Detection of morphometric differentiation in Sattar snowtrout, *Schizothorax curvifrons* (Cypriniformes: Cyprinidae) from Kashmir Himalaya using a truss network system

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Abstract: *Schizothorax curvifrons* is a morphometrically and meristically most variable and economically valuable promising fish food from Kashmir Valley. Since there is a lack of information on stock structure of wild populations on this species, this study was aimed to investigate the intraspecific variation of this important snowtrout. For this, two rivers and one lake in Kashmir Himalaya were sampled from January 2011 to October 2012. Fish body measurements were taken and morphometric characters using the truss network system was constructed. Altogether, 506 fish specimens were collected. Data were subjected to principal component analysis, discriminant function analysis and univariate analysis of variance. The first principal component explained 63.44% of total variation, while second and third components explained 8.34% and 5.31%, respectively. The step-wise discriminant function analysis retained two variables that significantly discriminated the populations. Using these variables 83.4% of the original specimens were classified into their correct groups and 81.1% of the cross-validated (leave one out procedure) specimens were classified into their correct groups. All of the total 31 transformed truss measurements exhibited highly significant (p<0.001) differences between the populations. This represents the first attempt on stock structure of *S. curvifrons*; therefore, this study will hopefully guide fisheries taxonomists about its current stock structure and would help in its management and conservation programme across Kashmir Himalaya area. Rev. Biol. Trop. 62 (1): 119-127. Epub 2014 March 01.

Key words: truss morphometrics, *Schizothorax curvifrons*, Kashmir Himalaya, India.

The cyprinid *Schizothorax curvifrons* (Heckel, 1838), commonly known as Sattar snowtrout is a good prized indigenous omnivorous coldwater teleost of Kashmir Valley, where it inhabits rivers, lakes and swamps. Besides India, it is also found in Afghanistan, Pakistan, China and Iran (Coad, 1995). This species can always be recognized by the combination of large high scale count, high gill raker number and thin lips (Mir, Shabir, & Mir, 2012); besides, the maximum size reported is 56cm (Berg, 1964) and 1 250g in weight (Talwar & Jhingran, 1991). Considering reproductive behaviour, mature adults undertake spawning migration to incoming streams, and breeding takes place amidst gravel and sandy beds (Raina & Petr, 1999); the spawning period is extremely protracted, from May until the beginning of August.

Several techniques have been proposed for stock identification, an interdisciplinary field that involves the recognition of self-sustaining components within natural populations. It is a central theme in fisheries science and management (Cadrin, Friedland, & Waldman, 2004; Mir, Sarkar, Dwivedi, Gusain, & Jena, 2013a). Stock identification can be viewed as a prerequisite for any fishery analysis, just

as population structure is considered a basic element of conservation biology (Thorpe et al., 1995). Stock identification methods have developed in parallel with the advancement of morphometric techniques. The earliest analyses of morphometric variables for stock identification were univariate comparisons, but these were soon followed by bivariate analyses of relative growth, to detect ontogenetic changes and geographic variation among fish stocks. As the field of multivariate morphometrics increased, a set of multivariate methods was applied to quantify variation in growth and form among stocks (Cadrin, 2000).

More recent advances have been facilitated by image processing techniques, more comprehensive and precise data collection, more efficient quantification of shape, and new analytical tools, landmark-based techniques of geometric morphometrics (Bookstein, 1991). These techniques pose no restrictions on the directions of variation and localization of changes in shape; and furthermore, they are very effective in capturing information about the shape of an organism (Cavalcanti, Monteiro, & Lopez, 1999). Image analysis systems played a major role in the development of morphometric techniques, boosting the utility of morphometric research in fish stock identification (Cadrin & Friedland, 1999). Morphometry based on truss network data has been used for stock identification (Sajina, Chakraborty, Jaiswar, Pazhayamadam, & Sudheesan, 2011; Mir et al., 2013a, b & c), ontogeny (Hard, Winans & Richardson, 1999) and functional morphology (Dean, Huber, & Nance, 2006).

The stock structure analysis of different fish species by employing truss network system has been done in India recently (Khan, Miyan, & Khan, 2012; Mir et al., 2013a, b and c), but there is no information regarding the stock structure of Sattar snowtrout. The present study was therefore taken as a step with the aim to explore stock structure of this species based on morphometric characteristics using the truss network system for its successful development and management in Kashmir Himalaya.

