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# Elliptic Fourier analysis in describing shell shapes of three species of *Lambis* (Gastropoda: Strombidae)

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ABSTRACT

A popular example in morphological evolution studies is the molluscan shell. Four species of shells, L. millipeda (Linnaeus, 1758), L. scorpius (Linnaeus, 1758), L. lambis(Linnaeus, 1758) and Strombus sinuatus (Humphrey, 1786) under Strombidae collected from Lugait, Misamis Oriental were used as samples. The study was conducted to describe the shell shape of the four species using Elliptic Fourier Analysis. Results revealed less percentage variance between species but most variations are based on the marginal digitation of the outer lip of the shells. Based from the principal component scatter plot, all four species' shell shape belong to the same group and cluster analysis showed 100% similarity between two groups, grouping L.millipeda with Strombus sinuatus and L. scorpius with L. lambis. These implies that the four species possess the same shape and classification of species through shell shape is less appropriate to be use in taxonomy in classifying the organism.



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## INTRODUCTION

The diversity of molluscan shell external morphologyis most obvious evidence of evolution because of its geometrically simple, yet diverse form (Liew and Schilthuizen, 2013). An obvious example is the Family Strombidae or the true conchs composed of five genera (*Strombus, L., Tibia, Terebellum, Rimella*) and 75 species (Abbott, 1960). These conchs are found to inhabit shallow water, on sandy, muddy or rubble bottoms or on marine grassflats distributed in tropical to subtropical regions. Four morphologically misidentified species are commonly collected for food and of economic importance locally since it now commands a higher price in the market. These are *Lambis millipeda*, *L. scorpius*, *L. truncata*, and *S. sinuatus* (Fig. 1). Theshells are solid and thick, with a relatively large body whorl, and a wide range of shell morphologies, with some of the most extreme (e.g. *L.*).

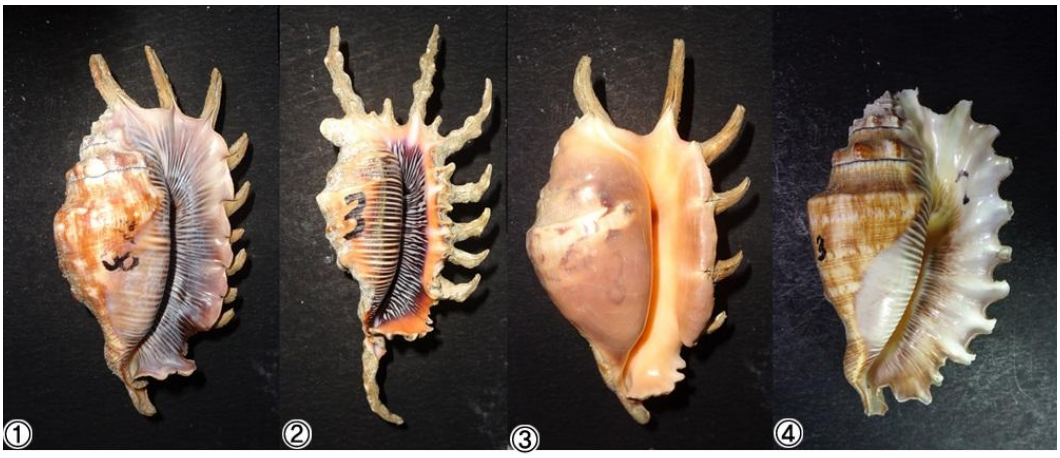

**Fig. 1:** Four species under Family *Strombidae*, (1) *L. millipeda* (Linnaeus, 1758), (2) *L. scorpius* (Linnaeus, 1758), (3)*L. lambis* (Linnaeus, 1758) and (4) *Strombus sinuatus* (Humphrey, 1786) ventral view.

