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Research Article

Applications of geometric morphometric analysis in describing sexual dimorphism in shell shapes in *Vivipara angularis* Muller (Family Viviparidae)

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Abstract. Prior studies have shown different insights about sexual shell dimorphism in the snails of family Viviparidae. This study was conducted to evaluate and determine the occurence of sexual dimorphism and shape variation in the shells of the viviparid snail, *Vivipara angularis* Muller, from three characters (ventral/aperture, dorsal, and top/whorl) using geometric morphometric analyses. All the three shell characters exhibited significant sexual shell dimorphism, this may be due to adaptations for brooding of the viviparid snails. Results indicate employment of different shell characters subjected to geometric morphometric analysis other than the aperture, in order to discriminate between sexes of the snails accurately.

Key Words: sexual dimorphism, shell shape variation, *Vivipara angularis*, geometric morphometric analysis.

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Introduction

Sexual dimorphism is the determining feature of organisms in which male and female differ. It can be defined as a systematic distinction in phenotypic traits between individuals of different sex in the same species. It is common in nature and often attributable to sexual selection, which indirectly results in the ornament expression in both sexes as a consequence of genetic correlation (Johnsen *et al* 2003). While this difference is common across living organisms, Purchon (1977) believed it to be rare in mollusks (see citation in Minton & Wang 2011). Sexual dimorphism in these phyla is traditionally quantified through straight-line measurements and ratios. Recently, geometric morphometric analysis has been applied for examinations of snail shell dimorphism, to provide descriptive analyses of shell shape (Samadi *et al* 2000; Galliguez *et al* 2009; Minton & Wang 2011). Applications of geometric morphometric methods have the 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).

One group of mollusks where sexual dimorphism has been explored in detail is the family Viviparidae, a freshwater brooding snail. Distinction between sexes in *Viviparus*, a member of family Viviparidae, has been documented as differences in size. However, shape differences are considered to be non-existent as suggested by Falniowski *et al* (1998) a finding the researchers reported "… confirmed the fact that there is no sexual dimorphism in *Viviparus* as far as the shell is concerned" as cited by Minton & Wang (2011). Nevertheless, Minton & Wang (2011) test the statement made by Falniowski *et al* (1998) using geometric morphometric analysis of the *Viviparus subpurpureus* shell shape and accounted "…shape variables without potential confounding effects of size were explored in *Viviparus subpurpureus*, sexual dimorphism was apparent" (Minton & Wang 2011), suggesting presence of sexual dimorphism in *Viviparus*. On the other note, a study of Galliguez *et al* (2009) of *Vivipara angularis*, also a member of freshwater brooding snail of family Viviparidae in the Philippines, using geometric morphometry through modularity and shell shape integration confirmed the absence of sexual dimorphism in the species and reported "… the same pattern of modularity is observed in both male and female populations of *V. angularis* Muller indicating the absence of sexual dimorphism in terms of modularity and integration of the shell" (Galliguez *et al* 2009).

With this background, this study was conducted to test different statements made by Falniowski *et al* (1998), Minton & Wang (2011), and Galliguez *et al* (2009) regarding sexual dimorphism of the mollusks of family Viviparidae. It aimed to explore the potential for sexual dimorphism and understand variations in the viviparid snail, *Vivipara angularis* based on its shell (apertural/ventral, dorsal, whorl/top) shape patterns. Examination of the shell shape variation was made using geometric morphometry by means of Relative Warp Analysis, Principal Component Analysis, Elliptic Fourrier Analysis and Discriminant Function Analysis.

Material and Method

A total of seventy two (33 females & 39 males) *V. angularis* snails were obtained randomly from Guillian stream Balangao, Diplahan, Zamboanga Sibugay (Fig. 1).

Figure 1. Map showing the study area, Guillian stream Balangao, Diplahan, Zamboanga Sibugay.

Shells were photographed by a digital camera. Images of the shell will always be in the same position with the columella at 90^0 of the x-axis in an aperture view or in the orientation in which the apex is visible. Obtained images were then subjected to geometric morphometric methods. In this study, landmark and outline-based geometric morphometric method were used to obtain detailed shell shape pattern information. In general, geometric morphometric methods provide greater power than the traditional methods because the position of the landmarks can be retained and can be graphically reconstructed. Meaning, it preserves geometry of object studied and it allows visualization of shape differences between specimens and between group means in specimen shape.

Digital images (ventral, dorsal and top view) were taken for each sample using a standardized procedure (Fig. 2).

