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STUDY OF GENETICAL DIVERSITY OF MAHSEER (*TOR TOR*) FROM RANA PRATAP SAGAR DAM, KOTA (RAJASTHAN) INDIA

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ABSTRACT

Mahseer (*Tor tor*) is an economical important fish but due to anthropogenic factors it is subjected to over exploitation and is threatened simultaneously. In Rana pratap sagar dam of Chambal, Kota (India) it is found in good number and despite of thermal pollution it has adapted itself. A present study was aimed to identify the molecular deviation of species within the morphologically akin population. The study reveals occurrence of two genetical subgroups with single nucleotide replacement and its parallel origin. B.oth the subgroups were found to be stable and fertile *inter se.*

KEYWORDS: Chambal, Cytochrome Oxidase I, Mahseer, Phylogenetical Tree, Ranapratap Sagar Dam, Time Tree

INTRODUCTION

The Chambal River is a part of Gangetic drainage system and a tributary of the Yamuna River in central India. The river run northeast through Madhya Pradesh, flow through Rajasthan, and then forms the boundary between Rajasthan & Madhya Pradesh before moving southeast to unite the Yamuna in Uttar Pradesh (Jain *et al.*, 2007). A series of multi-use dams at Gandhi Sagar (M.P.), Rana Pratap Sagar (Rajasthan), Jawahar Sagar (Rajasthan) and Kota barrage (Raj.) have been built in the greater reaches of the Chambal River. The Rana Pratap Sagar (RPS) dam has been constructed on the river Chambal at 24° 35 N lat. and 73° 35 E of river in Rawatbhata Kota (Rajasthan) India, which is well acknowledged for fish production as well as energy and irrigation. Recorded depth of this water reservoir is 37 m. with covering area of 21,300 ha.

Water pollution in India has now reached to a level of catastrophe due to unintended urbanization and hurried escalation of industrialization. Freshwater resources get contaminated due to various human activities viz. dumping of dead bodies, civic & industrial waste discharge, untreated sewage deposition and thermal effluents discharge etc. are major reason of ecological spoil and cause grave health hazards (Meitei *et al*., 2004; Duran and Suicnz, 2007). Due to water pollution the complete array of life in water is affected, including aquatic biodiversity which affect freshwater fauna and fishes directly or indirectly (Vyas and Singh, 2004; Verma *et al*., 2008). With the growing human interference in the natural aquatic ecosystems and especially of lakes and reservoirs sustenance of Mahseer has become an issue of vital concern in India (Saini *et al*., 2013).

In Kota (Rajasthan) the perepherial water bodies are continuously by heated water discharged by Rajasthan atomic power station. Due to thermal pollution, temperature of water rises by 2.53deg C beyond the ambient temperature including the current study site RPS. The vertical spread is limited to 22.5m depth. Though, it is stated that there have been no instances of massive fish kill at the site due to synergism, entrainment and impingement (Gurg *et al*., 1979).

Genealogy of *Labeo rohita* (Hamilton, 1822) from mitochondrial DNA 6/8 gene of Chambal river have been studied formerly (Luharia *et al*., 2013). Aquatic biodiversity of RPS have also been reported and morpho physiographic characters of Tor mahseer and *Labeo calbasu* (Ham.) from RPS have been previously reported (Saini *et al*., 2013; Choudhary *et al*., 1991).

Tor Mahseer forms an important component of fish catch from RPS Reservoir but due to thermal pollution its pool is changing drastically. As per IUCN list it's a threatened species and needs to be conserved but due to lack of molecular taxonomical parameters it forms a lacuna in conservation strategies hence the present study was planned to characterize the molecular diversity.

MATERIAL AND METHODS

DNA Extraction, Amplification

For isolation of DNA fin-clip of random mahseer samples were collected from Rana Pratap Sagar, Kota (Rajasthan). 90% ethanol was used to preserve the collected tissue and DNA was isolated using Phenol Chloroform method. Fish F1 and Fish R1 primers were used to amplify *Cytochrome Oxidase I (COI*) gene. 25 µl reaction mixture was prepared to amplify 50 ng. DNA template 1X PCR buffer, 2 mM $MgCl_2$, 10 picomoles of each primer, 0.25 mM of dNTP mix and 0.25 U Taq polymerase together were mixed. The PCR cycle set of initial denaturation (94ºC, 3 min) followed by 35 cycles of denaturation (94ºC, 30s), annealing (54ºC, 30s), and extension (72ºC, 1 min). Amplified product was directly sequenced using BigDye Terminator v3.1 Cycle Sequencing kit with FishF1 primer only. The latter product was purified using sodium acetate purification method. PCR product obtained in lyophilized form was sequenced with 310 genetic analyzer (Applied Biosystems).