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*L. millipeda* (Linnaeus, 1758), or the millipede spider conch is commonly found on various shallow-water bottoms, from low in the intertidal zone to a depth of a few meters. Appears frequently in the local markets of the central Philippines though having a bitter taste and less often eaten than the other *L.* species. This species is mainly collected for its shell. *L. scorpius* (Linnaeus, 1758), or the scorpion spider conch inhabits protected areas of coral reef flats, under or among dead coral slabs and boulders. It is found in shallow waters, from low tide levels to shallow subtidal zone to a depth of about 5 m. This species is also collected for shell trade. *L. lambis* (Linnaeus, 1758), or the common spider conch is common on reef flats and on coral-rubble bottoms or in mangrove areas, usually associated with fine red algae on which it feeds. Occupies shallow water, from low tide levels to a depth of about 5 m. These strombids appear in markets in the northern Philippines and commonly bought as an expensive food source (Carpenter and Niem, 1998). *Strombus sinuatus* (Humphrey, 1786), or the Laciniate conch are commonly found on sand bottoms with broken corals and algae, in relatively clear waters, from low in the intertidal zone to a depth of about 20 m. (Randall, 1964).Lambis and Strombus are the two most species-rich of traditionally defined strombid genera (Abbott 1960, 1961). They are herbivores associated with shallow-water reefs and grass beds and both possess similar soft tissue anatomies, egg masses, and radulae. Kronenberg (1998) suggested that Lambis and Strombus belong together in a group within the Strombidae, even though their shells show striking morphological differences. The relationship between spatial patterns of morphological diversity and species richness in *Strombus* and *Lambis* was examined (Roy *et al.*, 2001). However, species richness of individual clades of strombid gastropods related to their morphological diversity could not be examined because of the lack of a well-supported phylogeny.

What is interesting with these four species of strombids is their growth is determinate, allowing a clear identification of adult specimens, including fossils (Abbott, 1960; Vermeij and Signor, 1992). The morphology of the adult shell is extremely variable (Savazzi, 1991). The adult body size span a very large range (20-375 mm), and size frequency distributions show striking differences between the Indo-Pacific and Eastern Pacific/Western Atlantic (Abbott 1960, 1961) although the anatomy of the soft parts is quite uniform making them excellent candidates for constructional morphology studies (Savazzi, 1991). With the development of geometric morphometrics (GM), a statistical analysis of shape has made possible for a fast and reliable way of studying biological forms (Adams, 2004; Marcus *et al.*, 1996; Rohlf and Marcus, 1993; Tabugo *et al.*, 2012) thus it is used in this study. The tool was found to be useful inquantitatively describing shape variations in organisms especially between species and population within species (Hassall *et al*., 2008; Rohlf and Archie, 1984; Diaz *et al*., 1989; Tabugo *et al.,* 2012). Elliptic Fourier descriptors (EFDs), originally proposed by Kuhl and Giardina (1982) is one of the tools in GM that can delineate any type of shape with a closed two dimensional contour. The principal component scores obtained can be used as observed values of morphological features in subsequent analysisof the shapes of biological organs thus is used in the current study in describing the shapes of four species of true conchs.

## MATERIALS AND METHODS

*Collection of Samples:*

A total of 48 shells were collected from the coastal area of Lugait, Misamis Oriental (Fig. 2). Processed shells were preserved in the Department of Biological Sciences, Mindanao State University-Iligan Institute of Technology. To examine the variation in shapes of the four species, the outline of the ventral shells of the four species of strombids were analyzed in chain coding technique using the software package SHAPE v.1.3 (Iwata and Ukai, 2002). Chain code is a coding system for describing geometrical information about contours in numbers from 0 to 7. Image acquisition was done using a Digital Camera (Sony, DSC-W730). The full colored images of the shells produced were converted to 24-bitmap type, binary (black and white color) images. Herewith, the objects of interests were distinguished via segmentation techniques through a “threshold procedure” where a parameter called the brightness threshold is manually chosen from brightness histogram and applied. Undesirable marks also termed as “noise” were consequently eliminated by erosion dilation filter process. After noise reduction, the closed contour shape of each shell was extracted via edge detection and the contour were stored as chain codes (Dalayap *et al.,* 2011). Chain coding technique was used which relied on a contour representation to code shape information. This method tracks the shape of the shells and represents each movement by a chain code symbol ranging from 0-7. The set of possible movement depends on the type of contour representation, a pixel based contour representation were used in this study. Normalized Elliptic Fourier Descriptors (EFD) obtained from the chain codes were calculated using Elliptic Fourier transformation as suggested by Kuhl and Giardina (1982). Normalization of data obtained from chain codes used the first harmonic ellipse as a basis which corresponds to the first Fourier approximation and utilized the 20 harmonics number to be calculated as suggested by Iwata and Ukai (2002). Principal component analysis was used to summarize independent shape characteristics. The differences in shape among shells were determined and subjected to Multivariate Analysis of Variance and Principal Component Analysis (MANOVA/PCA). Wilks’ lambda and Pillai trace values and p values were obtained. Box- and whiskers and scatter plots showing the variations were generated, this is to visualize the distribution of shape variation using the principal component scores. Cluster analysis was also used to determine species' groupings based on the mean of coefficients.


