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Detection of morphometric differentiation among *Schizothorax* species complex (Teleostei: Cyprinidae) in Kashmir Himalayas (India) and their taxonomic status.

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ABSTRACT

Schizothorax are the most commercially important food fish in Kashmir Himalayas. The present study focused on the morphometric variations and taxonomic relations of five Schizothorax species namely, S. niger, S. curvifrons, S. esocinus, S. labiatus and S. plagiostomus collected from Jhelum river and Dal lake of Kashmir. The Bray-curtis similarity index showed higher similarity between S. labiatus & S. plagiostomus (0.983) and S. niger & S. curvifrons (0.9827). However S. esocinus shows least similarity with the other species. The Principal component analysis revealed five discriminating variables viz, max depth, head length, caudal fin length, caudal peduncle length and pectoral - pelvic length that promised 98% accuracy in classification of Schizothorax species. The present study confirms the specific status of the five species and revealed that morphological measurements may be taken into consideration by researchers and also by farmers to identify these Schizothorax species. The results can prove effective for the management and sustainable exploitation of the genetic resources of the five species.

Key words:, ***Scizothorax, Principle component analysis, Similarity index, Cluster analysis***

I.INTRODUCTION

The *Schizothorax* Heckel is one of the largest and most diverse group of fish in Asian waters. They are highly specialized for high elevation rivers and shows number of adaptations for surviving in the torrential mountain streams and plateau lakes of central Asia, Himalayas and Tibetan plateau. This genus is represented in Kashmir Himalaya by a number of species. The most common species include *S. niger, S. curvifrons, S. esocinus, S. labiatus* and *S.plagiostomus*. Their taxonomy has been studied by many workers especially by Heckel (1838), Mcclelland (1839), Hora (1936), Silas (1960), Talwar and Jhingran (1991), Kullander *et al*. (1999). However, still there is difference of opinion regarding the number of species of this genera due to several overlapping morphological characters. Heckel (1838) described ten species from Kashmir waters into newly created genera *Schizothorax*. Misra (1962) described eleven species in to a new genus *Schizothoraichthys* which were already

included under genus *Schizothorax* by Heckel. Later Talwar and Jhingran (1991) included two and Jayram (1999) four species in the *Schizothorax* Heckel (Krishanan and Tarana, 2010). However, still there is confusion regarding the exact number of species occurring in different aquatic habitats of the valley of Kashmir which has hindered many studies on this species complex. The hybrids of some of these species have further complicated their taxonomical and evolutionary concepts (Hora, 1936).

Investigations on morphometric variation among different populations and species may provide a basis for stock structure and may be applicable for studying short-term, environmentally induced variation that is necessary towards successful fisheries management (Murta, 2002; Pinheiro *et al*., 2005). The assessment of morphometric variations has also been useful to identify differences among fish populations (Cheng *et al.,* 2005; Kara and Bayhan, 2008**)**. Morphometrics describes complex shapes in a rigorous fashion and permits numerical comparison between different forms (Bookstein, 1991) and when combined with multivariate statistical methods viz. principal component analysis, cluster analysis etc) they offers powerful tool for testing and displaying differences in shape (Loy *et al*., 1993; Rohlf, 1996). The use of multivariate techniques such as principal components and discriminant analyses to quantify morphometric variables is receiving increased world-wide attention in fish stock and species identification (Kusznierz *et al.,* 2008; Bektas and Belduz, 2009; Specziar *et al*., 2009, Anvari Far *et al.,* 2011, Choudhury and Dutta, 2012).

It appears relevant database is not available on assessment of morphometric differentiation of *Schizothorax* species of the study area especially using multivariate mathematical approach. The present study therefore, aimed to examine the morphometric variations among the selected *Schizothorax* species. The study provides data-base for the identification, rational management, breeding, conservation and to reveal the taxonomic relationships of the collected *Schizothorax* species of the study area.

II.MATERIALS AND METHODS

River Jhelum, the tributary of the Indus River, flows in the Western Himalayan region of India with a total length of about 813 km. It originates from Verinag Spring in the southeastern part of Kashmir in India. The Dal Lake, a shallow open drainage type water body spread over an area of 11.4 km^2 , is an important fishery resource to the people of the Kashmir valley since ancient times. Specimens were collected from commercial catches from four different collection sites of River Jhelum and Dal lake in Kashmir valley. Table 1 depicts the details of collection sites. The collected species (*S. niger, S. curvifrons, S. esocinus, S. labiatus* and *S. plagiostomus)* were identified following Talwar and Jhingran, (1991), Jay ram (1999) and Day (1878). Fourteen measurements were taken from the lateral side of the fish on a continuous scale using vernier caliper. All lengths were taken parallel to the anterior-posterior body axis except for the body depth and Caudal peduncle depth that were taken perpendicular to the body axis (Manimegalai *et al.,* 2010). The sex of the individuals were not taken in consideration during analysis of data. The mean, standard deviation and standard error of the data for each species were calculated and used for further analysis (Table 2). All measurements have been normalized in order to correct relative differences in size (Table 3). The similarities among the species were calculated by Bray-Curtis similarity index. Principal components analysis was applied for morphometric data reduction (Veasey *et al.,* 2001), for extracting a number of independent variables for population differentiation (Samaee *et al.,* 2009)

