Article

Relative warp analysis in determining morphological variation and sexual dimorphism between sexes of flathead goby (*Glossogobius giuris*)

C.C. Cabuga, Jr.[1], **C.B.E. Masendo**[1], **B.J.H. Hernando**[1], **C.C.D. Joseph**[1], **J.P.B. Velasco**[1], **M.K.A. Angco**[1], **M. A. Ayaton**[1], **O.L.P. Obenza**[1], **J. H. Jumawan**[1], **J.C. Jumawan**[1], **E.A. Requieron**[2], **M.A.J. Torres**[3], **H.C. Havana**[4]

[1]Biology Department, College of Arts and Sciences, Caraga State University- Main Campus-Ampayon, Butuan City 8600, Philippines

[2]Science Department, College of Natural Sciences and Mathematics, Mindanao States University – General Santos City Campus, 9500 Fatima, General Santos City, Philippines

[3]Department of Biological Sciences, College of Science and Mathematics, Mindanao State University - Iligan Institute of Technology, Iligan City, Philippines

[4]Cagbas Elementary School, Bayugan City, Agusan Del Sur, 8502, Philippines

E-mail: cresenciocabugajr@gmail.com

Received 2 June 2016; *Accepted 20 June 2016*; *Published online 1 September 2016*

Abstract

Landmark-based geometric morphometrics is an effective tool for measuring the biological shape, shape variation and covariation both for biotic and abiotic elements. It ensures graphical illustrations of shape changes that are visually interesting and instinctual. Using the results from relative warp analysis, histograms, CVA and PCA were generated to visualize morphological variations and sexual dimorphism. A total of 60 individuals (30 males and 30 females) were subjected to landmark-based analysis. This application will demonstrate morphological variation and sexual dimorphism on the body shape of *Glossogobius giuris* using geometric morphometry by means of Relative Warp Analysis. Sixteen landmarks generated 19 relative warps for each sample and showed slight morphological variation were females obtained the highest percentage than males. The MANOVA test value shows non-significant value (P=0.1655) on the left lateral side between sexes. This indicates that there were no shape variations and may be due to behavior and ecology of the organism to compete with others for food haunting, mating and to buffer environmental condition. While the right lateral side shows significant value (P<0.05) between sexes this reason explains that they were sexually dimorphic. That was essential for its adaptation and reproduction. The results of Principal Component Analysis (PCA) and Canonical Variance Analysis (CVA) show no significant body variations between sexes. This study was employed to identify the importance or relative warp analysis in detecting and morphological variations and sexual dimorphism of *G. giuris* collected at Lower Agusan River, Butuan City, Philippines.

Keywords relative warp analysis; landmark-based; sexual dimorphism; *Glossogobius giuris*.

Computational Ecology and Software
ISSN 2220-721X
URL: http://www.iaees.org/publications/journals/ces/online-version.asp
RSS: http://www.iaees.org/publications/journals/ces/rss.xml
E-mail: ces@iaees.org
Editor-in-Chief: WenJun Zhang
Publisher: International Academy of Ecology and Environmental Sciences

1 Introduction

Morphometrics is the numerical study of biological shape, shape variation and covariation of shape with other biotic and abiotic aspects. This method presents a consistency into the description and difference amongst morphologies. The function of morphometric technique contributes advances in any research field that depends on upon comparative morphology like systematics, evolutionary biology and developmental biology (Webster and Sheets, 2010).Geometric morphometric (both outline and landmark-based) is effective and established tool because of the evidence concerning the spatial relationship between landmarks on the organism is enclosed within the data. It enables to draw suggestive illustrations of morphological transformations or dissimilarities, proposing an instantaneous image of shape and the spatial localization of shape variation. Such graphical demonstration is easier to instinctively recognize than a table of numbers (Webster, and Sheets, 2010). It helps to illustrate commonly shared characteristics of an organism between sexes and will identify the differences. In addition, Geometric morphometrically comprises of 2D or 3D Cartesian landmark coordinates (relative to some arbitrarily chosen origin and axes). It consists of landmarks that propose locations or points of correspondence on each sample that match among and inside the populations or, equally, biologically homologous anatomical loci noticeable on all specimens in some studies (Bookstein, 1991; Dryden and Mardia, 1998). Moreover, the importance of geometric morphometry on describing shape has prompted innovative mechanism in terms of biometric analysis (Rohlf and Marcus, 1993; Bookstein, 1996). It was constantly used as an indicative tool in order to describe the shape and size factors from any biological entities (Rohlf, 1993; Bookstein, 1991). Employing landmark-based methods was efficient way unlike others because they operate equivalences biologically both structures that match landmarks (Ibañez et al, 2007). Various publications deals extensively with the theory, development and application of land-mark based geometric morphometrics, i.e. Kendall, 1984; Bookstein, 1986, 1989, 1991, 1996b; Rohlf and Bookstein, 1990; Goodall, 1991; Marcus et al., 1996; Small, 1996; Dryden and Mardia, 1998; Elewa, 2004; Zelditch et al., 2004; Claude, 2008). Also, utilizing the importance of landmark-based geometric morphometric techniques to different research fields has been widely acknowledged (Rohlf, 1990, 1998; Rohlf and Marcus, 1993; Bookstein, 1996a; O'Higgins, 2000; Roth and Mercer, 2000; Richtsmeier et al., 2002, 2005; Adams et al., 2004; Slice, 2007; Lawing and Polly, 2010). This study focuses on landmark analysis which purposes in copying the location of a definite important points or it describe the shape of the object in its plainest form, eliminating irrelevant details such as size variances, location, and variation, thus, simplifying the differences between the two objects pictured via thin-plate splines (Requiron et al, 2012). It also identifies variation in shape which at present continuously uses because of its significant approach of differentiating individuals (Truong et al, 2005). Thus, the advancement of this technique carries better understanding to analyze the differences of shape among the organisms based on anatomic markers defined by Cartesian coordinates (x and y). Hence, the outcome of this study will eventually discuss the characteristics of sexual dimorphism and morphological differences in the organism standing as an important knowledge in the behavior, history and its ecology (Requiron et al, 2012). This study utilizes *Glossogobius giuris*, a freshwater organism that is widely found in the rivers of Mindanao specifically in Lower Agusan River, Butuan City and commonly subjected to experimental procedures. Thus, the goal of this research was to establish and aimed to identify sexual dimorphism and morphological variations on the body shape of *Glossogobius giuris* using geometric morphometry by means of Relative Warp Analysis.

