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Research Article

Stock assessment of *Schizothorax richardsonii* (Gray, 1832) using geometric morphometrics and mitochondrial marker COX1 from tributaries of the Chenab River, India

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Abstract

Schizothorax richardsonii (Gray, 1832) is extensively dispersed in the Indian Himalayan region and is recognized as an important source of animal protein. Geographical isolation between river ecosystems is believed to promote phenotypic and genotypic variation, ultimately leading to changes in the stock structure of aquatic species. With this aim of the study, 190 specimens of *S. richardsonii* were collected from four different tributaries, Rajouri Tawi, Chingus Tawi, Nowshera Tawi, and Ans River, of the Chenab River, to assess the intraspecific phenotypic and genotypic variations among stocks through landmark-based geometric morphometrics and mitochondrial marker COX1 gene. Three stocks were identified from four different tributaries of the Indus River basin. Principal Component Analysis (PCA) revealed that the first two principal components explained 76.89% of the total variance (PC1 = 67.67% and PC2 = 9.22%). The PC1-based shape wireframe showed that landmarks 5, 7, and 8 were crucial in distinguishing different morphotypes. The Canonical Variates Analysis (CVA) confirmed the existence of three morphologically distinct stocks within the Indus River basin. The COX1 sequence-based phylogenetic analysis also showed clear genetic differentiation among the stocks, supported by the Fst score-based heat map. These variations could be attributed to several factors, including uncommon hydrological conditions in these drainages, evolutionary pressures, and reproductive isolation among stocks. This study represents the first attempt to examine the stock structure of *S. richardsonii* using an integrated approach. The findings thoroughly explain species diversity and evolutionary dynamics in the Northwestern Himalayas and emphasize the importance of preserving genetic diversity and targeted conservation strategies to protect this ecologically significant species.

Keywords: COX1 gene, Genetic diversity, Indian Himalayas, Luss, Morphometric analysis

INTRODUCTION

Schizothorax richardsonii, commonly referred to as 'Luss' in the Northwestern Himalayas, is a freshwater fish species that lives in the cold, fast-flowing streams of the Indian Himalayas. It is recognized as the major source of animal protein in the regions, constituting 60-70% of the total fish catch in the upland riverine system (Mohanty, 2015). Despite being classified as "Vulnerable" by the International Union for Conservation of Nature (2010), the populations of this species are at risk due to unscientific fishing methods, including hammering, dynamiting, the use of bleaching powder, insecticides, and electric current, which are responsible for the mass mortality of fish of all sizes. The introduction of the alien species also poses a significant threat to native fish populations. In Kashmir, the common carp has nearly exterminated the indigenous schizothoracids (Agarwal *et al.*, 2009). Similarly, brown trout, introduced for sport fishing, inversely affect native snow trout by preying on their young, contributing to their decline. Additionally, climate change is predicted to reduce the habitat of *S. richardsonii* by 16% in the next 30 years and by 26% by 2070 (Sharma *et al*., 2021a). To ensure the sustainability of snow trout, conservation efforts are crucial, particularly in the Indian Himalayas, which serves as a critical breeding and feeding ground, especially in the Indus River basin (Agarwal *et al*., 2004; Gandotra *et al*., 2009). Understanding the genetic and morphometric diversity of *S. richardsonii* populations in this area is crucial for their conservation and management. Phenotypic and genotypic variations within a species are vital for its adaptability to changing environmental conditions and resilience against diseases and other stressors (Li *et al.,* 2013; Rayal *et al.,* 2019).

Understanding the natural phenotypic and genetic variation within wild populations is crucial for assessing their status and developing effective conservation and management strategies (Carvalho and Hauser, 1994; Kumar and Singh, 2019). The study, involving measuring body shapes and sizes, is a fundamental method for assessing phenotypic diversity in fish populations. Various morphometric approaches, viz., traditional, truss-based morphometrics and geometric morphometrics, have been developed to identify distinct morphological traits that can differentiate stocks and indicate adaptations to specific environmental conditions (Cavalcanti, *et al.,* 1999; Ingram, 2015; Reiss and Grothues, 2015). Among all the morphometric approaches, “Geometric Morphometrics” is a highly effective analytical method that utilizes distinct anatomical landmarks to accurately capture and measure organisms' shapes (Echem, 2016). It is commonly utilized in fish biology to study stock structure, species identification, and evolutionary relationships, offering valuable insights into phenotypic diversity and adaptations to environmental conditions (Dwivedi, 2022).

Geometric morphometric analysis, combined with the use of the mitochondrial DNA marker cytochrome oxidase subunit I (COX1), provides a complete picture of variation at both phenotypic and genotypic levels in fishes (Mandal *et al.,* 2021). The mitochondrial DNA (mtDNA) marker, especially the COX1 gene, is highly conserved and powerful for gaining insights into genetic diversity and phylogenetic relationships among stocks (Lakra *et al.,* 2011). Sequencing the COX1 gene, often called DNA barcoding, assists in identifying fish species and understanding the genetic structure of fish populations, which can inform conservation strategies (Ward *et al.,* 2005). However, in recent years, several studies have highlighted the declining populations of *S. richardsonii* species and the need for urgent conservation measures (Sharma *et al*., 2021b). These studies have underscored the importance of preserving genetic diversity as a buffer against environmental changes and anthropogenic pressures.

However, keeping in view limited information on the morphometric and genetic diversity of *S. richardsonii* populations in the Indian Himalaya (Negi and Negi, 2010; Sharma and Metha, 2010; Mir *et al*., 2013; Ali *et al*., 2014; Dwivedi, 2022), a comprehensive understanding of body shape variation among wild stocks of *S. richardsonii* across the Northwest Himalaya particularly in tributaries of Indus River basin had never been investigated before. The present study is the first investigation using the landmark-based “Geometric Morphometrics” and “Mitochondrial marker COX1” methods to assess phenotypic and genotypic variations, respectively, in *S. richardsonii* inhabiting four tributaries of Chenab River viz. Rajouri Tawi, Chingus Tawi, Nowshera Tawi, and Ans River.

