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Morphometric, Meristic, and Genetic Variability of Golden Mahseer (Tor putitora): An Integrated Assessment

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Abstract

A keystone freshwater fish of the Himalayas, the Golden Mahseer (Tor putitora) has seen a sharp decline in population as a result of habitat fragmentation, overexploitation, and genetic erosion. Morphometric, meristic, and RAPD-based genetic analyses are combined in this study to evaluate stock structure and population variability. 120 specimens in all were examined at three different sampling sites. Ten meristic characters and twenty morphometric traits were noted using standard ichthyological procedures. 14 morphometric characters showed significant variation ($p < 0.05$), but meristic traits were mostly conserved. Principal Component Analysis (PCA) revealed population-level differentiation by classifying samples into three separate clusters.Samples were divided into three separate clusters using Principal Component Analysis (PCA), which demonstrated population-level differentiation. Ten primers were used for RAPD profiling, which produced 78.4% polymorphism and genetic similarity values between populations ranging from 0.62 to 0.88. According to the combination of morphological and genetic markers, T. putitora populations exhibit moderate-to-high differentiation, which is probably influenced by environmental heterogeneity and river fragmentation. Implications for conservation include the necessity of basin-specific management techniques and genetically informed hatchery practices.

Keywords: Mahseer, Tor putitora, morphometry, meristic traits, RAPD markers, population structure, conservation genetics

Introduction

In South and Southeast Asia, mahseer fishes of the genus Tor are among the most significant freshwater cyprinids in terms of ecology, culture, and economy. These species, which are frequently referred to as the "King of Rivers," are essential to maintaining traditional heritage, sport fisheries, local livelihoods, and riverine ecosystem processes (Pinder et al., 2019; Nautiyal, 2013). The Golden Mahseer (Tor putitora) is a flagship species for freshwater conservation in the Himalayan region because of its large body size, migratory nature, and sensitivity to ecological disturbances (Bhatt et al., 2016).Due to overfishing, river fragmentation, pollution, hydropower development, and habitat modification, the species has experienced significant population declines throughout its range despite its ecological prominence (Sarkar et al., 2015; Baruah, 2023). In particular, hydroelectric projects impede migratory routes necessary for spawning and disturb river continuity, which ultimately reduces gene flow between populations (Baruah, 2023; Sarma et al., 2022). As seen in a number of freshwater fish species exposed to environmental heterogeneity, this ecological isolation frequently encourages morphological divergence and genetic structuring (Kendall et al., 2007; Solem& Berg, 2011).It has long been known that morphometric and meristic analyses are effective methods for population discrimination and stock identification (Helfman et al., 1997; Ihssen et al., 1981). While meristic counts are comparatively stable and helpful for taxonomic diagnosis, morphometric traits are highly malleable and frequently reflect ecological adaptation (Clayton, 1981). Morphometric indices have been shown to be useful in differentiating populations of Channa (Mohd. Hussain, 2007), Oreochromis (Muhd. Naeem et al., 2011), Etropusmaculatus (Manimegalai et al., 2010), and different cyprinids (Dhinakaran et al., 2011; Bagherian&Rahmani, 2009).In a similar vein, molecular markers like mitochondrial DNA and RAPD offer information about population structure, genetic diversity, and evolutionary independence. Taxonomic ambiguity in Tor, Neolissochilus, and Naziritor has been clarified by recent studies, highlighting the significance of combining morphology and molecular data (Khare et al., 2014; Jaafar et al., 2021; Johnson et al., 2023). It is crucial to determine whether Golden Mahseer populations show quantifiable morphological and genetic differentiation because they are dispersed throughout environmentally different river systems.Thus, the current study integrates morphometric, meristic, and RAPD-based genetic analyses to evaluate the diversity of Tor putitora across three river systems. The study specifically aims to compare variations in body shape, assess meristic trait stability, estimate genetic polymorphism levels, and identify population clustering patterns. The research aims to provide scientific support for the conservation and rehabilitation of Golden Mahseer in Himalayan Rivers, as well as to clarify population structure and identify possible management units

2. Materials and Methods

2.1 Sample Collection

A total of 120 specimens were collected from:
River 1 (Upper Himalayan zone)
River 2 (Mid-altitude zone)
River 3 (Foothill zone)
Specimens were preserved on ice and transported to the laboratory for analysis.

2.2 Morphometric Analysis

Digital Verniercalipers were used to take twenty morphometric measurements. Total length, standard length, head length, body depth, eye diameter, snout length, dorsal fin base length, etc. were among the characteristics.

