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Population Structures of *Vivipara Angularis* Muller from Lake Lanao, Mindanao, Philippines

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ABSTRACT

Native species of freshwater gastropod Vivipara angularis *have been widely distributed along Lake Lanao. The pattern of the morphological shell variation of this indigenous species was analyzed in the four populations sampled along three sites in Lake Lanao and in Balutmasla Island, one of its two islets located centrally in the lake. Variation in the shell outline was assessed using elliptic Fourier analysis of the captured two-dimentional outline of the shell. The results showed that the variations were detected along the spire-body whorl length and its aperture opening. The variations among the populations were due to the variations observed within the populations and these were argued primarily due to the phenotypic plasticity.*

Keywords: EFA, Indigenous Species, Vivipara, Gastropod, PCA

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INTRODUCTION

Vivipara angularis Muller commonly known as pond snail, is a native or indigenous gastropod species in Lake Lanao. It is used as food by some of the community people around the lake. Its shell is also a source of the high quality animal protein, used as calcium in animal formulated feeds, making asthrays, scouring powder, ceramic materials and ornaments [1]. The species shares the same habitat, food sources, calcium availability and spawning sites of the other introduced predatory invasive snails like *Pomacea canaliculata* (Golden apple snail) and *Melanoides tuberculata* [2, 3]. The presence of these two species has been argued to have affected the population structures of the native species. Their presence could have provided pressures on the species' populations that in order for the species to survive, changes in their phenotypes could have occurred. Some studies have shown that for snails, a longer spire in relation to aperture height, a longer shell length than shell width with large operculum serve as the defense mechanism against the predation [4-6], to avoid the dessication [7] and as an offensive weapon or an anchor for the locomotion on the substrate [8]. It has also been hypothesized that the existence of the conchological variation in shapes among the populations can be due to the phenotypic plasticity and allometry as a response to the environmental pressures and predation. It has also been argued that the common patterns of the allometric growth in gastropods that include increased thickness and variations in the apical angle resulting in the doming of the shell are the apparent anti predation adaptations [9, 10]. It has also been possible that the variations in the shell shape may also be due to non-allometric plasticity as a low gene flow may permit the local populations to evolve through the genetic drifts or adaptation to the local conditions. To be able to explore the current populations of the species in the lake, phenotypic variations were evaluated applying the tools of geometric morphometrics specifically Elliptic Fourier analysis. The tools were found to be competent in quantitatively analysing shape variations in organisms notably between the species and populations within the species [11-16].

METHODOLOGY

Lake Lanao (Figure 1) has two islets known as Balt masla and Balt maito, and Balt maito has been estimated to be about 5.3-5.6 million years old [17] and one of the world's 19 ancient lakes. It has been theorized that the lake was formed by the tectonic-volcanic damming of a basin between two mountain ranges, and the collapse of a large volcano[18]. It has a surface area of 35,468 hectares, a maximum depth of 112 meters, and a maximum depth of 60.3 meters. The basin is shallowest towards the north and gets deeper progressively towards the south.

A total of 120 adult shells were collected from the three sites along Lake Lanao namely; Tugaya, Bacolod Grande, Madamba and Balt masla Island (Fig. 2). The samples collected were washed, placed into separate polythene bags, preserved with 70% ethyl alcohol and were brought to the laboratory for further studies. Digital images of the ventral side of the shells were photographed using a DSLR camera. The raw images were subjected to Adobe Photoshop CSS software to obtain the outline of the ventral shape, and they were converted to bitmap files for Shape analysis software that was used in the study. The software package SHAPE v. 1.3 [19] was used in order to analyze the variations in shape by chain coding technique. A one step erosion dilation filter process was then applied in the images to eliminate the undesirable marks termed as "noise". Closed contours of the ventral shapes were obtained from the binary images, then, the contours were chain-coded [20]. Each contour was represented as a sequence of x and y coordinates of the ordered points that were measured counter-clockwise from an arbitrary starting point. The set of the movements depended on the type of contour representation. Chain coder outputs chain code file was analyzed by Che2Nef [21]. Elliptic Fourier transformation suggested by [22] was used to calculate the normalized Elliptic Fourier Descriptors (EFD) obtained from the chain code. EFA decomposed the outline of a species into a series of closed curves (called harmonics). Eight harmonics were sufficient to capture most of the shapes and variances in a specimen as suggested by [23]. Using too few harmonics would result in the loss of morphologic details, and an excessive number can add high-frequency noise to the outline. The first harmonic ellipse obtained from the chain codes of normalization of data was used as a basis which corresponded to the first Fourier approximation, and utilized the 30 harmonics number to be calculated as suggested by [19]. It was the first largest harmonic that could describe the overall length of the specimen, and the other following harmonics provided increasingly detailed information about its complexity.

Fig. 1. Map showing the study area: Tugaya, Bacolod Grande, Madamba and Balt Island (one of the two islets in Lake Lanao).

