Article

Phenotypes variability in *Glossogobius guiris* population using Geometric Morphometric Analysis with notes of physico-chemical parameters from Lake Mainit, Caraga Region, Philippines

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Abstract
Shape, size, and structures are fundamental elements for species identification. While phenotypic variability among species of the same populations usually occurs. To understand this phenomenon modern techniques like geometric morphometrics were employed to distinguishbody shape differences. This study aimed to investigate the phenotypic variability of *Glossogobius guiris* with notes of physicochemical parameters in Lake Mainit, Caraga, Philippines. A total of 75 samples (35 males and 35 females) of the same size were collected and subjected to Symmetry and Asymmetry Geometric Data (SAGE) Software. Results showed that the physico-chemical parameters are within the standard levels suggested by the Department of Environment and Natural Resources (DENR) Environmental Management Bureau (EMB) in the freshwater category. It implied that the condition of the water in the Lake was suitable for all aquatic life. On the other hand, Procrustes ANOVA indicated a significant difference (P<0.0001) between female and male populations across the factors analyzed (Individuals, Sides, Individual x Sides) inferring phenotypic variations. On the other hand, Principal Component Analysis (PCA) revealed a high percentage of Fluctuating Asymmetry (FA) in the male populations (75.86%) and female populations (64.74%). This indicates a wide range of morphological dissimilarities and these significant levels of FA are attributed to the modifications in the genetic composition of the populations resulting in observable physical traits. Ultimately, the advancement of using geometric morphometric analysis provides important evidence showing how species of the same population differ morphologically.

Keywords freshwater; coordinates; landmarks; morphology; shape.

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1 Introduction

Interest in species identification is an essential aspect of biology. It served as a tool for population discrimination and plays a role in biodiversity conservation among the fauna communities. Across the globe, the importance of naming organisms promotes the management of natural resources, including fishery. Species identification is often used to categorize organisms belonging to the same taxon. Neither fecundity, growth, mortality, trophic, and morphology are factors to consider when investigating biological traits (Ibanez, 2007). The traditional approach in determining species characteristics is widely employed to establish a meristic and morphological basis (Casselman et al., 1981; Ihssen et al., 1981; Cadrin, 2000). However, this technique creates erroneous reliability concerns in shape, size, and structure (Jerry and Cairns, 1998; Swain and Foote, 1999; Murta, 2000). While this method only uses linear measurements, ratios, and counts that are known to produce unspecified shape differences (Adams et al., 2004). On the other hand, several studies had used Geometric Morphometric (GM) a modern method broadly used in Biology for shape analysis using landmark data sets (Chen et al., 2011; Crews and Hedin, 2006; Recasens et al., 2006).

Moreover, GM provides a measurement and homologies associated with landmark coordinates. It resulted in statistical analysis that distinguishes the shape spaces of the specimen (Dryden and Mardia, 1998). This technique had vast applications across organisms like Insect wings (Rohlf, 1990; Cabuga et al., 2018), Asian citrus psyllid (Lashkari et al., 2013), *Scylla serrate* (Presilda et al., 2018), Freshwater fishes (Cabuga et al., 2016, 2017, 2018, 2019). Water hyacinth (Cabuga et al., 2018), fish scales (Ibañez, 2007), and golden apple snail (Cabuga et al., 2017). On the other hand, GM is also used to predict the environmental health condition and organism morphology (Requiron et al., 2010). Likewise, it has enhanced the complexity of quantitative biological shape analysis. Thus, formulating and efficiently analyzing data to answer questions concerning the shape of the phenotype (Kendall, 2014).

Nonetheless, the significance of using Geometric Morphometrics paves the way for systematics and taxonomy as two fields of Biology concerning species identification. GM is an extensively used application to investigate shape differences between populations (Santos and Quilang, 2012). Further, this tool presents a set of landmarks that merged to make complete information about the shape of samples (Bookstein, 1996). Likewise, this application draws variations between sexes of the same taxa. Since fish communities are more diverse in terms of structure it is difficult to identify their shape manually. The study suggested assessing the fish population through phenotypic differentiation using morphometric identification (Torres et al., 2020). In relation, body shape fluctuations of a fish usually create differences within species. It might be associated with genetics, selection on the species' history, and environmental variables that interplay (Cadrin, 2000).

The present study employs *Glossogobius guiris* (Pidjanga) a native fish from Lake Mainit in Caraga Region. The lake serves as the primary fishery resource in the region. Because of its location, the lake has been disturbed by various pollution from rice fields using pesticides and is surrounded by numerous households along the lake. This condition influences the water condition and its inhabitants. Accordingly, the growing population (Rabadon and Corpuz, 2021), numerous pollution (Wu et al., 2019), and climate change (Qui et al., 2019) have impacted the freshwater ecosystem and fishery resources globally. Studies showed that any organisms' physical modifications are associated with their environment (Cabuga et al., 2019; Jumawan et al., 2016; Lecera et al., 2015). While an organism's shape offers significant information about how it adapts to both evolutionary and functional changes (Singleton, 2002; Nicholson and Harvati, 2006).

