Malaysian Journal of Microscopy Vol.8 (2012) pg 159-163

PICTURESQUE OSSICLES FROM *Stichopodidae* FAMILY

S. H. Yahya[1*], S. Omar[2], A. F. M. Noor[3], and F. C. Ghazali[1*]

[1]*School of Health Sciences, Health Campus, Universiti Sains Malaysia, Kubang Kerian, Kelantan, MALAYSIA.*
[2]*Department of Development,Health Campus, Universiti Sains Malaysia, Kubang Kerian, Kelantan, MALAYSIA.*
[3]*School of Materials and Mineral Resources, Engineering Campus, Universiti Sains Malaysia, Nibong Tebal, Pulau Pinang, MALAYSIA.*

There are seven species of the Stichopodidae family of sea cucumber taxonomically identified as 'Gamat' species in Malaysia. These sea cucumbers are classified as endangered to sea cucumber population globally. Taxonomical identification of sea cucumbers is difficult and a dilemma. Little is also known of these said sea cucumber ossicle. These mineralized structures may not be a prima facie pathognomic taxonomical entities. A histological and electron microscopical observation was conducted to interpret the ossicles, presented here as spicules, rod and rosette under the Light Microscope while under the SEM they are appear either as spearhead proturbances and as aggregation of platelet-like structures forming a grape-like acinus.

Keywords: Stichopodidae, Sea cucumbers. Taxonomy, Ossicles

INTRODUCTION

Taxonomical features of sea cucumbers are complex and difficult effort [1, 2]. Among the many taxonomical entities utilized is the mineral ossicle. Mineralized ossicles are often used as an identification tool in taxonomic studies in order to recognize different genus and species of an invertebrate echinoderm [3, 4]. Ossicles are minute microscopical mineralised structure or calcareous pieces or plates of calcium carbonate that are widely distributed in the invertebrate mutable connective tissue leathery integument. These calcareous ossicles are composed at up to 80% of its dry weight of the body wall in some species, while few species may lack ossicles entirely. Calcified spicule, schlerite or ossicle resembling primitive microscopic endoskeleton. It is embedded within the integument tissue of this marine invertebrate and in echinoderm morphology is considered an endoskeleton. The shape of these ossicles varies. They are either shapes as rod, rosette, button, thin slices and hook. In tandem to these, sea cucumber ossicles can also be presented as a wondrous variety of well-defined delicate shapes of meshwork and are thus purported as a taxonomical identification feature of these families of echinoderms. The study of the formation of mineralized structures in organisms offers opportunities for understanding some intriguing aspects of cell and developmental biology. Examples of biomineralization are presented: (1) the structure of skeletal ossicles composed of calcium carbonate in some sea cucumber *Stichopodidae* family, (2) the development of skeletal spicules of sea urchin embryos. In this paper, some comparative study based on literature reviews regarding the biomineralization of these structures and present electron microscope studies are discussed.

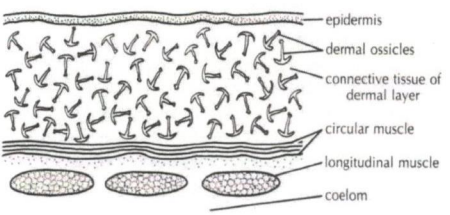


Fig.1. Schematic cross section of the body wall of a typical holothurian, showing the arrangement of the musculature and location of the ossicles. Separation and size reduction of the ossicles permits holothurians to undergo major shape changes; the body wall, musculature, and ceolom from a functional hydrostatic skeleton. (Source: Jan A. Penchenik, 2005)

**Corresponding author: Tel. + 6(09)7677729; Fax. + 6(09)7677515*
E-mail. shizuka_aja89@yahoo.com (Siti Hajar Yahyal); farid@kb.usm.my (Farid Che Ghazali)

