THE DIVERSITY, DISTRIBUTION AND MORPHOLOGICAL DESCRIPTIONS OF STICHOPODIDAE (ECHINODERMATA: HOLOTHUROIDEA) IN THE VICINITY OF MALAYSIAN WATERS

by

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KEPELBAGAIAN, TABURAN DAN DESKRIPSI MORFOLOGI STICHOPODIIDAE (ECHINODERMATA: HOLOTHUROIDEA) DI SEKITAR PERAIRAN MALAYSIA

Abstrak

Suatu kajian terhadap kepelbagaian dan taburan timun laut dari famili Stichopodiidae telah dijalankan di kawasan terumbu karang di perairan Selat Melaka, Laut China Selatan, Laut Sulu dan Laut Sulawesi. Sejumlah sembilan spesies dari dua genera (Stichopus dan Thelenota) telah dikenalpasti. Tujuh spesies berasal dari genus Stichopus iaitu S. chloronotus, S. horrens, S. ocellatus, S. rubermaculosus, S. herrmanni, S. vastus dan satu spesies yang tidak diketahui spesies diberikan nama Stichopus sp.. Dua spesies berasal dari genus Thelenota iaitu T. ananas dan T. anax. Identifikasi spesimen dilakukan berdasarkan morfologi luaran dan juga analisa spikul dari empat bahagian stichopodid iaitu permukaan dorsal, papillae, tentakel dan kaki bertiub. Satu spikul baru dijumpai di tentakel S. rubermaculosus dan satu di permukaan badan dorsal S. herrmanni yang belum pernah dilaporkan. Spesies S. chloronotus dan S. vastus mencatatkan taburan paling meluas dan dijumpai di kebanyakkan kawasan kajian. Laut China Selatan mencatatkan kepelbagaian stichopodid tertinggi berbanding the dengan laut-laut lain. Akan tetapi, Pulau Payar di utara Selat Melaka mencatatkan kepelbagain tertinggi antara kesemua lokasi penyampelan, dengan catatan empat spesies dan indeks kepelbagaian Shahnon-Wiener sebanyak Analisa (H')1.17. Non-metric multidimensional scaling (NMDS) menunjukkan Selat Melaka diklusterkan sebagai laut yang berbeza berbanding laut-laut lain berdasarkan taburan stichopodid. Timun laut stichopodid dijumpai di antara kedalaman 5 hingga 22 meter di permukaan berpasir kecuali *T. ananas* di atas permukaan karang mati beralga dan *S. chloronotus*

di permukaan campuran pasir dan karang mati. Ancaman terhadap habitat stichopodid dapat diperhati khususnya di kawasan terumbu karang Laut Sulu yang disebabkan oleh kegiatan dan kaedah perikanan destruktif. Selain itu, peningkatan tekanan terhadap penangkapan timun laut di utara Selat Melaka dan Laut Sulawesi juga menjadi ancaman kepada taburan dan kepelbagaian stichopodid. Taman laut telah dikenalpasti berupaya melindungi kepelbagaian dan taburan stichopodid dengan keadaan terumbu karang yang sihat dan kegiatan perikanan yang terkawal.

THE DIVERSITY, DISTRIBUTION AND MORPHOLOGICAL DESCRIPTIONS OF STICHOPODIDAE (ECHINODERMATA: HOLOTHUROIDEA) IN THE VICINITY OF MALAYSIAN WATERS

Abstract

A study was carried out on the diversity and distribution of sea cucumbers from the family Stichopodidae at the shallow coral reefs of the Straits of Malacca, South China Sea, Sulu Sea and Sulawesi Sea. A total of nine species of stichopodids from two different genera (Stichopus and Thelenota) were identified. Seven species were from the genus Stichopus, namely S. chloronotus, S. horrens, S. ocellatus, S. rubermaculosus, S. herrmanni, S. vastus and one unknown species named Stichopus sp.. Two species belong to the genus Thelenota namely T. ananas and T. anax. Identification of each species was done by description of their morphology as well as calcareous spicules examination from four parts of the body that were the dorsal body, papillae, tentacles and tube feet. A new spicule was described on the tentacles of S. rubermaculosus as well as a pseudo-table on the dorsal body of S. herrmanni that was not observed before. The most widely distributed species were S. chloronotus and S. vastus. The South China Sea was the most diverse area compared to the other seas with a total of six different species found. However, Pulau Payar at the northern Straits of Malacca recorded the highest diversity among all sampling locations with four species found and the Shahnon-Wiener diversity index (H') of 1.17. Non-metric multidimensional scaling (NMDS) analysis showed clear division in the distribution of stichopodids between the Straits of Malacca and the rest of the seas. The stichopodids were found in water depths ranging from 5 to 22 metres mostly on sandy substrate except for *T. ananas* on dead corals with algae and S. chloronotus on mixture of sand and coral rubble. Threats to the habitat of stichopodids were noted especially at coral reefs of the Sulu Sea due to destructive fishing methods while increasing fishing pressure were seen on the Straits of Malacca and the Sulawesi Sea. Marine Protected Areas were observed to harbour more diversity and distribution of stichopodids due to their undamaged reefs and regulated fishing activities within the gazetted area.