Nevertheless, fish often serve as the best model for evaluating environmental conditions. Since they are susceptible to different pollutions. While, they are considered as the biomarker of ecological alterations (Galbo and Tabugo et al., 2014). It is also used for scientific investigation due to its availability and precise species ID. Assemblages of fish and ecological traits of aquatic habitats are deemed good markers of ecosystem health

(Sheaves et al., 2012; Huang et al., 2019). Considerably this study associates water quality as an essential abiotic factor that regulates the growth and development of aquatic organisms. This study aims to analyze the phenotypes variability of *G. guiris* populations by using geometric morphometrics with notes of water quality parameters from Lake Mainit, Jabonga, Agusan Del Norte, Philippines.

2 Materials and Methods

2.1 Study area

The study area was Lake Mainit in Jabonga, Agusan Del Norte. It geographically lies between 9°32'6.00" N 125°31'22.80" E. It serves as the major fishery resource that houses several freshwater fishes and is commercially sold within the region. The lake is the fourth largest lake in the Philippines and measures 173.40 square kilometers (66.95 sq mi). Alongside it was a protected area under E-NIPAS Act (RA 7586) (Apduhan et al., 2014).

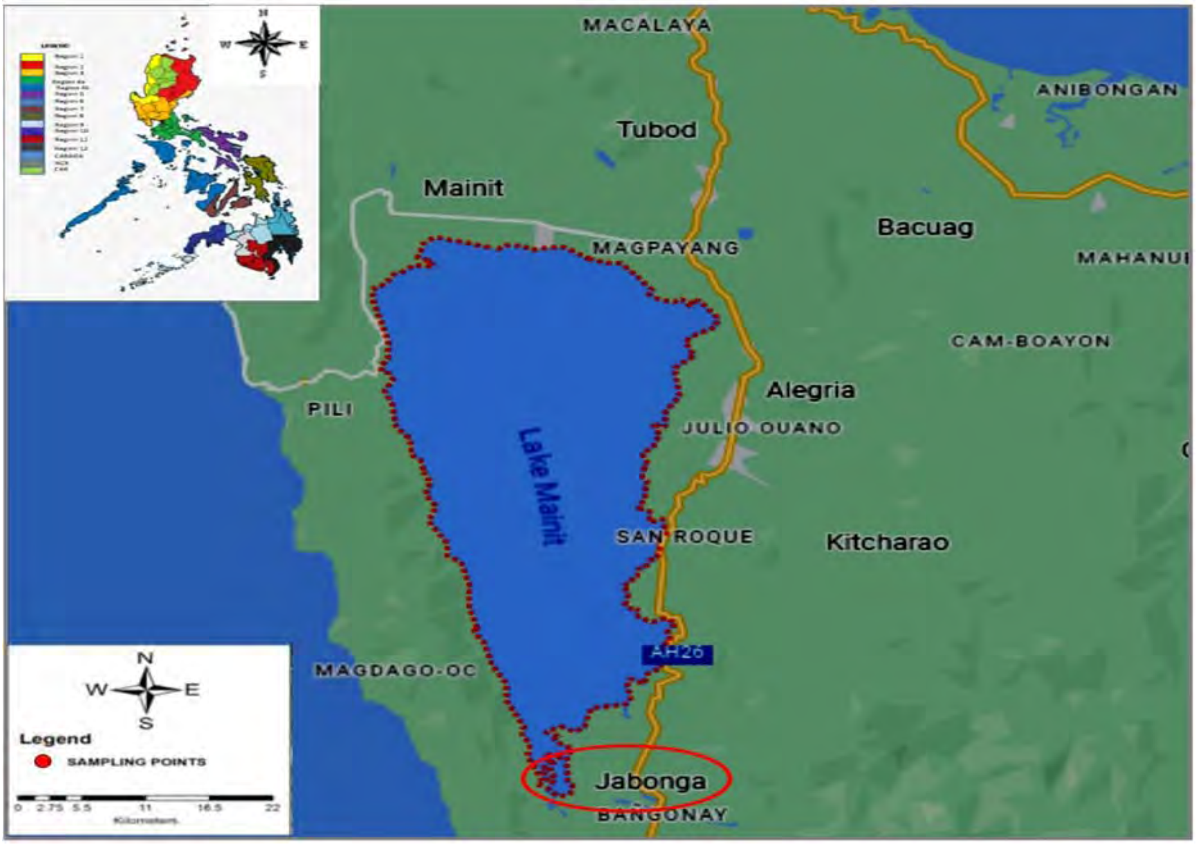

Fig. 1 Map of the study area, Lake Mainit, Caraga, Philippines.

