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Jhun Joules M. Mahilum
Department of Biological Sciences, College of Science and Mathematics MSU- Iligan Institute of Technology, Iligan City, Philippines

Cesar G. Demayo
Department of Biological Sciences, College of Science and Mathematics MSU- Iligan Institute of Technology, Iligan City, Philippines

Correspondence:
Cesar G. Demayo
Department of Biological Sciences, College of Science and Mathematics MSU-Iligan Institute of Technology, Iligan City, Philippines

Describing Lake Populations of the golden apple snail, *Pomacea canaliculata* using landmark-based geometric morphometric analysis

Jhun Joules M. Mahilum and Cesar G. Demayo

Abstract
This study was conducted to describe the shell shape differences among intra- and inter-lake populations of *P. canaliculata* using the geometric morphometric analysis. The shell shape variation among all populations collected is common in the spire, shell body and apertural area morphology. It was found out that minimal sexual dimorphism was observed on the dorsal portion of the shell where male and female populations tend to group separately. While on the ventral portion, male and female populations grouped uniquely. Results have shown significant variations validated by showing the superimposition with the expansion maps among all populations which showed the occurrence of variation of the both dorsal and ventral portion of the shell. This is either caused by the organism’s phenotypic responses (plasticity) or particularly which act during ontogenetic development or directly affected by the environmental factors.

Keywords: plasticity, morphometrics, phenotypes

1. Introduction

P. canaliculata is believed to be the most variable species among the group Ampullariidae [1] where intra- and inter-populational variations in size and shapes are qualitatively recognizable. Sexual dimorphism in its phenotype are generally based on qualitative descriptions of selected morphological characteristics like the size, shape, shell color and banding pattern and others. Evidently, females are much larger than males [2-5]. The shell of the female adult golden apple snail curves inward and the male shell curves outward [6]. Furthermore, the observed cosmopolitan distribution of the snail is argued to be affected by genetic and environmental factors [7-9]. The species can tolerate wide freshwater environment from canals to rice fields and even to extreme environments with much broader food preference. Samples collected from a wide type of environment were morphologically diverse indicating adaptability and the high phenotypic plasticity caused confusion to its proper identification Intraspecific morphological diversity existing throughout the wide distributions of the species has compounded the problem [5]. Since the observed morphological variation in this species are based on qualitative descriptions, it argued that variations should be further analyzed using more advanced quantitative tools. New developments in imaging, geometry and statistics known as geometric morphometrics are considered useful to further advance quantitative descriptions of morphological structures thus improving our understanding of variations [10]. The use of geometric morphometric analysis to describe shell shape variation has been useful in describing the shell at the population level and between sexes of *P. canaliculata* [11-14]. Since it was argued that the shell shape of this species is influenced genetically and environmentally, variations in morphometric traits may provide information useful in understanding the problematic issues on morphological variation on shell shape especially those that have invaded big lakes. We therefore use the method of geometric morphometrics specifically landmark-based geometric morphometric analysis on the shell shape of *P. canaliculata* collected from different lakes in Mindanao, Philippines. The three lakes were geographically isolated and it is not known how the snails get into these body of water thus this study will provide new information as to how variable the snails are and how different are from the three lakes.

2. Materials and Methods

The samples were obtained from three different lakes in Mindanao, Philippines (Lake Lanao, Lake Dapao and Lake Wood) (Fig. 1). A total of 180 samples were randomly collected, each comprising 30 males and 30 females per lake population. The shells were boiled with water and then cleaned with tap water after the meat was removed with a pin. Shells with cracks and eroded spires were discarded. The Images of the shells were captured using a DSLR camera, Nikon D5100. The shells were oriented is such a way that the spire was at 90° of the x-axis in a 2D orientation with the ventral side of the shell facing the top. All shells were captured in the same position. The camera, with constant 55 mm focal length, was mounted on a tripod to maintain a constant distance from the top of the shell and in order to obtain good images to minimize measurement error.

