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Interior Shell Patterns among the Spondylid Species (Bivalvia: Spondylidae) in Mindanao, Philippines

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Abstract

The variations among the species of the genus *Spondylus* were assessed based on its interior shell pattern using landmark- based geometric morphometry. Samples of the Spondylid species were collected and photographed and 15 landmarks from internal shell morphology were quantified and analyzed. Using the Canonical Variance Analysis (CVA) it was able to differentiate into clusters the local Spondylid species. The most distinct characteristic among the Spondylid species is the distance of the pallial line towards the ventral tip of adductor/muscle scar and the size of the cardinal tooth. Multivariate analysis of variance further showed significant differences in the internal shell characters among the Spondylid species across then sampling sites ($p<0.05$). The internal shell characters among the Spondylid species exhibit disparity despite its great morphological similarity. These shell characters can be used in the identification while conservation efforts are still in the initial stage of the development. The presence of notable dissimilarities in the internal shell characteristics among the Spondylid species indicates that even slight deviations can result in noticeable morphological distinctions. This discovery enhances the understanding of the species divergence within this group and underscores the significance of incorporating internal shell characteristics into taxonomic and ecological investigations.

Keywords: Landmark-based morphometry, Relative warps, Multivariate analysis

1. Introduction

The Lianga Bay Surigao del Sur in the Philippines has an abundant supply of spiny oysters (genus Spondylus) in the local market, restaurants and resorts. Economically, it becomes a good source of income for fishers living nearby the coastal areas of the Bay. Besides, it's pleasant taste compared to other oyster meat, it attracts more tourists to visit the Province because of the delicious oyster meat. However, anecdotal observation is that the unregulated, uncontrolled, and constant harvest of the spiny oyster is now being threatened. The oyster catch in the province has drastically declined. In the year 2017, the total mean catch rate of the Spondylus fishers is only 14.175 kg/gleaner/hour (with shell), which is far from 40- 50 kg/gleaner/hour for the past ten years (Mongado et al., 2016). Though, the Local Government Unit (LGU) is on the initial stage in crafting action in ecological, economical, and legal aspects in the fishery of Spondylus in Lianga Bay Surigao del Sur. The over-exploitation of this important reef bivalve could lead to an imbalance in the ecosystem where they play a vital role. Being both ecologically and economically important, the threats to this reef bivalve

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needs an immediate attention.

However, we cannot conserve what we do not know, considering these important local oysters has still no established taxonomy. The Spondylus is the only genus in the family Spondylidae. This species inhabits rocky reef areas from the intertidal area to 5- 25 meters in depths. Many taxonomic literatures in the Spondylus have been extremely different in their nomenclature. Some of the literature has so many revisions of names for a given species. In the studies of (Lamprell, 1986; Lamprell and Kilburn, 1995; Lamprell and Willan, 2000; Lamprell 2006) in Europe, Australia, and other Indo-Pacific countries members of this groups have been well documented and empha-sized. This species undergoes dispute and has rectification for its taxonomy (Lamprell and Willan, 2000; Lamprell 2006) be-cause of so closely related characters in terms of its morphology.

The concept of determining the variability among the local Spondylid species is the primary aim of the paper using inexpensive, reliable and simple method using morphometric approaches. Using expensive methods such as molecular techniques and other morphological approaches create a better conclusion but remain ambiguous for certain taxa. The landmark- based geometric morphometry is a low-cost technique. It was designed to effectively avoid 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 (Webster and Sheets, 2010).

This study is the first to report the morphometry of the Spondylus species in this field of science. This study was able to analyzed and determined the variability of the anatomical structure and landmarks of the interior shell patterns (left valve) among the local Spondylid species in Mindanao, Philippines. The interior shell was focused instead of the external shell because it is difficult to find sufficient number of distinct landmarks in the bivalve species (Lee et al., 2018). Besides, the Spondylid species comprises of highly dense epibionts attached on the shell's surface. Because of their generally large intra-specific variability, the taxonomy of Spondylus is under permanent revision and the potential for hybridization is assumed (Lamprell, 1986; Lamprell and Kilburn, 1995; Lamprell, 2006).

