Morphological Plasticity in a Wild Freshwater Fish, Systomus Sarana (Cyprinidae) from India: A Glimpse Through Advanced Morphometric Toolkits

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Research article

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Abstract

Background

Body morphology supposed to underpin a wide differences of animal performance that can be used to understand diversification of characters. Further, identifying fish population with unique shape due to variations in their morphometric characters enables better management of these subunits. Advanced statistical toolkits of morphometry called truss network system and geometric morphometrics have been increasingly used for detecting variations in morphological traits. Present study was carried out with the objective of determining whether there are morphological characteristics that separate freshwater fish *Systomus sarana* from different groups.

Methods

In the present study, 154 specimens of olive barb, *S. sarana* were collected from four distantly located rivers covering the northern (Ganga), southern (Godavari), central (Narmada) and eastern (Mahanadi) regions of India. Truss-network system and geometric morphometrics have been utilized. Fourteen landmarks were digitized uniformly on each specimen, In the present study, truss network system yielded size-corrected morphometric characters were subjected to univariate and multivariate statistical assessment.

Results

Analysis of variance (ANOVA) presented significant differences among 63 out of 90 variables ($p < 0.05$). Truss approach includes principal component analysis (PCA) and discriminant function analysis (DFA) while geometric approach includes PCA, DFA, canonical variate analysis (CVA), partial least square (PLS), the relative warp (RW) and wireframes. CVA extracted Mahalanobis and Procrustes distances among groups found to be highly significant ($p < 0.0001$). In linear DFA, the overall assignment of correctly classified individuals into their original groups was 86.2% for Ganga, 86.1% for the Godavari, 93.9% for the Narmada and 92.9% for Mahanadi population.

Conclusions

The results revealed significant variations in the morphometric characters which were reflected in the shape of different body features of the studied populations. Both methods revealed analogous results, and significant differences among groups in examined features. Our results suggest that *S. sarana* shows morphological plasticity across different rivers in India. This study supports the concept that geographical isolation among fish populations can lead to morphological variations.

Introduction

Morphologically similar populations thriving together in a region are not easily distinguishable. Therefore, it is essential to recognize characters that differentiate populations. Morphological characters are
capable of offering a foundation for population structure (Ihssen et al. 1981; Petrtyl et al. 2014). Further, the study of these characters with intends to differentiate fish population units has recently got much attention in ichthyology (Yusuf and Belduz 2009; Dwivedi 2019). Environmentally induced morphological variations provide clues related to fish population identities (Clayton 1981) which is essential from diverse perspectives including evolution, ecology, conservation, and also for managing water resources (AnvariFar et al. 2011). Morphological variations among populations can be assessed by traditional morphometric analysis (Turan 1999). Though this traditional approach is considered as a standard technique for species characterization, this method might not be useful for discriminate species when there is morphological plasticity (Takács et al. 2016). Advance statistical toolkits such as the truss network system and geometric morphometrics allow discrimination of species/population through the shape analysis of a whole or only fragment of structure to find the unidentified pattern of importance. These techniques have gained wide acceptance as tools in population studies and can potentially be used as low cost, accurate and precise tools, (Bookstein 1991; Rohlf 1990; Rohlf and Marcus 1993, Strauss and Bookstein 1982).

