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Genetic differentiation among Himalayan and local Mahseer populations

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Abstract
This study of genetic differentiation among Himalayan & local Mahseer concerns with a conservation plan is a fundamental part of the hydro projects still in the pipeline. *In-situ* conservation and gene banking also serve as the best medium for the same. Despite conservation programs, re-examination and revision of these conservation areas to update the number and lists of endangered status are mandatory.
Fish samples collected from 8 different waterbodies {Chambal River, Rana Pratap Sagar Dam, Bassi Dam, Kali Sindh River, Parwan River, Badi Lake, Daya Dam, and Madar Tank} representing two major drainage systems (the Bay of Bengal and the Arabian Sea) of India.
For the cytochrome oxidase subunit I, a total of 32 sequences was generated, whereas, for the ATPase 6 gene, a total of 66 sequences were generated. Two mitochondrial regions COI and ATPase6 were amplified using the set (Forward & Reverse primer set) of published primers (COI-Fish F-1/Fish R-1.
BOLD-IDS was used to test the efficiency of DNA barcoding as a species identification tool using a blind sampling test, the sample selected & sequenced were known only to the submitting individual. Mismatch distributions to evaluate the hypothesis of recent population growth with 99,999 permutations as implemented in ARLEQUIN 3.0.1 was used. This distribution is unimodal in populations that have passed through a recent demographic expansion and are multimodal in stable populations. A DNA sequence of Himalayan Mahseer (*Tor tor*) was downloaded from NCBI Gene Bank and the sequences generated in the present study were compared (Using MEGA7 Software) with the downloaded sequence.
It's derived that the stocks of Rajasthan Mahseer are quite distinct from the Himalayan stocks. The minimum genetic (116.06) distance was noticed for both Daya dam and Madar Tank and the highest (125.35) was for Bassi Dam. The evolutionary history was inferred using the Minimum Evolution method. The optimal tree with the sum of branch length 123.79668059.
The significantly higher genetic distance between Rajasthan and Himalayan stocks could be due to geographic location and mainly isolated aquatic resources. Similar findings have also been reported by earlier workers.

Keywords: DNA sequence, Himalayan Mahseer, genetic diversity, endangered status

Introduction

The conservation of genetic diversity is not only imperative for the sustainable fishery, but also known to play an important role in national development. A proper taxonomic identification using different molecular markers is a vital step towards the conservation of endangered Mahseer. Since all the species of Mahseer are considered endangered, careful consideration of all the stakeholders is needed to guard them against a further drop in numbers. The use of species-specific novel markers to assess genetic diversity has become the need of the hour, which can play an important role in the execution of potential conservation gambits [1].

The Mahseer conservation plan should be a fundamental part of the hydro projects that are still in the pipeline. In-situ conservation and gene banking can also serve as the best medium for the conservation of Mahseer species. It is essential to identify various areas across the country that being committed to the conservation of Mahseer by coordinating various activities in those areas. Despite conservation programs, re-examination, and revision of the defined conservation areas to update the number and lists of endangered status are mandatory. It is important to reignite the global interest towards Mahseer.

A formal taxonomical clarification is essential for this fish. Though Mahseer is known by its common name throughout the world, however, many reviews have reported confusion over the name. This requires scientific attention along with the aimed research by collecting the DNA. Taxonomic identity is important for the conservation approaches for fishes having a higher

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risk of extinction [2]. With this view, a study related to Genetic differentiation among the Himalayan and local Mahseer population was carried out.

Material and Method
In the present study, fish samples were collected from eight different aquatic systems {Chambal River (CR), Rana Pratap Sagar (RPS), Bassi Dam (BD), Kali Sindh River (KSR), Parwan River (PR), Badi Lake (BL), Daya Dam (DD), and Madar Tank (MT)} representing two major drainage systems (the Bay of Bengal and the Arabian Sea) of India. For the Cytochrome Oxidase subunit, I, a total of 32 sequences was generated, whereas, for the ATPase 6 gene, a total of 66 sequences were generated. Two mitochondrial regions, i.e. COI and ATPase6 were amplified using the set (Forward & Reverse primer set) of published primers (COI-Fish F-1/Fish R-1). Sequence Analysis Software Version 5.2 (Applied Biosystems, CA, USA) was used to generate sequence trace files and contiguous read lengths. The standard protocol prescribed by ABI systems for operating ABI310 Genetic Analyzer was followed for sequencing ATPase6 and COI genes of *Tor* sp.

Further, the sample identification based on the sequence similarity approach was carried out using the BOLD system. BOLD-IDS (BOLD Identification System) [3] was used to test the efficiency of DNA barcoding as a species identification tool, where a blind sampling test was conducted, and in the samples, identities unknown except to the submitting individual were selected and sequenced. We used mismatch distributions to evaluate the hypothesis of recent population growth with 99,999 permutations as implemented in ARLEQUIN 3.0.1 [4]. This distribution is commonly unimodal in populations that have passed through a recent demographic expansion and are multimodal in stable populations.

Result and Discussion
Species validation through DNA Bar-coding
The mitochondrial cytochrome oxidase I (COI) region of all samples was successfully amplified using PCR. Summary of identification based on each sample barcoded sequence. The following are shown (Table 1) using the BOLD Identification Method (BOLD-IDS) and the BLASTN search from GenBank.

