Sexual dimorphism in the shell shape of the golden apple snail, *Pomacea canaliculata*

[1]Fervil F. D. Tripoli, [1]Jessie Genecera, [1]Marc N. Matela, [1]Keeneth J. D. Fanuga, [1]Dailyn G. M. Velasco, [1]Rosemarie S. Landero, [1]Renalyn B. Cataluña, [2]Mark A. J. Torres, [3]Elani A. Requieron, [3]Ronaldo T. Bigsang

[1] Department of Secondary Education, College of Natural Science and Mathematics, Mindanao State University, General Santos City, Mindanao, Philippines; [2] Department of Biological Sciences, College of Science and Mathematics, Mindanao State University – Iligan Institute of Technology, Iligan City, Philippines; [3] Science Department, College of Natural Sciences and Mathematics, Mindanao State University Barangay Fatima, General Santos City, Philippines. Corresponding author: F. F. D. Tripoli, simplifire11@yahoo.com

Abstract. *Pomacea canaliculata* is a gastropod species that is highly recognized as invasive agricultural pests of rice in Asia particularly in the Philippines. This study was conducted to identify the possible sexual dimorphism and describe the extent of variations in shell shapes of *P. canaliculata* using conventional measurements of the geometric morphometric analysis. Using TPS software, the GAS samples collected in Barangay Lagao, General Santos City, Philippines were landmarked and processed. The output data was then analyzed using relative warp analysis, canonical variance analysis (CVA), Kruskal-Wallis and discriminant function analysis (DFA) of PAST software to determine the differences in the shell with respect to their sexes. Based on the results gathered, the MANOVA p-value is all 1, which means that there is no significant difference between male and female. This implies that there is no shape variation between sexes according to dorsal, ventral and whorl morphology.
Key Words: Landmark-based, geometric morphometric analysis, shape morphology, gastropods, Mindanao.

Introduction. *Pomacea canaliculata* is a common species that belongs to the family of Ampullariidae, which is also known as golden apple snail. This species have various adaptations that enable them to withstand a wide range of environmental conditions, primarily in tropical and sub-tropical areas where they inhabit swamps, ditches, ponds, lakes and rivers (Bronson 2002). Also it is said to be one of the worst invaders in recent time in the Southeast Asian region, and also introduced as a food to be eaten (Cowie 2002; Dapar et al 2014). It has been found in Taiwan since early 1980s (Liu et al 2006), in Japan since 1981 (Fujio et al 1991), in the Philippines since either 1980 or 1982 (Mochida 1991). *P. canaliculata* is one of the pests found in the rice fields and cause major problems to the farmers (Catindig & Heong 2006). Since then, there have been claims that *P. canaliculata* infestation has decreased due to the spread of integrated management approaches (Cagauan & Joshi 2003). *P. canaliculata* is easy to determine in a way that they group together making them easy to see, they also have characteristics that are very unique for a snail unlike the other family.

The snail is globular in shape, and can grow to 40-60 mm in height and 40-75 mm in wide and it could reach 150 mm in length. Environmental condition is a great factor in their growth, and it varies on certain climate and season. Mostly grows rapidly during spring and summer, due to the presence of water. Some apple snails are usually yellow, brown and green. Its whorls could be five or six, and separated by a deep intended suture. The aperture is large and oval shaped with males having a rounder aperture than females. However, females in the adult stage are overall larger than males. Operculum which is a great help for them to survive is moderately thick, corneous, concentric and light to dark brown in color. The operculum is retractable at the shell opening. The body

of the snail can vary in color from yellow to brown and almost black. Siphons are yellow in color and its tentacles are curled when in rest. *P. canaliculata* is similar to other family of canaliculata but has some variations when it comes to the color of egg, shell size and its angle of opening (Tamburi & Martin 2009).

P. canaliculata egg may rise from 200-600 in numbers. The eggs are attached to an object above the water surface and red in color, that is group together in compact. Incubation last for two weeks, then 12-25 days they become juvenile. *P. canaliculata* becomes full grown and sexually mature 45-59 days later after they are hatched. Summer is peak season when it comes to their reproduction and food availability can affect their mating. 2 months - 3 years is the period for their life cycle to reproduce. Their life cycle last depending on the environmental conditions they live. If the conditions are favorable they continue to reproduce which results shortening of their life span. During tough condition, they focus more on surviving, thus they bury their self in mud and decrease their metabolism which enables them to prolong their life (Holswade & Kondapalli 2013). Unlike the other types of freshwater snails namely: ramshorn (*Planorbarius corneus*), trumpet snail (*Melanoides tuberculata*), and pond snail (*Lymnaea stagnalis*), *P. canaliculata* is not hermaphrodite. Because of this, it is hard to determine whether they are male or female. Shell morphology has various details in their architecture that contributes effectively to gastropod identification and classification (Moneva et al 2012a,b,c). The *P. canaliculata* is considered to be a variable species with shell shape variability despite conflicting environments (Torres et al 2013; Torres 2008). Geometric morphometrics could be a great help to determine the gender of the said species and to determine the sexual dimorphism of *P. canaliculata*.