2.3 Meristic Analysis

Ten meristic traits were recorded:
Dorsal fin rays
Anal fin rays
Pectoral fin rays
Pelvic fin rays
Lateral line scales

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Pre-dorsal scales
Gill rakers

2.4 RAPD Analysis

Fin tissue was used to extract genomic DNA.
There were ten RAPD primers used.
Binary matrices (1 = presence, 0 = absence) were used for band scoring.
Jaccard's coefficient was used to calculate similarity indices, and UPGMA cluster analysis came next.

Results

The results of morphometric, meristic, and RAPD-based genetic analyses of Tor putitora populations collected from three distinct river systems are presented in this section. The findings seek to ascertain the degree of genetic variability, meristic trait stability, and morphological divergence between populations. Principal Component Analysis (PCA) and ANOVA were used to statistically analyze morphometric measurements in order to assess population-level differentiation. RAPD markers were used to evaluate genetic polymorphism and cluster relationships, and meristic traits were analyzed to determine possible taxonomic stability. Together, the results provide a comprehensive understanding of the genetic and phenotypic structure of T. putitora populations.

3.2 Description of Morphometric Results

Table 1. List of Morphometric Characters Measured in *T. putitora*

Code	Morphometric Character
TL	Total Length
SL	Standard Length
HL	Head Length
BD	Body Depth
ED	Eye Diameter
SnL	Snout Length
CPL	Caudal Peduncle Length
CPD	Caudal Peduncle Depth
PFBL	Pectoral Fin Base Length
AFBL	Anal Fin Base Length
DFBL	Dorsal Fin Base Length
PrDL	Pre-dorsal Length
PrAL	Pre-anal Length
PrPL	Pre-pectoral Length
PrVL	Pre-ventral Length
MW	Maximum Body Width
HW	Head Width
JLW	Jaw Length (Upper)

JLL	Jaw Length (Lower)
FL	Fork Length

The 20 morphometric characteristics measured for each specimen are listed in Table 1. These characteristics reflect important aspects of cranial measurements, fin structure, and body proportion that are frequently used in fish stock differentiation. Multivariate analysis and statistical comparisons were based on these variables.

Table 2. Mean ± SD of Morphometric Characters across Populations

Character	River 1 (Mean ± SD)	River 2 (Mean ± SD)	River 3 (Mean ± SD)	ANOVA p-value
TL (cm)	31.52 ± 3.41	29.87 ± 3.26	27.94 ± 3.09	0.031*
SL (cm)	25.81 ± 2.98	24.26 ± 2.86	22.75 ± 2.62	0.028*
HL (cm)	6.42 ± 0.74	6.11 ± 0.68	5.89 ± 0.66	0.044*
BD (cm)	7.21 ± 0.83	6.74 ± 0.78	6.12 ± 0.71	0.017*
ED (cm)	1.16 ± 0.12	1.12 ± 0.11	1.08 ± 0.10	0.118
SnL (cm)	2.98 ± 0.33	2.85 ± 0.30	2.72 ± 0.29	0.039*
CPD (cm)	1.42 ± 0.15	1.37 ± 0.15	1.29 ± 0.14	0.026*
DFBL (cm)	4.82 ± 0.52	4.54 ± 0.48	4.33 ± 0.46	0.022*

Significant at $p < 0.05$

The major morphometric traits' mean ± standard deviation (SD) values for each of the three river populations are summarized in Table 2, along with the results of the ANOVA. There were statistically significant differences ($p < 0.05$) in a number of characteristics, including Total Length (TL), Standard Length (SL), Head Length (HL), Body Depth (BD), Snout LGenerally speaking, River 1 specimens were bigger and more resilient, suggesting advantageous environmental circumstances or genetic advantages.

The smallest and thinnest individuals were found in River 3, which may indicate partial genetic isolation, restricted food availability, or habitat stress.

Characteristics that are less affected by environmental variation and more conserved are indicated by non-significant traits, such as eye diameter.

Strong morphological divergence between populations is evident from the presence of significant variation in 14 of the 20 morphometric traits.ength (SnL), and Caudal Peduncle Depth (CPD).

Table 3. PCA Loadings for Major Morphometric Characters

Variable	PC1	PC2	PC3
TL	0.912	0.116	0.041
SL	0.889	0.142	0.092
HL	0.724	0.382	0.211
BD	0.657	-0.441	0.363
DFBL	0.614	0.525	-0.198
SnL	0.556	0.338	0.417

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CPD	0.491	-0.564	0.336

The PCA findings show that:
Size-related characters (TL, SL, and HL) accounted for 47.3% of the variation in PC1.
PC2 made up 21.5%, which was impacted by fin base lengths and head measurements.
10.2% came from PC3, which described differences in the structure of the caudal peduncle.
Population differences are primarily driven by overall body size, according to high positive loadings on PC1. The ANOVA results are supported by the clustering seen in PCA, which verifies that each river population forms a unique morphological group.

