Article

Describing the body shape variation of spotted barb, *Puntius binotatus* (Valenciennes 1842) using fluctuating asymmetry from Tubay, Agusan del Norte, Philippines

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Abstract

This study aims to assess the condition of the water ecosystem in Tubay, Agusan del Norte by showing the level of FA on the bilateral symmetry of *Puntius binotatus* with respect to its sex that is known to be abundant in the area. This will show the status of *P. binotatus* in Tubay, Agusan del Norte that is known for having a mining activity in the area. Yet, there were no reports in the area that concerns with the developmental instability of *P. binotatus* and other fish inhabiting in the area using FA. Fluctuating Asymmetry (FA) defined as a subtle random deviation between the left and right side of symmetrically bilateral organisms that measures the inability of organisms to maintain homeostasis or measures the developmental instability of organisms. A total of 200 individuals (100 male and 100 females) were subjected to FA Analysis. Landmark analyses were obtained using Thin – plate Spline (TPS) series with 16 landmarks generated for each sample and loaded into Symmetry and Asymmetry in Geometric Data (SAGE) software. Procrustes ANOVA shows that individual symmetry has no significant difference. Sides and Interaction showed a high significantly difference ($P<0.0001$) which suggest being asymmetrical in the bilateral symmetry of *P. binotatus* population. The result of Principal Component Analysis (PCA) shows a level of asymmetry with 77% in female and 73% in the male. FA in female fish can be seen in snout tip, anterior and posterior insertion of the dorsal fin, midpoint, or lateral line, dorsal base of pelvic fin, and dorsal base of pectoral fin. In male samples, affected landmarks can be seen in the anterior and posterior insertion of the dorsal fin, dorsal insertion of caudal fin and dorsal base of the pelvic fin. The level of FA specifies a good indicator of stressors in the environment that causes a decline in reproduction and population.

Keywords Fluctuating Asymmetry (FA); Tubay; Agusan del Norte; Spotted Barb (*Puntius binotatus*).

