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Research Article

CHARACTERIZATIONOFFRESHWATERMUSSELSPECIES,*MARGARITIFERA MARGARITIFERA* LINNAEUS 1758 USING OUTLINE-BASED GEOMETRIC MORPHOMETRIC ANALYSIS

*Roldan T. Echem, Danica P. Piñero and Al-Axel O. Joaquino

Department of Biology and Natural Science, College of Science and Mathematics, Western Mindanao State University, Normal Road, Baliwasan, Zamboanga City 7000, Philippines

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ABSTRACT

The characterization of freshwater mussels (Bivalvia) through morphological variability between and within sexes of the same species (*Margaritifera margaritifera* L.) is not possible due to morphological similarities. Classic morphometric measurements (e.g. shell length, shell width, shell height) are not sufficient to determine sexes within species. Thus, outline-based geometric morphometric analysis has been employed to characterize individual species, independent of its size. The study aimed to test the usefulness of this geometric morphometric tool to analyze shell shape divergence between and within sexes of freshwater mussel *Margaritifera margaritifera* L. A total of 75 landmarks were established for the outline curveof the outer shell contour of the mussels. Principal Component Analysis (PCA) revealed that components 1, 2, 3, and 4 provided significant summary of the characteristics of freshwater mussels and relative warp analysis will visualized shape variations by the transformation of grids. Relative warps 1 and 3 showed shape variations in the left shell valve and right valve for females and males at p-value <0.000. These shape variations may be attributed to the kind of habitat, feeding habits and inhabit a variety of substrates. Shape variations will also be attributed to the reproductive capacity of mussels that may affect the external morphology.Shape elongation of mussel is considered as a function of environment and shell obesity is influenced by individual growth.

INTRODUCTION

For the past 90 years, there has been a rapid decline in the freshwater mussel populations all over the globe. The International Union on the Conservation of Nature (IUCN) regarded freshwater mussels as one of the most decreasing species in terms of population and *argaritiferamargaritifera* fresh water mussel as one of those critically endangered species. The overall global loss is 61.5% (IUCN, 2011). Its population is generally threatened with 126 species found in the red list of the IUCN (International Union for Conservation in Nature) in 2007. Among the many reasons for their decline in the population are global climate change, habitat disturbance and destruction, acidification of water bodies such as rivers, and depletion of mussels through pearl farming activities (IUCN, 2011). Regarded as highly threatened and some already specified as endangered species, the quest for restoration and conservation of freshwater mussels has been a major challenge to the different countries and recognized organizations aiming for ecological preservation of their present status (Degermanet al., 2009). Belonging to phylum Mollusca, class Bivalvia, order Unionoida and family Margaritiferidae is represented by 180 genera worldwide, 53 of which is found in the North America and Greenland (Burton-Kelly and Hoffman, 2014). Several studies regarding freshwater mussels were already conducted and most studies aims to determine shape variations between and within groups of mussels. Geometric morphometrics is a method mostly utilized to determine how shapes vary and their covariance with other variables. The data will serve as a basis for future plans on conservation and preservation of the freshwater mussel *M. margaritifera* species. Lastly, the information may also contribute as knowledge for mussel culture.

Corresponding author: Roldan T. Echem,
Department of Biology and Natural Science, College of Science and Mathematics, Western Mindanao State University, Normal Road, Baliwasan, Zamboanga City 7000, Philippines.

METHODS

*Margaritiferamargaritifer a*samples were collected from Malubal, R.T. Lim, Zamboanga Sibugay Province (07° 42'N 122°28'E) Western Mindanao, Philippines. The study site is approximately 12 kilometers from Surabay R.T Lim, ZamboangaSibugay Province (Figure 1).

