Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 8, No. 3, p. 55-63, 2016
http://www.innspub.net

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RESEARCH PAPER

OPEN ACCESS

Relative warp analysis on shell shapes of *Faunus ater* in Guihing River Hagonoy, Davao del Sur

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Article published on March 16, 2016

Key words: Pachychilidae, Oviparous, Shell morphology, Geometric morphometric.

Abstract

This study aims to characterize the relative warp analysis on shell shapes of the *Faunus ater*. A total of 60 individuals were analyzed based on their shell shape and was deliberated using a landmark-based methodology. The following are the total landmarks stated and used: ventral/aperture=27, dorsal=17 and top of the whorl=147. Procrustes ANOVA showed the significant level of symmetry of *F. ater* on its ventral/aperture=0.0392123; dorsal=0.1069 and top of the whorl=1.20366 portion. To further sustain the analysis, the upper five percent (5%) value of PCA was performed to visualized shape variation landmarked subject together with the histogram, and also used to investigate patterns of variation in the positions of landmarks: ventral/aperture=100.00%; dorsal=16.67% and top of the whorl=96.702%. The designated relative warps by its overall shape variation and percentage variance of *F. ater* confirms (a)16.66671667%; (b)16.6666%; (c)20.0001% for (a)ventral/aperture, (b)dorsal and (c)top of the whorl courtesy of relative warp box plot and histograms. The results of this study suggest that Relative Warp Analysis (RWA), Principal Component Analysis (PCA) of *F. ater* species mostly differ in the latter part and their body depth. The study of morphological differences in sizes and pattern using the ventral/aperture, dorsal, and top of the whorl characteristics of the shell clearly indicates its shell shape variation. In this manner, *F. ater* was quantitatively defined.

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Introduction

Faunus ater is from the Greek word "*Faunus*" Roman god, patron of farming and livestock and "*ater*" Latin word which means "black", a type of freshwater gastropod from the family Pachychilidae that is extremely abundant and widely distributed in mouths or low reaches of freshwater streams with brackish influence in parts of the Indo-West Pacific; *F. ater* commonly dispersed in India, Sri-Lanka, Andaman Islands, Myanmar, both sides of the Thai and Malay Peninsula, Singapore, Indonesia, New Guinea, western and southern Pacific Islands, northern Australia, China and in the Philippines (Swennen *et al.*, 2001; Kohler *et al.*, 2004; Sri-aroon *et al.*, 2005, 2006; Schäfer, 2010; Tan and Woo, 2010; Yap *et al.*, 2010; Strong, 2011; Lok *et al.*, 2011).

In the Philippines, *F. ater* is considered as a native delicacy consumed by the residents not only in Hagonoy but all over Davao del Sur. Due to the abundance of the species in Guihing, settlers near by the river harvest the shells and sell them to nearby cities and municipalities.

The ecology of *F. ater* is poorly known. The classification of these species remains unresolved due to the scarcity of constructive characteristics of the organism. In determining gastropods, the shell is usually used in its classification and taxonomic descriptions. Shell Morphometry is considered as a crucial method for species distinction (Kohler, 2003; Moneva, 2012).

Since the external shape of the shell was the major character used in the species-level taxon of gastropods and having a qualitative description is difficult to achieve, geometric morphometrics (GM) was introduced. GM is a method where species are determined based on its quantitative result by analysing shell shape variation using Cartesian geometric coordinates indicating curves, outlines, landmarks and surfaces. Quantitative descriptions of the species are expressed statistically which allows researchers to interpret the collected data. Therefore, in this study, *Faunus ater* was defined quantitatively by relative warp analysis of based on shell shape variation.

Materials and methods

Study area

The study was conducted within months of August to October (2015) in the Municipality of Hagonoy, province of Davao Del Sur situated in south-central Mindanao geographically located between 6.75° N and 123.36° E; 5 meters elevation above sea level.

