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Describing body shapes of the white goby, *Glossogobius giuris* of Lake Buluan in Mindanao, Philippines using landmark-based geometric morphometric analysis

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Abstract

In this study, landmark-based geometric morphometrics was used to determine morphological variation in body shapes of the white goby, Glossogobius giuris (Hamilton and Buchanan, 1822), an economically important fish species of Lake Buluan in Mindanao. A total of 60 images for both male and female were digitized and subjected for geometric morphometric analysis. Several dimorphic traits were revealed by relative warp analysis. These are deeper-bodies resulting to stout body outline in females relative to the more slender body outline of the male population. Females also exhibit greater degree of body curvature. Males however have longer gape length and caudal peduncle and larger pectoral and anal fin bases. MANOVA further revealed significant body shape differences between sexes.

Keywords: Landmarks, geometric morphometrics, relative warps.

Introduction

In fishery, understanding the genetic structure of fish species is necessary for the formulation of proper management programs. Morphological variation in body shapes between sexes could be one area that could be considered. Within a species, adult shape is conserved and phenotypically integrated^{1,2}, often persisting throughout the geographical range of the species and over extended geological time³. Different reproductive life histories between males and females could constrain the extent of shape dimorphism among populations⁴. However, diversifying selection can facilitate subtle morphological differences between the sexes⁵. The body shape of the white goby, Glossogobius giuris (Hamilton and Buchanan, 1822) was examined exploiting the techniques of geometric morphometrics, which allow the precise and detailed analysis of shape change and shape variation in organisms on the basis of positions of homologous anatomical landmarks or shapes of outlines⁶. Geometric morphometric methods also allow for the graphic presentation of results for visual display and comparison of shape changes among the forms of interest. These methods represent an interesting alternative to traditional morphometrics, based on measured distances, angles, and ratios, and have demonstrated to be particularly useful to studies involving the comparison of form in fishes^{3,7,8}

General observations show that males of *G. giuris* have longer dorsal fins and their overall color is brighter than the females thus this study was conducted to determine whether sexual differences in body shapes are exhibited by *G. giuris* using geometric morphometrics.

Material and Methods

Fish Specimens: A total of sixty *G. giuris* (30 per sex) were sampled from Lake Buluan, Lutayan, Sultan Kudarat from June to December 2008 and February to March 2009. Only sexually mature fishes were used to reduce the amount of intrapopulation shape variation based on ontogeny. The samples were transported in styropore box with ice and processed immediately. Sexing was based on external morphology and was later confirmed by direct examination of the gonads⁹. Both the left and right sides of the fish samples were scanned at uniform dpi (600) using a CanoScan model D646U flatbed scanner.

Landmark Selection and Digitization. A total of twenty landmarks (equivalent to 20 X and 20 Y Cartesian coordinates) were selected to provide a comprehensive summary of the morphology of the fishes (figure 1). The landmarks digitized in this study are standard points used in fish morphometrics and are said to have both evolutionary and functional significance^{10,11,12,13,14}. The landmarks were digitized on both sides of each specimen image using the TpsDig ver. 2.10^{15} . Digitization was done in tri-replicates for each fish sample.

Shape Analysis. The X and Y coordinate of the landmark points that were digitized from the left and right images of the fishes contain both shape and non-shape (differences in the position and orientation of the fishes in the flatbed scanner; size differences) components of variation^{16,17}. Since this study focused only on shape differences, the non-shape components were necessarily removed prior to analysis via Generalized Procrustes Analysis (GPA) using TpsRelw 1.36¹⁸. GPA aligned

in all the specimens in morpho-space, eliminating size and rotational/translational differences. GPA proceeded as follows: first the landmark coordinates were translated to a common centroid at the origin of the reference coordinate system or at point (X=0, Y=0). Then, the set of landmark coordinates of each fish sample were scaled to unit centroid size, thereby removing size differences and permitting analysis of body shape. Finally, the landmark configurations of all fishes were rotated to minimize the sum of squared distances between corresponding landmarks². This step removed residual translational and rotational differences among the samples resulting from differences in the way the fishes were oriented in the flatbed scanner when the digital images were taken.

