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Single Genetic Stock Revealed by Microsatellite Markers Among Wild Populations of *Cirrhinus mrigala* from Peninsular India

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Abstract

Cirrhinus mrigala (mrigal) is one of the Indian major carps widely cultured in the whole Indian subcontinent. Population genetic structure of this species from Peninsular Rivers of India is lacking. Among DNA markers, microsatellites are excellent tools to evaluate genetic variation of populations. In this study, genetic variation of six peninsular riverine mrigal populations was evaluated using seventeen microsatellite loci. In analyzing 288 samples, the number of alleles ranged from 4 to 27; observed heterozygosity from 0.595 to 1.00, expected heterozygosity from 0.586 to 0.959 and inbreeding coefficient (F_{IS}) ranged from -0.034 to 0.02. Exact test for Hardy Weinberg disequilibrium revealed that one locus was not in equilibrium across the rivers except one. The AMOVA analysis revealed the main source of genetic variation to be within the population (94.54%) than among the populations (5.46%). The Nei's genetic distance and structure analysis depict river Narmada and Mahi populations are different from the four east coast rivers. The overall Fst (0.05462) data showed moderate differentiation among the six populations. The results of this study provide essential information to resource recovery and help in delineating populations for fishery management. Besides, the data will provide a valuable baseline for further investigations on the geographic distribution of this commercially important fish species.

Introduction

The Indian carps are the most widely cultivated species throughout the peninsular region. Mrigal (*Cirrhinus mrigala*), a carp endemic to Indo-Gangetic riverine systems, is one of the three Indian major carp species cultivated widely in Southeast Asian countries. The species was transplanted in the peninsular riverine systems of India, where it has established itself. Subsequently, it has spread over whole of India. According to FAO (2005-2020) contribution of aquaculture sector is one third of the Indian's total fish production i.e, 9.06 million tonnes during 2012-2013. The total aquaculture production was valued at US$ 3.5 billion of which carp alone was responsible for as much as 4.18 million tonnes. Whereas, aquaculture production of only mrigal carp in 2009 was 497,493 tonnes that translates to approximately 0.9% of global aquaculture production, stated by FAO (2012). Wild capture fishery of mrigal is, however, exhibiting a declining trend. Comparison of capture survey data from 1958-1994 indicates an 87% decline in mrigal fishery in the Indian rivers (Payne *et al*., 2004). In global context India and Bangladesh both belongs to the major producer category of mrigal.

The lack of knowledge about the genetic structure of mrigal populations may result in the differential harvest of the populations that will ultimately have a

drastic and long-term effect. To overcome this, there is always a need for investigations encompassing the genetic variations at the intra and inter-population levels as well as at the intra and inter- specific levels of the fish and shellfish resources of any nation (Allendorf and Utter, 1979). With the depletion of mrigal fishery and rapid increase in hatchery production, it is necessary to understand the genetic composition to evaluate the genetic structure and intermixing of gene pool.

A microsatellite, also known as simple sequence repeat (SSR), consists of multiple copies of tandemly arranged repeats ranging in size from one to six base pairs. Microsatellites are the most versatile ones among molecular markers and are co-dominant in nature (Tautz, 1989). Although identifying microsatellite in a species of interest can be time consuming but the techniques employed are well established and reliable. Microsatellite markers have proven to be an exceptional indicator of genetic variation within and between populations of many fishery animals, including invertebrates (Aranishi and Okimoto, 2005; An *et al*., 2013a; Kim *et al*., 2013). The principal drawbacks of this marker are requirement of species specific primers, a relatively large number of microsatellites might be necessary to achieve sufficient statistical power, which can be labor intensive and expensive (Olafsson *et al*., 2010) and to overcome this problem cross species amplification technique is being used for achieving cost effectiveness. The success rate of cross-species microsatellite amplification is directly related to evolutionary and relatedness of loci isolated and the species to which the heterologous loci are being applied (Primmer CR *et al*., 1996). A few pairs of highly polymorphic microsatellites can be enough if the objective is to address genetic diversity related questions. Only few studies have characterized the population genetic structure of mrigal fishery; one is through microsatellite and allozyme (Chauhan *et al*., 2007) but from Himalayan region and others are mitochondrial *Cyt b* gene, ATPase-6 gene and truss morphometrics (Das *et al*., 2014. 2018 and Behera *et al*., 2015). The microsatellite markers exhibited a common gene pool in mrigal population from Himalayan region where as mitochondrial marker analysis showed a low level of genetic variation with a single genetic stock throughout the peninsular region. Therefore, to protect the mrigal population and to promote its sustainable exploitation, the present status of its genetic diversity and the relatedness of populations throughout its geographic range must be determined using molecular genetic analysis.

