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Intraspecific evaluation in the morphology of *Glossogobius guiris* using geometric morphometric analysis from lake Mainit, Agusan del Norte, Philippines

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Abstract

Morphological variations among organism have been baseline information to which species may vary from one and another. This study has been conducted to determine the intraspecific evaluation in the morphology of *Glossogobius guiris* using geometric morphometric analysis from Lake Mainit, Agusan del Norte, Phils. About 60 individuals (30 males and 30 females) were collected and subjected to analysis. Digital imaging was prepared and loaded to the tpdsdig2 program. Standard landmarks were applied in the fish morphology. Utilizing thin plate spline (tps) series, landmark examination were obtained and subjected to symmetry and asymmetry geometric data (SAGE) software. There were three factors applied to identify shape variations: individuals, sides and individuals vs. sides. In Procrustes ANOVA, results showed a highly significant difference of (P<0.0001**) in both male and female samples demonstrating FA in *G. guiris*. The asymmetry detected in the fish samples indicating a variation in the morphology among the female and male samples. While other correlate this phenomenon due to genetic composition and environmental perturbations. Principal Component Analysis was applied to investigate affected landmarks in the fishes and showed that females have the highest cumulative scores (82.9304%) while males (78.9154%). The data obtained revealed that morphological variations have been depicted between female and male samples yet they are in the same species. Thus, employing geometric morphometric to determined shape variances widely acknowledges and performs as a cost-effective tool.

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Introduction

Morphological assessment often investigates body shape variation in many biotic organisms specifically in fishes. This allows identifying species differences based on the phenotypes both interspecific and intraspecific. Indeed, morphology serves as a foundation for which taxonomist categorically distinguished every single species.

Modern approach has been widely acknowledged to differentiate structures of organisms (Cabuga *et al.*, 2017). For instance, geometric morphometric (GM) typically applied to evaluate dissimilarities of species outline.

These benefits to understanding the entire species formation. GM not only used in taxonomy and another related discipline however as an efficient tool to identify the source of phenotypic modifications (Klingenberg, 2011).

It comprises the Procrustes superimposition-based procedures that suggest an advancement rather than a traditional analysis of determining shape patterns (Bookstein, 1996; Rohlf, 1998).

While, the shape of an organism plays significant information on how it responds both on the functional adaptation and evolutionary change (Singleton, 2002; Nicholson & Harvati, 2006). In contrast, the conventional method in detecting organism morphology have need that samples be assessed and eventually taxonomic labels assigned through experts (Cope *et al.*, 2012). Further, this type of technique involves of the two approaches: outline-based analysis (utilizing the specimen margin) and landmark-based analysis (utilizing set of landmarks to define the specimen) (Richtsmeier *et al.*, 2002). Geometric morphometric known to be effective and cost-efficient to discriminate shape in a various organism (Addis *et al.*, 2010; Albutra *et al.*, 2012; Requiron *et al.*, 2012; Meyer *et al.*, 2006; Singh *et al.*, 2013). Also, species morphology plays an essential factor in phenotypic differentiation and a primary component to quantify shape and shape variations (Cabuga *et al.*, 2017). Furthermore, GM usually applied in various studies to determine shape deviations especially in fish samples (Velasco *et al*, 2016; Joseph *et al*, 2016; Cabuga *et al.*, 2016; Jumawan *et al.*, 2016. Moreover, the study aims to identify the shape variation between male and female samples of *Glossogobius guiris* collected from Lake Mainit, Agusan del Norte, using geometric morphometric analysis.

Materials and methods

Description of the area

The study was conducted in Lake Mainit, Agusan del Norte, Philippines (Fig. 1). Geographic coordinates are 9°20'33"N, 125°24'29"E. The collection of fish samples was done on the month of August 2018 with the aid of local fisherman in the study area.

Sample collection and processing

A total of 60 samples consisting of 30 males and 30 females were randomly collected. The samples were brought to the laboratory for further processing. Individually, the fish positioned in the top of the Styrofoam was a 10% formaldehyde applied in the fins to make it hardened through a small paint brush. Each of the samples was then photographed by a digital camera (DSLR). After which, a ruler was placed below of the fish samples to obtain the bilateral symmetry (Left and Right side).

Sex determination

To be able to determine the sex of the samples, its undergone external examination by looking the genitalia. In females, it was observed with yellow to orange granular textures while the male samples were observed by its testes where it is smooth and whitish with non-granular in texture (Requiron *et al.*, 2010).

