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Geometric morphometric analysis in determining phenotypic variability of Bugwan,(*Hypseleotris agilis,* Herre) in Lake Mainit, Philippines

Clevin P. Libay[1*], Virgilio B. Ratunil Jr.[1], Gideon A. Ebarsabal[1], Gregorio Z. Gamboa, Jr.[1], Emmylou A. Borja[1], Judy Ann A. Ga[1], Mary Grace E. Eclipse[1], Dexter Q. Mahomoc[1], Cresencio C. Cabuga Jr.[2]

[1]*Surigao State College of Technology-8400 Surigao City, Philippines*

[2]*Caraga State University – Main Campus, Ampayon, Butuan City 8600, Philippines*

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Abstract

The environment and genetic composition highly attribute to the phenotypes of an organism. This study was carried out to assess the phenotypic variability in the body shapes of *Hypseleotris agilis* or locally known as Bugwan from Lake Mainit, Philippines using Geometric Morphometric Analysis. Sixty samples consisting of 30 males, and 30 females were randomly collected and subjected to Symmetry and Asymmetry Geometric Data (SAGE) Software. A significant variation was observed in the body shapes among the two sexes from the data provided by Procrustes ANOVA, Principal Component Analysis (PCA) while supported in the visualization of Implied Deformation and Histogram. A difference in the morphology was observed among the two sexes indicating a body shape variations. Such dissimilarities were associated and considered to be the result of adaptation and selection pressure coming from the environmental condition. Thus, the use of geometric morphometric analysis helps to quantify phenotypic variation within the community of species.

* **Corresponding Author:** Clevin P. Libay ✉ clevinplibay@gmail.com

Introduction

Fish are commonly used as a biomarker of pollutants in the aquatic environ. They serve as the best sample for detecting environmental condition since they inhabit were most of the effluent occurs. The presence of these effluents can affect to its physiological activities and later may express to its morphology. Ecological risks such as agricultural runoff, industrial sewage, and anthropogenic activities may pose unfavorable condition both in the environment and the organisms (Natividad *et al.*, 2015). Over the decades, aquatic pollution has become a significant problem that has a wide range of effects (Dikshit *et al.*, 1990). In existence, pollutants can be a factor to modify the genetic makeup of an organism and resulted in diversity and variation in the population (Trono *et al.*, 2015). It causes intolerable effects, damaging environmental state and leads to mortality (Duruibe *et al.*, 2007). Pollutants are vital components that can alter morphological traits of the aquatic organism (Jumawan *et al* 2016). These are contributing factors that directly affects its state of well-being. The effect of these pollutants may describe as morphological asymmetries through imperfect development (Jumawan *et al.*, 2016). Lake Mainit is located between the Surigao del Norte and Agusan del Norte Philippines, holds the once endemic *Hypseleotris agilis* which locally known as "Bugwan." It is endemic to the lake and the distribution occurs from South Africa to Japan regions and in Southeast Asia. *H. agilis* is one of the abundant species found in Lake Mainit. Although not that commercially viable when compared to other bigger fishes like tilapia and carp, is shown to be important in the biodiversity of the lake and has been recommended for conservation (De Guzman, Gorospe and Openiano, 2009). The present study utilize Fluctuating Asymmetry (FA) a subset of Geometric Morphometric commonly uses to evaluate environmental stress and developmental variability of different biotic elements. FA was employed to assess the flux of pollutants in the aquatic environment and its effects on the organism. This mechanism provides a reliable result as it repeatedly used by numerous studies around the world. FA said to be an excellent means for determining environmental health (Lecera *et al.*, 2015). Fluctuating Asymmetry extensively used as a potential indicator for describing developmental instability (Ducos and Tabugo, 2015).On the other hand, FA plays an essential mechanism for distinguishing morphological similarity and differences (David Polly, 2012). It is also a significant instrument in the field of biology as it reveals a population's state of adaptation and co-adaptation (Jumawan *et al.*, 2016). Furthermore, fluctuating asymmetry identified to be a useful tool for giving vital information over another biomarker of developmental variability (Clarke, 1993). Thus, the study aims to determine and compare the morphological variation in terms of body shape of "Bugwan" (*Hypseleotris agilis*) based on the geometric morphometric analysis in Lake Mainit, Philippines.