Sequence Analysis *Studies*

Collected nucleotide sequences from lab studies were converted into protein sequence via ExPASy-Translate tool. ExPASy's ProtParam tool (http://web.expasy.org/protparam/) was used to analyze physico-chemical properties viz., amino acid composition (%), molecular weight, theoretical isoelectric point (pI), number of positively charged (Arg + Lys) and negatively charged residues (Asp + Glu), instability index, aliphatic index and grand average of hydropathy (GRAVY). Mega 6.0 program was used to create maximum-liklihood phylogenetic tree, time tree and.

RESULTS AND DISCUSSIONS

Through *in silico* comparative sequence study using local alignment revealed that RPS mahseer samples differed in single amino acid sequence in mitochondrial COI region. Substitution of Leusine was noted in RPS-2 sample, which evidently supported single nucleotide polymorphism (SNP) due to this RPS-2 sample clustered separately when maximum likelihood phylogenetic tree was created. Amino acid chain length differed from 188 to 206; so did its molecular weight which varied from 19924.65 to 21901.86. Isoelectric point also varied in different samples from 4.93 to 5.30. Total number of negatively charged residues (Asp + Glu) was constant in all samples (9), except in RPS-5 and RPS-6 which was 7 and 8 respectively. Whereas total number of positively charged residue (Arg + Lys), was constant in all samples (4). All the proteins were stable and their instability index ranged from 25.07 to 28.11. Aliphatic index varied in all the samples and their range was observed from 121.70 to 126.60. GRAVY values were observed from 0.828 to 0.906 (Table 1).

The phylogenetic tree and time-tree was prepared using MEGA 6. From Maximum Likelihood method based

phylogenetic tree three clusters were observed, where RPS-2 formed a separate cluster, RPS-1 and RPS-3 together formed second cluster and RPS-4, 5 & 6 formed the third cluster. Whereas from Maximum Likelihood method based time tree it can be easily inferred that all samples evolved at the same time except RPS-4 (Figure 1).

Time tree was created using the RelTime method (Tamura *et al*., 2012). Divergence times for all branching points in the topology and evolutionary history were calculated using the Maximum Likelihood method based on the JTT matrix-based model (Jones *et al*., 1992). The estimated log likelihood value of the topology shown is -147.9403. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 200.0000)). The rate variation model allowed for some sites to be evolutionarily invariable ([+*I*], 0.0000% sites). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the relative number of substitutions per site (next to the branches). The analysis involved 6 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 28 positions in the final dataset (Figure 2).

The molecular clock test was performed by comparing the ML value for the given topology with and without the molecular clock constraints under Jones-Taylor-Thornton (1992) model (+G+I) (Jones *et al*., 1992). Differences in evolutionary rates among sites were modeled using a discrete Gamma (G) distribution (shape parameter shown) and allowed for invariant (*I*) sites to exist (estimate of percent invariant sites shown). The null hypothesis of equal evolutionary rate throughout the tree was rejected at a 5% significance level (*P* = 0) (Table 2).

A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories, [+*G*]). Mean evolutionary rates in these categories were 0.90, 0.96, 1.00, 1.04, 1.10 substitutions per site. The amino acid frequencies are 7.69% (A-Alanine), 5.11% (R-Arginine), 4.25% (N-Aspargine), 5.13% (D-Aspartate), 2.03% (C-Cystein), 4.11% (Q-Glutamine), 6.18% (E-Glutamate), 7.47% (G-Glysine), 2.30% (H-Histidine), 5.26% (I-Isoleusine), 9.11% (L-Leusine), 5.95% (K-Lysine), 2.34% (M-Methionine), 4.05% (F-Phelanine), 5.05% (P-Proline), 6.82% (S-Serine), 5.85% (T-Threonine), 1.43% (W-Tryptophan), 3.23% (Y-Tyrosine), and 6.64% (V-Valine). Maximum percentage of Leusine (L-9.11%) was observed where as lowest was for Tryptophan (W-1.43%). For estimating ML values, a tree topology was automatically computed. Evolutionary analyses were conducted in MEGA6 (Tamura *et al*., 2013) (Figure 3). The existence of two genetically clusters with single nucleotide substitution and existence of stable protein marks the adaptability of this fish to the heated Chambal environment, which in turn is a positive indicator for conservation strategies (Ambili *et al.*,2014). In future, it may lead to new germ line adapting itself for the pertaining environments.