MATERIALS AND METHODS

Study area: The Indian Himalayas are drained by 19 main rivers among which three major river system i.e., Ganga, Indus and Brahmaputra are the largest. The Indus Basin system is the longest river system that starts from Western Himalaya (160 000km^2) and consist of five rivers (Hora, 1954). River Jhelum originates from Verinag Spring situated at the Southeastern part of Kashmir valley in India, is the tributary of Indus basin and flows in Western Himalayan region of India; it has a total length of about 813km. The Lidder River is the second largest tributary of river Jhelum and is a 73km long river in the Kashmir region of India; its source (Kolhoi Glacier) is located at a height of 4 653masl. These constitute the two least explored rivers of India. Furthermore, the Dal Lake has been an important fishery resource to the people of Kashmir valley since ancient times (Shafi & Yousuf, 2012); it is a shallow open drainage type water body spread over an area of 11.4km^2 (Fig. 1).

Sampling and digitization: A total of 506 *S. curvifrons* samples were collected from two different rivers including 138 specimens from River Jhelum at Baramulla (2 650masl; 34°20' N - 74°35' E) with a total length range of 12.5-49.5cm, and 217 specimens from River Lidder at Pahalgam (2 740masl; 34°02' N - 75°20' E) having length range of 10.8 - 51.2cm, and 151 specimens from Dal Lake at Hazratbal (1 584masl; 34°07' N - 74°52' E) in a length range of 18.5-52.6cm from Kashmir valley, during January 2011 to October 2012 and analyzed for morphometric variations.

For digitization, the sampled specimens were first cleaned with running water, drained and placed on a flat platform with graph paper as a back ground, which was used for calibrating the coordinates of digital images. The fins were erected and placed on the platform to make the origin and insertion points visible. Each individual was labeled with a specific code to identify it. A Cyber shot DSC-W300
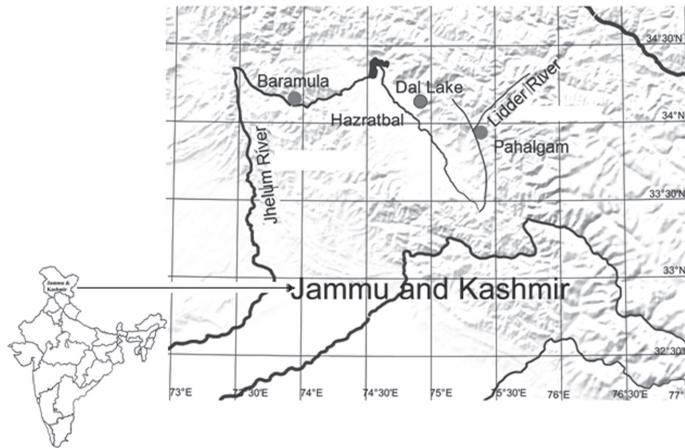
Fig. 1. Sampling locations of *Schizothorax curvifrons* on different rivers of Ganga basin.

digital camera (Sony, Japan) was used for capturing the digital images.

Digital records of specimens provide a complete archive of body shape and allow the opportunity for making repeated measurements if required (Cadrin & Friedland, 1999). After image capture, each fish was dissected to identify the sex of the specimen by microscopic examination of the gonads. Gender was used as the class variable in ANOVA to test for the significant differences in the morphometric characters, if any, between males and females of *S. curvifrons*.

The truss protocol used for the *S. curvifrons* in the present study was based on 14 landmarks, and the truss network was constructed by interconnecting them to form a total of 31 measurements (Fig. 2). The extraction of truss distances from the digital images of specimens was conducted using a linear combination of three software platforms, tpsUtil, tpsDig2v2.1 (Rohlf, 2006) and Paleontological Statistics (PAST) (Hammer, Harper, & Ryan, 2001). A box truss of 31 lines connecting these landmarks was generated for each fish to represent the basic shape of the fish (Strauss &
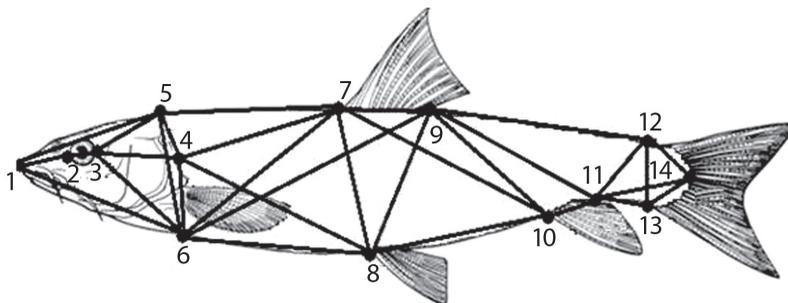