One of the advanced methods developed in studying images of biological structures is the method of geometric morphometrices which is involves image analysis using the tools of computer science, statistics, geometry and bioinformatics (Unito-Ceniza et al 2012). GM is defined as the study of form of a certain species in two or three dimensional spaces allowing in-depth investigation of morphological change. GM intends to study changes in size and shape, taking as a point of departure displacements in the plane (2D) or space (3D) of a set or morphometric landmarks (Requieron et al 2012; Yousif 2012). Landmark-based geometric morphometric methods begin with the collection of two- or three-dimensional coordinates of biologically definable landmarks (Adams et al 2004). Using GM, we may able to determine if the snail is male or female. Compared to traditional linear measurement, geometric morphometrics gives a higher morphological that is accurate and precise in terms of acquiring the desirable exact shape of the subject. This study focuses on the shape and variation of *P. canaliculata* and limited only in knowing its sexual dimorphism.

Material and Method

Study area. The study was conducted from August to October 2014, along the Conel Crossing Road Brgy. Lagao, General Santos City, Mindanao that is geographically lying 6° 8'36.18"N and 125°11'22.53"E (Figures 1 & 2).
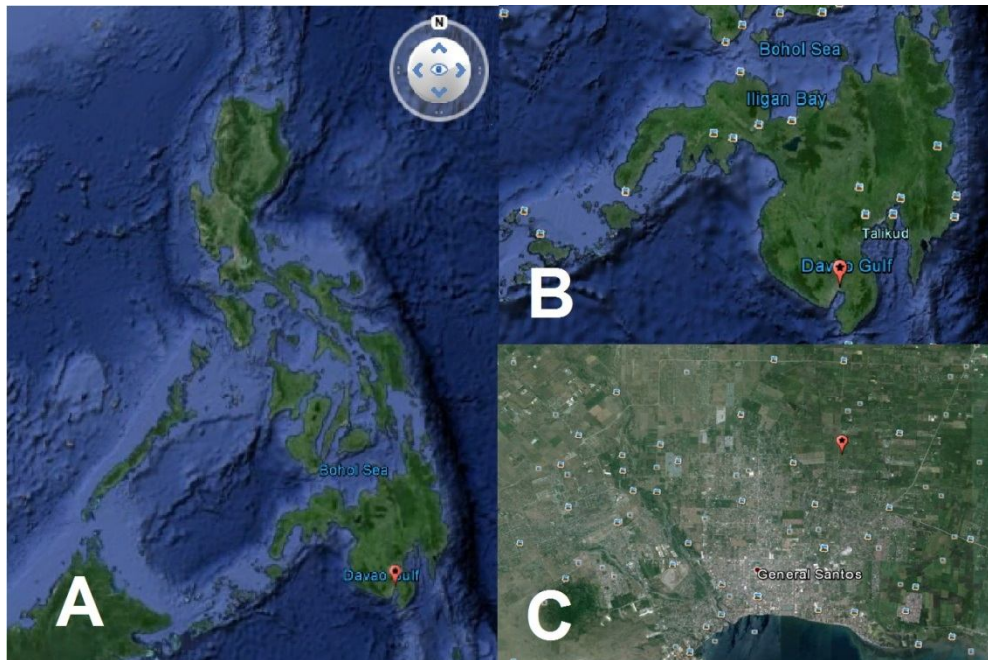
Figure 1. Study area Philippines (A), Mindanao island (B), and General Santos City (C) (Source: Google Earth 2015).
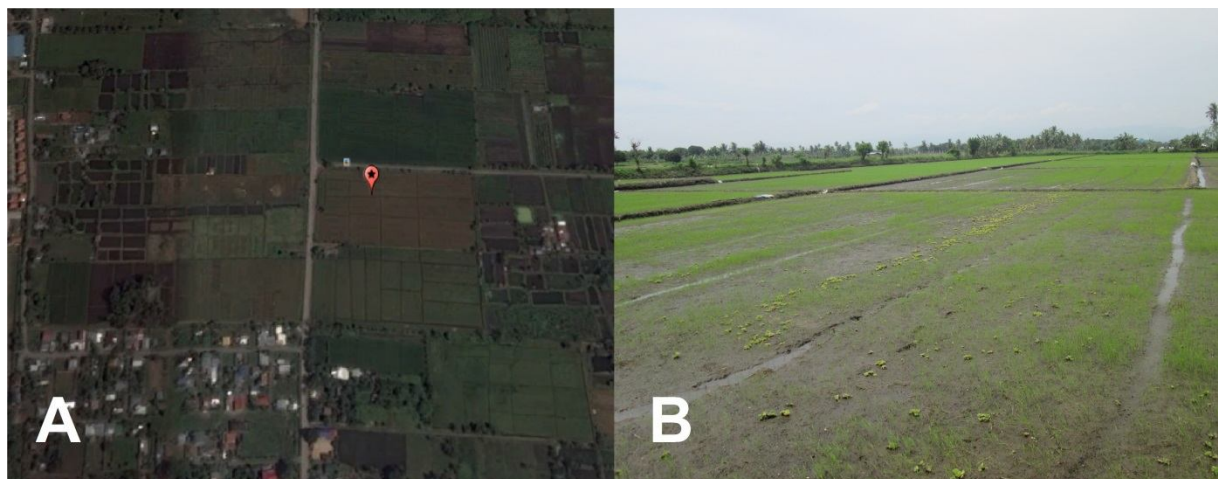

