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Full Length Article

Genetic Diversity in Different Populations of Mahseer (*Tor putitora*) in Pakistan: A RAPD Based Study

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Abstract

Random Amplified Polymorphic DNA (RAPD) based PCR amplification of nuclear DNA was carried out from collected samples of Golden mahseer (*Tor putitora:* Hamilton, 1822) from Rivers Poonch, Jhelum and Mangla Reservoir of Azad Jammu and Kashmir, Rivers Swat, Indus (Khyber Pukhtunkhwa) and Hingol (Baluchistan) of Pakistan. A total of 197 bands were generated by 16 RAPD primers with 87.73% polymorphic and 43.75% unique bands. Swat River's population exhibited the highest level of polymorphism and held the highest genetic diversity (73%) followed by population of Mangla reservoir (57%), River Indus (54.31%), River Jhelum (44.67%), River Poonch (37.06%) and River Hingol (2.03%). Heterogeneity (Ht, 0.19), genetic diversity within population (Hs, 0.13), between populations (Dst, 0.05) and genetic differentiation constant (Gst, 0.022) were low. Gene flow between populations (3.22) was high. Analysis of Molecular Variance (AMOVA) ascribed higher variability (79%) within population factors. UPGMA dendrogram based on Nei's genetic similarities and distances separated two main clusters of populations; cluster one subdivided into two; 1) were identified the populations of Poonch River, Jhelum River and Mangla Reservoir, and 2) swat River and Indus River. Population of Hingol River was appeared as the second cluster.

Keywords: *Tor putitora;* Genetic diversity; Nei's index; Shannon index; Polymorphism; Heterozygosity; AMOVA

Introduction

Golden mahseer (*Tor putitora*: Hamilton, 1822), a large **(**5–8 kg; specimens 2.7 m long, 30–54 kg recorded; Talwar and Jhingran, 1991; Mortuza and Rahman, 2006) economically valued sport freshwater carp, is widely distributed in trans-Himalayan rivers, streams and lakes with rapid streams of water and rocky substratum (Talwar and Jhingran, 1991; Hasan *et al*., 2015), from Myanmar, Bhutan, Bangladesh, India, China, Nepal to Pakistan(Chen *et al*., 2006; Nautiyal *et al*., 2008). Owing to its size, color, taste and market price golden mahseer has been overharvested and also by barriers to migration resulted in a decline in its numbers throughout the Himalayan stretch (Nautiyal *et al*., 2008; Jha and Rayamajhi, 2012). Thus it is regarded as endangered in India (Ingram *et al*., 2005), Bangladesh (Ameen *et al*., 2000) and Threatened in Nepal (Rai, 2008).

In Pakistan, golden mahseer has been a common fish species till late 20th century, almost with continuous range of distribution in all five rivers and associated tributaries of the Indus drainage system. Isolated populations were also present in southern rivers of Balochistan (Hingol, Porali Gaj and Anamber), which drain independently into the Arabian Sea. Fish moved to the northern parts of the rivers and streams to breed in colder waters during summer and return back to southern parts during non-breeding winter season (Rafiq and Najamul Huda, 2012). Construction of dams, barrages and water diversion for irrigation and habitat degradation through aquatic pollution have emerged as barriers to free movement of this species to/from breeding grounds. Contraction in distribution range under declining population and emergence of barriers to free movements have now restricted golden mahseer population of Indus rive system to isolated pockets (Irshad *et al*., 2008). Anglers suggest that viable populations of mahseer are now limited to Swat-Malakand, Indus north of Terbella Reservoir, Poonch River up to Mangla Reservoir, Jhelum River, Harro River (Attock), Swan River (Rawalpindi), Chenab River and Hingol River.

Present distribution suggests that golden mahseer population is segregated into isolated breeding sub-populations and probably this isolation is gradually increasing with gradual decline in the population levels and creation of physic-chemical-habitat barriers. Such isolation can add another factor to seal the fate of this species through genetic fixation, bottleneck effect and genetic drifts

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occurring in smaller subpopulation, even if overharvesting is controlled through administrative measures and habitat is restored through wiser pollution management. There is a need to understand the present genetic structure of golden mahseer population and level of isolation existing between its subpopulations to develop the future management strategy for this species.

