INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 12, No. 1, p. 82-89, 2018

RESEARCH PAPER OPEN ACCESS

Describing shell shapes of *Venerid bivalves* using elliptic fourier analysis

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Key words: Veneridae, CVA, MANOVA, Variation, Elliptic Fourier Analysis.

http://dx.doi.org/10.12692/ijb/12.1.82-89 Article published on January 12, 2018

Abstract

In this study, Elliptic Fourier (EF) analysis was used to describe phenotypic variation among the selected venerid bivalve species *Meretrix lyrata* (lyrate asiatic hard clam), *Chamelea striatula* (striped venus clam) and *Tapes dorsatus* (turgid venus clam). Thus, to describe possible phenotypic diversity, a total of 90 venerid bivalves (30 specimens for each species) were photographed on their right valves. Canonical variate analysis (CVA), multivariate analysis of variance (MANOVA), principal component analysis (PCA) and Kruskal-Wallis tests showed significant difference between species based on the Fourier coefficients. This study showed significant variations in the shape of the right external valve of the shell. CVA plot generated show separation of populations examined indicating significant difference between groups. Observed differences in the external shell shapes of different venerid bivalve species were based in the umbo, anterior margin, shell width, length depth and anterior margin depression. Results suggest hypothesized variable factors behind the disparity of the external shell shape which include habitat differences, environment and genotype interactions. Herewith, the use of Elliptic Fourier (EF) analysis proved to be useful in effective quantification of inter-specific variation between species.

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Introduction

There has been an enormous challenge pertaining to systematics since traditional conchology-based ideas on relationships among venerids were contested by recent studies and modern approaches (Chen *et al.*, 2011). Hence, this study will provide a novel means to describe shell shape variations on bivalve species challenging traditional morphology-based approaches. In this study, Veneridae bivalves are considered because they are of high ecological and economic value and one of the least understood and most poorly defined molluscan taxa. Common species has been harvested for example quahog, Pismo clams, venus clams, hard clams and Manila clams (Mikkelsen *et al.*, 2006). However, Morton 1993 stated that Lam (1980) reviewed the taxonomy of these and related species and concluded that there remains to be a dilemma as such is the taxonomy and lot of work need to be done for that matter. An example of this type of problem is the taxonomy of the *venerid* Veremolpa, a very important group of deposit feeding bivalves.

This species was cited as *V. micra* from Japan and has been named as *V. scabra* an Indo-Pacific species. In Hongkong, there appears to be a single species and in Japan 6 species were described and recognized in literature. Horikoshi and Thompson 1980 noted in Japan that this clam Veremolpa was classed as a mid bay species but in Tolo channel appeared to be in the mouth of the bay. Based on the assessment of physical characters, there lies a taxonomic confusion in the species but inferred to be due to ecological difference. As such, there lies to be unresolved taxonomic problem especially of closely related species present in a region which could also be polymorphic. Another example for instance is Tapes clams which were found to be highly polymorphic. In *Tapes philippinarum,* morphometrics allowed it to be distinguished but still considerable confusion exist.

Likewise, the striped venus clam *Chamelea striatula* are commercially important bivalves inhabiting European and North African coastal waters. The taxonomic status of these taxa has been the subject of debate for decades (Garcia *et al.*, 2017).

Meanwhile, the lyrate Asiatic hard clam, *Meretrix lyrata* occurring along the coasts of the Philippines, Vietnam and Southern China is an edible marine bivalve known for its economic value however, past studies considered only shell morphology (Yoosukh and Matsukuma, 2001) yet remained taxonomically confusing. Additional studies revealed that shell shape and color patterns showed remarkably intra-specific variability. Herewith, there remains systematic confusion with regard to the species and that the specific name *M. lyrata* pertain to various species (Yoosukh and Matsukuma, 2001). Traditional shell shape morphology and color pattern are often very similar leading to erroneous identification and notation in shell books.

