E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2019; 7(2): 287-294
© 2019 IJFAS
www.fisheriesjournal.com
Received: 18-01-2019
Accepted: 22-02-2019

Virgilio B Ratunil
Surigao State College of Technology, Surigao City, Philippines

Clevin P Libay
Surigao State College of Technology, Surigao City, Philippines

Emmylou A Borja
Surigao State College of Technology, Surigao City, Philippines

Gideon A Ebarsabal
Surigao State College of Technology, Surigao City, Philippines

Gregorio Z Gamboa
Surigao State College of Technology, Surigao City, Philippines

Dexter Q Mahomoc
Surigao State College of Technology, Surigao City, Philippines

Jerry B Manongas
Surigao State College of Technology, Surigao City, Philippines

Cresencio C Cabuga
Caraga State University – Main Campus, Ampayon, Butuan City, Philippines

Correspondence
Virgilio B Ratunil
Surigao State College of Technology, Surigao City, Philippines

Analysis of body shape variation in *Glossogobius giuris* (Hamilton 1882) sampled from Lake Mainit, Philippines, using geometric morphometrics

Virgilio B Ratunil, Clevin P Libay, Emmylou A Borja, Gideon A Ebarsabal, Gregorio Z Gamboa, Dexter Q Mahomoc, Jerry B Manongas and Cresencio C Cabuga

Abstract
Fluctuating Asymmetry (FA) is a tool to assess morphological differences from different bioindicator species. This study was conducted to evaluate the body shape variation of *Glossogobius giuris* sampled from Lake Mainit, Philippines, using geometric morphometrics. A total of 60 specimens was collected with 30 each sex. Landmarking techniques were established and applied to the fish morphology. Samples were subjected to tps Util and Symmetry and Asymmetry Geometric Data (SAGE) software to identify the body shape analysis. Among the factors analyzed, Procrustes ANOVA results shown a highly significant difference (P<0.0001) in the female and male samples and thus, indicating a shape differences between the sexes. While Principal Component Analysis (PCA) displayed a higher percentage in female samples (83.1614%) than of the male (79.3984%) denoting body shape variations between the populations indeed, females are highly inclined to perturbations since they need a buffer from various environmental requirements and primarily through the reproduction process. Thus utilizing geometric morphometric enables to determine morphological variations of species among and within the taxa.

Keywords: Fluctuating asymmetry, freshwater fishes, body shape analysis, bioindicator

1. Introduction
In the Philippines, Lake Mainit serves an important natural fish resource and classified as the fourth largest lake. It is a shared resource by the provinces of Agusan Norte and Surigao del Norte in Northeastern Mindanao, bordered by eight municipalities that comprise the Lake Mainit Watershed. While there were twenty eight (28) river system that is considered as tributaries making the increase of water level of the Lake and thus emptied by the 29km Kalinawan River and runs to Butuan Bay [1], however, this freshwater ecosystem had been exposed to several human-induced activities such as domestic wages, industrial and agricultural practices that affects the lake. On the other hand, two species of goby identified, the *Glossogobius giuris* or locally known as "Pijanga" and *Hypseleotris agilis* as "Bugwan," considered as a significant fish commodity of the lake, and still occurs but in much-decreased richness in the late 1990s [2]. The dominance of these species in the lake ecosystem implies to be a good bioindicator in assessing the lake's condition.

Geometric Morphometric (GM) often utilizes as a significant mechanism to understand shape variations. It pertains to the quantitative analysis of form, a concept which includes size and shape [3]. GM, introduced new techniques on how to accomplish, present, classify and evaluate morphological parts of species. This mechanism constantly applied to visualize and interpret shape differentiation. Subsequently, GM provides evidences in shapes that could analyze through comparing coordinates in the aspects of orientation, position and size [4-5].

Further, to understand the morphological variations in *G. giuris,* Fluctuating Asymmetry (FA) was employed to identify the different character traits of female and male populations. Indeed, FA was an effective tool to evaluate the developmental variability of an individual species as its represents the total population [6]. It serves as a significant mechanism to assess pollutants in the environment that alter the species traits [7]. It also identified to be an efficient instrument for quantifying environmental condition [8]. As well as, it is also a potential and quantitative approach in assessing if the environment is capable of providing ecological growth towards species [9].

In addition, FA was simple and reliable means of identifying developmental instability [10]. It is widely known to describe indiscriminate nonconformities based on morphological traits [11]. This application widely recognized as it can deliberately identify the effects of several effluents through species morphology [12]. Furthermore, FA is one of the most recognized scientific mechanisms because it can represent quantitative function and analyze morphological shape [13]. This study aims to evaluate the body shapes variation of *G. giuris* between male and female population using geometric morphometric in analyzing fluctuating asymmetry from Lake Mainit, Philippines.