Landmark selection and digitation

The obtained photographs were categorized through its sexes while converted into TPS file using the tpsUtil. Further, digitation of the samples was prepared using tpsDig2 (Rohlf 2004). A total of sixteen (16) anatomical landmarks point (Table 1) were applied to digitize the specimen (Fig. 2).

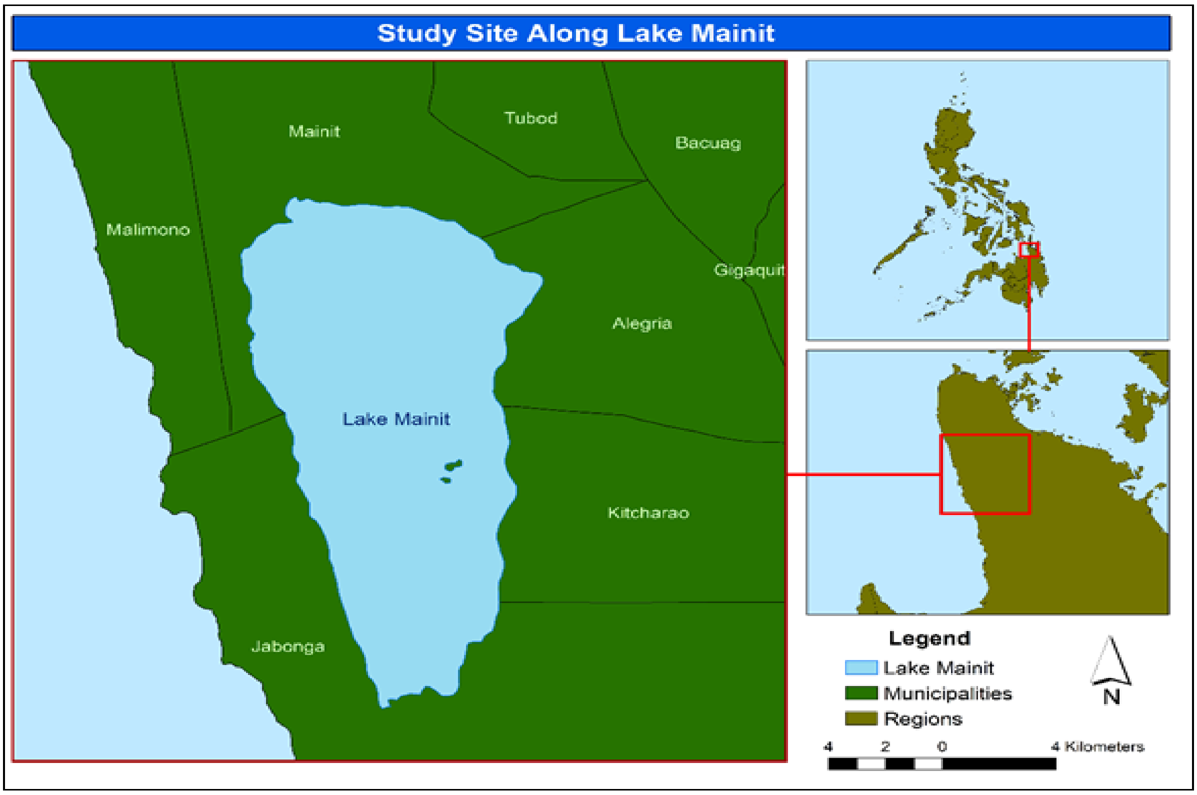

Fig. 1. Map of the Study Area, Lake Mainit, Agsusan del Norte. Phils. (Photo credit: Joseph *et al.*, 2016.).

Shape analysis

The samples were tri-replicated to minimize the measurement error. The left and right side of the samples was digitized. While the landmark points were transported to Symmetry and Asymmetry in Geometric Data (SAGE, version 1.04) by Marquez, 2007 (Fig. 3).

The obtained data specifies report from the principal components of individual symmetry which shows in a deformation grid (Natividad *et al.*, 2015). Further,

Procrustes ANOVA was employed to determine the significant difference in the symmetry of the three factors analyzed: individual, sides, and interaction of individuals and side.

The level of significance was tested at P<0.0001. Alongside, the differences of the side and the evaluation of directional asymmetry similarly examined. Further, the degree of fluctuating asymmetry (FA) was analyzed and shown in percentage (%) thus compared among the female and male samples (Natividad *et al.*, 2015).