2.2 Fish sample collection and sex determination

The fish collection was done in the month of May 2023. Freshly caught *G. guiris* were obtained directly from a local fisherman through an indigenous cast net fishing technique known as a *Pokot.* A total of 70 fish samples (35 males and 35 females) of the same size were randomly collected. Sex determination of a fish showed that females exhibit body shapes that are geared to support a large number of eggs (yellowish granular textures) ensuring reproductive rate while males exhibit a moreslender body (whitish and smooth texture) and are generally smaller than females (Requiron et al., 2010).

2.3 Water quality parameters

The water quality parameters were assessed on the same date mentioned above during the fish collection. From the study area, three sampling sites with a 100 meters distance each with three replicates were randomly selected for the measurements. The multi-parameter (HANNAH-HI98194) was used to determine the variables. Data were presented as mean ± standard error mean (SEM) and were calculated using the Graph Pad Prism 8.0.1. One-way Analysis of Variance (ANOVA) was also used for testing the significant difference in water quality parameters between the sampling areas.

2.4 Laboratory processes

The fish samples were then sorted according to their size by using a ruler. Individually, the fish was placed at the top of Styrofoam. The fish fins were pinned and applied with a 10 percent formaldehyde solution using a small paintbrush. Each fish sample was captured by using a digital camera in 3 replicates to minimize the error.

2.5 Landmark selection and digitization

The photographs of the female and male samples were separated based on their sex. Afterward, it was loaded to a TPS file and converted through tpsUtil. Landmarking was followed and utilized the tpsDig Version 2.0 (Adams et al., 2004) (Fig. 2) The left and right sides of the fish samples which digitally captures were subjected to replicates using the same software. The digitation process of the body shape of the fish samples was done using the sixteen anatomical landmark points (Table 1).

Table 1 Description of the landmark points adapted from 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

2.6 Shape analysis and data generation

The coordinates of the fish samples were utilized and loaded to Symmetry and Asymmetry in Geometric data analysis (software version 1.04, Marquez, 2007) (Fig. 3). Procrustes ANOVA test was utilized to define the components analyzed (Individual, sides, and interaction of individual and sides) and tested for significant difference ($P<0.0001$). While this was mused to determine the principal component analysis (PCA) of each fish sample's symmetry and asymmetry (interaction).

Fig. 2 Digitized sample of *G. guiris* with anatomical landmarks points.

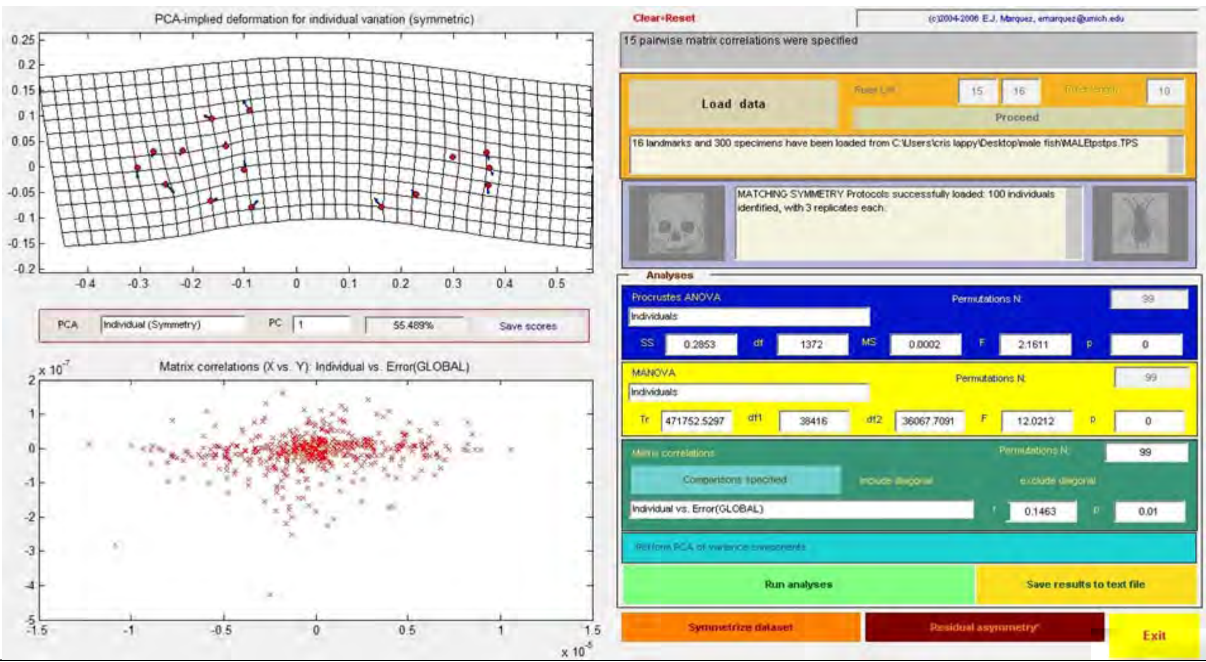

Fig. 3 Overview of the schematic flow of shape analysis using Symmetry Asymmetry Geometric Data Software (SAGE).

