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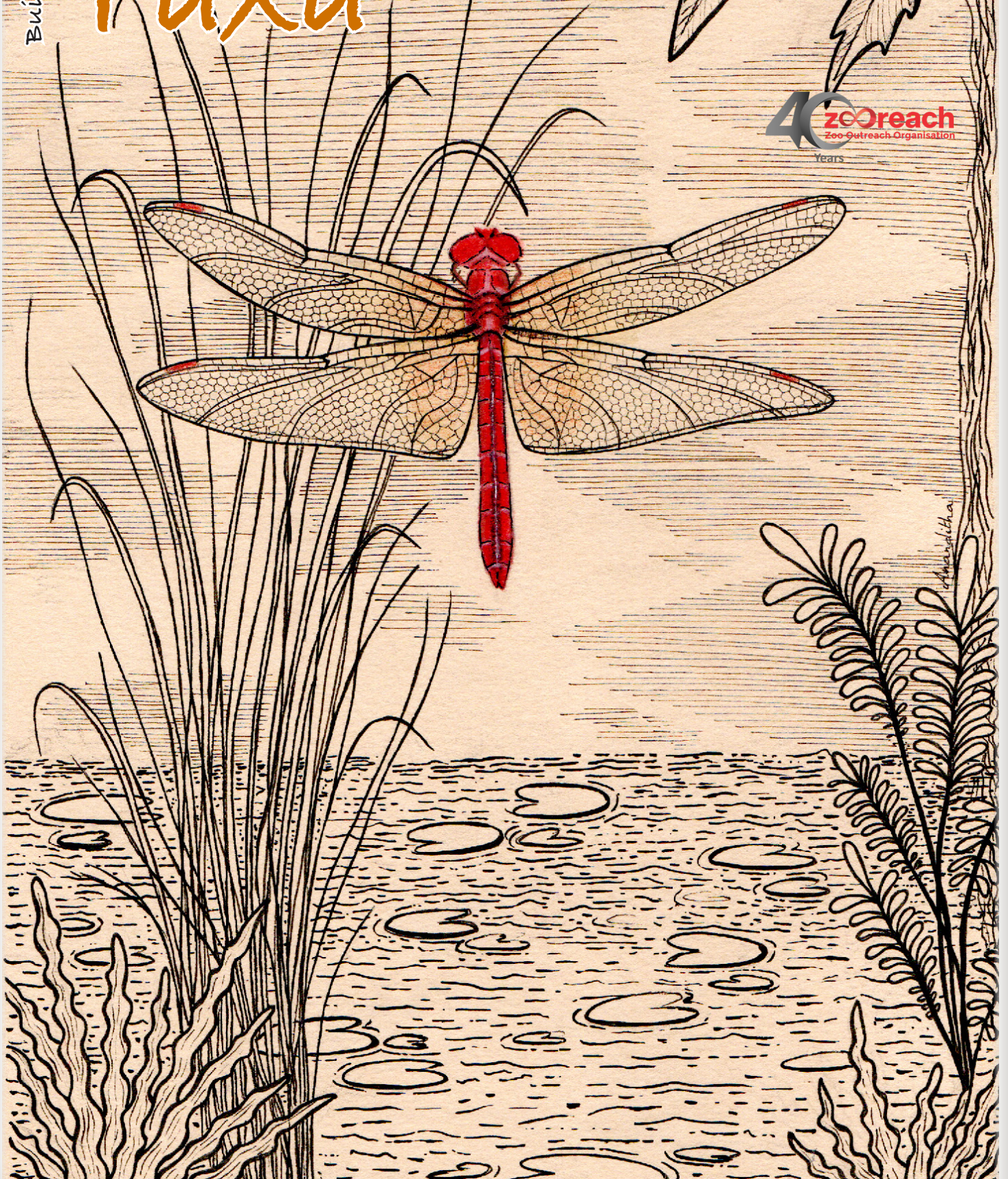
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Cover: A male Scarlet Skimmer perching on vegetation by the banks of a waterbody. Ink and watercolour illustration by Ananditha Pascal.



INTRODUCTION

Fish contribute 18% of animal protein intake globally (Devlin & Nagahama 2002), and in 2020 worldwide aquatic production reached approximately 178 million tonnes, as reported by the Food and Agriculture Organization of the United Nations (FAO 2022). *Schizothorax richardsonii*, also known as “Asela” in central Himalaya, is a member of the Cypriniformes order within the Cyprinidae family and the Schizothoracinae subfamily (Mir et al. 2013). It is found in the cold waters of the rivers, streams, tributaries, and lakes of the Himalayan and sub-Himalayan mountains and foothills at elevations of 1,000–3,300 m, is an important cold-water fish species widely distributed across India, Tibet, Nepal, Bhutan, Pakistan, and Afghanistan (Vishwanath 2010; Qi et al. 2012; Xiao et al. 2020). In the recent past, populations of this species have been under severe threat due to rapid industrial growth, alterations in the natural habitat due to physicochemical changes, and various human activities, leading to a significant decline in numbers and genetic diversity (Mir et al. 2013).

Morphometry is one of the most commonly utilized and cost-effective methods for assessing fish stocks and examining phenotypic variation. Many fish stocks have been successfully discriminated by traditional and truss morphometric approaches, which account for size and shape variation, but have recently been criticized for their concentration along the body axis with depth, breadth measurements, and size (Turan et al. 2004; Ingram 2015; Reiss & Grothues 2015). To overcome the limitations of traditional and truss-based morphometric methods, image-processing techniques like ‘Geometric Morphometrics’ have been developed to analyze shape variations across different populations. This approach has proved effective in identifying stocks, population structure, and species identification. It also enhances the biological understanding necessary for effective fish stock management (Cadrin et al. 2005). It has been predicted that rates of morphological and genetic changes should be positively correlated with rates of species emergence in several evolutionary theories (Rabosky 2013). Thus, in addition to the morphometric study, a genetic assessment was conducted to provide a comprehensive understanding of the stock structure of *S. richardsonii*. Mitochondrial DNA (mtDNA) marker, particularly the cytochrome oxidase subunit I (COX1) gene, is a powerful tool for genetic analysis in fish (Ward et al. 2005). The COX1 gene is an established and reliable genetic marker for identifying highly diversified ichthyofauna at the molecular level. It provides valuable

information on genetic diversity and phylogenetic relationships among populations (Lakra et al. 2011).

In recent years, several studies have highlighted the decline in populations of *S. richardsonii* and the need for urgent conservation measures (Sharma et al. 2021). These studies have underscored the importance of preserving genetic diversity as a buffer against environmental changes and anthropogenic pressures. The genetic diversity of *S. richardsonii* in various river systems has been linked to their resilience and ability to adapt to changing conditions (Mir et al. 2013). However, there is limited information on the phenotypic plasticity and genetic structure of *S. richardsonii* in the Indian Himalaya, necessitating comprehensive studies in this region (Negi & Negi 2010; Sharma & Metha 2010; Mir et al. 2013; Rajput et al. 2013; Dwivedi 2022).

In this study, an integrated approach combining geometric morphometrics with the mitochondrial COX1 gene marker was used to assess the phenotypic and genotypic variability of *S. richardsonii* from the Indian Himalayas, specifically in tributaries of the Ganga River basin. This study represents the first attempt to investigate these variations in this region. The findings will help clarify the phenotypic and genotypic complexity of the stocks, which may aid in developing effective management plans for these stocks. Similar integrative approaches combining morphometric and genetic data have informed conservation for native fish species such as *Silonia silondia* (Mandal et al. 2021), in Indian river systems.

MATERIALS AND METHODS

Collection and identification of samples

Freshly dead fish specimens were collected from local fishermen from February 2022 to April 2023. One hundred ninety adult specimens were collected from four tributaries of the Ganga River: Mandakini, Nandakini, Pindar, and Alaknanda (Figure 1). The taxonomic keys of Day (1878), Talwar & Jhingran (1991), and Mirza (1991) were used to identify the collected specimens. All the specimens were collected after the spawning season and before the breeding season. After photographs were taken for morphometric analysis, the fish specimens were preserved in 10% formalin. For molecular analysis, 100 mg tissue samples from dorsal muscle and fins were preserved in a 1:5 ratio with 95% ethanol and stored at 4°C. Voucher specimens of *S. richardsonii* preserved in a 10% formalin solution were also deposited in the museum of the Department of Zoology, HNB Garhwal

University, Srinagar (Garhwal), for future reference.