Present study has been based on the hypothesis that under existing barriers different subpopulations of golden mahseer (*Tor putitora*) in Pakistan are isolated from one another to different degrees. To test the hypothesis we subjected samples of nuclear genome collected from six geographic subpopulations of golden mahseer to RADP-based genetic analysis for intra-population genetic similarity and inter-population variability.

Materials and Methods

Sampling

A total of 60 freshly captured specimens of golden mahseer was collected during 2010–2012 from 6 localities (Fig. 1), viz., Poonch River (northern reaches and its tributaries; n=22), Mangla Reservoir (Poonch River pocket; n=21), Jhelum River (Arja Stream, Mahl Nullah; n=4), Swat River (Dir Nullah and Malakand; n=6) Indus River (upstream of Turbela Reservoir; n = 5) from Indus system and one population was collected from Hingol River (n=2). We packed each specimen in a separate polythene bag, and transported in ice box to Molecular Ecology Laboratory, Bioresource Research Centre, Islamabad (Pakistan). We identified each specimen as *Tor putitora* through morphological characters (Mirza and Javed, 1985; Mirza and Alam, 1994; Jayaram, 1999), confirmed through bar coding using cytochrome oxidase sub-unit 1 (COI) mitochondrial gene (Khaliq *et al*., 2015). Size of the sample being large for Poonch River and Mangla Reservoir, we randomly selected 6 specimens from each of these two samples and analyzed 39 specimens for molecular analysis, which were given reference numbers and grouped into 6 subpopulations, designated as: Poonch (1–12), Jhelum (13–16), Mangla (17–28), Swat (29–33), Indus (34–37) and Hingol (38–39).

DNA Extraction and Amplification

DNA was extracted from muscle tissues by phenol-chloroform protocol (Penzo *et al*., 1998; Sambrook and Russell, 2001), followed by proteinase K (2 mg/mL) and 25 µL dithiothreital (DTT) treatment. Presence of DNA was confirmed by agarose gel electrophoresis, stained with ethidium bromide and observed/photographed using gel-doc system (Pro-Alpha, 200). Purity of extracted DNA was determined by spectrophotometer (8415A Diode Array, USA) at 260–280 nm, following Linacero *et al*. (1998) and calculated DNA concentration using formula:

$$\text{DNA } (\mu g/\mu L) = [(A\ (260) \times DF \times 50)]/10{,}000$$

Where A (260) is absorbance at 260 nm and DF is dilution factor. DNA solution was diluted with de-ionized water to obtain final working concentration of 25 ng/µL.

Sixteen primer markers were used (FA and Operon series, Fermentas, USA, randomly selected based on 60% GC contents; Table 1) for PCR amplification of reproducible golden mahseer nuclear DNA in sprint thermo cycler (Thermo Hybaid, SPR 220362, USA) performed under conditions optimized for each marker (Shafi *et al*., 2015). PCR amplification was carried at initial denaturation temperature of 95ºC for 5 min (stage 1 of cycle 1) and 94ºC for 20 sec, followed by annealing (depending upon marker) for 20 sec and initial extension at 72ºC for 25 sec (stage 2 for 39 to 42 cycles), and final extension at 72ºC for 5 min (Esa, 2009). Again amplified PCR product was checked by gel electrophoresis on 2% agarose.

Data Generation for Genetic Analysis

The computer software (POPGENE version 1.31) was applied for genomic analysis. Binary data matrix was developed based on presence (1) and absence (0) of bands and estimated the number of RAPD fragments and polymorphic, monomprphic and unique bands for each primer. Pair-wise matrix of genetic distance was developed, following Fitzpatrick (2009) and Nei and Li (1979). The discriminatory power for each marker was developed using three parameters:

$$\text{Polymorphic information content (PIC)} = 2f_i\ (1\text{-}f_i)$$

Where f_1= Frequency of marker bands present (Silva *et al*., 2013).