Materials and methods

Description of the area

This study was conducted in Lake Mainit, Philippines (Fig. 1). Lake Mainit is the fourth largest lake in the Philippines, having a surface area of 173.40 square kilometers (66.95 sq mi). It is also distinguished as the deepest lake in the country with a maximum depth reaching 223 meters (732ft). It is located in the part of northeastern section of Mindanao and shared between the provinces of Surigao del Norte and Agusan del Norte in Caraga region. Its geographical coordinates are 9°32'6" North, 125°31'23" East. Fig. 1. Map showing the study area in Lake Mainit, Philippines.

Sample collection and processing

A total of Sixty (60) samples of *H. agilis* was collected. Thirty (30) were females, and thirty (30) males. Sample prepare for photo processing were each of the samples was flank on Styrofoam with fins pinned to show its point of origin. To preserve the fish, a 10% formalin was used to make the fins harden to obtain a decent image of the fish and its point of origin. The captured image is converted into thin-plate spline (TPS) file using tpsDig2 program version 2.0.

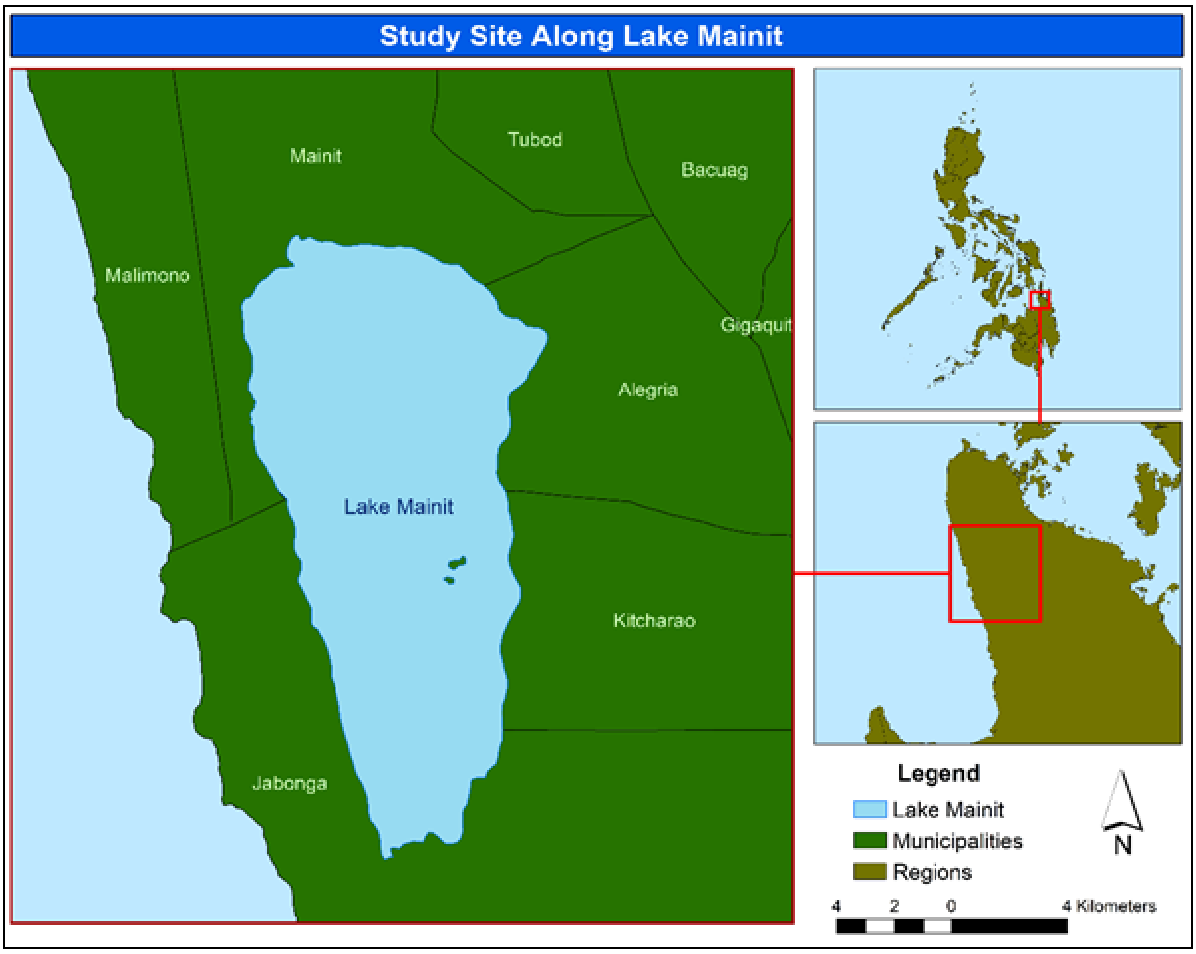

Fig. 1. Sampling area, Lake Mainit, Philippines. (Photo credit: Joseph *et al*., 2016).

Sex determination

After capturing digital images, sex of *H. agilis* was then determined by dissection process. Males were identified by observing its testes; testes were typically smooth, whitish and non-granular in appearance. Females were recognized by exploring its ovary, eggs are yellow or orange color.

Landmark selection and digitation

TPS series, landmark analyses was used to get its distorted features within the images. Landmarks were designated to have a homogenous outline of fish body shape using software tpsDig2.

A total of 16 markers (equivalent to 16 X and 16 Y Cartesian coordinates) to recognize the best representation of the external form of the body (Fig. 3). Landmark description was shown in Table 1. X and Y coordinates of the landmarks on the image were obtained for analysis. Digitization was accomplished three replicates for each fish samples to minimize the error in plotting the landmark points were used.

Shape analysis

