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Species Identification and Relationship of Sea Cucumber species from Pulau Tinggi and Sedili Kechil, Johor Based on Ossicle Shape

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Abstract: Sea cucumbers are an abundant echinoderm group in Malaysia which provides commercial and medicinal values to local people. Due to the lack of data on sea cucumbers status in Johor coastal seawaters, this study was conducted. This study aimed to identify the species and relationship of sea cucumber from two selected Johor coastal seawater; Pulau Tinggi and Sedili Kechil based on ossicle shape. The current study recorded 53 individuals of sea cucumbers, of which 23 individuals were from Pulau Tinggi, Mersing District and 30 individuals were from Sedili Kechil, Kota Tinggi District. A number of 10 species of sea cucumber were found in the coastal waters of Pulau Tinggi, Mersing and three species in Sedili Kechil, Kota Tinggi, Johor. A total of 10 ossicle shapes were observed and extracted from the sea cucumber samples namely button, perforated plate, table, C-shaped rod, rosette, anchor, spider, I-shaped rod, boomerang, and J-shaped rod. The species relationship based on ossicle shapes was analysed using clustering option of PAST (PAleontological STatistics) software version 4.03. The analysis concluded that two groups of sea cucumber families i.e. Holothuriidae and Stichopodidae were present, although *S. chloronotus* clustered closer with the members of family Holothuriidae. Although more specimens are required to generate better conclusive results, the outcomes provided updated data on sea cucumber species in Johor seawater.

Keywords: Sea cucumber, ossicles shape, PAST software, Pulau Tinggi, Sedili Kechil

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1. Introduction

Sea cucumbers are marine invertebrates in the phylum Echinodermata and class Holothuroidea. Globally, another names for sea cucumbers are cuke, holothuroid, and holothuria. In Malaysia, sea cucumber is commonly referred to as *timun laut*, *balat*, *bat*, *brunok*, and *gamat* while the Chinese community usually call it *hoi sum*. This marine invertebrate has a soft, cylindrical body and longitudinally elongated body [1]. The oldest fossil evidence of sea cucumbers found 400 million years ago, dating from the late Silurian period [2]. Holothuroidea has six orders that have been recorded to date, namely Apodida, Elasipodida, Aspidochirotida, Molpadiida, Dendrochirotida, and Dactylochirotida; and the orders have been found around the world with approximately 1400 to 2000 distribution species among orders [3]. Morphological classification of sea cucumber is the primary method that has been practised by identifying the ossicle shapes into a smaller group. Detection of similar ossicles shape will determine the closely related species if they have recently derived from a common ancestor [2].

The presence of more than 80 species of sea cucumber was found by several ecological studies conducted and aimed at the distribution of sea cucumbers in Malaysia seawaters [3]. Since 1999, the traditional morphological approaches and molecular ecological studies have been used for sea cucumber identification and species documentation purposes. Currently, the most diverse in terms of species richness or species number is order Aspidochirotida within the Indo-Pacific region [4]. In Malaysia, order Aspidochirotida has been the highest diversity reported and found in the ocean [5]. *Holothuria, Stichopus, Bohadschia, Thelenota* and *Actinopyga* are the five genera that make up the big order Aspidochirotida in Malaysian coastal areas [6]. There were nine species of order Dendrochirotida found in the coastal region of Malaysia [7]. From the six orders, most members that live in deep-sea and coastal waters i.e. Elasipodida and Dactylochirotida are rarely surveyed. Based on a previous research study in Johor, the determination of sea cucumber species has only been done in Pulau Besar, Johor, by which the most abundant species of the genus *Stichopus* still needs to be identified and studied [8]. In Pulau Aur, Johor; the most abundant species were *Holothuria (Halodeima) edulis*, Lesson, 1830 and *Stichopus chloronotus* Brandt, 1835 [9]. The earlier studies have shown the lack of research on sea cucumber in Johor seawaters. Hence, this study aimed to identify sea cucumber species at both selected coastal seawaters i.e. Pulau Tinggi and Sedili Kechil in order to update a new data on sea cucumbers species in Johor. PAST (PAleontological STatistics) software version 4.03 was used to identify the species relationship of sea cucumbers found in both areas. Finally, this study has contributed to more data on sea cucumbers in Johor.