CHAPTER 1

INTRODUCTION

1.1 Classification of Holothuroidea

Class Holothuroidea or commonly known as sea cucumbers are an abundant and diverse group of worm-like which typically cylindrical in shape, elongated and usually soft-bodied echinoderm. They can be found in almost all marine environments from the shallow intertidal areas to the deepest floor of oceanic trenches (Pawson, 1982; Hickman *et al.*, 2006). Deichmann (1957) regards them as the most aberrant group of extant echinoderms. One of the earliest accounts of sea cucumbers can be seen is isolated spicules from fossils of sea cucumber from the Silurian since 400 million years ago (Gilliland, 1993). The early classification of holothurians which falls under the class of the Echinoderms did not exist as they are all classified in the class of Vermes by Linneus (1758). It is not until Bruguiére (1791) subdivided the Vermes and established an order named Echinodermata. Bruguiére however included asteroids, ophiuroids and echinoids in this order but failed to recognise holothuroids as part of it (Smith, 1984). It is Lamarck (1801) later added the holothuroids to the Echinodermata eventually it is not until Leuckart (1854) successfully established the Echinodermata as a distinct phylum.

Sea cucumbers are all placed in the Class Holothuroidea (Figure 1.1) which is further divided into three subclasses: Dendrochirotacea, Aspidochirotacea and Apodacea. Dendrochirotacea which consists of two orders namely the order Dendrochirotida and order Dactylochirotida always has an introvert and introvert retractor muscles present. Subclass Aspidochirotacea is the largest group of holothurians with a distinctive peltate shape on their tentacles with two members in this subclass namely

the order Aspidochirotida and order Elaspodida. The subclass Apodacea is very distinctive where both order Apodida and order Molpadida has very reduced tube feet to absent. Their tentacles shaped pinnate or digitate. Table 1.1 illustrates descriptions and characteristics of each order. The family Stichopodiidae belongs to the order Aspidochirotida along side with two other members: the family Holothuriidae which is the largest family with the most species and the family Synallactidae which are restricted to species found only in deep water (Canon and Silver, 1986). There are only two genera in the family Stichopodiidae occurring in the Indo-Pacific region namely *Stichopus* and *Thelenota* (Clark and Rowe, 1971; Arnold and Birtles, 1989; Byrne, 2010).

Figure 1.1: Classification of the family Stichopodidae with the two genus *Stichopus* and *Thelenota* which was found in Indo-West Pacific (based on Clark and Rowe, 1971; Arnold and Birtles. 1989).

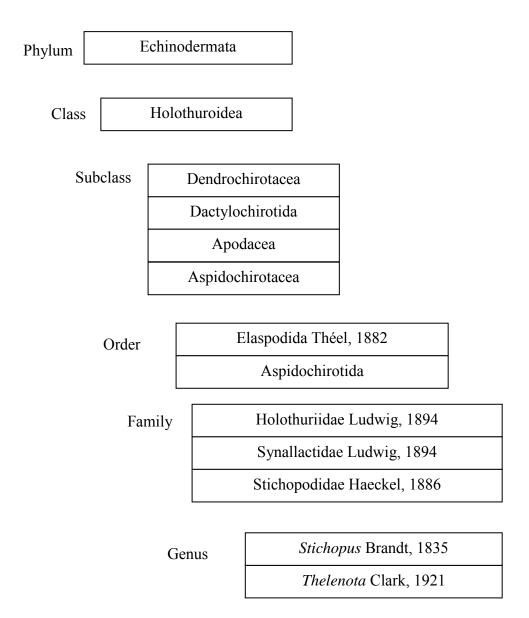


Table 1.1: Descriptions of sea cucumber classified into three subclasses and six orders (modified from Arnold and Birtles, 1989).

Subclass	Order	Description of Order
		There were 10-30 tentacles highly branched
	Dendrochirotida	or shaped in the form of dendritic. Ten
Dendrochirotacea		calcareous plates forming simple or with
		complex posterior process calcareous ring.
		They are usually sedentary, attached or
		burrowing
		Tentacles were not branched but digitate with
	Dactylochirotida	8-30 articles. It had U-shaped body enclosed
		in a test of imbricating plates. Calcarous ring
		was simple and lack of complex posterior
		process. Mostly found only in the deep-sea.
	A '1 1' '1	Very large group with very diverse and
A .1 1.	Aspidochirotida	abundant in shallow tropical habitats.
Aspidochirotacea		Tentacles always in the shape of peltate.
		Respiratory trees present. Three families
		belong to this order with one specific family (Synallactidae) restricted in deep waters.
	Elaspodida	Lackof respiratory tree in body cavity. Strongly bilaterally symmetrical with
	Elaspoulda	commonly complicated and elaborate
		projections like frills, veils and sails on their
		gelatinous body. Only found in deep sea at
		abyssal depths.
	Apodida	One of the simplest forms of sea cucumber. It
		has very long and worm-like body wall
Apodocea		without any tube feet present on it except for
		the tentacles. They also have soft smooth and
		soft body. Respiratory tree absent.
	Molpadiida	Tentacles are digitate. Stouted body with
		some slightly curved body to some curved
		liken a ball with tapering to a more or less
		conspicuous tail. No tube feet present in the
		body wall and mostly find in soft muddy
		substrates.

