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Investigating body shape variation of *Oreochromis mosambicus* (Peters, 1852) sampled from Lake Mainit, Mindanao, Philippines

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Abstract

Ecological differentiation in terms of spatial and temporal condition highly attributes changes of the organism from genotypic to phenotypic composition and thus affecting morphological structures. This study aims to investigate the body shape variation in the population of *Oreochromis mosambicus*; Peters 1852 sampled from Lake Mainit, Mindanao, Philippines using Geometric Morphometric analysis. The endemic *O. mosambicus* constitute a significant fishery resource in the study area. Thus, they are economically important fish species. A total of 60 individuals comprises of 30 males, and 30 females were collected. The sample collection was done in July 2019. Female and male samples were subjected to Symmetry and Asymmetry Geometric Data Software (SAGE). Results show that both female and male samples obtain a highly significant difference $P<0.0001$ from the data provided through Procrustes ANOVA and Principal Component Analysis (PCA) showing high fluctuating asymmetry (FA) levels in female 57.52% when compared in males 52.78%. This show a body shape variation among the sexes while the natural settings play a major component for shifting shape and forms of organims. Thus, the importance of using Geometric Morphometric analysis allows to scrutinize the shape variation of fishes among and between populations and thus clearly defines structures.

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Introduction

Shape, structure, and formation of the organism functions as the key elements for biological identification. While ecological differentiation in terms of spatial and temporal condition highly attributes changes of the organism from genetics to morphological structures. It is essential to understand organisms morphology based on their habitat preference. Thus, the incapacity of a body to buffer environmental changes contributes to developmental modification (Chau, 2008). While, suddenly changed of natural settings likely alter the genomic composition and thus affects phenotypic component of an organism (Trono *et al.*, 2015). Fish often utilize as an efficient bioindicator of ecological causation. Indeed, they are the significant biomarker of pollution occurence mostly in aquatic realm while inhabiting high trophic levels (Blasco *et al.*, 1998; Agah *et al.*, 2009). Likewise, using fish as a sample for various research works has been acknowledged worldwide (Cabuga *et al.*, 2017). Morphological traits have been usually utilized in fisheries biology to quantify discreteness and correlation amongst numerous taxonomic categories (Bookstein, 1991). Moreover, it also noted that freshwater fishes were mostly susceptible to aquatic modification (Laffaille *et al.*, 2005; Sarkar *et al.*, 2008; Kang *et al.*, 2009). While the changed of the environmental condition later affects the growth and development of each organism's population (Cabuga *et al.*, 2019).

On the other hand, freshwater ecosystem, i.e., lakes denoting to be diverse in terms of its resources and thus frequently exploited around the globe (Sala *et al.*, 2000; Cabuga *et al.*, 2016). Anthropogenic activities were considered as an essential reason for alteration of this type of resource and thus affecting its biodiversity. Hence, morphological and biological transformations of the organisms were connected to the changeability of the environment (Cazzaniga, 2002; Torres *et al.*, 2013). To further understand the suitability of habitat towards the development of the organism, modern technology applied to measure the quantitative analysis of developmental variability. In the present time, Geometric Morphometric (GM) widely employed to determine the body shape variation among biotic elements, specifically plants, and animals. This technique is known to be advanced and suitable to assess two-dimensional shapes for quantitative comparison and while this is repeatedly used landmarks and semi-landmarks as a quantitative approach, thus employed by a different author as a mean for systematic resolution in both flora and fauna (Vieira *et al.*, 2014). Substantially, this modern approach evaluates the relative position of anatomical landmarks and the set of points to estimate surfaces and curves to quantify the size and shape of biological entities (Jensen, 2003).

Further, this also utilizes to discriminate the morphological variation between male and female fish population. While, it widely applied as a tool to examine body shape structure of fish species (Cabuga *et al.*, 2016; Joseph *et al.*, 2016; Jumawan *et al.*, 2016; Cabuga *et al.*, 2017). Consequently, this often used to biologies such as anthropology, agriculture, medicine, conservation biology, and genetics (Graham *et al.*, 2010).

The present study employs *Oreochromis mosambicus*, Peters, 1852, a freshwater fish found in Lake Mainit in Mindanao, Philippines. The endemic *O. mosambicus* constitute a significant fishery resource in the study area. Thus, they are economically important fish species. There was no study yet perform to quantify shape analysis in the fish sample using Geometric Morphometric analysis. Therefore, the outcome of the study would serve as baseline information for further fishery resource management in the region.