This study will bridge the gaps and issues on the uncertainty of Spondylus taxonomy which morphometric studies can help to provide additional evidence for or against a particular taxonomic group. By analyzing the physical characteristics of organisms, morphometry studies can identify subtle differences in shape and size that may be useful for species. This paper can provide valuable information to fishery scientists and managers, helping them to make more informed decisions about the management and conservation of Spondylus populations.

2. Materials and methods

2.1 Sample Collections and Processing

The Lianga Bay, Surigao del Sur is famous in terms of spiny oyster (*Spondylus*) fishery in the Region. The expensive and high demand of its meat to the local markets and its beautiful shell compared to other shell when properly polished were used as crafts and jewelry. However, their exploitation has caused a drastic decrease in natural populations leading the Local Government Unit to act immediately on the restrictions in *Spondylus* fishing.

The Bay consists of 60 Barangays with 36 coastal Barangays. Overall, the Lianga Bay municipalities occupy a total land area of 1,007.50 km^2. It is located in the central part of the province of Surigao del Sur lies within a geographical coordinate of $8^{o}34'00"$ and $8^{o}25'06"$ latitude and $125^{o}59'00"$ and $126^{o}22'00"$ longitude. It is bounded on the north by the Pacific Ocean (Fig. 1).

The *Spondylus* specimens were sourced partly from the gleaners who collect them using basic implements. Additional samples were also collected during our field sampling at the coastal Barangays of

Marihatag, San Agustin, Lianga, and Barobo. Afterward the collected specimens were brought to Surigao del Sur State University- Lianga Campus- Biology Laboratory for processing. The live Spondylid was initially narcotized with magnesium chloride solution 3.5%. The samples were cleaned to detach the epibionts on the surface of the shells.

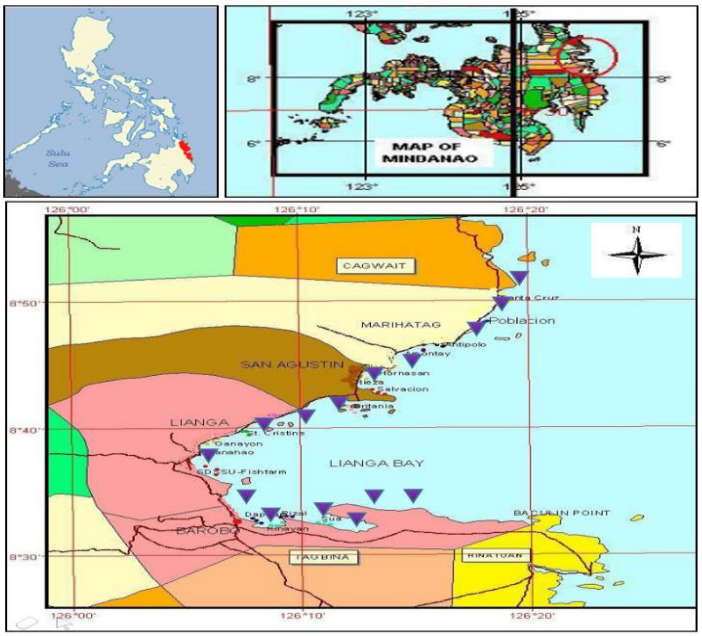

Fig. 1 Map of the study area

2.2 The local Spondylid species subjected for Geo Morphometry

Four (4) species including the two morphotypes of *Spondylus* were qualitatively diagnosed in detail. The spines were characterized into various types including broad, spathate spines and fine interstitial spines. The examination of the valves was also established, the left valve has the adductor muscle scar mostly to the left of the midline, and the right valve has a scar mostly to the right of the midline. Also, the hinge teeth size and color in the left valve was examined. Likewise, the color of the marginal band around the circumference of the inner margin of the shell was also noted.