Morphological analysis

Sample collection and data generation

Collected specimens were cleaned under running water, dried with blotting paper, and placed on a flat surface with laminated graph paper as the background for digital imaging. The fins were erected to aid in clear display of insertion points, and each specimen was assigned a unique identification code. A Nikon D3400 digital camera was used to capture lateral images of the left side of each specimen. To maintain consistency and minimize errors, all photographs were taken by the same individual from the same angle and height. Further morphometric data were generated by employing fourteen landmarks on lateral side photographs of each fish (Image 1). This data was generated with the help of software tpsUtil ver. 1.52 (Rohlf 2008a) and tpsDig ver. 2.16 (Rohlf 2008b). The landmarks-based data were

converted to shape coordinates through Procrustes superimposition (Rohlf & Slice 1990), standardizing each specimen to unit centroid size, which estimates overall body size (Bookstein 1991).

Statistical procedures

The morphometric data were analyzed to identify and describe potential morphological differences among the four populations. To focus solely on shape information, procrustes superimposition was used to remove variations related to size, position, and orientation (Rohlf & Slice 1990; Bookstein 1991). Procrustes ANOVA was conducted to assess the significance of overall size and shape variations. Shape variables were then used for further analysis. Principal component analysis (PCA) was employed to investigate shape variation's key characters, and explore relationships among the specimens (Veasey et al. 2001). Canonical variates analysis (CVA) was used to identify groups of populations, and discriminant function

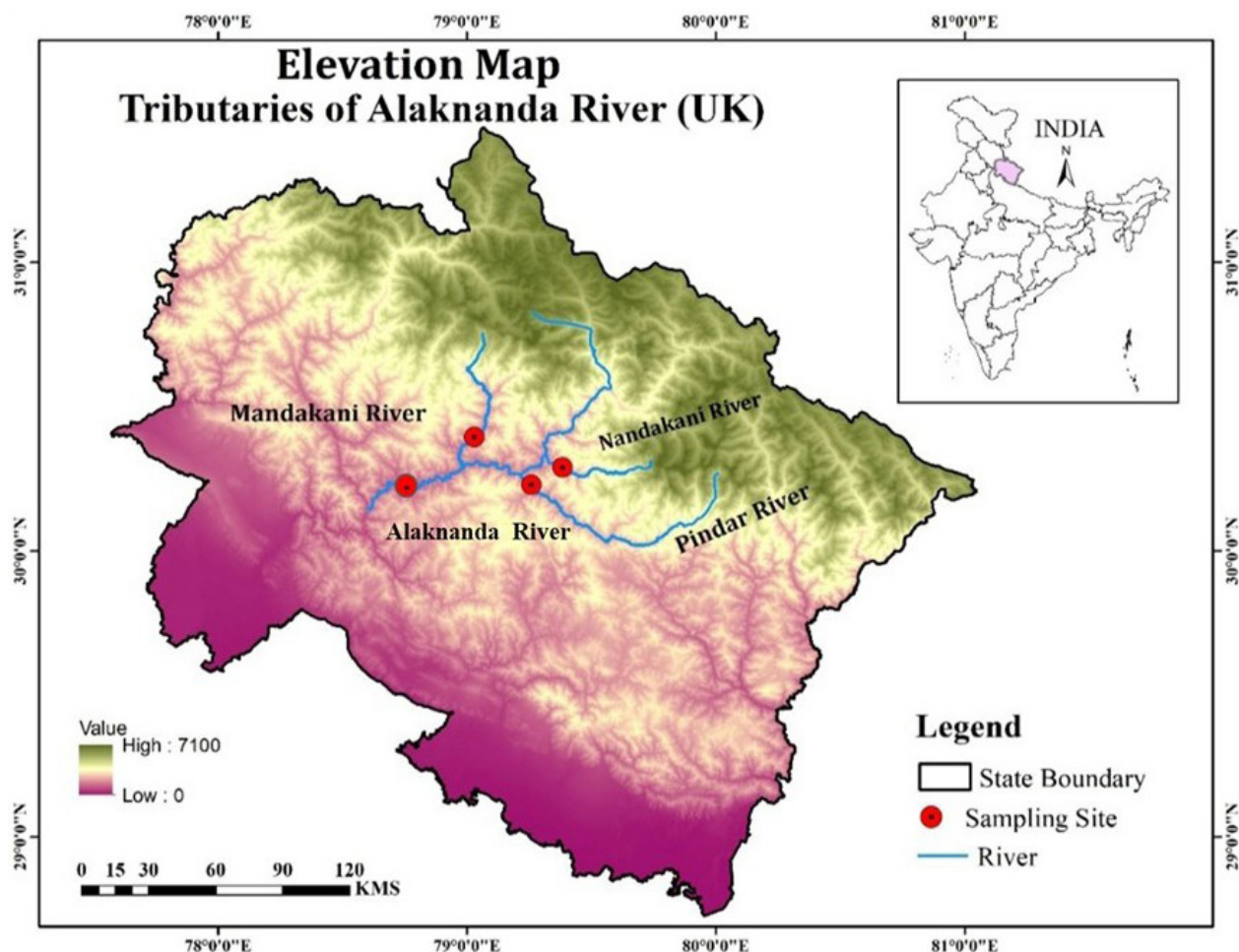


Figure 1. Map showing four sampling sites on the tributaries of the Ganga River basin.

analysis (DFA) was also applied to compare body shape differences between the populations. Additionally, specimens were classified into their original groups. All analyses were conducted using MorphoJ version 1.06d (Klingenberg 2011), a software package designed for geometric morphometrics.

Molecular analysis

DNA extraction, amplification, and sequencing: Approximately, 25 mg 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), 50 mM MgCl₂ (2 µl), 0.05 mM dNTP (0.25 µl), 0.01 mM primer (0.5 µl), Taq polymerase (1.5 IU), and 200 ng genomic DNA template (2 µl). Amplifications were carried out in the Veriti 96 fast thermal cycler from Applied Biosystems, Inc., USA. The primer pair utilized for the COX1 was FishF1 5'TCAACCAACCACAAAGACATTGGCAC3' and FishR15'TAGACTTCTGGG TGGCC AAAGAATCA3' (Ward et al. 2005). The temperature conditions for PCR for COX1 involve an initial denaturation period of 3 minutes at 94°C. Following the initial denaturation, there are 35 cycles of 1 min 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, as 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 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 work was carried out at the DNA Barcoding Laboratory of the Indian Council of Agricultural Research (ICAR) National Bureau of Fish Genetic Resources (NBGFR) in Lucknow,

India.

Genetic data analysis

For the analysis of sequence composition, genetic variation, and constructing a phylogenetic tree, the COX1 gene of all 12 samples from the Ganga River basin was sequenced. The forward sequence and inverted (reversed and complemented) reverse 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, included in 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 to NCBI GenBank. The accession numbers for the sequences range from PQ134998 to PQ135009 (Table 5). For phylogenetic analyses, COX1 partial gene sequences of *Schizothorax richardsonii* populations were generated in this study, along with additional sequences retrieved from NCBI (Table 5). Phylogenetic trees were constructed using the maximum likelihood (ML) method in MEGA 11 with 1000 bootstrap replications. The best-fit nucleotide substitution model was selected based on the Akaike information criterion (AIC) in MEGA X, and the Hasegawa-Kishino-Yano (HKY) model was identified as optimal. Since the analysis was based only on the COX1 gene, codon partitioning (1st, 2nd, and 3rd positions) was applied to account for variation in substitution rates across codons. Further haplotype diversity, nucleotide diversity, genetic differentiation, Fst values, and demographic history were calculated using DnaSP v.5.10.01 (Librado & Rozas 2009) and Arlequin 3.5.2.2 (Excoffier & Lischer 2010). Heat maps showing genetic differentiation among populations were generated using pairwise Fst scores from an online database (<http://www.hiv.lanl.gov/content/sequence/HEATMAP/heatmap.html>).