Interestingly, studies on intra-species morphological differentiation are essential in solving the problems related to species recognition, as it is agreed that insufficient information on intra-species geographic dissimilarities can lead to incorrect species identification (Ishihara 1987). Taxonomy is important to fishery scientists for the delineation of fish resources, and aids in developing balanced conservation strategies (Sangster et al. 2014). The taxonomic significance of the variation observed in the present study has to be assessed concerning the available taxonomic information on the species. Worldwide taxonomy of the *Puntius* including other Cyprinidae species has been dubious (Kortmulder 1972; Nagpure et al 2003; Kullander and Fang 2005; Balaraj and Basheer 2012; Sukham et al. 2015), and this has been the case for *S. sarana* in Asia. Hamilton in 1822 had described this species from the Ganga River and named *Cyprinus sarana*, and afterward, it was synonyms with *Puntius sarana*. Likewise, *Puntius sarana* have many synonyms assigned by various authors (*Puntius sarana sarana*, *Puntius sarana subnasutus*, *Puntius sarana spilurus*, *Puntius subnasutus*, *Systomus immaculatus*, *Barbodes sarana*, *Barbodes sarana subnasutus*, *Puntius saberi*, etc) these synonyms undoubtedly generate confusions in the identification of this species (Pethiyagoda 1991). So far five sub-species of *S. sarana* were identified worldwide; *P. sarana orphoides*, *P. sarana subnasutus* and *P. sarana sarana* from India and *P. sarana spilurus* from Sri Lanka (Irfan and Gunawardhara 2011). *S. sabnasutus* is referred to as *S. sarana*, although very recently it is categorized as a different sub-species (Biswal et al. 2018). These subspecies further adding taxonomic complexity of this species. Due to a lack of proper systematic studies and having phenotypic resemblance among subspecies, Pethiyagoda (1991) has recommended extensive population studies on these species by accompanying intra-species/population delineation studies (Talwar and Jhingran 1991, Irfan and Gunawickerma 2011).

Our organism of interest, *Systomus sarana* belong to the subfamily Barbinae, is a taxonomically diverse and complicated group of freshwater fish for studying morphological differences due to their wide distribution (Talwar and Jhingran 1991) and flipping systematic status i.e. many species formerly placed in *Puntius* have been moved to other genera (Kottelat 2013; Pethiyagoda et al. 2012; Raghavan et al.
It is characterized by a deep and moderately compressed body with a dorsal profile elevated. The maximum length of fish is 42.0 cm TL (FishBase). *Systomus sarana* (then allocated to *Puntius*) is an ecologically important, profitable, and cultivable candidate fish species (Gopakumar et al. 1999; Chakrabory et al. 2003). In India, this species is distributed widely excluding peninsular India-south of Krishna River and is also found in Afghanistan, Bangladesh, Bhutan, Nepal, and Pakistan (Talwar and Jhingran 1991). Previous studies have indicated that it is abundantly available however recent reports and observations indicate a decline in their wild population owing to their overexploitation (Hossain et al. 2009; Dahanukar 2010; Hussain and Mazid 2004). Consequently, considered as vulnerable species (Mijkerjee *et al.* 2002; Dahanukar 2010), some researchers also categorized them as critically endangered (Ameen et al. 2000; Hussain and Mazid 2004). Although unintentional selective fishing causing depletion of fish biodiversity hitherto, there is no published report on the fish population structure of *S. sarana* from Indian rivers based on morphometric characters. Moreover, only a little work has been done to delineate *S. sarana* population outside India (Irfan and Gunawickrama 2011; Siddik *et al.* 2016; Kabir *et al.* 2015).

Considering the above context, this study aims to find the morphological divergence of *S. sarana* populations from Ganga (North), Godavari (South), Mahanadi (East), and Narmada (Central) based on morphometric measurements by utilizing the following toolkits, truss network system and geometric morphometrics. This allows quantitative analysis of morphological divergences and may provide insight into microevolution.

**Materials & Methods**

**Study area**

For the present study, four rivers have been selected *viz.* Ganga (2600 km), Narmada (1312 km), Godavari (1465 km), and Mahanadi (900 km). The Ganga River originates in the Garhwal Himalayas from the Gaumukh glacier in Uttrakhand, India, and drains into the Sunderbans delta in the Bay of Bengal. The Narmada River originates from the Amarkantak, located in the Shahdol district of Madhya Pradesh, India and drains into the Arabian Sea. The Godavari River is also known as Dakshina Ganga, originating from the Nasik district of Maharashtra, India, and drained into the Bay of Bengal. The Mahanadi River, a major river in east-central India, originated Dandakaranya in Raipur district of Chhattisgarh, India empties itself into the Bay of Bengal. All the rivers taken into account are east flowing except the Narmada River, which is west-flowing.

**Sample Collection**

A total of 154 specimens of *S. sarana* were collected from Kanpur site of the river Ganga, Adilabad site of river Godavari, Haushangabad site of river Narmada, and Nadigaon site of Mahanadi river in two years duration (2016 to 2018). The specimens were caught before the breeding season and after the spawning
period to avoid a bias toward size difference. The fish samples were collected with the help of hired local fishermen. The identification of the fish was based on standard taxonomic keys of Talwar and Jhingaran (1991) and Jayaram (2010). Samples collection details and geographical coordinates of sites have been mentioned in Table 1 and Fig. 1.

