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ARTICLE

Characterization of genetic variance within and among five populations of *Sperata seenghala* (Skyes, 1839) revealed by random amplified polymorphic DNA markers

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Heterozygosity (H_T);
AMOVA;
Genetic polymorphism (P);
Conservation management

Abstract The genetic diversity among five populations (Bhadbada reservoir, Mohinisagar reservoir, Bansagar reservoir, Bargi reservoir and Gandhisagar reservoir) was revealed using random amplified polymorphic DNA markers. 10 random primers screened, 5 primers revealed various banding patterns and yielded 71 total loci as an average of which 39.60 (55.77%) were polymorphic between the population and 86.84% within the population of *Sperata seenghala*. Population wise the highest genetic polymorphism was obtained in Bhadbada reservoir as 67.61% whereas the lowest was in Gandhisagar reservoir as 49.30%. However, Analysis of Molecular Variance indicated low genetic diversity (H_{pop} = 0.0921 ± 0.1249; I = 0.1584 ± 0.1942) in Bansagar reservoir. Relative genetic differentiation (G_{ST} = 0.3993) and restricted gene flow (N_m = 0.7523) as an average indicated low gene diversity among the fish populations. The un-weighted pair group method with averages (UPGMA) dendrogram showed 05 major clusters, each cluster representing a population. Fish population of Mohinisagar reservoir showed high genetic distance (0.3981) with respective Bargi reservoir population and highest genetic identity (0.8846) reflected between Bansagar and Gandhisagar reservoir. Highest genetic distance between Mohinisagar and Bargi reservoir fish populations shows no significant correlation between genetic and geographical distance of the genotypes collected from different lentic and geographical isolated water bodies. This investigation indicated that lowest genetic diversity existed in different geographic populations of *S. seenghala*.

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All the five populations were found to be low in genetic variation, which is useful information for future conservation measures of *S. seenghala* confined in natural water bodies of Madhya Pradesh.

1. Introduction

Biological diversity has undergone restrictions due to direct and indirect anthropogenic activities. The reduction in the site of natural populations may have led to a reduction in evolutional options in the face of environmental changes due to loss of genetical diversity [1]. Most of the fishes used for human consumption are obtained from wild areas such as rivers and major lentic water bodies [2,3], therefore, natural populations are going toward threat. The management of the wild populations comprising commercial or sport fisheries presents genetic depletion that are unique to fisheries management and reduction of the genetic resources of natural fish populations has become an important fisheries management problem nowadays [4]. Successful conservation and effective management of a species, including developmental strategies for maintaining genetic diversity, is important to determine the levels of genetic changes or gene flow of genetic information which assist in solving problems of identifying and defining conservation unit for a species [5].

Sperata seenghala is also known as *Mystus seenghala* and *Aorichthys seenghala* and is mainly riverine fish, although it also inhabits in freshwater habitats [3,6]. This species is distributed throughout India, Pakistan, Bangladesh, Afghanistan and Nepal [7]. It is the most preferred fish species for eating in the north and north-western states of India because of its tasty flesh and the low number of intramuscular bones [3]. The entire demand for this fish in the domestic market is met through capture from rivers, thus, this species is going toward threats. Therefore, present investigation was performed to delineate the principles of the population genetics, testing the basic assumptions for population genetic analysis, departures from Hardy–Weinberg expectation, linkage disequilibrium between the loci, estimation of the genetic differentiation within and among populations using genetic distance, *FST* and gene flow analyses, determination of frequencies of genes and genotypes and estimation of effective population sizes has been outlined.

2. Materials and methods

2.1. Geographical localities and sampling

Sixty individuals from five feral populations of *S. seenghala* were caught from different lentic water bodies of Central India i.e., Bhadbhada reservoir ($n = 8$) at Bhopal, Mohinisagar reservoir ($n = 11$) at Gwalior, Bansagar reservoir ($n = 15$) at Shehdol, Bargi reservoir ($n = 11$) at Jabalpur and Gandhisagar reservoir ($n = 15$) at Neemach representing Kolans River, Sindh River, Sone River, Narmada River and Chambal River respectively (Table 1 and Fig. 1). The specimens were kept in iceboxes and brought to the laboratory for molecular investigation. The muscle, liver and brain tissues were removed from freshly caught fishes and preserved at −20 °C for molecular studies.

