

Landmark-based geometric analysis in describing the shell of the freshwater gastropod *Vivipara angularis* (Gastropoda: Viviparidae) from Lake Dapao, Pualas, Lanao del Sur, Mindanao, Philippines

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Abstract. This study was conducted to describe the shell shapes of *Vivipara angularis* collected from four different locations in Lake Dapao, one of the deepest lakes in Mindanao, Philippines. Geometric morphometric tools specifically relative warp (RW) and euclidean distance matrix analysis (EDMA) were used to describe the shapes. Results showed there was no population differentiation in the snail as the shell shapes in all four populations were concentrated towards the means shape revealed by relative warp analysis. A closer look at variations in shell shapes based on the results of EDMA revealed that individual variations were due to differences in selected interlandmark distances observed along the spire, body whorl and aperture of the shell. Observed variations in shell shapes can be argued to be due to ecological factors, developmental differences and/or maybe environmentally induced.

Key Words: EDMA, PCA, relative warp analysis, histogram.

Introduction. Lake Dapao is one of the deepest lakes in the Philippines (BIMP-EAGA 1997) and second largest lake in the Province of Lanao del Sur, as old as Lake Lanao (Figure 1). The area is located 7°48" North latitude and 124°03" East longitude. The pH value ranges from 6.5-6.7, and the dissolved oxygen level from 7.8 to 8.2 p.p.m. (Haribon Foundation 2012). This lake is an isolated and restricted body of water where *Vivipara angularis*, one of the most common and widely distributed freshwater gastropod can be found. Populations of the snail inhabit the lake's vicinity and are also used as food by some of the local residents in the area. At present however, an introduced species known worldwide as *Pomacea canaliculata* was found to be increasingly populating the lake. It is hypothesized that the population structures of freshwater snails around the lake might be affected by the introduction of the invasive *P. canaliculata* since they out-compete the native species populations for calcium resources, habitat, and spawning sites. To be able to test this hypothesis, selected populations were analyzed by describing morphological shapes of the shell using geometric morphometric methods, specifically landmark-based analysis to capture shape variation in shells (Torres et al 2011; Moneva et al 2012a). Similar study of the patterns of variation within populations of this species was analyzed from another lake using relative warps and it was observed to show significant shell shape variations (Moneva et al 2012b). Studies have shown that within species variations could be due to environmental differences like long-term stability of habitats (such as lakes vs. farms), the availability and concentrations of calcium sources in the area, and also the presence of predators. For example, intraspecific differences of the shell shape of *Viviparus georgianus* species complex were argued to be possibly due

to environmental factors (Katoh & Foltz 1994). In a study of the shell morphology of the African freshwater snail, *Biomphalaria pfeifferi*, shape differences is affected by ecological factors, such as the stability of the African Great Lakes over a long period of time, since populations in these lakes tend to develop their own characteristics, such as blunt keels and deflected apertures (Dupouy et al 1993). Furthermore, studies on individual variation in the shell shape of *Physa gyrina* showed these are affected by the presence of predators in local populations (Dewitt et al 1999). Studying the viviparid snail *V. angularis* in another lake such as the old deep Lake Dapao is interesting since the lake is quite stable unlike the nearby big Lake Lanao where an earlier study of the species was conducted (Moneva et al 2012b). This lake is not greatly disturbed by anthropogenic factors since there are a few human settlements in the area. Only possible competition for resources such as calcium by the introduced *P. canaliculata* and their uses as food by some of the community people the lake may have affected the population structures of this species. In the current study of the population structure of *V. angularis*, quantitative description of the shape of the shell using relative warp analysis in *P. canaliculata* (Torres et al 2011) and in *V. angularis* from Lake Lanao (Moneva et al 2010b) was used including another geometric morphometric tool the Euclidean Distance Matrix Analysis (EDMA).

