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Research article

PHENOTYPE AND GENOTYPE DIFFERENTIATION BETWEEN TWO STOCKS OF *TOR PUTITORA* (HAMILTON) POPULATION (PISCES: *CYPRINIDAE*) FROM HIMACHAL PRADESH, INDIA.

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ABSTRACT: This study aimed to study the phenotype and genotype differentiation and to compare the amount of differences in phenotype based on morphometric character indices and meristic counts with the amount of differences in genotype based on random amplified polymorphic DNA (RAPD) fingerprinting between two stocks of golden mahseer, *Tor putitora* (Hamilton) population. The results showed that there were no significant differences in most of the morphometric character indices and the meristic counts between the reservoir and the natural stocks of golden mahseer. In addition, Genotype analysis based on RAPD fingerprint showed low genetic dissimilarity (0.211) between the reservoir and the natural stocks. These results confirmed that the amount of similarity in genotype reflected the same amount of similarity in phenotype between the two different stocks. Therefore, based on these results it was concluded that both the stocks from two different river systems follow the similar migratory route during their migration period and shared the similar parental gene pool. Furthermore, we could conclude that for conservation of this endangered sport and food fish of the country, it would be cultured at various reservoirs and lakes for restocking in the natural environment of Himachal Pradesh.

Key words: Phenotype, genotype, *Tor putitora*, RAPD, Himachal Pradesh.

INTRODUCTION

Golden mahseer, *Tor putitora* is an important game fish, in most of the South Asian countries including India, Nepal, Bangladesh, Pakistan, Afghanistan, Sri Lanka and Myanmar. It is a large sized fish attaining a maximum length of 274 cm and weight of over 50 kg . The golden mahseer occurs naturally in rivers and streams and is also stocked in most of the reservoirs. Currently the genus *Tor* comprises of eight species (*Tor putitora, T. tor, T. khudree, T. progenies, T. kuklkarni, T. musullah, T. mosal and T. neilli*) in different parts of India [29]. Different methods are used for fish identification but phenotype based on morphometric and meristic is considered as earliest and authentic methods for the identification of fish species in fish biology to measure discreteness and relationships among various taxonomic categories. There are many well documented morphometric studies which provide evidence for stock discreteness [5, 25, 11, 4, 50, 55, 9, 16, 37]. Morphometric is the external measurements of an organism, while meristic counts means serial counts of body elements [47]. Morphological characters including meristic counts and body proportions often vary clinically (that is along a geographic gradient) [34, 27, 46, 3] reported that phenotypic adaptations do not necessarily result in genetic changes in the population and thus, the detection of such phenotypic differences among populations cannot usually be taken as evidence of genetic differentiation. Studies of morphological character variation are, therefore, vital in order to elucidate patterns observed in phenotypic and genetic character variation among fish populations [12]. The morphometric analysis can be used to assess the well being of individuals and to determine possible differences between separate unit stocks of the same species [31]. The technique of random amplified polymorphic DNA (RAPD) marker [52, 53] has been successfully exploited for stock identification and population analysis in fish [42, 10, 17, 1, 21]. In Himachal Pradesh, limited information is available on the comparative phenotypic and genotypic analysis between the reservoir and natural stocks of this important fish..

Therefore, the aim of this study was to study the phenotype and genotype differentiation and to compare the amount of differences in phenotype based on morphometric character indices and meristic counts with the amount of differences in genotype based on RAPD fingerprinting between the two different stocks of golden mahseer available in Himachal Pradesh

MATERIALS AND METHODS

This study was carried out at biotechnology research laboratory, School of biology, Shoolini University, Solan, Himachal Pradesh, India.

