Phylogeny Based on Truss Analysis in Five Populations of Freshwater Catfish: *Clupisoma Garua*

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Abstract: ***The experiment was conducted by observing the 13 landmark-based morphological variations to evaluate the population status of five different stocks of freshwater catfish Clupisoma garua. A total of 91 C. garua were collected during August 2013 to June 2014. On the basis of truss analysis univariate analysis of variance showed all significant morphometric measurements ((P<0.05) in five groups of C. garua out of 78 characters studied. In linear discriminant function analysis (DFA), the overall assignment of individuals into their original groups was 90.6%. The proportion of individuals correctly classified into their original groups was 89%. The principal component analysis (PCA) scatter plot of component scores between all populations showed 91 fish specimens grouped into five areas but with a relativity high degree of overlap between five populations appeared. The dendrogram showed three major clusters between Yamuna river, Betwa river and Gomti river Stocks. Son river and tones River were closely related. There was a high degree of variation in morphological characteristics among five different stocks of C.garua.***

Keywords: *C.garua*, Morphological variations, truss measurement, Discriminant Function Analysis.

1. Introduction

Many freshwater catfish species are currently threatened by direct and indirect influences of human activities such as habitat destruction and fragmentation (Yamamoto et al., 2006). Although there is evidence that Schilbid catfish *Clupisoma garua* may be threatened with overfishing in some areas (e.g. southern West Bengal), current evidence indicates that it is still a widespread and abundant species. It is therefore assessed at Least Concern here. However, given its widespread use as a food fish, close monitoring of harvest levels and its effects on population size is critically needed. Although Patra et al**.** (2005) report a mean decline of 30.4% in catch for this species in the Sundarbans (Ganges-Brahmaputra estuary) for the period 1960-2000 and Mishra et al**.** (2009) report a decline of 27.7% in catch for this species in southwestern Bengal for a similar period, there is insufficient data from other areas where this species is naturally distributed. However, indications are that this is still a relatively common and abundant species. This species is heavily utilized as a food fish, overfishing is a potential major threat. It is commonly known as Garua Bacha. Indeed, published data for parts of its natural distribution suggest that overfishing is a major cause of local population declines (Patra et al**.**, 2005; Mishra et al**.**, 2009). Other threats to this species are unknown, since there is no information on the biology of this species and therefore the impact of potential threats (especially those of an anthropogenic nature) remains unknown. The current threats to aquatic biodiversity in all of its known distribution have also not been adequately identified. Morphometric and meristic characters of fish are the measurable and countable characters, respectively common to all fishes. Landmarks refer to some arbitrarily selected points on a fish's body and with the help of these points, the individual fish body shape can be analyzed. In other words, a landmark is a point of correspondence on an object that matches between and within populations (Barlow W, 1961, Swain DP, 1999). Truss network systems constructed with the help of landmark points are powerful tools for stock identification. A sufficient degree of isolation may result in notable morphological, meristic and shape differentiation among stocks of a species which may be recognizable as a basis for identifying the stocks. The characteristics may be more applicable for studying shortterm, environmentally induced disparities and the findings can be effectively used for improved fisheries management (Ihssen PE. 1981, Templeman W. 1983, Smith PJ. 1986, Turan C. 2004). The present study deals with the population structure from a phenotypical point of view to determine the morphological characters among five stocks of *C.garua*.

2. Materials and methods

2.1 Sampling

The specimens of *C.garua* used for this study were randomly collected in period of one year during August 2013 to June 2014 from different places from their natural habitats. A total of 91 specimens were used to study the morphological variations. The 13 landmarks used to infer morphological differences among populations.1 Tip of snout; 2 center of eye;3 forehead (end of frontal bone); 4 end of operculum; 5 dorsal origin of pectoral fin; 6 origin of dorsal fin; 7 origin of pelvic fin; 8 termination of dorsal fin; 9 origin of anal fin; 10 termination of anal fin; 11 dorsal side of caudal peduncle, at the Nadir; 12 ventral side of caudal peduncle, at the nadir; 13 end of lateral line (Adapted from Strauss &Bookstein, 1982 and Bookstein, 1991).

2.2 Laboratory work

A total of 78 distance measurements between 13 landmarks were surveyed using the truss network system according to Bookstein (1991) and Strauss & Bookstein (1982) with minor modifications for this species. Truss network measurements are a series of measurements calculated between landmarks that form a regular pattern of connected quadrilaterals or cells across the body form (Turan, 1999). The fish were placed on a graph paper with dorsal and anal fins. The right body profile of each fish was photographed with digital camera. Images were saved in jpg format and analyzed with TPS dig (v.2.04; Rohlf, 2005) to coordinates of 13 landmarks. A box truss of 26 lines connecting these landmarks was generated for each fish to represent the basic shape of the fish (Cardin &Friedland, 1999). All 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 (Turan, 1999). ANOVA was used to test for the significant differences in the morphometric characters.

