

**MORPHOLOGICAL CHARACTERIZATION
OF SELECTED
PULAU PERHENTIAN SEA CUCUMBERS**

by

SITI FATIHAH BINTI ARIFFIN

Dissertation submitted in partial fulfillment
of the requirements for the Degree
of Bachelor of Health Sciences (Biomedicine)

April 2005

CERTIFICATE

This is to certify that this dissertation entitled

“MORPHOLOGICAL CHARACTERIZATION OF SELECTED PULAU PERHENTIAN SEA CUCUMBERS”

is the bonafide record of research work done by

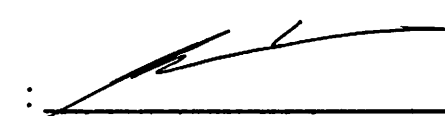
Ms. Siti Fatimah Binti Ariffin

During the period from August 2004 to March 2005 under our supervision

Signature of Supervisor

:

Name and address
of Supervisor

: 
Prof. Syed Mohsin Syed Sahil Jamalullail,
Deputy Dean,
School of Health Sciences.

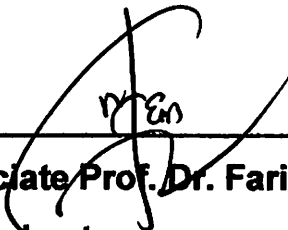
Date

: 7 April 2005

Signature of Co- Supervisor

:

Name and address
of Supervisor

: 
Associate Prof. Dr. Farid Bin Che Ghazali,
Senior Lecturer,
School of Health Sciences.

Date

: 7 April 2005

ACKNOWLEDGEMENTS

Bismillahirrahmanirrahim..... in the name of Allah The Most Gracious, The Most Merciful, as well as Shalawat and Remembrance for the Holy Prophet, Muhammad s.a.w.

Alhamdulillah, with the given strength and fate from the Almighty Allah, finally I succeed to complete this research undertaking to as a requisite for the Bachelor of Health Science in Biomedicine. There are many experiences and memory that I will always shared and remembers during this study. I would like to take this opportunity to thank the following individuals for the assistance and support that they had provided in the completion and excellence at this study and finally the thesis.

Firstly, I want to thank to my supervisor, our deputy dean, Prof. Syed Mohsin Syed Sahil Jamalullail for giving me a chance to do a work under his guidance. Thanks for the support and brilliant innovative ideas for this undertaking to finally produce an excellent thesis.

Special thanks to my co-supervisor, Associate Professor Dr. Farid Che Ghazali for his careful attention to detail and who had generously shared his ideas and experience regarding electron microscope. Thank for his advice, suggestion and comment during finishing this project. Every word that his said always gives me a spirit to finish this study without complaining. He was always

there to help me in many ways either in lab work to be a good researcher or during discussions on how to write a good thesis.

I personally thank everybody from Unit Kemudahan Makmal (UKM) that had facilitates material, equipment and support during this undertaking. Especially I want to thanks to En. Mohd Anizan, Encik Nik Fakuruddin and En Jamaruddin for the help. Appreciative thank also to the Forensic lab for allowing me the use of stereomicroscope.

The study needs a scanning electron microscope (SEM) to complete the thesis. Thank you to the EM unit at School of Biology in USM Penang for their cooperation and assistance provide in using the SEM especially Puan Jamilah and Encik Johari. My personal thank to Murni and Nor Azlin for their helping's hand during various preparation the specimens and handling the microscope. They always give comment and suggestion during work with them. Also thank to other friends for their support and valuable comment.

Additionally but not the least my loving thank to my family for their everlasting support especially my beloved sister for her cooperation to locate me to a scanning electron microscope.

Siti Fatihah Binti Ariffin, 2005

LIST OF CONTENT

Title page	i
Certificate	ii
Acknowledgements	iii
List of Content	v
Abbreviations	vii
Terminologies	viii
List of Figures	ix
List of Tables	xi
List of Plates	xii
Abstrak	xiv
Abstract	xvi
1.0 Introduction	1
1.1 Morphology	2
1.1.1 Anatomy of sea cucumber	2
1.1.2 Comparative anatomical characterization	4
between each species of sea cucumber	
1.1.3 The anatomy of Malaysian sea cucumber	11
1.2 Body tissue	12
1.2.1 Connective tissue	12
1.2.2 Muscle	15
1.2.3 Epithelium	16

1.2.4 Nervous tissue	24
1.3 Important of the sea cucumber body to human	26
2.0 Lacunae	29
3.0 Objective	30
4.0 Limitation of the study – pre-research	31
5.0 Inclusion and exclusion of the studies	32
6.0 Flow chart of the research methodology	33
7.0 Methodology	35
7.1 Processing after harvesting	35
7.2 Direct morphological observation using stereo photograph	35
7.3 Selected biopsies of body areas and tissue	36
7.4 Tissue processing	36
8.0 Result	37
8.1 Digital Camera	37
8.2 Light Microscope	50
8.3 Variable Pressure Scanning Electron Microscope	61
9.0 Discussion	66
10.0 Summary	67
11.0 Limitation of the study- post-research	68
12.0 Suggestion / recommendation and future work	69
References	70
Appendices	77

ABBREVIATIONS:

Abbreviation	Definition
DCT	Dense connective tissue
DHG	Depolarized holothurian glycosaminoglycan
EM	Electron Microscope
GFSKLYFa	Gly-Phe-Ser-Lys-Leu-Tyr-Phe-NH ₂
HIV	Human Immunodeficiency Virus
LM	Light microscope
LMBW	Longitudinal muscles of body wall
PBS	Phosphate buffer solution
PPSK	Pusat Pengajian Sains Kesihatan
SEM	Scanning electron microscope
USM	University Science Malaysia
USMKK	University Science Malaysia, Kubang Kerian
VPSEM	Variable Pressure Scanning Electron Microscope
5-HT	Serotonin

TERMINOLOGIES:

Terminology	Definition / Explanation
Atresia	Undischarged germ cell disintegration process
Cloaca	A common passage for fecal, urinary and reproductive discharge in lowest vertebrate
Cloacal muscle	Pertaining to the cloaca
Coelom	Body cavity of sea urchin
Coelothelium	Luminal epithelium
Crevice	A thin crack example in rock or structure
Detritivore	Animal that consumes decomposing organic particles, deriving nutrition primarily from microbes on the particles.
Neurotransmitter	A substance released from an axon terminal of a pre-synaptic neuron on excitation, which will diffuse across the synaptic cleft to either excite or inhibit the target cell.
Ossicle	A bony spicule or small plate bone
Pedicellaria	A small, snapper-like skeletal element that are used by the echinoderm to keep small organism settling on its body.
Protuberance	A projecting part of prominence surface or area
Retractile	Susceptible to of being drawn back
Spicule	A sharp, needle like body elevation
Stereom	Fine networks of calcium carbonate

