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Genetic Diversity between Populations of the Genus *Schistura* McClelland from the Garhwal and Kumaun Region Using RAPD Marker

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ABSTRACT

Genetic based study provide emphasis on the population study of geographically isolated population, gene flow among them and evolutionary history of the species. In the present study, we aimed to detect the genetic variations in species of genus *Schistura* from Uttarakhand. Very little is known for the pattern of distribution and genetic diversity of the species of genus *Schistura* from the Garhwal and Kumaun region. Out of six primer only four primers were used to generate the fragments patterns from the samples collected. Polymorphism within and between the populations were assayed and total 60 bands were amplified ranging from 1-150 kb. Maximum number of bands was observed in *Schistura montanus* from the Srinagar, Garhwal and Uttarkashi regions. Total 60 bands were amplified in the all four primer with high percentage of polymorphic loci 50% *Schistura montanus* from Garhwal region and 28.8% in the *S. montanus* from the Kumaun region were observed. The level of heterozygosity were found 0.006-0.18 in the all three species. The percentage of polymorphism was 50% within the populations and high heterozygosity the sample of *S. montanus* when compared with other species. Observed pattern of RAPD markers reveals that the samples from the Garhwal region exhibit high diversity and used four primers are sufficient to distinguish the different population in the both region except the samples of the *S. gangiticus* of Garhwal region.

Key words: Population structure, heterozygosity, phylogenetic, Uttarkashi, Srinagar

INTRODUCTION

Understanding of the genetic diversity of fish species, especially those are in trade and aquaculture stocks is essential for effective management of these populations or stocks. Molecular methods such as Random Amplified Polymorphic DNA (RAPD) and mitochondrial genes have been applied to understand the genetic diversity, population structure and other aspects of the fish species (Bartish *et al*., 2000; Alam and Islam, 2005; Ma *et al*., 2012; John *et al*., 2013). Because the molecular markers are validated to investigate and monitoring of the genetic conditions in both native and captive population (Alam and Islam, 2005). These markers can be analysed by Polymerase Chain Reaction (PCR) and helpful to study the patterns of genetic variability due to their advantages over other molecular methods, such as less complex and labour-intensive procedures and more arbitrary sampling of the genome (Williams *et al*., 1990). Very little is known for the pattern of distribution and genetic diversity of the in the species of genus *Schistura* from

the Garhwal and Kumaun region. The genus *Schistura* belongs to the family Balitoridae comprises of total 180 valid species (Eschmeyer, 2012) distributed in South and Southeast Asia (Kottelat and Leisher, 2012). The genus *Schistura* McClelland (1838) were summarized by Kottelat (1990) distributed from Thailand (Plongsesthee *et al*., 2011) and other parts of Southeast Asia (Vidthayanon and Jaruthanin, 2002; Ou *et al*., 2011). The family Balitoridae, play an important in freshwater ecosystem as cleaning of aquatic system and the species of genus *Schistura* under this family have been exploited for the aquarium fish (Vishwanath, 2010) due to its small size and striking colour pattern on the body. The morphological plasticity of species is some cases make identification difficult. Most of systemic studies on fish fauna were conducted relying on biometry such as morphometric and meristic characters (Dhanya *et al*., 2004; Zafar *et al*., 2002). However, such characteristics of limited values for identification and differentiation purpose at the species level because these biometric shows a considerable intra-specific variation (Callejas and Ochando, 1998). Therefore, understanding of population genetic structure of threatened or commercially important fish species' are crucial to develop management strategy because such population/or species can suffer severe genetic erosion (bottleneck, genetic drift, inbreeding, founder effect) without being detected by the traditional demographic monitoring approach (Nasren *et al*., 2009). For the genus *Schistura* no study was carried out on the fish taxonomy in this region using molecular markers, hence the aim of the study is to obtain a genetic diversity between two populations of genus *Schistura* from the Garhwal and Kumaun region of Uttarakhand state, India.

MATERIALS AND METHODS

Fish samples were collected from six sampling sites (Table 1) from the four river basins of Garhwal and Kumaun region. During the sampling the three species were reported *S. montanus* (SM), *S. gangeticus* (SG) and *S. rupecola* (SR). All the targeted species were collected and were preserved in the absolute ethanol. The 83 genomic DNA from muscle tissues were sampled using the commercial available Genei PureID DNA isolation mammalian kit according to manufactures instruction (GeNei, Bangalore, India). Total six primers were employed to perform the amplification reactions (Table 2). The reaction mixture (20 µL) contained 10 mM Tris-HCI pH 7.5, 50 mM KCI, 1.5 mM $MgCl_2$, 0.1 mM dNTPs, 15 pmoles of primer, 20 ng gDNA and 0.8 U of Taq DNA polymerase (GeNei, Bangalore, India). Amplification was carried with 30 cycles,