3 Results and Discussion

The determination of the water quality analysis was done on-site in Jabonga, Lake Mainit, Agusan Del Norte (Table 1). From the data presented it was observed that subjected parameters (Conductivity, Dissolve Oxygen, pH, Salinity, Temperature, TSS, and TDS) were within the standard set by the Department of Agriculture-Environmental Management Bureau. These variables play an important aspect in the physical composition of the water. The obtained value ranges from 166 uS/cm -180 for conductivity. Dissolve Oxygen (6.36 mg/L - 8.03 mg/L), pH (8.22-8.57), Temperature (29.47-30.70), TSS (0.07 mg/L-0.08 mg/L), TDS (70.00-80.00 mg/L). These are the recorded values across three sampling stations. Further, conductivity known to be an aqueous solution is not only a common index to measure water quality but also can reflect the number of ionizable substances in water. Because of its simple test, it has been widely used in earthquake monitoring and water quality analysis (Tan et al., 2019). The goal of measuring the conductivity of water is a very important index to measure water quality, which can reflect the degree of dielectric in water. The increases in ionization are due to the water containing inorganic acid, alkali, or salt, the conductivity is escalated (Xianhong, 2021).

Table 1 Results of physico-chemical parameters in Lake Mainit, Caraga Region, Philippines.

Water Quality Parameters	**Standard DENR-EMB, 2006**	**Station 1** (09.3500^{0}N; 125.5218^0 E) **Mean ± SEM**	**Station 2** (09.3517^{0}N; 125.5219^{0}E) **Mean ± SEM**	**Station 3** (09.3532^{0}N; 125.6216^{0}E) **Mean ± SEM**	**Interpretation of Data**
Conductivity	100-2000 uS/cm	180.67 ± 0.33	166 ± 7.51	179 ± 1.15	Within the Standard
DO	≥5mg/L	6.36± 0.10	8.03 ± 0.25	6.36 ± 0.15	Within the Standard
pH	6.5-8.5	8.57 ± 0.02	8.24 ± 0.03	8.22 ± 0.1	Within the Standard
Salinity	<0.5ppt	0 ± 0	0 ± 0	0 ± 0	Within the Standard
Temperature	3^{0}C rise[a]	29.47 ± 1.09	30.67 ± 0.00	30.70± 0.19	Within the Standard
Total Suspended Solid (TSS)	<50 mg/L	0.08 ± 0	0.07 ± 0	0.08 ± 0	Within the Standard
Total Dissolve Solids (TDS)	1,000 mg/L	80.00 ± 0	70.00 ± 0	70.00 ± 0	Within the Standard

Conversely, dissolved oxygen (DO) is necessary for the survival of all organisms and it is known as the amount of oxygen present in the water bodies (Martinez and Galera, 2011). It is required by fish to perform metabolic activities and respiration. Decreased levels of dissolved oxygen are usually associated with fish kill incidents. However, high levels can develop into good growth, thus resulting in a high yield of production. Thus, the obtained value of DO in Lake Mainit is a good indication that can support aquatic organisms.

At the same time, assessing the pH level of water represents acidity and basicity. Such the vital of each pH level can affect the overall water quality. Water that has low or high pH could be dangerous to fish and other aquatic life. For instance, low-pH toxic metals like aluminum can penetrate the water in high concentrations while chemical such as nitrogen tends to be more toxic. This affects the metabolic activity of the fish. Neither, water with a pH under 5-6.5 can prevent reproduction and mortality. Thus, juvenile fish and other aquatic animals are vulnerable (Brady and Ray, 2002). Moreover, the salinity of Lake Mainit was in the range of zero as the data presented. This showed that no salinity level was detected by the equipment and validated that the Lake cannot contain seawater. It is also necessary to assess the salinity hence freshwater organism has a different metabolic process and can be affected by the changes in water components. The recorded total dissolved solids (TSS) in Lake Mainit ranges from 0.07 – 0.08, and this is too far when compared to the standard level set by the DENR-EMB. As observed when TSS is higher it implied a lower ability of the water to sustain life because of decreased light penetration affecting photosynthesis (EMB, 2014). While, TSS concentrations range from hundreds to thousands of mg/L and tend to be harmful to fish and other organisms (Poole and Berman, 2001).