LIST OF FIGURES

<u>Figure</u>	<u>Title</u>	<u>Page</u>
Figure 1	Artist illustration at the various internal structure of a sea cucumber	7
Figure 2	Internal structure of sea cucumber, <i>Thyone (sclerodactyla) briaereus</i> .	8
Figure 3	Anatomy of the sea cucumber <i>Sclerodactyla</i>	9
Figure 4	The North Atlantic sea cucumber, <i>Cucumaria frondosa</i> .	10
Figure 5	Skin ossicles from the White-spined Sea cucumber	17
Figure 6	Skin ossicles and segments of the calcareous ring from the Tar Spot Sea Cucumber	18
Figure 7	A calcareous ossicle from the skin of a White-spined Sea Cucumber (<i>Parastichopus leukothele</i>) photographed with a scanning electron microscope	19
Figure 8	Microscopic ossicle of sea cucumbers. (After Bell)	19
Figure 9	Artist illustration of sea cucumbers ossicle	20
Figure 10	Picture show the tentacles of sea cucumber	21
Figure 11	Macroscopic gonad characteristics in <i>S. chloronotus</i>	23
Figure 12	Section through anterior end of <i>Ocnus planci</i>	24
Figure 13	Flow chart of research methodology	34

Figure 14	Light photomicrograph of a muscle at anus of specimen A (x100 objective)	50
Figure 15	A well representation light photomicrograph of muscle-like structure at anus of specimen A	51
Figure 16	Light photomicrograph of representation on an ossicle located at the integument of the anus of the specimen A	52
Figure 17	Another region at the anus revealing more ossicles presence in specimen A	53
Figure 18	Another light photomicrograph representation of an area close to the located at anus of the specimen A	54
Figure 19	Light photomicrograph of Milligan stained tissue at mouth region of the specimen A	56
Figure 20	Light photomicrograph of Milligan trichrome stained tissue at the mouth region of the specimen A	57
Figure 21	Light photomicrograph of muscle biopsies out at an area close to the mouth of specimen A	58
Figure 22	Light photomicrograph of tissue at anus of specimen B	59
Figure 23	A scanning electron micrograph using back scattered mode showing part of the tissue at mouth of specimen A	61
Figure 24	A scanning electron micrograph using secondary electron mode showing part of the integument at anus of specimen A	62

Figure 25	A scanning electron micrograph using secondary electron mode showing part of the integument at anus of specimen	63
Figure 26	A scanning electron micrograph using secondary electron mode showing part of the muscle at the anus of specimen A	64
Figure 27	A scanning electron micrograph using secondary electron mode showing part of the muscle at anus of specimen A	65

LIST OF TABLES

<u>Table</u>	<u>Title</u>	<u>Page</u>
Table 1	Tabulated summary of the various noted structures observed from the various body areas of the sea cucumbers at this research study	60

LIST OF PLATES

<u>Plate</u>	<u>Title</u>	<u>Page</u>
Plate 1:	Digital photograph of whole body of specimen A	37
Plate 2:	Digital photograph of blunt raised nodule observed before dehydration with no spear head protuberance on integument	39
Plate 3:	Digital photograph of spear head protuberance within minute dehydration at room temperature	40
Plate 4:	Digital photograph show tube feet area of specimen A	41
Plate 5:	Digital photograph show three row of tube feet	42
Plate 6:	Digital photograph of medial row- two rows with intermediate integument in between	43
Plate 7:	Higher magnification of the observed laterals row of the tube feet	44
Plate 8:	Digital photograph of thru and thru pore connecting the internal visceral structure to the outer surface integument	45
Plate 9:	Digital photograph of pleural-like space located between dermis and underlying connective tissue covering the visceral organ	46
Plate 10:	Digital photograph of whole body of specimen B	47
Plate 11:	Digital photograph of dorsal part with no homogenous dot	48

Plate 12:	A prominent sulcus with gyri-like structures at a lateral border of the body	49
Plate 13:	The mouth region of specimen B with no tentacles present	49

ABSTRAK

Pengenalan: Timun laut merupakan invertebrata laut yang mempunyai kaitan dengan tapak sulaiman dan 'sea urchins'. Terdapat hampir 2,000 spesies timun laut telah dijumpai termasuk di laut dalam. Di Asia, timun laut dikenali sebagai *Stichopus* spp dimana ianya dikenali sebagai gamat di Malaysia. Manakala, spesies yang sering dijumpai di Amerika Utara dan Eropah adalah *Cucumaria*, *Holothuria*, *Thyone* dan *Leptosynapta* (Barnes, 1991). Kajian yang dilakukan terhadap haiwan marin ini samada badan atau ekstraknya adalah untuk menjelaskan morfologi timun laut yang dijumpai di Pulau Perhentian dengan lebih tepat. Bentuk geografi dan biodiversiti merupakan faktor penting untuk mengenalpasti spesis dengan lebih spesifik dan mempunyai nilai dalam makanan tempatan, perubatan dan Obstetrik dan genekologi.

Matlamat dan tujuan kajian: Kajian ini dijalankan untuk menjelaskan dan menghuraikan ciri-ciri timun laut yang terpilih dari Pulau Perhentian dengan mengkaji morfologi luar dan dalam. Matlamat mengenalpasti dengan jelas taksonomi dan anatomi setiap sampel adalah untuk kajian selanjutnya di masa hadapan di dalam kesan farmakologi ekstrak tisu timun laut.

Bahan dan kaedah: Sampel telah di ambil dari Pulau perhentian dan kajian morfologi adalah berdasarkan pemerhatian menggunakan stereomikrograf, mikroskop cahaya dan SEM.

Keputusan dan perbincangan: Morfologi luar dan dalam setiap sampel yang dikaji mempunyai perbezaan morfologi yang pelbagai. Orientasi dan kepadatan kolagen 'mutable' adalah berbeza dari segi histologi terutama apabila perbandingan dibuat antara oral dan anus. Terdapat kehadiran osikel dan sel morula pada tisu dan integumen sampel A.

Kesimpulan: Spesimen A dicadangkan sebagai *Cucumaria frondosa* di mana hanya dijumpai di Amerika Utara, tetapi tidak mustahil dijumpai di Asia jika berlaku perubahan iklim secara global

ABSTRACT

Introduction: Sea cucumber is a marine invertebrate that is closely related to the starfish and sea urchins. There are about 2,000 species of sea cucumbers found everywhere including the deep sea. In Asia, sea cucumber is known as *Stichopus* spp. which known as 'gamat' in Malaysia. Besides it, common species in North America and Europe are *Cucumaria*, *Holothuria*, *Thyone* and *Leptosynapta* (Barnes, 1991). The interest of this marine animal either their extracts or bodily is to really define the morphological of sea cucumber that found in Pulau Perhentian. Geographical and biodiversity is important factor to properly identified specific species that is really having potential value in local delicious, medical importance and Obstetric & Genocology.