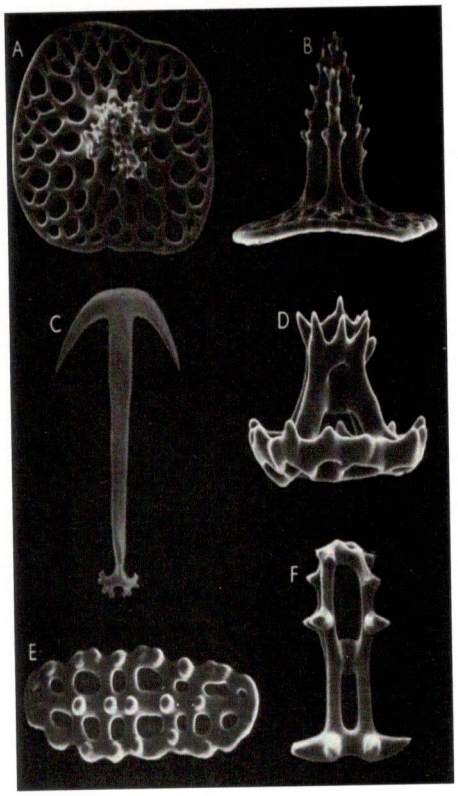
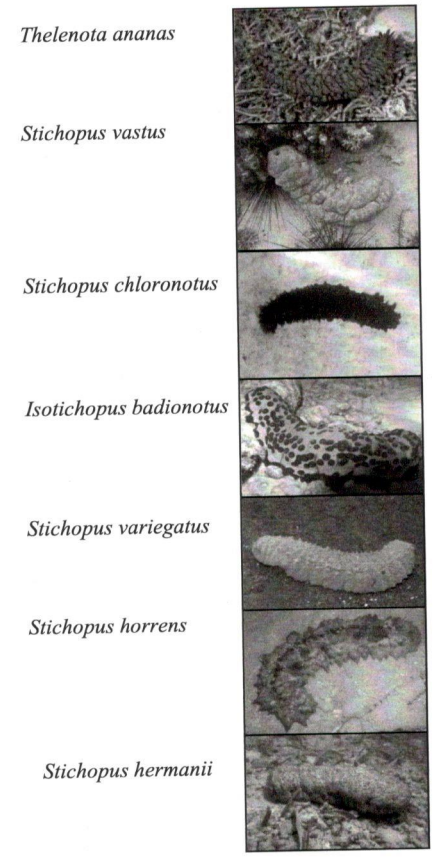


Fig.2. Scanning electron micrograph of the microscopic ossicles removed from the body walls of several holothurian species. Ossicle morphology plays a major role in species identification. (A, B) *Eostichopus regalis* (Cuvier), dorsal and lateral views; (C) *Euapta lappa* (Muller); (D) *Holothuria* (Cystipus) occidentalis Ludwig; (E) *Holothuria* (Cystipus) pseudofossor Deichmann; (F) *Holothuria* (Semperothuria) surinamensis Ludwig. These ossicles range from 60 – 400 µm (micrometers) in longest dimension. Source: Harbor Branch Oceanographic Institution, Inc. Fort Pierce, Florida.

The local Malaysian Sea Cucumber *Stichopodidae* family



(Source: Farid C. Ghazali, 2008)

MATERIALS & METHODS

Slices of 1 cm^2 x 1 mm integumental thickness were dissected from the dorsal epidermis of *Stichopus horrens* identified as '*Gamat Emas*' in Malaysia. Each sample was placed in a small test tube with 3 ml of commercial bleach (NaOCl). Samples were left undisturbed for approximately 30 minutes or until the body wall was observed to have completely dissolved and thus hypothetically the mineralised ossicles should have settled to the tube bottom, resembling fine white sediment. By means of a pipette, the precipitated spicules were then transferred to a cleaned microscope slide, covered

with a cover slip and examined either under a light microscope at 40 or 100 x magnifications [5].

RESULTS AND DISCUSSION

This ossicles may be the biomineralization site of this invertebrates. Little is known about the formation of this biomineralised structures especially the local sea cucumber and its differentiation to better understand the echinoderm cell biology. Although information on dentritine and bone of this mineralised structures is still a lacunae. It is believed that the various phase mineralised involved in this ossicles are identical to this hydroxite sulphite and carbonates commonly meet by bacteria and fungi. Hence, the definition of classification is now more mention as biomineralization [6]. Better understanding of mineralised present echinoderm not only create tangiblelities in cell biology but elucidate usable material properties for clinical replacement and remodeling. Thus, the formation of this mineralised structures must be studied further for its molecular and biological features.

It is important to mention here that, although calcelarous spicules is plate of calcium carbonate in nature [7], the finding of very unstable form of calcium carbonate called amorphous calcium carbonate is found in many incencus and this amorphous calcium carbonate does not diffract X – rays, i.e., is not crystalline. More studies are needed to associate possible relationship between amorphous calcium carbonate to this mineralised ossicles. The spicule can be cleaned with NaOCl to remove all organic materials from the surface of the crystalline spicule thus the spicule can be mineralised with constellation of survival protein removed [8, 9]. It is thus very important that the ossicles and possible biomineralization related be carefully studied to illustrate the diversity scenarios in this better understanding the local sea cucumber.

We believed that the various trace mineral interrelated to the biological origin of the ossicles and the molecular characterization is extraordinary diverse and can only be answered with detailed characterization studied. Finally, if all generalisation can be tragedies from the biomineralisation understanding, better conservative efforts Phylum can be better adapted and identified protien and biocompounds from the specific echinoderm can be better explored without detrimental effect.

