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Variability in Populations of Golden Apple Snail, *Pomacea canaliculata,* (Lamarck, 1822) in selected locations from The Philippines

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Abstract

The Pomacea canaliculata, also known as Golden Apple Snail (GAS), is considered to be a variable species with shell shape variability despite conflicting environments. Samples of GAS were collected from different locations and ecological environments in the Philippines to describe the extent of variations in shell shapes using conventional measurements and the tools of geometric morphometrics. Using TPS software, the GAS samples collected were landmarked and processed. The output data was then analyzed to determine the differences in the shell among different populations with respect to their sexes. Results showed that among populations, the main variations in the shell shape occurred in the apex of shell aperture and operculum. It also showed that there were no high variations between the shell shape among populations of male and females. The results indicate that shell shape variations among populations can be affected geographically. Ecological factors such as substrates and water flow could have affected also the growth patterns of the shell.

Keywords: *Pomacea canaliculata, g*olden apple snail, shell shape, geometric morphometrics, CORIANDIS.

Introduction

The Golden Apple Snail (GAS), *Pomacea canaliculata* (Lamarck, 1822), popularly known in the Philippines as "golden kuhol", was introduced into the Philippines between 1982 and 1984. Originally, it came from South America via Taiwan. At first, its high nutritive value as food and farm animals generated interest among both public and private sectors to propagate the production of this organism. However, the people easily got disinterested with this organism and abandoned it. Since then GAS invaded ponds, swamps, irrigated rice fields, canals, lakes and water-togged areas including polluted waters with low oxygen levels. This indicates that GAS has the capacity to invade new environments and has been nominated as number 73 of the “world’s worst” alien invaders[1]. It has become one of the worst pest of rice in the country and is believed to have even displaced other freshwater gastropod species.

GAS is known to be a very variable species showing shell shape variability having high phenotypic plasticity scale despite conflicting environments. Study shows both morphological and biological features have high variability and adaptability to wide variation in environment[2]. Since growth and life history traits of animals often vary geographically, body size frequently varies with latitude and altitude. Organisms respond to variation in temperature through the direct effect of temperature on phenotypes, or through long-term adaptation to temperature[3,4,5].

Since variability within organisms can be observed through its phenotype and measurable characteristics such as shape, size, color and other morphological aspects, it is argued that GAS will vary in its shell shape between sexes and among locations thus this study was conducted. This study on variability in shell shape among populations of GAS has important fitness consequences because this may explain the ability of the snail to occupy habitats successfully[6,7]. This study examined variations among shell shapes in selected populations of GAS collected from different ricefields, creeks and a river in the Philippines.

Material and Methods

Samples of Golden apple snails (GAS) *Pomacea canaliculata* were collected from different irrigated rice fields (Clarin, Lala, Ramon Magsaysay, Manaoag), creeks (Barangay Bunga and Iligan City) and a river (Layawan) in the Philippines (figure 1). GAS adults were handpicked and placed in plastic containers.

The flesh of the GAS samples was removed and dried. Each shell was measured three times on its total height and width and its operculum height (h) and width (w) using a Vernier caliper. Figure 2 shows how to measure the width and height of GAS.

For landmark and outline data acquisition, the images of the GAS samples were captured using a HD digital camera. Careful capturing was practiced to reduce parallax and reorient bad images. There were two designated orientations of the shell.

First, keeping the shell in upright position pointing the tip of the shell on top and second is in dorsal view facing the operculum out (apertural view). tpsDig ver.2[8] was used to landmark the samples (table 1).

The sexes were determined first based on the available references[9]. Using the TPS Utility software[8], metafiles of the photographs of the shells were made. Fourteen landmarks (LM) were assigned in the perimeter of the shell (figure 3-A). In order to reduce the measurement error, all specimens were digitized and done in three replicates[10]. For the top view, Outline data acquisition was done by having the landmark points placed on images and scale factors were recorded using TPS Dig ver.2[8]. This software was used to place 100 curve points in the whorl of the shell (figure 3-B). This curve points constitute the outline that covers the outer suture ending up to its apex.

