

Scanning Electron Microscopic Investigation of the Eviscerated Strands of Perhentian Island *Bohadschia* spp.

Mohsin, SSJ*, Nik Norliza, NH, Nik Fakurudin, NA, Jamaruddin, MA**, Ismail, AA** & Wan Rosli, WI.

*School of Health Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

**School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

*Corresponding author: Tel. + 6(09)7672362; Fax + 6(09)7672351 E-mail smohsin@kb.usm.my

ABSTRACT

As a defense reaction, the species *Bohadschia grafeii* and probably also another species, which we temporarily named as *Bohadschia* spp., eviscerate plenty of noodle-like threads which stick to the intruder's body. These species belonging to the order holothuridae, are also known as 'timun laut kuning' and 'timun laut batik' amongst the local populace at the resort islands of Perhentian, Terangganu, Malaysia. The eviscerated materials are probably collagen-based materials exhibiting unique features when observed under the scanning electron microscope (SEM). The threads, measuring 0.3 to 2.5 mm in diameter with variable length are white in colour, shiny and are sticky in nature. Under the SEM, the features which confer the ability for the threads to stick to many different types of surfaces are clearly displayed. The presence of cup-like structures distributed along the threads do provide possible explanation of the possible vacuum suctional ability that makes the threads stick fast to almost any surface. The role of this defense system, at least in the two species mentioned here, has not been described earlier.

Keywords: Eviscerated Strands, *Bohadschia* species, Scanning Electron Microscope (SEM)

INTRODUCTION

Studies related to the biodiversity, medicinal and pharmacological properties and other aspects of different species of sea cucumbers have been published since 1985. Some of these investigations include the coelomic fluid as an antioxidant (Ridzwan, 1993; [Hawa et al, 1999](#)), anti-proliferative activities (Althunibat, 2009), collagen characterization of *Stichopus japonicas* ([Cui et al, 2007](#)), population patterns and distribution of *Holothuria grisea* ([Mendes, 2006](#); Nash, 2006) and asexual reproduction of *Bohadschia marmorata* (Lakminarana, 2006).

The species *Bohadschia* is readily found in the seas around Malaysia. They belong to the genus *Holothuria* and the most commonly found species in the Perhentian Islands off the town of Besut in Terangganu, is the *Bohadschia graeffei* (fig.1). Recently, we found a specimen which presented itself with a colourful brownish and yellowish exterior while the main structures were similar to the species *B. graeffei*. This species was temporarily named *Bohadschia* spp. pending its proper identification. These two species belonging to the order holothuridae and are also known as the 'timun laut kuning' (fig. 1) and 'timun laut batik' (fig.2) amongst the local populace.

When taken out from their habitat, these organisms from the *Holothuria* genus, family *sticopodidae*, will inevitably shoot out an abundance of thread-like materials (fig. 3) which stick to the surface which they are in contact with. The removal of the threads is difficult as they strongly stick on to the surface of the intruder or even on to the wooden platform of the boat. While the victim of the evisceration is busy trying to dislodge the sticky threads, a little bit of time is available for the organism to initiate an escape. This act of the species could well be to distract the intruder from paying attention to the organism while giving it a chance to flee.



Figure 1. *Bohadschia graeffei*



Figure 2. An unknown species belonging to the *Bohadschia* genus.



Figure 3. Evisceration threads of the *Bohadschia* ssp. The white threads as seen here sticking even to its own body.

MATERIALS AND METHODS

Sample preparation

Fresh eviscerated threads of the sea cucumber were collected and placed in a solution of phosphate buffer (PBS). The samples were later fixed in the laboratory in 10% formalin prior to processing for SEM observation.

