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Research Article

Stock discrimination of *Sperata aor* from river Ganga using microsatellite markers: implications for conservation and management

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Abstract – High genetic variation is an important requirement for long term survival of wild populations through adaptations to changing environmental conditions. High levels of variability and the potential to isolate large number of loci make microsatellites the marker of choice to study intraspecific genetic variation in fish. Seventy *Sperata aor* samples each from four sites along the river Ganga were successfully cross amplified at six loci. All the selected loci were highly polymorphic showing a slight heterozygosity excess. The mean observed and expected heterozygosities across all investigated populations were 0.971 and 0.913, respectively. F_{ST} statistics, Bayesian model-based clustering analysis and analysis of molecular variance (AMOVA) revealed three different genetic stocks of *S. aor* (Narora–Kanpur, Varanasi and Bhagalpur), showing moderate genetic differentiation among them (total $F_{ST} = 0.069$). Genetic differentiation was significantly correlated with the distance among stocks. The populations of *S. aor* are currently stable in the river Ganga as evident from high levels of genetic variability and no inbreeding. However, a precautionary approach to fishery management and conservation of *S. aor* should be undertaken at priority in view of the anthropogenic as well as natural threats to the fishes of river Ganga.

Key-words: Cross-species amplification / Genetic variability / River Ganga / *Sperata aor* / Stock discrimination

1 Introduction

The inland water ecosystems are facing serious anthropogenic threats to biodiversity and ecosystem stability (Suski and Cooke, 2007; Sarkar et al., 2008; Januchowski-Hartley et al., 2011). Therefore, information on the basic biological parameters related to natural fish populations such as population structure and life history traits should be assessed for the specific water bodies. Several factors, such as environmental degradation due to urbanization, habitat loss, water diversions for irrigation and hydropower generation, overfishing, pollution and the introduction of exotic species, have resulted in drastic reduction of freshwater riverine fish genetic biodiversity (Dudgeon et al., 2006; Sarkar et al., 2008). The freshwater fishes exhibit global extinction rate greater than the higher vertebrates except amphibians; mainly because of the threats challenging the balance and functioning of freshwater ecosystems (Bruton, 1995; Sarkar et al., 2008). The actual obstacles to freshwater fish conservation are the lack of required management plans and protected areas (Barletta et al., 2010; Januchowski-Hartley et al., 2011). Hogan (2011) reported that freshwater fishes do not receive the same level of protection and management strategies as marine fishes. In fact, the necessary inputs required to devise the management strategies and decipher the population dynamics of target fish species are not undertaken for most of freshwater fish species across the globe and especially in India. The existing management strategies are specific to the type of water bodies and not to the fish populations or its stocks. In India, most of the freshwater bodies do not have effective fishery resource management strategies that can sufficiently address a sustainable use of resources. This may result in overfishing, decreased catch per unit effort, increasing conflict between fishing communities or regions and subtle changes in yield. The Blue Green Initiative of FAO supports sustainable management of freshwater fishery resources and conservation in an economically and environmentally organized form. In order to sustain the genetic composition of freshwater fishes in their natural conditions techniques and proper strategies should be designed to achieve successful management and conservation. Hence,