Material and Method. Populations of *V. angularis* were collected from four locations surrounding Lake Dapao namely, Yaran, Apo, Tanaon and Imbang in December, 2012 (Figure 1). Images of four hundred forty (440) shells were captured using a digital camera. Care was taken to align the plane of the aperture parallel to the work surface. The shells were positioned in such a way that the columella is at 90° of the x-axis in the aperture view and in the orientation in which the apex is visible. About two to three images were captured for each shell and the best images were used in the analyses. The digitized images were then save in TPS file and then subjected to a landmark acquisition to established x and y coordinates of landmarks. Landmarks are often referred to as "homologous points" that are structurally consistent loci which can have evolutionary, ontogenetic, and/or functional significance, and must be consistently present on all forms under consideration in order to be useful in analysis (Lele & Richtsmeier 1991). Fixed anatomical landmarks and sliding landmarks describing the curvature on the shell were applied to capture variation in the shell shape. The shell shape studied using landmark-based methodology eliminates the effect of variation in the location, orientation, and scale of the specimen.

A total of twenty three (23) anatomical landmarks located along the outline of the ventral/aperture portion (Figure 2) were defined and used. The chosen landmarks are described in Table 1.

Table 1

Descriptions of the anatomical landmark (LM) points in *V. angularis*

Landmark no.	Description of landmarks
1-12	LMs located in the aperture margin
13-14	LMs which was the most external point on the last whorl at the right profile of the body whorl of the shell
15	LM at the right border of the profile of the shell at the end of the upper suture of the penultimate whorl
16	LM at the right border of the profile of the shell at the end of the upper suture of the second penultimate whorl
17	Apex of the shell
18	LM at the left border of the profile of the shell at the end of the upper suture of the second penultimate whorl
19	LM at the left border of the profile of the shell at the end of the upper suture of the penultimate whorl
20-21	LMs which was the most external point on the last whorl at the left profile of the body whorl of the shell
22	LM with a line from LM4 below the most external point of the shell
23	LM with a line from LM5 touching the aperture margin

The landmarks digitized using the TpsDig ver.2 (Rohlf 2004a) were subjected to superimposition or procrustes fitting using the Generalized Procrustes Analysis (GPA) in TpsRelw, ver. 1.41 (Rohlf 2004b). Procrustes-based analyses shows the variation of the entire shapes. With the removal of size and coordinate system, shape is being measured as displacement in each landmark relative to all other landmarks. This procedure was used to standardize the size of the structure and optimize their rotation and translation to ensure minimal distances between corresponding landmarks (Bookstein 1991; Dryden & Mardia 1998; Zelditch et al 2004).

