

Pattern of Phenotypic Variation Among Three Populations of Indian Major Carp, *Catla catla* (Hamilton, 1822) Using Truss Network System in the Ganga Basin, India

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Abstract *Catla catla* (Hamilton, 1822) is a commercially important freshwater cyprinid distributed widely in India and adjacent countries. In total 142 specimens of *C. catla* were collected from three geographically separate watersheds of Ganga basin. A truss network was constructed by interconnecting 12 landmarks to yield 30 distance variables that were extracted from digital images of specimens using tpsDig2 and PAST software. Transformed truss measurements were subjected to univariate analysis of variance, factor analysis and discriminant function analysis (DFA). A total of 28 distance variables exhibited significant differences between the populations. The principal component analysis generated seven components explaining 86.40 % of total intraspecific variance in populations. By applying step-wise DFA, 100 % of the specimens were classified into their original populations (98.5 % under a ‘leave-one-out’ procedure). The occurrence of distinct populations may be due to differences in physical and ecological parameters of the three tributaries of the Ganga basin.

Keywords *Catla catla* · Shape morphometrics · Truss box network · Discriminant function analysis

Introduction

Among methods of stock identification, the analysis of morphometric characters is the most commonly used one. There are many studies that provide evidence that fish stocks can be discriminated on the basis of differences in morphometric characters [1–4]. Morphometric methods may be used to discriminate between ‘phenotypic stocks’ defined as a group of individuals with similar growth, mortality and reproductive rates [3].

Traditional morphometric methods are associated with some limitations in characterizing fish shape [5]. To overcome the inherent weakness in these traditional morphometric methods the Truss Network System [6] has been proposed. The latter is commonly being used for the purpose of stock identification and stock differentiation. In addition, the development of digital imaging systems, computer aided image analysis and advances in analytical methods have increased the power of morphometric analysis for stock identification [7].

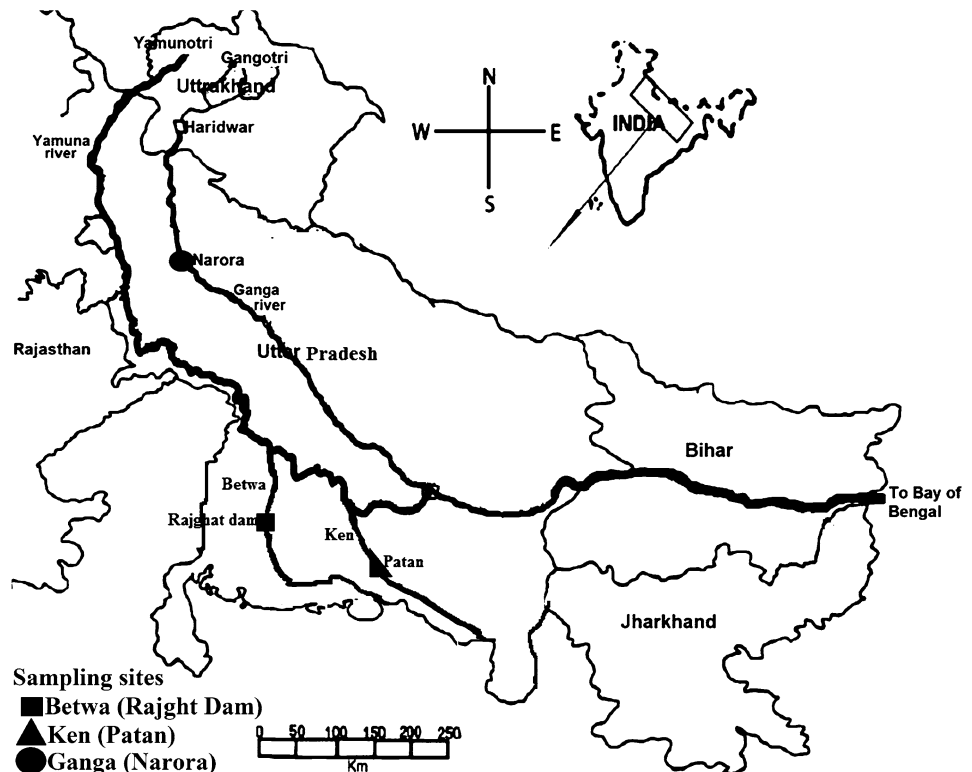
The cyprinid *Catla catla* is a commercially important Indian major carp that is widely distributed in India, Bangladesh, Pakistan and Myanmar [8]. In India, *C. catla* occurs naturally in the Indo-Gangetic river system [9]. The species is important for fisheries and aquaculture [10]. Recently, there has been a decline in the *C. catla* populations of the Ganga basin because of the introduction of alien species, destruction of breeding grounds, overfishing, dam construction and pollution [11]. In addition, farmed *C. catla* might be escaping and intermingling with wild stocks, leading to the loss of genetic variation [9].