Resolving power (Rp) = $\sum$ IB, where IB is for Band information (Pérez de la Torre and Escandón, 2006).

$$\text{Marker index(MI)} = PIC \times EMR$$

Where, EMR= Effective multiple ratio (Maras *et al*., 2008). The degree of similarities and dissimilarities of RAPD fragments were calculated between individuals and populations using Computer POPGENE (version1.31) software (Yeh *et al*., 1999; Kassam e*t al*., 2005).

Statistical Analysis

Genetic diversity analysis: Genetic diversity was projected by using POPGENE 1.32 software (Nei, 1978). We calculated total number of observed (na) and effective (ne) alleles sharing gene diversity, mean expected heterozygosity (observed and expected), Shannon's index (h) and trend line analysis for Nei's diversity index (Gaudeul *et al*., 2004). And also determined heterozygosity in genome of different populations in term of homozygosity (H), heterozygosity (Ht), genetic variation within population (Hs), genetic variation between populations (Dst) and genetic variation coefficient (Gst = Ht – Hs) by using

POPGENE 32 version 3.1. We estimated levels of gene flow between populations (Nm) as:

Nm = 0.5*(1 - Gst)/Gst (Setti *et al.*, 2012)

All amplified markers were tested for their neutrality by using the Ewens–Watterson test (Bagley *et al*., 1999).

Analysis of molecular variance (AMOVA) was calculated by GenA1Ex 6.4 (Gaudeul *et al*., 2004) for significance intra-population and inter-population variance (Fitzpatrick, 2009). AMOVA was used to judge significance of variance in pair-wise genetic distances (phist Φ_{ST}) between different populations, which were used to estimate gene flow (Nm= 1/ Φ_{ST} - 1) -1, using FAMD 1.5 software (Frankham *et al*., 2010) between populations.

Population clustering: For cluster analysis dendrogram was constructed by putting data into NTSYS-2.1pc program (Rohlf, 2004; Yao *et al*., 2007; Atalay and Babaoğlu, 2012) using un-weighed pair group mathematical averages (UPGMA), based on the genetic similarities and distances (Kassam *et al*., 2005). The nested cluster of dendrogram was used for Cophenetic correlation (CC) analysis for pair-wise genetic distance and genetic similarities through Mantel test.

Results

Genetic Characters

A total of 197 score-able bands were identified with a mean of 12.31 (SEM) bands/ marker (Table 3), ranging between 8 (OPA19; 4.06% of gene pool) and 21 (OPA4; 10.66% of gene pool) for different markers. Band sizes ranged between 114 bp (OPA4) and 2,000 bp (FA1). Majority of the bands (172; 87.31%; 10.75/marker) were polymorphic, while 25 (12.69%; 1.56/marker) were monomorphic (Red lines; Fig. 2). 13 (average 0.88) unique bands were identified and appearing only in specific individuals/populations (Table 2, Fig. 2).

Genetic Diversity

Values of genetic diversity constants, viz. heterozygosity (Ht, estimated under Hardy-Weinberg Equilibrium; 0.19), genetic diversity within populations (Hs; 0.13), genetic diversity between populations (Dst; 0.05,) and genetic diversity within populations (Gst; 0.022), in the overall sample of golden mahseer were low, indicating low genetic variability. On contrary, the overall sample of mahseer population exhibited higher values of genetic differentiation (Rst; 0.75) and gene flow (Nm; 3.22) constants between populations, suggesting a lower level of genetic segregation between sub-populations (Table 3).

Inter-population Variability

Genetic diversity constants in different populations, generated for 16 RAPD loci (Table 4), suggest a high polymorphism in Swat, Mangla and Indus populations and a very low polymorphism in Hingol population, as indicated by observed and effective number of alleles at different loci, and values of Shannon and Nei diversity indices were low (Table 4). Genetic distances between different populations (Table 5) were generally low (<16%). Hingol population showed larger genetic distance and lower similarities with all other populations. Three populations of Jhelum River System (Poonch, Mangla and Jhelum) revealed the lowest inter-population genetic distances (0.0171–0.0291).

AMOVA showed significant variation and attributed larger part of the variance (79%) to intra-population variability, while a smaller part (21%) was attributed to inter-population variability. Variance within populations was (*Fst* 0.214) significant ($p < 0.01$).