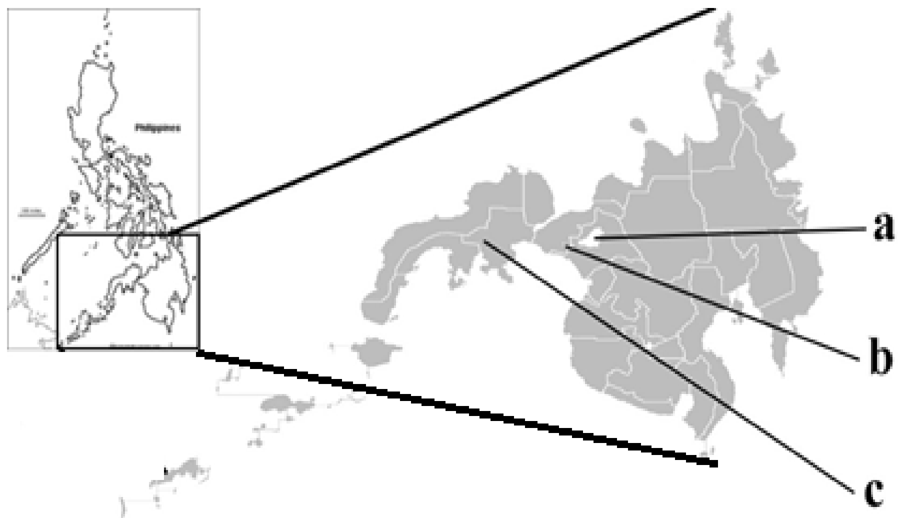

Fig 1: Map locating the study areas (a) Lake Lanao, Lanao del Sur, (b) Lake Dapao, Lanao del Sur and (c) Lakewood, Zamboanga del Sur.

From the acquired shell images, landmark-based methodology was used to study the shell shape of *P. canaliculata* which automatically transformed all 2D coordinates determined for each shell specimen into Procrustes average configuration. This was accomplished using image analysis and processing software Tps Dig freeware 2.12. Tp.Dig facilitated the statistical analysis of landmark data in morphometric by making it easier to collect and maintain landmark data from digitized images [10].

Seventeen (17) anatomical landmarks were selected as previously defined [12], located along the outline of the dorsal (Fig. 2a) portion of the shell and twenty-one anatomical landmarks located along the outline of ventral/apertural (Fig. 2b) portion of the shell were used. The X and Y coordinates were outlined with landmark points along the contour of the shell into the obtained images. Relative warps analysis was performed using tpsRelw version 1.48 [15]. Then, the generalized orthogonal least squares Procrustes average configuration of landmarks was computed using the Generalized Procustes Analysis (GPA) superimposition method. GPA was performed using the software tpsRelw, ver. 1.48 [15]. After GPA, the relative warps (RWs, which are the principal components of the covariance matrix of the partial warp scores) were computed using the unit centroid size as the alignment-scaling method [16-17]. The relative warp scores from 2D coordinates were then transferred to Microsoft Excel application for the organization of the data into groups (based on shell portions and eventually segregated into male and female). To clearly show the visible shell shape variation of the *P. canaliculata*, superimposition and Thin-plate Splines expansion factors were also performed. Visualization grid of mean shell shape on each population was also performed. To further understand and describe the variations reflected on the relative warp analysis and visualization grid, the superimposition and the expansion grid of the shell shape was also shown.

The goal of the superimposition is to position corresponding landmarks as close together as possible and conforms to a common centroid size and then minimizes the sum of squared distances between corresponding landmarks and the consensus (grand mean) via rigid rotation [18].

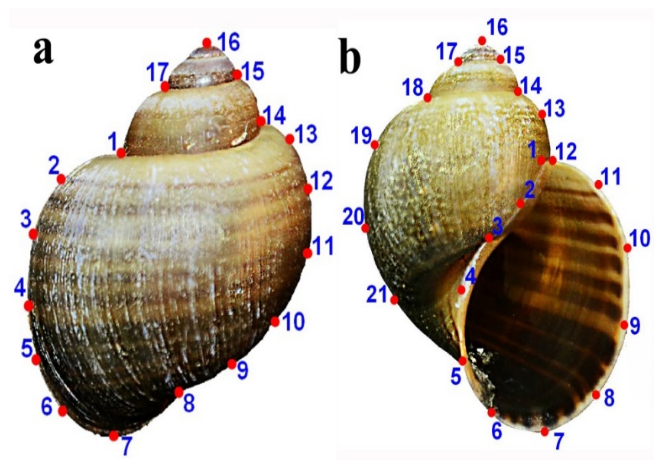

Fig 2: Landmarks used to describe the shape of (a) dorsal and (b) ventral/apertural view of the shell of *P. canaliculata.*