The present study was undertaken to generate basic information relating to intraspecific variation of *C. catla* inhabiting the river Ganga and its important tributaries within the Yamuna river system. Populations were distinguished on the

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Fig. 1 Map showing collection sites of *C. catla* from three Indian rivers (Source Khan et al. [12] with slight modifications)



basis of morphometric characters using the truss network system.

Material and Methods

Study Areas

The river Ganga originates in the Garhwal Himalayas ($30^{\circ}55'N$, $70^{\circ}7'E$) at an elevation of 4,100 meters above mean sea level from the Gaumukh glacier in Uttarakhand, India. It flows some 2,525 km before reaching the sea. River Yamuna originates from the Yamunotri glacier (Saptarishi Kund) near Bander punch peaks ($380^{\circ}59'N$, $780^{\circ}27'E$) at an elevation of 6,320 meters above sea level (masl) in the Mussoorie range of the lower Himalayas in Uttarkashi district of Uttarakhand, and runs some 1,336 km before merging into the river Ganga at Allahabad, Uttar Pradesh, India [12]. The rivers Betwa and Ken are the major tributaries of river Yamuna (major tributary of Ganga) in northern India. River Betwa, with a total length of around 1,370 km, is the largest tributary of the Yamuna (Fig. 1). The Betwa originates in the Raisen district in Madhya Pradesh at an elevation of 475 masl and joins the river Yamuna near Hamirpur in Uttar Pradesh, traveling a total distance of about 590 km. The river is regulated by three large dams (Rajghat, Matatila, and Parichha) and two small dam/weirs in the middle and upper stretch of the

river. The Ken river has its origin from the Ahirgawan village on the northwest slopes of the Kaimur hills in the Jabalpur district of Madhya Pradesh at an elevation of about 550 masl. The total length of the river from its origin to confluence with the river Yamuna is 427 km. The river joins the river Yamuna near village Chilla in Uttar Pradesh at an elevation of about 95 masl (Fig. 1). The river is the last tributary of Yamuna before the Yamuna joins the Ganga [11].

Sample Collection and Digitization

In total, 142 specimens of *C. catla* were collected from three rivers in the Ganga basin watershed during the period of June 2009–November 2011 before the dawn. They include 31 specimens from river Betwa at Rajghat dam, 58 specimens from river Ken at Patan and 53 specimens from river Ganga at Narora (Table 1).

Sampled specimens were bathed in running water, drained and placed on a flat platform with graph paper as a background, which was used for calibrating the coordinates of the digital images. Each individual was given a specific code for identification. A digital camera (Sony, Japan) was used to capture the digital images, which provided a complete archive of body shape and allowed a repeat of the measurements when necessary [7]. After image capture, each fish was dissected to examine the gonads for sex determination. The gender was used as the class variable in

Table 1 Rivers, collection sites, GPS coordinates, sample size, size range with standard error (SE) (based on the standard length) and total weight range with of *C. catla* from three different watersheds of Ganga basin, India

Rivers	Sampling sites	GPS coordinates	Sample size (<i>n</i>)		Size range (SE; cm)		Total weight range (g)	
			Male	Female	Male	Female	Male	Female
Betwa	Rajghat dam	24°45'N, 78°14'E	18	13	22–83.5 (6.3)	25.5–97.5 (11.4)	150–5,000	230–7,000
Ken	Patan	23°17'N, 79°41'E	35	23	24.5–66.0 (9.6)	22.5–78.0 (10.3)	175–3,750	160–3,500
Ganga	Narora	28°12'N, 78°23'E	33	20	20.5–69.0 (8.5)	21.5–67.0 (7.6)	150–3,800	165–3,500

analysis of variance (ANOVA) to test for significant differences in morphometric characters, if any, between male and female *C. catla*.