2. Materials and Methods

2.1 Locale of the Study: The sampling location was in Lake Mainit, Philippines (Figure 1). The collection of fish samples was completed in February 2018 through the effort of local fisherman. Appropriate fish preservation methods were applied to the collected samples and directly taken to laboratory for further procedures.

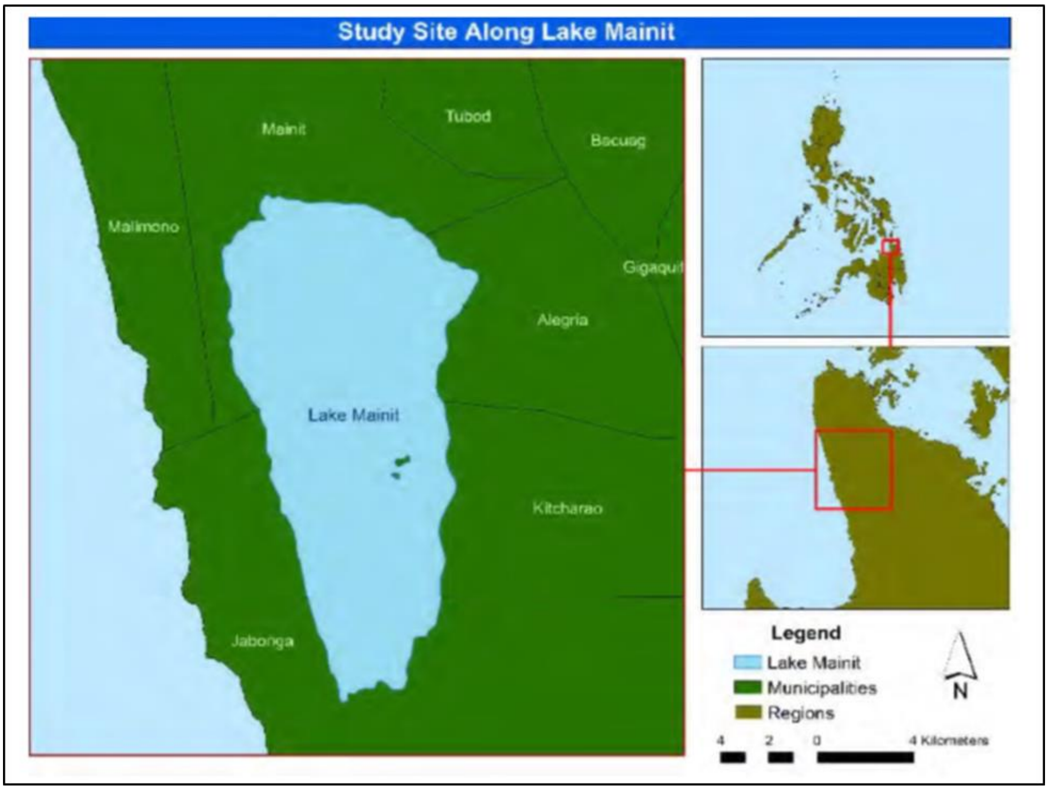


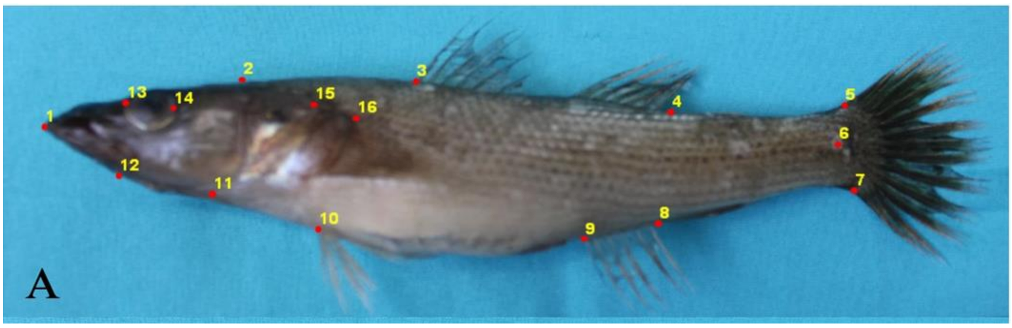
Fig 1: Sampling area, Lake Mainit, Philippines. (Photo credit: Joseph *et al*., 2016) [14].

2.2 Processing of Fish Samples and Sex Determination

There were sixty (60) specimens of *G. giuris* (Pijanga) was sampled comprising of 30 each male and females. Each of the fish was placed in the Styrofoam where a small pin utilized to stretch the fins. To harden the fins, a 10% formaldehyde were applied by using the small paint brush and dry for an hour. Subsequently, all the samples were placed to another Styrofoam and detached the pins. The portion left, and right of the fishes was taken an image three times utilizing a DSLR camera (Figure 2). A dissection procedure was done for the sex determination of the samples. Females were identified based on the presence of ovaries in a granular texture with a color of yellow to orange. Then the males in the presence of testes in whitish color and a non-granular texture.