2.2. Extraction of genomic DNA

Total genomic DNA was extracted as protocol provided by [8] with using some modifications. One hundred mg tissue sample was taken in a pre-chilled eppendorf tube (1.5 ml capacity) and the tissue was grinded with the help of micro pestle within the tube. During grinding, added 0.5 ml of digestion buffer (100 mM Tris–HCl with pH 8.0, 10 mM EDTA with 8.0, 1.4 M NaCl, 1% SDS and 0.2% β-Mercaptoethanol) in tubes and added remaining 0.5 ml after grinding. Incubated samples at 50 °C for 30–60 min on dry bath with occasional shaking and then centrifuged at 5000 rpm for 10 min at room temperature. Collected supernatant in a fresh eppendorf tube and added equal volume of phenol:chloroform:isoamyl-alcohol (25:24:1) to the samples. Centrifuged again at 10,000 rpm for 10 min at 4 °C and transferred top aqueous layer to a new tube. Added half volume of 7.5 M ammonium acetate and 2 volumes of 100% chilled ethanol. Tubes were kept in deep freezer for 1–2 h and centrifuged at 10,000 rpm for 10 min at 4 °C. Added 1 ml of 70% ethanol in the tubes for washing and centrifuged for 10 min at 10,000 rpm at 4 °C. Discarded upper aqueous layer and dried the pellet for 1–2 h at room temperature. Added 50 µl of Tris–EDTA buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.6) and stayed for 2 h to dissolve the pellets.

2.3. Quantification of extracted genomic DNA and integrity checking

The yield of extracted DNA from fish tissues in ng/µl was measured using a UV Spectrophotometer (ND-1000) at 260 and

Table 1 Details of the locations of *Sperata seenghala* (Skyes, 1839) genotypes sampled for DNA fingerprinting along with their coordinates.

S. No.	Sample code	Locality/Reservoir	Constructed on	Geographical coordinates	Samples size (n)
1.	BBR	Bhadbhada Reservoir	Kolans River	23°12′30″N 77°22′44″E	08
2.	MSR	Mohinisagar Reservoir	Sindh River	23.32°N 77.97°E	11
3.	BSR	Bansagar Reservoir	Sone River	24°11′30″N 81°17′15″E	15
4.	BRR	Bargi Reservoir	Narmada River	22°56′30″N 79°55′30″E	11
5.	GSR	Gandhisagar Reservoir	Chambal River	24°42′24″N 75°33′12″E	15

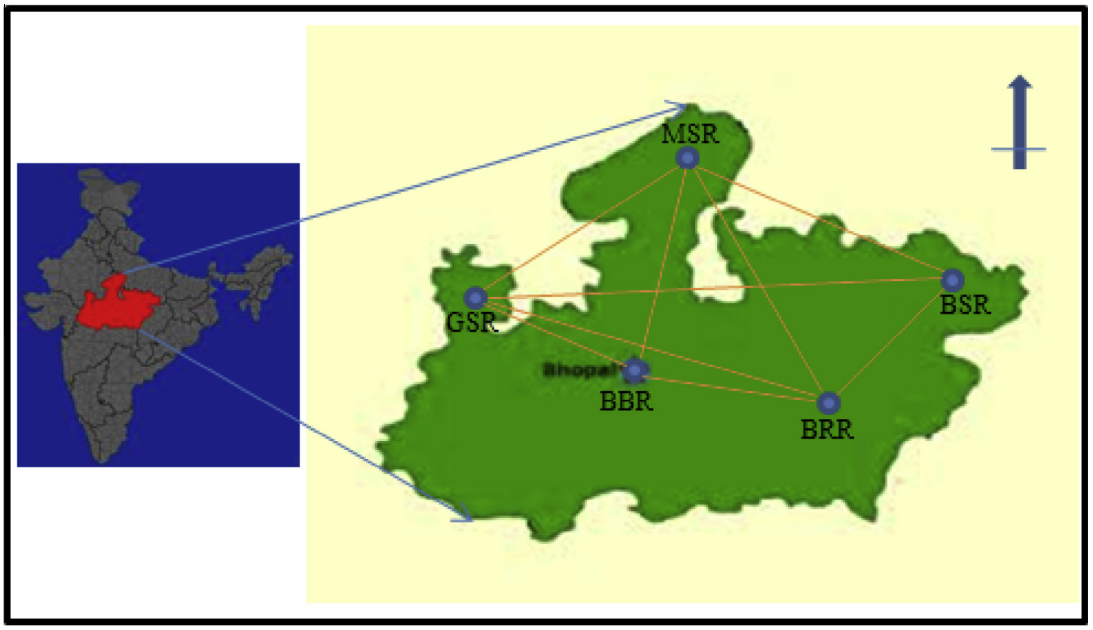

Figure 1 Map showing sampling sites for five populations of *Sperata seenghala*. BBR = Bhadbada reservoir (Bhopal), MSR = Mohinisagar reservoir (Shivpuri), BSR = Bansagar reservoir (Sehdol), BRR = Bargi reservoir (Jabalpur) and GSR = Gandhisagar reservoir (Neemach).