RESULTS

Geometric morphometrics analyses

A total of 190 fish specimens were analyzed, comprising 87 males and 103 females, with 50 specimens each from the Mandakini, Nandakini, and Pindar rivers. Forty specimens were collected from the Alaknanda River. Shape variations were examined using coordinates derived from a two-dimensional landmark

dataset and aligned through Procrustes transformation. This alignment process removed size effects, as indicated by the Procrustes ANOVA results, which revealed a non-significant difference in overall size ($F = 2.37$, $p > 0.05$) but a significant difference in shape coordinates ($F = 4.52$, $p < 0.05$) among sites. This suggests that size-related variation was largely minimized. Partial least squares (PLS) analysis of the superimposed shapes and log centroid sizes revealed a significant correlation ($R = 0.62$; $p < 0.05$) between groups, indicating a notable positive relationship between shape and size. In PCA, the first two PCs explained 52.5% of the total variance, with PC1 accounting for 36.1% and PC2 accounting for 16.4% (Figure 4). Most variations observed in the shape wireframe based on PC1 were related to landmarks 7, 8, 9, 10, and 12 (Figure 5). However, there was considerable overlap among populations along the first and second PC axes in the PCA plot (Figure 4), indicating minimal shape variation between them. Further analyses were performed using CVA and DFA. The shape coordinate data yielded three CVs. The first Canonical Variate (CV1) explained 57.91% of the total variance, while the second and third canonical variates (CV2 and CV3) accounted for 24.29% and 17.79%, respectively (Table 1). The CVA plot revealed a clear separation between populations based on shape (Figure 5). The Mahalanobis distances (Table 2) and Procrustes distances (Table 3) extracted from CVA were found to be significant ($p < 0.001$) among all four populations of *S. richardsonii* from Alaknanda,

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

CVs	Eigenvalues	% Variance	Cumulative %
CV1	2.52177150	57.910	57.910
CV2	1.05795057	24.295	82.204
CV3	0.77493328	17.796	100.000

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

	Mandakini	Nandakini	Pindar	Alaknanda
Mandakini		< 0.0001	< 0.0001	< 0.0001
Nandakini	3.5456		< 0.0001	< 0.0001
Pindar	2.6739	3.3538		< 0.0001
Alaknanda	3.1472	4.4769	2.8788	

Mandakini, Nandakini, and Pindar Rivers, indicating shape heterogeneity among the populations of these four tributaries.

The DFA accurately classified 87.4% of individuals into their original groups. A cross-validation test using the leave-one-out procedure confirmed that 73.7% of individuals were correctly classified into their original groups. Moderate mixing of individuals was also observed between the Alaknanda & Mandakini rivers,

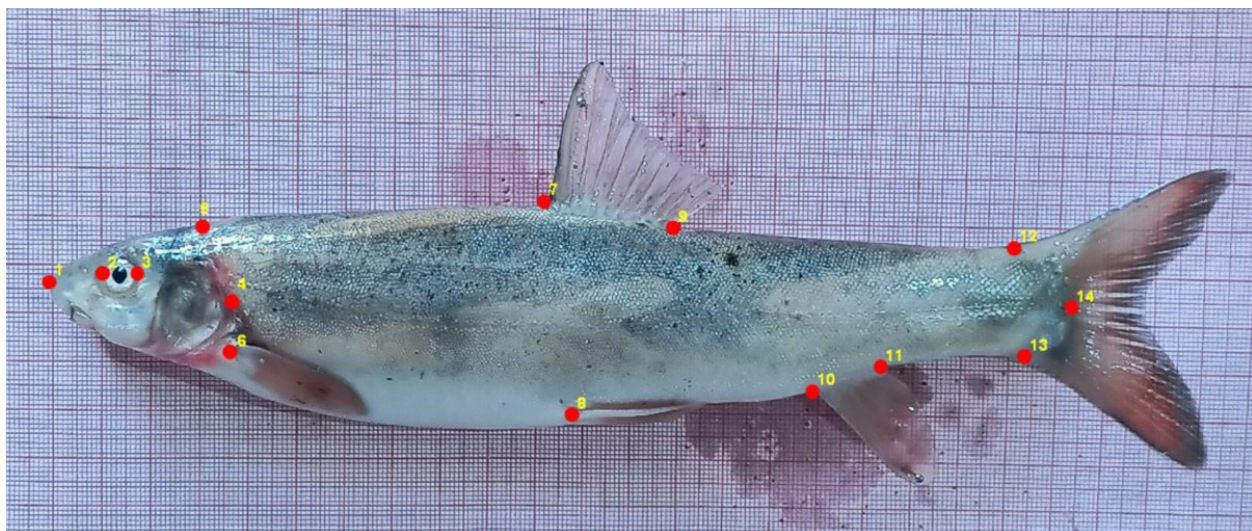


Image 1. Image of *Schizothorax richardsonii* showing the 14 landmarks used to compare among the populations: 1—tip of snout | 2—anterior border of the eye | 3—posterior border of the eye | 4—posterior border of operculum | 5—forehead (end of frontal bone) | 6—pectoral-fin origin | 7—dorsal fin origin | 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.

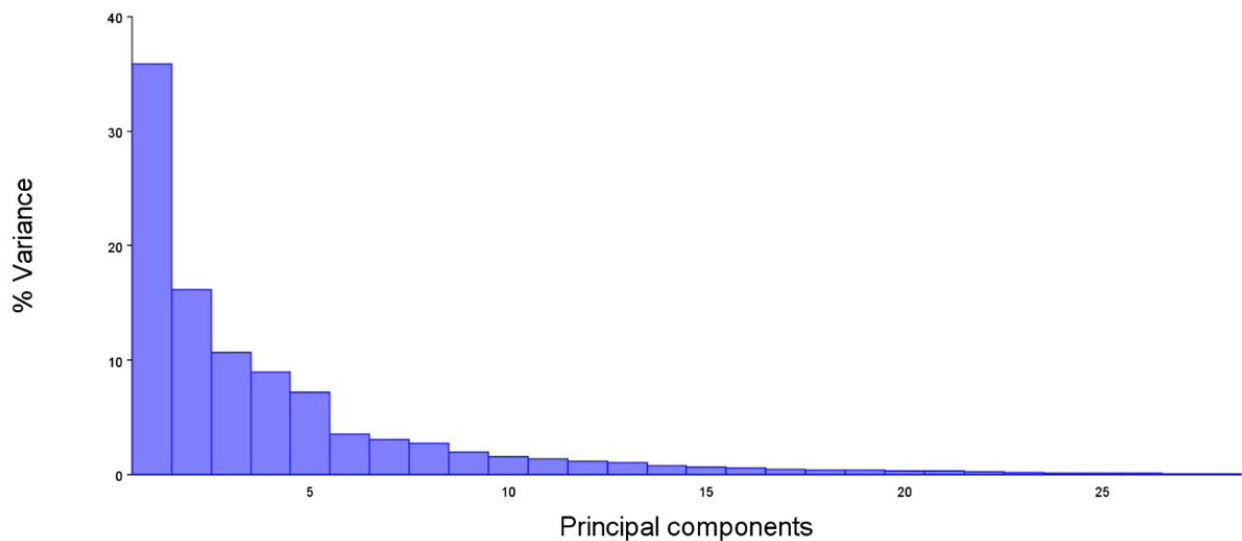


Figure 2. Scree plot of percentage variance and principal components in *Schizothorax richardsonii*.

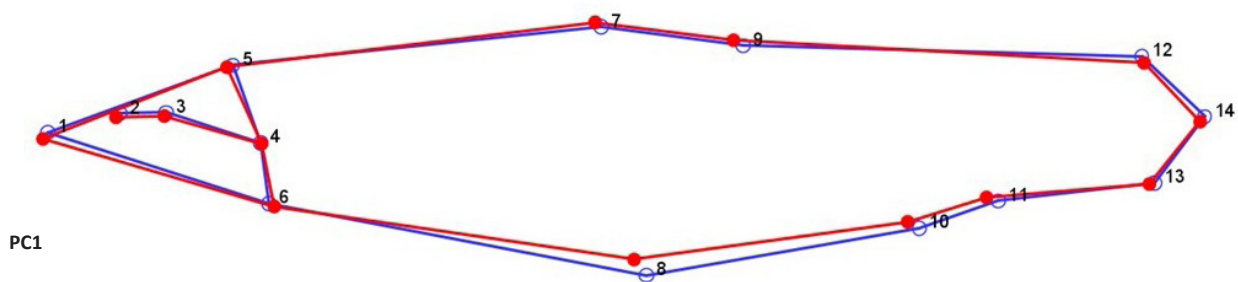


Figure 3. Wireframes displaying the shape changes associated with PC1 of *Schizothorax richardsonii* populations; red wireframe shows the original position of landmarks; blue wireframe shows variation in landmark position.