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1 Introduction

Fluctuating Asymmetry (FA) defined as a subtle random deviation between the left and the right side of symmetrically bilateral organisms that measures the inability of organisms to maintain homeostasis or measures the developmental stability of organisms (Muallil et al., 2014; Ducos and Tabugo, 2015; Van Valen, 1962; Palmer, 1994). Either this deviation is an expression of genetic or environmental stresses occur (Ayoade et al., 2004). Thus, FA is an essential tool to describe the differences or deviation of the ideal morphology of a symmetrically bilateral organism. It also measures the degree of differences between the left and right side of symmetrically bilateral species in response to the different stressors (Natividad et al., 2015; Ducos and Tabugo, 2015; Waddington 1942; Clarke 1998). The degree of FA exposes the level of stressors in the environment (genetic and environmental) that can disrupt the desired development of individuals (Muallil et al., 2014). According to Angtuaco and Leyesa, 2004 as the level of the stressor increases the capability of organisms to maintain the homeostasis at a different level may be affected. Therefore, the level of FA specifies a good indicator of stressors in the environment that causes a decline in reproduction and population. In addition, FA can measure the fitness of an individual to the harshness of the environment and its susceptibility to infections that leads to mortality. Individuals with random alterations from bilateral symmetry are predicted to have reduced fitness; it causes a lower rate of survival (Muallil et al., 2014). Moreover, FA measures the capacity of organisms to buffer its developmental pathways against any stressors in the environment and genetic stressors. The presence of the different stressors may weaken the effectiveness of their buffering mechanism and may affect the developmental process of an individual (Trono et al., 2015). In the Philippines, Fluctuating Asymmetry was utilized in quantifying the deviations from the bilateral symmetries by determining the level of FA in various traits of different bioindicator (e.g. fish, bivalves, etc.) in many water ecosystems in the Philippines. It also employed to examine the environmental conditions, health, and quality of fish (Ducos and Tabugo, 2015). *Puntius binotatus* (spotted barb) locally known in the Philippines as “gabot” belongs to Family Cyprinidae that is widely distributed in streams, river, and lake in Brunei Darussalam, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines, Thailand and Vietnam (Jenkins et al., 2009), native to Asia (Talwar and Jhingran, 1991) and introduced species to Singapore, and Palau (Jenkins et al., 2009). It is a valuable food and a common ornamental fish in some countries that is produced massively and intensively cultured (Lim et al., 2013). *P. binotatus* is distinguished by its phenotypic characteristics of having four barbels, silvery gray in color with a darker shade dorsally and pale shade on throat and belly, round body markings on the caudal base on the large specimen, and a broad-tipped snout. It is known to be a benthopelagic species that can live with a pH range of 6.0 - 6.5 and can stand at the temperature of 24°C - 26°C in tropical countries. They feed mostly on zooplanktons, larvae of insects and vascular plants present in the area. They are commonly available in large numbers and it has been utilized as a bio-indicator in assessing the quality of habitat they inhabit and status of the freshwater resources (Lim et al., 2013; Baumgartner, 2005; Zakeyudin et al., 2012). Tubay, Agusan Del Norte lays in the southern part of the Philippines with coordinates of 9º9’37’ to 9º18’N latitude and 125º31’ to 125º37’E longitude, with a total land area of 13,800 ha comprising of eight coastal and five inland villages (Patricio and Alima, 2010). Gold mining and small scale are widespread in Agusan Del Norte especially in Cabadbaran, Santiago, Jabonga, and Tubay. Water bodies in these sites serve as a basin of mining wastes that causes siltation of the coastal areas. Continuing mineral extraction activities and practices have brought substantial environmental destructions. Thus, more mining company and other industries will be developed; the devastation of environment will be prominent (Caraga Watch, 2009). Tubay encounters a major problem concerning the declining of fish catch in the coastal areas. Inhabitants state that the cause of these sudden changes is due to the mining activities on industrial mining and other destructive human activities in the site (Patricio and Alima, 2010).

2 Materials and Methods

2.1 Study area

The study was conducted in Tubay, Agusan Del Norte (Fig. 1). It is in the province of Agusan del Norte in Caraga, which is part of the Mindanao group of islands. Its geographic coordinates are 9° 10' 1" N, 125° 31' 26" E. The municipality of Tubay is located at the southern part of the Philippine archipelago. Fish collection was done in the month of October 2015.