280 nm wavelength. The purity of DNA was determined by calculating the ratio of absorbance at 260–280 nm. The ratio of absorption at 260 nm vs. 280 nm should be 1.8 which is commonly used to assess the purity of DNA with respect to protein contamination, since protein (in particular, the aromatic amino acid) tends to absorb at 280 nm. The DNA sample is considered as pure when the 260 to 280 ratio comes near 1.8. But the DNA sample having ratio of 1.5 to 2.0 can be easily used for PCR. After checking the quality and quantity of DNA, the dilutions were made as desired for PCR amplification as 50 ng/µl or samples were treated with proteinase-K or RANase to get the pure DNA in the samples.

2.4. PCR amplification

Ten commercially available random arbitrary primers (Make Bangalore Genei, India) were used to initiate PCR amplifications. Primers were randomly selected on the basis of their reproducibility for RAPD-PCR amplifications. After initial screening with all 10 primers, 5 primers (RAn-3, RAn-4, RAn-5, RAn-6 and RAn-8 with accession numbers AM765834, AM750059, AM750052, AM765829 and AM765833 respectively) were used for final amplification who gave positive and scorable results. The PCR reaction mixture (25.50 µl) was composed of 12.50 µl of Red Dye, 1.0 µl of primer, 11.00 µl of sterile distilled water and 1.0 µl of template DNA. After preheating for 5 min at 94 °C, PCR was run for 45 cycles. It consisted of a 94 °C denaturation step (0.45 min), 37 °C annealing step (1 min) and 72 °C elongation step (1.5 min) in a thermal cycler (Eppendorf, Germany). At the end of the run, a final extension period was appended (72 °C for 10 min) and then PCR products were stored at 4 °C until gel electrophoresis was performed.

2.5. Visualization of DNA pattern

The amplified DNA fragments were separated on 1.2% agarose gel and stained with Ethidium Bromide. A low range DNA marker was run with each gel (100, 200, 300, 600, 1000, 1500, 2000, 2500 and 3000 bp, make Bangalore Genei, India). The amplified pattern was visualized on an UV transilluminator and photographed by gel documentation system (Alpha-Innotech, USA) and after which scoring of the fingerprints and molecular weight was also performed by gel documentation system (Alfa View Software).

2.6. Statistical analyses

The RAPD fragments were scored for the presence (1) and absence (0) of fragments on the gel photographs and RAPD fragments were compared among the *S. seenghala* populations. RAPD banding patterns were recorded and analyzed using Population Statistics Analysis of Molecular Variance (AMOVA) and Fst [9–11] was calculated using Popgene Software ver. 1.31 [12] to clarify molecular patterns and variations between and within the five populations for the 60 isolates. All calculations were performed using Arlequin 3.01 software [11]. The total variance was partitioned into genetic variance between and among geographical areas and among isolates within geographical areas. POPGENE ver. 1.31 [12] was used to calculate allele frequencies, observed (*Ho*) and expected (*HE*) heterozygosity and effective number of alleles.

2.7. Data Generation & Genetic analyses

Observed number of alleles (na), effective number of alleles (ne), gene diversity (h), Shannon's Information Index (*I*), total

number of loci and their percentage were estimated using a 'POPGENE' software package ver. 1.31 [12]. Gene flow (N_m), intra-population heterozygosity (H_S), total heterozygosity (H_T), relative differentiation (G_{ST}), and estimate gene flow (N_m) were also calculated to characterize the gene diversity and the distribution of the variation using 'POPGENE' program. Nei's unbiased measurement of genetic identity and genetic distance was calculated [13].

3. Results and discussion

In the present investigation, we used RAPD analyses on 60 individuals of *S. seenghala* (Skyes, 1839) collected from five different reservoirs encompassing almost entire aquatic ecosystem of Madhya Pradesh, because, these reservoirs are constructed on different rivers flowing in the state. Among all the five reservoirs, minimum geographic distance of 190 km was between Bargi and Bansagar reservoirs while, maximum distance of 576 km was between Gandhisagar and Bargi reservoirs (Fig. 1 and Table 5). 10 commercially available oligonucleotide RAPD primers (Bangalore Genei, India) were used to initiate PCR amplifications of which 5 primers RAn-3 (AM765834), RAn-4 (AM750059), RAn-5 (AM750052), RAn-6 (AM765829) and RAn-8 (AM765833) that gave better results were used for final amplification to delineated genetic diversity in five populations. Due to high polymorphism and variation in fragment intensities, many RAPD fragments could not be confidently compared between samples and between gels and were discarded from further analyses in the construction of the presence/absence of matrix.