1.2 The Morphology and Anatomy of Sea Cucumbers.

1.2.1 The General Morphology of Sea Cucumbers

Generally all sea cucumber take the shape of cylindrical body and elongated orally-abodally (Arnold and Birtles, 1989). Differing from their cousins in the phylum Echinodermata, holothurians have their pentamerous radial symmetry axis horizontally where the other members of the phylum have their axis vertically. The ambulacra which runs longitudinally across from the oral to aboral according to the pentamerous arrangement in such a way that two can be described as dorsal and three as ventral (Clark and Rowe, 1971). Such pentaradiate arrangement condition may not be obvious to certain group especially in the Aspidochirotida or some in the Dendochirotida due to the modified dorso-ventrally which is flattened and equipped with locomotory podia formed by three ventral ambulacra known as trivium (Clark and Rowe, 1971). The two other ambulacra are arched on the dorsal side scattered with sensory podia know as bivium.

Podia which are normally restricted to the areas of ambulacra arise from the water vascular system but sometimes they may be scattered irregularly and extending towards the interambulacra area. However some holothurians from the order Apodida completely lack of podia on their body and the family Psolidae have a very distinctive plate or sole derived from podia (Forbes, 1841; O'loughlin and Maric, 2008). These are sometimes called tube feet due to their cylindrical shape and are categorized into two groups based on their functions and position. Papillae are podia specialized for sensory at the dorsal side of the body. The number of papillae and prominence varies with age where increasing to maturity and perhaps decreasing during senescence and therefore they cannot be relied on for specific character (Clark, 1922). Podia at the flattened ventral surface are modified with suction cups

for locomotory functions are called pedicels. The number of pedicels like the papillae increases with age and are of little in taxonomic importance (Clark, 1922).

Tentacles surrounding the mouth in the oral opening are also highly modified podia. The structure of tentacles (Figure 1.2) differs tremendously between different groups of holothuroids rendering them useful for separation at the level subclass and order. They assume the forms of from richly branching (dendritic) to shield-shaped, to featherlike, to digitiform (fingerlike). This modification of shape and structure of the tentacles also constitutes their function either for suspension feeding or deposit feeding. For example the suspension feeders in sea cucumber have tentacles which are highly branched and consist of numerous minute finger-like structures to capture suspended organic particles from moving waters. The peltate tentacles are adapted for deposit feeders where their compact plate-like tentacles sweeps substrate en masse into the mouth (Rowe, 1969). The number of tentacles are typically 20 but it is not uncommon to have species with tentacles ranging from 15 to more than 20 tentacles though it is speculated that specimen with less than 20 tentacles are normally juveniles and those with more than 20 are individual variants making number of tentacles is of no value for distinguishing a species (Clark, 1922). The position of the oral opening also dependent on the feeding habits where suspension feeder will have their oral opening on the dorsal side and oral opening for deposit feeders normally faces ventrally or sometimes terminal. Aboral opening or the anus are always terminal.

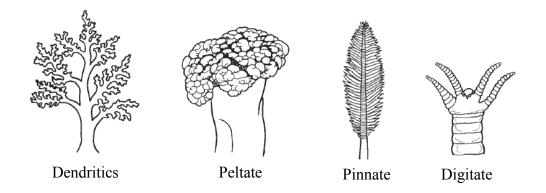


Figure 1.2: The different structures of tentacle in sea cucumber (modified from Conand, 1998).

1.2.2 The General Anatomy of Sea Cucumber

The general internal anatomy of sea cucumbers is shown in Figure 1.3. The mouth or oral in the anterior of body is surrounded or bordered by circlet of tentacles. Depending on the species, the mouth is either curved dorsally or ventrally. The mouth then leads to the pharynx that is situated in the centre of the aquapharyngeal bulb which goes through the calcareous ring as well as the water-vascular ring (Khanna & Yadav, 2005). The calcareous ring is normally made out of 10 calcareous plates (five radial and five interradial plates) that support the pharynx, nerve ring and the water rings (Borradaile & Potts, 1963). Calcareous ring also serves as a point of insertion for longitudinal muscles and retractor muscles whenever present (Miller & Pawson, 1984) where they are attached specifically at the radial plates which are larger than the interradial plates (Arnold & Birtles, 1989). Longitudinal muscles run from the calcareous ring along the body to the aboral end of the body This longitudinal muscles along with the circular muscles in the body allows the sea cucumber to expand and contract its body.