Table 1: Sample collection sites for genus *Schistura* (McClelland) in different region of Garhwal Himalaya of Uttarakhand State

Name of sampling sites	Longitude	Latitude	Elevation (ft)	Region
Khanda	30°11'26.71"	78°46'47.69"	2449	Srinagar (Garhwal)
Kirtinagar	30°13'8.22"	78°44'47.19	1828	
Bageswar	30°7'45.49"	78°34'59.39"	1432	
Uttarkashi	30°44'38.47"	78°21'31.39"	3308	Uttarkashi (Garhwal)
Chinyalisod	30°33'10.89"	78°19'12.12"	2774	
Moriyana Gad	30°30'11.32	78°16'0.73"	5591	
Haldwani	29°15'41.27"	79°32'53.65"	1635	Kumaun
6Ramnagar	29°23'19.93"	79°07'58.53"	1133	

Table 2: List of RAPD primer selected for the present study

Name	Primer sequence (5'-3')	Melting temperature (Tm)	PCR amplification success
M06	ACTGGCCGAGGG	42	79/83
M50	ATTGGTGCAGAA	34	23/83
M90	ACTGAGCAACAA	34	83/83
M98	GACGGTTGTACA	36	10/83
NTU11	CGGCCCCTGT	36	81/83
NTU31	TCGATGAAGAACGCAGC	52	80/83

RAPD: Random amplified polymorphic DNA, PCR: Polymerase chain reaction

each consisting of a denaturing step of 1 min at 94°C, followed by annealing step of 1 min at 34-51°C and an extension step of 2 min at 72°C. The last cycle was followed by 5 min of extension at 72°C. Extracted DNA and amplified PCR products were electrophoresed on 0.8 and 2% agarose gel respectively and patterns of bands were visualized under the transilluminator and gel were photographed.

Data analysis: The banding pattern RAPD markers were compared within and between the sampling sites. These banding were metricize on the excel sheet in 1, 0 in which 0 show absence of band and 1 shows presence of band. Only reproducible bands were scored on the gel photographs. Recorded bands on spreadsheets were used to determine Nei (1978) gene diversity, number of polymorphic loci and genetic distance and to construct an Unweighted Pair Group Method of Arithmetic Mean (UPGMA) dendrogram among populations using POPGENE 1.32 (Yeh *et al*., 1997). After that tree was edited in the MEGA 6 software.

RESULTS

DNA was successfully extracted from all the samples with good quality of DNA. Based on the gel visualization on 0.8% agarose and extracted DNA was contained fragment ranged from the 1 kb. PCR amplification of RAPD was carried out at different concentration of DNA ranged from 1-50X dilution and good amplification was achieved with high intensity band at 1:20 dilution of original template. Out of six RAPD primer selected for the amplification only 4 primers (M06, M90, NTU11 and NTU31) provides readable bands from both the population of Kumaun and Garhwal regions where two primer (M50 and M98) were not consistent for the analysis and were not taken for analysis (Fig. 1). The observed banding pattern of the different species of *Schistura* is summarized in Table 3. Total 60 bands were amplified with high percentage of polymorphic loci 50% in the *Schistura montanus* from Garhwal region and 28.8% in the *S. montanus* from the Kumaun (Fig. 2). Matrix of Nei genetic identity were ranged from 0.332-0.999 in the total three species from 6 population of each species (Table 4). The sequence similarity was found commonly lower that observed in other studies of different population (Wasko *et al*., 2003). The overall mean heterozygosity were observed in all three species from all population was (0.021 SE 0.021) (Table 5). The observed heterozygosity was ranged from the 0.006-0.18, in *S. montanus* from Uttarkashi site-I which show high heterozygosity (0.182) and whereas, minimum heterozygosity was reported in *S. gangeticus*. The phylogenetic tree was differentiated into two major clades named Kumaun and Garhwal region (Fig. 3). The phylogenetic tree further split into the population

Table 3: Number of individuals and RAPD primer used and number bands observed in this study

RAPD primer used	No. of samples	Species	Total bands	Polymorphic bands	Monomorphic bands
NTU11	31	*S. montanus*	4-5	1	4
	21	*S. rupecola*	4-5	2	4
	31	*S. gangeticus*	4-5	2	4
M90	31	*S. montanus*	4	1	3
	21	*S. rupecola*	4	1	3
	31	*S. gangeticus*	3	1	2
M06	31	*S. montanus*	7-8	2	6
	21	*S. rupecola*	7-8	3	6
	31	*S. gangeticus*	7	2	5
NTU31	31	*S. montanus*	5	1	4
	21	*S. rupecola*	4	1	3
	31	*S. gangeticus*	5	2	3