2 Materials and Methods

2.1 Study area

This study was conducted in Lower Agusan River Basin, Butuan City, which lies between 8059'26.35"

N125031’31.16” E in Agusan Del Norte, Philippines. Fish collection was done in the month of May 2016.

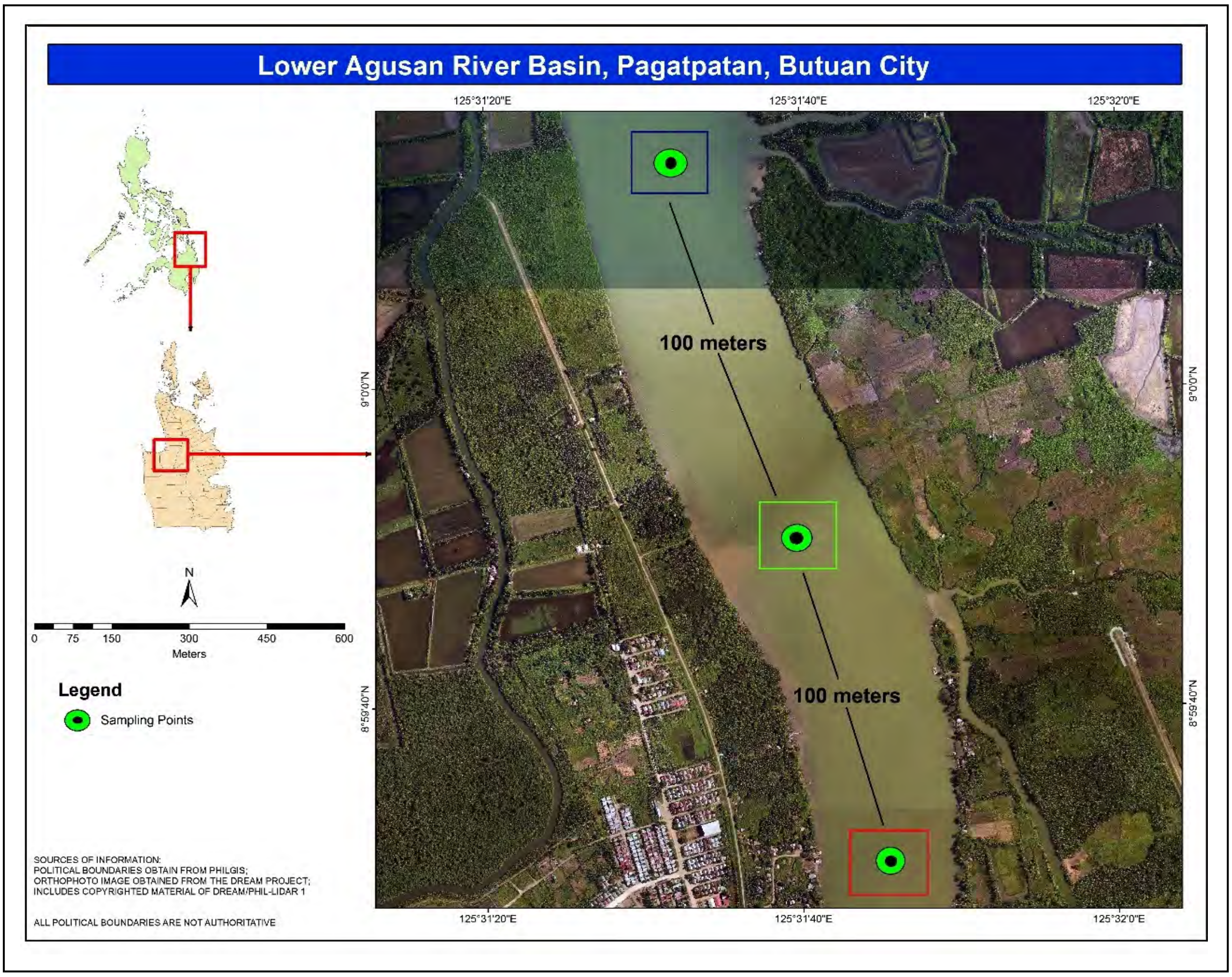

Fig. 1 Map showing Lower Agusan River Basin, Butuan City, Agusan del Norte, Philippines.