Shell shapes were studied using a landmark-based methodology that eliminates the effect of variation in the location, orientation, and scale of the specimens. Twenty anatomical landmarks located along the outline of the ventral or apertural portion (Fig. 2a) of the shell and also twenty anatomical landmarks along the dorsal portion (Fig. 2b) of shell were defined and used. This was made possible using an image analysis and processing software Tps Dig freeware 2.12. Tps Dig facilitates the statistical analysis of landmark data in morphometrics by making it easier to collect and maintain landmark data from digitized images (Rohlf 2008).

These coordinates were then transferred to Microsoft Excel application for organization of the data into groups (based on species). 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 (Rohlf & Slice 1990 and Slice 2001). GPA was performed using the software tpsRelw, ver. 1.45. 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 (Tabugo *et al* 2010).

The top or whorl (Fig. 2c) portion of the shell were outlined with 199 outline points using tpsDig program and the tps curve outline was converted to landmarks (corresponding x and y) using tpsUtil ver. 1.38. The collective coordinates of all individuals were then subjected to different multivariate analyses, which include Principal Component Analysis (PCA), Elliptic Fourier Analysis (EFA), and Discriminant Function Analysis, using geometric morphometric computer application Paleontological Statistics (PAST) software developed by Hammer *et al* (2009). PCA was used to summarize the information of the variations and mean shapes contained in the coefficients of landmark descriptors. Moreover, the shapes of snail's shell were compared using the method (EFA) this is to observe shape variation. The Fourier coefficients were then used as variables for another multivariate method of statistical analysis in the form of (DFA), to calculate the percentage of sexual dimorphism in the shell shape pattern of *V. angularis*.

Results and Discussion

Geometric morphometrics were applied in order to determine sexual dimorphism and shape variation in the shells of *V. angularis* from three characters (ventral/apertural portion, dorsal portion, and top/whorl portion). To distinguish shell shape variation based on the characters, relative warp analysis was used (Torres *et al* 2010). Figures 3 illustrate the summary of the geometric morphometric analysis (N=72) showing the consensus morphology (ventral/apertural and dorsal portion) produced by the relative warps (RW). Projections on the left side of the histogram are considered to be variations in shell shape foreseen as negative deviations of the mean in the axis of the relative warps. Then, on the right side are variations in shell shape foreseen as positive deviations of the mean in the axis of the relative warps. The topmost figure is the mean shape of the samples obtained. The consensus morphology and variation in the (a) ventral/apertural and (b) dorsal shell shape patterns of *V. angularis* population from Guillian stream, Balangao, Diplahan, Zamboanga Sibugay was shown in Figure 3.

As depicted above (Fig. 3a), the RW explains more than 7% of the overall variation in *V. angularis* apertural shell. The first relative warp axis (RW1) explains 39.43% of the total variation observed, which describes differences in the shape of the shell opening and sizes based on shell shapes. Samples with low negative RW1 score have narrow shell opening and larger size relative to the shell shape. On the other hand, those with high positive scores along the first relative warp axis have wider shell opening and relatively small size based on the shell shapes. The second relative warp axis (RW2), which attributed to differences in the whorls, explains 22.92% of the total variance in shape. A low negative RW2 score means that a shell has less pronounced whorls.

