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Multi-Locus Genetic Analysis in Silver Carp Populations of Indus River as Influenced by Restocking Programs

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TS conducted the experiment and wrote manuscript. KA planned the research layout and finalized the manuscript. MSA and JK helped in collecting the fish individuals. SN and HA assisted in results interpretation. All authors have read and approved the manuscript in its final form.

Key words
Restocking programs, Genetic diversity, Microsatellite markers, Natural aquatic species

ABSTRACT

The genetic integrity of fish populations has been continuously threatened due to various anthropogenic actions, particularly restocking programs. These losses in genetic diversity present erosion of global biodiversity. The genetic status of *Hypophthalmichthys molitrix* in the Indus River was assessed by using microsatellite markers. For the purpose, samples were collected from five selective Indus River sites, and DNA extraction was done by proteinase-K and standard phenol/chloroform DNA isolation method. Genomic DNA was PCR amplified by *Labeo rohita* cross-species amplification of *H. molitrix*. The results showed a low-to-moderate level of genetic diversity. The number of alleles on each locus ranging from 2.0 to 6.0, with an average of 3.48 was observed at various loci. The average observed and expected heterozygosities ranged from 0.664 to 0.76 and 0.631 to 0.664, respectively. For all tested loci, population combinations showed significant deviation ($p<0.05$) from HWE. The AMOVA indicated that most of the variation lied within individuals in populations of *H. molitrix*. Based on Nei's genetic distance, UPGMA dendrogram was constructed that resulted in two clusters: one cluster consisted of JB and CB while other consisted of GG, GB and TB. The findings of this research will be useful for restoring, conservation and monitoring the natural aquatic fish species in Pakistan.

INTRODUCTION

In a constantly changing environment, genetic diversity ensures the fitness of species by providing them the ability to face the natural selection pressure and ecological problems efficiently. Any species' biological diversity depends upon its phenotypic plasticity as well as the ability of its populations to adopt the natural environment and human disruptions. During last few decades, the sustainability of fish species has affected severely due to various environmental hazards mainly floods, habitat loss, eutrophication and human interruptions such as dam construction, overfishing, pollution and introduction of new species (Vandewoestijne *et al*., 2008). Introduced fish species has played a considerable part in decrease and loss of native fish populations. As a consequence of these introductions in populations, diseases are spread that are extremely detrimental to the health of indigenous fish species. Local fish populations are also reduced due to inter-breeding and displacements after introducing non-native fish species (Qadeer, 2017).

A species native to China, silver carp (*Hypophthalmichthys molitrix*) is now commonly cultured in Asia, contributing 22% (> 3 MMT) of global carp aquaculture production (FAO, 2005). Belonging to family Cyprinidae, silver carp is among the most valuable aquaculture fish species and is filter-feeding in nature (Fu and He, 2012). It is one of most intensively cultured species in China as well as worldwide. It has excellent values not only for food fish but also for biological control of cyanobacterial bloom formation in rivers, streams, reservoirs and ponds (Ke *et al*., 2009). Due to the rapid growth rate, easier cultivation, high feed efficiency ratio and high nutritional value, its commercial production has increased dramatically in the past few years. Annual worldwide silver carp production was 4.0 MMT (FAO, 2010), but according to recent statistics, it has gone beyond 10.0 MMT and is the second major aquaculture species produced across the world (FAO, 2018).

However, over-fishing and habitat fragmentation

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have significantly reduced the natural populations of silver carp in Asian countries. Due to long term propagation and improper broodstock management, production traits are also reduced (Shi *et al*., 2009). The disappearance of egg-laying and fry nursing grounds is also caused by rapid industrialization and corresponding aquatic pollution. Furthermore, the improper raising of fry has severely minimized the genomic heterozygosity of farmed silver carp, causing a decline and reduction in its growth performance. Besides this, the introgressive hybridization has become a usual problem between silver carp and its closely-associated bighead carp. Therefore, the genetic status of silver carp must be conserved and examined (Lamer *et al*., 2010).