Laboratory Procedure

The truss protocol used for *C. catla* in the present study was based on 12 landmarks. The truss network was constructed by interconnecting these landmarks to form a total of 30 measurements (Fig. 2). The extraction of truss distances from the digital images of specimens was conducted using a linear combination of three software platforms viz. tpsUtil, tpsDig2 v2.1 [13] and Paleontological Statistics (PAST) [14]. A box truss of 30 lines connecting these landmarks was generated for each fish to represent the basic shape of the fish [6]. 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 [15].

Multivariate Data Analysis

As most shape measurements are in some way related to size, any heterogeneity in the size across the specimens will result in heterogeneity in the shape, but without providing information on differences in body proportions among populations [16]. Several univariate and multivariate analyses such as regression analysis, allometric methods, multiple group principal component analysis (PCA), etc. can be used to remove the size effect of the specimens. The allometric methods are significant help in achieving the size and shape separation and reasonably meet the statistical assumption [17]. Significant correlations were observed between size and morphometric characters of the specimens. All measurements were standardized following Elliott et al. [18], to eliminate any variation resulting from allometric growth. The calculation of size adjusted measurement has been done by the following formula.

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

where *M* is the original measurement, *M_{adj}* is the size adjusted measurement, *L_o* is the standard length (SL) of the

fish, and *L_s* is the overall mean of the SL for all fish from all specimens in each analysis. Parameter *b* was estimated for each character from the observed data as the slope of the regression of log *M* on log *L_o*. The transformed data were checked for efficiency by testing the significance of the correlation between the transformed variables and standard length. SL was excluded from the final analysis.

The results derived from the allometric method were confirmed by testing the significance of the correlation between the transformed variables and SL following Mir et al. [19]. Univariate ANOVA was performed for 30 morphometric characters to evaluate the significant difference among the three tributaries. The transformed data were subjected to PCA and discriminant function analysis (DFA) to examine any phenotypic differences between the populations. The eigenvectors and eigenvalues were obtained from the covariance matrix in the PCA, which allowed the largest part of the variance of original variables in a low number of factors. This analysis enabled the evaluation of the relation between the populations by means of proximity in the space defined by the components. The DFA was used to calculate the percentage of correctly classified (PCC) fish and a cross-validation using PCC was done to estimate the expected actual error rates of the classification functions. All statistical analyses were carried out using MS EXCEL and SPSS (version 16.1.0).

Results

There was no significant correlation between any of the transformed morphometric variables and SL (*p* > 0.001), indicating that the size effect was successfully removed.

Univariate ANOVA showed that 28 out of 30 morphometric characters were significantly different among specimens (*p* < 0.001; Table 2). These are particularly noticeable for the body height distances and measurements of the caudal peduncle region (*p* < 0.001). The morphometric characters between both sexes did not differ significantly (*p* > 0.05), hence the data for both sexes were pooled for all subsequent analyses. PCA of 30 morphometric measurements extracted seven factors with eigenvalues >1, explaining 86.4 % of the total variance.

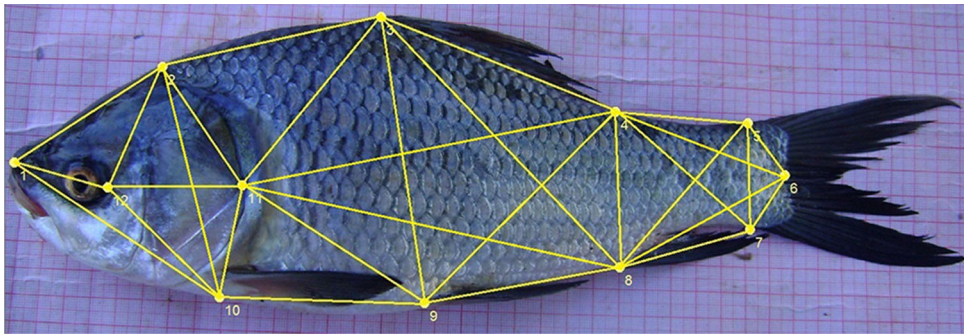


Fig. 2 Locations of 12 landmarks used for shape analysis. Land marks refer to 1 anterior tip of snout at upper jaw; 2 most posterior aspect of neurocranium (beginning of scaled nape); 3 origin of dorsal fin; 4 90° of the origin of anal fin; 5 anterior attachment of dorsal

membrane from caudal fin; 6 posterior end of vertebrae column; 7 anterior attachment of ventral membrane from caudal fin; 8 origin of anal fin; 9 insertion of pelvic fin; 10 insertion of pectoral fin; 11 end of operculum; 12 posterior end of eye

