INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 23, No. 2, p. 240-254, 2023

RESEARCH PAPER OPEN ACCESS

Ossicle morphology of Sandfish (*Holothuria scabra* Jaeger) at different stages of maturity

Jason N. Tersol*, Maribel V. Gabuyo, Lymuel May M. Palmones, Fraxie D. Manipon, John Raymund D. Torres

Don Mariano Marcos Memorial State University, Bacnotan, La Union, Philippines

Key words: *Holothuria scabra*, Morphology, Ossicle

http://dx.doi.org/10.12692/ijb/23.2.240-254 Article published on August 08, 2023

Abstract

Holothuria scabra Jaeger was investigated for its ossicle morphology by the use of spicule analysis. Buttons, tables, and rods are the most observable ossicles in all weight ranges. Different kinds of rod ossiclessuch as I-shape rods, spiny branched rods, and less spiny branched rods were observed in some weights. Weights ranged in 20-59g, 60-89g, and 90g and above respectively. I-shape rods was observed in the ventral region of specimens weighing 65g, and 93g, while it was also observed in the dorsal region of specimen weighing 252g. Bothspiny and less spiny branched rods were observed mostly on the specimens weighing 40g, and 50g. Other ossicles observed were branched rods, ring-like ossicle, and ellipsoid in buttons. Results obtained in this study show that buttons are the most abundant ossicle observed in the adult stage of *H. scabra* Jaeger. It is advised that future research use samples weighing between 20 and 250 grams to examine the ossicle formations present on these weights, measure the sizes of the ossicles to be observed with the suggested weights of sample species, and conduct additional sampling from other sites to determine whether a particular ossicle morphology first appears at a particular stage of maturity and may be absent in succeeding stages of maturity.

* **Corresponding Author:** Jason N. Tersol ✉ jrtorres@dmmmsu.edu.ph

Introduction

Sea cucumbers are benthic marine invertebrates that belong to the taxonomic class Holothuroidea that fall under the Phylum Echinodermata (Al- Yagout *et al.*, 2021). They are related to other well-known echinoderms such as sea stars, sea urchins, and sand dollars (Ross, 2019). These marine invertebrates are elongated or cylindrical in shape, resembling a large cucumber plant that contains a single branched gonad, and has leathery and spiny skin (Pangestuti & Arifin, 2017; Ross, 2019; Susetya *et al.*, 2020). Benthic organisms like sea cucumbers have an importance on the ecosystem. Ecologically, they recycle benthic organic matter through the consumption of sediments, sea grasses, organic detritus, and produce fecal (Dar & Ahmad, 2006; Wolkenhauer *et al.*, 2010). Some sea cucumbers, like those which belong to the genera *Holothuria, Actinopyga,* and *Bohadschia*, are edible and commonly a source of food (Torres *et al.*, 2019). The body walls, gonads and intestines of these marine creatures are consumed because of their nutritional values (Purcell *et al.*, 2012). Some studies have also shown multiple biological properties of sea cucumbers (Althunibat *et al.*, 2009; Bordbar *et al.*, 2011; Mamelona & Pelletier, 2010; Torres *et al.*, 2021). Aside from being a source of food, these marine organisms have also been used as a medicinal ingredient since ancient times (Mohsen & Yang, 2021). Since the sea cucumbers are utilized as food and medicine, overexploitation 2 of the species might lead to them being endangered and in worst cases, might become extinct.

Sea cucumbers or Holothurians are diverse and consist of six orders and nearly 1,400 species (Dabbagh & Sedaghat, 2012). In the Philippines, the species of sea cucumbers are highly diverse, which is threatened by overfishing and habitat loss (Pitogo *et al.*, 2018). There are 170 species identified in the country wherein 25 to 41 species of these are mostly from the members of the families Holothuroidea and Stichopodidea and they are commercially valued wherein they are commonly exploited for trade (Gamboa *et al.*, 2004; Jontila *et al.*, 2014; Olavides *et al.*, 2010). Meanwhile, sea cucumbers are also traded for aquarium purposes (Bordbar *et al.*, 2011). In China, there are about 20 species of edible sea cucumbers that have been considered as traditional medicine and tonic food for many years. Nutrient analysis shows that the body walland intestines of sea cucumbers have a high nutrient value (Conand, 2004). Most of the commercially important sea cucumbers have been fished with increasing intensity (Wolkenhauer *et al.*, 2010). They are vulnerable to harvesting because of their high abundance and easy access to their habitats (Purcell *et al.*, 2012).

Sea cucumbers have unique names from different places. “Beche-de-mer” is a French word that means marine food product. Locally, sea cucumbers are known as “balat” or “balatan”. It is also known as “balat kagisan” or “putian” (Perez, 2011). Dried sea cucumbers are known as “trepang” in Indonesia.

One of the species of sea cucumbers is the *Holothuria scabra* Jaeger. It is considered one of the most commercially valuable species for beche-de-mer production and has been widely fished in the tropics. Beche-de-mer is processed from sea cucumbers 3 belonging to the families Holothuroidea and Stichopodidea. These species are distributed worldwide as found in the Indo- West Pacific region including the Islands of the Western Indian Ocean, Mascarene Islands, East Africa, and Madagascar, Red Sea, Southeast Arabia, the Persian Gulf, West coast of India and Pakistan, Maldives, and the Lakshadweep, Sri Lanka, Bay of Bengal, the East Indies, North Australia, the Philippines, China, Southern Japan, South Pacific Islands and the Hawaiian Islands (Dabbagh *et al.*, 2012). The A-grade classification of beche-de-mer from sandfish (*Holothuria scabra* Jaeger) commands one of the highest prices on the international market (Agudo, 2006). Due to global overexploitation by intensive commercial extraction, they are listed as endangered by the International Union for the Conservation of Nature. Most heavily exploited sea cucumber populations suffer rapid declines in their abundance and population densities with the onset of commercial exploitation. The declining numbers of sea cucumbers have serious consequences in the ecosystem such as habitat structure alteration, as they are the seas' bioturbators and recyclers (Wolkenhauer *et al.*, 2010).

Holothuria scabra Jaeger is commonly known as sandfish because of its ability to thrive in calm sandy or muddy coastal areas. They are characterized to tolerate low saline environments and are sometimes found to proliferate in some estuarine environments (Junus *et al.*, 2018). Sandfish (*Holothuria scabra*) move with the help of tube feet densely distributed on the ventral surface of the body wall and also through muscular action of the body wall (Hame *et al.*, 2001). Echinoderms may be able to thrive as a result of this to interact with the surroundings from every angle (Yoshimura *et al.*, 2012).