Specimen collection

Fishes were obtained by sampling during March-May and August-October, 2011 for captive stocks of Tor putitora population from Pong Reservoir (30^0 01' N 76^0 05E, Kangra) and natural stocks from Seer stream (31^0 -26'-59"N and 16^0 -43'-11"E, Bilaspur) from Himachal Pradesh, India. Two samples, each of 30 specimens of *Tor putitora* of variable size were taken from each locality. These were collected with cast net in natural stream and gill net in reservoir with the help of local fishermen. Collected fishes were preserved in 10% formaldehyde solution and stored in plastic containers. These were blotted dry on a paper towel and weighed on an electronic balance AD-500 B (readability 0.01 g). Body length measurements were taken by using wooden tray fitted with a measuring scale and Vernier Calliper to the nearest 0.01 cm.

Quantitative phenotype analysis

A total of 23 morphometric characters and 8 meristic counts (Table1) were recorded within each stock as described by [26, 48, 43, 13, 16]. The measurements were subjected to correlation and regression analysis using SPSS version 16.0. The coefficient of correlation (r) and regression (b) were tested for significance. The data was used to compute the regression equation for each dependent variable to fit the straight line equation (Y=a+bX), where 'Y' is the dependent variable, 'a' the intercept, 'b' the slope of regression line and 'X' is the independent variable. The various morphometric characters were then classified on the basis of range into genetically (1-9.99 or <10%), intermediate (10-14.9 or <15%) and environmentally (>15%) controlled characters (Johal *et al*, 1994). For the calculation of regression equation, standard deviation (SD), coefficient of correlation (r) and graphs, the computer software SPSS version 16.0 was used.

The cluster analysis using unweighted pair group average method (UPGMA: Sneath and Sokal, 1973) was performed in order to depict hierarchical differences between the reservoir and natural stocks of *Tor putitora.*

Statistical analysis

Data significance of the morphometric character indices and meristic counts were analyzed using unpaired Student's t-test ($P<0.05$) according to [45].

Genotype analysis

Genotype analysis was performed based on RAPD fingerprinting. DNA was extracted from preserved blood samples of each stock of golden mahseer according to the method described.. In this study ten base long oligonucleotide primers were used to initiate polymerase chain reaction (PCR) amplifications. Primers were randomly selected on the basis of GC content and annealing temperature for RAPD-PCR amplification (Table 2). PCR amplifications were performed according to the procedure of Williams et al. (1990, 1993). The reaction (25 µl) was carried out in a mixture consisting of 0.8 U of Taq DNA polymerase (Fermentas), 25 pmol dNTPs, and 25 pmol of random primer, 2.5 µl 10X Taq DNA polymerase buffer and 40 ng of genomic DNA. The final reaction mixture was placed in a DNA thermal cycler (Labnet). The PCR programme included an initial denaturation step at 94°C for 5 min followed by 45 cycles with 94°C for 30 s for DNA denaturation, annealing as mentioned with each primer (Table 2), extension at 72°C for 1 min and final extension at 72°C for 4 min were carried out. Samples were cooled at 4°C. The amplified DNA fragments were separated on 1.3% agarose gel and stained with ethidium bromide.100bp DNA Ladder marker was used in this study. The amplified patterns were visualized on an UV transilluminator and photographed by gel documentation system (Alpha Innotech).

RAPD patterns were analyzed and scored from photographs. For the analysis and comparison of the patterns, a set of distinct, well separated bands were selected. The genotypes were determined by recording the presence (1) or absence (0) in the RAPD profiles. Genetic similarity (GS) between the reservoir and the natural stocks was calculated according to the formula given by Nei and Li (1979): Bij=2 Nij/(Ni + Nj), where Nij is the number of common bands observed in individuals i and j, and Ni and Nj are the total number of bands scored in individuals i and j respectively, with regard to all assay units. Thus, GS reflects the proportion of bands shared between two individuals and ranges from zero (no common bands) to one (all bands identical). Genetic dissimilarity (GD) was calculated as: GD = 1- GS [10].