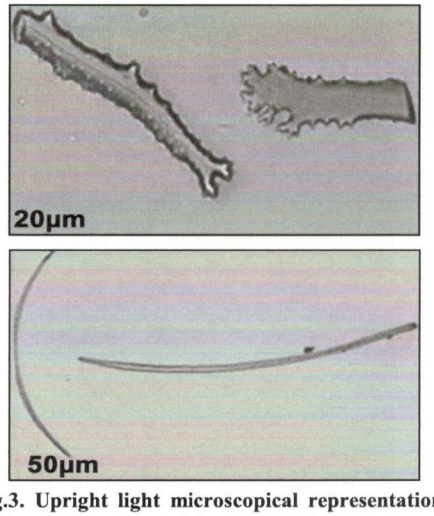


Fig.3. Upright light microscopical representation *Stichopus horrens* ossicle presented here as rod spicules with blunt protuberances of its corpus and a branching end or tip. Right, are fine needle shaped ossicles with a fine projected sharp end. Scale : 20 µm, 50 µm.

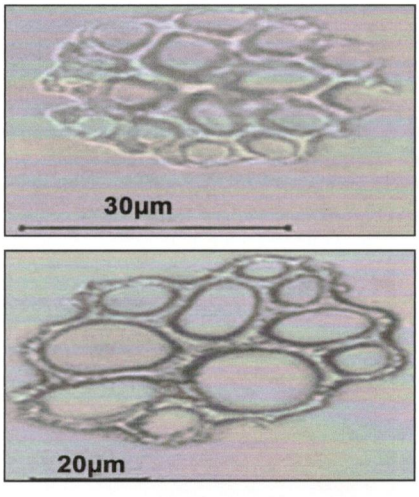


Fig.4. Upright light microscopical representation *Stichopus horrens* ossicle presented here as plate spicules with hollow well-defined lumens. Scale : 20 µm, 30 µm

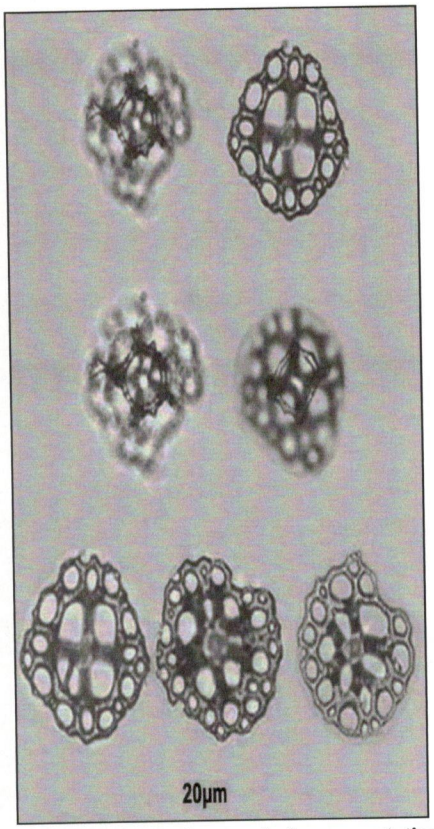


Fig.5. Upright light microscopical representation the meshwork structure from *Stichopus horrens* ossicle presented here as knotted button and table spicules, (bottom and top view). Scale : 20 µm

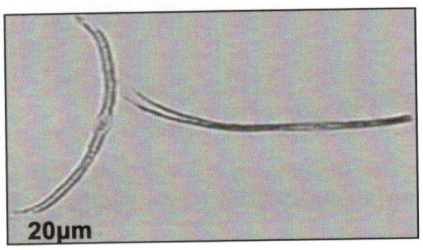


Fig.6. Upright light microscopical representation *Stichopus horrens* ossicle presented here as C-shaped semi-spiral spicules. Scale : 20 µm

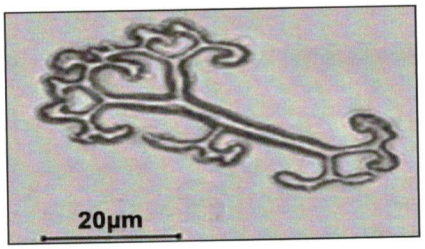


Fig.7. Upright light microscopical representation *Stichopus horrens* ossicle presented here as a Rosette spicule. Scale : 20 µm

Scanning electron microscope (SEM) observation of the various architecture buildups of the ossicles as observed in various anatomical parts of different species of sea cucumbers; *H. edulis* and *S. vastus*:

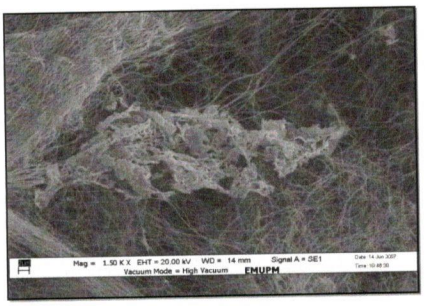


Fig.8. Scanning electron microscope (SEM) representation *Holothuria edulis (dorsal)* ossicle presented here as a Rosette spicule.

