

The Body Wall Spicule Formations of Mature *Holothuria impatiens* Found in La Union, Philippines

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Abstract – Describing spicule formations in sea cucumbers is necessary to establish an extensive data to fully characterize the species. The present research described the spicule formations from the mid-dorsal and mid-ventral body wall of a mature *Holothuria impatiens* collected from Poro Point, San Fernando City, La Union. The characterization was done by spicule analysis. Investigation showed the presence of different spicule formations in *Holothuria impatiens* which includes C-shaped rods, spinous rods, tables, and buttons of varying forms. Button is the most abundant spicule formation for both the mid-dorsal and mid-ventral body wall. Present investigation shows an uncommon record of varying button formations with more than three inside holes.

Keywords – body wall, *Holothuria impatiens*, spicule, tentacle

INTRODUCTION

Sea cucumbers are marine organisms that play important functions in the marine ecosystem (Purcell, 2010). They belong to the phylum Echinodermata which includes an estimated number of more than 1,400 species. They can be classified taxonomically through their anatomical features like observing microscopic skeletons or spicules present in their body walls (Tehranifard, Oryan, & Vosoghi, 2011). Some species are famous in the country due to their high market value (Choo, 2008). It was reported that there are about a hundred species of sea cucumbers in the Philippines and twenty five of which are exploited for trade (Gamboa, Gomez, & Nievaes, 2004). Species under the genera *Holothuria*, *Actinopyga*, and *Bohadschia* are commonly fished for food. Some traders also sell live species for aquarium purposes (Toral-Granda, Lovatelli, & Vasconcellos, 2008). People also trade sea cucumbers for medicinal and pharmaceutical purposes (Torres, 2017; Bordbar, Anwar, & Saari, 2011).

Utilization of sea cucumber for human consumption is already worldwide. The sea cucumber or beche-de-mer trading industry started several decades ago concerning countries like China, Japan, Ecuador, Malaysia, Indonesia, and the Philippines (Lovatelli, Conand, Purcell, Uthicke, Hamel, & Mercier, 2004). In a report by the Food and Agriculture Organization (FAO)

of the United Nations, Asian region is the primary supplier of sea cucumber or beche-de-mer contributing approximately 93% of the world's production. Southeast Asian countries like Indonesia and Philippines are one of the biggest contributors in the sea cucumber production and trade (SEAFDEC, 2012). Unfortunately, incidents on overexploitation and poaching were already reported in the Philippines (Brown, Perez, Garces, Ragaza, Bassig, & Zaragosa, 2010; Uthicke & Conand, 2005).

One common reported problem on the commercially valuable species today is enough information on their identification. In the Philippines, it was reported that references on sea cucumber species are only limited to local descriptions. Studies are also difficult to compare since different local names are used (Gamboa et al., 2004). The change in appearance makes identification through inspection and photo comparison quite difficult for the customs officials or local law enforcers to verify illegal cargo especially when they are not familiar on species of sea cucumber. Spicule analysis is an alternative way to taxonomically classify sea cucumbers. It was reported that these spicules can remain undamaged and fully distinguishable even if the sea cucumber is already in processed state. It was also noted that there is no distinct change on size and shape of the spicules of samples processed at different stages to that of spicules observed in the fresh or live sea cucumber of the same species (Toral-Granda, 2005). Thus, describing

spicule formations in sea cucumbers is necessary to establish an extensive data to fully characterize species since previous investigation suggest that environment and developmental stage may influence spicule formation (Rasolofonirina & Jangoux, 2005). Furthermore, there is a need to develop a guide to help researchers and custom officials in the easy and fast identification of species especially the commonly exploited genera in the fresh, salted, and dried form.

OBJECTIVES OF THE STUDY

The study aimed to describe and/or re-describe the spicule formations in the *Holothuria impatiens* found in San Fernando City, La Union, Philippines using spicule analysis. Specifically, it aimed to characterize the spicule formations found in the body wall region at mid-dorsal and mid-ventral which may serve as reference guide for the identification of some sea cucumber species from San Fernando City, La Union using calcareous spicule analysis.

MATERIALS AND METHODS

After obtaining Gratuitous Permit from the Bureau of Fisheries and Aquatic Resources (BFAR), mature *Holothuria impatiens* were collected from Poro Point, San Fernando City, La Union through diving at shallow water region or deeper areas with ranging depths of 15-20 feet. The collected live sample species were initially stored inside coolers. Identification was done with the help of an expert from the College of Fisheries of the Don Mariano Marcos Memorial State University, La Union, Philippines.

Dissection of Sea Cucumber for Slide Preparation

The methodology used in the dissection of sea cucumber samples and the preparation of slide for spicule observation were mainly based on the procedure described by Toral-Granda (2005) and Dabbagh & Kamrani (2011).