Fig. 2. Schematic image of *Schizothorax curvifrons* depicting the 14 landmarks and associated box truss used to infer morphological differences among populations. 1 Tip of snout; 2 end of eye towards mouth; 3 end of eye towards tail; 4 end of operculum; 5 forehead (end of frontal bone); 6 dorsal origin of pectoral fin; 7 origin of dorsal fin; 8 origin of pelvic fin; 9 termination of dorsal fin; 10 origin of anal fin; 11 termination of anal fin; 12 dorsal side of caudal peduncle, at the nadir; 13 ventral side of caudal peduncle, at the nadir; 14 end of lateral line (Adapted from truss box, after Strauss & Bookstein 1982 and Bookstein 1991).

Bookstein, 1982). All the measurements were transferred to a spreadsheet file (Excel 2007), and the X-Y coordinate data transformed into linear distances by computer (using the Pythagorean Theorem) for subsequent analysis.

Size dependent variation was corrected by adapting an allometric method as suggested by Elliott, Haskard and Koslow (1995).

$$M_{adj} = M \ (L_s/L_0)^b$$

Where M is original measurement, M_{adj} is the size adjusted measurement, L_0 is the standard length of the fish, L_s the overall mean of standard length for all fish from all samples in each analysis, and *b* was estimated for each character from the observed data as the slope of the regression of log *M* on log L_0 using all fish from every group. The results derived from the allometric method were confirmed by testing significance of the correlation between transformed variables and standard length (Turan, 1999). Univariate analysis of variance (ANOVA) was performed for 31 morphometric characters to evaluate the significant difference among the three locations. In the present study, linear discriminant function analyses (DFA), principal component analysis (PCA) and factor analysis (FA) were employed to discriminate the three populations. Principal component analysis helps in morphometric data reduction (Veasey, Schammass, Vencovsky, Martins, & Bandel, 2001), in decreasing the redundancy among the variables (Samaee, Mojazi-Amiri, & Hosseini-Mazinani, 2006) and in extracting a number of independent variables for population differentiation (Samaee, Patzner, & Mansour, 2009). The Wilks' λ was used to compare the difference between all groups. The DFA was used to calculate the percentage of correctly classified (PCC) fish. A cross-validation using PCC was done to estimate the expected actual error rates of the classification functions. Statistical analyses for morphometric data were performed using the SPSS version 16.1.0 software package and Excel (Microsoft Office 2007).

RESULTS

None of the standardized truss measurements showed a significant correlation with the standard length of the fish, indicating that the effect of the body length had been successfully removed by the allometric transformation. Among three populations, mean values of all the truss measurements of *S. curvifrons* were found to be significantly (p<0.001) different in univariate analysis of variance. The truss characters between two sexes did not differ significantly (p>0.05) hence; the data for both sexes were pooled for all subsequent analysis.

By applying principal component analysis on 31 truss measurements four factors with eigenvalue >1 were extracted, explaining 80.68% of the variance. The first principal component (PC1) accounted for 63.44% of the variation, second (PC2), third (PC3) and fourth (PC4) for 8.35, 5.31 and 3.58, respectively (Table 1), the most significant loadings on PC1 were 1-5, 1-6, 2-3, 3-4, 3-5, 3-6, 4-5, 4-7, 4-8, 5-6, 5-7, 6-7, 6-8, 6-9, 7-8, 7-9, 7-10, 8-9, 8-10, 9-10, 9-11, 9-12, 10-11, 11-12, 11-13, 11-14, 12-14 and 13-14 (Table 1). In this analysis, the characteristics with an eigen values exceeding 1 were included and others discarded.

The Wilks' λ tests of discriminant analysis indicated significant differences in morphometric characters of all the populations, both the two functions showed highly significant differences (p<0.001).