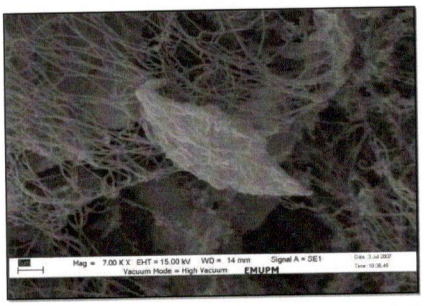


Fig.9. Scanning electron microscope (SEM) representation *Holothuria edulis (ventral)* build up ossicle.

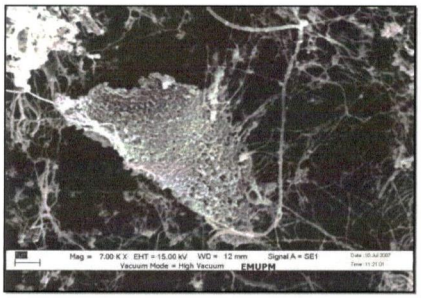


Fig.10. Scanning electron microscope (SEM) representation *Stichopus vastus (dorsal)* ossicle.

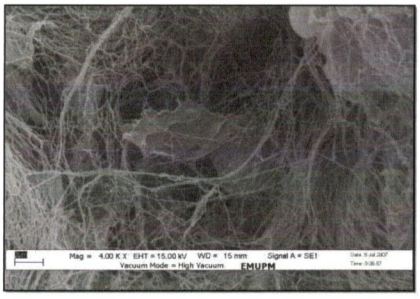


Fig.11. Scanning electron microscope (SEM) representation *Stichopus vastus (ventral)* ossicle presented here as a Rosette spicule.

CONCLUSIONS

A catalogue identifying the key external characteristics as well as the spicules present in each of the commercially important species could be of great help to aid conservation efforts and encourage sustainable trade in these species.

REFERENCES

[1] Conand C. (1998). Holothurians. p. 1157–1190. In: Carpenter K. and Niem V. (eds). FAO species identification guide. The marine living resources of the Western Central Pacific. Vol 2 Cephalopods, Crustaceans, Holothurians and Sharks. Journal citation: Hench, L.L. (1992). Bioceramics, J. Am. Ceram. Soc. 81 (7) 1705.

[2] Conand C. (2004). Doc. 2. *Sea cucumber biology: Taxonomy, distribution, biology, conservation status.* Technical workshop on the conservation of sea cucumbers in the families *Holothuridae* and *Stichopodidae.* Kuala Lumpur (Malaysia), 1–3rd March 2004. Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

[3] Rowe, F. W. E. & Dotty, J. E. (1977). The shallow water holothurians of Guam. Micronesia, 13(2): 217 – 250.

[4] Pawson, D. L. (1980). Holothuroidea. Dlm Echinoderms – notes for a short course. Brodhead, T. & Waters, *J. E. (ed.).* Univ. Tennessee. 235 P.

[5] Hickman C.J. (1998). A field guide to sea stars and other echinoderms of Galápagos. Sugar Spring Press, Lexington, VA, USA. 83 pp.

[6] Fred H. W. (2005). Review: Developmental biology meets materials science: Morphongenesis of biomineralized structures. *Developmental Biology* 280, 15 – 25.

[7] Lowenstam, H., Weiner, S. (1989). On biomineralization. Oxford Univ. Press, New York.

[8] Killian, C. E., Wilt, F. H. (1996). Characterization of the proteins comprising the integral matrix of Strongylocentrotur purpuratus embryonic spicules. *J. Biol. Chem.* 271, 9150 – 9159.

[9] Addadi, L., Raz, S., Weiner, S. (2003). Taking advantage of disorder: Amorphous calcium carbonate and its role in biomineralization. *Adv. Mater.* 15, 959 – 970.

*[10]*Pechenik, J. A. (2005). Biology of the invertebrates. McGraw-Hill Companies. Fifth edition. New York, United States.

*[11]*Farid Bin Che Ghazali, (2008). Gamat: The Malaysian Aquatic Sea Cucumber. Utusan Publisher Sdn. Bhd. ISBN: 978-983-44051-1-3. (Final draft submitted and accepted for review to Utusan Publisher Sdn. Bhd.)