The Principal Component analysis (PCA) of the variance- covariance matrix [23, 24] was used to summarize the independent shape characteristics. Those PCs of which eigenvalues represented more than the 5% of the total variance were retained [25]. The average plus minus 2 standard deviation (SD) shapes for each site were reconstructed from the mean values of EFDs using the inverse Fourier transformations (provided by SHAPE- Print Print software) done to appreciate the effects of each PC on the ventral shape. Then to justify the results, whether there was statistically significant (ρ same value < 0.05) difference between and among snails, Kruskal-Wallis Test was done using the Paleontological Statistics (PAST) Software version 2.0 [26]. Non-parametric form of the multivariate analysis of the variance (MANOVA), and Canonical Variance Analysis (CVA) were used in order to compare the patterns of the population variation as well as Box plot and XY graph which were also visibly presented to observe the results graphically.

RESULT AND DISCUSSION

Multivariate analysis of variance (MANOVA) of the principal component scores generated from the PCA of Fourier descriptors showed significant differences between the 4 populations of the snails [Wilk's Lambda = 0.4023, df1 = 30, df2 = 303, F = 3.674, and p(same) = 3.569E-09]. The distribution of individuals has been shown in the CVA plot (Fig. 2). It can be seen from the plot that the variations in shell shapes between the populations of the snails were attributed to the variations observed within the populations. To be able to describe the nature of the variations in the shape of the shells within (Table 1) and between the populations (Table 2) based on the significant principal components of the PCA of the normalized Elliptic Fourier descriptors showed how these were reflected in the graphical presentation shown in Figure 2. The box plots shown in Figure 4 have visualized the distribution of the individuals with their corresponding shell shapes.The variations between the shell shapes of the four populations showed Balt Island population differing significantly with the coastal populations of *V. angularis.*

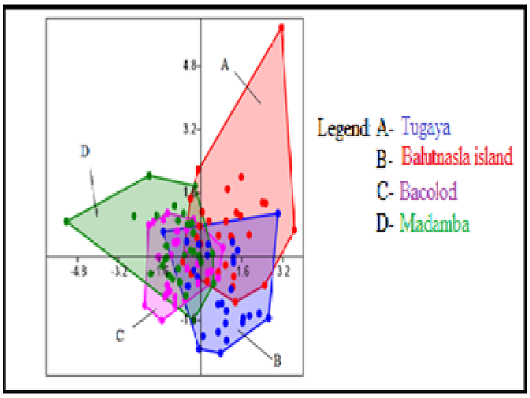

Fig. 2. Distribution of the individuals in a scatter plot generated from the CVA of the landmark scores.

The overall variation in the shell shape was also evaluated by analyzing the PC scores with a nonparametric version, namely the Kruskal-wallis test. The results showed a significant relationship between the four locations and shell shapes. Shell population from Balt Island and Madamba were shown to be the most significantly different as compared to the other populations. It was 83.62% correctly classified as shown in figure 3.

Table 1. The Eigenvalues and percentage variance explained for each significant principal component for the shell variation of *V. angularis* from Balutmasla Island, Tugaya, Bacolod, and Madamba

Principal Component	Eigenvalue	Proportion (%)	Cumulative (%)
A-Tugaya			
1	6.647188 E-00	37.2684	37.2684
2	4.135710 E-00	23.1874	60.4558
3	2.232235 E-00	12.5153	72.9711
4	1.793076 E-00	10.0531	83.0243
B-Balt Island			
1	5.990586 E-00	36.0061	36.0061
2	3.216967 E-00	19.3354	55.3415
3	2.182715 E-00	13.1191	68.4605
4	1.485592 E-00	8.9291	77.3896
C-Bacolod			
1	9.076198 E-00	43.6671	43.6671
2	4.265282 E-00	20.5210	64.1880
3	1.875037 E-00	9.0211	73.2091
4	1.344885 E-00	6.4705	79.6796
D-Madamba			
1	9.839102 E-00	43.8821	43.8821
2	3.233961 E-00	14.4234	58.3054
3	2.560263 E-00	11.4187	69.7241
4	1.560263 E-00	6.5608	76.2849
5	1.471044 E-00	5.3994	81.6843

Table 2. Pooled Eigenvalues and percentage variance explained for each significant principal component of the shell variation of *V. angularis*

Principal Component	Eigenvalue	Proportion (%)	Cumulative (%)
1	7.731281 E-00	35.6389	35.6389
2	3.388980 E-00	15.6222	51.2611
3	2.385517 E-00	10.9965	62.2576
4	2.106344 E-00	9.7096	71.9672
5	1.286539 E-00	5.9306	77.8978