Using the thin-plate spline equation and the standard formula for uniform shape components, a weight matrix (containing uniform and non-uniform shape components) from the aligned specimens were generated². Variability in body shapes was then examined via relative warp (RW) analysis of the set of uniform and non-uniform components of shape using TpsRelw 1.36¹⁸. The RW scores were then subjected to Multivariate Analysis of Variance (MANOVA) and Canonical Variate Analysis (CVA)

to test for sex differences in body shapes in *A. testudineus* using the PAST software ((PAleontological Statistics, version 1.27)¹⁹.

Results and Discussion

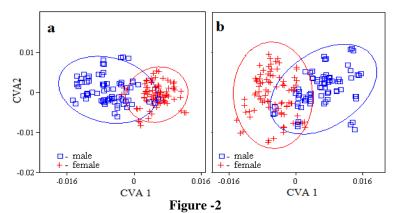
Significant differences in body shapes of the two sexes of *G. giuris* (right image) can be seen based on the distribution of the samples along the first two canonical variate axes (Wilks' lambda = 0.1617; Pillai trace = 0.8383; P-Value = 1.559E-35). As shown in figure 2, the first axis explains much of the variation between the sexes and accounts for nearly 100% of the variance (Eigenvalues for CV1 and CV2 are 5.183 and 9.852E-16, respectively).

Figure 2b shows significant differences in body shapes of the two sexes of *G. giuris* (left image) based on the distribution of the samples along the first two canonical variate axes (Wilks' lambda = 0.1585; Pillai trace = 0.8415; P-Value = 4.524E-36). The first axis explains much of the variation between the sexes and accounts for nearly 100% of the variance (Eigenvalues for CV1 and CV2 are 5.31 and 1.11E-15, respectively).



Figure -1

Landmarks' description of the White Goby, *G. giuris* (A. female, B. male): (1) snout tip; (2) and (3) anterior and posterior insertion of the dorsal fin; (4) and (6) points of maximum curvature of the peduncle; (5) posterior body extremity; (7) and (8) posterior and anterior insertion of the anal fin; (9) insertion of the pelvic fin;(10) insertion of the operculum at the lateral profile; (11) posterior extremity of premaxillar; (12) centre of the eye; (13) superior insertion operculum; (14) beginning of the lateral line; (15) point of maximum extension of operculum on the lateral profile; (16) and (17) superior and inferior insertion of the pectoral fin; (18) and (19) superior and inferior margin of the eye; (20) superior margin of the preoperculum



Distribution of the two sexes of G. giuris (a) right image and (b) left image along the first two canonical variate axes

Sexual dimorphism in *G. giuris* was tested using Multivariate Analysis of Variance (MANOVA). MANOVA reveals that there is a significant sexual dimorphism on its overall body shape. This is based on the results of the two statistics calculated from MANOVA which are the Wilks' lambda and Pillai trace. These results are further summarized by the canonical variate analysis (CVA). Canonical Variate Analysis is one of the more interesting applications of multivariate statistics. The technique is used to examine interrelationship between a number of populations simultaneously with a goal of objectively representing the interrelationships graphically in few dimensions (ideally two or three). In this study, two dimensions were utilized. The axes of variation are chosen to maximize the separation between the populations 20 .

In all the CVA scatter plots presented, both male and female points overlap around zero of the first and second axes. The CVA produces a scatter plot of specimens along the first two canonical axes, producing maximal separation and second to maximal separation between the two sexes of each fish species evaluated¹⁹. Thus, the results from MANOVA and visualized by the CVA scatter plots revealed significant differences between sexes of *G. giuris* based on their body shapes.

White Goby, *G. giuris* is a benthopelagic fish and native in Lake Buluan. The body is brownish yellow with rounded spots on the sides. Its dorsal fins are light with brownish spots. Pectoral and caudal fins are usually gray and are hyaline. Males are longer than females and possess longer dorsal fins. In addition, male goby has straight, thin and pointed genital papilla while female has short, fleshy and circular one²¹.

In this study, patterns of intra-specific differences in body shapes within each sex were summarized via frequency histograms of the RW scores (figures 3 and 4). Looking at the results of the analysis for the females (figure 3), the entire relative warps generally indicate continuous variation in body depth among the female fishes. The trend in shape variation

among the females reflects selection towards bodies capable of supporting large number of eggs.