3. Results and Discussion

Figures 3 and 4 shows the superimposition of the landmarks in the dorsal and ventral configuration of the shell and with the expansion grid that detected the areas of the shell which shows the expanding part or compressing part of the shell. For the dorsal configuration, Fig. 3a shows the superimposition of the landmark points for all the shells of individuals in all 3 lake populations. Fig. 3b shows the superimposition of all the 3 consensus shapes of the shell of the 3 lake populations of *P. canaliculata*. The expansion map or "parrot plot" of dorsal configuration of the shell is shown in Fig. 3c, where the color hues from yellow to red indicates expansion of the grid while as the color hues goes close from green to blue indicates compression [19]. It can be seen from the figures that shell shape deformations between lake populations of snail are detectable in the spire and the base part of the shell which appeared to be compressing while the body of the shell appeared to be expanding.

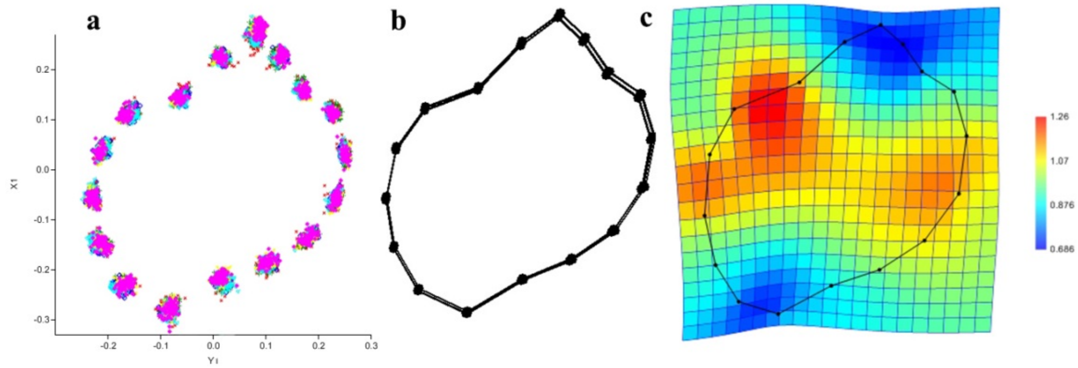

Fig 3: XY graph of the superimposition on the dorsal portion of the shell of *P. canaliculata* showing (a) landmark points superimposition of pooled data, (b) superimposition of the relative warps and (c) expansion map/ "parrot plot" where color hues represent relative proportional variation in areas of the shell (yellow to red: expansions and green to blue: compression).

For the ventral configuration, the XY graph of the superimposition of landmark points (Fig. 4a), relative warps (Fig. 4b), and the expansion map (Fig. 4c) are shown. Fig. 10a shows the superimposition of all the landmark points along the contour of the shell, Fig. 4b shows superimposed consensus shapes of the 3 lake populations, and Fig. 4c shows the expansion map on ventral portion of the shell of *P. canaliculata*. In this configuration, the spire and the basal lip of the aperture of the shell appeared to be compressing while the body of the shell appeared to be expanding. The Inner lip of the shell on the ventral portion appeared to be expanding.