2.2 Relative warp analysis of *Glossogobius giuris*

2.2.1 Sample processing

About 60 individuals (30 males and 30 females) were collected in the area with the aid of local fisherman. The collected samples will then be processed for image capturing and analysis. The sampled fish was placed in a flat styrofoam for the pinning of its fins to make it wider and to clearly see the samples point of origin for the land-marking process. About 10% Formalin was applied in all the fins of the fish samples to make it hardened using a small brush. Digital image of the left and right lateral side of each sample was taken using Canon camera (14 mega pixels).

2.2.2 Sex Determination

Sex of the samples was also determined by its genitalia. Females were identified by the presence of its eggs and ovaries which are in general yellow or orange and granular in texture. The males were identified based on the presence of its testes which were typically smooth, whitish and non-granular in texture ((Requieron et al., 2010). Images were assorted according to sex and digitized using the tpsDig2 program (version 2.0, Rohlf, 2004) and were saved as TPS file.

2.2.3 Landmark selection and digitization

Digital images were assorted according to sex and converted to tps files using tpsUtil. Landmarking of the samples were digitized using the tpsDig version 2 (Rohlf, 2004). A total of 16 anatomical landmarks points (Table 1) were located in right lateral view to represent the external shape of the body of the sample for male and female goby (Fig. 2).

Table 1 Description of the landmark points according to Dorado et al. (2012).

Coordinates	**Locations**
1	Snout tip
2	Posterior end of nuchal spine
3 & 4	Posterior & anterior insertion of 1st dorsal fin
5 & 6	Posterior & anterior insertion of 2nd dorsal fin
7 & 9	Dorsal and ventral insertion of caudal fin
8	Lateral line
10 & 11	Posterior & anterior insertion of anal fin
12	Insertion of the pelvic fin
13	Insertion of the operculum at the lateral profile
14	Posterior extremity of premaxillar
15	Anterior margin through midline of orbit
16	Posterior margin through midline of orbit

