Comparative morphological descriptions of interior shell patterns of the Venerid bivalves: *Meretrix lyrata*, *Mercenaria mercenaria* and *Venerupis philippinarum* using Landmark-based Geometric Morphometric Analysis

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**Abstract.** The shell is the most distinct feature of bivalves, which exhibits a large degree of variation in terms of morphology. Recognition of bivalve variability is important for the effective culture of these organisms. Traditional approach has been made to study shell morphology using differences in length and color. However, conventional methodology can be strengthened by applying additional quantitative tools. A more recent and advanced approach in investigating morphological shell shape patterns were incorporated in the present study. Using Geometric Morphometric (GM) Analysis, the right and left valve interiors of the three species of bivalves from family Veneridae - Lyrate asian hard clam (*Meretrix lyrata*), Quahog or little neck (*Mercenaria mercenaria*), and Manila clam (*Venerupis philippinarum*) from Tubod, Lanao del Norte, Philippines were explored for phenotypic variations. Fifteen (15) anatomical landmark test was performed – covering characteristics of the umbo, hinge, muscle scars, pallial line and pallial sinus. Multivariate Analysis of Variance (MANOVA)/Canonical Variance Analysis (CVA) scatter plot was used to graphically present the clustering of each species. Moreover, Relative Warp Analysis (RWA) and Kruskal–Wallis Test, was used to obtain consensus morphology of the three species and their variations along the negative and positive axes, and to test if there are significant differences on the anatomical landmark of the three species, respectively. Results in MANOVA yielded significant differences in the three species, CVA showed a distinct clustering of species population, and Kruskal–Wallis supported this clustering by obtaining significant results. The analyses showed that the umbo position, orientation of the muscle scar, relative deepness of the pallial sinus, and pallial line length are significantly different in *M. lyrata*, *M. mercenaria* and *V. philippinarum*. The morphological differences on their anatomical landmarks are consequences adaptation to their specific environmental preferences. The results of the present study can provide more information about the ecology and characteristics of the three species and use this information to devise techniques that can manipulate production yield without the expense of the life of other animals and the whole biodiversity.

**Key Words:** bivalve variability, shell morphology, shell shape patterns, anatomical landmarks, biodiversity.

**Introduction.** The shell in general, is the most discernible feature of the molluscan body. It also exhibits a large degree of variation in terms of morphology, even among individuals of the same species (Marquez et al 2010). Bivalve shell morphology is linked to a series of endogenous (genetic and physiological) and exogenous factors (biotic and abiotic interactions). The interactions between endogenous and exogenous factors result to phenotypic plasticity of aquatic shells. Moreover, the variation in shell phenotypes hinders their correct identification. Most current descriptions of freshwater shells are based on subtle differences in length and color, but only a small percentage of these identifications are valid. The clarification of which taxa constitute valid species is mainly due to advances in genetics. Still, the identification of species cannot be obtained solely...
on genetics because of the genetic boundaries between taxa but morphological aspects are also fundamental for routine identification (Morais et al 2013).

However, paradigm shift on how morphometric studies perceived by researchers resulted from recent studies in this field. Morphometrics, the study of shape variation and co-variation among variables, was traditionally restricted to a set of multivariate methods applied to linear measures such as the analysis of linear distances and ratio variables in a standard multivariate framework (Marquez et al 2010). But assessment of shell shape variation using these methods has some statistical drawbacks of which results are sensitive to particular distances and chosen ratios. Furthermore, there is also difficulty in acquiring size-free shape variables from healthy individuals since these measurements are highly correlated with size (Morais et al 2013). Geometric morphometric approach effectively avoids confusion between size and shape by preserving shape variables and main geometric properties of the specimens while producing a visual representation and determining shape variables that can be statistically interpreted throughout the analysis (Webster & Sheets 2010).

