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Relative Warp Analysis of Shell Shapes of *Vivipara Angularis* Muller Collected from Four Lakes in Mindanao, Philippines

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ABSTRACT

Studying variation and phenotypic plasticity in the shell shape of Vivipara angularis *Muller, a native freshwater species from lakes, has been interesting in order to understand the evolutionary processes in fragile environments. In this study, the population structures of the snails were investigated based on their shell shapes. The snails were collected in Lake Apo in Bukidnon, Lake Wood and Dasay in Zamboanga and Lake Holon, South Cotabato, Philippines. Geometric morphometric tools such as relative warps, superimposition, thin-plate splines and cluster analysis were employed. The results revealed significant variations in shell shapes among V. angularis populations from different lakes. Most variations in the shells were detected in the spire, body whorl and inner lip of the aperture which indicated that the degree of dissimilarity varied from population to population. Meanwhile, Lakes Apo, Dasay and Wood shared the most similarities. Significant differences in shell shapes were observed in lake populations, but what was significant in this study was that the variations could not be attributed to the geographic distances between the lakes but maybe to the ecological and environmental differences between the lakes resulting in the developmental modifications of the shell shapes.*

Keywords: Geographic Distance, Predation, Similarities, Thin-Plate Spines, Viviparid, Vivipara Angularis

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INTRODUCTION

A better way to understand the evolutionary processes in the fragile environments such as lakes is understanding the ecological and geographical interactions using morphology [1]. The variations in morphological characters within and among the populations may describe different evolutionary units over the geographic spaces [2] and looking through the geographic variation in the process of evolutionary divergence may be a breakthrough to observe the course of speciation in action [3] or the geographic isolation may lead to a major cause of the evolution of new taxa [4].

It has also been argued that the documentation of the inter-populational and intraspecific variation is significant to further understand the impacts of the environmental factors such as those on native species in tectonic and crater lakes found geographically distant from one another. Since many lakes may also be affected by the agricultural or anthropogenic environmental alterations and climate change, these may also affect the structure of the populations based on their phenotypes. For gastropods, the shell shapes provided important information in their systematics and taxonomy especially variations based on the geographical locations. It was on this premise that this study was conducted to determine both intra- and interpopulation variations in shell shapes using the tools of geometric morphometrics (GM). GM has been recognized reliable to assess the interspecific variations in form, particularly for taxon whose systematics has proven to be challenging [5-9].

MATERIALS AND METHODS

Study Area

The study area comprised of six different geographically isolated lakes in Mindanao, Philippines (Fig. 1). These lakes were either described as tectonic or crater (Table 1). The surface areas and elevation including location and distance between lakes were also presented.

Collection and Processing of Samples

A total of thirty (30) mature *Vivipara angularis* individuals were obtained from each lake in the littoral zone through the opportunistic sampling. 2D images of the shells were obtained by individually photographing using a Canon 70D digital single lens reflex camera. The camera was set in a constant focal length and mounted on a tripod to maintain a constant distance from the top of the shell in order to obtain good images to minimize the measurement error. The photographs of the ventral view were taken wherein the apex was visible. Then, the obtained images were subjected to the geometric morphometric methods.