Besides the ability to determine the relatedness among and within species, good natural populations need an observable ecology with environmental aspects likely to influence the individuals fitness. Estimation of species genetic variations is necessary for many reasons as it guarantees the fitness and development of a population in a constantly fluctuating environment. Therefore, the information is essential to circumvent the environmental damage and to make effective management for exploited populations (Ullah *et al*., 2015).

Molecular markers occur naturally; they are inherited inevitably, furnish a foundation for statistical data analysis and thus, they are preferably suitable for estimation of genetic status and variability in feral populations. Molecular markers are utilized to find out morphologically mysterious species, evaluate populations in regions of population socialization (Hauser *et al*., 2006), describe population boundaries and roots of juveniles in their lifetime migrations (Bowen *et al*., 2006). A promising benefit of molecular data is to assess population parameters, e.g. size of population (Turner *et al*., 2002), or to restructure demographic records of population development or bottlenecks in population size. Knowledge of responses of historic environmental or climatic disruptions can provide clues to how populations encounter with upcoming challenges. Data achieved by molecular markers are especially useful for assessment of kinship among individuals in fish populations (Banks *et al*., 2003), for determination of reproductive success and forensic identification (Birstein *et al*., 2000).

Among the molecular markers, microsatellite DNA markers have already been proved to be robust tools for population genetic analysis due to high abundance, even distribution throughout the genome, high polymorphism and amenability to PCR. The purpose of the study was to estimate the magnitude of genetic diversity in populations of *H. molitrix* in Indus River as well as to assess the current population status in *H. molitrix* stocks in Indus River.

MATERIALS AND METHODS

Sampling and DNA isolation

Sampling of *Hypophthalmichthys molitrix* (silver carp) was done from five particular sites of River Indus viz. Chashma Barrage (CB), Taunsa Barrage (TB), Guddu Barrage (GB), Ghazi Ghat (GG) as well as Jinnah Barrage (JB). Total 125 individuals were collected; 25 from each sampling site. The collected individuals were kept in ice and transported immediately to the laboratory where they were stored at -20°C. DNA extraction from dorsal muscle tissues was done by proteinase-K and standard phenol/chloroform DNA isolation methods of Sambrook and Russell (2001), with slight modifications.

The quality of extracted DNA was appraised through 0.8% AGE incorporated in TAE buffer with bromophenol blue loading dye. 1µL DNA sample was used for checking the concentration of isolated DNA by nanodrop. Concentration was adjusted at 50ng/µL for PCR by mixing the stock solution of DNA with nuclease-free water.

Amplification of targeted loci

Genomic DNA was PCR amplified by *Labeo rohita* cross-species amplification of *H. molitrix*. Five primers viz. Lr22, Lr40, Lr21, Lr38 and Lr12 were used taken from gene link (Singh *et al*., 2008). The microsatellite primers characteristics are given in Table I. PCR reaction was conducted in 25µL reaction mixture that contained the following ingredients: 2µl of each primer set, 12µl of PCR master mix 2X (Thermo Scientific, USA), 3µl of 50ng template DNA and 6µl nuclease-free water. The PCR cycles were as follows: Three min initial denaturation at 94°C followed by 30 cycles of 1 min at 94°C, 30 sec at a primer-specific annealing temperature for 1 min at 72°C, and final elongation for 4 min at 72°C.

Following the PCR amplification, the amplicons were confirmed for the amplification through 0.8% agarose gel electrophoresis. The PCR products were isolated on a 5% non-denaturing PAGE containing 19:1 acrylamide: bis-acrylamide and then visualized by silver staining method of Sanguinetti *et al*. (1994). Electrophoresis was carried out using a SequiGen sequencing gel electrophoresis method. For the visualization of DNA bands, silver-staining methods were used. After gel imaging in the gel documentation system (UVCI, Major Science, USA), the allelic bands were scored manually and compared with the mixed range DNA ladder (Thermo Scientific, USA) to determine the size of allelic bands.

Table I. SSR markers of *H. molitrix* with details.