Accordingly, with this in mind it is pertinent to search for a tool to delineate species. In the past, there has been poor literature and poor taxonomic methodology has been employed and many of bivalve identification taken from text that was applicable in one region and may not be applicable in another region (Morton, 1993). However, seeking identification through morphological aspects of an organism is still of fundamental importance (Morais *et al.*, 2013). With the emergence of advanced and effective statistical tool in computational biology integrating statistics, computer imaging and geometry, confusion in delineation between shape and size by maintaining the shape variables and main geometric properties of the specimens used in the analysis of variation has come to its solution (Marquez *et al.*, 2010; Webster and Sheets, 2010; Morais *et al.*, 2013; Dapar *et al.*, 2014).

One of the best and effective geometric morphometrics approach is the utilization of Elliptic Fourier descriptors (Kuhl and Giardina, 1982) to quantitatively describe mathematical approach in the overall shape by transforming coordinate information concerning its contours into Fourier coefficients.

In addition, principal component analysis for summarizing the elliptic Fourier descriptors was suggested by Rohlf and Archie (1984). Consequently, Iwata and Ukai (2002) come to the development of a program package for the quantitative evaluation of biological shapes on the basis of elliptic Fourier descriptors, hence, the SHAPE software package. In addition, increasing awareness on sympatric sibling species is important for species delineation.

Thus, Elliptic Fourier Analysis was employed in this study. This study described the morphology of the right external valve shell shape of selected venerid bivalve species, *Meretrix lyrata* (lyrate asiatic hard clam), *Chamelea striatula* (striped venus clam) and *Tapes dorsatus* (turgid venus clam) and identified key characters for delineation process respectively.

Materials and methods

Collection and preparation of bivalves

A total of 90 venerid bivalves of which 30 specimens for each of the following identified species: *Meretrix lyrata* (lyrate asiatic hard clam), *Chamelea striatula* (striped venus clam) and *Tapes dorsatus* (turgid venus clam) (Fig. 1) were acquired from Iligan City, Philippines.

The samples were cleaned off with their soft tissues and disinfected with detergent before the shells were sun-dried. The two valves of each sample were slowly separated by carefully tearing their ligament.

Image processing and elliptic fourier analysis

The external right valves were photographed using Sony Cyber-shot DSC-RX100 II digital camera. For outline analysis of the shell shape, the software package SHAPE v.1.3 was used (Iwata and Ukai, 2002) based on the methodology of Elliptic Fourier descriptors to describe each type of two-dimensional shape with a closed outline, in terms of harmonics. All images were saved in. bmp format (24bit) and were binarized with Chain Coder that converts outlines into series of chain codes, a coding system that describes the geometrical information of the shapes. Then, the Chain-code file was transformed into a Normalized Elliptic Fourier file with Chc2Nef, using 20 harmonics. It allows detailed analysis of fine-scale morphological variation in the outline of the right external shell shape.

The matrix of the harmonic coefficients underwent normalization based on the first harmonic, the data transformed into shape variables. Subsequently, a PCA was performed on the variance covariance matrix of normalized coefficients (elliptic fourier descriptors) using PrinComp, which gives a graphical output of the average shape ± the standard deviation (Magrini and Scoppola, 2010).

Principal component scores were further subjected to Kruskal-Wallis test, a non-parametric version of one-way ANOVA, to determine if the populations differ significantly from each other based on the shape of its external right valve. Boxplot was used to visualize the distribution of different venerid bivalves. Multivariate and statistical analysis were done using the software PAST version 3.0 as platform (Hammer *et al.*, 2001). The outline of elliptic fourier analysis is illustrated in Fig. 2.

Results and discussion

In this study, Elliptic Fourier Analysis was employed. It described the morphology of the right external valve shell shape among selected venerid bivalve species, *Meretrix lyrata* (lyrate asiatic hard clam), *Chamelea striatula* (striped venus clam) and *Tapes dorsatus* (turgid venus clam).

Table 1. MANOVA results for significant variation in the right valve shape of the venerid bivalves.