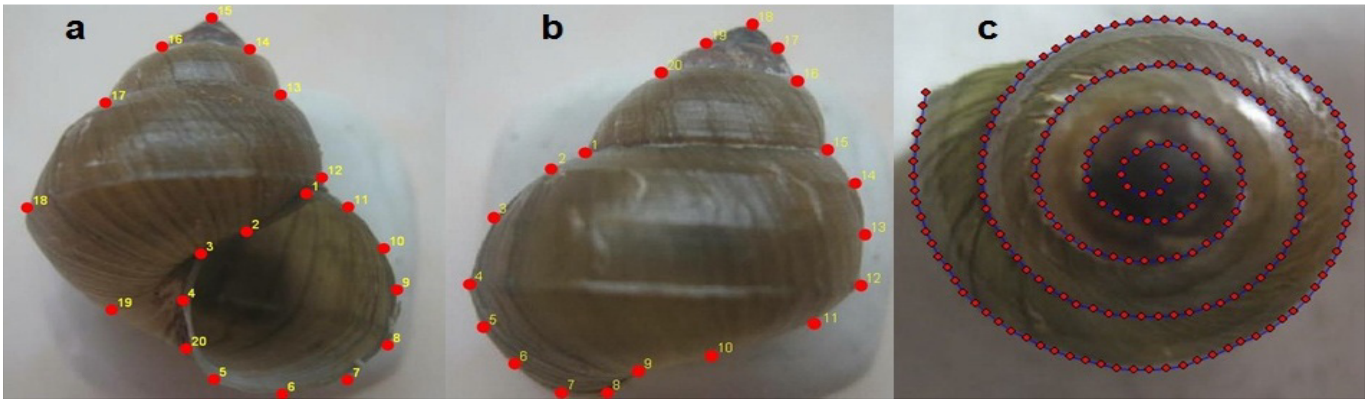

Figure 2. Landmarks used to describe the shape of the (a) ventral/aperture (b) dorsal and (c) top/whorl view of the shell of *Vivipara angularis*

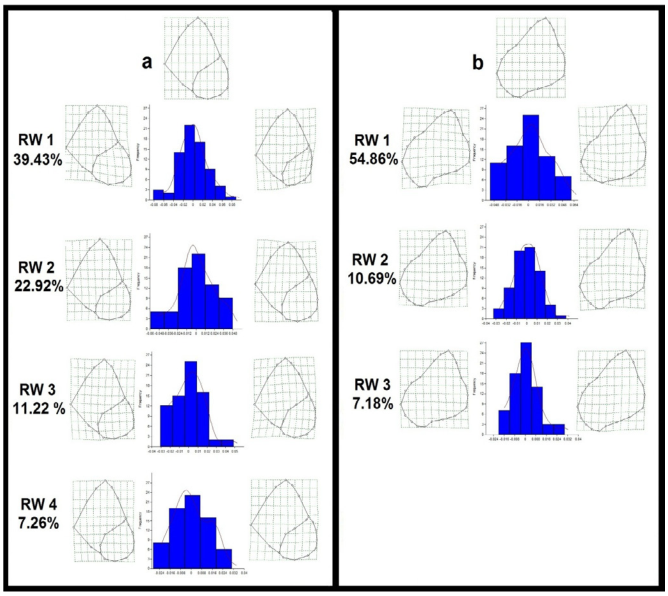

Figure 3. Summary of geometric morphometric analysis showing consensus morphology and variation in (a) ventral/apertural and (b) dorsal shell of *V. angularis* produced by Relative Warps.

Table 1. Results of the F and T test conducted on the landmark sets of the aperture and dorsal shell

Shell character		F value	p (same variance)	t value	P (same variance)
Ventral/ aperture	RW 1	1.4475	0.28744	0.96156	0.33958
	RW 2	1.0278	0.9439	0.05534	0.95602
	RW 3	1.1061	0.77575	0.52136	0.60376
	RW 4	1.6108	0.17081	0.52136	0.54068
Dorsal	RW 1	1.211	0.58331	-0.6148	0.30415
	RW 2	1.1981	0.60488	0.40007	0.69032
	RW 3	1.4737	0.25129	0.55698	0.57931

Table 2. Results of the MANOVA conducted on the landmark sets of the aperture and dorsal shell

Ventral/aperture		**Dorsal**	
	Female		Female
Male	0.943846	**Male**	0.596598

Furthermore, a sample with a high positive RW2 score has shell with less pronounced whorls. Lastly, the third and fourth relative warp (RW3 and RW4) accounts for 11.22% and 7.26% of the total shape variation respectively. For the dorsal part of the shell, relative warp analysis shows three significant relative warp scores (RW1-RW2) (Fig. 3b). RW1 explains differences in the posterior margin of the outer dorsal lip and accounts for 54.86% of the total shape variation. Samples with high positive RW1 score have the posterior margin of the outer dorsal lip depressed towards the center of the shell opening. The RW2 explains 10.69% of the total variance and describes differences in whorls.

Samples with low negative RW2 score have shell with less pronounced whorls, while high positive RW2 score has more pronounced whorls. Finally, RW3 accounts for 7.18% of the total shape variance accounting for more than 7% of the overall variation in *V. angularis dorsal* shell.

Results from the ventral/aperture (Fig. 3a) and dorsal (Fig. 3b) shell shape variation produced by the relative warps in *V. angularis* illustrates that almost all population are concentrated towards the mean shape, as demonstrated in the histogram.