Aim and purpose of the study: This study was designed to clarify and characterized the selected Pulau Perhentian sea cucumber by examined their external and internal morphology. The aim to clearly identify each sample taxonomy and anatomy for further future work in pharmacologic effects of their tissue extract.

Material and methodology: The sample was harvested at Pulau Perhentian and the morphological study was based on observation using stereomicrograph, light microscope and scanning electron microscope.

Result and discussion: There seem to exist in a variable difference in the external and internal morphology at from each specimen observed. The orientation and density of the 'mutable' collagens was in different histological feature especially when comparing at the mouth and its anus. There are highly suggestive present of ossicles and morula cells at the tissue and integument of sample A.

Conclusion: Specimen A was highly suggestive as *Cucumaria frondosa* which reported only found in North America, changes in global climate may not make its finding in Asia impossible.

1.0 INTRODUCTION

Sea cucumber known as *gamat* in the Malay language or *haisom* in Hokkien or *bêcher-de-mer*, is a remedy derived from a marine animal closely related to starfish and sea urchins. It is also called *brunok*, *bat* or *balat* in various South East Asian languages. The extracts from sea cucumber have been used as a therapeutic remedy by the Malays and are well-known in most local ethnic community especially in South East Asia. It is claimed to reduce pain in ailments of inflammatory origin or stiffness in arthritis sufferers (Mohsin *et al.*, 2003)

The interest in sea Urchin or sea cucumber either to their tissue extract or bodily present is a global research and commercial interest that is economically lucrative and stills an open potential for further evidence based research to proving of more possible pharmacotherapeutic element and values that is still pertaining to certain species of the sea cucumber as part of healthy lifestyle package.

Geographical and biodiversity together with better morphological technique is now looked upon as parameter to properly identify specific species that is really of potential value as foodstuff, genecology and therapeutic value.

This thesis is part of an ongoing research investigation and interest of a team of researchers based the School of Health Science (PPSK) with the focused objective to properly characterize the therapeutic value and benefit derived from the sea cucumber. To achieve this is legally right to properly investigate their geographical habitant and associating biodiversity with the anatomically correct description.

Sea cucumbers classified under the phylum echinoderms, are marine form animal. They are link to an ancient group of biological structure back standing to the Cambrian period. Most of this sea cucumber was given their name based on their external features and protuberance observed present. However, although numerous literatures have shown its link and associated excellent fossil record, the origin and early evolution of echinoderm or sea cucumber are knowledge areas that are still obscure. Echinoderms have no ability to osmoregulated. They occur in all oceans of the world and at all depth (Hickman, *et al.*, 1996).

Physically Holothurians or sea cucumbers are greatly extended in the oral-aboral axis. They are oriented in an axis parallel to the substrate and are usually observed lying on one side. While there are reported that sea cucumber are deposit feeders (Hickman, *et al.*, 1996).

1.1 MORPHOLOGY

1.1.1 Anatomy of sea cucumber.

The sea cucumbers actual morphological measurable size can vary dramatically especially when measured in hydrated and a dehydrated state. Review of literatures have shown a maximum size of less than a 2 cm long, and the largest can reach more than a meter (3 feet) in length and be nearly one feet in diameter (24cm) (Barnes, 1991). Sea cucumber has a ventral (superior) and dorsal (anterior) surface. The bodies of the sea cucumbers were observed as have been elongated, leathery and at more reported muscular. Its mouth and

anus are located and oriented dorsally at the two ends of the long axis of its body. Some sea cucumber was reported to be oriented in U-shaped morphology (Barnes, 1991).

A circumoral (around the mouth) nerve ring lies near the base of its tentacles. This nerve ring supplies nerves axons to its tentacles calcareous-ringed pharynx. Sensory cells are located in the epidermis, and are most abundant at the ends of the animal. The pentamerous radial symmetry is the most striking characteristic of the group where the body can be dividing into five parts arranged around the central axis (Barnes, 1991).

Generally the mouth of the sea cucumber is surrounding by a modified tube feet identified as tentacle which can be at 8 to 30 in numbers. These tentacles are arranged in five double rows of tube feet with tiny suction cups present along its body. The use of tube feet is for crawling along the seabed or anchoring to a rock. The epidermis of its body wall is non-ciliated and covered by a variable thick or thin cuticle. The underlying dermis consisted of either a thick or a thin layer of connective tissue surrounding the microscopic ossicles beneath with an associated a layer of circular muscles arranged in five bands of longitudinal fibers. Respiratory tree is use for breathing by pumping seawater in and out of an internal organ. The respiratory trees are located in the coelom on the right and left side of the digestive tract.

Calcium carbonate plates and spines are the building frame at the skeleton of Echinoderm. This skeleton is enclosed by the epidermis and is thus known as an endoskeleton. The plates in sea urchin are fitted together tightly. Where else the plates in star fish are more loosely bound. These plates are usually microscopic in size. These minute plates of echinoderms have a very

typical microstructure only revealed by electron microscopy as fine networks of calcium carbonate forming a structure known as stereom.

Each skeletal element of an echinoderm is a single crystal of calcium carbonate occasionally finely branched and well structured. A number of special structures protrude was observed place between the skeletal plates. All echinoderms have a water-vascular system which is a set of water-filled canals branching from a ring canal that encircles the gut. The echinoderm used tube feet that act as sucker-like appendages to move, grip the substrate, or manipulate objects. These tube feet are extended and retracted by hydraulic pressure in the water-vascular system. The echinoderm has pedicellaria to keep small organisms from settling on its body.

1.1.2 Comparative anatomical characterization between each species of Sea cucumber

Sea cucumber belongs to Class of Holothuroidea and Order of Aspidochirotida. Under the Order of Aspidochirotida, the family of Holothuridae and Stichopodidae are the most research investigated and described in publication, that taxonomic relation are still disputed. Most sea cucumber is black, brown, or olive green in color. The smallest species are less than 3 cm in length (oral to body length), whereas Stichopus from the Philippines may attain a length of 1 m and a diameter of 24 m. The common species of sea cucumber found in North America and Europe Seas are *Cucumaria*, *Holothuria*, *Thyone* (*Scerodactyla*), and *Leptosynapta*. (Barnes, 1991).

There exists some confusion over the morphotypes of *Stichopus variegatus* found in the South China Sea. Zulfigar Yasin, *et al*, (2000) have established the characteristics and description required in the classification of this species of sea cucumber. The sea cucumber *Stichopus variegatus* (also known as *teripang jagung* in Indonesia) is a commercial species found sporadically in the South China Sea.

Recently, a specimen that was thought to be *S. variegatus* was confirmed instead to be *S. horrens*. Therefore, the common *S. variegatus* has been renamed as *S. hermani*. These authors observed and revealed that several types of Stichopodids resembling *S. hermani* are found in the South China Sea but show differences in colour variations and body wall patterns. In addition, there were behavioral differences between the two subspecies. *S. hermani* is a diurnal species while the other specimens were strictly nocturnal. (Zulfigar *et al.*, 2000).