Table 2 The results of univariate ANOVA for morphometric measurements of *C. catla* from three different watersheds of Ganga basin, India

Truss character	<i>F</i>	df1	df2	Sig.
1–2	2.857	2	139	0.060
1–10	1,274.176	2	139	0.000
1–12	3,191.409	2	139	0.000
2–3	30.006	2	139	0.000
2–10	496.266	2	139	0.000
2–11	629.801	2	139	0.000
2–12	758.234	2	139	0.000
3–4	20.611	2	139	0.000
3–8	108.268	2	139	0.000
3–9	302.079	2	139	0.000
3–11	438.160	2	139	0.000
4–5	23.842	2	139	0.000
4–6	60.815	2	139	0.000
4–7	68.500	2	139	0.000
4–8	86.120	2	139	0.000
4–9	175.439	2	139	0.000
4–11	258.590	2	139	0.000
5–6	78.235	2	139	0.000
5–7	73.907	2	139	0.000
5–8	100.657	2	139	0.000
6–7	28.348	2	139	0.000
6–8	20.769	2	139	0.000
7–8	38.308	2	139	0.000
8–9	92.426	2	139	0.000
8–11	52.456	2	139	0.000
9–10	29.456	2	139	0.000
9–11	115.972	2	139	0.000
10–11	71.808	2	139	0.000
10–12	2.034	2	139	0.134
11–12	131.777	2	139	0.000

Character description given in Fig. 2

Principal component 1 (PC1) accounted for 35.38 % of total variance and the second (PC2), third (PC3), fourth (PC4), fifth (PC5), sixth (PC6) and seventh (PC7)

components accounted for 14.32, 11.81, 7.95, 6.52, 5.89 and 4.53 % respectively (Table 3). All these components were positively correlated to some variables and negatively

Table 3 Component loadings of seven principal components for truss morphometric characters in *C. catla* collected from three different watersheds of Ganga basin, India

Truss character	Components						
	PC1 (35.38 %)	PC2 (14.32 %)	PC3 (11.81 %)	PC4 (7.95 %)	PC5 (6.52 %)	PC6 (5.89 %)	PC7 (4.53 %)
1-2	-0.883	-0.024	-0.153	0.084	0.152	-0.144	-0.039
1-10	0.147	-0.005	0.017	0.943	0.181	-0.030	0.036
1-12	0.448	-0.100	-0.153	0.192	0.543	0.081	0.186
2-3	-0.200	0.120	0.000	-0.001	0.036	-0.053	0.896
2-10	0.752	0.022	0.084	0.603	0.004	0.067	0.022
2-11	0.884	0.079	0.011	-0.102	0.286	0.151	0.054
2-12	0.943	-0.017	0.007	0.023	0.122	0.136	0.084
3-4	-0.330	0.126	0.194	-0.030	0.723	0.188	0.363
3-8	0.426	0.726	0.307	-0.152	0.224	0.141	-0.172
3-9	0.740	0.024	0.409	-0.081	0.224	-0.056	-0.216
3-11	0.731	0.043	0.117	-0.216	0.349	0.268	-0.311
4-5	-0.178	0.042	0.199	0.127	-0.782	0.197	0.298
4-6	0.368	0.190	0.623	-0.081	0.409	-0.180	-0.333
4-7	0.794	0.212	0.182	0.092	-0.365	-0.140	-0.050
4-8	0.635	0.652	0.283	-0.069	0.115	0.064	-0.201
4-9	0.883	0.020	0.219	0.024	-0.026	-0.163	-0.294
4-11	0.883	0.065	0.061	-0.083	0.150	0.157	-0.317
5-6	0.129	0.180	0.885	-0.024	-0.019	0.025	0.012
5-7	0.781	0.138	0.163	0.092	-0.282	-0.194	0.018
5-8	0.557	0.630	0.442	-0.030	-0.127	0.165	0.018
6-7	0.253	0.305	0.616	-0.003	-0.340	0.304	0.326
6-8	0.207	0.761	-0.014	-0.085	-0.011	0.414	0.226
7-8	0.341	0.435	0.559	-0.032	-0.334	0.326	0.164
8-9	0.187	-0.642	0.602	-0.135	-0.018	0.063	0.009
8-11	0.262	-0.855	-0.228	-0.066	0.119	0.099	-0.158
9-10	-0.087	0.014	-0.145	0.705	-0.092	0.618	-0.035
9-11	0.065	0.150	0.121	-0.184	0.087	0.884	0.004
10-11	0.099	0.187	0.344	-0.734	0.090	0.362	-0.033
10-12	0.140	-0.049	-0.120	-0.894	0.150	0.030	0.019
11-12	0.136	0.072	0.509	0.136	-0.185	0.515	-0.115