**Fig. 2:** Sampling Area.

## RESULTS AND DISCUSSION

Principal component analysis of the normalized Elliptic Fourier descriptors of the ventral view of thefour species of*Strombidae* showed 11, 9, 3 and 3 significant components (PCs) (Tables 1-4). Figure 3 shows the mean shell shape of the four species based on the principal component. Significant differences in shell variations on four species as explained by each of significant principal components isgraphically shown in Fig. 4. These are based on the principal component (PC) scores that defined shape differences. The standardized elliptic Fourier coefficients calculated were used to reconstruct the consensus morphology and the positive (+) and negative (-) deviations from the mean shape was used to determine subtle shape variations of the shells. This is an experimental procedure to compare the mean shapes and interpret the underlying relationships within and between the four species. Descriptions of variations in the shell shapes within species are summarized in Table 5. It can be seen from the results that the shell shape of the four species are phenotypically plastic as most of the variations are based on the anterior canal and marginal digitations of the outer lip which are observable in all species. It is argued that the marginal digitations in the outer lip of the shell plays an important role in lowering the vulnerability of the shells towards predators. A set of long spines surrounding the outer lip, increases the footprint of the shell and reduces the likelihood of overturning during the shells' locomotion which are very frequent in strombids of all geological ages, except during the Cretaceous (Savazzi, 1991). To compare between species differentiation, principal component scatter plot andcluster analysis was done (Fig. 4 and 5). Results show the four species are morphologically differentiated.While the four species have variations in the anterior canal and marginal digitations in the outer lip, these variations differ from species to species (Table 5).

**Table 1**: The Eigenvalues and percentage variance explain by each significant principal component for the shell variation of *L. millipeda* (Linnaeus, 1758).

| Principal Component | Eigenvalue | Proportion (%) | Cumulative | Total Variance |
|---|---|---|---|---|
| Overall<br>1 | 4.86E-03 | 28.4641 | 2.85E+01 | 1.71E-02 |
| 2 | 3.96E-03 | 23.2179 | 5.17E+01 | |
| 3 | 2.56E-03 | 14.994 | 6.67E+01 | |
| 4 | 1.71E-03 | 10.0005 | 7.67E+01 | |
| 5 | 1.25E-03 | 7.3308 | 8.40E+01 | |

**Table 2:** The Eigenvalues and percentage variance explain by each significant principal component for the shell variation of *L. scorpius* (Linnaeus, 1758).

| Principal Component | Eigenvalue | Proportion (%) | Cumulative | Total Variance |
|---|---|---|---|---|
| Overall | | | | |
| 1 | 1.04E-02 | 34.1511 | 34.1511 | 3.06E-02 |
| 2 | 5.06E-03 | 16.5653 | 50.7164 | |
| 3 | 4.48E-03 | 14.6772 | 65.3936 | |
| 4 | 3.66E-03 | 11.9861 | 77.3797 | |
| 5 | 1.87E-03 | 6.1092 | 83.4889 | |

**Table 3:** The Eigenvalues and percentage variance explain by each significant principal component for the shell variation of *L. lambis*(Linnaeus, 1758).

| Principal Component | Eigenvalue | Proportion (%) | Cumulative | Total Variance |
|---|---|---|---|---|
| Overall | | | | |
| 1 | 3.44E-03 | 56.7291 | 56.7291 | 6.06E-03 |
| 2 | 1.40E-03 | 23.1475 | 79.8767 | |
| 3 | 1.22E-03 | 20.1233 | 100 | |

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**Table 4:** The Eigenvalues and percentage variance explain by each significant principal component for the shell variation of *S. sinuatus* (Humphrey, 1786).