Finally, the Total Dissolve Solids (TDS) signify the clarity of the water. TDS with a color concentration of 50 mg/L and beyond indications of turbid water can be caused by natural disturbances such as increased sedimentation during the rainy season or anthropogenic disturbance (Sargaonkar and Deshpande, 2003). While the data collected from Lake Mainit only revealed that the lake has no turbidity and this is associated with good water condition. Thus, the importance of evaluating the water condition both physical and chemical relates to knowledge and understanding of the overall.

Table 2 Procrustes ANOVA on the body shape of *G. guiris* in terms of sexes from Lake Mainit, Caraga, Region, Philippines.

FACTORS	SS	DF	MS	F	P-VALUE
Female					
Individuals	0.3373	952	0.0004	5.1084	0.0001
Sides	0.0290	28	0.0001	14.9377	0.0001
Individual x Sides	0.0660	952	0.0001	6.9058	0.0001
Measurement Error	0.0197	1960	0	--	--
Male					
Individuals	0.1715	952	0.0002	1.7813	0.0001
Sides	0.0558	28	0.0002	19.6935	0.0001
Individual x Sides	0.0963	952	0.0001	12.0924	0.0001
Measurement Error	0.0164	1960	0	--	--

(P<0.0001) highly significant.

The observed body shape variations of *G. guiris* in terms of sexes were presented in Table 2. Procrustes ANOVA resulted in significant differences (P<0.0001) detected across the factor analyzed (Individuals, Sides, and Individuals x Sides) and between the female and male samples. The obtained data implied an asymmetry among the populations. This is also supported by the study conducted on the same fish species in that both males and females exhibit significant differences in morphology (Cabuga et al., 2019). Further, the development of asymmetry could also be associated with ecological changes hence species have to be adaptive within the environment. Also, when individuals and populations performed fluctuations this is usually attributed to genetic and environmental stresses (Sadeghi et al., 2009; Yuto et al., 2016).

Further, assessing the shape differences can be figured out by measuring the portion of the organism's phenotype i.e. *individual x sides* interaction which is the inconsistency of the individuals to be similar from side to side. Results implied that such variations often occur due to adaptation and this lead to speciation (Ricklefs and Miles, 1994). Moreover, phenotypic variations have led to fluctuating asymmetry and are known to be an indication of developmental instability and thus act as a bioindicator of ecological stress (Tabugo et al., 2013). A previous study showed that *Glossogobius guiris* collected in the same area shows high levels of FA both in male and female samples (Jumawan et al., 2016). Additionally, morphological differences could be established to a wide range of environmental factors i.e. food deficiency, pesticides, parasitism, mobility, inbreeding and climatic condition (Mpho et al., 2000). Nonetheless, significant levels of fluctuating asymmetry are attributed to the modifications in the genetic composition of the populations resulting in observable physical traits (Yuto et al., 2016).

In addition, the relevance of FA was because of the mobility and feeding habits of an organism (Webb 1982; Caldecutt and Adams, 1998). Raising the water temperature, swimming patterns, feeding habits, and lifestyle are the notable reasons that develop body shape variations in fishes (Marcil et al., 2006; Cullen et al., 2007; Rincón et al., 2007). As well as, the previous study proves that phenotypes are significantly affected by several ecological conditions, and thus fishes situated in a high trophic level had the most fluctuated (Cabuga et al., 2019).

Table 3 Principal component scores of the values of symmetry and asymmetry scores with the summary of the affected landmarks from Lake Mainit, Caraga Region, Philippines.

PCA	Individual (Symmetry)	Sides (Directional Asymmetry)	Interaction (Fluctuating Asymmetry)	Affected Landmarks
Female				
PC1	53.01%	100%	34.16	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16
PC2	21.82%		18.38	3,4,5,6,7,9
PC3	7.74%		11.78	1,4,5,6,7,8,9,11,15,16
Total	**82.57%**		**64.74%**	
Male				
PC1	54.76%	100%	44.90%	1,2,3,4,5,6,7,8,9,10,11,
PC2	11.37%		13.69%	1,2,3,4,5,6,8,9,10,12, 14,15,16
PC3	7.58%		9.93%	2,3,4,6,7,8,9,12,14,15,16
PC4	5.70%		7.34%	1,2,3,6,7,8,9,10,13,15,16
Total	**79.41%**		**75.86%**	

In order to understand the effect of FA on the body shape of *G. guiris*, Table 3 was provided. Here, there are data presented to visualize the quantitative evaluation between the female and male populations. In males, there were four principal components (PCA) accounting for 79.41%, and the FA (Interaction) for 75.86% and highest when compared to females with only 3 principal components (PCA) accounting for 82.57% and the FA (Interaction) 64.74% These significant levels of FA were manifested and associated with the degree of physical dissimilarities. The level of FA is often times utilized to evaluate the developmental variability of populations and individual species. As bilateral characters demonstrate variability in shape and size of the left-right side, these would determine the asymmetrical observation (Moller, 1997). Variances in the level of FA may be associated with the ability of the phenotypes to shield developmental changes (Dahm et al., 2007). Consequently, the ecological state gives a part in the complete fitness of the organism and might increase its form to repel alterations.