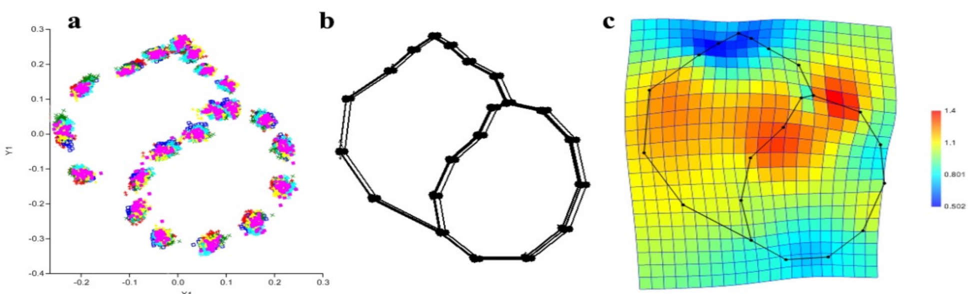

Fig 4: XY graph of the superimposition on the ventral portion of the shell of *P. canaliculata* showing (a) landmark points superimposition of pooled data, (b) superimposition of the relative warps and (c) expansion map/ "parrot plot" where color hues represent relative proportional variation in areas of the shell (yellow to red: expansions and green to blue: compression).

To have a clearer quantitative descriptions of the variations between the three lake populations, a comparison of the consensus shape or mean morphology visualization was done based on the scatter plot generated from CVA (Figures 5 and 6). Visible differences in shell shapes can be observed. While sexual dimorphism within lake populations was visible, geographical differences cannot be ascertained.

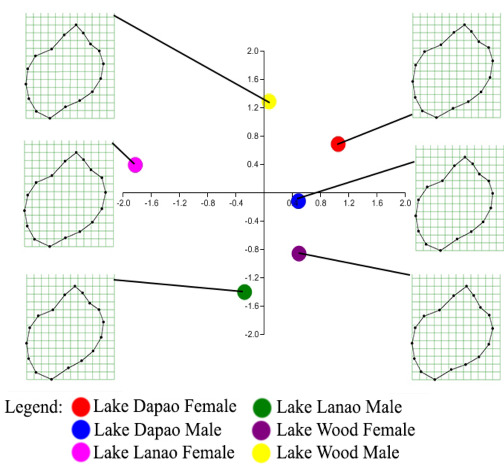

Fig 5: Consensus morphology visualization grid of dorsal portion of *P. canaliculata* shell shape pattern derived from relative warp axes 1 and 2. (a) Lake Dapao Female; (b) Lake Dapao Male; (c) Lake Lanao Female; (d) Lake Lanao Male; (e) Lake Wood Female; (f) Lake Wood Male.

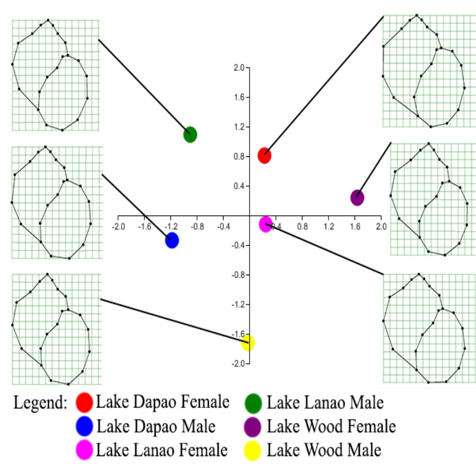

Fig 6: Consensus morphology visualization grid of ventral portion of the *P. canaliculata* shell shape pattern derived from relative warp axes 1 and 2. (a) Lake Dapao Female; (b) Lake Dapao Male; (c) Lake Lanao Female; (d) Lake Lanao Male; (e) Lake Wood Female; (f) Lake Wood Male.

Differences in the three lake populations described as a result of the Relative Warp Analysis (RWA) are presented in Table 1. Five relative warps were found to be significant in describing both dorsal and ventral configuration of the shells. For the dorsal portion, 78% of the total shell shape variations are explained by the 5 significant warps while more than 72% of the total shell shape variation was listed as significant warps for the ventral configuration of the shell. The descriptions of the variations are presented in Table 2. The differences between lake populations are graphically presented in Figure 7. It can be seen from the results that while most variations are observed in the spire and basal and inner lip of the aperture, the extent of differences vary from population to population.