Dendrograms constructed using UPGMA exploiting genetic distances (Nei, 1972: Fig. 3) and Jaccard similarity coefficient (Fig. 4) divided six populations into two clusters. First cluster represented all five populations of Indus River Drainage System.

Table 1: RAPD primers sequences tried for PCR amplification of golden mahseer DNA

Primer	Sequence	G+C (%) content
FA-1	5'---CAATCGCCGT--3	60
FA-2	5---ACCTGAACGG---3	60
FA-3	5---CTCTGGAGAC—3	60
FA-4	5---AGCGCCATTG---3	60
FA-5	5---GGGGTGACGA---3	70
FA-6	5---CTTCCCCAAG---3	60
FA-7	5---ACCCGGTCAC---3	70
FA-8	5---TTCGAGCCAG---3	60
FA-9	5---GGGGGTCTTT---3	60
FA-10	5---GTGCCTAACC---3	60
OPA - 4	5---AATCGGGCTG—3	60
OPA -11	5---CAATCGCCGT---3	60
OPA-17	5---GACCGCTTGT---3	60
OPA-19	5---TCTGTGCTGG---3	60
OPA- 20	5---GTTGCGGATCC-3	70
OPN -04	5---GACCGACCCA--3	70
OPN -11	5---TCGCCGCAAA--3	60
OPMN-13	5---AGCGTCACTC---3	60
OPN -19	5---GTCCGTACTG---3	70
OPN -20	5---GGTGCTCCGT---3	70

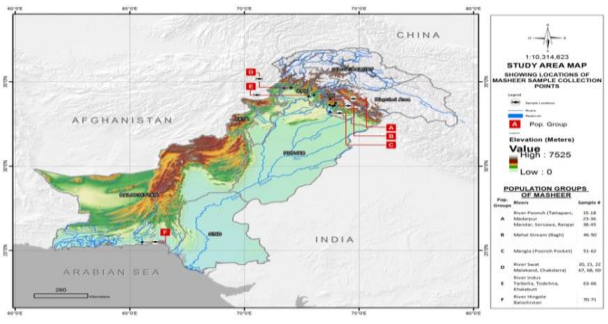

Fig. 1: Map showing sampling streams and rivers of six different Populations of golden mahseer

Table 2: Description of amplicon for different RAPD markers in golden mahseer nuclear genome

Marker	Bands						% Gene Pool	Loci Range (bp)
	Total (#)	Unique	Monomorphic		Polymorphic			
			#	(%)	#	(%)		
FA1	11	2	3	27.27	8	72.73	5.58	400-2000
FA3	14	0	2	14.29	12	85.71	7.11	333-
FA4	15	2	0	0	13	100.00	7.61	142
FA5	9	1	1	11.11	8	88.89	4.57	466-
FA6	10	0	1	10.00	9	90.00	5.08	250-
FA7	9	0	1	11.11	8	88.89	4.57	236-
FA8	12	0	1	8.33	11	91.67	6.09	248-
FA10	11	0	0	0	11	100	5.58	281-
OPN13	16	0	3	18.75	13	81.25	8.12	229-
OPA4	21	0	2	9.52	19	90.48	10.66	114-
OPA11	13	0	4	30.77	9	69.23	6.60	464-
OPA17	9	3	3	33.33	6	66.67	4.57	426-
OPA19	8	2	2	25.00	6	75.00	4.06	360-
OPN04	9	2	1	11.11	8	88.89	4.57	205-
OPN11	17	0	0	0	17	100.00	8.63	445-
OPN20	13	1	0	0	12	100.00	6.60	254-
Total	197	13	25	-	170	-		**114-2000**
Mean	12.31	0.88	1.56	12.69	10.69	87.31		
SEM	0.90	0.26	0.30		0.92			

Table 3: Genetic diversity constants for different RAPD loci in golden mahseer population of Pakistan