The DFA revealed two morphological indices describing 78.6 and 21.4% of the morphological variation among the populations (Table 2). The truss distances with meaningful loading on first factor (DF1) were 1-6, 2-3, 3-4, 3-5, 3-6, 4-5, 4-6, 4-7, 4-8, 5-6, 5-7, 6-7, 6-8, 7-8, 7-9, 7-10, 8-9, 8-10, 9-10, 9-11, 9-12, 10-11, 11-12, 11-13, 11-14, 12-13, 12-14 and 13-14, this factor explained 78.6% of the total variance. All these 28 distances characterize the measurements covering the fish transversely as well as longitudinally. The second factor (DF2) explained 21.4% of total variation, and the meaningful truss distance loadings on this factor were 1-2, 1-5 and 6-9, these loadings

<table>
<thead>
<tr><th rowspan="2">Truss measurement</th><th colspan="4">Principal Components</th></tr>
<tr><th>PC1 (63.4%)</th><th>PC2 (8.3%)</th><th>PC3 (5.3%)</th><th>PC4 (3.5%)</th></tr>
</thead>
<tbody>
<tr><td>1-2</td><td>-.748</td><td>-.597</td><td>.656</td><td></td></tr>
<tr><td>1-5</td><td>-.801</td><td></td><td>.436</td><td></td></tr>
<tr><td>1-6</td><td>-.940</td><td></td><td></td><td></td></tr>
<tr><td>2-3</td><td>.897</td><td></td><td></td><td></td></tr>
<tr><td>3-4</td><td>.982</td><td></td><td></td><td></td></tr>
<tr><td>3-5</td><td>-.945</td><td></td><td></td><td></td></tr>
<tr><td>3-6</td><td>-.959</td><td></td><td></td><td></td></tr>
<tr><td>4-5</td><td>.933</td><td></td><td></td><td></td></tr>
<tr><td>4-6</td><td>.856</td><td>.614</td><td>.441</td><td></td></tr>
<tr><td>4-7</td><td>-.974</td><td></td><td></td><td></td></tr>
<tr><td>4-8</td><td>-.926</td><td></td><td></td><td></td></tr>
<tr><td>5-6</td><td>-.641</td><td>.615</td><td></td><td></td></tr>
<tr><td>5-7</td><td>.858</td><td></td><td></td><td></td></tr>
<tr><td>6-7</td><td>.641</td><td>.534</td><td></td><td></td></tr>
<tr><td>6-8</td><td>.901</td><td></td><td></td><td></td></tr>
<tr><td>6-9</td><td>-.884</td><td></td><td></td><td></td></tr>
<tr><td>7-8</td><td>-.800</td><td></td><td></td><td></td></tr>
<tr><td>7-9</td><td>.946</td><td></td><td></td><td></td></tr>
<tr><td>7-10</td><td>-.737</td><td>.487</td><td></td><td></td></tr>
<tr><td>8-9</td><td>.555</td><td>.479</td><td></td><td></td></tr>
<tr><td>8-10</td><td>.924</td><td></td><td></td><td></td></tr>
<tr><td>9-10</td><td>.975</td><td></td><td></td><td></td></tr>
<tr><td>9-11</td><td>.500</td><td></td><td></td><td>.613</td></tr>
<tr><td>9-12</td><td>-.828</td><td></td><td></td><td></td></tr>
<tr><td>10-11</td><td>.927</td><td></td><td></td><td></td></tr>
<tr><td>11-12</td><td>.717</td><td></td><td></td><td></td></tr>
<tr><td>11-13</td><td>.953</td><td></td><td></td><td></td></tr>
<tr><td>11-14</td><td>-.979</td><td></td><td></td><td></td></tr>
<tr><td>12-13</td><td>.332</td><td></td><td></td><td>.648</td></tr>
<tr><td>12-14</td><td>.900</td><td></td><td></td><td></td></tr>
<tr><td>13-14</td><td>.591</td><td></td><td></td><td></td></tr>
</tbody>
</table>

TABLE 1

Results of factors extraction in principal component analysis after Varimax normalized rotation

TABLE 2

Contribution of morphometric measurements to discriminant functions of *Schizothorax curvifrons* collected from Kashmir Himalaya

