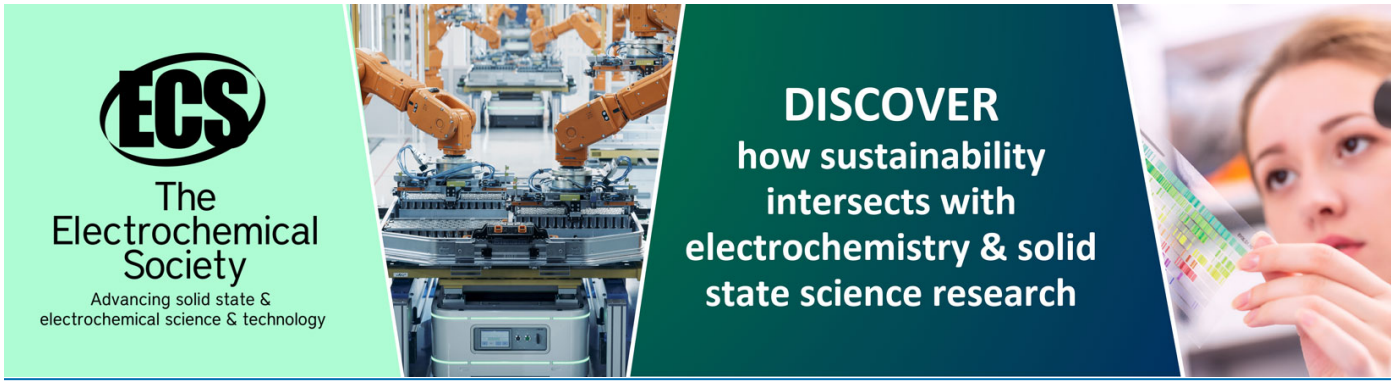
The diversity of echinoderms in the seagrass meadows of Penang Island, Malaysia



The diversity of echinoderms in the seagrass meadows of Penang Island, Malaysia

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Abstract. Many studies had been done on the diversity of echinoderms in Malaysia, especially on holothuroids, but only a handful was conducted on seagrass meadows. The lack of studies conducted on echinoderms in seagrass meadows coupled with the steadily declining area of seagrass meadows represents a serious threat to the documentation of Malaysia's echinoderm biodiversity. This study was conducted to provide a checklist of echinoderms found in the seagrass meadows of Penang Island. Four sampling trips were conducted from October 2019 to February 2020 and specimens were collected by hand during low tide in two sites, Pulau Gazumbo and Middle Bank. Additional specimens in the collection of Centre for Marine and Coastal Studies, Universiti Sains Malaysia (CEMACS, USM) were also included in this study. Ossicles of holothuroids were extracted, observed under a light microscope, and illustrated. A total of 31 specimens were examined and identified, comprised of 15 species: one from the class Asteroidea; seven from the class Ophiuroidea; two from the class Echinoidea; and five from the class Holothuroidea. The results would be able to serve as a basis for future studies on the taxonomy, diversity, and distribution of echinoderms in the seagrass meadows of Malaysia.

1. Introduction

The phylum Echinodermata are exclusively marine, mostly benthic invertebrates [1]. Currently, there are approximately 7000 extant living species and 13,000 worldwide [2]. They can be found at all known depths and habitats of the ocean [3], including seagrass meadows. Seagrass meadows are shallow-water marine environments that provides important ecosystem services [4–5]. Despite their importance, seagrass meadows are still being lost globally and have been declining at a rate of 7% per year of the total global seagrass area since 1990 [4–5].

Many studies have been done in Malaysia on the diversity and distribution of echinoderms [6–8], especially on holothuroids [9–17]. However, only a handful of studies on echinoderms in seagrass meadows were conducted in Malaysia [18–21] and Singapore [22–26]. The lack of studies of echinoderms in seagrass meadows and the increasingly declining area of seagrass meadow poses a threat to the documentation and our understanding of Malaysia's echinoderm diversity. As such, there was a need to conduct this study to provide a checklist of echinoderms in the seagrass meadows of Penang Island with hope that it would be able to become the basis of future studies, especially on the community

structure, diversity, distribution, ecology, and taxonomy of echinoderms in the seagrass meadows of Malaysia.

2. Materials and Methods

Four sampling trips were conducted from October 2019 to February 2020. Two sampling trips were conducted on 29th October 2019 and 29th November 2019 at Pulau Gazumbo while another two sampling trips were conducted on 13th December 2019 and 10th of February 2020 at Middle Bank. All specimens were deposited in CEMACS, USM, Penang, Malaysia. The general sampling location is shown in Figure 1.