Procrustes ANOVA was used to determine the individual symmetry and sides of L-R size and shape. X and Y coordinates serve as the starting point in examining or analyzing the Fluctuating Asymmetry (FA) of the fish. Left and right phase landmarks of TPS image processed in Symmetry and Asymmetry in Geometric Data (SAGE) software (version 1.04, Marquez 2007) (Fig. 2). SAGE software is useful in shape conformation of individual's variation including the probable covariance condition. The percentage (%) of FA was obtained, and the differences between sexes of fish was determined. The three factors were considered; individuals, sides, and interaction of individuals x sides in measuring FA of *H. agilis*.

Results and discussion

Procrustes ANOVA was used to identify the morphological variation of *H. agilis* (Table 2). There were factors analyzed; individuals, sides, individual by sides, measurement error among the male and female samples. In females result showed a high significant difference (P<0.0001) among the factors considered. On the other hand, males result shows a high significant difference (P<0.0001) in the factor of individual by sides however sides and individuals denoting a value of P<0.1913 and P<0.0821, respectively.

Table 1. Description of the landmark points of *Hypseleotris agilis* based on Paña *et al* (2015).

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

Table 2. Procrustes ANOVA on body shape of *Hypseleotris agilis* in terms of sexes.

Factors	*SS*	*DF*	*MS*	*F*	*P-VALUE*
		Female			
Individuals	0.2426	812	0.0003	1.3021	<0.0001**
Sides	0.031	28	0.0011	4.8244	<0.0001**
Individual x Sides	0.1864	812	0.0002	8.9491	<0.0001**
Measurement Error	0.0862	3360	0	--	--
		Male			
Individuals	0.1447	812	0.0002	1.0632	0.1913
Sides	0.0066	28	0.0002	1.4013	0.0821
Individual x Sides	0.1361	812	0.0002	3.7964	<0.0001**
Measurement Error	0.1484	3360	0	--	--

** (P<0.0001) highly significant.

The Principal Component Analysis (PCA) further described the scores of symmetry and asymmetry and its affected landmarks of *H. agilis* population. Result showed that female population generated five (5) PC scores accounting to 81.68% and Interaction (Fluctuating Asymmetry) of 79.33%. The common affected landmarks were 1 (Snout Tip), 9 (Anterior insertion of anal fin), 10 (Dorsal base of pelvic fin), 12 (Posterior end of maxilla) and 16 (Dorsal base of pectoral fin).