S. No	No. of locus	Repeat sequence	Gene bank accession No.	Sequence of primer (5`-3`)	T_a (°C)	No. of alleles
1	Lr12	$(CA)_{13}$	AJ507524	F: CACCGCTGCTGTCCATCA R: AGGTCGGCCAGATACACG	58	4
2	Lr21	$(CA)_{11}$	AJ831436	F:GATCAGAGGGTCAATGTGG R: CAGCAGAGTACTATGGAAGA	58	6
3	Lr22	$(TG)_{19}$	AM285342	F: GATCTGTGTGTGTGTGTGC R: GGTGGCGACACAACAAATG	58	4
4	Lr38	$(GT)_{12}$	AM269528	F: ATAGCATCACCATCTGTTGGTG R: TCTGCTTCAGTCACTCAGCAC	59	2
5	Lr40	$(GT)_{15}$	AM269530	F: GATCAATCTTACAGTAATCTTG R: AGACGGAGATATGATGAACTG	54	2

Genotypic data analyses

The microsatellite data set was analyzed for detecting probable genotyping errors (i.e. null-alleles, large allele dropout and stuttering bands) with the software Micro-Checker 2.2.1 (Oosterhout *et al*., 2004). To describe the genetic characteristics of a population, allele frequencies, number of alleles (Na), effective number of alleles (Nae), allelic richness (Ar), observed (Ho) and expected (He) heterozygosity, as well as inbreeding coefficient (F_{IS}) were estimated with FSTAT Version 2.9.3.2 (Goudet, 2002).

Genetic divergence among populations was assessed with F_{ST} (Weir and Cockerham, 1984). By AMOVA, the hierarchical partition of genetic diversity was assessed using ARLEQUIN (Excoffier *et al*., 2005). Using software TFPGA Version 1.3 (Miller, 1997), UPGMA dendrogram based on Nei's (1972) unbiased distance was assessed. Population structuring was also assessed with the software Structure 2.3.2 (Falush *et al*., 2003), using a burn-in length of 50,000 and 100,000 MCMC (Monte-Carlo Markov Chain) iterations. Five independent runs were conducted for each k-value and the number of genetic clusters was determined according to Evanno *et al*. (2005) with Structure Harvester (Earl and Vonholdt, 2012).

RESULTS AND DISCUSSION

In the current study, the "Micro-Checker" software was applied to the genotypic data obtained for *Hypophthalmichthys molitrix* populations that showed no scoring errors related to large allele, stuttering bands and presence of null alleles at all the loci employed for genotyping. All *H. molitrix* populations had screened microsatellite loci that were found to be polymorphic. The patterns of genetic variability varied depending on the screened microsatellite loci and examined fish population.

Genetic diversity

Microsatellite diversity indices for each population have been outlined in Table II. The average allele frequency and allele size ranging from 0.004 to 0.552 and 140 to 174bp, respectively were observed at various screened loci in *H. molitrix* populations. All the loci were found to be polymorphic. The number of alleles (Na) and allelic richness (Ar) per locus ranging from 2.00 to 7.00, with an average of 3.2 to 3.4 and 3.2 to 3.4, respectively, were observed. Regarding the number of alleles and allelic richness, the largest average value was calculated in the population of GB as well as TB and minimum in the population of GG. The average values of effective number of alleles (Nae) were observed ranging from 2.404 to 3.02 in various studied *H. molitrix* populations.

Heterozygosity (H) level was observed moderate in all examined *H. molitrix* populations. The average observed heterozygosity (H_O) were measured ranging from 0.56 to 0.816. The fish population JB revealed the lowest value of H_O and GB population showed the highest value as compared to others. Average values of expected heterozygosity (He) were measured, ranging from 0.58 to 0.649. The largest H_e value was observed for TB population whereas the lowest for JB population.

The values of 1-Ho/He were found positive mostly at all the screened SSR loci with exception at some loci where negative values were also observed. On the average base, populations showed positive average values for 1-Ho/He except one population, i.e. CB.