Figure 2. Location of the sampling site (Source: Google Earth 2015).

Collection of P. canaliculata. A total of 80 individuals consisting of 40 male and 40 female were collected from an irrigation of a specific rice field at DBP home Subdivision, Napal 23, Conel Crossing Road, Barangay Lagao, General Santos City, Philippines (Figure 2). The specimens were imaged using Canon Power Shot A3500 IS in a consistent orientation with the dorsal, ventral and top view of its shell and a ruler at the top to measure its length (Figure 3). To minimize the error and to have a precise data, careful image capturing and shell measurement acquisitions were practiced. The internal structure of a male and female *P. canaliculata* is shown in the Figure 4. Meta files of the photographs of the shells were made possible by using the package TPS version 2 software (Rolfh 2008).

Figure 3. Photograph of male (up) and female (bottom) *Pomacea canaliculata* dorsal, ventral and top view of its shell (Original).
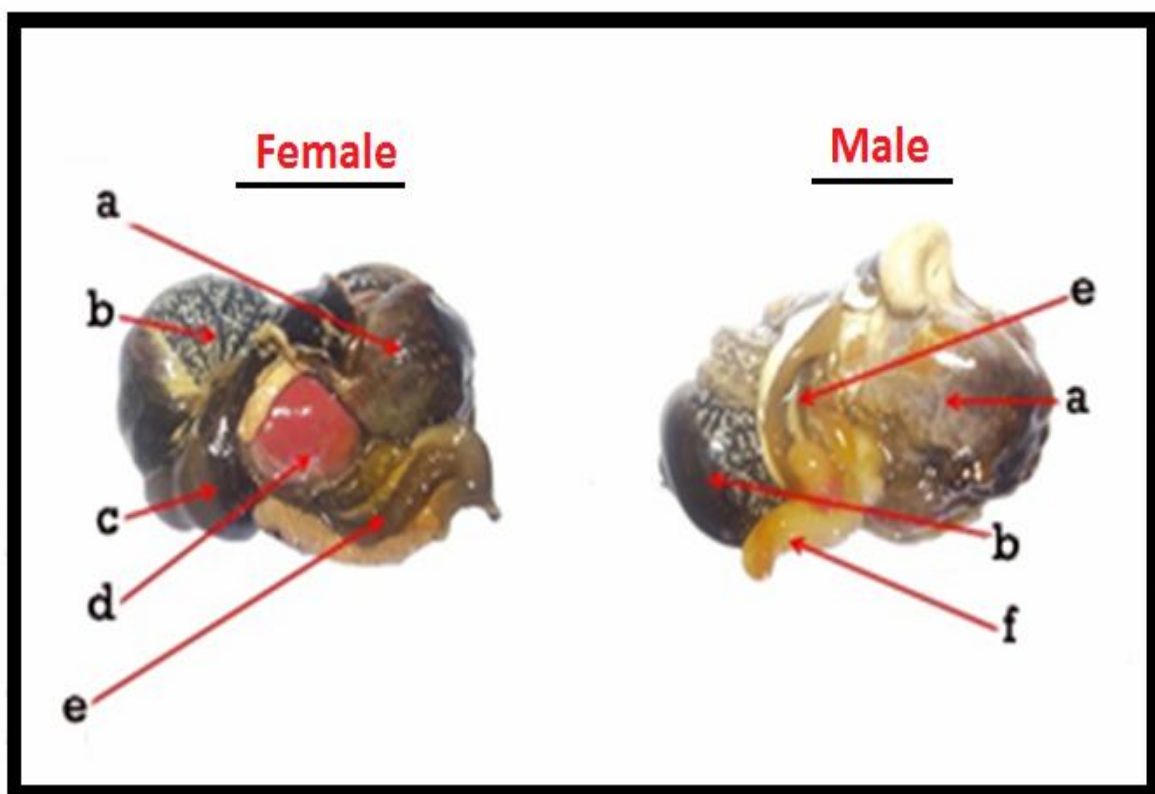

Figure 4. Photograph of female (left) and male (right) *Pomacea canaliculata* internal parts namely: (a) mantle, (b) digestive gland, (c) stomach, (d) kidney, (e) gill, (f) penile sheath (Original).

