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Genetic status of *Bagarius bagarius* in Indus River and its implications for conservation

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Genetic integrity of commercially important fish species is being compromised due to ongoing anthropogenic interventions. Hitherto, there are limited number of studies addressing the questions related to population structure of dwindling fish populations. Studies were undertaken to assess the genetic status of *Bagarius bagarius* populations from Indus River System by using five microsatellite DNA markers. DNA was extracted by PCI method and genotypic data was generated by PCR amplification of targeted loci. Results showed low-to-moderate level of genetic diversity in terms of number of alleles (*Na*), allelic richness (*Ar*), effective number of alleles (*Nae*) and heterozygosity (*Ho* and *He*). The observed heterozygosity (*Ho*) declined in comparison to the expected heterozygosity (*He*) in the wild stocks, with mean values ranging from 0.667 to 0.718 and 0.786 to 0.853, respectively. Significant deviation from H-W test was observed in 6 out of 25 tests. The population differentiation (F_{ST}) estimates were low (0.0013 to 0.0367) and insignificant. *AMOVA* showed that 62.38% genetic variation existed within individuals while 4.93% between populations. All of the populations were divided into two clusters by the *UPGMA* dendrogram constructed for genetic relatedness analysis. The study will be helpful in understanding the impact of genetic integrity on fish populations and, for sustainable management of fish genetic resources.

Keywords: Genetic status, *Bagarius bagarius,* freshwater catfish, microsatellite DNA markers.

INTRODUCTION

Natural populations of many fish species have been suffering decline at multiple scales due to anthropogenic activities including habitat alterations, over-exploitation and pollution, thus threatening the ecological stability and long-term survival of these species. Overharvesting may diminish population size to the point that loss of genetic variations and inbreeding become dreadful or lead to extinction of local populations (Liermann *et al.*, 2012). Many factors, including deforestation, watershed erosion and silting, are destroying fish habitats and disrupting the breeding, nursery and spawning grounds in natural water bodies. Remnant fish populations are additionally stressed by agricultural runoff, sewage, pesticides and chemical pollutants (Scharbert and Borcherding, 2013). Extensive damming or diversions tend to block the migratory fishes' natural dispersal routes and prevent gene flow between populations. Native populations decline following the introduction of exotic fish as they keep up with each other for resources, causing an imbalance of species' interaction (David *et al.*, 2015).

A freshwater catfish, *Bagarius bagarius* (Hamilton, 1822), is important in Pakistan, Nepal, Bangladesh, Bhutan and India both as food and game fish and has is no exception to genetic degradation. It is a carnivorous fish that consumes small fish, prawns, frogs, insects, and other small animals. As a result, *B. bagarius* plays a vital role in the top-down management of the riverine food chain. In both, the 2000 and 2015 IUCN Red Lists, it was designated as a Near Threatened (NT) species (Saha *et al.*, 2021). In the wild, species classified as NT in the red list categories have a high risk of extinction. So, government officials, researchers, conservationists, nature enthusiasts, and partner organizations must take prompt conservation efforts for NT listed species.

In spite of high market demand, catfish aquaculture is still in infancy, relying primarily on wild seed, which exerts a pressure on natural stocks. Fishermen have concentrated their efforts to gather seed from open water as a result of the cumulative effect of overfishing, habitat degradation, aquatic

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pollution, and so forth, and the availability of this species in nature has deteriorated day by day. That's why, effective management of the available natural stock is critical for the species and its populations survival. In this appalling condition, immediate actions to safeguard and conserve the species are required (Jousy *et al.*, 2017).

The genetic resources are drying up and genetic deterioration are amplifying. Despite of being the commercially significant species, investigation on population structure of *B. bagarius* is in its primary phase. A significant knowledge gap exists about the population structure and genetic diversity of dwindling fish populations, leaving a critical knowledge gap in conservation efforts. So, there is dire need for conservation and management of this species to resurrect its natural stocks (Li *et al.*, 2007). Genetic diversity is thought to be a principal factor for the management and conservation as it helps in sustainability of species. Low genetic diversity, having a negative relationship with the ability to adapt to changing environmental conditions, may pose a threat to endangered species' survival (Andayani *et al.*, 2001). Correspondingly, baseline data on declined species' fine-scale genetic population structure is required for identifying and conserving populations that could be used as a potential source for future natural restocking programs and for saving genetically depauperated populations from extinction.

