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Morphology

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DETERMINATION OF SEXUAL DIMORPHISM AND MORPHOLOGICAL VARIATION OF POOL BARB, *PUNTIUS SOPHORE* (CYPRINIFORMES, CYPRINIDAE), USING LANDMARK BASED GEOMETRIC MORPHOMETRIC ANALYSIS

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Determination of Sexual Dimorphism and Morphological Variation of Pool Barb, *Puntius sophore* (Cypriniformes, Cyprinidae), Using Landmark Based Geometric Morphometric Analysis. Roy, S., Ray, S. & Saikia, S. K. — Geometric morphometrics is an efficient way to quantify biological shape variation and its covariation with other biotic or abiotic factors. The geometric information of an object is measured in a non-linear shape space after removing size, orientation, and position-related effects. This study was aimed to determine sexual dimorphism and morphological variation in body shape using landmark-based morphometrics by means of relative warp analysis. The indigenous freshwater fish *Puntius sophore* (Hamilton, 1822) (Cyprinidae) was selected as the model organism for the study. Adult male and female individuals were collected during July–August 2019 (breeding season) and March–April 2019 (non-breeding season) from the ponds of Bolpur, West Bengal, India. Prior to analysis, the images were digitised using the appropriate fourteen landmarks and relative wrap analysis was performed using appropriate software. The results were then analysed, and the maximum relative displacements of adjacent landmarks were interpreted using canonical variate analysis (CVA) and principal component analysis (PCA). Significant body shape differences were found to be present in the non-breeding season and breeding season (male and female) populations of the species. Thus, geometric morphometrics can be used to understand the sexual dimorphism in natural populations of small fish species.

Key words: freshwater fish, *Puntius sophore*, morphological variation, sexual differences, geometric morphometrics.

Introduction

Morphometrics is the study of variations in the linear measurements of an organism. Within traditional morphometric studies, a single measurement can be perceived as a variable. However, independent analysis may at times result in diverging conclusions during biological interpretation. Geometric morphometrics, on the other hand, visualizes shape as a configuration of the relative positions of landmarks in a non-linear space (Zelditch et al., 2012). The accurate identification of each individual specimen is imperative in order to investigate biological characteristics such as growth, trophic relationships, ontogenetic events, and more. The fine-scale shape assessment may possess significant value in identifying the patterns of morphological variation within a species (Dorado et al., 2012). An additional advantage lies in the incorporation of the covariance between the landmark configurations in the analysis. Consequently, quantification of shape becomes more realistic, unveiling the adaptive value of shape, while the quantitative information permits a more comprehensive understanding of evolutionary processes. Sexual dimorphism may arise because of varying selection pressures on males and females, stemming from their distinct reproductive responsibilities, foraging strategies, energy intake, and energy allocation (Nitychoruk, 2013). The varying trait values can differentially influence the success of mating in each sex, ultimately leading to the evolution of sexual dimorphism in morphological characteristics (Silos, 2015).

Aquatic ecosystems are acknowledged as a potent factor in shaping the physical structure of an organism throughout its development (Solis et al., 2015). Thus, differentiation populations hold significant value in management and the preservation of aquatic biodiversity (Ibañez et al., 2007). Determining the characteristics of sexual dimorphism in terms of size, shape, and behaviour is a crucial aspect that must be taken into account in fisheries management. This is because such variations between the sexes can potentially result in the excessive exploitation of one sex, leading to an imbalance in the population's sex ratio (Nitychoruk, 2013). Besides, morphometric studies is a pre-requisite to standardize any indicator parameter for climate change and small fishes has recently projected as a potential indicator for climate change (Zhang et al., 2022).