Data collection

The specimens of *F. ater* were collected in Guihing River, Municipality of Hagonoy. A total of 60 individuals were analyzed for shell shape differences. The shell is unique on account of its expanded size, fine conical profile, and especially by its privy aperture. The periostracum protects the shell from the acidic environment of its habitat. The broad, muscular snout, large buccal mass and robust radula indicates that this species grazes on coarse substrata. The reproductive biology of *F. ater* were unidentified; although they are known to be oviparous, their large geographical distribution suggests an extended free swimming larval stage (Houbrick 1991, Kohler *et al*, 2004; Lok *et al.*, 2011). The shells were in the position of ventral and dorsal side then on the top of the whorl. Digital images were obtained using a Nikon 16MP digital camera as shown (Fig. 2).

Land marking

The shells positioned where the head is at 90□ of the X-axis in an aperture view in which the apex is visible. Acquired images were then used as subject to geometric morphometric method. Digital images (ventral, dorsal and top of the whorl view) were taken for each sample using a standardized procedure (shown in Fig. 3).

Shell shape was deliberated using a landmark-based methodology that purges the effect of variation in the location, orientation, and scale of the *F. ater*. Twenty anatomical landmarks were sited along the outline of the (a) ventral or aperture portion of the shell seventeen anatomical landmarks along the (b) dorsal portion and one hundred forty seven anatomical landmarks within the (c) top of the whorl of shell were stated and used. This was possible using an image analysis and processing software, the Tps Dig freeware 2.12. Tps Dig abridges the statistical analysis of landmark data in morphometric by making it easier to gather and uphold landmark data from digitized images (Rholf, 2008).