1.1.2.1 Genus *Holothuria*

Holothuria atra or the Black Sea Cucumber can found at Indo-Pacific, Red Sea to Hawaii. Detritivore often found in shallows, close to shore. It size is about two feet in length. *Holothuria edulis* also known as the Edible Sea Cucumber which found at Indo-Pacific including Hawaii. Their characteristic is skin soft, pink underneath and blackish above. They usually found in shallows which close to shore and general detritivore. It length is about eight inches and edible but not considered delicious (<http://www.itmonline.org/arts/seacuke.htm>.)

Holothuria hilla or the Light-Spotted Sea Cucumber is usually found partly exposed at night times in rocky and sandy settings at Indo-Pacific region including Hawaii Island. *Holothuria mexicana* (Ludwig, 1875) or the Donkey Dung Sea Cucumber live in Tropical West Atlantic. They are detritivorous and found singly on grass and sandy beds. Their length is twenty inches in length. Whereas *Holothuria thomasi* (Pearson, 1914) or the Tiger Tail Sea Cucumber found in Tropical West Atlantic, in holes, crevices and beneath rocks. This species can move quickly with their size about two meters in length (<http://www.itmonline.org/arts/seacuke.htm>.)

1.1.2.2 Genus Stichopus

Stichopus chloronotus (Brandt, 1835), the Black Sea Cucumber always found in Indo-West Pacific; eastern Africa to Hawaii and the South Pacific. This species usually use for reef aquariums when small. It size about one foot in length. *Stichopus parvimensis*, or the Southern California Sea Cucumber live in cool to coldwater only. (<http://www.itmonline.org/arts/seacuke.htm>.)

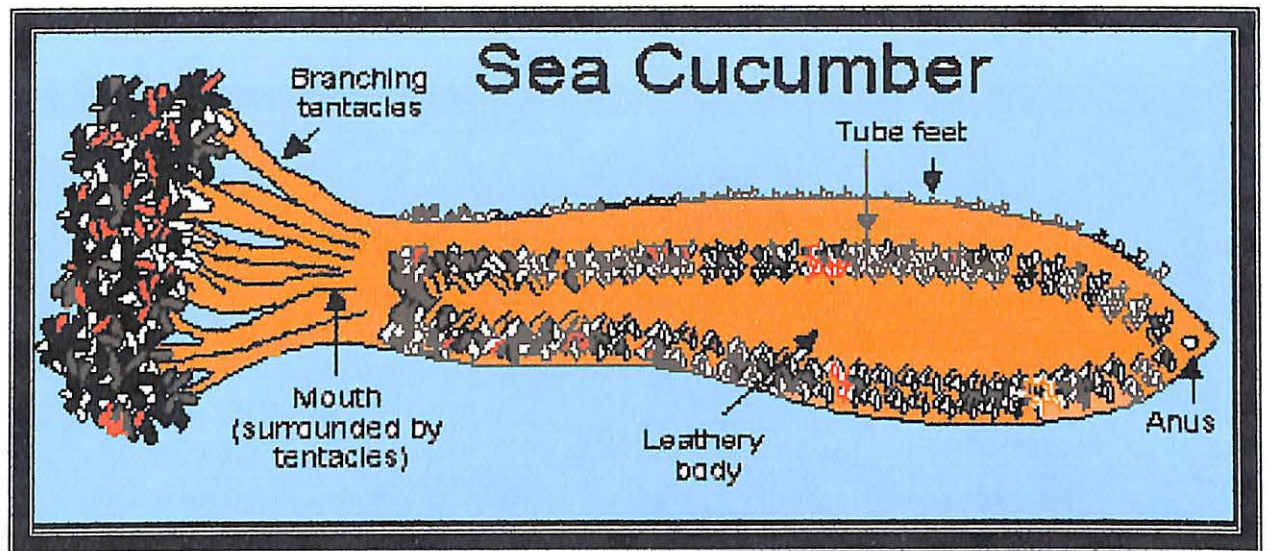


Figure 1: Artist illustration at the various internal structure of a sea cucumber
(Image was adapted, taken then modified from Sea cucumber Printout-Enchanted Learning .com)