2.3 Coordinates Selection, Digitation and Shape Investigation

The specimens photographed were categorized based on its sexes and loaded to TPSutil to covert the file while the digitation procedures were applied utilizing TPSdig2 [36]. The sixteen (16) structural coordinates shown in Table 1 was employed in the fish populations. While the shape analysis was also used to error, the standard metric traits (left-right) portions of the fish samples were digitized quantify the morphological variation among the sexes and the later were tri-replicated to decrease the measurement and loaded to SAGE (Symmetry and Asymmetry Geometric) (Figure 3) Software version 1.04 [37].



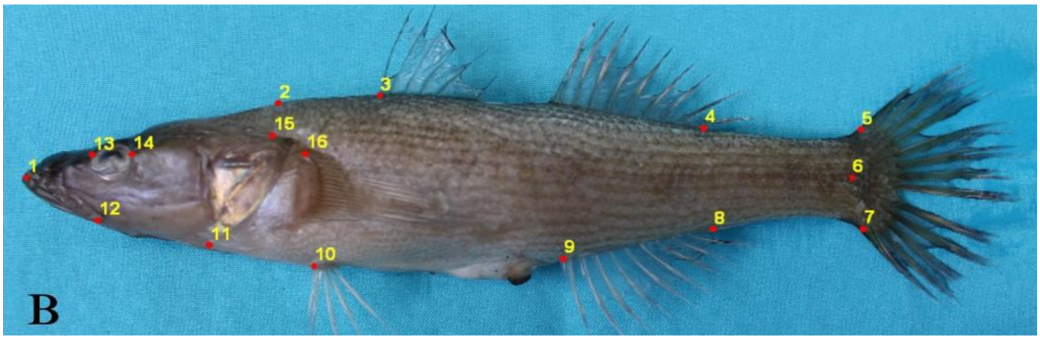


Fig 2: Actualized digitized sample and landmark points used to describe the body shape of *G. giuris.* (A) Male, (B) Female.

Table 1: Depiction of the landmark coordinates of *Glossogobius giuris* adapted on Paña *et al.* (2015) [15].

No	Description
1	Snout tip
2	Posterior end of nuchal spine
3	Anterior insertion of dorsal fin
4	Posterior insertion of dorsal fin
5	Dorsal insertion of caudal fin
6	Midpoint of caudal border of hypural plate
7	Ventral insertion of caudal fin
8	Posterior insertion of anal fin
9	Anterior insertion of anal fin
10	Dorsal base of pelvic fin
11	Ventral end of lower jaw articulation
12	Posterior end of maxilla
13	Anterior margin through midline of orbit
14	Posterior margin through midline of orbit
15	Dorsal end of operculum
16	Dorsal base of pectoral fin

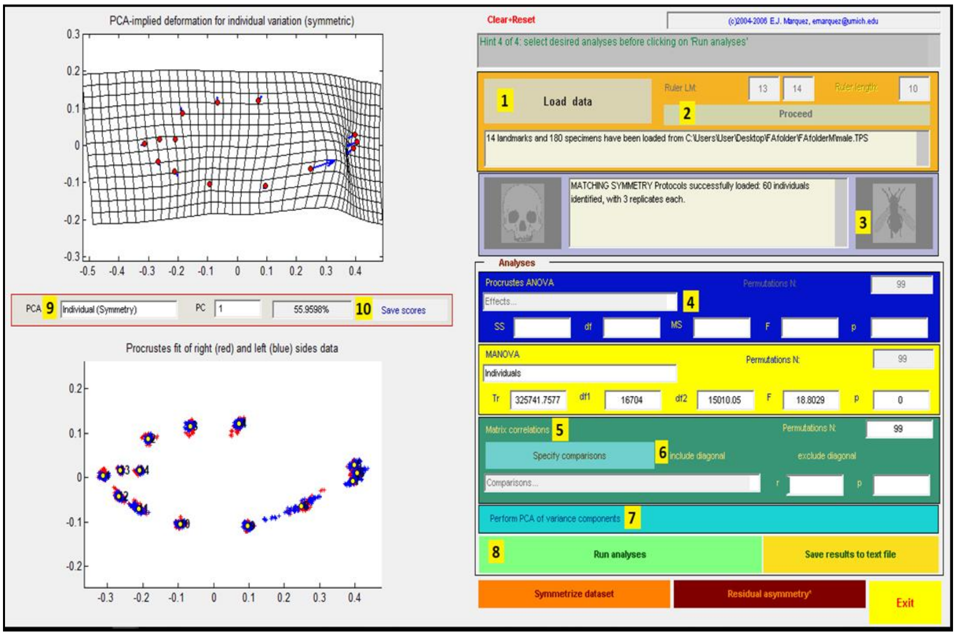


Fig 3: Diagram of shape analysis using Symmetry and Asymmetry Geometric Data Software**.**

3. Results and Discussion

Table 2: Procrustes ANOVA on body shape of *G. giuris* between male and female samples.