In the present study, we investigated the genetic diversity within and between six mrigal populations along with the genetic structure of this species in peninsular India using 17 microsatellite loci. This genetic information will be useful for resource management and conservation of this economically important fish species.

Material and Methods

Sample Collection and DNA Extraction

This study examined 288 wild *Cirrhinus mrigala* from peninsular riverine systems of India. We have collected samples from six riverine sources i.e. Mahanadi (Cuttack), Godavari (Rajahmundry), Krishna (Vijaywada), Kaveri (Bangalore), Narmada (Varuch) and Mahi (Aanand) (Table-1). The live fish samples were caught by gill net or cast net and morphological identification of species was done based on Talwar and Jhingran (1991). Fin clipping was done from each individual fish, preserved in 95% ethanol and stored frozen at -20°C until DNA extraction. Total DNA was isolated from fin tissues by Proteinase K digestion followed by standard Phenol-Chloroform method by Sambrook and Russel, 2001. The DNA samples were subsequently dissolved in 1X TE buffer. The purity and concentration of the DNA samples were estimated in 260/280nm wavelength by a Spectrophotometer.

Isolation of Microsatellite Markers

A set of polymorphic microsatellite markers for this species was developed by cross amplifying highly informative rohu repeat sequences already developed in our laboratory earlier (Das *et al*., 2005; Patel *et al*., 2009; Sahu *et al*., 2012). Out of 150 rohu microsatellite loci, seventeen microsatellite loci were chosen based on specific amplification in mrigal from screening of more than 37 rohu loci. This primer panel of 17 was fluorescence labeled (either FAM or HEX) and used for population genetic studies (Table - 2).

Cross-species Amplification

One hundred and fifty rohu microsatellite loci developed earlier as mentioned above were selected for cross amplification and polymorphism screening in a set

Table 1. Geographical co-ordinate of locations along with sample sizes and year of sampling.

Rivers	Sampling sites	n	Year of sampling
Mahanadi	Cuttack (20.27ºN85.52ºE)	48	2009
Godavari	Rajahmundry (16.59ºN81.47ºE)	48	2010
Krishna	Vijayawada (16.31ºN80.37ºE)	48	2011
Kaveri	Mysore (12.18ºN76.38ºE)	48	2011
Narmada	Varuch (21.7ºN72.97ºE)	48	2012
Mahi	Anand (22.57ºN72.93ºE)	48	2012

Table 2. List of microsatellite loci amplified in wild population of *C. mrigala.*