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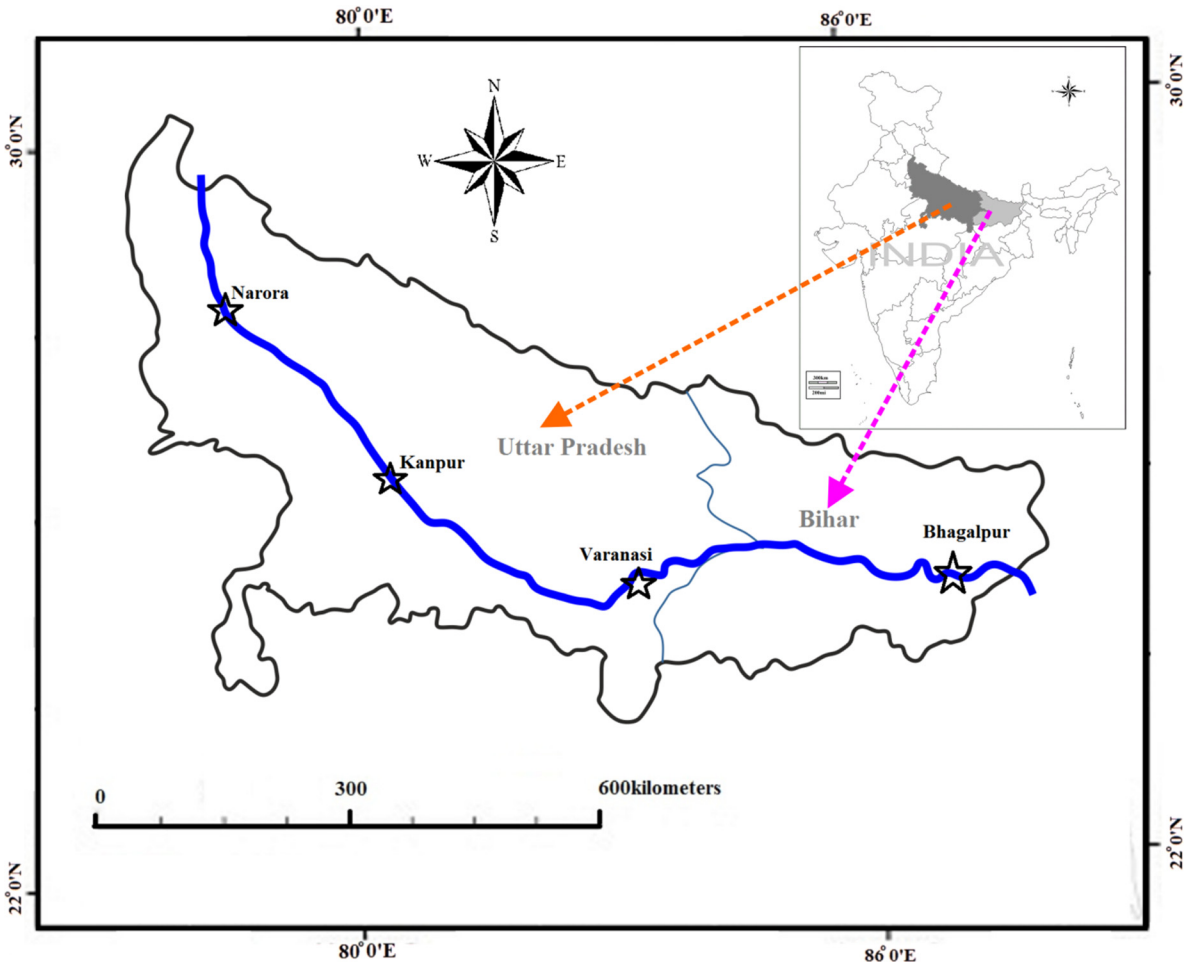

Fig. 1. Map showing the sampling sites of *Sperata aor* across the river Ganga.

studies on genetic variation are of vital significance to develop conservation and management strategies with positive long-term impact.

Stocks are discrete group of fishes with spatial or temporal integrity, sufficient to self-reproduce with members of each group having similar life history traits (Hilborn and Walters, 1992). Stock identification limits the boundaries between stocks of the same species and most population models assume that the groups of individuals have the same growth, reproductive and mortality rates; hence they should be managed separately. In order to conserve the natural gene pool of fish stocks it is very important to identify genetic stocks so that stock-specific management policies and conservation strategies can be prepared (Begg et al., 1999).

High genetic variation is an important feature of natural populations for long term survival through adaptations to changing environmental conditions (Ferguson et al., 1995). The molecular markers provide important information on fish genetic stock structure, gene flow between populations and forewarning of genetic bottleneck. Microsatellites are commonly used to study genetic variation within and between populations of fish species due to their high variability, relatively small size, and abundance in the genome as compared to other vertebrates and invertebrates (Zane et al., 2002). Single locus microsatellites showing codominance are frequently used for population studies (Abdul-Muneer et al., 2009). Genetic variation analysis using microsatellite markers demands species-specific primers but cross-species amplification of existing primers of closely related species can also be employed successfully. Cross-species amplification is utilized due to the fact that many microsatellite loci surrounded by flanking regions are conserved among closely related species despite high mutation rate (Kathirvelpandian et al., 2014). The low rate of evolutionary divergence among the closely related species justifies the success of cross-species amplification (Gupta et al., 2013). The information provided by microsatellite markers on fish genetic stock structure, genetic connectivity and inbreeding are very important for a successful genetic conservation and a sustainable management of the fishery resources (Abdul-Muneer et al., 2009).