For the top/whorl portion of the shell, the landmark coefficients (199 landmarks) were calculated using Elliptic Fourier Analysis (EFA) and interpreted using the multivariate Principal Component Analysis (PCA) to identify sources of variation from the whorl shell shape pattern (Torres 2008). Figure 4 illustrates the mean shell shape (top/whorl portion) of *V. angularis* (a) female and (b) male. A principal deformation from the mean shape of viviparid top/whorl part of the shell explains differences in sizes based on the shape.

The top/whorl portion of the shell obtained nine principal components (PC1-PC9) for female and ten principal components (PC1-PC10) for male individuals of *V. angularis*. These components were considered to be significant since their Eigenvalues are above the Jollifee cut-off score. However, the first and second principal components (PC1 and PC2) were only considered since the first two components provide a good summary of the variation for the top/whorl portion of the shell. PC1 and PC2 for both the female and male top/whorl portion of the shell accounted for 77% and 72% of the total variance respectively, as presented in Table 3.

To test whether sexual dimorphism based on the shell shape (ventral/apertural, dorsal, and top/whorl portion) pattern is statistically significant Discriminant Function Analysis (DFA) was performed.

Figure 4. Principal deformations from the mean shape of *V. angularis* top/whorl shell: (a) female and (b) male.

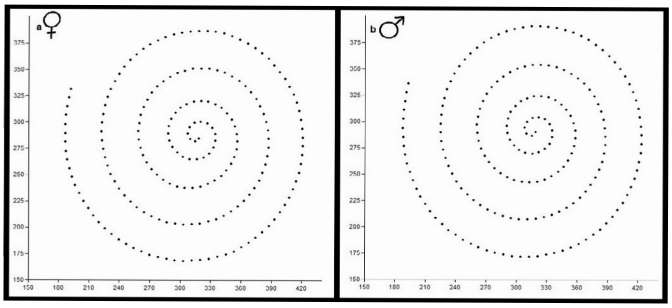

Table 3. % variance value of the significant components in the top/whorl portion of the shell of *V. angularis*

	Female		Male	
	Eigenvalue	Variance (%)	Eigenvalue	Variance (%)
Principal components				
PC1	80046.4	44.138	72597.5	40.041
PC 2	59611.3	32.87	59126.2	32.611
TOTAL		77		72

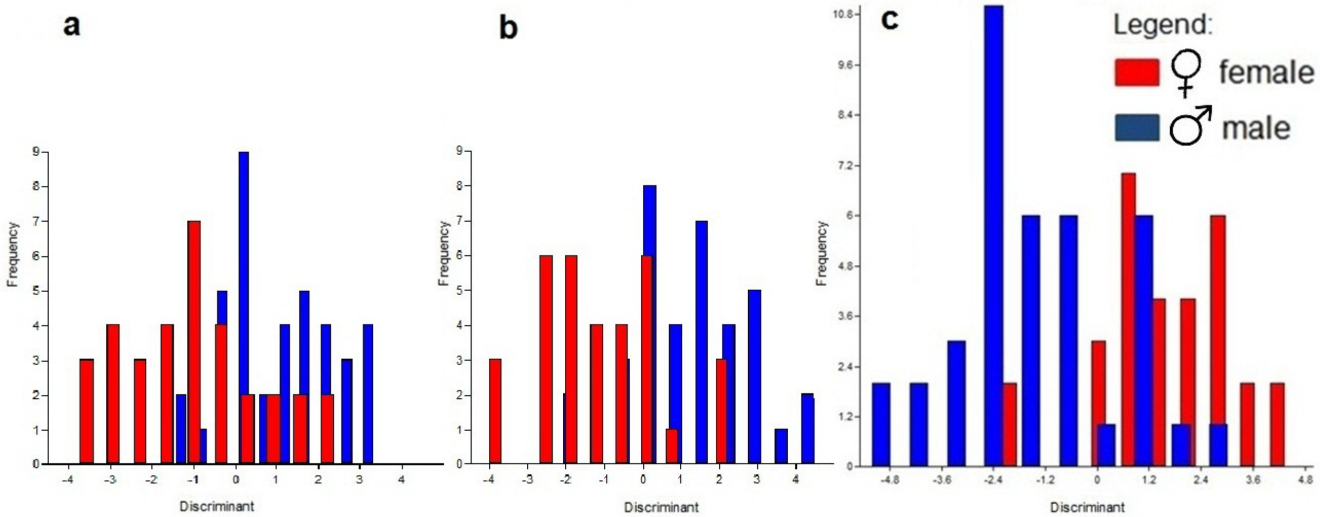

Fig. 5. Frequency distribution histogram showing the variation in the (a) ventral/apertural portion, (b) dorsal portion, and (c) top/whorl portion in the shell pattern of the female (red) and male (blue) species of *V. angularis*