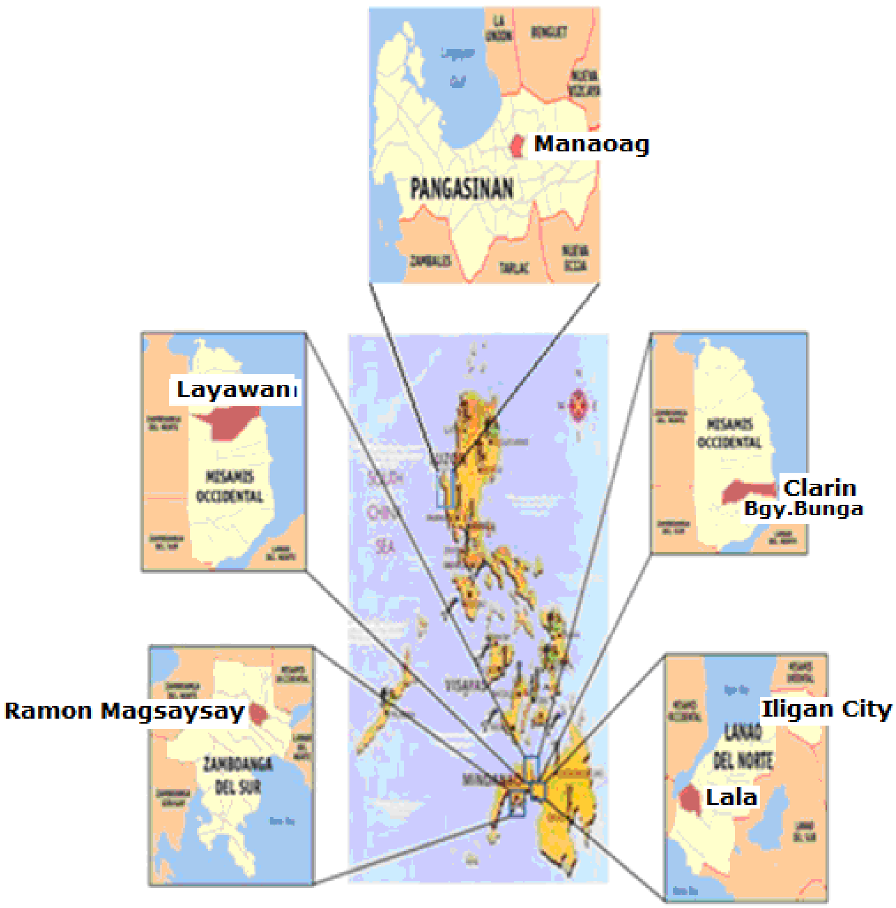

Figure - 1
Map of the Philippines where the samples of Golden Apple Snails were collected

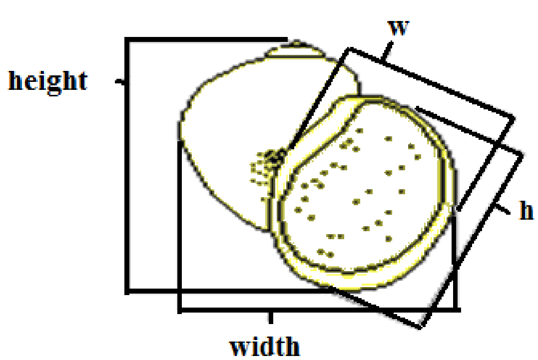

Figure - 2
The peripheral measurements of the apertural view

Data Analysis: The relative warp analysis [11] was performed using the tpsRelw ver.1.46 [12]. This type of analysis corresponds to a Principal Components Analysis of the covariance matrix of the partial warp scores, which are different scales of a thin-plate spline transformation of landmarks [13]. Relative warps were the main components of the matrix that combine partial warps and uniform components. The most informative were the first and second relative warps according to Hammer *et al.* [14]. Differences in shell shapes of GAS between sexes were analyzed using Kruskal-Wallis test using the Paleontological Statistical Analysis Software [14]. Box plot was used to visualize the distribution of shape variation among the populations of GAS. Correlation Analysis Based on Distances (CORIANDIS) ver. 1.1 Beta [15] was used to determine similarities and/or differences between population of GAS with respect to the different multivariate data -two landmark data and one non-landmark data.