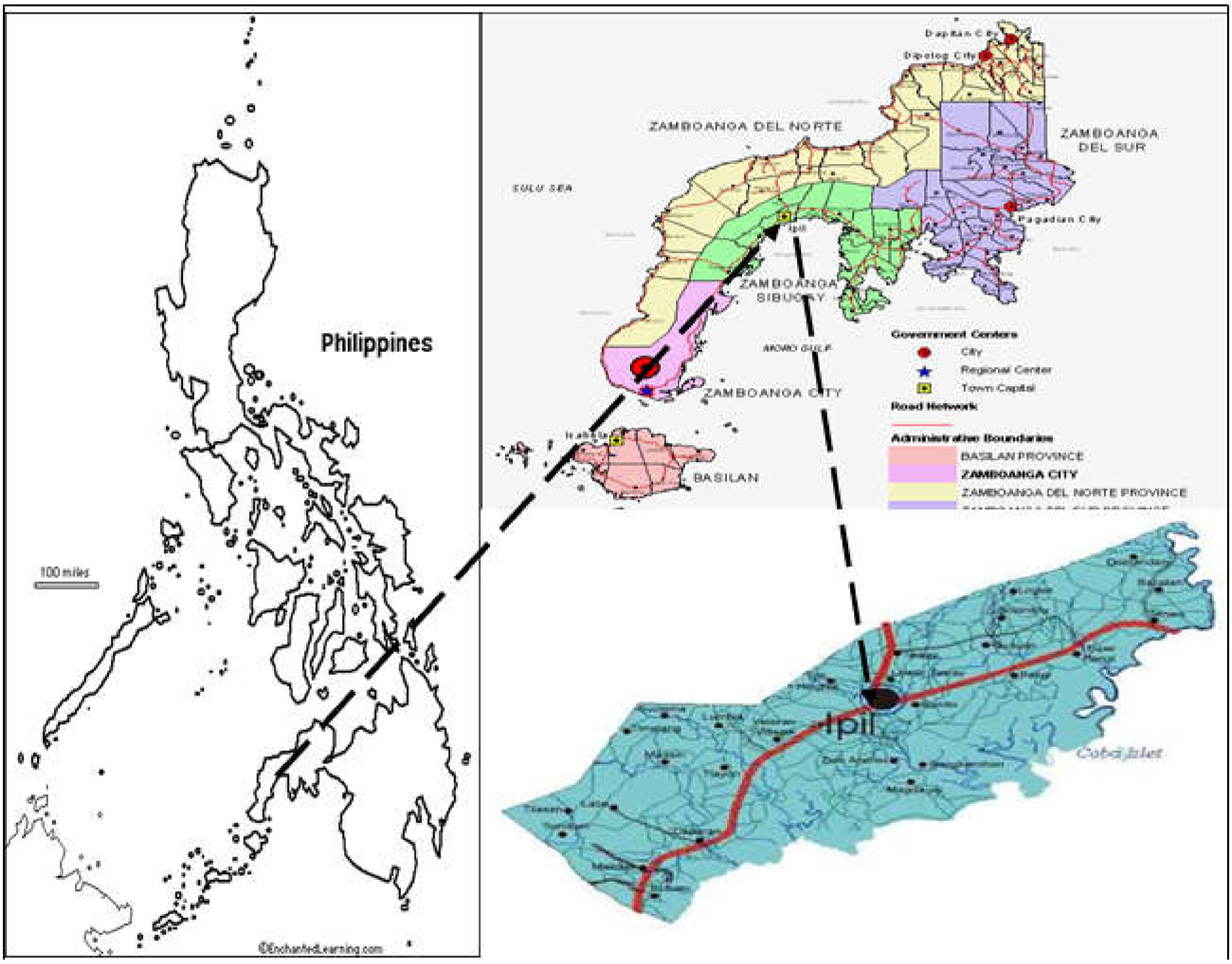


Figure 1. Map of the Philippines showing the study sites

(A) (B)

Figure 2. (A)Mussels on the onset of spawning. (B) Mussels show cloudy water indicating the presence of male gametes. The observable yellow andspherical particlesindicatethepresence of eggs for the females

Determination of Sexes

Sexes were determined using induced spawning method adapted from Allan *et al.,* 2013 by direct gonadal examination. Samples were placed in separate individual vessels to prevent uncontrolled fertilization.1 ml of 0.5 M Potassium chloride (KCl) was introduced to the mantle cavity of individual mussels through injection. Mussels with injected KCl were placed in the separate vessels with the filtrated water. Mussels were then observed for a 4-hour period.Onset of spawning was determined by the presence of eggs for females and characteristic pungent odor and cloudy milky white appearance of a substance for males. Eggs are identified as small and pale orange spheres at the base of the beaker. High output of male spawning is identified by cloudy water or a characteristic pungent odor (Figures 2A-B).

A total of 150 samples of *M. margaritifera* vary in greenish-black to brownish-black in colour with a characteristic pearly white umbo. Samples were categorized into 75 left shell valves and 75 right shell valves each sex. These were subjected into thin plate software for digitization. The morphology of *M. margaritifera* showing the pallial line, lateral side umbo and pseudocardinal tooth area (Figure 3).

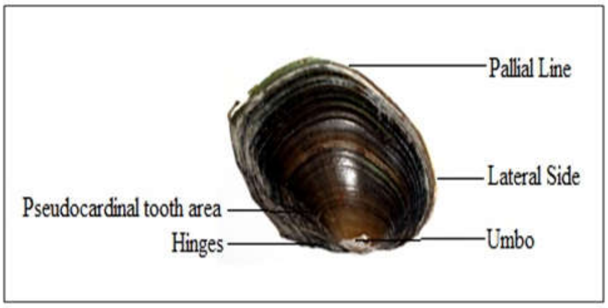


Figure 4. Outlined of the (A) left shell and (B) right shell valve of *M.margaritifera* L

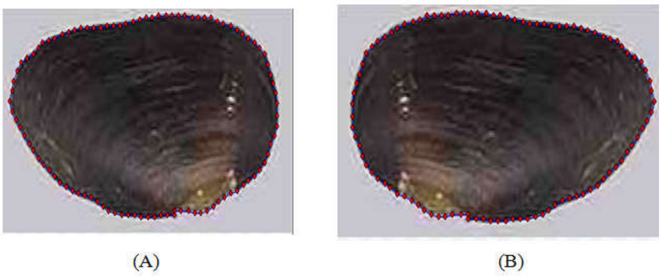


Figure 4. Outlined of the (A) left shell and (B) right shell valve of *M.margaritifera* L

Outline-based Geometric Morphometric Analysis was used to illustrate morphological variations in the shell contour of the outer face of the left and right valves of the freshwater mussels. Differences in the outlines of the outer face of the left and right valves of *M. margaritifera* were used to determine variations between sexes of the fresh water mussel. A total of 150 samples of *M. margaritifera* vary in greenish-black to brownish-black in color with a characteristic pearly white umbo. Samples were characterized into 75 left shell valves and 75 right shell valves for the males and females. These were subjected into thin plate software for digitization (Figure 4A-B). The relative warps analysis was performed using the tpsRelw version 1.46 (Rohlf, 2008) to generate deformation grids. Relative warp scores obtained will be recorded and subjected to multivariate statistical analysis.