Long-whiskered catfish, *Sperata aor* (Hamilton, 1822), is a demersal fish species, which inhabits rivers, ponds, lakes, tanks, channels and reservoirs. It is reported that *S. aor* attains a maximum length of 180 cm and distributed in India, Pakistan, Bangladesh, Nepal and upper Myanmar (Talwar and Jhingran, 1991). The adult *S. aor* is a bottom and column feeder carnivore with piscivorous and predaceous nature whereas the juveniles feed mainly on insects. *S. aor* is very difficult to make spawn artificially as it is highly sensitive to water quality changes and is easily stressed (Rahman et al., 2005). Saigal (1964) reported

March to August as the breeding season with spawning peaks in April and June in Ganga river. It is also well documented that the *S. aor* shows fractional spawning (Saigal, 1964; Ramakrishniah, 1992). *S. aor* is one of the most commonly exploited fish species for food throughout river Ganga and it fetches a higher price than Indian major carps. The fish has high food value with quality protein content and a low number of intramuscular bones (Talwar and Jhingran, 1991). Until 1950s, *S. aor* was found in the middle and lower stretches of the river Ganga (Menon, 1954) but due to a perceptible shift in the distribution pattern of fishes leading to range extension the species is now reported from the upper cold region of Haridwar stretch (Sarkar et al., 2012). On a commercial scale the supply of *S. aor* depends on capture fisheries, as captive breeding and cultural techniques have so far not been standardized (Khan et al., 2016). In view of the current anthropogenic threats to the freshwater ecosystems, and in order to develop strategies for stock-specific management and conservation, effective steps should be undertaken to identify the genetic stocks of *S. aor* (Sarkar et al., 2008). Since there is no published report on the stock structure of this species, the present study was conducted to discriminate the *S. aor* stocks inhabiting the river Ganga using microsatellite markers.

2 Material and methods

2.1 Sampling

Fish samples were collected from four sites on the river Ganga: Narora (28°11′ N, 78°23′ E), Kanpur (26°27′ N, 80°20′ E), Varanasi (25°18′ N, 83°0′ E) and Bhagalpur (25°16′ N, 87°02′ E) (Fig. 1). In the river Ganga the stretch between Brijghat to Narora is a protected area (Ramsar site). A total of 280 fish samples, 70 each from four selected sites were collected monthly from April 2013 to December 2015. A small piece (5–7 g) of anal fin was excised and placed in 95% alcohol at the sampling site, transported to laboratory in frozen state and stored at −20 °C until DNA extraction.

2.2 DNA extraction and amplification

Genomic DNA was extracted from the fin tissue using DNeasy® Blood and Tissue Kit, Qiagen, Valencia, CA, USA with minor modifications. The extracted DNA concentration was estimated by 1% agarose gel stained with ethidium bromide and by UV spectrophotometer with optical density at 260 and 280 nm.

A total of 15 microsatellite primers previously developed for *S. aor* and closely related species were used for screening in the selected fish species (Supplementary data S1). Polymerase chain reaction (PCR) amplifications were performed in a 25 µL reaction mixture consisting of 1× PCR buffer (10 mM Tris, 50 mM KCl, 0.01% gelatin, pH 9.0, and 1.5 mM $MgCl_2$), 200 mM dNTPs, 5 pmol each primer, 2 U Taq DNA polymerase (Sigma-Aldrich, Missouri, USA), and 25–50 ng DNA. The PCR amplification was carried out in a T100™ thermal cycler (Bio-Rad, Hercules, California, USA). The reaction mixture was preheated at 94 °C for 5 min followed by 25 cycles at 94 °C for 30 s, a primer-specific annealing temperature for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 4 min. The PCR products were separated on a 10% polyacrylamide gel electrophoresis and then visualized using silver staining. The Image Lab 4.1 software in the Gel Doc system (Molecular Imager® Gel Doc™ XR+, Bio-Rad, Hercules, California, USA) was employed to calculate the molecular weight of amplified fragments with reference to 50 bp DNA ladder Mol Bio™ Himedia.

