

Comparing Compartmentalization in the Shell of Two Populations of Golden Apple Snails, *Pomacea canaliculata* Lamarck (Gastropoda: Ampullariidae)

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Abstract: This study was conducted to describe compartmentalization in the shell of *P. canaliculata* using geometric morphometric approach. Modularity and Integration Tool (MINT) was used in the analysis of geometric morphometric data. A total of 150 points were used to outline the margins around the contour of the shell. Several models were hypothesized to determine the best fitted model. Result show the shell is divided into contours based on its shells boundaries. These compartments also referred to as modules are (1) the spire, (2) body whorl and (3) the whole space occupying the whole apertural area. There was consistency in the best-fit model generated in both sexes and populations indicating the same morphological pattern followed. The resulting fair consistency of the observed patterns of hypothesized developmental modules implies that shell of *P. canaliculata* is highly conserved and that these three parts of the organism's shell are coordinated in their sizes and shapes to make up a functional whole. It can be argued from this study that the spire, body and aperture parts of the GAS shell are integrated with each other because they develop, function and evolve jointly.

Keywords: Integration, lake dapao, lake lanao, modularity, modules

INTRODUCTION

The introduction in the Philippines of the golden apple snail *P. canaliculata* showed its unparalleled success of breeding spreading from canals, streams, rivers, rice fields and even to a remote bodies of water, the lakes (Joshi *et al.*, 2003). Studies have shown that this invasive species exhibit wide morphological differences (Torres *et al.*, 2011; Moneva *et al.*, 2012; Demayo *et al.*, 2011a). Within and among populations of the species are assumed to be genetically unstable as its gene pool is reduced by strong founder effects (Allendorf and Lundquist, 2003; Huey *et al.*, 2005). The observations of intraspecific variations in many populations make this species a good model for inferring the mechanisms behind population differentiation as this organism is believed to have evolved into a complex of morphologically divergent populations in response to ecologically diverse habitats (Trussel and Etter, 2001; Torres *et al.*, 2011; Rosin *et al.*, 2011; Cazzaniga, 2006). Many studies on biological structures using data on size and shape variations have contributed in establishing additional reliable criteria in determining population differentiation. Developmental processes for example, produce morphological structures such as modules in which covariation between traits can have substantial implications for understanding genetic variation and the potential for evolutionary change (Klingenberg, 2008).

It is for this reason that modularity is suggested to be both the result of evolution or facilitates evolution (Wagner and Altenberg, 1996; Wagner, 1996). Modularity is considered a prerequisite for the adaptation of an organism and their evolvability (Raff, 1996; Gerhart and Kirschner, 1997; Calabretta *et al.*, 2000; Sole and Valverde, 2013) and these modules are internally integrated by developmental interactions (Klingenberg *et al.*, 2002; Demayo *et al.*, 2011b; Coronel *et al.*, 2012). Studies on both lotic and lentic populations of *P. canaliculata* for example showed differences in shell shape (Estebenet *et al.*, 2006) thus understanding the nature of the shell shape in this snail species in lakes is important. In this study, identification of the homology in the patterns of shell development among individuals of *P. canaliculata* is viewed through defining its modules as basis to best account the variation structure within the overall phenotypic scheme. Using newly developed software, Modularity and Integration (MINT) analysis tool (Marquez, 2008), the concept of modularity and integration has been studied using geometric morphometrics to prove that even if, through time while isolated in the lake, shell shape traits of *P. canaliculata* are high in evolvability or there is still conservation of said traits. While there are several different approaches in the analysis of morphological integration and modularity, popular are those conducted with geometric morphometric approaches (Klingenberg and Zaklan,

2000; Klingenberg *et al.*, 2001a; Bookstein *et al.*, 2003; Klingenberg *et al.*, 2003; Badyaev and Foresman, 2004; Bastir, 2008; Bastir and Rosas, 2005; Monteiro and dos Reis, 2005; Goswami, 2006b; Young, 2006; Young and Badyaev, 2004; Cardini and Elton, 2008a; Zelditch *et al.*, 2008; Klingenberg, 2009; Ivanovic and Kalezic, 2010; Jamniczky and Hallgrímsson, 2011; Jovic *et al.*, 2012). In this study we made use of the modularity and integration tool developed for geometric morphometric data by Marquez (2008).