FACTORS	SS	DF	MS	F	P-VALUE
Female					
Individuals	0.1848	812	0.0002	1.1225	<0.0499
Sides	0.0066	28	0.0002	1.162	<0.2582
Individual x Sides	0.1646	812	0.0002	10.474	<0.0001**
Measurement Error	0.065	3360	0	--	--
Male					

Individuals	0.2601	812	0.0003	1.445	<0.0001**
Sides	0.0414	28	0.0015	6.6624	<0.0001**
Individual x Sides	0.18	812	0.0002	7.0923	<0.0001**
Measurement Error	0.105	3360	0	--	--

** (P<0.0001) highly significant

The Procrustes ANOVA is shown in Table 2 in the analysis of shape variation between the sexes of *G. giuris*, from Lake Mainit, Philippines. In females, the factor (individual x sides) show a highly significant difference (P<0.0001) while individuals (P<0.0499) and sides ((P<0.2582). While in males, the data have shown a highly significant difference (P<0.0001) among the factors analyzed. It denotes a high fluctuating asymmetry among of the samples tested. In relation, the occurrence of FA on the fishes highly associated with the ecological condition and genetic composition [16-17]. At the same time study suggests that rigid relationship of fluctuating asymmetry and developmental instability highly correlated to ecological perturbations and genetic influences [18-19]. Indeed, a more significant level of FA could be due to environmental stress and increased homozygosity [20]. While the study shows that the ability of organisms to Corresponds with various ecological disturbances could lead to stress and later affects FA level [10]. Relative to this, the significant degree of FA in a community of fishes may show that individuals had great difficulty in sustaining specific development ensuing in undesirable outcomes on the population through time [21].

Moreover, the incidence of FA in the population of *G. guiris* could also be associated with different anthropogenic activities in the study site. The area had surrounded by numerous households, mining activities and agricultural practices that contribute to the augmentation of pollutants and that could be possible affects the species developmental stability. From the literature, the obtained FA of the fishes could be linked to the distressed environment mainly towards various contaminants. Environmental state greatly disturbs species phenotypic characteristics, and thus pollution had an immense impact that eventually interrupts growth and development [22]. In addition, freshwater ecosystem commonly altered by countless substances and specifically fish biological stages emerge and this highly contribute their normal development [23]. Similar studies also demonstrated that ecological status may directly influence and altered phenotypic variability of fishes hence they consider as top consumer of the food tropic [24].

The advantage of using geometric morphometric (GM) often understands how organisms differ from one and another, especially in the morphology. They are also employed to assess for strong relationships amongst body shape formation and environmental traits or to determine the significance of phylogenetic correlation and shape comparison. While closely related taxa are likely to be comparable to one another than they would be without collective evolutionary information [25-27]. Further, geometric morphometrics has used in numerous investigations on fish assemblages, distinction and identification [28]. Also, this approach widely utilized from numerous studies to evaluate phenotypic variability of freshwater fishes i.e. *Glossogobius giuris* [24&29], *Mugil cephalus* [30], *Ambassis interrupta* [31], *Johnius vogleri* [12]. Nonetheless, the significant contribution of fluctuating asymmetry (FA) in assessing fish structures has widely acknowledged from various studies [32]. As well as, this tool is further advantageous than traditional morphometrics since all geometric information is retained throughout the analyses. Additionally, GM further analyze shape discrimination and thus constitute to compare shape variation and co-variation in a way of graphical representation [33-35]. Thus, the significant contribution of geometric morphometrics in the field of biological sciences widely recognized in detecting morphological variations.

Table 3: Principal component analysis (PCA) showing the values of symmetry and asymmetry scores of *G. giuris* with affected landmarks.

PCA	Individual (Symmetry)	Sides (Directional asymmetry)	Interaction (Fluctuating asymmetry)	Affected landmarks
Female				
PC1	38.927%	100%	29.6296%	1,2,4,5,6,7,8,9,10,11,12,13,14,15,16
PC2	19.6211%		23.6872%	1,2,3,4,5,6,7,8,9 10,11,12,13,14,15 16
PC3	10.0051%		14.2763%	1,3,4,5,6,7,8,9,10,12,14,15,16
PC4	8.0452%		10.7929%	1,2,4,5,6,7,8,9,12,13,15,16
PC5	6.2885%		4.7754%	1,2,4,5,6,7,8,9,10,11,12,13,14
	82.8869%		83.1614%	
Male				
PC1	40.7623%	100%	35.8756%	2,4,5,6,7,8,10,11 15,16
PC2	22.4493%		15.8117%	1,2,3,4,5,7,8,9,10,11,13,14,15,16
PC3	9.9829%		13.5237%	1,3,6,7,8,9,10,11 16
PC4	5.5798%		7.5296%	1,3,4,5,6,7,8,9,11 13,15,16
PC5	5.09%		6.6578%	1,2,3,4,5,6,7,8,9 10,11,12,15,16
	83.8643%		79.3984%	