3.1. RAPD-PCR phenotypes and their polymorphism

A comparable number of polymorphic markers of each type were found for each population. The combination of markers was more than sufficient for the identification of 60 individuals of *S. seenghala* from all locations. Each genotype was unique to a single population. Total of five RAPD primers were able to identify a large percentage of unique multilocus genotypes than the same number of polymorphic DNA fingerprints. Primer RAn-3 was screened on 60 individuals and produced bands from 260 to 1200 bp with a range of total loci as 4–11 ± 14 (Fig. 2). Table 2 shows genetic polymorphisms between and among 05 populations of *S. seenghala* which reflected that, RAn-3 primer gave minimum polymorphic bands as 3 (75.00%) in Bargi reservoir population, while maximum polymorphic bands as 9 (100%) in Bansagar reservoir. RAn-4 produced band ranges from 150 to 1800 bp sizes with a range of total loci as 9–14 ± 16 out of which, minimum polymorphic bands were as 8 (88.89%) in Gandhisagar reservoir and maximum as 13 (92.86%) in Bhadbada reservoir. RAn-5 produced band ranges from 200 to 1500 bp sizes with a range of total loci as 5–11 ± 13 out of which, minimum polymorphic bands were as 5 (83.33%) in Bhadbada reservoir and maximum as 11 (100%) in Bargi reservoir. RAn-6 produced band ranges from 110 to 1400 bp sizes with a range of total loci as 8–11 ± 14 out of which, minimum polymorphic bands were as 6 (75.00%) in Mohinisagar reservoir and maximum as 11 (91.67%) in Bansagar reservoir. Similarly, RAn-8 produced band ranges from 200 to 1466 bp sizes with a range of total loci as 3–14 ± 14 out of which, minimum polymorphic bands were as 2 (66.67%) in Bansagar reservoir and maximum as 13 (92.86%) in Bhadbada reservoir.

Overall observations clearly indicated that, the minimum total numbers of loci as 2 were produced by RAn-8 in Bansagar reservoir while maximum were produced by RAn-4 and RAn-8 as 13 in Bhadbada reservoir. However, RAn-3, RAn-5 RAn-6 and RAn-8 give 100% polymorphism in Bansagar, Bargi, Gandhisagar and Bargi reservoirs respectively.

3.2. Genetic variation within and between populations

The genetic distance (D) estimates of pair-wise comparisons within and between populations were used for constructing UPGMA clusters to analyze genetic variation. We obtained 71 loci from all the populations screened by five RAPD primers (Table 2) in which, minimum number of polymorphic loci as 35 with 49.29% polymorphism was obtained in Gandhisagar reservoir population. Though, the maximum number of polymorphic loci as 48 was obtained in Bhadbada reservoir with 67.60% polymorphism. In this observation, genetic distance were high (0.142–0.827) and within the limit expected for population level comparisons. The largest genetic distance as 0.827 was observed between samples from Bargi reservoir (BRR) of Jabalpur (Table 2).

The populations of Bhadbhada reservoir (BBR) amplified by these selected 5 primers generated 56 loci of which 48 were polymorphic and 8 (14.29%) were monomorphic between the population. However, among the population of Bhadbhada reservoir the polymorphic band were obtained as 48 with 85.71% polymorphism and a maximum genetic distance (D) as 0.694. On screening of Mohinisagar reservoir (MSR) populations, 48 loci were obtained of which 40 were polymorphic and 8 were monomorphic representing 83.33% polymorphism with maximum genetic distance (D) as 0.756. The populations of Bansagar reservoir (BSR) revealed 41 loci on amplification of which 36 were polymorphic with 87.80% polymorphism and 5 were monomorphic loci with maximum genetic distance 0.814. Similarly, Bargi reservoir (BRR) population revealed 43 loci of which 39 were polymorphic and 4 were monomorphic with 90.70% polymorphism and maximum genetic distance (D) as 0.827. However, Gandhisagar reservoir (GSR) fish population gave total of 40 loci of which 35 (87.50%) were polymorphic and 5 were monomorphic with a maximum genetic distance of (D) 0.750.

3.3. Gene diversity and Shannon Information Index

Statistical analysis as AMOVA (Analysis of Molecular Variance) shows maximum Gene diversity (H_{pop}) and Shannon's Information Index (I) within population in Mohinisagar Reservoir as $H_{pop} = 0.2069 \pm 0.2052$, $I = 0.3074 \pm 0.2927$ and the minimum value was found in Bansagar reservoir as $H_{pop} = 0.0921 \pm 0.1249$, $I = 0.1584 \pm 0.1942$ (Table 3).