and in decreasing the redundancy among the variables (Samaee *et al.*, 2006). A multivariate cluster analysis according to ward's method was also conducted using the normalized morphometric data. Confusion matrix was used to access the accuracy of classification. Statistical analyses were performed using the XLSTAT 2014 software package, PAST and Origin Pro software.

III.RESULTS

Table 2 depicts descriptive data for the mean, standard deviation and standard error of selected fourteen morphological lengths of collected specimens of the study area and their normalized data is shown in Table 3. According to the Bray-Curtis similarity index (Table 4) the similarity was found highest between *S. labiatus* and *S. plagiostomus* (0.9830) followed by *S. niger* and *S. curvifrons* (0.9827) and the least similarity was found between *S. esocinus* and *S. curvifrons* (0.912) followed by *S. esocinus* and *S. labiatus* (0.919). The similarity index between all the five species was >0.91 indicating close relationship among the species. The cluster analysis dendogram constructed from the normalized data of all selected morphometric parameters showed the relationship among these species (fig 1). The dendogram clustered *S. niger* and *S. curvifrons* in to one group and *S. labiatus* and *S. plagiostomus* in to second group and *S. esocinus* forms a separate cluster. Confusion matrix revealed a total of 98% accuracy for the morphometric characters indicating proper classification of individuals in to their original taxa. The percent accuracy obtained from cross-validation test showed 92% accuracy (Table 5).

In order to determine the morphometric measurements that most effectively differentiates the species, the contributions of variables to principal components (PC) were examined. Principal component analysis of 14 morphometric measurements extracted three factors with eigenvalues >1, explaining 98.37% of the variance (Table 7). The first principal component (PC1) accounted for 59.08% of the variation and the second principal component (PC2) for 29.55% (Table 7), and the most significant loadings on PC1 were 5 and 10 and on PC2 were 3, 9 and 12 as shown in Table 6.

The plot of PC1 versus PC2 scores revealed that collected specimens of the study area were grouped into five different areas, but with a some degree of overlapping between *S. niger* and S. *curvifrons, S. curvifrons* and *S. labiatus, S. labiatus* and *S. plagiostomus.* However no overlapping was found with *S. esocinus* in terms of morphometric characters (Fig 2).

IV.DISCUSSION

Morphometric assessments have been widely and successfully applied for both taxonomic purposes of fish and to appraise their biodiversity (Yakubu and Okunsebor 2011, Kohestan-Eskandari *et al.*, 2013). *Schizothorax* species are identified conventionally on morphological and meristic characters, relying mainly on pigmentation pattern, skin coloration and meristic counts. However these approaches cannot be used to establish the taxonomic relationship among the species. Thus in systematics, variations in morphometric characters are used to identify species and highlight their phylogenetic relationships (Clabaut *et al.*, 2007). This study provided a useful tool for the identification and assessment of stock status of *Schizothorax* species by using only five

morphometric characters (variables with high discriminating power) that are easy-to-operate with and are easily accessible in field work.

The five discriminating variables max depth, head length, caudal fin length, caudal peduncle length and pectoral - pelvic length promised 98% accuracy in classification of *Schizothorax* species. Cross-validation with confusion matrix method also indicated more than 92% overall success rate of the classification (table 5).