| Principal Component | Eigenvalue | Proportion (%) | Cumulative | Total Variance |
|---|---|---|---|---|
| Overall | | | | |
| 1 | 1.01E-02 | 59.5317 | 59.5317 | 6.06E-03 |
| 2 | 3.75E-03 | 21.988 | 81.5198 | |
| 3 | 3.15E-03 | 18.4802 | 100 | |

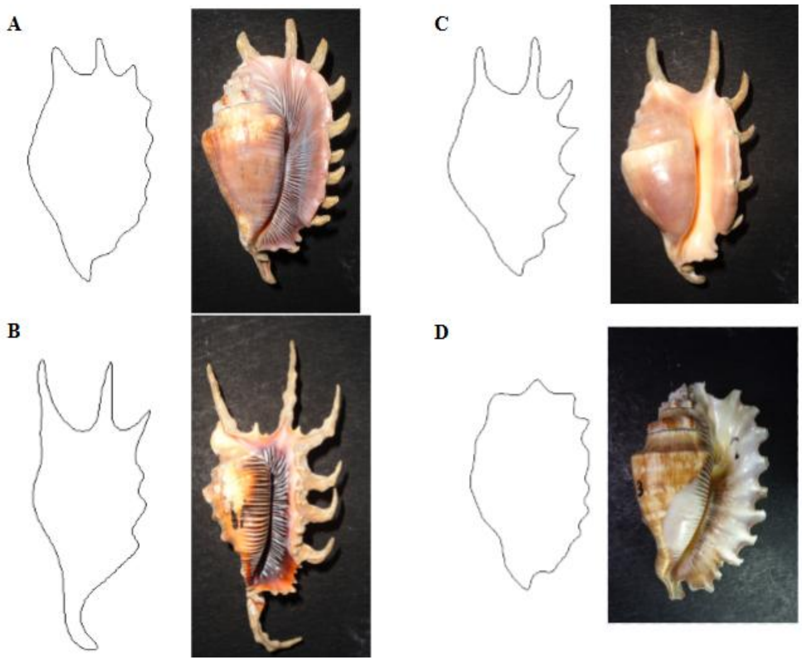


**Fig. 3:** Mean shape of the shells under Strombidae, (A) *L. millipeda* (Linnaeus, 1758), (B) *L. scorpius* (Linnaeus, 1758), (C) *L. lambis* (Linnaeus, 1758) and (D) *Strombus sinuatus* (Humphrey, 1786) ventral view.

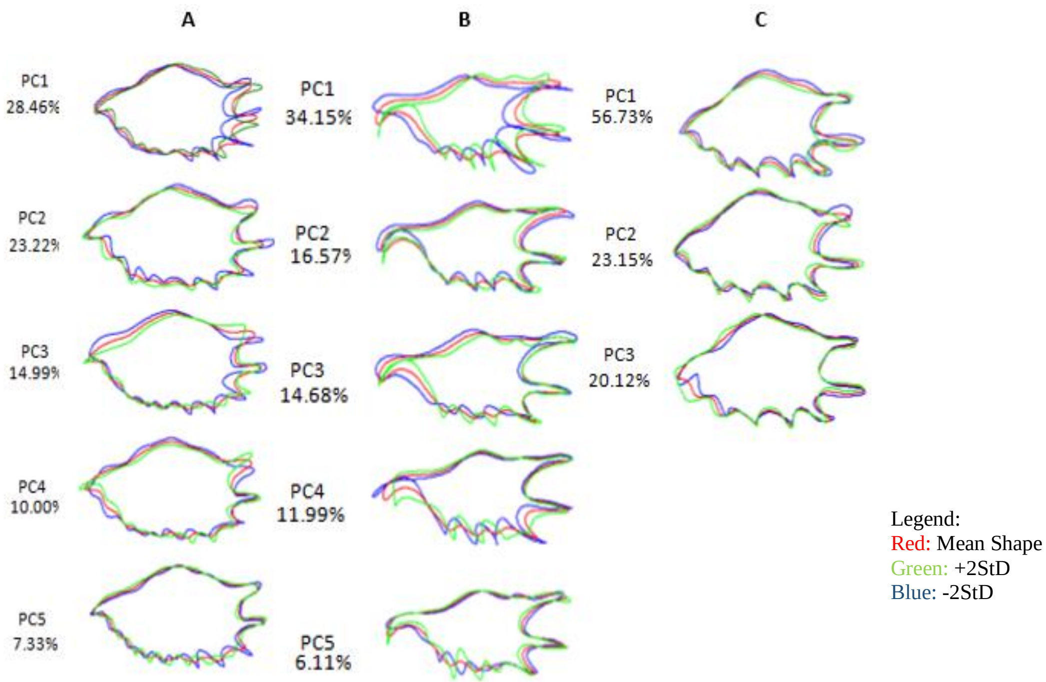


**Fig. 4:** Significant principal component showing shell variations of (a) *L. millipeda*, (b) *L. scorpius (c) L. lambis (d) S. sinuatus.*