Sandfish (*Holothuria scabra* Jaeger) undergo a metamorphic phase that transforms from larva to adult and undergoes a complex development and regeneration of internal 4 organs. The newly emerged juveniles sink to the bottom in order to complete their development and take on the adult form but will continue to grow until they reach the full adult size (Al-Rashdi *et al.*, 2012). The morphological features used to identify holothurian species are the ossicles. Sea cucumbers have ossicles that form part of their skeleton. The ossicles are small parts of iron-rich calcified materials that are made of calcium carbonate which are infused with the tissue and covered by the epidermis. It varies on shape and size which creates a diversity of species. Some studies stated that describing the morphology of ossicle formation or shapes is useful for species identification and is necessary for establishing extensive data for characterizing the sea cucumber species (Kamarudin *et al.*, 2017; Torres *et al.*, 2019). Their shapes, in fact, have continued to be an important characteristic for the morphological identification of sea cucumbers (Torres *et al.*, 2019). According to Sticker (1986), among the shapes of ossicles include perforated rods, buttons, and tables. The formation of the ossicles occurs on the multinucleated syncytia of sclerocytes on the dermal layer of the body wall (Sticker, 1986). The ossicles of sea cucumbers occur scattered throughout the dermal layer of the body wall. Presumably, it helps to increase the overall stiffness of the body wall. Ossicles could lead to such a stiffening by acting analogously to the various types of biological "filler particles" that have been studied in other invertebrates. Aside from probably increasing the overall stiffness of the body wall, these ossicles may form discrete coverings that help to protect internal structures from mechanical damage. Furthermore, ossicles may also function as antipredatory devices by making juvenile and adult sea cucumbers less palatable (Sticker, 1986).

According to the United Nations' Food and Agriculture Organization (FAO), Asia is the world's leading supplier of sea cucumber, accounting for roughly 93 percent of global production. Southeast Asia represents the global market "hotspots" for sea cucumber trade due to their known "mega biodiversity". Many sea cucumbers are gathered for human consumption and some are cultivated in aquaculture systems (Pangestuti & Arifin, 2017). The number of commercially exploited species varies widely, with the highest number of species in Asia (52 species) and Pacific (36 species) regions, partially due to the higher natural diversity in these areas. The majority of sea cucumbers are exported for the beche-de-mer market, with a few species for the live trade (aquarium) market (Han *et al.*, 2016). The Philippines was already recognized as one of the largest exporters of commercially important sea cucumber species for many years (Torres, 2015). However, in spite of its growing local and international trade and the spread of some information for their utilization, there is still a dearth of knowledge on their systematics and macro biota (Macfadyen *et al.*, 2009). Information about most holothuroid species' biology and ecology is still limited in our country (Torres, 2015; Torres *et al.*, 2019). References on sea cucumber species are only confined to local descriptions in the Philippines, according to reports. It is also difficult to compare studies because different regional names are utilized (Torres *et al.*, 2019). According to a report by the Bureau of Fisheries and Aquatic Resources (BFAR), sea cucumber species are vulnerable to exploitation and extinction due to a lack of information, which is usually manifested by a decrease in the number of catches, particularly for commercially important species found near the described mainland fishing areas (Brown *et al.*, 2010).

Age is important for animal biology and is fundamental for estimating baseline parameters such as growth rate, population age structure productivity, mortality rate, and recruitment (Sun *et al.*, 2019).The body weight and size of the sea cucumber (*Holothuria scabra* Jaeger) sub-adult stage can be distinguished. Sexually mature sea cucumbers, on the other hand, are difficult to identify only by their weight and size, however, most developed sea cucumbers weigh around 250 grams. The size when they reach sexual maturity varies by region (Hamel *et al.*, 2001; Purcell *et al.*, 2012). Since sea cucumbers are commonly poached or illegally traded, there is a need for confirmation and establish a standardized fast, easy to be applied and cheap protocol for taxonomic identification such as spicule analysis to effectively evaluate traded marine organisms, especially in the processed state of sea cucumber. It is therefore important to confirm and to study if the ossicle morphology is changing during the stages of maturity of *Holothuria scabra* Jaeger in order to provide a guide for developing fishery management plans, conservation, or recovery strategies. Spicule analysis is a different approach to characterize a sea cucumber based on the morphology of ossicles. It was reported that these ossicles can remain undamaged and fully distinguishable even if the sea cucumber is already in a processed state (Torres *et al.*, 2019).

In this light, the researchers aimed to contribute to describing and distinguishing the morphology of ossicles present in the dorsal and ventral regions of *Holothuria scabra* Jaeger in order to determine if there is a change in the ossicle morphology as the sandfish matures.

Material and methods

Time and Place of the Study

The study was conducted within the span of February to May 2022. The dissection and observation processes were performed at the Systematics Laboratory of the College of Arts and Sciences at Don Mariano Marcos Memorial State University – South La Union Campus, Agoo, La Union.

Collection of Samples

After the researchers obtained a consent letter from the municipal hall of Sto. Tomas, La Union, a total of nine (9) samples of live sea cucumbers, specifically the species of sandfish (*Holothuria scabra* Jaeger) having different sizes and weights were collected with the help of some fishermen at Sto. Tomas, La Union. The live sample species were weighed using a digital weighing scale and measured their length using a tape measure and classified them into three ranges having three samples per range. After the measurements, the samples were stored in styro boxes with enough amount of seawater and sand. The sea cucumber samples were transferred to the glass jars and were soaked in 95% ethyl alcohol for preservation.

Dissection of Samples for Ossicle Preparation, and Preparation of Sample Slides

Using a scalpel, forceps, and dissecting pan, each sample species was dissected in its dorsal and ventral regions randomly to have 1cm^2 x 1mm thick slices that were used as a sample specimen for spicule analysis (see Fig. 1). The incised portions from the dorsal and ventral regions of the samples were washed in 5 ml plastic vials filled with 3ml of 95% ethanol for about 5 seconds. After washing the specimen, it was transferred to another empty vial and added a 3ml of commercial bleach (NaOCl) or household bleach, and set aside for at least 30-40 minutes or until the tissues were dissolved (Toral-Granda, 2005; De Jesus-Navarrete *et al.*, 2018).

The precipitated ossicles that were settled at the bottom of the vial are usually in the form of fine white sediment. After the tissues were dissolved, the specimen was rinsed with distilled water using a plastic pipette, wait for at least five seconds until the ossicles were settled down. Once settled, the excess distilled water was carefully removed using a plastic pipette. The procedure was repeated four times. By means of a plastic pipette, the precipitated ossicles were engulfed gently, waited for a few seconds for them to settle then they were transferred to a clean microscope slide and were covered with a clear glass coverslip. The specimens were utilized for the spicule analysis.