MATERIALS AND METHODS

Sample collection and identification

A total of 199 freshly dead adult male and female individuals were collected from local fishermen across four different tributaries of the Chenab River basin: Rajouri Tawi, Chingus Tawi, Nowshera Tawi, and Ans River (Fig. 1) from April 2022 to March 2023. All these tributaries were free of dams and were not altered by human activities in their structure or environmental settings. Collected samples were immediately transferred to an ice box and brought to the laboratory for morphometric analysis. Firstly, the specimens were identified with the help of the taxonomic keys of Day (1878), Talwar and Jhingran (1991), Mirza (1991), and Kullander *et al.* (1999). All these specimens were collected before the breeding season and after the spawning season. To fix and preserve the samples that were morphometrically analyzed, a 10 % formalin solution was used. However, for molecular analysis, 100 mg tissue from dorsal muscle and fins was preserved in a 1:5 ratio of 95% ethanol and stored at 4°C. Voucher specimens of *S. richardsonii* preserved in 10% formalin solution were also deposited in the museum of the Department of Zoology, H.N.B. Garhwal University, Srinagar (Garhwal) for future reference.

Animal ethical approval statement

As freshly dead specimens were collected from the commercial catch, ethical guidelines for the experimental use of fish do not apply to the present study.

Morphological analysis

Digital imaging of specimens

The specimens were rinsed in running water, dried with blotting paper, and placed on a flat platform with laminated graph paper as a background for digital imaging. The fins were erected to highlight the insertion points, and each individual was labelled with a specific identification code. Nikon D3400 digital camera was used to capture the digital lateral image of each individual's left side. All the images were taken from the same angle and height to maintain uniformity and minimize errors.

Data generation

To create a thin plate spline (TPS) file from the photographs, we utilized tpsUtil (version 1.52, Rohlf, 2008a). Fourteen homologous landmark points (Fig. 2) were digitized on the lateral-side photographs of each fish using the software tpsDig (version 2.16, Rohlf, 2008b). The scale factor for each specimen was established using the set scale option. Two-dimensional x, y coordinate data for the 14 landmarks on all specimens were obtained using ImageJ (version 1.50i) and saved in TPS format. Procrustes superimposition (Rohlf and Slice, 1990) was employed to convert the landmarks into shape coordinates, thereby standardizing each specimen to a unit centroid size, which estimates overall body size (Bookstein, 1991).

Statistical procedures

The data were analyzed to describe and detect possible morphological variability among the four populations. Procrustes superimposition was used to remove non-shape components, such as variations in orientation, position, and size of the samples (Rohlf and Slice, 1990; Bookstein, 1991), resulting in exclusively shape information. Procrustes Analysis of Variance (ANOVA) was conducted to evaluate the significance level in overall size and shape. The shape variables were subsequently utilized for further analyses. Principal Component Analysis (PCA) was used to analyze significant shape variation and identify patterns of relationships within the specimens (Veasey *et al.,* 2001). Canonical Variates Analysis (CVA) was used to identify shape differences among groups, and Discriminant Function Analysis (DFA) was used to classify individuals into their original groups. Additionally, shape changes of each stock relative to the mean shape were visually presented in a graphical format. These analyses were performed in MorphoJ version 1.06d (Klingenberg, 2011), an integrated software package for geometric morphometrics.

Molecular analysis

DNA extraction, amplification, and sequencing

Approximately 25mg of tissue was utilized for DNA isolation using a modified version of the standard phenol: chloroform: isoamyl alcohol method, with some adjustments made during the initial homogenization step. After DNA isolation, the DNA pellet was dissolved in TE buffer, which consists of a 10 mM Tris–HCl and 0.1 mM EDTA solution with a pH of 8. In the PCR reaction for COX1 amplification, a 50 µL volume was used. The reaction mixture included 10X Taq polymerase buffer (5 µl), 50mM $MgCl_2$ (2 µl), 0.05mM dNTP (0.25 µl), 0.01mM primer (0.5 µl), (1.5 IU) Taq polymerase, and 200ng genomic DNA template (2 µl). Amplifications were carried out in the Veriti 96 fast Thermal cy-

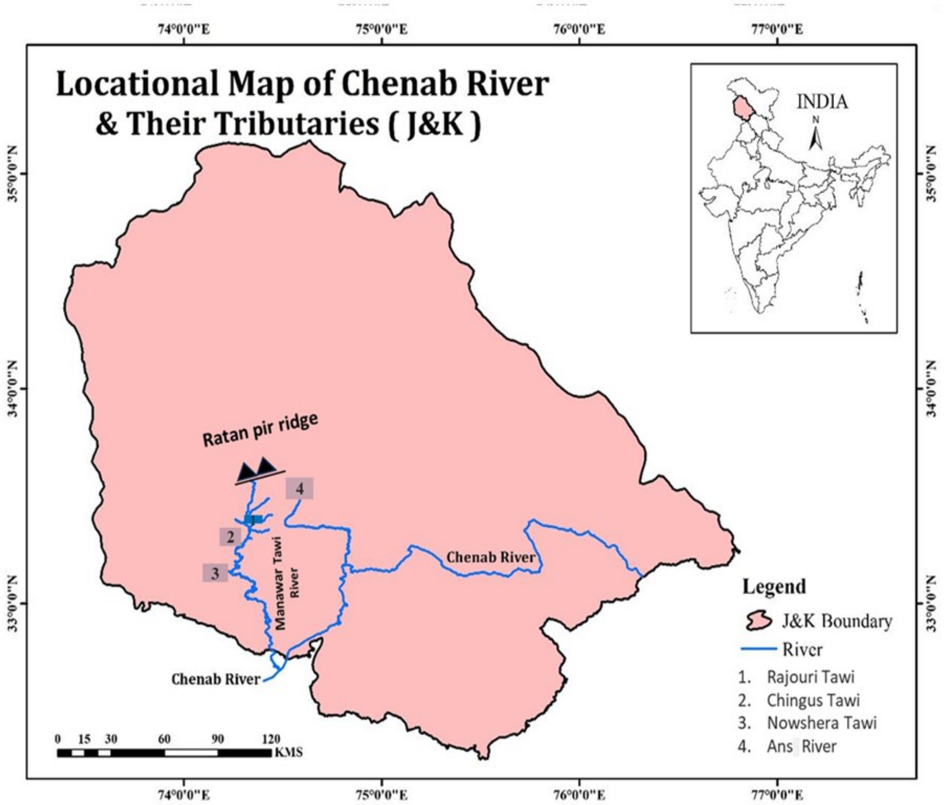

Fig. 1. *Map showing the four sampling sites located on the tributaries of the Chenab River*