2. Materials and Methods

Sea cucumbers were collected around coastal seawater during low tide from two different locations which were in Johor state (Fig. 1); Pulau Tinggi (Fig 1(a)) and Sedili Kechil (Fig 1(b)). All specimens were put into the soft nets or sealed plastic bags filled with seawater once the sea cucumbers were successfully collected. On site, each specimen was soaked with 5 % Magnesium chloride ($MgCl_2$) for preservation. The specimen was then dry-packed and sealed in a plastic bag and left in a freezer at the site area. The specimens were wrapped with old newspaper before put into an icebox to be brought back to the lab in the faculty. For long-term storage, the specimens were stored in the -20°C chest freezer with proper cataloging.

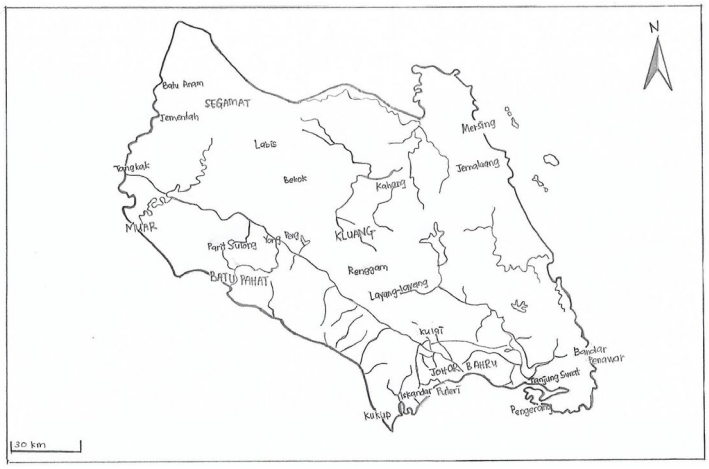

Fig. 1 - State of Johor, Malaysia

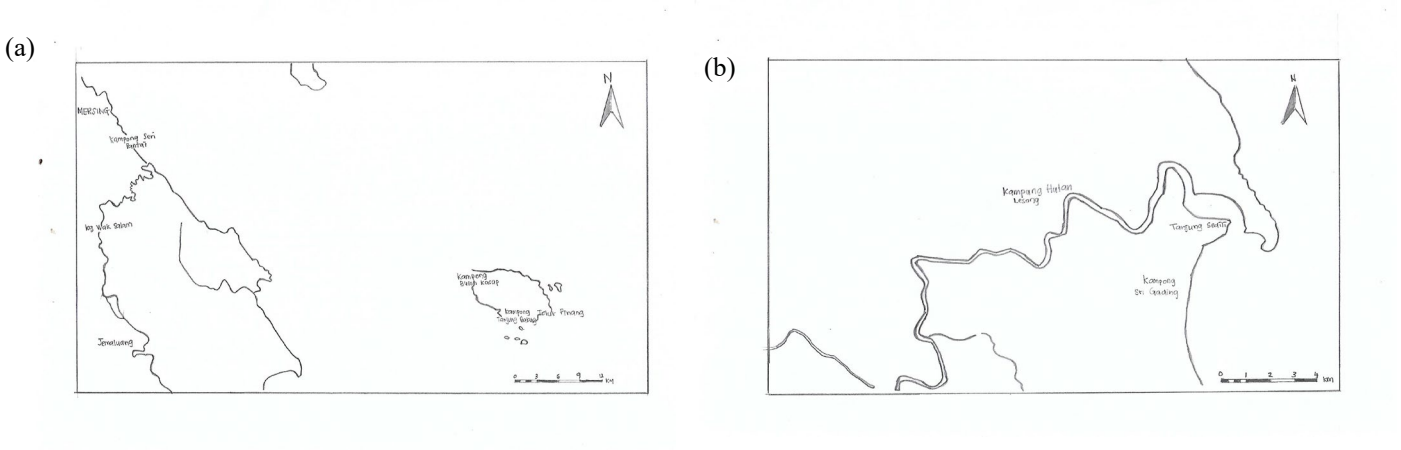

Fig. 1 - (a) Pulau Tinggi, Johor; (b) Sedili Kechil, Kota Tinggi, Johor

2.1 Morphological Species Identification

Ossicles extraction was done according to Kamarudin and Rehan [10] guidelines with slight modification. A small piece of tissue was cut from the body part of a sea cucumber with a sterile blade. Then, the tissue was placed in a glass slide. A few drops of liquid household bleach were used to dissolve the soft tissue and to leave the hard ossicles on the glass slide. The prepared ossicles were observed under a compound microscope and the images of ossicles were saved for the morphological observations.

