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RESEARCH PAPER

Microsatellite Diversity and Population Structure of *Hypophthalmicthys molitrix* in Hatchery Populations of Punjab

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Abstract

The genetic integrity of the commercially important fish species is being compromised mainly due to artificial propagation. The present study evaluated the extent of genetic variability and assessed the population structure of Silver Carp (*Hypophthalmicthys molitrix*) in hatchery stocks of Punjab province, Pakistan using 16 species-specific microsatellite loci. The results revealed low-to-moderate scale of genetic variability in the hatchery stocks. The level of average observed heterozygosity varied from 0.3770 to maximum of 0.5874 in all the stocks. Most of the hatcheries showed heterozygote deficit. The average values of inbreeding coefficient ranged from 0.174 to 0.441. Most of the locus-population combinations showed significant departures from Hardy-Weinberg equilibrium. The AMOVA revealed significant genetic structuring in hatchery populations of *H. molitrix*. The UPGMA dendrogram showed the populations clustering irrespective of their geographical proximities. The current research work provides a comprehensive knowledge regarding the genetic structure and genetic diversity of *H. molitrix* populations which may be applicable for the management of the concerned populations to maintain their genetic quality in Pakistan.

Keywords: Silver Carp, DNA markers, population genetics.

Introduction

Like indigenous carps, artificial propagation of exotic carps is also posing a big threat to the genetic integrity of fish species including Silver Carp (*Hypophthalmicthys molitrix*) in Pakistan. For instance, hatchery stock deterioration is much debated issue in fisheries sector due to genetic erosion which can be explained by insufficient number of effective breeders, negative selection, poor broodstock management, hybridization and inbreeding among exotic fish species (Hauser & Seeb, 2008). The alterations in the hydrography and human interventions in the natural water bodies have degraded the nursing and breeding sites of fish (Diana, 2009). Deterioration of water quality and disruption in migration channels resulted in the ecological problems (Van Overzee & Rijnsdorp, 2015). Major cause behind the diminishing exotic fish stocks is overfishing and degraded fish biodiversity, making them more susceptible to climatic variations (Paukert *et al*., 2016).

With the expansion of aquaculture in Pakistan, the demand for fish seed is also increased. About 99% of fish seed is produced through artificial propagation by public and private hatcheries in Pakistan to meet requirement of expanding aquaculture practices (Khan, Shakir, Khan, Abid, & Mirza, 2008). Generally, stakeholders maintain few small sized brooders to keep production cost minimum; therefore, there is always a risk of producing poor quality seed (Munilkumar & Nandeesha, 2007). Utilization of small number of fish brooders, generation after generation, may cause reduction in genetic variability and result in indiscriminate hybridization, inbreeding and loss of genetic purity of Chinese fish species (Ellergern & Galtier, 2016). Therefore, regular monitoring of genetic variability in fish hatchery stock is essential to detect whether breeding programs are affecting genetic integrity by reducing genetic variation (De-Cara, Villanueva, Toro, & Fernandez, 2013) which is lacking in Pakistan. By upgrading and managing standards of breeding activities and brood stock in hatchery operations, aquaculture outputs can be enhanced and their influence on capture fisheries biodiversity can be reduced (Xu *et al*., 2015).

DNA markers techniques are extensively utilized to investigate different issues pertaining to genetics of aquaculture species (Khoo, Lim, & Phang,

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2011). Selectively neutral markers can provide an insight into dissemination of genetic diversity within and among the fish populations (Avise, 2010) and could be helpful to assess the level of genetic diversity in cultured stock (Askari, Shabani, & Miandare, 2013). Microsatellite markers have been considered for analysis of hatchery fish stocks (Defaveri, Viitaniemi, Leder, & Merila, 2013). Moreover, the microsatellite markers have been developed for Silver carp for monitoring the influence of conventional hatchery practices on genetic integrity of species (Liao *et al*., 2007) to provide valuable information for their effective management in hatcheries. The main objective of the current study was to evaluate the extent of genetic diversity of *H. molitrix* in hatchery populations of Punjab by using microsatellite markers.