Truss measurement	Function	
	DF1 (78.6%)	DF2 (21.4%)
1-2	.147	.179(*)
1-5	-.188	.215(*)
1-6	-.326(*)	.165
2-3	.545(*)	-.134
3-4	.500(*)	-.146
3-5	-.324(*)	.132
3-6	-.369(*)	.115
4-5	.604(*)	-.175
4-6	.440(*)	-.188
4-7	-.354(*)	.148
4-8	-.333(*)	.080
5-6	.071(*)	.039
5-7	.549(*)	-.274
6-7	.592(*)	-.182
6-8	.518(*)	-.129
6-9	-.173	.187(*)
7-8	-.224(*)	-.017
7-9	.612(*)	-.185
7-10	-.153(*)	.139
8-9	.505(*)	-.195
8-10	.497(*)	-.307
9-10	.484(*)	-.085
9-11	.591(*)	-.234
9-12	-.397(*)	-.240
10-11	.597(*)	-.265
11-12	.615(*)	-.267
11-13	.571(*)	-.083
11-14	-.392(*)	.129
12-13	.214(*)	-.057
12-14	.545(*)	.017
13-14	.513(*)	.071

were mostly restricted to head and middle region of fish. The DF1 *vs* DF2 plot explained 100% of total variance among the samples and showed clear separation between riverine and lake populations, however slight inter-mingling was observed between the riverine stocks (Fig. 3). Discriminant function analysis showed 83.4% correct classification of individuals into their original populations and the cross-validation test results were comparable to the results obtained from PCC. The percentage of correctly classified fishes was highest in all three populations. The slight intermingling was observed between riverine stocks (Table 3).

DISCUSSION

The results obtained from the truss-based morphometrics indicated that the *S. curvifrons* showed significant phenotypic heterogeneity
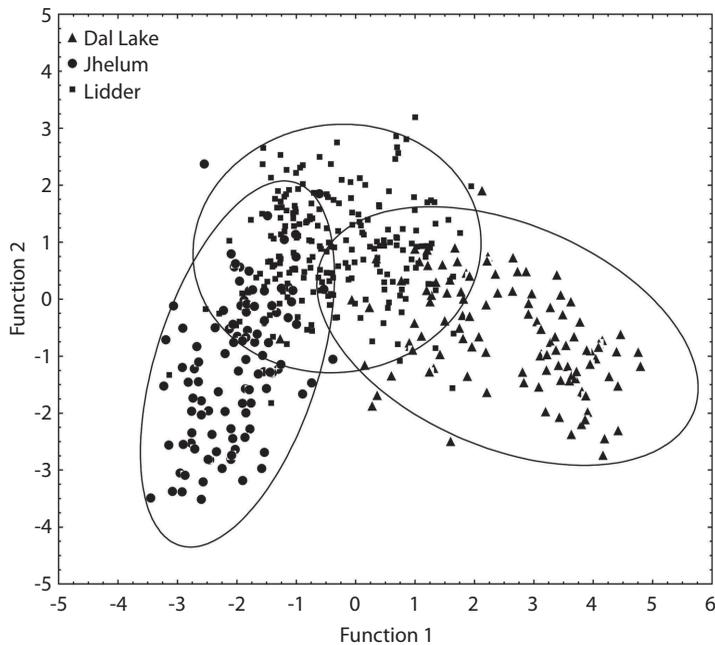
Fig. 3. Discriminant analysis plot where 31 morphometric variables were used for *Schizothorax curvifrons* from Kashmir Himalaya.

TABLE 3

Percentage of specimens classified in each group and after cross validation for morphometric measurements for *Schizothorax curvifrons* from Kashmir valley (83.4% of original grouped cases correctly classified, 81.1% of cross-validated grouped cases correctly classified)

			Predicted Group Membership			Total
			Dal lake	Jhelum	Lidder	
Original	%	Dal lake	83.6	3.6	12.7	100.0
		Jhelum	.0	81.3	18.7	100.0
		Lidder	3.7	12.0	84.3	100.0
Cross validated	%	Dal lake	80.0	4.5	15.5	100.0
		Jhelum	.0	79.4	20.6	100.0
		Lidder	4.6	12.9	82.5	100.0

among three populations and limited overlapping between two rivers. Discriminant function analysis (DFA) could be a useful method to distinguish different stocks of the same species (Karakousis, Triantaphyllidis, & Economidis, 1991). In the present investigation, 83.4% of individuals were correctly classified into their respective groups by DFA, indicating slight intermingling among the populations. Turan, Erguden, Gurlek, Basusta, and Turan (2004) studied the anchovy (*Engraulis encrasicolus*) from different areas of the Mediterranean Sea, and found significant morphometric heterogeneity among different populations by applying DFA and this to the migration of the fish. The DFA segregation was partly confirmed by PCA, where the graphs of PC1 and PC2 scores for each sample revealed that, among three populations slight overlapping was observed between two rivers, whereas, the population of Dal lake was distinct. The rivers Jhelum and Lidder are tributaries of the River Chenab (a