2.3 Statistical analysis

The mean number of alleles, mean effective number of alleles, mean observed and expected heterozygosities (H_O and H_E) and percentage of polymorphic loci for each population were calculated using GenAlEx 6.1 (Peakall and Smouse, 2006). Linkage disequilibrium among different loci and departures from Hardy–Weinberg equilibrium (HWE) were tested using Genepop version 4.0 (Raymond and Rousset, 1998; Rousset, 2008). Locus conformance to HWE was determined using Fisher's exact tests (using default Markov chain method). Sequential Bonferroni correction was used for multiple comparisons at adjusted significant levels (Rice, 1989). All the genotypes of the loci showing deviation from HWE were tested for null alleles and the expected frequency of null alleles was evaluated using Micro-Checker 2.2.3 (Van Oosterhout et al., 2004).

The coefficient of genetic differentiation (F_{ST}), inbreeding coefficient (F_{IS}), Nei's unbiased genetic distance, and gene flow (N_M) based on the private alleles method were calculated using Genepop version 4.0 (Nei, 1978; Wright, 1978; Weir and Cockerham, 1984; Barton and Slatkin, 1986). The hierarchical analysis of molecular variance (AMOVA) was performed using Arlequin version 3.0 (Excoffier et al., 2005). To analyse quantity of genetic variation, AMOVA estimations were carried out at three levels of the population structure: among populations, among individuals, and within individuals (Chaturvedi et al., 2011).

Genetic stock structuring was investigated using the Bayesian model-based clustering algorithms implemented in STRUCTURE 2.3.4 (Pritchard et al., 2000). Admixture model where α (the degree of admixture) was inferred from a uniform prior, with initial $\alpha = 1$, max = 10.0 and standard deviation = 0.025. A correlated allele frequency model was employed amongst populations. This uses a Markov Chain Monte Carlo (MCMC) algorithm to assign individuals to their most likely genetic cluster (K) and estimates membership probability (Q) for each individual to these clusters. Each run had a burn-in period of 50 000 followed by 1 000 000 MCMC repetitions (Araripe et al., 2013). We explored a range of K from 1 to 10 with 10 runs of each K value. The most likely value of K was evaluated using both the maximal average value of $\ln P(D)$, a model choice criterion that estimates the posterior probability of the data and ΔK, an ad hoc statistic based on the second order rate of change of the likelihood function with respect to K (Evanno et al., 2005).

The Mantel test was used to test correlation between genetic distances, expressed as $F_{ST}/(1 - F_{ST})$, and geographic distance, expressed as $\log K_m$, with the program Isolation By Distance Web Service (Jensen et al., 2005).

3 Results

Successful cross-species amplification of six out of fifteen primer pairs was obtained in *S. aor* whereas, the nine primer

Table 1. Sequences, annealing temperature and size of alleles of amplified microsatellite primers in *Sperata aor*.

Primers	Sequence 5′–3′	Annealing temp for each primer (°C)	Size range of alleles (in bp)	GenBank accession number
SA03	F CGAACACGCCACAGAGAGTA R CCACACCCACAACACCATAA	58	150–216	DQ780021
SA04	F CCAGCAACCCACATAATTGA R CAGCTCAGGGCCAAAAGTAG	56	128–188	DQ780022
SA05	F AGGTAGTGCACAGTAGCGC R TGCACACACACACACACAC	57.8	170–238	DQ888903
SA08	F AAAGAAGAAAGCGGTTTTAT R ACGGCAGTAGTCTTTCCTC	55.5	170–228	EU439603
SA10	F CAAGTGCAAAGACAGACAGA R TCTCTAAGGCTATCCATCCA	56	156–210	AY207448
SA13	F CTTCTCCTCTGTTTCGCTGT R GGAGTGAGCTGGAGGACT	55	180–272	AY462582

Table 2. Genetic diversity population statistics across six microsatellite loci and four populations: number of alleles (N_A); number of effective alleles (N_E); observed (H_O) and expected (H_E) heterozygosity; inbreeding coefficient (F_{IS}).

