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Original Article

Phylogeographic Divergence and Evolutionary Dynamics of Labeo rohita in Central Indian River Basins Based on COI Gene Analysis

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Abstract

Commonly Rohu, which can be referred to as Labeo rohita (Hamilton, 1822), is a fish species that is regarded as highly valuable in the Indian fish markets especially freshwater fishes It becomes essential to understand genetic structure of this fish in order to conserve and sustain aquaculture practices like fishery agriculture. The use of COI gene sequences technique allowed us to study & explore the evolution and morphological relationships of L. rohita populations in the Narmada and Godavari river basins in comparison with each other. Here The Tamura-Nei model was employed to analyze six sequences using maximum likelihood (ML) and neighbor-joining (NJ) methods. There were 665 nucleotide positions contained in the final dataset Two distinct clusters were identified by phylogenetic analysis, with one consisting of Narmada samples (MP04, Narmada-5) grouped with the Godavari sample, and the other consisting of Narmada samples (MP01, MP02, MP03.) Nucleotide composition was biased towards AT (56%) and there were a nucleotide diversity ($\pi = 0.118$) and Tajima's D (0.981) that indicated neutral evolution and potential population stability. The Narmada exhibits genetic structuring within the basin, and there could be historical or anthropogenic connectivity between the river basins, as determined by the results The findings give crucial information about the biology and genetic preservation of L. rohita in Central India.

Keywords: *COI: Cytochrome c Oxidase subunit I; L.rohita: Labeo rohita, MP: Madhya Pradesh*

Introduction

The Labeo rohita is a key species in Indian fish aquaculture and inland fisheries due to its high economic and nutritional value and it consumed in large quantity it is one of the Indian major carp in Labeo family. Overexploitation, habitat degradation/fragmentation, and hatchery intrusion can all significantly increase the risk of extinction, resulting in an erosion of population size, connectivity, and genetic integrity. The protection of natural populations cannot be achieved without managing fishing efforts, protecting/restoring habitat, and tightly controlling stocking practices. In mammals and many protists, the mtDNA COI gene is frequently utilized for researching genetic diversity, population structure, and phylogeography as it is easy to amplify, quickly evolve, and has maternal inheritance without recombination. By comparing matched Narmada and Godavari populations, COI reconstruction can aid in separating the geological sources of connectedness between these two central Indian basins godavari and Narmada.

The objective of this study is to:

1. Look at the phylogenetic connections between Labeo rohita populations.
2. Assess the variety and neutrality of nucleotides.
3. Study how evolutionary patterns differ across codon positions.

Materials and Methods:

Labeo Rohita Sample was collected from Local fish Market where it arrived from godavari river tributary. Next step was DNA Extraction, Amplification and Sequencing the process of DNA extraction was easy initially we took fish samples of fish that are fins and tail parts and stored in absolute alcohol (70-80%) DNA was extracted from fin using a standard extraction kit (Qiagen method). After this we obtained mitochondrial COI gene region (~658 base pairs) which was further amplified by Polymerase Chain Reaction (PCR) using universal primers

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- **FishF1:** 5′-TCAACCAACCACAAAGACATTGGCAC-3′
- **FishR1:** 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′

Sequences were edited, aligned, and confirmed using BioEdit, BLAST, and MEGA software, & obtained COI Sequence of Godavri River was Submitted to NCBI Portal with accession number PZ158824

1. **Sequence Data**

We studied six COI gene sequences of the fish L. rohita The analysis of six COI gene sequences of each L. rohita, consisting of five COI sequences from the Narmada River basin and one from the Godavari River basin the samples from Narmada river basin which were named as MP01, MP02, MP03, MP04, and Narmada-5 respectively while sample from Godavari River as it is as Godavari River sample the COI Sequences data was obtained from the NCBI portal. The Accession numbers of both samples of Narmada River and godavari River on the NCBI portal, which were JQ912095, JQ912094, JQ912093, JX983352, and JX983350 and JQ983350, respectively for Narmada River basin. The Accession number PZ158824 of was Godavari River sample which we have obtained by submitting COI Sequence at NCBI Portal.