Materials and Methods

Specimens of *H. molitrix* were collected from eight selected hatcheries from different districts of the Punjab province of Pakistan (Table 1) designated in map (Figure 1). Thirty individuals from each population were collected in ice boxes and transported to the laboratory for preservation at -20 ºC till DNA extraction. DNA extraction from dorsal muscle tissues was done with slight modifications in the methods of Yue & Orben (2005) and that of Green & Sambrook (2012).

The quantitative and the qualitative assessment of DNA was done on 0.8% agarose gel. To get optimum PCR amplification, the concentration of each DNA sample was quantified for dilution. The quantification of DNA samples was done using UV spectrophotometer (NanoDrop 2000c, Thermo Scientific, USA). The final concentration of the DNA samples was adjusted to almost 50 ng/mL using TE to have uniform concentration of each fish sample.

Sixteen species-specific microsatellite markers were used for genotyping the *H. molitrix* individuals at specific temperature (Liao *et al*., 2007) (Table 2). The primers were purchased from the company Gene-Link, USA. The PCR reaction was carried out in a 20 µl reaction mixture in labeled PCR tubes using thermal cycler (Multigene, LabNet, USA). The mixture included 0.8 µl of each primer set of 10 µM concentration, 0.4 µl dNTPs of 10 mM concentration, 2.0 µl of 10 x PCR buffer (20 mM), 1.5 µl $MgCl_2$ (20 mM), 0.4 µl (2 U/ µl) *Taq* DNA polymerase (Fermentas, USA) and about 50 ng of the template DNA. The PCR cycles were adjusted for 5 minutes denaturation at 94˚C, 32 cycles of 1 minute at 94˚C, 30 seconds at a primer-specific temperature for annealing, 1 minute at 72˚C and final elongation for 4 minutes at 72˚C. The amplicons were tested using 2% agarose (Pei, Costumbrado, Hsu & Kim, 2012) and finally resolved on polyacrylamide gel followed by silver staining (Qu., Li, Wu, & Yang, 2005). The gel imaging was done in gel imaging system (UVCI, Major Science, USA)

The allelic data obtained through individual genotyping were analyzed by using different analytical programs/software. FSTAT (ver. 2.9.3.2)

Table 1. Sampling details of *H. molitrix* from selected hatcheries of Punjab, Pakistan

Sr. No	Population Name	Code	District	Collection date
1	Central Fish Seed Hatchery, (Manawan)	LHR	Lahore	14.11.2014
2	Fish seed hatchery, Mureedkey	GWH	Gujranwala	19.03.2015
3	Fish seed hatchery, Satiana Road,	FSD	Faisalabad	03.06.2014
4	Fish seed hatchery, Mian Chanu	KWH	Khanewal	14.01.2014
5	Fish seed nursing unit, Farooqabad	SHK	Sheikhupura	27.01.2015
6	Fish seed nursing farm, Sargodha	SGD	Sargodha	30.10.2014
7	Fish Seed Nursing Farm, Sialkot	SKT	Sialkot	02.09.2014
8	Fish seed nursing farm, Toba Tek Singh	TTS	Toba Tek Singh	19.06.2014

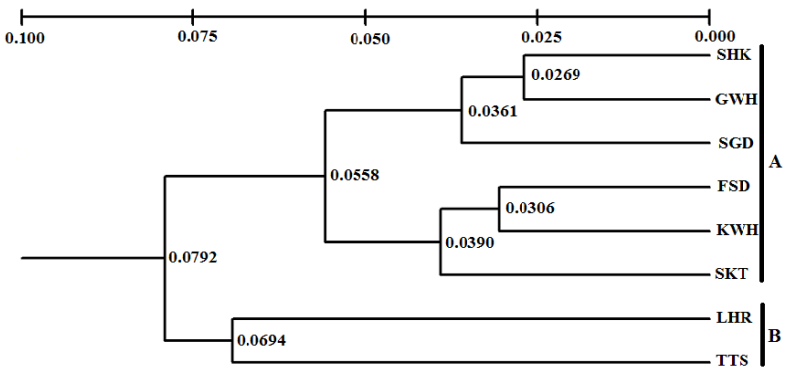

Figure 1. UPGMA dendrogram based genetic relatedness between the hatchery populations of H. Molitrix.