Marker	Heterozygosity (Ht)	Genetic diversity		Genetic differentiation		Gene flow (Nm)
		Hs	Dst	Gst	Rst	
FA1	0.16	0.14	0.02	0.15	0.88	3.26
FA3	0.13	0.11	0.02	0.15	0.85	4.01
FA4	0.12	0.10	0.02	0.15	0.83	5.04
FA5	0.13	0.12	0.01	0.10	0.92	5.42
FA6	0.17	0.15	0.02	0.12	0.88	4.21
FA7	0.23	0.19	0.04	0.16	0.83	2.90
FA8	0.17	0.11	0.06	0.23	0.65	3.85
FA10	0.30	0.09	0.21	0.65	0.30	0.95
OPMN13	0.34	0.15	0.19	0.51	0.44	1.09
OPN04	0.15	0.12	0.03	0.16	0.80	3.20
OPN11	0.10	0.09	0.01	0.10	0.90	4.65
OPN20	0.18	0.14	0.04	0.20	0.78	2.69
OPA4	0.22	0.16	0.06	0.24	0.73	1.75
OPA11	0.19	0.16	0.03	0.17	0.84	2.69
OPA17	0.17	0.14	0.03	0.17	0.82	2.94
OPA19	0.22	0.12	0.10	0.27	0.55	2.95
Mean	0.19	0.13	0.05	0.22	0.75	3.22
S.E	0.02	0.01	0.02	0.04	0.04	0.32

Hs =genetic diversity within population, Dst= genetic diversity between population, Gst = genetic differentiation between populations, Rst = genetic differentiation within population

This cluster was further divided into two sub-clusters. One of these sub-clusters included three populations of Jhelum River system (Poonch, Mangla and Jhelum), while the other sub-cluster included two populations of Indus River (Swat and Indus). The second main cluster included Hingol population.

Discussion

The genetic distance between populations of golden mahseers was calculated based on an unbiased measure, i.e., Nei's diversity index (Nei, 1972; Parveen *et al*., 2011). Fishes exhibit high levels of population differentiation across geographic areas (Gottelli and Colwell, 2001; Hanfling and Brandl, 1998), by mean of shifting in genetic structure and diversity (Gonzalo and Dopico, 2006). It is therefore, important to describe and monitor the fish population genetic structure and diversity threats attributable to the human activity. Measure of genetic diversity and magnitude of genetic variability in a population is fundamental source of biodiversity; hence wild populations of fishes have received the foremost concentration from molecular biologists. Over the recent years variety of techniques has been developed for the analysis of population discreteness and related models for analysis of genetic distribution and gene flow. The most accepted application in molecular biology is the PCR–based tools for the investigation of population structure together with micro-geographic isolation and the molecular description of allelic variants among population (Tiwari *et al*., 2013).

Table 4: Average genetic diversity constants in different golden mahseer populations generated for 16 RAPD loci

Population	N	Poly (%)	na	ne	h	I
Poonch	12	37.06	1.37	1.07	0.05	0.10
Mangla	12	57.36	1.57	1.21	0.14	0.23
Jhelum	4	44.67	1.45	1.22	0.14	0.22
Swat	5	73.10	1.73	1.41	0.25	0.38
Indus	4	54.31	1.54	1.30	0.18	0.28
Hingol	2	2.03	1.02	1.01	0.01	0.01
Mean		44.76	1.45	1.20	0.13	0.20
SEM			0.10	0.06	0.04	0.05

Poly=Polymorphism, na=observed alleles, ne=effective alleles, h=Nei index, I=Shannon index

Table 5: Genetic similarity (upper) and distances (lower) between different populations of golden mahseer

Population	Poonch	Mangla	Jhelum	Swat	Indus	Hingol
Poonch	****	0.9871	0.9830	0.9536	0.9472	0.8758
Mangla	0.0171	****	0.9713	0.9604	0.9385	0.8724
Jhelum	0.0171	0.0291	****	0.9458	0.9369	0.8576
Swat	0.0475	0.0404	0.0558	****	0.9529	0.8799
Indus	0.0543	0.0634	0.0652	0.0482	****	0.8551
Hingol	0.1326	0.1365	0.1536	0.1280	0.1565	****