the Nandakini & Mandakini rivers, and the Nandakini & Pindar rivers. A lower level of mixing was observed between individuals from the Alaknanda & Nandakini, and the Alaknanda & Pindar rivers (Figure 6). These findings were congruent with the variations depicted by the deformed shape wireframe of the average shape, which highlighted differences among the four populations of *S. richardsonii*. The shape differences observed between populations of the Alaknanda and Mandakini rivers were primarily based on landmarks 6 and 3–4; for Alaknanda and Nandakini populations 3–4, 7, 8, and 9; for Alaknanda and Pindar populations variations were seen at landmarks 2–3, 7, 8, and 9; differences between Mandakini and Nandakini populations were based on landmarks 6, 7, 8, 9, 12, 13, and 3–4; for Mandakini and Pindar populations 2–3, 7, 8, 9, 12, and 13; lastly for Nandakini and Pindar 2–3, 3–4, 6, and 8 (Figure 7). It was observed that most of the variations occurred in the diameter of the eye, the anterior and posterior origins

of the dorsal fin, and the origins of the pelvic and caudal fin. These morphometric measurements indicate that they are useful for describing morphological variation and offer insights into population distinctiveness within the tributaries of the Ganga River basin. The CVA results aligned with the DFA results, highlighting variations in body shape among the *S. richardsonii* populations. Overall, both analyses indicated the presence of four distinct populations of *S. richardsonii* in the selected rivers, based on their shape: 1. Alaknanda, 2. Mandakini, 3. Nandakini, and 4. Pindar.

Genetic diversity and phylogenetic tree

After excluding the primer sequences and performing equal-length alignment, each sequence was 655 bp. No insertions, stop codons, or deletions were detected, confirming that all amplified sequences derived from a functional mitochondrial COX1 gene. Analysis of the COX1 sequences revealed the average nucleotide composition

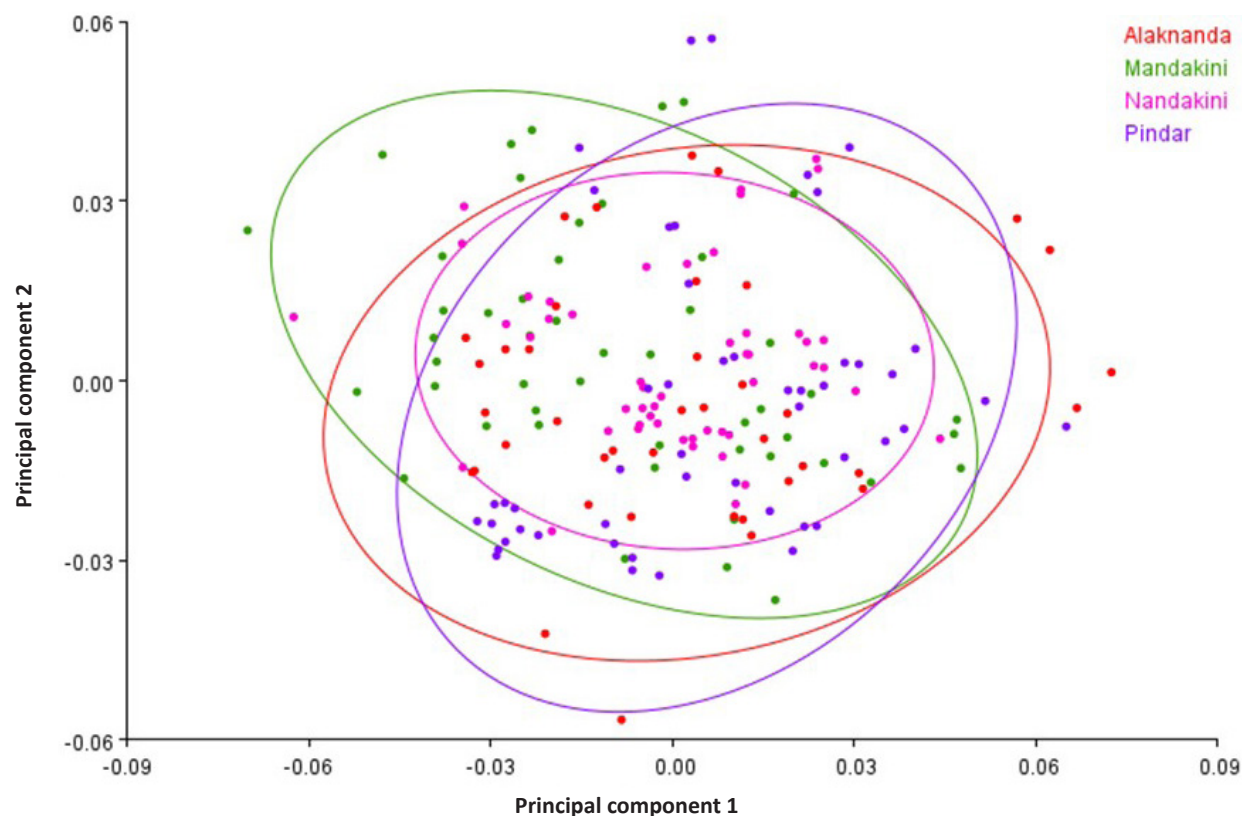


Figure 4. The principal component analysis plot of *Schizothorax richardsonii*, showing loadings of each sample on the first two principal components.

Table 3. Procrustes distances (lower diagonal) and p-value (upper diagonal) of canonical variates analysis among *Schizothorax richardsonii* populations.

	Mandakini	Nandakini	Pindar	Alaknanda
Mandakini		< 0.0001	< 0.0001	< 0.0001
Nandakini	0.0156		< 0.0001	< 0.0001
Pindar	0.0222	0.0165		< 0.0001
Alaknanda	0.0214	0.0273	0.0227	

in *S. richardsonii* from the Ganga River tributaries as 25.79% (A), 27.79% (T/U), 28.17% (C), and 18.25% (G). The COX1 gene analysis identified three variable polymorphic sites and three parsimony-informative sites in the specimens from the Ganga River tributaries. Five distinct haplotypes were observed among the *S. richardsonii* populations in the present study. The highest haplotype (h) diversity and nucleotide diversity (π) (0.66667 & 0.00105) were found in the Mandakini River (Table 4). Further, a phylogenetic tree was constructed by MEGA 11, using the maximum likelihood (ML) method with 1000 bootstrap replications, based on

Table 4. Intrapopulation, haplotype (individuals' frequency), haplotype (h), and nucleotide (π) diversities for the COX1 mitochondrial partial gene in four riverine populations of *Schizothorax richardsonii*.

Locations	Sample size (N)	Haplotype (individuals frequency)	Haplotype diversity (h)	Nucleotide diversity (π)
Mandakini	3	Hap_1(1), Hap_2 (2)	0.66667	0.00105
Nandakini	3	Hap_1 (1)	0.00000	0.00000
Pindar	3	Hap_4 (3),	0.20000	0.0060
Alaknanda	3	Hap_5 (3) Hap_2 (1)	0.40000	0.00063
Overall	12	Hap_1-Hap5	0.844848	0.00212

the Hasegawa-Kishino-Yano (HKY) model, keeping *Rita rita* (NC023376) as an outgroup to provide an external reference point for the tree root. While other highly specialized Schizothoracine (NC025650, NC024537) and specialized Schizothoracine (NC021420) species were incorporated to strengthen the evolutionary framework and improve the resolution of relationships within *Schizothorax richardsonii* populations. The

