



# Body Shape Variation in the Goby, *Glossogobius giuris* Collected in Selected Areas in the River of Norzagaray Bulacan Using Landmark-Based Geometric Morphometrics

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**Abstract.** The study was conducted to analyze body shape variations between sexes of *G. giuris* in different collection sites in Norzagaray River, Bulacan, Philippines using the tools of *geometric morphometrics* (GM), a combination of advanced different statistical tests to describe variations in shapes. The comparative study of *G. giuris* population structure using landmark-based morphometrics can be the reliable tool to describe variations in population structures of the fish from different areas of the river. The fish were collected and scanned for image capturing. After image capture, the fishes collected were preserved, dissected following the protocol of identification, authentication and gender determination by the Bureau of Fisheries and Aquatic Resources (BFAR) - Region III, Maimpis City, Pampanga, Philippines. The images of collected individuals of *G. giuris* were subjected to landmarking to obtain the x and y coordinates of the landmarks using the TpsRelw software. The raw coordinates were subjected to procrustes-fitting to superimpose the different orientation and position of the specimens. Relative warps Score generated by the TpsRelw were subjected to different statistical tests such as MANOVA, Discriminant Function Analysis and Canonical Variate Analysis. Results showed no differences in body shapes between sexes of *G. giuris* although variation within sexes were found to be the major contributor for the differences observed populations. This study has shown that GM is a useful tool to be able to quantitatively describe variations in populations.

**Keywords.** Geometric morphometrics; MANOVA; CVA; Relative warps

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## 1. Introduction

*Glossogobius giuris* (Hamilton, 1822), a member of the Gobiidae (Perciformes), lives in sea, brackish- and fresh waters. It is common in inland freshwater bodies in several Asian countries, where it occurs in streams, canals, ditches and ponds with rock, gravel or sand bottoms, feeding on small insects, crustaceans and small fishes [1]. In Bulacan, Philippines, gobies are fished for food and are commanding a good price in the market. Observations show the gobies sold were of different qualitative morphological attributes. Few attempts have been made to study morphological variations in this group of freshwater gobies in the Philippines [2] since there are several factors that can affect morphological characteristics of fish such as flow rates, depth level of the river, temperature and pollutions, major threats to this species are not currently known in the area. It is hypothesized that potential threats may include the damage and or modification of the river systems such as the creation of dams which may adversely affect recruitment. Another is of the utilization of the fish as food and is considered a localized threat and may have affected the population structure as reflected in the differences in sizes of caught fish from the different parts of the river. Since morphometric analysis of fish is an important key in ichthyology for it can be used to quantify a trait of evolutionary significance and detecting changes in the shape, deduction of their ontogeny, function or evolutionary relationships, the use of advanced tools like geometric morphometrics can help statistically test hypotheses about the factors that affect their body shapes. Traditional morphometric method shows some significant error and inaccurate results thus the importance of utilizing more appropriate quantitative tools in morphometric analysis. Using the advance technologies such as digital imaging, computer softwares and different statistical applications, the tools of Geometric morphometrics (GM) using landmark-based analysis was used to quantitatively analyzed and describe variations in body shape of the fish. The application of GM in describing variations in body shapes and exterior forms using automated softwares has been a welcome respite in the quantitative description of variations that cannot be done by traditional morphometry such as manual measurements that may lead to different errors. GM eliminates the effect of size and only attribute the variations in the fish to the shape of the body, tools in geometric morphometrics is applied [3, 4]. This analysis will help understand the nature of morphometric variations in *G. giuris*, which is perceived the most reliable means of assessing the evolutionary adaptation of a species to its environment. The study also helps determine morphological variations in body shapes between sexes of *G. giuris* which could be an important biological information useful not only in biodiversity studies but also in the implementation of proper fishery management practices for the fish [5].