2.3 Inner Shell Diagnosis: Landmark-Based Geometric Morphometric Analysis

Shell specimens were mounted on modeling clay to establish standard orientation before to photography or measurement (Shaw, 1957). The scale bar (ruler) was attached to the modeling clay since it is essential in the meristic presentation of various landmark configuration to be computed. The upper valves were photographed (DSLR Canon EOS750D) dorsoventrally in a constant position where all internal shell structures are clear and distinct, with the umbo oriented vertically and upward positions.

All digitally photographed samples were transferred to a computer for storage and enhancement using photo editing software (Adobe Photopshop). Also, the shell was photographed and digitized twice to assess measurement error (Viscosi and Cardini, 2011). Each shell was yielded 15 landmarks on its interior shell patterns. The establishments of these land-marks were based on Neubauer et al., (2013) on the Venerid bivalve species of 15 land-marks (Fig. 2). The landmarks include the umbo, teeth, size of the muscle scar, and the pallial sinus. The edited images were digitized using the TPS software (TPSDig 2232) to obtain the landmarks data. Afterward, images that came from TPSDig were uploaded to PaSt v3.01 (Hammer et al., 2001) to produce a variety of coordinates. This software was packaged with geometric and multivariate analyses, including the relative warp and thin plate splines and warps (2D). Aside, the scores were directly analyzed using the MANOVA and Canonical Variates Analysis (CVA).

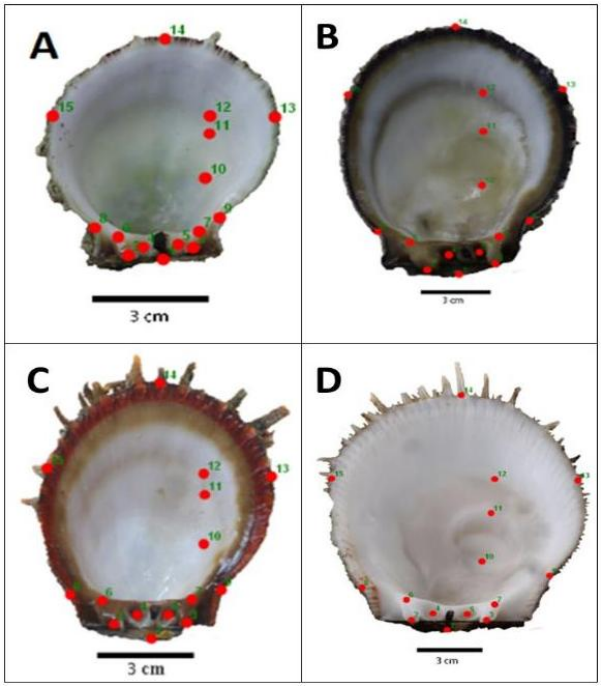

Fig. 2 Landmarks used to describe the left valve internal morphology of the Spondylid species: **(A)** *S. squamosus*; **(B)** *S. limbatus*; **(C)** *S. versicolor*; **(D)** *S. varius*: *Landmark configuration*: 1. umbo; 2. dorsal tip of anterior cardinal tooth; 3. dorsal tip of anterior cardinal tooth; 4. Posterior socket; 5. Anterior socket; 6. Ventral tip of posterior cardinal tooth; 7. Ventral tip of anterior cardinal tooth; 8. Postero-dorsal shell curvature; 9.Antero-dorsal shell curvature; 10.Dorsal tip of adductor/retractor muscle scar; 11. Junction of ventral tip of adductor-retractor muscle scar; 12. Pallial line; 13. Anterior end of the shell; 14.Ventral end of the shell; 15. Posterior end of the shell.

3. Results

A total of 200 specimens were subjected for landmark-based geometric morphometric analysis. The sample sizes ranged from 50 individuals per species.