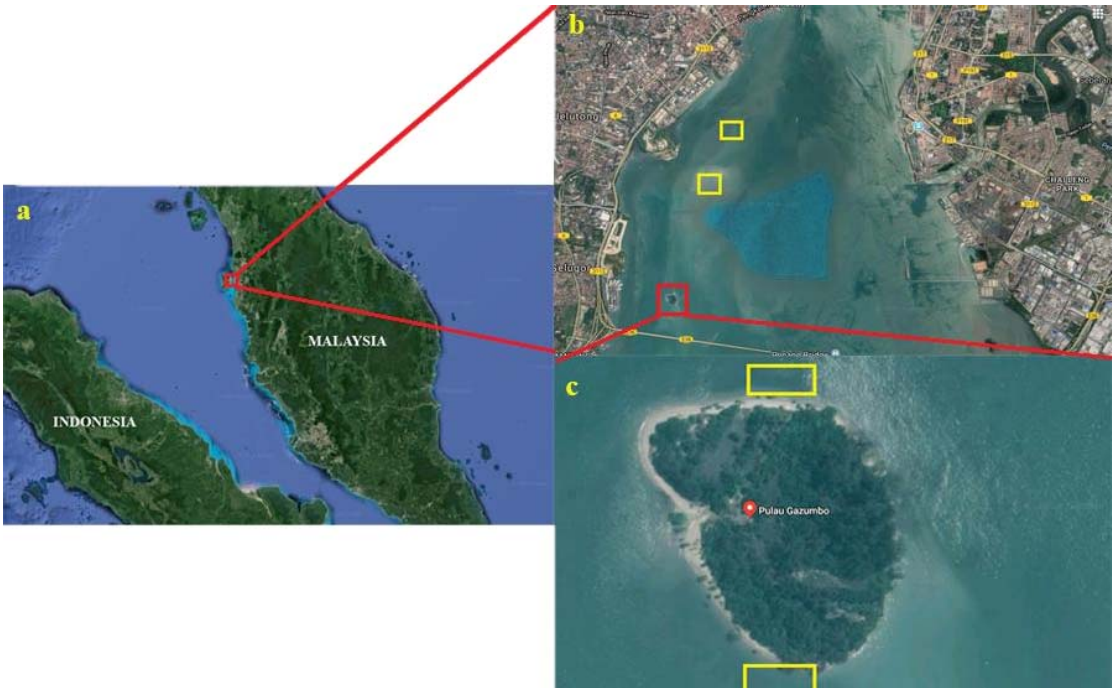

Figure 1. Study area in Pulau Gazumbo and Middle Bank (in yellow box); a. location of Pulau Gazumbo and Middle Bank; b. Middle bank; c. Pulau Gazumbo.

2.1. Sampling Method

Specimens were collected by hand while walking on the exposed seagrass bed. Additionally, a hand shovel was used to acquire infaunal specimens by digging the substrate. All relevant data including ID code, location, date, name of collector, colour of specimen, substrate, and general terrain formation were recorded. In-situ photographs were taken prior to collection of specimen. Field specimens collected were stored temporarily in individual plastic bags filled with seawater.

2.2. Preparation and Preservation Method

Collected specimens were anaesthetized with an 7.5% magnesium chloride solution. Specimens were fully submerged into the solution and given enough time to be fully anaesthetized. Specimens were determined to be fully anaesthetized when the specimens show no reaction upon prodding. Specimens were photographed against a white or black background, fixed, and preserved in 95% ethanol. A piece of waterproof label paper with the ID code, location, date, name of collector, and specimen colour was placed into the jar containing the preserved specimen.

For regular echinoids, half of the spines on the test were detached by soaking the specimen partially in 10% domestic bleach solution and held in place for five minutes. The detached spines were collected, examined, and preserved in 95% ethanol.

For holothuroids, the ossicles were extracted by dissolving the dermal tissue. A solution was made by mixing 10% domestic bleach solution. A $5mm^3$ piece of tissue sample was cut from the dorsal body wall and further cut into smaller pieces. Tissue samples from the papillae, tube feet, and tentacle were cut as well. Tissue samples were placed in individual labeled test tubes and filled with 5ml of the domestic bleach solution, fully submerging the tissue samples. Each test tubes were left for ten minutes for the tissue to be fully dissolved. Test tubes were shaken every three minutes to facilitate the dissolving process. If tissue samples were not completely dissolved after ten minutes, 5ml more domestic bleach solution was added into the test tube. After the tissue samples were fully dissolved, the ossicles were rinsed four times using distilled water. The ossicles were transferred to a glass slide with a glass dropper. The glass slides were left overnight to dry. After drying, cover slips were placed onto the glass slides with Eukitt® Quick-hardening mounting medium.