Locus name	Forward and Reverse Sequence (5' - 3')	Poduct size	Accession no.	Primer Label	Motif
Lr-206	F- GAAGTGTTTGTGTGGCTGGA R- CCGGTGGGATCTGTGTATG	182	JN581273	Fam	(TG)25
Lr-298	F- TGGTCCCATAACGTGATGAAT R- GAAAACAGGCATCTGAACACAA	246	JN581361	Fam	(AC)17
Lr-36	F- AGC GTG TCT GAT GTG TGA AAG G R- TCA GAT GCC TCC TGC ATT CTG	181	AM269526	Hex	$(CA)_{10}$
Lr-162	F- GCGAAACAGCAGCAACACT R- AAGAGAAGGCTTCACCTGGA	209	JN581239	Fam	(AC)34
Lr-485	F- TCTACAGCACACTGACCCTG R- AGTAACCGACAATCTGTGGC	170	JQ862222	Fam	(TC)12
Lr-231a	F- CTATCGTTTGCTTGTTCGTTTG R- GATGGACGAATCGACAGATAGA	202	JN581295	Hex	(ATCT)6
Lr-237	F- CAGCAGGACTGAAGAAATGTATG R- TGTAGGGCGATAGAAAGTACGG	199	JN581335	Fam	(CA)10
Lr-545	F- ACTCCTCCTGACACCTTGAG R- ACGAACAGTGCAAAGACGTG	236	JQ862282	Hex	(TGA)9
Lr-251a	F- GAGGTCAGTTGGTCAGAGTTCA R- ACCCTTTCACACCCCTCTTATT	193	JN581315	Fam	(CA)15
Lr-547	F- CTGTAGATGCTGGTCTGGGG R- ACCTTACATTGGCTGTTGGTC	238	JQ862284	Hex	(CAT)10
Lr-432	F- GGTTGGGATAGGGGACAGAC R- GCTCCCCAGAATGCCTAAAC	138	JQ862170	Hex	(GT)13
Lr-158	F- CAGCAAACACACTCCTGTCAAA R- GACGCGCAAATCAAAGTGAG	274	JN581271	Fam	(AC)15
Lro-32	F- ACC CTC TTT GTT TTG GCT CTC R- TCT CTT ACC CTG TTT CTC TGT	136	AM184148	Hex	(GT)17
Lr-51a	F- CCCATAACGTGATGAATACC R- AGGCATCTGAACACAAGAACAC	172	JN581361	Fam	(CA)9
Lr-189	F- GATCAGAGCAATATTGGGGTTT R- AGAAGCTGCTGTGTGCAGAA	219	JN581257	Fam	(CA)13
Lro-41	F- GACTTCAGCTTCTCCACTCAA R- CTGCGTTTAACCAATCACAA	144	AM184156	Hex	(CTT)2 (GT)8 (CT)7
Lr-63	F- CTTGGAATCCCCTCAATTAGC R- ACTGATAGGGAGACAGAAAG	154	JN581148	Fam	(TC)9

of 3-5 individuals initially from six different populations. PCR was performed in a 10 µl reaction volume containing 5 ng genomic DNA, 2.5 pmol of each forward and reverse primers, 200 µM of each *d*NTP, 1.5 mM $MgCl_2$ and 0.25 units *Taq* DNA polymerase (Bangalore Genei). Amplification was carried out with the touchdown PCR profile on a GeneAmp 9700 thermal cycler (Applied Biosystem, USA) with the following temperature setting for all primers: Initial denaturation of 94°C for 5 min (1 cycle) followed by 45 s at 94°C (denaturation), 45 s annealing at required temperature and 1 min extension at 72°C for each cycle. Annealing temperature started from 60°C to 55°C in successive 1°C difference in each step. Two cycles of amplification were set for each annealing temperature but 25 rounds of amplification were performed at final annealing temperature making the total number of cycles 35. The PCR products were then checked for amplification and informativeness in 3% agarose gel electrophoresis.

Multiplex PCR Optimization and Genotyping

PCR reactions for 17 fluorescence labeled primers were carried out in 10 µl reaction volumes containing 1x *Taq* DNA polymerase reaction buffer, 5 pmol of each primer, 200 µM dNTPs, 0.25 units of T*aq* DNA polymerase and 20 ng genomic DNA as template. All 17 loci were grouped into 6 multiplex panels considering their product size (Table - 2). Amplification was performed in a GeneAmp 9700 Thermocycler (Applied Biosystems, USA) programmed for initial denaturation of 4 min at 94°C followed by 30 cycles of 45s at 94°C, 1 min at annealing temperature of corresponding primer and 2 min at 72°C, and finally a 7 min extension at 72°C. PCR amplification was carried out following Das *et al.*, (2005). PCR products were 150 times diluted before taking 1µl each to be mixed with 11µl of Hi-Di formamide and 0.1µl of GeneScan™-500 LIZ™ as internal size standard (Applied Biosystems, USA). The mixtures were denatured at 95º C for 5 min and loaded in 310 Genetic Analyzer (Applied Biosystems, USA).