Results and Discussion

Relative warps analysis was used to determine the similarities and differences for every populations of male and female Golden Apple Snails (GAS). Relative warps detected the differences in shell shapes between populations. RW1 to RW4 describes difference in the morphometry of the shell aperture based from the corresponding landmark points of the shell (figure 4).

Table - 1
Landmark Points and their Corresponding Anatomical Location on *Pomacea canaliculata,* Lamarck (1822), Apertural View

Landmark Number	Anatomical Location On The Gas Apertural View	Type of landmark
1	right border of the profile of the shell at the end of the upper suture of the last whorl	Type 1
2	apex of the Shell	Type 1
3	left border of the profile of the shell at the end of the upper suture of the last whorl	Type 1
4	most external point below the last whorl at the left profile of the Shell	Type 2
5	most external point on the last whorl at the left profile of the shell on a perpendicular line to the axis from LM 10	Type 2
6	umbilicus of the Shell	Type 1
7	with a line from LM 2, touching the lower aperture margin	Type 2
8	with a line perpendicular from LM 12	Type 2
9	with a line across LM 5 was the outermost point of the external part of the outer lip	Type 2
10	just above LM 9 was the outermost point of the external part of the lip	Type 2
11	right border of the profile of the shell below the end of the lower suture of the last whorl	Type 2
12	last whorl of the Shell	Type 1
13	with a line below LM 1 was the left profile of the outer lip	Type 2
14	just above LM 6 place on the left profile of the lip	Type 2

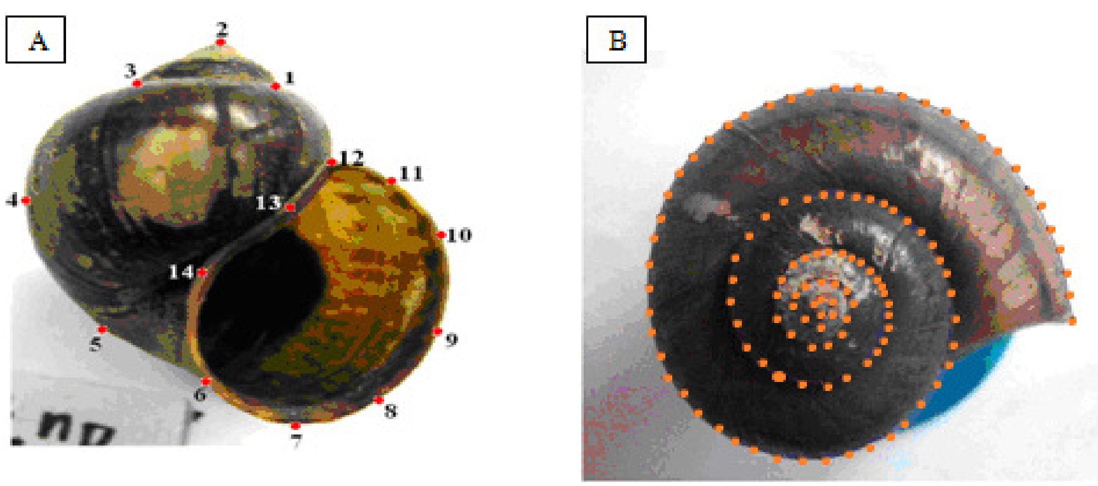

Figure - 3
Photographs of Golden Apple Snail, *Pomacea canaliculata* Lamarck: (A) Aperture of the shell with assigned five Type 1 or true landmarks and nine Type 2 or pseudo-landmark. (B) Top view with 100 curve points

The disparity in distances of anatomical landmark points and differences in shell shapes among populations indicate that geographical locations play an important role in the observed variations. As shown by the box plots, visualized differences can be seen by the first four effective warps (figure 4).