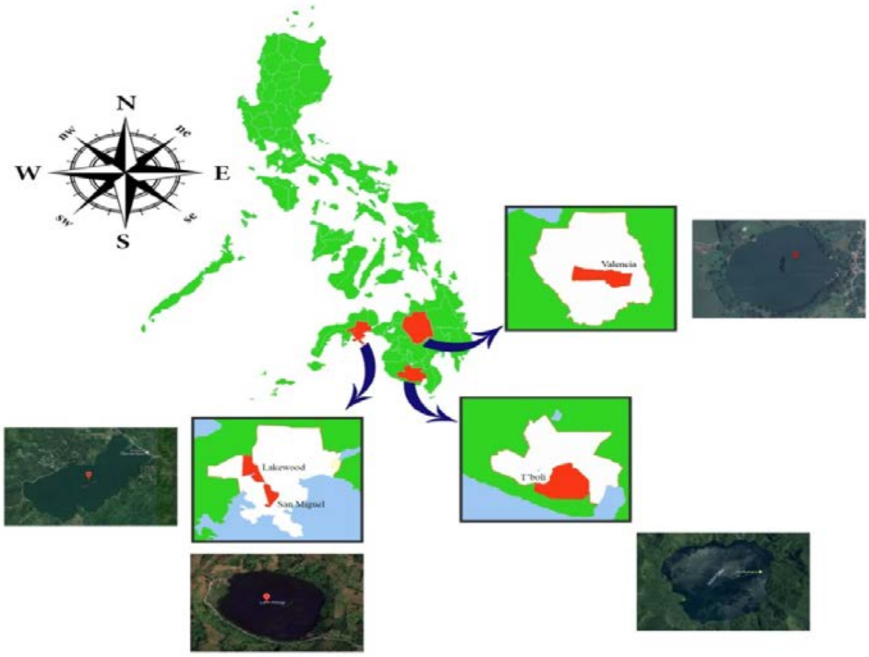

Fig. 1. Map of the Phillipines showing the locations of the four lakes.

Table 1. Characteristics of the four Lakes showing variations in surface area, elevation and distances between them

Lake	Type of Lake	Surface Area (ha)	Surface Elevation (m)	Coordinates
Apo	Crater	24	640	7°52′45″N; 125°0′21″E
Wood	Tectonic	738	320	7°50′36″N;123°9′47″E
Dasay	Tectonic	40	230	7°39′43″N;123°15′11″E
Holon	Crater	317	1,338	6°6′5″N;124°53′20″E
		Distance (km)		**Distance (km)**
Apo ← → Wood		362	Wood ← → Dasay	65.9
Apo ← → Dasay		348	Wood ← → Holon	408
Apo ← → Holon		321	Holon ← → Dasay	394

Landmark Selection, Digitization and Analysis

Twenty (20) anatomical landmarks along the outline of the ventral (Fig. 2) image of the shell were identified in order to get the x and y coordinates. This was done using TpsDig, an image analysis and processing software [10]. To organize the data into the populations, the two-dimensional coordinates of the landmarks for each shell specimen were moved to Microsoft Excel application. Using the Generalized Procrustes Analysis (GPA) superimposition method through tpsRelw software [11], generalized orthogonal least squares and Procrustes average configuration of the landmarks were computed. The generated relative warps were computed using the unit centroid size as the alignment-scaling method. Using PAST software [12], histogram and box plots were made from the relative warps of the shell shapes. Kruskal-Wallis test was used to analyze whether or not the species differ significantly with regards to their shell shapes. Meanwhile, to compare the patterns of population variation, Canonical Variance Analysis (CVA) was also completed.

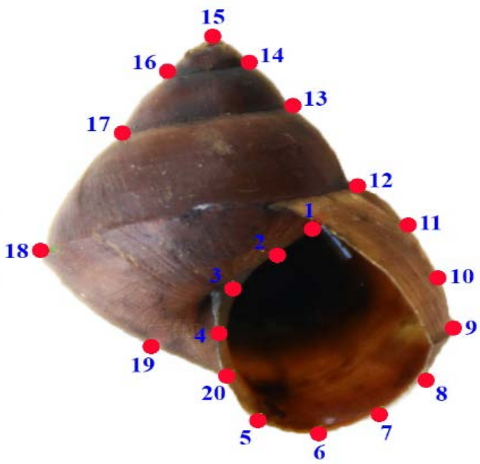

Fig. 2. Landmarks of the shells used in characterizing and describing shapes.

Superimposition and Thin-plate Splines expansion factors were also performed to show the visible shell shape variation of the *V. angularis* and further understand and describe the variations reflected in the relative warp analysis and visualization grid. The goal of the superimposition was to position corresponding landmarks as close together as possible, and conform to a common centroid size and then minimize the sum of squared distances between corresponding landmarks and the consensus via rigid rotation [13]. Cluster analysis was done to determine the degree of similarity between lake populations based on the RW scores.

RESULTS AND DISCUSSION

Canonical variate analysis (P≥4.941E-324) showed significant variations in shell shapes among the lake populations of *V. angularis*. The regions in the shells where the variations can be observed have been shown in Fig. 3b. The differences between the lake populations of the snails have been likewise graphically presented in Fig. 4 as a result of the cluster analysis of RW scores. The interesting thing about the results was that the geographic distance was not a factor for the differences between the populations. Lake Lakewood and Lake Dasay were geographically close but the shapes of the shells were significantly different, while Lake Holon and Lake Apo which were geographically distant had shells that were not significantly different from each other.