Procedure of SEM sample preparation (Biological sample)

Samples were fixed in the fixation buffer-glutaraldehyde buffer (2.5% GA + 4% PFA/0.1M phosphate or cacodylate buffer) for 4hr at room temperature and then the samples are passed through the following steps:

The first step is rinsing in osmium tetroxide buffer (OsO₄/0.1M phosphate or cacodylate 3X for every 15 min. at room temperature. Then it undergoes washing in ascending concentration of ethanol as follows: 30% EtOH 10 min, 50% EtOH 10 min, 70% EtOH 10 min, 85% EtOH 20 min, 95% EtOH 20 min, 100% EtOH 20 min, 100% EtOH 20 min, 100% Acetone 20 min and 100% Acetone 20 min. Sample is then ready for drying. The samples were dried in Balzers CPD 030 critical point dryer (CPD) under CO₂ fluid with critical point of 73.8 bar and 31°C. Subsequently, dried samples were affixed to aluminum specimen stubs using carbon paint. The dried samples were coated with a thin layer of gold in a vacuum evaporator (Baltex SCD005 Sputter Coater, Hi-Tech, Germany) for viewing on a Leo 1455 VPSEM under 5.5 Pascal pressure, using the secondary electron mode, at a working distance of 9-10 mm at 5.0 kV (Inca Oxford).

RESULTS & DISCUSSION

The eviscerated strands of *Bohadschia spp.* has been reported as a form of defense mechanism or as a distraction device were found to be between 0.3 to 2.5 mm in diameter and their length varies at 8 to 25 cm. The eviscerated strands were shiny, very sticky and will stick fast to almost any surface. Removal of these strands requires some effort to dislodge and to pull them off from the surface of attachment. They do break upon strong pulling. The nature of defense that these strands confer can only be conjectural presently, as they require a separate study to determined its intentions. However, it could be postulated that the act of releasing the strands causes the intruder to spend time on getting it off while giving time for the organism to escape.

It is also of interest that the people of Palau and nearby islands west of Papua New Guinea utilized these strand as a protective natural stoking like garment used when wading in stony beaches with dead coral and sharp debris. These natives agitate the sea cucumbers to release the sticky strands and they wrapped it around their feet to form a kind of sock.

Under the SEM the strands were of solid cylindrical shape and are seen to also occasionally branch (Fig. 4 and 5) and at 500, 1000 and 10 000 magnifications the surface of the strands show a thick pile carpet-like features (Fig. 6, 7 and 8) with many different types of protrusions showing several types of endings.

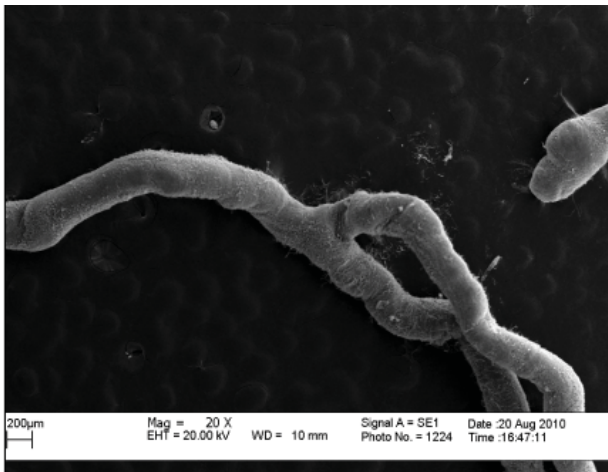


Figure 4. SEM image of the strands of *Bohadschia spp.* at 20 X magnification

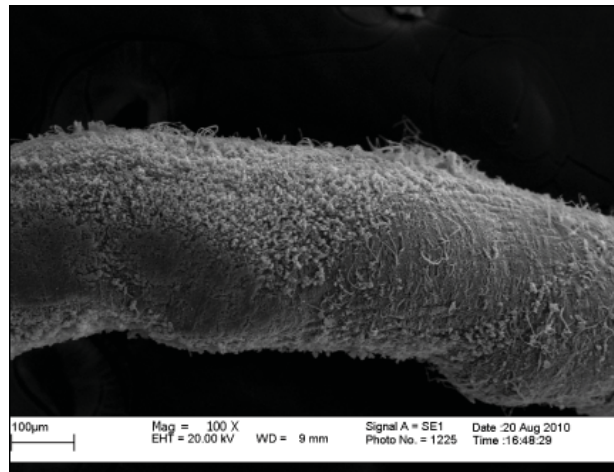


Figure 5. SEM image at 100 X magnification of a strand showing areas of flattened surfaces, some of which are fibrous with many micro threads on the surface.