Table I: Quantitative phenotype traits based on morphometric characters and meristic counts used for differentiation analysis between reservoir and natural stocks of *Tor putitora*

Characters	Acronyms
Morphometric analysis	
Total length	SL
Standard length	TL
Head length	HL
Head depth	HD
Pre-orbital distance	Pr-OD
Post-orbital distance	Po-OD
Eye diameter	ED
Inter orbital distance	IOD
Pre-dorsal distance	Pr DD
Post-dorsal distance	Po DD
Length of dordal fin	LDF
Depth of dorsal fin	DDF
Length of anal fin	LAF
Depth of anal fin	DAF
Pre-anal distance	Pr AD
Length of pectoral fin	LPF
Length of pelvic fin	LpF
Minimum body width	Min BW
Maximum body width	Max BW
Distance between pectoral & ventral fins	Dist.pec.&vent.
Distance between pelvic & anal fins	Dist. pel.& anal
Length of caudal fin	LCF
Length of caudal peduncle	LCP
Meristic analysis	
Lateral line scale count	LLSC
Scales above the lateral line	SALL
Scales below the lateral line	SBLL
Dorsal fin rays count	DFRC
Pelvic fin rays count	Pel FRC
Pectoral fin rays count	Pec FRC
Anal fin rays count	AFRC
Caudal fin rays count	CFRC

Table 2. The sequences, GC% and the annealing temperatures of the primers used.

Primer Sequence 5`- 3`	GC%	Annealing Tm°C /Sec
1 AGACGGCTCC	70	34/30
2 AGAGCGTACC	60	32/30
3 CCTGGGTCAG	60	34/30
4 GGCGAGTGTG	70	34/30
5 GTAAGCCCCT	60	32/30
6 GGGATGACCA	60	32/30
7 TGCGCCCTTC	70	34/30
8 GTCCACACGG	70	34/30
9 CTGCTGGGAC	70	34/30
10 CATCGCCGCA	70	34/30

RESULTS
Quantitative phenotype analysis
In proportion to total length

Various body measurements in relation to total length of *Tor putitora* were compared on the basis of correlation coefficient and regression coefficient (Table3).

Table3: Values of different morphometric characters in *Tor putitora* from various collection sites of Himachal Pradesh State, India

Parameters	SEER STREAM					PONG RESERVOIRT				
	Regression equation Y=a+Bx	Mean	SD	Range Difference	Correlation coefficient (r)	Regression equation Y=a+Bx	Mean	SD	Range Difference	Correlation coefficient (r)
In %age of Total Length										
Standard length	Y=-0.286+0.752X	73.61	1.29	4.66	0.958**	Y=-1.745+0.855X	77.41	1.88	7.13	0.991**
Predorsal length	Y=-1.732+0.688X	40.20	0.49	2.3	0.978**	Y=1.735+0.349X	43.08	1.82	6.88	0.975**
Post dorsal length	Y=0.417+0.378X	59.18	1.03	3.51	0.976**	Y=4.678+0.409X	62.82	3.58	12.76	0.994**
Length of dorsal fin	Y=-0.171+0.205X	19.58	0.33	1.33	0.965**	Y=-0.601+0.253X	22.56	0.68	2.77	0.987**
Depth of dorsal fin	Y=-1.627+0.224X	13.43	0.68	2.17	0.941**	Y=0.263+0.180X	19.31	0.77	2.62	0.958**
Length of anal fin	Y=-3.617+0.324X	12.34	1.25	3.59	0.969**	Y=-0.479+0.179X	15.75	0.40	1.53	0.996**
Depth of anal fin	Y=-2.567+0.278X	13.58	0.91	3.36	0.976**	Y=0.915+0.091X	13.44	0.82	2.66	0.926**
Pre-anal distance	Y=-2.606+0.335X	61.59	2.25	6.92	0.935**	Y=0.251+0.162X	61.38	5.32	18.29	0.985**
Length of pectoralfin	Y=-3.441+0.313X	12.24	1.23	4.3	0.958**	Y=1.590+0.087X	16.15	1.23	4.21	0.969**
Length of pelvicfin	Y=-3.821+0.333X	12.14	1.44	4.66	0.952**	Y=2.198+0.034X	13.73	1.63	5.69	0.913**
Minimum body width	Y=-1.489+0.130X	5.26	0.51	1.85	0.979**	Y=-0.002+0.087X	8.75	0.53	1.70	0.913**
Maximum body width	Y=-1.521+0.134X	15.72	0.34	1.7	0.973**	Y=-1.294+0.296X	23.59	1.07	3.86	0.990**
Distance between pectoral & pelvic	Y=-3.124+0.358X	18.46	1.31	4.42	0.944**	Y=-0.060+0.274X	21.13	0.42	1.54	0.991**
Distance between pelvic & anal	Y=-4.110+0.431X	20.34	1.49	4.1	0.963**	Y=0.717+0.226X	26.04	1.53	6.37	0.933**
Length of caudalfin	Y=-3.383+0.392X	20.48	1.23	4.04	0.974**	Y=1.572+0.175X	24.92	1.53	5.01	0.944**
Length of caudal peduncle	Y=-3.732+0.269X	6.24	1.27	4.18	0.963**	Y=-0.731+0.149X	11.50	1.26	5.09	0.849**
In %age of Head Length										
Head depth	Y=1.517+0.057X	76.22	6.06	19.88	0.975**	Y=0.858+0.042X	86.94	3.23	12.5	0.986**
Pre-orbital distance	Y=0.598+0.868X	67.28	4.79	16.68	0.964**	Y=0.178+0.701X	74.29	4.76	17.0	0.954**
Post-orbital distance	Y=0.02+0.510X	51.68	2.82	8.89	0.943**	Y=0.356+0.404X	48.62	3.43	12.5	0.945**
Eye diameter	Y=0.108+0.220X	25.59	0.82	3.37	0.978**	Y=0.180+0.193X	23.46	1.47	5.33	0.961**
Inter- orbital distance	Y=-1.205+1.091X	69.81	6.09	18.94	0.991**	Y=-1.274+1.119X	83.82	6.19	21.4	0.985**