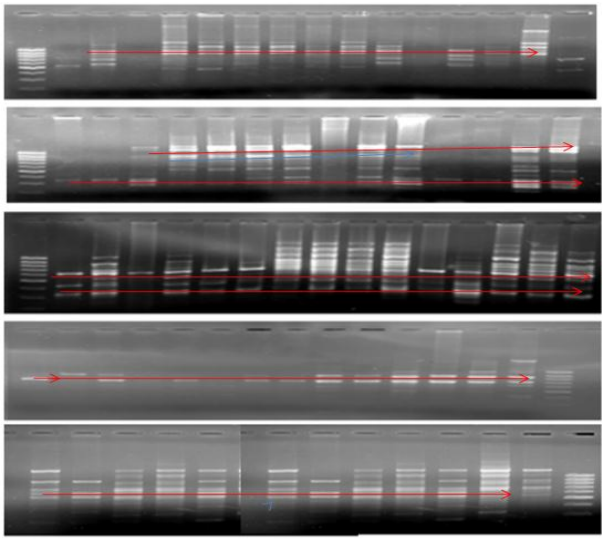

Fig. 2: Representation of some gel doc. of PCR amplification product of golden mahseer genome with RAPD markers; FA- 1, FA- 6, FA-7, OPA-17and OPN-20
(Red lines point out/illustrate the monomorphism within most of the population)

Distribution of genetic variability in a natural fish population depends upon migration and mating occurring between adjacent populations, which decides the level of sharing of a common gene pool. When only few individuals exchange genes between populations, under low migratory potentials of the species or effectiveness of physical/ecological barriers, there are higher chances of genetic segregation of populations. Populations are then exposed to higher level of inbreeding, resulting in genetic fixation and consequent loss of heterozygosity (Thorpe *et al*., 2000). Molecular techniques analyze heterozygosity in the populations and thence genetic distances and gene flow between sub-populations.

RAPD is one such technique used by molecular biologists for the analysis of population structure and allelic diversity in the species (Gottelli and Colwell, 2001) with little expenditure, time and effort (Hardys *et al.*, 1992). It has also been successfully used in fisheries research to determine species/subspecies/population genetic diversity in many different species, including, guppy (*Poecilia reticulata*; Suresh *et al*., 2013), brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*; Parveen *et al*., 2011), largemouth bass (*Micropterus salmoides*; Wang *et al*., 2012), ictalurid catfishes (Gjedrem and Baranski, 2009), common carp (*Cyprinus carpio*; Chiu *et al*., 2009), Indian major carps (*Labeo rohita*; Parveen *et al*., 2011), and a migratory freshwater fish (*Prochilodus marggravi;* Sanches *et al*., 2012).

Present results revealed a low intra-population (21%) and a high inter-population (79%) variation. Similar results have been previously reported by Nguyen *et al*. (2006), for *Tor douronensis*, sampled from tributaries of Limbang River, Bunan River and Layar River (Sarawak, Malaysia).Although there were some type of barriers to the free movement of mahseer, the rates of gene flow between population was high (3.22). We believe that periodic floods and opening of spillways allowed fish migration across drainage systems, as indicated by Wang *et al*. (2000) for *Arossocheilus paradoxus.*

High level of polymorphism (87%) is an indication of variability in genetic structure of golden mahseer, which has been supported by high values of Nei and Shannon (1978) diversity indices. This suggests that genetic fixation is still not a serious problem for the present mehseer populations. High rate of gene flow (Nm; 3.22; i.e.>1) indicates inter-population migration of individuals, low probability of inbreeding and lower chances of the genetic drift in these populations. This allows us to suggest that the barriers have not been working effectively to isolate golden mahseer populations till the recent past. The area faces heavy/floods at different intervals, when the ecological/or physical barriers may break, reducing the efficacy of such barriers created under the anthropogenic influences.

The genetic similarity indices between different populations suggest a high level (80%) of inter-population similarity in confirmation to a higher rate of gene flow and lower heterogeneity within population. The genetic similarity is very high between three populations of Poonch River System (97–99%; Poonch, Jhelum and Mangal). These three populations exhibited almost equally high genetic similarities (94–95%) with two other populations of Indus drainage system (Swat and Indus). Similarities between five populations of the Indus river system, located in the northern parts of Pakistan, shared higher similarities (> 94%) compared with that shared with the Hingol River population.