RESULTS AND DISCUSSION

In this study, shell shape of *M. margaritifera*awas visualized before Procrustes fitting showing a thick and very shaded outline of the shell contour. However, after being subjected to Procrustes fitting representing the mean shapes of the shell valves showed a less shaded outline by removing the biases and unrelated points. Mean shapes of the left shell valves of the pooled *M. margaritifera* showing difference in the thickness of the shape outline before and after procrustes fit (Figures 5). Mean shapes of the right shell valve of the pooled *M. margaritifera* showing difference in the thickness of the shape outline before and after procrustes fit (Figures 6).

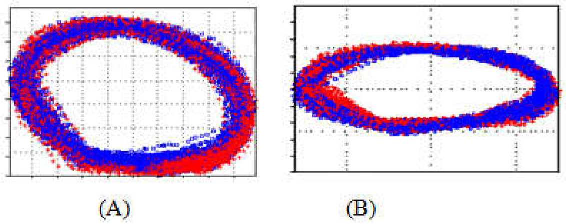


Figure 5. Mean shape of the pooled left shell valve of *M. Margaritifera* (A) before and (B) afterprocrustes

The shape variations at RW1 occur in the left shell valve of the male and female *M. margaritifera* was found at the lateral sides along the hinges of the shell contour pallial line.The second ranked variation of the left shell valve was found on the right lateral side of the hinges at RW2. The least variation is located at the pseudocardinal tooth areaat RW3 (Figure 7).

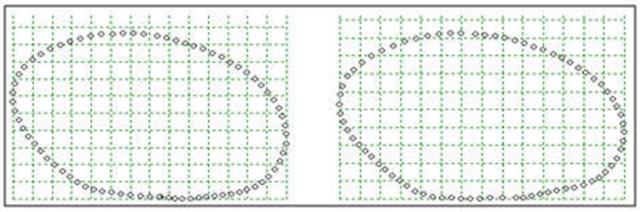


Figure 7. Mean shapes of the left shell valves of both sexes of *M.margaritifera*

The variation at RW1 of the right shell valves of the male and female *M.margaritifera* was found at the lateral sides along the hinges of the shell contour pallial line. The second ranked variation of the right shell valve was found on the right lateral side of the hinges at RW2. The least variation at the RW3 of the right shell valve of the male and female *M. margaritifera* species was found at the lateral side of the pallial line (Figure 8).

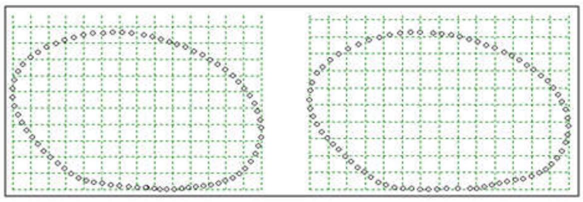


Figure 8. Mean shapes of the right shell valves of both sexes of *M. margaritifera* L

Based from the consensus morphologies showing the mean shapes of the left shell valve of both male and female and right shell valve of both male and female, the differences between the outer shell contours were highly significant.

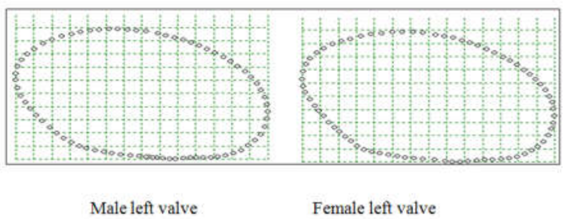


Figure 9. Mean shapes of the pooled shell valves of both sexes of *M. Margaritifera*

The consensus morphologies with the left shell valve and right shell valve of both male and female shows that the points associated with 75 landmark curves each exhibit a tendency to have the same magnitude and frequency and showed highly significant differences between sexes (Figure 9).

To further show the distinction of the shell contour between the left and right valve of the male and female *M. margaritifera*, the data were subjected to the Relative Warp Analysis to produce a scatter plot of the individual specimens in two-dimensional space. The spread of the points were used to visualize distances between points which represent the specimens and consequently showing the amount of similarity and differences between the male and female populations. The result of the scatter plot showed an overlapping of points which can be interpreted as the presence of sexual dimorphism in the shell contour of the *M. margaritifera*. Figure 10 showed the Relative Warp Analysis of the left shell valve of the male and female *M. margaritifera*generating a scatter plot of points depicting the spread of individual specimens.