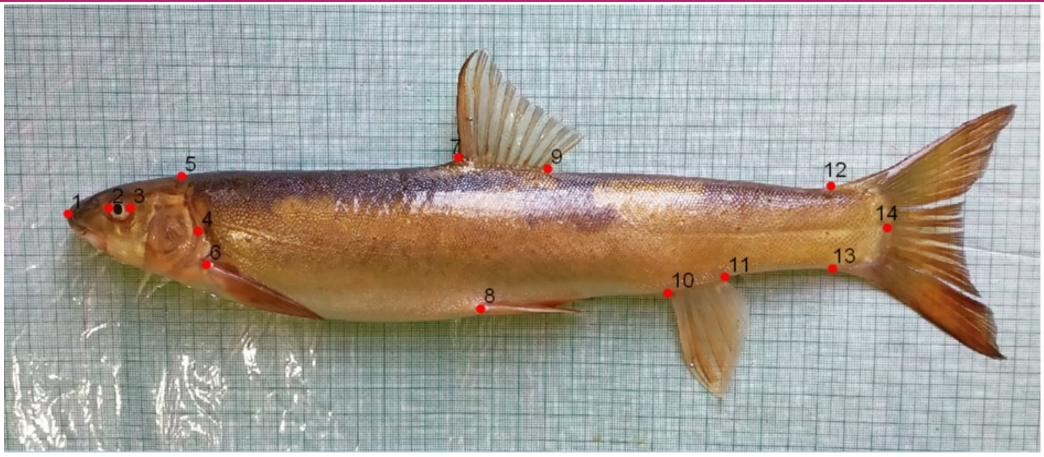

Fig. 2. *Schematic image of Schizothorax richardsonii showing 14 landmarks (1 tip of snout; 2 anterior border of the eye; 3 posterior border of the eye; 4 posterior border of operculum; 5 forehead (end of frontal bone); 6 pectoral-fin origin; 7 dorsal fin origin; 8 pelvic fin origin; 9 dorsal fin termination; 10 origin of anal fin; 11 termination of anal fin; 12 dorsal side of caudal peduncle, 13 ventral side of caudal peduncle; 14 termination of lateral line) used to compare among the stocks.*

cler from Applied Biosystems, Inc., USA. The primer pair utilized for the COX1 was: FishF1 5'TCAACCAACCACAAAGACATTGGCAC3' and FishR1 5'TAGACTTCTGGGTG GCCAAAGAATCA3' (Ward *et al*., 2005). The temperature conditions of PCR for COXI involve an initial denaturation period of 3 minutes at 94 °C. Following the initial denaturation, there are 35 cycles of 1min at 94 °C, followed by annealing at 54 °C for 45 s, extension at 72 °C for 1 min, and final extension at 72 °C for 10 min. A 1.5% agarose gel stained with ethidium bromide was prepared to visualize the PCR products of COX1 using a gel documentation system (Biovis). The PCR products were sequenced using the di-deoxynucleotide chain termination method described by Sanger *et al.* (1977). The sequencing was performed on an automated ABI-3500 Genetic Analyzer. The PCR products were fluorescently labelled using the BigDye Terminator V.3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.). The composition of the cycle sequencing PCR reaction of 10 µl involved the use of Big Dye reaction mix (2.5 ×) 4 µl, sequencing buffer (5 ×) 2 µl, purified PCR product (50 ng/µl) 1 µl, primer (10 µM) 0.5 µl and nuclease-free water of 2.5 µl. The PCR cycle sequencing conditions involved a series of temperature changes to facilitate amplification, i.e., 25 cycles of 96 °C for 20 s, 50 °C for 15 s, and 60 °C for 4 min. This research was conducted at the DNA Barcoding Laboratory of the ICAR-National Bureau of Fish Genetic Resources (NBGFR) in Lucknow, India.

Genetic data analysis

In this study, the COX1 gene of all 18 samples from the Chenab River basin was sequenced to analyze sequence composition, genetic variation and construct a phylogenetic tree. The forward sequence and inverted (reversed and complimented) sequences were aligned to make a consensus sequence for each sample. Ambiguous bases were checked manually against the raw sequencing electropherogram files and corrected accordingly. Sequence alignment was performed using Clustal-W, an integrated feature of the Molecular Evolutionary Genetics Analysis (MEGA) software, version 11 (Tamura *et al.,* 2021). The obtained consensus sequences were blasted in the National Centre for Biotechnology Information (NCBI) GenBank for the nearest similar sequence matches and submitted in NCBI GenBank. Their accession numbers range from PQ135010 to PQ135026 and PQ13944. The phylogenetic tree was also constructed by using the MEGA 11. The sequences were pooled to know the Genetic differentiation using Fst values with the help of softwares DnaSP v.5.10.01 (Librado and Rozas, 2009) and Arlequin 3.5.2.2 (Excoffier and Lischer, 2010). The heat maps for genetic differentiation among stocks were determined by an online database using pairwise Fst scores among the stocks (http://www.hiv.lanl.gov/ content/ sequence/HEATMAP/heatmap.html).

RESULTS

Geometric morphometrics

The shape variation of *Schizothorax richardsonii* was explored using the shape coordinates extracted from the two-dimensional landmark dataset, using Procrustes alignment. The shape coordinates were superimposed to eliminate the size effect. This is supported by the results of the Procrustes ANOVA, which indicated a non-significant difference ($F = 2.08$, $p > 0.05$) in overall size while showing a significant difference ($F = 3.85$, $p < 0.05$) in overall shape coordinates among the sites. This suggests the fish body images were successfully superimposed, minimizing the size effect. Furthermore, the PLS analysis for superimposed shape and log centroid size demonstrated a significant positive correlation ($R=0.61$, $p < 0.05$) between groups, indicating a strong relationship between shape and size. PCA revealed shape variation in *S. richardsonii*, with the first two principal components (PCs) accounting for 76.89% of the total variance. PC1 explained 67.67%, while PC2 accounted for 9.22% (Fig. 3). The results showed that most variations were primarily attributed to PC1. The average shape derived from the PCA data indicated that variations were based on landmarks 3, 7, 8, 9, and 12, all visible in the wireframe shape (Fig. 4). However, there was a low level of overlap and a moderate level of variance among stocks observed on the first and second PC axes of the PCA plot (Fig. 5). Further analysis was conducted using Canonical Variate Analysis (CVA) and Discriminant Function Analysis (DFA) for additional verification. The shape coordinate data were examined with CVA, resulting in three Canonical Variates (CVs). The first Canonical Variate (CV1) explained 70.46% of the total variance, while the second (CV2) and third (CV3) Canonical Variates accounted for 22.25% and 7.28% of the total variance, respectively (Table 1). The scatter plot from the CVA analysis showed distinct, non-overlapping stocks between the Ans River and Rajouri Tawi, alongside the rest of the stocks, with 90% confidence in the CVA ellipses (Fig. 6). The Mahalanobis distances (Table 2) and Procrustes distances (Table 3), derived from the Canonical Variates Analysis (CVA), were found to be significantly different ($p < 0.001$) among the three stocks of *S. richardsonii* from the Rajouri Tawi, Nowshera Tawi, and Ans River. This indicates heterogeneity in their shape, except for the Chingus Tawi stock.