### Table 1

| Rivers  | Sampling sites                  | Site code | GPS location       | Sample size |
|---------|---------------------------------|-----------|--------------------|-------------|
| Ganga   | Kanpur barrage, U.P.            | GA        | 26.50°N 80.31°E    | 29          |
| Godavari| Adilabad Telangana              | GO        | 18.79°N 79° 90°E   | 36          |
| Narmada | Haushangabad M.P.               | NA        | 22.35°N 77.13°E    | 33          |
| Mahanadi| Nadigaon Chhattisgarh            | MA        | 21.70°N 83° 83.38°E| 56          |

#### Digitization Of Samples And Morphometric Measurements

The freshly caught sampled specimens (only undamaged) were placed with the left side up on a water-resistant paper and the body posture and fins were teased into a natural position to make the landmark points visible. Each individual was labeled with a specific code for identification and archiving purposes. Images of the specimens were taken by a camera (Canon IXUS145), set on a tripod stand directly above the specimen and the camera lens was adjusted and each image included a scale to normalize the individual sizes and additional scaling was applied in tpsDig making use of the millimeter gridiron in the graph paper.

#### Landmark-based Truss Analysis

Fourteen homologous anatomical landmarks (Winans and Nishioka 1987) were selected for the analysis (Fig. 2). A box-truss network was developed to give 91 morphometric variables through interconnection among these landmarks. Software including tpsUtil, tpsDig (Rohlf 2006), and software PAST (Hammer et al. 2001) was employed for generating truss data from the digital images. Since the standard length (SL) of fish specimens were different, it was necessary to remove dissimilarities due to size variations (Reist 1985). The truss measurements were standardized to account for size variation through the method described by Elliott et al. (1995) to eliminate the size component from the shape measurements: \( \text{Madj} = M \cdot (\text{Ls}/\text{Lo})^b \), Where M denotes original measurement, Madj is the size-adjusted measurement, Lo is the SL of the fish, and Ls is the overall mean SL for all fish from all samples in each analysis. Parameter b was calculated for each character from the observed data as the slope of the regression of log M on log Lo. SL (character code 1–6) was excluded from the final analysis because SL was used as a basis for
transformation (Mamuris et al. 1998) and thus 90 morphometric variables were retained for further analysis. The transformed data were validated for efficiency by testing the significance of the correlation between standard length and the transformed variables. The SL was excluded from the final analysis. Univariate ANOVA was performed for each morphometric character to assess the significant variation among the four populations (Gomez–Rodriguez 2010). The transformed data representing characters that showed significant variation between populations were analyzed using PCA. This analysis was applied to determine the linear combinations of variables that responsible for a large amount of the variation in the data and to identify influential variables (Johnson and Wichern 1998). PCA plot was formed by using components that confirmed high variance. In PCA, Jolliffe's rule with eigenvalues of at least 0.7 was applied to retain principal components (Dunteman 1989) and factor loading greater than 0.30 is considered significant, 0.40 more important, and 0.50 or greater very significant (Nimalathasan 2009). In the present study, only those factors were considered as significant that having loadings above 0.50. The Wilks’ k was used to compare the differences. Further, a stepwise procedure was employed to lessen the number of variables to meet the requirement of a reduced set of characters for the DFA. Standardized canonical discriminant function coefficients and coefficients in the structure matrix were used as the criteria to identify the discriminating variables between two populations. DFA was used to assign individuals to their original group and to compute the percentage of correctly classified (PCC). Cross-validation (leave-one-out method) employing PCC was done to approximate the expected actual error rates of the classification functions. Statistical analyses were performed with the computer software programs MS-Excel (vers.2007), SPSS 16.0, and PAST 1.47.