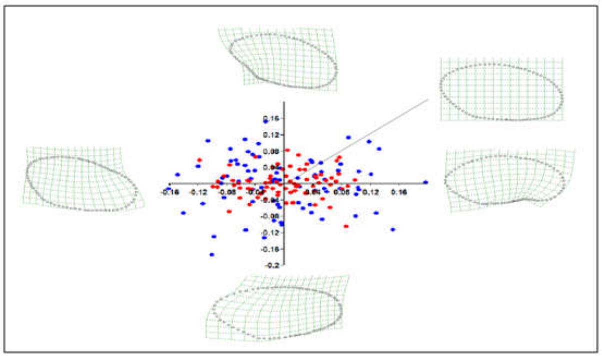


Figure 10. Scatter plot of the first two relative warps of the pooled left shell valve of the *M. margaritifera*L. species

Figure 11 showed the Relative Warp Analysis of the right shell valve of the male and female M. *margaritifera* generating a scatter plot of points depicting the spread of individual specimens.

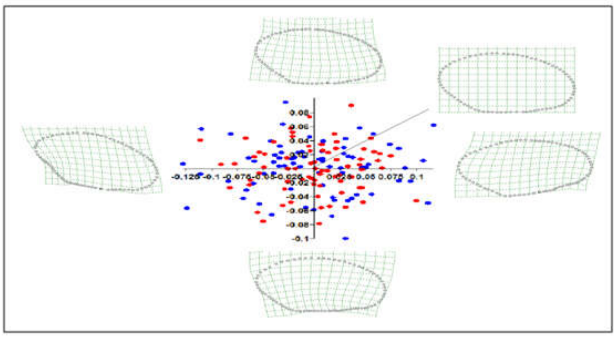


Figure 11. Scatter plot of the first two relative warps of the pooled right shell valve of the *M. margaritifera* species

To help support the results of the scatterplot method, Discriminant Function Analysis (DFA) was utilized to confirm shape variations between the two sexes. This is seen in the disparity in the distribution of male and female discriminant scores which strengthen the probability that variationsismanifested in the outer shell contour of the pooled left shell valvesM. Margaritifera (Figure 12).

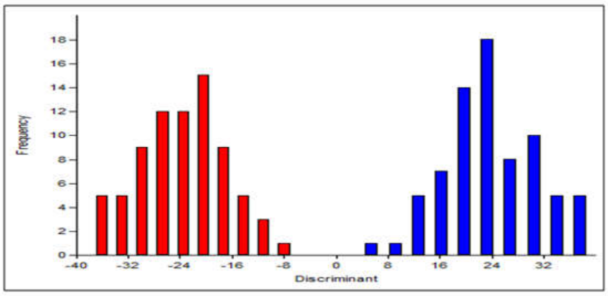


Figure 12. A histogram of shape characters of the pooled left shellvalve of *M. margaritifera* L. manifesting sexual dimorphism

Discriminant Function Analysis (DFA) was utilized to confirm shape variations between the two sexes. The disparity in the distribution of male and female discriminant scores revealed that variation is manifested in the outer shell contour of the pooled right shell valves *M. margaritifera* (Figure 13).

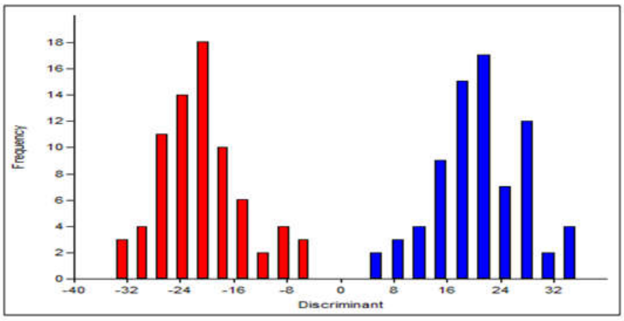


Figure 13. A histogram of shape characters of the pooled right shell valve of *M. margaritifera* L. manifesting sexual dimorphism

Furthermore, Multivariate Analysis of Variance (MANOVA) of the relative warp scores shows that the variation in the shape of outer shell contour of the left shell valves between sexes is highly significant (Table 1). Principal Component Analysis (PCA) was determined to support the Relative Warp Analysis by providing the Eigen values relative to the shape deformation and variances.The PCA finds the Eigen values of the variance of the correlation matrix. The smallest Eigenvalues correspond to the thinnest directions having the least variation while the largest Eigen values correspond to the thickest direction having the most variation. Results revealed shell shape variation in the left shell valves of *M. margaritifera* L. Principal Component 1 has the highest percent variance (37.05%) in the male and (41.45%) for the female (Table 2). Principal Component Analysis (PCA) was used to analyze shell shape variation in the right shell valves of *M. margaritifera*. Results revealed that Principal Component 1 has the highest percent variance (40.82%) in the male and (31.51%) for the female (Table 3). To further compare the two groups, shell contour of the left shell valve and right shell valve of the male and female, Kruskal-Wallis test was used determine which relative warps are statistically significant. The results revealed that the left valves and right valves of both sexe of *M. margaritifera* have a significant effect. Kruskal-Wallis shows that there were high variations of the left shell contour in the Relative Warp 1 ($P = \leq 0.000$) and Relative Warp 3 ($P = \leq 0.000$) at □ = 0.05 level of confidence (Table 4).