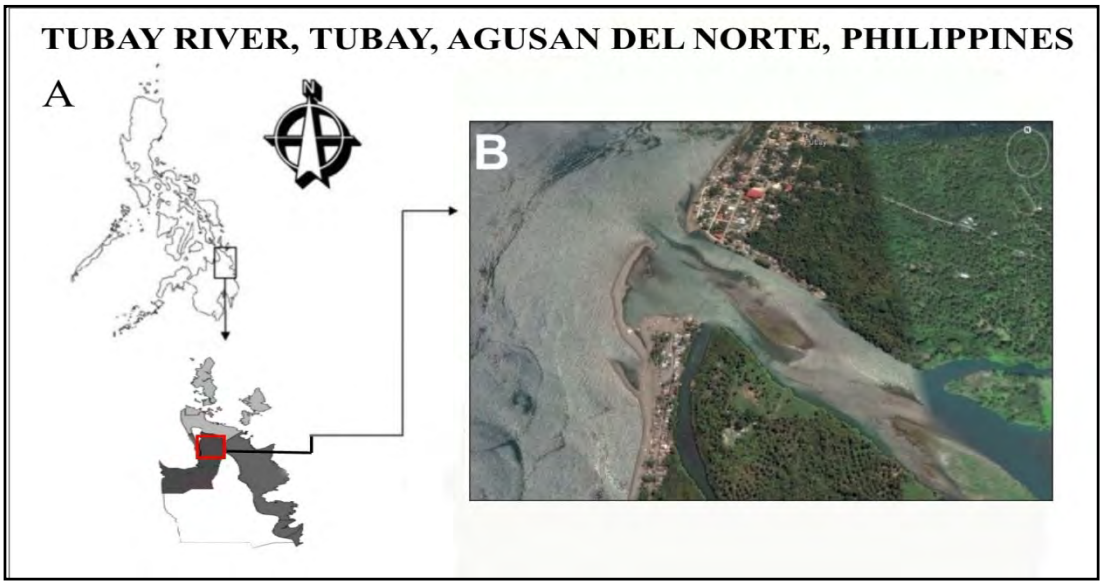

Fig. 1 A. Map of the Philippines showing the map of Mindanao, B. Tubay, Agusan del Norte.

2.2 Processing of fish samples

One hundred male and one hundred female fish individuals of *P. binotatus* were collected and analyzed. Samples were placed in styro box prior to processing for data gathering. Samples were placed in flat styro for the pinning of its fins and 10% formalin were applied to harden all the fins to clearly seen the samples point of origin in land marking process using a small brush. Digital image of the left and right lateral side of each sample was taken using Olympus T – 100 (12 megapixels) with a ruler parallel to its length. Both sides were tri-replicated to determine the digitize error prior to the asymmetry analysis. Using tpsUtil program, captured images were the converted to TPS format and digitized using tpsDig2 program. Sexes were identified by dissecting and observing the genitalia of each specimen. Female fish samples were identified by the presence of its eggs and ovaries, generally yellow or orange in color with a granular in appearance. The males, on the other hand, were determined based on the presence of their testes, which were typically smooth, whitish in color, and non-granular in appearance (Requieron et al., 2010).

2.3 Landmark selection and digitization

Using thin-plate spline (TPS) series landmark analysis was obtained to incorporate all the curving part of the specimens' images. Standard forms of digitized landmarks in fish morphometrics were applied (Table 1). A total of landmarks were used to obtain a homogenous outline in the body shape of the specimen as shown in Fig. 2 and describe in Table1 (Natividad et al., 2015; Jumawan et al., 2016).

Table 1 Landmarks used to digitize the images of the freshwater fish samples collected.

COORDINATES	LOCATIONS
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 or lateral line
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 the premaxilla
13	Anterior margin through midline of orbit
14	Posterior margin through midline of orbit
15	Dorsal end of opercle
16	Dorsal base of pectoral fin

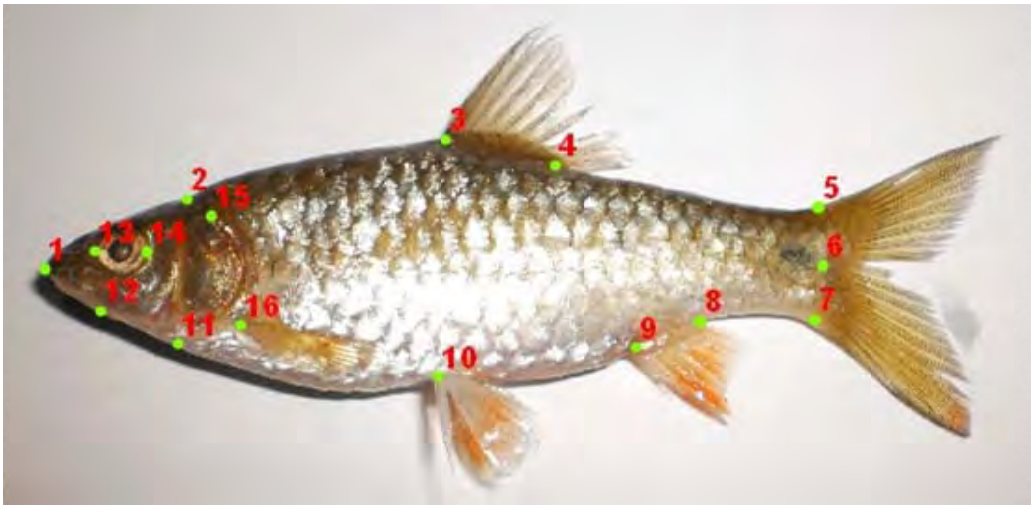

Fig. 2 Landmark points of *P. binotatus* through digitations using tpsDig2 from TPS program.