DNA markers are widely utilized to explore several issues pertaining to genetics of aquaculture species. Selectively neutral markers can assist in measuring the extent of genetic diversity of farmed stocks as well as dissemination of genetic diversity among and between fish populations (Khoo *et al.*, 2011). For its uniform distribution across the genome and PCR-ability, microsatellite DNA markers have proven reliable tools for population genetic studies. These are the arrays of DNA sequences, composed of tandemly repeating units of mono-, di-, tri-, and tetranucleotide, distributed throughout most eukaryotic species' genomes. Microsatellites are codominant in nature, highly polymorphic, and inherited in a Mendelian pattern, making them ideal for exploring population structure and pedigree analysis, as well as discovering variations between closely related species (Muneer, 2014).

Microsatellite markers have been developed from the genome of several catfish species, and used for the study of population genetic structure. As microsatellite markers have not been isolated from *B. bagarius*, whereas the markers developed from *B. yarrelli* were used as cross-species amplification to reveal the genetic diversity and compare the genetic variation in five different riverine populations of *B. bagarius* in Pakistan. Thus, the present study was aimed to evaluate the genetic diversity and population structure of the threatened catfish *B. bagarius* from five sites of River Indus. The findings show a low-to-moderate level of genetic diversity, suggesting a possible decrease in the genetic integrity of the subject species. Moreover, significant deviations from the expected heterozygosity and population differentiation underline the urgent need for conservation measures. The study offers insights into the sustainable management and conservation of fish genetic resources in the Indus River System. The findings underline the significance of conserving *B. bagarius*' genetic diversity and highlight the need to implement conservation strategies to protect the species' future and preserve the ecological integrity of the Indus River System.

MATERIALS AND METHODS

***Sample collection of fish*:** One-time sampling of *B. bagarius* was carried out from five selected sites (Fig. 1) of River Indus viz. Chashma Barrage (CB), Taunsa Barrage (TB), Guddu Barrage (GB), Ghazi Ghat (GG) as well as Jinnah Barrage (JB) from January to April, 2018. The sampling sites were chosen based on their ecological significance, accessibility, and to cover a broader range of habitats and environmental conditions. With the cooperation of local fishermen, 30 genotypes from each reservoir were obtained by using cast and fyke nets, ensuring an adequate representation of the appraisal species. The samples were stored in ice for transporting to the laboratory, where they were stored at -20°C.

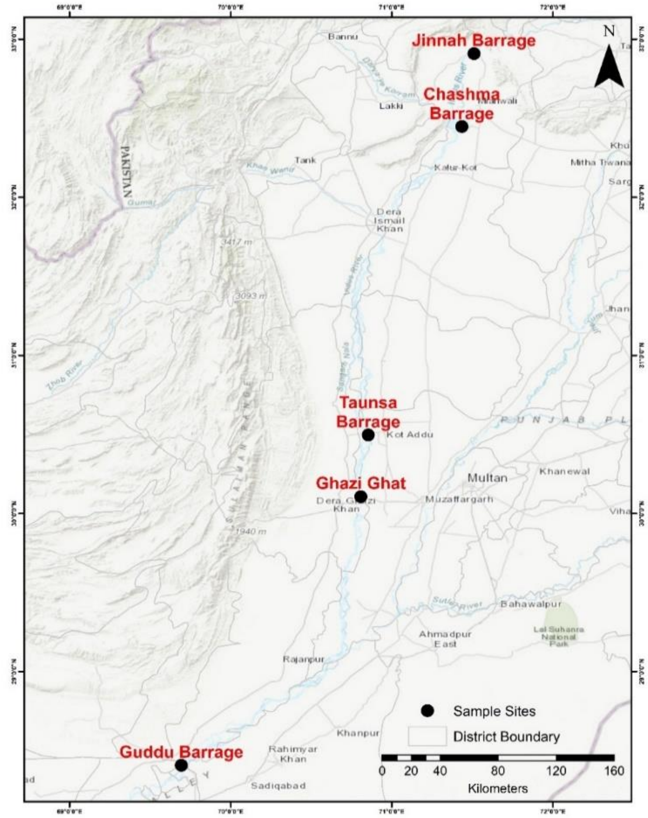

Figure 1. Map showing the sampled sites of Indus River System

Table 1. Characteristics of microsatellite loci and PCR conditions used to genotype *B. bagarius* samples.