RAPD: Random amplified polymorphic DNA

Table 4: Pairwise population matrix of Nei genetic identity in all population of Schistura from Garhwal and Kumaun region of Uttarakhand State

Populations	KHSG	UKISG	UKIISG	UKIIISG	KMISG	KMIISG	KHSM	UKISM	UKIISM	UKIIISM	KMISM	KMIISM	KHSR	UKISR	UKIISR	UKIIISR	KMISR	KMIISR
KHSG	1.000																	
UKISG	1.000	1.000																
UKIISG	1.000	1.000	1.000															
UKIIISG	0.999	1.000	1.000	1.000														
KMISG	0.870	0.871	0.871	0.877	1.000													
KMIISG	0.892	0.893	0.893	0.900	0.978	1.000												
KHSM	0.459	0.461	0.461	0.459	0.389	0.359	1.000											
UKISM	0.595	0.596	0.596	0.594	0.512	0.478	0.767	1.000										
KMISM	0.537	0.539	0.539	0.536	0.457	0.424	0.854	0.908	1.000									
UKIIISM	0.523	0.524	0.524	0.522	0.479	0.449	0.781	0.739	0.911	1.000								
KMISM	0.462	0.464	0.464	0.462	0.531	0.500	0.718	0.671	0.844	0.953	1.000							
KMIISM	0.462	0.464	0.464	0.462	0.531	0.500	0.718	0.671	0.844	0.953	1.000	1.000						
KHSR	0.391	0.393	0.393	0.398	0.332	0.357	0.725	0.655	0.718	0.632	0.571	0.571	1.000					
UKISR	0.391	0.393	0.393	0.398	0.332	0.357	0.725	0.655	0.718	0.632	0.571	0.571	1.000	1.000				
UKIISR	0.355	0.357	0.357	0.362	0.367	0.393	0.689	0.615	0.680	0.629	0.607	0.607	0.964	0.964	1.000			
UKIIISR	0.379	0.381	0.381	0.382	0.350	0.376	0.712	0.643	0.708	0.655	0.633	0.633	0.952	0.952	0.988	1.000		
KMISR	0.391	0.393	0.393	0.390	0.332	0.357	0.718	0.655	0.718	0.665	0.643	0.643	0.929	0.929	0.964	0.994	1.000	
KMIISR	0.391	0.393	0.393	0.390	0.332	0.357	0.718	0.655	0.718	0.665	0.643	0.643	0.929	0.929	0.964	0.994	1.000	1.000

KMI: Kumaun region site I and II followed by species name *S. rupecola*, *S. gangiticus*, *S. montanus*, UKI: Uttarkashi Site I, II and III, KH, Khanda, Srinagar S

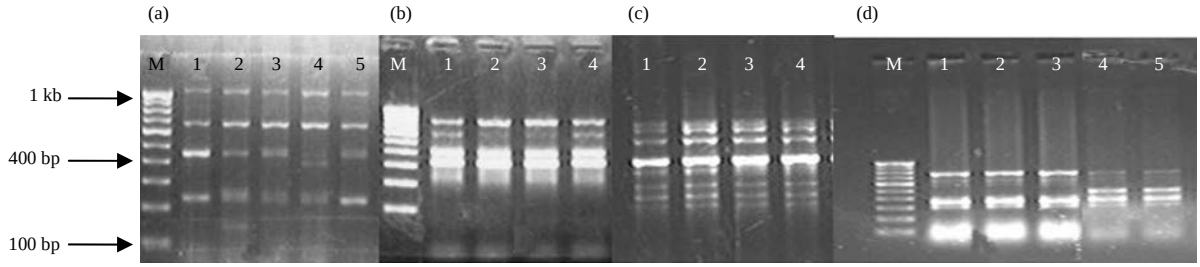

Fig. 1(a-d): Polymerase chain reaction amplification banding pattern of RAPD markers with the samples of *Schistura* on 2% agarose gel, RAPD profiles for (a) NTU11, (b) M90, (c) M06 and (d) NTU11 with different individual