correlated with others, showing that there was variation in body shape. The high component loadings were from the characters 1-2, 2-10, 2-11, 2-12, 3-9, 3-11, 4-7, 4-8, 4-9, 4-11, 5-7 and 5-8 to the first principal component, 3-8, 4-8, 5-8, 6-8, 8-9 and 8-11 to the second, 4-6, 5-6, 6-7 and 8-9 for third, 1-10, 2-10, 9-10, 10-11 and 10-12 to fourth, 3-4 and 4-5 to fifth, 9-10 and 9-11 to sixth and 2-3 to seventh component (Table 3).

Wilks' λ tests of discriminant analysis indicated significant differences in morphometric characters of all populations ($p < 0.001$). Forward stepwise discriminant analysis of the 30 variables produced two discriminating functions (DFs). The first canonical discriminant function of the discriminant analysis explained 98.7 % of the total variance while the second one accounted for 1.3 % of the

total variance. The plot of the two canonical variables showed a complete separation among three rivers (Fig. 3). The morphometric truss measurements viz. 1-12, 1-10, 2-12, 2-11, 2-10, 3-11, 3-9, 9-11 and 8-9 showed highest variation on DF1 while 3-4, 5-8, 4-8, 4-7, 3-8, 5-6, 2-3 and 5-7 contributed to DF2 (Table 4), showing that these characters were the most important in distinguishing the populations. The DF1 versus DF2 plot explained 100 % of total variance among the specimens and showed wide distinction among *C. catla* stocks from the Ganga basin (Fig. 3). DFA showed 100 % correct classification of individuals into their original populations, and the cross-validation test results were comparable to the results obtained from PCC. The PCC fishes was 100 % in all the populations.

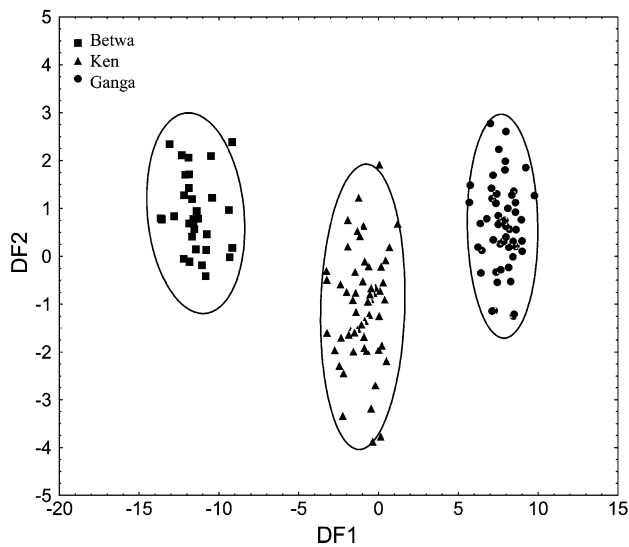


Fig. 3 Coordinate plots of three populations of *C. catla* by plotting first two discriminant functions from morphometric data analysis

Discussion

The observed phenotypic divergence among *C. catla* specimens revealed the existence of three morphologically differentiated stocks viz., the Betwa river population, the Ken river population and the Ganga river population. The distinction among the specimens may suggest a relationship between the extent of phenotypic heterogeneity and geographic distance. This might be due to the geographic isolation and environmental condition of the rivers. These intraspecific morphological differences in *C. catla* were related to body shape and not to the size effects which were successfully removed for by allometric transformation.