The genus *Puntius* holds great importance in terms of biodiversity and taxonomy, as numerous new species under this genus have been identified in South East Asia, especially India (Pethiyagoda et al., 2012). There are reviews, articles, and experimental studies on *Puntius sophore* about its feeding habit, morphometrics, reproductive biology, distribution pattern, and so on (Pethiyagoda et al., 2012; Roy et al., 2020; Roy et al., 2023; Sarkar et al., 2019). *Puntius sophore* (pool barb, spotfin swamp barb, or stigma barb) is a small freshwater indigenous fish (SIF) of the family Cyprinidae, found in rice fields and associated ponds of many Asian countries like in India, Bangladesh, Bhutan, Nepal, China, Pakistan, Afghanistan, and Sri Lanka. They are benthopelagic in nature and become adults at 7–8 cm of length (Talwar & Jhingran, 1991). SIFs are a rich source of micronutrients for rural households. Further, their introduction into polyculture systems has proven to increase overall fisheries production (Mohanty et al., 2013). However, their natural populations have been documented as experiencing a decline owing to the destruction of their habitats and the presence of pollution (Aziz et al., 2021; Mandal & Nandi, 2015). Hence, the present study was conducted to investigate the morphological divergence and sexual dimorphism in the adult populations of *Puntius sophore* (Hamilton, 1822). Identifying the selection pressures on morphological divergence can help us better understand the species reproductive biology and population trends. Shape differences between males and females in the population were visualized and analysed using landmark-based geometric morphometric analysis. Also, a correlation was established with the ecological insights derived from existing literature.

Material and Methods

A total of 55 *P. sophore* individuals were collected from local ponds in Bolpur, West Bengal, during July–August 2019 (breeding season) and March–April 2019 (non-breeding season). 15 female, 16 male and 15 non-breeding season specimens were used. Collected samples were immediately transferred to an ice box and brought to the laboratory for morphometric analysis. All ethical guidelines have been followed while sampling and processing fish for study.

In this study, male fish with an average weight of 5.68 gm and an average length of 7.55 cm and female fish with an average weight of 12.95 gm and an average length of 9.45 cm were used for analysis. Only adult specimens were used in the analysis to reduce the ontogenic effect on shape. The male and female individuals were primarily identified by external characters and later by dissection. Left-side images of the specimen were taken for each individual (fig. 1, a). The images were further digitized and analyses were done using MorphoJ (https://morphometrics.uk/MorphoJ_page.html) and Thin Plate Spline (Tps) series of software (https://www.sbmorphometrics.org/soft-tps.html).

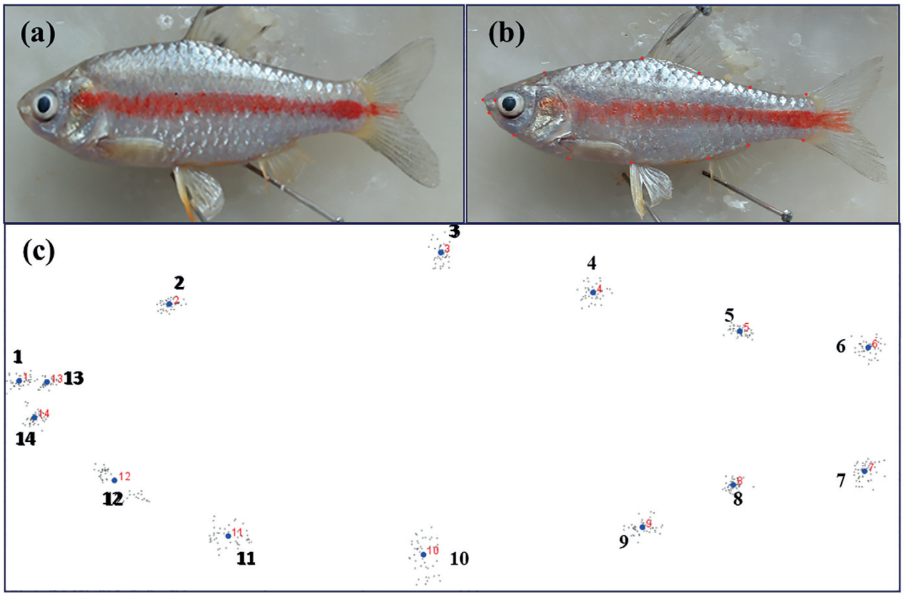

Fig. 1, a — male individual in breeding season; b — digitized image of *P. sophore* with the 14 landmarks (red points) used for the geometric morphometric analysis: c — scatter plot of 14 landmarks configurations after Procrustes Superimposition.