Sr.#	No. of locus	Repeat sequence	Sequence of Primer (5-3)	T_a (°C)	No. of alleles
1	*Baya*153	(TTTTA)6	F: CACCGCTGCTGTCCATCA R: AGGTCGGCCAGATACACG	57.8	4
2	*Baya*155	(TTTTC)5	F:GATCAGAGGGTCAATGTGG R: CAGCAGAGTACTATGGAAGA	58.2	3
3	*Baya*166	(CCTCT)6	F: GATCTGTGTGTGTGTGTGC R: GGTGGCGACACAACAAATG	55.7	5
4	*Baya*160	(CGCTG)5	F: ATAGCATCACCATCTGTTGGTG R: TCTGCTTCAGTCACTCAGCAC	57.8	6
5	*Baya*161	(GAGGT)5	F: GATCAATCTTACAGTAATCTTG R: AGACGGAGATATGATGAACTG	56.8	2

***Total genomic DNA extraction*:** Total genomic DNA from dorsal muscles was extracted following phenol-chloroform protocol provided by Sambrook and Russell (2001), with slight modifications. 0.2 g of tissue samples were taken in an eppendorf tube and grinded with the help of micro pestle. A volume of 700µL tissue lysis buffer was added, mixed gently for fifteen minutes and incubated at 55-60˚C overnight. DNA was purified by PCI extraction, resulting extracted DNA was suspended in TE solution.

***DNA quality controls*:** The yield of each DNA sample was ascertained using a nanodrop and the purity of extracted DNA was tested by electrophoresis on 0.8 % agarose gel (Fig. 2). After ensuring that the DNA extract is of good quality and quantity, it was diluted to a desired concentration of approximately 50ng/µL for use in polymerase chain reaction (PCR).

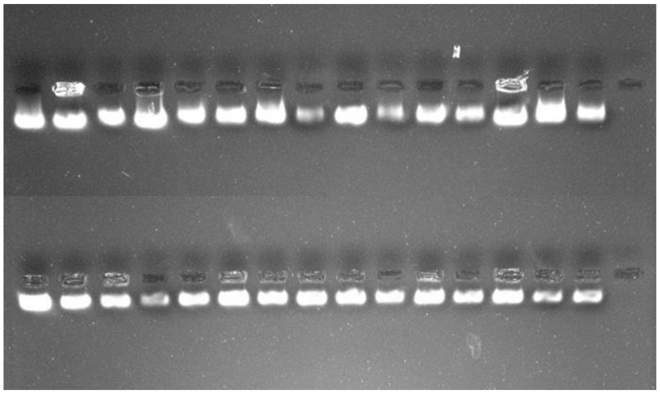

Figure 2. The sample radiograph of extracted DNA as tested by agarose (0.8%) gel electrophoresis.

***Molecular analysis*:** In this study, five microsatellite loci developed *Bagarius yarrelli* were used (taken from Gene Link) for screening. The microsatellite primers (Du *et al.*, 2015) characteristics are given in Table 1. PCR reaction mixture (25µL) was composed of 3µL of template DNA (50ng/µL), 2µL of each primer (forward and reverse), 6µL of nuclease free water and 12µL of PCR Master Mix 2X (Thermo Scientific, USA). After preheating at 94ºC for 5 minutes, amplification profiles (in a MultiGene OptiMax Thermal Cycler, Labnet, USA) were as follows: initial denaturation of 1 min at 94ºC; annealing temperature (specific for each respective primer) for 30sec followed by elongation for 1 min at 72°C. Amplicons were kept at 4°C until gel electrophoresis was done after a final extension period (72°C for 5 min) was appended at the end.