Materials and methods

Description of the area

The study was conducted in Lake Mainit, Mindanao, Philippines (Fig. 1). A fourth largest lake in the Philippines with a depth of 223 meters (723 ft). It is located in the Caraga region situated in the north-eastern portion in Mindanao. Known as an excellent fishery resource and thus it serves as an economical source in the provinces of Agusan del Norte and Surigao del Norte.

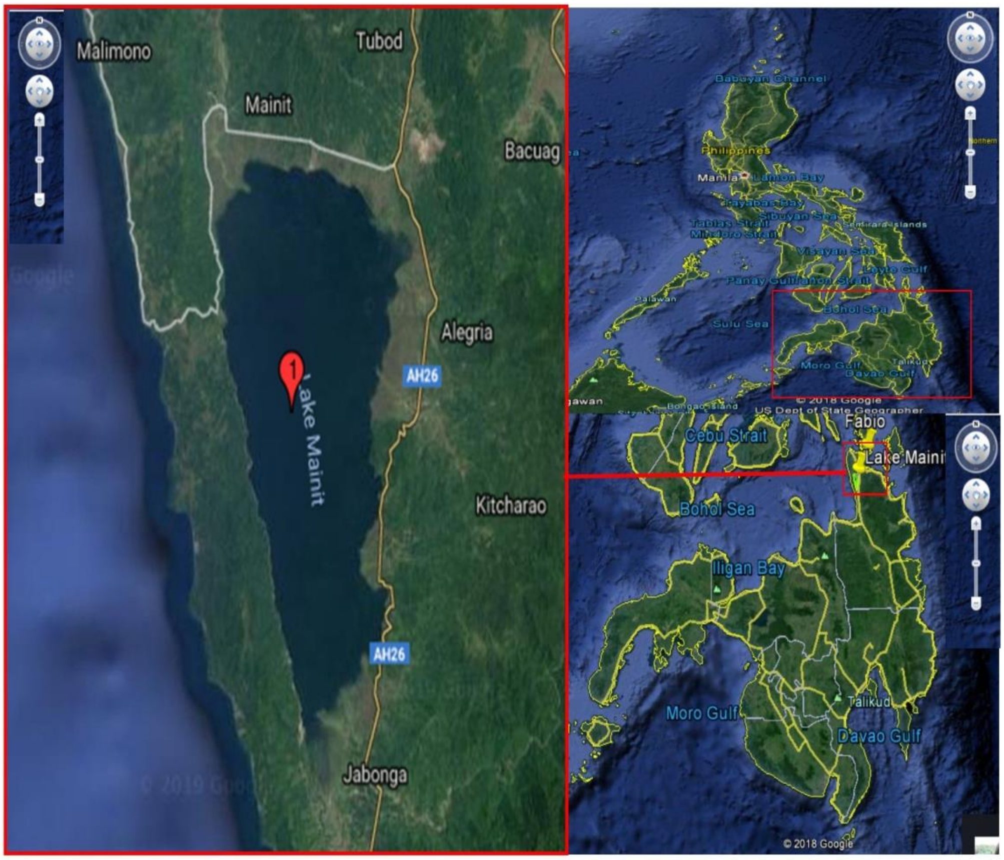

Fig. 1. Map of the Study Area, Lake Mainit, Mindanao. Philippines.

Sample collection and processing

There were 60 fish individuals of *O. mosambicus* comprising of 30 males and females were collected from the study site. The collected samples were subjected to laboratory protocols. A 10% formaldehyde applied in the fish fins to make it hardened and individually the fish placed in the top of the Styrofoam. After which the fish samples were photographed three times while obtaining the left and right portion and a ruler was placed below it.

Sex determination

A dissection process were applied for the sex determination of the fish samples through internal examination. Females were observed by its ovary in a yellow to orange granular texture while males were observed by its testes in a whitish, smooth and non-granular texture (*Requiron et al.*, 2010).

Landmark selection and digitation

The digital images of *O. mosambicus* were separated by sexes and converted into a TPS file using tps Util. After which tpsDig2 (Rohlf 2004) were utilized for the digitation with sixteen (16) anatomical coordinates (Table 1) were applied to digitize each sample (Fig.2).

Table 1. Description of the landmark points of *O. mosambicus* 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
16	Posterior margin through midline of orbit

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

Shape analysis

The digitized fish samples were loaded and subjected to Symmetry and Asymmetry Geometric Data (SAGE, v1.04) by Marquez, 2007 (Fig. 3).