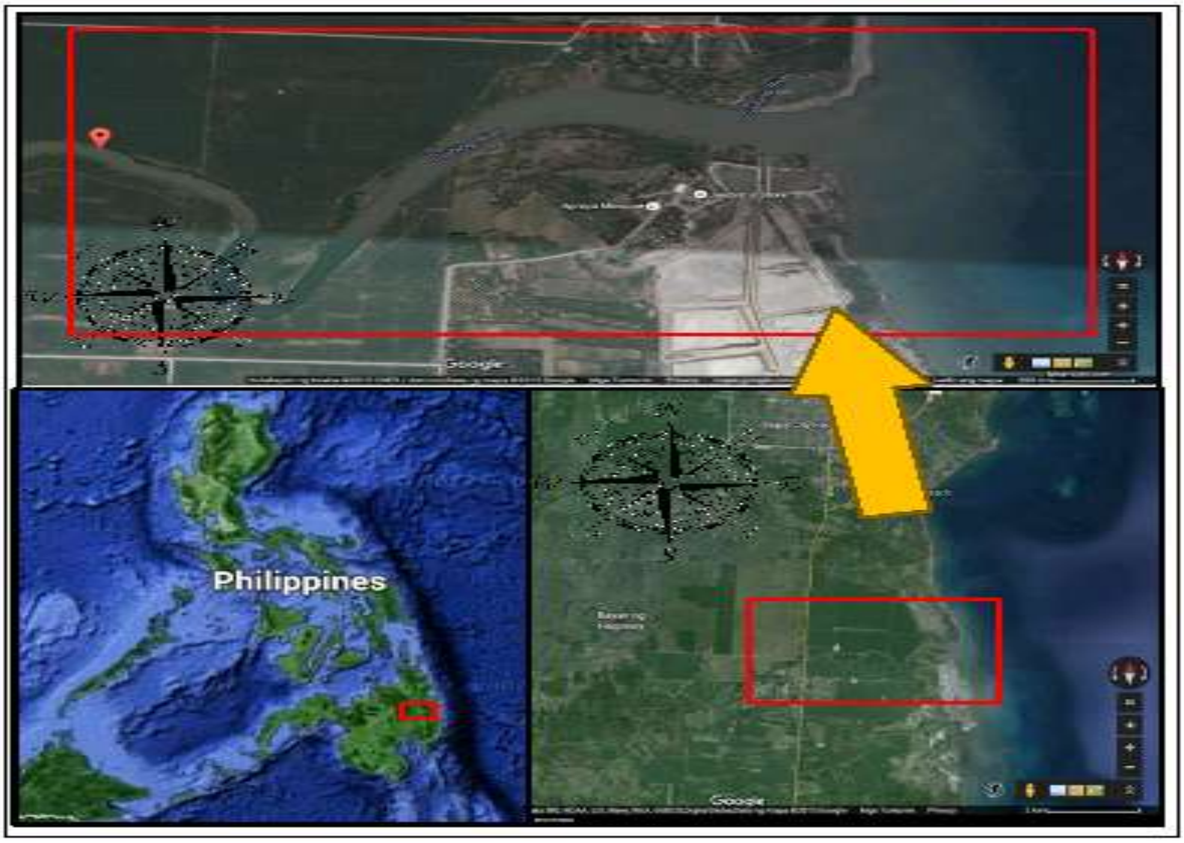


Fig. 1. Map of the Philippines (Left) showing Davao Del Sur in Mindanao; (right) displaying Municipality of Hagonoy Davao del Sur; (up) enhanced map showing Guihing River in Davao Del Sur. (www. Google.com).

These coordinates were then conveyed to Microsoft Excel application for organization of the data into groups. The two-dimensional coordinates of these landmarks were done for *F. ater* specimen. The relative warps were computed using the unit centroid size alignment-scaling method.

Histogram and box plots were made using PAST software (Hammer *et al.*, 2009) from the relative warps of the shell shapes. Histogram and box plots are powerful displays in associating distributions. It provides a condensed view of the data and how it is allocated over the range of the variable. The collective coordinates of all individuals were then exposed to Principal Component Analysis (PCA) using geometric morphometric computer application Paleontological Statistics (PAST) software developed by Hammer *et al.* in year 2009. PCA was used to abstract the information of the variations and mean shapes bounded in the coefficients of landmark descriptors.

Results and discussion

Procrustes ANOVA showed a significant level of symmetry in individuals which is *F. ater* (Table 1). To further sustain the analysis, the upper five percent (5%) value of PCA was performed (Table 2) to visualized shape variation in Fig. 4 landmarked subject together with the histogram, and also used to investigate patterns of covariation in the positions of landmarks (Dryden and Mardia, 1998).

Table 1. Procrustes ANOVA analysis for *F. ater*. The factor in each test was in sample locations: dF - degrees of freedom; SS - sum of squares; MS - mean of squares; F - ratio.

	VENTRAL				DORSAL				WHORL			
WITHIN	SS	dF	MS	F	SS	dF	MS	F	SS	dF	MS	F
GROUPS	0.00083	5	0.00016	1.542	0.003115	5	0.000623	2.126	6.04956	5	1.20991	0.00030161
	0.03837	354	0.00010	-	0.103784	354	0.000293	-	1.2036	30	0.40119	-
TOTAL	0.0392123				0.1069				1.20366			

Table 2. Upper 5% principal components analysis (PCA) in Faunus ater. Legend: PC- Principal Component, EV-Eigenvalue, V- Variance.

VENTRAL			DORSAL			WHORL		
PCA	EV	V%	PCA	EV	V%	PCA	EV	V%
PC 1	0.000305632	46.988	PC 1	0.00078244	44.526	PC 1	1.02852	89.68
PC 2	0.00016979	26.104	PC 2	0.000501695	28.521	PC 2	0.27863	96.26
PC 3	6.37206E-05	9.7965	PC 3	0.000256211	14.565	PC 3	0.15143	98.20
PC 4	5.41647E-05	8.3273	PC 4	0.000112514	6.3963	PC 4	0.11748	99.37
PC 5	4.12105E-05	6.3357	PC 5	5.6265E-05	3.1986	PC 5	0.08608	100.00
PC 6	1.59278E-05	2.4488	PC 6	4.9125E-05	2.7927	-	-	-
TOTAL		100.00			16.67			96.702

Projections on the (A) representation of the histogram in Figure 3 are considered to be variations in shell shape foreseen as negative deviations of the mean in the axis of the relative warps. Extrinsic factors due to environmental conditions also induce variation in the shell shape of the *F. ater*. The shape of the apertural opening where the operculum is located also contributes to its variation. Differences in shape of the aperture with respect to the body whorl could be linked to predatory defense.

Table 3. Description of the designated relative warps by its overall shape variation and percentage variance of *F. ater*.