2.4 Shape analysis

Generated x and y coordinates served as a baseline data in evaluating fluctuating asymmetry of *P. binotatus*. Symmetry and Asymmetry in Geometric Data (SAGE, Marquez, 2007) were used for the left and right platform landmark coordinates of the TPS. The symmetrized data sets and residuals from symmetric components were generated by Symmetry and Asymmetry in Geometric Data (SAGE). This was used for the identification of geometric data of the object with essential on its symmetry see (Fig. 3). Procrustes ANOVA was used to determine significant differences in the symmetry of the factors considered. These factors were individuals, sides, and interaction of individuals and sides of *P. binotatus*. The level of significance was tested

at P<0.0001. The variation between the side and the measure of directional asymmetry also indicated. Percentage (%) FA was obtained and compared between the sexes (Natividad et al., 2015).

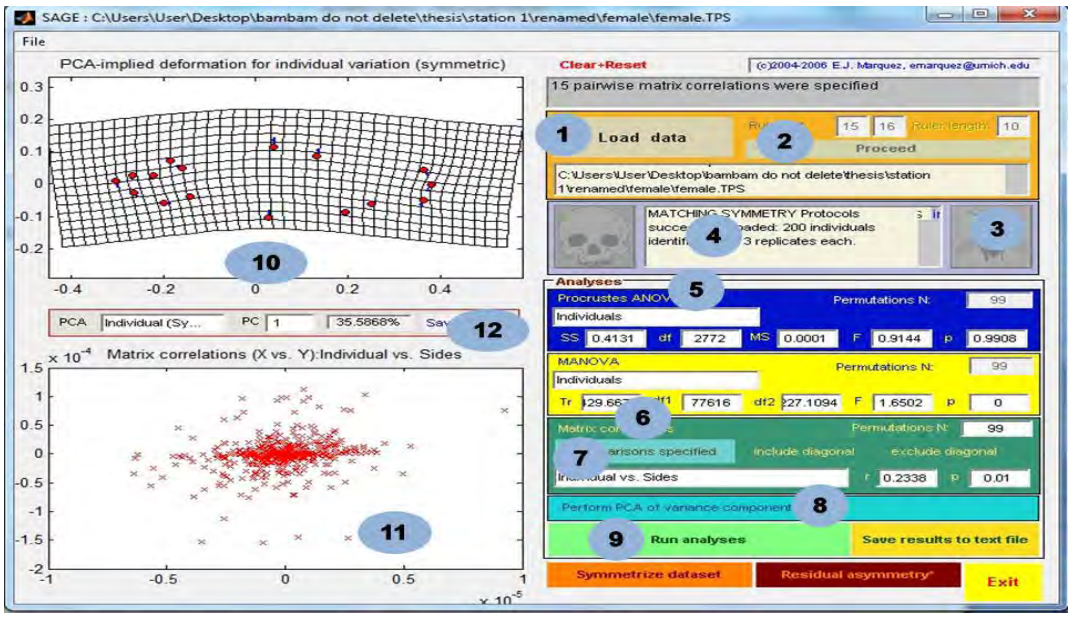

Fig. 3 Overall schematic diagram of shape analysis using SAGE software.

3 Results and Discussion

A total of 200 collected samples of *P. binotatus* in Tubay, Agusan del Norte (100 each sex) were collected. *P. binotatus* was abundant in the sampling areas, where the fish samples were collected.

Table 2 Procrustes ANOVA on body shape of *P. binotatus* in terms of sexes in Tubay, Agusan del Norte.