The data provides a report from Procrustes ANOVA and Principal Component Analysis with history of deformation grid. Further, the results evaluate the significant difference P<0.0001 among the factors considered: individual, sides, and interaction of individual and side.

Nonetheless, the extent of fluctuating asymmetry (FA) between fish samples (male and female) was examined and further shown in percentage (Natividad *et al.*, 2015).

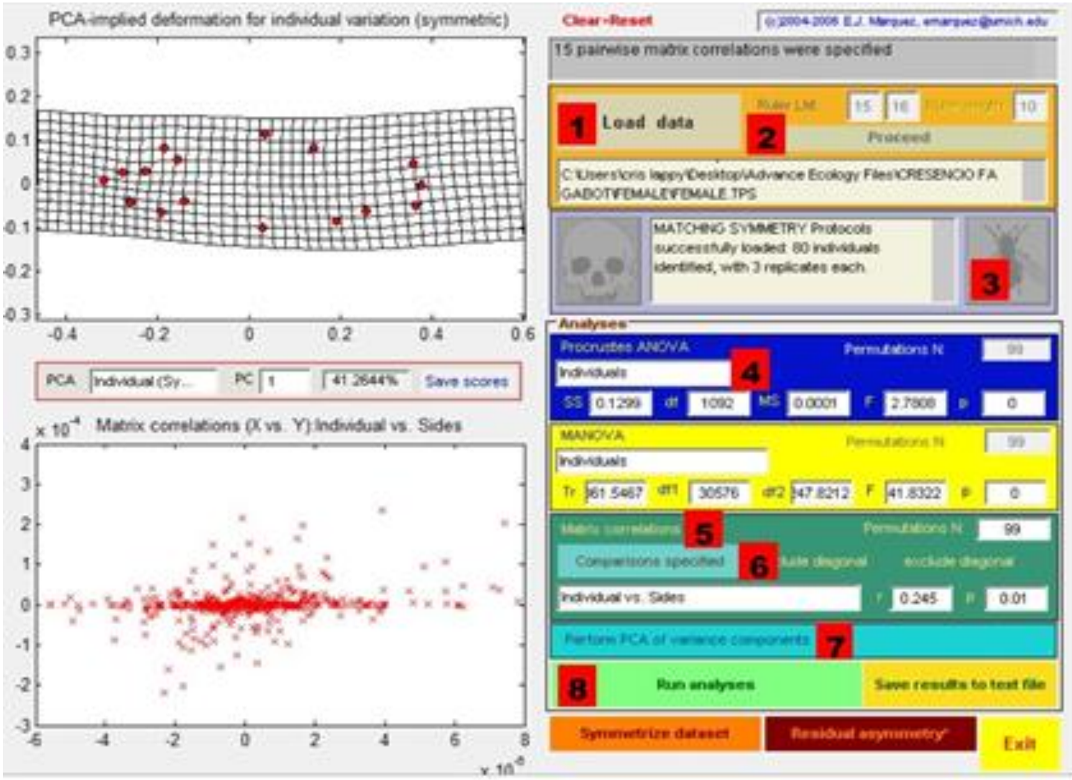

Fig. 3. Overview of the schematic flow of shape analysis using SAGE.

Results and discussion

Procrustes ANOVA was utilized to determine the significant difference P<0.0001 from the factors analyzed within and among the male and female samples of *O. mosambicus* (Table 2). The result shows that a highly significant difference (P>0.0001) were observed and thus indicating a body shape variation. This also implies that a high level of fluctuating asymmetry (FA) were observed and attributed from the mobility and feeding habits of an organism (Webb 1982; Caldecutt and Adams,1998). While the natural settings is a significant component for shifting shape and forms of organisms from its ontogeny stage. Besides, fast, flowing water could also improve swimming behavior of a fish when compared to stationary water and thus expecting indifferent maneuverability creating shape deformation (Libay *et al.*, 2019). Indeed, female fishes projected to have an exceptional level of FA due to metabolic changes from sustaining homeostasis and reproduction state (Requiron *et al.*, 2012). While other studies suggest that differences in FA levels were associated with wih various traits and its capacity to buffer developmental perturbations (Graham *et al.*, 1993; Lens *et al.*, 2002), also, water depth could enhance swimming ability and thus results in different body depth specifically in fishes (Rincon *et al.*, 2007).