Further, in males, the commonly affected landmarks (encircled with red) among the four PCs are 2 (Posterior end of the nuchal spine), 3 (Posterior insertion of 1st dorsal pin), 8 (Lateral line), 9 (Dorsal and Ventral insertion of caudal fin), 15 (Anterior margin through the midline of the orbit) and 16 (Posterior margin through the midline of the orbit). Along with these affected anatomical points indicates body shape variations within the male samples across the fish populations. These anatomical landmarks points were located in the regions where it was constantly used for swimming, food hunting, predator escape, and mobility. Studies showed that feeding habits are the foremost environmental aspect prompting morpho space restructuring. The discerning ability of omnivores with taller bodies, shorter caudal peduncles, and herbivores with small heads, predators with elongated bodies these attributed to swimming and larger mouths and head mouths (Cavalcanti et al., 1999; Kassam et al., 2003).

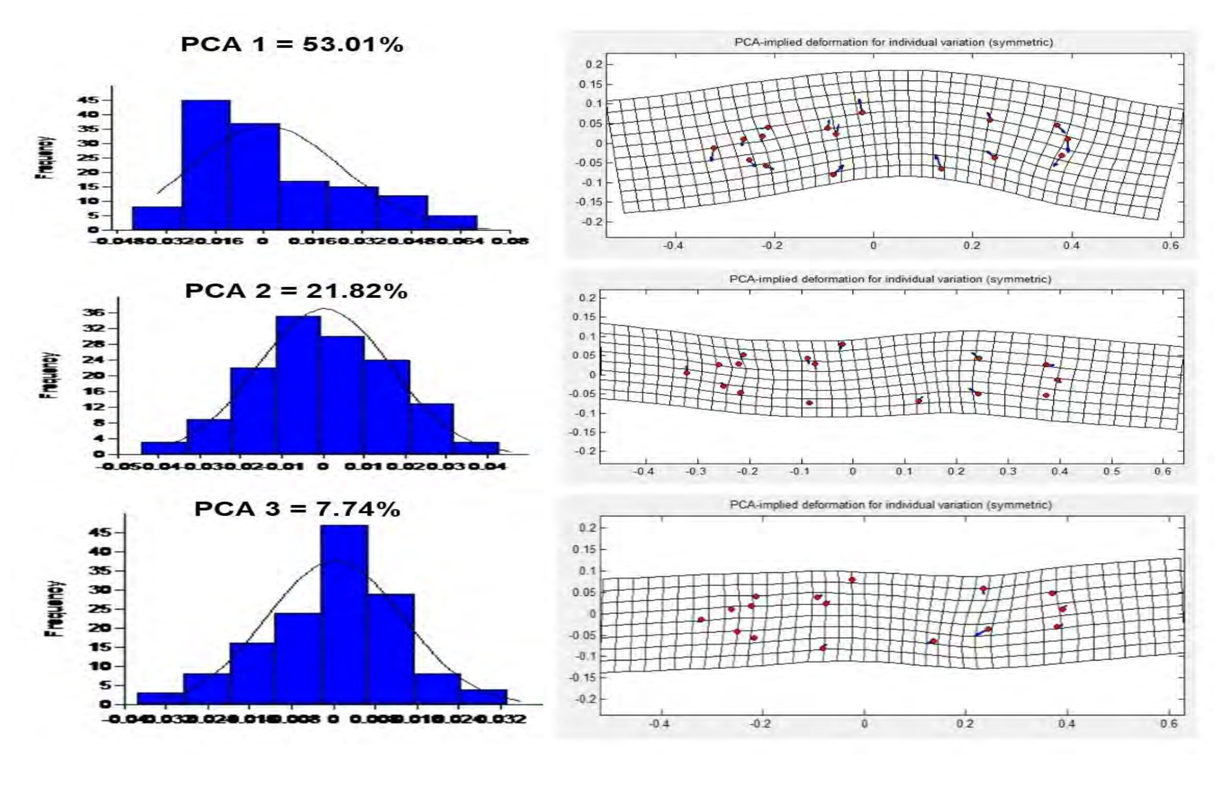

Fig. 4 PCA-implied deformation grid female *G. guiris* populations.