Following PCR amplification, the amplicons were verified for amplification by electrophoresis on 0.8 % agarose gel. The pattern of bands was photographed under UV light. The PCR products from each sample were resolved electrophoretically on a 5% polyacrylamide gels stained with silver nitrate (Sanguinetti *et al.* 1994). DNA ladder (Thermo Fisher Scientific, USA) was electrophoresed alongside the amplified samples as a reference marker for allele size determination. Electrophoresis was carried out using a SequiGen sequencing gel electrophoresis method. For the visualization of DNA bands, silver-staining methods were used. The allelic bands were manually scored and compared to the mixed range DNA ladder after imaging in the gel doc (UVCI, Major Science, USA).

Scoring and analysis of microsatellite data: Probable genotyping errors such as strutting bands, null alleles and large allele dropout were analyzed with software MICRO-CHECKER ver. 2.2.3. (Oosterhout *et al.*, 2004). Allele frequency, number of alleles (*Na*), allelic richness (*Ar*), inbreeding coefficient (F_{IS}) and pair-wise F_{ST} were estimated using FSTAT (Goudet, 2001). ARLEQUIN was used to analyze AMOVA's hierarchical partition of genetic diversity. AMOVA was incorporated using ARLEQUIN (ver. 3.5.2.2) to clarify the differences and similarities in molecular patterns across and within the five populations (Excoffier and Lische, 2010).

Effective number of alleles (*Nae*) observed (*Ho*) and expected (*He*) heterozygosity were also calculated to characterize the diversity and the distribution of genetic variations using 'POPGENE' program (Yeh *et al.*, 1999). Deviations from *HWE*, unbiased measurement of genetic distance (*GD*) based on Nei (1972) were drawn in TFPGA ver. 1.3 (Miller, 1997). The software STRUCTURE 2.3.2 (Falush *et al.*, 2003) was used to examine population structuring, with burn-in lengths of 50,000 and 100,000 MCMC iterations. Five independent runs for each k-value were performed and it was determined how many genetic clusters were there using STRUCTURE HARVESTER (Earl and Vonholdt, 2012).

RESULTS

***Allelic and genotypic variability*: ** No strutting bands, null alleles or drop out of large alleles at all the loci employed for genotyping were shown when MICRO-CHECKER software was applied. Five wild populations of *B. bagarius* were screened for genetic diversity at five microsatellite loci. All the screened loci were found to be polymorphic.

Table 2 shows the microsatellite diversity indices for each population. Across all the loci, average allele size and allele frequency ranged from 123 to 404bp and 0.073 to 0.503, respectively. The number of alleles (*Na*) and allelic richness (*Ar*) per locus ranging from 2.00 to 6.00 with an average 3.4 to 4.0 and 3.4 to 4.0, respectively were observed. Largest

Table 2. Genetic diversity at microsatellite loci in *B. bagarius* populations.