Table 2. Procrustes ANOVA on the body shape of *O. mossambicus* in terms of sexes collected from Lake Mainit, Mindanao, Philippines.

Factors	SS	DF	MS	F	P-VALUE
Female					
Individuals	0.1823	812	0.0002	1.8121	<0.0001**
Sides	0.01	28	0.0004	2.8909	<0.0001**
Individual x Sides	0.1006	812	0.0001	13.4701	<0.0001**
Measurement Error	0.0309	3360	0	--	--
Male					
Individuals	0.1941	812	0.0002	2.2274	<0.0001**
Sides	0.0086	28	0.0003	2.8479	<0.0001**
Individual x Sides	0.0872	812	0.0001	5.4287	<0.0001**
Measurement Error	0.0664	3360	0	--	--

** (P<0.0001) highly significant

Nonetheless, contributing factors that develop shape variation of fishes are raising the water temperature, swimming patterns, feeding habits and lifestyle (Marcil *et al.*, 2006; Cullen *et al.*, 2007; Rincón *et al.*, 2007). In this study, water depth is expected to account for much variation since Lake Mainit is a deep lake with an average depth of 223 meters. Additionally, change in some parts of the fishes accredited from prey capture kinematics (Leysen *et al.*, 2011). From the same study also concluded that body shape is a parallel indicator of fish ecology, swimming activity, and foraging fish behavior (Sparks, 2004). Nonetheless, the relation between morphology and diet in fish population is postulated through feeding routine (Loy *et al.*, 2001) and therefore morphology and shapes are impacted on a fish feeding potential (Prosanta, 2006). In the previous work identified that fish phenotypic plasticity permits them to counteract due to environmental modification by changing in the physiology and behavior and that later affect the morphology, reproduction state and even survivorship from results of ecological variation (Blake 1983).

Moreover, differences in the body shape of female and male *O. mosambicus* were shown in the principal component analysis (PCA) (Table 3) and deformation grid (Fig. 4 and 5). In females, three PCA scores generated accounting to 78.66% while an interaction or FA with 57.52%. The typically affected landmarks among the 3PC's were 11 (Posterior & anterior insertion of the anal fin), 12 (Insertion of the pelvic fin) and 15 (Anterior margin through the midline of the orbit). At the same time, males generated three principal component scores accounting to 75.94% while an interaction or FA with 52.78%. The typically affected landmarks among the 3PC's were 1 (Snout tip) and 5 (Posterior & anterior insertion of the 2nd dorsal fin). The affected markers for two sexes were dissimilar, and these indicate variation in some body parts. As cited, the dorsal portion of fishes is highly corresponded lesser to environmental stress when compared to the body side and thus a good indicator for fish stock differentiation (Reis *et al.*, 2006).

In this study, frequently affected landmarks among the male and female samples were posterior and anterior region, and that signifies that this part was usually used for swimming and hunting procedures. Study shows that the exhibition of body shape variation due to maneuverability and habitat preference (Libay *et al.*, 2019). While different taxa can buffer environmental condition and that depending on their traits.

Moreover, related literature also confirms that phenotypes are greatly influenced through various ecological state and thus fishes situated in a high trophic level (Cabuga *et al.*, 2019). Therefore, geometric morphometric take advantage of identifying dissimilar traits of the organism in a similar population (Ratunil *et al.*, 2019). Finally, the use of geometric morphometric exhibits significant shape variation among the tested fish samples.

Table 3. Principal component scores showing the values of symmetry and asymmetry scores with the summary of the affected landmarks among female and male *O. mosambicus*.

PCA	Individual (Symmetry)	Sides (Directional asymmetry)	Interaction (Fluctuating asymmetry)	Affected landmarks
Female				
PC1	47.6506%		27.3276%	1,11,12,14,15,16
PC2	21.915%	100%	19.0301%	1,5,6,7,9,10,11,12,13,14,15,16
PC3	9.0913%		11.1649%	2,3,9,10,11,12,13,15
	78.6569%		57.5226%	
Male				
PC1	60.8791%		27.105%	1,5,6,10,11,12,14,15,16
PC2	8.7244%	100%	13.4838%	1,2,3,4,5,6,7,9,12,15
PC3	6.3328%		12.196%	1,2,3,5,6,7,10
	75.9363%		52.7848%	