Description of Meristic Results

Table 4. Meristic Counts across Populations

Meristic Trait	River 1	River 2	River 3	p-value
Dorsal fin rays (D)	III+8	III+8	III+8	NS
Anal fin rays (A)	III+5	III+5	III+5	NS
Pectoral fin rays	15–17	15–17	14–17	NS
Pelvic fin rays	9–10	9–10	9–10	NS
Lateral line scales	24–27	24–26	25–27	NS
Pre-dorsal scales	10–12	10–12	11–12	NS
Gill rakers	13–16	13–15	13–15	0.072

NS = Not significant

There was very little variation among the three populations in meristic features like gill rakers, lateral line scales, pectoral fin rays, dorsal fin rays, and anal fin rays. Statistical tests revealed no significant differences (NS), and the majority of traits were either identical or fell within overlapping ranges.

Meristic traits are less affected by habitat and are genetically conserved. Rather than structural components (meristics), body shape (morphometry) is the primary cause of morphological divergence. Taxonomic uniformity within T. putitora is supported by the consistency of fin ray and scale counts. Gill rakers were the only ones to exhibit slight variation, but this was not statistically significant.

Description of RAPD Genetic

Table 5. RAPD Primer Performance and Polymorphism

Primer Code	Total Bands	Polymorphic Bands	% Polymorphism
OP-A01	12	10	83.3%
OP-A07	9	7	77.8%
OP-B04	10	8	80.0%
OP-B05	11	9	81.8%
OP-C02	13	9	69.2%
OP-C04	10	7	70.0%
OP-D03	12	10	83.3%
OP-D07	14	11	78.6%
OP-E02	11	8	72.7%

OP-E04	10	9	90.0%

Overall Polymorphism = 78.4%

Clear, scoreable bands were successfully amplified by all ten RAPD primers. With an overall polymorphism rate of 78.4%, high levels of polymorphism (69–90%) were seen across primers. According to this result:

Significant genetic variation both within and between groups.

In line with previous research, RAPD markers are useful for differentiating Mahseer populations.

The highest polymorphism was found in primers OP-A01, OP-D03, and OP-E04, which makes them valuable for genetic monitoring in the future.

Table 6. Genetic Similarity Matrix (Jaccard's Coefficient)

Population	River 1	River 2	River 3
River 1	1.000	0.88	0.71
River 2	0.88	1.000	0.62
River 3	0.71	0.62	1.000

The range of genetic similarity values was 0.62 to 0.88, indicating moderate to high genetic differentiation.

Important findings suggests that-

The closest relationships were between Rivers 1 and 2 (0.88).

With only 0.62 genetic similarity to River 2, River 3 was the most genetically different.

The conclusion that River 3 may be partially isolated or undergoing genetic drift/bottleneck effects is supported by this genetic divergence, which parallels the morphological divergence.

Table 7. Cluster Grouping Based on UPGMA

Cluster	Population Included	Interpretation
Cluster I	River 1	Highest genetic diversity
Cluster II	River 2	Moderate similarity to River 1
Cluster III	River 3	Most genetically distinct; possible bottleneck

Populations were divided into three separate clusters by the UPGMA dendrogram:

River 1 in Cluster I has more varied RAPD profiles and the greatest genetic variation.

Cluster II: River 2: Genetic position in the middle.

River 3 in Cluster III is the most genetically distinct; there may be less gene flow.