Landmarking the homologous points. The shell shape was studied using a landmark-based methodology that eliminates the effect of variation in the location, orientation, and the scale of the specimens (Demayo et al 2012). Seventeen (17) landmarks were located

along the dorsal region (Figure 5a), fourteen (14) landmarks were at the ventral region of the shell (Figure 5b) and thirteen (13) landmarks for the top view of the shell (Figure 5c).

The landmarks were carefully selected for it to provide a comprehensive summary of morphology and shape disparities among the samples. Some of the landmarks represent distinct anatomical loci that were homologous from one form to the other. Some of the points represented conserved topological positions relative to other landmarks including those that lie on junctions between two structures.

The landmarks used in this study fall into three classes. Type 1 landmarks were those located on discrete juxtapositions of structures such as junctions of the spires in the GAS shell. Type 2 landmarks that represent points at maximal curvatures were also collected such as those that lie in some parts of the GAS shell. Helping points or Type 3 landmarks include extremal points or points identified (or constructed) by reference to other features/landmarks such as points that are found furthest from another structure or point, intersection or division of hypothetical planes or the top of the shell.

The shell of the apple snail is spherical or heliciform or elongate ovate shell form having three to five sutures with wide oval or circular aperture. It has no siphonal canal and the outer lip of the aperture is not reflected. The shapes of the curves in the shell of the snails provide important biological information but lack obvious points at which they should be samples. Such points were regarded as "deficient" or "semi" landmarks in recognition of the fact that the dimensionality of their coordinate locations exceeds that of their biologically relevant content (Torres et al 2011). To solve this problem, "semi" landmarks were allowed to slide in the preprocessing step along directions of arbitrary variability to minimize bending energy using the sliding-landmark support in the tpsRelw software (Torres et al 2011).

Seventeen (17) anatomical landmarks were selected as this was previously defined (Mahilum & Demayo 2014) and are located along the outline of the dorsal (Figure 5A) region. Landmarks 1 and 14-17 represent the whorl of the shell; 2-8 are located on the outer lip while 9-13 were located on the outer whorl.

At the ventral region, landmarks 1 to 3 were located on the whorls of the shell. Landmarks 4 and 5 were used to represent the outer whorl. Landmarks 6-14 define the outline of the shell opening. A total of 14 landmarks were used to summarize the ventral shape of the shell (Figure 5B). The first three landmarks are located on the whorls of the shell. Landmarks 4 and 5 describe the shape outer whorl. These landmarks were treated as pseudolandmarks and were allowed to slide during the preprocessing stage to minimize bending energy. Landmarks 6 to 14 define the outline of the shell opening. Of these, landmarks 7 to 11 are located on the outer lip while the landmarks 13 and 14 are found on the inner lip. Most of these landmarks were as pseudolandmarks.

Thirteen landmarks were used in the analysis of the shape of the shell top (Figure 5C). Of these, only three are true landmarks which include 1 and 8 on the shell opening and 13 on the last whorl of the shell top. The other landmarks were treated as pseudolandmarks and were also allowed to slide during the preprocessing stage to reduce bending energy.
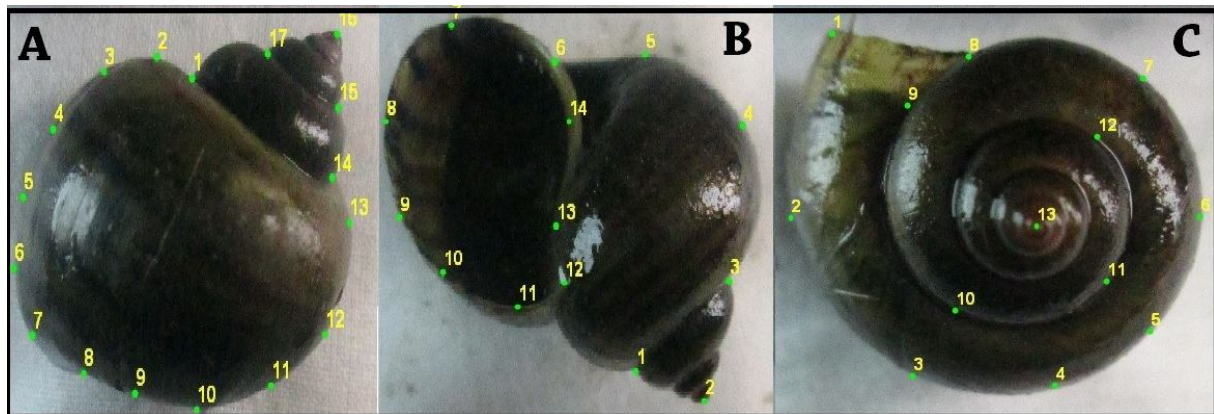
Figure 5. Landmarks used to (A) dorsal, (B) ventral, and (C) top view of the shell (Original).