The discriminant function analysis indicated a high percentage of specimens correctly classified, achieving an overall accuracy of 78.7% for individuals placed into their original groups (Table 4). A moderate mixing of individuals was observed between the Nowshera Tawi and Chingus Tawi rivers. In contrast, low mixing levels were noted among the individuals from the Ans River and those from Rajouri Tawi, Chingus Tawi, and Nowshera Tawi (Fig. 7). A deformed wireframe representing the average shape (Fig. 8) revealed a significant difference in body shape among the stocks of *S. richardsonii*. The morphometric measurements that contributed most to the variation between the stocks from the Ans River, Chingus Tawi, and Nowshera Tawi were primarily based on landmarks 3, 5, 7, 8, 9, and 12. Specimens from the Ans River were larger than those from Chingus Tawi. The differences between the Ans and Rajouri Tawi stocks were identified based on landmarks 2, 3, 10, 11, 12, and 13. The relevant landmarks for the Rajouri Tawi and Chingus Tawi stocks were 2, 3, 7, 9, 11, 12, and 13. In the case of Nowshera Tawi and Rajouri Tawi stocks, the landmarks were 11, 12, and 13, while for Chingus Tawi and Nowshera Tawi stocks, landmarks 7 and 8 were significant (Fig. 8). Most of the observed variations in body shape stretch it dorsoventrally. Key morphometric measurements, such as the origin of the pelvic fin, the anterior and posterior origins of the dorsal fin, eye diameter, head length, and caudal peduncle width, are important morphological descriptors. These measurements provide valuable insights into the distinct characteristics of fish stocks within the tributaries of the Indus River basin. The Canonical Variate Analysis (CVA) results were consistent with those of the Discriminant Function Analysis (DFA), highlighting variations in body shape among the various stocks of *S. richardsonii*. Overall, the CVA and DFA identified three distinct stocks of snow trout in the selected rivers based on their shape: 1. Rajouri Tawi, 2. Chingus and Nowshera Tawi, and 3. Ans River.

Genotypic data analyses

The sequence alignment resulted in 655 base pairs per sequence after excluding the primer regions and ensuring a consistent length across alignments. No insertions, deletions, or stop codons were detected, which confirms that all amplified sequences originated from a functional mitochondrial COX1 gene. Analysis of the COX1 sequences indicated that the average nucleotide composition for *S. richardsonii* from the tributaries of the Chenab River was as follows: 25.68% Adenine (A), 27.95% Thymine (T/U), 28.21% Cytosine (C), and 18.16% Guanine (G) (Table 5). The nucleotide sequences of the COX1 gene were found to be A+T rich, composing 53.63% of the total. The calculated Transition/Transversion bias (R) was 5.997. The transitional substitution ratios were as follows: A to G at 58.19, C to T at 0.06, G to A at 41.23, and T to C at 0.06. In contrast, the transversion substitution ratios were A to T at 0.06, T to A at 0.06, A to C at 0.06, T to G at 0.06, C to A at 0.06, C to G at 0.06, G to C at 0.04, and G to T at 0.04 (Table 5). The phylogenetic tree was constructed using MEGA 11, employing the Maximum Likelihood (ML) method and the Tamura 3-parameter model distance method. *Barbus barbus* (NC008654) was used as the outgroup. A bootstrap consensus tree generated from 1000 replicates illustrated the evolutionary relationships among the analyzed stocks. The phylogenetic tree revealed one lineage and 2 groups of *S. richardsonii* from the Rajouri Tawi, Nowshera Tawi, Chingus Tawi, and Ans Rivers (Fig. 9). The genetic distance observed between the stocks was 0.00153. The prepared heat map based on Fst scores clearly illustrates genetic differentiation among stocks (Fig. 10).

DISCUSSION

Adapting to environmental conditions is essential for the survival of organisms. These conditions shape the body form of each organism during its anatomical development and ontogeny. Fish can adapt to their environments (Nacua *et al.,* 2010). They encounter various environmental conditions affecting many traits, includ-

Table 1. Eigenvalues and total variance explained by three canonical variates extracted from four riverine stocks of *Schizothorax richardsonii*

CVs	Eigenvalues	% Variance	Cumulative %
CV1	3.90702097	70.46	70.461
CV2	1.23402890	22.25	92.716
CV3	0.40390597	7.28	100.000

Table 2. Mahalanobis distances (lower diagonal) and p values (upper diagonal) of canonical variate analysis among *Schizothorax richardsonii* stocks

Sampling site	Rajouri Tawi	Chingus Tawi	Nowshera Tawi	Ans River
Rajouri Tawi		<0.0001	<0.0001	<0.0001
Chingus Tawi	3.0157		<0.0001	<0.0001
Nowshera Tawi	2.5106	1.8164		<0.0001
Ans River	5.0143	5.1463	5.1259	

Table 3. Procrustes distances (lower diagonal) and p values (upper diagonal) of canonical variate analysis among *Schizothorax richardsonii* stocks

Sampling site	Rajouri Tawi	Chingus Tawi	Nowshera Tawi	Ans River
Rajouri Tawi		<0.0001	<0.0001	<0.0001
Chingus Tawi	0.0787		0.1165	<0.0001
Nowshera Tawi	0.0855	0.0121		<0.0001
Ans River	0.0785	0.1493	0.1579	

Table 4. Number and percentage of correctly classified specimens of *Schizothorax richardsonii* into their original stocks from the Indus River basin