FACTORS	SS	DF	MS	F	P-VALUE
		F E M A L E			
Individuals	0.4131	2772	0.0001	0.9144	0.9908^{ns}
Sides	0.0272	28	0.001	5.9652	0.0001**
Individual x Sides	0.4518	2772	0.0002	14.9846	0.0001**
Measurement Error	0.1218	11200	0	--------	----------
		M A L E			
Individuals	0.413	2772	0.0001	1.0415	0.1425^{ns}
Sides	0.0462	28	0.0016	11.5295	0.0001**
Individual x Sides	0.3966	2772	0.0001	5.7229	0.0001**
Measurement Error	0.28	11200	0	------	------

** (p < 0.0001**) – highly significant, ns – not significant

Table 2 shows the analysis of individual symmetry and FA of Left-Right body size and shape of fish samples through Procrustes ANOVA that was employed to both male and female species in Tubay, Agusan del Norte. Three factors were considered in the analysis, the individuals; sides; and the interaction of individuals and sides. The results verify the fluctuating asymmetry for the three factors considered which

occurs in the body shape of both sexes (P<0.0001). The individual symmetry among samples showed no significant differences in shapes between sexes. It was observed that sides and interaction of individuals and sides of both sexes shows a highly significant difference (P<0.0001). The asymmetry in the morphology of the three factors among males and females may be an indication that the species in the study area is under environmental stress.

The differences in shapes between the left and right sides of the fishes can be an evidence of asymmetric morphology of the fish due to exposure of different pollutants in its environment (Natividad et al., 2015). Prolonged exposure to stressed and polluted water will eventually lead to undesired development in the phenotypic, as the stressors affect the individuals during the development of the fish species (Barrett, 2005; Bonada and Williams, 2002). Thus, body asymmetry is a manifestation of the inability of the species to thrive and buffer environmental disturbances (Van Valen, 1962). The developmental instability of *P. binotatus* eventually resulted to fluctuating asymmetry in the species. At present, Tubay has gold mining that might contribute to the environmental destruction in the area that ended with a major problem concerning the declining of fish catch in the area (Caraga Watch, 2009; Patricio and Alima, 2010).

Principal component analysis was employed to determine the affected landmarks using the symmetry and asymmetry scores of the fish samples. There were five principal components (PC) considered in female samples and six PCA in male. Table 3 shows the five and six highest PC scores that determined landmarks were commonly affected in fluctuating asymmetry. Skewness of the histogram was reflected in every PC score along with the deformation grid to determine affected landmarks in the body form of the sampled species, shown in Figs. 4 and 5.

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

PCA	Individual (symmetry)	Sides (directional symmetry)	Interaction (fluctuating asymmetry)	Affected Landmarks
		F E M A L E		
PC1	35.5868	100%	33.0139	1,2,3,4,5,6,7,9,10,11,12,13,14,15
PC2	16.0341		21.5931	1,3,4,6,8,9,10,11,14,15,16
PC3	9.7512		9.6916	1,3,4,6,7,8,9,10,11,13,16
PC4	8.2637		6.7746	3,4,5,6,8,9,10,11
PC5	5.384		5.6821	1,3,4,7,8,9,10,16
	75.0198		76.7553	
		M A L E		
PC1	29.529	100%	33.33	1,2,3,4,5,6,7,8,9,10,11,12,16
PC2	16.6754		15.4399	1,2,3,4,5,6,10,11,12,13,14,15,16
PC3	10.5353		10.7477	1,3,4,5,6,7,8,9,10,15,16
PC4	7.6512		7.3076	1,3,4,5,6,7,8,9,10,13,16
PC5	6.817		6.0384	1,2,4,5,6,8,9,10,12,15,16
	71.2079		72.8636	