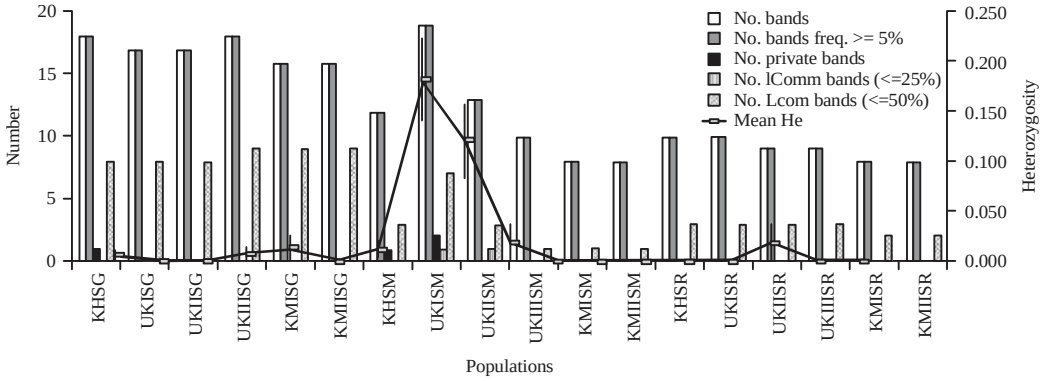

Fig. 2: Number of bands and heterozygosity observed in the different species of *Schistura* from Garhwal and Kumaun regions

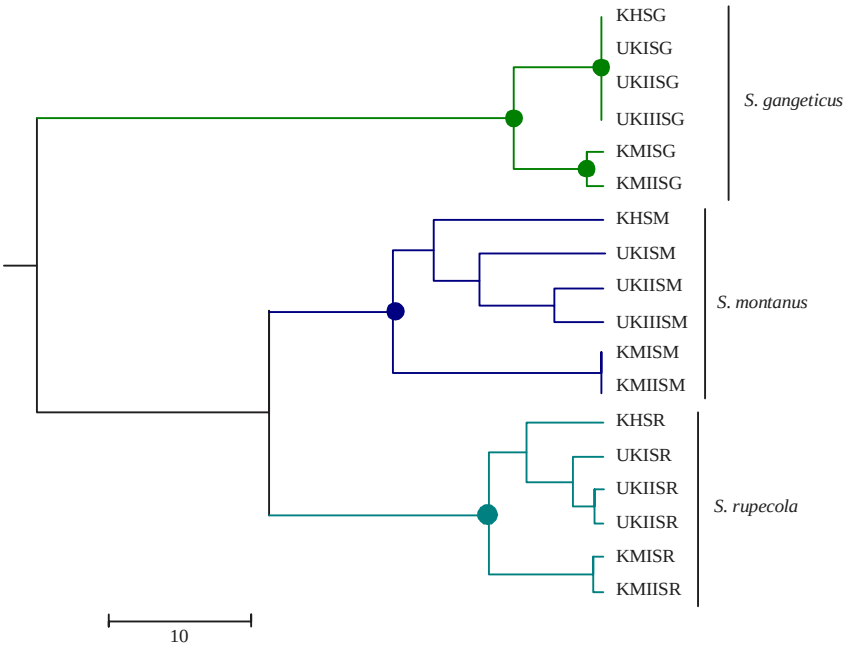

Fig. 3: Unweighted pair group method of arithmetic mean dendrogram based on Nei (1978) constructed in the popgene V1.2 and edited in MEGA 6 based on the three RAPD markers

Table 5: Different statistical values observed using three RAPD markers showing mean and SE over loci for each