3.4. Relative differentiation (G_{ST}) and gene flow (N_m) between geographic sites

Gene diversity in subdivided population was calculated with the help of Popgene Software on the basis of RAPD DNA fingerprints. Overall Intra-population (H_S) observed was 0.2509 ± 0.0249, Total heterozygosity (H_T) was

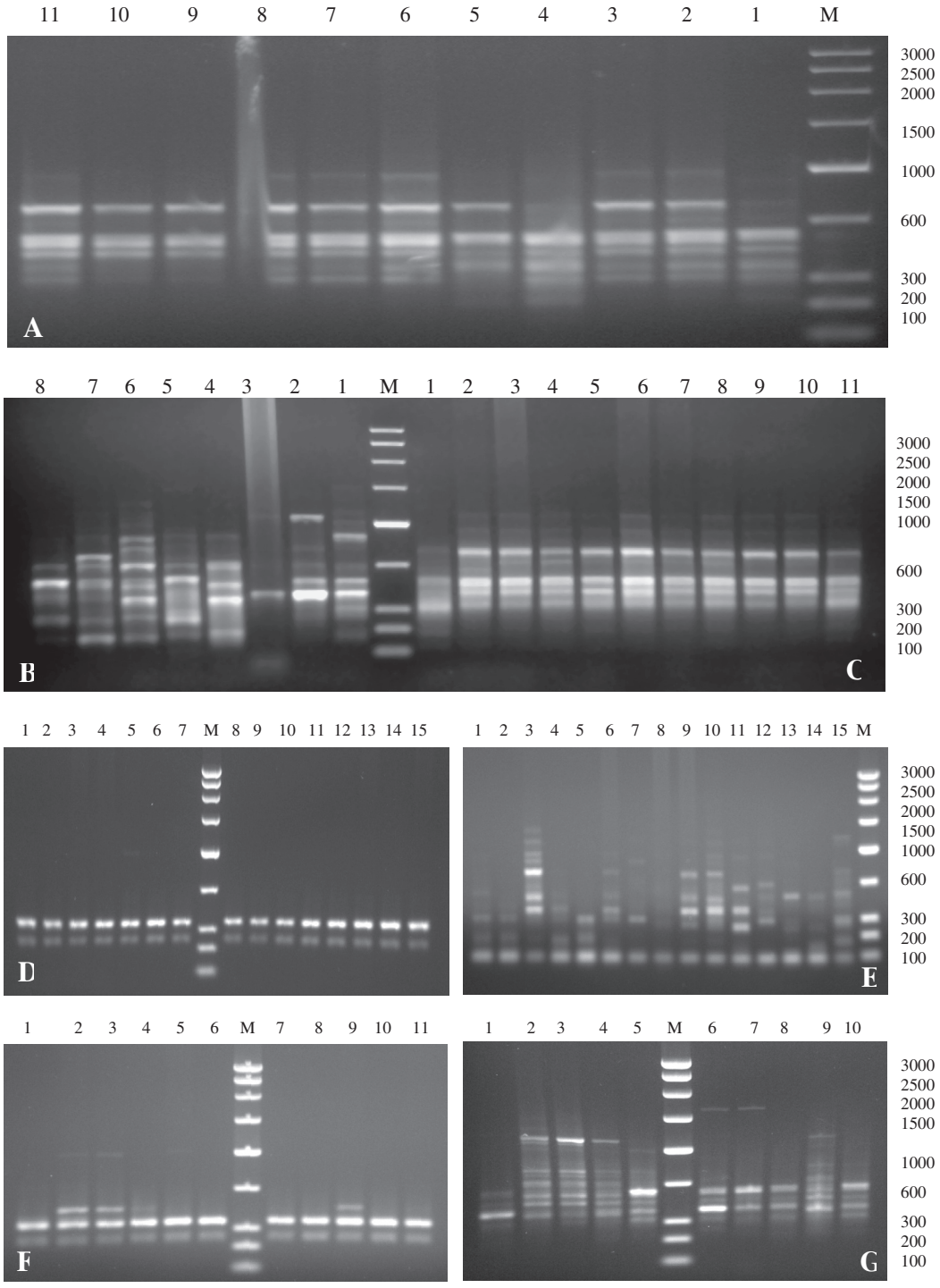

Figure 2 Random amplified polymorphic DNA (RAPD-PCR) banding patterns produced by different primers. DNA fingerprints generated in A = individuals of Mohinisagar reservoir (n = 11) using RAn-4, B = individuals of Bhadbada reservoir (n = 15) using RAn-6, C = individuals of Mohinisagar reservoir (n = 15) using RAn-6, D = individuals of Gandhisagar reservoir (n = 15) using RAn-3, E = individuals of Bansagar reservoir (n = 15) using RAn = 6, F = individuals of Bargi reservoir (n = 11) using RAn-3 and G = individuals of Bargi reservoir (n = 11) using RAn-4. M is the molecular marker (bp) of low range DNA ladder.