Table 1. MANOVA test for significant variation in the shape of the left shell valve between male and female *M. margaritifera* L

Source of Variation	Wilk's lambda (Λ)	df1	Df2	F	P (same)
Left shell Valve	0.010	146	3	1.94	0.33

Table 2. The Principal Component Analysis of the left shell valves of *M. margaritifera* L

Left shell valves					
Male			Female		
PC	Eigen Values	% of Variance	PC	Eigen Values	% of Variance
1	0.005	37.05	1	0.002	41.45
2	0.004	26.58	2	0.001	18.08
3	0.001	8.74	3	0.000	7.75
4	0.000	5.29	4	0.000	5.96
5	0.007	4.61	5	0.000	3.92

Table 3. The Principal Component Analysis of the right shell valves of *M. margaritifera* L

Right shell valves					
Male			Female		
PC	Eigen Values	% of Variance	PC	Eigen Values	% of Variance
1	0.003	40.82	1	0.002	31.51
2	0.001	18.75	2	0.001	20.38
3	0.000	9.83	3	0.000	9.87
4	0.000	5.69	4	0.000	7.73
5	0.000	4.28	5	0.000	5.14

Table 4.Kruskal-Wallis of the left shell contour of *M. margaritifera* L

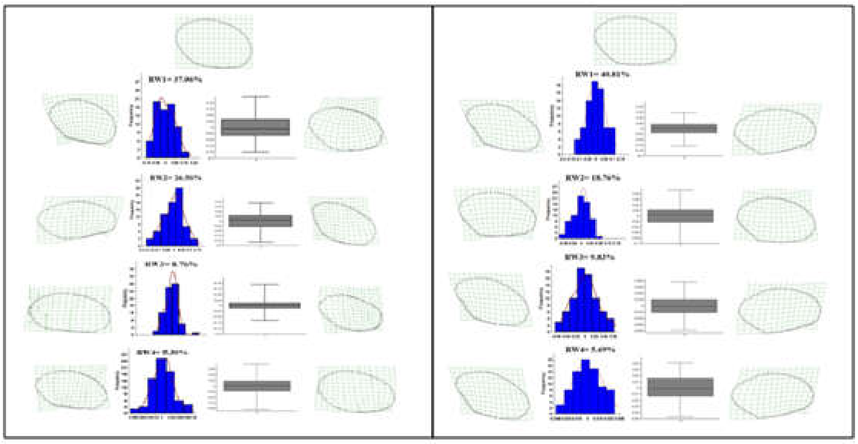
PC	H	p-value	Remarks
1	15.49	<0.00	Extremely significant
2	4.28	0.03	Not significant
3	65.58	<0.00	Extremely significant
4	6.64	0.009	Not significant

Table 5.Kruskal-Wallis of the right shell contour of *M. margaritifera* L

PC	H	p-value	Remarks
1	15.49	<0.00	Extremely significant
2	4.28	0.03	Not significant
3	65.58	<0.00	Extremely significant
4	6.65	0.009	Not significant

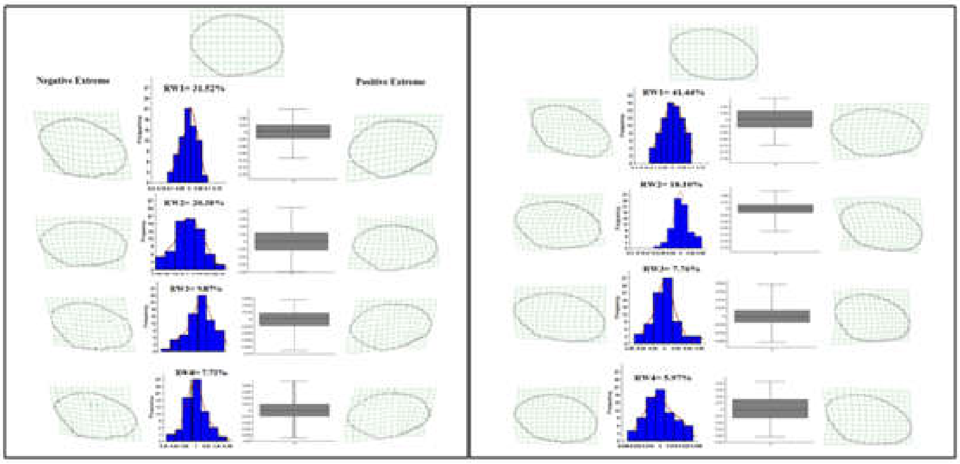
Table 6. Variability in the outer shell contour of the left and right shell valves of of *M. margaritifera*

Relative Warp	Description of Variations			
	%	Left Valve	%	Right Valve
1	31.71%	Significant variations along the right pallial line were observed from the negative to the positive extremes of RW1	35.98%	The positive extreme showed distortion in the pallial line contour while the negative extreme showed constriction in the pseudocardinal tooth area near the hinges at the lateral sides
2	27.42%	Thepositive extreme showed a constriction near the hinge area to the right lateral side.	21.35%	The positive extreme showed an expansion of the pallial line surrounding the shell contour while the negative extreme showed distortion in the left lateral side of the shell valve
3	9.80%	The positive extreme showed a constriction at the pseudocardinal tooth areawhile the negative extreme showed a variation at the pallial area	9.04%	The positive extreme showed significant variation along the pallial line.
4	6.77	A very significant distortion can be seen on the hinges of the valve while the negative extreme showed a distortion along the pallial line.	6.18%	The positive extreme showed a slight constriction of the hinges while the negative extreme showed a sharpening of the pallial line along the left lateral line close to the hinges