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**Table 5:** Description of shapes for ventral shells based on significant Principal Component.

| | *L. millipeda* | *L. scorpius* | *L. lambis* | *Strombus sinuatus* |
|---|---|---|---|---|
| PC1 | (28.46%) Variations at the anterior canal ranging from a more pointed to a blunt end and digitation of the outer lip ranging from more defined to slender digitation. | (34.15%) Very slender to blunter anterior canal and shorter to longer posteriormost digitation are the observed variations. | (56.73%) Shorter to longer anterior canal and less recurved to more recurved base shape | (59.53%) Less to more prominent digitations and shape base varies from having unnoticeable to larger protrusions. |
| PC2 | (23.22%) Posteriormost digitation of outer lip ranging from a more pointed to a blunt end and blunter to sharper marginal digitations. | (16.57%) Shorter to longer shell base, more pointed to blunter posteriormost digitation and less to more defined number of digitation of outer lip. | (23.15%) Posteriormost digitation that extends from inward to outward direction and more pronounced to less defined anterior canal. | (21.99%) Blunter to sharper posteriormost digitation and rough to rougher base shape are the variations observed. |
| PC3 | (14.99%) Variations from slender to a wider anterior canal and more pronounced to less defined stromboid notch. | (14.68%) Smaller to larger base shape and very slender, recurved to blunter anterior canalare the observed variations. | (20.12%) Less prominent to more prominent anterior canal is the observed variation. | (18.48%) Less to more prominent digitations are the observed variations. |
| PC4 | (10.00%) More to less pronounced posteriormost digitation and regular to irregular base shape are the observed variations. | (11.99%) Less number of digitations to a more pronounced digitations and straight, wide to recurved, slender anterior canal are the variations observed. | | |
| PC5 | (7.33%) Regular to irregular base shape and blunter to sharper digitations are the observed variations. | (6.11%) More recurved to less recurved anterior canal and more defined to less defined digitations are the observed variations. | | |