A total of 77%of the cumulative variation represents in five principal components (PC) in female samples. PC1 has the highest variation accounted for 33.01%. The asymmetry can found immensely in the area of

landmarks: 1 (snout tip), 3 (anterior insertion of dorsal fin), 4 (posterior insertion of dorsal fin), 6 (midpoint or lateral line), 10 (dorsal base of pelvic fin), and 16 (dorsal base of pectoral fin). The most common affected landmarks but not present in all PC score were 5, 7, 8, 9, and 11. It was observed that in females, the heavily affected landmarks were the portion of the head (snout tip) and fins. In male samples, the five PC constituted to 73% of the cumulative variation. PC 1 contributed the highest accounted variation with 33.33%. The affected landmarks in male samples were landmarks 3 (anterior insertion of dorsal fin), 4 (posterior insertion of dorsal fin), 5 (dorsal insertion of caudal fin), and 10 (dorsal base of pelvic fin). The most affected landmarks were portions of the fins of the sampled fish. These affected landmarks were further shown in the deformation grid and histogram of the values revealed skewness suggesting an asymmetry in body form (Figs. 9 and 10). Yellow marks represented the different fluctuation on the affected landmarks.

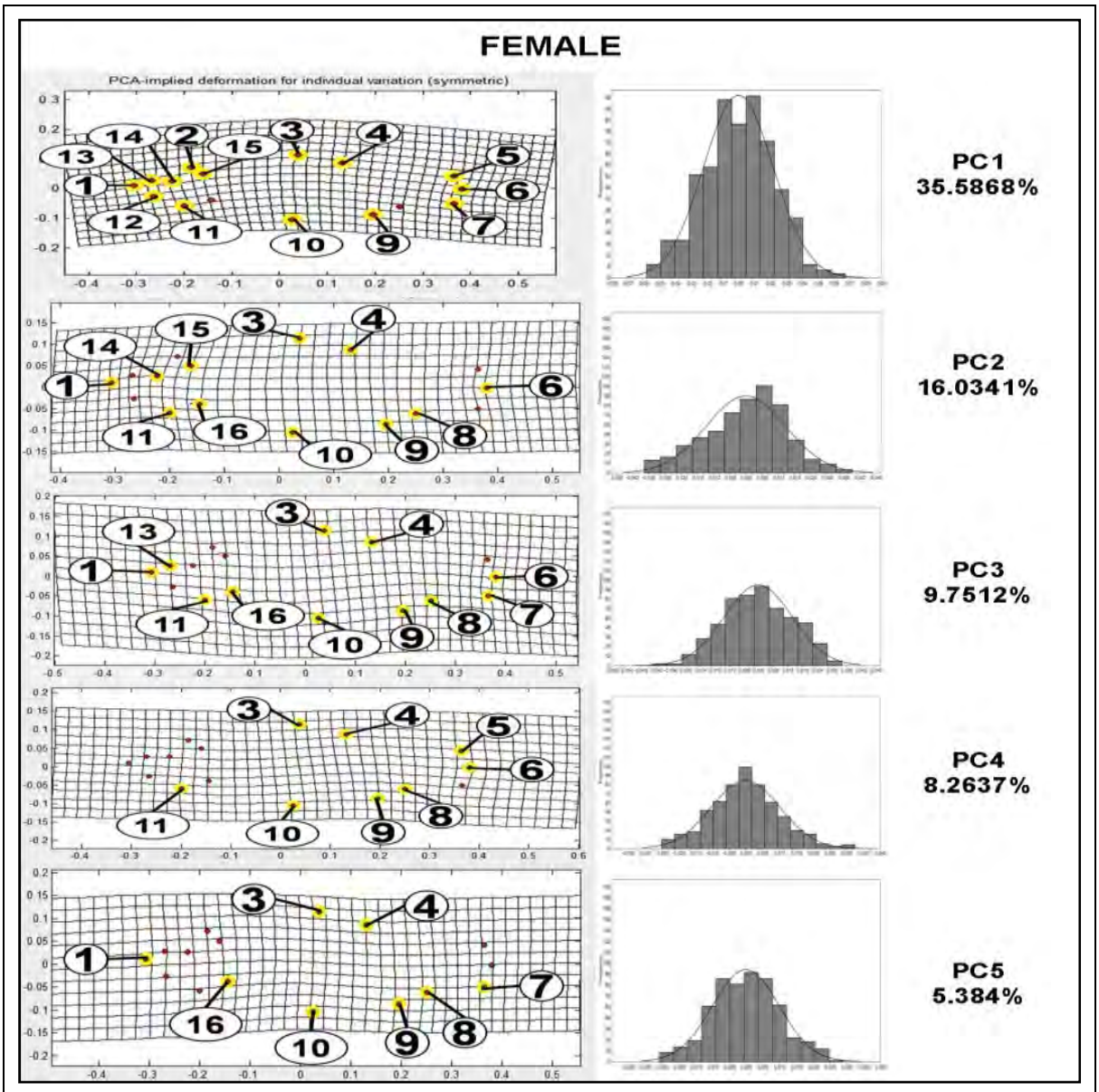

Fig. 4 Principal components (PC) implied deformation grid and a histogram of individual (symmetric) in *P. binotatus* female species

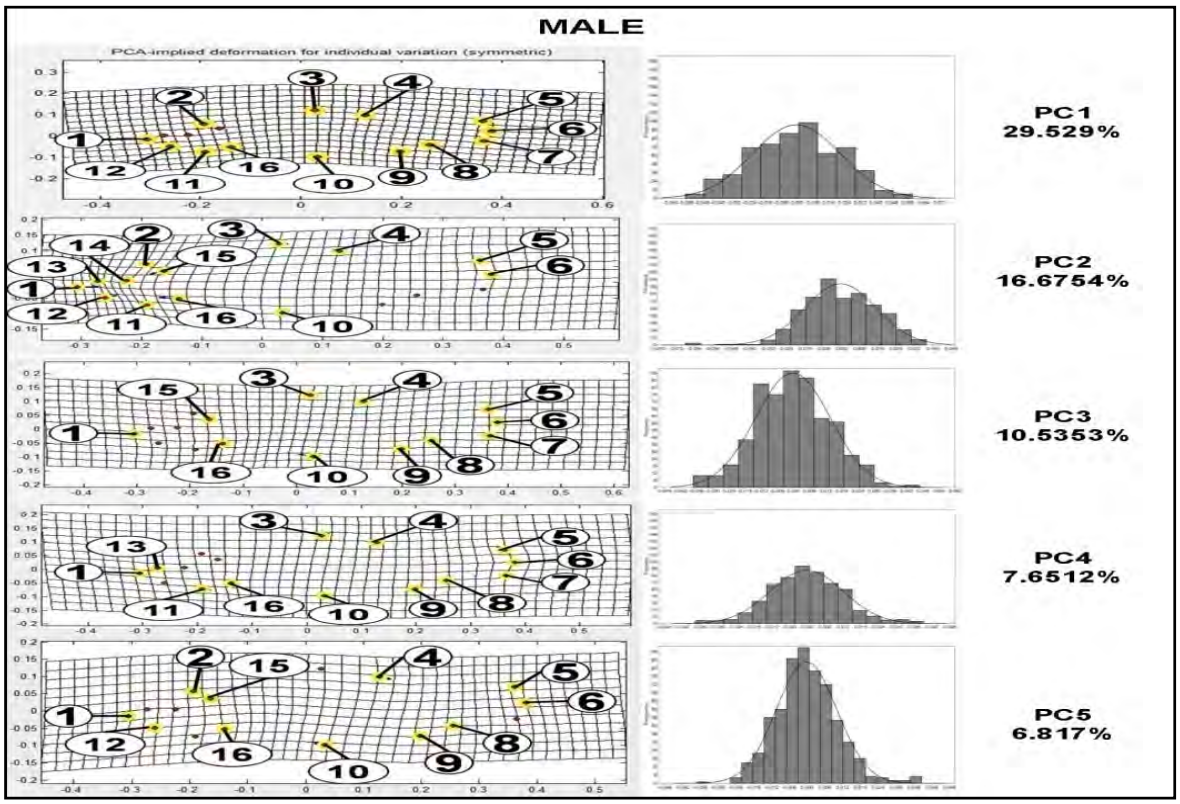

Fig. 5 Principal components (PC) implied deformation grid and histogram of individual (symmetric) in *P. binotatus* male species.