Population	Parameters	Loci					Average
		Baya-153	Baya-155	Baya-166	Baya-160	Baya-161	
GG	*Na*	4	3	4	6	2	3.8
	Ar	4	3	4	6	2	3.8
	Nae	3.921	2.965	3.821	5.806	1.997	3.702
	Ho	0.757	0.674	0.75	0.841	0.507	0.706
	He	0.833	0.833	0.7	0.833	0.766	0.793
	1-Ho/He	-0.099	-0.0236	0.067	0.01	-0.509	-0.153
	F_{IS}	0.102	0.241	0.069	0.01	0.523	0.189
	PHWE	1.000^{NS}	0.234^{NS}	0.100^{NS}	1.000^{NS}	0.008*	-
CB	*Na*	4	2	5	5	2	3.6
	Ar	4	2	5	5	2	3.6
	Nae	3.887	1.98	4.972	4.878	2	3.543
	Ho	0.755	0.503	0.812	0.808	0.508	0.677
	He	0.833	0.7	0.833	0.8	0.8	0.793
	1-Ho/He	-0.103	-0.391	-0.025	0.009	-0.574	-0.217
	F_{IS}	-0.105	-0.4	-0.026	0.011	-0.589	-0.134
	PHWE	0.6875^{NS}	0.0611^{NS}	1.0000^{NS}	1.0000^{NS}	0.0027*	-
JB	*Na*	3	2	5	6	2	3.6
	Ar	3	2	5	6	2	3.6
	Nae	2.98	2	4.8	5.714	2	3.499
	Ho	0.675	0.508	0.805	0.839	0.508	0.667
	He	0.7	0.733	0.833	0.866	0.8	0.786
	1-Ho/He	-0.035	-0.442	-0.035	-0.033	-0.573	-0.224
	F_{IS}	0.037	0.453	0.036	0.034	0.589	0.23
	PHWE	1.0000^{NS}	0.0262^{*}	1.0000^{NS}	1.0000^{NS}	0.0027^{*}	-
TB	*Na*	4	3	4	4	2	3.4
	Ar	4	3	4	4	2	3.4
	Nae	3.092	2.965	3.846	3.896	2	3.16
	Ho	0.688	0.674	0.752	0.755	0.508	0.675
	He	0.733	0.866	0.766	0.833	0.8	0.8
	1-Ho/He	-0.065	-0.285	-0.018	-0.102	-0.573	-0.209
	F_{IS}	0.067	0.292	0.019	0.104	0.589	0.214
	PHWE	0.7076^{NS}	0.2341^{NS}	0.6875^{NS}	0.0653^{NS}	0.0027*	-
GB	*Na*	4	3	5	6	2	4
	Ar	4	3	5	6	2	4
	Nae	3.938	2.980	2.880	5.678	2.000	3.495
	Ho	0.758	0.675	0.810	0.837	0.508	0.718
	He	0.866	0.833	0.833	0.933	0.800	0.853
	1-Ho/He	-0.142	-0.233	-0.027	-0.113	-0.573	-0.218
	F_{IS}	-0.145	-0.238	-0.028	-0.116	-0.589	-0.078
	PHWE	0.3755^{NS}	0.2341^{NS}	1.0000^{NS}	1.0000^{NS}	0.0027*	-

value was observed in GB whereas smallest in TB population. The average values of *Nae* ranged from 3.16 to 3.702. The decreasing order of *Nae* was as follows: 3.702, 3.543, 3.499, 3.495 and 3.16 in GG, CB, JB, GB and TB, respectively. The largest value was observed in GG while the lowest in the TB population.

Across all the loci, heterozygosity level was found to be low-to-moderate. The average values of H_O were noted ranging from 0.667 to 0.718. The largest value of H_O was observed in GB population while the minimum in population of JB. Similarly, the average values of expected heterozygosity (*He*) were noted, ranging from 0.786 to 0.853. JB population showed lowest while GB population exhibited largest values of *He*.

On average, the inbreeding coefficient (F_{IS}) values were found to be positive across all the studied populations, except CB and GB that showed negative mean values. Average F_{IS} values ranging from -0.134 to 0.23 were recorded. Highest average F_{IS} value was measured for JB (0.23) while the lowest for CB (-0.134) population. Significant deviations from *HWE* (p<0.05) were detected at various screened loci following the application of multiple test correction. Out of 25 tests, a total of 6 tests were found to be deviate from *H-W* test.

Genetic structure: The pair-wise population genetic differentiation (F_{ST}) among *B. bagarius* populations was analyzed by FSTAT across all the microsatellite loci. Most of the population pairs showed statistically significant (p<0.05) differentiation, indicating that most of the populations are genetically non-homogeneous. F_{ST} values ranged from 0.0013 (CB-JB) to 0.0367 (CB-TB) among the population pairs (Table 3). Nei's unbiased genetic distance (GD) across populations-pairs was estimated which suggested significant variation (p<0.05). Highest GD was calculated 0.0758 between CB-TB whereas the smallest 0.0036 was noted between CB-JB populations (fig. 3).

Table 3. Nei's unbiased genetic distance (below diagonal) and pair-wise population differentiation (F_{ST}) (above diagonal).

Population	GG	CB	JB	TB	GB
GG	-	0.0226*	0.0294	0.0050*	-0.0039
CB	0.0461*	-	0.0013**	0.0367*	0.0208*
JB	0.0604	0.0036*	-	0.0268*	0.0251*
TB	0.0041*	0.0758	0.0510*	-	0.0084*
GB	0.0163*	0.0403*	0.0485*	0.0097	-

*Significant at *P<0.05*

According to AMOVA, the majority of the variation percentage was observed within individuals (62.38%). Variation were measured 7.31% and 32.69% between populations and between individuals within populations, respectively (Table 4).