	Variation%	Ventral/Aperture Observation	Variation%	Dorsal Observation	Variation%	Whorl Observation
RW1	46.988%	There are variations in the head region. In the negative extreme, there is inflation in the head region. It also shows that there is putruding edge in between the body and the head. While in the positive extreme, it described to be straighten. The latter also expresses constriction in the positive extreme.	44.526%	There are variations in the head region. In the negative extreme, there is inflation in the body region. While in the positive extreme, there's an obvious pultrude in the upper section the image. The latter also expresses constriction in the positive extreme.	85.696%	There's an obvious difference between the two extremes, left extreme manifest large inflation in size along its mass as well, where as in positive extreme the constriction of its extent is palpable.
RW2	26.104%	The negative extreme manifests enlargement of the head region. On the other hand, positive extreme displays tighten head. It also signifies that in the negative extreme there is an obvious flaunt between the head and the belly while in the positive extreme it shows clearly plain designation.	28.521%	There are variations head. The negative extreme manifests enlargement of the body. On the other hand, positive extreme displays tighten head. It also signifies that in the negative extreme there is broadening of the head portion while in the positive extreme it shows clearly flaunt in the top of its head.	8.757%	It manifest the palpability of it's enlarge extent from the negative extreme specifically on the left side. On the other hand the evident of the constriction of its mass are expressed in detail on the positive extreme
RW3	9.7965%	There are variations in the head region. The negative extreme shows that the head region is enlarge while the latter part is somehow manage to expand its size. On the other side, the positive extreme exhibits a petite body length.	14.565%	There are variations in the head region. The negative extreme shows that the head region is enlarge while the latter part is somehow manage to expand it size. On the other side, the positive extreme exhibits a petite body length.	3.084%	In the negative extreme, it shown that there is an alteration in size which is the mass while on the other hand it expresses the tightening of its extent (positive extreme)
RW4	8.3273%	There are variations in the head region. In the negative extreme, it has a greater head inflation and curvature	6.3963%	There are variations in the head region. In the negative extreme, it has a greater head expansion	1.4883%	It demonstrates the expansion of its amount as well as its elongation in

		between the body and the head compare to the positive extreme. In contrast, positive extreme shows that it has a broader depth in the terminal area.		along with its body compare to the positive extreme. In contrast, positive extreme shows that it has a broader depth in the terminal area.		length, while in the positive extreme it manifest full condensed physique on the centre.
RW5	6.3357%	There are variations in their head region. We can see a difference in their head region because the negative extreme manifests compression in the cranium portion and its expanded size to which pertain to the body due to the pultrude section in the intersection of the head and the body. While the positive extreme shows that the head is constricted and it has somehow small body depth.	3.1986%	There are variations in their head region. It shows difference in their head region because the negative extreme manifests expanded size. While the positive extreme shows that the head is being constricted and the body was fused. Compare to the negative extreme.	0.9752%	-
RW6	2.4488%	There are variations in the head region. The negative extreme has a more constricted head and clearly manifest a curvature in the inner lip than the positive extreme yet it has a large amount of space in the mouth.	2.7927%	There are variations in the head region. The negative extreme has a more spaces in the body length while in the positive extreme it shows to be condensed to look like small in shape.	-	-

It has been reported by DeWitt (2000) that the shape of aperture is the best way to deter shell entry as frequently performed by the predators such as decapods. Narrow apertures are potentially important defense in freshwater. Conversely, wider aperture indicates vulnerability to predation (Williams, 2005). It could be inferred that predators may affect apertural shape of the freshwater snails.

