



# Describing Scale Shape Morphology of *Glossogobius Giuris* Using Landmark-Based Geometric Morphometrics

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**Abstract.** In the conservation of biodiversity, natural resources, and fisheries management, species identification and population discrimination are necessary. In freshwater fishes, the scale morphology and squamation is important because this can be helpful in the identification of fishes up to the extensive levels of species, sex determination and pathology of fish. *Glossogobius giuris* is an endemic species and considered important in Balo-i, Lanao del Norte and Mandulog River in Iligan City, Philippines. It is locally known as “kadurog”. The fish is highly commercialized because it provides food for the community, thus this study was conducted. We used scale morphology to distinguish male and female of *G. giuris* using landmark-based geometric morphometrics. Results of the study have shown that there is no sexual dimorphism in the fish based on scale shapes. However, variations of the scales do exist within sexes based on the results of the relative warp analysis. The variations observed can be attributed to environmental factors affecting the development of the fish and thus resulting to phenotypic plasticity in the patterns of variation in the scale shapes among individuals.

**Keywords.** *Glossogobius giuris*; Geometric morphometrics; Relative warps; Phenotypic plasticity

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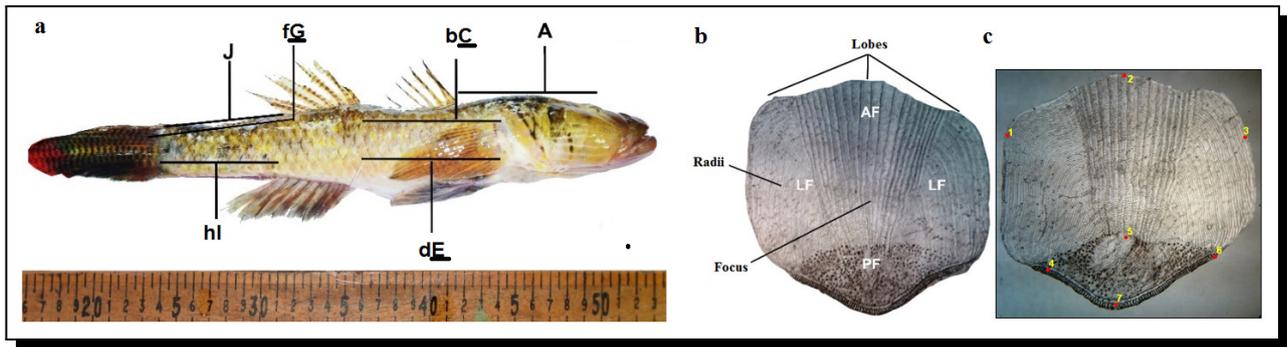
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## 1. Introduction

*Glossogobius giuris* is a freshwater fish [1,2]. It is an endemic and considered an important species that is highly commercialized in the local market. It is considered a suitable candidate for aquaculture. Thus, there is a need for a clearer understanding of the many aspects of its biology [3]. For freshwater fishes like the *G. giuris*, it is argued that describing scale morphology and squamation is important because this can be helpful especially in the discrimination of the fish up to the extensive levels of populations or stock but also in sex determination and pathology. This paper describes and understand the nature of morphometric variation in the scales within and between sexes of *G. giuris* using landmark-based geometric morphometrics. This biological tool was developed to evaluate the differences of shape based on the geometry and statistical analysis of anatomic landmarks defined by Cartesian coordinates (x and y). Multivariate analysis with accurate consideration of spatial relations of parts is considered a powerful and flexible tool to possibly investigate morphological differences in species [4,5].

## 2. Materials and Methods

Since sexes in *G. giuris* is difficult to be determined based from external morphology, identification was based on their gonads. For the scale shape analysis, fish body regions were determined first (Figure 1a) and are used as reference for area of scale collection [6]. Scales were removed from each six regions for both left and right sides of the fish. To get rid of the odor and clear up all the foreign matter of the scales, samples were soaked with Joy dishwashing liquid solution for 24 hours. This made the scales clean and distinguishable enough under the microscope. Finally, scales were mounted in the glass slides (each slide contains 6-7 scales), photographed and captured using the camera Sony Xperia Z1 with 20.7 megapixels (Figure 1b). Digital images captured were then transferred for image processing. Seven homologous landmarks were assigned to the scales of the species (Figure 1c) Seven homologous points were digitized using the software tpsDig2 version [7] and extract the x and y coordinates of the landmarks on the images. Since, the coordinates (x and y) would vary from image to image, this was standardized by Procrustes superimposition in order to extract biases in size, shape and rotation [4]. Such method translates the centroid of the shapes to (0,0), bended landmark coordinates which shows the positive and negative deformation from the consensus scale shape as illustrated in the grid square. Paleontological/Statistical analysis (PAST) software [8] was used in analyzing intraspecific variation in scales at distinct 6 body regions of each sex. The change in spline shape was described between sexes of each species. The relative warp scores were used to achieve the values that will constitute the multivariate shape data. Additionally, the Discriminant Function Analysis was used to describe scale shape variation between sexes and the compared groups was tested using Hotelling's t-squared with a level of significance  $p$  value,  $< 0.05$ . Multivariate Analysis and Canonical Variate Analysis were performed to assess the differences between the six body regions of the species.