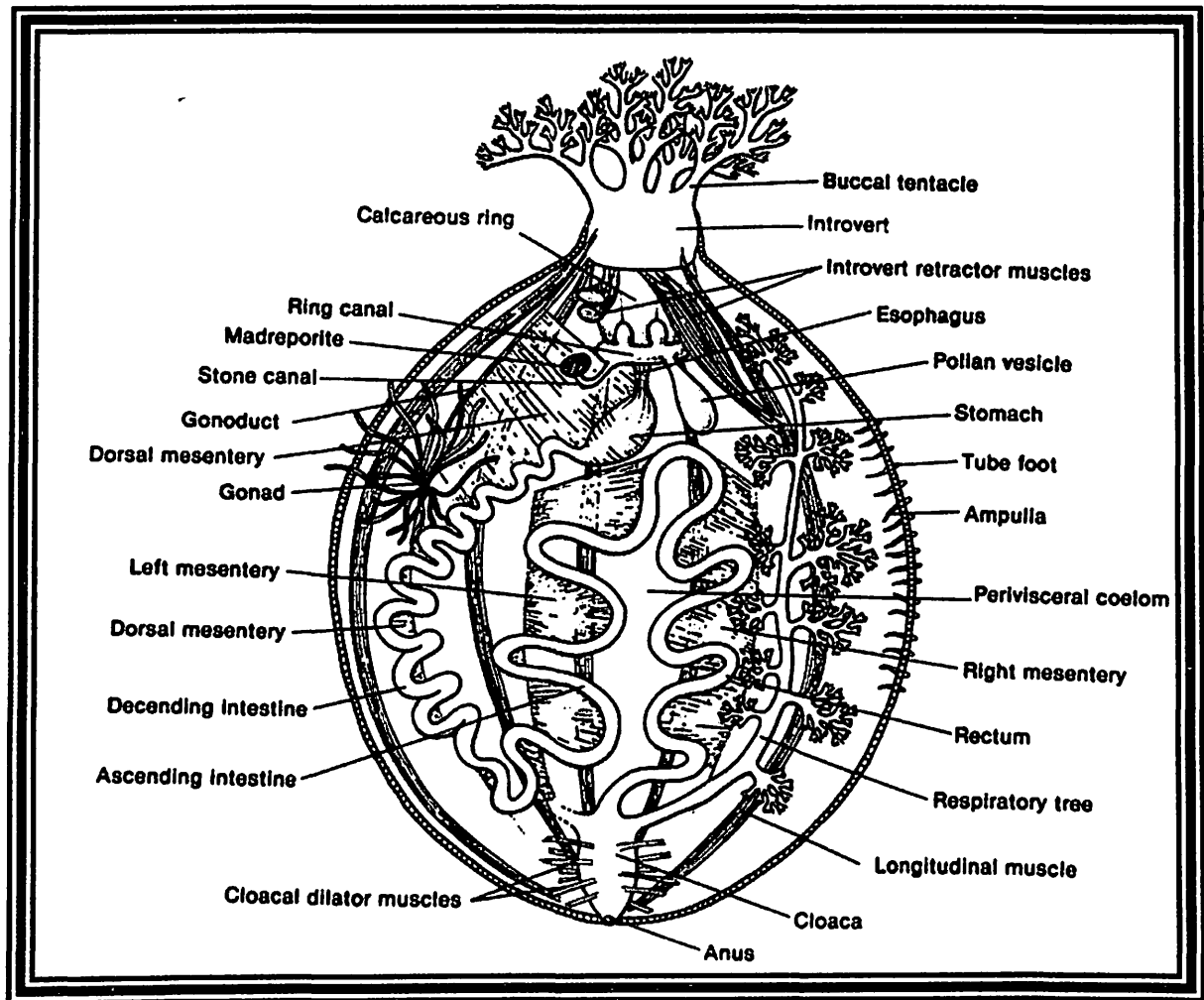


Figure 2: Internal structure of sea cucumber, *Thyone (sclerodactyla) briaereus*.

(Image was scanned, modified and adapted from Invertebrate Zoology, Barnes R.D 1991)

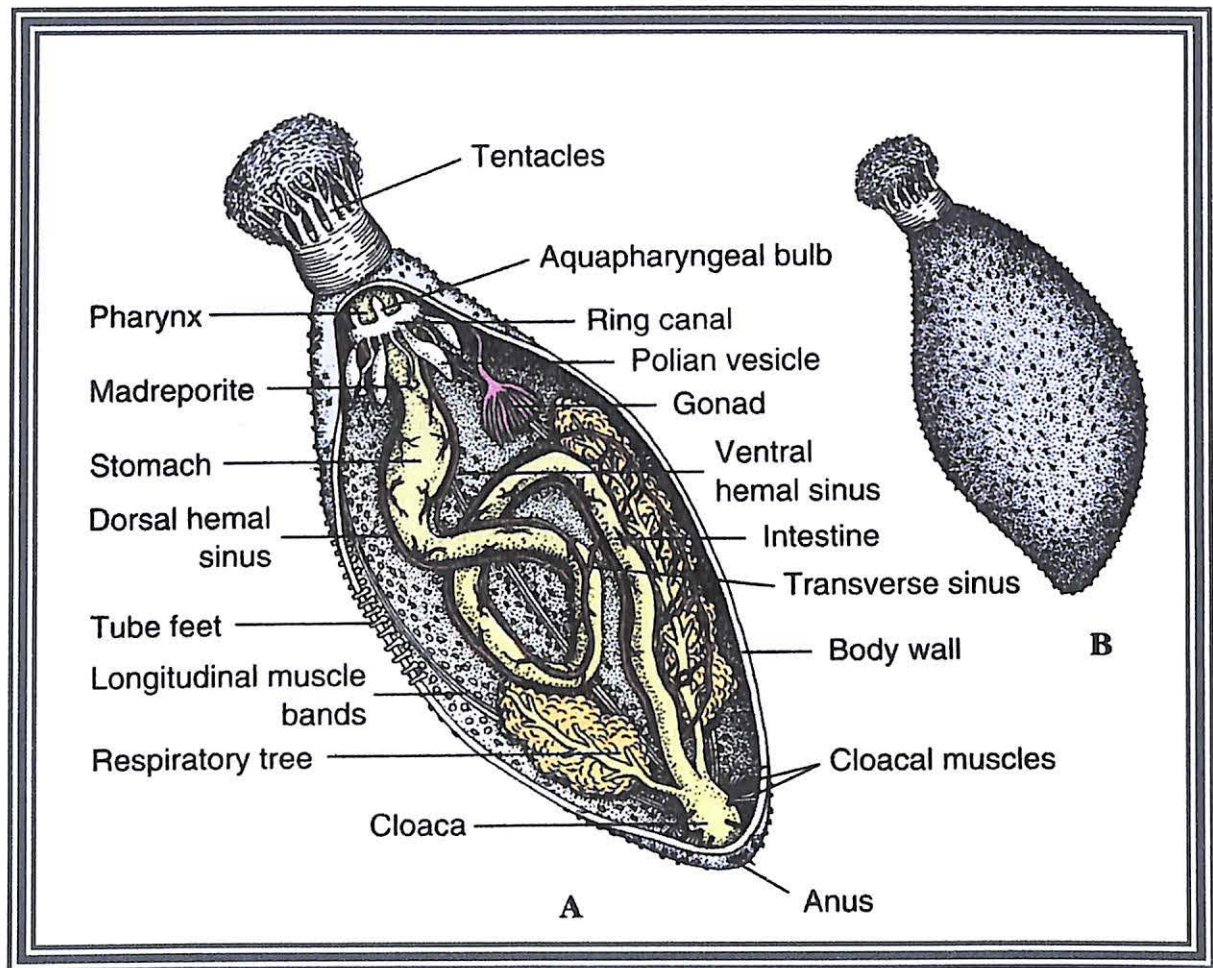


Figure 3: Anatomy of the sea cucumber *Sclerodactyla*

(Image was scanned, modified and adapted from Integrated Principles of Zoology, Hickman et.al., 1996)