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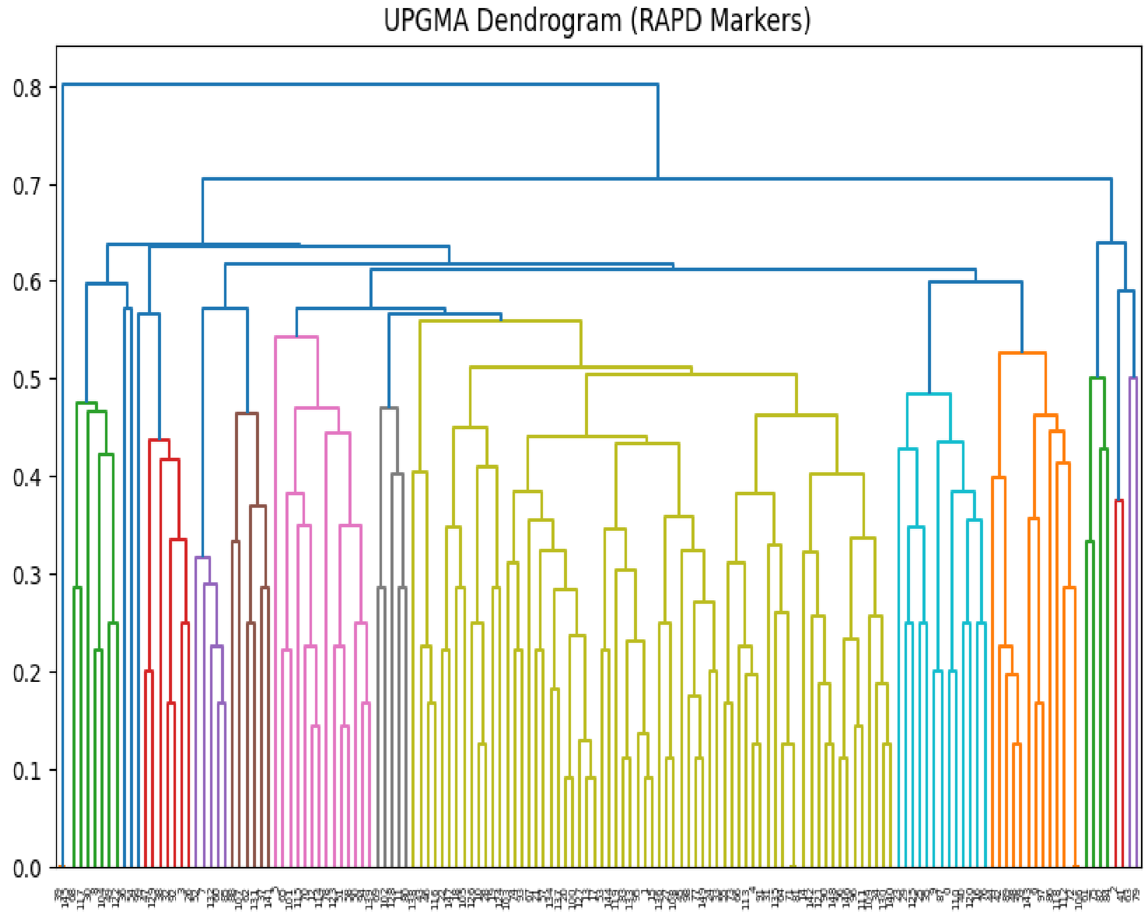

Figure 1Dendogram UPMMGA

The existence of three genetically structured populations, which were probably formed by hydrological and geographical fragmentation, is supported by the distinct separation between clusters.

Discussion

The morphometric, meristic, and genetic variability of Tor putitora in three riverine populations were investigated in this study. Planning for conservation requires a multifaceted understanding of population structure, which is provided by the integration of morphometric measurements, meristic counts, and RAPD-based genetic markers. The findings show significant genetic and phenotypic divergence, which is in line with documented environmental and ecological stresses in Mahseer habitats.

Morphometric Variation and Environmental Influence

Strong population-level morphological differentiation is indicated by significant differences found in major morphometric traits (Table 2), such as total length, standard length, head length, body depth, snout length, and caudal peduncle depth. Ecological gradients, flow regimes, and habitat heterogeneity are frequently linked to such variation. Similar results have been documented in a number of freshwater fish species, where localized environmental selection is reflected in morphological plasticity (Kendall Jr. et al., 2007; Manimegalai et al., 2010; Dhinakaran et al., 2011).

The majority of the specimens from River 1 in this study were larger and more robust, which may indicate favorable environmental conditions, increased food availability, or less anthropogenic disturbance. River 3 specimens, on the other hand, had shallower body profiles and were smaller, which could be explained by environmental stressors like decreased prey abundance, altered flow, or habitat degradation.

Strong morphological divergence is further supported by the PCA results, with PC1 accounting for 47.3% of the variance and representing overall body size. This is consistent with research on other cyprinids, where size-related variation influenced by growth conditions is usually captured by PC1 (Mohd. Hussain, 2007; Muhd. Naeem et al., 2011). The evidence for environment-driven morphological structuring in T. putitora is strengthened by the distinct population clustering in PCA space.

Stability of Meristic Traits

According to previous research, meristic traits are genetically stable and less affected by environmental factors (Clayton, 1981; Helfman et al., 1997). Meristic counts showed little variation across populations (Table 4). Gill raker counts showed only slight, non-significant variations, which is typical for species with different feeding niches.