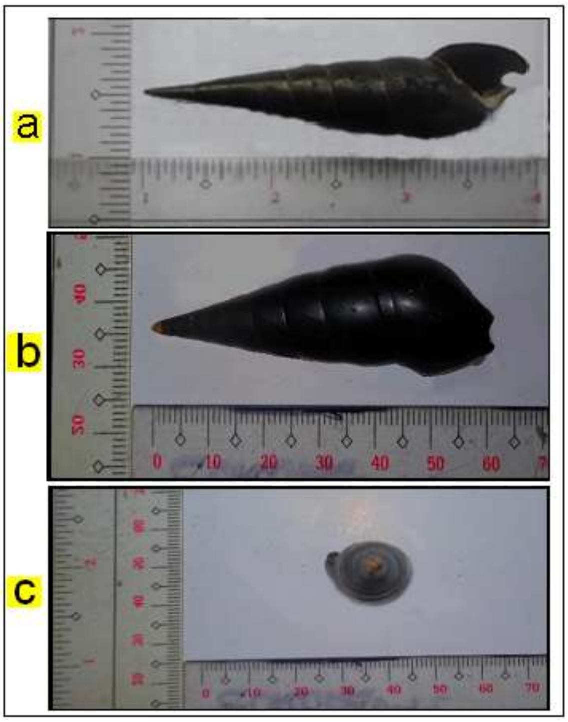


Fig. 2. *Faunus ater*. (a) Ventral, (b) Dorsal and (c) whorl, hermaphrodite (Images obtained by Nikon 16MP Digital camera).