Populations	N	Na	Ne	I	He	Uhe
KHSG						
Mean	6.000	0.679	1.007	0.011	0.006	0.006
SE	0.000	0.104	0.007	0.011	0.006	0.006
UKISG						
Mean	5.000	0.607	1.000	0.000	0.000	0.000
SE	0.000	0.094	0.000	0.000	0.000	0.000
UKIISG						
Mean	5.000	0.607	1.000	0.000	0.000	0.000
SE	0.000	0.094	0.000	0.000	0.000	0.000
UKIIISG						
Mean	5.000	0.679	1.008	0.012	0.007	0.007
SE	0.000	0.104	0.008	0.012	0.007	0.007
KMISG						
Mean	5.000	0.607	1.019	0.019	0.012	0.014
SE	0.000	0.107	0.019	0.019	0.012	0.014
KMIISG						
Mean	5.000	0.571	1.000	0.000	0.000	0.000
SE	0.000	0.095	0.000	0.000	0.000	0.000
KHSM						
Mean	6.000	0.500	1.014	0.021	0.011	0.012
SE	0.000	0.121	0.009	0.015	0.008	0.009
UKISM						
Mean	5.000	1.179	1.329	0.269	0.182	0.202
SE	0.000	0.171	0.081	0.057	0.041	0.045
UKIISM						
Mean	5.000	0.750	1.213	0.175	0.120	0.134
SE	0.000	0.168	0.067	0.053	0.037	0.041
UKIIISM						
Mean	5.000	0.393	1.035	0.025	0.018	0.020
SE	0.000	0.107	0.035	0.025	0.018	0.020
KIMISM						
Mean	5.000	0.286	1.000	0.000	0.000	0.000
SE	0.000	0.087	0.000	0.000	0.000	0.000
KMIISM						
Mean	5.000	0.286	1.000	0.000	0.000	0.000
SE	0.000	0.087	0.000	0.000	0.000	0.000
KHSR						
Mean	1.000	0.357	1.000	0.000	0.000	0.000
SE	0.000	0.092	0.000	0.000	0.000	0.000
UKISR						
Mean	5.000	0.357	1.000	0.000	0.000	0.000
SE	0.000	0.092	0.000	0.000	0.000	0.000
UKIISR						
Mean	2.000	0.321	1.000	0.000	0.000	0.000
SE	0.000	0.090	0.000	0.000	0.000	0.000
UKIIISR						
Mean	3.000	0.357	1.034	0.024	0.017	0.021
SE	0.000	0.106	0.034	0.024	0.017	0.021
KMISR						
Mean	5.000	0.286	1.000	0.000	0.000	0.000
SE	0.000	0.087	0.000	0.000	0.000	0.000
KMIISR						
Mean	5.000	0.286	1.000	0.000	0.000	0.000
SE	0.000	0.087	0.000	0.000	0.000	0.000
Total						
Mean	4.611	0.506	1.037	0.031	0.021	0.023
SE	0.056	0.027	0.007	0.006	0.004	0.004

KMI: Kumaun region site I and II followed by species name *S. rupecola*, *S. gangiticus*, *S. montanus*, UKI: Uttarkashi Site I, II and III, KH, Khanda, Srinagar S, Na: No. of different alleles, Ne: No. of effective alleles, I: Shannon's information index, He: Diversity, Uhe: Unbiased diversity, SE: Standard error, RAPD: Random amplified polymorphic DNA

level except for the *S. gangeticus* where samples from the Srinagar Garhwal and Uttarkashi region were not separated into different clade and form monophyletic clade which show low genetic variability in this species where it is separated into separate Garhwal and Kumaun populations. Other two species, *S. rupicola* and *S. montanus* were separated into the separate clades (Fig. 3).

DISCUSSION

In the present investigation, the genetic diversity were found higher in the Garhwal regions when compared to the Kumaun region. The banding pattern of the primers shows low level of polymorphic bands in the different species of *Schistura* that reveals different levels of genetic variability within and between the population of the Kumaun and Garhwal region. However, there was almost no polymorphic bands and high number of monomorphic bands were observed within the population except in the *S. montanus* from Srinagar, Garhwal and can be used in population genetics as similar finding were observed in (Baradakci and Skibinski, 1994; Ertas and Seker, 2005). But the band-sharing using the different indices such as similarity and heterozygosity are sufficient to distinct some of the sampling locality in the Garhwal and Kumaun region. But within population it is difficult to detect the level of genetic diversity using the RAPD marker in our study and similar in other study which suggested the use of mitochondrial markers (Ali *et al*., 2004; Cadena *et al*., 2011). The UPGMA analysis permitted the clustering of different individuals of three *Schistura* species from Garhwal and Kumaun region with distinguished clade at population level but unable to distinguished individuals of the same population and similar finding were also reported by (Rodrigues *et al*., 2007). Garhwal region samples indicating a high genetic similarity among *S. gangiticus* population (Fig. 3), while individuals from *S. montanus* were not clustered on a single unit, demonstrating a higher genetic heterogeneity. As heterozygosity is an important evolutionary indicator in determining the dynamics and survival of populations (Reed, 2009). All the samples from the Kumaun regions are separated in the separated clade with similarity matrix of 0.92. Hence, further more detailed investigation using the mitochondrial and nuclear genes are needed to explore the phylogenetic relationship of these species and to understand their evolution history in these river systems. Out of the six primer used only four primer provide readable band as similar kind of the success rate have been obtained in (Santis *et al*., 2007) where they have got success only in 5 primers out of 14 primers used. Where low number (2) of RAPD primer also been used in assessing the genetic diversity in the gold fishes and these population were separated (Prasad, 2014).

CONCLUSION

All three species of *Schistura* exhibiting good population structure from large geographic region in which only *S. gangiticus* not splits in the Garhwal regions and indicating low level of genetic diversity. The *S. montanus* indicate high level of genetic diversity when compared to other of species and over all the samples from the Garhwal region show high genetic diversity.

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