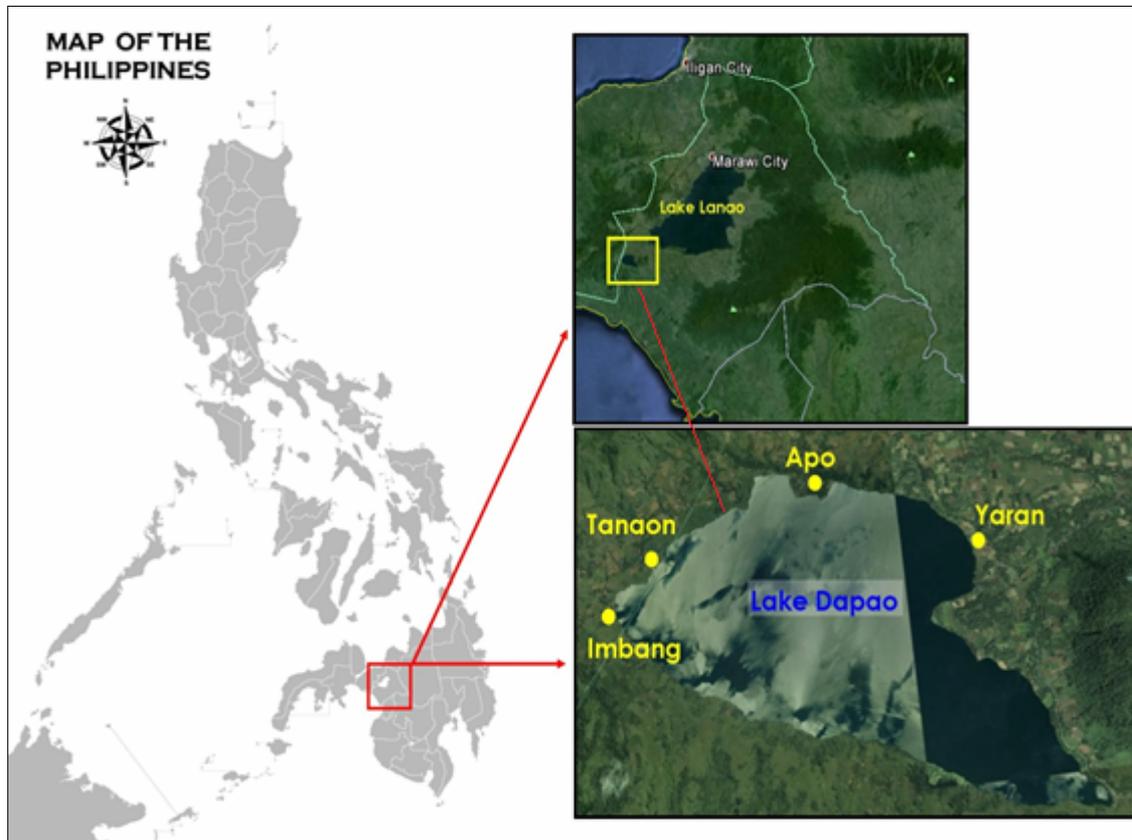


Figure 1. Map of Lake Dapao showing the study area (Source: <http://maps.google.com/maps?hl=en>. Retrieved: Sept.20, 2013).

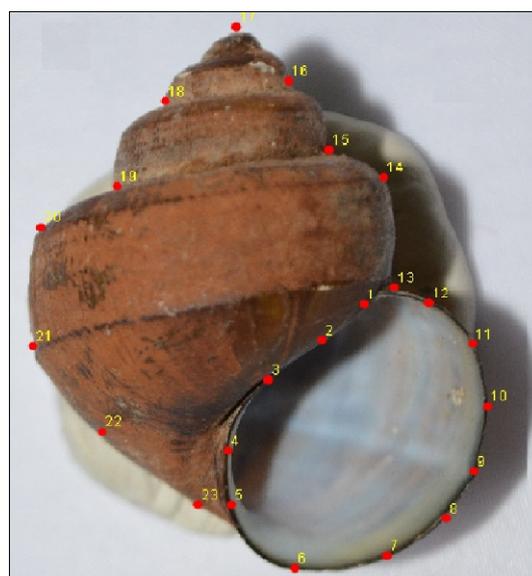


Figure 2. Ventral view of the snail showing assigned landmarks in *V. angularis*.

The data obtained from procrustes fitting or superimposition method were then analyzed using the different geometric morphometric methods: Relative Warp (RW) and EDMA.

After GPA, the relative warps, which are the principal components of the covariance matrix of the partial warp scores are computed using the unit centroid size as the alignment-scaling method. The primary strength of relative warp analysis is its unique ability to provide both a descriptive and a quantitative description of shape. Deformation grids using thin-plate splines were used to graphically portray the patterns of shape variation among the landmarks. Histogram and box plots were generated using PAST software (Hammer et al 2001) from the relative warps of the shell shapes. Histogram and box plots are a powerful display for comparing distributions and were previously used in the study of this species from another lake (Moneva et al 2012b). They provide a compact view of where the data are centered and how they are distributed over the range of the variable. The shape variables obtained as partial warp scores from the aligned landmark configuration produced by GPA are suitably employed in traditional multivariate statistical analyses of variation in shape (Adams et al 2004) and were used to ordinate the data in a principal component analysis (PCA). This analysis involves a simple reduction of the shape and size variables to only two variables (two most common components), for plotting and clustering purposes. It also displays the relative effects of the most important sources of shape variation (Moneva et al 2012a). The first principal component will explain more of the shape variation than any other component (Hammer et al 2002).