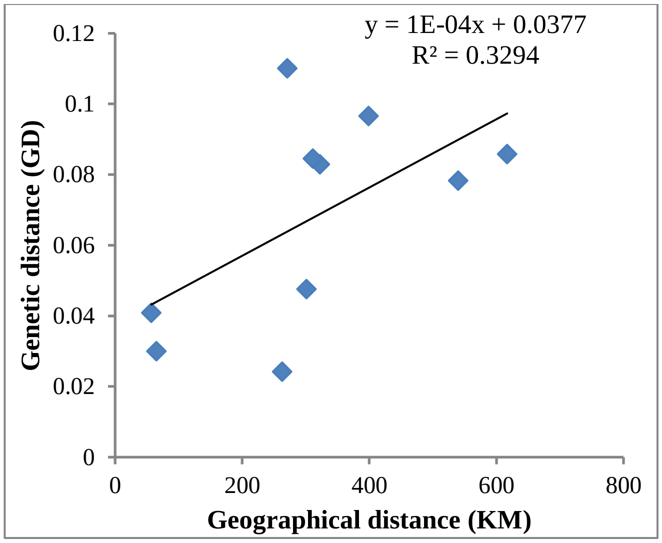

Figure 3. Correlation of geographical distance (KM) and genetic distance (GD) values in *B. bagarius* populations

Table 4. Analysis of molecular variance (AMOVA) for *B. bagarius* populations

SOV	df	MSS	Variance	% variation
Between populations	4	10.734	0.1551	04.93
Between individuals within Populations	145	1.4221	0.5455	32.69
Within individuals	150	2.5133	2.5133	62.38

The genetic relatedness of all populations was analyzed using the UPGMA dendrogram, which was based on Nei's genetic distance (1972). The segregation of three populations (GG, TB, and GB) from the other two populations (CB and JB) was suggested by this analysis (Fig. 4).

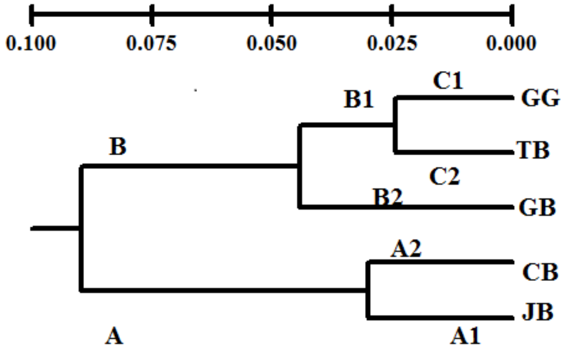

Figure 4. UPGMA dendrogram based on Nei's genetic distance for *B. bagarius* populations

For the populations of *B. bagarius*, data analyses by the STRUCTURE grouping algorithm method proposed the occurrence of two discrete genetic groups. For each K value, constant results were obtained across the 6 independent runs.

STRUCTURE HARVESTER admixture model interpretations showed highest estimated log-likelihood mean value and delta-k value for K = 5 in this study (Fig. 5).

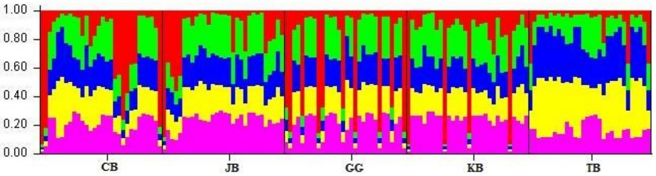

Figure 5. Genetic structuring patterns among *B. bagarius* populations as revealed by STRUCTURE analysis. Distinct colors of the column represent the estimated probability of belonging to all populations

DISCUSSION

The current study is the first to uncover the genetic issues that have gone unnoticed in the subject species. The lack of understanding regarding genetic concerns related to fisheries management, as well as the skills necessary for genetic analysis, are evident factors. This study is the first thorough attempt in Pakistan to investigate the genetic structure of *B. bagarius* in the River Indus, Punjab to determine the influence of various human interventions in natural fish stocks. The abundance of this fish is dropping over the world as a result of a myriad of anthropogenic influences, causing conservationists to be concerned (Saha *et al.*, 2021). The loss of fish productivity due to genetic deterioration has become a serious fisheries management concern that threatens the aquaculture industry's long-term viability, and the consequences cannot be mitigated instantly (Naz and Abbas, 2022).