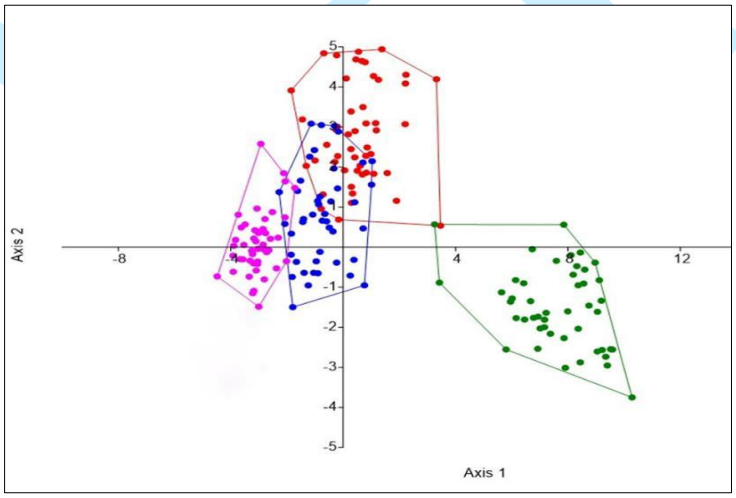

Fig. 3 CVA scatter plot of the shape variation of the interior ventral left valves. *S. squamosus* (pink); *S. versicolor* (red); *S. limbatus* (blue) and *S. varius* (green)

Distinct clusters among the four (4) Spondylid species of the Bay were noticed when projected onto two-dimensional plane (Fig. 3) as defined by their relative warp coordinates. The *S. squamosus* (pink dots) population was mainly clustered at the negative x-axis and slightly overlapped with the *S. limbatus* (blue dots). While the *S. versicolor* (red dots) population was located at the positive y– axis and almost half of the species overlapped with *S. limbatus* (blue). The *S. varius* (green dots) was mainly clustered in the fourth quadrant. The CV axis 1 and 2 accounted for 87.4 % of the total variation (Table 1) and the distinct separation of the sample

species. This suggests that the first CV accounts for a substantial amount of variation in the dataset and represents the most significant axis of differentiation among the Spondylid species.

Table 1 Eigen values and percentages of variance for the first three CV's from Canonical Variates Analysis (CVA) for Spondylids species

CV	Eigen Value	Total Variances (%)
1	17.89	72.87
2	3.56	14.53
3	0.74	3.12

3.1 Interior Shell Patterns

It is clear from the ensuing analysis that the interior shell pattern can be used to classify the *Spondylus* species in Lianga Bay (Eastern Mindanao, Philippines). Aside, it detects variations within the same species. Further analysis of the data using the Multivariate Analysis of Variance (MANOVA) showed significant differences with regards to the shell shapes of the five populations of Spondylid species (Wilk's lambda= 0.00188; Pillai trace= 2.902). Overall, the result indicates that there were significant differences in the internal shell characters of sampled Spondylid species (p-value < 0.05).