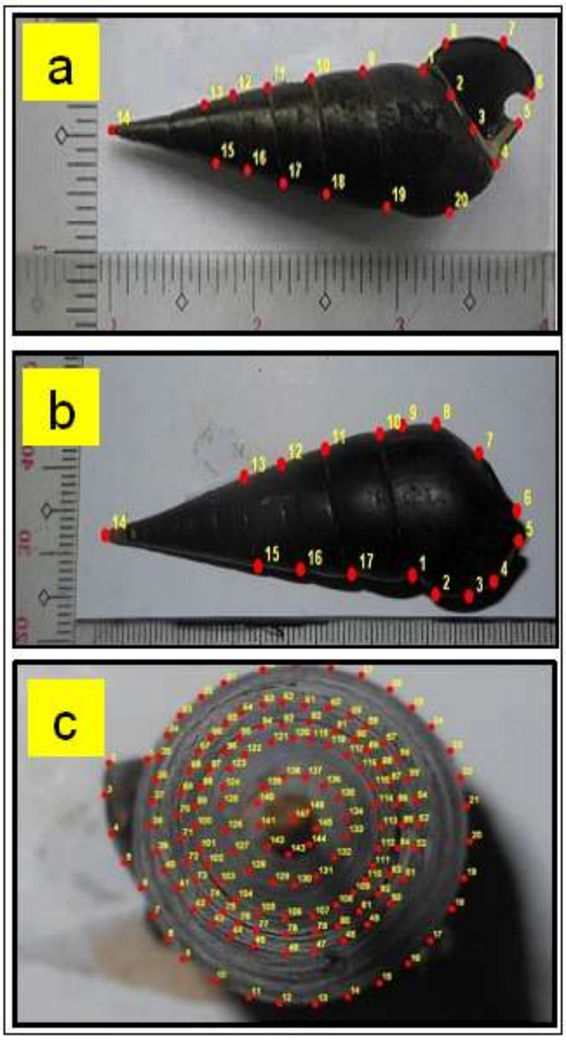
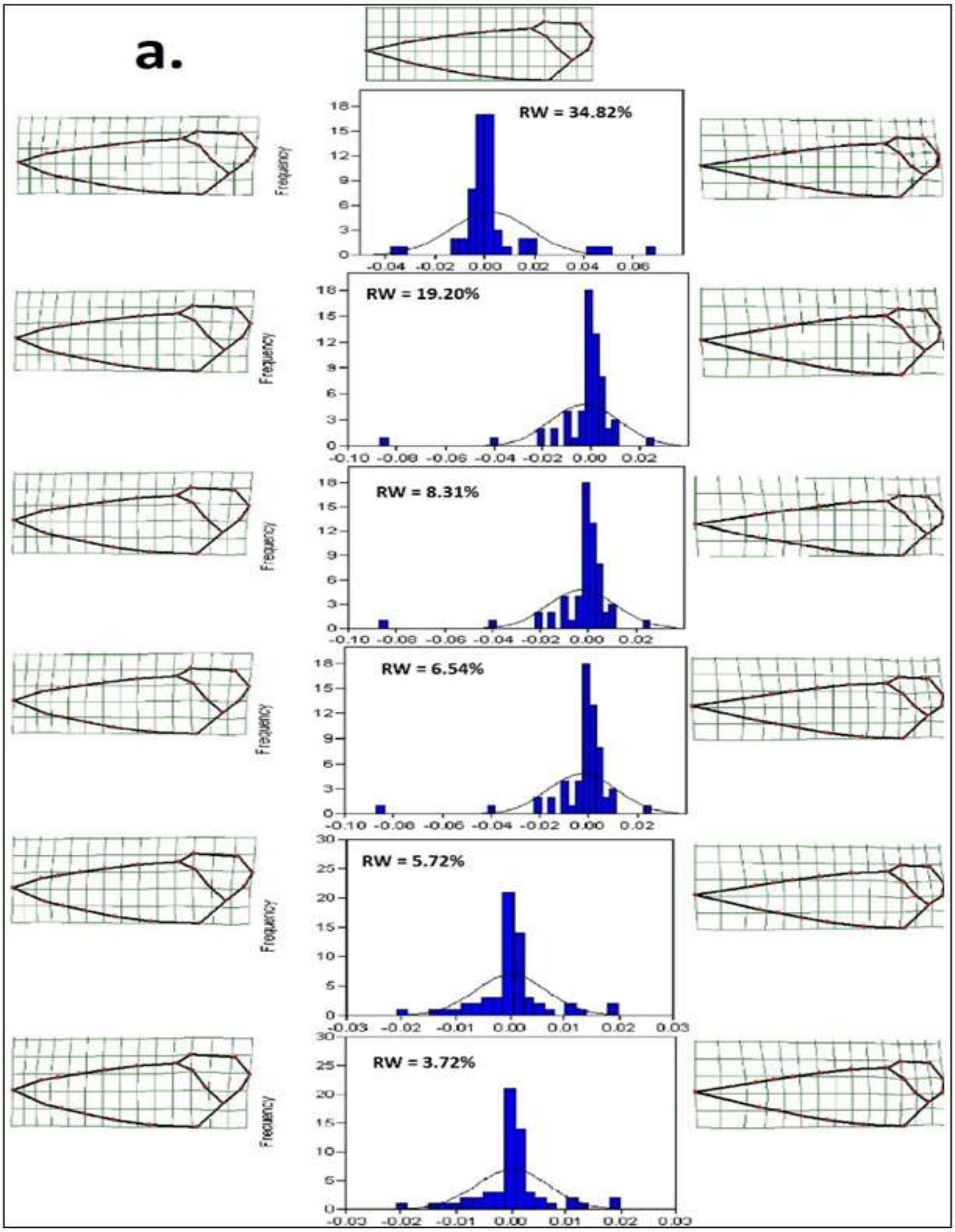