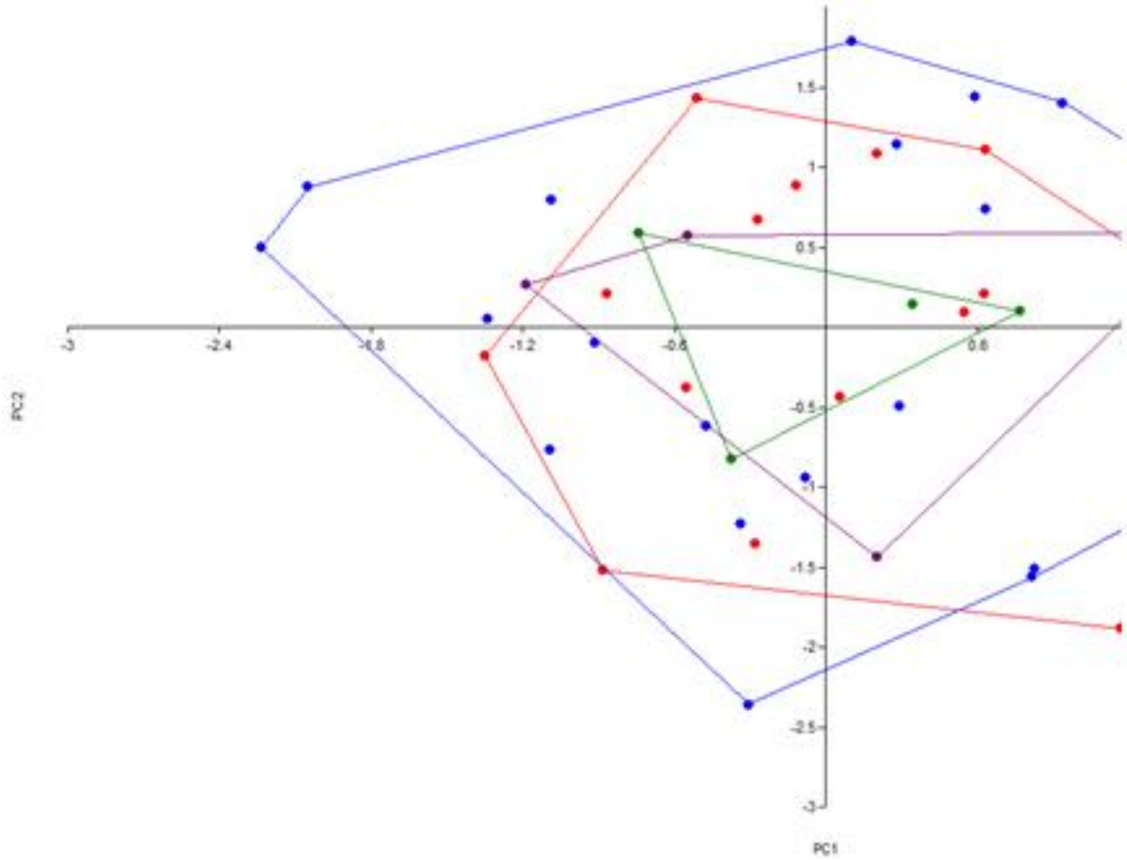


**Fig. 5:** Plots of the first two principal component of the four species under Strombidae (Wilks' lambda: 1; p-value: 1; Pillai trace: 1.772E-07; p-value: 1) using Principal Component.

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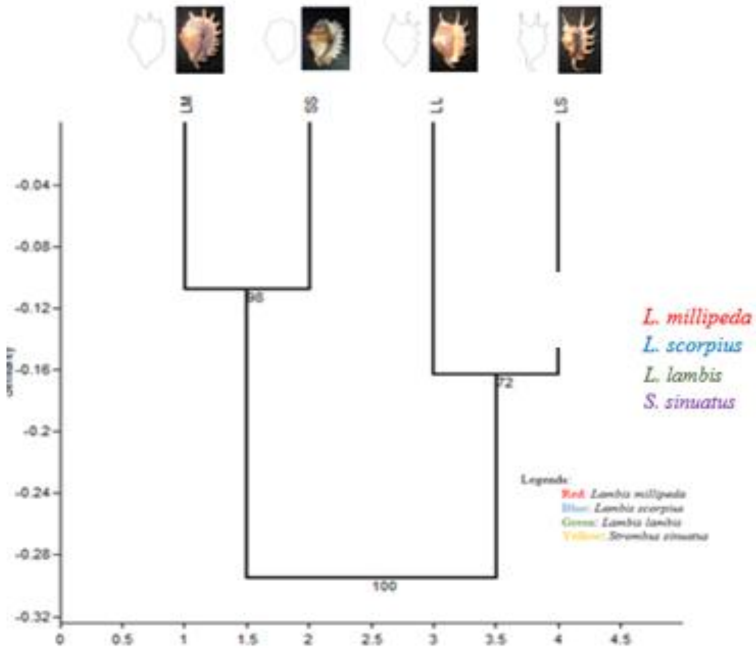


**Fig. 6:** Cluster analysis of the four species under Strombidae, (LM*) L. millipeda*, (LS) *L. scorpius*, (LL) *L. lambis* and (SS) *Strombus sinuatus* based on shell shape.

*Conclusions:*

In this study, Elliptic Fourier Analysis was used in describing the shell shape under Strombidae. Results revealed that there is less percentage variation between species as supported by the different parameters analyzed. However, the primary variations observed are based on the marginal digitation of the outer lip of the shells. Cluster analysis showed two groupings with 100% similarity, grouping *Lambis millipeda* and *Strombus sinuatus* and *L. scorpius* with *L. lambis.* In conclusion, the four species under Strombidae generally have similarshapes therefore classification of species through shell shape is less appropriate in taxonomy. Furthermore, the study revealed that Elliptic Fourier Analysis can be applied in describing the shell shape of Gastropods, specifically on the four species under Strombidae.

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