Data Analysis

All the samples from six wild populations of *C. mrigala* were genotyped using the microsatellite marker panels. Fragment size was measured according to the GeneScan™-500 LIZ™ size standards (ABI) using GeneMapper v.3.7 software (Applied Biosystems, USA). General diversity measures of mrigal population such as

number of alleles, observed heterozygosity (HO), expected heterozygosity (HE) and probability test for Hardy-Weinberg Equilibrium (HWE) were studied using GDA software (Lewis and Zaykin 2000). Pairwise linkage disequilibrium (LD) among loci was tested using allele frequency for loci that were in HWE and genotypic frequencies were used to calculate LD of loci that deviated from HWE to prevent interference from within-locus disequilibrium. For null alleles and scoring errors MICRO-CHECKER program (van Oosterhout *et al*., 2004) was used to check microsatellite data. Analysis of molecular variance to measure genetic variation within and among populations, genetic differentiation and pairwise Fst values were calculated using ARLEQUIN version 3.11 (Excoffier *et al*., 2005). STRUCTURE version 2.3 (Pritchard *et al*., 2000; Falush *et al*., 2003, 2007) was used to predict the number of clusters (K) by using Markov chain Monte Carlo methods (MCMC) to estimate allele frequencies and the probability of individual assignment to each cluster. The parameter sets assumed were admixture allele models with correlated allele frequencies and with no prior population location information. The number of clusters was set from K = 1 to 10 with four simulations for each K. Each run consisted of a burn-in period of 50,000 steps followed by 100,000 MCMC replicates.

Results

Marker Development

Development of repeat markers, particularly microsatellites, from a species of interest is a tedious process of constructing partial genomic library involving restriction enzyme digestion, cloning, colony hybridization, sequencing of positive clones and finally primer designing from flanking regions. Though the Next Generation and high throughput sequencing technologies are available now to sequence a portion of a genome and search for microsatellite markers, in either case it is costly and time consuming. However, a primer designed from one species may work well in closely related species through cross species amplification. Successful cross amplification of microsatellite loci has been reported in catla (Mohindra *et al*., 2001), rohu (Das *et al*., 2005), Indian snow trout (Barat *et al*., 2011), mahseer species (Esa *et al*., 2008). In the present study, rohu microsatellite loci have been used in mrigal. Rohu, catla and mrigal being closely related to each other offer the possibility to take advantage of cross species amplification. Cross species amplification of 150 polymorphic rohu loci in mrigal resulted in good amplification at 37 loci. Polymorphism check of these on 3% agarose gel resulted in seventeen loci that were polymorphic in mrigal. The results indicated that about 25% of the rohu microsatellite markers can be cross amplified which can be utilized for different studies in mrigal.

Genetic Variation

High throughput multiplex genotyping of these loci showed polymorphism in all the 6 populations. All the mrigal populations were genetically diverse, with high allelic richness. Number of alleles per locus ranged from 4 to 27, observed heterozygosity from 0.595 to 1.00, expected heterozygosity from 0.586 to 0.959 and inbreeding coefficient (F_{IS}) ranged from -0.034 to 0.02. The average number of alleles, observed heterozygosity (Ho), expected heterozygosity (He) and p value for HWE per locus per population are presented in Table-3. Observed heterozygosity values were found to be relatively higher and within the range (0.05–0.07).

Exact tests (3,200 iterations) for Hardy Weinberg equilibrium (HWE) showed Lr-36 in all population except Mahanadi and Lr-231a in all population except in Kaveri followed by Lro-32 in Mahanadi, Godavari and Kaveri, Lr-251a in Mahanadi, Godavari and Krishna, Lr-545 in Mahanadi, Godavari and Kaveri, Lr-485 in Godavari and Narmada, Lr-237 in Mahanadi and Godavari, Lro-41 in Godavari and Kaveri, Lr-206 in Kaveri, Lr-432, Lr-63 and Lr-51a in Godavari and Lr-162, Lr-547 and Lr-158 in Mahanadi population to be significantly deviating from HWE at 5% significance level (Table - 3).