The present investigation revealed the relationships among the five *Schizothorax* species and has grouped them into clusters on the basis of their morphometric variations. *S. niger* and *S. curvifrons* are put in to one group indicating *S. niger* and *S. curvifrons* are closely related to each other. Some experts have separated these two species in to a new genus *Schizopyge* (Khan and Sabah, 2013). *S. labiatus* and *S. plagiostomus* form a second group and *S. esocinus* makes a separate cluster . *S esocinus* seems to be phylogenetically distant from the other four species as it showed least similarity with other species (Table 4). *S. esocinus* also differs morphologically from the other species due to its attainment of large size, light ground colour and contrasting black spots and a unique shape of mouth. Silas (1960) considered two species, *S. niger* and *S. curvifrons*, as two varieties or sub species of *s. niger*. The plot of PC1 versus PC2 scores revealed that specimens were grouped into five different areas (Fig 2), thus confirming the specific status of five *Schizothorax* species on the basis of morphometric characteristics. The present study revealed that the two species *S. niger* and *S. curvifrons* can be treated as two distinct species. Yakubu *et al*., (2011) correctly classified overall 98.0% of *Oreochromis niloticus* Linnaeus and 97.0% of *Lates niloticus* Linnaeus individuals on the basis of three strong discriminating variables. Choudhary *et al*., (2011) reported the similarity index and relationship of six species of genus *Puntius*. The five discriminating variables obtained from the study might not be so exhaustive but may be the indicative of first hand classification. The present study is supported by the biochemical genetic and karyomorphological studies of the five *Schizothorax* species (Ganai *et al.*, 2014). The investigation is under progress to generate more evidences in terms of molecular markers that will consolidate the information obtained from morphometric variability.

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Tables

Table 1. Collection sites of the five *Schizothorax* species

Species	Collection site	Latitude/Longitude
S. niger	Dal lake, Srinagar	$34^0$08´05.58´´N/$74^0$50´29.75´´E
S. curvifrons	River Jhelum , srinagar	$34^0$05´34.70´´N/$74^0$46´46.35´´E
S. esocinus	Dal lake, Srinagar	$34^0$08´05.58´´N/$74^0$50´29.75´´E
S. labiatus	River Jhelum Kakapura	$33^0$57´06.75´´N/$74^0$55´41.33´´E
S. plagiostomus	River Jhelum, srinagar	$34^0$05´34.70´´N/$74^0$46´46.35´´E

Table 2 Stastical analysis of the morphometric measurements

Species	*S.plagiostomus*			*S. niger*			*S. curvifrons*			*S. labiatus*			*S. esocinus*		
Morphometric Variables	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
Total length (1)	31.5	5.45	1.72	32.5	8.14	2.57	30.32	7.24	2.56	30.78	6.57	2.07	37.5	7.72	2.44
Standard length (2)	26.8	5.33	1.65	27.9	7.23	2.28	26.2	6.48	2.29	26.12	6.12	1.93	32.93	7.10	2.24
Max depth (3)	5.34	0.40	0.12	7.04	1.64	0.52	5.8	0.83	0.29	5.54	0.84	0.26	6.35	1.20	0.38
Preanal length (4)	19.8	5.00	1.58	22.2	5.50	1.74	19.85	6.45	2.28	19.92	4.71	1.49	25.98	6.78	2.14
Head length (5)	5.48	0.60	0.19	6.11	1.39	0.44	5.26	1.49	0.52	5.47	1.02	0.32	9.13	1.88	0.59
Predorsal length (6)	12.4	2.22	0.70	15.1	3.85	1.21	13.36	3.13	1.10	12.82	3.00	0.94	16.83	3.34	1.05
Postdorsal length(7)	14.3	3.16	1.00	12.4	4.04	1.27	13.12	3.14	1.11	13.19	3.09	0.97	15.65	3.85	1.21
Caudal peduncle depth (8)	2.92	0.59	0.18	2.79	0.67	0.21	2.51	0.86	0.30	2.9	0.60	0.19	3.48	0.71	0.22
Caudal peduncle length (9)	5.05	0.67	0.21	4.16	1.02	0.32	4.68	0.65	0.23	4.74	0.95	0.30	5.06	0.54	0.17
Pectoral - Pelvic length (10)	8	1.52	0.48	8.48	2.29	0.72	8.71	1.58	0.55	8.85	0.84	0.26	8.63	1.19	0.37
Pelvic - Anal length (11)	7.1	1.24	0.39	7.58	2.08	0.65	6.91	1.58	0.56	7.14	0.70	0.22	7.38	1.12	0.35
Caudal fin length(12)	5.3	0.68	0.21	4.58	1.79	0.56	5.03	0.79	0.28	5.3	0.65	0.20	5.11	0.49	0.15
Pectoral fin length (13)	4.4	0.60	0.19	4.13	0.94	0.30	4.05	0.97	0.34	4.34	0.53	0.16	4.39	0.59	0.18
Pelvic fin length (14)	3.8	0.52	0.16	3.73	0.90	0.28	3.7	0.94	0.33	3.67	0.62	0.19	4.03	0.50	0.15

SD (Standard deviation), SE (Standard error)