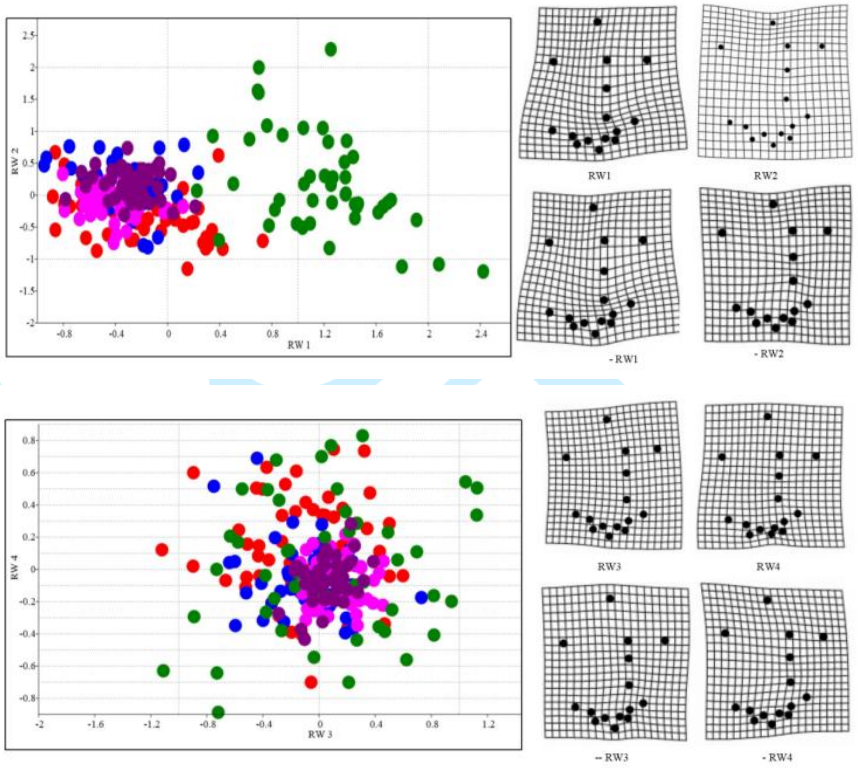

Fig. 4 Relative warps analysis showing the first four warps and the associated deformation grids at the approximate minimal and maximal occupied values of the Spondylid species

To determine which character may have influenced the classification, the first four (4) relative warps, thin-plate spline images for the approximate minimum and maximum occupied values per warp are presented (Fig. 4). The groups/population of the Spondylid species in RW1 (36.87%), RW2 (19.29%), RW3 (10.27%) and RW4 (7.85%) was best differentiated. Higher percentages indicate that a greater amount of variation in the morphological characters is captured by that particular relative warp.

The groups or populations of the Spondylid species were best differentiated using the information captured by RW1, RW2, RW3, and RW4. The relative warps captured the most significant sources of variation in the morphological characters analyzed, leading to effective differentiation between the groups or populations of the species. The RW1 is correlated with the shell length as *S. varius* was remarkably

distinguished among the groups. The negative values for RW2 indicated the increasing size (length) of the cardinal teeth of the Spondylid species. There was a shorter dorsal tip of adductor/retractor muscle scar to the junction of the ventral tip of the adductor-retractor muscle scar. There was increasing size of the palial line down to the ventral tip of the adductor-retractor muscle scar. The RW3 reflected the sizeable cardinal teeth but less pallial line. The low values of RW4 corresponded to the rotation of cardinal tooth, making it distinctly elongated.

4. Discussion

4.1 *Landmark-based geometric morphometry results*

While individual measurement of internal shell characters differed only slightly, the collective difference when analyzed collectively using CVA resulted in the clustering of the specimens into species within minimal tools. It was observed that most of the attributes of the *Spondylus* shared the same character within. Like, the slight overlap in the positive y- axis (Fig. 3) of *S. versicolor, S. limbatus* and *S. squamosos* showed that some shell characters/landmarks have similar morphological attributes. Among the *Spondylus* species, the *S. varius* is the most distinguishable Spondlylid species. Aside, from its exceptional size which is relatively large, it has distinct spines, pure white in outside and inside and has color red- orange spots on its umbo and cardinal area of its hinge.

According to Kobayashi (1967) the shells of individual specimen have the same basic morphological types that can be found in all species. The adductor muscle scar and the cardinal area of the hinge made it comparable to other species. In terms of color, these species have diverse marginal color band. The *S. limbatus* has deep color purple margin band while the *S. versicolor* has deep color orange. However, Comfort, (1951) suggested that color pigmentation in bivalves was due to the secretion of metabolic waste products. In *S. americanus* the coloration can have no primary camouflagic function, as the shell surface is usually heavily encrusted with epibiont.

The epibionts attached and cemented on the outer shells makes uncertain on the identification and characterization. The taxonomic and biological literature has been exceedingly disparate in the use of names for a given species (Lodeiros et al., 2016). Its delinquent taxonomy of the Spondylus groups is due to its extreme intraspecific variation (Lamprell, 2006). The genus, *Spondylus* is commonly distorted in shape because of crowding of epibionts or it is attached to the rocky substratum. The habitat preference and epibionts are some of the factors of hybridization that caused confusion on the identification of the species (Martonos et al., 2019). This study suggests that the most distinguishable characters of the *Spondylus* are on the internal shell.

