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Describing Populations of *Pomacea canaliculata* Lamarck from Selected Areas in Mindanao, Philippines using Relative warp analysis of the whorl shell shape

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ABSTRACT

Pomacea canaliculata is a gastropod species that has been highly recognized as a fast growing and invasive serious agricultural pests of rice in Asia particularly the Philippines. The rapid invasion of this species in many variable habitats and in the past several years attempts were made to clearly understand the nature of this pest. It was therefore the objective of the study to describe geographic variations in this pest especially the snail has been found not only in agricultural rice farms but also in canals and ponds. The adaptation could be inherent in the pest and could be reflected in the phenotype. Thus to describe possible phenotypic diversity in this organism, the shape of the shell was quantitative analyzed and described using relative warp analysis, the principal components of the covariance matrix of the partial warp scores generated. Results showed that *P. canaliculata* shells vary in whorl shell shape between geographical locations. There were many factors hypothesized that may have affected the shapes of the shell such as habitat types and other environmental gradients. While the use of the relative warp analysis was useful in the quantitative description of the shape of the shell, more studies are needed to relate these variations to factors that may influence variations in shapes.

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INTRODUCTION

Pomacea canaliculata Lamarck commonly known as the "golden apple snail" (GAS) has received much attention when it became established as a serious rice pest in Asia (Cowie, 2006; Cagauan and Joshi, 2003; Joshi *et al.*, 2005; and Joshi, 2005; Guerrero, 1989; Kho, 2011). The golden apple snail's growth and distribution vary indicating this species has high adaptability to form a new population (Dong *et al.* 2011). In temperate areas, the shell growth of this species occurs mainly in spring and summer, while it stagnates in fall and winter (Ghesquire, 2007). The species also thrive in tropical environments and was argued to have evolved accordingly, such that they are capable of closing off their shells completely during dry periods. *P. canaliculata* is extremely polyphagous, feeding on vegetal (primarily macrophytophagous, floating or submersed higher plants), detrital, and animal matter. Diet may vary with age, with younger smaller individuals feeding on algae and detritus, and older, bigger (15mm and above) individuals later shifting to higher plants (Estebenet & Martin, 2002). These traits of the snail could explain for higher survival and adaptability to variable environments and has since then became an invasive species.

To be able to understand the degree of adaptation and differentiation in this pest species, geographical variations between populations was investigated. Traditionally, studies on population differentiation were done qualitatively. With the advent of advanced methods in biology, statistics, geometry and computer science, studies on phenotypic variations have improved and descriptions of variations have become more quantitative. One of the advanced methods is geometric morphometrics particularly landmark-based analysis. This technique is highly advantage against traditional measurements by eliminating the effects of variation in location, orientation, scale, and position biases of the specimens (Bookstein 1991; Chiu *et al* 2002; Rohlf 2003; Zelditch *et al* 2004). The use of GM analysis has been applied in describing shell shape patterns (Samadi *et al* 2000; Galliguez *et al.* 2009; Minton & Wang 2011). In this study the method is applied to describe geographic

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variations in shell shape patterns especially in the apple snail's coiling pattern as reflected in the whorl shapes. We examined the whorl of the shell of the golden apple snail collected in selected geographically distant populations specifically North Cotabato, Dipolog City and Lanao del Norte in Mindanao, Philippines. The information generated by the study could be useful in understanding the nature of the pest species which may be important in its management as an agricultural pest.

MATERIALS AND METHODS

A total of 90 specimens of the golden apple snails, *P. canaliculata* were obtained from North Cotabato (del Carmen), Dipolog City and in the province of Lanao del Norte (Kapatagan) (Fig. 1). Shells were photographed by a digital camera. Digital images of top view or whorl were taken for each sample using a standardized procedure (Fig. 2). Shell shape was studied using a landmark-based methodology that eliminates the effect of variation in the location, orientation, and scale of the specimens. Thirteen (13) anatomical landmarks located along the outline of the whorl or top view (Fig. 2) portion of the shell and the obtained digitized images of the snails' shell were then outlined with sample points around its contour in order to get the x and y coordinates. This was made possible using an image analysis and processing software Tps Dig freeware 2.12 (Rohlf 2008a). TpsDig facilitates the statistical analysis of landmark data in morphometrics by making it easier to collect and maintain landmark data from digitized images (Rohlf 2008a). These coordinates were then transferred to Microsoft Excel application for organization of the data into groups (based on locations). The two-dimensional coordinates of these landmarks were determined for each shell specimen. 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. 1.46 (Rohlf 2008b). 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. Histogram and box plots were generated using Paleontological Statistics (PAST) version 1.91 software from the relative warp scores. Histogram and box plots are a powerful display for comparing distributions. They provide a compact view of where the data are centered and how they are distributed over the range of the variable. Kruskal-Wallis test was used to analyze whether or not the species differ significantly with regards to its shell shape (Demayo *et al* 2011). The results were then analyzed and interpreted accordingly from the percentage variance and overall shape variation as explained by significant relative warps.