On average, the inbreeding coefficient (F_{IS}) values were found to be positive, except one population CB that showed negative mean value. F_{IS} values ranging from -0.149 to 0.088 were recorded at various screened SSR loci. Highest average F_{IS} value was measured for TB (0.088) while the lowest for CB (-0.149) population of *H. molitrix.* The mean values of F_{IS} in GG, JB and GB were observed as 0.013, 0.016 and 0.036, respectively.

Table II. Genetic diversity at different SSR loci in *H. molitrix* populations collected from riverine sites.

Population	Parameters	Loci					Average
		Lr-12	Lr-21	Lr-22	Lr-38	Lr-40	
JB	Na	3.000	7.000	3.000	2.000	2.000	3.4
	Ar	3.000	6.880	3.000	2.000	2.000	3.376
	Nae	2.723	2.777	2.777	1.854	1.891	2.404
	Ho	0.720	0.440	0.560	0.560	0.520	0.56
	He	0.645	0.653	0.653	0.470	0.480	0.58
	1-Ho/He	-0.116	0.326	0.142	-0.191	-0.083	0.016
	F_{IS}	-0.118	0.331	0.145	-0.196	-0.083	0.016
	PHWE	0.434^{NS}	0.421^{NS}	0.421^{NS}	0.409^{NS}	1.000^{NS}	
TB	Na	4	5	4	2	2	3.4
	Ar	4	4.999	4	2	2	3.4
	Nae	3.644	3.655	3.834	1.996	1.971	3.02
	Ho	0.64	0.6	0.64	0.72	0.72	0.72
	He	0.74	0.741	0.754	0.509	0.502	0.649
	1-Ho/He	0.135	0.19	0.151	0.414	-0.434	0.091
	F_{IS}	0.106	0.194	0.122	-0.426	0.445	0.088
	PHWE	1.000^{NS}	0.421^{NS}	0.150^{NS}	0.410^{NS}	0.043*	--
GG	Na	3	5	4	2	2	3.2
	Ar	3	4.999	4	2	2	3.2
	Nae	2.302	3.709	3.041	1.996	2	2.61
	Ho	0.52	0.72	0.68	0.56	0.6	0.616
	He	0.577	0.745	0.684	0.509	0.51	0.605
	1-Ho/He	0.098	0.033	0.005	0.1	-0.176	0.012
	F_{IS}	0.101	0.035	0.007	0.102	-0.18	0.013
	PHWE	1.000^{NS}	0.082^{NS}	0.010*	0.699^{NS}	0.440^{NS}	--
CB	Na	3	6	4	2	2	3.4
	Ar	3	5.96	4	2	2	3.392
	Nae	2.51	4.058	3.698	1.891	1.923	2.816
	Ho	0.52	0.76	0.72	0.6	0.8	0.68
	He	0.613	0.769	0.744	0.48	0.489	0.619
	1-Ho/He	0.151	0.011	0.032	-0.25	-0.635	-0.138
	F_{IS}	0.156	0.012	-0.002	-0.254	-0.655	-0.149
	PHWE	1.000^{NS}	0.190^{NS}	0.675^{NS}	0.392^{NS}	0.002**	--------
GB	Na	4	5	4	2	2	3.4
	Ar	4	5	4	2	2	3.4
	Nae	3.731	4.18	2.997	1.987	1.95	2.969
	Ho	0.84	0.92	0.88	0.92	0.52	0.816
	He	0.746	0.776	0.68	0.506	0.497	0.641
	1-Ho/He	-0.126	-0.185	-0.294	0.818	-0.046	0.033
	F_{IS}	-0.128	-0.19	-0.302	0.846	-0.047	0.036
	PHWE	1.000^{NS}	0.183^{NS}	0.115^{NS}	0.001*	1.000^{NS}	--

Na, number of alleles; Ar, allelic richness; Nae, effective number of alleles; Ho, observed heterozygosity; He, expected heterozygosity; Fis, inbreeding cofficient values; CB, Chasma Barrage; TB, Taunsa Barrage; GB, Guddu Barrage; GG, Ghazi Ghat, JB, Jinnah Barrage.

Out of 25 tests, a total of 4 tests were found to deviate from HWE significantly after applying multiple test correction. At various screened loci, the pairwise p-value was found significant at $p < 0.05$.