Population Genetic Differentiation

AMOVA analysis showed that out of total variation, only 5.46% was due to among population and 94.54% was due to within population differences (Table - 4). The pairwise F_{ST} values ranged from -0.00284 (Krishna and Kaveri) to 0.14863 (Kaveri and Mahi) (Table - 5). All the pair-wise comparisons except Krishna-Kaveri and Narmada-Mahi showed significant genetic differentiation among population. The UPGMA dendrogram based on Nei's (1972) genetic distance showed three clusters (Fig. 1). The Mahanadi and Godavari populations were in one cluster, Krishna and Kaveri were in one and Narmada and Mahi populations were in the other cluster. The clustering result is pertinent to their geographic distribution. To investigate the population structure at the regional level, we applied a Bayesian model-based clustering algorithm implemented in the STRUCTURE program, which estimates the shared population ancestry of individuals purely on the basis of genetic data without considering population location information. The whole dataset provided a best fit model of cluster determination detected three clusters at ΔK=5, suggesting that the 288 individuals partitioned into three clusters (Fig. 2). STRUCTURE analysis separated samples with respect to their geographical distribution and the result is similar to the dendrogram obtained from the genetic distance.

Discussions

Genetic diversity is the key feature for any trait improvement whereas; genetic structure analysis is a

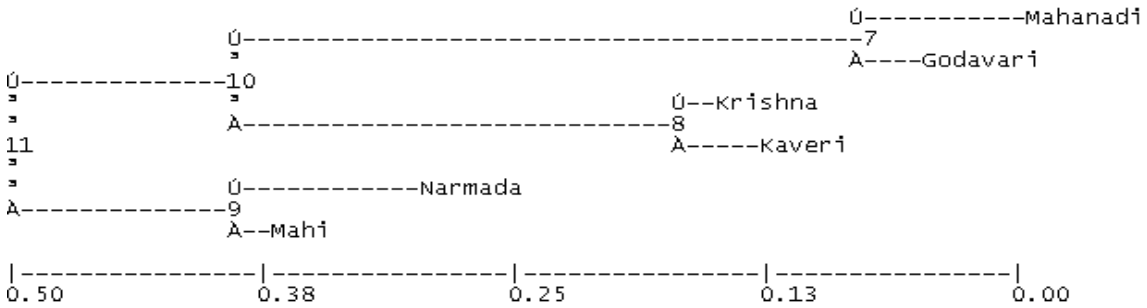

Figure 1. Dendrogram based Nei's genetic distance summarizing variations among 6 populations of *C. mrigala* using microsatellite markers.

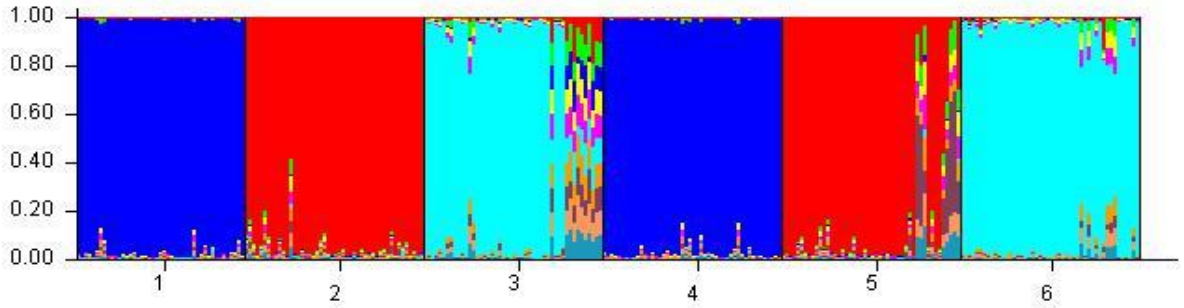

Figure 2. Genetic Structure visualization for the Bayesian population assignment test for 6 populations of *C. mrigala* using 17 microsatellite markers.

Created with STRUCTURE for K = 5; each population represented as a single, vertical bar (1= Mahanadi population, 2= Krishna population, 3= Narmada, 4= Godavari, 5= Kaveri & 6= Mahi Populations), is partitioned into K colored segments. 288 individuals clustered into three different genetic groups. The proportions of the colour bars represent the admixtures in the varieties. The *Y*-axis shows the estimated ancestry of each genotype from a particular group

raw material for genetic variability, population differentiation with respect to evolution and geographical range of stocks. In the present investigation, genetic diversity study as well as population structure of six wild riverine stocks of mrigal were studied.