Furthermore, Principal Component Analysis (PCA) in (Table 3) presented the scores of symmetry and asymmetry with the affected landmarks among of the fish samples. Results revealed that female population, generated five (5) PC scores accounting to 82.88% and Interaction (Fluctuating Asymmetry) of 83.16%. It was observed that the common affected landmarks among the 5 PC's were 1(Snout tip), 4 (Posterior insertion of dorsal fin), 5 (Dorsal insertion of caudal fin), 6 (Midpoint or lateral line), 7 (Ventral insertion of caudal fin), 8 (Posterior insertion of anal fin), 9 (Anterior insertion of anal fin) and 12 (Posterior end of maxilla). On the other hand, male population generated five (5) Principal Component Analysis with valued scores (83.86%) and Interaction (79.39%). The common affected landmarks among the 5 PC's were 7 ((Ventral insertion of caudal fin), 8 (Posterior insertion of anal fin, 11 (Ventral end of lower jaw articulation) and 16 (Dorsal base of pectoral fin). It was noted that the affected landmarks among the male and female fishes were alike however there were set of coordinates that varies. While it was observed that the percent of fluctuating asymmetry (FA) were higher than of the female this proposing shape variability among species of the same lineage. Besides, these suggests that sexual orientation also influence the buffering mechanism towards developmental instability, Indeed, figure 4 and 5 were presented to visualize the metric traits deformation of the sample tested. These identifies the body shape variations occur in the two sexes while the incidence of phenotypic variation was attributed to genetic components and as well as from environmental causation [24].