Table 2. Characterization of microsatellites for *H. molitrix* (Liao et al., 2007)

Locus	Motif	Primer sequence (5′-3′)	Annealing temperature (˚C)
BL42	(TG)14	F:TGCCGATGTTATGTTTGCT R:TGCTTGTGGGTGAGTTTCT	52
BL56	(AC)16	F: TTAGGTGAACCCAGCAGC R:AAGAAGCATTAGTGCAGATGAGTAC	54
BL64	(TG)23	F: GCCAGGCTAGAAGAACCACC R: TTGCAGCACAGTTACCAAGACA	54
BL67	(AC)17	F: GGCAGGCTTCAAAGGACA R: CTCGCCCTCATTGTCGTA	54
BL73	(AC)6	F: TGACTTTACACGGCTCCA R: TTACTCTGTTATGGTGGGTCA	54
BL83	(AC)6	F: CTATCCGCCCTGTTCTGA R: ACCAAACATCCCTCAAGC	54
BL90	(CA)19	F: ATGCGAGGGTGGATGATGGG R: GGAAAGCAAAGCCTGGACTA	52
BL106-2	(AC)14	F: TTTAATTCTTCTAGCTGGACACG R: CACTCCTCTTCCCTCGTAAAT	54
BL108	(GT)9	F: GATGAATCGCAGGGCGTGAGG R: GCAGAACACGCACAATGGAGA	54
BL109	(TG)21	F: GTGTCCTGGATTCTAGCCG R: CATGAGAGAAACACCTGAACA	54
BL116	(CT)15	F: GCGGGATGAGTTTGAAGAA R: TATGGACTGGACTGCTGGAT	54
BL123	(TG)9	F: GCGACAGGAACAGTGAAAAC R: CAAAGAAGGCACAAAGGATT	54
BL132	(AC)10	F: CTTTGACTGCTGGTTGGTTGT R: TTTCTTGTCTTCCTGGCTTCT	54
BL138	(TG)8	F: ACTGAAAACATCACTGCCACG F: ACTGAAAACATCACTGCCACG	54
BL145	(TG)12	F: GTGATTGGACGGGATGAACTA R: TCTTTCTTTTCTGTCCGAGGG	52
BL167	(AC)6	F: AGTGCGCCTAAAGTAAAAG R: AACCAGGACGAATCAAGTA	52

(Goudet, 2002) and POPGENE (ver. 1.31) (Yeh, Yang, Boyle, Ye, & Mao, 1999) softwares were implemented to calculate the allelic richness (*Ar*), allelic frequency, expected and observed heterozygosity (*He* & *Ho*) and inbreeding coefficient $F_{IS.}$ The population differentiation F_{ST} for sampling locations was calculated following Weir and Cockerham (1984). To adjust the statistical significance of deviations from Hardy-Weinberg equilibrium (*HWE*) sequential Bonferroni correction (Rice, 1989) was used and for each locus sample type-1 error of $\alpha = 0.05$ was maintained. The presence of null alleles was calculated by MicroChecker 2.2.3 (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004). Analysis of molecular variance (AMOVA) was conducted using ARLEQUIN ver. 3.1 (Excoffier, Smouse, & Quattro, 1992) to analyze molecular variance as well as to estimate the hierarchical partitioning in genetic diversity. The software TFPGA ver. 1.3 (Miller, 1997) was employed to construct UPGMA dendrogram based on Nei's (1972) unbiased distance.

Results

The present study assessed the level of genetic diversity and the population structure of Silver Carp (*Hypophthalmicthys molitrix*) in eight hatchery populations in different districts of Punjab, Pakistan.

In the hatchery stocks of *H. molitrix*, genetic diversity was measured in terms of allelic richness (*Ar*), observed (*Ho)* and expected (*He)* heterozygosity, inbreeding coefficient (F_{IS}) and Hardy-Weinberg equilibrium (*HWE*) over the sixteen microsatellite loci. The data set was analysed for presence and frequency of null alleles at all the loci. There were certain loci which yielded null alleles in an inconsistent pattern. No one of the loci showed null allele frequency more than 10%. All the screened loci were found to be polymorphic by following allele frequency criteria at *P* 0.95 criterion. The highest average values of allelic diversity were observed in the LHR (*Ar* = 6.522) to a minimum for TTS population (*Ar* = 4.732). The highest value for the allelic diversity (*Ar* = 11.93) was observed in the LHR hatchery population of *H. molitrix* over the locus BL109, while the lowest value at the locus BL67 (*Ar* = 2.00) in SHK population of *H. molitrix* (Table 3).