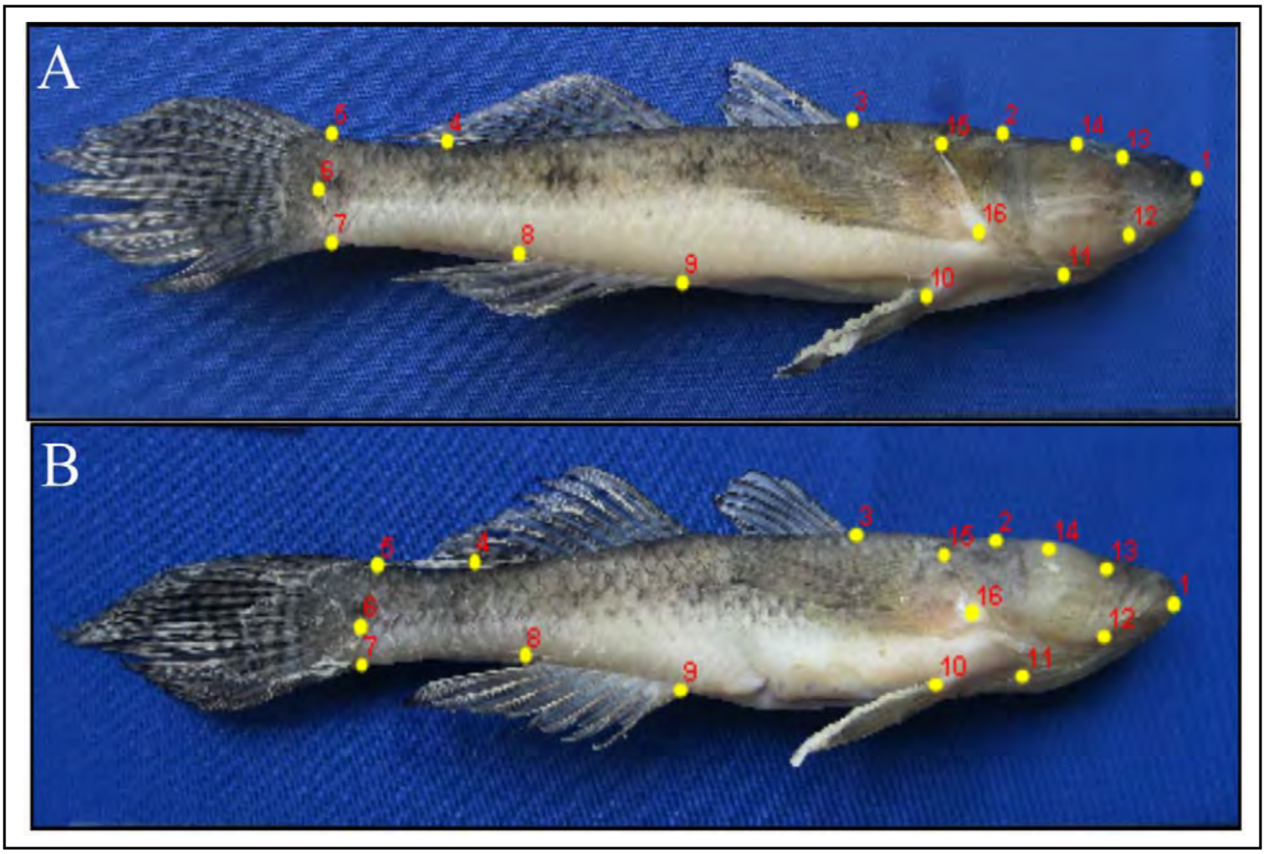

Fig. 2 Landmarks' description of the *Glossogobius guiris* (A. male, B. female):

2.2.4 Shape analysis

The converted tps files with the anatomical landmarks was then processed in tpsRelw to get relative warp analysis and to obtained X and Y coordinates for further analysis. The coordinates were then first transferred to Microsoft Excel 2010 application before transporting to PAST software (Hammer et al., 2009). Histograms were generated which serves as presentations for comparing patterns of sexual dimorphism. It generated from the relative warp scores of the fish shapes which were computed and analyzed by PAST software. This software provides valuable information on the distribution of the data from the mean over the range of the variable. Collected coordinates were then subjected to MANOVA, Canonical Variance Analysis (CVA) and Principal Component Analysis (PCA) using PAST software (Hammer et al., 2009) from the relative warps of the morphology of the flathead gobies.

3 Results and Discussion

MANOVA was used to show the significant body variations in the body shapes of *Glossogobius guiris* (Table 2) for both sexes. The result shows a non-significant value of (P=0.1655) on the lateral side of the sample between sexes. This could happen because organisms shared common characteristics to be used as a mechanism to adapt to the environment which they inhabit. The reasons might be to their behavior, ecology and life history. Perhaps, adaptation and survival were considered to be aspects of non-variation in the shape of organisms that will be used within the environment. A changed condition might affect organism shape and this may lead to mortality. Alongside, organisms must keep in shape so that they can be coordinated to the environmental conditions which typically affect their health status. On the other hand, right lateral side for both sexes of *G. giuris* showed significant value of (P<0.05) this is contradictory with the lateral side, however this may be due to condition that organism may hold a shape variations in order to fit within the environment or it will provides a manifestation of adaptation. The result shows that *G. giuris* were slightly sexual dimorphic and were observed in its right lateral portion for both sexes. This happens because it acts as a mechanism to be capable of reproduction and maintain its population. This is also used to be more adaptable in the environment towards changes in nature. According to Casselman and Schulte-Hostedde (2004), sexual dimorphism contributes in reproduction that has been manifested in its morphology. Thus, male organism necessary for adaption and keeping its superiority in competing with their mates while the female organism essentially adaptive for producing offspring.

Table 2 Results of MANOVA test for body shape sexes of *G. giuris*.

	Wilk's Lambda	**df1**	**df2**	**F**	**p(same)**
Between sexes	0.9563	5	174	1.589	0.1655^{ns}

** (P<0.05) highly significant; ns= not significant

	Wilk's Lambda	**df1**	**df2**	**F**	**p(same)**
Between sexes	0.9563	5	174	5.422	0.001161**