Population genetic structure

Using windows-based program FSTAT, the pair-wise population differentiation (F_{ST}) across all the screened microsatellite loci among various *H. molitrix* populations was analyzed and found to be significant (*P<0.05*) statistically between most of the population pairs. The significant findings regarding population genetic differentiation for most of the stocks revealed genetically non-homogenous groups. The pair-wise estimates of F_{ST} indicated a moderate level of population genetic differentiation among the studied *H. molitrix* populations. Highest level of differentiation was found 0.0724 in GG-GB, while the lowest 0.0084 between the populations of CB and JB.

Using the windows-based software TFPGA, the genetic distance (GD) was calculated based on the allele frequency data by considering all the examined populations. Among pairs of populations, the unbiased genetic distance indicated considerable variation (*P<0.05*) in magnitude. The highest value of genetic distance was examined 0.1226 in JB-GB while, the minimum 0.0115 between the TB-GB, as shown in Table III.

Table III. Nei's unbiased genetic distance (below diagonal) and pairwise population differentiation (*FST*) (above diagonal) between populations of *H. molitrix*.

Populations	GG	CB	JB	TB	GB
GG	--	0.0396*	0.0432*	0.0196*	0.0724
CB	0.0210	--	0.0084**	0.0179*	0.0116*
JB	0.0678	0.0280	--	0.0143*	0.0122*
TB	0.0115	0.0287	0.0640	--	0.0287*
GB	0.0143	0.0440	0.1226	0.0154	--

*Significant at *P<0.05*. For abbreviations see Table II.

Table IV. Analysis of molecular variance (AMOVA) table for *H. molitrix* populations.

SOV	df	MSS	Variance	% Variation
Between populations	4	8.502	0.1388	6.7839
Between individuals within populations	120	1.559	0.3481	17.0112
Within individuals	125	2.256	2.2560	76.205

The AMOVA indicated low variation percentage (17.0112%) between individuals within populations and revealed that most of the variation (76.205%) lied within the individuals. The AMOVA further specified that 6.7839% variation was contributed due to the variation between populations in this study (Table IV).

Across all the screened microsatellite loci, the gene flow (Nm) rate in various examined populations of *H. molitrix* was measured by using the windows-based program. The largest value of *Nm* was observed 13.2605 at locus *Lr-38* while the lowest value of *Nm* was noted 4.1487 at locus *Lr-22*. Overall SSR loci, the average value of Nm was observed 8.0037 (Table V).

Table V. Gene flow (*Nm*) at different SSR loci in *H. molitrix* populations.

Locus	Sample size	Nm
Lr-12	250	8.1440
Lr-21	250	5.6312
Lr-22	250	4.1487
Lr-38	250	13.2605
Lr-40	250	8.8343
Mean	250	8.0037

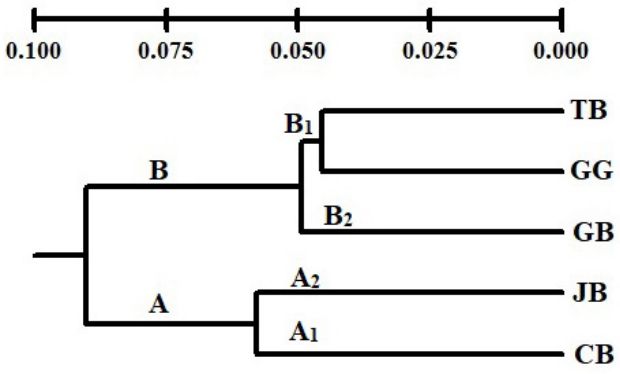

Fig. 1. UPGMA dendrogram based on Nei's genetic distance showing the relationship and clustering patterns between *H. molitrix* populations.

Genetic relatedness was further investigated by constructing UPGMA dendrogram (Fig. 1). Two major clusters or clades A and B were observed which predict that the populations in both clusters had shown a close relationship. Cluster A further divided into cluster A_1 and A_2. The riverine population of *H. molitrix* CB was present in cluster A_1 while, the population of JB in the cluster A_2. Cluster B was divided further into B_1 and B_2. B_1 was further clustered in two groups containing TB and GG whereas B_2 consisted of GB.