Table 3. Normalization data of fourteen morphometric characters

	S.Plagiostomus	*S.niger*	*S.curvifrons*	*S.labiatus*	*S.esocinus*
1	0.96863	0.99938	0.93235	0.94649	1.15314
2	0.95893	0.99643	0.93571	0.93286	1.17607
3	0.88793	1.1706	0.96442	0.92118	1.05587
4	0.91879	1.03016	0.92111	0.92436	1.20557
5	0.87122	0.97138	0.83625	0.86963	1.45151
6	0.88281	1.06986	0.94658	0.90832	1.19243
7	1.04655	0.90182	0.95418	0.95927	1.13818
8	1	0.95548	0.85959	0.99315	1.19178
9	1.06585	0.87801	0.98776	1.00042	1.06796
10	0.93743	0.99367	1.02062	1.03703	1.01125
11	0.98644	1.0487	0.956	0.98783	1.02103
12	1.05906	0.90157	0.99016	1.04331	1.00591
13	1.03238	0.96903	0.95026	1.0183	1.03003
14	1.02052	0.98106	0.97317	0.96528	1.05997

Table 4. Bray-Curtis similarity index of the *Schizothorax* species

	S.Plagiostomus	*S.niger*	*S.curvifrons*	*S.labiatus*	*S.esocinus*
S.Plagiostomus	1				
S.niger	0.94527	1			
S.curvifrons	0.96799	0.98265	1		
S.labiatus	0.98306	0.95384	0.95592	1	
S.esocinus	0.92397	0.92648	0.91199	0.9187	1

Table 5 Confusion matrix summarizing the reclassification of observations.

from \ to	*S. esocinus (%)*	*S. labiatus(%)*	*S. niger(%)*	*S. plagiostomus(%)*	*S.curvifrons(%)*
Confusion matrix for the estimation sample:					
S. esocinus	100.00	0	0	0	0
S. labiatus	0	90.00	0	10.00	0
S. niger	0	0	100.00	0	0
S. plagiostomus	0	0	0	100.00	0

S.curvifrons	0	0	0	0	100.00

Total Correct 98%

Confusion matrix for the cross-validation results:

S. esocinus	100.00	0	0	0	0
S. labiatus	0	90.00	0	10.00	0
S. niger	0	0	90.00	0	10.00
S. plagiostomus	0	0	0	100.00	0
S.curvifrons	0	20.00	0	0	80.00

Total Correct 92%

Table 6. Results of factors extraction in PCA after Varimax normalized rotation

	Factor 1	Factor 2
1	-0.2928	-0.1044
2	-0.4103	-0.1873
3	0.78016	-2.1864
4	-0.6437	-0.6159
5	-2.5336	-0.1998
6	-0.2959	-1.3293
7	-0.2341	1.08178
8	-0.9672	0.88074
9	0.4194	1.3534
10	1.41705	-0.3429
11	0.75623	-0.4061
12	0.95068	1.22462
13	0.60201	0.61808
14	0.45197	0.21344

Table 7. Eigenvalues, percentage of variance and percentage of cumulative variance for Principal components in case of morphometric measurements for *Schizothorax*

Factor	Eigenvalues	Percentage of variance	Percentage of Cumulative variance
PC1	14.56	59.082	59.08
PC2	7.28	29.556	88.64
PC3	2.39	9.731	98.37
PC4	0.4	1.631	100

Figures

Fig. 1 Multivariate cluster analysis of five *Schizothorax* species based upon their morphometrics

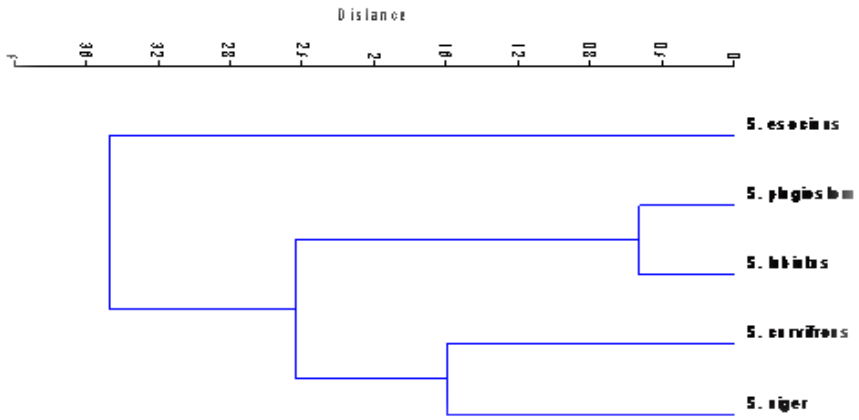

Fig. 2 Chart representing the observations on the factor axes extracted from the morphometric variables.