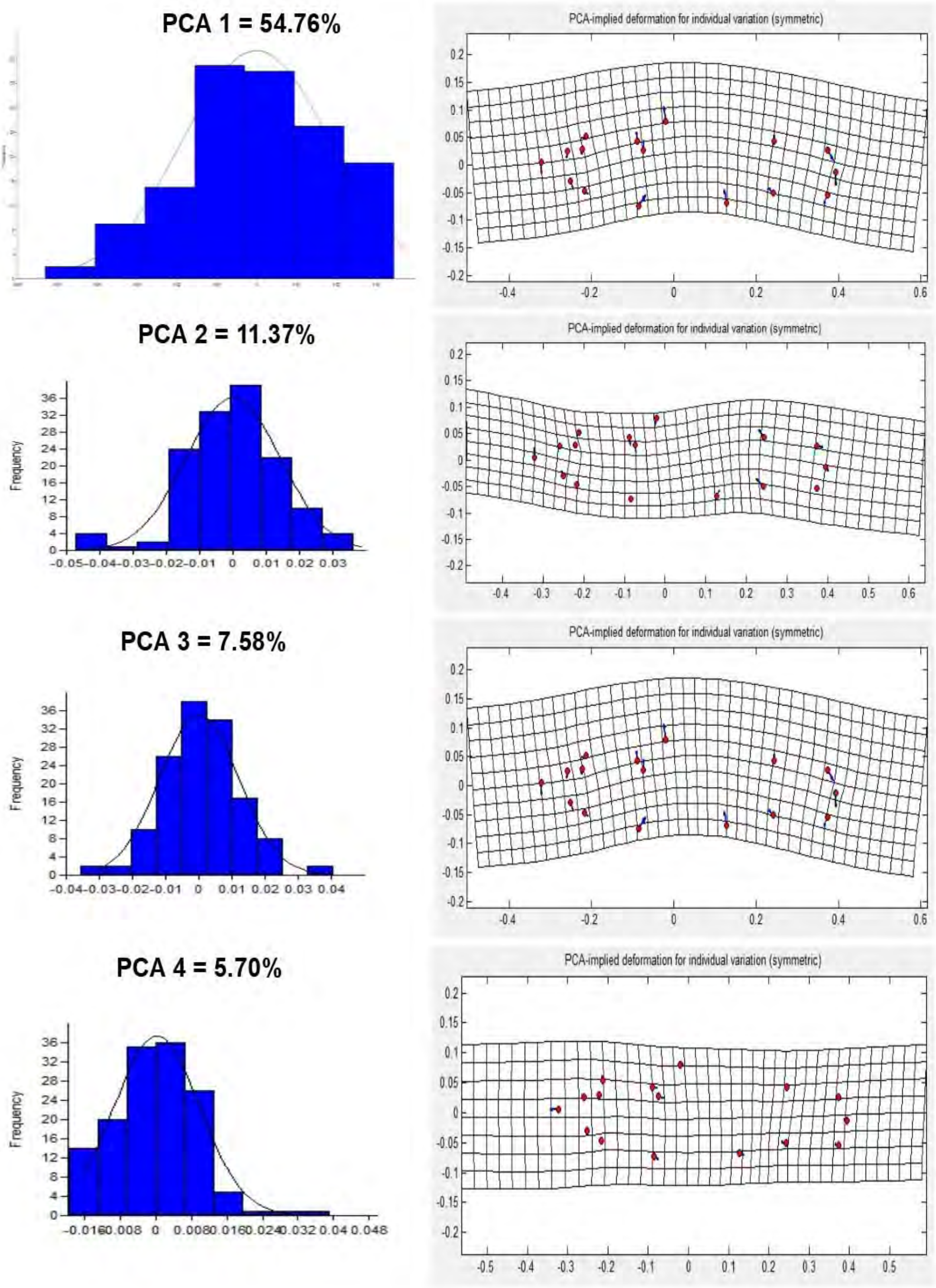

Fig. 5 PCA-implied deformation grid female *G. guiris* populations.

Moreover, in females, the commonly affected landmarks (encircled with red) among the 3 PCs were 4 (Anterior insertion of 1st dorsal fin), 5 & 6 (Posterior Anterior insertion of 2nd dorsal fin), 7 & 9 (Dorsal and ventral insertion of caudal fin). As observed, these regions were located in the fins that were the most essential areas necessary used for mobility. While if we compared the affected landmarks between the male and female samples it differs significantly suggesting that each fish has its own mechanism to buffer physical modifications. This situation has been linked to the fish the aging process, swimming ability, and food hunting. For instance, needlefishes utilized their feature compact set of fins to modify the flows shaped by body movement, an important trait of their locomotion (Liao, 2002). Nonetheless, the body shape change is also altered by habitat: fish with rounded-shaped are adapted to low activity, living in generalist habits, while elongated-shaped fish swim in the water column areas (Clabaut et al., 2007; Farré et al., 2015).