For the populations of *H. molitrix*, microsatellite data analyses by the Structure grouping algorithm method proposed the occurrence of two discrete genetic clusters. For each K value, constant results were obtained across the 6 autonomous runs. Structure Harvester admixture model inferences showed highest estimated log-likelihood mean value and delta-k value for K= 2 in this study (Fig. 2).

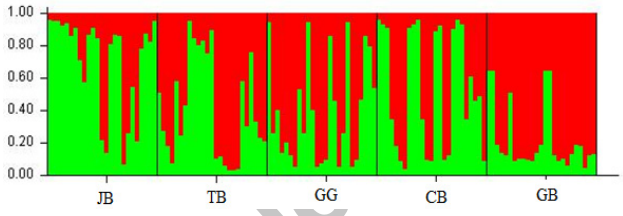

Fig. 2. Genetic structuring patterns among populations of *H. molitrix* as revealed by structure analysis. The two distinct colors of the column represent the estimated possibility of belonging to two populations, and each vertical column represents one individual. Distinct colors in the same individual indicate the %age of the genome shared with each cluster.

DISCUSSION

The assessment of genetic diversity in aquaculture or wild fish stocks is essential for effective management, interpretation and conservation of fish stocks (Hallerman, 2003). Genetic diversity enables the ecological adaptations to ensure the chances of survival of a single species or a population and is thought to be vital for the survival of a species. Due to direct and indirect anthropogenic activities, biodiversity has been subjected to restrictions. Reducing the sites of natural populations may have resulted in reduced evolutionary options in the face of environmental modifications owing to the loss of genetic diversity (Artoni *et al*., 2009). Most of the fish used for human consumption come from wild sources such as rivers and major lentic water bodies (Saini *et al*., 2008; Agostinho *et al*., 2008); therefore, natural stocks are at risk. The commercial fishing in natural stocks has caused genetic depletion that is peculiar to fisheries management and the line of the genetic resources of natural fish populations has now become a major issue in fisheries management (Yilmaz and Okumuu, 2002).

The current study was designed to underpin the genetic diversity of *H. molitrix* in Indus River by using molecular markers. The protocols followed were fairly efficient for the genomic DNA isolation from muscle tissues, its analysis and successive PCR-based genotyping of the fish individuals at target loci. SSR markers have

proven to be very robust for determining genetic status of *H. molitrix* in sampled populations. With countless applications in population genetics, conservation biology, and evolutionary biology, microsatellites are the most common and diverse genetic markers (Muneer, 2014). Liu and Cordes (2004) emphasized the significance of molecular markers in aquaculture genetics as they make genetic variability observable throughout the genome.

In the current study, allelic diversity level was observed low-to-moderate in *H. molitrix*, ranging from 2.0 to 7.0. When compared to our study, Liao *et al*. (2007) revealed number of alleles varying from 4 up to a maximum of 15 in *H. molitrix* by using the same markers. The obvious basis for reduced allelic diversity in *H. molitrix* population is the invasiveness as the species is not native to Pakistan and has recently been introduced in the country. The genetic status of the silver carp populations was also assessed by Ji *et al*. (2009) by using SSR markers and they reported that conventional breeding approaches in hatcheries has resulted in allelic loss primarily owing to low effective population size.

The average value of effective number of alleles in the *H. molitrix* populations, ranging from 2.40 to 3.02, was examined. In the fish population sampled from TB, the highest value of *Nae* was noted while the lowest in the JB population. Compared with the observed number of alleles, less *Nae* was noted indicating loss of alleles in different populations examined. When the actual number of alleles is greater in value relative to the effective number of alleles, which shows that all allele frequencies are not equivalent. Using microsatellite markers, limited effective number of alleles was reported in *C. catla* by Hansen *et al*. (2006).