Classification Results	Sampling site	Predicted Group Membership				Total
		1	2	3	4	
		Original				
Count	1.Rajouri Tawi	41	8	1	0	50
	2. Nowshera Tawi	10	37	9	0	56
	3. Chingus Tawi	1	11	43	0	55
	4. Ans River	2	0	1	38	41
Percentage (%)	1.Rajouri Tawi	82.0	16.0	2.0	0.0	100.0
	2. Nowshera Tawi	17.9	66.1	16.1	0.0	100.0
	3. Chingus Tawi	1.8	20.0	78.2	0.0	100.0
	4. Ans River	4.9	0.0	2.4	92.7	100.0
		Cross-validated				
Count	1.Rajouri Tawi	35	13	1	1	50
	2. Nowshera Tawi	11	29	16	0	56
	3. Chingus Tawi	1	19	35	0	55
	4. Ans River	3	0	1	37	41
Percentage (%)	1.Rajouri Tawi	70.0	26.0	2.0	2.0	100.0
	2. Nowshera Tawi	19.6	51.8	28.6	0.0	100.0
	3. Chingus Tawi	1.8	34.5	63.6	0.0	100.0
	4. Ans River	7.3	0.0	2.4	90.2	100.0

78.7% of originally grouped cases correctly classified; 67.3% of cross-validated grouped cases correctly classified

Table 5. Maximum composite likelihood estimates pattern of nucleotide substitution and base composition (%) for *Schizothorax richardsonii*

	A	T	C	G
A	-	*0.06*	*0.06*	**41.23**
T	*0.06*	-	**0.06**	*0.04*
C	*0.06*	**0.06**	-	*0.04*
G	**58.19**	*0.06*	*0.06*	-
Base composition	25.68	27.95	28.21	18.16

Different transitional substitution rates are shown in **bold** and transversion substitutions are shown in italics

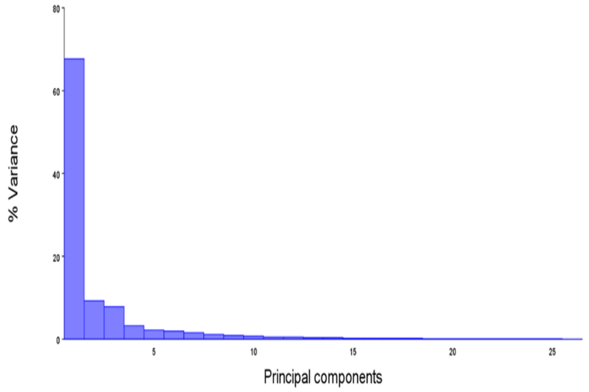

Fig. 3. *Scree plot showing percentage variance explained by different principal components in Schizothorax richardsonii*

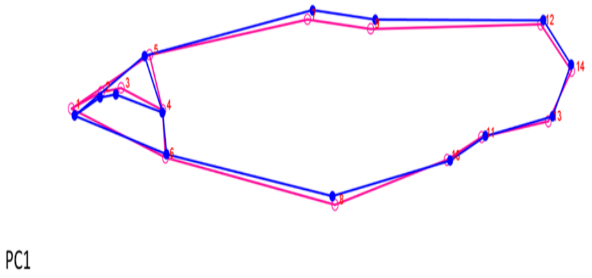

Fig. 4. *Wireframes showing the shape changes associated with PC1 of S. richardsonii populations. (The red wireframe shows the original position of landmarks, and the blue wireframe shows variation in landmark positions)*

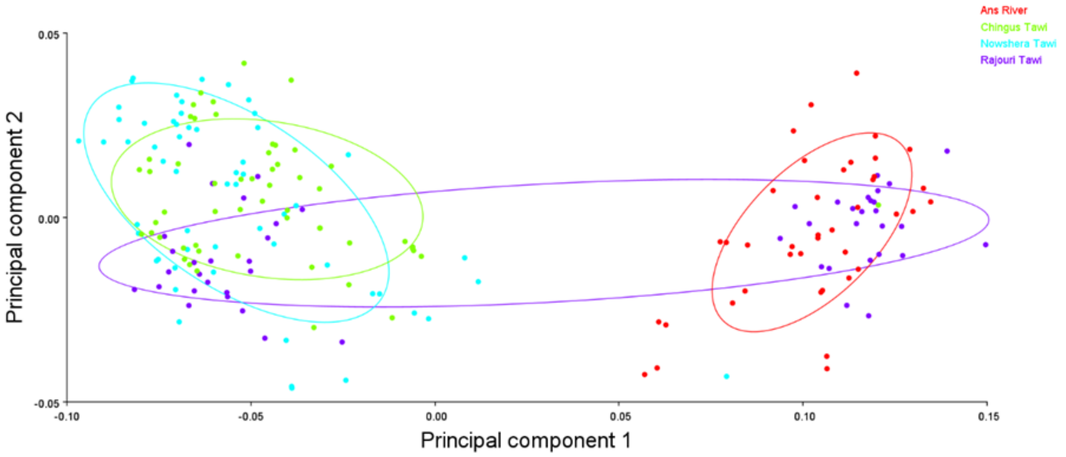

Fig. 5. *Scatter plot of Principal Component Analysis (PCA) in Schizothorax richardsonii showing loadings of each sample on the first two principal components*

ing body shape and genetic characteristics (Costa and Cataudella, 2007). The present study provides a fresh perspective on management, conservation, and fish production by presenting a combined analysis of morphology and genetics in wild stocks of *S. richardsonii*. This analysis utilized Geometric Morphometrics (GM) alongside the mitochondrial DNA marker COX1, sourced from the tributaries of the Chenab River. The findings of this study indicated significant intraspecific plasticity among wild populations of the vulnerable snow trout, *S. richardsonii*, located in the tributaries of the Chenab River in the northwestern Himalayas. The PCA scatter plot demonstrated a high level of phenotypic plasticity in the Rajouri Tawi, Chingus Tawi, and Ans River. This phenotypic variation is typically a result of adaptation to the local ecological conditions and the significant isolation between these sites. Such isolation may lead to reproductive separation and the development of distinct spawning areas.