2.3 Data Analysis

Size dependent variation was corrected by adapting an allometric method as suggested by (Elliott et al., 1995)

Madj= M (Ls/Lo)b

where M is original measurement, Madj is the size adjusted measurement, Lo is the standard length of the fish, Ls 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 Lo using all fish from all five the groups. 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 each morphometric character to evaluate the significant difference between the five locations (Zar, 1984), and those morphometric characters which showed significant variations (P>0.05) In the present study, linear discriminant function analyses (DFA), principal component analysis (PCA) and cluster analysis (CA) were employed to discriminate the five populations. Principal component analysis helps in morphometric data reduction (Veasey et al., 2001), in decreasing the redundancy among the variables (Samaee *et al*.2006) and in extracting a number of independent variables for population differentiation (Samaee *et al*.2009). The Wilks' k 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 software package.

3. Result

The correlation between transformed morphometric variables and standard length was non-significant (P>0.05) which confirms that size or allometric signature on the basic morphological data was accounted for. Statistically significant differences among five populations were observed in 78 morphometric characters out of 78 studied. Of these 78 characters, almost characters are highly significant (P>0.05) and were used further for multivariate analysis. In order to determine which morphometric measurement most effectively differentiates populations, the contributions of variables to principal components (PC) were examined. A univariate analysis of thirteen morphometric characters shown that all morphometric measurements and truss measurements were significantly differed to varying degrees. In the present study, linear discriminant function analyses (DFA) are given in Table.2, principal component analysis (PCA) plot are shown in Fig.1 and cluster analysis (CA) means dendogram Fig.3 were employed to discriminate the five populations in *C. garua* . Anova of 78 variables which is generated by 13 landmark of fish species. Variables 1-2,1-3,1-4,1-5,1-6,1-7,1-8,1-9,1-10,1-11,1-12,1-13, similarly 2-13, 3-13,4-13,5-13,6-13,7-13,8-13,9-13,10-13,11-13,12-13. 1 Tip of snout; 2 center of eye; 3 forehead (end of frontal bone); 4 end of operculum; 5 dorsal origin of pectoral fin; 6 origin of dorsal fin; 7 origin of pelvic fin; 8 termination of dorsal fin; 9 origin of anal fin; 10 termination of anal fin; 11 dorsal side of caudal peduncle, at the Nadir; 12 ventral side of caudal peduncle, at the nadir; 13 end of lateral line. Eigen values of First 4 canonical discriminant functions were used in the analyses which are given in Table 1.

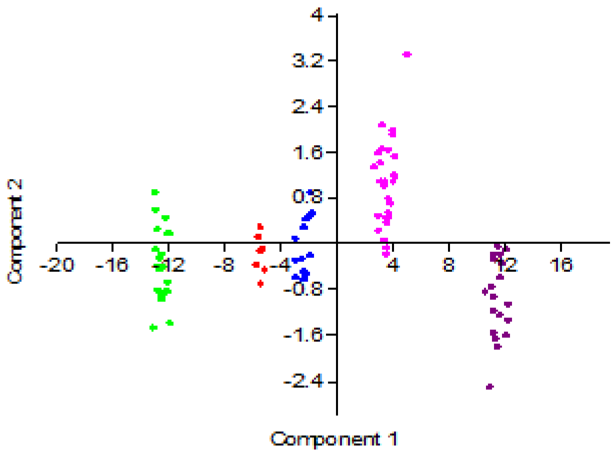

Figure 1

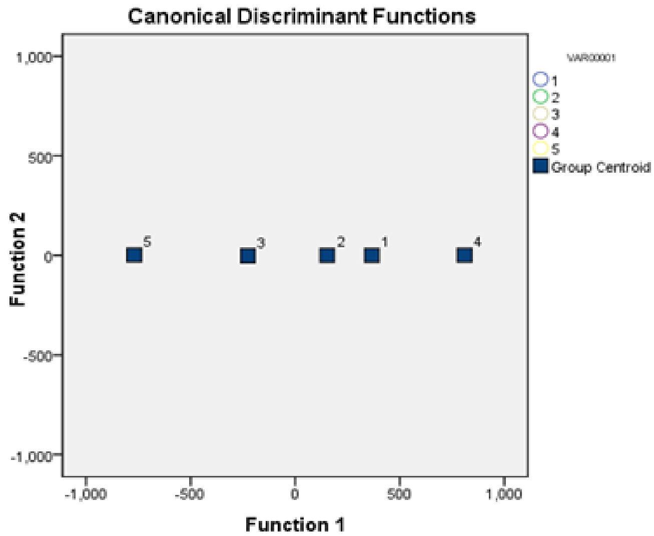

Figure 2

Figure1: PCA plot of 5 population of *Clupisoma garua* which were collected from -Son river (Red), Tones river (Blue), Yamuna river (Pink), Betwa river (Green), Gomti river (Purple).