** $p < 0.05$ level of significance.

Among the seer stream population, the most highly correlated body parameters in relation to total length were, minimum body width (min BW) (r= 0.979), pre-dorsal distance (pr DD) (r= 0.978), post-dorsal distance (po DD) (r= 0.976) and depth of anal fin (DAF) (r= 0.976) and least correlated were, pre-anal distance (pr AD) (r= 0.935), depth of dorsal fin (DDF) (r= 0.941) and distance between pectoral where as compared to the Pong reservoir stocks, the most highly correlated body parameters in relation to total length were LAF (r= 0.996), SL (0.991), po DD (r= 0.994), max BW (r= 0.990) and dist. pect.& pelv. (r= 0.991) and least correlated parameters were LPF (r= 0.913), min BW (r= 0.913) and LCP (r= 0.849). It has been observed that all the characters follow linear relationship for Seer stream and Pong Reservoir populations and high degrees of correlation (r> 0.91) with less than one percent level of significance (p< 0.01), indicates that morphometric characters increase in direct proportion to each other.

In proportion to head length

The body measurements inter-orbital distance (IOD) (r= 0.991), eye diameter (ED) (r= 0.978) and head depth (HD) (r= 0.975) to head length, were the most highly correlated parameters with p< 0.01 level of significance and least correlated parameter was the post- orbital distance (post OD) (r= 0.943) in Seer stream population, comparable to Pong reservoir samples with most highly correlated body parameters as HD (r= 0.986) and IOD (r= 0.985) and least correlated parameter po OD (r= 0.945) (Table3). However, all the characters were showing correlation with head length and almost all the characters followed linear relationship for natural and artificial stocks of *T.putitora* populations. The results showed also that no significant differences were detected in the morphometric indices between the Seer stream stock and Pong reservoir stock (Table 3).