Rajput *et al.* (2013) identified strong correlations be-

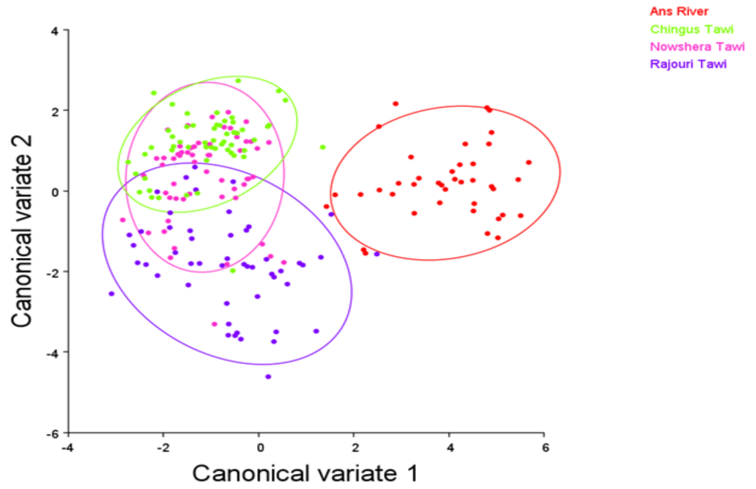

Fig. 6. *Scatter plot of Canonical Variates Analysis (CVA) of Schizothorax richardsonii showing the frequency of specimen distribution in respective groups on the first two axes*

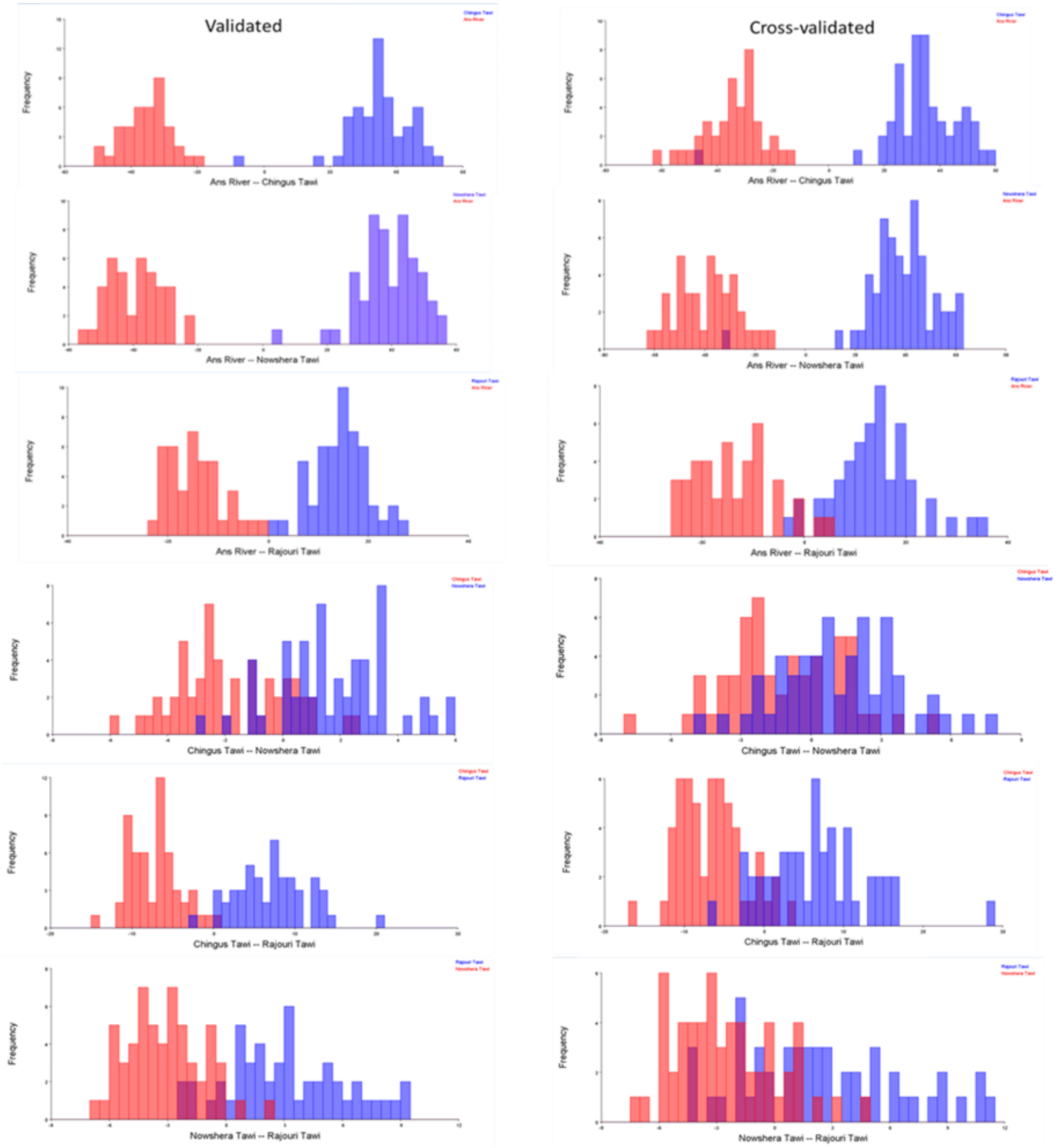

Fig. 7. *Graph of Discriminant Function Analysis (DFA) depicting the differentiation among Schizothorax richardsonii stocks from four Chenab River basin tributaries*

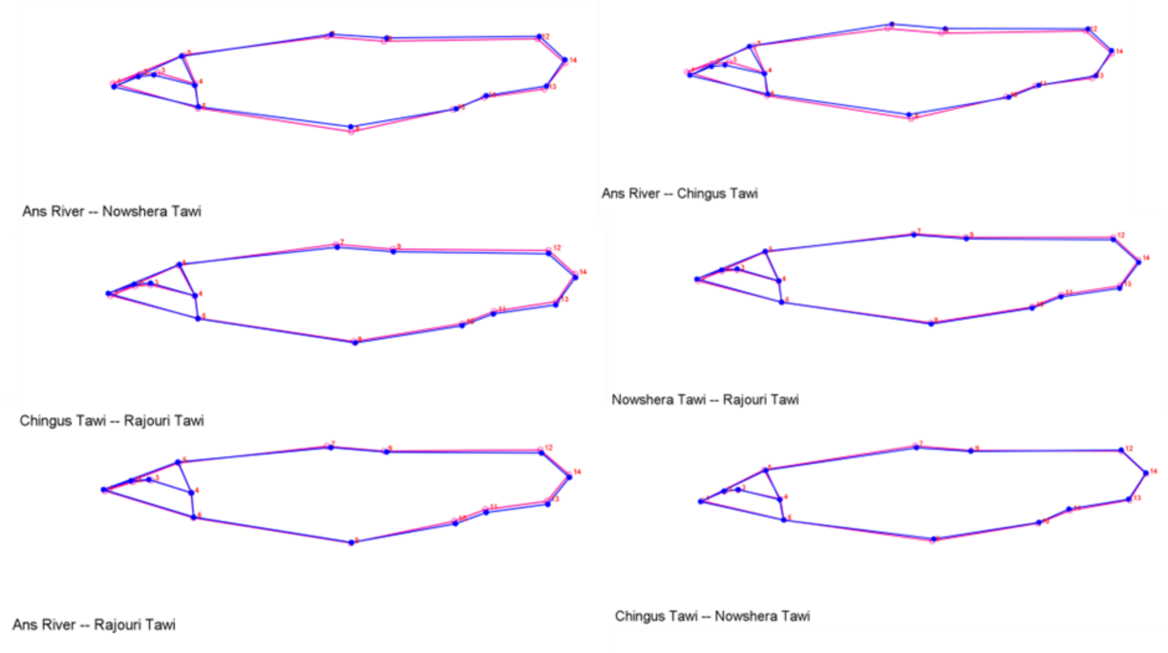

Fig. 8. *Wireframes illustrate the shape changes associated with discriminant scores among the four stocks of Schizothorax richardsonii (the blue wireframe shows the original positions of the landmarks, and the red wireframe indicates variations in landmark positions)*