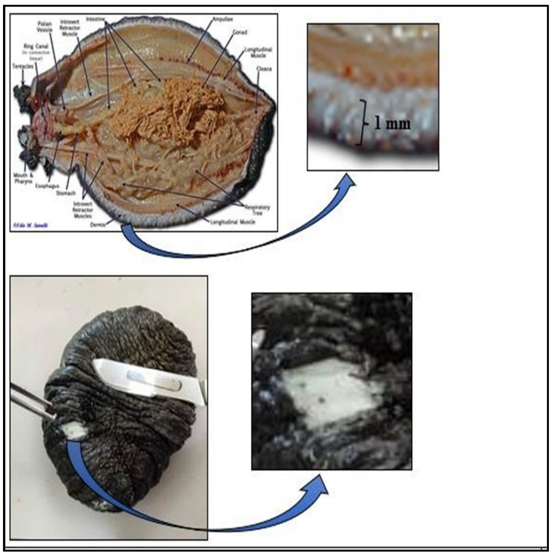

Fig. 1. Diagram of dermis part of the body wall of *H. scabra* Jaeger with a depth to be cut (top) and photoof incised portion of the sample (bottom).

Morphological Observation

The observation of the ossicles present on the dorsal and ventral regions of the prepared slide was done using a compound microscope at 40x magnification. The observation of the slides was done by preparing 5 slides from the dorsal region and 5 slides in the ventral region having a total of 10 slides per sample. After it was observed, the prepared slides were sealed with a transparent cuticle polish and labeled accordingly.

Spicule analysis

This study used spicule analysis in describing and identifying the morphology of sandfish (*Holothuria scabra* Jaeger). Spicule analysis is a currently used procedure in establishing and identifying species identification commonly in sea cucumbers. This procedure is cost-effective time saving and also easy to perform by biologically untrained personnel. Spicule analysis is used for many studies suggesting the usefulness of ossicle shapes for the species identification of sea cucumbers based on morphology.

Ossicle Morphology as Described in Previous Studies

Ossicles such as buttons, tables, rods, plates, and I-shape rods are the previously reported ossicle morphology of *Holothuria scabra* Jaeger and are expected to be seen on the sample species in this study. Buttons were described in the previous reports by Yaghmour and Whittington-Jones (2017) as nodulous buttons that possess three to four pairs of holes with some larger buttons comprising fiveto seven pairs of holes.

On the other hand, buttonswere described by Massin *et al.* (2000) as smooth buttons with numerous holes. Tables in the investigation of Yaghmour and Whittington-Jones (2017) and Massin *et al.* (2000) revealed its appearance to have a disc with undulating rims, the disc is perforated by one central hole and 8-16 peripheral holes ofvarious sizes.

The spire consists of four pillars bridged together by a single cross-beam or 4-5 cross beams to which the spire ends in a crown having a blunt spine. The crown is never as wide as thedisc. Rods in the previous study of Yaghmour and Whittington-Jones (2017) and Massin *et al.* (2000) were described as smooth rods, branched rods, and perforated rods that are 124 um long. Plates on the observation of Massin *et al.* (2000) were described asperforated and rounded.

I-shape rods were observed by Kamarudin *et al.* (2017) that they were very minimal in quantity and stated that they may represent the uniqueness of *Holothuria scabra* Jaeger species. These are the basis to describe the ossicle morphology of *Holothuria scabra* Jaegersamples.

Results and discussion

Morphology of Ossicles Present in the Dorsal and Ventral Regions of Holothuria scabra Jaeger

Different forms of ossicles were observedin the dorsal and ventral regions of nine samples. These samples were categorized into three weightranges: 20-59g, 60-89g, and 90g and above respectively. Range 1 includes samples with weights of 21g, 40g, and 50g. Range 2 includessamples weighing 60g, 65g, and 85g. And range 3 includes samples with weights of 93g, 125g, and 252g.

Table 1. Observed Ossicles in the Dorsal and Ventral Regions of 20 – 59 g, 60 – 89 g, and 90 g and Above Fresh Weights of *Holothuria scabra* Jaeger.

Ossicle Type	B	T	Rd	P	IR	BR	SR	Ro	Eb	BRs
Weight										
21g (D)	+	+								
21g (V)	+	+								
40g (D)	+	+	+	+			+		+	
40g (V)	+	+	+	+			+			
50g (D)	+	+	+			+			+	
50g (V)	+	+	+							
60g (D)	+	+	+			+				
60g (V)	+	+	+							
65g (D)	+	+	+			+			+	
65g (V)	+	+	+		+		+			
85g (D)	+	+	+	+						
85g (V)	+	+	+							
93g (D)	+	+	+			+				
93g (V)	+	+	+		+					
125g (D)	+	+	+			+				
125g (V)	+	+	+							+
252g (D)	+	+	+		+	+		+	+	
252g (V)	+	+	+							

Legend: B = Buttons, T = Tables, Rd = Rods, P = Plates, IR= I- shape Rods, BR = Branched Rods, SR = Spiny Rods, Ro = Ring- like ossicle Eb = Ellipsoid Buttons, BRs = Branched Rosettes, D = Dorsal, V = Ventral

Morphology of the Ossicles Found in 21 grams Samples of Holothuria scabra Jaeger

The fig. 2 shows the images of ossicles observed in the dorsal and ventral region of 21 g fresh weight of *H. scabra* Jaeger. Fig. 2 (A-D) are the buttons observed in the dorsal region. The button in Fig. 2 (A) is described as a smooth button by Massin *et al.* (2000). It has one (1) pair of oblong-shaped holes in the center and has a hole on one side and two holes on the other side. While buttons in Fig. 2 (B-D) are described as nodulous buttons by Yaghmour & Whittington-Jones (2017). Fig. 2 (B) has 3 pairs of holes which is a common number of pairs a button had. In Fig. 2 (C), the button has 4 pairs of holes, and in Fig. 2 (D) is composed of 9 holes. The buttons in the ventral region (Fig. 2 E-G) were considered nodulous buttons. They also have 3 pairs of holes except Fig. 2 (G) which consists of 12 holes. Fig. 2 (H-J) are tables that are observed in the top view position showing the regular shape of disc and considered as a fully developed table (Fig. 2H) as described by Massin *et al.* (2000) that the four large central holes will later become narrower and surrounded by numerous circles of small holes. Fig. 2 (K) was observed laterally, and the crown itself was visible. These tables are observed in the dorsal body wall. The tables (Fig. 2L) in the ventral body wall were observed on its lateral view and the spire on its crown was also visible. Based on the observation, a few buttons with one pair of holes and fully developed tables are present in the dorsal region and absent in the ventral region. And plates are absent in the dorsal region but present in the ventral region. While buttons with 3 or more pairs of holes and tables could be observed in both dorsal and ventral regions.