This analysis above perform better when coupled with EDMA, which is a linear distance-based method which compares linear distances that connect landmark pairs in one form with corresponding linear distances in another form and provide information pertaining to the difference in length of these linear distances. Results from EDMA are based solely on what can be known about the form of an object and the difference in form between objects, and takes into account the non-estimability of rotation and translation parameters. The form of matrix does not change, no matter where the form is or how it is oriented (Lele & Richtsmeier 1991; Lynch et al 1996). Figure 3 shows the interlandmark distances of the snail of its ventral orientation.

Kruskal-wallis test, a nonparametric test was also used to compare independent groups of simple data and in this case determine the significance of difference (at 0.0.5 level of significance) in the shape variation of shells (Anies et al 2013).

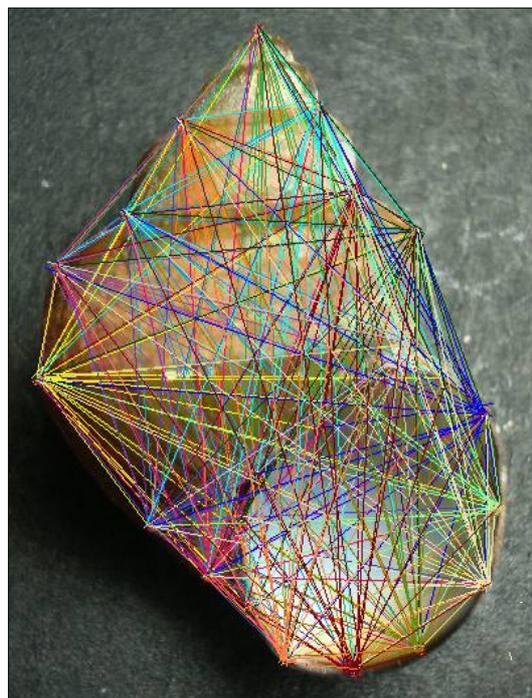


Figure 3. EDMA interlandmark distances of the ventral orientation of *V. angularis*.

Results and Discussion. A summary of the variations in shell shapes as well as consensus morphology in *V. angularis* is shown in Figure 4. More than 47% of the overall variation is associated with differences in the shell aperture. The first relative warp (RW1) explains 28.57% of the total variation observed, describing difference in the shape of the shell's apertural opening and size. Shells with low negative RW1 score have narrow shell opening and larger size relative to the body whorl shell shape. On the other hand, those with high positive scores along the first relative warp axis have wider shell opening and small size based relative to the shell shape. The second relative warp (RW2), explains 19.39% of the total variance in shape shown by observed differences in the height of the spire located in the apical portion of the shell. Shell samples found along the positive axis have a highly elevated spire compared to samples along the negative axis which showed a shorter spire. The third relative warp (RW3) explains 13.19% of the total variation describing differences in the shape of the margin of the lip. Those with negative scores have shells with broader opening and the anterior portion of the inner lip is showing depression towards the center of the shell opening. Shells with a positive RW3 score has shorter and narrow shell opening. Lastly, the fourth and fifth relative warps (RW4 and RW5) accounts for 6.69% and 5.45% of the total variation in shape space, respectively. Shell samples with low negative RW4 and RW5 scores describes those shells with broader body whorl in contrast with those having higher positive scores where the shells have narrower body whorl. It is important to note however that while individual variations were observed in the spire, body whorl and the apertural region of the shells, these variations were not enough to differentiate between those collected from different locations. Results of the relative warp analysis show that the four populations assume the average ventral/apertural shell shape (Figure 4). The pattern of variation observed within populations was similar to all the populations investigated. Results of the plot shows a leptokurtic curve which is an indicative that the populations have low morphological variability.