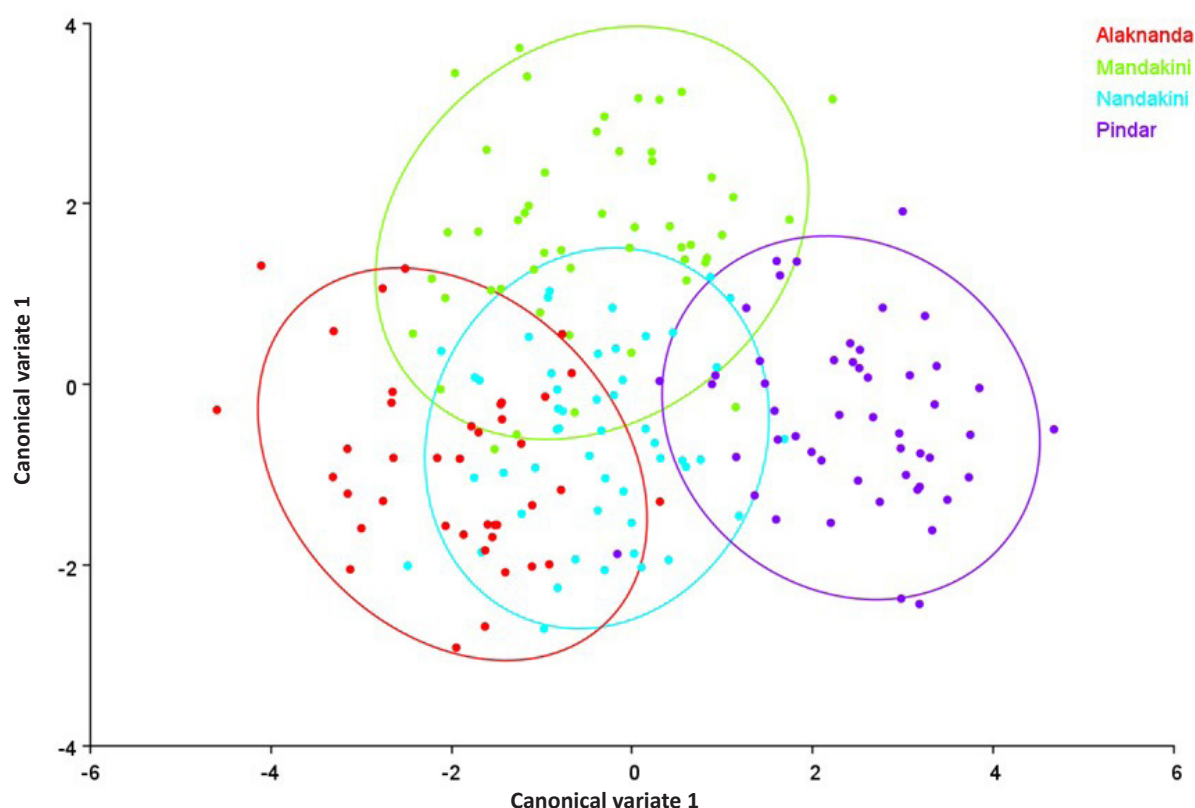


Figure 5. Geometric morphometric-based canonical variates analysis plot of *Schizothorax richardsonii* showing the frequency of specimen distribution in respective groups on the first two axes.

Table 5. Accession numbers and voucher specimen numbers of *Schizothorax richardsonii* individuals collected from tributaries of the Ganga River Basin, and reference sequences retrieved from NCBI.

	Accession No.	Voucher specimen
1	PQ134998	SrHNBGUM1
2	PQ134999	SrHNBGUM2
3	PQ135000	SrHNBGUM3
4	PQ135001	SrHNBGUN3
5	PQ135002	SrHNBGUN5
6	PQ135003	SrHNBGUN6
7	PQ135004	SrHNBGUP1
8	PQ135005	SrHNBGUP4
9	PQ135006	SrHNBGUP8
10	PQ135007	SrHNBGUA2
11	PQ135008	SrHNBGUA4
12	PQ135009	SrHNBGUA7
13	OQ130193	<i>Schizothorax richardsonii</i> (NCBI)
14	PV643387	<i>Schizothorax richardsonii</i> (NCBI)
15	PV643388	<i>Schizothorax richardsonii</i> (NCBI)
16	PV643389	<i>Schizothorax richardsonii</i> (NCBI)
17	PV643390	<i>Schizothorax richardsonii</i> (NCBI)

ML phylogenetic tree based on mitochondrial COX1 sequences revealed four distinct groups within a single clade of *S. richardsonii*, representing populations from the Alaknanda, Mandakini, Pindar, and Nandakini rivers. Notably, NCBI retrieved sequences of *S. richardsonii* clustered with the Alaknanda and Nandakini populations (Figure 8). Based on the F_{st} scores, the heatmap shows clear genetic differentiation among the Pindar & Alaknanda, Nandakini & Alaknanda, and Mandakini & Pindar populations (Figure 9). Tajima's D neutrality test ($D = 1.72912$, $P = 0.10$) provides a positive but non-significant value, indicating a weak tendency toward balancing selection, population contraction, or a potential bottleneck effect.

DISCUSSION

Species population structure and composition are crucial indicators for assessing freshwater biodiversity (Turek et al. 2016). Fish serve as excellent model systems for studying interspecific and intraspecific divergences, providing insights into the ecological factors driving

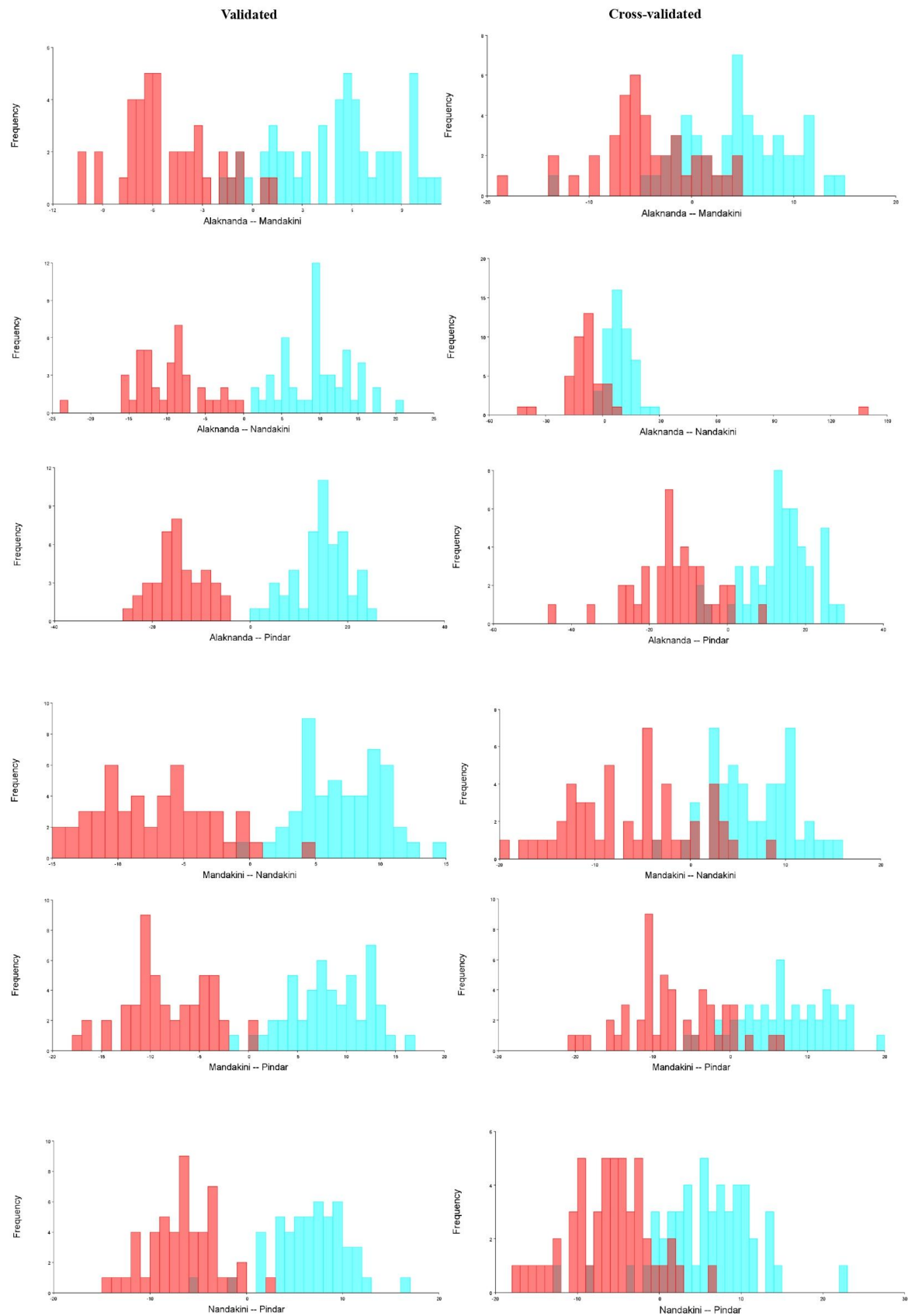


Figure 6. Graphs of discriminant function analysis between the *Schizothorax richardsonii* populations from four tributaries of the Ganga River basin.