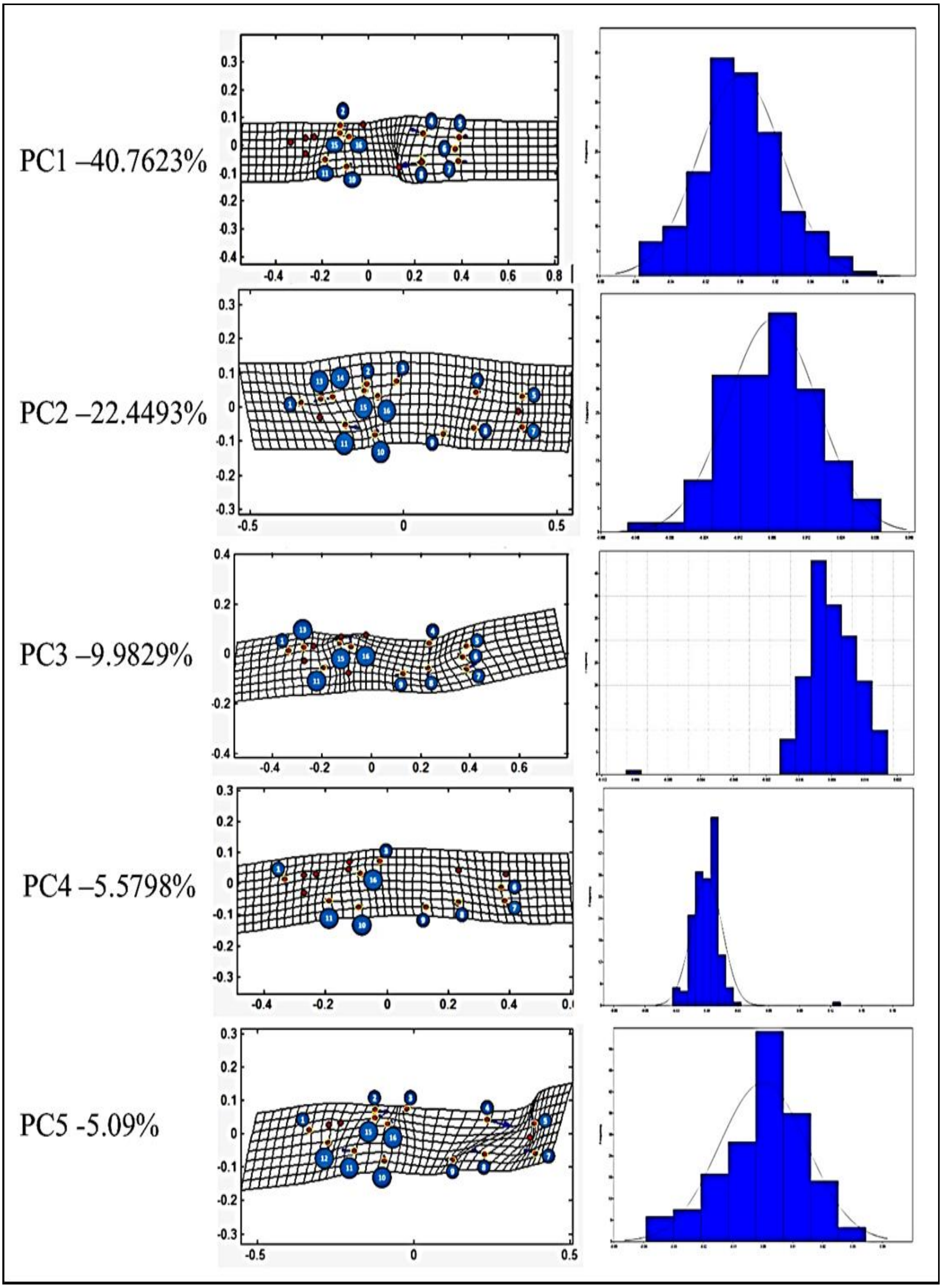


Fig 4: Male (*G. giuris*) implied deformation grid and a histogram of individual symmetric from principal component analysis scores.

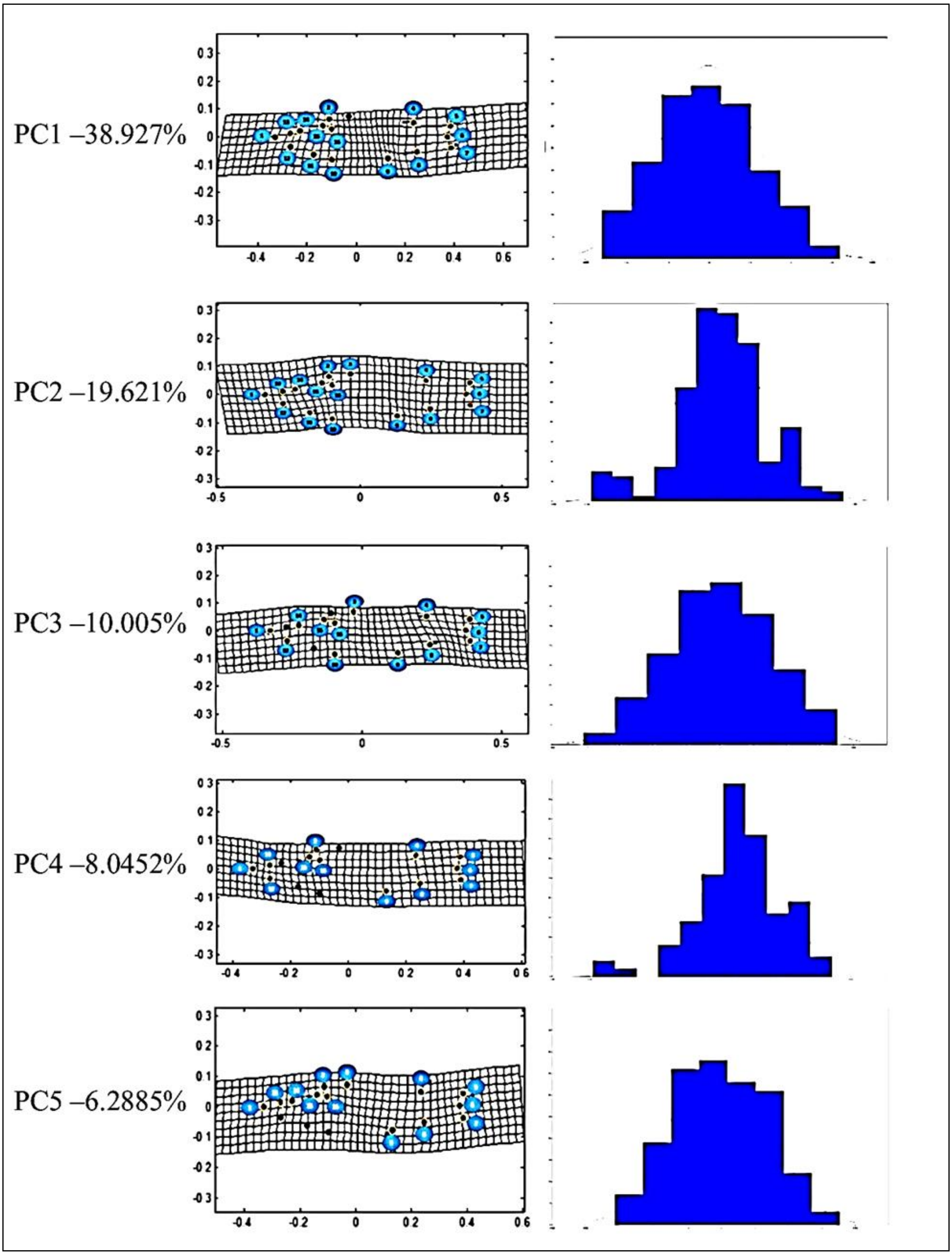


Fig 5: Female (*G. giuris*) implied deformation grid and a histogram of individual symmetric from principal component analysis scores.