Table 4. Proportion of variation with the discriminant function scores of the ventral/apertural, dorsal, and top/whorl portion of the *V. angularis* shell

	Ventral/Apertural shell			Dorsal shell			Top/Whorl shell		
	Female	Male	correctly classified (%)	Female	Male	correctly classified (%)	Female	Male	correctly classified (%)
Female	26	7	78.8	27	6	81	28	5	85
Male	8	31	79.5	8	31	79.5	8	31	79
	Total		79	Total		80	Total		82
Hotelling's t2: p (same)			0.9438	Hotelling's t2: p (same)		0.5966	Hotelling's t2: p (same)		

Figure 5 graphically showed the frequency histogram of the degree of sexual dimorphism of *V. angularis* based on three shell shape characters: (a) ventral/apertural portion, (b) dorsal portion, and (c) top/whorl portion of the shell.

In all cases, the ventral/aperture (Fig. 5a), dorsal (Fig. 5b) and top/whorl (Fig. 5c) portion of the shell and the discrimination of the female (in red) from male (in blue) are not separated along the discriminant axis. Overlapping between female and male *V. angularis* is evident. However, there are instances wherein sexual dimorphism could still be present even if the overlapping occurs in the histogram. The overlap observed on the shell shape pattern from ventral/apertural, dorsal, and top/whorl portion were indicated by misclassification of the Discriminant scores between the sexes.

Illustrated in Table 4 are the percent correctly classified data based on the discriminant function scores of the ventral/apertural, dorsal, and top/whorl portion of the shell. This percentage shows how correctly female shells are classified as female and how male shells are classified as males.

Results in Table 4 have shown that there are 79% and 80% correctly classified discriminant function scores for both the ventral/aperture (78.8% of females; 79.5% of males) and dorsal view (81% of females; 79.5% of males) of *V. angularis* respectively, which is considered to be significant. These percentages are significant since values greater than or equal to 75% of the correctly classified percentage is considered to be a cut-off for variation in structures, leading to sexual dimorphism. Moreover, the discrimant function correctly classified 82% of the top/whorl portion of the shell (85% of females; 79% of males). All the three shell characters exhibited sexual shell dimorphism in *V. angularis* collected from Guillian stream Balangao, Diplahan, Zamboangoa Sibugay.

It has been accounted by Falniowski *et al* (1998) and as cited by Minton & Wang (2011) that no sexual dimorphism in shape exists in *Viviparus* sp. of family Viviparidae. However, in a separate study of Minton & Wang (2011) sexual dimorphism in *V. subpurpureus*, also a member of family Viviparidae, was evident. In the present study, results of the Geometric morphometric analyses of shell shape indicate the presence of sexual dimorphism in *V. angularis* apertural, dorsal, and whorl portion portion. This morphological distinction in the whorl/top shell may be due to adaptations for brooding of the viviparid snails. Female viviparids brood their young in the pallial oviduct situated on the right side of the snail's body. Females may then assume a more globose shape, producing a wider aperture and a more obese body whorl. This shape variation may allow females to brood more successfully (Dillon 2000 and Jokinen *et al* 1982 as cited by Minton & Wang 2011).

Prior to this study, Galliguez *et al* (2009) reported no sexual shell dimorphism based on the modularity and integration in the shape of *V. angularis* shell. Differences in the results of sexual shell dimorphism in the viviparid snail species could be attributable to the process being applied to investigate shape dimorphism were not sensitive enough to detect it. Geometric morphometric methods have greater advantage over traditional morphometry in the determination of shape variables (Reyment 1991). It involves novel approaches in analysis of multiple multivariate datasets that can be used as a supplement in the determination of shape variables.

Conclusion

Geometric morphometric analyses of three shell characters (ventral/aperture, dorsal, and top/whorl portion) indicated sexual dimorphism in the shape in *V. angularis*. The results of the study suggests that Relative Warp analysis, Elliptic Fourrier Analysis and Discriminant Function Analysis can be used as a tool for observing morphological difference in the snail's shell using various characters like ventral/aperture, dorsal, and top/whorl potion. It strongly implies that determination of sexual shell dimorphism in the viviparid snail does not only involved one character. Employment of three or more characters could elucidate shell shape variation that is useful in the determination of sexual dimorphism. Further studies using correlation of different shell shape characters is highly recommended in order to determine sexual shell dimorphism in Viviparidae.

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