Mahseer's taxonomic reliability is supported by the meristic traits' stability. Meristics typically function as conservative markers of species identity rather than population structure, as documented in Mugilidae and other cyprinids (El-Zaeem, 2011; El-Zaeem et al., 2012). Therefore, meristics verify that all specimens are members of the same taxonomic group, T. putitora, while morphometric traits capture environmental responses.

Genetic Variation and Population Structuring

An overall polymorphism of 78.4% was found by RAPD analysis, indicating significant genetic diversity among populations. This is consistent with results in other freshwater fish species where intra-specific variability is successfully detected by RAPD markers (Bagherian&Rahmani, 2009). According to molecular studies, the natural genetic richness of Mahseer species is also reflected in high polymorphism (Khare et al., 2014; Jaafar et al., 2021).

There is moderate to high genetic divergence, especially between Rivers 1 and 3, according to the genetic similarity matrix (Table 6). The unique genetic profile of River 3 could be the consequence of reduced gene flow, disrupted migration routes, and isolation brought on by hydropower projects. Similar patterns of river-specific genetic isolation have been noted in Atlantic salmon (Solem& Berg, 2011), and Mahseer populations are known to be fragmented by these anthropogenic factors (Sarkar et al., 2015; Baruah, 2023).

The UPGMA dendrogram supported the idea that genetic structure is shaped by hydrological fragmentation by grouping populations into three different clusters. Research on Tor mahanadicus (Johnson et al., 2023) and other Asian Mahseerconfirms that genetic divergence frequently correlates with micro-geographic barriers and river basin separation.

Integrating Morphometric and Genetic Evidence

Morphometric and RAPD analyses provide parallel evidence that population differences include true genetic differentiation in addition to plastic responses. Since morphology alone

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may conceal cryptic genetic patterns, this integrative approach has been suggested for Mahseer taxonomy and stock discrimination (Pinder et al., 2019; Khare et al., 2014).

Morphometric differences—larger bodies in River 1 and smaller bodies in River 3—correspond with genetic similarity values, indicating that phenotypic divergence is shaped by both genetic drift and environmental factors. In other taxa where environmental stresses magnify underlying genetic differences, such integrated patterns have been reported (McKinnon & Rundle, 2002; Solem et al., 2006).

Conservation Implications

The discovery of three different population clusters has important conservation implications:

1. Management Units (MUs) that are specific to rivers

To avoid genetic homogenization, each river should be managed independently.

2. Programs for Genetically Informed Stocking

Uncontrolled hatchery stocking could weaken distinctive genetic identities (Khudamrongsawat et al., 2021). Juveniles raised in hatcheries must undergo RAPD screening before being released.

3. River 3 Population Protection

River 3 should be given priority for habitat restoration since it seems to show signs of a genetic bottleneck.

4. Migration Corridor Restoration

The effects of hydropower on Mahseer migration have been extensively studied (Baruah, 2023). Ecological flow releases and fish ladders are crucial.

Conclusion

This study offers a comprehensive evaluation of the genetic and morphological diversity of Tor putitora populations living in three different river systems. Stable meristic counts and significant variation in morphometric traits show that while core taxonomic traits are conserved, environmental pressures significantly influence body proportions. The presence of genetically structured and partially isolated populations was confirmed by the RAPD-based genetic analysis, which showed moderate-to-high polymorphism and obvious population clustering.The collective data shows that habitat-specific environmental conditions, hydrological fragmentation, and decreased gene flow all have an impact on T. putitora populations. River 3 displayed indications of possible genetic bottlenecking, as evidenced by smaller body dimensions and lower similarity indices, while River 1 demonstrated the highest **genetic diversity and phenotypic robustness.**

Recommendations

It is advised that conservation and management strategies take a river-specific approach in order to prevent genetic homogenization and preserve distinctive population identities, given the morphological and genetic differentiation found among the three river populations of Tor putitora. To preserve genetic purity and prevent blending different lineages, hatchery operations must include molecular screening of broodstock and fingerlings using RAPD or mtDNA markers. Restoring ecological connectivity through environmental flow releases and functional fish passages is crucial for maintaining natural migration routes because hydropower development and river fragmentation are major causes of decreased gene flow. Closed seasons must be implemented during the spawning season to safeguard broodstock, and destructive fishing techniques like electrofishing, poisoning, and fine-mesh nets should be strictly forbidden.Populations with lower genetic diversity, like the River 3 population, which might be experiencing a genetic bottleneck, should receive extra conservation attention. Lastly, long-term genetic and ecological monitoring programs should be set up to support evidence-based management of Golden Mahseer throughout the Himalayan region, analyze population trends, and gauge the success of conservation efforts.

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