** (P<0.05) highly significant; ns= not significant

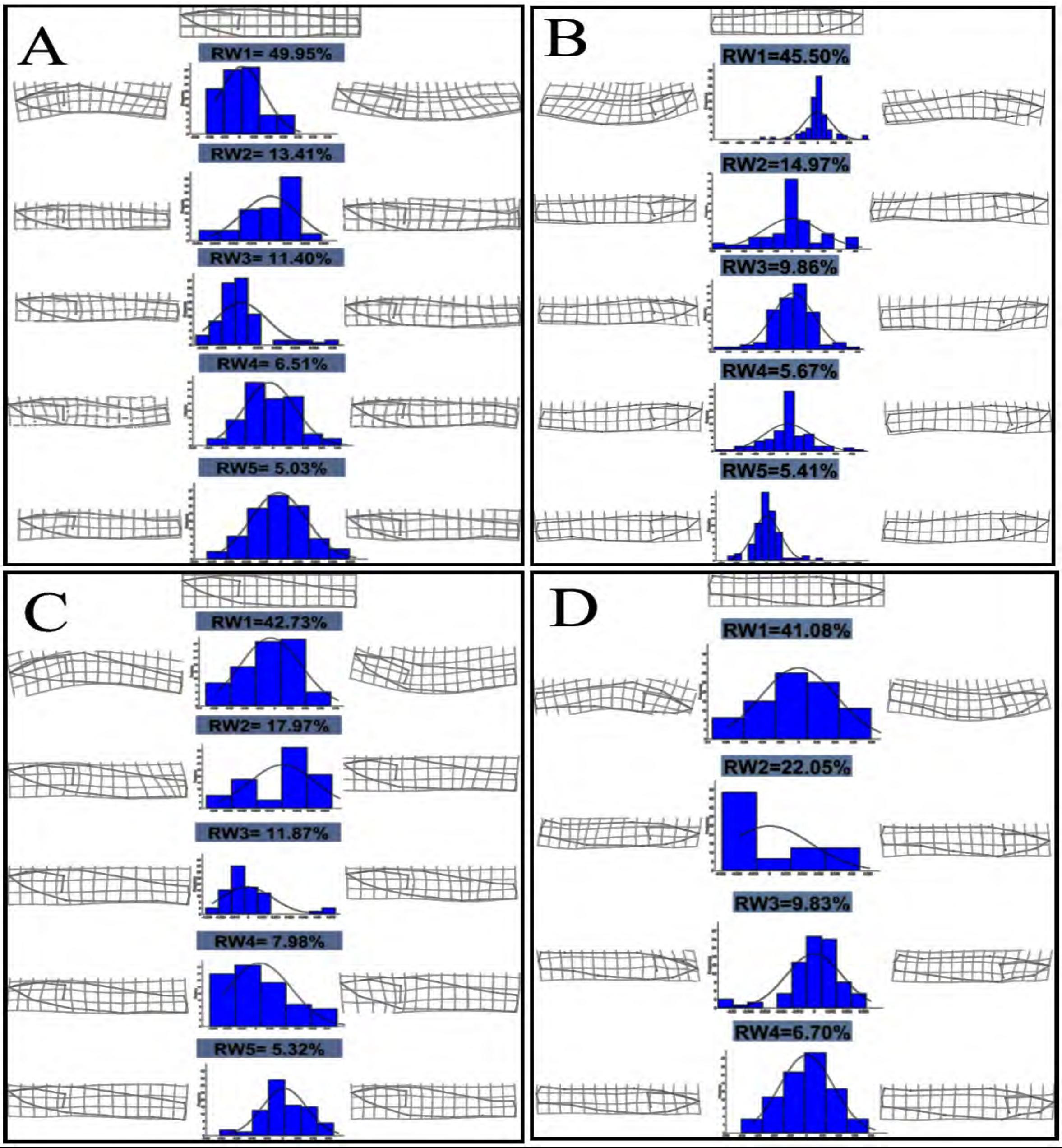

Fig. 3 Summary of the geometric morphometric showing consensus morphology and the variation in the body shape of female and male population of *G. giuris*, (**A**) female left, (**B**) female right (**C**) male left, (**D**) male right.

It was observed that the females obtain the highest values of relative warp analysis (RW1=49.95%) (RW2=13.41%) (RW3=11.40%) (RW4=6.51%) (RW5=5.03%) for the left and (RW1=45.50%) (RW2=14.97%) (RW3=9.86%) (RW4=5.67%) (RW5=5.41) for the right side of the body shapes (Fig. 3). This is because the female was more vulnerable in many changes towards environmental condition and the most considerable factor is the reproductive ability of the females to uphold offspring. In females, changes in shape are very significant to sustain its capability to resist changes and maintain homeostasis and metabolic rate for reproduction. In the study conducted by Requiron et al. (2012), it states that a shape has a big role in depicting biological studies. Also, shape variations are generated because of several progressions like pathology, ontogeny, and adaptability to geographic changes. Furthermore, diverse in shapes suggests a different useful way (Requiron et al., 2012). On the contrary, males generated the lowest values of relative warp analysis (RW1=42.73%) (RW2=17.97%) (RW3=11.87%) (RW4=7.98%) (RW5=5.32%) for the left side and (RW1=41.08%) (RW2=22.05%) (RW3=9.83) (RW4=6.70) for the right sides of the body shapes. This is because males were more adaptive in nature than females. Males were inclined to suppress shape variations coming from different environmental changes. They were constantly adaptable to be more efficient in food haunting and mating process. The lowest the body shape variations, the organism are more flexible for the incoming and outgoing changes occur in the environment. In the aspects of morphological variations and sexual dimorphism it can be illustrated in the Principal Component Analysis (Fig. 4) and Canonical Variance Analysis (Fig. 5). It shows that male and female *G. guiris* shared common characteristics (male in blue, female in red). It represents, the degree of similarities in both sexes although from the result of MANOVA test right lateral side indicates significant values (P<0.05). In this, determining morphological variations of *Glossogobius giuris* cannot be illustrated clearly since the actual results show non-significant and significant values that are contradictory. However, it can be due to its nature, ecology and innate characteristics of the organism. Subsequently, morphological variation in the fishes could be attributed due to its feeding adaptation like in bigger head region in order to maximize buccal volume and suction velocity (Caldecutt and Adams, 1998) while deep bodies increase maneuverability when foraging (Webb, 1982).Likewise, male in particular must have wider anal fin base than the females to be able to promote success in male-male competition or female choice as observed in the genus *Pretotilapia* by Kassam et al (2004).