2.3. Identification Method

Specimens were observed with an Olympus SZX16 stereomicroscope with Olympus DP72 camera attached. Photographs were taken for external morphological structures that were critical for identification of the species. Ossicles extracted from holothuroids were examined using an Olympus BX50 light microscope equipped with Olympus DP21 camera. Apart from specimens collected in this study, additional specimens in the collection of CEMACS, USM were also examined in this study. Specimens were identified mainly using Clark & Rowe [27] and other relevant references.

3. Results and Discussion

A total of 31 specimens were examined and identified, comprised of 15 species: one from the class Asteroidea; seven from the class Ophiuroidea; two from the class Echinoidea; and five from the class Holothuroidea. The results were summarized in Table 1. Part of the specimens collected were displayed in Figure 2. Records of echinoderm species from this study and other studies [19–26] were listed in Table 2. The species composition of echinoderms in this study were found to differ from other studies [19–26]. This suggests that while the sampling sites were identical in which all were intertidal seagrass meadows, the echinoderm diversity differs geographically, as this study was conducted on the northern part of Malaysia while the other studies were conducted on the southern tip of Malaysia.

There were fewer sea star species found in this study (one species) compared to the study done by [19] (five species) and [21] (seven species). Incidentally, there were more seagrass species present in Merambong Shoals compared to Pulau Gazumbo, with ten different species of seagrass reported in Merambong Shoals [28] while only two seagrass species were reported in Pulau Gazumbo [29].

Although this initially suggests that there may be an association between the diversity of seagrass and asteroids, [19] has remarked in their study that there were no particular association between species of asteroids and specific species of seagrass from field observations. [19] further remarked that the composition of asteroids was higher in abundance within areas with seagrass species with lower canopy height compared to seagrass species with higher canopy height. According to [29] the dominant seagrass species in Pulau Gazumbo is *Halophila ovalis* (R. Br.) Hook. *f.*, which suggests that there should be more species of asteroids within this study site as the overall seagrass canopy height is low. Unfortunately, the number of asteroid specimen collected from Pulau Gazumbo is far too little to come up with something more conclusive.

Based on field observations in this study, it is likely that rather than an association between ophiuroids with seagrass, it is more likely that ophiuroids are more associated with soft-bottom substrates, hard surfaces, or sessile invertebrates. All specimens of ophiuroids in this study were collected from muddy surfaces between seagrass patches, within debris such as driftwoods, or within sessile invertebrates such as sponges and sea pens. No ophiuroid specimens in this study were collected directly from the surface of seagrass.

Table 1. Echinoderms collected from the seagrass meadows of Penang Island.

Class	Family	Species	Locality
Asteroidea	Luidiidae	*Luidia hardwicki* (Gray, 1840)	Pulau Gazumbo
Ophiuroidea	Amphiuroidea	*Amphioplus* sp.	Middle Bank
	Ophiotrichidae	*Macrophiothrix speciosa* (Koehler, 1898)	Pulau Gazumbo
		Ophiothrix (Acanthophiothrix) spinosissima (Koehler, 1905)	Pulau Gazumbo
		Ophiothela venusta (de Loriol, 1900)	Middle Bank
	Ophiactidae	*Ophiactis carnea* (Ljungman, 1867)	Pulau Gazumbo & Middle Bank
		Ophiactis fuscolineata (H. L. Clark, 1938)	Pulau Gazumbo & Middle Bank
		Ophiactis savignyi (Müller & Troschel, 1842)	Pulau Gazumbo
Echinoidea	Diadematidae	*Diadema setosum* (Leske, 1778)	Pulau Gazumbo
	Astriclypeidae	*Echinodiscus truncatus* (L. Agassiz, 1841)	Pulau Gazumbo
Holothuroidea	Cucumariidae	*Actinocucumis longipedes* H.L. Clark, 1938	Pulau Gazumbo & Middle Bank
	Phyllophoridae	*Stolus buccalis* (Stimpson, 1855)	Pulau Gazumbo
		Phyllophorella thyonoides (H. L. Clark, 1938)	Pulau Gazumbo & Middle Bank
	Holothuriidae	*Holothuria (Mertensiothuria) leucospilota* (Brandt, 1835)	Middle Bank
		Holothuria (Metriatyla) martensii (Semper, 1868)	Middle Bank

Table 2. List of records of echinoderms collected in intertidal and seagrass meadows based on this study and other studies. Asterisks (*) denotes species recorded within seagrass meadows of Penang Island for the first time in this study.