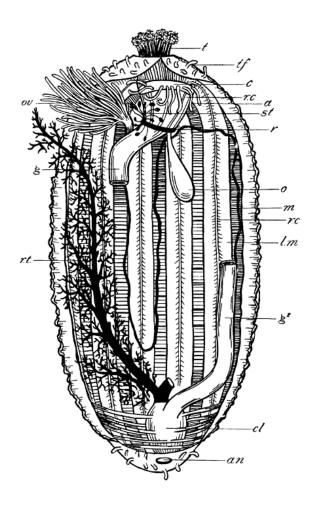


Figure 1.3: A general anatomy of a sea cucumber revealed by dissection, t, tentacles; tf, scattered tube-feet; c, calcareous ring; a, ampullae of tentacles; r, circular vessel; st, stone canal; o, Polian vesicle; rc, radial canals; lm, longitudinal muscles; g-g¹, intestine; m, mesentery; cl, cloaca; an, anus; rt, respiratory tree; ov, gonad (modified from Thomson, 1916).

The water-vascular ring in the posterior of the calcareous ring gives off five radial canals which connect to two kinds of appendages: polian vesicles and stone canals. The stone canal is a short calcareous tube that connects the madreporite to the radial canal. Stone canal opens on the mid-dorsal surface through a hydropore but once achieving adulthood, the connection with surface is lost except for few holothurians especially from order Elasipodida (Miller & Pawson, 1984; Khanna & Yadav, 2005). Madreporite termination forms a sac shaped round to elongated form

that lies in the coelom. As Madreporite in Holothurians are usually found either internal or is in contact with the exterior by means of a pore-canal (Miller & Pawson, 1984). In other Echinoderms, Madreporite always occur external of the body. Madreporite functions as pressure regulator for the radial canal by the influx or outflow of water through the pore.

Figure 1.3 shows the detail structure of radial canal that is connected to the water-vascular ring. The radial canals extend out to each side lateral branches of podial canal and gives rise to podia.

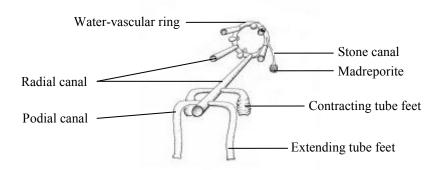


Figure 1.4: Extension of the water-vascular ring forming radial canal that give rise to tube feet (modified from Ross, 2009).

The respiratory tree is connected directly to the cloaca and acts as the "lung" for sea cucumbers. It is a hollow and highly branched organ situated in body cavity at either side of the intestine. But the respiratory tree is absent in two order: Apodida and Molpadiida. Cloaca is a muscular cavity made out of circular muscles called sphincter which aids them in the process of respiration. The cloaca is expanded to allowing oxygenated water to fill up the cavity through the anus. Subsequently the posterior sphincter shuts together with the contraction of the cloacal muscles to force the water up into the respiratory tree (Lambert, 1997). The thin membrane of the

respiratory tree aids the transfer of oxygen into the fluids of body cavity. Body wall then contracts to force the oxygen depleted water out of the trees through the same cavity and anus out of the body.

Cuvierian tubules as shown in Figure 1.5 is a system consisting of many short and whitish tubules whose proximal ends are attached to the basal part of the respiratory trees and their distal ends are left floating freely in the coelomic cavity (VandenSpiegel & Jangoux 1987; VandenSpiegel et al., 2000). The Cuvierian tubules are used as a defensive mechanism to protect itself from predation and attacks. Sea cucumber will curve itself with the aboral end facing towards the irritating object and undergo a strong contraction. This will force the Cuvierian tubules to be expelled through the anus together with the coelomic fluid. The emitted tubules lengthen, instantly become sticky and rapidly immobilize and trap most organisms in contact (VandenSpiegel and Jangoux, 1987). Ejected tubules will be generated (Hyman, 1955) and this mechanism is explained in details by (VandenSpiegel et al., 2000). This special structure of Cuvierian tubule only can be found in some species in the Order Aspidochirotida from the genera of Actinopyga, Bohadscia and Holothuria only. Generally Cuvierian tubules are found to be absent in the genus Stichopus, Thelenota and Astichopus from the family Stichopodiidae as noted by Clark (1922).

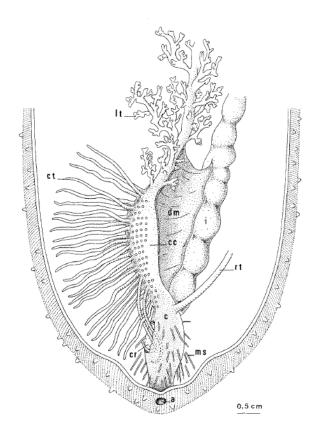


Figure 1.5: The anatomy of the posterior of *Holothuria forskali* in the attachment of the Cuvierian tubules, a: anus; c: cloaca; cc: Cuvierian chamber; cr: Cuvierian rupture; ct: Cuvierian tubule; dm: digestive mesentery; i: intestine; lt: left mesentery; ms: mesenteric strand; rt: right respiratory tree (modified from VandenSpiegel & Jangoux, 1987).