2. **Phylogenetic Analysis**

Two widely recognized methods were used to construct phylogenetic trees in order to explore evolutionary relationships among the L. rohita fishes. We first utilized the Maximum Likelihood (ML) method as a basis for the Tamura-Nei nucleotide substitution model. We considered implementing the Neighbor-Joining (NJ) methodology and the Maximum Composite Likelihood approach as our second Step To measure the statistical confidence of the tree topology and the reliability of the identified clades, & also we conducted an online bootstrap analysis with 500 replications in MEGA 12 Software to get following Phylogenetic Result

3. **Data Treatment:**

The final analysis included 665 base pairs of COI gene sequences obtained from Mitochondrial DNA, and the pairwise deletion option was used to remove any gaps and missing data in the alignment in MEGA 12 Software. We considered all codon positions (1st, 2nd, and 3rd) as well as non-coding regions in the analysis. MEGA 12 a software version was used for all phylogenetic analyses and sequence alignments

4. **Statistical Analysis**

A number of essential parameters were calculated to gain a greater understanding of the inheritable variation in the dataset. According to the analysis, there were 156 segregating sites (S) that indicated the number of positions in the sequences where variation took place. The total amount of inheritable variation among the sequences studied was estimated at 0.118296. This shows a high level of nucleotide diversity (π). Tajima's D value was measured at 0.981791. This value gives insight into the evolutionary forces that affect the population. To explore evolutionary connections, a Tajima's relative rate test was carried out on three sequences, which enabled a comparison of mutation rates between lineages.

(**b**)

(**a**)

Figure 1. Fig.1 Labeo Rohita Fish Image

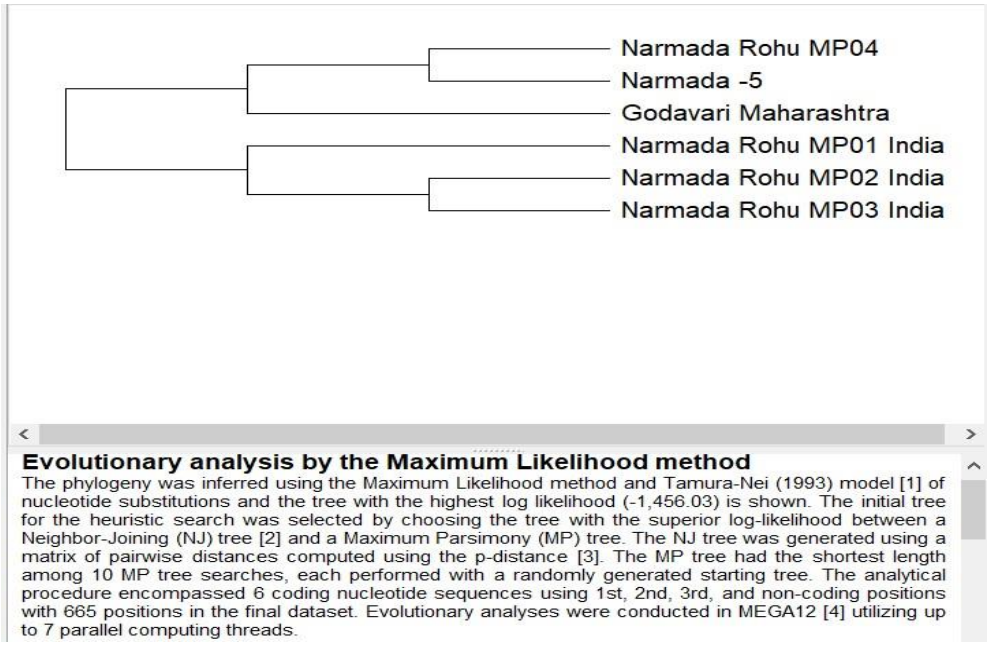
Results

1. **Phylogenetic Relationships:** A tree with a log-liability value (log-likelihood = −1456.03) was generated by the phylogenetic analysis using the Maximum Likelihood (ML) system. The performing tree was capable of separating the sequences into two major clusters, revealing distinct inheritable groupings in the dataset.

The ML revealed two major clusters:

- **Cluster I:** Narmada samples (MP04, Narmada-5) grouped with the Godavari sample
- **Cluster II:** Narmada samples (MP01, MP02, MP03)