Results and discussion

Procrustes ANOVA was applied to draw the fluctuating asymmetry (FA) in the morphology of *G. Guiris* (Table 2).

Table 1. Description of the landmark points of *G.guiris* adopted from Paña *et al.,* 2015.

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

There were three factors analyzed: individuals, sides, and the interaction of individuals by sides. Results show a highly significant difference (P<0.0001) among the female samples (individual and individual × sides).

While male samples are shown (P<0.0001) in the individual x sides.

Table 2. Procrustes ANOVA on the morphology of *G. guiris* in terms of sexes from Lake Mainit, Agusan del Norte, Philippines.

Factors	SS	DF	MS	F	P-VALUE
			Female		
Individuals	0.2079	812	0.0003	0.8872	0.0001**
Sides	0.0044	28	0.0002	5502	0.9726
Individual x Sides	0.2344	812	0.0003	25.9398	0.0001**
Measurement Error	0.0374	3360	0	-	-
			Male		
Individuals	0.2121	812	0.0003	0.9083	0.9147
Sides	0.0034	28	0.0001	0.416	0.9970
Individual x Sides	0.2336	812	0.0003	33.2628	0.0001**
Measurement Error	0.0291	3360	0	-	-

** (P<0.0001) highly significant.

Table 3. Principal component scores showing the values of symmetry and asymmetry scores with the summary of the affected landmarks of *G. guiris* from Lake Mainit, Agusan del Norte, Philippines.

PCA	Individual (Symmetry)	Sides (Directional Asymmetry)	Interaction (Fluctuating Asymmetry)	Affected landmarks
		Female		
PC1	39.6174%	100%	47.7873%	1,2,3,4,5,6,7,8,9,10,11,12,13,14,16
PC2	23.8366%		14.0605%	1,2,3,4,5,6,7,8,9,10,12,13,14,15
PC3	7.1370%		12.1716%	1,2,8,9, 10, 11, 12,15,16
PC4	6.4586%		5.8074%	1,2,3,8,9,10,11,13,15
PC5	5.8808%		5.3429%	1,2,7,8,16
	82.9304 %		85.1697%	
		Male		
PC1	35.5259%	100%	48.77%	1,2,3,4,5,6,7,8,9,10,11,12,13,14,16
PC2	23.0101%		13.12%	1,2,3,4,5,6,8,10,15
PC3	7.7699%		11.23%	1,4,8,9,11,15,16
PC4	6.4351%		6.87%	1,4,7,8,9,10,11,15,16
PC5	6.1774%		5.63%	1,2,3,6,7,8,9,10,13,14,16
	78.9184%		85.62%	

This indicates that fluctuating asymmetry (FA) were observed in both samples. Studies show that the incidence of FA could be associated with ecological requirements hence species have to be adaptive within the habitat (Sadeghi *et al.*, 2009; Yuto *et al.*, 2016). Genetic aspects of organisms such as in fishes have been established to be adaptive prior to its exposure from different environmental perturbation and thus affects its history patterns (Hernando *et al.*, 2014). While reproductive differences among male and female species highly associated with shape dimorphism between populations. The increased of homozygosity or ecological stress results to the high level of fluctuating asymmetry and thus affects the morphology (Galbo and Tabugo, 2014).

Further, study shows that there is a strong relationship amongst FA and developmental instability and FA was believed to reveal a species capacity to manage with genetic and ecological stress (Parsons 1990; Graham *et al.*, 2010). Increased levels of FA result from dissimilarities in the genetic structure of the population ensuing in various stress tolerance (Ducos and Tabugo, 2014). Also, it was suggested that the correlation between FA and stress is because species needs the energy to respond to different stressors (Ducos and Tabugo, 2015). Nonetheless, FA consequently lessens energy both for reproduction and growth thus later influence the lineage (Koehn and Bayne, 1989).

Additionally, stress defined as the key factor promoting energy reduce that eventually affects developmental accuracy (Sommer, 1996).

Fig. 2. Landmark points used to describe the body shape of *G. guiris*. Top (Female), Bottom (Male).