A set of five primers developed from *B. yarrelli* were screened for cross-species PCR amplification. In closely related species, microsatellite loci can be cross-amplified by the primers designed for one species (Estoup and Angers, 1998). Yue *et al.* (2003) successfully cross-amplified the microsatellite loci in catfishes (*Clarias macrocephalus*, *C. fuscus*, *C. gariepinus*, *Heteropneustes fossilis*, *H. longfilis* and *Phractocephalus hemioliopterus*) developed for *C. batrachus*. Similarly, Sultana *et al.* (2015) tested cross-amplification of five primers developed from *C. batrachus* for *H. fossilis* and Ali *et al.* (2021) for *Rita rita*.

Five wild populations of *B. bagarius* were screened for genetic diversity at five microsatellite loci, that were found polymorphic. Comparable conclusions were prescribed by Islam *et al.* (2007) who detected all screened loci to be polymorphic in case of *C. batrachus* while working with microsatellite markers. Parallel findings to ours were also given by Na-Nakorn (2004) in case of *C. macrocephalus* natural populations.

Low-to-moderate genetic diversity was revealed from the obtained results. At five microsatellite loci, 2 to 6 number of alleles (*Na*) and allelic richness (*Ar*) were observed with highest average value in GB population while the lowest in TB population. Obtained *Na* are lower than reported for *Liobagrus obesus* by Lee *et al.* (2014), *Glyptothorax laosensis* by Jin *et al.* (2012), *Glyptosternum maculatum* by Guo *et al.* (2009), *Horabagrus brachysoma* by Gopalakrishnan *et al.* (2006) and *Sperata seenghala* by Acharya *et al.* (2019). Maximum average value of *Nae* was seen in GG whereas minimum in TB population. Results are consistent with the findings of Alam and Islam (2005), Barroso *et al.* (2005), Hansen *et al.* (2006), Basak *et al.* (2014) and Zhou *et al.* (2021), but contrary with Aung *et al.* (2010).

Heterozygosity is a key factor in determining population dynamics and viability since it acts as an indicator of evolutionary potential (Reed, 2009). The observed heterozygosity (*Ho*) declined in comparison to the expected heterozygosity (*He*) in the wild stocks, with mean values ranging from 0.667 to 0.718 and 0.786 to 0.853, respectively. Homozygote excess or limited heterozygosity in the wild populations could be described by a Wahlund effect (formation of intra-population structure) or founder effect or by over-generation stocking effects or bottleneck population or population under stress due to pollution, overfishing or habitat destruction (Alam and Islam, 2005). The findings of our study are in corroboration with Na-Nakorn *et al.* (2004) that analyzed genetically the *C. macrocephalus* populations taken from different sites of Thailand. Wenne *et al.* (2016) and Islam and Alam (2004) deduced the parallel results about genetic analysis of *Labeo rohita* but Jewel *et al.* (2006) reported contradictory results.

The *1-Ho/He* values were negative in TB and GB, stating that populations have not lost heterozygosity and individuals are termed as naturally outbred, instead of bottlenecked. Comparable results about negative values were also given by Islam *et al.* (2007) in *C. batrachus*, referring the recent bottleneck activity in populations. The positive values of *1-Ho/He* at GG, CB and JB refer that studied populations were deficient in heterozygosities. However, heterozygote excess in the populations is not as common as heterozygote deficiency.

To measure the frequency of observed heterozygosity as compared to expected heterozygosity when a population is under *HWE* is called inbreeding coefficient (F_{IS}). On the average base, the F_{IS} values were found positive in all the examined natural populations except the populations collected from CB and GB that showed negative mean values. Highest average F_{IS} was measured in wild population sampled from TB while the lowest in CB population. Negative mean value of F_{IS} indicated heterozygosity excess and revealed this population has not lost the heterozygosity and individuals of this population could be considered as outbred. Positive F_{IS} values confirm the homozygosity excess and significant deviation from the *HWE* in a population (Abbas *et al.*, 2010). Similar findings about *HWE* deviation were described by Yang *et al*. (2013).