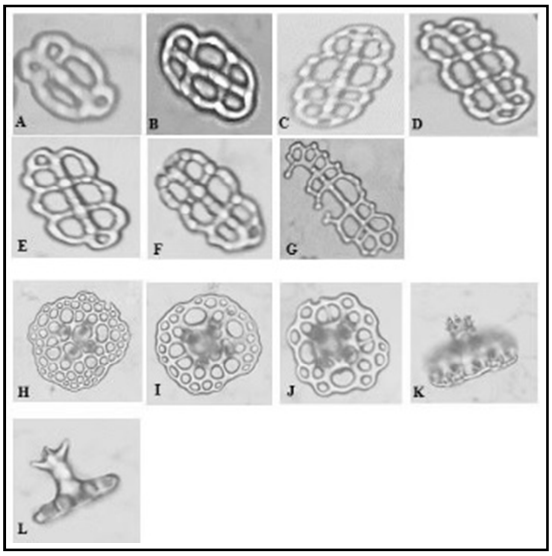

Fig. 2. Morphology of the Ossicles Found in 21 grams Samples of *Holothuria scabra* Jaeger. (A-D) Buttons in dorsal region, (E-G) Buttons in ventral region, (H-J) Tables in dorsal region (top view), (K) Table in dorsal region (lateral view), (L) Table in ventral region (lateral view).

Morphology of the Ossicles Found in 40 grams Samples of Holothuria scabra Jaeger

In Fig. 3, the observable types of ossicles in the dorsal and ventral region of 40 g fresh weight of *H. scabra* Jaeger are buttons, tables, plates, and rods. Buttons in the dorsal region have 3 pairs of holes (Fig.s 3A&3C) which are the common pairs to be observed, and there are also more than 3 pairs (Fig.s 3B&3D). Fig. 3 (E-F) are buttons in the ventral region where 3 pairs of holes (Fig. 3E) and 5 pairs of holes (Fig. 3F) are observed. Tables are characterized by four central large holes and surrounded by numerous circles on a disc, and it has an x-shape (Massin *et al.*, 2000) or a crown on the center if it is viewed from the top by adjusting the fine adjustment knob of the microscope. Dorsal tables shown in Fig. 3 (G- I) are in top and lateral view showing the disc (Fig. 3G), crown (Fig. 3H), and pillars and crown (Fig. 3I). Tables shown in Fig. 3 (J- N) were observed in the ventral region showing a top view (Fig. 4J), fully developed (Fig. 3K), and not fully developed (Fig. 3L-N). Plates observed in the dorsal region (Fig. 3O) have15 holes, and in the ventral region (Fig. 3P) have numerous holes. Perforated rods were observed in the dorsal region (Fig. 3Q) and the in ventral region (Fig. 3R) with few holes attached. Ossicles observed in both dorsal and ventral region comprises buttons and tables, limited number of plates, and few rods.

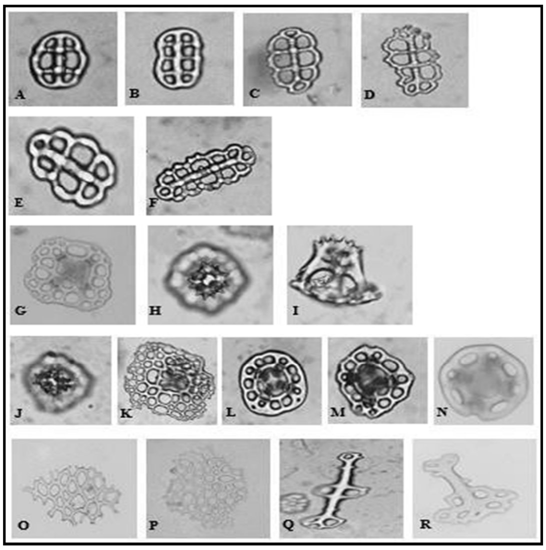

Fig. 3. Morphology of the Ossicles Found in 40 grams Samples of Holothuria scabra Jaeger. (A-D) Buttons in dorsal region, (E-F) Buttons in ventral region, (G-I) Tables in dorsal region, (J-N) Tables in ventral region, (O) Plates in dorsal region, (P) Plates in ventral region, (Q) Perforated rod in dorsal region, (R) Perforated rod in ventral region.

Morphology of the Ossicles Found in 50 grams Samples of Holothuria scabra Jaeger

Fig. 4 presents the different ossicles observed in the dorsal and ventral region of 50g fresh weight of *H. scabra* Jaeger. The observed dorsal buttons are comprising with 3 pairs ofholes (Fig. 4A&D), with 6 pairs of holes (Fig. 4C), and with a perforated rod-like buttons (Fig. 4B, E, F, & G). These perforated rod-like buttons have a total of 13 holes (Fig. 4B&4G), 11 holes (Fig. 4E), and 18 holes(Fig. 4F). Some smooth buttons were observedin the ventral region having 3 pairs of holes in bigger size (Fig. 4H), and narrow holes (Fig. 4I). Most of the tables observed in the dorsal region are in top view position (Fig. 4J-M) while other are in lateral view (Fig. 4N-O). Thelateral view of the tables shows some parts such as the pillars, cross-bridge, spire of the crown. Tables observed in the ventral region were consist of more than 13 holes around a disc. Perforated rods were also observed in this sample on their dorsal region were smaller than others (Fig. 4Q), and on the ventral region having several holes attached randomly (Fig. 4R).

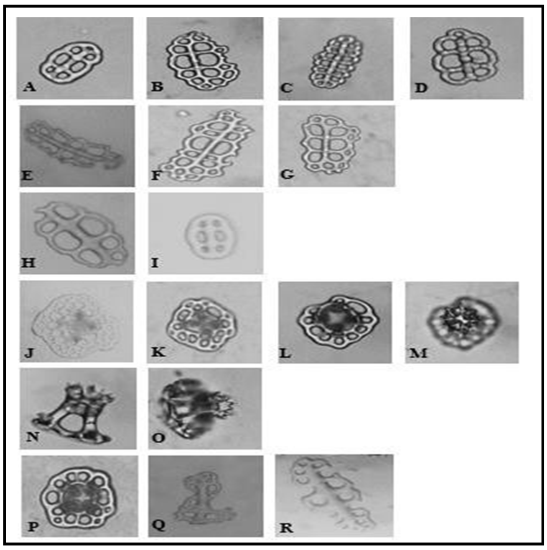

Fig. 4. Morphology of the Ossicles Found in 50 grams Samples of *Holothuria scabra* Jaeger. (A-G) Buttons in dorsal region, (H-I) Buttons in ventral region, (J-M) Tables in dorsal region (top view), (N- O) Tables in dorsal region (lateral view), (P) Tables in ventral region, (Q) Perforated rods in dorsal region, (R) Perforated rods in ventral region.

