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Outline-based Analysis of Sexual Dimorphism in the Shell of the Freshwater Mussel (*Margaritifera margaritifera* L.)

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Abstract

This study was conducted to determine the patterns of sexual dimorphism in the vulnerable species of freshwater mussel *Margaritifera margaritifera* L. The method of Elliptic Fourier Analysis (EFA) was employed to extract shape information from a total of one hundred (100) points collected from digitized images of the shells. The EFA coordinates where then subjected to statistical analyses such as Kruskall-Wallis and Discriminant Function (DFA) test. Results showed differences in the shape of the lateral sides along the hinges of the shell contour pallial line, the pseudocardinal tooth area and at the umbo. Aside from revealing information on sexual dimorphism, the result of this study is discussed in relation to using patterns of sex differences in shell shape to assess the health of aquatic habitats.

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Introduction

The freshwater Pearl Mussel *Margaritifera margaritifera* L. is listed in the International Union for the Conservation of Nature (IUCN) Red List as one of the vulnerable species of shells worldwide because its populations are said to be declining in size in the past years (Mollusc Specialist Group, 1996). The IUCN Red List website explains several factors that led to this dramatic decline. These include eutrophication of rivers, intensification of agriculture, land drainage and afforestation.

The life history of this mussel is said to be complex. In fact, *M. margaritifera* is capable of shifting from being dioecius in highly dense populations to being self fertile hermaphrodites in small population sizes. This capability confers the ability of low density populations to increase in numbers to recover from episodes of bottleneck and survive various forms of selection pressures. This ability is not a unique feature of this species as it can also be observed in the unionoid mussel *Anodonta anatina* (Zieritz and Aldridge, 2011). In both species, being dioecius also means that the morphological attributes of males can sometimes be different from that of the females. Thus, dioecius populations may exhibit patterns of sexual dimorphism.

An important observation in *A. anatine* is that the degree of sexual dimorphism between populations is said to be constrained by the health of the environment. Under "good" habitat conditions, female/hermaphroditic mussels are expected to be more fecund, resulting in swollen gills and shells that are more inflated than the males (Zieritz and Aldridge, 2011). This phenomenon of environmentally constrained sexual dimorphism can also be observed in gastropods (Goodwin and Fish, 1977) and beetles (Stilwell and Fox, 2007).

Perhaps one of the challenges in measuring the degree of sexual dimorphism in the shapes of the shells in mussels is the limited power of traditional morphometrics to extract such information.

However, the advent of Geometric Morphometrics makes it possible to detect global and local differences in the shapes of biological structures between sexes (Adajar *et al*., 2011; Albutra *et al*, 2012). For two-dimensional structures of organisms, either landmark-based or outline-based geometric morphometric approaches are being utilized. For the latter, Elliptic Fourier descriptors are typically used to analyze closed contours and have been effectively utilized in studying shape variation in flora and fauna (Ferson *et al*., 1985; White *et al*., 1988; Diaz *et al*., 1989; Laurie *et al*., 1997; Iwata *et al*., 1998; Yoshioka *et al*., 2004). The Elliptic Fourier Analysis (EFA) decomposes the contour shape into a series of harmonics with four Fourier coefficients (Rohlf and Archie, 1984).

In this study, the outline based geometric morphometric method of Elliptic Fourier Analysis was used to determine sex differences in the shapes of the shells of the freshwater pearl mussel *M. margaritifera*. Specifically, the present study determines and describes the variability in the shape of the left and right valves between males and females of the species.

Materials and methods

Collection of samples and segregation of sexes

Samples were collected from Malubal, R.T. Lim, Zamboanga, Sibugay Province (07° 42'N 122°28'E) (Fig. 1). Sexes were determined via analysis of the gonads of the mussels. Mussels were placed individually in containers to prevent unintended fertilization. Then, 1 ml of 0.5 M Potassium Chloride (KCl) were injected through the mantle cavity of each sample to induce spawning. After which, the mussels were placed in containers with filtrated water and observed for period of four hours. During this period, males were distinguished from the females by the characteristic cloudy appearance of the water surrounding it. Females on the other hand, produce eggs that are small and whitish to pale orange in color.