Species	This study	[19]	[21]	[22]	[20]	[24]	[23]	[25]	[26]
Class Asteroidea									
Family Archasteridae									
Archaster typicus Müller & Troschel, 1840		x							
Family Goniasteridae									
Goniodiscaster scaber (Moebius, 1859)		x	x						
Stellaster childreni Gray, 1840 olim *Stellaster equestris* (Retzius, 1805)		x	x						
Family Astropectinidae									
Astropecten sp.			x						
Astropecten vappa Müller and Troschel, 1843			x						
Family Luidiidae									
Luidia maculata Müller & Troschel, 1842		x	x						
Luidia hardwicki (Gray, 1840)	x*								
Family Oreasteridae									
Anthenea aspera Döderlein, 1915			x						
Protoreaster nodosus (Linnaeus, 1758)		x	x						

Table 2. Continued.

Species	This study	[19]	[21]	[22]	[20]	[24]	[23]	[25]	[26]
Class Ophiuroidea									
Family Amphiuridae									
Amphipholis misera (Koehler, 1899)				x					
Amphiura (Ophiopeltis) phalerata (Lyman, 1874)				x					
Amphioplus sp.	x*								
Family Ophiotrichidae									
Macrophiothrix hybrida H. L. Clark, 1915				x					
Macrophiothrix speciosa (Koehler, 1898)	x*								
Ophiothrix (Acanthophiothrix) spinosissima Koehler, 1905	x			x					
Ophiothela venusta (de Loriol, 1900)	x*								
Family Ophiactidae									
Ophiactis delagoa Balinsky, 1957				x					
Ophiactis carnea Ljungman, 1867	x*								
Ophiactis fuscolineata H. L. Clark, 1938	x*								
Ophiactis modesta Brock, 1888				x					
Ophiactis savignyi (Müller & Troschel, 1842)	x			x					
Family Ophionereididae									
Ophionereis dubia dubia (Müller & Troschel, 1842)				x					

Table 2. Continued.

Species	This study	[19]	[21]	[22]	[20]	[24]	[23]	[25]	[26]
Class Echinoidea									
Family Diadematidae									
Diadema setosum (Leske, 1778)	x*								
Family Astriclypeidae									
Echinodiscus truncatus (L. Agassiz, 1841)	x*								
Class Holothuroidea									
Family Psolidae									
Psolidium helenae Ong & O'Loughlin 2019									x
Family Cucumariidae									
Actinocucumis longipedes H.L. Clark, 1938	x*								
Colochirus quadrangularis Troschel, 1846					x				
Cercodemas anceps Selenka, 1867					x				
Cucumaria sp1.					x				
Cucumaria sp2.					x				
Mensamaria intercedens (Lampert, 1885)						x			
Family Sclerodactylidae									
Globosita murrea Cherbonnier,1988					x				
Afrocucumis africana (Semper, 1868)								x	
Havelockia sp.								x	

Table 2. Continued.

Species	This study	[19]	[21]	[22]	[20]	[24]	[23]	[25]	[26]
Family Phyllophoridae									
Stolus buccalis (Stimpson, 1855)	x							x	
Phyllophorella thyonoides (H. L. Clark, 1938)	x*								
Family Holothuriidae									
Actinopyga lecanora (Jaeger, 1833)								x	
Holothuria sp1.						x			
Holothuria sp2.						x			
Holothuria (Lessonothuria) pardalis Selenka, 1867								x	
Holothuria (Mertensiothuria) leucospilota (Brandt, 1835)	x							x	
Holothuria (Mertensiothuria) sp.								x	
Holothuria (Metriatyla) martensii Semper, 1868	x							x	
Holothuria (Metriatyla) scabra Jaeger, 1833						x		x	
Holothuria (Thymiosycia) impatiens (Forskål, 1775)								x	
Family Caudinidae									
Acaudina molpadioides (Semper,1867)						x	x		
Acaudina leucoprota H. L. Clark, 1938							x		
Acaudina rosettis O'Loughlin & Wong, 2015							x		
Family Synaptidae									
Anapta gracilis Semper, 1867						x			
Synaptula recta (Semper, 1867)						x		x	