Table 3. Principal component scores showing the values of symmetry and asymmetry scores with the summary of the affected landmarks.

PCA	*Individual (Symmetry)*	*Sides (Directional asymmetry)*	*Interaction (Fluctuating asymmetry)*	*Affected landmarks*
		Female		
PC1	34.3109%	100%	42.0745%	1,2,7,8,9,10,11,12,15,16
PC2	23.6926%		15.0026%	1,2,3,4,5,6,7,8,9,10,11,12,14,15,16
PC3	10.7392%		9.323%	1,2,3,4,5,6,7,8,9,10,12,13,14,16
PC4	7.0363%		6.9397%	1,9,10,11,12,15,16
PC5	5.9052%		5.9936%	1,2,3,4,5,7,8,9,10,11,12,15,16
	81.6842%		79.3334%	
		Male		
PC1	45.435%	100%	33.8273%	1,2,3,4,5,6,7,8,9,11,12,15,16
PC2	15.3467%		17.8213%	1,2,7,8,9,10,11,12,14,15,16
PC3	10.5448%		11.3494%	1,2,3,4,8,9,10,11,16
PC4	7.7897%		9.2573%	1,2,3,4,5,8,9,11,15,16
PC5	5.1707%		6.0232%	2,3,4,7,9,11,15,16
	84.2869%		78.2785%	

In male population, PC scores were also five (5) accounting to 84.29% with Interaction (Fluctuating Asymmetry) of 78.28%. The common affected landmarks were 2 (Posterior end of nuchal spine), 9 (Anterior insertion of anal fin), 11 (Ventral end of lower jaw articulation) and 16 (Dorsal base of pectoral fin). Figure 4 and 5 further showed the consensus morphology and the variation in the body of female and male population of *H. agilis*.