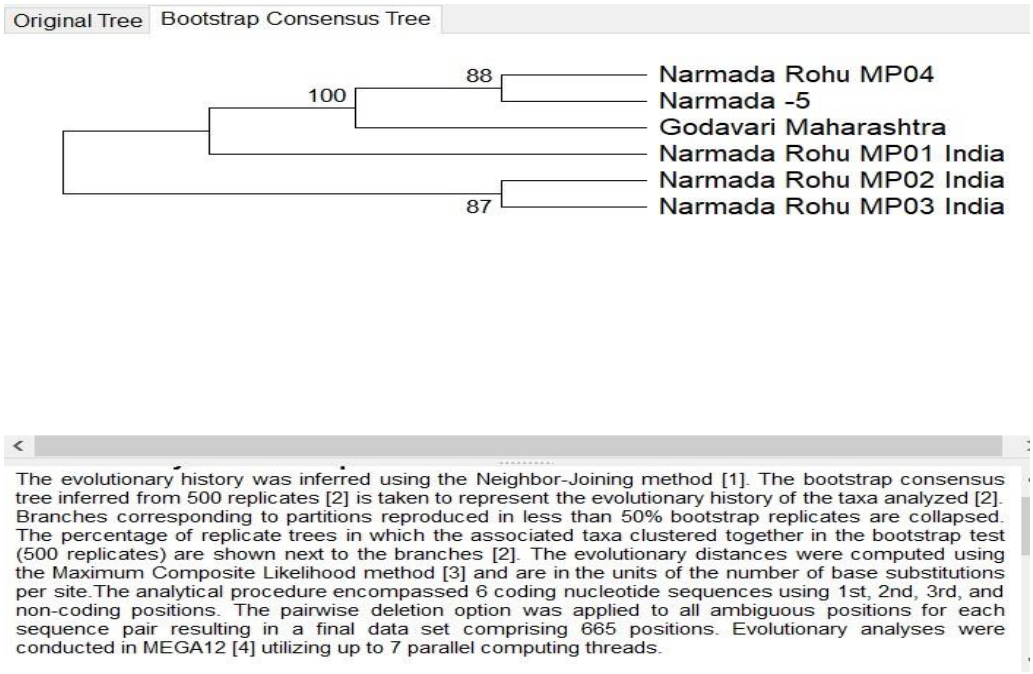
According to this clustering pattern, it is evident that Narmada receptacle populations experience conspicuous inheritable isolation The comparison of certain Narmada samples with the Godavari sample suggests that there might be a connection between these two River systems at the same time



2. **Bootstrap Support**

To validate the trustability of the observed phylogenetic connections, a Neighbor- Joining (NJ) tree was constructed and estimated using bootstrap analysis. The results showed strong statistical support for the major groupings linked in the ML tree. A bootstrap value of 100 was entered for the Narmada-Godavari clade, which indicates a high level of confidence in this grouping. The MP04 – Narmada- 5 subclade was supported by a bootstrap value of 88, buttressing their close inheritable relationship. The bootstrap value of 87 was found in the grouping of MP02 and MP03, which suggests a well-supported association between these samples. Overall, these high bootstrap values strengthen the credibility of the phylogenetic structure and confirm that the linked clades are statistically robust.

These values indicate strong statistical support for clade formation.



3. **Nucleotide Composition**

An AT bias of 56% was observed, typical of mitochondrial DNA.

Codon-wise G distribution:

1st position: 8.4%

2nd position: 28.1%

3rd position: 13.0%

Table 1 Average nucleotide frequencies.

Sample ID	T (%)	C (%)	A (%)	G (%)	G% (1st Codon)	G% (2nd Codon)	G% (3rd Codon)
Godavari MH	28.1	27.4	26.4	18.1	7.8	30.9	15.5
Narmada MP01	29.3	28.3	28.3	14.0	10.7	22.3	8.9
Narmada MP02	29.3	28.3	28.0	14.3	10.7	23.3	8.9
Narmada MP03	29.3	28.3	28.0	14.3	10.7	23.3	8.9
Narmada MP04	28.6	26.9	26.9	17.5	6.5	30.7	15.3
Narmada-5	28.5	26.9	27.3	17.3	7.8	30.1	14.0
Average	28.7	27.5	27.3	16.5	8.4	28.1	13.0

(Table 1 Graphical representation of Average nucleotide frequencies)

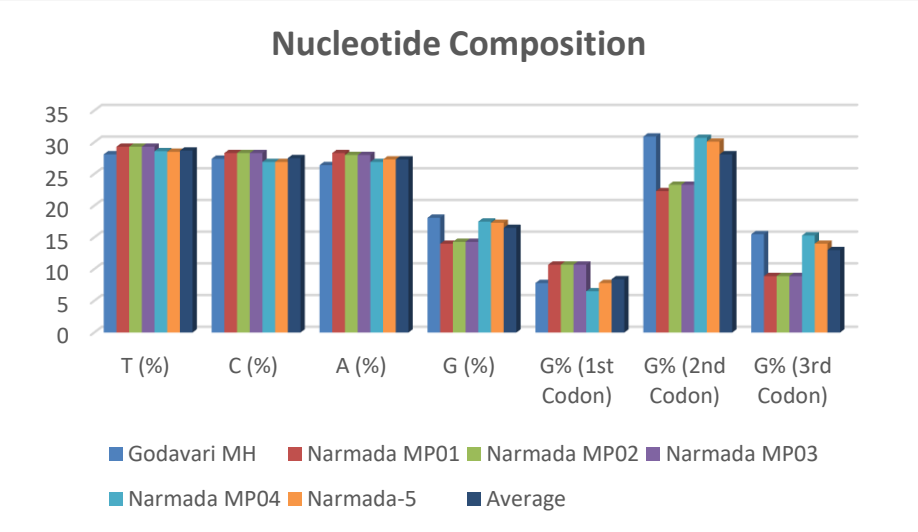
4. **Genetic Diversity and Neutrality**:

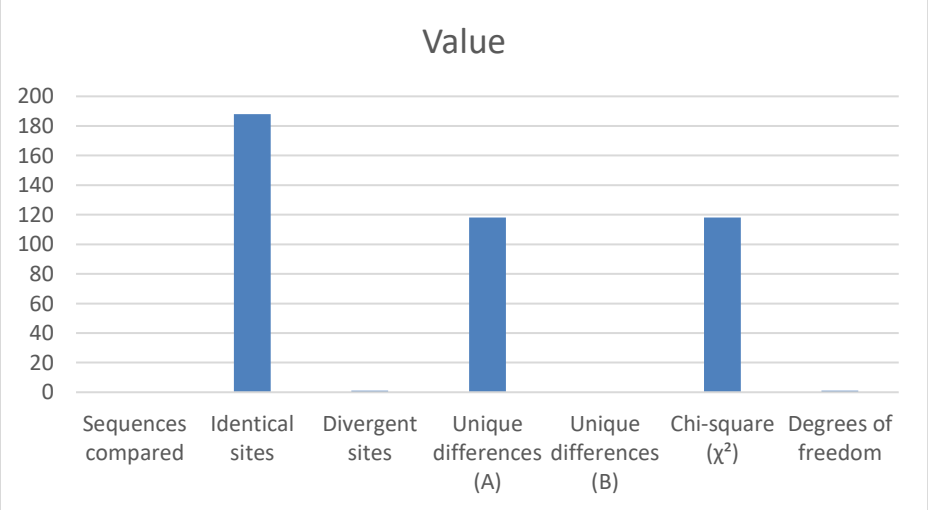
Segregating sites (S): 156

- Nucleotide diversity (π): 0.118
- Tajima's D: 0.981

The positive Tajima's D suggests neutral evolution or population equilibrium, although not strongly significant.

Parameter	Value
Number of sequences (m)	6
Total sites analyzed (n)	665
Segregating sites (S)	156
Proportion of segregating sites (ps)	0.234586
Theta (Θ)	0.102739
Nucleotide diversity (π)	0.118296
Tajima's D	0.981791





5. **Relative Rate Test:**

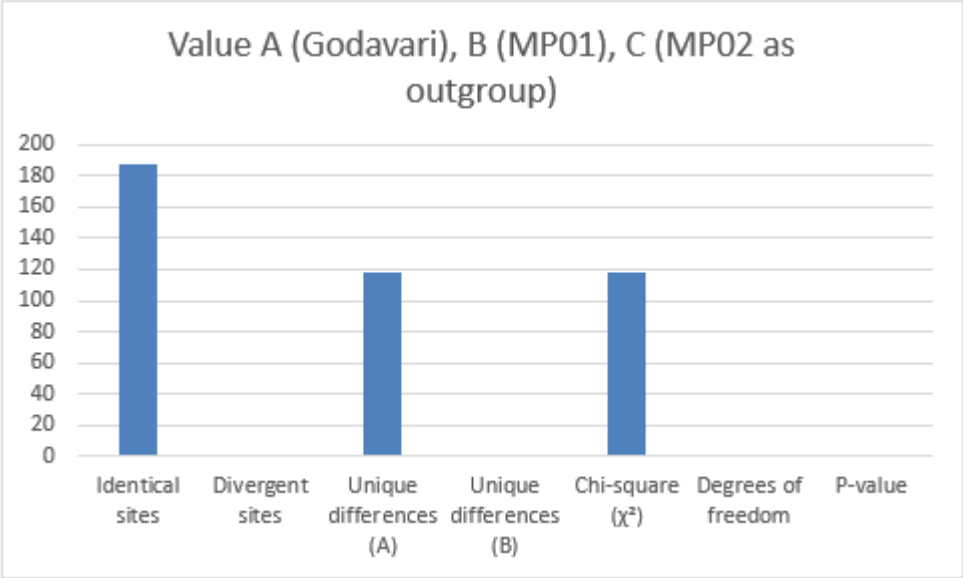
To examine whether the sequences have evolved at similar rates, a Tajima's relative rate test was performed. This analysis test compared sequence A (Godavari) and sequence B (MP01), using sequence C (MP02) as an outgroup to provide a reference point for evolutionary divergence. There was a clear distinction in the number of unique changes between the sequences in the results. All three sequences had 188 identical sites, which indicates a high level of genetic background. However, only one site showed general divergence, in sequence A (Godavari), there were 118 sites that showed unique differences, but sequence B (MP01) did not show any unique differences. This imbalance can be observed statistically through a chi-square (χ^2) value of 118.00.00 with 1 degree of freedom The observed difference was highly significant and unlikely to have been caused by chance, with an associated p-value of 0.00000 ($P < 0.05$).