	A. *Meretrix lyrata*	B. *Chamelea striatula*	C. *Tapes dorsatus*
A. *Meretrix lyrata*	-	3.62274E-35*	1.28464E-46*
B. *Chamelea striatula*	1.08682E-34*	-	3.23579E-21*
C. *Tapes dorsatus*	4.85393E-46*	9.70738E-21*	-

*significant, $p<0.05$

Results yield highly significant difference in the right external shell shape of the three populations examined based on the distribution of the individuals along the first two canonical variate axes. Herewith, CV1 and CV2 accounts and explains the overall variation and indicate a clear separation among species examined based on overall shell shape (Fig. 3). Moreover, Multivariate Analysis of Variance (MANOVA) and the percentage of variance with the overall shape variation and the Kruskal-Wallis test for significant difference are presented in Table 1 and 2 respectively showing further support for significant variation between species examined. For the Principal component analysis, it further shows that described variations were based specifically in the umbo, anterior margin, shell width, length depth and anterior margin depression with PC1- 49.0861% and PC2- 25.6865% respectively (Fig. 4 and Table 2). Based on the results, this shows that observed variation may be due to adaptive responses to environmental gradients over large population scales of various venerid bivalve species.

Table 2. Percentage of variance with overall shape variation and Kruskal-Wallis test for significant difference of right valve of the venerid bivalves by each of the significant principal components.

PC	Variation	Kruskal-Wallis testp-value	Remarks
1 (49.0861%)	Variation in the size of umbo, anterior margin and shell width, length and depth.	3.72E-17	significant
2 (25.6865%)	Variation in between the umbo and the anterior margin depression	2.56E-13	significant
3 (6.28850%)	Slight variation in the shell margin	0.61070	not significant
4 (4.60250%)	Variation in the size of the posterior margin	0.02584	significant
5 (3.05610%)	Slight variation in the anterior margin	0.12150	not significant
6 (2.55810%)	Slight variation in the shell length	0.60780	not significant
7 (1.97820%)	Slight variation in between umbo and anterior part depression	0.77730	not significant
8 (1.58550%)	Variation in the length and the size of the umbo	0.76780	not significant

This may also be attributed that *M. lyrata* is commonly inhabiting tidal flats, estuaries and sandy beaches making them as hard clams (Yamakawa and Imai, 2013) while *C. striatula* and *T. dorsatus* both are venus clams, which are marine bivalve mollusks of small to large saltwater clams which colonize the sandy ocean bottom, and their populations are often dense and large (Whitehead, 1977).

Fig. 1. Three identified bivalve species, namely lyrateasiatic hard clam, *Meretrix lyrata*; striped venus clam, *Chamelea striatula*; and turgid venus clam, *Tapes dorsatus* (Bivalvia: Veneridae).

Moreover, results coincide to previous studies where shells largely demonstrate a degree of variation in morphology and even among individuals of the same species (Marquez *et al.*, 2010).

Shell morphology and structural traits are the principal characters used in traditional taxonomy of bivalves (Coan *et al.*, 2000; Vadopalas *et al.*, 2010).