The values of coefficient of correlation have been found to be highly significant at p< 0.01 for all the morphometric characters from all the sampling sites (natural and reservoir), however, in proportion to head length; all the values have high correlation coefficient (r= 0.96) in natural stock except post OD (r= 0.94) comparable to Pong reservoir samples all the values have shown high correlation coefficient (r= 0.95) except post OD (r= 0.94). From these observations it is evident that most of the characters included in present study increase in direct proportion to each other.

Moreover, the results of meristic counts showed that dorsal fin rays (iv-8), pectoral fin rays (i-14), pelvic fin rays (i-8), anal fin rays (iii-5), caudal fin rays (17-19), lateral line scales (25-27), scales above the lateral line (4½) and scales below lateral line (2½-3½) remained constant in both groups of fish having different body length (Table 4). It means that in both groups of fish having different body length, meristic counts are independent of body size and there is no change in meristic counts with increase in body length (Talwar and Jhingran, 1992; Vladykov, 1934).

Table 4: Means and standard deviation of quantitative phenotype traits based on meristic counts used for differentiation analysis between two stocks of *Tor putitora* population.

S.No.	Parameters	Natural Stock (Seer Stream)		Reservoir Stock (Pong Dam)		t-test
		MEAN	SD	MEAN	SD	
1	Lateral line scale count	25.66	0.479	26.5	0.508	*
2	Scales above lateral line	4½	0.00	4½	0.00	NS
3	Scales below lateral line	2½	0.00	3½	0.00	*
4	Dorsal fin ray count	4’8”	0.00	4’8”	0.00	NS
5	Pectoral fin ray count	1’14”	0.00	1’14”	0.00	NS
6	Pelvic fin ray count	1’8”	0.00	1’8”	0.00	NS
7	Anal fin ray count	3’5”	0.00	3’5”	0.00	NS
8	Caudal fin ray count	17	0.00	19	0.00	*

t-test: * P<0.05 NS: not significant.

Genotype analysis

All the ten different primers used in this study produced different RAPD band patterns (Table 5). The number of amplified bands detected varied, depending on the primers and samples of different water system; in addition to ensure that the amplified DNA bands originated from genomic DNA and not from primer artifacts.

Also, negative control was done for each primer/species combination. No amplification was detected in the control reactions. All amplification products were found to be reproducible when reactions were repeated using the same reaction conditions (Table 5 and Figure 1). The RAPD fingerprint was used for the detection of the genetic diversity between the reservoir and natural stocks of *Tor putitora* population. The results showed moderate genetic dissimilarity range (0.00 to 1.00) with an average of 0.211 using different random primers (Table 5 and Figure 2).

Table 5. Total number of band, polymorphic bands and genetic dissimilarity between natural and reservoir stock of *Tor putitora* population using different random sequence of primers.

Primer number	Total band	Polymorphic band	Genetic dissimilarity
1	14.00	3.00	0.21
2	16.00	6.00	0.37
3	12.00	2.00	0.16
4	16.00	2.00	0.12
5	15.00	6.00	0.40
6	19.00	3.00	0.15
7	16.00	0.00	0.00
8	14.00	3.00	0.21
9	9.00	3.00	0.33
10	15.00	2.00	0.13
Average	-	-	**0.211**