As shown in table 3, different populations of male and female geometrically vary with each other. The populations with corresponding values of less than or equal to 0.05 have significant difference in the shape of aperture. Based from Kruskal-Wallis test, it was observed that some populations of male and female GAS with corresponding values are highly significant with each other in the morphometry of aperture. In addition, statistical analysis of the data of samples were obtained and showed that some populations of GAS are evenly distributed. In each warp, it revealed that both male and female populations of GAS showed significant differences in shell shape from other populations (table 3). This variability in shell shape was considered to be an indicator that geographic location of populations of GAS was a factor to the said difference.

The apertures in the shell of GAS populations were observed and analyzed to indicate if variations have occurred. Based on the geometric morphometry analysis, it was found out that the difference in the shell shape occurred mainly at the apex and operculum for male and female populations of GAS as shown in table 2. The shell aperture and operculum was the major characteristics of GAS, thus these were the most influenced traits in the occurrence of variations. Generally, table 2, which determined the specific characteristic of the shell shape of GAS where every population of both sexes differs, correlate with table 3.

Snail plasticity has focused either on presence or absence of a single factor, or has looked at responses to environmental gradients over large geographical scales. We therefore looked into variations in populations of the snails looking into several characters using correlation analysis based on distances. The total height of each bar resulted from the addition of the squared distances of each trait separately, making it a measure of trait disparity (figure 5). The figure showed that among males, the location in which traits departs the most were Lala for aperture, Pangasinan for shell outline, Bunga for shell height, shell width and operculum height, and Clarin for operculum width. While among females, the most departed area for aperture, shell height and shell width was Pangasinan, shell outline for Iligan City, and operculum height and width for Clarin. Differences of traits among populations of male and female *Pomacea canaliculata* indicate that geographical location has significant effect on the variability of their shell shape.

Minor differences of the shell shape can be observed also among populations of GAS and seen as differences in the aperture and outline based on the stacked bar graphs in Figure 5. Likewise, minor size difference can also be observed in the shell shape of the males and females with respect to the shell height, shell width, operculum height and operculum width. The results suggested that variability of GAS may also be determined from specific characteristics of its shell shape.