Number of alleles per locus ranged from 4 to 27, observed heterozygosity from 0.595 to 1.00, expected heterozygosity from 0.586 to 0.959 and inbreeding coefficient (F_{IS}) ranged from -0.034 to 0.02. For each population, expected heterozygosity (He) was generally lower than the observed heterozygosity (Ho) leading to negative inbreeding coefficients (Fis). Several factors such as wide geographical distribution, migration and mutation etc. are responsible for maintaining genetic variability within a species. Observed heterozygosity values were also relatively high and within the range (0.05–0.07) reported for teleost fish species described by Nevo (1978). As reported for several vertebrates (Nevo *et al*., 1984) and plants (Frankham, 1996), populations of widespread species often show significantly higher heterozygosity estimates than for populations of species with more restricted distribution.

No significant (P>0.05) linkage disequilibrium was detected between different genotypes at each of the different microsatellite loci. Particularly with microsatellites, one possible explanation for these observations may be the presence of null alleles that do not amplify allowing heterozygotes difficult to distinguish (Van Oosterhout *et al*., 2004). But, the analysis of data using Micro-Checker did not give any evidence for null allele homozygotes in mrigal populations.

It is elsewhere suggested that if the Fst value between a pair is less than 0.05, the populations are expected to be one. Considering the range of significant Fst values in the present study, it is apparent that 4 populations (Mahanadi, Godavari, Krishna and Kaveri) were having low genetic differentiation (Fst ranging from 0.00281 to 0.01362, though statistically significant) indicating common ancestry in the prehistoric period and possible gene flow among these populations. This also holds good for Krishna-Kaveri and Narmada-Mahi suggesting possible connectivity and gene flow among them. Similar findings were reported in wild common carp (Li *et al*., 2007) and grass carp populations (Liu *et al*., 2009) in China using microsatellites. While studying mrigal from rivers belonging to Indus, Ganges and Brahmaputra basins using allozymes and microsatellites, Chauhan *et al*. (2007) reported low genetic differentiation. Also, studies using mitochondrial DNA of mrigal showed concordant results (Das *et al*., 2014,

Table 3. No. of individuals (n), no. of alleles (A), alleles per locus (Ap), expected heterozygosity (He), observed heterozygosity (Ho), Fis value (f) and p value for testing Hardy–Weinberg equilibrium in six populations of *C.mrigala*.