0.1507 ± 0.0078. Estimated relative differentiation (G_{ST}) was 0.3993 and Estimate gene flow (N_m) was 0.7523 (Table 4).

Analysis within population showed maximum no. of loci from the accessions from Bhadbhada (56) with maximum no. of polymorphic and monomorphic loci as 48 and 8 respectively. Minimum no. of loci was obtained from the accessions of Gandhisagar reservoir (40) with minimum polymorphic loci 35. Minimum monomorphic loci were found in Bargi as 4. Maximum polymorphic % of 90.70 with genetic distance 0.82759 was obtained from Bargi dam and minimum polymorphic % of 83.33 with genetic distance 0.694 was obtained from Bhadbhada. Analysis between populations showed maximum

Table 2 Genetic polymorphism between and within populations of *S. seenghala*.

Locations	Between populations		Within population			
	Total loci	Polymorphic loci (%)	Total loci	Monomorphic loci (%)	Polymorphic loci (%)	Genetic distance (*D*)
BBR	71	48 (67.60)	56	08 (14.29)	48 (85.71)	0.400–0.694
MSR	71	40 (56.33)	48	08 (16.67)	40 (83.33)	0.142–0.756
BSR	71	36 (50.70)	41	05 (12.20)	36 (87.80)	0.272–0.814
BRR	71	39 (54.93)	43	04 (09.30)	39 (90.70)	0.267–0.827
GSR	71	35 (49.29)	40	05 (12.50)	35 (87.50)	0.278–0.750
Average	71	39.6 (55.77)	45.6	06 (13.16%)	39.60 (86.84%)	–

Table 3 Population wise genetic analyses among five populations.

Population genetic parameters	BBR	MSR	BSR	BRR	GSR
Samples size (*n*)	8	11	15	11	15
Observed number of alleles A (na)*	1.6761 ± 0.4713	1.5634 ± 0.4995	1.5070 ± 0.5035	1.5493 ± 0.5011	1.4930 ± 0.5035
Effective number of alleles (ne)*	1.3143 ± 0.3450	1.3586 ± 0.3885	1.1278 ± 0.1976	1.2274 ± 0.2907	1.1759 ± 0.2755
Nei's (1973) gene diversity (H_{pop})*	0.1928 ± 0.1817	0.2069 ± 0.2052	0.0921 ± 0.1249	0.1474 ± 0.1651	0.1145 ± 0.1547
Shannon Information Index (*I*)*	0.3007 ± 0.2566	0.3074 ± 0.2927	0.1584 ± 0.1942	0.2347 ± 0.2444	0.1859 ± 0.2290
Total number of loci	71	71	71	71	71
Number of polymorphic loci	48	40	36	39	35
% of polymorphic loci (*P*)	67.61	56.34	50.70	54.93	49.30

* Mean values with standard deviation (SD).

Table 4 Overall Nei's (1987) analysis of gene diversity in subdivided populations.

Population genetic parameters	Obtained values
Intra-population (H_S)	0.2509 ± 0.0249
Total heterozygosity (H_T)	0.1507 ± 0.0078
Relative differentiation (G_{ST})	0.3993
Estimate gene flow (N_m)	0.7523

no. of polymorphic loci from the accession from Bhadbhada (48) with maximum polymorphic % as 67.60. Minimum no. of polymorphic loci were obtained from the accessions from Gandhisagar (35) with minimum % of polymorphism was obtained as 49.29.

3.5. Phylogenetic analysis

Two different tree building methods 'Phylogeny' and 'Neighbor' were done through Mega (ver. 5) and Tools population genetic analysis (TFPGA) softwares to determine the DNA fingerprints based phylogenies of the 05 populations of *S. seenghala*. In the case of phylogeny, binary matrix of all random primers along with estimated parameters were used to derive likelihood trees through genetic distance matrixes and Jaccard's coefficient, These molecular phylogenetic studies suggested that all five populations are genetically distinct to each other (Fig. 3) which are distributed in five major groups with their respective individuals except population of Bargi reservoir (BRR). Two individuals of Bargi reservoir (BRR-03 & BRR-04) are exclusively different from other 09 individuals and made a separate clade. In addition, the 04 populations i.e., Bhadbhada reservoir (BBR), Bansagar reservoir (BSR), Mohinisagar reservoir (MSR) and Gandhisagar reservoir (GSR) made separate clusters and conjugated with a main branch. Whereas, individuals of Gandhisagar reservoir has did not support the monophyly and made separate cluster with all individuals are directly associated with main branch. Thus, there is much disagreement in the literature on Gandhisagar reservoir population genetic status of various individuals of *S. seenghala*. This ambiguous genetic status has serious consequences for studies targeting. *S. seenghala* has been used extensively as food in India and abroad also. In addition, taxonomic status often dictates conservation priorities; thus this situation will also confound conservation effort for *S. seenghala*.