Geometric morphometric is often explained as a revolution in the area of morphometric and has been successfully applied in fields of biological studies such as ecology, evolution, systematics, and taxonomy (Morais et al 2013). There are two approaches in geometric morphometric techniques namely: a) contour/outline analysis, where the border of a specimen is analyzed, and b) landmark analysis, where the change in the position of homologous points identified in each specimen is quantified. Both approaches offer different types of information and can be applied in distinct circumstances. An ideal group for the application of geometric morphometric methods is bivalves due to their hard and stable shells. Primarily, geometric morphometric studies on bivalves mainly employed contour-based methods. Many current researches used landmarks, a combination of both approaches and sliding semi-landmarks to analyze curves. These methods have been successfully applied to differentiate between similar species of bivalves between wild and aquaculture populations and between fossil and modern taxa, and also to identify developing changes in shape and to analyze geographic variation in shape.

Asian hard clams (Meretrix lyrata) are commercially important bivalves in East and Southeast Asia. This species commonly inhabits tidal flats, estuaries and sandy beaches (Yamakawa & Imai 2013). The external shell is pearly-white in color, while the interior is pale-yellow to off-white.

Quahogs/hard clams (Mercenaria mercenaria) occupy intertidal and sub-tidal habitats, and burrow into substrate in various depths (Roberts et al 1989). The Quahog has two thick, equal-sized valves that are elliptical in shape and marked with concentric rings (Eversole 1987). A dark brown external ligament found below the anteriorly-inclined umbo joins the valves dorsally. A distinct heart-shaped lunule is found opposite the hinge. External colors range from off-white to tan. The internal shell is white or pale yellow with an indigo margin. Adductor scar may also be purple.

Venerupis philippinarum, commonly called the Manila clam, is a species of the family Veneridae, native to East and Southeast Asia and introduced throughout the world in the early 1900s (Shean 2011). The Manila clam’s preferred growing ground which is in the higher part of the intertidal zone, in the sand-gravel substrate. It also lives at a shallow depth within the ground (Jones et al 1993). This species has multi-colored external valves, that usually have a purple hue along the umbo and the inner rim of the shell (Shean 2011).

Economically, clams and bivalves constitute a large portion of the marine invertebrates and fauna that are being sold in the markets as protein food sources, primarily to the people living near water bodies. They make up a significant proportion of the world fishery of edible bivalves. The Philippine territories are homes to several genera and species of Venerids that are dispersed across the country.

The primary aim of the study is analyze and determine if there are significant differences and variations in the anatomical structure/landmarks on both valves of the three Venerid species. Main statistical tools used were Relative Warp analysis, Multivariate Analysis of Variance (MANOVA), Canonical Variance Analysis (CVA), and
Kruskal–Wallis test. Results of the study could help wildlife and conservation biologists to devise techniques to monitor on Venerids and Bivalves by understanding their biology and dynamics in our ecosystem.

**Material and Method.** The Venerid bivalves were randomly acquired from Pala-o Wet Market in Iligan City, Philippines. Originally, the samples were taken from the marshes in the municipality of Tubod, located in Lanao del Norte, Philippines. A total of 148 specimens were used comprising 50 of *M. lyrata*, 50 of *M. mercenaria* and 48 of *V. philippinarum*).

The samples were cleaned off their soft tissues before the shells were sun-dried. The two valves of each sample were slowly separated by carefully tearing their ligament. The left and right valves were documented dorso-ventrally. The ventral aspect was documented in a constant position where all internal shell structures are clear and distinct, with the umbo oriented vertically and upward (Figure 1).

All photographs were then transferred to a computer for storage and enhancement. The programs used to enhance the photos were Photoscape v3.6.5 and Adobe Lightroom 5.3.

Obtained images were then subjected to geometric morphometric analysis. To cover as many morphological differences as possible, a landmark-based morphometric method that makes use of homologous points in the shell was applied. This technique eliminates the effect of variation in the location, orientation, and scale of the specimens (Moneva et al 2012). The technique made use of landmarks in the shell’s internal features. Landmarks used were based on the study of Neubauer et al (2013), having a total of 15 landmarks. The landmarks, in general, were used to describe the umbo, the features of the cardinal teeth, the size of the muscle scar and the pallial sinus.