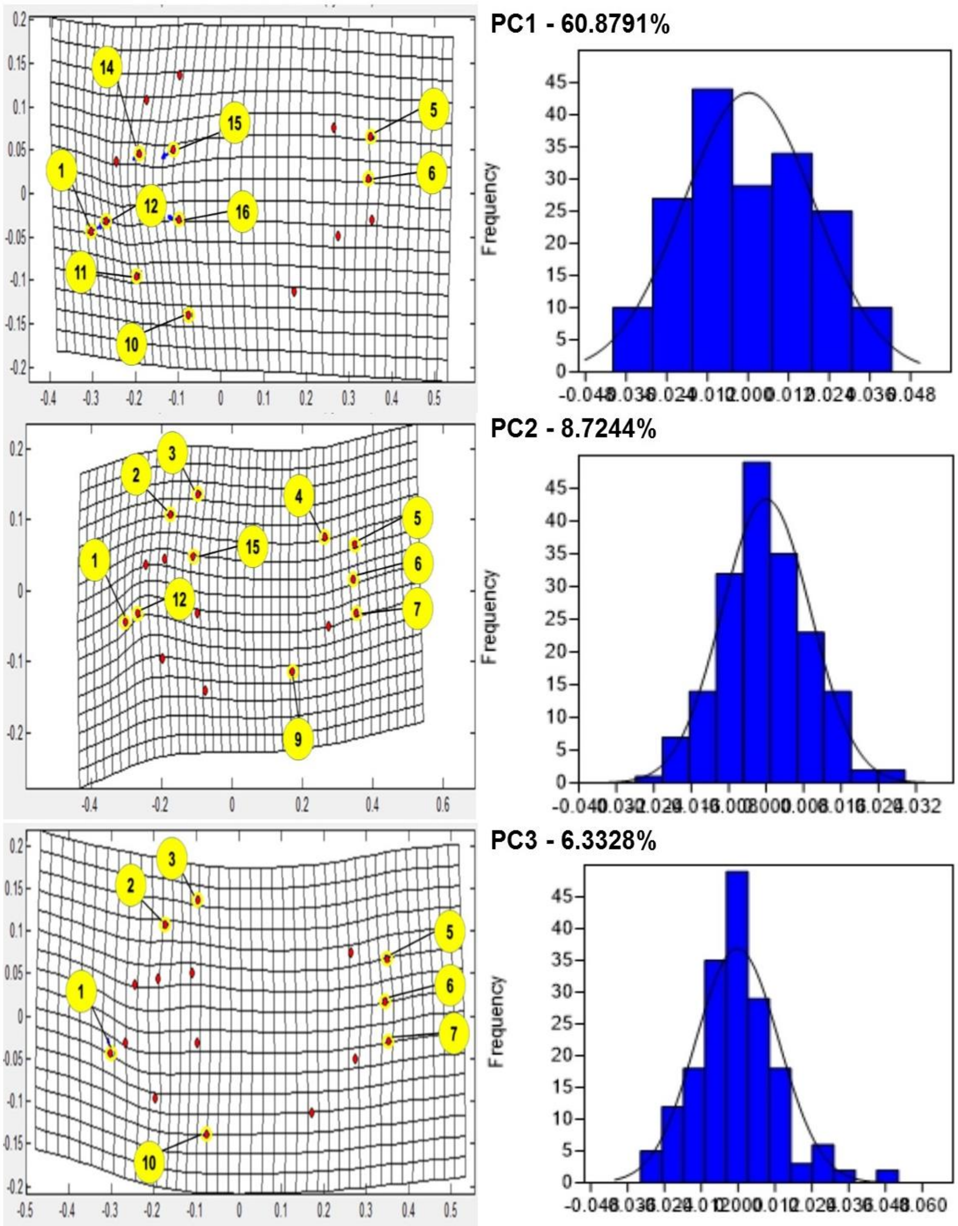

Fig. 4. Principal Component (PC) implied deformation grid and histogram of individual (Fluctuating Asymmetry) of female *O. mosambicus*.