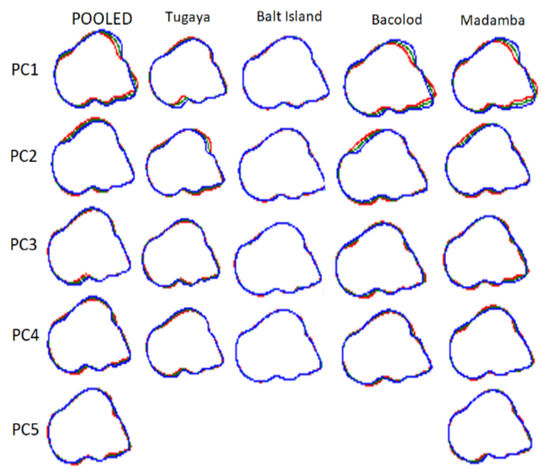

Fig. 3. The summary of the results of the mean shapes of the four pooled populations and their percentage variance explained by each significant principal component of the shell variation of *V. angularis.*

The most common variations in shape observed, which were common to all the populations and described by PC1, were correlated to the differences in the apical whorls (apex- body whorl length) relative to the rest of the shells. The decrease in the height of the apical whorls resulted in the widening of the aperture. These variations could be attributed to allometry and plasticity of the snails. Phenotypic plasticity has been always present in nature as a result of the environmental and ecological interactions in which the amount may differ when exposed to the same environmental changes. However, the environmental changes without genetic changes can create distinct non-genetic changes in the shell morphology. In addition, plasticity also influences the evolution and the adaptive responses of the organisms by altering the relationship between the phenotype, which have been the target of the selection and genotype [27].

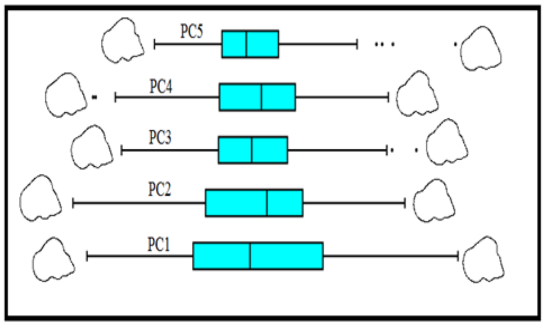

Fig. 4. Box plot comparing the variations between the populations based on the principal component analysis from the four populations of *V. angularis.*

Population within the species from Balt Island was observed to have the least percentage variance or almost a similar shape from spire to aperture opening. The less variation or little difference could be explained by a combination of geographic isolation and possible environmental similarities since the island has been isolated in this isolated lake located between the two towns, Bacolod Grande in one side of the lake, and Binidayan in the opposite side of the lake as seen in Figure 1. The population grown in the same area across the geographic range showed a substantial variation. Moreover, for the population existing in the isolated habitats such as Balt Island, it was expected that the interpopulation shell shape variance in morphology would be much lesser than the intrapopulation variance.

Since the gastropods collected were from sites near each other such as those three coastal populations, they may share the same gene pole, climatic, biotic and abiotic factors. According to [28], the genetic variation may motivate the magnitude and expression of phenotypic plasticity which could be performed by natural selection making it as an adaptation to allow the organisms to compete their phenotype to be better in succeeding the environmental conditions. Shell shape and appearance may change in response to the environment, and the species vary over time because of the genetic changes. Since the water chemistry was closely uniform throughout the lake's shore where the gastropods were collected from its three sites and four sides of Balt Island, they experienced quite similar environmental conditions. Hence, some believed and argued that the lake environment itself was the cause of the phenetic variations of the gastropods. Overall, the results obtained in this study and those observed in other freshwater gastropod species such as in Lake Dapao [2] with less shell variability suggested that the shell morphological differences were an adaptive trait with an important genetic basis and a plastic potential.

It could be seen from the above results that the conchological variations of the shell shape reflected the phenotypic plasticity and maybe the developmental stability [29]. The environmental differences that included availability and concentrations of calcium sources, pH, long-term stability of the habitats, the presence of predators, and the presence of invasive Golden apple snail (GAS) and *M. tuberculata* in the lake may have contributed to the variations observed between the individuals. The two invasive species that were accidentally introduced in the lake may have acted as a competitor of the indigenous or native *V. angularis* in terms of the food sources, calcium availability, pH, habitat and spawning sites [30]. The amount of calcium in the habitat of the gastropod shell greatly affected its morphology. The low calcium restricted species diversity, controlled the growth rate, and increased the vulnerability to predation. Thus, calcium as one of the environmental factor has been needed physiologically by Vivipara snails for the repair and construction of their shells. Moreover, as cited by [31], the aquatic molluscs have been known to achieve calcium requirements by the absorption of calcium from the external medium, although calcium existed in the food was also consumed.

CONCLUSION

The study indicated that phenotypic plasticity has been widespread in aquatic gastropods. The results showed that the conchological variability of the shell shape could be due to the phenotypic plasticity and that a genetic component of its shell morphology existed, their shell characteristics changed through the ontogeny according to the environmental conditions since this lake has been isolated for many decades. The differences could also be attributed to allometry, climatic, biotic and abiotic factors.

The study further revealed that Elliptic Fourier Analysis could be applied for determining the shell shape of the gastropods. It was efficient in capturing the complete shell shape even when few or no landmarks were available, and it could delineate any type of shape with a closed two-dimensional contour.

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