution in priming sites that cause misleading priming [26].

The additional advantage of RAPD fingerprinting lies in the fact that it is a sequence independent approach and each primer – DNA annealing will produce different spectrum of fragments from the PCR generating a species- specific fingerprint [32]. Actually, the co-migrating dominant markers may not be homologous with one another. Secondly the source of bands use presence/absence, although single change in the primer sequence or single substitution, insertion or deletion in gene sequence would alter the product of PCR while scoring that bands as presence/absence characters causes statistical error [33]. Moreover, in the present study, the genetic distance is more between genus than between species that clearly reflected the use of primers for discrimination of fish at genetic level is fruitful. Similar to this present study, RAPD assay also been used to construct phylogenetic tree for resolving taxonomic problems in many organisms [34,35].

4. CONCLUSION

The present study indicates that comparatively higher level of genetic variation exists in the populations of carp fishes collected from three different freshwater resources located in North Maharashtra region. Despite some limitations, the RAPD analysis can be used effectively for initial assessment of genetic variation among fish species, particularly in fish for which the morphological variance is little. This study represents a first step towards the generation of DNA markers for species diagnosis and assessment of genetic diversity of economically important carp fish species of Cyprinidae family.

The present study may also serve as a reference point for future examinations of genetic variations within the populations of other fishes which are commercially important and the possible use of DNA markers in the future may create new avenues for fish molecular biological research. Further molecular studies, comprising more molecular markers, samples and other populations are still required to precisely evaluate the genetic structure of carp fishes found in freshwater bodied of Maharashtra and other part of India.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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