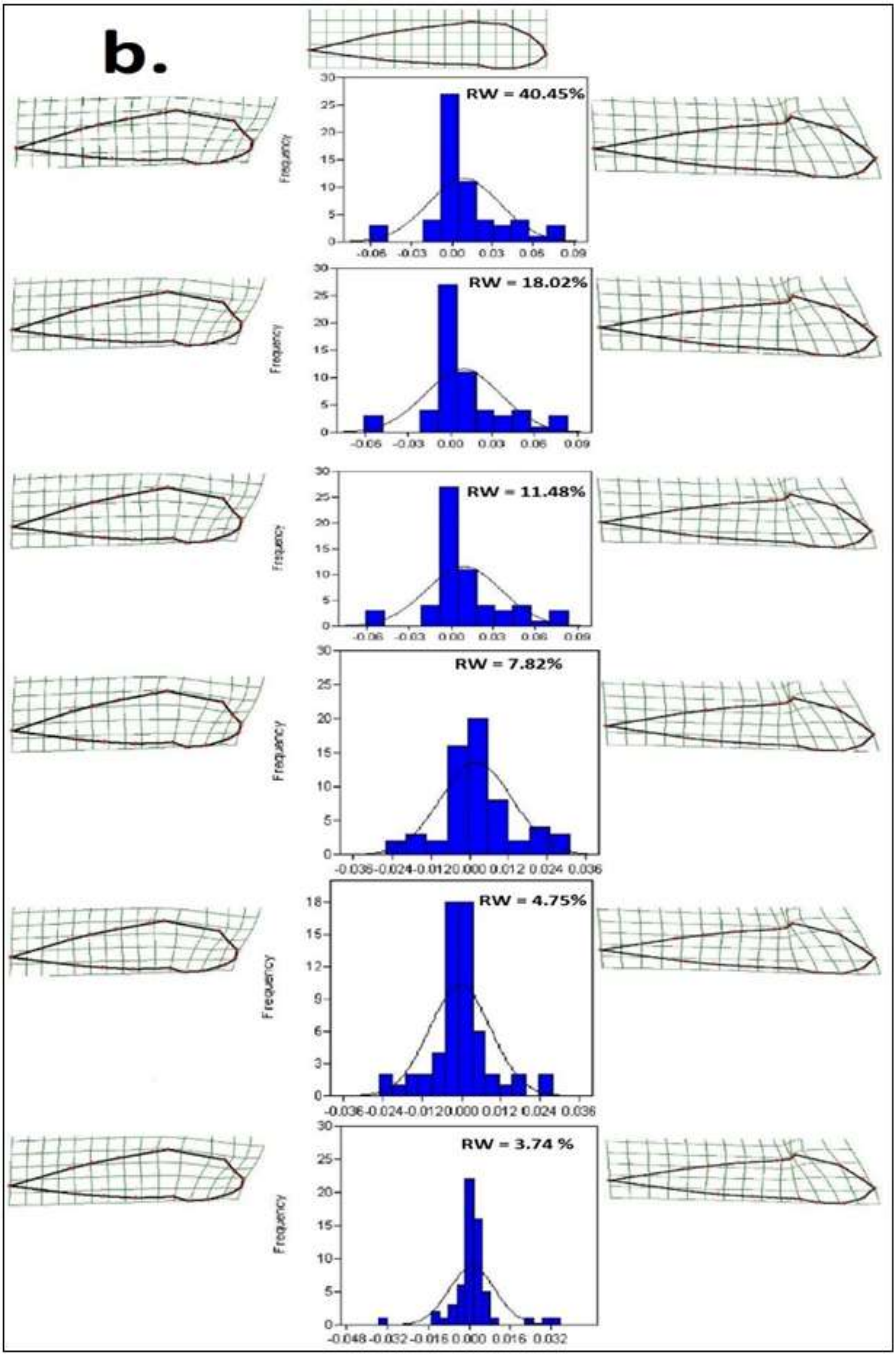