To further describe the variations in the shell shapes of *V. angularis*, the calculated distances between landmarks following EDMA, were then subjected to PCA analysis. Table 2 shows the result of the PCA analysis featuring the eigenvalues and the percentage variance of the significant components. Only the first component was considered significant, since the eigenvalue of the second component is below the Jolliffe cut-off score and which was used to determine the interlandmark distances that were considered significant (Table 3).

Table 2
Variance associated with most significant principal components in *V. angularis*

<i>Principal component</i>	<i>Eigenvalue</i>	<i>% Variance</i>
1	948796	85.501
2	81725.3	7.3647

Jolliffe cut-off: 1.689E0

Only 19 out of the 276 interlandmark distances were found significant to describe the variations between shell shapes of the snails (Table 3 and Figures 5 and 6). Landmark 17 corresponds to the apex/tip portion of the snail while the other landmarks correspond to the aperture. It can be seen from the results that variations in shells can be attributed to differences in the sizes of the spires as well as the apices, the body whorls and the aperture. Comparing variations between populations of *V. angularis* show that Apo and Imbang were similar but Imbang and Yaran showed some differences in 2 to 3 other populations in selected interlandmark distances (Table 4).

Table 3

Identified significant interlandmark distances and their corresponding PCA loading values

<i>Interlandmark distances</i>	<i>Values</i>
7-17	0.1205
8-17	0.1201
6-17	0.1152
9-17	0.1139
8-18	0.1108
7-18	0.1088
9-18	0.1072
7-16	0.1065
8-16	0.1047
9-20	0.1044
8-20	0.1031
10-17	0.1026
6-16	0.1024
5-17	0.1023
6-18	0.1021
8-19	0.1017
17-23	0.1013
10-20	0.1013
9-19	0.1007

