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Article

Uncommon ossicle in *Holothuria* (*Mertensiothuria*) *leucospilota* from Teluk Nipah, Pangkor Island

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***Abstract*— The high popularity of a commercial sea cucumber species on Pangkor Island (i.e. *Stichopus horrens*) has resulted in a lack of studies on *Holothuria* (*Mertensiothuria*) *leucospilota* from Pangkor Island, Perak, Malaysia. *S. horrens* is the main ingredient of *gamat*-based traditional medicine on the resort island, while *H. leucospilota* is the most dominant species. For that reason, this study aimed to isolate ossicles from a specimen of morphospecies *H. leucospilota* collected from Teluk Nipah Beach, Pangkor Island; and to determine the shapes of the ossicles for species verification. A number of six ossicle shapes were observed. Beside buttons and tables as the common ossicle shapes; I-shaped rod, long I-shaped rod, branched rod, and spider-shaped rod were also documented. Nonetheless, the spider-shaped rod is an unusual ossicle shape in *H. leucospilota* as there was no previous record on it, thus it could be considered as a new finding. In summary, the results of this research indicate the uniqueness of the specimen of morphospecies *H. leucospilota* as a result of the presence of the spider-shaped rod. More studies with more samples, more morphological approaches as well as more molecular techniques need to be incorporated in future for a better insight.**

***Keywords*— *Holothuria* (*Mertensiothuria*) *leucospilota*; mitochondrial rRNA genes; ossicle shape; Pangkor Archipelago; spider-shaped rod**

I. INTRODUCTION

Sea cucumber is an echinoderm from the class Holothuroidea. Its great values in the traditional medicine, modern-formularised health food, beauty care, and trepan, or beche-de-mer industry since years ago has made the marine-dwelling organism as one of the Malaysian heritages [1]-[2]. *Gamat* and *timun laut* are the two main categories of Malaysian sea cucumber [2]-[3]. Generally, the members of family Stichopodidae e.g. *Stichopus vastus* and *Thelenota ananas* (the prickly redfish) are called *gamat* while the others are known as *timun laut* (non-*gamat*) species e.g. *Actinopyga lecanora* (the stonefish) and *Pearsonothuria graeffei* (the flowerfish). However, some Malaysians also regard a few *timun laut* species, for example *Holothuria (Metriatyla) scabra* (the sandfish), as the *gamat* species. Regarding the species richness, a study found in [2] recorded the presence of 52 sea cucumber species in Malaysia and order Aspidochirotida was the most dominant order. In addition, a study found in [4] documented that 13 *timun laut* species and six *gamat* species were of commercial importance in Malaysia.

Beche-de-mer industries in Sabah, Malaysia are exploiting *timun laut* and *gamat* species as food. Approximately 139 tonnes of sea cucumbers were landed in Sabah from the year 2000 until 2005 [5]. In contrast, the Peninsular Malaysia markets are very popular with *gamat*-based traditional products, e.g. lipid extracts (i.e. *minyak gamat*) and body fluid extracts (i.e. *air gamat*). The *gamat*-based products are also being manufactured through modern technologies. Langkawi Island, Kedah and Pangkor Archipelago, Perak are the two main production sites of *gamat*-based traditional products in Peninsular Malaysia [2]. Both resort islands are in the northern part of Peninsular Malaysia.

The presence of a commercial *gamat* species, *Stichopus horrens* and a *timun laut* species, *Holothuria (Mertensiothuria) leucospilota* were reported in Pangkor Archipelago, Perak, Malaysia [6]. *H. leucospilota* is considered as the most dominant species in Malaysia [2]-[3]. *Lintah laut* and *bat puntil* are among its local names in Malaysia. The *timun laut* species has been listed by [4] as one of the commercial species of sea cucumbers in Malaysia, Thailand, Indonesia, the Philippines, China, and Vietnam. Known as the white threads fish in English, this sea recycler is black, tubular, long, and its slithery body is surrounded by short tube feet or podia. Ossicles have been extracted from various external and internal body parts of *H. leucospilota* [6]. Interestingly, a study found in [6] showed the absence of ossicle only in the gastrointestine.

In view of that, the aims of this study were to isolate ossicles from a specimen of morphospecies *H. leucospilota* collected from Teluk Nipah Beach, Pangkor Archipelago, Perak, Malaysia; and to determine the shapes of the ossicles for species verification. In summary, the outcomes of this study exposed an uncommon ossicle shape, i.e. the spider-shaped rod in the *H. leucospilota* specimen, consequently proposed the uniqueness of *H. leucospilota* from Teluk Nipah Beach, Pangkor Archipelago, Perak, Malaysia.

II. MATERIALS AND METHODS

A. Study Site and Sampling

A specimen of morphospecies *Holothuria* (*Mertensiothuria*) *leucospilota* was collected from Teluk Nipah Beach, Pangkor Archipelago, Perak, Malaysia (Global Positioning System (GPS) position - 4º 14' 03.3"N 100º 32' 41.4"E, Fig. 1)). The sampling was done during the low tide (0817 dated 2nd March 2016). The specimen was labelled HL1 (Fig. 2).

Fig. 1 The collection site of morphospecies *Holothuria* (*Mertensiothuria*) *leucospilota* in Teluk Nipah Beach, Pangkor Archipelago, Perak, Malaysia (4º 14' 03.3"N 100º 32' 41.4"E) highlighted with the yellow mark. Adapted from [7]

Fig. 2 Specimen of *Holothuria* (*Mertensiothuria*) *leucospilota* or white threads fish from Teluk Nipah Beach, Pangkor Archipelago, Perak, Malaysia

Species identification of the specimen was done prior to microbial isolation from various parts of its body. Approximately 2 cm^2 tissue portion of the specimen was cut and preserved in 70% ethanol prior to the transportation by ferry and then by car to the Science Research Lab 3.2 (SRL 3.2), Faculty of Science and Technology (FST), Universiti Sains Islam Malaysia (USIM), Nilai, Negeri Sembilan, Malaysia. The tissue sample was stored in -20ºC chest freezer in the SRL 3.2 for long-term storage with proper cataloging.