Morphology of the Ossicles Found in 60 grams Samples of Holothuria scabra Jaeger

Fig. 5 shows the observed ossicle in the dorsal and ventral region of 60 g fresh weight of *H. scabra* Jaeger. Fig. 5 (A-C) are the observed buttons in the dorsal region. The button in Fig. 5 (A) was characterized as a smooth button by Massin *et al.* (2000). It has one pair of holes in the center and two pairs of holes on both sides. Buttons in fig. 5 (B-C) were characterized as nodulous buttons described by Yaghmour & Whittington-Jones (2017). Fig. 5 (B) has 3 pairs of holes which is the common number of pairs of holes. Button in Fig. 5 (C) has an irregular size of holes where it consists of 14 numbers of holes. The buttons in the ventral region (Fig. 5D-F) were considered as smooth buttons; they also have 3 pairs of holes. Fig. 5 (G-J) is considered a knobbed button where Fig. 5 (G) consists of 3 pairs of holes, and Fig. 5 (H) consists of 6 pairs of holes. Fig. 5 (I-J) consists of irregular shapes of holes. Dorsal tables shown in Fig. 5 (K-L) are in top and lateral view showing the disc (Fig. 5K) and a crown (Fig. 5L). Table shown in Fig. 5 (M) was observed in the ventral region is in top view showing the disc considered as a fully developed table and Fig. 5 (N) is in lateral view showing a crown shape. Perforated rods were observed in the dorsal region (Fig. 5O) and in the ventral region (Fig. 5P) with few holes attached. The ossicles observed in Fig. 6 showed that both the dorsal and the ventral regions consist of smooth and nodulous buttons, tables with an equal number and a limited number of rods present.

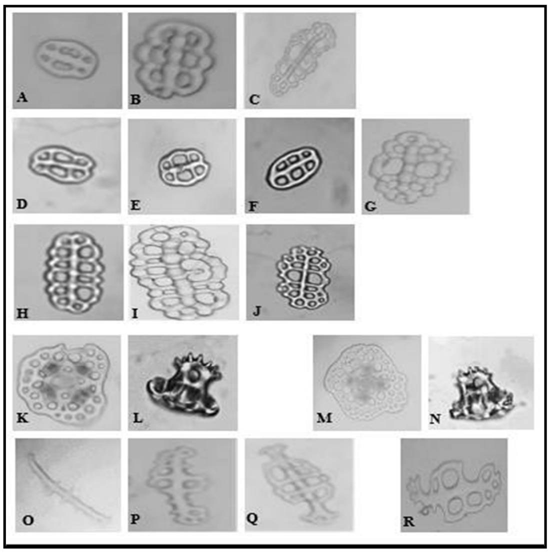

Fig. 5. Morphology of the Ossicles Found in 60 grams Samples of *Holothuria scabra* Jaeger. (A-C) Buttons in dorsal region, (D-J) Buttons in ventral region, (K) Table in dorsal region (top view), (L) Table in dorsal region (lateral view), (M) Table in ventral region (top view), (N) Table in ventral region (lateral view), (O-Q) Perforated rods in dorsal region, (R) Perforated rod in ventral region.

Morphology of the Ossicles found in 65 grams Samples of Holothuria scabra Jaeger

Fig. 6 shows the common ossicle found on the 65 g fresh weight of *H. scabra* Jaeger are buttons, tables, and rods. The buttons on the dorsal region of this sample show smooth and nodulous buttons with varying pairs of holes ranging from three to several pairs (Fig. 6A-C). Meanwhile, buttons on the ventral regions are smooth, lengthy and have several holes that range from three to seven pairs (Fig. 6D-I). The table ossicles on the dorsal region of the sample are small, with spires and crown with blunt spines as described by Yaghmour & Whittington-Jones (2017) (Fig. 6J). On the other hand, the tables on the ventral side are similar to those on the dorsal region. The table has large holes surrounding the disc, has a spire that consists of four pillar bridges with a single cross- beam, it also has a blunt crown with spines (Fig. 6K&6L). The perforated rods on the dorsal region have several holes on its side, and on their top and bottom part (Fig. 6M). Meanwhile, the rods on the ventral region are similar to those on the dorsal region (Fig. 6N).

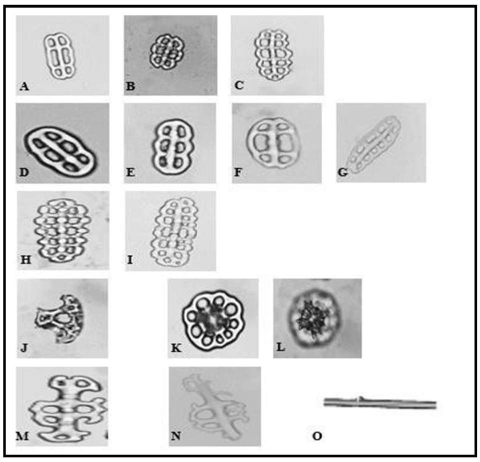

Fig. 6. Morphology of the Ossicles found in 65 grams Samples of *Holothuria scabra* Jaeger. (A-C) Buttons in dorsal region, (D-I) Buttons in ventral region, (J) Table in dorsal region (lateral view), (K-L) Tables in ventral view (top view), (M) Perforated rod in dorsal region, (N) Perforated rod in ventral region, (O) I- shape rod.

Morphology of the Ossicles Found in 85 grams Samples of Holothuria scabra Jaeger

Fig. 7 shows the different morphology of ossicles observed in an 85 g fresh weight of *H. scabra* Jaeger collected samples. The buttons, tables, and perforated rods are the common spicules found on the dorsal region and ventral region. The button-typed spicule on the dorsal side varies from its size and number of holes. Some buttons are smooth and small compared to others, with three pairs of small holes(Fig. 7A), while the others have several large pairs of holes, smooth and are nodulous (Fig. 7B-E). On the ventral region, some buttons are similar to the buttons on the dorsal region, smoothand small (Fig. 7F & 7G), while the others expresses large pair of holes and are nodulous. There are also extra holes aside from the pairs (Fig. 7H-J). The table on the dorsal region are perforated around the disc (Fig. 7K), and sometables have large holes that surrounds the disc (Fig. 7L). The table has spires with four pillars bridged together by a cross-beam, and a crown with spines (Fig. 7M-O).