**Landmark-based Geometric Morphometric Analysis**

Shape coordinates were superimposed to successfully eliminate the size effect, which was apparent from Procrustes analysis (Procrustes sums of squares: 0.363 and Tangent sums of squares: 0.361). Also, partial least square (PLS) revealed a non-significant covariance between superimposed shape and log centroid size \((R = 0.54; P > 0.001)\), resulting in overlap among populations (Fig. 4). The deformed wireframe of average shape also showed variations between individuals and between populations (Fig. 5). Relative warp (RW) analysis illustrated deformation in shape (Fig. 6) from the reference that corresponds to selected positions in the ordination. The deformed wireframe was drawn on the shape among four populations to interpret shape changes that support the RW analysis.

The PCA extracted 24 components with a 100.00% variance. The first two principal components (PCs) account for 40.22% of the total variance (22.48% for PC1, 17.74% for PC2). Overlap among the specimens obtained from four rivers is evident in the PCA plot of PC1 and PC2 (Fig. 7). A low level of variance and a high level of overlapping in the PCA demands further verification through CVA and DFA to determine shape variations. The CVA based upon 14 landmarks showed four groups with slight overlap among populations (Fig. 8). The larger part (82.98%) of the total variance (100.00%) was explained along the first two canonical variates (CVs): CV1 and CV2 explained 55.47% and 27.50% of the total variance, respectively, while CV3 explained only 17.016% of the total variance. CVA extracted Mahalanobis and
Procrustes distances among four groups found to be highly significant \( (p < 0.0001) \) (Tables 6, 7). Classification results of CVA indicated that all the specimens of each group were allotted to their respective groups with a slight misclassification rate. The classification of individuals into their cross-validated groups showed a low level of mixing between the populations (Table 8; Fig. 9). These results go well together with those depicted by the deformed wireframe of average shape.

Table 6
Mahalanobis distances based on geometric morphometrics. Pair wise matrix of Mahalanobis distances among groups (upper diagonal) and \( p \) value (lower diagonal) of canonical variate analysis.

| Groups     | Ganga | Godavari | Narmada | Mahanadi |
|------------|-------|----------|---------|----------|
| Ganga      | 5.8149| 4.0786   | 3.9214  |          |
| Godavari   | < .0001| 4.1109   | 4.5639  |          |
| Narmada    | < .0001| < .0001  | 3.3623  |          |
| Mahanadi   | < .0001| < .0001  | < .0001 |          |

Table 7
Procrustes distances based on geometric morphometrics. Pair wise matrix of Procrustes distances among groups (upper diagonal) and \( p \) value (lower diagonal) of canonical variate analysis.

| Groups     | Ganga | Godavari | Narmada | Mahanadi |
|------------|-------|----------|---------|----------|
| Ganga      | 0.0357| 0.0272   | 0.0269  |          |
| Godavari   | < .0001| 0.0241   | 0.0265  |          |
| Narmada    | < .0001| < .0001  | 0.0189  |          |
| Mahanadi   | < .0001| < .0001  | < .0001 |          |
Table 8
Discriminant function analysis based on geometric morphometric. Misclassification of specimens between groups extracted from discriminant function analysis.

| Classification | Groups | Ganga | Godavari | Mahanadi | Narmada |
|----------------|--------|-------|----------|----------|---------|
| Validated      | Ganga  | 0     | 2        | 0        |         |
|                | Godavari | 0    | 1        | 0        |         |
|                | Mahanadi | 1    | 1        | 0        |         |
|                | Narmada | 0    | 0        | 0        |         |
| Validated      | Ganga  | 1    | 6        | 4        |         |
|                | Godavari | 4    | 4        | 2        |         |
|                | Mahanadi | 3    | 4        | 4        |         |
|                | Narmada | 3    | 1        | 3        |         |