Morphological Observation

Morphological observation was done by looking at the spicules present around the body walls on mid-ventral and mid-dorsal regions of the sea cucumber. The precipitated spicules were transferred from the well to the microscope slide using a micropipette. The microscope slide was covered with a glass cover slip and observed under an electric microscope (Nikon Eclipse

E200, Japan) at 40x magnification. Slide observation was done by preparing nine slides for every body part sampling region.

RESULTS AND DISCUSSION

The occurrence of specific type of spicule formation in a sea cucumber is one unique way of distinguishing species from one another (Dabbagh & Kamrani, 2011). Certain species will possess specific kind of spicule formations, varying characteristic abundance for each spicule type, and to some with added little modification.



Fig. 1. Mid-dorsal and mid-ventral body wall spicule formations in *Holothuria impatiens*. C exemplifies simple large spinous rod. A, B, and D represents C-shaped rods. E, F, G, and H (side view) and I and J (top view) show table formations.

James (1992) and Deichmann (1957) described the same species with the presence of tables. Deichmann (1957) expressed table formations which usually arranged in crowded manner and found to have four upright rods and two cross beams. Likewise, James (1992) descriptions also match the observed table spicule formations (Fig. 1, E, F, G and H). In addition, James (1992) also mentioned the occurrence of disc sub-quadrangle formations commonly possessing nine holes with central hole larger than other holes. Deichmann (1957) described it as squarish disk with eight marginal holes with a spire ending in numerous short teeth. These descriptions by James (1992) and Deichmann (1957) match the present observation of table disks or saucer-like appearance of spicules with around nine holes (Fig. 1, I and J). Moreover, occurrence of C-shaped rods as a different form of rod type spicule in *H. impatiens* (Fig. 1, A, B, and D) was also found to be similar in the rods

declared by James (1992), specifically describing these rods to be slightly curved. However, another form of rod formation was observed in this particular study (Fig. 1, C). The rod is a regular kind of rod which is characterized by spinous appearance, not curved unlike the C-shaped rods. The said observed rod formation does not have too many slight undulations compared to the C-shaped rods. The former is described to have smoother outer lining too (Fig. 1, C).



Fig. 2. Button spicules observed from the body wall mid-dorsal and mid-ventral regions of *Holothuria impatiens*.

Holothuria impatiens body wall is greatly dominated by the presence of button type spicule or ossicles. Figure 2 (A-G) shows the different forms of button type spicules found in the sample species. This finding is somehow similar to the previous spicule analyses of Deichmann (1957) and James (1992). Deichmann (1957) specifically described button type spicules as oval shaped and with three large pairs of oblong hole inside. To James (1992), the button spicules are also oval in shape around 0.084 mm to 0.10 mm length and 0.040 mm to 0.049 mm width. Ovals were described to have mostly three pairs of holes with slight undulating margins, obtuse ends and very rarely with more than three holes on each side. Descriptions from the mentioned studies are similar at some point and would justify the occurrence of button type of rod with specific form observed in this study (Fig. 2, A, B, C, and D). However, the present investigation observed other forms of button spicule (Fig. 2, E, F, and G). Obviously, observed new spicules exceeded the number of holes found inside the oval shaped button spicules. Some had seven holes with slight formation of a new small hole near the periphery (Fig. 2, G); some have 4 pairs of holes (Fig. 2, E), and with more than 10 holes (Fig. 2, G). The latter form of button spicule can be one of those said to

be rarely seen form of spicule (i.e. with more than three holes in each side) described by James (1992).

The different forms exhibited by the variety of spicule types are another way to further differentiate species of sea cucumber. In addition, there were reports that spicule formation may vary as the organism ages or simply may disappear or appear when certain species of sea cucumber reaches specific stage in its life cycle (Rasolofonirina & Jangoux, 2005). For example, buttons in Figure 2 show different constructions which mainly differ in the number of holes they contain. The argument here is that the size of the spicule increases as the number holes increases, which may also correspond to the maturity of the organism. Some reports say that as the organism matures, the formation of spicules also changes or other formations may appear or disappear (Rosolofonirina & Jangoux, 2005). However, this claim must be further investigated especially in the case of the sample species.

CONCLUSION AND RECOMMENDATION

The use of spicule analysis to characterize sea cucumber like *Holothuria impatiens* is economically worthwhile. It does not need expensive chemicals to analyze the specimen. In addition, it is not time consuming and a very effective method to use, because it can provide you result within a short period of time. Consequently, it is really an ideal method to be use during fast inspection or raids in cargo shipments for the sea cucumber species identification.

The researcher recommends further investigation of the occurrence of new forms of spicule types which was not described in the previous studies. Identifying or characterizing *Holothuria impatiens* by spicule analysis using samples with different levels of maturity is necessary. This will help confirm the constant occurrence of spicule formations across the body of a sea cucumber as it matures. In this regard, a more effective and reliable guide will be created which will be helpful in future researches and for law enforcers as basis in identifying the species.

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