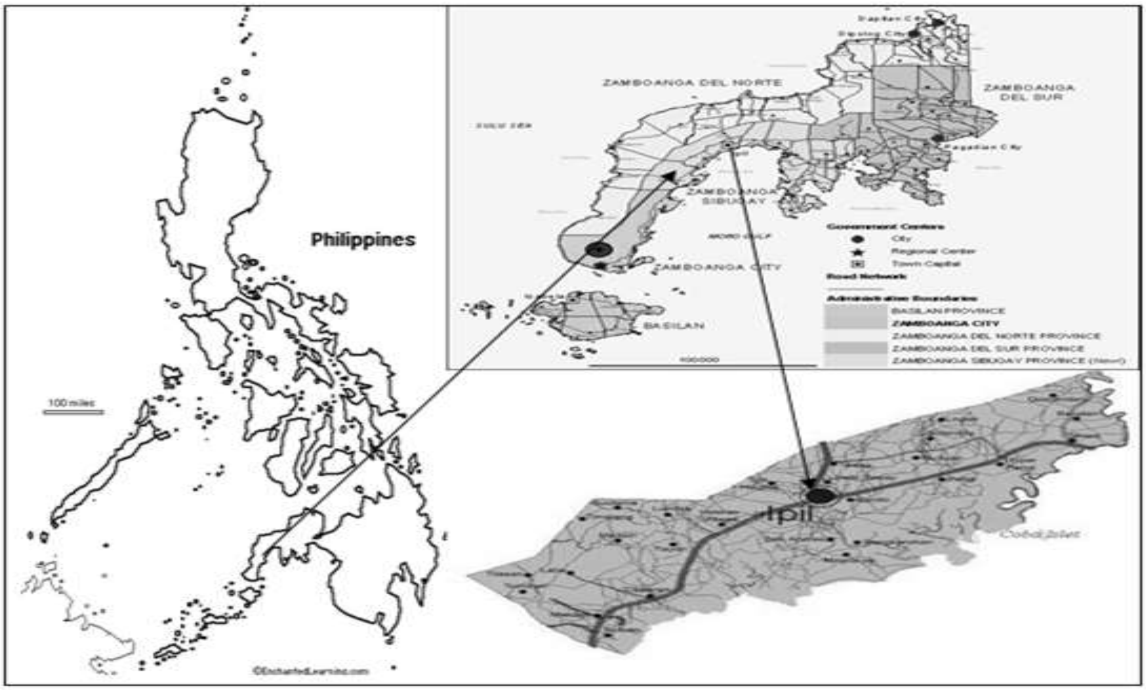

Fig. 1. Map of the Study Site.

Image processing and generation of outline points

Images of the samples generated using a DSLR, Canon EOS 110D were used in this study. A total of one hundred fifty (150) images were loaded in the tps Util 1.38 (Rohlf, 2008) which were used for digitization. The outlines of the left and right valves of the shells were traced by laying a total of one hundred (100) closely connected points using tpsDig 2.12. These points cover hinges, pallial line and lateral sides (Fig. 2 and 3).

Analysis of elliptic fourier descriptors

Subjecting the Cartesian (or x and y) coordinates of the 100 outline points to Elliptic Fourier Analysis returned a total of 20 harmonics which was sufficient to correctly depict the shell outlines. Then, the coefficients of the first twenty harmonics were selected for Principal Component Analysis (PCA). The analysis was performed using the PAST ver. 2.17 program (Hammer *et al.*, 2001). To differentiate between sexes, the X and Y coordinates were plotted to reconstruct the shapes of the shells of the males and females. Then, the principal component scores were subjected to Kruskall-Wallis test for significant differences in means and Discriminant Function analysis. All statistical analyses were carried out using PAST ver. 2.17.

Results and discussion

The reconstructed outlines in Fig. 1 appear to demonstrate variation in the shapes of the shell in *M. margaritifera* especially in the lateral sides along the hinges of the shell contour pallial line, the pseudo cardinal tooth area and at the umbo.