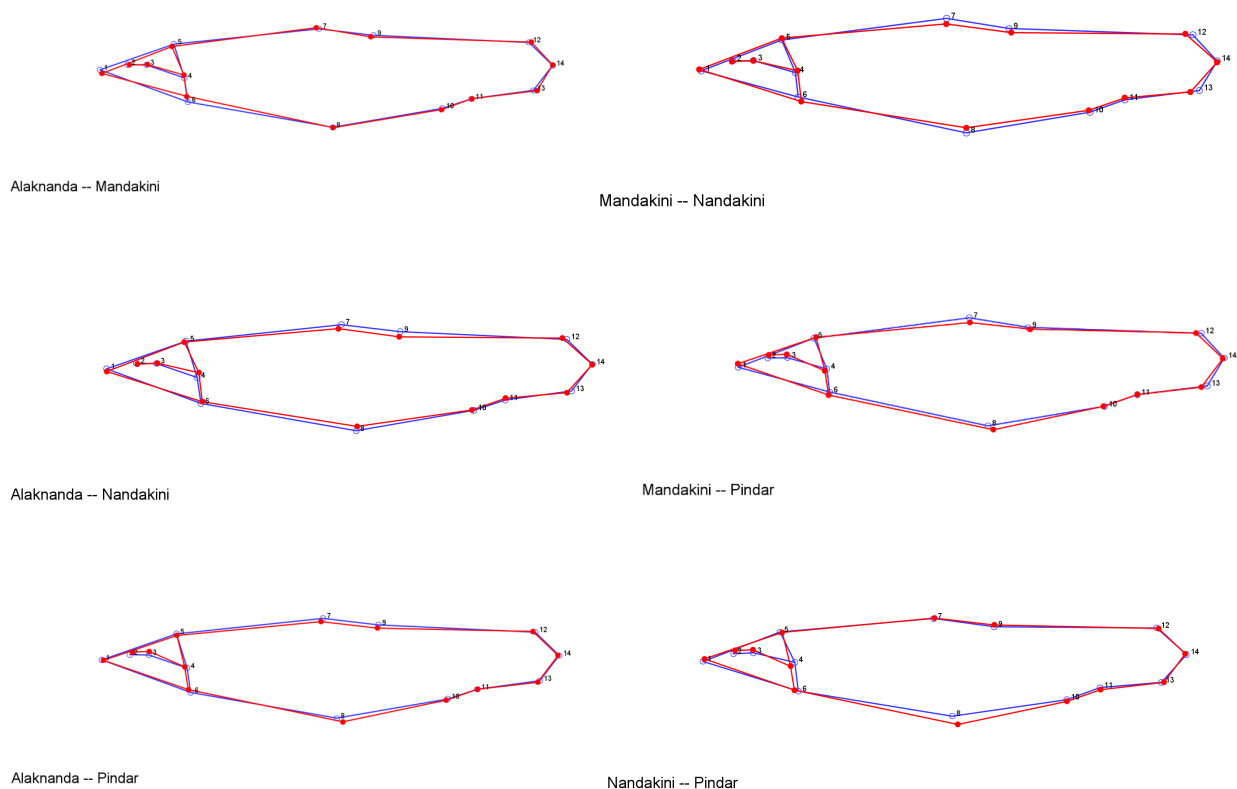


Figure 7. Wireframes showing the shape changes associated with discriminant scores among four populations of *Schizothorax richardsonii* (blue wireframe shows the original position of landmarks, the red wireframe shows variation in landmark position).

morphological and genetic diversification. Neglecting to address stock complexity within management units has resulted in the depletion of spawning components, leading to a loss of genetic diversity and potentially other ecological effects (Begg et al. 1999). The present study combines geometric morphometric and mitochondrial DNA (COX1) analyses to evaluate phenotypic and genetic variations among wild populations of *S. richardsonii* from the Ganga River basin.

The study results revealed morphological differences among *S. richardsonii* populations from four tributaries of the Ganga River basin. The PCA indicates phenotypic plasticity, with the first three principal components accounting for a combined variance of 63% among the four populations. The PC1 described shape variation mainly associated with shifts in the pelvic fin, caudal peduncle, and anal fin positions. These differences were most pronounced among the four phenotypic stocks, suggesting population-level morphological differentiation. These measurements likely reflect adaptations to distinct ecological conditions in their habitats, such as different flow regimes, predation pressures, and food availability. Geometric morphometrics effectively delineates populations based

on shape variations using CVA (Cadrin & Silva 2005; Maderbacher et al. 2008).

Overall, CVA shows a significant difference among populations of *S. richardsonii* from four distinct tributaries of the Ganga River basin, and four different populations were identified phenotypically. The CVA plot further indicated that the Pindar population showed greater morphological divergence from the Mandakini and Alaknanda populations than from the Nandakini, suggesting stronger phenotypic differentiation among populations inhabiting more geographically isolated rivers. The CVA plot also distinguished the Pindar and Alaknanda populations from the others, possibly due to anthropogenic disturbances such as water diversion for irrigation and domestic use, as well as the extraction of construction materials from riverbeds, which are common in these regions. Intense human intervention also resulted in habitat loss and degradation of the freshwater ecosystem, thus affecting the fish species, especially in regions with high water demand (Sarkar et al. 2012). Dwivedi (2022), while studying the phenotypic variation in *S. richardsonii* from the Indian Himalaya, also revealed the existence of four different stocks from seven Indian rivers using CVA and DFA. However, he

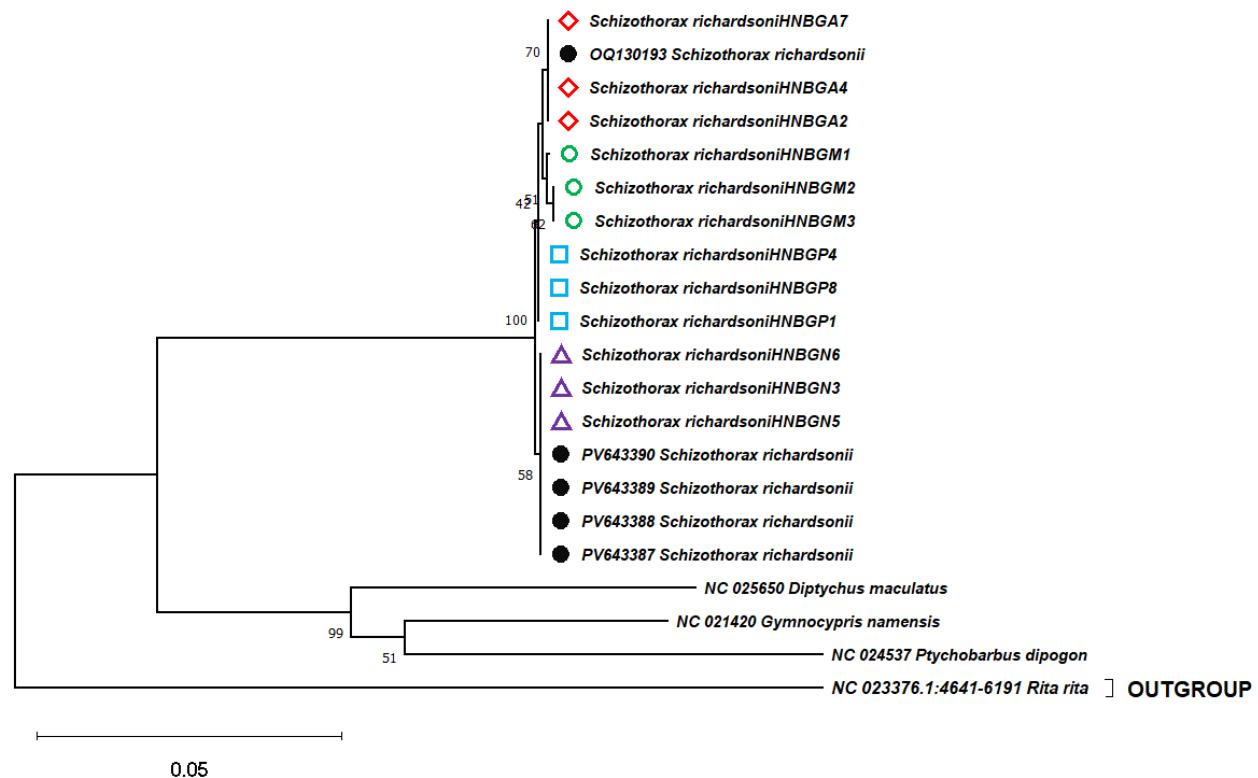


Figure 8. Maximum likelihood type of phylogenetic tree for the natural population of *Schizothorax richardsonii* based on mitochondrial COX1 partial gene sequences from four different tributaries of the Ganga River basin: circle represents the populations from the Mandakini River, Triangle Nandakini River, Square Pinder River, diamond Alaknanda river and Black drop represents the reference sequences retrieved from NCBI.

did not specify the key morphometric measurements that differentiate these populations. In this study, the Mahalanobis and Procrustes distances confirm the heterogeneity among these populations. The results of the present study align with those of Mejia & Reis (2024), who found notable morphological differences among *Otocinclus cocama* populations in Amazon River tributaries and suggested that environmental factors play a crucial role in the isolation and movement of fish stocks.