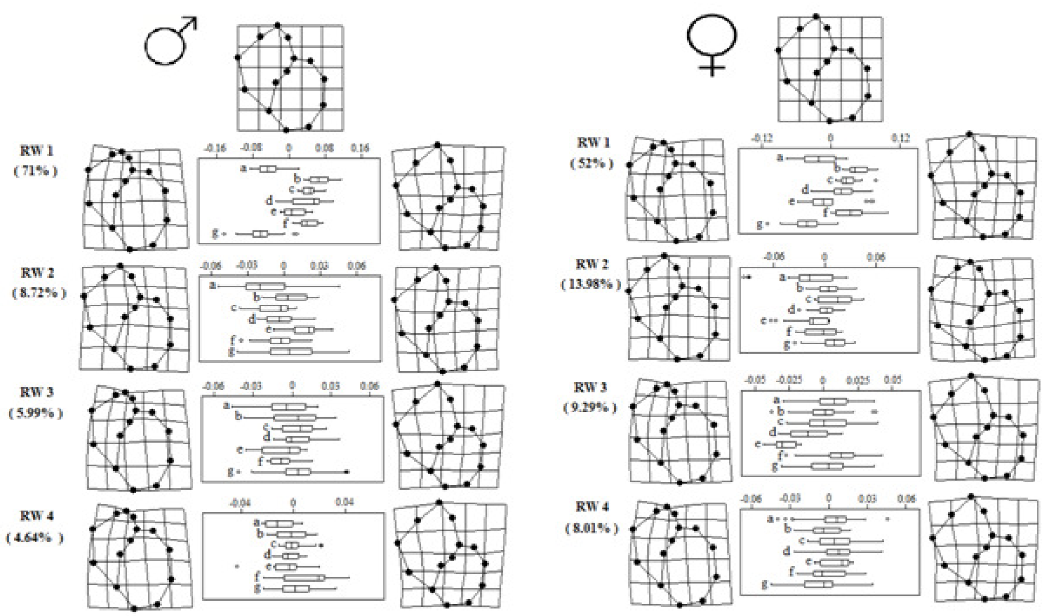

Figure - 4

Summary of the geometric morphometric analysis showing the consensus morphology (*uppermost panel*) and the variation in aperture among male and female populations of *Pomacea canaliculta* found in different locations in the Philippines: a – Pangasinan, b – Iligan City, c – Lala, d – Barangay Bunga, e – Layawan, f – Clarin, g – Ramon Magsaysay

Table - 2
Descriptions of the shape of aperture as shown by the relative warps

RW	Aperture	
	Males	**Females**
RW1	Variations in the apex and operculum of the shell aperture show significant results among populations of Lala, Bunga, Layawan, Clarin, Pangasinan, and Ramon Magsaysay	The apex and operculum of the shell aperture show significant variations among populations of Iligan City, Lala, Barangay Bunga, and Clarin.
RW2	Outermost point of the external part of lip and outer lip show significant variation among populations of Pangasinan, Iligan City, Lala, and Layawan.	Variations in the apex and operculum of the shell aperture show significant results among populations of Pangasinan, Lala, Layawan, and Clarin.
RW3	The apex and operculum of the shell aperture show significant variations among populations of Pangasinan, Iligan City, Lala, Barangay Bunga, and Layawan.	Variations in the apex and operculum of the shell aperture show significant results among populations of Iligan City, Lala, Barangay Bunga, and Layawan.
RW4	Variations in the apex and operculum of the shell aperture show significant results among populations of Pangasinan, Barangay Bunga, Layawan, and Clarin.	The apex and operculum of the shell aperture show significant variations among populations of Pangasinan, Iligan City, Layawan, Clarin, and Ramon Magsaysay.