Table 2 Variations observed in the body shapes of female *G. giuris.*

RW (%)	Left	RW (%)	Right
RW1 = 49.95 %	Differences in the observed on the head , trunk and tail regions. Its body shows slanted position.	**RW1 = 45.50%**	Variations occurs relating to the curvature in the head trunk and tail regions. Its body showing twisted formation.
RW2 = 13.41%	Observable body shape differences relating to the head specifically in its pelvic fin and operculum.	**RW2 = 14.97%**	Variability in the body shape occurs in the dorsal-caudal fin.
RW3 = 11.40%	Variations in the body shape occurs in the head and trunk regions. its body has minimal deformities.	**RW3 = 9.86%**	Differences in the body shape happens into the head region, making it downward position.
RW4 = 6.51%	Variability in the mouth, trunk and tail regions. Its body shape slightly skewed.	**RW4 = 5.67%**	Observable variations involving its head making it upward position. Its body shape slightly twisted**.**
RW5 = 5.03%	Differences in the head and tail regions are observed. Its body shape slightly deformed.	**RW5 = 5.41%**	Variability in the body shape occurs in the head region making upward position. Its body shape slightly slanted.

Table 3. Variations observed in the body shapes of male *G. giuris.*

RW (%)	Left	RW (%)	Right
RW1 = 42.73%	Variations in the body shape occurs in the head, trunk and tail regions. Its head in upward position and its body in a curvature form.	**RW1 = 41.08%**	Observable body shape variations occurs in the head, trunk and tail regions. Its head in upward formation a its body is slanted.
RW2 = 17.97%	Changes occurs in the body depth trunk, head and tail regions. Specifically its tail pointing downward.	**RW2 = 22.05%**	Differences occurs in the regions of the head and in the dorsal and ventral insertion of caudal fin.
RW3 = 11.87%	Variations associated with the head region. Its trunk gets shorter and the tail region pointing downward.	**RW3 = 9.83%**	Variability in the body shapes take place in its head region and its tail becomes narrower.
RW4 = 7.98%	Observable changes in the body shape occurs in the head region, making it upward. While its tail in downward position.	**RW4 = 6.70%**	The fourth relative warp (RW4) accounts for variations linked with the head region.
RW5 = 5.32%	The fifth relative warp (RW5) manifests changes associated with the head region projecting upward. Its dorsal portion slightly slanted.		

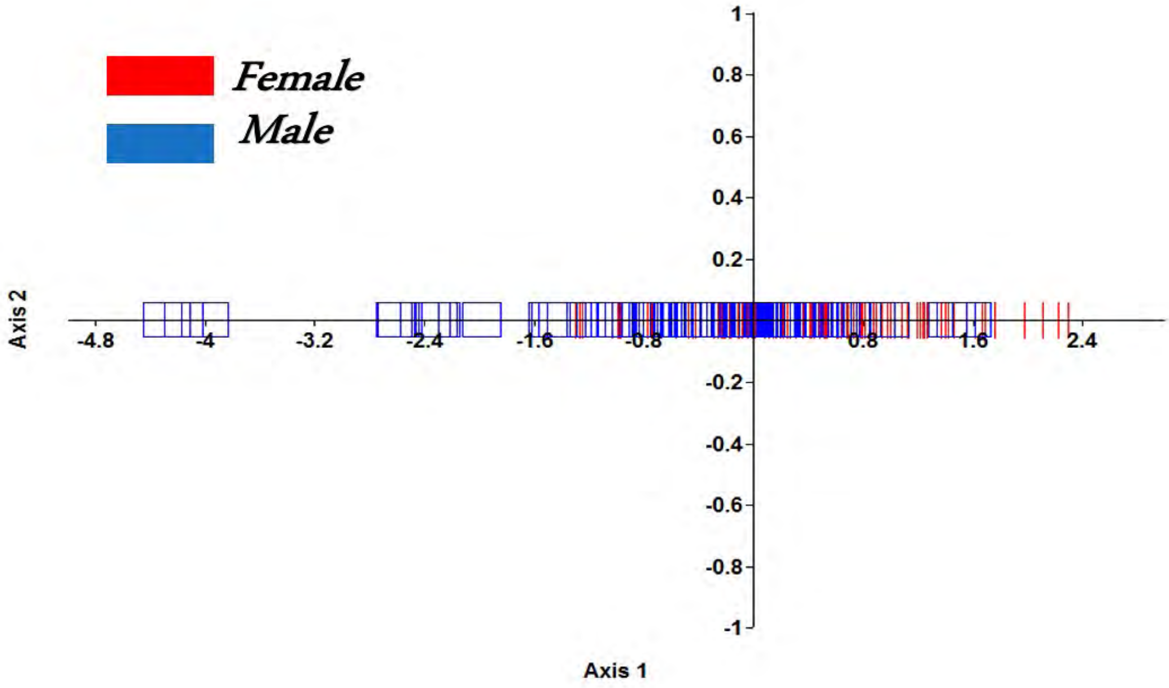

Fig. 4 PCA scatterplot of the landmark coordinate data of female and male body shape variation of *G. giuris*.