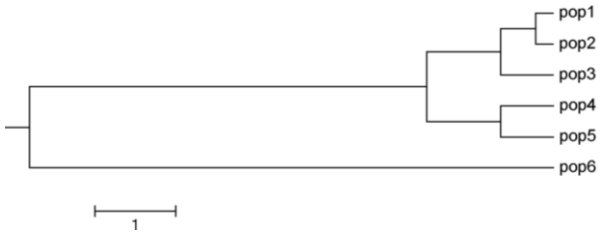

Fig. 3: UPMGA cluster analysis based on genetic distance (Nei, 1972), calculated on band amplification at16 RAPD markers in different populations of golden mahseer
Pop1= Poonch, Pop2=Jhelum, Pop3=Mangla, Pop4=Swat, Pop5=Indus, Pop6=Hingol

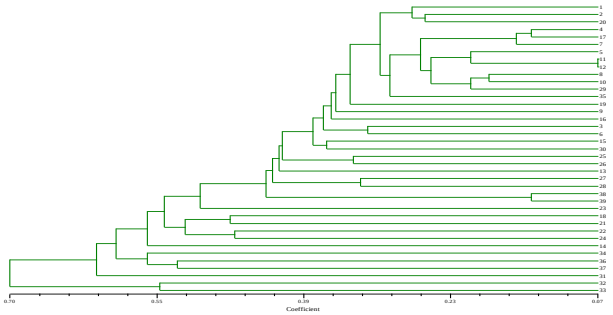

Fig. 4: UPMGA generated clustering of different RAPD genotypes of golden mahseer based on Jaccard similarities coefficient. Populations: 1-12=Poonch, 13-16=Jhelum, 1-28=Mangla, 29-33=Swat, 34-37=Indus, 38-39=Hingol

Cluster analysis supported such a pattern of inter-population genetic affinity. Such clustering of populations of migratory fish species, *Brycon lundii* suggested 100% sharing of bands between un-isolated populations and 27.3% sharing with the recently isolated population (Sanches *et al*., 2012).

Hingol River maintains isolation from the Indus drainage system since last Pleistocene glaciations periods but maintains a common mahseer lineage with the Indus river system mahseer population. Longer separation of Malaysian population of golden mahseer has been indicated previously by Esa (2009). Historic interconnection of majority of rivers systems of Peninsular Malayasia during Tertiary and Quaternary periods (10–5 Million years ago) attributed a common lineage of Endau-Rompin *Tor douronensis* (Esa and Rahim, 2013).

Golden mahseer is migratory fish species, which moves longer distances for the breeding purposes (Nautiyal *et al*., 2008). Under such movement fair amount of inter-population gene flow probably persisted till recent times between populations occupying different rivers of Indus river system. Damming of water at different places and pollution caused barriers are recent events; therefore effects of such isolations do not appear in present analysis. Hingol River population appears to have a longer isolation from other golden mahseer populations of the Indus river system. Similar studies, using RAPD markers in *Prochilodus marggravii* (Hatanaka and Galletti Jr., 2003) and Korean cat fish (*Silurus asotus;* Yoon and Kim, 2001) indicated significant differences between different population, attributed to different habitat conditions of different sampling sites and thence ecological isolations. Isolation resulted in high rates of inbreeding within population in damsels fishes (Tamang *et al*., 2012).

Conclusion

There still exist sufficient heterogeneity in the individual populations of golden mahseer and the populations are not systematically isolated from one another. Man-made reservoirs, dams and barriers are not very effective and the possible isolation is broken through floods in freshwater systems during different parts of the year. Three populations of AJK are probably a single population, with very frequent migration of individuals between different populations. The creation of Mahseer National Park at Poonch River will support protection of golden mahseer population genetic diversity, and hence probably special efforts are not required for facilitating inter-population gene exchange. Nonetheless Hingol population has distant relation from other populations of golden mahseer present in the Indus River system.

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