tween environmental factors and the morphological characteristics of *S. richardsonii* in their study conducted in the upper Ganga River basin. They found that the dorsal and pectoral fin were the most significantly affected morphometric traits. The current study shows that principal component analysis (PC1) identifies the dorsal fin, pelvic fin, and eye diameter as the most significant morphological features. Fish morphology is closely linked to habitat use (Videler and Wardle, 1991) and body shape, both of which are crucial for performing essential tasks such as swimming and feeding (Triantafyllou *et al.,* 2000). The CVA identifies a significant difference in the average shape among three of the four stocks.

Specifically, the stocks are categorized to be Rajouri Tawi as first stock, Chingus and Nowshera Tawi combined as a second stock, and Ans River as the third stock. The morphological differences in *S. richardsonii* among the tributaries may be due to a lack of connectivity and variations in topography. Additionally, human activities such as increased urbanization, agricultural runoff, and industrial discharge notably impact the Rajouri Tawi River. The selected site along the Ans River experiences minimal human interference upstream and maintains a lower temperature due to its proximity to the Pir Panjal Mountain range (Ahmed and Riaz, 2022). Additionally, the turbulent water conditions in the Ans River increases resistance for fish during swimming, which may contribute to the phenotypic plasticity observed in the *S. richardsonii* stock. A stressed population may struggle to adapt to early maturation and other rapid life-history traits (Trippel, 1995). Similarly, Mir *et*

Fig. 9. *Maximum Likelihood (ML) type of phylogenetic tree based on mitochondrial COX1 partial gene sequences for a natural population of Schizothorax richardsonii from four different tributaries of the Chenab River ● (Represent stock from the Chingus 🟩 Tawi, ▲ Nowshera Tawi, ♦ Rajouri Tawi, and Ans River)*

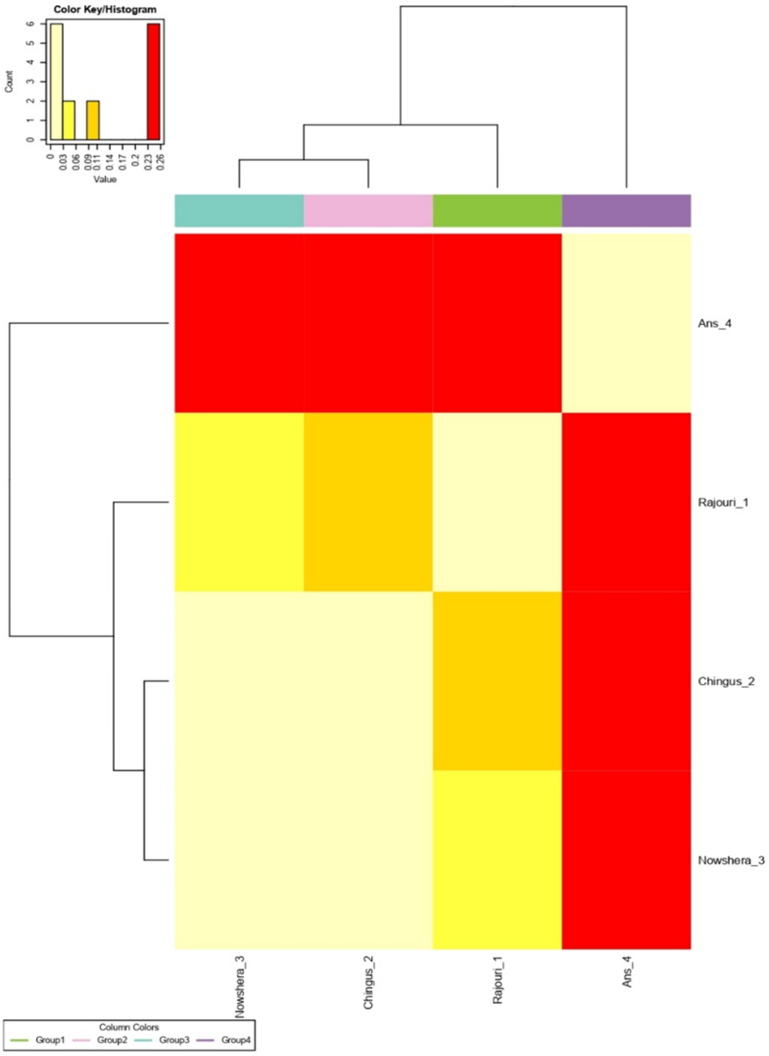

Fig. 10. *Heatmap based on the Fst scores illustrating genetic differentiation among Schizothorax richardsonii stocks*

al. (2014) reported morphological differences among the stocks of *Schizothorax esocinus* from the Kashmir Himalaya, attributing these variations to differences in environmental factors and levels of human interference. The results from the Mahalanobis and Procrustes distances highlighted shape-based differences among these stocks. Notably, the Nowshera Tawi and Chingus Tawi stocks appeared similar, likely because of the close proximity of their geographical locations. In a previous study, Dwivedi (2022) examined shape variations in the body of *S. richardsonii* from the Indian Himalayan region but did not identify the key morphometric characters that could serve as morphological descriptors. In contrast, the present study identified five key morphometric characters based on warp scores from discriminant function analysis (DFA) and principal component analysis (PC1), contributing to the phenotypic differentiation among these stocks. These characteristics are eye diameter, the origin of the pelvic fin, the anterior and posterior origins of the dorsal fin, the length of the frontal bone, and the width of the caudal peduncle. We found that specimens from the Ans River were larger than those from other tributaries. These morphometric traits elongate the body shape dorsoventrally, influencing body height.