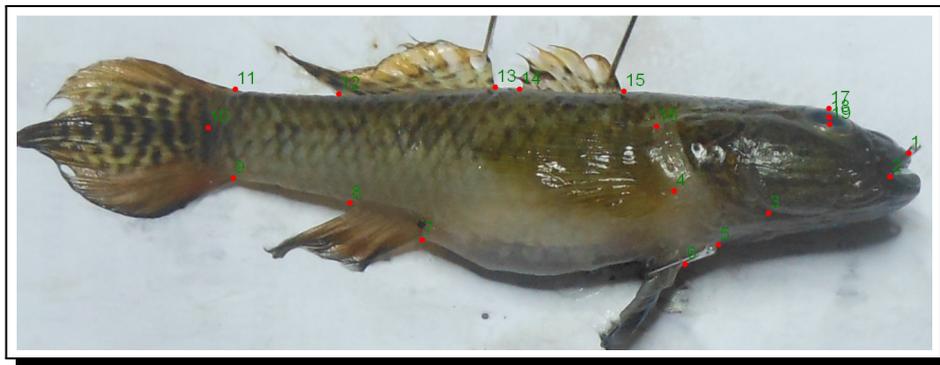
## 2. Methodology

*G. giuris* samples were collected in five stations of the Norzagaray River located in Barangay Maticic, Norzagaray, Bulacan, Philippines. The stations and its map coordinates were determined using GPS mobile device. Fish collection was done using back pack electrofishing to stun fish before they are caught by fishing nets. Proper care in handling of the fish during collection is necessary to ensure specimens are in good condition before image capture.

**Table 1.** Collected samples in each station

Station	Male	Female
Kanyakan	18	12
Calumpang	9	10
MaticticIbayo	13	17
Bakas	14	11
Laog	5	7
	N = 59	N = 57
	Total of 116	

For image capture, individual fish was laid on the dissecting pan side up with fins spread to make sure that the landmarks can be easily detected. A ruler was placed on the dissecting pan for scaling. Tags were placed on the pan alongside the fish for identification. Fish were photographed with the camera parallel to the plane of the dissecting pan, with the ruler and the tag in the field of view of the digital scanner [6]. Determination of sex was through the gonads identified upon dissection of the fish. The collected specimens were then delivered to Bureau of Fisheries and Aquatic Resources (BFAR) City of San Fernando, Pampanga Philippines for authentication and identification of fish.



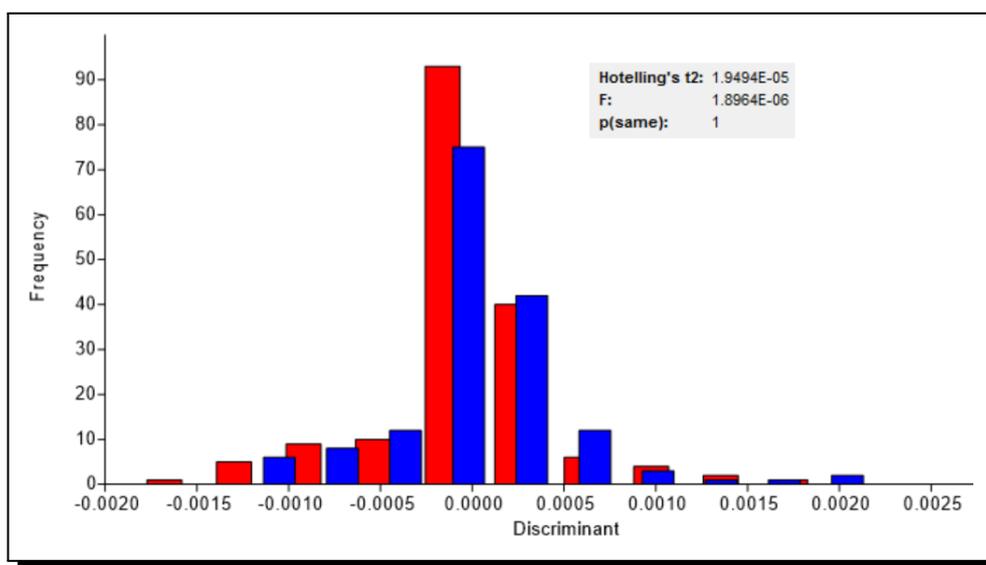
**Figure 1.** A scanned image of *Glossogobius giuris*, digitized with the 19 landmarks as follows: (1) snout tip; (2) and (3) anterior and posterior insertion of the first and second dorsal fin; (4) and (6) dorsal and ventral region of the caudal peduncle where there is the greatest curvature; (5) posteriormost body extremity; (7) and (8) posterior and anterior insertion of the anal fin; (9) insertion of the pelvic fin; (10) insertion of the operculum on the lateral profile; (11) posterior extremity of premaxillar; (12) centre of the eye; (13) superior insertion of operculum; (14) point of maximum extension of operculum on the lateral profile; (15) and (16) superior and inferior insertion of the pectoral fin; (17) and (18) superior and inferior margin of the eye; (19) superior margin of the pre-operculum