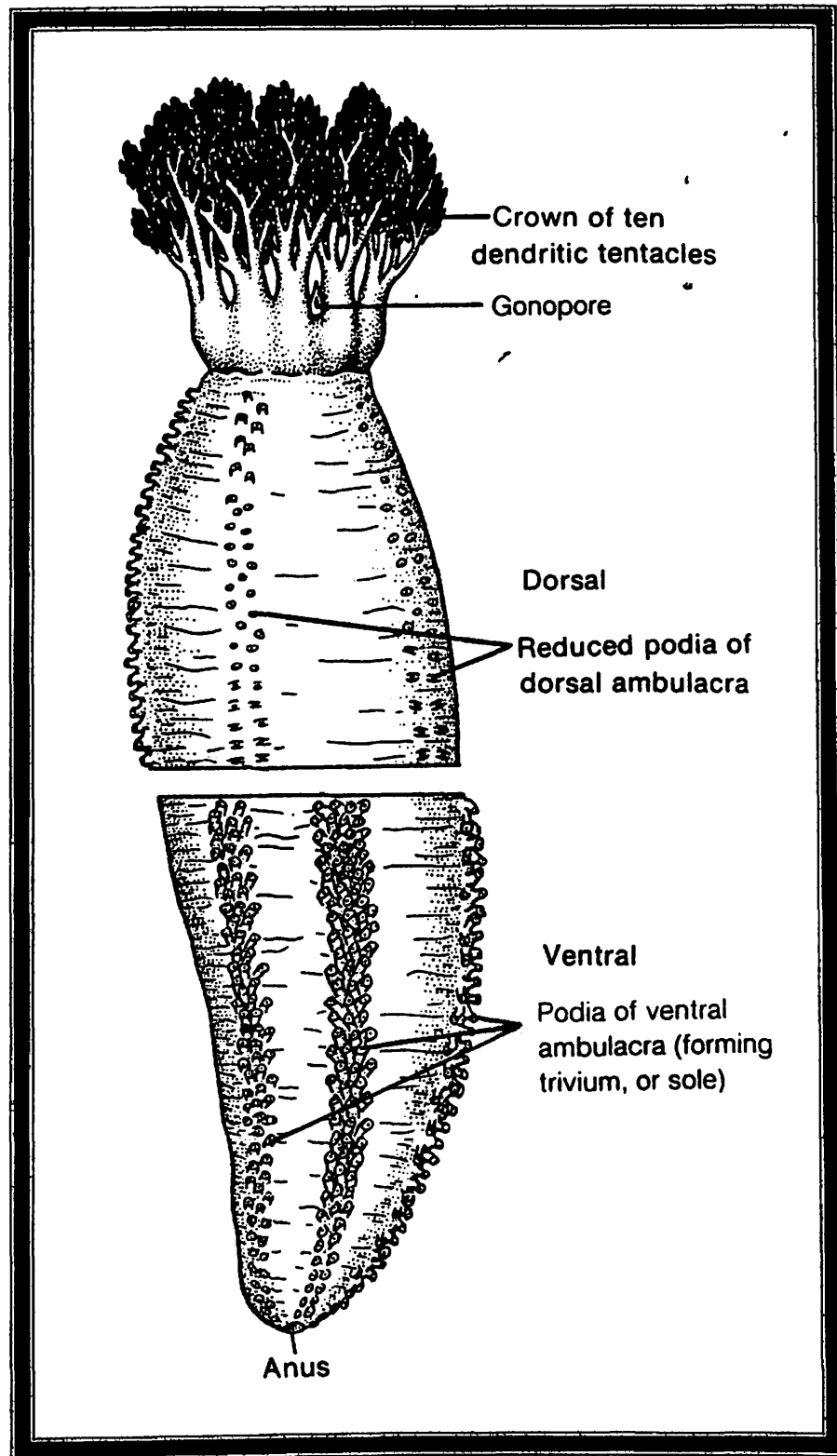


Figure 4: The North Atlantic sea cucumber, *Cucumaria frondosa*.

(Image was scanned, modified and adapted from Invertebrate Zoology, Barnes, 1991)

1.1.3 The anatomy of Malaysia sea cucumber

About 2,000 species of sea cucumbers are found throughout the world, but only 30 species are found in Malaysian seas. Out of that 30 species, only *Stichopus variegatus*, *Stichopus chloronotus*, *Stichopus badionatus* and *Stichopus horrens* are believed to be of medicinal value but vastly unexploited commercially. It is believed that the number of species from the genus *Stichopus* is at higher incidence than the number of species from other genera of the same order. *Stichopus* is considered the main dominant genus (Zulfigar *et al.*, 1999).

Stichopus horrens is known as Teatfish sea cucumber. Its body is covering by teat-shape papillae which suited its name. The body is very firm but can rapidly integrate when this sea cucumber is at stressed. But if the animal is promptly returned to the water and left alone, the process reversed. When attached, the sea cucumber will led parts of the body in contact with the predator. The length of this sea cucumber is reported to be at 35 cm length (Gerald & Roger, 1996).

Stichopus Chloronotus is known as Green fish. It is roughly square in cross-section with large prominent papillae at each corner of the square. However its body surface is smooth. There are many tubes arranged in three rows on its underside. The color of this sea cucumber is either very dark green or almost appearing black. The papillae are orange-tipped. They inhabits the reef flats or on broken-coral rubble, at depths of 0 to 5 m in areas where there is much water movement. Average density is several hundred individual per

hectare. The length is 10-30cm and 0.2-0.4 kg of weight (<http://www.sea-ex.com/fishphotos/greenfish.htm>)

Variegated Sea Cucumber is a common name for *Stichopus variegatus*. It lives in sand and rubble with general water depth at 0 – 30m (98ft). Their size is about 900mm (35 in). They feed on passing sand and coral detritus through its intestines to extract the organic material nourishment from. It often hosts the slim-bodied pearl fish (*Corapus sp*), which takes refuge in the sea cucumber body during the day. It actually enters the cloaca. The imperial shrimp may also reside on its surface.

1. 2. BODY TISSUE

The human body is composed of four basic tissues, namely the epithelium, connective tissue, muscle and the neurons. For research discussion, the body tissue of the sea cucumber will be discussed using the classification.

1.2.1 Connective tissue

The principal component of the body wall of the sea cucumber *Cucumaria frondosa* is a dermis consisting of collagen fibrils, micro fibrils, proteoglycans and other soluble and insoluble components. A major structural constituent of the dermis is a network of 14 nm diameter micro fibrils, which surrounded and penetrates bundles of collagen fibrils. However collagen fibrils are still the main principle source for mechanical strength in the 'mutable' dermis of the sea cucumber *Cucumaria frondosa*, this is generally true for all sea cucumber.

The mechanical properties of the body wall of many holothurians have fascinated researcher especially physiologists for a long time, and are well described for *S. chloronotus* (Motokawa, 1982, 1984). Connective tissue in holothurians (and other echinoderms) is named "catch-connective-tissue" (Motokawa, 1984) or "mutable collagenous tissue" (Wilkie, 1984). These tissues may contract or expand almost instantaneously without the action of muscles, presumably under the control of the nervous system (Wilkie, 1984). The function of the catch connective tissue may mainly be associated with locomotion.

Peptides will control the stiffness of the connective tissue of a sea cucumber. The dermis of a sea cucumber was reported to contain bioactive peptide, which is a neuropeptides. Holokinins are homologous with bradykinin and effect the softened of the dermis (R. Birenheide, *et al.*, 1998).

The fibrils of echinoderms are relatively short (less than 2 mm or so) and symmetrically spindle shaped (Matsumura 1973a, Matsumura 1973b and Matsumura 1974). Vertebrate connective tissues may contain either bipolar fibrils (Birk, *et al.*, 1996 and deVente, *et al.*, 1997) or both unipolar and bipolar fibrils (Holmes, *et al.*, 1994, Kadler, *et al.*, 1996 and Graham *et al.*, 2000). Unipolar fibrils are those in which the collagen molecules are in same orientation, whereas in bipolar fibrils the molecules at one end have the opposite orientation to those at the other end. Bipolar fibrils contain a short stretch, 5–10 D-periods long, in which the molecular polarity is reversed (Holmes, *et al.*, 1994, Thurmond and Trotter 1994, Birk, *et al.*, 1996 and deVente, *et al.*, 1997). (Holmes, *et al.*, 1994 and Graham, *et al.*, 2000) found that the transition region may occur at any axial position along a bipolar fibril,

whereas (Birk, *et al.*, 1996) found that transition regions occur preferentially in the central third of fibrils from embryonic chick tendon, skin, and cornea.