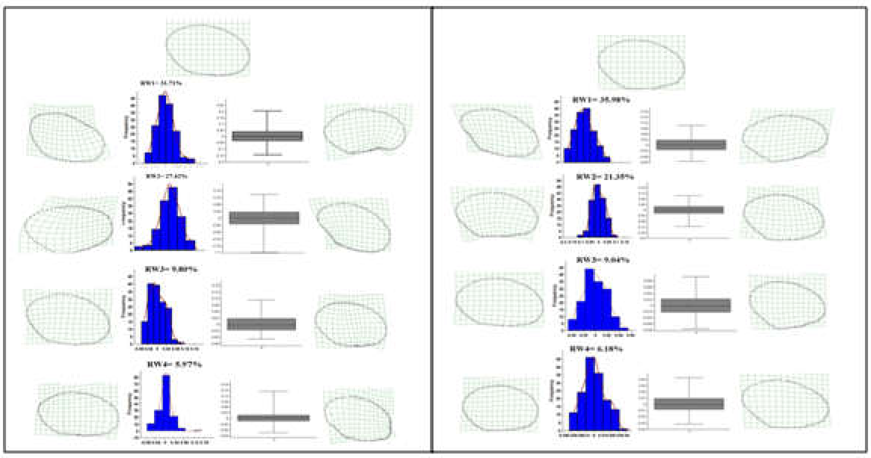
(A)

Figure 9. (A) Summary of the geometric morphometric analysis showing the concensus morphology and frequency histogram of each relative warps of the left and right valves of the male *M. margaritifera* L. species



(B)

Figure 9. (B) Summary of the geometric morphometric analysis showing the concensus morphology and frequency histogram of each relative warps of the left and right valve of the female *M. margaritifera* L. species



(C)

Figure 9. (C)Summary of the geometric morphometric analysis showing theconcensus morphology and frequency histogram of each relativewarps of the pooled left and right valve of both sexes of *M. margaritifera* L. species

Table 7. The Principal Component Analysis of the left shell valves of *M. margaritifera*

Left shell valves					
Male			Female		
PC	Eigen Values	% of Variance	PC	Eigen Values	% of Variance
1	0.005	37.05	1	0.002	41.45
2	0.004	26.58	2	0.001	18.08
3	0.001	8.74	3	0.000	7.75
4	0.000	5.29	4	0.000	5.96
5	0.007	4.61	5	0.000	3.92

Table 8. The Principal Component Analysis of the right shell valves of *M. Margaritifera*

Right shell valves					
Male			Female		
PC	Eigen Values	% of Variance	PC	Eigen Values	% of Variance
1	0.003	40.82	1	0.002	31.51
2	0.001	18.75	2	0.001	20.38
3	0.000	9.83	3	0.000	9.87
4	0.000	5.69	4	0.000	7.73
5	0.000	4.28	5	0.000	5.14

Table 9.Kruskal-Wallis of the left shellcontour of *M. margaritifera*

PC	H	*P*-value	Remarks
1	15.49	<0.0000	Extremely significant
2	4.282	0.03853	Not significant
3	65.58	<0.0000	Extremely significant
4	6.649	0.00923	Not significant

Table 10.Kruskal-Wallis of the right shell contour of*M. margaritifera*

PC	H	P-value	Remarks
1	15.49	<0.0000	Extremely significant
2	4.28	0.03853	Not significant
3	65.58	<0.0000	Extremely significant
4	6.65	0.00923	Not significant

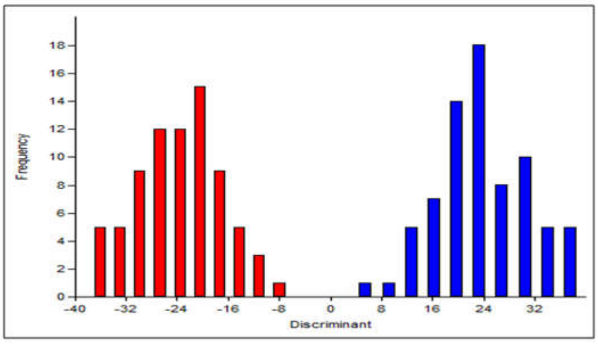


Figure 12. A histogram of shape characters of the pooled left shell valve of *M. margaritifera* manifesting sexualdimorphism

Table 11. Reclassification of *M. margaritifera* into male or female based on the landmark analysis of the outer shell contour of the left shell valve

	Male	Female	Total
Male	75	0	75
Female	0	75	75
Total			150

% correctly classified: 100%

Table 12. MANOVA test for significant variation in the shape of the left shell valve between male and female *M. margaritifera* L

Source of Variation	Wilk's lambda (Λ)	df1	Df2	F	P (same)
Left shell Valve	0.010	146	3	1.94	0.33