4. Conclusion

Geometric Morphometric Analysis through Fluctuating Asymmetry was utilized to identify the body shape variation among the population of *G. guiris* collected from Lake Mainit, Philippines and detected of having morphological differences. The data from Procrustes ANOVA also suggests a highly significant difference (*P*<0.0001) among the factors analyzed (sides, individuals and individual x sides) denoting shape differences between the male and female samples. Further, the data from Principal Component Analysis (PCA) also shows that the percentage of fluctuating asymmetry (FA) were higher in female than of the male suggesting phenotypic variability. Indeed, the dissimilarities are correlated to shape variations in the populations. Thus, the essential contribution of geometric morphometric using fluctuating asymmetry advances as an efficient mean of quantifying and illustrating shape differences among fishes of the same population.

5. References

1. De Guzman AB, Uy WH, Gorospe JG, Openiano AE, Acuña RE, Roa RL *et al*. Sustainable Fisheries Management Program for Lake Mainit. Phase II:

Comprehensive Resource Assessment. Final Report. MSU at Naawan Foundation for Science and Technology Development, Inc, 2009, 1-73
2. Galicia AM, Lopez NA. The Biology and Fishery of Indigenous Gobies of Mainit Lake, Philippines. Paper presented at the International Workshop on Reservoir and Culture Based: Biology and Management, Maruay Garden Hotel, Bangkok, Thailand, 2000.
3. Chiappe LM. A new Late Mesozoic *Confucius ornithid* Bird from China, Journal of Vertebrate Paleontology. 1999; 19(1):1-7.
4. Adams DC, Rohlf JF, Slice DE. Geometric Morphometrics: ten years of progress following the “revolution”. Italian Journal of Zoology. 2004; 71:5-6.
5. Moneva CS, Demayo CG, Torres MA. Applications of geometric morphometric analysis in describing sexual dimorphism in shell shapes in *Vivipara angularis* Muller (Family Viviparidae). Animal Biology & Animal Husbandry. 2012; 4(1):14-19.
6. Bergstrom CA, Reimchen TE. Geographical variation in asymmetry in *Gasterosteus aculeatus.* Biological Journal of the Linnean Society. 2002; 77:9-22.
7. Tomkins JL, Kotiaho JS. Fluctuating asymmetry. Encyclopedia of Life and Sciences. Macmillan Publishers Ltd, Nature Publishing Group, 2001, 1-5.
8. Lecera JMI, Pundung NAC, Banisil MA, Flamiano RS, Torres MAJ, Belonio CL *et al*. Fluctuating asymmetry analysis of trimac *Amphilophus trimaculatus* as indicator of the current ecological health condition of Lake Sebu, South Cotabato, Philippines. AACL Bioflux. 2015; 8(4):507-516.
9. Angtuaco SP, Leyesa M. Fluctuating Asymmetry: An Early Warning Indicator of Environmental Stress. Asian Journal of Biology Education. 2004; 2:35-38.
10. Ducos MB, Tabugo SRM. Fluctuating asymmetry as bioindicator of stress and developmental instability in *Gafrarium tumidum* (*Ribbed venus* clam) from coastal areas of Iligan Bay, Mindanao, Philippines. AACL Bioflux. 2015; 8(3):292-300.
11. Swaddle JP. Fluctuating asymmetry, animal behavior and evolution. Advances in the Study of Behavior. 2003; 32:169-205.
12. Jumawan JH, Cabuga CCC Jr, Jumawan JC, Cortez EMB, Salvaleon SMN, Gamutan KJS *et al.* Probing the exposure to environmental stress using fluctuating asymmetry of metric traits in *Johnius vogleri*, (Bleeker, 1853) from lower Agusan River basin, Butuan City, Agusan del Norte, Philippines. AACL Bioflux. 2016; 9(1):122-132.
13. David Polly P. Geometric morphometrics. Biology and Anthropology University, Department of Geology, Indiana, 2012.
14. Joseph CCD, Jumawan JH, Hernando BJ, Boyles LZ, Jumawan JC, Velasco JPB *et al*. Computational Ecology and Software. 2016; 6(2):55-65.
15. Paña, BH, Lasutan, LG, Sabid J, Torres MA, Requieron E. Using Geometric Morphometrics to Study the Population Structure of the Silver Perch, *Leiopotherapon plumbeus* from Lake Sebu, South Cotabato, Philippines. AACL Bioflux. 2015; 8(3):352-361.
16. Sadeghi S, Adriaens D, Dumont HJ. Geometric morphometric analysis of wing shape variation in ten European populations of *Calopteryx splendens* (Harris, 1782) (Zygoptera: Odonata). Odonatologica. 2009; 38:343-360.
17. Hernando BJ, Demayo CG, Caasi-Lit M, Manting MM. Quantitative Descriptions of head shapes of three different instar-larvae of the Asian corn borer *Ostrinia furnacalis*. Journal Applied Science & Agriculture. 2014; 9(11):257-262.
18. Parsons PA. Fluctuating asymmetry: an epigenetic measure of stress. Biology Review Cambodia Philosophy Society. 1990; 65(2):131–145.
19. Graham JH, Raz S, Hagit H, Nevo E. Fluctuating Asymmetry: methods, theory and applications. Symmetry. 2010; 2:466-495.
20. Galbo KR, Tabugo SRM. Fluctuating asymmetry in the wings of *Culex quinquefasciatus* (Say) (Diptera: Culicidae) from selected barangays in Iligan City, Philippines AACL Bioflux. 2014; 7(5):357-364.
21. Markow TA. Evolutionary ecology and developmental instability. Annual Review of Entomology. 1995: 40:105–120.
22. Bonada N, Williams DD. Exploration of utility of fluctuating asymmetry as an indicator of river condition using larvae of caddis fly Hydropsyche morosa (Trichoptera: Hydropsychidae). Hydrobiologia. 2002; 481, 147-156
23. Conclu PV. Guide to Philippine Flora and Fauna: Fishes. IX. 104-105 Ross AH, Slice DE and Williams SE. 2010. Geometric Morphometric Tools for the classification of Human Skulls, 1986, 1-59.
24. Cabuga CCC, Milloria MB, Lanes JR, Varona CJL, Pondang JMD, Raules JJ *et al*. Intraspecific evaluation in the morphology of *Glossogobius guiris* using geometric morphometric analysis from Lake Mainit, Agusan del Norte, Philippines. International Journal of Biosciences. 2019; 14(1):379-387.
25. Felsenstein Joseph. Phylogenies and Comparative Method. The American Naturalist. 1985; 125(1):1-15.
26. Rueber L, Verheyen E, Meyer A. Replicated evolution of trophic specializations in an endemic cichlid fish lineage from Lake Tanganyika. Proceedings Natural Academy Science USA. 1999; 96:10230-10235.
27. Rosenberg NA, Pritchard JK, Weber JL, Cann HM, Kidd KK, Zhivotovsky LA *et al*. Genetic Structure of Human Population. 2002; 298:2381-2384.
28. Caldrin SX, Friedland KD. The utility of image processing techniques for morphometric analysis and stock identification. Fisheries Research. 1999; 43:129-139.
29. Portillo JN, Patulili RR. Lucas MMA, Alaijos O, Demayo CG. Body Shape Variation in the Goby, *Glossogobius giuris* Collected in Selected Areas in the River of Norzagaray Bulacan Using Landmark-Based Geometric Morphometrics. Journal of Informatics and Mathematical Sciences. 2017; 9(4):1109–1116.
30. Cabuga CCC, Seronay RA, Busia MA, Billuga NP, Ayaton MA, Angco MK *et al*. Geometric morphometric and heavy metals analysis of flathead grey mullet (*Mugil cephalus*) from Agusan River, Butuan City, Philippines. Journal of Biodiversity and Environmental Science. 2017; 11(1):134-151.
31. Medrano MGT, Jumawan JC. Landmark-based geometric morphometric analysis of body shape variation within population of Ibis fish, *Ambassis interrupta*, collected from Masao River, Butuan City, Philippines. International Journal of Advances in Chemical