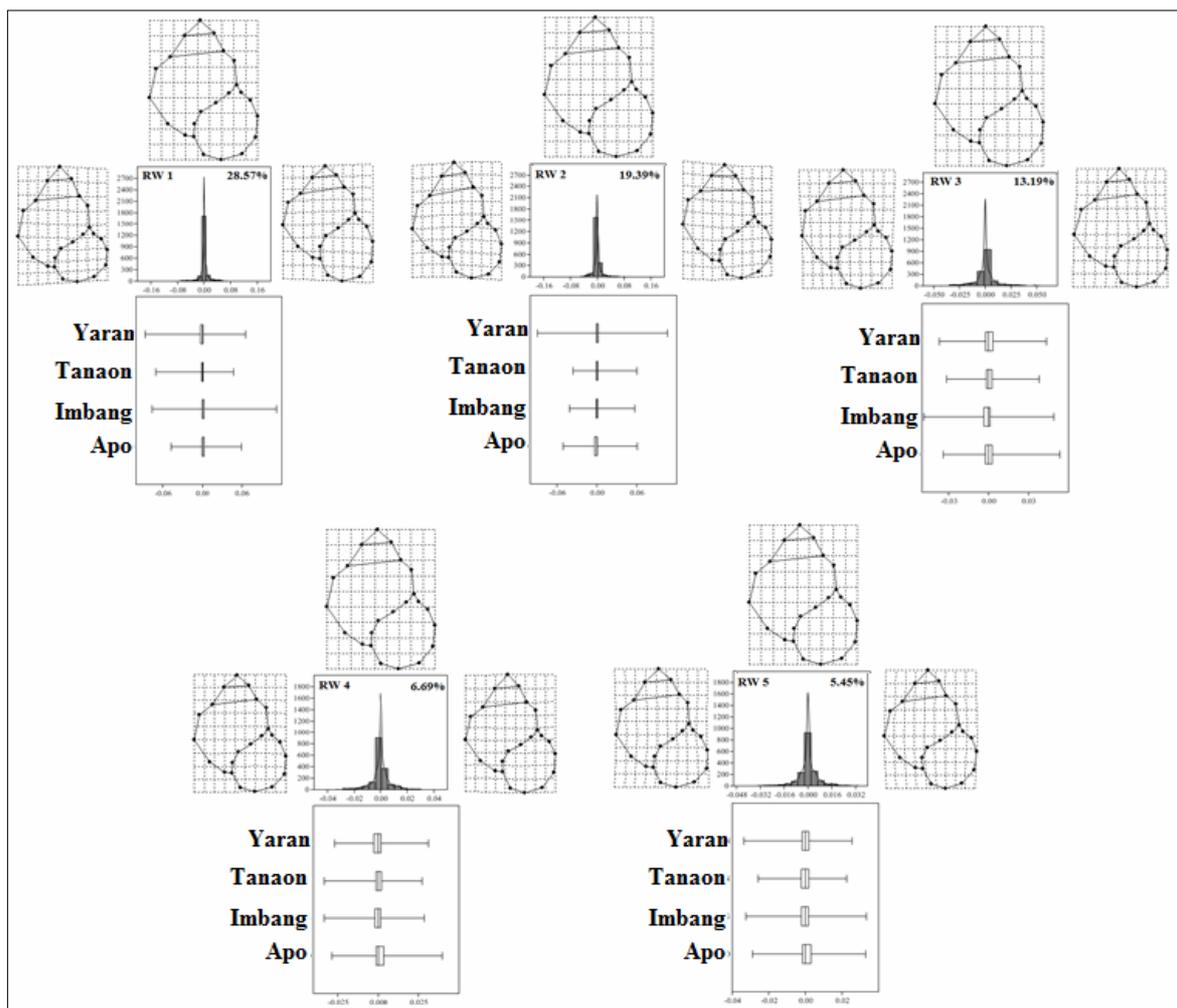


Figure 4. Box plots and histograms showing variations in the shape of ventral/aperture portion of shell of *V. angularis* in Apo, Imbang, Tanaon and Yaran based on the results of RWA.

Table 4

Pairwise comparison showing differences in the linear morphometries of the shells of *V. angularis*

<i>Landmark Distance</i>	<i>Population</i>	<i>Imbang</i>	<i>Tanaon</i>	<i>Yaran</i>
7-17	Apo	0.558	0.126	0.003
	Imbang		0.336	0.000
	Tanaon			0.000
8-17	Yaran			
	Apo	0.430	0.036	0.022
	Imbang		0.166	0.002
6-17	Tanaon			0.000
	Yaran			
	Apo	0.731	0.339	0.001
9-17	Imbang		0.508	0.000
	Tanaon			0.000
	Yaran			
8-18	Apo	0.241	0.002	0.259
	Imbang		0.066	0.023
	Tanaon			0.000
7-18	Yaran			
	Apo	0.266	0.003	0.236
	Imbang		0.066	0.023
9-18	Tanaon			0.000
	Yaran			
	Apo	0.594	0.335	0.001
7-16	Imbang		0.611	0.000
	Tanaon			0.000
	Yaran			0.000
8-16	Apo	0.263	0.011	0.131
	Imbang		0.143	0.011
	Tanaon			0.000
9-20	Yaran			
	Apo	0.450	0.001	0.002
	Imbang		0.000	0.000
8-20	Tanaon			0.857
	Yaran			
	Apo	0.419	0.048	0.023
10-17	Imbang		0.219	0.003
	Tanaon			0.003
	Yaran			0.000
6-16	Apo	0.313	0.003	0.138
	Imbang		0.000	0.003
	Tanaon			0.000
5-17	Yaran			0.056
	Apo	0.650	0.560	0.000
	Imbang		0.835	0.000
6-18	Tanaon			0.000
	Yaran			0.000
	Apo	0.320	0.003	0.720
8-19	Imbang		0.039	0.165
	Tanaon			0.000
	Yaran			
17-23	Apo	0.613	0.001	0.000
	Imbang		0.000	0.000
	Tanaon			0.652
8-17	Yaran			
	Apo	0.513	0.072	0.016
	Imbang		0.223	0.002
6-18	Tanaon			0.000
	Yaran			
	Apo	0.844	0.000	0.000
8-19	Imbang		0.000	0.000
	Tanaon			0.591
	Yaran			
17-23	Apo	0.521	0.263	0.001
	Imbang		0.584	0.000
	Tanaon			0.000
7-17	Yaran			
	Apo	0.209	0.007	0.007
	Imbang		0.000	0.000
	Tanaon			0.880