PAST (PAleontological STatistics) software version 4.03 was used in executing a range of standard numerical analysis and operations used in quantitative paleontology by using ossicle shapes for each specimen. Multivariate statistics were chosen and hierarchical clustering graph was constructed by using binary "1" for presence and "0" for absence based on the ossicle shapes character in the PAST integrated spreadsheet-type data entry. The resulting data were submitted for cluster analysis using the Unweighted Pair Group Method with sample Arithmetic Mean (UPGMA) [11].

3. Results and Discussion

There were a total of 53 individuals or samples of sea cucumbers recorded in this study, of which 23 individuals were from Pulau Tinggi and 30 individuals were from Sedili Kechil. These individuals comprised 12 species from two families i.e. Holothuriidae and Stichopodidae as shown in Figure 2. International Union for Conservation of Nature (IUCN) Red List of Threatened Species status of the species was summarized in Table 1.

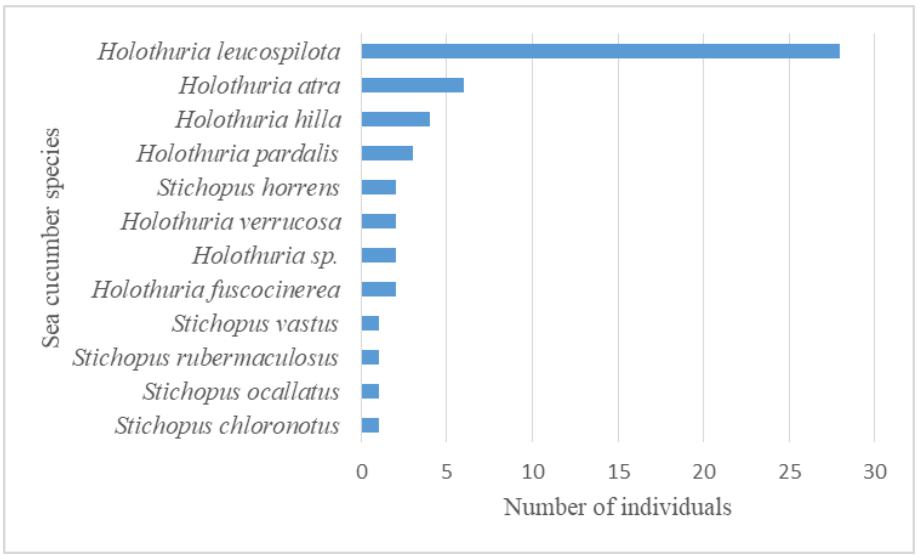

Fig. 2 - The checklist of sea cucumber species surveyed in Pulau Tinggi and Sedili Kechil

Table 1 - Family status and IUCN status of sea cucumbers observed in Pulau Tinggi and Sedili Kechil.

No	Family	Scientific Name	IUCN	Total No. of Individuals
1	Holothuriidae	*Holothuria (Mertensiothuria) leucospilota* (Brandt, 1835)	LC	28
		Holothuria (Mertensiothuria) hilla Lesson, 1830	LC	4
		Holothuria (Lessonothuria) pardalis Selenka, 1867	LC	3
		Holothuria (Stauropora) fuscocinerea Jaeger, 1833	LC	2
		Holothuria (Lessonothuria) verrucosa Selenka, 1867	LC	2
		Holothuria (Halodeima) atra	LC	6
		Holothuria sp.	-	2
2	Stochopodidae	*Stichopus rubermaculosus* Massin, Zulfigar, Hwai & Boss, 2002	DD	1
		Stichopus chloronotus Brandt, 1835	LC	1
		Stichopus horrens Selenka, 1867	DD	2
		Stichopus ocellatus Massin, Zulfigar, Hwai & Boss, 2002	DD	1
		Stichopus vastus Sluiter, 1887	LC	1