Low-to-moderate level of average observed heterozygosity was found in the present study that varied from 0.377 (TTS) to a maximum of 0.5875 LHR population of *H. molitrix.* The values of average

Table 3. Genetic structure analysis of *H. molitrix* in the hatchery stock selected from various districts of Punjab, Pakistan

Populations	Parameters	Loci																
		BL42	BL56	BL64	BL67	BL73	BL83	BL90	BL106-2	BL108	BL109	BL116	BL123	BL132	BL138	BL145	BL167	Average
LHR	A_a	6	5.998	7.993	8.926	3.933	4.933	8.853	7.867	3.997	11.93	3.933	6	6	6	7.997	4	6.522
	H_o	0.4000	0.4333	0.6667	0.4	0.7	0.6333	0.6333	0.6333	0.6333	0.7	0.4667	0.6.000	0.5667	0.7333	0.6667	0.5333	0.5875
	H_e	0.5272	0.6428	0.7222	0.7594	0.6217	0.6178	0.7211	0.73	0.6694	0.8661	0.5606	0.7628	0.7383	0.7728	0.7983	0.7161	0.7055
	F_{IS}	0.244	0.325	0.094	0.486	-0.109	-0.008	0.138	0.149	0.071	0.209	0.184	0.23	0.248	0.068	0.181	0.271	0.174
	HWE	0.0490 *	0.0590 ns	0.7060 ns	0.0002 ***	1.0000 ns	0.4814 ns	0.0205 *	0.1572 ns	0.4467 ns	0.3829 ns	0.1320 ns	0.2162 ns	0.4540 ns	1.0000 ns	0.1016 ns	0.1259 ns	
SHK	A_a	3.966	4.867	2.997	2	4	5	5.933	6	3.997	10.8	3.933	6	6	6	4	4	4.968
	H_o	0.2333	0.3	0.5333	0.4	0.7	0.7	0.7	0.4	0.6	0.6333	0.4333	0.6	0.4667	0.6	0.7	0.4333	0.527
	H_e	0.5594	0.5533	0.5267	0.42	0.6367	0.7306	0.7056	0.7172	0.62	0.825	0.5322	0.7211	0.7472	0.7078	0.6367	0.6683	0.6442
	F_{IS}	0.583	0.471	0.004	0.065	-0.083	0.059	0.025	0.456	0.049	0.248	0.202	0.184	0.39	0.169	-0.083	0.366	0.194
	HWE	0.0022 **	0.0225 *	0.7241 ns	1.0000 ns	1.0000 ns	0.7060 ns	0.4467 ns	0.0010 ***	0.2870 ns	0.2162 ns	0.0546 ns	0.1572 ns	0.2162 ns	0.1549 ns	1.0000 ns	0.0068 **	
GWH	A_a	5	5.93	2.933	2.933	4	5.997	8.86	6.997	3.997	10.926	3.933	7	6	7	5.997	5	5.781
	H_o	0.4667	0.4333	0.6333	0.4333	0.5	0.6	0.6	0.4667	0.5667	0.7	0.5333	0.4	0.6333	0.7667	0.5667	0.5333	0.552
	H_e	0.605	0.59	0.5128	0.455	0.545	0.7528	0.7817	0.76	0.5539	0.8661	0.5739	0.7556	0.7694	0.8333	0.6928	0.7489	0.6747
	F_{IS}	0.208	0.262	-0.219	0.065	0.099	0.219	0.248	0.4	-0.006	0.208	0.088	0.484	0.193	0.097	0.198	0.303	0.178