The digestive system of most sea cucumber possesses oesophagus and stomach but some with the pharynx opens directly to the intestine as shown in Figure 1.6. The intestine is elongated, coiled and loops within the body cavity before terminating at the cloaca at the posterior end of the coelom. First the intestine runs towards the posterior (descending) from the stomach with attachment to the body wall by dorsal mesentery. Until it reaches the posterior part, the intestine makes a loop and continues to elongate in the reverse direction back to the anterior part. This ascending part of intestine is connected to the body wall by left mesentery. Upon

reaching at the anterior end of the body cavity, it makes another loop and reversing towards the cloaca forming the rectum that is connected to the body wall through the right mesentery. The cloaca is attached to the surrounding body wall by an abundance of threadlike radial cloacal dilator muscles (mesenteric strands). Both openings between the rectum and cloaca as well as the cloaca to the exterior via anus are closed by a sphincter. The respiratory tree is very much associated with the intestines.

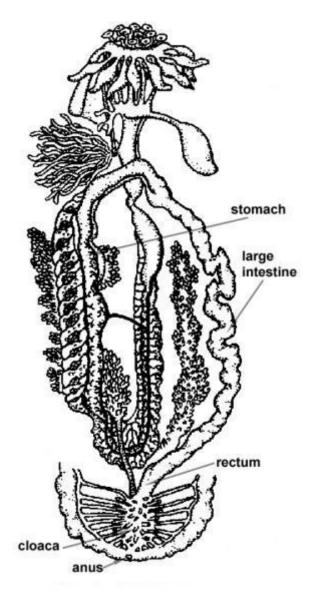


Figure 1.6: The organs involved in the digestive system of a sea cucumber (modified from Myers *et al.*, 2006)

The respiratory organ consists of two respiratory trees that originated from the cloaca. They are hollow and highly branched evaginations of the wall of the cloaca rendering them an efficient gaseous exchange site. The rhythmic contraction and expansion of the muscular body wall draws and expels water though the anus so that respiration can take place (Hyman, 1955). Some sea cucumbers like the Apodids which are lacking of the respiratory tree depends on the thin body wall for breathing where oxygen are able to pass through this extremely thin body wall.

1.3 The Life Cycle of Sea Cucumber

Holothuroids are predominantly gonochoric, which means they exists as two separate sex male or female. They are very rarely hermaphroditic except to a few species as found in *Holothuria atra* (Harriot, 1982), the genus *Leptosynapta* (Everingham, 1961; Sewell, 1994) and from the genus *Peniagone* (Tyler *et al.*, 1985). For sexual reproduction, they are broadcast spawners where the female sea cucumber will produce oocytes which are released into the water and fertilized externally by motile sperm cells released by another male. When an egg is fertilized and hatches, the tiny planktonic larva drifts with the ocean currents. It will eventually settle onto the sea floor and develop into an adult (Figure 1.7).

Upon fertilization, a free-swimming larva is quickly formed starting at blastula stage after the first 24 hours, a hatched gastrula by the 64th hour then to a microalgae-feeding auricularia larva about 6-13 days after fertilization (Cameron and Frankboner, 1989). Metamorphosis then happens to first form a doliolaria stage occurring at 65-125 days and then to a pentacula stage about two days after doliolaria stage which they remain pelagic before settling on various substrata upon reaching the final larval stage (Cameron and Frankboner, 1989).

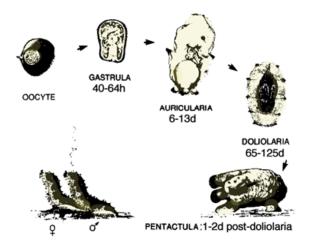


Figure 1.6: A diagram to show the general life cycle of a sea cucumber (modified from Cameron and Frankboner, 1989).

1.4 The Ecological, Economical and Medical Potentials of Sea Cucumber

1.4.1 The Ecological Importance and Roles of Sea Cucumber in the Ecology

Holothurians play important roles ecologically in keeping the benthic environment healthy especially in the coral reefs (Birkeland, 1989) mainly as suspension feeders, detritivores and a prey as their main diet consist of bacteria, diatoms and detritus (Yingst, 1976; Massin, 1982; Moriarty, 1982). Suspension feeding sea cucumbers can have substantial effect to a local ecosystem as demonstrated by Velimirov *et al.* (1977), where two species of holothurians alone represent nearly half of the filter feeding biomass in South African kelp forests. Massin (1982) also demonstrated that filter feeding habits of sea cucumber regulates water quality at the environment by affecting the carbonate content as well as the pH of the water. Behairy *et al.* (1985) showed in their study that there is a reduction of aragonite concentration in the faeces relative to the gut content suggests that mineral alteration and utilization occur during feeding and therefore important in the phenomenon of controlling carbonate mineralogy in a local area.