Population/locus		SA03	SA04	SA05	SA08	SA10	SA13	Mean (SE)
Narora ($N=70$)	N_A	18	21	13	15	12	15	15.66 (1.35)
	N_E	12.87	11.20	9.36	12.20	9.87	12.25	11.29 (0.57)
	H_O	0.982	0.957	0.920	0.965	0.931	0.985	0.956 (0.004)
	H_E	0.922	0.911	0.893	0.918	0.899	0.918	0.910 (0.005)
	F_{IS}	−0.084	−0.098	−0.119	−0.089	−0.113	−0.089	−0.099 (0.006)
Kanpur ($N=70$)	N_A	14	21	15	24	10	16	16.66 (2.06)
	N_E	11.62	11.36	10.66	14.32	8.49	13.22	11.61 (0.83)
	H_O	0.983	0.985	0.956	0.985	0.955	0.961	0.970 (0.003)
	H_E	0.914	0.912	0.906	0.930	0.882	0.924	0.912 (0.007)
	F_{IS}	−0.094	−0.096	−0.103	−0.075	−0.133	−0.082	−0.097 (0.008)
Varanasi ($N=70$)	N_A	18	17	21	19	12	15	17 (1.29)
	N_E	13.29	13.24	12.40	12.69	10.43	12.38	12.41 (0.42)
	H_O	0.975	0.984	0.971	0.988	0.991	0.973	0.980 (0.004)
	H_E	0.925	0.924	0.919	0.921	0.904	0.919	0.919 (0.003)
	F_{IS}	−0.081	−0.082	−0.088	−0.086	−0.106	−0.088	−0.088 (0.004)
Bhagalpur ($N=70$)	N_A	19	10	23	14	14	19	16.50 (1.91)
	N_E	11.96	7.96	13.82	10.21	10.91	14.08	11.49 (0.94)
	H_O	0.985	0.934	1.000	1.000	1.000	0.957	0.979 (0.006)
	H_E	0.916	0.874	0.928	0.902	0.908	0.929	0.910 (0.008)
	F_{IS}	−0.091	−0.144	−0.078	−0.108	−0.101	−0.030	−0.092 (0.015)

SE is the standard error.

pairs did not amplify or exhibited irregular amplification or the peaks were not scorable. The optimum annealing temperature to get scorable bands were different from that reported for resource species (Tab. 1). All the amplified loci was highly polymorphic in the selected fish populations.

The mean allele number and the mean effective number of alleles were between 15.66–17.00 and 11.29–12.41, respectively. The mean observed heterozygosity ranged from 0.956 to 0.980 and the mean expected heterozygosity ranged from 0.910 to 0.919 (Tab. 2). Significant deviation from HWE ($P < 0.001$) were detected at all loci in all populations and remained significant after sequential Bonferroni corrections. The mean estimates of the inbreeding coefficient ranged from −0.099 to −0.088 (Tab. 2), indicating a slight excess of heterozygotes. The estimated null allele frequency was not significant ($P < 0.05$) at all six loci using different algorithms, indicating the absence of null alleles and false homozygotes. No linkage disequilibrium was detected which indicates that the assortment of alleles is independent at all the six loci.

The Fisher's pair-wise value of the coefficient of genetic differentiation (F_{ST}) was 0.087 between Narora and Bhagalpur populations and 0.001 between Narora and Kanpur populations (Tab. 3). The Nei's unbiased genetic distance was least (0.110) between Narora and Kanpur and highest

Table 3. Fisher's pair-wise F_{ST} (above diagonal), gene flow N_M (between parenthesis), and Nei's unbiased genetic distance (below diagonal) of all the studied loci for populations of *Sperata aor*.

Populations	Narora	Kanpur	Varanasi	Bhagalpur
Narora	–	0.001(16.13)	0.066*(4.84)	0.087*(3.23)
Kanpur	0.110	–	0.039*(5.14)	0.073*(3.32)
Varanasi	0.384	0.302	–	0.048*(4.66)
Bhagalpur	0.682	0.533	0.371	–

* $P < 0.001$.

Table 4. Analysis of molecular variance of *Sperata aor* stocks delineated from the river Ganga.

Source of variation	df	Sum of squares	Variance component	% of variation	Coefficient of genetic differentiation
Among stocks	2	62.31	0.205	6.00	$F_{ST} = 0.069$*
Among individuals within stocks	207	521.80	0.000	0.00	
Within individuals	210	628.50	2.993	94.00	

* $P < 0.001$.

(0.682) between Narora and Bhagalpur populations. The AMOVA test indicated a significant genetic differentiation among *S. aor* populations. A value of 94% was observed within individuals, whereas 6% of the variation was observed among stocks of *S. aor* across river Ganga (Tab. 4).