*Notes: LC= Least Concern and DD = Data Deficient according to IUCN Red List Website [12]

A total number of two sea cucumber families were morphologically observed and recorded in the study area. The first family that had been identified belonged to family Holothuriidae with a total of seven species including one unknown species named *Holothuria* sp. This family had 47 individuals, by which the highest individual number of species was *Holothuria (Mertensiothuria) leucospilota* with six individuals in Pulau Tinggi and 22 individuals in Sedili Kechil. The second highest individual number of species found in this family was *Holothuria (Halodeima) atra* with six individuals and this species was found only in Sedili Kechil. The third rank belonged to *Holothuria (Mertensiothuria) hilla* with four samples, while the fourth rank belonged to three other species which were *Holothuria (Stauropora) fuscocinerea*, *Holothuria (Lessonothuria) verrucosa*, and *Holothuria* sp. with only two individuals for each species recorded. *H. hilla*, *H. fuscocinerea*, and *H. verrucusa* were found in Pulau Tinggi seawaters and *H.* sp. from Sedili Kechil. Family Holothuriidae is usually called as sea cucumber or *timun laut* by local people.

The second sea cucumber family recorded was the family Stitchopodidae. This family had 5 different species. The highest individual number of species from this family was *Stichopus horrens* with two individuals. Meanwhile, the other species were *Stichopus chloronotus*, *Stichopus rubermarculosus*, and *Stichopus ocellatus* with only one individual for each species. All species were discovered and recorded in Pulau Tinggi. The species from this family are generally called *gamat* and the most precious species in this family is known as *S. horrens* (*gamat emas*) due to their beneficial properties for medicinal uses.

In general, this study recorded two orders of sea cucumber consisting of two genera in Pulau Tinggi. The total number of sea cucumber species consisted of five species from the genus *Holothuria*, followed by five species of *Stichopus. H. leucospilota*, the most dominant species in Pulau Tinggi, was observed and documented at all collection sites around the mangrove area to the Kampung Tanjung Balang area in Pulau Tinggi. This species was the most dominant species in the marine waters of Malaysia [13]. A number of five species from the genus *Stichopus* were collected namely *S. rubermaculosus*, *S. horrens*, *S. vastus*, *S. ocellatus*, and *S. chloronotus*. Most of the species were found at the sandy bottom, rubble, and rocky areas along Pulau Tinggi from the mangrove area near the Shaz Resort to the jetty of Kampung Tanjung Balang during the low tide via random surveying.

The number of species in Pulau Tinggi was lesser compared to the study conducted in Pulau Aur in 2007 by Zulfigar and his research team [9] in which 20 specimens were recorded. Previously, the data on the distribution of sea cucumber in Pulau Aur, Johor in 2005 by several researchers covered three different sites and depth in Sultan Iskandar Marine Park. There were four sites including Pulau Dayang, and in this area, the total species of sea cucumber were 12

species. The second site was Pulau Aur by total species found were 12 species [9]. The third was in Pulau Pinang, with a total of species found were 12 species. The last site was in Pulau Lang, where six species were found on this site [9]. It covered different zonation for different sites, from the shallow area until a deeper area where approximately 30 m deep to see the different composition of the sea cucumbers for each zonation [9]. The differences between the findings of the previous study and this current study was expected due to the method used, the area covered, and the depth of sea level where the survey was conducted. Firstly, the method used in the previous survey in the Aur group consisting of Pulau Dayang, Pulau Pinang, Pulau Lang, and Pulau Aur exhibiting higher biodiversity of sea cucumber population compared to Pulau Tinggi was done using scuba diving up to 0 – 30 m depth and the survey was conducted both during night and day since most of the sea cucumber species are nocturnal [9]. Meanwhile, the current survey incorporated random surveying where this method was conducted in the shallow water only. Colour photographs of living specimens were taken after the specimens were collected and transferred into a container filled with seawater for relaxing purposes and to achieve a natural body form. Good colour photographs of living organisms taken with scale were of particular importance when identifying species since preserved materials often lose colour and shape.