Mean shapes using relative warp analysis show sexual dimorphism in the body shapes of G. giuris. Females are deeper-bodied resulting to stout body outline in comparison to the more slender body outline of the male population (figure 4). In addition, females have shorter standard length than males. Males have longer gape length and caudal peduncle. Gape length is an important feature that relates to varied foraging techniques of fishes¹⁷. A shorter or longer peduncle may reflect the different swimming ability of the fish. These body shapes may not necessarily represent the phylogenetic history of populations but rather adaptations to local trophic and environmental conditions²². In addition, males were observed to have larger pectoral and anal fin bases. The frequency histograms of the relative scores (RW) also reveal that females exhibit greater degree of curvature of the body than the males. This could be attributed to the bulkier bellies of females as a consequence of their reproductive role. Similar curvature of the body but related to size and independent of genetic and sexual dimorphism was also observed in the body shapes of trout Salmo trutta²³.

One prediction of sexual dimorphism is that the differences in the reproductive roles occupied by the sexes should influence patterns of selection and thus should ultimately lead to sex differences in morphology. Males should have adaptations that increase the probability of acquiring mates and of success at male-male competition. Females should be under selection to acquire process and store energy to facilitate the production of offspring²⁴. These were also observed in other fishes suggesting that such patterns of body shape variation are associated with the maneuverability of fishes 25,26,27 . On the other hand, shallower body in most freshwater fishes can be a function of reduced predation pressure as observed in most freshwater sticklebacks³. In addition, larger fins should aid in stability and control during swimming and may help males position themselves optimally relative to females during spawning in order to maximize fertilization success²⁴.

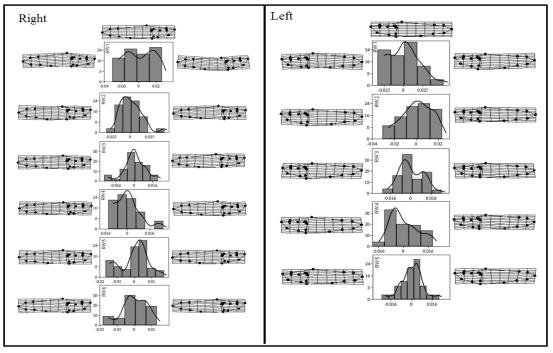


Figure -3

Summary of the geometric morphometric analysis showing the consensus morphology (*uppermost panel*) and the variation in body shape among the female population (right image) of *G. giuris*, produced by the first six relative warps RW explaining 83% of the total variation. [Right image: (RW1=31.71%,RW2=21.24%, RW3 =9.67%, RW4 =8.28%, RW5=6.63%, RW6 = 5.47%)], left image [(RW1=37.30%,RW2 =16.58%, RW3 =8.10%,RW4 =7.62%, RW5 = 6.48%)]

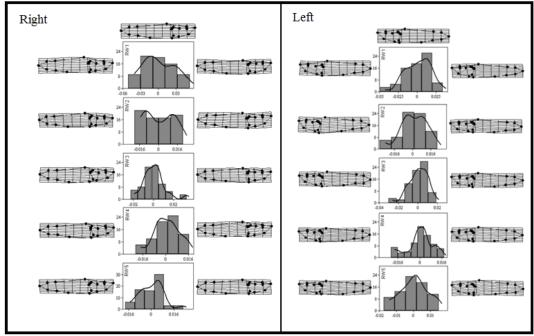


Figure-4

Summary of the geometric morphometric analysis showing the consensusmorphology (*uppermost panel*) and the variation in body shape among the male population (right image) of *G. giuris*, produced by the first five relative warps (RW) explaining more than 82% of the total variation. [Right image: (RW1 = 51.99 %, RW2 = 10.76%, RW3 = 8.04%, RW4 = 6.22%, RW5 = 5.05%)] [Left image: (RW1 = 33.65 %, RW2 = 15.79%, RW3 = 10.87%, RW4 = 9.14%, RW5 = 7.16%)

Conclusion

The results of the study underlie the utility of the method of Geometric Morphometrics in describing shape differences sexes in *G. giuris*. In this study, landmark-based geometric morphometrics was very useful in describing body shapes of *G. giuris*. Significant sexual dimorphism in body shapes of this fish is argued to be brought about by differences in the patterns of variation within each sex. Female population exhibits deeperbodies resulting to stout body outline and greater degree of body curvature relative to the more slender body outline of the males. On the other hand, males have longer gape length and caudal peduncle and larger pectoral and anal fin bases than their female counterparts. Such traits of males are believed to enhance their mating opportunities.

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