The landmarks were plotted on digitized images using the program tpsDig2 v 2.12 (Rohlf 2008a). The different coordinates produce by tpsDig2 were subjected to the program tpsRelw v.1.46 (Rohlf 2008b) which this program shows the Relative Warps among the three specimens. Relative warp analysis displays a plot of relative warp scores.
and provides "visualization window" that displays the estimated shape for arbitrary points in the ordination (Rohlf 2008b). Relative Warp scores were then saved in a Matlab file and loaded in Microsoft Excel for organization based on significant relative warps and populations.

All relative warp scores were subjected to Paleontological Statistics (PAST) software v2.17c and v3.01 (Hammer et al 2001) for Multivariate Analysis Of Variance (MANOVA) to test the significant difference on the anatomical landmarks in the three species including the significant and non–significant relative warps, and Canonical Variance Analysis (CVA) for scatter plot elucidation. CVA scatter plots show different clustering patterns of the population variations of different species on different axes. Kruskal–Wallis Test was used to determine if there were significant differences on the anatomical structures/landmarks on the left shell valves of the populations of different species. Other graphical presentations include box plots and histograms, generated from the Paleontological Statistics PAST software, to display where the different populations are centered and their differing distributions over the range of variables.

Results and Discussion. Canonical Variance Analysis (CVA) scatter plots of the pooled individuals of three different venerid species showed significant variation in the patterns of shell interior morphology (Figure 2).

![Figure 2. CVA scatter plot of the shape variation of the interior ventral left valves (A) and right valves (B) of M. lyrata (red), M. mercenaria (blue), and V. philippinarum (green).](image)

CVA scatter plot shown in Figure 2A are clustering patterns of different populations in left interior valves of the three venerid species on different axes, defined by their relative warp coordinates. Species A or M. lyrata (red) population is clustered at the negative x-axis. Cluster of species B or M. mercenaria (blue) population are majorly located at the 4th quadrant and slightly overlaps with species C or V. philippinarum (green), which is located and confined within the 1st quadrant.

For the right shell interior morphology, CVA scatter plot shown in Figure 2B, species A (M. lyrata) measures highly positive on the X-axis. While species B (M. mercenaria) and C (V. philippinarum) measures negatively on the X-axis. Species A measures more positive on the Y-axis in comparison to the mostly negative readings of species B. In addition, it is shown that M. mercenaria and V. philippinarum slightly overlapped in the CVA scatter plot both in the left and right interior ventral valves.

Table 1 summarizes the values of the MANOVA. Overall, the values indicate that there are significant differences among the different Venerid species.
Table 1
Results for the comparison of species A, B, and C data multivariate analysis of variance (MANOVA) p-values

<table>
<thead>
<tr>
<th>Left interior valve</th>
<th>M. lyrata</th>
<th>M. mercenaria</th>
<th>V. philippinarum</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. lyrata</td>
<td>-</td>
<td>2.32E-70*</td>
<td>5.13E-63*</td>
</tr>
<tr>
<td>M. mercenaria</td>
<td>6.97E-70*</td>
<td>-</td>
<td>3.97E-22*</td>
</tr>
<tr>
<td>V. philippinarum</td>
<td>1.54E-62*</td>
<td>1.19E-21*</td>
<td>-</td>
</tr>
<tr>
<td>Right interior valve</td>
<td>M. lyrata</td>
<td>M. mercenaria</td>
<td>V. philippinarum</td>
</tr>
<tr>
<td>M. lyrata</td>
<td>-</td>
<td>1.80352E-64*</td>
<td>1.99544E-68*</td>
</tr>
<tr>
<td>M. mercenaria</td>
<td>5.41057E-64*</td>
<td>-</td>
<td>3.34198E-16*</td>
</tr>
<tr>
<td>V. philippinarum</td>
<td>5.98633E-68*</td>
<td>1.00259E-15*</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: significant values denoted by (*).