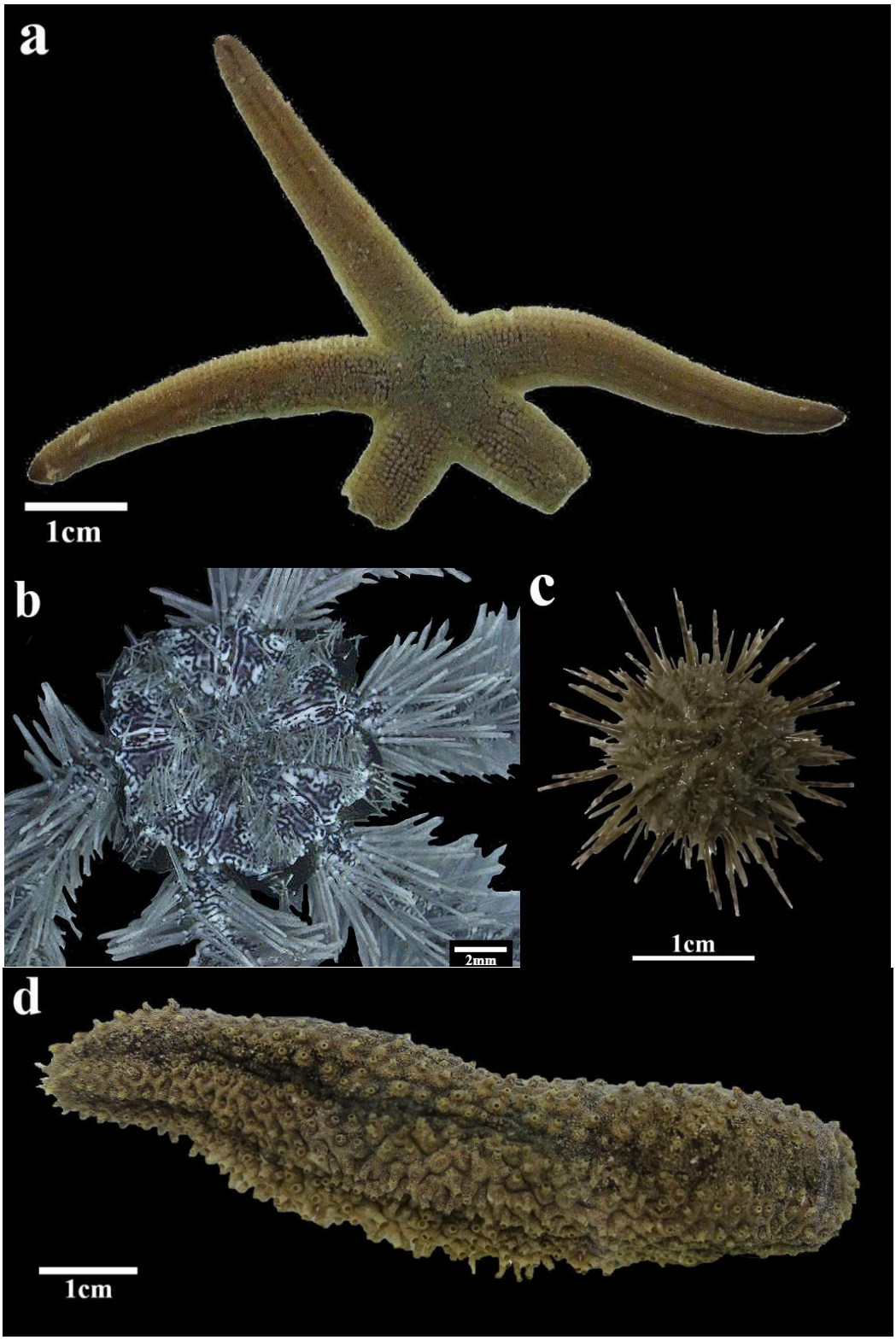

Figure 2. Echinoderms collected from the seagrass meadows of Penang Island: a. *Luidia hardwicki*, PG058; b. *Ophiothrix (Acanthophiothrix) spinosissima*, PG020; c. *Diadema setosum*, PG018; d. *Actinocucumis longipedes*, MB015.