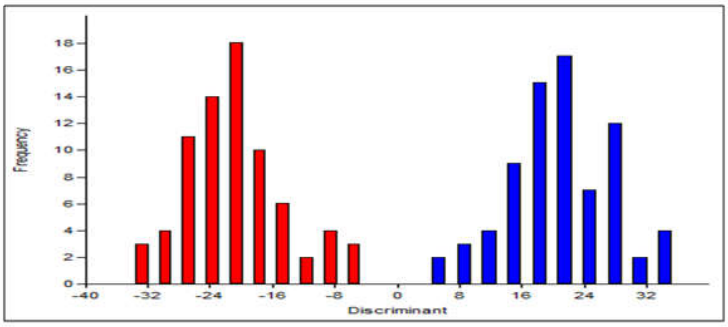


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Table 8.Reclassification of *M. margaritifera* L. into male or female based on the landmark analysis of the outer shell contour of the right shell valve

	Male	Female	Total
Male	75	0	75
Female	0	75	75
Total			150

% correctly classified: 100%

Table 9.MANOVA test for significant variation in the shape of the right shell valve between male and female *M. margaritifera* L

Source of Variation	Wilk's lambda (Λ)	df1	Df2	F	P (same)
Right shell Valve	0.004	144	5	8.72	0.01

Kruskal-Wallis shows that there were high variations of the right shell contour in the Relative Warp 1 (P =≤0.000) and Relative Warp 3 (P = ≤0.000) at α = 0.05 level of confidence (Table 5). Variations in the shell valves between male and female populations of *M. margaritifera* as visualizedinthe transformation grids supported by the frequency histograms and boxplots have furthermore supported uniformity to slight variation in the outer shell contour of the left and right valves between sexes. The description of variation between the sexes of the *M. margaritifera* is explained and tabulated corresponding to its respective significant relative warp. Significant differences between sexes were clearly observed as the shell shapes from the negative to positive extremes on each significant relative warp vary.

The main variations that occur in male and female freshwater mussels are found at the lateral sides of the hinges, both for the left and right shell valves. Broadness and constrictions in the umbo and lateral sides were observed as visualized in the transformation grids.Results revealed that the variation in each valve of both sexes of *M. margaritifera* is found at the lateral sides of the hinges of the shell contour. These shape variations may be attributed to the kind of habitat and feeding habits. As adult, mussels inhabit a variety of substrates. Most species either thrive in an environment where particle sizes vary from silt to boulder. These particles can affect not only the reproductive capacity of mussels but may also affect the external morphology mechanically (Landis et al., 2012). Traditionally, shape elongation is considered as a function of environment and shell obesity is influenced by individual growth (Aldridge, 1999; Zieritz and Aldridge, 2009; Zieritz et al., 2010; Zieritz et al*.,* 2012; Widarto, 2007). In their study they found out that mercury is one of the causes of the thinning of its shells and consecutively causes the disappearance of freshwater mussel populations. Umbo's sculpture and projection are also considered as adaptation to environmental conditions (Watters, 1994).In addition, an irregular shell growth (in length or in height) was shown in the freshwater mussel, *M. margaritifera*as an indication that these growth parameters have rhythmic character (Zotin, 2015).

Moreover, studies carried out by Zieritz and Aldridge (2011) on *Anodonta* species show that female *Anodonta anatine* specimens are generally more inflated than males in order to increase the volume of the branchial chambers for glochidia brooding. The Relative Warp Analysis provided the shape deformations and the percentage of shape variations of each relative warp contributed to the overall variation between sexes of *M. margaritifera*.

RW 1 has the highest significant variation (31.71%) for the left valve attributed along the right pallial line. RW 1 has the highest significant variation (35.98%) for the right valve showing a distortion along the pallial line contour and a constriction in the pseudocardinal tooth area near the hinges at the lateral sides (Table 6). The summary of the geometric morphometric analysis showing concensus morphology of the shell shapes and the variation among within sexes of *M. margaritifera* (Figure 9A-C). Principal Component Analysis (PCA) was used to analyze shell shape variation in the left shell valves of *M. margaritifera*. Results revealed that Principal Component 1 has the highest percent variance (37.05%) in the male and (41.45%) for the female (Table 7). Principal Component Analysis (PCA) was used to analyze shell shape variation in the right shell valves of *M. margaritifera*. Results revealed that Principal Component 1 has the highest percent variance (40.82%) in the male and (31.51%) for the female (Table 8).