MATERIALS AND METHODS

Collection and processing of samples: Snails were randomly obtained from two separate lakes in Mindanao, Lakes Lanao and Dapao (Fig. 1). Since there were limits the number of samples to be analysed using the software, only a total of 120 samples ($N = 60$: 30M; 30F in Lake Lanao and $N = 60$: 30M; 30F in Lake Dapao) were collected. Shells were cleaned with tap water after the meat was removed with a pin. Shells with cracks were discarded.

Images of the shells were captured using a DSLR camera, Nikon D5100. The shells were oriented in such a way that the spire is at 90° of the x-axis in a 2D

orientation with the ventral side of the shell facing the top. All shells were captured in the same position. The camera with constant 55 mm focal length was mounted on a tripod to maintain a constant distance from the top of the shell and in order to obtain good images to minimize measurement error.

Analysis of *P. canaliculata* modules: Geometric morphometric tools that include outline-based methods were used to generate and harvest shape information from coordinate data using a set of steps which eliminates variation and reflection in scale, orientation and position using tpsUtil and tpsDig2 (Rohlf, 2008, 2009). A total of 150 points was digitized around the generated outline of the ventral portion of the shell (Fig. 2). The line around the contours of the shell constitutes the points were always resampled to maintain consistency within the population.

The data loaded to the MINT will test the acceptability of hypothetical modules which assumes that the data themselves have a modular structure. Covariance matrices expected under models of modularity were computed based on the modified data resulting from partitioning the entire data space into orthogonal subspaces or modules.

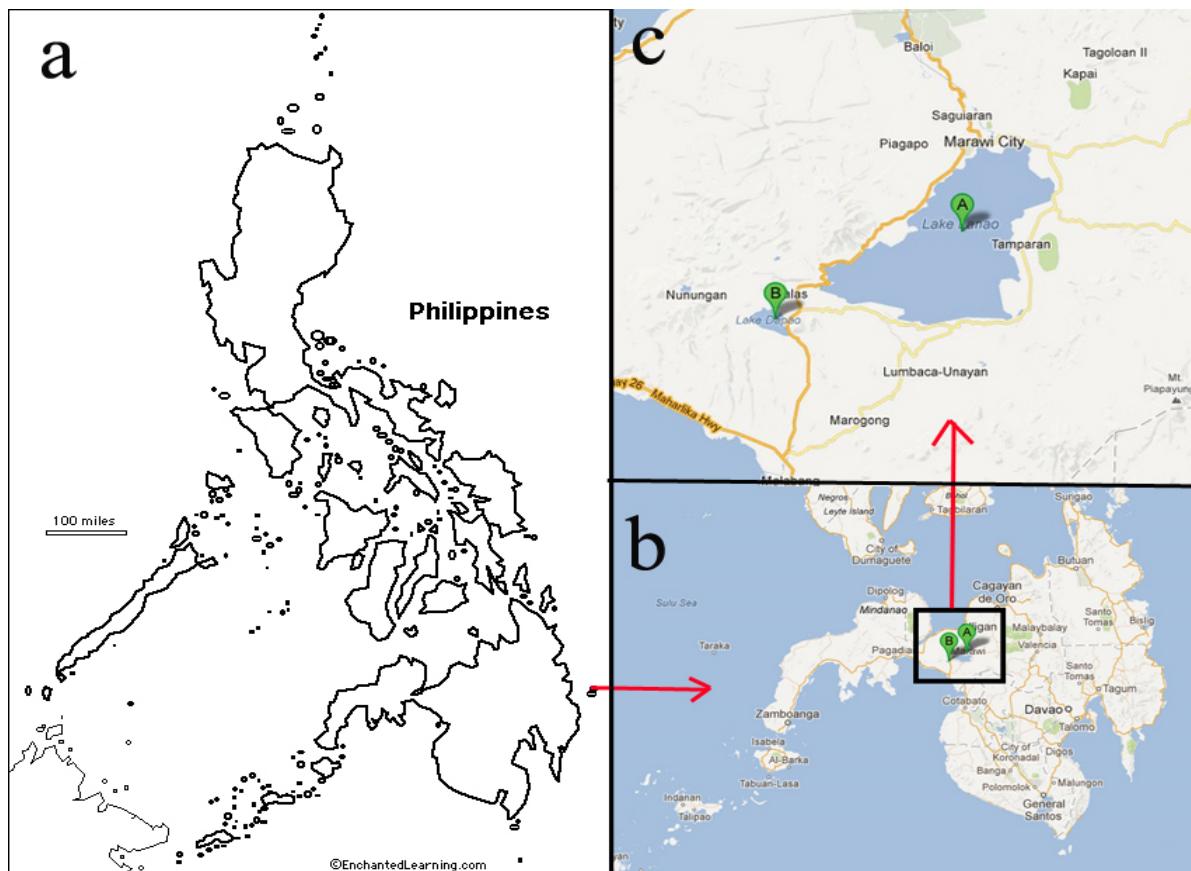


Fig. 1: Map of the (a) Philippines, (b) Mindanao and (c) Location of Lake Lanao (A) and Lake Dapao (B) Source: <https://maps.google.com/>