Canon LiDE 50 with configuration of 1200 DPi was used to get a fine detail of the image. Landmarking of the image made use of the software “TPSDIG” software [7] for scaling, taking of the x-y coordinates of the identified landmarks (Figure 1). Generalized Procrustes Analysis were used to orient the differences of position of the fish after image acquisition followed by Multivariate analysis of variance (MANOVA), Canonical Discriminant Analysis (CVA) and

Cluster analysis. The software used for the statistical analysis is the paleontological statistics software (PAST) capable of handling large amounts of data and can perform all of the analyses covered in the text and much more it is a free software environment for statistical computing and graphics [8].

### 3. Results and Discussion

Canonical Variate Analysis, a multivariate technique which is concerned with determining the relationships between groups of variables in a data set was also used to explore the interrelationship between a number of populations simultaneously. The goal was of objectively representing the interrelationships graphically in few dimensions which in this study, two dimensions were utilized to maximize the separation between the populations relative to the variation within each of the populations [9]. CVA results (Wilk’s lambda=1, F=1.762E-06, P=1.0) (Figure 2) and discriminant analysis (Hotelling’s t2: 1.9494E-05, P=1) (Figure 2) which were not significant. Only 51.56% was correctly classified indicating that the populations of *G. giuris* collected show no sexual dimorphism.



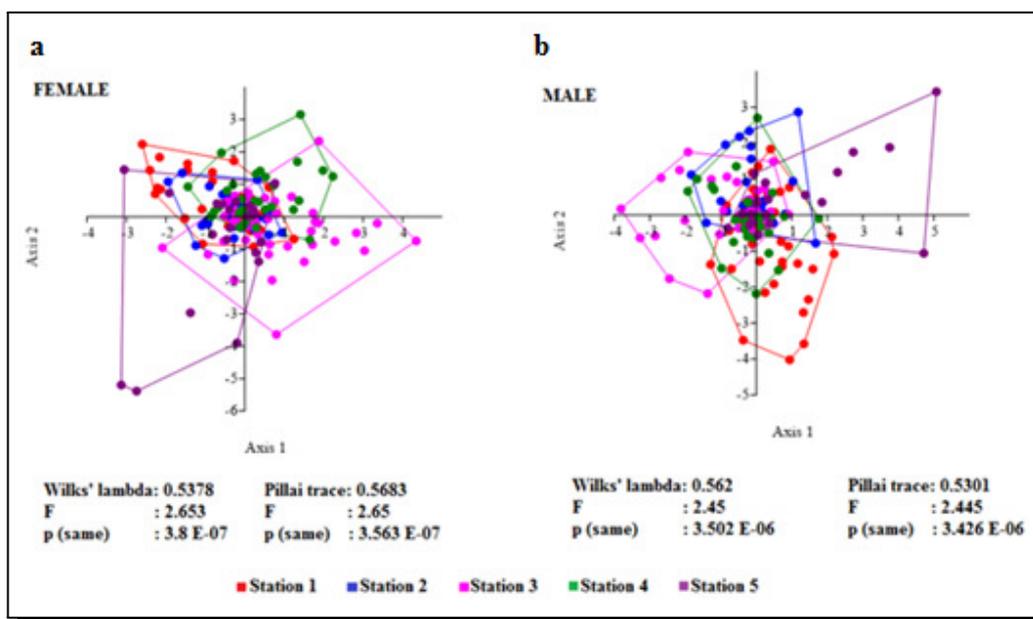
**Figure 2.** Distribution of individuals in the two sexes of *G. giuris* based on discriminant analysis