Relative Warp Analysis was used to interpret the morphological differences revealed by the CVA. Only the significant relative warps, those that were above 5% variation, were analyzed. The analysis obtained three significant and two significant relative warp axes for the right and left valve interior view respectively. Results of the significant relative warps are summarized and presented in Figure 3. The figure contains boxes that include histograms and box plots, both describing the distribution of the Venerid populations. Histogram shows the number of modes and peaking frequencies in each relative warp; kernel densities are also displayed. Box plots convey the ranges of species along the axis relative to other species. Variations on the negative axis are shown in the left side of each box while the variations on the positive axis are shown in the right. Mean or consensus morphology is displayed at the top of the box.

Presented in Figure 3a is the relative warp box plot and histogram showing variations in the left valve interiors of the Venerid bivalves. Shown on the first half (left), RW 1 is bimodal, having two peaks at the positive and negative values. Range of M. lyrata (species A) spreads along the negative values of the axis while the ranges of M. mercenaria (species B) and V. philippinarum (species C) are on the positive values of the axis. RW2 is unimodal, having a single peak at 0, indicating that most individuals have morphologies identical or similar to the consensus. Box plot graphs for the species in the RW2 are relatively similar in distribution, supporting the histogram that the three populations fall in the 0 value or consensus morph.

Shown in Figure 3b is the relative warp box plot and histogram presenting variations in the right valve interiors. There is a bimodal distribution of the population in the first relative warp which strongly suggests two separate normally distributed populations. M. lyrata draw towards the positive axis, while both M. mercenaria and V. philippinarum lies along the negative axis. Box plot for RW2 shows that the range of M. lyrata is almost uniformly distributed in the positive and negative values of the axis. M. mercenaria is contained within the negative values but slightly deviates to the positive values of the axis. The range of V. philippinarum is located in the positive axis but also slightly extends to the negative axis. RW3 box plot graph shows that M. lyrata fall in the zero value, while M. mercenaria is found lying in the negative axis and gradually extends to the positive. Lastly, V. philippinarum is positioned in the positive values but slightly deviates to the negative axis. Histograms for RW2 and RW3 are unimodal in distribution, indicating that most individuals have morphologies identical to the mean shape. Variation percentages and detailed differences in morphological variations on different axis of the two significant warps are discussed in Table 2.
Figure 3. Relative warp box plot and histogram showing variations in the left (a) and right (b) internal shell of: (A) *Meretrix lyrata*, (B) *Mercenaria mercenaria* and (C) *Venerupis philippinarum*.

Table 2

<table>
<thead>
<tr>
<th>RW % variation</th>
<th>Description</th>
<th>RW % variation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 67.89</td>
<td>At the extremity of RW1 positive axis, the distance between anterior cardinal tooth and the dorsal tip of the anterior adductor/retractor muscle scar is shorter but longer for the negative values, both compared from the consensus morphology. The distance between the dorsal and ventral tips of the posterior adductor/retractor muscle scar is shorter for the values in the RW1 negative axis. The pallial sinus in the positive RW 1 axis is more concave and shallower pallial sinus of the values along the extremity of the RW1 negative axis. The pallial line in the RW1 negative axis is slightly longer compared to the consensus. The distance between the deepest point of the pallial sinus and the junction of the ventral tip of the anterior adductor/retractor muscle scar and the pallial line is shorter in the RW1 positive axis. In the RW1 negative axis, the umbo is orthogyrous and located at the median. The posterior adductor/retractor muscle scar is also directed anteriorly.</td>
<td>1 68.78</td>
<td>Scoring a high positive on RW1 indicates that the umbo significantly in vertical position, the dorsal tip and the junction of the ventral tip of the retractor muscle moves down. The deepest part of the pallial sinus also becomes less pronounced. A high negative score, in contrast, indicates that the umbo and teeth move to the left (pointed anteriorly) with the dorsal tip and the junction of the ventral tip of the retractor muscle moves closer to the teeth. The deepest part of the pallial sinus also becomes more noticeable.</td>
</tr>
</tbody>
</table>
The pallial line of the individuals along the RW2 negative axis is slightly shorter compared to the consensus shape. The pallial sinus is also slightly shallower. The deepest point in the pallial sinus of RW2 positive axis is nearer to the pallial line and the distance between the dorsal tip of the posterior/retractor adductor muscle scar and ventral tip of the posterior cardinal tooth is slightly shorter.