	HWE	0.4655 ns	0.0597 ns	0.4613 ns	0.6817 ns	0.0219 *	0.0017 **	0.1016 ns	0.0068 **	1.0000 ns	0.5886 ns	0.2881 ns	0.0017 **	1.0000 ns	1.0000 ns	0.0116 *	0.1284 ns	
TTS	A_a	3.999	2	2	3	4	4.933	4.933	5	5	10.993	3.933	5.997	5	4.996	5	4.93	4.732
	H_o	0.4333	0.0667	0.1	0.1333	0.4	0.6667	0.3	0.5	0.6	0.5333	0.3333	0.3667	0.2333	0.5333	0.4333	0.4	0.377
	H_e	0.5517	0.5	0.4861	0.545	0.6406	0.7156	0.6433	0.7428	0.7161	0.8633	0.6083	0.7372	0.6678	0.6717	0.7006	0.5711	0.6475
	F_{IS}	0.219	0.866	0.8	0.763	0.39	0.085	0.546	0.342	0.179	0.397	0.465	0.515	0.66	0.222	0.396	0.205	0.441
	HWE	0.1332 ns	0.0000 ***	0.0000 ***	0.0000 ***	0.0037 **	0.0546 ns	0.0010 **	0.1016 ns	0.0219 *	0.0117 *	0.0118 *	0.0000 ***	0.0002 ***	0.1259 ns	0.0020 **	1.0000 ns	
FSD	A_a	5	2.997	2	4	4	5	4.997	5	3	10.99	3.997	7	6	5.997	6	4	4.9987
	H_o	0.3333	0.5	0.4667	0.4	0.5333	0.5333	0.5667	0.5	0.4	0.5667	0.5667	0.5667	0.4667	0.7333	0.4667	0.5	0.5062
	H_e	0.5367	0.5228	0.4911	0.5861	0.6344	0.755	0.665	0.71	0.5761	0.8667	0.6072	0.7506	0.7661	0.7828	0.7472	0.7117	0.6693
	F_{IS}	0.318	0.06	0.067	0.333	0.176	0.309	0.164	0.311	0.321	0.361	0.084	0.261	0.405	0.08	0.39	0.313	0.247
	HWE	0.0490 *	0.4814 ns	0.7270 ns	0.0749 ns	0.0315 *	0.4179 ns	0.4789 ns	0.0324 *	0.1320 ns	0.0598 ns	0.1549 ns	0.0225 *	0.0219 *	0.2162 ns	0.0004 ***	0.4485 ns	
SGD	A_a	4	3	3	3.933	4	5	4.997	5	3.997	8.996	3.933	6	5	4.997	5.933	4	4.7366
	H_o	0.4333	0.4667	0.5333	0.4	0.5667	0.5667	0.6	0.5667	0.5667	0.6333	0.4333	0.6	0.4	0.6333	0.5333	0.4	0.5208
	H_e	0.61	0.5228	0.56	0.5061	0.6261	0.7117	0.6428	0.7428	0.6128	0.8611	0.5428	0.7994	0.7178	0.6978	0.6789	0.6939	0.6579
	F_{IS}	0.305	0.077	0.065	0.226	0.112	0.22	0.083	0.253	0.092	0.28	0.218	0.265	0.456	0.109	0.231	0.437	0.214
	HWE	0.1549 ns	0.7149 ns	1.0000 ns	0.4485 ns	0.2881 ns	0.0205 *	0.4789 ns	0.4179 ns	0.7234 ns	0.5886 ns	0.1259 ns	0.0730 ns	0.0002 ***	0.4540 ns	0.0315 *	0.0563 ns	
KWH	A_a	4.998	2	2	2.933	4	5	5	5	4	10	3.933	7	6	6	6	3	4.804
	H_o	0.4333	0.3	0.3667	0.4	0.6333	0.5333	0.4.000	0.5333	0.5333	0.5667	0.4667	0.4333	0.5333	0.7333	0.5667	0.4667	0.4937