At first, images were converted to. tps files using the Tps Utility version 1.7 programme (Rohlf, 2016). Then landmark points with functional significance (reference) were digitized on each specimen image by TpsDig2 version 2.26 software (Rohlf, 2016). A landmark is a point of correspondence on each object, similar to every specimen (fig. 1, b). A total of 14 landmarks (equivalent to 14 X and 14 Y Cartesian coordinates) were selected (table 1), and the whole set of information was recorded for a particular shape (fig. 1, c). The shape comparisons were between the matching configurations of landmarks and changes in their relative positions. Here, an individual landmark has no significance (Park et al., 2013; Zelditch et al., 2012).

Finally, the shape information was extracted by generalized Procrustes analysis (GPA) using TpsRelw 1.65 (Rohlf, 2016). Before that, all the non-shape components like size, position, and orientation-related variations were eliminated from the dataset based on landmark coordinates (Klingenberg, 2011; Dorado et al., 2012; Park et al., 2013). Multivariate statistical analyses were done by using the coordinates of the superimposed landmarks. Superimposition was conducted through a three-step process. First, the matrix of landmark coordinates was translated to a common centroid located at the point (X, Y = 0, 0). Second, the centroid size was scaled to one. Lastly, the configurations of the landmarks were rotated in order to minimize the sum of squared distances between the corresponding landmark points (Bookstein, 1991; Dorado et al., 2012; Zelditch et al., 2012). At the end of this process of centering, scaling, and rotating, the loss of degrees of freedom was observed. However, the 28 landmark variable coordinates remained intact as the reduction in degrees of freedom affected all coordinates equally. This is the initial advantage of utilizing tps software (Zelditch et al., 2012). Following the superimposition, a weight matrix consisting of both uniform and non-uniform components was produced (Bookstein, 1991). The relative warp (RW) scores were used in Canonical Variate Analysis (CVA) to test for sexual discrimination in external body shapes within *P. sophore* by using the MorphoJ software, version 1.06d. Further, it was used to do principal component analysis (PCA) of covariance matrix of the digitized samples.

Table 1. List of landmarks used for geometric morphometric analysis

Number	Anatomical points
1	Anterior tip of upper snout
2	Dorsal occiput
3	Anterior insertion of the dorsal fin
4	Posterior insertion of the dorsal fin
5	Points of maximum curvature of the peduncle
6	Dorsal region of the caudal peduncle
7	Ventral region of the caudal peduncle
8	Posterior insertion of the anal fin
9	Anterior insertion of the anal fin
10	Anterior insertion of the pelvic fin
11	Inferior insertion of the pectoral fin
12	Ventral insertion of operculum
13	Anterior orbit of the eye
14	Posterior extremity of lower jaw

Results

Evaluation of sexual and seasonal effects by Canonical variates analysis (CVA)

Significant body shape differences were present in the non-breeding season and breeding season (male and female) populations of *P. sophore* along the first two canonical variate (CV) axes. The first axis explains 70.387 % of variation, and the second axis explains 29.613 % of variation (fig. 2). The Mahalanobis distances and procrustes distances among groupsare described in table 2.