On the basis of the inter-population genetic distance, the neighbor-joining (NJ) dendrogram was constructed (Fig. 4) using TFPGA (Tools for Population Genetic Analysis) which indicated that the samples from Mohinsagar reservoir (MSR) were clustered in one group, while the Bhadbhada reservoir (BBR), Bansagar reservoir (BSR), Bargi reservoir (BRR) and Gandhisagar reservoir (GSR) populations were in another group (Fig. 4).

The random polymorphic DNA (RAPD-PCR) analyses are adequate for elucidation of genetic variation within and between populations of the freshwater fish *S. seenghala*. Clarification of taxonomic designations often needs to be addressed before appropriate conservation programs can be successfully implanted [14]. *S. seenghala* is a riverine fish although it is also found in freshwater lentic habitats and the fish is distributed in Pakistan, Bangladesh, Afghanistan and Nepal including India [3,6]. Characterization of genetic variation among the populations and identifying distinct population is important for developing a conservation plan [14]. Our data show the genetic differentiation among *S. seenghala* populations revealed by random primers. Random amplified polymorphic DNA-polymerase chain reaction technique is a useful tool for estimating DNA polymorphism of genetic diversity and differentiation of related fish species [15]. Therefore, we had chosen random

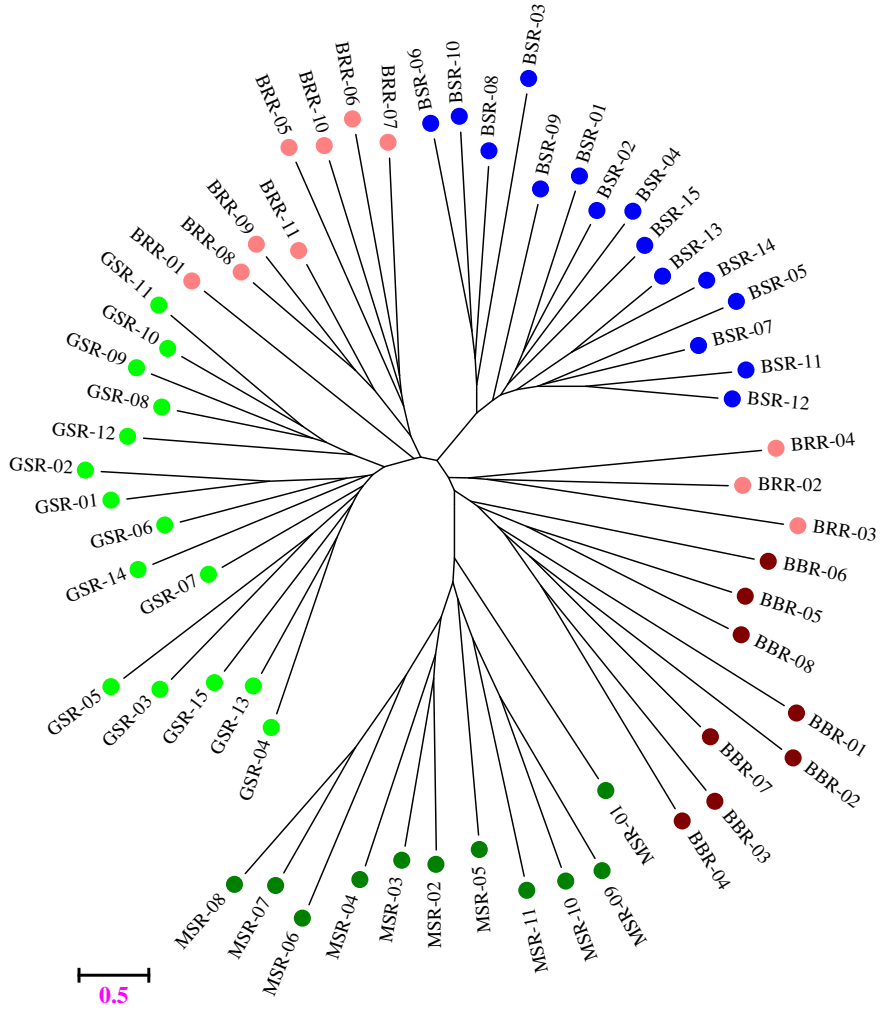

Figure 3 Neighbor-joining cluster analysis based on Nei's genetic distance (*D*) among five populations of *S. seenghala*. 1 = BBR = Bhadbada reservoir (BBR), 2 = MSR = Mohinisagar reservoir (MSR), 3 = BSR = Bansagar reservoir (BSR), 4 = BRR = Bargi reservoir (BRR), 5 = GSR = Gandhisagar reservoir (GSR).