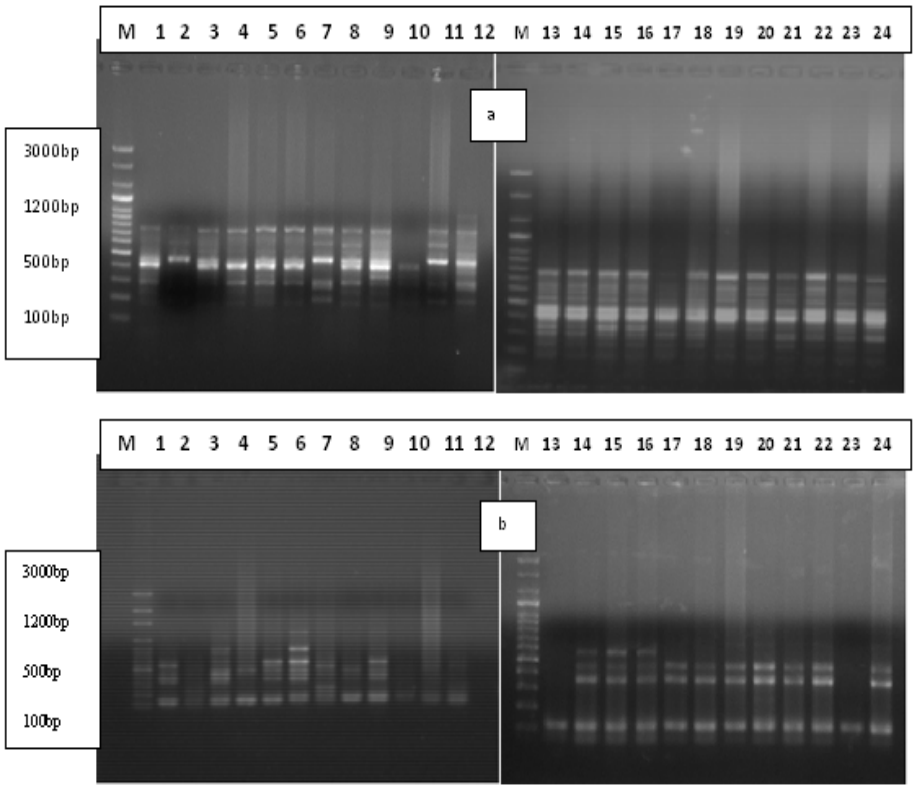

Figure1. Example of RAPD amplification products with primer1 & 2 fig.a &b respectively. Lane M: 100 bp DNA marker, lanes 1-12 (natural stock) and lanes 13-24 (reservoir stock).