B. Ossicle Extraction and Shape Observation

The methods by [6] were used with small modifications. Approximately 20 mg tissue from the dorsal cuticle part of the specimen was used for the microscopic observation using the Olympus culture microscope model CKX41 with 400X magnification. The observation was done without definite microscopic size measurement. The tissue portion was placed on a clean glass microscope slide and several drops of liquid household bleach were applied onto it in order to dissolve away the soft tissue. The mixture was left at room temperature for 30 minutes until a white pellet of ossicles was clearly formed in the solution. The images of the ossicle shapes were recorded for morphological identification.

III. RESULTS AND DISCUSSION

The ossicles from the morphospecies *H. leucospilota* from Teluk Nipah Beach, Pangkor Archipelago, Perak, Malaysia were successfully extracted. Fig. 3 depicts a number of six ossicle shapes which were microscopically observed without their size measurement, i.e. buttons, tables, I-shaped rod, long I-shaped rod, branched rod, and spider-shaped rod. The buttons and tables (Fig. 3(a)~(b)) were common ossicle shapes in *H. leucospilota* [8]. Interestingly, four additional ossicle shapes, i.e. the I-shaped rod, the long I-shaped rod, the branched rods, and the spider-shaped rod were also recorded in this study (Fig. 3(c)~(f)). A study in [6] also recorded the presence of I-shaped rods in the tentacles and respiratory trees of *H. leucospilota* specimens from Pangkor Archipelago, Perak, Malaysia. Nonetheless, the branched rod and the spider-shaped rod were not listed. The branched rod as shown in Fig. 3(e) could be the broken part of the spider-shaped rod as shown in Fig. 3(f) but further analyses are required to confirm the status. The spider-shaped rod could be considered as a new finding due to its unusual presence in *H. leucospilota* and it was not reported in previous studies.

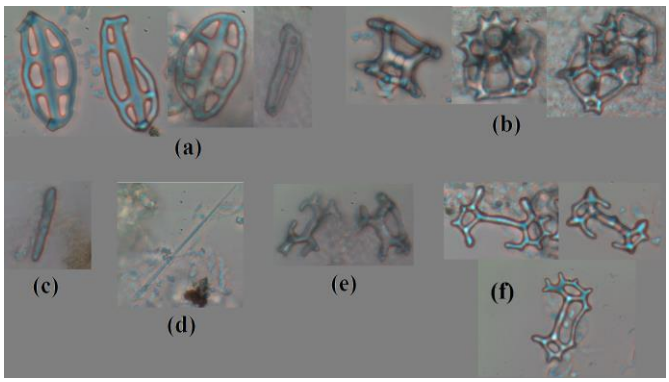

Fig. 3 Ossicle shapes in morphospecies *Holothuria* (*Mertensiothuria*) *leucospilota* or white threads fish from Teluk Nipah Beach, Pangkor

Archipelago, Perak, Malaysia: (a) buttons, (b) tables, (c) I-shaped rod, (d) long I-shaped rod, (e) branched rod, and (f) spider-shaped rod

By taking into account the observation of two common ossicle shapes in the morphospecies *H. leucospilota* specimen and its physical appearance, this study suggested the species status of the specimen as *H. leucospilota*. *H. leucospilota* is regarded as one of the *timun laut* species [2]. *H. leucospilota* was among the Malaysia's commercial species of sea cucumbers [4]. Furthermore, the white threads fish is not regarded as endangered or at a risk of extinction based on the International Union for Conservation of Nature (IUCN) Red List for aspidochirotid holothuroids [9]. The species status subsequently suggested the uniqueness of *H. leucospilota* from Teluk Nipah Beach, Pangkor Archipelago, Perak, Malaysia due to the presence of the spider-shaped rod as depicted in Fig. 3(f). Nonetheless, the size of the spider-shaped rod could not be measured due to the specifications of the Olympus culture microscope used in this study. In other words, size measurement of the spider-shaped rod needs to be done in the future in order to determine its variation.

Generally, additional approaches are still required to support and then to confirm the species identity of the *H. leucospilota* specimen from Teluk Nipah Beach, Pangkor Archipelago, Perak, Malaysia even though there were common ossicle shapes in the microscopic observation. Additionally, the outcomes of this study also discovered an uncommon ossicle shape, i.e. the spider-shaped rod in the *H. leucospilota* specimen from Teluk Nipah Beach, Pangkor Archipelago, Perak, Malaysia. Further studies with more samples, more morphological approaches and more molecular techniques need to be done in order to get a better view and verification.

IV. Conclusions

In brief, ossicles from the morphospecies *H. leucospilota* specimen from Teluk Nipah Beach, Pangkor Archipelago, Perak, Malaysia were well extracted. A number of six ossicle shapes, i.e. buttons, tables, I-shaped rod, long I-shaped rod, branched rod, and spider-shaped rod were observed and recorded. In fact, the buttons and tables are common ossicle shapes in *H. leucospilota*. Nevertheless, the spider-shaped rod is an unusual ossicle shape in *H. leucospilota* since there was no previous report on it, thus it could be considered as a new finding. The ossicle shape observation and the physical appearance of the morphospecies specimen suggested the species status of the specimen as *H. leucospilota*, subsequently highlighted the uniqueness of *H. leucospilota* from Teluk Nipah Beach, Pangkor Archipelago, Perak, Malaysia due to the presence of the spider-shaped rod.

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