Deposit feeders too are very important in bioturbation which occurs when sea cucumber ingests sediments (Massin 1982) as Holothuroids especially in the tropics are mainly epibenthic sediment feeders forming an important part of the many shallow reefs (Kerr *et al.*, 1993). One example is the feeding on tiny dead planktons and organic particulate that settles on the benthic of the ocean floor that creates mud on the sea floor bed (Lambert, 1996). The displacement and mixing of the sediment particles will alter the stratification and stability of muddy and sandy bottoms of all grain size as holothuroids do not have special preference on grain sizes but rather on the availability and amount of organic matter present in the sediment (Hammond, 1982; Behairy *et al.*, 1985). Some species exhibit burrowing behaviour in the mud and therefore aerating the bottom and recycling nutrients as they feed (Lambert, 1996). Extirpation of holothurians from an ecosystem may cause hardening of sea floor that may avert the potential habitat for other benthic organism (Bruckner *et al.*, 2003) and the bioturbation activity recycles nutrient in oligotrophic environments from trapping in the sediments (Uthicke, 2001).

Spatial variation of holothuroid population can reveal patterns of spesies interaction in a community (Kerr *et al*, 1993). One of those interactions can be seen where sea cucumber supports other communities of organisms as prey especially when they are consumed by fishes, sea stars and crustaceans (Francour, 1997). They are not just important links in the web chain both in the coral reef in the tropics to the temperate waters (Birkeland, 1982; Francour, 1997) but to the shallow to deep sea as well (Massin, 1982).

1.4.2 Sea cucumber fisheries and trade in Malaysia

Sea cucumber fisheries in the Peninsular Malaysia are very small scale and limited to only few locations comparative to the East Malaysia especially Sabah with mostly artisanal fishing methods (Choo, 2008). This is because most of the islands in the Peninsular are gazetted as Marine Protected Area where fishing and collection of aquatic organisms are not permitted. The four main island clusters gazetted are Pulau Payar located in the Straits of Malacca and three clusters in the South China Sea (Redang, Tioman and Johor group of islands). Harborne et al. (2000) suggested the fishing pressure is very low at the East Coast of Peninsular Malaysia where edible species of sea cucumbers are still frequently and abundantly found during their survey at the Redang Islands, Tioman Islands and Tinggi Islands. Therefore sea cucumber fisheries in the Peninsular Malaysia only concentrated in ungazetted islands of Langkawi group of islands, Sembilan group of islands and Pangkor group of islands. All of these islands situated at the west coast of Peninsular Malaysia in the Straits of Malacca. Choo (2004) illustrated a small commercial fishery mainly on the species Stichopus horrens for traditional medicinal products exists in the north western of Pangkor island. Baine and Choo (1999) also elaborated that the sea cucumber fishery in Pangkor island mainly is sustainable because it is only run by one family and sea cucumbers are only collected at low tide approximately five days a month at each catch reaching up to a total of two gallon-drums per day. Another site for sea cucumber fisheries can be found in Langkawi Island. The target species in Langkawi Island are also from the genus Stichopus. The fishery in Langkawi Island dates back to the mid 1900s but markedly disappeared in the early 1940s when the target species Stichopus hermanni and Stichopus horrens are fished to depletion (Forbes and Ilias, 1999; Choo, 2004)

As compared to the Peninsular Malaysia, sea cucumber fisheries in the East Malaysia especially in the state of Sabah are larger and more important not due to the fact that it contributes to foreign exchange but rather because it provides livelihood, supplement incomes to poor coastal communities and also food for the indigenous people in the coastal area (Choo, 2004). Sea cucumbers are collected from the shallow reefs by hand picking, snorkelling as well as free diving. Though the fishing methods used seems harmless to the population of sea cucumbers, the intensity of collection as well as number of fishers is staggering. The Annual Fisheries Statistics of Sabah in year 2000 highlighted that there are only 217 registered sea cucumber collector but the actual number are expected to be at least five to six times higher (Choo, 2004). The landing tonnage of sea cucumbers captured in the Annual Fisheries Statistics, Sabah indicated that more than 90% of sea cucumber catches are landing from bycatch of trawlers with amount of about 139 tonnes in year 2005 (Choo, 2004). This number did not really reflect the actual amount of sea cucumber landing because those harvested by hand-picking are difficult to capture in the official fisheries statistics as artisanal fishing of these products are not required to report to the authority. Forbes and Ilias (1999) showed from their survey done in Sabah, East Malaysia from July 1996 to December 1999 that high value species such as Holothuria scabra are already rare. This result in the increase of pressure on middle to low value species including several species from the family Stichopodiidae namely Thelenota anas, Thelenota ananas and other Stichopus spp.. Fresh sea cucumber is processed to become beche-de-mer in the form of chilled, fresh or frozen before exported to target countries which include China, Hong Kong, Peninsular Malaysia and Singapore. China remains to be the main major market and consuming country for sea cucumber in the world amounting to about 5500 tonnes in

year 2001 (Ferdouse, 2004). A more recent report suggested that Hong Kong alone is responsible for 58% of global sea cucumber imports volume (Anderson *et al.*, 2011).