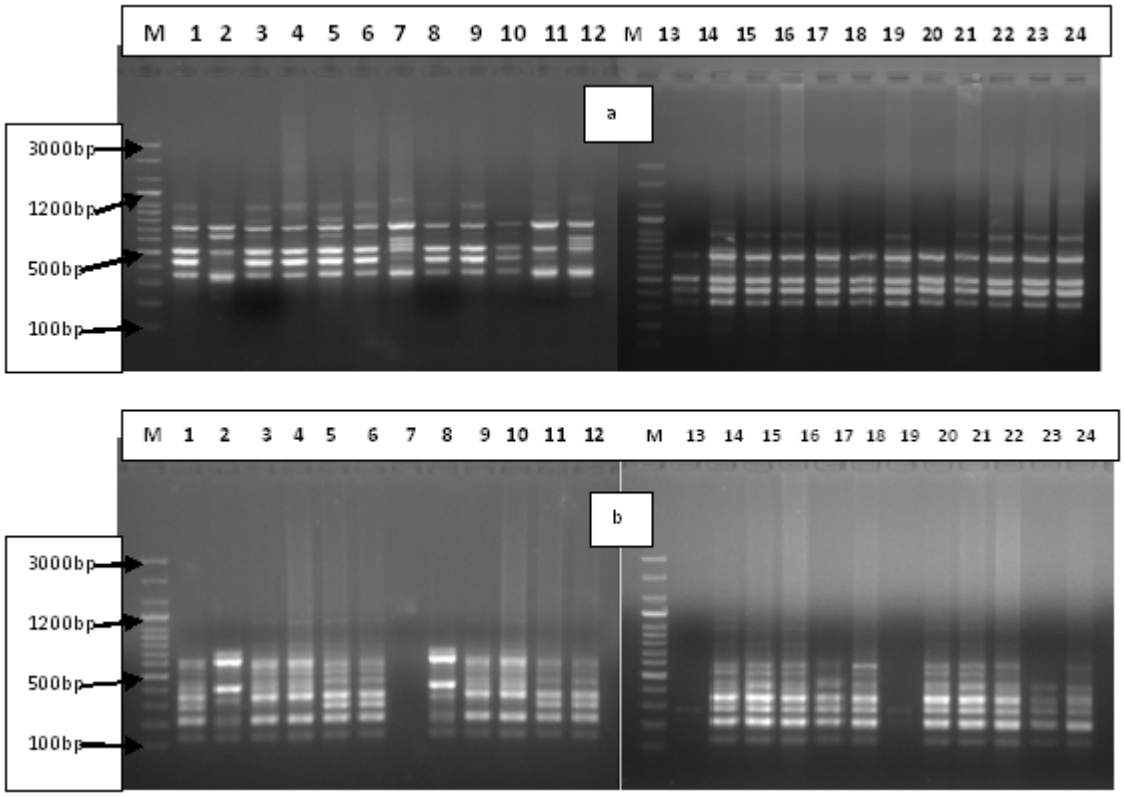

Figure2. Example of RAPD amplification products with primer5 & 10, fig.a &b respectively. Lane M: 100 bp DNA marker, lanes 1-12 (natural stock) and lanes 13-24 (reservoir stock).

DISCUSSION

Morphometric characters and meristic counts have been the most widely used tool in racial studies in fish taxonomy [4]. Meristic counts were much easier to evaluate and seem to be advantageous because most counts can be collected from live fish. However, meristic data alone may not provide the detail necessary to discern dissimilarities between different populations at the same species and sometimes at the same genus [40]. Various morphometric characters are categorized on the basis of range difference into genetically (<10%), intermediate (10-15%) and environmentally (>15%) controlled characters [30,], categorized morphometric and meristic characters as follows:

1. Genetically controlled characters; like number of caudal and ventral fin rays which are not subjected to environmental modifications.
2. Intermediate characters; which appear to be slightly modified by environment such as pectoral fin rays and gill rackers on the first brachial arch.
3. Environmentally controlled characters; strongly modified by environment, includes morphological characters, metamerism, and number of vertebrate, dorsal and anal fin rays, color bars, color spots and size of the fish.

In general, characters of the first category show minimum range of variation as compared to second and the third categories exhibiting moderate and maximum range of variation respectively. The present investigation also show that various morphometric characters in golden mahseer fall under the Vladykov's categories. During the present investigation, among Seer stream population 16 characters such as SL, pr DD, po DD, LDF, DDF, LAF, DAF, pr AD, LPF, LpF, minBW, maxBW, dist.pec.and pel., dist. pel.and anal, LCF and LCP in the percentage of total length have found to be genetically controlled, where as none of the character in percentage of total length included in the environmentally controlled and intermediate category.