Table 1: Significant relative warps that explain most of the variation observed in the shell shape morphology of *P. canaliculata.*

RW	Dorsal			Ventral		
	Eigenvalue	%	% Variance	Eigenvalue	%	% Variance
1	1.25727	37.94	37.94	0.79080	25.39	25.39
2	0.84961	17.33	55.27	0.66119	17.75	43.13
3	0.71876	12.40	67.67	0.58782	14.03	57.16
4	0.50138	6.03	73.71	0.48533	9.56	66.72
5	0.46069	5.09	78.80	0.39017	6.18	72.90

Table 2: Percentage variance and overall shape variation in the dorsal and ventral shell configurations of *P. canaliculata* as explained by the five significant relative warps.

RW	Dorsal Shell			
	% Variation	Female	% Variation	Male
1	39.98%	Variations are visible at the spire and body of the shell, where shells at negative extremes have pronounced and longer spire with narrow body as compared to the shells at positive extremes with short and wide body.	37.17%	Variations can be observed at the spire and body on the shell. Shells at negative extremes have shorter spire, wide body and longer body height as compared to the shells at positive extremes.
2	16.98%	Shells at negative extremes have pronounced and longer spires with narrow body.	17.23%	At negative extremes, shells have wider body.
3	14.21%	At negative extremes, shells have pronounced and longer spires with narrow body.	11.01%	At negative extremes, Shells have pronounced and longer spire.
4	5.55%	Shells at negative extremes have narrow body.	6.47%	At negative extremes, shells have wider body.
5			5.52%	At negative extremes, Shells have pronounced and longer spire.
RW	**Ventral Shell**			
	% Variation	Female	% Variation	Male
1	24.73%	Variations are visible at the spire and apertural area of the shell. Shells at negative extremes tend to have a shorter spire and wide apertural opening as compared to the shells at the positive extremes.	24.53%	Variations are visible at the spire, body and apertural area of the shell. Shells at the negative extremes have pronounced and longer spires, long body height and narrow apertural opening.
2	22.43%	Shells at negative extremes have a shorter spire and wide apertural opening.	15.53%	At negative extremes, shells have longer spires and narrow apertural opening.
3	13.14%	Shells at negative extremes have a shorter spire, wide apertural opening and long apertural height.	12.89%	At negative extremes, Shells have wider body.
4	10.89%	Shells at negative extremes have wide apertural opening.	9.82%	At positive extremes, shells have wider body and wider apertural opening.
5	5.08%	Shells at negative extremes have wide apertural opening.	7.11%	At negative extremes, Shells have wider body.