The model-based stock structure analysis showed that, after ten iterations, the highest value of K were for $K = 3$ (log likelihood ln $P(K)$ for $K = 3$ was −4381.69, from which ln $P(K)$ values began to fall and variance increased) (Supplementary data S2). Estimates of ΔK revealed the largest increase in the likelihood of the number of clusters at $K = 3$ (Fig. 2a) and three stocks were identified using structure analysis of the *S. aor* populations across the river Ganga (Fig. 2b). The Mantel test revealed a significant positive correlation between genetic distance and geographic distance across the sampling locations ($r = 0.905$; $P < 0.05$) indicating significant isolation-by-distance (Fig. 3).

The gene flow (N_M) was highest (16.13) between Narora and Kanpur populations and lowest (3.23) between Narora and Bhagalpur populations (Tab. 3).

4 Discussion

Environmental disturbances and fishing could result in bottlenecks, which favor losses of genetic variability and inbreeding in natural fish populations, and might result in decreased adaptability of species to their environments (Garcez et al., 2011). For example, low genetic variation at microsatellite loci and a deficiency of heterozygotes possibly due to inbreeding was observed in *Horabagrus brachysoma* (Abdul-Muneer et al., 2009). The *S. aor* populations from the river Ganga studied here displayed high levels of genetic variation expressed by number of alleles and heterozygosity, and no evidence of bottleneck effects were noticed. Reports of high levels of genetic variability are not uncommon for freshwater fishes, and have been observed in populations of as different species as *Salvelinus fontinalis* and *Gobiobotia brevibarba* (Pilgrim et al., 2012; Kim et al., 2014). In *S. aor*, the observed allele frequencies at all loci showed significant deviation ($P < 0.001$) from HWE even after sequential Bonferroni correction. This may be due to several reasons, such as transient increase in heterozygosity by founder effect, selection, or Wahlund effect (Abdul-Muneer et al., 2009; Simbine et al., 2014). The significant and negative F_{IS} values for each locus in all the four populations indicate no inbreeding and hence excess of heterozygotes. This could be due to wide distribution of the target fish species, its ability to occupy a variety of habitats and stable population trend (Rema-Devi and Raghavan, 2011).

Significant moderate genetic differentiation was observed for *S. aor* across the Ganga River. The observed amount of genetic differentiation is normal for the selected fish species as compared to other fish species which shows moderate to high genetic differentiation. Musammilu et al. (2014) reported moderate genetic differentiation for *Gonoproktopterus curmuca* ($F_{ST} = 0.067$) from three river systems of Southern Western Ghats of India. Also, moderate genetic differentiation was observed for *Siniperca chuatsi* ($F_{ST} = 0.105$) from the middle reach of the Yangtze River (Tian et al., 2013). Moderate to high genetic divergence was observed for *H. brachysoma* ($F_{ST} = 0.045$–0.219) and *Pararasbora moltrechti* ($F_{ST} = 0.089$–0.456) from three rivers in the Western Ghat region and central Taiwan, respectively (Abdul-Muneer et al., 2009; Chiang et al., 2011). The pairwise F_{ST} and Structure analysis revealed three different stocks of *S. aor*, i.e. Narora–Kanpur, Varanasi and Bhagalpur among the selected sampling sites. The genetic differentiation may be due to restricted migration (no published information on the active migration of the selected fish species) among the populations of three distinct stocks. The high levels of gene flow (N_M) between Narora and Kanpur revealed no restriction in fish movement thus they did not differentiate into distinct populations. The other factors besides migration that can influence the levels of gene flow and population structure are the presence of physical or ecological barriers, behavioural and life history traits. The differences in environmental