**Table 2.** Classification of body shapes based on discriminant analysis

	Female	Male
Female	53.80	46.20
Male	47.21	52.80
%correct classified	51.56%	

To determine whether populations of the fish from different locations were of the same structure, the body shapes were described by subjecting the relative warp scores generated from

Procrustes analysis to CVA. Results of the CVA of the relative warp scores show differences in body shapes between male populations of the fish from the five collection sites (Figure 3a). This is also true when females were compared (Figure 3b). The descriptions of the variations in body shapes are graphically shown in Figure 4. Comparison of populations of the fish show statistical differences between female populations [Wilk's lambda=0.5378, F=2.653, P(same)=3.8E-07; Pillai trace= 0.563, F=2.65, P(same)=3.563 E-07] (Figure 3a). Similar results were also observed for the male populations [Wilk's lambda=0.562, F=2.45, P(same)=3.502E-06; Pillai trace=0.5301, F=2.445, P(same)=3.426E-06] (Figure 3b). Looking at the distribution of individuals in the CVA plots show the nature of variations in the five populations. Those individuals found inside the overlaps of the convex hulls were those fishes that have similar body shapes and those that were not in the common hull were the individuals whose body shapes were different and have contributed to the differences between the populations. To be specific which of these populations differed, Kruskal-Wallis test was used (Tables 3 and 4). Results show males between station 1 and station 4, station 2 and station 5, station 3 and station 4, station 2 and station 5 were not different from each other. Other comparisons were significantly different. For the females, only females between station 1 and station 2, between station 2 and station 5, between station 3 and station 5 were not different. Other populations compared have shown significant differences. Summary of the geometric morphometric analysis show the consensus morphology (uppermost panel) and the variation in body shape among the female population of *Glossogobius giuris* produced by the first six significant relative warps (RW) explaining 73% of the total variation (RW1=23.21%, RW2=15.34%, RW3=14.42%, RW4=8.69%, RW5=6.78%, RW6=5.29%) are shown. The directions of expansion and compression of the different areas of the body of the female fish (Figure 4a) and the males (Figure4b) are shown by the different colors in the map.