Fig. 3. Landmarks were used for digitizing the image of the subject. (a) ventral/aperture (b) dorsal and (c) top of the whorl of the shell.

Then, on the (B) representation are variations in shell shape foreseen as positive deviations of the mean in the axis of the relative warps. The topmost figure (C) is the mean shape of the samples obtained. Looking at the overall body shapes of the shells, the results of the analyses manifested that all species exhibited a not significant levels of *F. ater* (Table 3).




b.
RW = 40.45%
RW = 18.02%
RW = 11.48%
RW = 7.82%
RW = 4.75%
RW = 3.74 %
Frequency

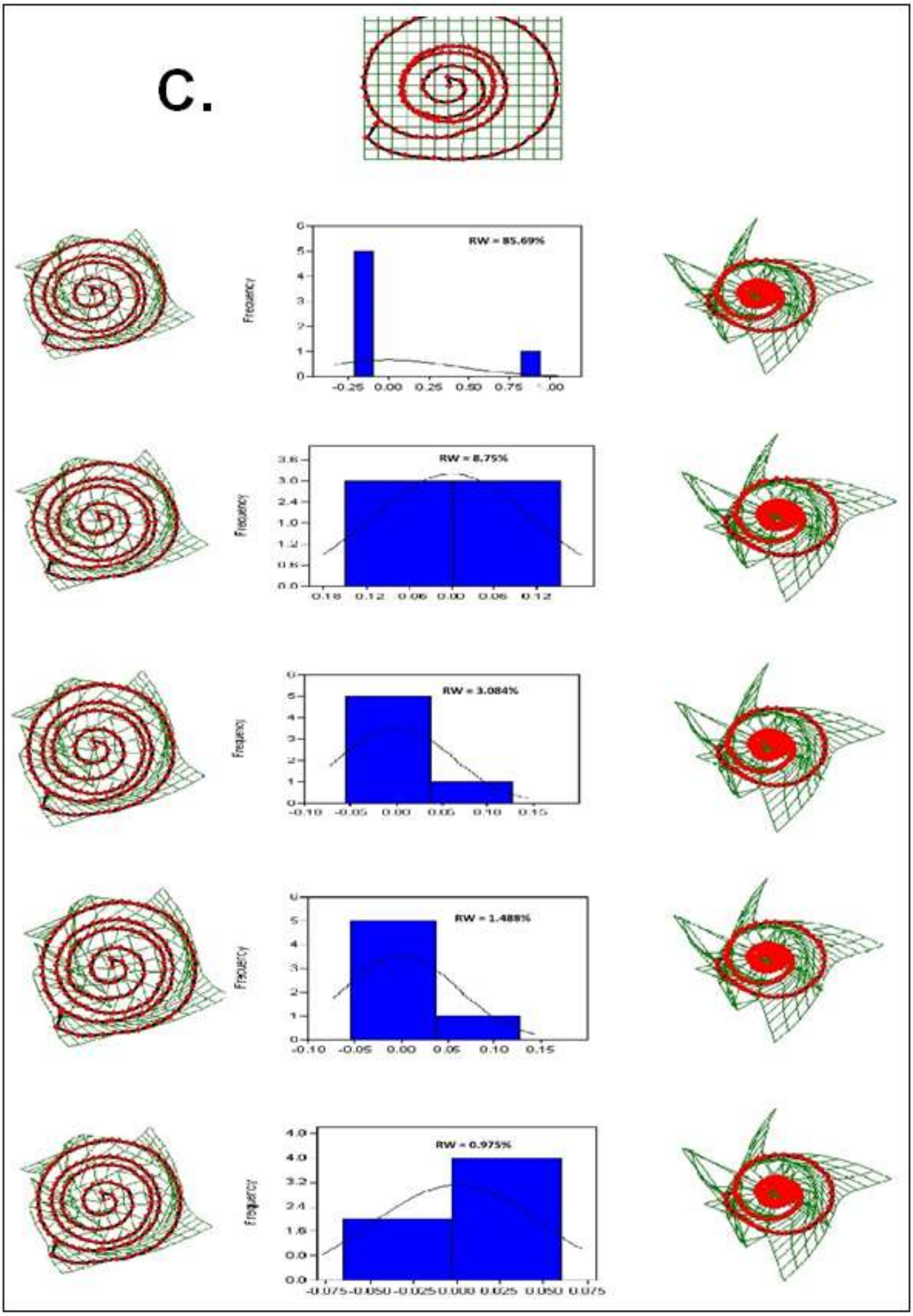


Fig. 4. Relative warp box plot and histogram showing variations in shape of the (a) ventral/aperture, (b) dorsal and (c) whorl segmentation in body shapes from negative extreme (A) towards positive extreme (B) as compared to the mean shape (C).

The patterns of intraspecific variations in the body shapes were summarized through the Relative Warp Scores and its corresponding frequency histograms. Based on the Relative Warp analysis results, the head region and the body depth varies from the top. Furthermore, this study reveals that positive extreme have greater curvature in their head down to its body length compared to negative extreme.

Body shell shape morphology in *F. ater*, it can be classified with its latter region and its enlargement of the size in the head region. Patterns of variation among species may also be symptomatic of processes occurring on several levels. Different proportions of individuals in the populations within the species imply genetic diversity, which in turn has implications for phenotypic diversity. It may also be due to varying strength of predation inducing different ecological and morphological strategies for survival. Autoregulatory developmental processes may also vary from species to species in their ability to buffer against environmental or genetic perturbations (Callier, 2006). Thus, genetic, ecological and developmental sources of phenotypic variation may have contributed to morphological differences within species of freshwater gastropods of the genus Pachychilidae.

Conclusion

The results of this study suggest that Relative Warp Analysis (RWA), Principal Component Analysis (PCA) of *F. ater* mostly differ in the latter part and their body depth. The study of morphological differences in sizes and pattern using the ventral/aperture, dorsal, and top of the whorl characteristics of the shell clearly indicates its shell shape variation. Thus, defining *F. ater* quantitatively was used to show shell shape variation.

Acknowledgement

The researchers would like to express their gratitude to Mindanao State University–General Santos City, College of Natural Sciences and Mathematics, Science Department and more importantly to Mindanao State University-Iligan Institute Technology.

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