Table 1. Results of the Kruskal-Wallis test for significant differences in the Principal Components scores between sexes.

VALVE	PC	H	P-VALUE	REMARKS
Left	1	15.49	<0.000	Extremely significant
	2	4.28	0.0385	Not significant
	3	65.58	<0.000	Extremely significant
	4	6.65	0.009	Not significant
Right	1	15.49	<0.000	Extremely significant
	2	4.28	0.039	Not significant
	3	65.58	<0.000	Extremely significant
	4	6.65	0.009	Not significant

However, the variation generated does not clearly separate the two sexes. By further subjecting the principal component scores (PCA) to statistical analyses, one can detect sexual dimorphism in the shape of the shell. In Table 1, the odd-numbered PCA scores were extremely significant between sexes. This result is also supported by the results of the Discriminant Function Analysis (DFA), where the histograms for the males and females are clearly separated.

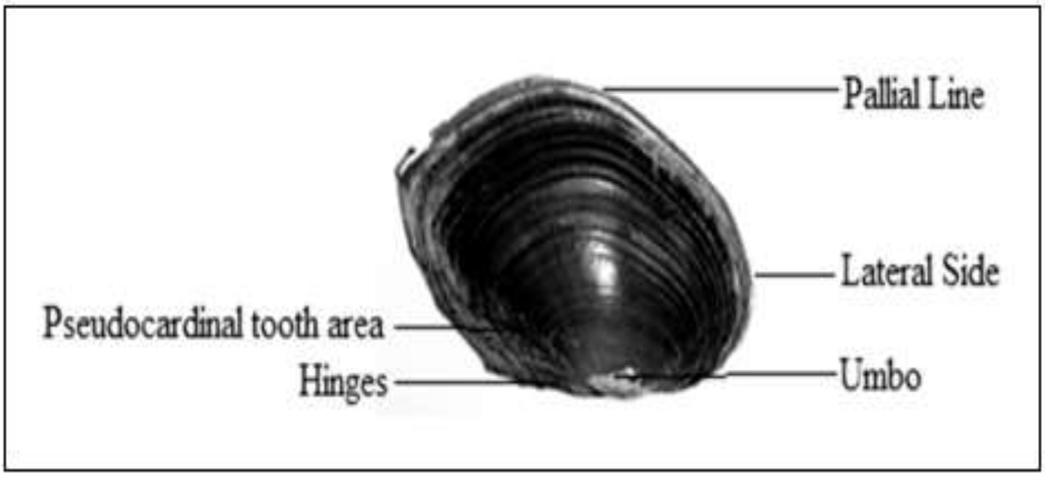

Fig. 2. Anatomical parts of the shell.

Just like other similar species, sexual dimorphism in *M. margaritifera* is not a universal phenomenon in all populations. Being dioecius or sexually dimorphic is only a characteristic of populations found in "good" habitat conditions or environments that are in the pink side of health (Zieritz and Aldridge, 2011). Those that are monoecius are thought to be manifested by populations in "poor" habitat conditions. Since dimorphism is evident in the population from Malubal, R.T. Lim, this implies that the aquatic environment here is in "good" condition.