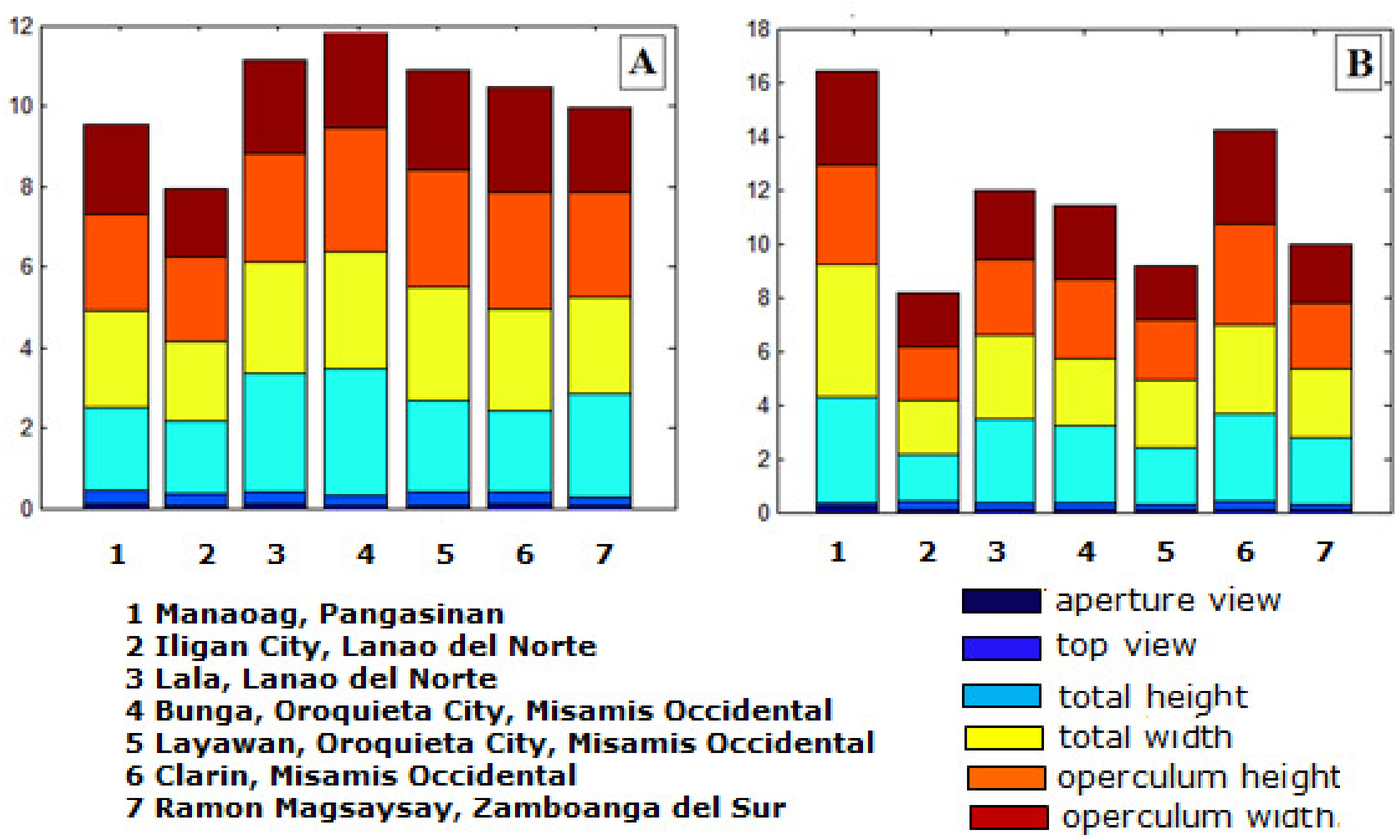

Figure - 5
Squared distances to centroid for individual sets for landmark and non-landmark traits for the [A] male and [B] female *P. canaliculata* Lamarck

Table - 3
Comparison of the shape of aperture among the populations of *Pomacea canaliculata* using Kruskal-Wallis test