Results and Discussion. The summary of the patterns of shape variation is shown through the Relative Warp Analysis. It shows the frequency histograms of the RW scores together with the grid plots of the landmark points showing the shape variation along the RW axes of male and female species (Figure 6, 7 & 8). It projects the negative and positive sides for the comparison of the variation. The topmost figure represents the mean shape of the species. The qualitative shape descriptions were summarized on Table 1 & 2. It only includes relative warp with rounded off to 5% and more variations. Moreover, descriptions of the shell variation produced by RW are shown in these tables. Table 3 shows the Kruskal – Wallis test for significant differences in the mean shapes of dorsal, ventral and whorl. Figure 9 shows the frequency distribution histogram of the degree of sexual dimorphism in *P. canaliculata* based on its shell. Figure 10 shows the CVA scatterplot of whorl of male and female *P. canaliculata*. The result of MANOVA p-value is 1, which means that there is no significant difference between male and female. This means that there is no shape variation between opposite sexes according to whorl morphology (Figure 10). These data were obtained using PAST software (Hammer et al 2009).
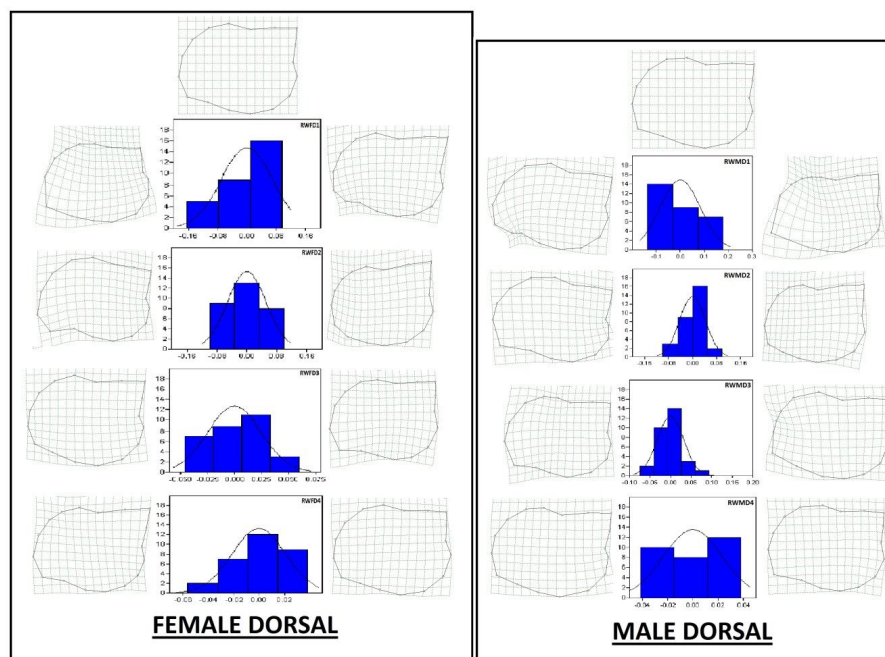