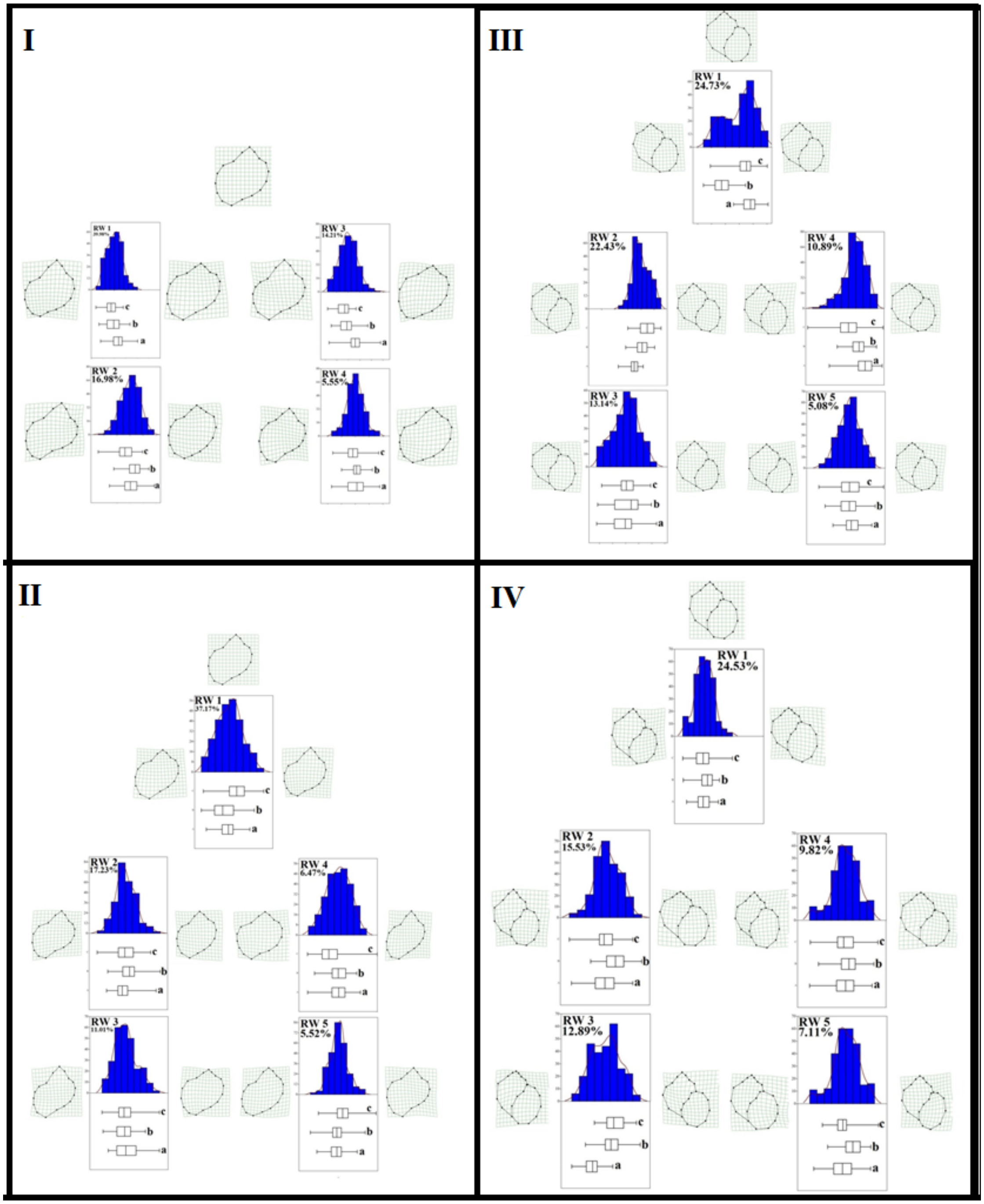

Fig 7: Summary of the geometric morphometric analysis showing the mean shape and variation in dorsal shell in Female (I and II) and male (III and IV) populations of *P. canaliculata* produced by relative warps. (a) Lake Dapao, (b) Lake Lanao and (c) Lake Wood.

The results of the relative warp analysis revealed that the shell shape of the three lake populations of *P. canaliculata* differ based on the extent of compression and expansion of the spire, body and aperture. These can be described using the consensus morphology visualization and superimposition. Relative warp analysis revealed visible morphological variations of the spire, body whorl and apertural area of the shell. Differences between sexes and lake populations include variations in spire length, wide and/or narrow body, wide and/or narrow aperture, and either long or short aperture height. The expansion map show between sex populations of *P. canaliculata* differ suggesting that sexual dimorphism exists but not serve as the only basis for the differences observed between lake populations. Results on the superimposition of the pooled data among populations revealed the differences can be due to compression on the shell's spire, body and aperture. This compression can be attributed to differences in the environment of the lakes such as low temperature. Temperature was argued to be one of the factors that can affect the shell formation and growth. The phenotypic variations in the shape of the shell of *P. canaliculata* as shown in this study continues to be an important area of research in evolutionary biology. While the observed sexual dimorphism within populations be attributed to genetic factors [4], this could not be the only basis for the variations observed between lake populations. It is suggested that the differences can be attributed to variations in the environment where the snails were found which may have acted during ontogenetic development [20].

4. Conclusion

Results of this study have demonstrated that geometric morphometrics especially relative warp analysis and superimposition can be used to quantify phenotypic variation in shell shape morphology of *P. canaliculata*. These variations can be brought about by either possible different environmental factors or as a result of genotypic activity that leads to phenotypic plasticity in this invasive species. It is however recommended that further genetic and ecology studies will be conducted to understand the nature of variations in the organism.

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