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# Shell Shapes of the Chinese Pond Mussel *Sinanodonta woodiana* (Lea, 1834) from Lawis Stream in Iligan City and Lake Lanao in Mindanao, Philippines

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**ABSTRACT**

This study aimed to determine morphological variation within and between two populations of *S.woodiana* (Lea, 1834) from Lake Lanao, Marantao and Lawis stream, Iligan City using elliptic Fourier analysis. Results showed morphological divergence between the two populations. The MANOVA/ CVA resulted to the rejection of allometry as the cause of variations between the two populations. The observed differentiation may reflect some underlying genetic basis. There was considerable difference in the distribution of the CVA scores between the two sites which is suggestive of varying selection pressure on the populations.

***Key words:*** elliptic Fourier, mussel, geometric morphometrics.

## Introduction

The Chinese pond mussel, *S. woodiana* (Lea, 1834) (Bivalvia: Unionida: Unionidae) originated from Eastern Asia [23]. They are intense blue green in color. This species primarily inhabited the ecosystems of two large Asian rivers – the Amur and the Yangtze. It is a large size representative of the Unionidae family, with populations distributed in both East and South- East Asia. Native distribution areas of this species include the basin of the Amur River, Hanka Lake, China, Hong Kong, Taiwan, Kampuchea, Thailand and Japan [13]. Species is invading worldwide with Tilapia fish culture. Procedures connected with fish cultivation have contributed to the spread of this species in southern Asia [24, 6, 8, 10, 23], Dominican Republic and Costa Rica [23], Europe [19], Romania [18], Italy [5] and the Iberian Peninsula [19], Poland [15], Serbia [16], Iberian Peninsula [19], Czech Republic [2] primarily associated with fish farms [15, 17].

The spread of these species in some parts of the Philippines like Lake Lanao and Lawis stream is mainly due to the introduction of fish species from Eastern Asia, where they are indigenous, acting as host to their parasitic larvae. Lake Lanao is a large lake and fishes have been introduced for culture purposes such as tilapia. The Lawis stream has also been infected with this invasive mussel due to the nearby fish culture in the area where tilapia was introduced. The areas where the species were observed were geographically distant and are of different physical attributes thus populations of the species are good candidates for investigation. Likewise, qualitative observation showed variations in the size and shapes of the Shell.

Comparative morphological studies on bivalves have focused on the diversity of shell shape and sizes. Also, the shapes of the bivalves have been used in the systematics and classification as these features provide phylogenetic signals and are taxonomically important in discriminating species. A new tool in morphometrics, Geometric morphometric (GM) analysis is one of the ways that can test variations within or between populations of a single species or different species thus is useful in describing variations in the shape of the Shell of *S. woodiana*. GM is a collection of approaches for the multivariate statistical analysis of Cartesian coordinate data, usually (but not always) limited to landmark point locations. It is the class of morphometric methods that preserve complete information about the relative spatial arrangements of the data throughout an analysis. As such, these methods allow for the visualization of group and individual differences, sample variation, and other results in the space of the original specimens [3, 7]. Traditionally, morphometrics was the application of multivariate statistical analyses to sets of quantitative variables such as length, width, and height. In the late 1980's and early 1990's however, a shift occurred in the way morphological structures were quantified and how

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the data were analyzed. This shift emphasized methods that captured the geometry of the morphological structures of interest, and preserved this information throughout the analyses [1]. In 1993 a review of the field of morphometrics called this new approach geometric morphometrics and suggested that this paradigm shift signaled a"revolution in morphometrics" [21].

Elliptic Fourier analysis (EFA) was the first geometric morphometric method to be used. While the bounding edge of a structure or region can be considered homologous across specimens, points collected to sample such curves do not have such clear correspondences. The approach usually used is to digitize points along an outline, fit the points with a mathematical function (usually some form of Fourier analysis), and then compare curves by using the coefficients of the functions as shape variables in multivariate analyses [1, 20]. In this method, outlines of structures can be captured and analyzed using shape variables generated. In the current study, morphological variations within and between the two populations of *S.woodiana* were analyzed using the outline-based method of EFA. The divergence in the shape of the shell and the allometric variation within and between the two populations of *S.woodiana* were the focus of the study.