Other two characters like post OD and ED categorized as genetically controlled and 3 characters like HD, pr OD and IOD are included in environmentally controlled category in percentage of head length (Table3), comparable to Pong reservoir samples of which, 14 characters like SL, pr DD, LDF, DDF, LAF, DAF, LPF, LpF, minBW, maxBW, dist.pec.& pel., dist.pel.&anal, LCF and LCP were genetically controlled, one character like po DD as intermediate and one character pr AD as environmentally controlled in relation to total length while other one character like ED as genetically controlled, two characters like HD and po OD as intermediate and two characters like pr OD and IOD as environmentally controlled in relation to head length(Table 3). In a similar study, Johal *et al* (1994) reported 13 characters in relation to total length to be genetically controlled in *Tor putitora* from Gobind Sagar reservoir in Himachal Pradesh. Vladykov maintains that in the fish species showing restricted distribution, majorly of morphometric characters show narrow range and are genetically controlled. On the contrary, in species which have wide range of geographical distribution, most of the characters are strongly influenced by the environment. *Tor putitora* have a restricted zoogeographical distribution, because of majority of their morphometric characters show narrow range differences from both the collection sites. In a similar study [39], have studied the variation in some morphometric characters of sympatric hill stream teleost *Barillius bendelensis* and *Barillius vagra* from khanda, a lesser Himalayan stream and revealed that among the 22 variables selected for the study, 12 were found to be genetic, 4 were intermediate and 6 were environmental. Among the oriental fishes showing similar distribution, [49] studied *Cirrhinus neba, Tor putitora* from Gobind Sagar reservoir and studied *Tor putitora* from foothill section of the Ganga. In *C. reba* 14 out of 19 and *G. chapra*, 13 out of 18 morphometric characters exhibited wide range differences and were hence environmentally controlled while in *T. putitora*, 9 were environmentally controlled characters (Tondon *et al*., 1993a, b). On contrary, in *T. putitora* restricted to Himalayan foothills, 13 out of 27 characters were genetically controlled, showing narrow range difference, 12 out of 20 were genetically controlled while 3 were environmentally controlled in the population of *T. putitora* inhabiting the foothill section of river Ganga [30], studied the population of *T. putitora* at Ramsar site of Pong dam reservoir in Himachal Pradesh, India and found that 12 out of 22 morphometric characters were genetically controlled, while 5 were environmentally controlled. These observations support the hypothesis of Vladykov, that fishes with restricted distribution have greater numbers of genetic characters, as is also evident in present investigation. High degrees of correlation ($r > 0.91$) with less than one percent level of significance ($p < 0.01$), indicates that morphometric characters increase in direct proportion to each other in relation to total length and head length (Table3) among all the two collection sites from Himachal Pradesh.[51] Uniyal, recorded high coefficient of correlation ($r = 0.9694$ to 0.9998) in a morphometric and meristic analysis in the population of *Tor chilinoides* in the river Nayar of Garhwal, Central Himalaya. The present study also revealed that no significant difference at $p < 0.05$ level of significance was observed between both the stocks of *T. putitora* population from Himachal Pradesh, India. Thence, concluded that all these stocks are homogenous and *Tor putitora* could be restocked from or to these water bodies for its conservation in near future to increase their number as is considered as endangered (FAO, 2008). The main advantages of RAPD markers are the possibility of working with anonymous DNA and the relatively low expense, also fast and simple to produce RAPD marker [20, 19, 2]. Moreover, RAPD analysis might be useful for systematic investigation at the level of species and subspecies [7], and more sensitive and technically easier to perform and produced results with low statistical error, whereas DNA fingerprinting detected greater genetic differentiation between Nile tilapia strains than other molecular techniques such as multilocus minisatellite marker [38]. A higher level of intra-species similarity index and lower proportion of polymorphic loci in *T. putitora* population reflect a relatively lower level of genetic variability within the various stocks available in the state. This may be attributed to the maintenance of a limited number of individuals introduced in Himachal Pradesh and their repeated propagation over a long period due to interruptions created on their migratory routes. The intraspecific similarity indices in hatchery population was found to be higher than those of wild populations of a particular species [28, 8, 25]. The different stocks of *T. putitora* are more similar to each other phenetically as well as genetically, thence we can also conclude that the variable stocks are sharing the similar parental gene pool and the natural stocks could be restocked by culture practices at various reservoirs and lakes of Himachal Pradesh under the in-situ conservation strategy for this legendary sport fish of the country. RAPD markers have been proved as effective tools to monitor the genetic variation in different organisms. Using only ten primers and 24 samples, the present study revealed a remarkable level of intraspecific genetic variability in *T.putitora*, though it is considered as an endangered species in India. A more definite conclusion, however, may be reached with larger samples including other rivers (if possible to collect) with faster evolving molecular markers such as microsatellite loci.

The level of genetic variation provides the raw material for the selective improvement of a stock for sustainable aquaculture production. The major limitation of morphological characters at the intra-specific level is that phenotypic variation is not directly under genetic control but subjected to environmental modification [14]. Therefore, it can be use either phenotype analysis based on a large number of morphometric character indices and meristic counts or genotype analysis based on RAPD fingerprinting to discriminate the various stocks of *Tor putitora* population available with the same results.

By this study, we have revealed, for the first time, the intraspecific genetic variability between the two stocks of *Tor putitora* population in Himachal Pradesh. The results of the present study can be used for further study involving this endangered species in the country.

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