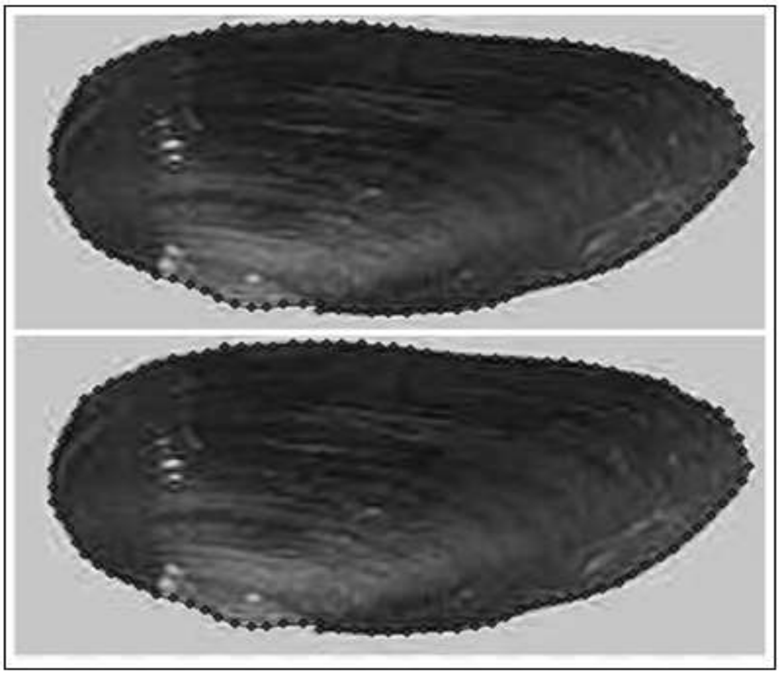

Fig. 3. Digitized image of the *M. margaritifera* showing the locations of the outline points.

The phenomenon of environmentally constrained sexual dimorphism in *M. margaritifera* lends an important application in determining the health not only of the population but also of the environment (Goodwin and Fish, 1977; Stilwell and Fox, 2007). In other species of Uninoids, species thrive in a variety of habitats characterized by an environment where particle sizes vary from silt to boulder.

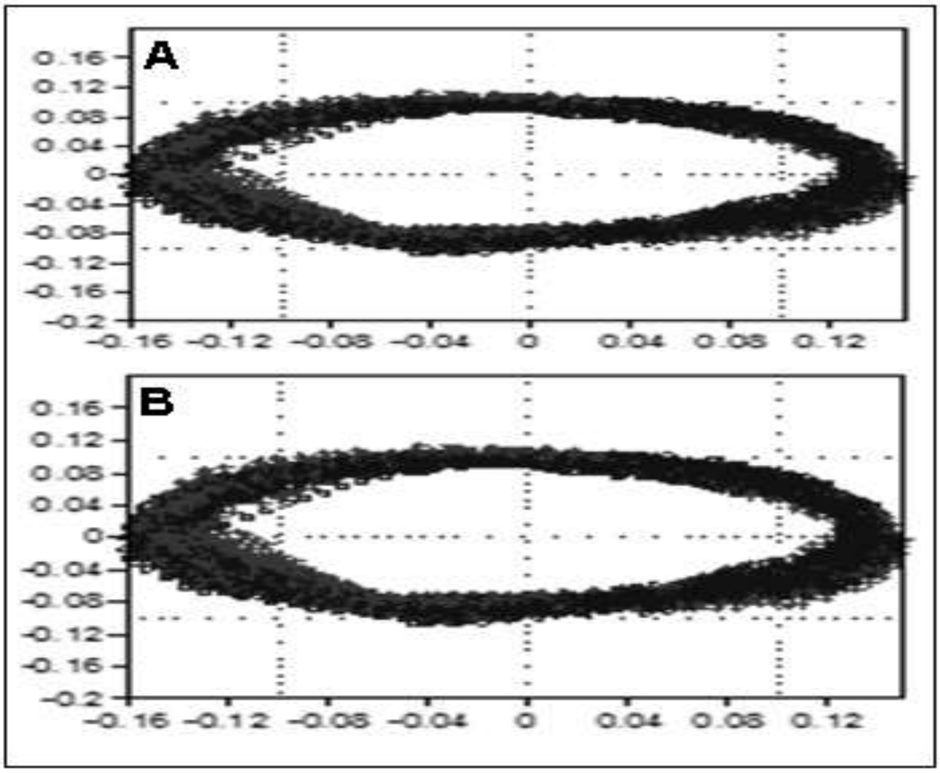

Fig. 4. Reconstructed outlines of the (a) left and (b) right valves of the shells of *M. margaritifera* L.