Fig. 1: Map showing the study area. (a) Dipolog City (b)Lanao del Norte (c) North Cotabato (Source: map.google.com)

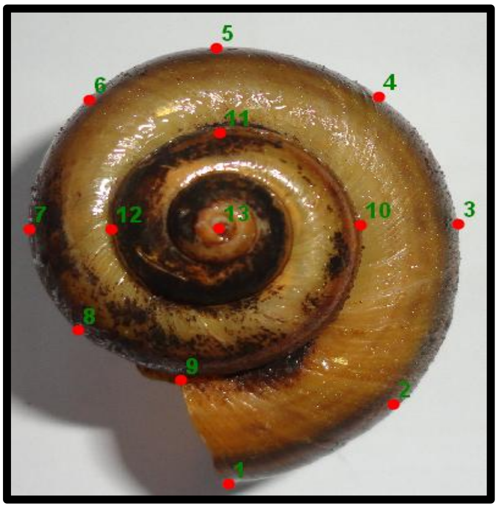

Fig. 2: Landmarks used to describe the shape of the top view/whorl of the shell of *P. canaliculata*

RESULTS AND DISCUSSION

Results of the relative warp analysis describes the variations in the whorl of the shell. The relative warp (RW) describes the total variance in shapes (Table 1). A low negative RW score means that a shell has less pronounced whorls while high positive RW score means has more pronounced whorls (Table 1). Specific descriptions of the variations observed are graphically presented in Fig. 3. Differences in mean shell shapes between populations were tested using Kruskall-Wallis test (Table2).

Tables 1: Percentage variance and overall shape variation in top view or whorl shell of golden apple snails as explained by the significant relative warps.

RW	% Variation	Whorl Shell
1	38.64%	The first relative warp illustrates the differences of the distance of the whorls on the upper part and the lower part of the whorls. Samples found on the positive RW1 axis has a spaced distance on the upper part of the whorl and slightly close/near on the lower part while negative RW1 axis has a close/near distance at the uppermost left part of the whorl and having a spaced distance on the lower part of the whorl. Based on the boxplot, it was revealed that samples from North Cotabato are toward the positive axis while samples from Lanao del Norte are toward the negative axis. However, samples from Dipolog City almost illustrates the mean shape.
2	28.57%	Second relative warp axis describes the distance of the topmost whorl and the whorl adjacent to the shell opening to the inner whorl. Positive RW2 axis has a centered topmost whorl when the spaced distance on the whorl adjacent to the shell opening is wide while negative RW2 axis has topmost whorl which tends to shift to the left when the spaced distance on the whorl adjacent to the shell opening is narrow. Samples from North Cotabato and Dipolog City are located slightly to the positive axis while samples from Lanao del Norte are located slightly to the negative axis.
3	11.16%	The third relative warp describes the distance of the whorl with regards to the whorl adjacent to the shell opening and the inner whorl. Positive RW2 axis has a whorl adjacent to the shell opening which has a distance near to the inner whorl but slightly misaligned from center to top while negative RW2 axis have a distance slightly away the inner whorl but almost aligned from center to top. Samples from North Cotabato follow slightly toward the positive axis while samples from Dipolog City follow slightly towards the negative. However, samples from Lanao del Norte are almost on the origin showing the mean shell shape pattern.
4	9.43%	The fourth relative warp describes the distance of the whorl on the left side part of the whorl and the right side part of the whorl. Positive RW4 has a close/near distance on the left side of the whorl but aligned from the center to top and the right side of the whorl is slightly spaced while the negative RW4, the right side part of the whorl is close/near and the left side of the whorl is spaced. Samples from Lanao del Norte follow toward the positive axis while samples from rom North Cotabato and Dipolog City are oriented toward the negative axis.