The cause of morphological discreteness between the populations is sometimes difficult to explain [20], but it is assumed that these differences may be genetically related or might be associated with phenotypic plasticity in response to different environmental factors in each area [21]. It may be assumed that in the present investigation, morphological variation could have been produced as a result of genetic variation between stocks or ecological differences between rivers. The main differences among these three populations were noted in the areas of the head, body depth and caudal peduncle. Morphological variations in the head and middle region of fish have been considered to occur due to differences in feeding regimes [19] and water quality parameters. Genetic analysis of the *C. catla* population from the Brahmaputra, Mahanadi, Sutlej and Bhagirathi rivers showed that there exist multiple population structure of *C. catla* [9]. While investigating the comparative morphometry of *Labeo rohita* from six Indian rivers, Mir et al. [19] reported highly significant variations in body width, eye diameter and caudal peduncle for the

Table 4 Pooled within-group correlations between discriminating variables and discriminant functions (DFs; variables ordered by size of correlation within function)

Truss character	Function	
	DF1 (98.7 %)	DF2 (1.3 %)
1–12	0.841 ^a	0.201
1–10	0.531 ^a	0.186
2–12	0.410 ^a	–0.141
2–11	0.373 ^a	–0.242
2–10	0.331 ^a	–0.084
3–11	0.310 ^a	–0.236
3–9	0.258 ^a	–0.169
9–11	0.114 ^a	–0.070
8–9	0.107 ^a	0.017
3–4	0.046	0.429 ^a
5–8	0.142	–0.401 ^a
4–8	0.131	–0.385 ^a
4–7	0.117	–0.345 ^a
3–8	0.150	–0.344 ^a
5–6	0.127	–0.318 ^a
2–3	0.073	0.312 ^a
5–7	0.123	–0.311 ^a
4–5	0.064	–0.294 ^a
4–11	0.237	–0.282 ^a
4–9	0.194	–0.282 ^a
4–6	0.112	–0.273 ^a
7–8	0.083	–0.225 ^a
1–2	–0.005	0.216 ^a
8–11	0.106	0.192 ^a
9–10	0.078	0.188 ^a
10–11	0.055	–0.174 ^a
6–8	0.071	–0.124 ^a
6–7	0.078	–0.102 ^a
10–12	0.021	–0.028 ^a
11–12	0.015	0.016 ^a

^a Denotes the largest correlation between each variable and DFs

fish sample from the Ganga basin in India and found water impoundments of rivers and environmental conditions of water responsible for the same.

Differentiation between specimens from adjacent stations may be due to the geographic isolation of stations by artificial obstacles from each other allowing morphological differentiation to proceed independently at each station [22]. The dams obstruct the migration of fish populations resulting in an ecological trap [23]. A series of barrages and dams have been commissioned in the upper segment of river Ganges from Rishikesh to Narora [24]. The river Betwa is regulated by three large dams and two small dam/weirs in the middle and upper stretch of the river [11] and Gangau Weir, Rangwan and Bariyarpur projects are

constructed on river Ken. The fragmentation of the river converts a free flowing river into reservoir habitat, affecting the ecosystem. The blockage of fish movements can have a very significant impact on fish stocks by obstructing the genetic exchange [25]. Also, the construction of a dam can lead to dramatic changes in the environment of a river and particularly affect fish communities. Dams can also alter the feeding habits of the species, availability of food items, growth pattern and reproductive strategy of fish species of a river [26]. The importance of such factors on producing morphological differentiation in fish species is well known [27].

The present study indicates that the *C. catla* present at the investigated localities within the Ganga watershed form distinct populations. Possible reasons for the differentiation could be the fragmentation of river impoundments resulting from the construction of dams, along with influences of genetic and environmental factors. Study involving use of molecular genetics will be useful to confirm the present findings based upon morphometric discrimination, and common garden experiments using offspring from broodfish of the three populations could shed additional light on the source and causes of the observed phenotypic differences. In conclusion, the present study provides basic information about morphometrics and shape variation of *C. catla* populations in the Ganga river system and suggests that the morphological variations observed in *C. catla* should be taken into consideration in fisheries management and stock management programmes.

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