Having a high positive RW2 score means that the interior of the right shell becomes wider. This difference is more pronounced in the posterior end. Also, the junction of the ventral tip of the anterior retractor muscles scar and pallial line moves closer to the dorsal tip of the anterior retractor muscle scar. Scoring a high negative on the RW2 graph indicates a shift in interior structure of the shell towards the left. The junction of the ventral tip of the anterior retractor muscles scar and pallial line moves further from the dorsal tip of the anterior retractor muscle scar.

A high positive on the RW3 score indicates that the interior of the right shell becomes wider, with more distinction in the posterior end. The pallial sinus is deeper. The junction of the ventral tip of the anterior adductor muscle scar and pallial line and the dorsal tip of the adductor muscle scar also moves further away from each other. On the other hand, a high negative score showsthat the interior of the right shell becomes narrower. The teeth and the umbo shifts its direction to the left while the junction of the ventral tip of anterior retractor muscle scar and pallial line shifts its direction toward the umbo and the teeth. The pallial sinus is shallower.

Table 3 summarizes the values of the result from Kruskal-Wallis test for the significant relative warps of the left and right shell interior view. It is shown that _M. mercenaria_, _V. philippinarum_, and _M. lyrata_ varied from each other in terms of internal shell morphology.

<table>
<thead>
<tr>
<th>RW 1</th>
<th>Left valve</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>RW 2</th>
<th>Left valve</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-</td>
<td>5.36E-09*</td>
<td>2.56E-11*</td>
<td></td>
<td>A</td>
<td>-</td>
<td>7.031E-8*</td>
<td>1.527E-17*</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1.61E-08*</td>
<td>-</td>
<td>1.60E-16*</td>
<td></td>
<td>B</td>
<td>2.109E-17*</td>
<td>0.09846</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>7.68E-11*</td>
<td>4.81E-16*</td>
<td>-</td>
<td></td>
<td>C</td>
<td>4.582E-17*</td>
<td>0.2954</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RW 3</th>
<th>Right valve</th>
<th>A</th>
<th>B</th>
<th>C</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>-</td>
<td>7.02E-18</td>
<td>7.02E-18</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>2.11E-17*</td>
<td>-</td>
<td>2.47E-09</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2.11E-17*</td>
<td>7.42E-09*</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>-</td>
<td>-</td>
<td>7.562E-07*</td>
<td>6.4E-05*</td>
</tr>
<tr>
<td>B</td>
<td>2.269E-06*</td>
<td>-</td>
<td>8.066E-13*</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.000192*</td>
<td>2.42E-12*</td>
<td>-</td>
<td></td>
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<tr>
<td>A</td>
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<td>0.08234</td>
<td>5.508E-09*</td>
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</tr>
<tr>
<td>B</td>
<td>0.247</td>
<td>-</td>
<td>9.952E-09*</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.652E-08*</td>
<td>2.986E-08*</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Note: significant values denoted by (*).

This finding is considered significant as the results in the Kruskal-Wallis test yielded significant values, suggesting that umbo position, muscle scar orientation, relative deepness of the pallial sinus, and pallial line length are significantly different in the Venerid bivalves being studied.