## Materials and Methods

The freshwater mussels, *S.woodiana*, were collected through random hand- picking. The first collection site was situated in Lake Lanao specifically in Marantao, Lanao del Sur. Lake Lanao is a large lake in the Philippines, located in Lanao del Sur province in the country's southern island of Mindanao. With a surface area of 340 km² (131 square miles), it is the largest lake in Mindanao, and the second largest lake in the Philippines. The second site is conducted in Lawis stream located in Iligan City. This stream is located nearby residential houses.

The mussels were boiled to discard the meat. Then, the samples were dried and the shells were then used in this study. The shells of the mussels were scanned at 300 dpi resolution using Microtek scanner. Both the two valves of the shell were scanned. The other half of the shell was flipped horizontally prior to the analysis. Samples of the scanned images are shown in Figure 1.

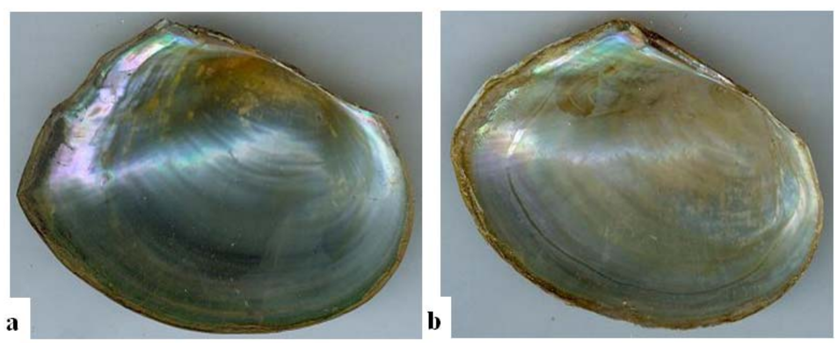


**Fig. 1:** Scanned images of *S. woodiana* from a) Lawis stream and b) Lake Lanao.

The scanned images of bivalves were built into tps file using the tpsUtil program. The purpose of this program is to provide various file conversion and other utility functions to help one manipulate the TPS files that are used in tps series of morphometric programs [22]. Curves were drawn on the outline of the shell using tpsDig version 2 [22]. The purpose of this program is to facilitate the statistical analysis of landmark data in morphometrics by making it easier to collect and maintain landmark data from digitized images. The TPS format was the output file and is a simple ASCII file that can be easily converted into other standard formats. The curves were resampled to 100 points by length. Two replicates were made on each half of the shell. There was a total of 843 samples outlined having 487 samples from Lake Lanao and 356 samples from Lawis stream. The curve points on the digitized image are shown in Figure 2.

Through the tpsUtil program, the tps curve files were converted to landmarks (points marked with the pencil tool in tpsDig as landmarks) [22]. The converted files were opened in the Palaeontological Statistics software (PAST) [12] used for morphometric data analysis. The shape of the shell from the two populations of the bivalve were analyzed using the Elliptic Fourier method that allowed various simple transformations so that the results will be invariant to size, location, rotation, and starting point of the digitized outline [9]. Multivariate analysis of variance (MANOVA) methods extend analysis of variance methods to cover cases where there is more than one dependent variable and where the dependent variables cannot simply be combined. The allometry or the relationship between the size and shape of the bivalves were studied through the use of MANOVA/ CVA. The shape used was taken from the first canonical variate axis which has the highest percentage of eigenvalue. The size was also determined using the PAST software.

The frequency distribution of the first canonical variate scores of the two populations of *S.woodiana* from Lake Lanao, Maranato and Lawis stream, Iligan City was plotted using Kernel density plots.