large tributary of the Indus), and the morphological discreteness among these rivers may be due to the selection of the sampling sites. Dal Lake is a lentic water body with profuse human interruption. Morphological parameters of *S. curvifrons* between two rivers may be attributed to the small geographic distances between these drainages and their similar environmental conditions. Such indications of stock structure arise from consideration of the first and second factors. This analysis confirmed that the variation in morphological measurements was evident in the head region, eye diameter, body depth and caudal peduncle, among different populations of *S. curvifrons*. Similar results were obtained by Mir et al. (2013b & c) in *Schizothorax esocinus* and *Schizopyge niger* from Kashmir Himalaya.

The variation among the stocks of three populations could be a consequence of phenotypic plasticity in response to uncommon hydrological conditions such as differences in alkalinity, current pattern, temperatures, turbidity, and land-use patterns among these drainages. The closeness between stocks may be due to their similar habitat attributes and to environmental impacts (Mir et al., 2013b & c). These results are similar to the findings of Boussou et al. (2010) in the *Chromidotilapia guntheri* from three coastal rivers of Africa where environmentally-induced morphological differences were found among the tributaries of the Tanoe River, which were geographically close to each other. The phenotypic variability may not necessarily reflect population differentiation at the molecular level (Ihssen et al., 1981). Apparently the fragmentation of river impounds can lead to an enhancement of pre-existing genetic differences, providing a high interpopulation structuring (Esguicero & Arcifa, 2010). Thus, there is the possibility that the observed morphological variations in the present study might be due to genetic differences among the populations.

The truss system can be successfully used to investigate stock separation within a species, as reported for other species in freshwater and marine environments. In this study, the truss protocol revealed a clear separation of *Schizothorax curvifrons* stocks observed in three water bodies of Kashmir Himalaya, suggesting a need for separate management strategies in order to sustain the stocks for future use. The observations in the present study can further be confirmed based on molecular and biochemical methods, which would provide further confirmation of the stock structure resolved in this study with the truss analysis. However, based on this morphometric study, development of proper guidelines for the implementation of appropriate mesh sizes in water bodies of Kashmir Himalaya may help in sustaining this resource for future use and it is suggested that morphological variations observed in this species should be considered in fisheries management and commercial exploitation of this species and any stock enhancement programme.

RESUMEN

Schizothorax curvifrons es un alimento para peces, morfométricamente y merísticamente más variable y de mayor valor económico del Valle de Kashmir. Dado que existe una falta de información sobre la estructura de las poblaciones silvestres de esta especie, este estudio tuvo como objetivo investigar la variación intraespecífica de esta importante *trucha de nieve*. Para ello, se tomaron muestras de dos ríos y un lago en Kashmir Himalaya, de enero 2011 a octubre 2012. Con las mediciones de los peces se construyó una *red truss*. En total, se recolectaron 506 muestras de peces. Los datos se sometieron a análisis de componentes principales, análisis de función discriminante y el análisis univariante de la varianza. El primer componente principal explicó 63.44% de la variación total, mientras que los componentes segundo y tercero explicaron el 8.34% y 5.31%, respectivamente. El análisis discriminante por etapas, retuvo dos variables que discriminaron significativamente las poblaciones. El uso de estas variables de 83.4% de los especímenes originales se clasificaron en sus grupos correctos y 81.1% de la validación cruzada (deja fuera un procedimiento) especímenes fueron

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clasificados en sus grupos correctos. Todas las mediciones para entramados transformados, del total de 31, mostraron diferencias altamente significativas (p<0.001) entre las poblaciones. Esto representa el primer intento de obtener la estructura de la población de *S. curvifrons*, por lo tanto, este estudio espera orientar a los taxonomistas de peces acerca de la estructura de la población actual y ayudar en su manejo y poder establecer un programa de conservación en el área de Kashmir Himalaya.

Palabras clave: morfometría truss, *Schizothorax curvifrons*, Himalaya, Kashmir, India.

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