RW	Population	A	B	C	D	E	F	G
	Male							
1	A		0.000	0.000	0.000	0.000	0.000	**0.025**
	B	0.000		**0.005**	**0.041**	0.000	0.000	0.000
	C	0.000	0.109		0.974	**0.001**	0.358	0.000
	D	0.000	0.854	1.000		**0.016**	1.000	0.000
	E	0.000	0.000	**0.021**	0.337		**0.001**	0.000
	F	0.000	**0.005**	1.000	1.000	**0.029**		0.000
	G	0.515	0.000	0.000	0.000	0.000	0.000	
2	A		0.000	0.083	**0.016**	0.000	**0.011**	0.000
	B	**0.002**		**0.021**	**0.031**	**0.019**	**0.040**	0.810
	C	1.000	0.439		0.700	0.000	0.675	0.103
	D	0.344	0.661	1.000		**0.001**	0.852	0.122
	E	**0.001**	0.408	**0.005**	**0.014**		0.000	0.055
	F	0.231	0.830	1.000	1.000	**0.003**		0.132
	G	**0.003**	1.000	1.000	1.000	1.000	1.000	
3	A		0.226	0.181	0.228	0.501	0.135	0.099
	B	1.000		0.753	0.975	0.124	**0.014**	0.833
	C	1.000	1.000		0.797	0.052	**0.003**	0.902
	D	1.000	1.000	1.000		0.162	**0.001**	0.795
	E	1.000	1.000	1.000	1.000		0.402	0.065
	F	1.000	0.288	0.053	**0.029**	1.000		**0.001**
	G	1.000	1.000	1.000	1.000	1.000	**0.015**	
4	A		**0.004**	**0.004**	**0.003**	0.192	0.000	0.000
	B	0.079		0.512	0.771	0.466	**0.009**	0.239
	C	0.082	1		0.542	0.230	0.074	0.606
	D	0.071	1	1		0.579	**0.012**	0.181
	E	1	1	1	1		**0.011**	0.062
	F	**0.001**	0.199	1	0.250	0.231		0.051
	G	**0.001**	1	1	1	1	1	
	Female							
1	A		0.000	0.000	0.000	0.370	0.000	**0.001**
	B	0.000		**0.001**	0.000	0.000	**0.013**	0.000
	C	0.000	**0.021**		0.110	**0.003**	0.836	0.000
	D	0.000	**0.001**	1.000		**0.008**	0.087	0.000
	E	1.000	**0.007**	0.053	0.176		**0.001**	**0.001**
	F	0.000	0.269	1.000	1.000	**0.011**		0.000
	G	**0.029**	0.000	0.000	0.000	**0.028**	0.000	
2	A		**0.001**	0.000	**0.005**	0.842	0.070	0.000
	B	**0.018**		0.154	0.389	**0.003**	0.096	0.103
	C	**0.002**	1.000		**0.043**	0.000	**0.006**	0.574
	D	0.104	1.000	0.895		**0.011**	0.334	**0.001**
	E	1.000	0.058	**0.007**	0.224		0.109	0.000
	F	1.000	1.000	0.118	1.000	1.000		0.000
	G	0.000	1.000	1.000	**0.018**	0.000	**0.007**	
3	A		0.076	0.287	0.000	0.000	0.087	0.153
	B	1.000		0.991	**0.006**	0.000	**0.003**	0.686
	C	1.000	1.000		**0.017**	0.000	**0.048**	0.986
	D	0.000	0.136	0.349		0.000	0.000	0.000
	E	0.000	0.000	0.000	**0.002**		0.000	0.000
	F	1.000	0.064	1.000	0.000	0.000		**0.003**
	G	1.000	1.000	1.000	**0.009**	0.000	0.067	
4	A		**0.026**	0.994	0.321	0.751	0.108	**0.002**
	B	0.539		0.062	**0.003**	**0.018**	0.562	0.692
	C	1.000	1.000		0.533	0.759	0.124	**0.023**
	D	1.000	0.054	1.000		0.620	**0.027**	0.000
	E	1.000	0.381	1.000	1.000		0.095	**0.016**
	F	1.000	1.000	1.000	0.560	1.000		0.335
	G	**0.034**	1.000	**0.492**	**0.003**	0.330	1.000	

Legend: A – Pangasinan, B – Iligan City, C – Lala, D – Brgy. Bunga, E – Layawan, F – Clarin, G – Ramon Magsaysay

Cluster analysis show the majority of the populations are located away from each other. Figure 6 shows the relationship among populations of male and female *Pomacea canaliculata*.

In figure 6-A, populations of male were divided into two groups according to their similarities of its shell shape and structure. The distance between locations had lesser significance to the similarities of their morphometry. On the other hand, figure 6-B showed that populations of female from different locations were closely related to each other with respect to the similarities of their morphometry. Moreover, in figure 6, among populations, females were group into three compared to populations of males which comprised only into two groups. Among populations of male GAS (figure 6-A), Ramon Magsaysay correlates and similarly grouped with Iligan City, Pangasinan, and Layawan with respect to their morphometry of the shell. While among populations of female GAS (figure 6-B) result shows that Ramon Magsaysay belong in a group with Lala, Barangay Bunga, and Clarin. As shown also in figure 6-A, population of male GAS from Iligan City was closely related to Pangasinan, whereas in figure 6-B, population of female GAS from Iligan City was closely related to Layawan. The relationship among populations of male GAS between Bunga and Clarin also differs among populations of female GAS from these two geograpgical locations.