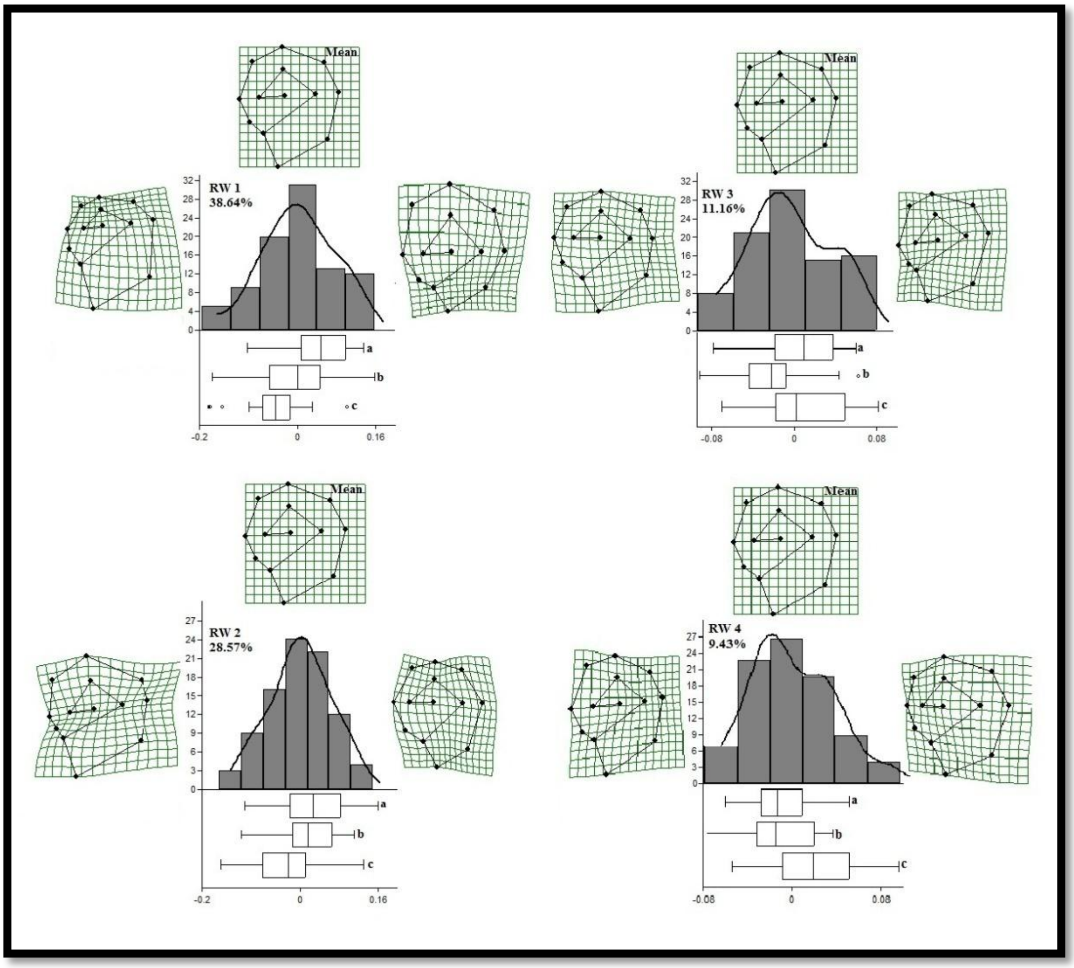

Fig. 3: Relative warp box plot and histogram showing variations in the shape of whorl shell portion of *Pomacea canaliculata* found in varying regions: (a) North Cotabato (b) Dipolog City and (c) Lanao del Norte Lanao del Norte

Table 2: Results of the Kruskal-Wallis test for significant differences in the mean shapes of the top view or whorl shell of *P. canaliculata*

Relative Warp (RW)	Population	North Cotabato	Dipolog City	Lanao del Norte
1	North Cotabato	-	0.2727	4.11E-07
	Dipolog City			0.01695
	Lanao del Norte			-
2	North Cotabato		0.6048	0.001767
	Dipolog City		-	0.004033
	Lanao del Norte			-
3	North Cotabato		0.001174	0.9234
	Dipolog City			0.006522
	Lanao del Norte			-
4	North Cotabato		0.5692	0.003419
	Dipolog City			0.00238
	Lanao del Norte			-

Macrogeographical trends of shell morphology in *P. canaliculata* could be attributed to differences in habitats as the samples were collected in different locations including canals, farm and stream. Related studies in selected species of gastropods such as on the geographical variation of *Cypraea annulus* in Zanzibar, East Africa by Orr (1959) revealed variations in four habitat types based on geological features (rock, muddy, sandy and bay reefs). Low temperature could also be an environmental factor that affect the size and shape of the shell of *P. canaliculata*. It was one of the factors that was argued to be affecting body size in some species of gastropods (Kay, 1961; Tissot, 1984; Irie & Iwasa, 2003) as well as in many other ectotherms (Ray, 1960; Atkinson, 1994), in the fruit fly Drosophila melanogaster (James, *et al* 1997) and the soil nematode *Caenorhabditis elegans*. It is argued that low temperature could decrease the speed of callus thickening in shells since calcium carbonate precipitation on shell surfaces is limited in cold waters (Graus, 1974; Clarke, 1983; Vermeij, 1987). Morphological variations along environmental gradients have been documented in a number of gastropod species (Reid, 1996; Saier, 2000), exploring causal mechanisms involved such as evolutionary (Johannesson, 1986), genetic differentiation (Johannesson *et al*, 1993; Johannesson & Johannesson, 1996; Kyle & Boulding, 1998), phenotypic plasticity (Trussell, 1996, 2000b), genotype and environment interactions (Trussell, 2000a). For *P. canaliculata*, there is still a need to explore these factors to be able to understand why these species have become adaptive and invasive in variable habitats.

Conclusion:

This study have shown significant geographical variations in shell shapes of *P. canaliculata* revealed by the landmark-based geometric morphometric analysis. Several factors were hypothesized to be influencing the differences observed such as plasticity brought about by habitat differences or could be due to environment and genotype interactions. More studies are recommended to further explore the basis for the differentiation of *P. canaliculata* populations.

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