Fish can have varying eye diameters depending on the light conditions in their habitat (Pakkasmaa *et al*., 1998). The amount of available light and depth influences their visual acuity. One adaptation to deeper waters, where light decreases significantly, is the enlargement of the eye and pupil area (De-Busserolles *et al.,* 2013). In the present study, stocks of *S. richardsonii* found in deeper, high-turbidity rivers (Chingus Tawi and Nowshera Tawi) had larger eyes than specimens from clear, shallow rivers (Ans River). Baumgartner *et al*. (1988) suggested that eye size may be linked to feeding behavior. Additionally, body depth and fin size are important morphological traits in stream fish that affect their ability to maintain a position and move effectively (Douglas and Matthews, 1992). Pelvic fins serve several functions; they help guide and stabilize the fish while swimming, act as brakes to stop movement, assist in maintaining a stationary position, and facilitate both forward and backward locomotion (Osburn, 1906). Harris (1936) observed that the dorsal fin rises during gliding in many teleost fishes and quickly changes direction. Additionally, Duff *et al.* (2019) suggested that an elongated caudal peduncle might be related to courtship behaviors. The dorsal fin and the caudal peduncle are crucial for swimming, as they provide directional control for the fish.

Negi and Negi (2010) reported that 90% of the variation in the morphometric traits of *S. richardsonii* populations from Uttarkashi, India, was attributed to genetic factors, while environmental factors accounted for the remaining 10%. In the current study, analysis of the COI gene revealed a distinct genetic difference between the stocks. Notably, the phylogenetic tree, constructed using the maximum likelihood method, indicated the presence of two groups. One group comprised populations from the tributaries of Manawar Tawi and the Ans River, suggesting that these populations still experience gene flow or retain ancestral haplotypes. The second group, originating from the Ans River, displayed newly emerged population-specific haplotypes, which underscore the need for distinct population-specific management strategies. Such differentiated populations may be at risk of genetic erosion, reduced genetic diversity, and other unforeseen ecological consequences (Begg *et al.,* 1999).

The findings of this study are consistent with those of Ali *et al.* (2014), who examined genetic variations in *S. richardsonii* populations using ATPase6/8 and COI gene sequences from mitochondrial DNA. Their research revealed three distinct groups among five high-altitude rivers in India, with some populations exhibiting gene flow while others evolved independently. In contrast, the present study showed that the genetic distance among these *S. richardsonii* stocks is 0.00153, which is significantly lower than the threshold values (0.6–2.0%) typically used for species discrimination through DNA barcoding (Hebert *et al.,* 2003; Ratnasingham and Hebert, 2013). Research on Neotropical fish indicates that average intraspecific distances do not exceed 1% (Anjos *et al*., 2020; Fagundes *et al*., 2020). Maximum Likelihood phylogenetic analysis and morphometric data indicated that the Chingus Tawi and Nowshera Tawi stocks were closely related, suggesting they share similar evolutionary histories and morphological traits. In our study, a genetic variation heatmap based on Fst scores revealed clear genetic differentiation between the stocks of the Ans River and those of other rivers isolated by geographic distance. This differentiation may be attributed to specimens' varying feeding habits and swimming patterns among these drainages. The significant flow fluctuations in Rajouri Tawi may lead to changes in biofilm composition. Additionally, hydropeaking and intermittent flow conditions further affect fish growth, potentially resulting in phenotypic and genotypic differentiation (Korman and Campana, 2009).

Furthermore, in this study, the base composition pattern of the COX1 gene in *S. richardsonii* showed slight differences from that reported by Ali *et al.* (2014) for the Central Indian Himalayan population of *S. richardsonii*. Specifically, the present analysis showed A+T = 53.63% and G+C = 46.37%, compared to their findings of A+T = 55.5% and G+C = 44.5%. The nucleotide sequence of the COX1 gene in the present study exhibits a deficiency in guanine, with guanine making up 18.16%, while adenine, cytosine, and thymine are present at 25.68%, 28.21%, and 27.95%, respectively. This nucleotide composition pattern is common across teleost fishes, with only minor variations (Zhu *et al*., 2012).

Despite identifying morphological differences in shape using geometric morphometry, the mtDNA COX1 sequence analysis revealed a high similarity among the tributaries of Manawar Tawi. In this study, *S. richardsonii* consisted of three morphotypes and two genotypes from the tributaries of the Indus River basin. Various environmental factors may influence the genetic and morphological variations among these stocks. These factors include unusual hydrological conditions in these drainages, differences in swimming patterns, predation pressures, food availability, varying habitat conditions, and reproductive isolation among the stocks.

Conservation programs and effective management strategies depend on understanding the extent of genetic divergence within and between species (Lakra *et al.,* 2009). Mitochondrial DNA, particularly the COX1 gene, is crucial for identifying distinct populations. This study used partial COX1 mtDNA sequences to assess intraspecific genetic diversity and identify genetic stocks of *S. richardsonii* from different drainages in the northwestern Himalayas. The findings will be helpful in stock identification and knowing the evolutionary dynamics of this species, which is crucial for their conservation and management. Comprehensive scientific analysis and trait-based research on the morphometric and genetic plasticity of this vulnerable Himalayan endemic snow trout will be essential for developing effective restoration and conservation strategies for fishery biologists and taxonomists in the future.

Conclusion

This study presents strong evidence of significant morphological and genetic variations within the *S. richardsonii* stocks among the tributaries of the Chenab River. Key morphometric measurements, such as dorsal fin origin, pelvic fin origin, eye diameter, and caudal peduncle width, are crucial descriptors of these morphological traits. The observed variations in morphometric and genetic data among *the S. richardsonii* stocks may be attributed to geographical isolation, hydrological conditions unique to each drainage, swimming patterns, predation pressures, food availability, substrate variability, and reproductive isolation among the stocks.The results from an integrated approach that combined Geometric Morphometrics and mtDNA COX1 analysis complement each other in delineating the stock structures from four different tributaries, Rajouri Tawi, Chingus Tawi, Nowshera Tawi, and Ans River of the Chinab River. This emphasizes the need for stock-specific conservation and management strategies, focusing on ecosystem-based approaches to safeguard this important genetic resource. These findings will be essential for ichthyologists and taxonomists in preserving genetic diversity and developing effective conservation and management strategies for these vulnerable fish within their natural habitats.

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Conflict of interest

The authors declare that they have no conflict of interest.

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