Results

Landmark-based truss analysis

After the allometric transformation, there was no significant correlation ($p > 0.05$) found between standardized truss measurements with the standard length (SL), indicating that the size effect had been effectively removed from the data. Hence, all the measurements were utilized for further calculations. Further, the morphometric characters did not differ significantly ($p > 0.05$) between both sexes, therefore the data for both sexes were pooled for all subsequent analyses. By applying ANOVA (one way) on 90 morphometric characters, only 63 showed a significant difference in their mean values ($p < 0.05$). Significant variables were subjected to principal component analysis (PCA) and DFA. PCA plot does not allow one to draw a conclusion about homogenous grouping based on visuals. By applying PCA, a total of 13 principal components were extracted explaining 93.311% of the total variance among populations. Principal component 1 (PC1) and PC2 contribute 24.412% and 19.028% of total variance respectively (Table 2). The high component loadings were from the characters (1–11, 1–12, 11–14, 6–12, 2–11, 6–11, 2–11, 12–14, 12–13, 11–13, 2–12, 1–13, 13–14, 6–13) to the first principal component, (4–13, 2–4, 3–4, 1–4, 4–14, 4–12, 4–6 and 4–11) to the second, 1–5, 5–14, 5–13, 2–5, 3–5, 5–12, 5–11, 5–8 and 5–9 for third component. The factor analysis extracted 6 factors having eigenvalues summed to ≥1. The results of factor analysis indicated that the first three factors together explained 89.6% of the total morphometric variation, with eigenvalues of 58.1, 28.8, and 13.1, respectively. Mahalanobis distances between the centroids of the clusters and the $p$-value of discriminant truss morphometric characters among four populations have been presented in Table 3.
Table 2

**Eigenvalue, Percentage of variance, Cumulative percentage.** Eigenvalues, percentages of variances, and cumulative percentages for the 13 principal components from a PCA in case of 63 morphometrics measurements from four populations of *Systomus sarana*.

| Components | Initial Eigenvalues |
|------------|---------------------|
|            | Eigenvalue | Percentage of variance | Cumulative percentage |
| 1          | 15.4       | 24.4                  | 24.4                  |
| 2          | 12.0       | 19.0                  | 43.4                  |
| 3          | 5.4        | 8.6                   | 52.0                  |
| 4          | 5.0        | 7.9                   | 60.0                  |
| 5          | 4.3        | 6.8                   | 66.8                  |
| 6          | 3.6        | 5.7                   | 72.5                  |
| 7          | 3.0        | 4.8                   | 77.3                  |
| 8          | 2.2        | 3.5                   | 80.7                  |
| 9          | 2.1        | 3.3                   | 84.0                  |
| 10         | 1.8        | 2.8                   | 86.9                  |
| 11         | 1.6        | 2.5                   | 89.3                  |
| 12         | 1.5        | 2.4                   | 91.7                  |
| 13         | 1.0        | 1.6                   | 93.3                  |

Table 3

Mahalanobis distances based on truss morphometrics. Pairwise matrix of Mahalanobis distances between the centroids of the population clusters (above diagonal) and corresponding p-values (below diagonal) from DFA of discriminant 63 truss-based morphometric characters distances among four populations of *Systomus sarana*.

| Groups   | Ganga | Godavari | Narmada | Mahanadi |
|----------|-------|----------|---------|----------|
| Ganga    |       |          |         |          |
| Godavari | <.0001|          |         |          |
| Narmada  | <.0001| <.0001   |         |          |
| Mahanadi | <.0001| <.0001   | <.0001  |          |

The Wilks’ k test revealed significant differences in morphometric characters among four populations ($p > 0.001$). Forward stepwise discriminant analysis of all the significant variables produced thirteen
discriminating variables (Table 4). These variables or morphometric truss measurements were found to be the most important characters in distinguishing the selected populations. The linear discriminant analysis produced an average percentage correct classification (PCC) of 90.3% for morphometric characters indicating a high rate of correct classification of individuals into their original populations (Table 5). The percentage of correct classification ranged from 86.1% (Godavari) to 93.9% (Narmada). It was highest for the population of river Narmada followed by river Mahanadi (92.9%), river Ganga (86.2%), and lowest for river Godavari (86.1%). The results attained from the PCC cross-validation test were analogous to the results. Additionally, the plot of the discriminant variables showed a pattern that reflects successful discrimination among populations of four rivers (Fig. 3).
Table 4
Summary of morphological features differentiating populations. Thirteen linear measurements were of major importance in the delineation of these populations.