The DFA can effectively differentiate stocks within the same species (Karakousis et al. 1991). In this study, the leave-one-out cross-validation test accurately assigned 73.7% of individuals to their original groups, indicating intermingling among some populations, i.e., Nandakini with Pindar and Mandakini with Nandakini. The Ganga River is an ancient river that originated in the late Pleistocene, while its tributaries formed more recently as lateral rivers (Daniel 2001). It has been suggested that fish stocks are distributed along a spatial gradient, leading to frequent fish mixing within the basin (Murta et al. 2008). However, in the present study, a close resemblance between the two populations from

Pindar and Nandakini was noticed, probably due to the proximity in terms of geographical location. The local migration of the species may also result in the mixing of the Nandakini population with Mandakini. Some other researchers also reported morphological closeness within the basin due to seasonal migration and similar ecological conditions between sites for spawning (Murta et al. 2008). Mir et al. (2013) identified three key morphometric characteristics, eye diameter, body depth, and caudal peduncle, that contribute to population variations in snow trout from the Indian Himalayas based on DFA, using a truss-based morphometric approach. In contrast, the present study found that the anterior and posterior origins of the dorsal fin, origin position of the pelvic fin, anal fin, caudal peduncle, and eye diameter exhibited significant variation based on discriminant scores using geometric morphometrics. These morphometric measurements serve as crucial morphological descriptors, offering valuable insights into the distinctiveness of populations within the tributaries of the Ganga River basin. The PC1-based shape wireframe also supported this result. Osburn (1906) noted that pelvic fins assist fish in maintaining

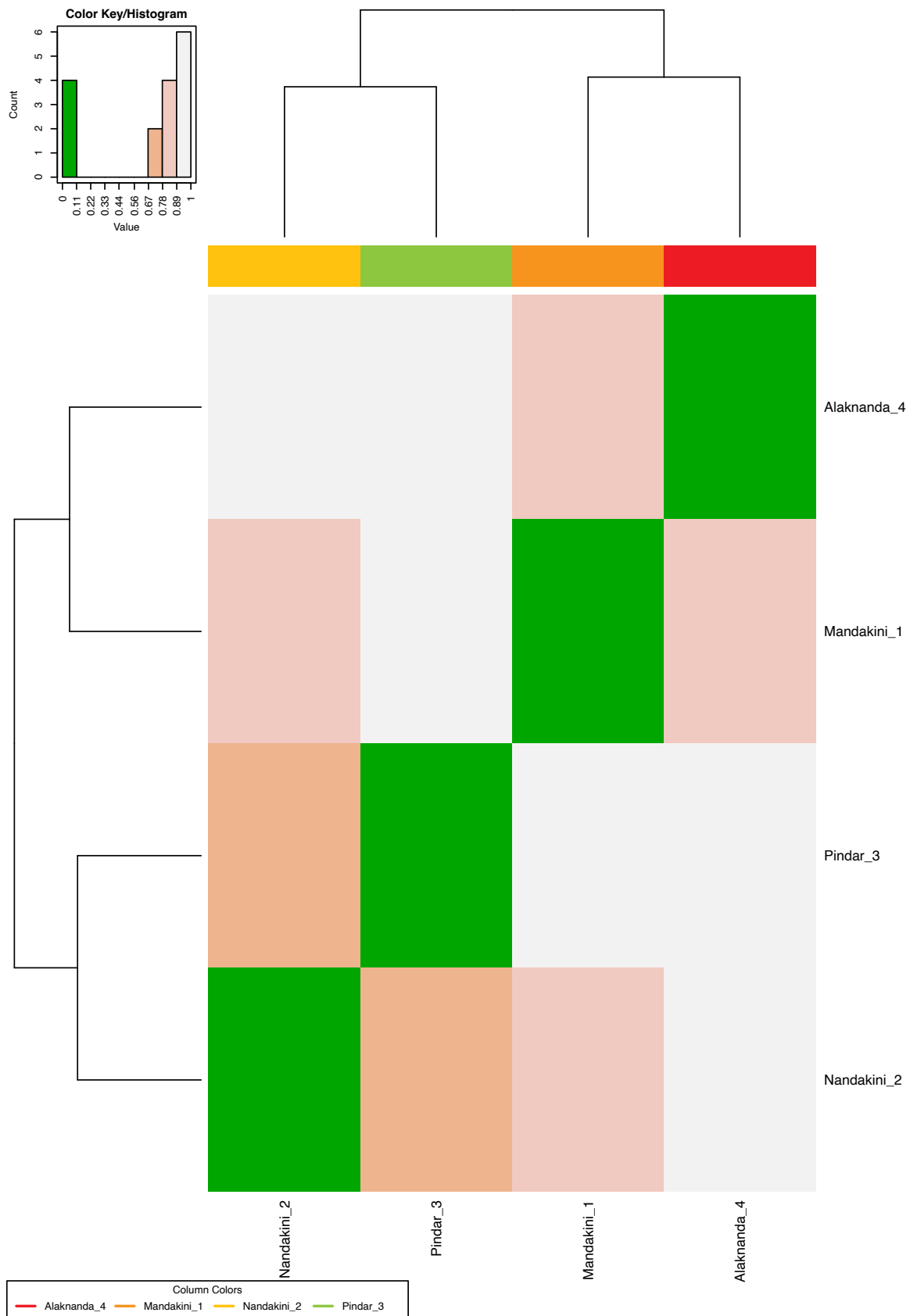


Figure 9 . Fst scores-based heatmap showing genetic differentiation among the *Schizothorax richardsonii* population based on the mitochondrial COX1 partial gene.

balance while swimming. Harris (1936) observed that many teleost fish raise their dorsal fins while gliding, enabling them to change direction quickly. This suggests that the dorsal and pelvic fins contribute to population-level morphological variations by influencing movement and control during swimming.

To assess genetic variability and establish the phylogenetic relationship among the *S. richardsonii* populations, this study used the mitochondrial marker COX1, which revealed three polymorphic sites, three parsimony sites, and five haplotypes. Interestingly, the highest genetic variability was observed in the Mandakini population, which generally indicates a stable and resilient gene pool crucial for adaptability and long-term survival. Genetic differentiation plays a vital role in comprehending the evolutionary dynamics of fish populations (Stange 2021). In the present study, the phylogenetic analysis based on the ML method showed one clade and four separate groups, depicting the clear distinction among the four populations of *S. richardsonii* from the Ganga River basin and justifying their separate management strategies. The genetic distance between these populations of *S. richardsonii* is very small, 0.020%, which is considerably lower than the standard threshold used for species discrimination through DNA barcoding (Hebert 2003). This suggests that the genetic differentiation between these populations has not reached the level required for speciation. Populations with such differentiation may be at risk of genetic erosion, loss of genetic diversity, and other potential ecological impacts (Begg et al. 1999). ML phylogenetic analysis also suggested that the Mandakini and Alaknanda populations were closely related to each other compared to other populations. All the sequences were adenine and thymine-rich, consistent with earlier reports in fish (Johns & Avise 1998). The average A+T content was 53.58%, and the GC content was 46.42%, similar to the results reported by Ward et al. (2005), Lakra et al. (2011), and Vineesh et al. (2013). Min & Hickey (2007) demonstrated a strong correlation between the GC content of the COX1 gene and that of the entire mitochondrial genome. Fst scores also indicated clear genetic differentiation among river Alaknanda & Pindar populations, Nandakini & Alaknanda, and Mandakini & Pindar populations based on mitochondrial COX1 partial gene. This differentiation could be due to the hydro-power projects built over the Alaknanda River in the central Himalaya, which have disrupted the natural habitat by blocking the fish migratory routes. The government of India has issued policies to exploit the riverine system of the Indian Himalaya, which is hypothetically proven to

cause serious damage to biodiversity and changes in the ecosystem (Pandit & Grumbiene 2012).