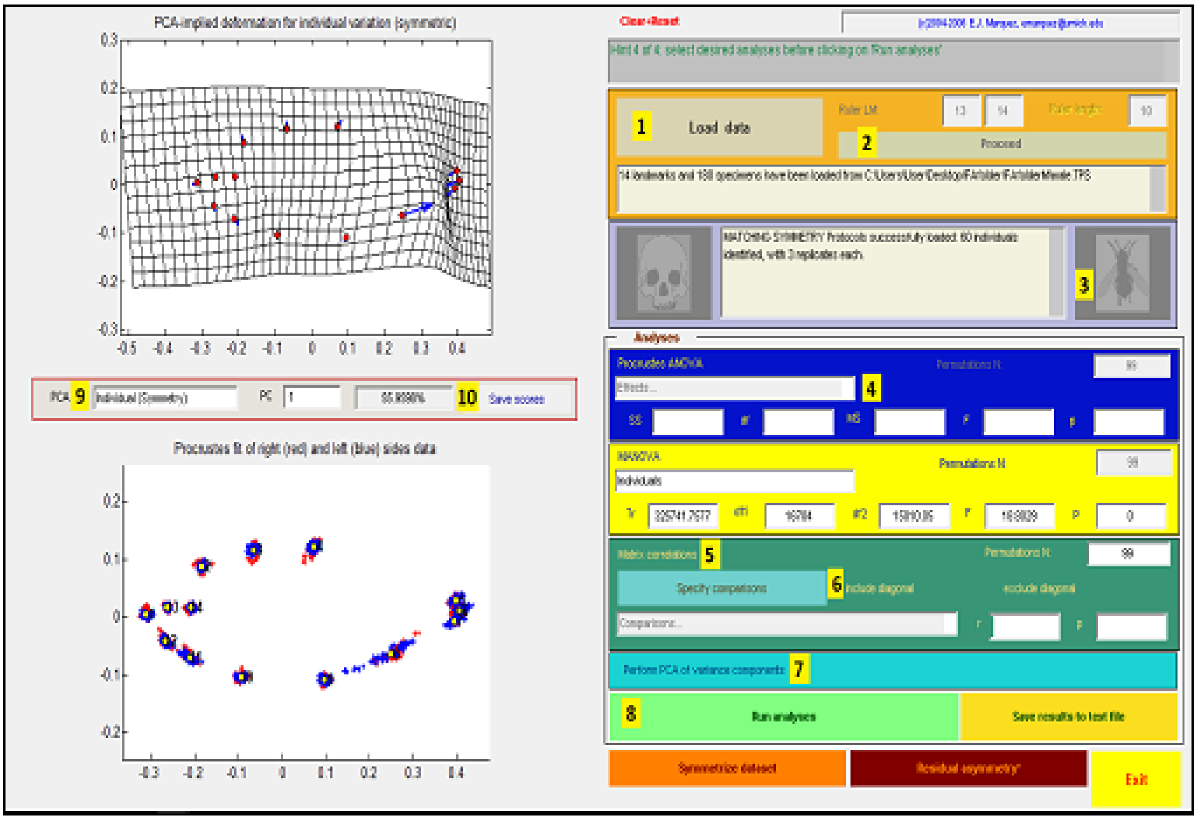

Fig. 2. Diagram of shape analysis using Symmetry and Asymmetry Geometric Data Software.

The obtained result shows a body shape variation between female and male *H. agilis* samples. Considerably, 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 significant role in depicting biological studies. Besides, detecting the forms of organisms also attributed to the ecological condition where species mostly inhabit.

Fig. 3. Landmark point of the *Hypseleotris agilis;* male in the upper part and female in the lower part.

In the same survey conducted by many research also suggested that shape variations mainly happen to freshwater fishes were in often altered by several anthropogenic activities (Medrano and Jumawan, 2016; Jumawan *et al.*, 2016; Portillo *et al.*, 2017; Cabuga *et al.*, 2017; Cabuga *et al.*, 2019). Indeed environmental perturbations greatly influence on the aspect of developmental stability leading to species inability to grow naturally.

Accordingly, fishes are efficient of adaptations which are essential for existence specifically when subjected to diverse environmental state. Also, the environment is primarily a driving force in shaping the morphology of organism throughout the ontogeny. Further, the manifestation of body shape is a marker of the mobility and swimming facility and habitat selection. Therefore, it is assumed that a swimming fish entity that dwells in fast flowing water expectedly shaped indifferently than of the fish living in stationary water thus consideration of their ecology. Nonetheless, the evidence of morphological differences between fishes and sexes could also be due to genetic aspect and thus sexual dimorphism constantly a way of predicting individuals and means of identifying behavior, the life history of species and ecology (Requiron *et al.*, 2012).It is indeed an effective way of using geometric morphometric to understand shape variations among samples of species. The maximum advantage over traditional morphometrics is the results accessible to visualized of the coordinates out from the original pattern of the species and thus represented as definite shape or shape formations than of the statistical scatter plots provided.