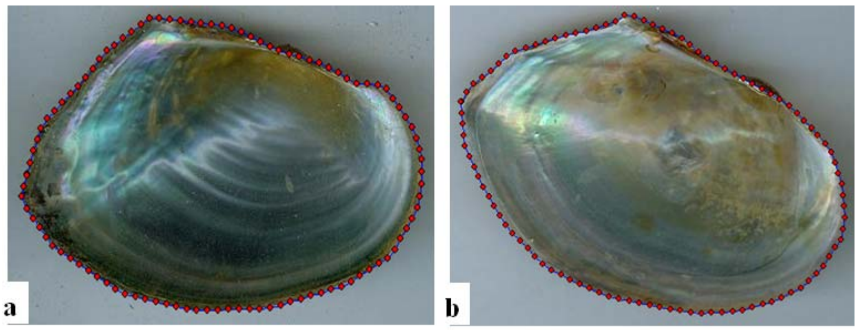


**Fig. 2:** Outlined images of *S.woodiana* a) Lawis stream and b) Lake Lanao.

## Results and Discussion

The resultant elliptic Fourier coefficients from the Elliptic Fourier Analysis were then subjected to Multivariate Analysis of Variance (MANOVA) and Canonical Variate Analysis (CVA). MANOVA was used to test the equality of the means of several multivariate samples that produced an ordination map based on maximal separation as shown in Figure 3. Morphological divergence on the shape was evident showing differences between the two populations of bivalve collected from Lake Lanao and Lawis stream.

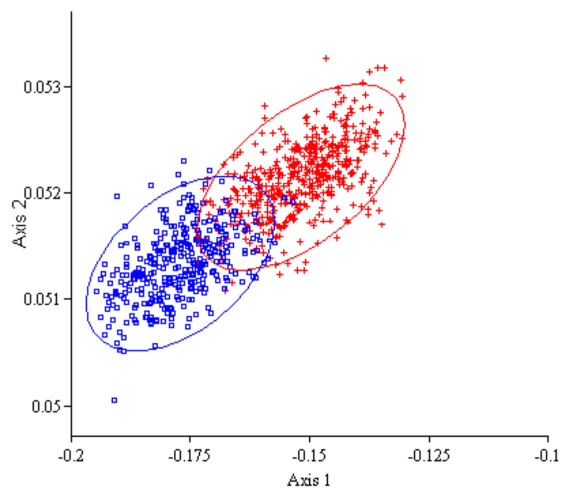


**Fig. 3:** Ordination map of the bivalve *Sinanodonta woodiana* from Lake Lanao, Marantao (red) and Lawis stream, Iligan City (blue).

The allometry within and between the two populations of the mussel was tested using multivariate analysis of variance (MANOVA) method. Two tests were used in this method, the Wilk's lambda and the Pillai trace. The corresponding *p* values of these two tests were used to indicate the significant difference of the shell shapes of the two populations of the mussel. Based on table 1, the *p* values from the two tests were the same and were less than 0.05 which means that the variation between the two populations was highly significant. The table also showed that the first eigenvalue was 100% in its linear combination while the second eigenvalue was 1.642E- 11%. Thus, the shape was taken from the first canonical variate axis showing the highest eigenvalue since the largest eigenvalues correspond to the dimensions that have the strongest correlation in the data set.

A scatter plot of specimens was produced by CVA along the first canonical axes, producing maximal and second to maximal separation between all groups. The axes are linear combinations of the original variables, like that of the PCA, in which the corresponding eigenvalues indicate the amount of variation explained by these axes. The results (Figure 4) in the CVA scatter plot showed that the variations within each population were due to allometry. The shape of the shell changed as the size increased due to growth. However, differences between populations can be observed and that it was not due to allometry.

**Table 1:** Result of MANOVA using the EFA coefficients.

| | df1 | df2 | F | p (same) |
|---|---|---|---|---|
| Wilk's lambda | 80 | 762 | 40.68 | 4.181E- 224 |
| Pillai trace<br>Eigenvalue 1=<br>Eigenvalue 2= | 80<br>4.271<br>7.014E- 13 | 762<br>Percent: 100%<br>Percent: 1.642E-11% | 40.68 | 4.181E- 224 |