These results clearly show that while geography affects the differences in the shell shape of GAS, results show geographically distant populations showed lesser variation in the characteristics of their shell and geographically close populations showed greater differences on their shell shapes for both male and females sexes. It is hypothesized that variations in shell shapes between loactions could be due to differences in environmental factors where the snails were collected. Rice fields in Clarin Lala and Manaoag were irrigated and treated with pesticides, those collected from Ramon Magsaysay were from a small isolated montane rice field, those from barangay Bunga in Oroquieta city and in Iligan City were from a creek where canals drained, and those from Layawan were collected from a flowing river. The differences could be due to variations in substrates and hydrological factors. Phenotypic plasticity as a result of ecological and environmental interactions is ubiquitous in nature[16]. The degree of phenotypic plasticity among organism may differ when exposed to the same environmental change. Phenotypic plasticity is a well-known phenomenon in freshwater mollusks, occurring in both bivalves[17,18] and gastropods[19,20,21] and may explain the results generated with the golden apple snail.

Conclusion

The results showed that while geographic location had a significant effect to the shell shape of GAS for both male and female sexes, the substrates and other hydrological factors could have affected also the growth patterns of the shell, resulting in uncontrollable variations in shell shapes thus needed further investigation.

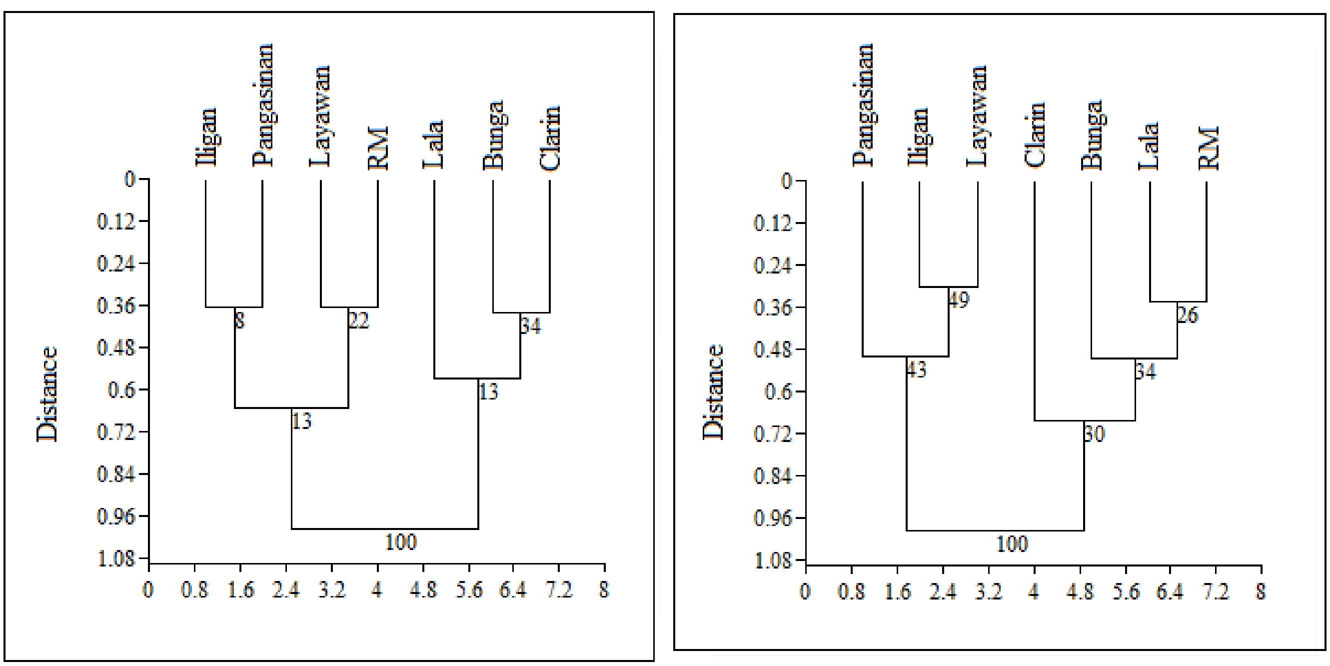

Figure - 6
Cluster diagram based on the compromise scores showing the systematic relationship of *Pomacea canaliculata* population of male (A) and female (B) collected from different locations in the Philippines

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