Figure 6. Summary of GM Analysis showing consensus morphology and variations in shape of dorsal shell by histogram and box plot of female and male *Pomacea canaliculata*.
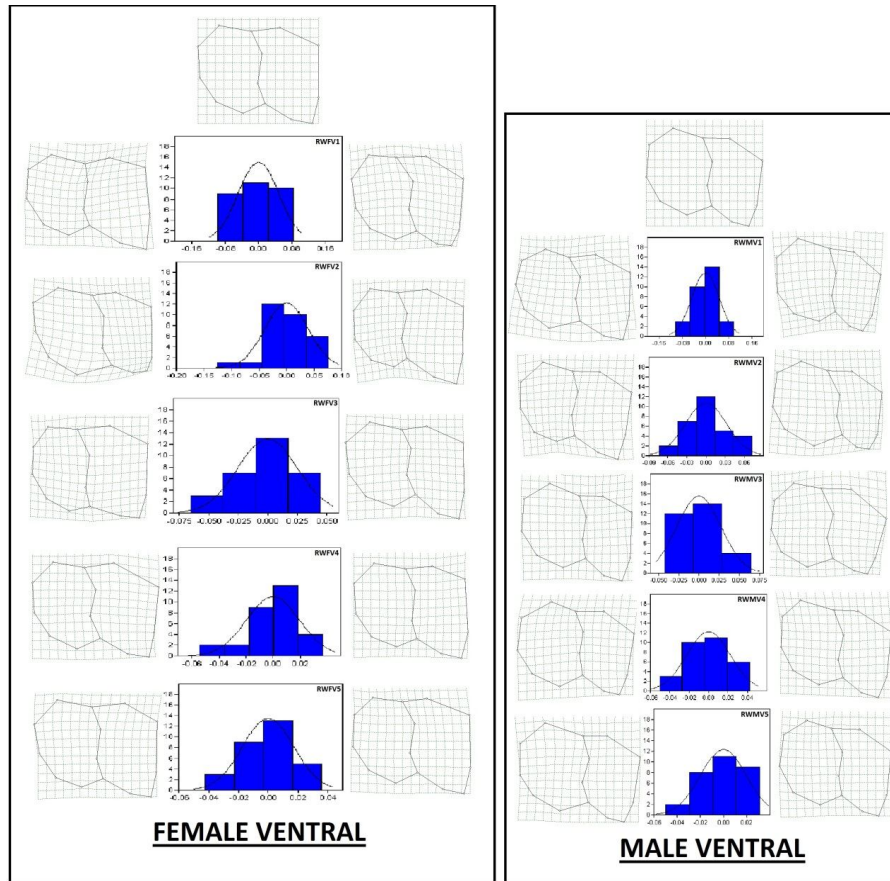
Figure 7. Summary of GM Analysis showing consensus morphology and variations in shape of ventral shell by histogram and box plot of female and male *Pomacea canaliculata*.
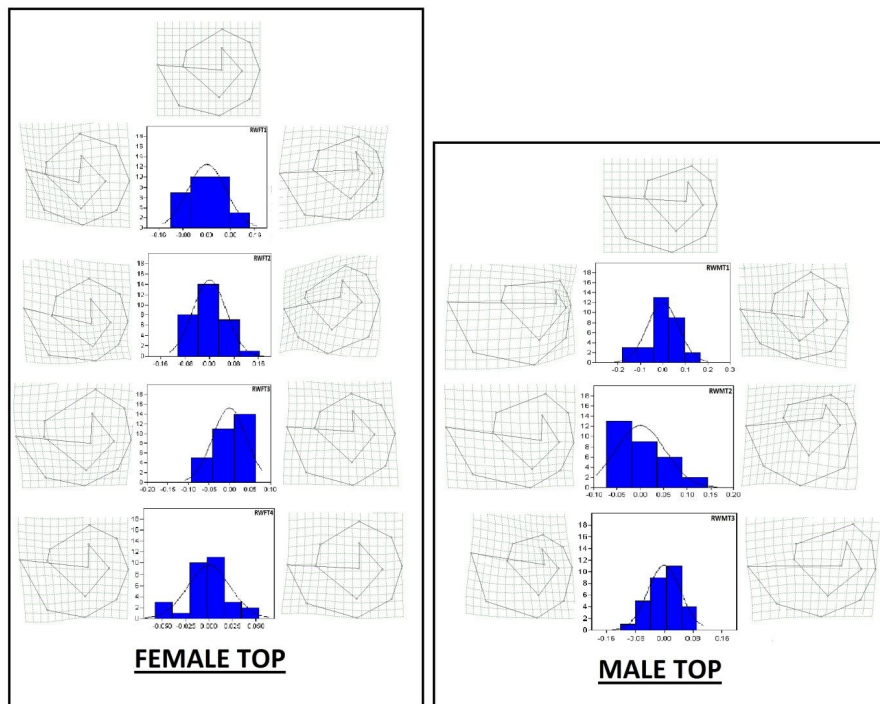

Figure 8. Summary of GM Analysis showing consensus morphology and variations in shape of top shell by histogram and box plot of female and male *Pomacea canaliculata*.
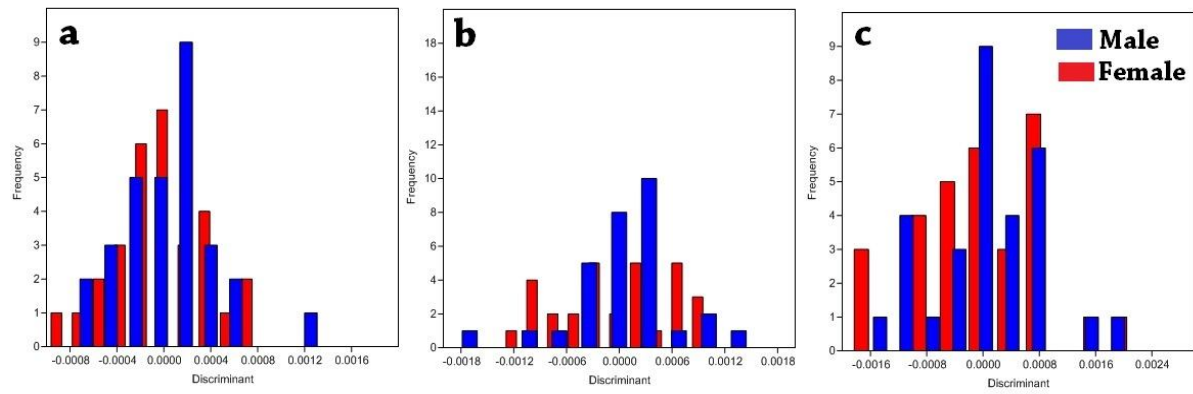
Figure 9. Frequency distribution histogram showing variation in dorsal (a), ventral (b) and whorl (c).