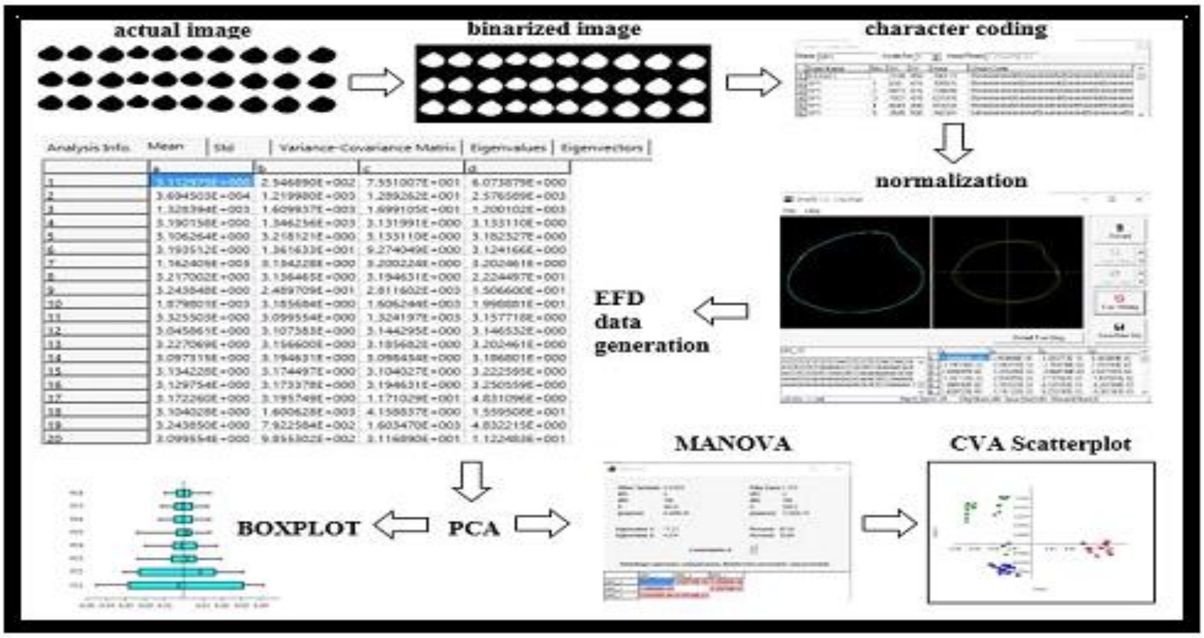

Fig. 2. Outline of the Elliptic Fourier Analysis of the right valve external shell shape of bivalves.

Herewith, external shape differences may suggest a number of factors or mechanisms that are involved in the evolution of these species in different geographical area or habitat differences. Several studies have shown that shell variations can be attributed to several factors inter-specifically that may include the ecological, genetic, and developmental variations (Dapar *et al.*, 2014; Camama *et al.*, 2014).

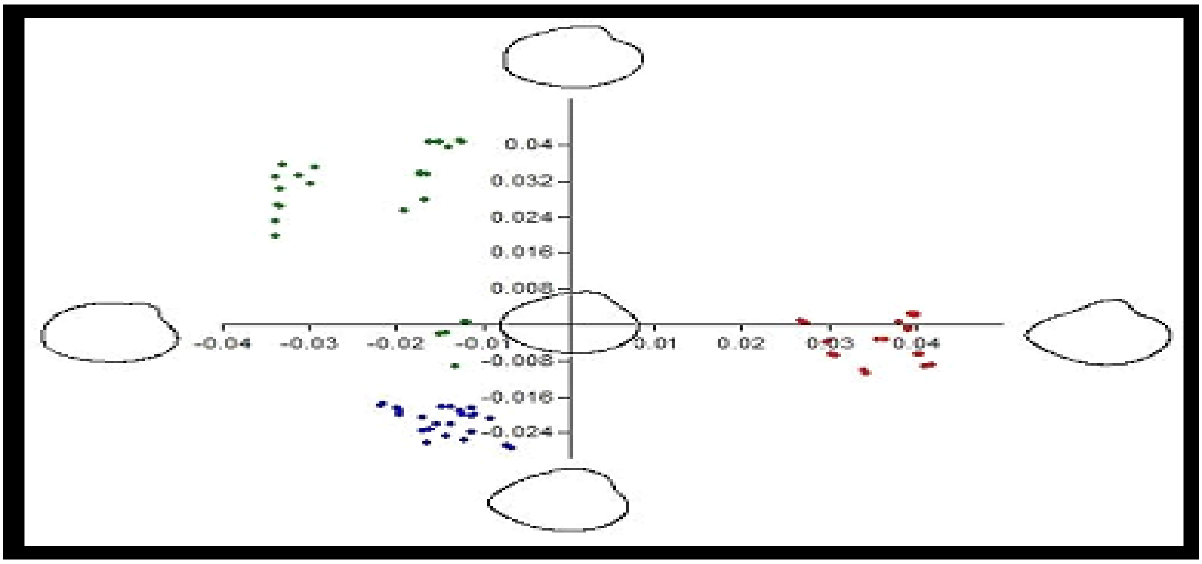

Fig. 3. Plots of the first two canonical axes showing significant difference in the right valve external shell shape of three venerid bivalve species: *Meretrix lyrata*, lyrate asiatic hard clam (red); *Chamelea striatula*, striped venus clam (green); *Tapes dorsatus,* turgid venus clam (blue).