10-20	Yaran	0.503		0.146	
	Apo		0.045		
	Imbang		0.161		0.038
	Tanaon				0.000
9-19	Yaran	0.271		0.001	
	Apo		0.015		
	Imbang		0.000		0.000
	Tanaon				0.158
	Yaran				

Note: Underlined and in bold - significant at $\alpha < 0.05$.

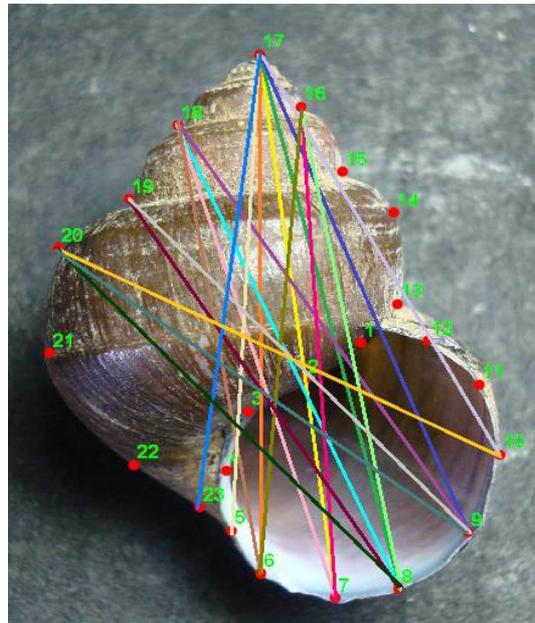


Figure 5. Identified significant interlandmark distances in *V. angularis*.

Results of this study have shown that while there are differences in some populations when compared to others based on selected interlandmark distances, relative warp analysis revealed that the shape of the shells of *V. angularis* are concentrated towards the mean shape. Individual variations can be attributed to a lot of factors that may include the ecological, genetic, and developmental variations. While it can be hypothesized that the snails from the different populations were from the same stock, observations show some of the snails collected showed erosion of the spire top which can be probably due to the low pH and low calcium concentrations (Jokinen 1982). Calcium is important environmental factor for the physiological needs of viviparid snails because it is necessary for the shell construction and repair. Variation in shell shapes might also be due to developmental or environmental effects. According to Krist (2002), changes in the freshwater snails are often accompanied by a change in spire height and aperture size; high narrow spires and small aperture may reduce predation in headwaters while low spires and large aperture may reduce dislodging and damage during tumbling (Haase 2003). Moreover, it is argued that species with high spire are able to burrow at soft substrate and could survive periods of droughts for months because they are hidden in the bottom of the mud. Differences in the shape of the shell opening or aperture could also be attributed to predatory defense of the snail. The aperture is the best way to prevent shell entry as frequently performed by predators such as decapods (DeWitt et al 2000). Narrow apertures are a potentially important defense in freshwater whereas, wider aperture indicates vulnerability to predation (Williams 2005). The predators (includes the indigenous and invasive species in the lake such as the Golden Apple snail *P. canaliculata*) affect the apertural shape of the freshwater snails.