Interestingly, seven species were listed in this study as new records for the Pulau Tinggi Marine Park as they were not reported or listed in the Pulau Aur group data. The species were *H. leucospilota*, *H. pardalis*, *H. fuscocinera*, *H. verrucosa*, *S. rubermaculosus*, *S. ocellatus,* and *S. vastus.* Most of the sea cucumber species were easily identified morphologically as they can be fully examined from the photographs and in situ observation.

The study in Sedili Kechil noted three species of sea cucumber from one order that consisted of the same genus i.e. *Holothuria*. The study in Sedili Kechil also recorded *H. leucospilota* as the most dominant species as predicted when referring to the earlier studies conducted by Kamarudin and his research team [13]. It reported that *H. leucospilota* was the most dominant species of sea cucumbers present in the marine waters of Malaysia. All the species were collected at the same collection site around Pantai Tanjung Buloh, Sedili Kechil. They were found in the shallow and calm water during low tide by random surveying. Most of *H. atra* body surface was covered with a fine film of sand except in some circular places on the dorsal surface and found exposed on sandy bottom. Meanwhile, *H. leucospilota* and the *H.* sp. were found in little deep on sandy bottoms and some were found under the rocks. All these species lived in shallow habitats, among rubble and coral reefs. Pantai Tanjung Buloh is one of the tourism spots in Sedili Kechil. During the weekends, the area will be packed with tourists.

There were three sampling sites in Sedili Kechil with different types of substrates which were rocky intertidal, sandy intertidal, and mixed of rocky and sandy intertidal. The first sampling site was the main tourism spot at the beach where human water-based activities take place. Every weekend, the beach crowded with people swimming around the area. There was no sea cucumber seen or collected within this area. This is probably because the area is a high-risk habitat for the sea cucumber to inhabit. It could make the sea cucumber being most vulnerable as there is a high probability for human direct contact. The second sampling site is located at rocky intertidal where no individuals of sea cucumber are seen or collected. This sampling site is isolated from people and far from any human disturbance, however, it might be unsuitable for sea cucumber to inhabit because of the strong underwater current and the lack of coral cover. All the individuals collected were found at mixed rocky and sandy intertidal zone where there was no, or little human disturbance taken place in the area. Therefore, it has less stressor to the sea cucumber habitat. This area is characterised by its sandy-muddy bottom with corals and seagrasses thriving on it. Some individuals of the dominant species, *H. leucospilota* were found with their body partially hidden under the rocks. A study by Purcell *et al* [14] figured that *H. leucospilota* inhabited shallow habitat on outer and inner reef flat, back reef and usually found in the sandy and muddy bottoms with rubble or coral reefs. The different substrates of these sites influenced the preferences of sea cucumber habitat. The research sites presented one genus and three species of sea cucumber, with one species still in need of further verification. The data gathered for the abundance of *H. leucospilota* was higher than the number of other species of the same genus from the same order. Meanwhile, a study by Siti and others [8] reported that *Stichopus* was the most abundant genus in Pulau Besar, Johor.

This current study reported ten species as new records for the Pulau Tinggi Marine Park. They were *H. leucospilota*, *H. pardalis*, *H. fuscocinera*, *H. verrucosa*, *H. hilla*, *S. horrens*, *S. chloronotus*, *S. rubermaculosus*, *S. ocellatus*, and *S. vastus.* Besides, three new species were regarded as a new record for Sedili Kechil, Johor as there had been no or little study on sea cucumber recorded for this area. The three new species were *H. leucospilota*, *H. atra,* and *H.* sp. This research study showed that there were different species ranges based on the location of sampling, ecology, and distribution of species for both Pulau Tinggi and Sedili Kechil coastal area. As this checklist of sea cucumber in Pulau Tinggi and Sedili Kechil was obtained, it enables us to require information on the availability of various species of sea cucumbers. The sea cucumbers collected were identified based on their morphological properties from the photograph and in situ observation.