Out of 25 tests, only 6 deviated significantly from *HWE* when applied multiple correlation test. Inbreeding, fishing pressure, non-random sampling, selection against heterozygote and Wahlund effect have been proposed as explanations for the divergence from *HWE* (Abbas *et al.*, 2010; Ruzafa *et al.*, 2006). Equivalent results were reported by Na-Nakorn *et al.* (2004) in case of *C. macrocephalus* populations where 6 out of 26 populations deviated from *HWE*.

Lack of genetic differentiation among adjacent populations is not so unexpected. However, when distant populations display less genetic differentiation, demographic and historical ecology are the most important factors in justifying such reduced genetic differentiation, which may involve ancient population connectivity (Steven, 2004). The population differentiation (F_{ST}) values were low (0.0013 to 0.0367) and insignificant. The fact that the populations are homogeneous is evidenced by this outcome, i.e., the populations are almost genetically identical, and the findings are congruent with those of Indo-Pacific King Mackerel (*Scomberomorus guttatus*) (Abedi *et al.*, 2011), Silver Pomfret (*Pampus argenteus*) (Archangi *et al.*, 2013) and Channel Catfish *(Ictalurus punctatus)* (Perales-Flores *et al.*, 2007), all inferred from microsatellite analysis. Low F_{ST} values (0.004-0.038) were detected among Indo-Pacific king mackerel population pairings, according to Abedi *et al.* (2011). In silver pomfrets, pairwise F_{ST} values demonstrated modest (0.040) genetic difference across five sampling sites in the Persian Gulf and Oman (Archangi *et al.*, 2013). The gene flow estimates indicated that there was considerable gene flow among populations, preventing regional genetic differentiation.

Analysis of molecular variance (*AMOVA*) is used to describe how genetic divergence is partitioned within and among groups. The outcomes of *AMOVA* in this study indicated that the majority of variation was found within individuals, indicating moderate genetic structuring in wild populations of *B. bagarius*. These findings are consistent with those of Feng *et al.* (2014).

The clustering order represents population relationships. According to our research, two major clusters or clades by constructing UPGMA dendrogram tree were yielded. CB and JB populations, indicating a closest genetic relationship, were in one cluster while the GB, GG and TB populations were in another cluster. This shows that geographic isolation is the most important factor in genetic differentiation. Biba *et al.* (2017) discovered the same thing with their model-based clustering method.

A structural grouping technique was used to investigate the genetic relationship further, revealing the presence of two separate clusters. The highest estimated log probability mean value and delta k value was yielded for k = 5. The number of groups existing in any population is determined by the no. of individuals genotyped and the quantity of markers used for genotyping. Evanno *et al.* (2005), on the other hand, claimed that computing the delta k with as few as five microsatellite markers could allow for the determination of the actual number of groups.

***Conclusion*:** Population genetics and genetic variation are the baseline information for conservation management of any fish species. The results of this study will aid in the identification of brood catfish with good genetic data for use in selective and induced breeding strategies. Reduction of pressure on the wild stock as well as providing protection to the existing populations are prime steps to get success in conservation of any fish species. Over exploitation of the wild stock to meet the market demand has been reported as one of the major reasons behind rapid declination of *B. bagarius* population. This problem can be solved by (i) banning fishing during its breeding season as well as by implementing size specific capture and (ii) by culturing this fish species in captivity. However, molecular diversity analysis and genetic characterization could be crucial in the breeding, conservation and sustainable aquaculture of the catfish species.

***Conflict of interest*:** The authors declare that there is no conflict of interests regarding the publication of this article.

***Authors contribution*:** Sadia Rabbani and Taqwa Safdar conducted the experiment and wrote manuscript. Khalid Abbas and Tanveer Ahmed planned the research layout and finalized the manuscript. Muhammad Sarfraz Ahmed and Jamal Kazam helped in collecting the fish individuals. Rabia Maqsood and Muhammad Waseem assisted in results interpretation. All authors have read and approved the manuscript in its final form.

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