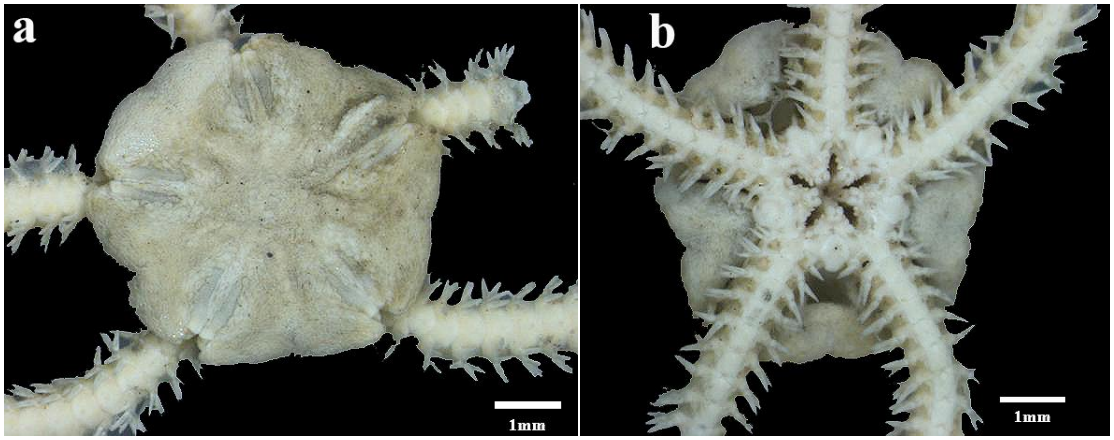

Figure 3. Amphioplus sp., MB007: a. dorsal surface; b. ventral surface.

The unidentified specimen *Amphioplus* sp. (Figure 3) resembles *Amphioplus (Amphioplus) lucidus* Koehler, 1922 based on the thin radial shields that were only contiguous distally, blunt spear-shaped oral shields, four oral papillae with a gap between the infradental and second oral papillae revealing the first oral tentacle scale, oval-shaped dorsal arm plates, and rectangular ventral arm plates that were longer than wide. However, the *Amphioplus* sp. specimen in this study had at most five arms spines and two large tentacle scales that were able to completely cover up the tentacle pore while Liao [30] described *A. lucidus* had 7–8 arm spines and two small tentacle scales that were unable to cover up the tentacle pore. Additionally, the *Amphioplus* sp. specimen in this study had hooks on the tips of all of the arm spine while [30] described that *A. lucidus* only had hooks on the second and third arm spine counting from the bottom.

Comparison between the species of ophiuroids identified in this study and [22] suggests that the species composition of ophiuroids seems to differ among different localities. Out of the seven species identified in this study, only two species (*O. spinosissima* and *O. savignyi*) were also found in the study by [22]. Both study sites (Pulau Gazumbo and Middle Bank) in this study were located in Penang Island, which was north of Malaysia, while the study site (Johor Straits) in the study by [22] were located near Singapore, which was on the southern tip of Malaysia.

It is likely that seagrass meadows may play an important role in the life cycle of *D. setosum*. The specimen of *D. setosum* identified in this study was a juvenile, and no adult specimens has been found in the study sites. This suggests that seagrass meadows may act as a nursing ground for juvenile *D. setosum*. Other examples of echinoderms utilizing seagrass meadows as a nursing ground has been documented in the past, as with the case for *A. typicus* [31].

The holothuroid species composition of this study differs greatly compared to the study done by [20] even though both study sites were identical, being seagrass meadows. No species of holothuroid were simultaneously found in both studies. This seems to indicate that there may be an association between the holothuroid species composition and the diversity of seagrass or the locality. However, some holothuroid species identified in this study were also found in the study done by [24] and [25] suggesting that locality as a factor influencing species composition of holothuroids in seagrass meadows may not be as significant.

This study differs from the [24] in that the latter study also collected specimens from intertidal mudflats and sandy shores rather than solely seagrass meadows. Hence the difference in holothuroid species composition between this study and the study done by [24] was likely due to the fact that some of the holothuroid species identified were more closely associated with mudflats and sandy shores but their ecological range may extend into seagrass meadows due to the close proximity of the habitats.

4. Conclusion

A total of 31 specimens were examined and identified, comprised of 15 species: one from the class Asteroidea; seven from the class Ophiuroidea; two from the class Echinoidea; and five from the class Holothuroidea. The species composition found in this study, which was conducted on the northern part of Malaysia, differs from the other studies that were done on the southern tip of Malaysia, suggesting that the species composition of echinoderms differs geographically even though both this study and the other studies were conducted on seagrass meadows. However, only 31 specimens were examined in this study, which was far too little compared to the amount of specimens examined in the other studies. More specimens should be collected from Pulau Gazumbo and Middle Bank to obtain a better representation of the species composition of echinoderms before further remarks can be made.

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