Based on the IUCN Red List of Threatened Species (Table 1), there were three species classified as Data Deficient (DD) status, which were *S. horrens*, *S. rubermoculosus*, and *S. ocellatus.* It was proven in this study because these species were rarely to be found with one or two samples were discovered during one month sampling session in Pulau Tinggi coastal area. Most species from family Holothuriidae were classified as Least Concern (LC) with two other species from family Stichopodidae, which were *S. chloronotus* and *S. vastus*.

A dendogram was constructed in this study based on ossicle shapes according to the guidelines by Manoko [15] and Yaradua and his research team [16] with slight modification. Some important shapes that could be used to differentiate the sea cucumbers species were the presence of button shape that can be only observed on *Holothuria* species while C-shaped rod and rosette were mostly observed on *Stichopus horrens* [10]. The finding was supported by Tehranifard and his research team [17] when there were numerous c-shaped rods and rosette with a variable development commonly observed in *S.hermanni* collected from Kish Island, Persian Gulf [17]. Besides that, smooth and knobbed button and table were recorded from *H. albiventer* from Johor Straits [18]. Based on Figure 3, two sea cucumber families were observed i.e. Holothuriidae and Stichopodidae, even though *S. chloronotus* were found closer to the members of family Holothuriidae. Table-shaped ossicle was the most common shape found in both Holothuriidae and Stichopodidae families [19]. Thus, this table shape was observed in all samples collected from Pulau Tinggi and Tanjung Sedili. In fact, it was shown that all sea cucumbers recorded from Pulau Tinggi and Tanjung Sedili could be identified either from Holothuriidae or Stichopodidae family. However, the table shape also often underwent evolution and reduced with age [20]. Wen *et al.* [21] suggested that table- and button-shaped ossicles were ancestral those evolved into rods and rosettes in Stichopodidae samples [21]. Furthermore, the table shapes were also not homogenous in early juveniles of Holothuriidae and would affect the identification of sea cucumbers [19]. In future, molecular approach is required as an additional complement for better insight and understanding of the relationship.