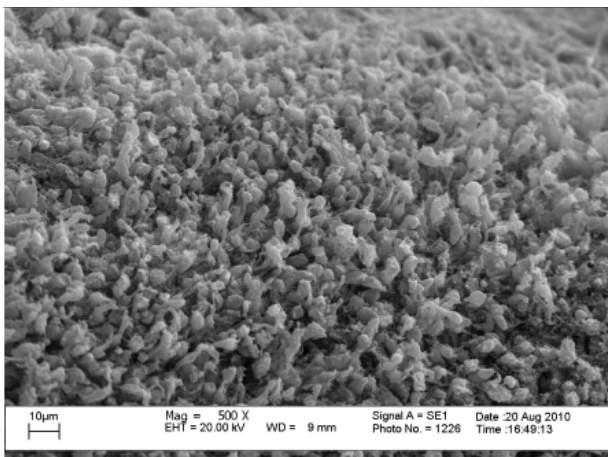


Figure 6. SEM image of the protrusions of the strands show a thick pile carpet-like features at 500 X magnification

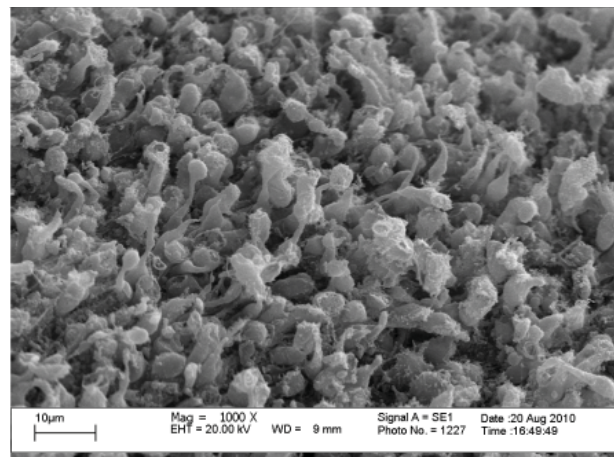


Figure 7. The endings of the protrusions could be in the form of solid beaded club-like, with sharp tapering ends, cup-shape ends or just blunt ends at 1000 X magnification.

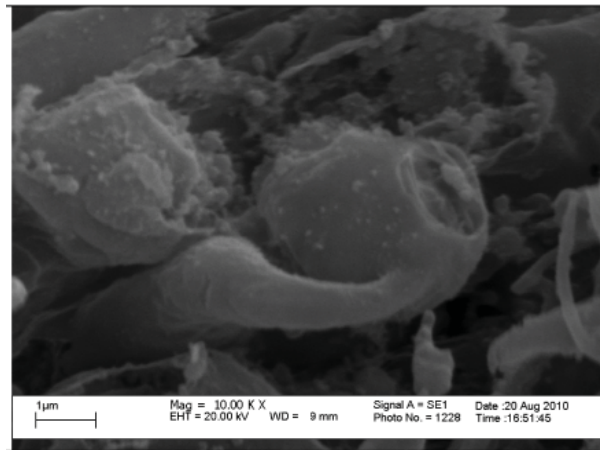


Figure 8. Shows the beaded cup-like endings with typical suctional surface - which may confer the stickiness property of the eviscerated strands.

CONCLUSION

In conclusion, the eviscerated strands of the sea cucumber from the *Bohadschia* species show several unique ultra-structural features which confer to it the ability to adhere strongly to almost any surface. These include hook-like, club shaped and suction cup shape protrusions organized on a thick pile carpet formation over the surface of the eviscerated strands. The function of the strands may well be protective or a deterrent or it could act as a possible detractor thus allowing the organism some time space in order to flee.

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