An interesting finding in this study is that the Pindar population shows highest morphometric and genetic variability among all the three tributaries of the Ganga River basin due to the difference in habitat conditions, environmental factors, and anthropogenic effects such as overfishing, household wastage, water withdrawal, and pollution from plastics among these tributaries. However, a low level of genetic diversity was observed in the Nandakini population. Interestingly, most of the sequences retrieved from NCBI clustered with the Nandakini population, while one sequence clustered with the Alaknanda population, likely due to its origin from the same stream, indicating genetic similarity. A decline in genetic variation within any population reduces the fish's ability to adapt to environmental changes and decreases the species' chances of long-term survival (Tickner et al. 2020). In our study, the COX1 marker clearly showed the population delineation of *S. richardsonii* from four tributaries of the Ganga River basin. As documented in previous studies, alteration in fish population structure can result from river fragmentation caused by physical barriers such as dams and barrages (Anvarifar et al. 2011). In the present study, Tajima's D analysis yielded a positive but non-significant value, suggesting a weak tendency toward balancing selection, population contraction, or a potential bottleneck effect. However, the results tentatively suggest recent expansion; we emphasize that broader sampling and the use of nuclear markers are needed to provide stronger evidence for demographic processes.

Kousar et al. (2025) studied mitochondrial DNA variability in *S. richardsonii* using the COX1 marker from tributaries of the Chenab river and reported limited gene flow between populations. In contrast, our results identified four distinct population groups and revealed no gene flow between the Pindar River and the remaining tributaries. Moreover, the COX1 base composition observed in the present study (A+T = 53.58% and G+C = 46.42%) differs slightly from that reported by Kousar et al. (2025) from the Western Indian Himalayan population (A+T = 53.63% and G+C = 46.37%). Overall, in the present study, the geometric morphometrics analysis based on multivariate analysis and mtDNA COX1-based sequences analysis revealed a clear phenotypic and genotypic heterogeneity among *S. richardsonii* populations from four distinct tributaries within the Ganga River basin.

CONCLUSION

The results of the present study provide compelling evidence of phenotypic and genotypic differences among *S. richardsonii* populations in the tributaries of the Ganga River basin. Key phenotypic traits such as the origins of the pelvic fin, dorsal fin, anal fin, and caudal peduncle were critical for morphological descriptions. Additionally, a genetically low percentage of nucleotide base composition was observed. These variations may be influenced by dam construction, anthropogenic disturbances like water diversion for irrigation & drinking, extraction of building materials from riverbeds, differences in flow regimes, genetic isolation, and evolutionary pressures. Integrating morphometric and genetic data enhances our understanding of the species diversity and evolutionary dynamics in the central Himalaya. It underscores the need for population-specific conservation and management strategies, including implementing the closed season during breeding periods for *S. richardsonii* in the Ganga River basin, emphasizing ecosystem-based approaches to protect this valuable genetic resource.

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Articles

Morpho-taxonomic studies on the genus *Fissidens* Hedw. (Bryophyta: Fissidentaceae) in Senapati District, Manipur, India

– Kholi Kaini & Kazhuhrii Eshuo, Pp. 27787–27796

Ecology and conservation concerns of *Indianthus virgatus* (Marantaceae): an endemic species of the Western Ghats–Sri Lanka Biodiversity Hotspot

– Shreekara Bhat Vishnu, Vivek Pandi, Bhathiya Gopallawa, Rajendiran Gayathri, B. Mahim, Deepthi Yakandawala & Annamalai Muthusamy, Pp. 27797–2805

An updated floral diversity of Tal Chhapar Wildlife Sanctuary, Rajasthan, India

– Sneha Singh & Orus Ilyas, Pp. 27806–27821

An updated checklist of the family Rosaceae in Arunachal Pradesh, India

– Pinaki Adhikary & P.R. Gajurel, Pp. 27822–27841

Restoring biodiversity: case studies from two sacred groves of Kozhikode District, Kerala, India

– K. Kishore Kumar, Pp. 27842–27853

A preliminary investigation on wing morphology, flight patterns, and flight heights of selected odonates

– Ananditha Pascal & Chelmala Srinivasulu, Pp. 27854–27862

Phylogenetic confirmation of generic allocation and specific distinction of Mawphlang Golden-cheeked Frog *Odorrana mawphlangensis* (Pillai & Chanda, 1977) (Amphibia: Anura: Ranidae) and its updated distribution records

– Angshuman Das Tariang, Mathipi Vabeiryureilai, Fanai Malsawmdawngliana & Hmar Tlawmte Lalremsanga, Pp. 27863–27873

Phenotypic and genotypic variability in the Snowtrout *Schizothorax richardsonii* (Cypriniformes: Cyprinidae) wild populations from central Himalayan tributaries of the Ganga River basin

– Yasmeen Kousar, Mahender Singh & Deepak Singh, Pp. 27874–27888

Avian composition and distribution in the bird sanctuary planning zone of Can Gio Mangrove Biosphere Reserve, Ho Chi Minh City, Vietnam

– Huynh Duc Hieu, Huynh Duc Hoan, Bui Nguyen The Kiet, Dang Ngoc Hiep, Nguyen Thi Phuong Linh & Nguyen Dang Hoang Vu, Pp. 27889–27896

Bat echolocation in South Asia

– Aditya Srinivasulu, Chelmala Srinivasulu, Bhargavi Srinivasulu, Deepa Senapathi & Manuela González-Suárez, Pp. 27897–27931

A checklist of the mammals of Jammu & Kashmir, India

– Muzaffar A. Kichloo, Ajaz Ansari, Khursheed Ahmad & Neeraj Sharma, Pp. 27932–27945

Communications

Notes on distribution, identification and typification of the Elongated Sweet Grass *Anthoxanthum hookeri* (Aveneae: Poaceae) with comparative notes on *A. borii*

– Manoj Chandran, Kuntal Saha, Ranjana Negi & Saurabh Guleri, Pp. 27946–27953

Conservation significance of Yelakundli Sacred Grove: a relic population of the endemic dipterocarp *Vateria indica* L.

– G. Ramachandra Rao, Pp. 27954–27959

A preliminary study of fish diversity in Sirum River of East Siang District, Arunachal Pradesh, India

– Obinam Tayeng, Leki Wangchu & Debangshu Narayan Das, Pp. 27960–27969

Preliminary investigation on morphometrics and habitat of the Indian Flapshell Turtle *Lissemys punctata* (Bonnaterre, 1789) (Reptilia: Trionychidae) in rural wetlands of Alappuzha, Kerala, India

– Sajan Sunny, Appiyathu Saraswathy Vijayasree, Nisha Thomas Panikkaveetil & E. Sherly Williams, Pp. 27970–27975

A preliminary assessment of avifaunal diversity in Parwati Arga Bird Sanctuary, Uttar Pradesh, India

– Yashmita-Ulman & Manoj Singh, Pp. 27976–27984

Sightings of the Rusty-spotted Cat *Prionailurus rubiginosus* (I. Geoffroy Saint-Hilaire, 1831) (Mammalia: Carnivora: Felidae) in Saurashtra Peninsula, Gujarat, India

– Raju Vyas, Pranav Vaghshiya & Devendra Chauhan, Pp. 27985–27991

Short Communications

Abundance and distribution of the Critically Endangered Giant Staghorn Fern *Platyserium grande* (A.Cunn. ex Hook.) J.Sm. in Maguindanao del Sur, BARMM, Philippines

– Marylene M. Demapitan, Roxane B. Sombero, Datu Muhaymin C. Abo, Nof A. Balabagan & Cherie Cano-Mangaoang, Pp. 27992–27996

***Bonnaya gracilis* a novel find for the flora of Uttarakhand, India**

– Monal R. Jadhav, Revan Y. Chaudhari & Tanveer A. Khan, Pp. 27997–28000

Notes

Crab eating crab: first record of the Horn-eyed Ghost Crab *Ocypode brevicornis* preying on the Mottled Light-footed Crab *Grapsus albolineatus* in Visakhapatnam, India

– Harish Prakash, M.K. Abhisree & Rohan Kumar, Pp. 28001–28003

First record of Greater Scaup *Aythya marila* in Farakka IBA near West Bengal & Jharkhand border, India

– Subhro Paul, Sudip Ghosh & J. Jiju Jaesper, Pp. 28004–28006

Filling the gap: first regional record of the Little Owl *Athene noctua ludlowi* (Strigiformes: Strigidae) from Uttarakhand, India

– Anuj Joshi, Dhanesh Ponnu, Vineet K. Dubey & Sambandam Sathyakumar, Pp. 28007–28010

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