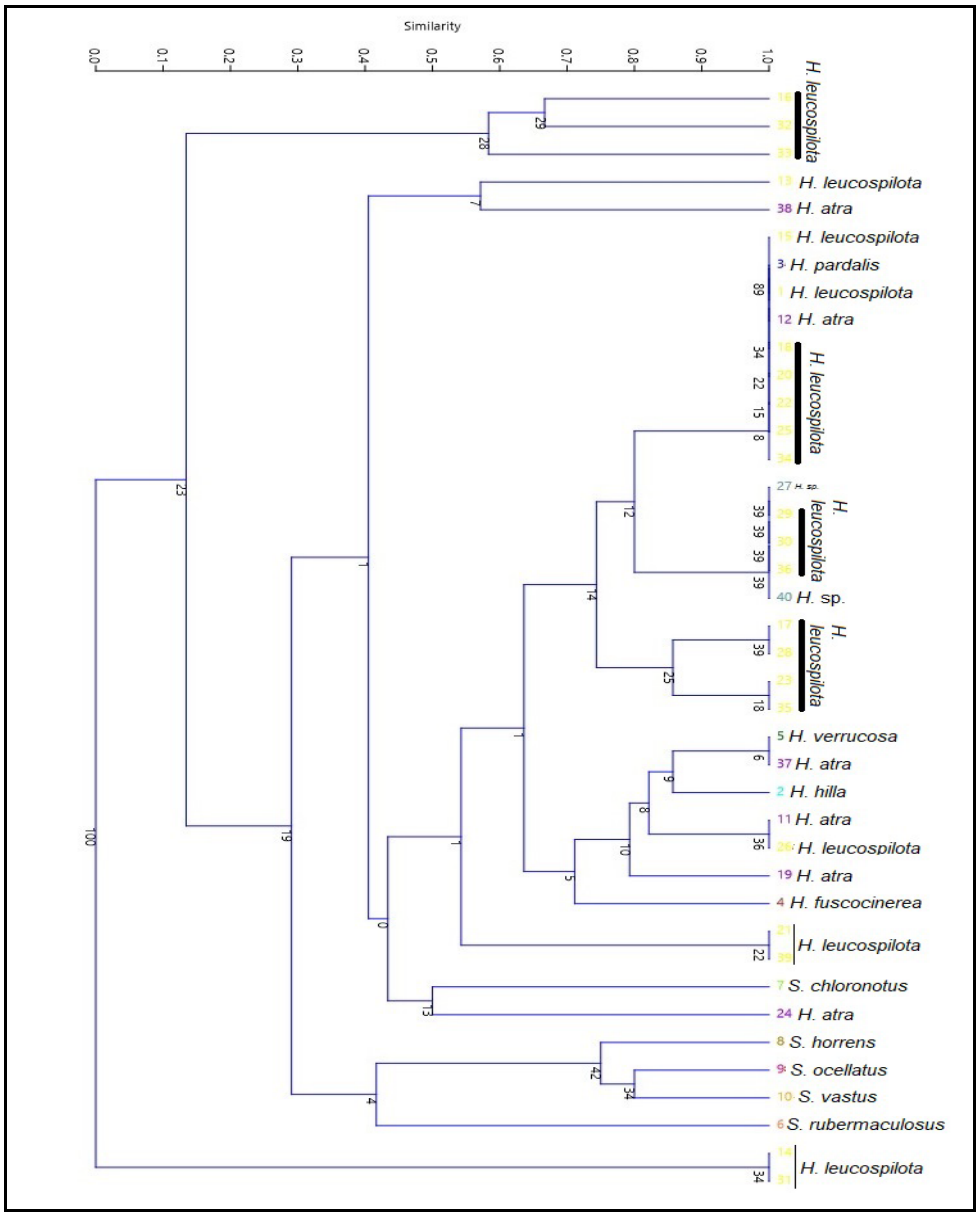

Fig. 3 - Hierarchical clustering using Paired Group (UPGMA) by using Bray-Curtis similarity index with 1000 bootstrap value for all ossicle shapes

4. Conclusion

In conclusions, 10 species of sea cucumber were recorded in the coastal waters of Pulau Tinggi, Mersing, and three species in Sedili Kechil, Kota Tinggi, Johor based on morphological species identification. All of these species in Pulau Tinggi were from genus *Holothuria* and genus *Stichopus*, namely *Holothuria (Mertensiothuria) leucospilota*, *Holothuria (Mertensiothuria) hilla*, *Holothuria (Lessonothuria) pardalis*, *Holothuria (Stauropora) fuscocinerea*, *Holothuria (Lessonothuria) verrucosa*, *Stichopus horrens*, *Stichopus chloronatus*, *Stichopus ocellatus*, *Stichopus rubermaculosus* and *Stichopus vastus* respectively. Meanwhile, all species in Sedili Kechil were from genus Holothuria namely *Holothuria (Mertensiothuria) leucospilota, Holothuria (Halodeima) atra,* and *Holothuria sp.* A total of 10 ossicle shapes were observed and extracted from the sea cucumber samples. Ossicle shapes such as button, perforated plate, table, C-shaped rod, rosette, anchor, spider, I-shaped rod, boomerang, and J-shaped rod were recorded. Species relationship based on ossicle shapes was analysed using PAST software and presented by Unweighted Pair Group Method with sample Arithmetic Mean (UPGMA). The analysis showed two groups of sea cucumber families i.e. Holothuriidae and Stichopodidae, although *S. chloronotus* was closer to the members of family Holothuriidae. Therefore, the outcomes of this study have assisted in updating status of sea cucumber in Johor and providing global references for future study of sea cucumber from Malaysia. This is the first record of sea cucumber species from Pulau Tinggi and Sedili Kechil. Further studies on genetic identification are recommended in order to support the identification of sea cucumber species present in Pulau Tinggi and Sedili Kechil. Additionally, more studies on ecological and biological aspects are required to understand the factors that affect the pattern of distribution of sea cucumber species and to implement appropriate management strategies for the existing species in order to prevent the declination of marine life's population in Johor seawaters.

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