Engineering & Biological Sciences (IJACEBS). 2016; 3(2):1-5.
32. Paña BHC, Lasutan LGC, Sabid JM, Torres MAJ, Requieron EA. Using Geometric Morphometrics to study the population structure of the silver perch, *Leiopotherapon plumbeus* from Lake Sebu, South Cotabato, Philippines. AACL Bioflux. 2015: 8(3):352-361.
33. DC Funk DJ. Morphometric inferences on sibling species and sexual dimorphism in Neochlamisus Morphometrics of eretmodine cichlids *Neochlamisus bebbianae* leaf beetles: multivariate applications of the thinplate spline. Systematic Biology. 1997; 46:180-194.
34. Caldecutt WC, Adams DC. Morphometrics of trophic osteology in the three-spine stickleback, *Gasterosteus aculeatus*. *Copeia*, 1998, 827-838.
35. Adams DC, Rohlf FJ. Ecological character displacement in Plethodon: Biomechanical differences found from a geometric morphometric study. Proceedings Natural Academy Science USA. 2000: 97:4106-4111.
36. Rohlf FJ. On applications of geometric morphometrics to studies of ontogeny and phylogeny. Systematics Biology. 1998; 47(1):147-67.
37. Márquez EJ, Knowles LL. Correlated evolution of multivariate traits: detecting co-divergence across multiple dimensions. Journal of Evolutionary Biology. 2007; 20:2334-2348.