The identified landmark points affected by FA were shown together with the actual digitized photograph of the fish sample (Fig. 6). The defined illustration was summarized using PC1 and PC2 landmarks, which the two highest accounted variations in all the principal components were collected. The high significant level of FA in the bilateral morphology of fish is the best assumption that the species in the certain area experiences stresses that disables them to maintain its developmental homeostasis. However, the poor quality of water prominently affects the growth and development of fishes that results to fluctuating asymmetry of the body shape of the species (Schlosser, 1991).

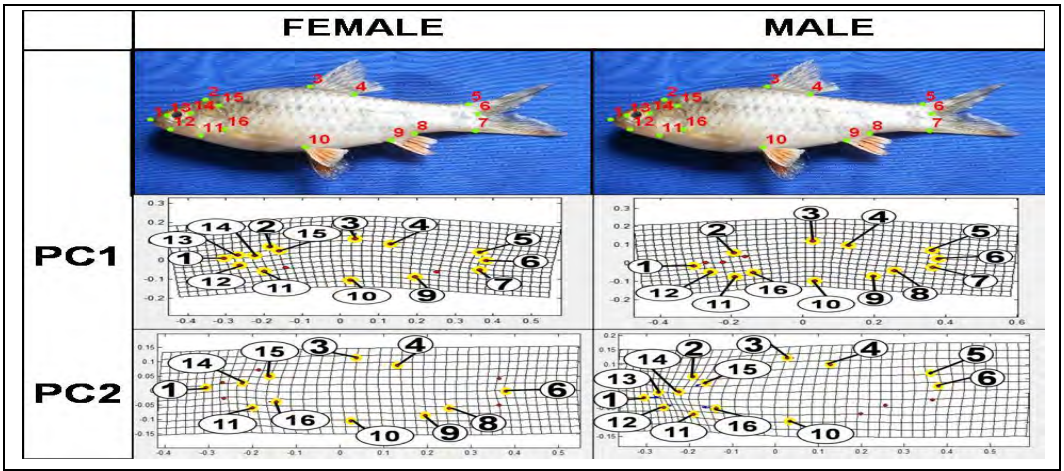

Fig. 6 Actualized picture of digitized male and female fish with the affected landmarks shown in PCA-deformation grid for PC1 and PC2.

4 Conclusion

The study verified the use of Fluctuating Asymmetry as a tool in examining the asymmetry level in the bilateral symmetry of *P. binotatus* due to environmental stressors in Tubay, Agusan del Norte Freshwater ecosystem. It has been reported that there is a major problem concerning the declining of fish catch that is may cause by the mining activities and other destructive human activities on the site. Yet, there were no studies conducted to assess the condition of *P. binotatus* that is may be exposed to possible pollutants from Tubay freshwater ecosystem. Procrustes ANOVA shows that Individual Symmetry represents no significant difference. Sides (Directional Asymmetry) and Interaction (Fluctuating Asymmetry) showed a high significant difference ($P<0.0001^{**}$) in both male and female sexes samples in the area. The PCA result shows the level of fluctuating asymmetry in male (PC1 – PC6) that comprises of 73% and female (PC1 – PC5) sexes that constitute 77%, as well as in pooled male and female samples shows a high level of FA which composed of 75% (PC1 – PC5). As a result, the study verifies the use of fluctuating asymmetry in determining and assessing the health of water ecosystem of the aquatic environment and discriminating sexes of *P. binotatus* by showing an evidence of fluctuation in the physiological characteristics of the fish samples.

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