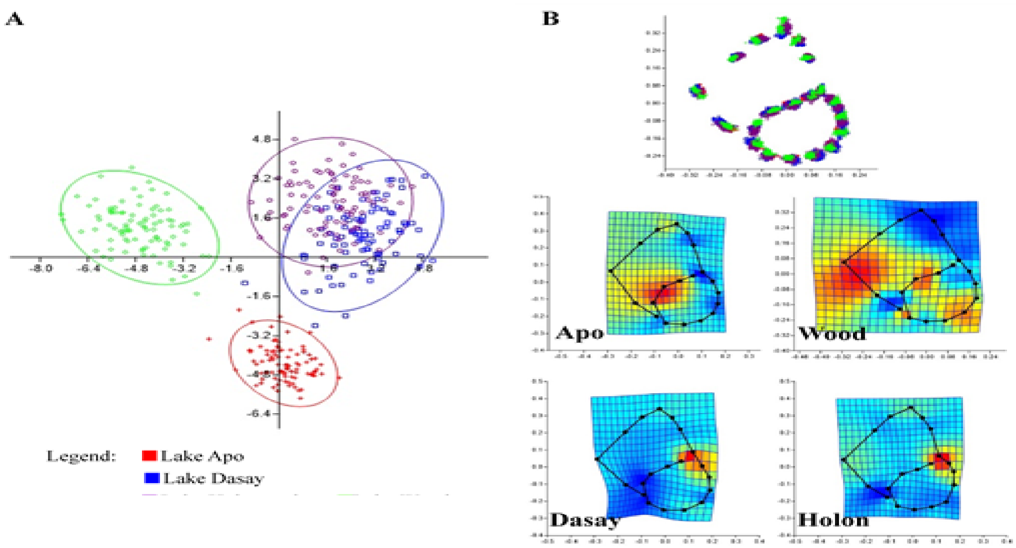

Fig. 3. (A) CVA scatter plot showing the distribution of individuals within and between the populations of the snails based on shell shapes and (B) a shape map showing the areas where the variations in shapes were determined.

The results of the current study were in contrast with that of [14] where they argued that the closer the geographic distance, the more populations tend to have a shared genetic similarity. Their study was focused on the populations within the same lakes but differed only in distance, thus the results could be due to the gene flow. The current study was done among the lake populations that were separated by land thus, the migration of the species would be impossible. The inter- and intrapopulational variability observed in the shell morphological shapes maybe the result of the disparity of responses from the ecological, environmental and biotic alterations within each lake [15], and the strong relation to a wide range of the preferred habitat characteristics, and the changing strength of the predation prompting different ecological and morphological approaches for survival [16].

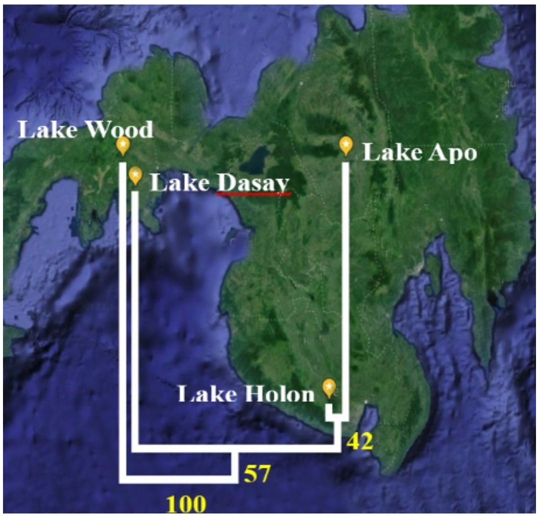

Fig. 4. Cluster analysis showing the relationships of the populations based on RW scores.

CONCLUSION

The results of the study demonstrated variations in the shape of the shells of *V. angularis* mostly in the spire, aperture and body whorl. Significant differences in the shell shapes were observed in the lake populations, but what was significant in this study was that the variations could not be attributed to the geographic distances between the lakes, but could be attributed to the ecological and environmental differences between the lakes resulting to the developmental modifications of the shell shapes.

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