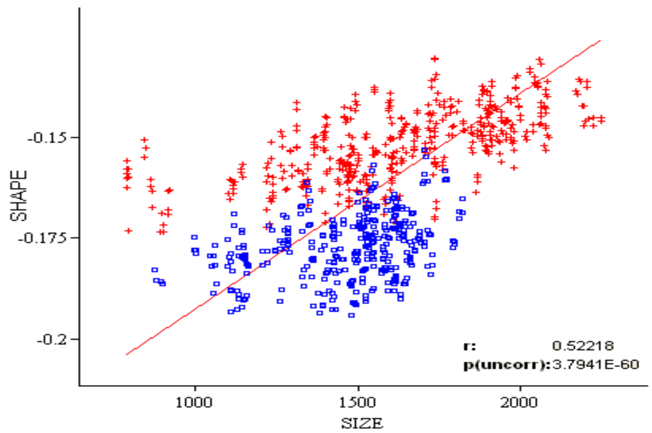


**Fig. 4:** Results of the test for allometry within and between the two populations of the bivalve *S.woodiana* from Lake Lanao (red) and Lawis stream, Iligan City.

The frequency distribution of two populations of bivalve was plotted using the histogram in Figure 5. The distribution of the CVA1 scores for the Lake Lanao, Marantao population deviated from normality (W = 0.9932, P = 0.027, Shapiro-Wilk's W test) which expressed a directional selection. Directional selection occurred because a certain allele had a greater fitness than others, resulting in an increase in frequency of that allele. This process continued until the allele was fixed and the entire population shared the fitter phenotype.

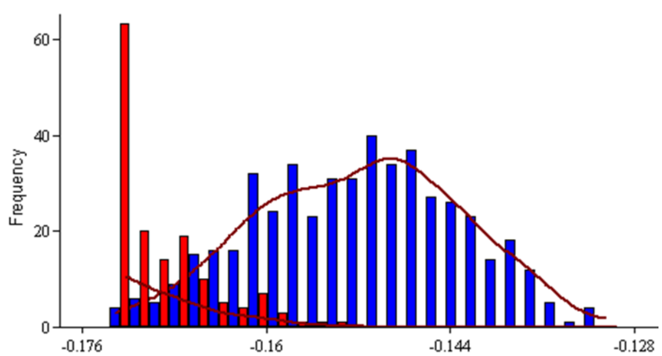


**Fig. 5:** Frequency distribution of the first canonical variate scores of the two populations showing a prevalence of balancing selection for the Lawis, Iligan (blue) population and directional selection for the Lake Lanao, Marantao (red) population.

On the other hand, the distribution of the CVA1 scores for the Lawis stream, Iligan City population followed a Gaussian distribution (W = 0.9924, P = 0.067, Shapiro-Wilk's W test) exhibiting a balancing selection. In this population, balancing selection did not result in fixation, but maintained an allele at intermediate frequencies in a population. This occurred through frequency-dependent selection, where the fitness of one particular phenotype depended on the distribution of other phenotypes in the population.

The results showed in this study manifestly expressed the distinction between the two populations of the bivalve species. The two populations of the mussel showed differences in the shape of the shell and a morphological divergence was evident. Allometry was also tested for the variations within and between the two populations of the species. The data showed that allometry was the cause of the morphological variations within the populations but not between the two populations. Shape variation observed within the species can be argued to be brought about by age [11]. Other factors like the habitat might have contributed to the variation between the two populations of the mussel [4]. Increasing sedimentation of organic matter ensure that the mussels had a constant supply of food. Heavily modified and artificial aquatic habitats, with high silting rates, were found to be especially suitable for population of *S.woodiana*. A mass occurrence of Chinese mussel can be observed in habitats where bottom substrata is predominantly composed of silt-clay [4](Cakic *et al*., 2006). The geographical factor was considered since the two sampling sites have different currents of water since the collection site in Lake Lanao is not flowing while in the Lawis stream the species was collected in flowing wáter and the current was strong [14]. The variation may also be a product of genetic differences brought about by varying environmental conditions [4]. Thermal conditions, water flow, and the character of the substrate determine the distribution and density of this species [14]. The mussels exhibited preference for warmer hábitats. The optimal thermal conditions are within a seasonal temperature range of 10 to 35ºC, within which the mussel occupy habitats with moderate water flow that guarantee them adequate access to food and make reproduction possible.

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