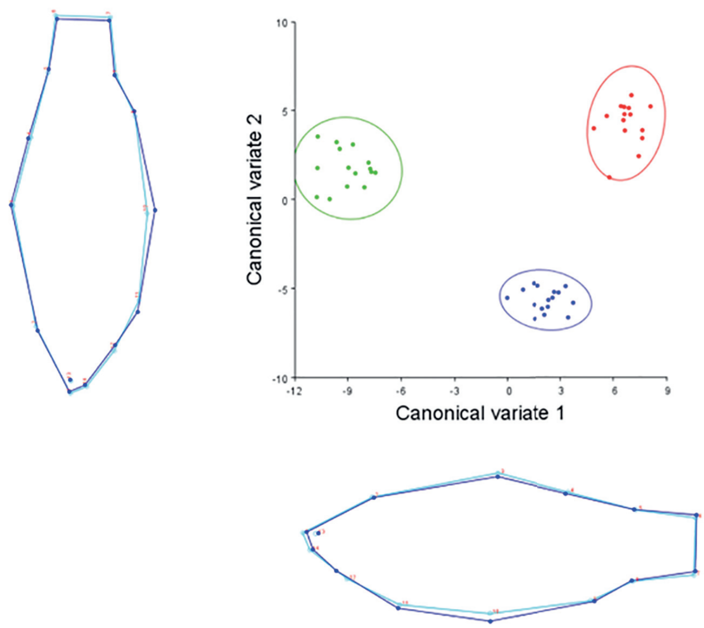

Fig. 2. Distribution of non-breeding season population and the breeding season (male and female) population along first and second canonical variate axes (female in red, male in blue and non-breeding season population in green).

Principal components analysis of body shape

Principal component analysis of breeding and non-breeding season populations showed a total of 64.41 % of variation explained by the first two axes (fig. 3, a, b). A total of 83 % of variations were explained by the first five axes (each contributing at least > 5 %). There was no external sexual difference between the sexes in the non-breeding population. But they formed morphologically different and distinguished cluster. Most of the common direction of sexual dimorphism is evident along PC1 and PC2 in the morpho-space (fig. 4). The highest sexual differences are mainly based on the anterior insertion of the pelvic fin, the operculum insertion region, and the dorsal and ventral regions of the caudal peduncle. The null hypothesis of equal change of external body shape in the sampled population was rejected at 95 % confidence level. Females of breeding season have a larger pelvic and head region than non-breeding season population.

Table 2. The Mahalanobis distances and Procrustes distances among groups

Mahalanobis distances	Female	Male	*p* value
Male	10.9334		< .0001
Non-breeding	15.9643	13.4223	< .0001
Procrustes distances	Female	Male	*p* value
Male	0.0389		< .0001
Non-breeding	0.0547	0.0454	< .0001

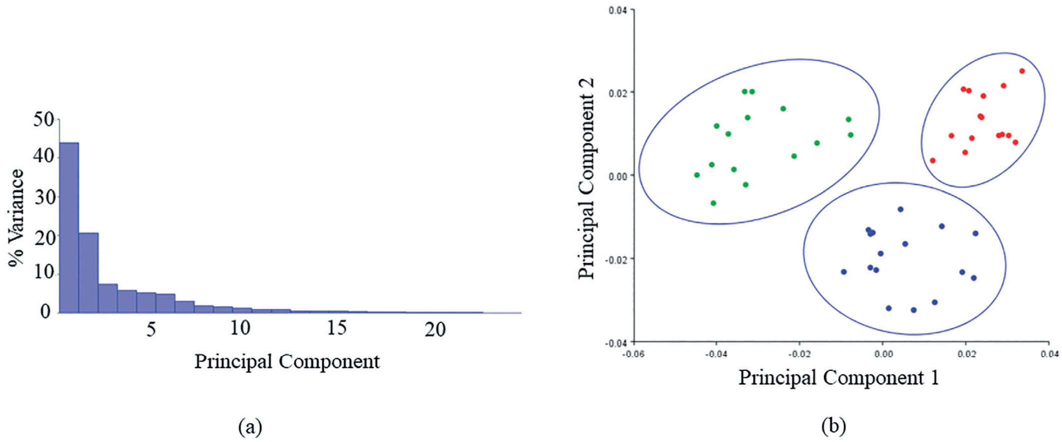

Fig. 3, a — eigenvalues plot of the proportion of variance described by each PC, b — scatter plot showing scores on the first two PCs for the sample of non-breeding season and breeding season fish population (female in red, male in blue and non-breeding season population in green).