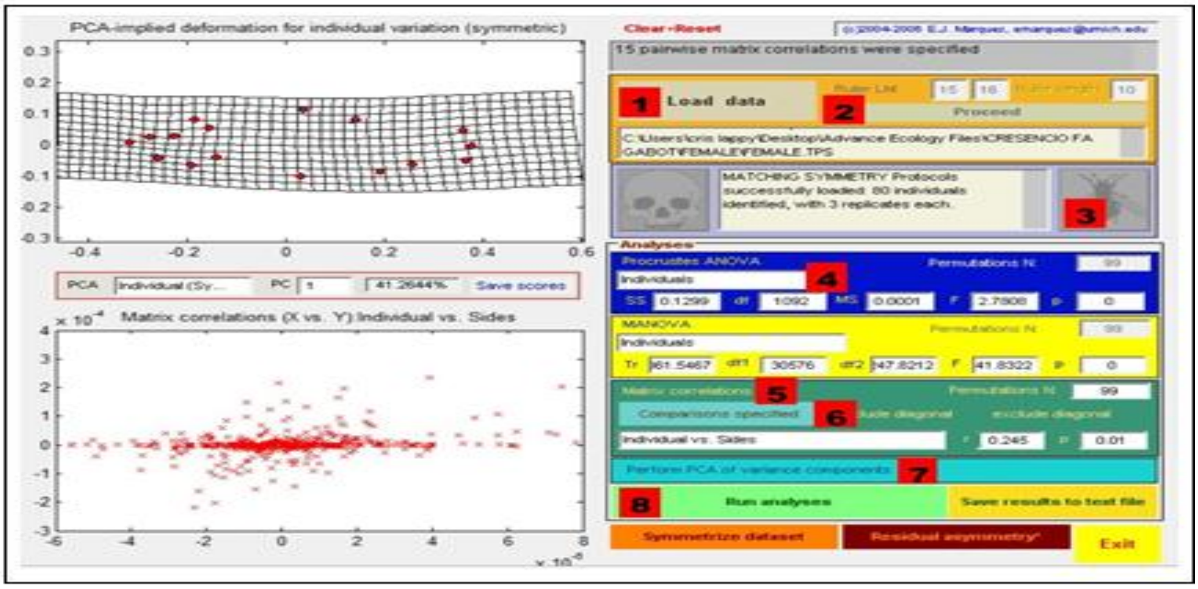

Fig. 3. Overview of the schematic flow of shape analysis using SAGE**.**

Comparative study shows that fishes are an efficient model for determining morphological variations. Indeed, Gobies were used as a biomarker of water quality owing to its various population throughout the country which includes 16 species known to locate only in the Philippines (Hoese and Allen, 2009; Lekshmi *et al.*, 2010). Along with, *G. celebius* has been employed as an indicator to detect developmental stability and evaluate environmental stress (Kark *et al.*, 2001; Mpho *et al.*, 2002; Velickovic 2004). Also from the study of Joseph *et al.*, 2016 in *Glossogobius guiris* collected in the same area shows high levels of FA both in male and female samples.