Evidently, fishes with elongated/flattened shapes (eels or flatfishes) are typically situated at the periphery of the morpho space (Friedman, 2010; Tuset et al., 2014). In this study, the phenotypic variability of *G. guiris* is manifested and can be seen in Figure 4 (female) and Figure 5 (male). Additionally, graphical methods are in general more intuitive and interpretable, a combination of different analytical methods, including numerical and graphical ones, is the best and most complete option for assessing morphological differences (Tuset et al., 2014; Farré et al., 2015). Representing such variability is correlated to their biological features (Loy et al., 2001; Rüber and Adams, 2001). Evidently, the selection of the sample of species in the graph is partly subjective, but biologically it is noteworthy since graphs are able to explain vital environmental traits of fish assemblages, such as structural complexity or interrelationships between species (Strogatz, 2001; Dale and Fortin, 2010). Thus, species that are close together interrelate in the same location and utilize the same resources would results in competition and relate to shapevariability because of nutrient availability. The present study identifies phenotypic variations between the female and male populations and revealed significant body shape differences. The use of geometric morphometrics and the combination of different methods is the best approach for analyzing the species occupation within morphospace (Perry et al., 2006; Van Bocxlaer and Schultheiß, 2010).

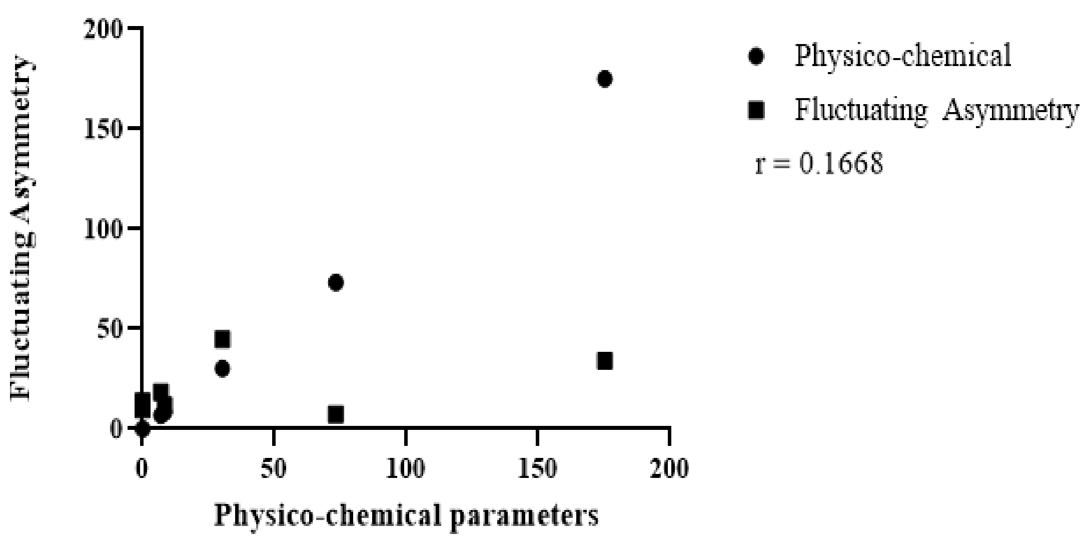

Fig. 6 Correlation of physico-chemical parameters vs. fluctuating asymmetry.

Furthermore, the interplay of biological resources where the species inhabits likely affects its physical characteristics. The combination of abiotic and biotic elements, food resources, competition, predation, and other ecological mechanism impacted the overall condition of the organism. This study intended to correlate the physico-chemical parameters versus the fluctuating asymmetry (FA) of *G. guiris* in order to identify its interrelatedness (Fig. 6), however, the results showed no correlation (r=0.1668) between the two variables subjected to analyses. This implies that the water has no effect on the body shape of the samples. It might be due to the reason that the fish maturity doesn't only correspond to the water of the study area. A possible explanation is that the fish collected had existed and grown for several months and even years in the Lake. The observed FA was not only associated with one factor but with several biological elements. Thus, the water quality merely doesn't affect the morphology leading to fluctuating asymmetry (FA). A study suggests that other causes of differing levels of FA among individuals in a population may be instigated by temporal changes (Lutterschmidt, 2016). Also, both land-use and chemical influences related to anthropogenic land-use and landscape scale developments can play a substantial role in defining stream, and lake conditions and thus establish developmental stressors ensuing in FA (Poff et al., 1997; Allan, 2004). Finally, the importance of using advanced methods like GM has been widely known to identify dissimilarities in shape in the individuals and populations thus understanding its morphological variation. Nevertheless, FA may be detected in meristic and metric characters and quantified based on the data presented yet the organisms have an adaptive mechanism for ecological survival.

4 Conclusion

The study identified phenotypes variability in the body shape of *G. guiris* populations by the use of Geometric Morphometric (GM) analysis collected from Lake Mainit, Caraga Region, Philippines. The evidence of the dissimilarities is revealed in its meristic and metric traits through anatomical landmark points. Procrustes ANOVA has shown a highly significant difference between the sexes. It was further visualized by detailing the Principal Component Analysis (PCA). Observable differences were clearly presented by affected coordinates and showed that male populations have the highest interaction when compared to females. Thus, the application of modern techniques further understands shape variations among biological organisms.

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