In this study, the average values of observed heterozygosity ranged from 0.56 up to a maximum of 0.816. The fish population taken from JB showed the least *Ho* whereas GB population showed the maximum value as compared to others. The decreased levels of *Ho* in the riverine populations might correspond to the restocking programs (Zhou *et al*., 2011).

The F_{IS} values were found positive on the average in all the populations, except for the population CB which exhibited negative mean values. In this study, the highest average F_{IS} value was noted in population TB while the lowest in CB population. Negative mean F_{IS} value in CB population indicated the excessive heterozygosity and revealed that this population does not lose heterozygosity and the individuals in this population could be termed as outbred. Positive F_{IS} values verify the excessive homozygosity and significant deviation in a population from the HWE (Zhang *et al*., 2015).

After applying multiple test correction, a total of 4 tests out of 25 were examined to deviate significantly from HWE. The deviation from HWE could primarily be attributed to heterozygote deficiencies, inbreeding and the existence of null alleles (Adams *et al*., 2011; Guo *et al*., 2013). Any deviation from HWE may show stratification of the population, genotyping issues as well as inbreeding. Li *et al*. (2010) and Keller *et al*. (2013) reported the similar results for an extensive range of 126 freshwater fish species.

In current study, pair-wise estimates of F_{ST} showed moderate population genetic differentiation among the *H. molitrix* populations studied. Maximum level of differentiation was found to be 0.0724 in the population GG-GB while, the minimum 0.0084 between the CB and JB. Dissimilar genetic origin was indicated by the highest level of genetic differentiation, while the lowest suggested similar genetic origin of these populations. Limited dispersal potential between freshwater fish exists due to anthropogenic activities, which becomes the reason for prominent genetic differentiation levels. Different evolutionary forces including individual migration, random genetic drift, mutual interactions or DNA mutation also affect the patterns of genetic distribution and genetic structure among populations. Ou *et al*. (2009) reported the low-to-moderate level of genetic differentiation in natural stocks of *Ctenopharyngodon idella*. Similar results were given by Zhu *et al*. (2015) in *H. nobilis* and Zhang *et al*. (2007) in *C. idella.*

Analysis of molecular variance (AMOVA) is an appropriate standard for evaluating the population genetic structure and determining genetic similarity and differentiation between populations (Excoffier, 2001). The AMOVA suggested that the majority of variations exist within individuals in riverine populations of *H. molitrix*. Chaturvedi *et al*. (2011) and Gopalakrishnan *et al*. (2009) detected similar patterns of genetic structuring in other freshwater fish species in aquatic systems of the region.

On the basis of Nei's unbiased genetic distance in the *H. molitrix* populations, the UPGMA dendrogram was constructed to investigate the genetic relationship in them. Partially following their geographic distribution, the dendrogram grouped the populations into two utmost groups showing their genetic relatedness. Despite greater geographic distance, populations in the same cluster with restricted genetic distance show the broodstock management patterns of the fisheries department in the province. This is due to the paucity of a genetic management plan in Pakistan for fish genetic resources (Haque and Hoq, 2016).

CONCLUSION

To minimize the genetic effects, it is just necessary

to enhance the genetic management related to aquatic species by examining the genetic integrity. Meanwhile, many *H. molitrix* alleles are at risk of extinction. For the conservation of natural sources and preventing the alleles from damaging, we just need to strengthen the monitoring and conservation of riverine resources; this can make the *H. molitrix* populations much stronger and overfishing must be prohibited in natural water system. The findings of this research will be useful for restoring, conservation and monitoring the natural aquatic fish species in Pakistan. To preserve the genetic integrity of *H. molitrix*, there is a dire need to develop a great genetic management policy.

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IRB approval

IRB was obtained from Institutional Biosafety and Bioethic Committee (IBC), Department of Zoology,Wildlife & Fisheries. UAF, Faisalabad, Punjab, Pakistan.

Ethics statement

This work was carried out by following all the guidelines of National Biosafety 2005, Punjab Biosafety Rules 2014 and Punjab Animal Health Act 2019.

Statement of conflict of interest

The authors have declared no conflict of interest.

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