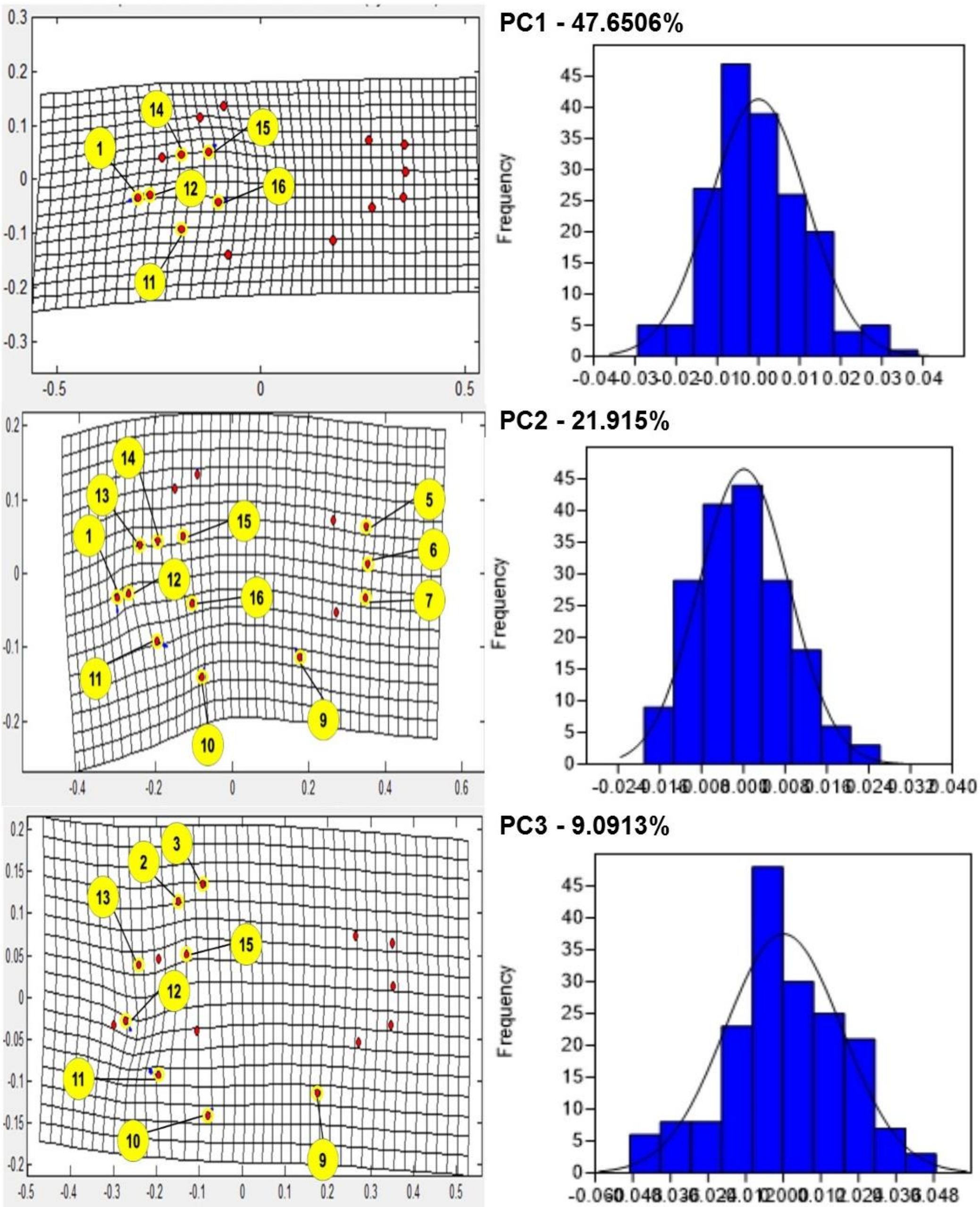

Fig. 5. Principal Component (PC) implied deformation grid and histogram of individual (Fluctuating Asymmetry) of male *O. mosambicus*.

Conclusion

The present study identifies the potential and efficacy of the use of geometric morphometrics as a tool for describing shape variation between the male and female population of *O. mosambicus* for detecting phenotypic differences. Indeed, geometric technique further investigates stock partition among taxa that allows, an extensive, accurate, and precise comparison of morphological development of species, though utilizing the same set of coordinates.

The data provided by Procrustes ANOVA and Principal Component (PCA) shows the evidence of shape variation. Significant differences ($P<0.0001$) were demonstrated from the factors analyzed individuals, sides, and interaction by sides while supported by the deformation grid.

These shape changes are most likely associated with habitat selection and feeding habits among the sexes. Thus, the study inferred the effectiveness of geometric morphometric technique as a tool for fishery management and its efficiency to scrutinize shape variation in the significant number of fish sample. Lastly, this mechanism entails stock identification and could enhance biological information through fishery management.

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