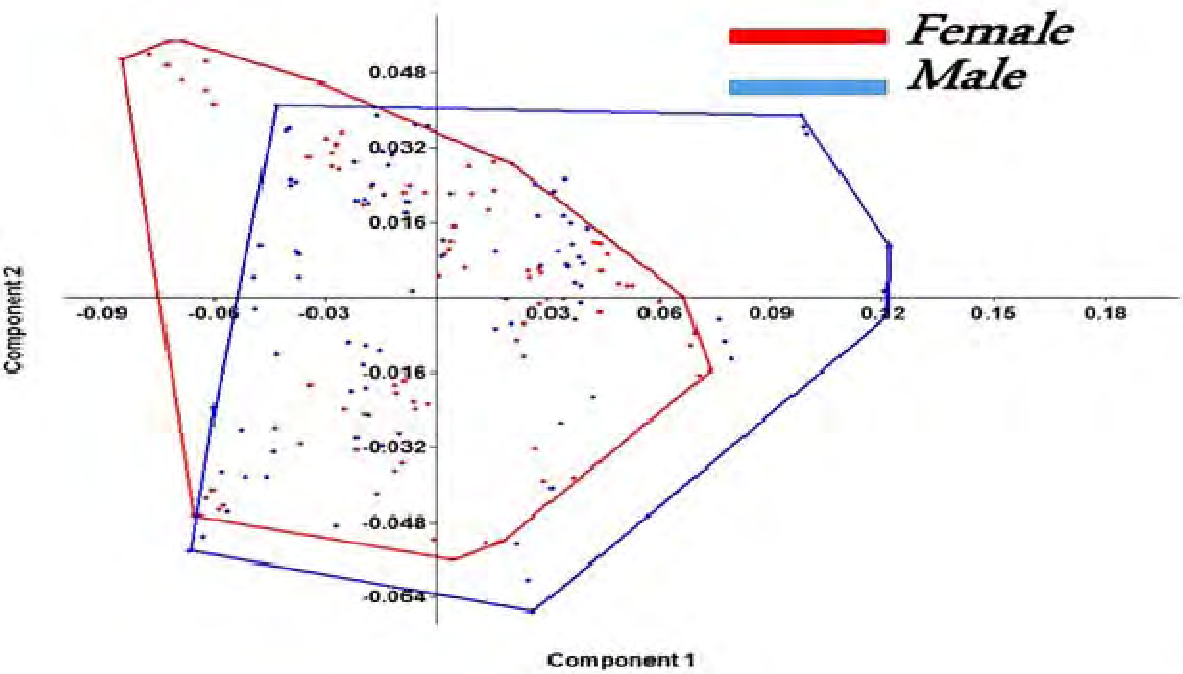

Fig. 5 CVA scatter plot showing the distribution of female and male *G.giuris* based on the body shape.

4 Conclusion

The fish flathead Goby (*G. iuris*) was utilized to determine the morphological variations and sexual dimorphism on the body shape using geometric morphometry by means of Relative Warp Analysis. To locate the standard landmark the metric traits of the fish were used. The results of MANOVA test revealed contradictory of non-significant value (P=0.1655) for the left lateral side while the right lateral side was significant (P<0.05). It might be due to the organism's innate characteristics. The morphological variations were drawn in the Relative Warp Analysis which is the highest values obtain in females it generated ten (10) relative warps while in males it has the lowest values and generated nine (9) relative warps. In the aspects of Principal Component Analysis (PCA) and Canonical Variance Analysis, it showed a non-significant degree of dissimilarities in both sexes. The used of landmark-based analysis provides insight to determine shape variation and sexual dimorphism in the organism.

Acknowledgement

The researchers would like to extend their gratitude to Mr. Anol Ligtas for providing the fish samples utilizes in the study.

References

Adams DC, Rohlf FJ, Slice DE. 2004. Geometric morphometrics: ten years of progress following the 'revolution'. Italian Journal of Zoology, 71: 5-16

Bookstein FL. 1986. Size and shape spaces for landmark data in two dimensions. Statistical Science, 1: 181-242

Bookstein FL. 1989. Principal warps: Thin-plate splines and the decomposition of deformations. IEEE Transactions on Pattern Analysis and Machine Intelligence, 11: 567-585

Bookstein FL. 1991. Morphometric Tools for Landmark Data: Geometry and Biology. Cambridge University Press, USA

Bookstein FL. 1996. Biometrics, biomathematics and the morphometric synthesis. Bulletin Mathematics and Biology, 58: 313-365

Bookstein FL. 1996A. Biometrics, biomathematics and the morphometric synthesis. Bulletin of Mathematical Biology, 58: 313-365