**Table 1:** Using the (BOLD-IDS) and the BLASTN search from GenBank

Sampling Location	No of Samples	Bold -IDS		GenBank (BLASTN)		Consensus (Morphological & Bar-coding Data)
		Species Identified	% similarity	Species identification	% Max identity	
Chambal river (CR)		*Tor tor*	100	*Tor tor*	99.06	*Tor tor*
Rana Pratap Sagar (RPS)		*Tor tor*	100	*Tor tor*	99.14	*Tor tor*
Bassi dam (BD)		*Tor tor*	1000	*Tor tor*	99.26	*Tor tor*
Kali Sindh river (KSR)		*Tor tor*	100	*Tor putitora*	97.65	*Tor tor*
Parwan river (PR)		*Tor tor*	100	*Tor tor*	99.05	*Tor tor*
Badi Lake (BL)		*Tor tor*	100	*Tor tor*	98.94	*Tor tor*
Daya dam (DD)		*Tor tor*	100	*Tor tor*	99.27	*Tor tor*
Madar tank (MT)		*Tor tor*	100	*Tor tor*	98.93	*Tor tor*

Genetic differentiation among Himalayan and local Mahseer populations
Mahseer is Himalayan fishes of cold waters, however, they are also disseminated in plains by some or other means and are well established in some regions. Keeping this point in mind, the present aspect of genetic differentiation among Himalayan and local Mahseer populations study was attempted. For this purpose, a DNA sequence of Himalayan Mahseer (*Tor tor*) was downloaded from NCBI Gene Bank (Accession No JX204431), and the sequences generated in the present study were used for comparison (Using MEGA7 Software) with the downloaded sequence. As such, the results obtained are presented in the following figures 1:

It is obvious from the above figure that the stocks of Rajasthan Mahseer are quite distinct from the Himalayan stocks. The minimum genetic (116.06) distance was noticed for both Daya dam (DD) and Madar Tank (MT). However, the highest (125.35) genetic distance was for Bassi Dam. Evolutionary history has been inferred using the Minimum Evolution Process. The optimal tree with the sum of branch length = 123.79668059 (see figure 2 below)
The significantly higher genetic distance between Rajasthan and Himalayan stocks could be due to geographic location and mainly isolated aquatic resources. Similar findings have also been reported by earlier workers [5, 6, 7].
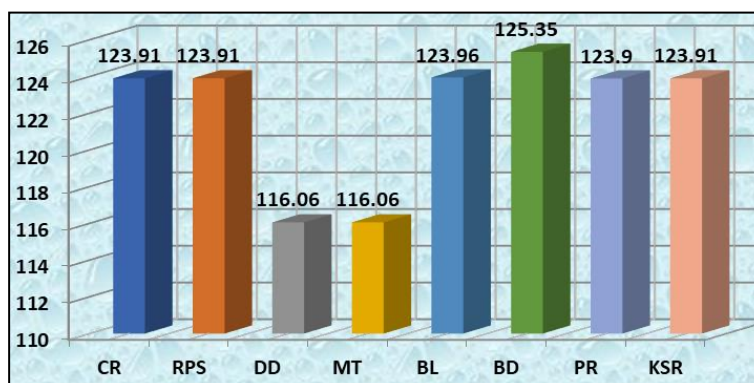
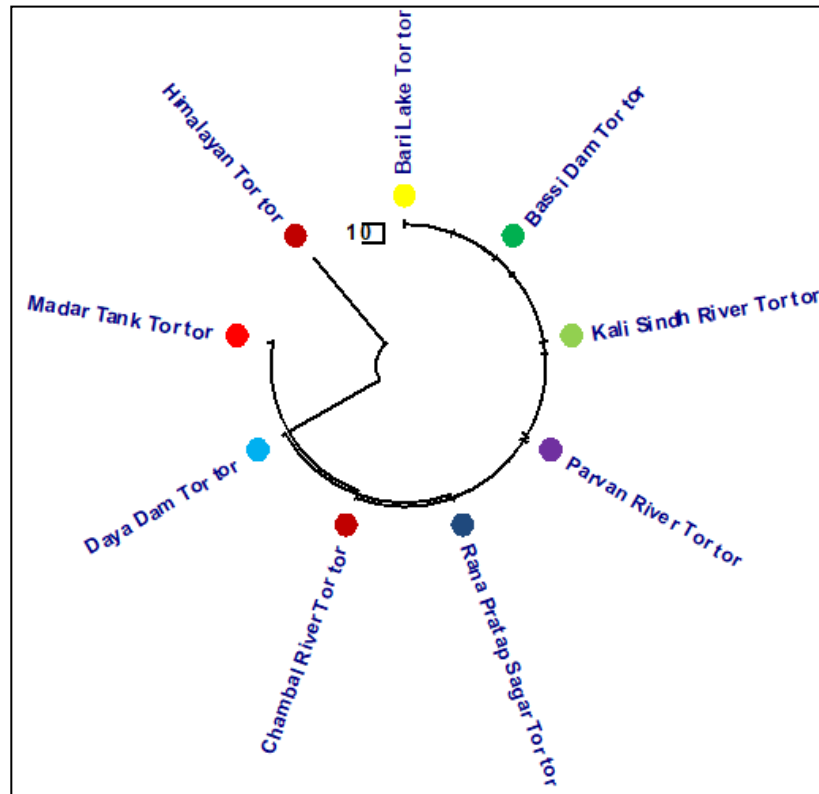**Fig 2:** Genetic distances in comparison to Himalayan *Tor tor*

Fig 3: Evolutionary relationships of taxa

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