Sea cucumber populations are particularly vulnerable to the anthropogenic threat of overfishing due to the effectiveness and increase intensity of harvesters to collect and capture them at the shallow reefs (Uthicke and Benzie, 2000). They are slow moving and most of the time seen exposed at shallow reefs especially during low tide. This make them easy target for harvesting using simple and cheap methods such as hand collecting, spear, hook or net while wading the water. The other factor has to do with their reproduction cycle where they have late maturity age, slow growth and low rates of recruitment causing the low rate of population restocking (Uthicke *et al.*, 2004).

1.4.3 The Medical Properties and Potentials of Sea Cucumber

Marine products are long believed to have high nutritional value and traditionally used as medicine. Sea cucumbers have been eaten as tonic food by the Chinese dating back to the Ming Dynasty (Chen 2004) and other Asians for centuries due to their curative and dietary properties (Conand, 1990; Conand 2006). Sea cucumber is believed to reduce joint pain and arthritis, help restore correct intestinal and urinary functions, reinforce the immune system and can treat certain cancers (Chen, 2004). One example of medicine produced in Malaysia is the "Minyak Gamat" where traditionally believed to heal cuts, skin rashes, joint pains and helps the recovering of post-natal. The "Minyak Gamat" derived from sea cucumber belonging to the family of Stichopodidae mainly curryfish, *Stichopus hermanni* and the warty sea, *Stichopus horrens* (Choo, 2004).

Jha and Xu (2004) elaborated the potentials and variety beneficial biomedical compounds in marine organism including from the group Echinoderms. Bordbar *et al.* (2011) reviewed extensively the potentials, nutrients and high value bioactive compounds found in various form in sea cucumbers by reporting from various previous studies. Till date, there are scores of potentially medicinal compounds has been isolated from holothurians species of those including antitumour, antiviral, anticoagulant and antimicrobial compounds (Kelly, 2005). Therefore the therapeutic and pharmaceutical value in sea cucumber holds significant future development of health care product as well as medicine. Lawrence *et al.* (2010) also found significant intraspecific variation between populations of the same species along different habitats suggesting greater potential for future bioprospecting. Conserving each population even of the same or different species becomes utmost important because even severely depleted populations recover, they might lack the genetic diversity and therefore potential bioprospecting uses (Lawrence *et al.*, 2010).

1.5 Research on the Taxonomy and Distribution of Stichopodids in Malaysia

One of the earliest comprehensive studies on the diversity and distribution of holothurians was done by Ridzwan and Che Bashah (1985). Then, Zulfigar *et al.* (2008) produced a field guide to sea cucumbers found in shallow waters and coral reef in Malaysia. The specific study of the taxonomy and distribution of sea cucumber from the family Stihchopodidae in Malaysia were done by Siti *et al.* (1999), Zulfigar *et al.* (2000) and Massin *et al.* (2002) which there were two new species described (*Stichopus rubermaculosus* and *Stichopus ocellatus*).

1.6 Objectives of the Study

In view of the current threats of overharvesting, degrading habitat and potential detrimental effects of climate change to the population of sea cucumbers, it is utmost important to understand the classification and taxonomy of these species in order to have protection measures in place. Waters around Malaysia sits on the centre of maximum biodiversity (Hoeksema, 2007; Veron *et al.*, 2009) having the most number of species of marine organism in the world. Therefore there are still undiscovered species of sea cucumber bound to be found besides resolving the many cryptic species.

This study aims to determine the diversity and distribution of sea cucumber focusing species from the family Stichopodidae by achieving objectives as follows:

- a) to produce a taxonomic account of sea cucumber from the family
 Stichopodidae found in waters around Malaysia
- to describe the distribution pattern of Stichopodids in the waters around Malaysia.

CHAPTER 2

METHODOLOGY

2.1 The study areas

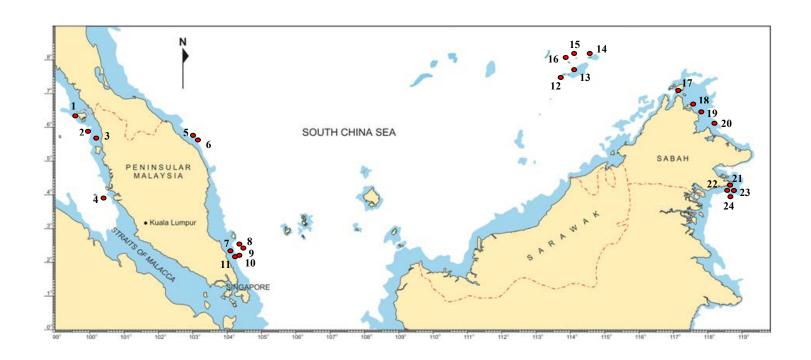
This study was carried out in several islands around the four main waters of Malaysia covering the Straits of Malacca, South China Sea, Sulu Sea and Sulawesi Sea, which were very different in their characteristics and geographical importance. The sampling sites were searched to cover as many representative habitats as possible from the outer reef to the associated coral reef lagoon in the sandy area and adjacent seagrass bed. The list of sampling areas and coordinate were listed in Table 2.1.