Bookstein FL. 1996B. Standard formula for the uniform shape component in landmark data, In: Advances in Morphometrics (Marcus LF, Corti M, Loy A, et al., eds). 153-168, NATO ASI Series A: Life Sciences Volume 284. Plenum Press, New York, USA

Caldecutt WJ, Adams DC. 1998 Morphometrics of trophic osteology in the three spine stickleback, *Gasterosteus aculeatus.* Copeia, 4: 827-838

Casselman SJ, Schulte-Hostedde AI. 2004. Reproductive roles predict sexual dimorphism in internal and external morphology of Lake Whitefish, *Coregonus clupeaformis*. Ecology of Freshwaters Fish, 13: 217-222

Claude J. 2008. Morphometrics with R. Springer, New York, USA

Dorado E, Torres MA, Demayo C. 2012. Describing body shapes of the white goby, *Glossogobius giuris* of Lake Buluan in Mindanao, Philippines using landmark-based geometric morphometric analysis. International Research Journal of Biological Sciences, 1(7): 33-37

Dryden IL, Mardia KV. 1998. Statistical Shape Analysis. John Wiley and Sons, Chichester, UK

Eelewa AMT. 2004. Morphometrics: Applications in Biology and Paleontology. Springer-Verlag, Berlin, Germany

Goodall C. 1991. Procrustes methods in the statistical analysis of shape. Journal of the Royal Statistical Society Series B (Methodological), 53: 285-339

Hammer O, Harper DAT, Ryan PD. 2009. PAST version 1.91: Paleontological Statistical Software package for education and data analysis. Paleontologia Electronica, 4(1): 9

Ibañez AL, Cowx IG, O'Higgins P. 2007. Geometric morphometric analysis of fish scales for identifying genera, species, and local populations within the Mugilidae. Canadian Journal Fishery Aquatic Science, 64: 1091-1100

Kassam D, Mizoiri S, Yamaoka K. 2004 Interspecific variation of body shape and sexual dimorphism in three coexisting species of the genus *Petrotilapia* (Teleostei: Cichlidae) from Lake Malawi. Ichthyol Research, 51: 195-201

Kendall DG. 1984. Shape manifolds, Procrustean metrics, and complex projective spaces. Bulletin of the London Mathematical Society, 16: 81-121

Lawing AM, Polly PD. 2010. Geometric morphometrics: recent applications to the study of evolution and development. Journal of Zoology, 280: 1-7

Marcus LF, Corti M, Loy A, et al. 1996. Advances in Morphometrics. NATO ASI Series A: Life Sciences Volume 284. Plenum Press, New York, USA

O'Higgins P. 2000. The study of morphological variation in the hominid fossil record: biology, landmarks and geometry. Journal of Anatomy, 197: 103-120

Requiron EA, Torres MAJ, Demayo CG. 2012. Applications of relative warp analysis in describing of scale morphology between sexes of the snakehead fish *Channa striata*. International Journal of Biological, Ecological and Environmental Sciences, 1(6): 205-209

Richtsmeier JT, Deleon VB, Lele. SR. 2002. The promise of geometric morphometrics. Yearbook of Physical Anthropology, 45: 63-91

Rohlf FJ, Bookstein FL. 1990. Proceedings of the Michigan Morphometrics Workshop. University of Michigan Museum of Zoology Special Publication Number 2, Ann Arbor, Michigan, USA

Rohlf FJ. 1990. Morphometrics. Annual Review of Ecology and Systematics, 21: 299-316

Rohlf FJ. 1993. Relative warp analysis and an example of its application to mosquito wings. In: Contributions to Morphometrics Vol 8 (Marcus LF, Bello E, Garcia-Valdecasas A. eds.). 131-159, Museo Nacional de Ciencias Naturales (CSIC), Madrid, Spain

Rohlf FJ, Marcus LF. 1993. A revolution in morphometrics. Trends Ecology Evolutionary. 8: 129-132

Rohlf FJ. 1998. On applications of geometric morphometrics to studies of ontogeny and phylogeny. Systematic Biology, 47: 147-158

Roth VL, Mercer JM. 2000. Morphometrics in development and evolution. American Zoologist, 40: 801-810

Slice DE. 2007. Geometric morphometrics. Annual Review of Anthropology, 36: 261-281

Small CG. 1996. The Statistical Theory of Shape. Springer Series in Statistics. Springer, New York, USA

Truong NT, Gwag JG, Park YJ, Lee SH. 2005. Genetic diversity of soybean pod shape based on elliptic Fourier descriptors. Korean Journal Crop Science. 50(1) 1-7

Webb PW. 1982 Locomotor patterns in the evolution of actinopterygian fish. American Zoologist, 22: 329-342

Webster M, David Sheets H. 2010. A practical introduction to landmark-based geometric morphometrics. In: The Paleontological Society Papers Volume 16 (John Alroy, Gene Hunt, eds). 163-188

Zelditch ML, Swiderski DL, Sheets HD, Fink WL. 2004. Geometric Morphometrics of Biologists: A Primer. Academic Press, San Diego, USA