Locus Name	Parameters	Mahanadi	Godavari	Krishna	Kaveri	Narmada	Mahi
Lr-206	n	48	48	48	48	48	48
	A	6	12	12	6	9	8
	A_p	6	12	12	6	9	8
	H_e	0.787	0.794	0.799	0.732	0.773	0.817
	H_o	1.000	1.000	0.976	0.958	1.000	0.928
	f	-0.274	-0.262	-0.224	-0.312	-0.297	-0.138
	P	0.098750	0.497188	0.156562	0.014687	0.419687	0.390000
Lr298	n	48	48	48	48	48	48
	A	8	7	11	11	12	12
	A_p	8	7	11	11	12	12
	H_e	0.808	0.748	0.876	0.849	0.884	0.819
	H_o	1.000	1.000	0.906	0.976	1.000	1.000
	f	-0.241	-0.340	-0.034	-0.151	-0.133	-0.223
	P	0.308125	0.226250	0.267813	0.132812	0.397813	0.621250
Lr36	n	48	48	48	48	48	48
	A	7	4	4	4	4	4
	A_p	7	4	4	4	4	4
	H_e	0.633	0.637	0.643	0.603	0.586	0.680
	H_o	1.00	1.00	0.818	0.833	0.918	1.00
	f	-0.589	-0.578	-0.276	-0.385	-0.580	-0.476
	P	0.056875	0.000000	0.030625	0.000313	0.013750	0.000000
Lr162	n	48	48	48	48	48	48
	A	5	8	5	5	9	8
	A_p	5	8	5	5	9	8
	H_e	0.728	0.825	0.607	0.648	0.801	0.764
	H_o	1.00	1.00	0.673	0.791	0.944	0.854
	f	-0.378	-0.214	-0.109	-0.223	-0.181	-0.118
	P	0.018750	0.107188	0.689375	0.308437	0.232500	0.219688
Lr231a	n	48	48	48	48	48	48
	A	8	9	10	10	10	19
	A_p	8	9	10	10	10	19
	H_e	0.770	0.837	0.797	0.782	0.820	0.860
	H_o	1.00	1.00	0.974	1.00	1.00	0.937
	f	-0.302	-0.197	-0.225	-0.281	-0.223	-0.091
	P	0.001250	0.000625	0.013437	0.253125	0.006875	0.001250
Lr485	n	48	48	48	48	48	48
	A	6	7	11	10	5	10
	A_p	6	7	11	10	5	10
	H_e	0.750	0.733	0.767	0.811	0.721	0.728
	H_o	1.00	1.00	0.872	1.00	1.00	1.00
	f	-0.337	-0.368	-0.138	-0.235	-0.394	-0.377
	P	0.081562	0.000000	0.820000	0.264062	0.001250	0.367812
Lr237	n	48	48	48	48	48	48
	A	5	6	10	10	11	11
	A_p	5	6	10	10	11	11
	H_e	0.791	0.817	0.827	0.797	0.796	0.832
	H_o	1.00	1.00	1.00	1.00	1.00	1.00
	f	-0.267	-0.225	-0.211	-0.257	-0.259	-0.204
	P	0.047500	0.001563	0.563125	0.364063	0.662500	0.079062
Lr545	n	48	48	48	48	48	48
	A	6	8	5	5	8	8
	A_p	6	8	5	5	8	8
	H_e	0.655	0.660	0.666	0.692	0.657	0.670
	H_o	1.00	1.00	0.840	1.00	0.944	0.979
	f	-0.535	-0.522	-0.264	-0.449	-0.445	-0.467
	P	0.000000	0.044062	0.279062	0.001250	0.192188	0.153750
Lr251a	n	48	48	48	48	48	48
	A	7	6	10	6	8	9
	A_p	7	6	10	6	8	9
	H_e	0.734	0.714	0.690	0.659	0.747	0.739
	H_o	0.955	1.00	0.844	0.812	0.953	0.978
	f	-0.304	-0.404	-0.225	-0.235	-0.279	-0.327
	P	0.025625	0.002188	0.026875	0.447500	0.214688	0.246250
Lr432	n	48	48	48	48	48	48
	A	10	6	15	12	11	12

***Table 3** continued*

Locus Name	Parameters	Mahanadi	Godavari	Krishna	Kaveri	Narmada	Mahi
	A_p	10	6	15	12	11	12
	H_e	0.717	0.770	0.845	0.852	0.862	0.827
	H_o	1.00	1.00	0.971	1.00	1.00	1.00
	f	-0.400	-0.303	-0.151	-0.175	-0.161	-0.211
	P	0.085000	0.015938	0.104063	0.246562	0.710625	0.503750
Lr547	n	48	48	48	48	48	48
	A	8	9	5	9	8	11
	A_p	8	9	5	9	8	11
	H_e	0.688	0.677	0.641	0.667	0.725	0.747
	H_o	0.825	0.851	0.864	0.895	0.941	0.869
	f	-0.201	-0.260	-0.355	-0.345	-0.303	-0.165
	P	0.005313	0.450313	0.112500	0.271250	0.506563	0.100625
Lr158	n	48	48	48	48	48	48
	A	27	22	18	22	22	22
	A_p	27	22	18	22	22	22
	H_e	0.959	0.937	0.936	0.931	0.926	0.922
	H_o	1.00	1.00	1.00	1.00	1.00	1.00
	f	-0.042	-0.067	-0.069	-0.074	-0.080	-0.085
	P	0.035625	0.156250	0.991250	0.586875	0.724063	0.556562
Lr51a	n	48	48	48	48	48	48
	A	9	8	17	11	10	11
	A_p	9	8	17	11	10	11
	H_e	0.789	0.836	0.768	0.728	0.837	0.829
	H_o	0.928	1.000	0.863	0.953	0.931	1.000
	f	-0.178	-0.198	-0.124	-0.314	-0.114	-0.207
	P	0.112187	0.034063	0.053125	0.670000	0.545625	0.338438
Lro32	n	48	48	48	48	48	48
	A	5	5	5	4	6	5
	A_p	5	5	5	4	6	5
	H_e	0.717	0.639	0.629	0.625	0.740	0.716
	H_o	0.888	0.729	0.871	0.790	0.733	0.785
	f	-0.242	-0.141	-0.391	-0.268	0.010	-0.098
	P	0.010625	0.000000	0.100937	0.044375	0.104375	0.303750
Lr198	n	48	48	48	48	48	48
	A	16	16	15	16	15	15
	A_p	16	16	15	16	15	15
	H_e	0.917	0.899	0.894	0.871	0.881	0.901
	H_o	1	1	1	1	1	1
	f	-0.090	-0.113	-0.119	-0.149	-0.137	-0.110
	P	0.701562	0.106563	0.298750	0.739375	0.798125	0.715938
Lr-63	n	48	48	48	48	48	48
	A	17	18	12	15	16	15
	A_p	17	18	12	15	16	15
	H_e	0.922	0.908	0.855	0.868	0.873	0.904
	H_o	1	1	0.955	0.979	0.944	1
	f	-0.084	-0.101	-0.118	-0.129	-0.082	-0.106
	P	0.643125	0.049688	0.426250	0.658438	0.327500	0.279062
Lro-41	n	48	48	48	48	48	48
	A	9	11	7	7	11	12
	A_p	9	11	7	7	11	12
	H_e	0.726	0.779	0.607	0.705	0.822	0.775
	H_o	1	1	0.595	0.75	1	0.937
	f	-0.382	-0.286	0.02	-0.063	-0.219	-0.211
	P	0.051250	0.005938	0.051250	0.008438	0.759062	0.847500