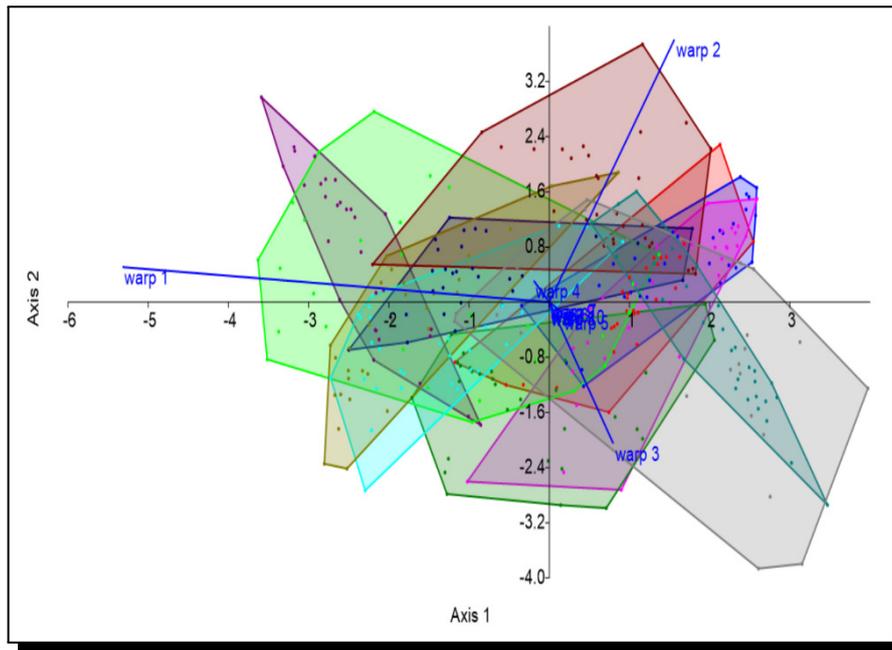


**Figure 1.** (a) Fish body regions where scale samples collected. [A=head region, Bc, De, Fg, Hi, J=body regions]; (b) Principal scale fields. AF, anterior field; LF, lateral field; PF, posterior field; (c) Landmarks used for digitizing image of scales: (1) left lateral tip, (2) center of anterior region, (3) right lateral tip, (4) left boundary between anterior and posterior region, (5) focus, (6) right boundary, and (7) center in the posterior region