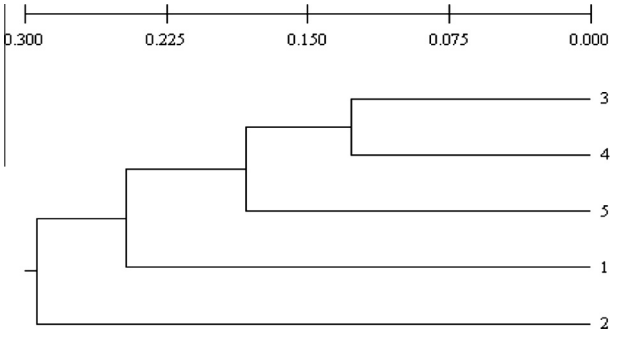

Figure 4 UPGMA cluster using Nei's (1972) original distance among five populations of *S. seenghala*. 1 = BBR = Bhadbada reservoir (BBR), 2 = MSR = Mohinisagar reservoir (MSR), 3 = BSR = Bansagar reservoir (BSR), 4 = BRR = Bargi reservoir (BRR), 5 = GSR = Gandhisagar reservoir (GSR).

primers to identify the genetic polymorphism among five populations representing the whole Madhya Pradesh.

The five RAPD-PCR primers provided a higher resolution of standard population genetic diversity and structure parameters than was possible with other molecular markers. As expected, measures of total heterozygosity (H_T) was 0.1507 ± 0.0078 notably lower than the same measures estimated in *Catla catla* (H_T = 0.232) [16] and higher percentage of polymorphism 67.61% in Bhadbada reservoir population as compared to *Tilapia* species reported [17]. The level of genetic diversity (H_{pop}) was reported as 0.1928 ± 0.1817 in Bhadhadar reservoir, 0.2069 ± 0.2052 in Mohinisagar reservoir, 0.0921 ± 1249 in Bansagar reservoir, 0.1474 ± 0.1651 in Bargi reservoir and 0.1145 ± 0.1547 in Gandhisagar reservoir which are very less than reported by [16] overall genetic diver-

Table 5 Geographical distances (km) between sampling sites.

Locations	BBR	MSR	BSR	BRR	GSR
BBR	–				
MSR	287	–			
BSR	398	376	–		
BRR	265	387	190	–	
GSR	235	283	576	488	–

Table 6 Nei's (1978) genetic distance (d) and genetic identity (i) measured between five populations of *S. seenghala*.

Populations compared	Distances (d)	Identity (i)	Unbiased distance (d)	Unbiased identity (i)
BBR vs. MSR	0.2606	0.7706	0.2406	0.7862
BBR vs. BSR	0.2405	0.7863	0.2166	0.8052
BBR vs. BRR	0.2697	0.7636	0.2498	0.7790
BBR vs. GSR	0.2277	0.7963	0.2068	0.8132
MSR vs. BSR	0.2081	0.8121	0.1913	0.8259
MSR vs. BRR	0.3981	0.6716	0.3852	0.6803
MSR vs. GSR	0.3073	0.7355	0.2934	0.7457
BSR vs. BRR	0.1267	0.8810	0.1099	0.8959
BSR vs. GSR	0.1809	0.8846	0.1632	0.8494
BRR vs. GSR	0.1845	0.8315	0.1707	0.8431

sity (H_{pop}) as 0.208 in *Catla catla* population. The average gene flow (N_m) was 0.7523 and relative differentiation (G_{ST}) was 0.3993 in present *S. seenghala* was lowest when compared to [16] and [18] studied on rock fish *Sebasticus marmoratus* revealed by SSR markers. Genetic distance (*D*) as calculated from combined data sets for 05 primers ranged from 0.142 to 0.827 (Table 2). A direct correlation was tested between different populations and geographic distance of pairwise population using the mental test (Table 5) obtained highest distance 576 km between Bargi reservoir (BRR) and Gandhisagar (GSR) is evidence for negatively correlated genetic distance (0.0778) with BRR and GSR as shown in Table 2 and Table 6. Our study certainly showed a relatively high level of genetic polymorphism in terms of the proportion of polymorphic loci, intra-population similarity indices and Nei's gene diversity (H_{pop}) in BBR as compared to other fish populations. In conclusion among different populations, the samples not only could be easily separated between the 5 geographical regions the results also indicated that, the low genetic diversity existed in different geographical populations of *S. seenghala*. All five populations were found to have low genetic variation; this information could be useful for making a plan for conservation of this species in Madhya Pradesh.

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