| S.No. | Character Code | Morphological Feature | Body part                      |
|-------|----------------|-----------------------|--------------------------------|
| 1     | 1–2            | distance between anterior tip of snout at upper jaw to most posterior aspect of neurocranium | Head length 1                  |
| 2     | 1–7            | distance between anterior tip of snout at upper jaw to anterior attachment of ventral membrane from caudal fin | Body length                    |
| 3     | 2–4            | distance between most posterior aspect of neurocranium to end of dorsal fin | Mid body length 1              |
| 4     | 2–5            | distance between most posterior aspect of neurocranium to anterior attachment of dorsal membrane from caudal fin | Mid Body length 2              |
| 5     | 2–10           | distance between most posterior aspect of neurocranium to insertion of pelvic fin | Max. Body depth                |
| 6     | 2–11           | distance between most posterior aspect of neurocranium to insertion of pectoral fin | Head depth                     |
| 7     | 5–12           | distance between anterior attachment of dorsal membrane from caudal fin to end of operculum | Mid diagonal length 1          |
| 8     | 5–14           | distance between anterior attachment of dorsal membrane from caudal fin to anterior end of eye | Mid diagonal length 2          |
| 9     | 6–7            | distance between posterior end of vertebrae column to anterior attachment of ventral membrane from caudal | Half base of caudal fin        |
| 10    | 6–8            | distance between posterior end of vertebral column to end of anal fin | Caudal peduncle length         |
| 11    | 7–14           | distance between anterior attachment of ventral membrane from caudal fin to anterior end of eye | Mid body length 3              |
| 12    | 11–12          | distance between insertion of pectoral fin to end of operculum | Portion of head depth          |
| 13    | 11–13          | distance between insertion of pectoral fin to posterior end of eye | Post orbital length            |
Table 5

**Discriminant function analysis based on truss morphometric.** Discriminant function analysis of on 13 truss-based morphometric distances characters among in four populations (90.3% of original grouped cases correctly classified and 83.8% of cross-validated grouped cases were correctly classified).

| Predicted Group Membership | Species | Ganga  | Godavari | Narmada | Mahanadi | Total  |
|----------------------------|---------|--------|----------|---------|----------|--------|
| **Original percentage (%)**|         |        |          |         |          |        |
| Ganga                      | 86.2    | 3.4    | 3.4      | 6.9     | 100.0    |
| Godavari                   | 2.8     | 86.1   | 5.6      | 5.6     | 100.0    |
| Narmada                    | 3.0     | 3.0    | 93.9     | .0      | 100.0    |
| Mahanadi                   | 3.6     | 1.8    | 1.8      | 92.9    | 100.0    |
| **Cross validated percentage (%)** |         |        |          |         |          |        |
| Ganga                      | 72.4    | 6.9    | 3.4      | 17.2    | 100.0    |
| Godavari                   | 2.8     | 80.6   | 8.3      | 8.3     | 100.0    |
| Narmada                    | 3.0     | 6.1    | 90.9     | .0      | 100.0    |
| Mahanadi                   | 7.1     | 3.6    | 1.8      | 87.5    | 100.0    |

**Discussion**

Several statistical methods have been employed to study morphological divergences among wild populations of *S. sarana* collected from different geographic regimes. This is the first study on the population delineation of *S. sarana* using truss network analysis with geometric morphometrics. The results revealed that heterogeneity exists among examined populations of *S. sarana* procured from the specific sites of rivers (Ganga, Godavari, Narmada, and Mahanadi). Significant variations were detected for most of the analyses. The PCA loadings (truss analysis) of principal components revealed distinctness between populations. Though, there was a slight overlap found in the characters which were examined among the four groups. This separation was corroborated by DFA (truss analysis), showed significant morphological heterogeneity among populations, the level of differentiation between most of them as evidenced by a slight overlap of statistical data on derived plots.

Using geometric morphometrics, CVA plot obtained, have shown a slight level of overlaps among groups with a high percentage of correct classification suggesting differentiation among the examined populations. The PCA (geometric analysis) and DFA (geometric analysis) further confirmed the morphological heterogeneity among populations of *S. sarana*. The higher misclassification (DFA) observed for the Ganga with Mahanadi River and least with the Narmada. The biological variations of morphometric characters based on DFA are majorly associated with head morphology, covering lateral body lengths and caudal peduncle regions. Shape differences have been visualized with the deformation grids using geometric morphometrics. Geometric morphometry-based deformations grids (wireframes and relative warps) of average shapes between populations correspond to the high values of statistical
distance between them and confirm the distinctness of populations in their immediate anatomical context.