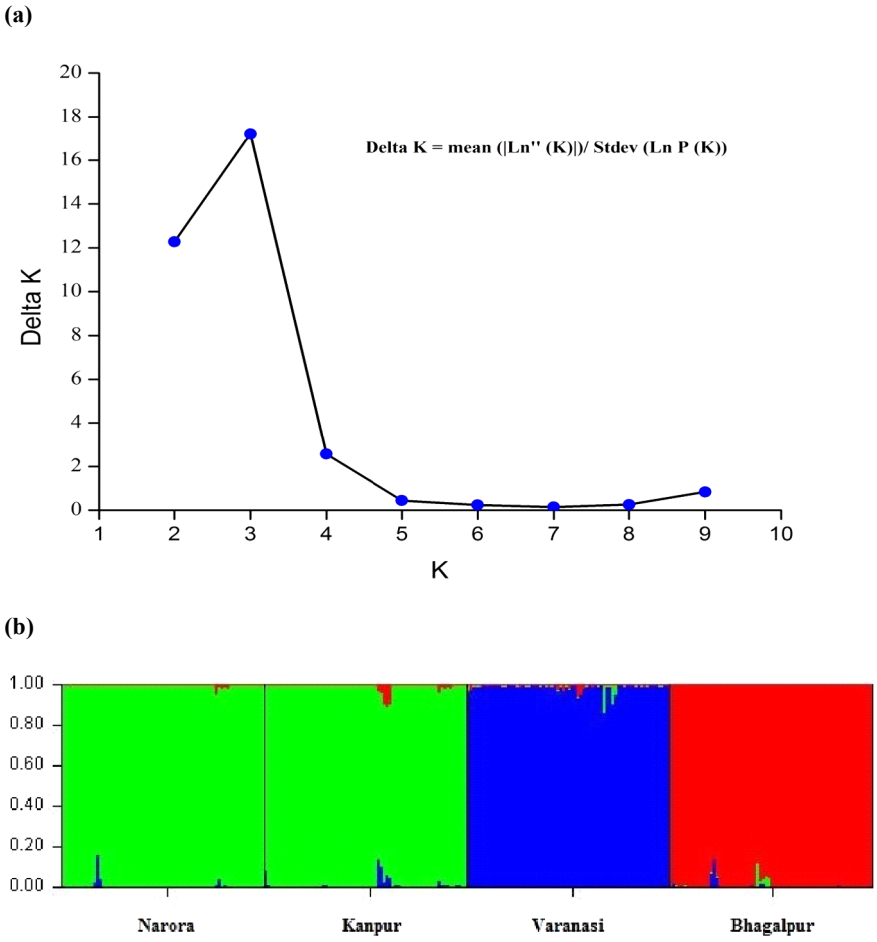

Fig. 2. Estimated population structure of *Sperata aor* across the river Ganga. (a) Bayesian inference of number of clusters (K) among four populations using ΔK. (b) Plot of assignment probability (Q-values) of individuals to three genetic groups (resolved within the model of $K = 3$) each represented by a different colour.

parameters (water chemistry, temperature and salinity) between sampling sites may also play an important role to determine the genetic structure which needs to be further examined using adaptive loci (Heithaus and Laushman, 1997). The Bayesian model-based analysis showed a single stock for Narora and Kanpur populations while Varanasi and Bhagalpur were delineated as different stocks. The gene flow among the *S. aor* populations of Narora and Kanpur region may be due to upriver migration as observed in a number of species of the migratory siluriform catfish (Araripe et al., 2013). A strong positive correlation between genetic and geographical distances (Mantel test: $r = 0.905$; $P < 0.05$) revealed a pattern of isolation-by-distance across the river Ganga. Narora site does not face significant pollution load compared to the other downstream sites that are overexploited and severely polluted. The Kanpur site is the most polluted among the selected sites. A large number of tributaries of river Ganga, such as Gomti, Ghaghara, Gandak and Son, merge with the river Ganga between Varanasi and Bhagalpur. Thus, the fish population from Bhagalpur may migrate towards tributaries instead of moving towards Varanasi because of poor water quality at the Varanasi site. Also, fish population from Varanasi will face a major obstacle in migrating towards Kanpur because of a barrage at Kanpur and merging of river Yamuna into the river Ganga at Allahabad that lies between Kanpur and Varanasi sampling sites. The fish population from Kanpur can migrate freely upstream towards Narora site because of no physical barrier. Thus, it can be inferred that the tributaries of river Ganga and the physical barriers might have promoted the differentiation between Bhagalpur, Varanasi and Kanpur whereas the populations of Kanpur and Narora show insignificant differentiation.