Table 1
Percentage variance and overall shape variation in the dorsal and ventral of female *Pomacea canaliculata* as explained by significant relative warps

	Variation %	Dorsal	Variation %	Ventral	Variation %	Whorl
1	49.67%	RW1 indicates differences in the height of spires. Samples along the positive axis exhibit elevated spires while samples along negative axis show shorter spire length.	36.75%	RW1 illustrates the distinction in the height of the spires in the apical region of the shell. Low negative scores have higher and pointed spire height compared to the samples with high positive scores which have shorter spires.	36.48%	RW1 samples along the negative axis possess a narrow opening with a more pronounced outline while the positive axis exhibits a wider opening.
2	26.81%	RW2 describes the outer lip. Low negative scores are narrower compared to the positive scores which are wider.	24.49%	RW2 suggests the difference in body whorl. High positive scores signify narrower body whorls while low negative scores mean broader body whorl.	26.32%	RW2 represents the tip of the opening of the shell. The negative axis displays a more pointed shell opening while the positive axis displays a blunter opening.
3	6.31%	RW3 suggests the difference in the outer lip margin of the shell. In contrast with the RW2.	10.17%	RW3 represents an observation that the positive scores have a narrower spires and having a wider shell opening compared to the negative ones.	15.27%	RW3 shows the largeness of the outer shells. Both negative and positive axis exhibits a larger outer shell compared to the median. The only difference is that the negative axis has a narrow opening while the positive has a wider opening.
4	4.60%	RW4 suggests that results are concentrated towards the mean shape and are unimodal in distribution.	6.13%	RW4 indicates a similar result with RW3 but with some differences. Negative scores suggest a more detailed outline than the positive.	4.89%	RW4 possessed similar result with RW3 but has some visible differences. Negative axis has a wider outer shell just below the lip opening while positive has a slimmer outer shell.

	Variation %	Dorsal	Variation %	Ventral	Variation %	Whorl
5	-	-	4.75%	RW5 accounts for the results that are also concentrated towards the mean shape.	-	-

Table 2
Percentage variance and overall shape variation in the dorsal and ventral of male *Pomacea canaliculata* as explained by significant relative warps

	Variation %	Dorsal	Variation %	Ventral	Variation %	Whorl
1	58.75%	RW1 indicates differences in the outer lip. Samples along the positive axis exhibit pointed structures while samples along negative axis show little rounds.	36.18%	RW1 illustrates the distinction in the spires in the apical region of the shell. High positive scores which have shorter spires compared to the samples with low negative scores that have higher spire height.	41.32%	RW1 indicates the difference in the lip opening of the shell. Negative axis has a long lip opening with a flat inner shell while the positive axis has a more round inner shell.
2	14.92%	RW2 represents the total shape variation. Low negative scores are broad compared to the positive scores which are petite.	17.33%	RW2 attributed to difference in body whorl. High positive scores signifies have narrower and less pronounced body whorls while low negative scores mean broader and more pronounced body whorl.	24.07%	RW2 illustrates the same structure with 1 with small variations. Negative axis has a wider, rounder inner shell while positive axis has a smaller but round inner shell.
3	8.67%	RW3 suggests contrast result with RW2. Positive scores illustrate pointed and higher height of spires.	12.38%	RW3 represents the difference in body whorl. Low positive scores signifies have narrower and less pronounced body whorls while high negative scores mean broader and more pronounced body whorl.	17.62%	RW3 still have some similarity with RW1. However, negative axis is smaller compared t median but has a wider opening. Positive axis on the other hand, is larger, with a wide opening and a flatter inner shell compared to the median.

	Variation %	Dorsal	Variation %	Ventral	Variation %	Whorl
4	4.54%	RW4 indicates differences in the height of spires. Samples along the negative axis exhibit elevated spires while samples along positive axis show shorter spire length.	8.46%	RW4 indicates that both positive and negative scores don't have much difference.	-	-
5	-	-	6.44%	RW5 accounts for the results that are also concentrated towards the mean shape.	-	-

Table 3

Results of Kruskal-Wallis test for significant differences in dorsal and ventral mean of both male and female *Pomacea canaliculata*