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APPENDICES

Table 1: In Silico Physic- Chemical Properties of Rana Pratap Sagar Mahseer Samples and Its Components

S. No.	PCP	RPS1	RPS2	RPS3	RPS4	RPS5	RPS6
1	NAA	205	206	206	206	188	192
2	MW	21754.68	21853.82	21901.86	21901.86	19924.65	20435.20
3	pI	4.94	4.94	4.94	4.94	5.30	4.93
4	TNNR (D + E)	9	9	9	9	7	8
5	TNPR (R + K)	4	4	4	4	4	4
6	II	28.11**	27.61**	28.02**	28.02**	25.07**	26.05**
7	AI	122.29	123.11	121.70	121.70	126.60	123.96
8	GRAVY	0.828	0.845	0.838	0.838	0.906	0.895

1. RPS- Rana Pratap Sagar Mahseer

2. PCP- Physicochemical Parmeter; NAA- Namber of Amino Acids; MW- Molecular Weight; PI- Isoelectric Point; TNNR (D+E) – Total number of negatively charged residues (Asp+ Glu); TNPR (R+ K)- Total number of Positively charged residues (Arg + Lys); II- Instabilityindex; AI- Aliphatic index and GRAVY- Grand of hydropathicity

3. *- unstable;**- stable

Table 2: Test of Molecular Clocks using the Maximum Likelihood Method

	ln*L*	Parameters	(+*G*))	(+*I*)
With Clock	-262189.148	7	200.000	0.00
Without Clock	-147.940	11	200.00	0.00

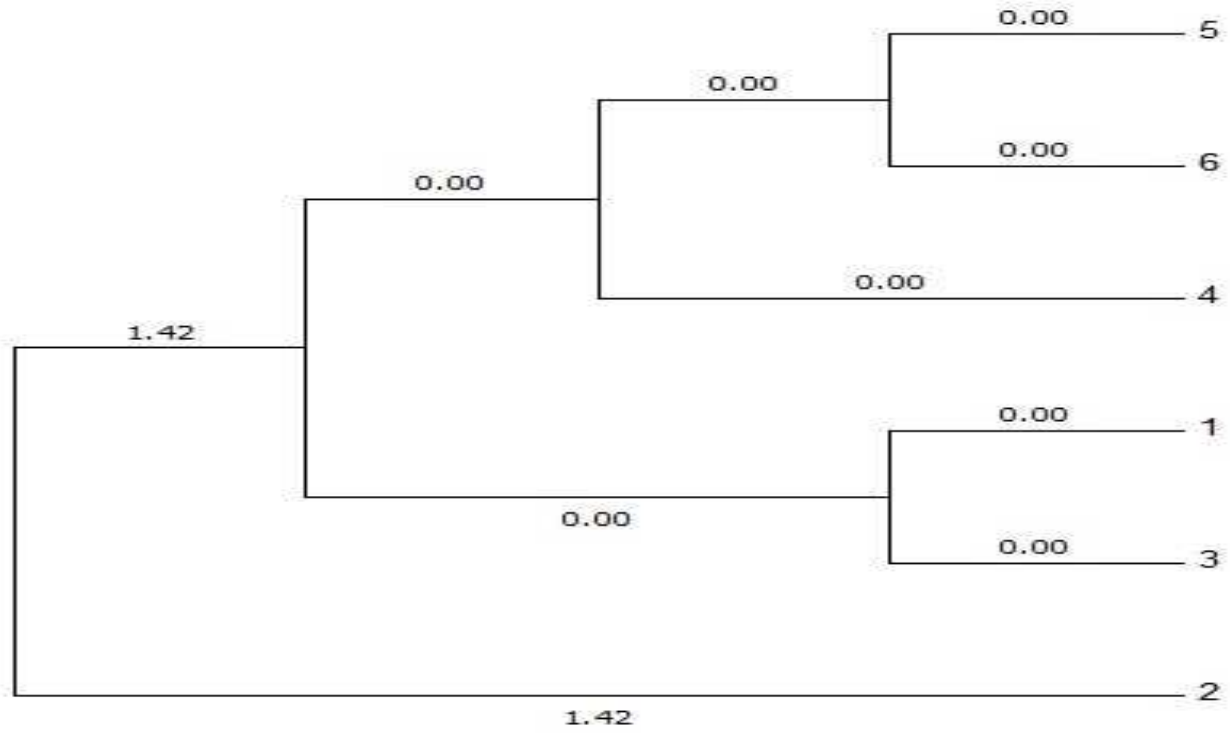

Figure 1: Molecular Phylogenetic Analysis of Mahseer Clustering by Maximum Likelihood Method