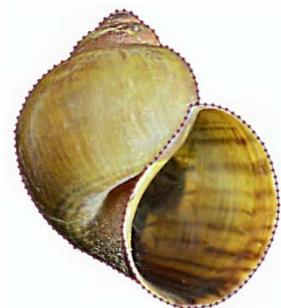


Fig. 2: Converted TPS images with landmarks

Defined (*a priori*) models were constructed including alternative models that were generated after individual defined models within loaded modules) were mixed by MINT (Modularity and Integration Analysis Tool) software (Marquez, 2009). The criteria for the choice of landmarks to be included in modules within every model of *P. canaliculata* are shown in Table 1. The null model assumes the absence of modules in the shell (Table 1 and Fig. 3).

Analyzing goodness of fit: Through the Goodness of Fit (GoF) tests, there is an assessment of whether a pre-defined model or hypothesis is good enough to explain variation in a dataset. Using the Mint software, the interest is for testing the GoF of models depicting tight associations within integrated sets of traits, the variational modules and no association with traits outside of those sets. p-values and γ -values were assumed to be the results of this GoF tests. In this approach, γ^* values were used to determine if the best-fitted model is the one from where the data was derived when fitted to the original set of 5 models and to the complete set of 10 possible model combinations for the

ventral side of the shell. A low (<0.05) p-value, closer to 0, indicates that the models generated are no longer different from the null model, thus a poorly fitting model. Models which correspond to large values of γ^* , would mean that a large difference between observed data and proposed model corresponds to the best fitted model (Marquez, 2008).

Determining model support: Jackknife support values were determined by re-sampling a total of 1000 replicates randomly dropping 10% of the individuals in each replicate. The confidence interval for the GoF statistic using the 95% level of confidence was added to the jackknife support and was also computed. Jackknife support values which are equal to 1 or closer to 1, indicates that each of the 1000 jackknife replicates for which a γ^* value was calculated. The model ranked as number 1 is the best fitting alternative (Manly, 2006; Marquez, 2008).

RESULTS AND DISCUSSION

A total of 5 alternative models (Fig. 4) (6-10) were generated after individual defined (*a priori*) models (2-5) within loaded modules (model 1-4) were mixed by MINT (Modularity and Integration Analysis Tool) software version 1.5 (Marquez, 2009). It can be seen from the generated models that models 3 and 7 were similar, also for models 4, 6 and 8 and lastly for models 5, 9 and 10.

Table 2 shows the gamma (γ^*) value and P-value computed for each model of *P. canaliculata*. The top three best-fitted models (models 5, 9, 10) (Table 2) for both sexes in both lake populations showed that these are the same models where the spire, body and aperture

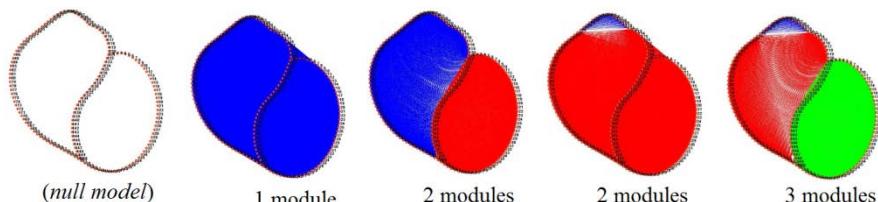


Fig. 3: Developmental modules tested in the study of modularity in *P. canaliculata*

Table 1: *A priori* developmental and genetic models tested in the study of male and female *P. canaliculata*