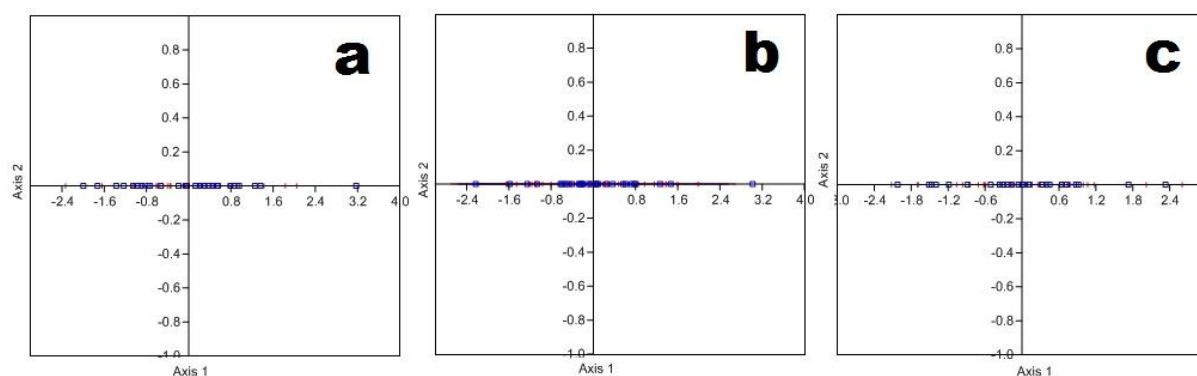| Relative warp	Dorsal value	Relative warp	Ventral value	Relative warp	Whorl value
		1	0.9498		
		2	1		
1	0.9519	3	0.9979	1	0.7528
2	0.9039	4	0.9226	2	0.9435
3	0.8011	5	0.668	3	0.5655
4	0.9226	6	0.9247	4	0.566
5	0.8133	7	0.9018	5	0.5642
6	0.8378	8	0.8337	6	0.4866
		9	0.9372		
		10	0.862		

Figure 10. CVA scatterplot of the dorsal (a), ventral (b) and whorl (c) of male (blue) and female (red) of *Pomacea canaliculata*.

Conclusions. The results of the current study strongly suggest that there is no significant sexual dimorphism exhibited by *P. canaliculata* snails in Brgy. Lagao, General Santos City in terms of their shell morphology. This was made possible as it was revealed by the landmark-based geometric morphometric analysis. Similarities may be environmental adaptation and not sexual dimorphism, since both male and female *P. canaliculata* are subjected under the same conditions. GM is an aid for identifying variation and similarities of shapes between sexes of the same species. It has tremendous advantage for it serves the purpose of visualization and interpretation of results. As a conclusion, several factors like sexual selection and the environmental pressures were hypothesized that these are not influencing the differences contributed to the sexual dimorphism of *P. canaliculata*. Hence, relative warp analysis had been a useful mean to determine morphological variations of the species.

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Authors:
Fervil Fritz Dedicatoria Tripoli, Mindanao State University, College of Education, Department of Secondary Education, Philippines, Barangay Fatima, General Santos City 9500, e-mail: simplifire11@yahoo.com
Jessie Genecera, Mindanao State University, College of Education, Department of Secondary Education, Philippines, Barangay Fatima, General Santos City 9500, e-mail: genecerajayssie@yahoo.com
Marc Noel Villareal Matela, Mindanao State University, College of Education, Department of Secondary Education, Philippines, Barangay Fatima, General Santos City 9500, e-mail: marc27kje@gmail.com
Keeneth Joy Diego Fanuga, Mindanao State University, College of Education, Department of Secondary Education, Philippines, Barangay Fatima, General Santos City 9500, e-mail: joy_heartatruck03@yahoo.com
Dailyn Grace Medilo Velasco, Mindanao State University, College of Education, Department of Secondary Education, Philippines, Barangay Fatima, General Santos City 9500, e-mail: dailyngracevelasco@gmail.com
Rosemarie Soldevilla Landero, Mindanao State University, College of Education, Department of Secondary Education, Philippines, Barangay Fatima, General Santos City 9500, e-mail: rosemarielandero@rocketmail.com
Renalyn Bonifacio Cataluña, Mindanao State University, College of Education, Department of Secondary Education, Philippines, Barangay Fatima, General Santos City 9500, e-mail: renalyncatalua@yahoo.com
Mark Anthony Jariol Torres, Mindanao State University – Iligan Institute of Technology, College of Science and Mathematics, Department of Biological Sciences, Philippines, Iligan City 9200, Andres Bonifacio St., e-mail: torres.markanthony@gmail.com
Elani Antonio Requieron, Mindanao State University, College of Natural Sciences and Mathematics, Science Department, Philippines, Barangay Fatima, General Santos City 9500, e-mail: elani_requieron2003@yahoo.com
Ronaldo Tilan Bigsang, Mindanao State University, College of Natural Sciences and Mathematics, Science Department, Philippines, Barangay Fatima, General Santos City 9500, e-mail: ronbigz@yahoo.com