Table 4. AMOVA of microsatellite markers of six Peninsular riverine populations of *Cirrhinus mrigala.*

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	5	58.426	0.10532 Va	5.46
Within populations	556	1013.528	1.82289 Vb	94.54
Total	561	1071.954	1.92821	100
Fixation Index FST : 0.05462				

Table 5. Fst Values of mrigal across six peninsular rivers

	Mahanadi	Godavari	Krishna	Kaveri	Narmada	Mahi
Mahanadi	0.00000					
Godavari	0.00330	0.00000				
Krishna	0.01780	0.00112	0.00000			
Kaveri	0.02146	0.01636	0.00565	0.00000		
Narmada	0.08027	0.08668	0.10662	0.09898	0.00000	
Mahi	0.08529	0.08973	0.09740	0.10082	-0.00644	0.00000

2018, Behera *et al*., 2015). In the present study, only Narmada and Mahi populations were highly differentiated from all others. This may be acceptable because these two peninsular rivers are geographically in west coast of India flowing to Arabian Sea and the other four rivers are in the east coast flowing to Bay of Bengal. Different environment induces population genetic structure because of the different habitats isolated regionally. Further, in freshwater fishes fragmented populations are expected to exhibit high genetic differentiation (Ward *et al*., 1994).

In spite of the popularity of microsatellites in the study of population genetics, their development requires substantial time, financial as well as technical resources. Cross-species amplification in the present study allowed using heterologous primers to describe genetic diversity and population structure of a potential carp species mostly cultivated in the country. The result established a single genetic stock of *Cirrhinus mrigala* in Peninsular regions of India inferred from microsatellite markers because during the Eocene period the genus Cirrhinus is believed to have entered India following migration of Indo-Malayan fishes via the Indo–Brahma River, flowing westward from Assam in the north-east to the present-day Arabian Sea (Daniels, 2001) specifying a common origin of this species in Indian rivers. Also, low genetic differentiation is most probably a result of gene flow due to human introduction of this species to rivers, unethical aquaculture practices, natural calamities, etc. in the absence of connectivity among the rivers. The results of this study will definitely update the breeders and conservationists while planning for genetic improvement by developing base population for long-term genetic gain and conservation of this species.

Ethical Statement

Not applicable.

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Author Contribution

S.P.D.: Conceptualization, Methodology, Data curation, Sample collection Writing - original draft. **S.K.S.**: Helped in Sample collection and Data analysis. **L.S.**: Sample collection. **J.K.J.:** Conceptualized. **P.D.:** Conceptualized, Supervision, Project administrator, review and editing.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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