The Tajima relative rate test showed:

- $\chi^2 = 118.00$ & $P < 0.05$

This indicates unequal evolutionary rates between compared sequences.

Parameter	Value
Sequences compared	A (Godavari), B (MP01), C (MP02 as outgroup)
Identical sites	188
Divergent sites	1
Unique differences (A)	118
Unique differences (B)	0
Chi-square (χ^2)	118
Degrees of freedom	1
P-value	0



Discussion:

1. **Phylogeographic Structure**

Labeo rohita populations can be geographically distributed and we can study evolutionary histories of Labeo rohita by analyzing phylogenetic patterns by clustering of the Godavari sample with specific Narmada sequences was one of the most intriguing findings. The pattern of results have indicated that swash systems were preliminarily connected conceivably during once geological events when swash courses were connected presumably it may also indicate fish migration caused by humans including common stocking practices in aquaculture and fisheries management. In the phylogenetic observation the presence of two distinct genetic clusters within the Narmada river basin indicated a distinct intro-basin structure and we know this type of differentiation is often caused by ecological variations habitat fragmentation, or geographical barriers that limit gene flow between populations over a period of time. The phylogenetic analysis was executed using established methods and parameters to ensure reliability and robustness in this research. Both Maximum Likelihood (ML) and Neighbor-Joining (NJ) approaches were applied The Tamura-Nei substitution model was employed in the ML analysis while Maximum Composite Likelihood distances were utilized by the NJ method. Statistical support for hypothesized relationships between fish species was provided by a Bootstrap analysis with 500 replicates. A pairwise deletion method was used to remove gaps in the sequence alignments, and analyses were performed for each codon position (first, second, third, and non-coding regions). All analyses were performed with MEGA Version 12.

clustering of the Godavari sample with Narmada sequences suggests:

Parameter	Setting
Phylogenetic methods	Maximum Likelihood (ML), Neighbor-Joining (NJ)
Substitution model (ML)	Tamura–Nei (1993)
Distance method (NJ)	Maximum Composite Likelihood
Bootstrap replicates	500
Gap treatment	Pairwise deletion
Codon positions analyzed	1st, 2nd, 3rd + non-coding
Software used	MEGA Version 12

2. **Evolutionary Dynamics**

AT-rich nucleotides are commonly observed in mitochondrial genomes. The third codon position was predicted to have a higher level of variability, which is in line with its lower functional constraints and resistance to mutations. The preservation of the first and alternate codon positions during the discrepancy is likely due to their importance in protein structure and function. The COI gene's functional importance is confirmed by these patterns, which support its use as a molecular marker for phylogenetic and population-level investigations.

3. **Population Genetics:**

The population's genetic variation is moderate based on the estimated nucleotide diversity (<unk>= 0.118). This suggests that although there is noticeable genetic diversity it is not sufficient to indicate deep or long-term divergence among these groups Tajima's d-value lends further support to this observation. The outcome does not show that the population is under a lot of selective pressure. However, it suggests a scenario of population stability that fails to demonstrate strong positive selection, recent population expansion, or bottleneck events

Conclusion:

The study showed that Labeo rosita populations from Central India, especially in Madhya Pradesh and Maharashtra, have strong phylogenetic patterns. Here, the identification of distinct genetic clusters within the Narmada basin points to internal population differentiation, while the observed association with the Godavari population suggests potential historical or can be human-mediated connectivity between these river systems. The practical implications of these findings are significant by ensuring the maintenance of genetic diversity it is possible to contribute to more intellectual conservation strategies and aid in the management of genetic resources which will supports the development of sustainable fish aquaculture practices and fish agriculture in upcoming days a Future research that focuses on these findings should incorporate larger sample sizes and nuclear DNA markers which would provide a more comprehensive understanding of population structure and evolutionary dynamics

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Conflicts of Interest: The study was conducted independently, without any financial, commercial, and without any personal relationships with any other that could influence the results or interpretation of these findings."

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