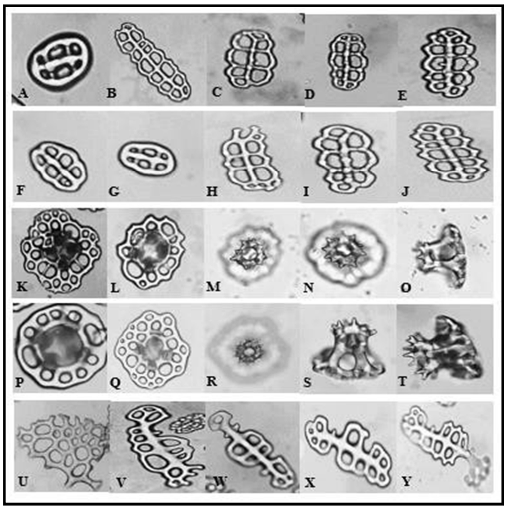

Fig. 7. Morphology of the Ossicles Found in 85 grams Samples of *Holothuria scabra* Jaeger. (A-E) Buttons in dorsal region, (F-J) Buttons in ventral region, (K-N) Tables in dorsal region (top view), (O) Table in dorsal region (lateral view) (P-R) Tables in ventral region (top view), (S-T) Tables in ventral region (lateral view), (U) Plate in dorsal region, (V) Perforated rod in dorsal region, (W-Y) Perforated rods in ventral region.

Meanwhile, sometables on the ventral side are round, with several large holes that surround the disc (Fig. 5P), while the others are perforated (Fig. 7Q). The top and side view of the tables on the ventral side are similar to those on the dorsal. However, somecrowns are smaller than those on the dorsal region (Fig. 7R-T).

The plate on the dorsal region is perforated with large and small holes (Fig. 7U).The perforated rods on the dorsal region have pairs of holes on the side, with extra holes on top and bottom side (Fig. 7V & 7W), while the rods on the ventral region are similar to those on the dorsal region (Fig. 7X & 7Y).

Morphology of the Ossicles Found in 93 grams Samples of Holothuria scabra Jaeger

Fig. 8 shows the observed ossicle extracted in the dorsal and ventral region of 93 g fresh weight of *H. scabra* Jaeger. There were at least three different shapes of ossicle observed in dorsal and ventral region. Fig. 8 (A-G) are the buttons in dorsal region. The button in Fig. 8 (A-D) is characterized as smooth buttons described by Massin *et al.* (2000) Fig. 8 (E-G) is characterized as nodulous buttons. Fig. 8 (E) has 3 pairs of holes which is a common number of pairs of buttons.

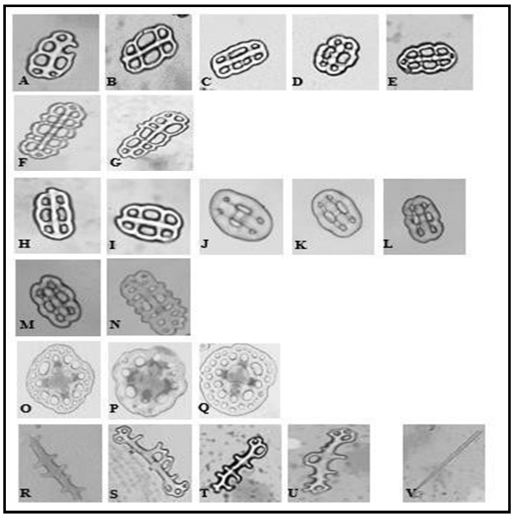

Fig. 8. Morphology of the Ossicles Found in 93 grams Samples of *Holothuria scabra* Jaeger. (A-G) Buttons in dorsal region, (H-N) Buttons in ventral region, (O-P) Tables in dorsal region (top view), (Q) Table in ventral region (top view), (R) Rods in dorsal region, (S) Perforated rods in dorsal region, (T-U) Perforated rods in ventral region, (V) I-shape in ventral region.

Fig. 8 (F) consist of 5 pairs of buttonswhile Fig. 8 (G) consist of 10 holes. The buttons in ventral region (Fig. 8H-L) were considered as smooth buttons consist of 1-3 pair of holes. Fig. 8 (N) is considered as nodulous button consist of 12 holes. Fig. 8O & 8P are tables that are observed in top view shows aregular shape of disc.

Fig. 8 (Q) is observed table in ventral region also observed in top view shows regular shape of disc. Fig. 8 (R) is the observed rods in dorsal region and perforated rods also observed in dorsal region (Fig. 8S). Fig. 8T & 8U were the observed rods in ventral region. In this present study, the ossicles observed in bothdorsal and ventral regions of 93 grams consist of buttons having 3 or more than pairs of holes, tables showing a regular size, and a limited number of rods.

Morphology of the Ossicles Found in 125 grams Samples of Holothuria scabra Jaeger

Fig. 9 presents the observable types of ossicles in the dorsal and ventral region of 125 g fresh weight of *H. scabra* Jaeger are buttons, tables, and rods. Fig. 9 (A-G) are the observedbuttons in the dorsal region, Fig. 9 (A-C) is characterized as smooth buttons consisting of 3 pairs of holes described by Massin *et al.* (2000), who also recorded the same observation.

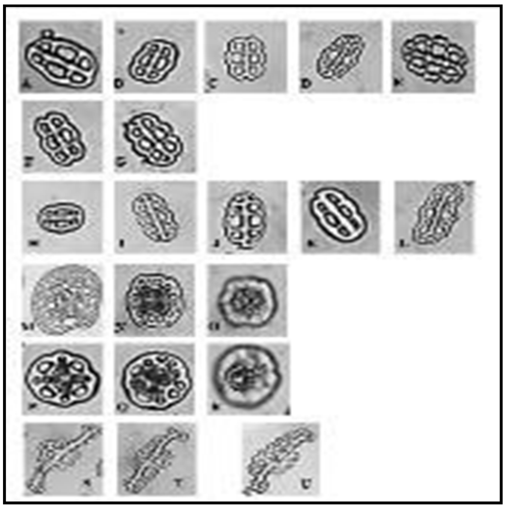

Fig. 9. Morphology of the Ossicles Found in 125 grams Samples of *Holothuria scabra* Jaeger. (A-G) Buttons in dorsal region, (H-L) Buttons in ventral region, (M-O) Tables in dorsal region (top view), (P-R) Tables in ventral region (top view), (S-T) Perforated rods in dorsal region, (U) Perforated rods inventral region.