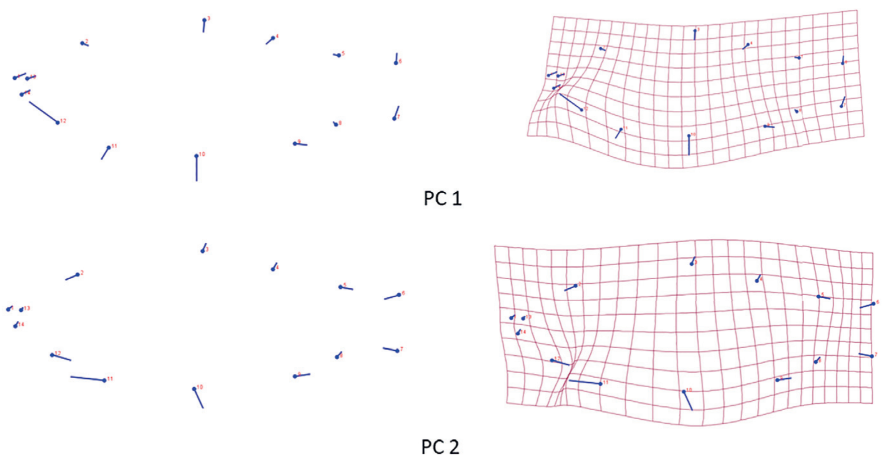

Fig. 4. Change of body shape along principal component axis (PC 1 = 43.827 %, and PC 2 = 20.578 %). Left side is the lollipop plots. Right side is the transformation grids of shape change.

Discussion

Sexual dimorphism is of three types: 1) monomorphic; 2) temporarily dimorphic or dichromic; and 3) permanently dimorphic. *P. sophore* belongs to the second class. They temporarily show colour differences in breeding seasons (Korbuly et al., 2007; Parés-Casanova, 2014). If sexual dimorphism is observable in the external body form or colouration of a species, it can have significant evolutionary implications for the process of mate selection and the overall reproductive fitness of individuals within that species (Gunawickrama, 2008). Based on the relationship between fish standard length (SL) and gonadosomatic index, it was found that female *P. sophore* matures at 4.2 cm of standard length. Also, the relative growth rate of fork length to standard length showed negative allometric growth at sizes 4 cm SL, indicating sexual maturity (Ahamed et al., 2015).

The study findings demonstrated the importance of geometric morphometrics in understanding the sexual dimorphism of *P. sophore* and identifying the variable regions that contribute to gender differences. Results of the CVA test revealed significant dimorphism in the overall body shapes (containing 14 landmarks) between the sexes of the breeding population as well as between the breeding and non-breeding populations. A CVA scatter plot was further used to summarize the results. The first two canonical axes produced maximal separation. Further analysis of body shape using the PCA technique also showed differences in fish body shape. In breeding season, the body shapes of males were more slender than females. Females had a deeper body, a bigger head region, and a shorter caudal peduncle region than males. The ecological interpretation is that the bigger head region is an adaptation for feeding in females. The wider buccal volume could help in maximizing prey consumption. The heavier abdominal region of females gets support from the convex curvature of the dorsal part of the tail region (Dorado, 2012). Deeper body height increases manoeuvrability during foraging (Roy et al., 2020). The wider anal fin base in males indicates sexual competition. The morphological trait aids stability during the fertilization process and swimming power (Dorado, 2012; Gunawickrama, 2008). This elongated caudal peduncle observed in males might potentially be linked to courtship behaviours (Duff, 2019).

Conclusion

This study also suggested the possibility of using geometric morphometric analysis as a tool for sexual dimorphism in small freshwater fish. The existence of such dimorphic traits is postulated to arise from the influence of different selective pressures. Today, freshwater ecosystems are at risk due to a variety of factors, including unsustainable resource consumption, altered wetland drainage, intensified land use, altered flow, urbanization, and climate change. These factors make freshwater species more vulnerable to extinction in the future than their terrestrial equivalents (Roy et al., 2021). Understanding species population trends like the male-female ratio can help restore their declining population. Geometric morphometric is a very useful technique. It can identify slight differences in body geometry that may not be revealed through traditional morphometrics. Although the process is time-consuming, and demands a strong grip on image analysis performance and statistical computation (Duff, 2019). To get more reliable results, it is advised to use more sampling sites and more samples. Employment of high-resolution cameras can produce sharp, high-quality specimen pictures to ease landmarking the specimens (Silos et al., 2015).

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Declaration of Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical statement

All attempts were made to maintain standard ethical norms for the fish (approval of institutional ethical committee ref. AEC/III-14/2020 dated 17/10/2020) in the subject during the experiment.

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