4.2 Interior Shell Patterns

There are basically two factors (environmental/ ecological and evolutionary) that explain the wide variation of the Spondylid groups. Attempting to comprehend the association between morphological and taxonomic diversification has become a central concern of paleobiology and ecology (Hadžiomerovi´c et al., 2023), considering that these species have great morphological similarity. The internal shell morphology of Spondylids could be a factor leading to the divergence of the species.

The distinguishable internal shell characters of the *Spondylus* compared with other similar specimens on the internal shell are the marginal band, adductor muscle scar and the cardinal area of the hinge made it comparable to other species. The two most distinct characteristic among the Spondylid species is the (1) distance of the pallial line towards the ventral tip of the adductor/muscle scar and the (2) size of the cardinal tooth (Fig. 4). This study showed that the pallial lines among the species depend on the size and shape of the adductor/muscle scars. In Venerid bivalves, the pallial line was the characteristic that differs among shells (Carboni et al., 2021).

The color of their hinge teeth is not adequate character for identification in Spondylid considering that most of all the specimens had brown hinge teeth. The size of the hinge teeth was considered for identification. The hinge plate in adults has two large corral teeth in the right (lower) shell, and the corresponding sockets in the left shell (Mackensen et al., 2012). The adductor muscle scar is also the most important character to be established, considering that the abductor muscle scar is mostly in the midline of the shell (Fedorˇcák et al., 2023). It is characterized by a large muscle scar, posterior to center of shell. The adductor muscle scar is mostly posterior to the midline of the shell. Thus, the left valve has the adductor muscle scar mostly to the left of the midline, and the right valve has a scar mostly to the right of the midline. The adductor/muscle scars of the species were almost the same in shape but relatively different in size. These internal shell characters could be applied as tentative IDs in the field.

The individual shell characters measurements posed some caveats. The shell shape differs across the habitat they occupied by the bivalves (Latiolais, 2005). It depends on the different physical and ecological conditions such as slope, wave energy, and wave exposures. The phenotypic plasticity of the Spondylid groups is possible across the habitats is evident. Bourdeau et al., (2015) defined this type of character as an environmentally contingent expression of phenotypes. He elaborated that the cause of this expression could be adaptive (like the development and behavior in response to environmental cues) and non-adaptive (for example, stressful environments or poor diets result in slow growth, low survival or low fecundity). The differences in shell shape across the sampling sites may be their mechanism for maintaining a wide distribution and maximizing local success in various environments (Yonge, 1973). In addition, Ruaza et al., (2015) detected significant differences in all shell characters across sampling sites, which means the location and habitat preferences may also be a factor. Certain shell characters exhibited differences due to their coping ability to the substrates or habitats (Ruaza and Dy, 2016).

4.3 Implications

This study provides significant advancement tool in biology and shell researches. This serves as baseline data that could help students, researchers, educators and general public alike for identification of *Spondylus* species. The novel use of geometric morphometric study as a minimal yet effective tool in *Spondylus* species identification is with great help in the realignment of previous perceptions of local constituents. Further, the recent study could help the government in their effort for protection and conservation measures of *Spondylus* species in the region.

5. Conclusions

This study provides additional information and understanding of the morphological variations of the Spondylid species in Lianga Bay, Surigao del Sur Philippines. The landmark- based geometric morphometry successfully determined the variation on the five Spondylid species. The disparity of their internal shell characters showed significant differences despite its great morphological similarity.

The significant differences observed in the internal shell characters among the Spondylid species demonstrate that even small variations can lead to distinct morphological differences. This finding contributes to our knowledge of species differentiation within this group, highlighting the importance of considering internal shell characters in taxonomic and ecological studies.

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Declaration of Conflict

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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