Anatomical and structural descriptions for the consensus morphology include anteriorly positioned and prosogyrous umbo; anterior adductor/retractor muscle scar is slightly larger/elongated compared to the posterior adductor/retractor muscle scar; and
deep pallial sinus. For the left interior valve, the negative values in RW1 have orthogyrous and medially positioned umbones; shallow pallial sinuses; and the posterior adductor/retractor muscle scars are oriented anteriorly. As shown in the CVA scatter plot, *M. lyrata* is located at the negative values of the axis which perfectly parallels to the visual systematic classification of structures. RW1 positive values have umbones that are prosogyrous and are anteriorly positioned; deep or pronounced pallial sinuses; and posterior adductor/retractor muscle scars that are oriented posteriorly. The characteristics also show high similarity to the existing descriptive characteristics for *M. mercenaria* and *V. philippinarum*. The differing characteristics for both are the pallial line length and relative deepness of their pallial sinus. *V. philippinarum* has more pronounced pallial sinus and longer pallial line. Similar results were also observed in the right interior ventral valves.

The three species are all found at the inter-tidal zones (Palomares & Pauly 2013; Bower et al 1986; Bower 1992). Inter-tidal zones are high-energy areas and to protect them, they require high mechanical stability and one adaptation to this is the circular/obovate shape and thick valves of their shell. Venerids do not possess lateral teeth, they rely largely on their cardinal teeth that provide strength and integrity between the valves to protect them against shearing stress, hence the 3 cardinal teeth present in the three venerids are often being semi-fused and medially positioned. The pallial sinus is also correlated with the depth of burrowing of organisms. Deep–burrowing bivalves require longer siphons to reach the bottom surface of the substrate to filter food and a larger space is required to house this relatively large siphon, hence the deep pallial sinus. *M. lyrata* has a relatively shallower pallial sinus (Anderson, N. D.) as compared to the other two. This implies that *M. lyrata* can burrow down the substrate but not as deep as *M. mercenaria* and *V. philippinarum*, that can burrow at deeper locations (Neubauer et al 2013; Roberts et al 1989; Eversole 1987). This is also supported by the thicker shell of *M. lyrata* to protect them from frequent encounter with tidal waves.

The results of the present research somewhat correlates to the studies of Carlton (1979) and Kraeuter & Castagna (2001) wherein the exterior appearances of the two species, *M. mercenaria* and *V. philippinarum* is also related except for the umbo is covered with series of growth rings for *M. mercenaria*, and *V. philippinarum* has triangular mottled markings that disperses outwards its shell.

**Conclusions.** Venerids are ubiquitous in our markets but are the least being assessed. Traditional methods include visual estimation and basic biometrics. A few studies only used Geometric Morphometric Analysis as a tool to describe variations in shape and anatomical landmarks of the Venerids, and they only cover less than half of the total species of Venerids. Results from the Geometric Morphometric Analysis, coupled with MANOVA, RWA and Kruskal–Wallis tests, on the three venerid species show that they are significantly different in terms of anatomical structure/landmark in their left and right valves. Significant values from MANOVA and Kruskal–Wallis test provided concrete supporting data. With the tests performed, we can safely concur that there is a significant phenotypic differences between *M. lyrata* and the populations of both *M. mercenaria* and *V. philippinarum*. The species sampled were located on the same environment yet they have significant phenotypic differences. Morphological differences among the three species are consequences from the environmental factors in their ecological niche as an adaptive mechanism. Geometric Morphometric Analysis was successful on the comparative anatomical landmark analysis by showing highly parallel results with the existing basic systematic classification on descriptive characteristics. This study provides additional knowledge and understanding on the morphological variations of different species of Venerids and their ecology. From this information, one can come up with efficient methods, such as proper culturing technique in relation to bivalves’ preferred habitat, so as to prevent possible disturbances that may directly affect its population. This study also boosts the importance factor of Geometric Morphometric Analysis Tools.
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