Table 3. Continued

H_e								0.7.00									
	0.5778	0.4861	0.4861	0.4994	0.6633	0.6967	0.6661	0	0.6994	0.8511	0.5606	0.7911	0.6933	0.7789	0.7567	0.5844	0.6557
F_{IS}	0.25	0.367	0.262	0.215	0.062	0.25	0.414	0.254	0.253	0.349	0.184	0.466	0.247	0.075	0.267	0.218	0.2583
HWE	0.1341 ns	0.0617 ns	0.2559 ns	0.2559 ns	0.1549 ns	0.0068 **	0.0205 *	0.0749 ns	0.0017 **	0.0730 ns	0.1320 ns	0.0038 **	0.0749 ns	1.0000 ns	0.0563 ns	0.2881 ns	
A_r	5	2.999	3	3.933	4	4	5	6	4	8.996	4	5.997	6	5	6	4	4.87
H_o	0.4333	0.3667	0.4667	0.4333	0.4667	0.7333	0.6333	0.6667	0.4667	0.7	0.5667	0.6333	0.6333	0.6667	0.5333	0.3	0.5437
H_e	0.5939	0.5294	0.5228	0.5139	0.6322	0.69	0.7344	0.7333	0.7039	0.825	0.6417	0.7506	0.7828	0.7711	0.7378	0.6917	0.6784
F_{IS}	0.223	0.297	0.124	0.173	0.278	-0.046	0.154	0.108	0.352	0.168	0.134	0.161	0.207	0.152	0.293	0.578	0.209
HWE	0.2747 ns	0.0597 ns	0.7060 ns	0.4485 ns	0.0315 *	0.7109 ns	0.4467 ns	0.1572 ns	0.1320 ns	1.0000 ns	0.4789 ns	0.2559 ns	0.1284 ns	0.3784 ns	0.0563 ns	0.0038 **	

Statistically significant values are marked with asterisks. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$; ns = non-significant

Here (A_r) designated for allelic richness, (H_o) observed heterozygosity, (H_e) expected heterozygosity, (F_{IS}) inbreeding index and (*HWE*) deviation from Hardy-Weinberg equilibrium

expected heterozygosity ranged from 0.6475 (TTS population) to maximum 0.7055 (LHR population).

Inbreeding coefficient (F_{IS}) for each hatchery population of *H. molitrix* was calculated across all the loci. The mean values of F_{IS} ranged from 0.174 in the LHR population to a highest of 0.441 for the TTS population indicating heterozygote deficiency in all populations. The $\chi 2$ values (for loci) clearly indicated the significant deviation from Hardy-Weinberg equilibrium (*HWE*) in all the hatchery populations of *H. molitrix*. Out of 128 tests, 38 tests showed significant deviation from *HWE* in the hatchery populations of *H. molitrix*. Non-significant deviations from *HWE* were observed at Locus BL138 in all the hatchery populations.

Across all the microsatellite loci, the pairwise genetic differentiation (F_{ST}) among hatchery stocks of *H. molitrix* was analysed by FSTAT. The study revealed statistically significant ($P<0.01$) differentiation among most of the population pairs which reflects that most of hatchery populations are genetically non-homogenous (Table 4). The values of F_{ST} among the population pairs ranged from 0.0014 (FSD-GWH) to 0.0297 (TTS-KWH). The results of AMOVA indicated that most of the variation percentage lie within the individuals (39.54%). The variation between populations and between samples within population was measured as 27.31% and 33.16%, respectively (Table 5).

The UPGMA dendrogram was constructed based on Nei's unbiased genetic distance among all the examined hatchery populations of *H. molitrix* by using the software TFPGA. The dendrogram showed hatchery populations of *H. molitrix* in two clusters/groups (Figure 2).

Discussion

In Pakistan, many studies have been conducted on nutrition, toxicology, physiology, growth performance and culture of Chinese carps but genetic issues pertaining to the subject species remained unattended. The available data on population genetics of *H. molitrix* is overwhelmingly reported from China and few studies from other regions. The study on genetic structure of *H. molitrix* was not reported from Pakistan so far. Hence, this is the very first information describing the population structure of *H. molitrix* in hatchery conditions of Punjab, Pakistan. Lack of expertise and infrastructure for undertaking genetic studies are the obvious reasons for the scarcity of work on genetic status of Chinese carp and even native fish species (King, Eackles, & Chapman, 2011).

The present study was conducted to underpin the genetic status of *H. molitrix* in selected hatchery populations of the Punjab province. The methodology used was fairly efficient for the extraction of genomic DNA from muscle tissues, its qualitative and quantitative analysis and subsequent PCR-based genotyping of the fish individuals at target microsatellite loci. The species-specific microsatellite markers employed for genotyping of the sample individuals proved to be robust tools for genetic monitoring of hatchery populations.