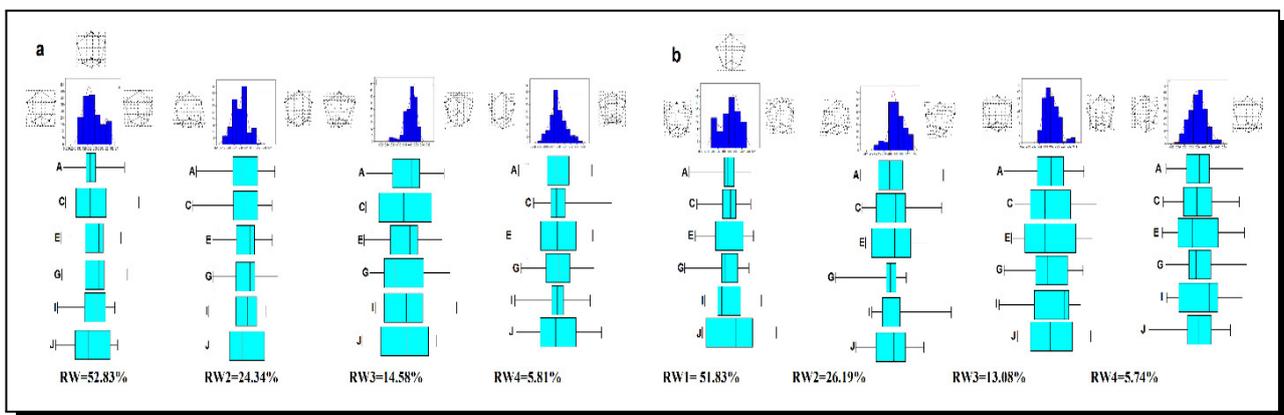
### 3. Results and Discussion

Results of the MANOVA of relative warp scores of all the scales in the body regions within sexes of the fish showed significant differences between the scale shapes of the different regions of *G. giuris* [Wilk's lambda (0.1113),  $F=7.906$ ,  $P=1.981E-98$ , Pillai trace (1.578),  $F=5.929$ ,  $P=9.356E-70$ ]. Comparison of scale shapes between regions were observed within sexes are shown in a scatterplot in Figure 2. Looking at the distribution of the individuals with scale shapes within and between regions of the 2 sexes show the differences among the regions with and between sexes are contributed by the 4 significant relative warps. A total of 97.56% variations in the male (RW1=52.83%, RW2=24.34%, RW3=14.58%, RW4=5.81%) and 96.72% for the female (RW1=51.83%, RW2=26.19%, RW3=13.06%, RW4=5.74%) (Figure 3). For the male fish, RW1 accounts for the variation in scales at the lateral and posterior region of the scale. There was a pointed lateral and blunted posterior regions of the scales. RW2 accounts for the variations at the left lateral tips location in the anterior region, RW3 for variations mainly at the lateral region of the scales and RW4 for the posterior region which is more blunted in focus. In the female, RW1 accounts for variations at the lateral and posterior region of the scales with a pointed scale shape than in male. RW2 accounts for variations at the anterior region with shape of the scales pointed at the anterior region through the posterior surface location. RW3 accounts for variations at the anterior region of the scales with shapes more blunted in the surface region. RW4 accounts minimal variations mainly at the posterior region where it is more inward in shape through the posterior region (Figure 3). While significant differences were observed in the scale shapes within and between regions within and between sexes (Table 1), confusion matrix revealed that only the scale shapes in the G and I regions for the 2 sexes were correctly classified ( $\geq 70\%$ ) (Table 1). Other regions have scale shapes that are more or less classified with other scale shapes of the other body regions. It is important to also note

here that sexual dimorphism was observed here based on these 2 regions. The female has scale shapes of the G and I regions which are significantly different from the scale shapes of the G and I regions of the males. These 2 regions thus can be used as diagnostic characters for sex in *G. giuris*. While sexual dimorphism is always attributed to the general idea that males are bigger than females and that males are more colorful than females, these have caused problems in identification of the sexes [9–11]. Body size is greatly affected by growth rate of the fish which could be altered by environmental factors and the physiology of the fish itself [12–14].



**Figure 2.** Scatterplot showing the distribution of individuals with differences in scale shapes from different body regions of the fish. Also shown are the significant relative warps where the variations were significantly observed. [Legend: red – A (female); blue – C (female); pink – E (female); green – G (female); violet – I (female); light green – J (female); dark blue – A (Male); light blue – C (Male); light green – E (Male); brown – G (Male); medium blue – I (Male); light grayish blue – J (Male)]



**Figure 3.** Variations in shapes and the box plots showing the mean and nature of variation in scale shapes in (a) male and (b) female *G. giuris* collected from Balo-i Lake and Mandulog River in Iligan City

**Table 1.** Confusion matrix between the scale shapes from the different body regions of the female and male *G. giuris*.

0	AF	CF	EF	GF	IF	JF	AM	CM	EM	GM	IM	JM
AF	40	16.67	23.33	10	-	-	-	3.33	-	-	-	6.67
CF	6.67	60	-	6.67	-	3.33	-	-	-	3.333	10	10
EF	16.67	6.67	30	10	-	-	-	-	-	-	13.33	23.33
GF	-	-	3.33	<b>83.33</b>	-	-	-	6.67	3.33	-	3.33	-
IF	-	-	-	-	<b>70</b>	3.33	3.33	20	3.33	-	-	-
JF	6.67	6.67	3.33	3.33	13.33	36.67	6.67	10	10	-	3.33	-
AM	6.67	-	6.67	-	6.67	16.67	50	6.67	6.67	-	-	-
CM	-	3.33	-	16.67	-	16.67	6.67	46.67	6.67	3.33	-	-
EM	-	-	-	-	6.67	10	3.33	6.67	66.67	6.67	-	-
GM	6.67	6.67	10	-	-	-	3.33	-	-	<b>73.33</b>	-	-
IM	13.33	10	-	-	-	-	-	-	-	6.67	<b>70</b>	-
IM	3.33	6.67	13.33	3.33	-	-	6.67	10	-	20	26.67	10

## 4. Conclusion

Results of this study have clearly shown the existence of sexual dimorphism in shapes of the scale in the fish. The shapes of the scales in I and G regions are considered diagnostic characters of the sex of fish. With the use of landmark-based geometric morphometrics, the statistical analysis (relative warps analysis) of shape, the shape of these scales in the 2 regions were found to be the distinguishing characters for sexual dimorphism.

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## 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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