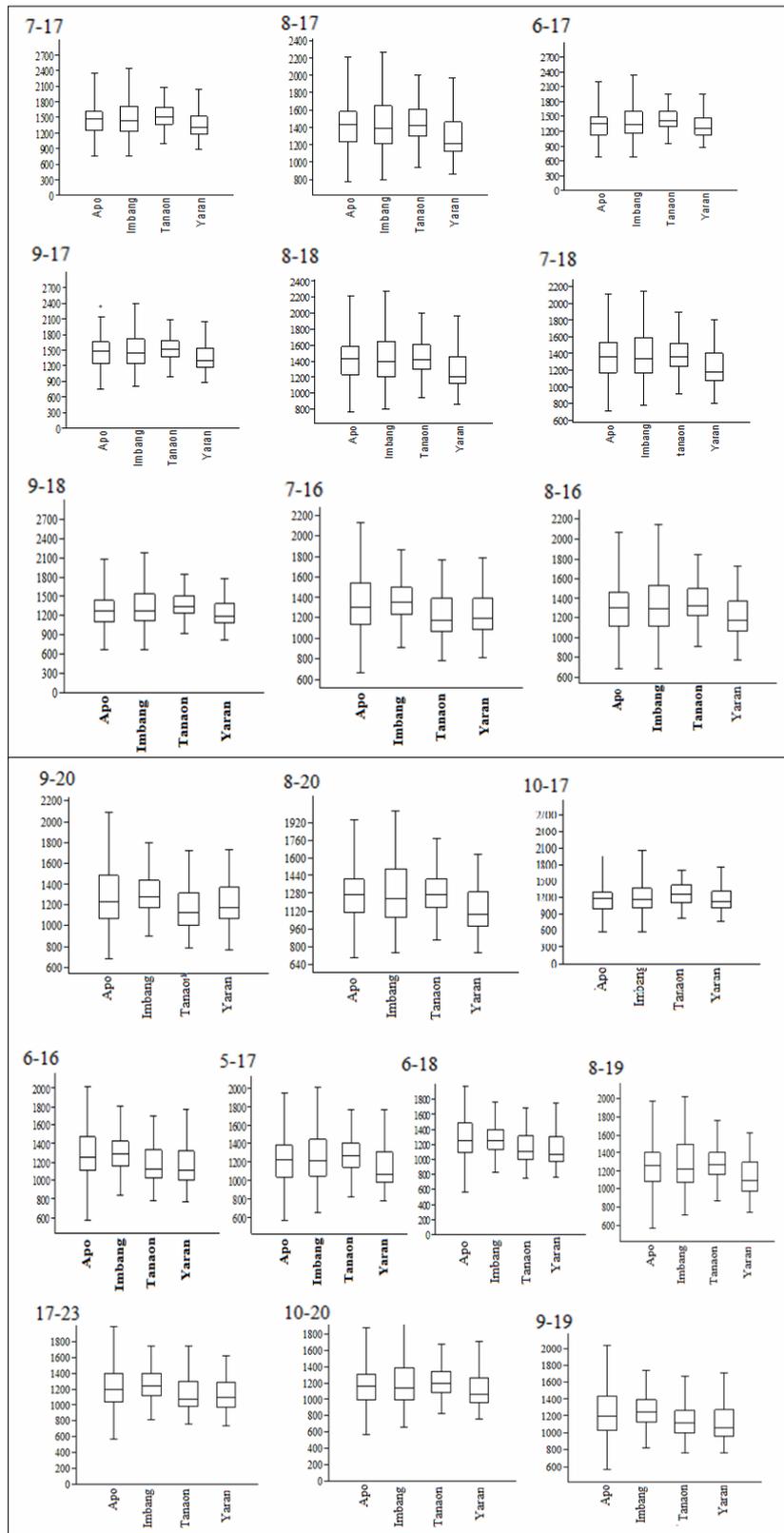


Figure 6. Box plots comparing variations between populations of *V. angularis* based on nineteen (19) interlandmark distances.

Conclusions. The results of the study show the shell shape of *V. angularis* are concentrated towards the mean shape. While there are observed variations in shell shapes of *V. angularis* mostly seen in the length of the snail from the apex/tip down to the aperture and to the body whorl, these can be attributed to differences in

environmental factors such as pH and sources of calcium for the development of the shells. The results of the study also suggest that relative warp analysis and Euclidean distance matrix analysis are useful tools in describing variations of shapes among populations representing a fundamental improvement for statistical detection of differences among groups and for the biological interpretation of shape variation.

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