The dermis of *C.frondosa*, a collagenous tissue with mutable mechanical properties, was found to have concentrations of Ca, Mg, Na, and K that resulted from its being in ionic equilibrium with seawater. Intact collagen fibrils isolated from *C.frondosa* dermis are aggregated in the ionic milieu of seawater by a fibril-binding glycoprotein, stiparin, which is the most abundant soluble protein in the dermis (Trotter, *et al.*, 1997)

The body wall of sea cucumber contained glycosaminoglycan, which is involved in the cation-induced change of body structure of *Stichopus japonicus*. It is also suggested that GAG is closely involved in the change of toughness of sea cucumber body wall. The body wall of sea cucumber also contained high amounts of sulfated glycans, which differed in structure from glycosaminoglycans of animal tissues and from the fucose-rich sulfated polysaccharides isolated from marine algae or from the jelly coat of sea urchin eggs. This compound was reported to be involved in maintaining the integrity of the sea cucumber's body wall, in analogy with the role of other macromolecules in the vertebrate connective tissues.

Microfibrils are essential structural components of the extracellular matrix and are widely distributed in both vertebrate and invertebrate tissue, where they impart elastic properties on all dynamic connective tissues. Microfibrils are becoming increasingly recognized as an important component of the extra-cellular matrix. However, almost nothing is known about their mechanical role in the diversity of tissues in which they are found. Where else Elastic fibers consist of two morphologically distinct components: elastin and

10-nm fibrillin-containing micro fibrils. During development, the micro fibrils form bundles that appear to act as a scaffold for the deposition, orientation, and assembly of tropoelastin monomers into an insoluble elastic fiber.

Micro fibrils play an important role in the organization of elastic tissues throughout the animal kingdoms. Thurmond & Trotter (1996) study showed that fibrillin-like microfibrils play a role in the elasticity of sea cucumber dermis. Fibrillin microfibrils may also be an important elastomer in mammals, independent of elastin, as has been suggested by Keene *et al.*, (1991) and others.

1.2.2 Muscle

The muscle system of holothurians include visceral (coelomic epithelium) and somatic (longitudinal muscle bands, retractors of aquapharyngeal complex) musculature. Visceral musculature regeneration is believed to be achieved by the transformation of myoepithelial cells via their dedifferentiation, migration, proliferation, and redifferentiation. During somatic muscle regeneration the new muscle bundles are formed due to dedifferentiation, migration, and immersion of the coelomic epithelial cells into the connective tissue (Dolmatov & Ginanova, 2001)

Body wall muscle of holothurian was distributed of 5-HT (serotonin). Serotonin was investigated which it give effects on longitudinal muscles of body wall (LMBW) of the sea cucumber *Apostichopus japonicus* (Inoue, *et al.*, 2002). Serotonin reacts as vasodilatation to the muscles. 5-HT-like immunoreactivity

was observed in LMBW and in mesentry using indirect immunohistochemistry and these result strongly suggested that both LMBW and mesentry contained 5-HT. (Inoue, *et al.*, 2002).

1.2.3 Epithelium

i. Skin Ossicles

Ossicles are a small bone, which located in the dermis of sea cucumber. The bone pieces in ora, anus, back, abdomen and tentacle of sea cucumber are different. They are the important bases to identify sea cucumber. The bone pieces are observed for the microstructure (Chen, *et al.*, 2002).

The ossicles are believed to be consistently different from species to species (Lambert & Rutherford, 1997). They are remnants of a solid skeleton typical of many other members of the echinoderms, such as sea stars and sea urchin. Each species has uniquely shaped ossicles. Some similar species can easily be confused, not only by the novice, but also by non-taxonomic researchers working on other aspects on biology.