Overall, the variations among the four groups in this study were largely owing to the dissimilarities of morphometric characters broadly associated to head, and body characteristics. However, the shape differences observed in this study presents little practical use in terms of discriminating fish populations in the field. The visualization of the body shape differences, associated with other groups of correlated morphological traits, allowed to obtain a clear diagnosis of fish morphology for each population (Viscosi and Cardini 2011; Orlofske and Baird 2014). Visualization tools might help to further study of the putative underlying mechanisms involved (Manacorda and Asurmendi 2018). The result of the present study is in line with other studies based on truss analysis (Dwivedi 2019; Khan et al. 2012; Mohaddasi et al. 2013a, Hanif et al. 2019) and geometric morphometrics (Mohaddasi et al. 2013b; Geladakis et al. 2018; Pérez-Quinonez et al. 2018) which have shown the fish species to have a distinctive morphology.

The highest percentage of correct classification for the Narmada River population indicates greater distinction from the other populations which may be possibly due to west word flow of Narmada River compared to east word flow of other rivers. Overall, the selected populations were geographically isolated from each-others which could have hindered the movement of fish from intermingling with populations in other rivers. Therefore, the variability of morphological characters among populations possibly accredited due to separate geographical locations, the distance between the rivers, as well as the environmental variability of the river experienced by each population which leads to the local adaptations (Paugy and Lévêque 1999, Pardo 2002). The morphological variation could probably be coupled with the variation of feeding regimes and habitat circumstances (Langerhans et al. 2007; Sajina et al. 2011; Drinan et al. 2012; Khan et al. 2012; Lostrom et al. 2015; Jearranaiprepame 2017). Additionally, different reports indicate variations of the whole fish body are mainly due to fish inhibiting in different flow regimes (Jearranaiprepame 2017; Shukla and Bhat 2017).

Earlier efforts have been made to differentiate S. sarana populations using traditional morphometry (Siddik, et al. 2016). In the case of genetic studies, S. sarana, have only been carried out in Bangladesh from three geographically distant locations, analyses on RAPD revealed some degrees of genetic diversity among populations (Kabir et al. 2015). Furthermore, the overall accuracy of population differentiation in the present study is comparable to that found in other studies for closely-related Indian fishes. Findings reported by Mir et al. (2013) and Shukla and Bhat (2017) indicating that the results of the present study are in agreement with previous studies. Contrary, Das et al. (2013) reported low morphometric divergences (truss-based study) despite Cirrhinus mrigala populations were collected from isolated geographic locations.

Morphological differentiation can enable individuals to survive with existing environmental variability (Senay et al. 2015). Fishes are excellent model systems for studies on inter as well as intra-specific divergences to understand ecological correlates of morphological diversifications. Some factors were assumed to be controlling the differences observed such as plasticity owing to habitat dissimilarities or
could be due to environment and genotype interactions. Earlier, it was assumed that the variation of morphometric characters was exclusively genetic, but recent studies have established its relation with environmental factors (Georga and Koumoundouros 2010; Nahar et al. 2015; Sharker et al. 2015) and role of epigenetics cannot be ruled out as suggested by many scientists, population differentiation associated with ecological factors have the main element as epigenetic (Felsenfeld 2014).

As mentioned above, intraspecific variability can have huge ecological effects (Fridley and Grime, 2010; Becks et al. 2010; Bolnick et al. 2011). Charles Darwin indicated that variations among individuals of species offer the raw materials for natural selection. All hereditary characters in the genotype are not expressed in the phenotype. Further, variation not attributable to genetic factors not necessarily is environmental. Interestingly, the environment is often made responsible for non-genetic variations in phenotypes but it could be because of meta-stable epigenetic regulation (Wong et al. 2005). Considering that morphological variations are raw materials, truss and geometric analysis techniques are the best approaches to delineate populations on the bases of morphological characters. Results from the present study show that geometric morphometrics can provide additional information for shape delineation between populations that might otherwise be unnoticed. Further, the use of both truss and geometric morphometrics can provide deeper insight into the pattern of shape variations. This study could not answer whether are the results of morphological plasticity, genetic difference, or interaction of either mechanisms or epigenetic related hence, to resolve this, additional studies such as common garden experiments and epigenetic and/or genetics studies can be performed. More precise results might be obtained if larger sample sizes with a greater geographical extent were available. Geometric morphometrics analyses that include other aspects of fish morphology could enhance the precision of results.