**Figure 3.** First two Canonical Variate Axes shown significant differences within population of male and female

**Table 3.** Comparison between male populations (stations) of *G. giuris*

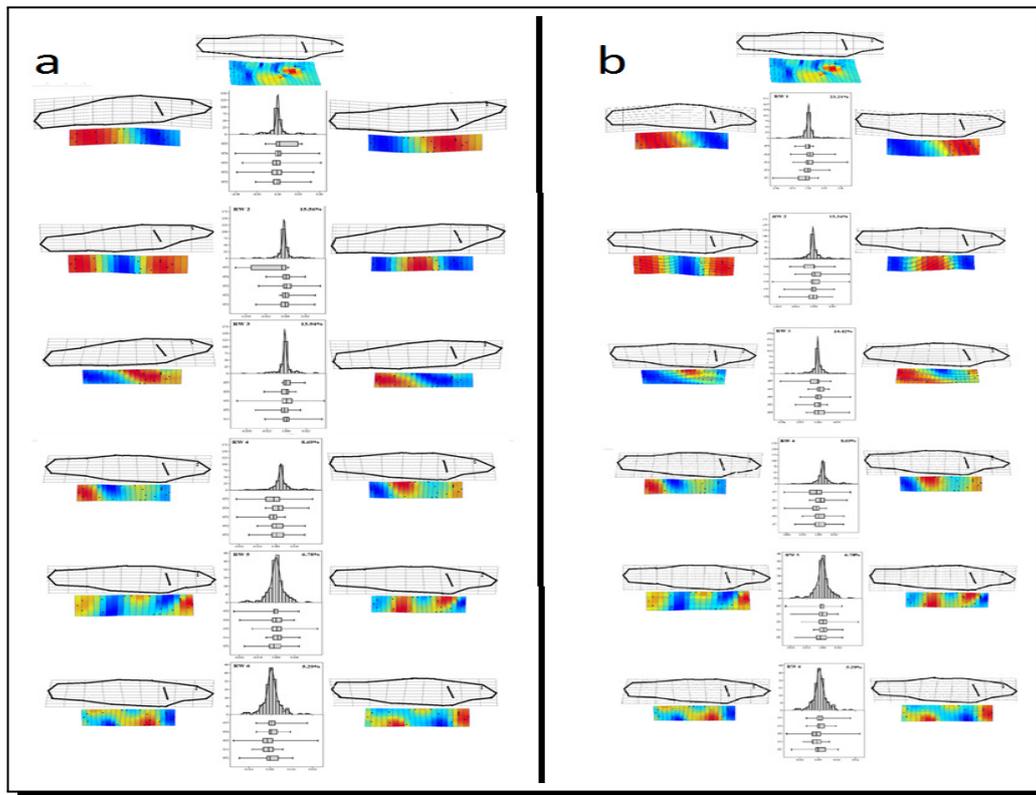
		ST2M1	ST3M1	ST4M1	ST5M1
MALE	STM1	<b>0.0178291</b>	<b>0.00332305</b>	0.108187	<b>0.0179394</b>
	STM2		<b>0.00332305</b>	0.26203	0.0657907
	STM3			0.0812009	<b>0.00152127</b>
	STM4				<b>0.0205206</b>
	STM5				

The values in red are significant. ST - station M - male

**Table 4.** Comparison between female populations (stations) of *G. giuris*

		STF2	STF3	STF4	STF5
FEMALE	STF1	<b>0.0863533</b>	<b>0.000798609</b>	<b>0.0539488</b>	<b>0.0415844</b>
	STF2		<b>0.0113105</b>	<b>0.042552</b>	0.0659824
	STF3			<b>0.0260305</b>	<b>0.0059166</b>
	STF4				<b>0.00515227</b>
	STF5				

The values in red are significant. ST - station F - female



**Figure 4.** Nature of body shape variations observed in the (a) male and (b) female populations of *G. giuris* as described by the 6 significant relative warps (the box and whisker plots from top to bottom – station 1-5)

Results shown in this study show variations in body shapes of fishes collected from different sources in the river can be attributed to variations in environmental conditions of the river. Typhoons and flooding which commonly occur in the Philippines may have affected the population structure of the fishes by altering dispersal of individuals across different change in landscapes [10–13]. In recent years for example, a number of genetic investigations have revealed freshwater migratory fish can exhibit population structuring, with different genetic populations within a single hydrographic system [14].

## 4. Conclusion

Geometric Morphometric of five different populations of *G. giuris* collected from different location in Norzagaray River show no sexual dimorphism in the fish. However, distribution of individuals of the two sexes of *G. giuris* along the first two canonical variate axes showed significant differences between populations. The differences are attributed to shape variations within populations. In this study, geometric morphometrics using landmark-based morphometry is a helpful tool to quantitatively describe shape differences between populations of the fish.

### Competing Interests

The authors declare that they have no competing interests.

### Authors' Contributions

All the authors contributed significantly in writing this article. The authors read and approved the final manuscript.

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