The present study revealed that the genetic diversity in hatchery stocks was found at low-to-moderate scale. The probable reason for the limited allelic diversity in hatchery populations of *H. molitrix* is the invasiveness because this species is not indigenous to Pakistan and was introduced in the

Table 4. Pairwise *FST* (above diagonal) and geographical distance (Km) (below diagonal) in the hatchery populations of *H. molitrix*

Populations	LHR	SHK	GWH	TTS	FSD	SGD	SKT	KWH
LHR	-	0.0114	0.0076*	0.0109	0.0249	0.004*	0.0132	0.0187
SHK	56	-	0.0062*	0.0120	0.0078*	0.002*	0.0138	0.008*
GWH	72	54	-	0.0128	0.0014*	0.002*	0.009*	0.002*
TTS	265	213	250	-	0.0254	0.006*	0.0224	0.0297
FSD	184	133	183	82	-	0.003*	0.002*	0.006*
SGD	189	139	175	160	92	-	0.009*	0.003*
SKT	143	138	52	304	223	229	-	0.008*
KWH	298	310	369	415	203	245	415	-

* = Significant statistically at $P<0.01$

Table 5. Hierarchical AMOVA in the hatchery populations of *H. molitrix*

Source of variance	df	MSS	Variance	% variation
Between populations	7	18.66	0.25	27.31
Between samples within Populations	232	6.43	1.23	33.16
Within individuals	240	4.21	3.32	39.54
Total	479	29.3	4.8	100

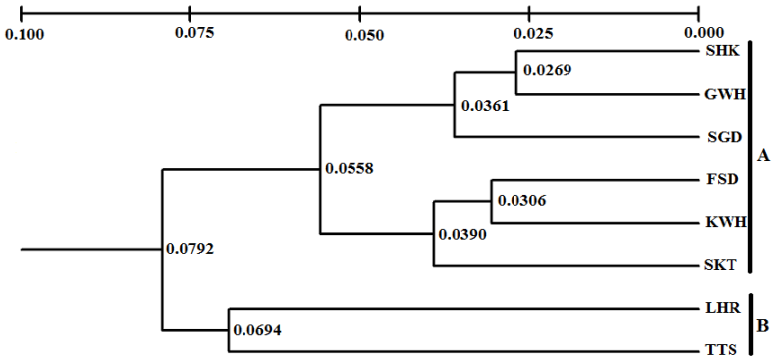

Figure 1. UPGMA dendrogram based genetic relatedness between the hatchery populations of *H. Molitrix.*

country during the recent past. During 1960s, the Chinese carps were introduced to Pakistan primarily in order to control the menace of vegetation in aquatic systems. Due to close ecological conditions, *H. molitrix* adapted well in Pakistan (DoF, 2005). Moreover, the technique of hypophysation for successful artificial spawning carps led to rapid expansion of Chinese carps in natural water bodies (Bondad-Reantaso, 2007). Although, there is no data available over the number of individuals of Chinese carps imported from China, the evidences suggest that the number was very small (in thousands). Secondly, the loss of allelic diversity due to mass production of fish seed with limited number of brooders in hatcheries is a common phenomenon in the Asian region (Aung, Nguyen, Supawadee, & Kamonrat, 2010). Microsatellite markers based studies on genetic structure of *H. molitrix* revealed that conventional breeding practices in hatcheries has resulted in allelic loss mainly due to low effective population size and other poor brood stock management practices (Ji, Gu, Mao, Zhu, & Sun, 2010; Ovenden *et al*., 2016).

Low to moderate level of allelic diversity (2-11) was observed in hatchery populations of *H. molitrix* in the present study. The reason of limited allelic diversity is directly influenced by bottleneck and founder effect because the hatchery managers kept limited number of brooders in artificial propagation for commercial reasons (Wang *et al*., 2008). Related findings about low to moderate level of the allelic diversity were reported by Liao *et al*. (2007) and allelic diversity ranged from 2 to 16 while developing primer for cross amplification of Silver Carp and Bighead Carp. This value can also be in the range of 2-7 in wild Silver Carp as described by Cheng, Liu, Yu, and Tong (2008).