Horreo and Garcia-Vazquez (2011) reported that the river basins are the gene highways where introgressions of unprotected fish populations enter protected areas upstream or downstream. In the present study, the fish populations from downstream unprotected areas may show movement towards the upstream protected area (Ramsar site, between Brijghat to Narora). The potentially detrimental effects of unprotected fish populations on the protected populations seems to be minimal, since the populations of *S. aor* are presently stable as evident from high genetic variability. However, it is desirable to study the status of Ramsar site regarding abundance and conservation status of fish species because, it does not, by itself, eliminate the plethora of anthropogenic threats on the fish populations in the river Ganga. The increase in human population resulted in dense human settlements along river

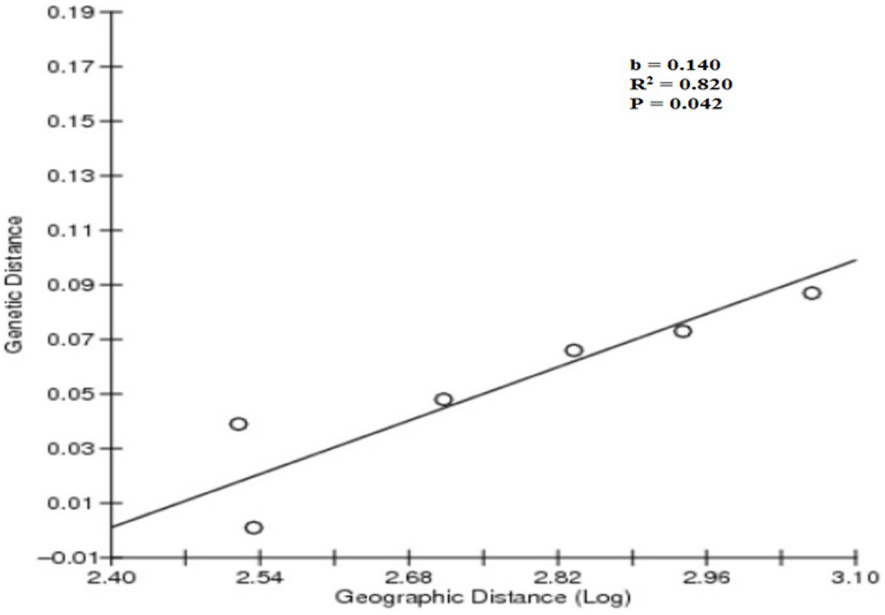

Fig. 3. Pairwise relationship between genetic ($F_{ST}/(1 - F_{ST})$) and geographic distance ($\log(K_m)$) among populations of *Sperata aor*.

Ganga which ultimately lead to growing demand for agriculture, shelter, food, developmental and religious activities. The actions to fulfill these demands have led to intense alterations in the riverine environment, mostly due to the release of untreated wastes. Moreover, sand from the river bed is extracted in enormous quantities for construction purposes and flood control. All together these factors have a direct or indirect impact on the riverine ecology and overall health of the ecosystem.

Presently, no published information is available on the exploitable potential of *S. aor* since fishery practices in the Ganges are mostly unorganized. Moreover, there has been an immense hunt for the selected fish species from the wild for the food and ornamental fish market. *S. aor* has been listed in the vulnerable category in India due to undesirable changes in its habitat environment and anthropogenic threats such as, destructive fishing practices, pollution, climate change and rapid sedimentation (Lakra et al., 2010; Sarkar et al., 2012). Sarkar et al. (2012) reported that the *S. aor* from the upper and middle stretch of the river Ganga is under severe threat due to the consequences of damming and water diversions projects. Keeping in view the current threats facing the fish populations in river Ganga due to anthropogenic activities a precautionary approach to *S. aor* conservation and management should be undertaken at priority. In the context of the conservation of *S. aor*, a non-destructive sampling procedure (e.g. fin clipping in the present study) that reduces the impact on wild populations should be utilized for any further study.

5 Conclusion

The present study provided evidence of three different stocks of *S. aor* across the river Ganga. The populations of Narora and Kanpur did not show significant variations whereas, the populations Narora–Kanpur, Varanasi and Bhagalpur show significant variations and represent three stocks as evident from pairwise F_{ST}, AMOVA and Structure analysis. The high genetic variability coupled with no inbreeding and the ability of the *S. aor* to occupy a variety of habitats indicates that the populations of the target fish species are presently stable. However, in view of the anthropogenic and natural threats facing the fish populations in river Ganga, the documentation of genetic variability of the fish populations should be undertaken at priority. Therefore, it is highly recommended to formulate precautionary strategies for the management and conservation of *S. aor* stocks across the river Ganga.

Supplementary Material

Supplementary file supplied by authors.
The Supplementary Material is available at http://www.alr-journal.org/10.1051/alr/2017033/olm.

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