Fig. 9 (D-G) is characterized as nodulous buttons described by Yaghmour & Whitting-Jones (2017) consist of 3 pairs of holes. The buttons in ventral region (Fig. 9H & 9K) are considered as smooth buttons consisting of 3 pairs of holes. Fig. 9 (I&J) are considered as nodulous buttons consisting of 3 pairs of holes and a nodulous button consisting of 12 unequal shapes of holes (Fig. 9L). Fig. 9 (M-O) are observed tables in the top view in the dorsal region showing the disc with x-shape in the center with numerous holes (Fig. 9M & 9N) showing the disc, crown (Fig. 9O). Fig. 9(P-R) are observed tables in the top view in the ventral region showing the disc with x-shape with 4 holes (Fig. 9P), several holes with unequal shape (Fig. 9Q) and a crown when (Fig. 9R). Fig. 7 (S-Y) is the observed perforated rods in the dorsal region with a few holes attached, while Fig. 9 (U) is observedperforated rod in ventral region.

Morphology of the Ossicles Found in 252 grams Samples of Holothuria scabra Jaeger

Fig. 10 shows the ossicles observed in 252g fresh weight of *H. scabra* Jaeger. Buttons, tables, and rods are observed in the dorsal and ventral region. Fig. 10 (A-G) are buttons that were observed in the dorsal region, where one paired of hole (Fig. 10A & 10D) is a rare kind of buttons, there are also 3 pairs of holes (Fig. 10B & 10C), and more than 3 pairs (Fig. 10E- G) which some are having irregular in size of holes.

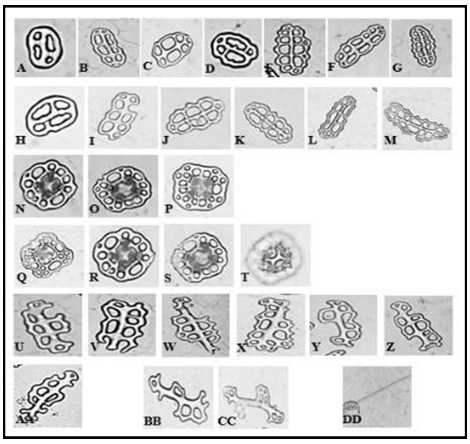

Fig. 10. Morphology of the Ossicles Found in 252 grams Samples of *Holothuria scabra* Jaeger. (A-G) Buttons in dorsal region, (H-M) Buttons in ventral region, (N-P) Tables in dorsal region (top view), (Q-T) Tables in ventral region (top view), (U-AA) Perforated rods in dorsal region, (AB-AC) Perforated rods in ventral region, (DD) I-shape rods.

Buttons observed in the ventral region (Fig. 10H-M) were having various sizes of holes just like in Fig. 10L

& 10M. The tables in the dorsal region (Fig. 10N-P) have a spherical shape with 4 huge central holes and fewperiphery holes on a disc perforation, a tall to low, slender spire terminating in a crown of spines with a tiny center hole or knobs. Fig. 10 (Q-S)are the tables observed in the ventral region weresimilar with dorsal table. Perforated rods observed in the dorsal region were bigger in size incorporated with 5-10 holes in various sizes (Fig. 10U-AA). Fig. 10 (BB-CC) are perforated rods observed in the central regionwherein they have 4-6 holes. A needle-like ossiclewas observed in the ventral region which is characterized by Kamarudin *et al.* (2017) as I-shape rods. Based on the findings observed within this sample weight, perforated rods in the dorsal region are few but they are bigger than those observed in the ventral region.

The observed ossicles on different stages of maturity of *Holothuria scabra* Jaeger that ranges from 21g to 252g showed similar characteristics. All samples have showed common type of ossicles namely button-typed ossicle, tables, and perforated rods. However, based on this study, some samples have showed different kinds of ossicle such as perforated plates which are only present only on the 40g sample and 85g sample. The ossicles on the dorsal regionof range 1 (21g to 59g) have similar characteristics. In buttons, the paired holes ranges from three to seven pairs of holes with extra holes on the side, bottom, or top. The buttons are also nodulous (Massin *et al.*, 2000). The tables, are large and perforated, with a spined crown. The rods are perforated and have a limited pair of holes on the side. There is also a presence of perforated plate on 40g. In the second range (60g to 89g), the button-type ossicles on the dorsal region are nodulous and smooth which consist of three to several pairs of holes. The tables are perforated and smaller compared to the tables on the range 1.The disc is surrounded by large holes. The rods are perforated and has pairs of holes on the side, and has holes on top and bottom part of the rod. On the third range (90g and above), the buttons are smooth and nodulous which consist of 2-3 pairs of holes, and occasionally several pairs of holes. It was also observed that the pairs are not linear to each other. The tables are smaller in size and perforated. However, some tables have large holes that surrounds the disc. The rods have several pair of holes on the side, top, and bottom.

The ossicles observed on the ventral region (21g to 59g) are the common ossicles namely buttons, tables, and rods. The buttons on range 1 express three to several holes and are nodulous. The tables have large holes, perforated, and round. The rods have pairs of holes. On the second range, the buttons are smooth or nodulous, with three to several pairs of holes. The tables areround and perforated, with large holes that surrounds the disc. The rods have several pairs of holes on the side, top, and bottom part. In range 3,the buttons are small, some are perforated, and has several holes. The tables are round. Someholes on the disc are arranged. The rods have several holes on the side, top, and bottom.

Interestingly, there is also the presence of ossicles which are rare and are not found on some weight samples. Aside from the common ossicle shapes such as button, tables, and rods, there wereother different ossicle shapes that were observed on the specimens of *Holothuria scabra* Jaeger.

Morphology of Additional Different Ossicle Formation Observed on Holothuria scabra Jaeger specimens

The fig. shows the additional ossicle formation per weight range. In the ventral region of 60g, the rod was a simple curved shaped (Fig. 11A), the I-shaped rods found on the ventral region of 65g, 93g, 252g, expresses a perfect needle-like shape (Fig. 11B). The branched rods which are found on the dorsal region of 50 grams are spiny (Fig. 11C). The branched rosettes can also be found on the species of *Actinopyga caerulea* (Fig. 11D). The less spiny rods, which can also be found on the species of *Actinopyga caerulea,* do not have a sharp end (Fig. 11E). The branched rod resembles the branches of the tree (Fig. 11F). The eight-shaped rod has a smooth lining (Fig. 11G). The spiny rods have four inside hole with spines on its lining (Fig. 11H-J). The ossicle in Fig. 11K is in a development stage of a table (Rosolofonirina & Jangoux, 2005). Fig. 11L shows a ring-like ossicle. The ellipsoid ossicle expresses a black color (Fig. 11M).