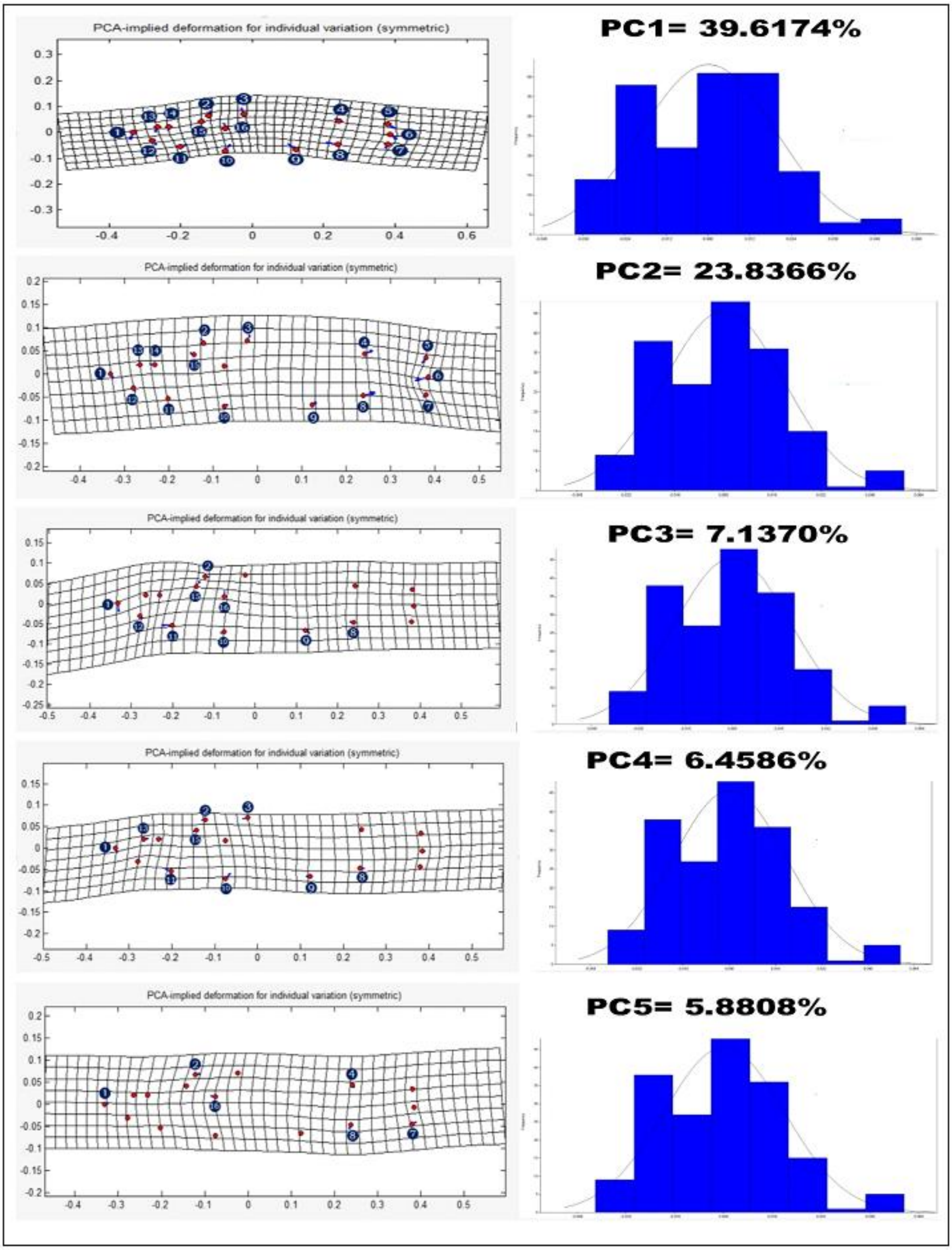

Fig. 4. Principal Component (PC) implied deformation grid and histogram of individual (Fluctuating Asymmetry) of female *G. guiris*.

Principal Component Analysis (PCA) among female and male samples were shown in Table 3 along with its Interaction or Fluctuating Asymmetry. As observed, the female has 85.17% of FA comprising about five principal component scores (PC) with a cumulative value of 82.93%. While, the commonly affected landmarks among the five PC were 1 (Snout tip), 2 (Posterior end of nuchal spine) and 8 (Lateral line). On the other hand, the male has 85.62% of FA comprising about five principal component scores (PC) with a cumulative value of 78.92% which were lower than the female samples. The commonly affected landmarks among the five PC were 1 (Snout tip) and 8 (Lateral line). Moreover, it was detected that among of the fish samples it obtained similar affected landmark points. Certainly, snout tip was normally used for food hunting and might be a reason why there was a commonality among the two. Relatively, the PCA implied deformation for both male and female samples were shown in (Fig. 4 & 5).