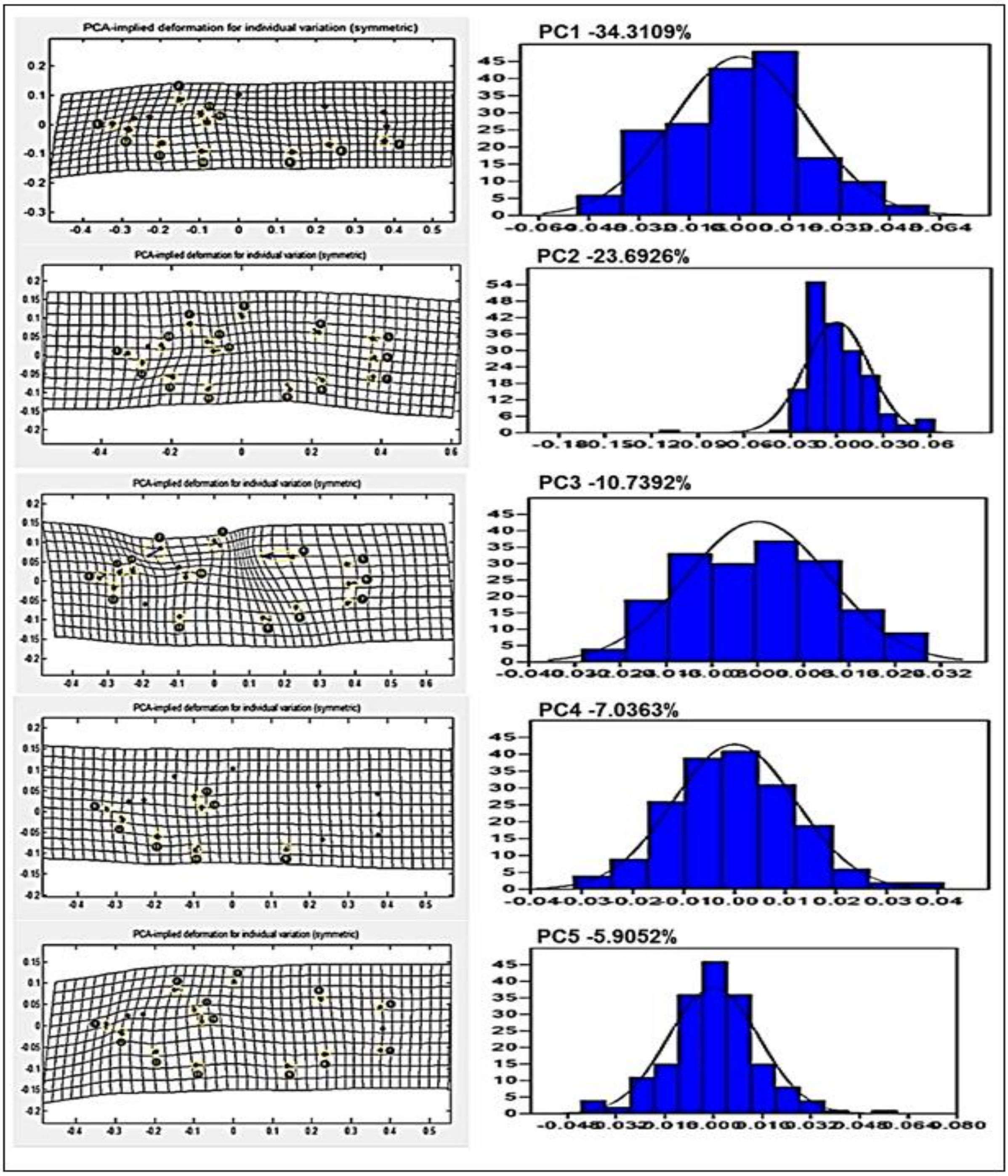

Fig. 4. Summary of the geometric morphometric showing consensus morphology and the variation in the body shape of female population of *H. agilis*.

Moreover, species body shape formation could also 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). Specifically, male species were inclined to suppress shape variations coming from different environmental changes. 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 it can be illustrated in the Principal Component Analysis in Table 2. It represents the degree of similarities and dissimilarities in both sexes of *H. agilis*.