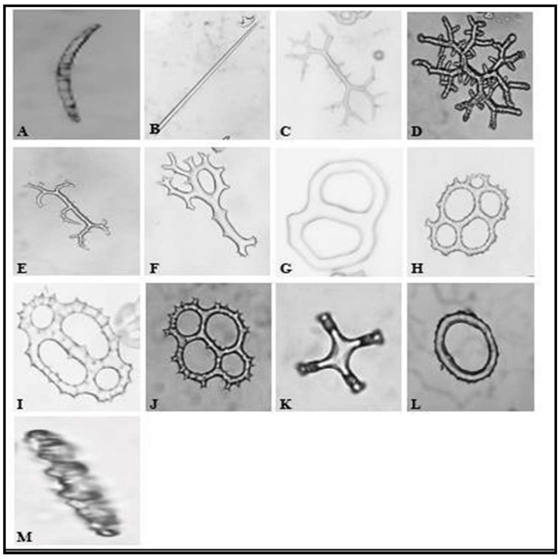

Fig. 11. Morphology of Additional Different Ossicle Formation Observed on *Holothuria scabra* Jaeger specimens. (A) Rod from the ventral region of 60g, (B) I-shaped rods from the ventral region of 65g, 93g, 252g, (C) Spiny branched rods in dorsal region of 50g, (D) Branched rosettes in ventral region of 125g, (E) less spiny rods on the ventral region of 65g, (F) Branched rods from the ventral region of 65g, (G) Rods in dorsal region 40g, (H) Spiny branched rods in dorsal region of 40g, (I) Spiny rods in ventral region of 40g, (J) Spiny rods in dorsal region of 40g, (K) table development on the dorsal region of 93 grams (L) ring-like ossicle on the dorsal region of 252g, (M) Ellipsoid in ventral region of 252g.

Distinction of H. scabra having different weights of 20g - 59g, 60g - 89g, 90g and above based on the morphology of ossicles present in the dorsal and ventral regions

Table 1 presents the different morphology of ossicles observed in the dorsal and ventral regions of *Holothuria scabra* Jaeger having weight ranges of 20g - 59g 60g - 89g, and 90g and above. The positive (+) sign represents the type of ossicles present in the dorsal and ventral regions of the individual samples of *H. scabra* Jaeger in each gram. Buttons, tables, and perforated rods are the three common type of ossicles observed per weight range. While the additional type of ossicles namely: plates, I-shape rods, simple rods, branched rods, spiny branched rods, ring-like ossicle, ellipsoid buttons, and branched rosettes were observed in some samples with specific weights.

In 21g fresh weight of *H. scabra* Jaeger, buttons and tables are the observable ossicles while perforated rods are not observed in both dorsal and ventral regions. In 40g fresh weight of *H. scabra* Jaeger, buttons, tables, and perforated rods are observed in the dorsal and ventral regions, while plates, spiny rods and ellipsoid buttons were the additional ossicle type observed on this sample having this weight. In 50g, 60g, and 65g fresh weights of *H. scabra* Jaeger, buttons, tables, and perforated rods were observed in both dorsal and ventral regions, while branched rods, ellipsoid buttons, and I-shape rods were the additional ossicles where branched rods were observed in the dorsal region of 50g, 60g, and 65g, ellipsoid buttons were observed in dorsal region of 50g, and 65g, and I-shape rods were observed in the ventral region of 65g. In 85g fresh weight of *H. scabra* Jaeger, plates were also observed only in the dorsal region aside from the observed three common types of ossicles in both regions. In 93g and 252g fresh weights of *H. scabra* Jaeger, the three common type of ossicles in both dorsal and ventral regions were observed while the I-shape rods are observed in the region of 93g and in the dorsal region of 252g, branched rods were also observed in the dorsal region of both weights, and ellipsoid buttons were observed in the 252g. In 125g fresh weight of *H. scabra* Jaeger, the three common type of ossicles were observed with the additional ossicle of branched rods in the dorsal region and branched rosettes in the ventral region.

The different forms of ossicles are important taxonomic features of a sea cucumber. There were reports that these parts can change during growth which involves the size or its structures (Rosolofonirina & Jangoux, 2005). An example is the size and number of holes of buttons of range 1 and range 3. Fig. 2 buttons show paired inside holes while in Fig. 10 buttons show unpaired inside holes. Some reports say that as the organism matures, some ossicle can appear or disappear (Rosolofonirina & Jangoux, 2005). In Fig. 10, the ring-like ossicle is present on the 252g sample which are not present on the range 1 and range 2 samples. Branched rosettes are observed on 125g sample. The spiny rods are present on the range 1 and 2 and absent on the range 3 samples. Further investigation should be done in order to confirm the absence or occurrence of the ossicles.

Conclusion and recommendation

Based on the findings of this study, the researchers concluded that there is no pattern to conclusively suggest that as the sea cucumber matures, the morphology of observed ossicles differs. However, in the observation, the researchers observed that buttons, tables, and rods are constant in all weight ranges except in the 21g where rods are not observed, while I-shape rods are described in previous reports. These ossicles are found in samples weighing 65g, 93g, and 252g. It was revealed in the investigation that the ossicle morphology on all weight samples was similar to each other. However, there were some changes in the sizes and inside holes of the ossicles as it matures. There were also observed ossicles which are present on some weight samples. These are the ring-like ossicle found only in 252g, ellipsoid buttons found in samples weighing 40g, 50g, 65g, and 252g, plates found in samples weighing 40g and 85g, branched rods found in samples weighing 50g, 60g, 65g, 93g, 125g, and 252g, spiny rods found in samples weighing 40g and 65g and branched rosettes found in sample weighing 125g. Although some of the ossicles found on *H. scabra* Jaeger can also be found on some other species, ossicles such as I-shaped rods and ring-like ossicle may present the uniqueness of the *H. scabra* Jaeger. Further research should be done in the future. The researchers suggest to use samples weighing below 20 grams and above 250 grams to examine the ossicle formations present on these weights, measure the sizes of the ossicles to be observed with the suggested weights of sample species, do further sampling from other sites to really see if this certain morphology of ossicles starts to appear at a specific stage of maturity and may be absent in the succeeding stages of maturity, compare the ossicle of *H. scabra* to other species, include parameters such as temperature and physicochemical analysis to know if there is an effect to the morphology of ossicles, and for conservation strategy, incise the sea cucumber's body wall and put the specimen on the vials and return the sea cucumber on the sea or in the sampling site.

Acknowledgements

The researchers like to whole-heartedly that the administration of Don Mariano Marcos Memorial State University, faculty, and staff for their contribution to make this research worthwhile and meaningful.

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