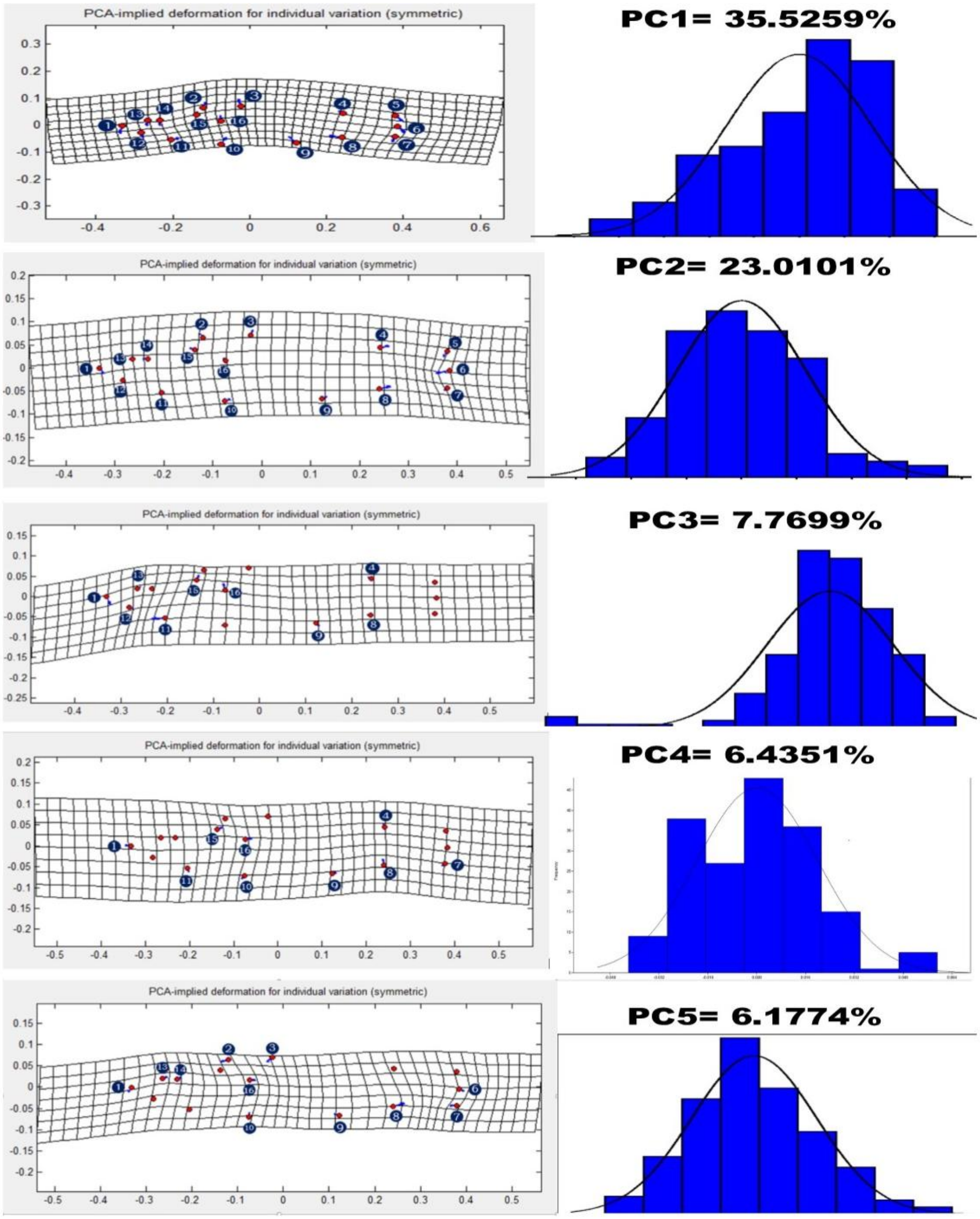

Fig. 5. Principal Component (PC) implied deformation grid and histogram of individual (Fluctuating Asymmetry) of male *G. guiris*.

The later demonstrate the affected landmark points of the fish samples resulting in fluctuating asymmetry in its morphology. Thus, body shape deformation could also be associated with genetic information and environmental requirements hence female fishes specifically were highly affected by the situation during and after reproduction (Requiron *et al.*, 2012).

Essentially, GM quantifies fluctuating asymmetry and conveniently applied in different studies (Savriama *et* *al*., 2012; Hermita *et al*., 2013). Nonetheless, the importance of geometric morphometric (GM) often times described as a development in the area of morphometric and has been effectively applied in fields of biological studies such as taxonomy, ecology, evolution and systematics (Morais *et al* ., 2013).

Conclusion

The obtained results from Geometric Morphometric Analysis that comprises with Procrustes ANOVA, Principal Component Analysis (PCA), Implied Deformation and Histogram shows that both male and female samples of *G. guiris* show significantly different in the body shape. While, the data provide from Procrustes ANOVA supports that each sexes was morphologically dissimilar. Phenotypic variations among organisms normally occur due to its genetic component and environmental requirements. Thus, Geometric Morphometric Analysis was able to differentiate from the assigned landmark points through the fish morphological structure. This study entails important knowledge to which variation of phenotypes were evident even species belonging to the same taxa. Thus, the advantage of using a modern and systematic approach helps to understand phenotypic variability.

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