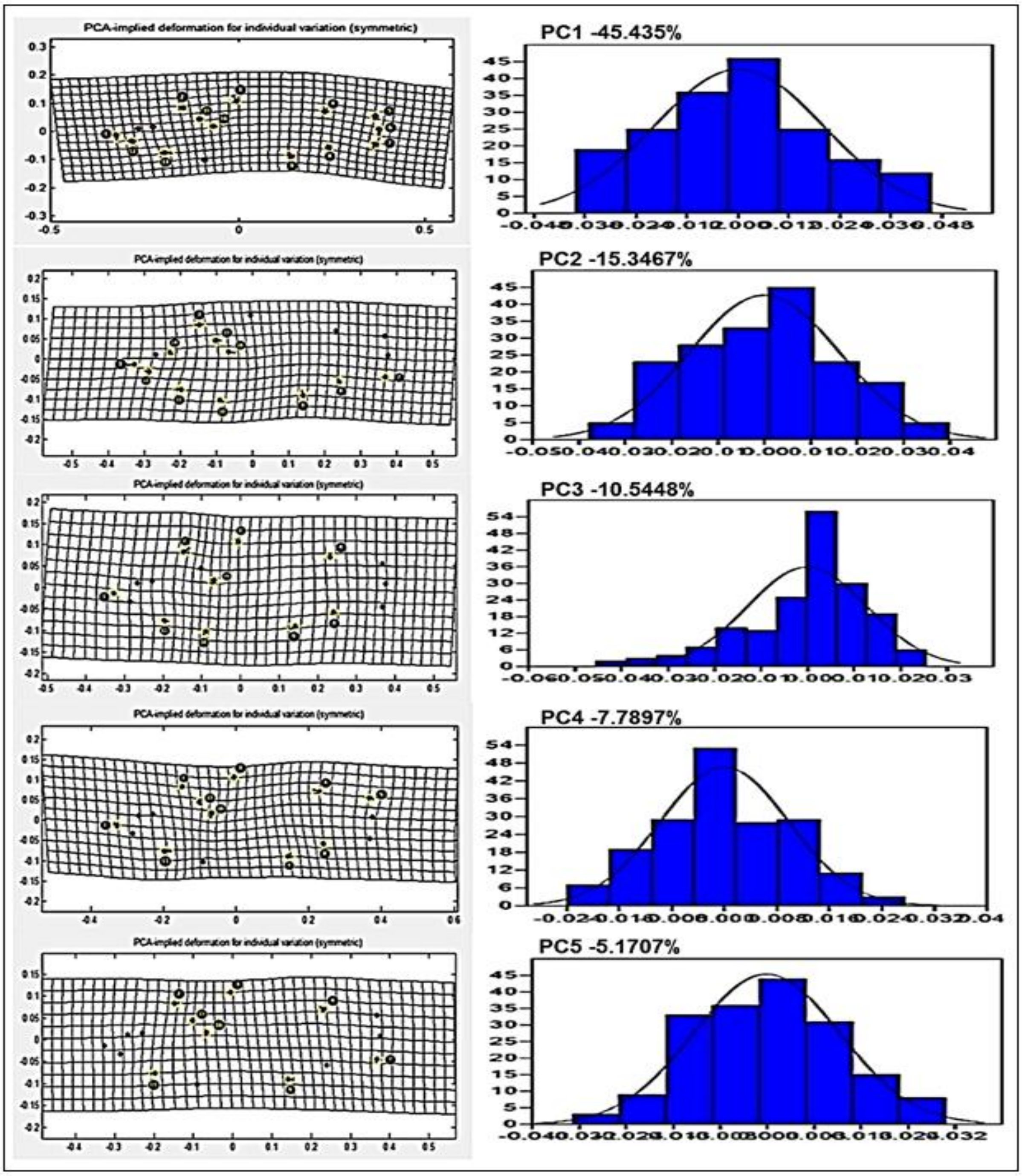

Fig. 5. Summary of the geometric morphometric showing consensus morphology and the variation in the body shape of male population of *H. agilis*.

Conclusion

The fish "Bugwan" (*H. agilis*) was utilized to determine the morphological variations on the body shape using geometric morphometric using fluctuating asymmetry analysis. Standard landmarks used to locate, the metric traits of the fish samples. Results of the Procrustes ANOVA test revealed not high significant in male's individuals and sides with P-value of ($P<0.1913$) and ($P<0.0821$), respectively. On the other hand, the female was highly significant in three factors considered individuals, sides and individual by sides with ($P<0.0001$).This indicating shape variation between the two sexes.

The morphological variations were drawn in the Principal Component Analysis by which the highest values obtained by males in individual symmetry while female in fluctuating asymmetry. In the aspects of Principal Component Analysis (PCA), also showed a degree of dissimilarities from the data of histogram and deformation grid in both sexes. The use of geometric morphometric analysis provides insight to determine shape variations within and between fish community.

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