Conclusion

To summarize, we quantified the morphological variation of populations of S. sarana from four major rivers of India. The basic characteristics of discrimination are overall body shape majorly associated with head morphology, covering lateral body lengths and caudal peduncle regions. Body morphology shows variation and could separate most populations, observed morphological variations provide good evidence for intraspecies heterogeneity between S. sarana populations. The study suggests that the S. sarana distributed across selected Indian rivers shows morphological plasticity. The high degree of classification accuracy of these two approaches advocates their extension to other problematic species and highlights their importance as exploratory tools in morphological based population studies.

Declarations

Ethics approval and consent to participate Fish specimens were obtained from the wild, directly from the commercial catches. The collection sites of fish specimens collected were fell outside Protected Areas (PAs). Fish were captured by gill nets. Fish if alive were euthanized with MS222 (Sigma) anesthesia and transported to the laboratory on ice to avoid damage to its morphological characters. The Research
Ethics Committee of the University of Lucknow, Uttar Pradesh, India has permitted the design and implementation. *Systomus sarana* is not considered a protected or endangered species in India and no special permits were required for handling and studying this fish species. All persons occupied in the capture, handling, holding, and processing of fish were aptly trained for their specific tasks during the procedure. All applicable international, national, and departmental guidelines for the care and use of animals were followed.

**Consent for publication** not applicable

**Availability of data and materials** The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Competing interests** The authors declare that they have no competing interests

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**Authors’ contributions** D.G. initiated the research, and together with M.T. and A.K.D. outlined the study. D.G. collected the samples and performed all preliminary analysis, investigation, methodology. M.T. provided guidance, done project administration and supervised the study. D.G. and A.K.D. assisted with data curation, software, validation, visualization. D.G. wrote the original draft of the manuscript, revised and rewrote it. D.G., A.K.D. and M.T. reviewed and edited the manuscript.

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**Figures**

![Map depicting sampling sites for the S. sarana populations from four rivers of India. (Sampling sites; 1: Ganga; 2: Godavari; 3: Narmada; 4: Mahanadi). The map was designed by Deepmala Gupta](image)

**Figure 1**

Map depicting sampling sites for the S. sarana populations from four rivers of India. (Sampling sites; 1: Ganga; 2: Godavari; 3: Narmada; 4: Mahanadi). The map was designed by Deepmala Gupta
Figure 2

Fourteen landmarks employed for analysis of morphological variation in S. sarana. Landmarks 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) end of dorsal 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) end of anal fin (9) origin of anal fin (10) insertion of pelvic fin (11) insertion of pectoral fin (12) end of operculum (13) posterior end of eye (14) anterior end of eye
Figure 3

Discriminant function plot based on DFA of 13 truss-based morphometric distances variables. (Group Centroids; 1: Ganga; 2: Godavari; 3: Narmada; 4: Mahanadi)
Figure 4

Scatter plot of the partial least square analysis in S. Sarana computed on shape (Block1 PLS1) and size (Block2 PLS1) variables
Figure 5

Deformation grid of wireframe graph showing the variation of the body shapes among populations of S. sarana (Light Blue: first river of each pair, Dark blue: second river of pair)
Figure 6

Deformation grid of relative warps graph showing the variation of the body shapes among populations of S. sarana

Figure 7

Plot of principal component analysis for S. sarana populations showing loadings of each sample on the first two principal components
Figure 8

Plot of canonical variate analysis for S. sarana populations showing frequency of specimen distribution in respective group on the first two axis
Figure 9

Discriminant function analysis from geometric morphometric variables showing original and cross-validation bar plots of S. sarana body shape between populations (Ganga- Godavari, Ganga-Narmada, Ganga-Mahanadi, Godavari-Narmada, Godavari-Mahanadi, Mahanadi-Narmada)