Kruskal-Wallis shows that there were high variations in Relative Warp 1 ($P = \leq 0.000$) and Relative Warp 3 ($P = \leq 0.000$) at □ = 0.05 level of confidence (Table 9). Kruskal-Wallis shows that there were high variations in Relative Warp 1 ($P = \leq 0.000$) and Relative Warp 3 ($P = \leq 0.000$) at □ = 0.05 level of confidence (Table 10). It can be observed in the histogram of the shape characters of the pooled left shell valves of *M. Margaritifera* that the bins did not overlap at some point implying complete separation of data sets. This entails that there is a variation between male and female left shell valve of *M. Margaritifera.* There were no shared characteristics between the pooled left shell valves of *M. Margaritifera* (Figure 12). The percentage of correctly classified (100%) values of the left shell valves of male and female *M. margaritifera* (Table 11). Multivariate analysis of variance (MANOVA) revealed highly significant variation with Wilk's lambda (Λ) value of 0.010 and $P = 0.33$ (Table 12). It can be observed in the histogram of the shape characters of the pooled right shell valves of *M. Margaritifera* that the bins did not overlap at some point implying complete separation of data sets. This entails that there is a variation between male and female right shell valves of *M. Margaritifera*. There were no shared characteristics between the pooled right shell valves of *M. Margaritifera* (Figure 13). The percentage of correctly classified (100%) values of male and female *M. margaritifera* (Table 8). Multivariate analysis of variance (MANOVA) revealed highly significant variation with Wilk's lambda (Λ) value of 0.004 and $P = 0.01$(Table 9).

Conclusion and R**ecommendations**

This study showed shape variations in the left and right shell valve of the male and female *M. margaritifera* L.The main variations that occur in male and female freshwater mussels are found at the lateral sides of the hinges, both the left and right shell valves. Broadness and constrictions in the umbo and lateral sides were found as visualized in the transformation grids. These shape variations may be attributed to the kind of habitat, feeding habits and inhabit a variety of substrates. Shape variations may alsoattributed to reproductive capacity of mussels and individual growth. The results revealed the accuracy of the outline-based geometric morphometric analysis in determining shape variations within sexes of the same species compared to the traditional method.

Therefore, it is recommended that the inner shell contour of the shell valves of the male and female *M. margaritifera*will be studied. Also, it is recommended that another geometric morphometric study will be conducted on the factors that affect the growth and development of the shell valves of the *M. margaritifera*.

REFERENCES

Aldridge, D., Hoffman, A. and Zieritz, A. 2009. Phenotypicplasticity and genetic isolation-by-distance in the freshwater mussel *Uniopictorum* (Mollusca: Unionida).

Aldridge, D. and Zieritz, A. 2009. Identification of ecophenotypic trends withinthree European freshwater mussel species (Bivalvia: Unionida) using traditional and modern morphometric techniques.

Aldridge, D. and Zieritz, A. 2011. Sexual, habitat-constrained and parasite-induced dimorphism in the shell of a freshwater mussel (*Anodonta anatina,*Unionidae).

Allan, G. and Burnell, G. 2013. Advances in aquaculture hatchery technology.

Aypa, S. 1990. Bureau of Fisheries and Aquatic Resources (BFAR).Mussel Culture.

Bolotov, I., Vikhrev, I., Bespalaya, Y., Artamonova, V., Gofarov, M., Kolosova, J., Kondakov, A., Makhrov, A., Frolov, A., Tumpeesuwan, S., Lyubas, A., Romanis, T., & Titova, K. 2014., Ecology and conservation of the endangered Indochinese freshwater pearl mussel, *Margaritifera laosensis*

Brown, A., Cao, Y., Huang, J., Cumming, K., A. Holtrop., 2013. Modeling changes in freshwater mussel diversity in an agriculturally dominated landscape.

Burton-Kelly, M. and Hartman, J. 2014. Comparing size of morphospace occupation among extant and cretaceous fossil freshwater mussels using Elliptical Fourier Analysis.

Cohen, A. and Weinstein, A. 2001. Zebra mussels calcium threshold and implications for its potential distributions in North America.

Degerman, E., Alexanderson, S., Bergengren, J., Hennrikson, L., Johansson, B., Larsen, B. Soderberg, H. 2009. The freshwater pearl mussels and its habitats in Sweden: Restoration of freshwater pearl mussels.

Gelsvartas, J. 2002. Geometric Morphometrics. Hammer, O., and Harper, D., 2001. PAST: Paleontological statistics software package for education. International Union on the Conservation of Nature (IUCN). 2011.List of threatened species.

Landis, A., Haag, W. and Stoeckel, J. 2013. High suspended solids as a factor in reproductive failure of a freshwatermussel.

Lea, 1863. in the Nam Pe and Nam Long rivers, Northern Laos.

Rohlf , J. 2008. tpsRelw. Ecology and evolution. State University of New York at Stony Brook. Version 1.46.

Rohlf, A., and D.Slice., 2004.Geometric Morphometrics: Ten Yearsof Progress Following the Revolution.

Schwaebe, L., Acharya, K. and Nicholl, M. 2013.Comparative efficacy of *Dreissenarostriformis bugensis* (Bivalvia: *Dreissenidae*) spawning techniques.

Siegele, R., Cohen, O. and Jeffree, M. 2001. Manganese profiles in freshwater mussels shells.

Waters, G., 1994. Form and function of *Unionoidean* shell sculpture and shape (Bivalvia).

Widarto, H. 2007. Shell form variation of a freshwater mussel *Velesunioambiguous* Philippi from the Ross River, Australia.

Williams, J., Butler, R., Zarren, G., and Johnson N. 2012. Freshwater mussels of Florida.

Zotin, A. 2014.Specific features of linear growth influencing morphometric parameters of the shell in *Margaritifera margaritifera* (Bivalvia: Margaritiferidae).

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