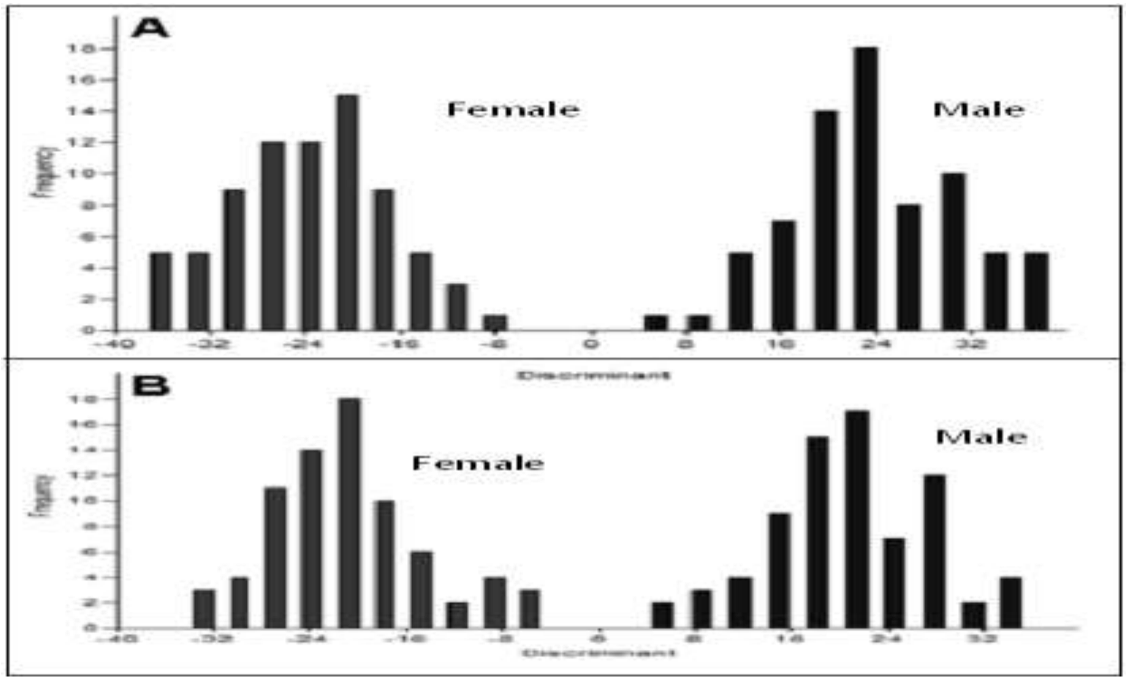

Fig. 5. Frequency distribution of the discriminant scores between sexes.

These particles are said to affect not only the reproductive capacity of mussels but may also affect the external morphology mechanically (Landis *et al.*, 2012). Traditionally, elongation is considered as a function of environment and shell obesity is influenced by individual growth (Zieritz and Aldridge, 2011). On the other hand, the umbo's sculpture and projection are also considered as adaptation to environmental conditions. One other possible reason would be on the trace elements found in the water where the mussels thrive. According to the literature, freshwater mussel shells can be even be used to determine the amount of mercury-related exterpations in its environment based on the degree of sexual dimorphism.

Conclusion

This study showed sexual dimorphism in the shape of the shell in the population of *M. margaritifera* from the collection site in Malubal, Zamboanga Sibugay. The disparity in shell shape is probably related to the kind of environment they thrive as "good" habitat condition permits the occurrence of dioecius states as females modify their morphologies to accommodate their increased fecundity.

In species highly tolerant of fluctuations in the health of the environment, it is interesting to note that shifting from hermaphroditic states to being sexually dimorphic can be used to monitor the status of the habitat. Regarded by IUCN as a species vulnerable to global climate change, habitat disturbance and destruction, acidification of water bodies, and harmful anthropogenic activities, the degree of sexual dimorphism in *M. margaritifera* can definitely find utility in assessing environment quality.

The findings of this study also revealed the potential of outline-based geometric morphometrics in detecting sexual dimorphism in shells. It is recommended that further studies be conducted to correlate the amount of sexual dimorphism in other species of freshwater mussels with known indicators of environmental health.

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