Figure2: Sample centroids of discriminant function scores based on morphometric measurements of five populations of *C.garua*

Table 1: First 4 canonical discriminant functions were used in the analysis

Eigen Value

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	3.310E5[a]	100	100	1
2	1.996[a]	0	100	0.816
3	.566[a]	0	100	0.601
4	.006[a]	0	100	0.079

Table 2: Classification result Linear discriminant function analyses (DFA) of five groups.

			Classification result [b,c]					
			Predicted Group Membership					
		VAR00001	1	2	3	4	5	Total
Original	Count	1	8	0	0	0	0	8
		2	0	13	0	0	0	13
		3	0	0	29	0	0	29
		4	0	0	0	21	0	21
		5	0	0	0	0	20	20
	%	1	100	0	0	0	0	100
		2	0	100	0	0	0	100
		3	0	0	100	0	0	100
		4	0	0	0	100	0	100
		5	0	0	0	0	100	100
Cross-validateda	Count	1	8	0	0	0	0	8
		2	0	13	0	0	0	13
		3	0	0	29	0	0	29
		4	0	0	0	21	0	21
		5	0	0	0	0	20	20
	%	1	100	0	0	0	0	100
		2	0	100	0	0	0	100
		3	0	0	100	0	0	100
		4	0	0	0	100	0	100
		5	0	0	0	0	100	100

4. Hierarchical Cluster Analysis

Dendrogram using Average Linkage (Between Groups)

Rescaled Distance Cluster Combine

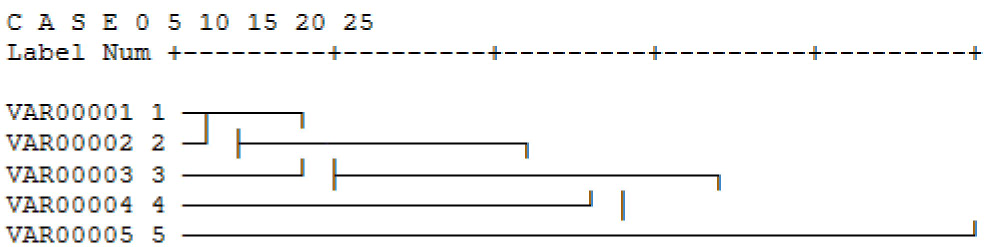

Figure 3: Dendrogram based on morphometric characters and landmark distances of five populations of *C. garua* i.e- var1. Son river, var2. Tones river, var3. Yamuna river, var 4. Betwa river, Var5.Gomti river

5. Discussion

In general, fish demonstrate greater variances in morphological traits both within and between populations than any other vertebrates and are more susceptible to these modifications ultimately change their morphology **(**Allendorf FW, 1987**)**. Morphometric differences among stocks are expected, because they are geographically separated and may have originated from different ancestors. Thus, it is not unlikely that obvious environmental variations. The causes of morphological differences between populations are often quite difficult to explain (Poulet et al., 2004). It has been suggested that the morphological characteristics of fish are determined by genetic, environment and the interaction between them (Swain & Foote, 1999; Poulet *et al.*, 2004; Pinheiro *et al.*, 2005). Fish are very sensitive to environmental changes and quickly adapt themselves by changing necessary morphometrics. Therefore, truss network measurements were employed in this experiment. Truss network systems are a powerful tool for identifying stocks of fish species **(**Turan C, 2004**)**. An unbiased network of morphometric measurements over a 2 dimensional outline of a fish removes the need to find the types of characters and optimal number of characters for stock separation and provides information over the entire fish form **(**Turan C, 2004**)**. The truss network system can effectively be used to distinguish between different stocks. Environmentally induced phenotypic variations however, may have advantages in the stock structure analysis of exploited species, especially when the time is insufficient for significant genetic differentiation to accumulate among populations. Genetic markers might not be sufficient to detect existing genetic variation among populations and also only a small proportion of DNA is analyzed by genetic markers. In this experiment, (Table2) DF analysis determined the dissimilarity among the stocks and significant correlations were observed between size and truss measurement characteristics among five stocks of *C. garua*. The dendrogram employed in this study resulted in 4 clusters: the Son river and the Tones river stocks were very close (Fig3). The differences between the river stocks may have been due to environmental as well as genetic variations. Plotting DFs revealed high isolation in morphometrics among the three stocks namely Betwa river, Yamuna river and Gomti river. Son and Tones stocks, however, broadly overlapped, while the Betwa river, Yamuna river and Gomti river stock clearly differed.

In conclusion, the present study provides basic information about the morphometric differentiation of *C. garua* populations of five rivers suggests that the morphological variations in *C. garua* should be considered in fisheries management and commercial exploitation of this species. This is one of the new studies of this endangered fish species which is IUCN red List of threatened species listed. The results of this study are useful as preliminary baseline information of *C. garua* populations for further studies.

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