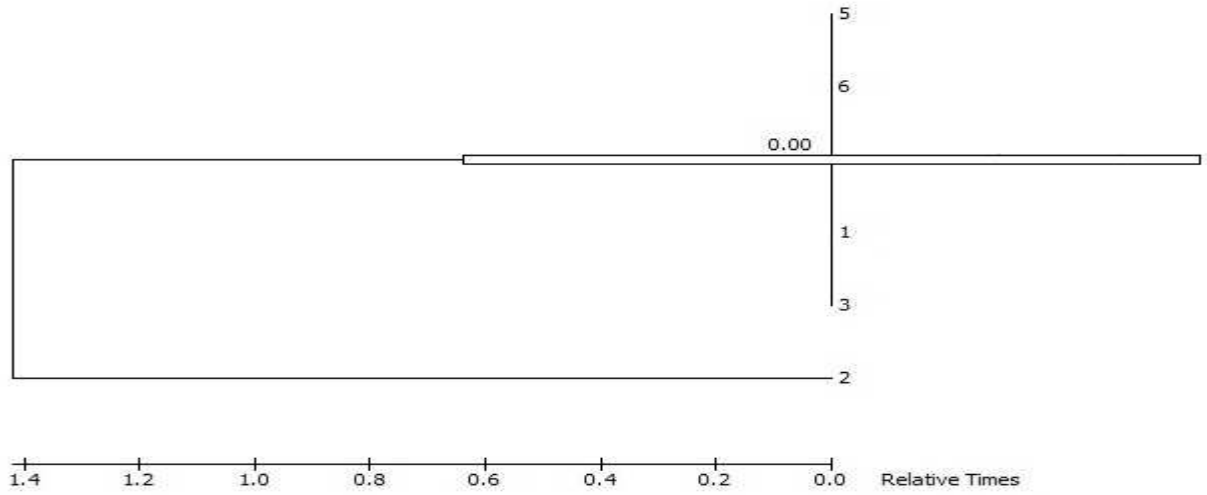

Figure 2: Molecular Time Tree Analysis in Six Samples of Mahseer Using Maximum Likelihood Method

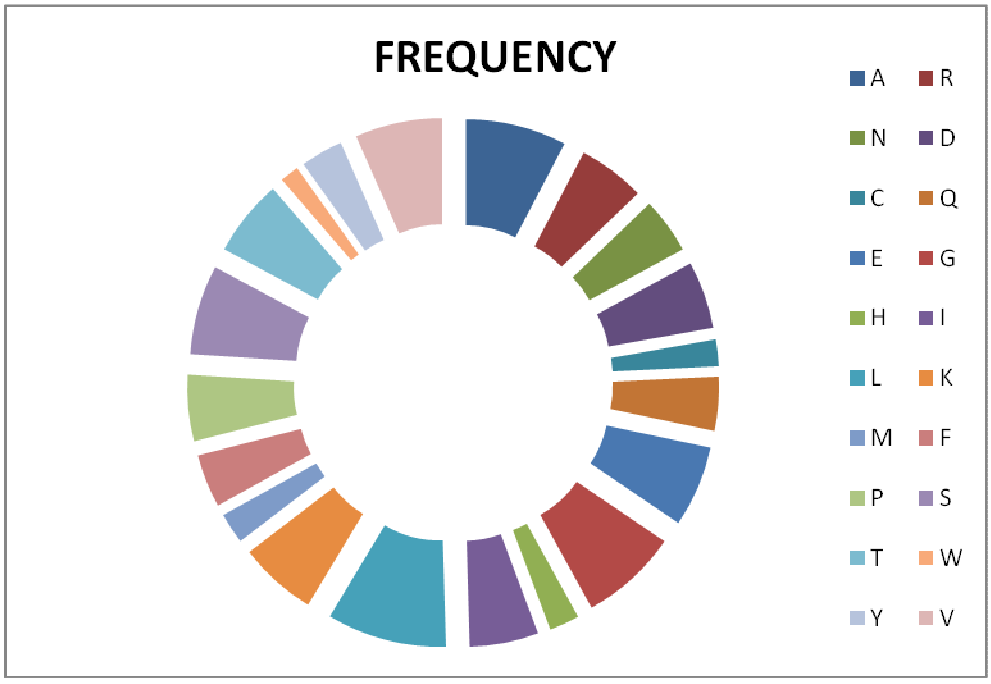

Figure 3: Comparative Percentage of Amino Acid Frequency in Mahseer Population