Several factors were hypothesized to be influencing the differences observed such as plasticity brought about by habitat differences or could be due to environment and genotype interactions (Johannesson *et al*, 1993; Reid, 1996; Trussell, 1996, 2000; Kyle and Boulding, 1998; Dapar *et al.*, 2014). Morais *et al.*, 2013 further explained that bivalve shell morphology is linked to a series of endogenous and exogenous factors which can result to phenotypic plasticity of aquatic shells (Rufino *et al.*, 2006).

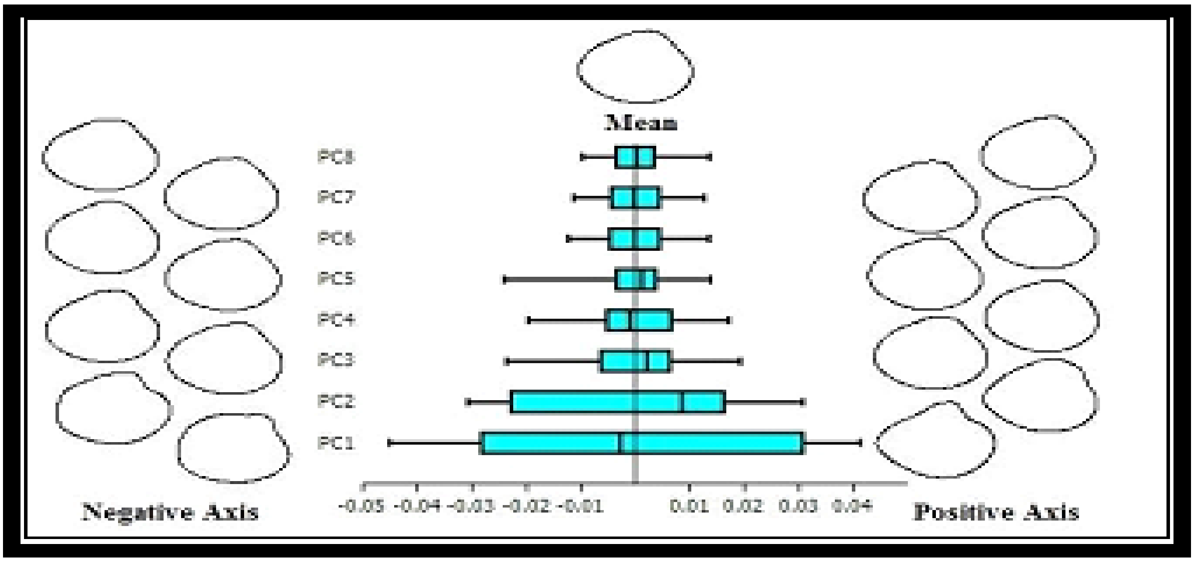

Fig. 4. Boxplots of the significant principal components in the right valve external shell shape of the three venerid bivalve species: *Meretrix lyrata* (lyrate asiatic hard clam), *Chamelea striatula* (striped venus clam), *Tapes dorsatus* (turgid venus clam).

Conclusion

Results showed that selected venerid bivalve species: *Meretrix lyrata* (lyrate asiatic hard clam), *Chamelea striatula* (striped venus clam) and *Tapes dorsatus* (turgid venus clam) have significant external shell shape variation. Variations observed were based in the size of umbo, anterior margin depression and shell width, length and depth as revealed in the principal component analysis. It revealed that quantitative analysis of the external right valve shape of the venerid bivalves using principal component scores obtained from standardized elliptic fourier descriptors can effectively quantify intra-specific morphological variations which is far more effective than traditional or conventional way. Thus, the application of elliptic fourier descriptors and principal component analysis proved to be useful for effective quantification of subtle differences and variation. Variable factors were hypothesized to be causing phenotypic variation such as habitat differences or environment and genotype interactions.

More studies are recommended to further explore the basis for the differentiation of bivalves from the family Veneridae in response to taxonomic crisis.

Acknowledgment

The researchers would like to express their heartfelt gratitude to their families and friends who become a considerable source of inspiration and determination.

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