		Description
H_0	No Modules	"Null" model, predicting the absence of modular structure; all covariances is hypothesized to be zero.
H_1	1 module {landmarks 1-150}	Only one module was considered in this model. The spire, whorls and body as one. (e.g. model 1)
H_2	2 modules { landmarks 53-120} { landmarks 1-52,121-150}	Spire and body whorl was considered are one module while the apertural area as the other module. (models 3 and 7)
H_3	2 modules {landmarks 96-111}{ landmarks 1-95,112-150}	The spire was considered as one module; and the body whorl and apertural area as the last module. (models 4, 6, 8)
H_4	3 modules {landmarks 96-111}{ landmarks 53-95,112-120}{ landmarks 1-52,121-150}	Partitions were determined based on the Model 0 of the <i>P. canaliculata</i> population. The spire being the first module; followed by the body whorl; and the last module was the one occupying the apertural area. Landmark points were of exclusive correspondence to a module for every model. The genes expressing the spire, whorls and apertural area are assumed to affect developmental and genetic modularity of the shell. (models 5, 9, 10)

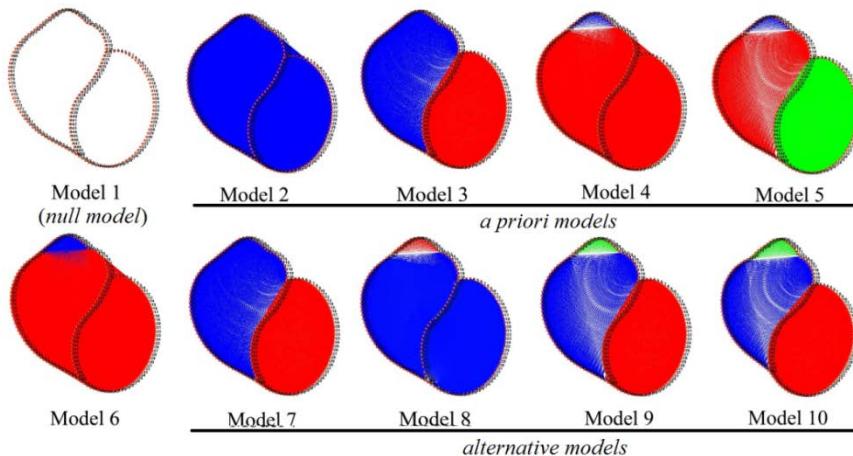


Fig. 4: Model 1 (Hypothesis model); 2-5 (A priori models) and models 6-7 are the alternative models

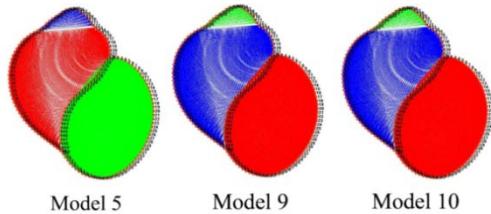


Fig. 5: Top three best fit models for both male and female of *P. canaliculata*. (Note: the 3 models were the same)

Table 2: Computed γ -value and P-value for the ventral shells of both male and female of Golden Apple Snails, *P. canaliculata* and the top three (3) best fit models

Sex	Location	Rank	Model	γ -Value	p-value
Male	Lake Dapao	1	5	0.22471	1
		2	10	0.22471	1
		3	9	0.22628	1
	Lake Lanao	1	9	0.26611	0.97
		2	5	0.26972	0.995
		3	10	0.26973	0.995
Female	Lake Dapao	1	5	0.20724	1
		2	9	0.20966	1
		3	10	0.20967	1
	Lake Lanao	1	9	0.22894	0.911
		2	5	0.29136	0.968
		3	10	0.291365	0.967

were separate modules (Fig. 5). The consistency observed in the best fit model for the shell indicates the absence of sexual dimorphism thus both sexes follow the same morphological pattern. The resulting fair consistency of the observed patterns of hypothesized developmental modules implies that shell of *P. canaliculata* is highly conserved and that these three parts of the organism's shell are coordinated in their sizes and shapes to make up a functional whole. It can be argued from this study that the spire, body and aperture parts of the GAS shell are integrated with each other because they develop, function and evolve jointly. Integration of these parts is inevitable because they share developmental precursors that are packed together

tightly in the shell, because different functions place demands on different parts of the shell and because all parts of the shell share an evolutionary history. Yet this integration is not total, but is structured as modules that are relatively independent within the overall integration of the shell as a whole (Klingenberg, 2008, 2010).

CONCLUSION

Results of the study show that the shell of *P. canaliculata* showed the same best supported model with the shell divided into 3 separate modules comprising the shell spire, body whorl and the whole apertural area. Integration of these parts shows no variations existed between sexes and populations indicating compartmentalization in the shell is highly conserved and that these three parts of the organism's shell are coordinated in their sizes and shapes to make up a functional whole.

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