The mean values of observed heterozygosity (*Ho*) varied from 0.3770 to 0.5874 in the hatchery populations of *H. molitrix*, in this study. The lower level of *Ho* in the hatchery stocks might correspond to the inbreeding and negative selection during hatchery propagation programs (Zhou, Zhao, Bi, & Hu, 2011). Secondly, allelic diversity plays a key role in determining the level of heterozygosity that was found limited in this study. The findings of this study about *Ho* are in accordance to the inferences reported by Pang, Yu, and Tong (2015).

who described higher level of *Ho* in natural populations of *H. molitrix* from China. In this study, the mean values of expected heterozygosity (*He*) were recorded as relatively higher in comparison to *Ho* that is in accordance with the findings of Zhang, XU, Liu, Duan, and Shi (2012). Coscia, Chopelet, Waples, Mann, and Mariani (2016) described that the value of *He* directly correlate with the effective size (*Ne*) of population especially in case of wild fish. While, in hatchery fish stock the relatively *He* implied to large number of breeding individuals. Therefore, it is necessary to maintain sufficient census number in relation to their *Ne* for the sustainable fish hatchery stock (Hare *et al*., 2011). Generally, real number of brooders that contributed in succeeding generations remain unknown due to large number of effective individuals utilized in hatcheries and short domestication history.

The average values of inbreeding coefficient (F_{IS}) ranged from 0.174 to 0.441 in the hatchery populations of *H. molitrix*. The impact of demographic processes in isolated small populations results in loss of genetic variability which increases the incidence of inbreeding depression which may lead to diminish population's existence probability Hayer *et al*. (2014).

The findings of the present study about F_{IS} are different from those reported by Guo, Yu, and Tong (2013) for *H. molitrix* due to inadvertent hybridization (that is an unnoticed phenomenon) in the hatcheries of Punjab that is adversely affecting the genetic integrity. The same has been reported by Sabbir, Khan, Sultana, Rahi, and Shah (2017) over the incidence of hybridization and introgression between the indigenous carps in the hatcheries of Bangladesh. The occurrence of hybridization in hatchery stocks is widespread than in the wild environments mainly caused by poor broodstock management practices in Asia.

The microsatellite loci screened in this study revealed low level of population differentiation among the hatchery populations *H. molitrix*. Generally, genetic differentiation is considered as

very weak with F_{ST} ranging from 0.00 to 0.05, medium with 0.05-0.15 and large genetic differentiation with 0.15-0.25 and higher for intra-specific groups (Wright, 1951). The range of F_{ST} values among all the hatchery population pairs of *H. molitrix* were observed from 0.0014 (FSD-GWH) to 0.0297 (TTS-KWH) and 0.0021 (FSD-KWH) to 0.0175 (TTS-GWH), respectively. Low gene flow and a large geographical distance (415 Km) between SKT-KWH population pair may have contributed to higher population differentiation. Conventional mixing of gene pools by human interventions may be the reason of lowest value of F_{ST} between the FSD-GWH population pair even with a larger geographical distance of approximately 183 Km. Low gene flow and a widespread geographical distance between population pair may be the possible reasons to higher population's differentiation (Li *et al*., 2011).

Over most of the loci in *H. molitrix* in hatchery populations, the $\chi 2$ values clearly indicated the significant deviation from *HWE*. The deficiency in heterozygotes can be caused by inbreeding, presence of null alleles and admixture of distinct populations (Guo *et al*., 2013). Comparable findings have been reported for a wide range of freshwater fish species by Keller *et al*. (2013).

Analysis of molecular variance (AMOVA) is used to delineate partitioning of genetic divergence within and among groups. In the present study, the results of AMOVA revealed that most of the variation existed at the inter-population level, showing significant genetic structuring in hatchery populations of *H. molitrix*. These results are in agreement with Feng, Yu, Fu, He, and Tong (2014).

Based on Nei's unbiased genetic distance among all the examined hatchery populations of Silver Carp, the UPGMA dendrogram was constructed to explore the genetic related among them. Mostly, the dendrogram clustered the populations onto two major branches showing their genetic relatedness partially following their geographic distribution. The populations lying in the same cluster with limited genetic distance despite larger geographic distance reveal the brood stock management structure of fisheries department in the province. This owes to lack of plan for genetic management of fish genetic resources in Pakistan (Haque & Hoq, 2016). For perspective studies, it is suggested to develop novel markers for the local populations. A holistic study with comprehensive sampling and employment of a combined panel of different marker systems can better explore the underlying genetic issues. Future research on hybridization and genetic introgression between Chinese carps and related taxa can add to the existing understanding over the genetic structure of subject species.

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