2.2 Sample collection and preservation

Collection of samples was carried out over a period from June 2009 to December 2011 at locations shown in Figure 2.1. SCUBA diving was employed as the main sampling method which collection can be at the shallow-waters up to the depth of 30 metres. Samples were also collected by handpicking during reef walk in the intertidal zone while trawl nets that dredge the deeper water by the fishermen were also searched. Samplings were done both at night as well as during the day when and wherever possible to search for nocturnal species. The numbers of individuals of all species found were noted as well as the total area sampled in each site to determine species abundance. Upon collection, individuals were kept in separate plastic bags underwater. The physical separation between each live specimen will minimize the potential antagonistic reaction of sea cucumber when in

contact with the other individual such as expulsion of Cuverian tubules and autoevisceration (Samyn *et al.*, 2004).

Sea cucumbers were relaxed before preservation to ensure tentacles, papillae and tube feet remain extended. Anesthetization was done using sea water which was added with menthol crystal. The specimens were prodded gently to ensure they were fully relaxed with no reaction being noticed. Dorsal and ventral pictures of each sample were taken before preservation. Larger specimens were injected with 95% ethyl alcohol into the body cavity before submerging them into the 95% concentration of alcohol (Rowe and Doty, 1977). For permanent preservation, specimens were transferred to 70% alcohol accompanied by a waterproof label paper with information of the specimen (species name, substrate, depth, date and name of collector).



Legends

• Sampling location

1 – Teluk Datai	5 – Pulau Bidong	9 – Pulau Dayang	13 – Terumbu Ubi	17 – Pulau Banggi	21 – Pulau Si Amil
2 – Pulau Payar	6 – Pulau Redang	10 – Pulau Mentinggi	14 – Terumbu Peninjau	18 – Bilean	22 – Pulau Mabul
3 – Pulau Songsong	g 7 – Pulau Tinggi	11 – Pulau Tinggi	15 – Terumbu Siput	19 – Mumiang	23 – Pulau Kapalai
4 – Pulau Sembilan	8 – Pulau Aur	12 – Pulau Layang-layang	16 – Terumbu Mantanan	i 20 – Pulau Lankayan	24 – Pulau Sipadan

Figure 2.1 Sampling locations in the Straits of Malacca, South Chinas Sea, Sulu Sea and Sulawesi Sea.

Table 2.1: Coordinates and types of zone of each sampling locations.

Sea	Location	Latitude and	Zone
		Longtitude	
	Teluk Datai	N 06° 26' 02.7"	Intertidal
		E 99° 40′ 54.8″	
-	Pulau Payar	N 06° 03' 49.6"	Subtidal
Straits of		E 100° 02' 32.7"	
Malacca	Pulau Songsong	N 05° 48' 31.2"	Subtidal
		E 100° 17' 38.0"	
-	Pulau Sembilan	N 4° 01' 46.8"	Subtidal
		E100° 32' 39.7"	
	Pulau Bidong	N 05° 37' 15.7"	Subtidal
<u>-</u>		E 103° 03' 25.2"	
	Pulau Redang	N 05° 44′ 01.2″	
		E 103° 01' 10.5"	Subtidal
		N 05°46' 22.7"	
-		E 103° 03' 35.2"	
	Terumbu Siput	N 08° 05' 53.7"	Subtidal
		E 114° 34' 39.5"	
		N 08° 95' 42.3"	Subtidal
		E 114° 07' 47.1"	
-	Terumbu Mantanani	N 07° 58' 04.6"	Subtidal
		E 113° 54' 41.6"	
		N 07° 57' 38.5"	Subtidal
		E 113° 55' 48.6"	
South	Terumbu Peninjau	N 08° 07' 07.4"	Subtidal
China Sea		E 114° 34' 39.5"	
_	Terumbu Ubi	N 07° 67' 51.8"	Subtidal
		E 113° 55' 48.6"	
-	Pulau Layang-	N 07° 22' 32.5"	Subtidal
	layang	E 113° 42' 27.8"	
		N 07° 22' 46.6"	
		E 113° 47' 27.8"	
-	Pulau Tinggi	N 02° 17' 33.9"	Subtidal
	<i>55</i>	E 104° 06' 06.8"	
-	Pulau Aur	N 02° 28' 09.8"	Subtidal
		E 104° 30' 27.5"	~ >
-	Pulau Dayang	N 02° 28' 14.8"	Subtidal
	- 4.44 24 4418	E 104° 30' 17.5"	~ 40 11441
-	Pulau Mentinggi	N 02° 16' 27.9"	Subtidal
	1 4144 1/101111111551	E 104° 06' 47.9"	Subtidat