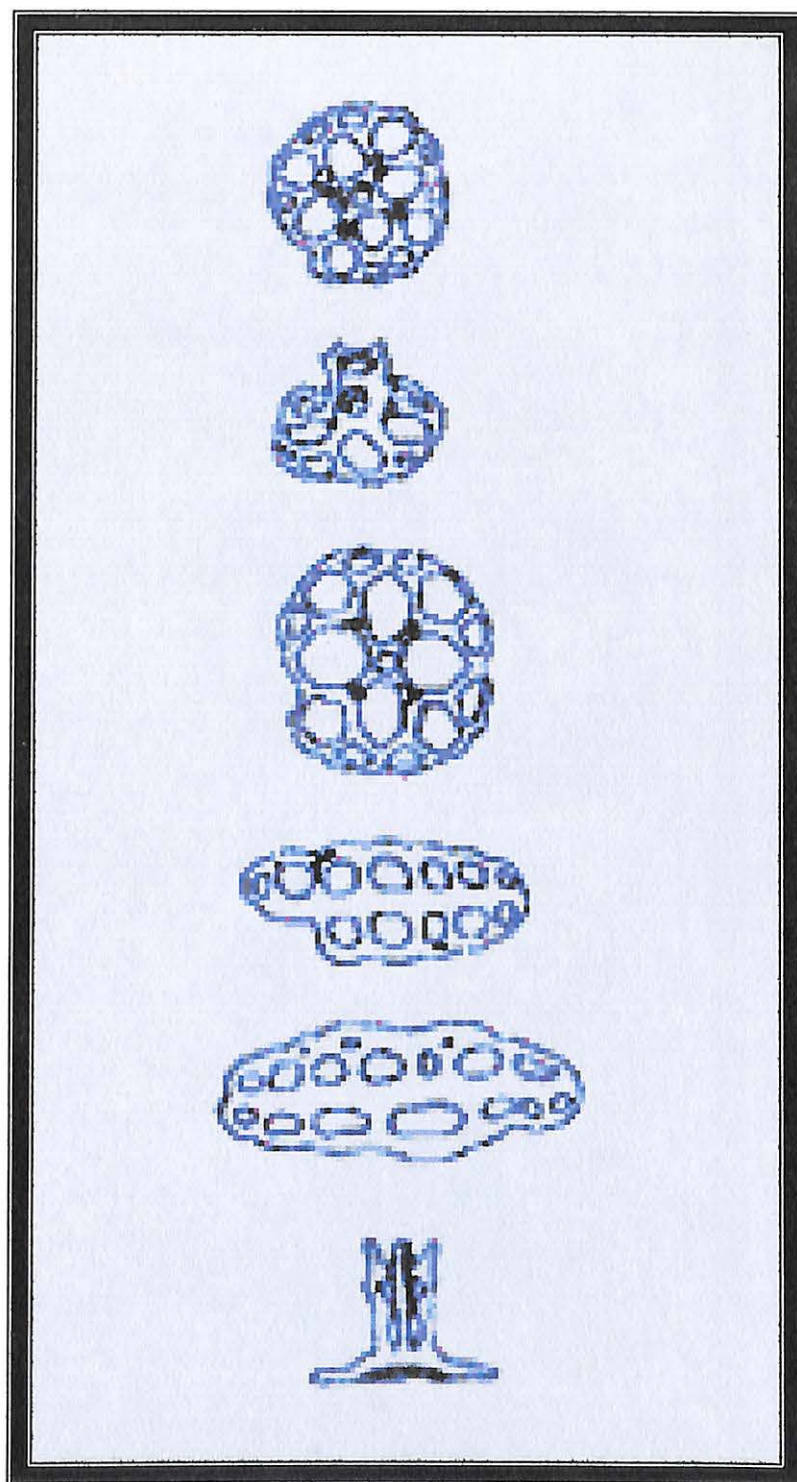


Figure 5: Skin ossicles from the White-spined Sea cucumber

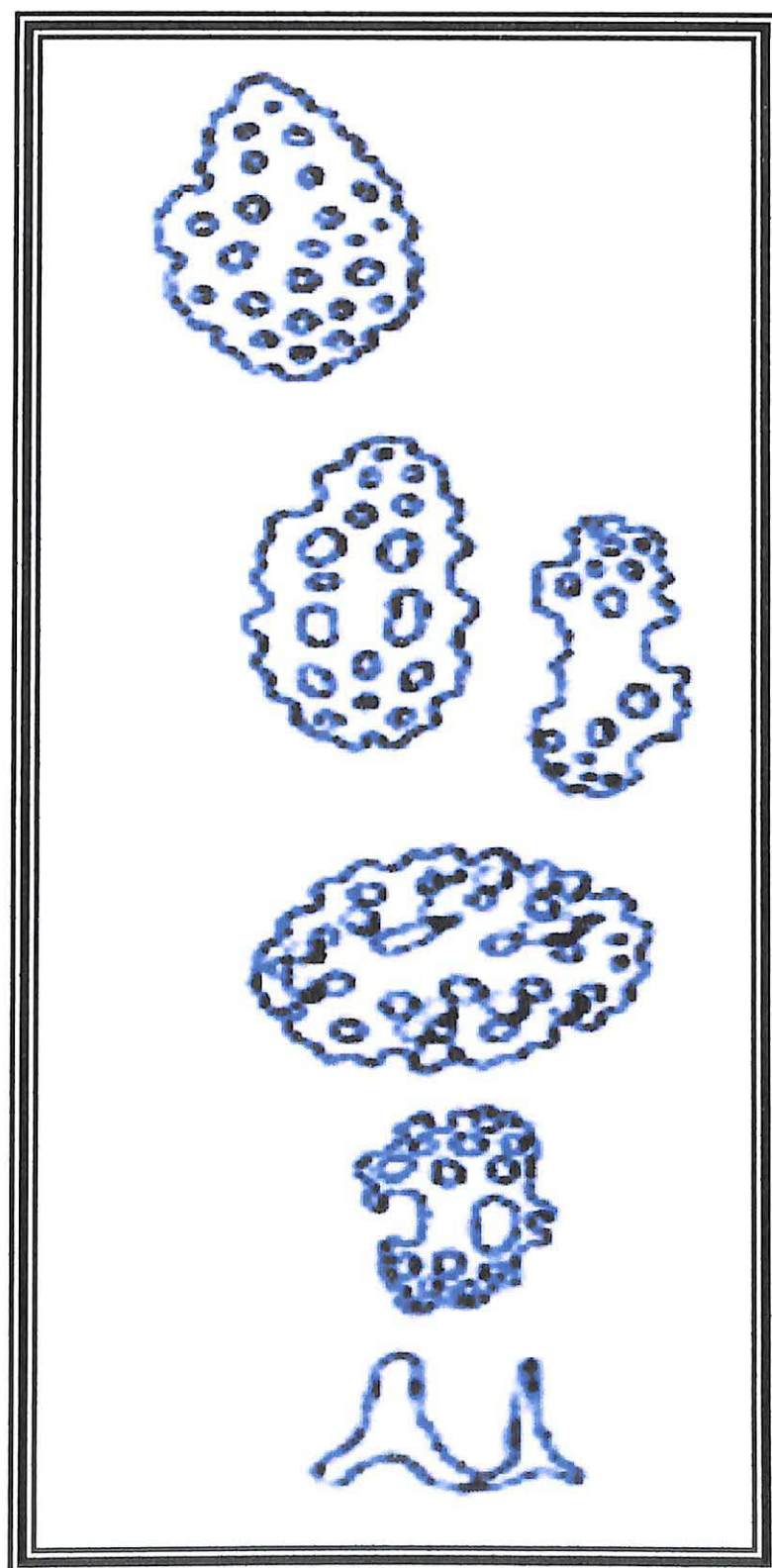


Figure 6: Skin ossicles and segments of the calcareous ring from the Tar Spot Sea Cucumber

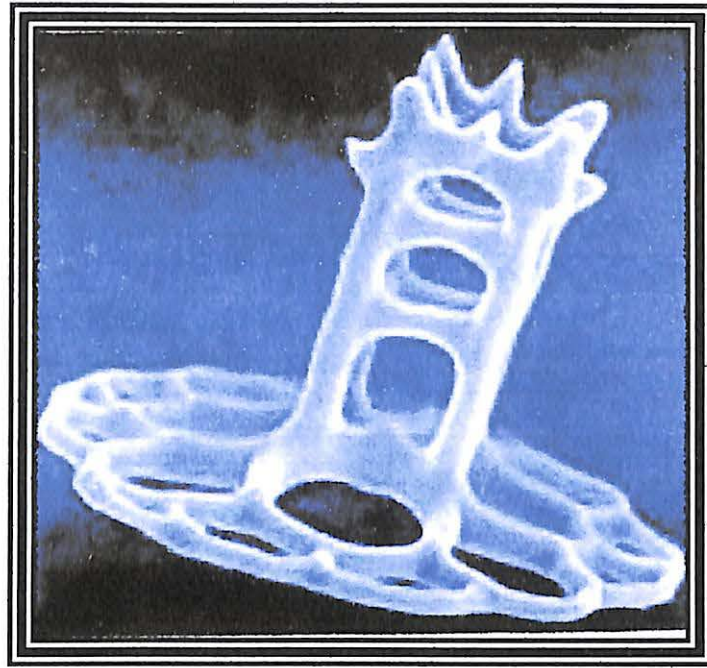


Figure 7: A calcareous ossicle from the skin of a White-spined Sea Cucumber (*Parastichopus leukothele*) photographed with a scanning electron microscope. Diameter of disk approximately 0.1 mm.

(Images was scanned, modified and adapted from Lambert P & Rutherford, J.C article)

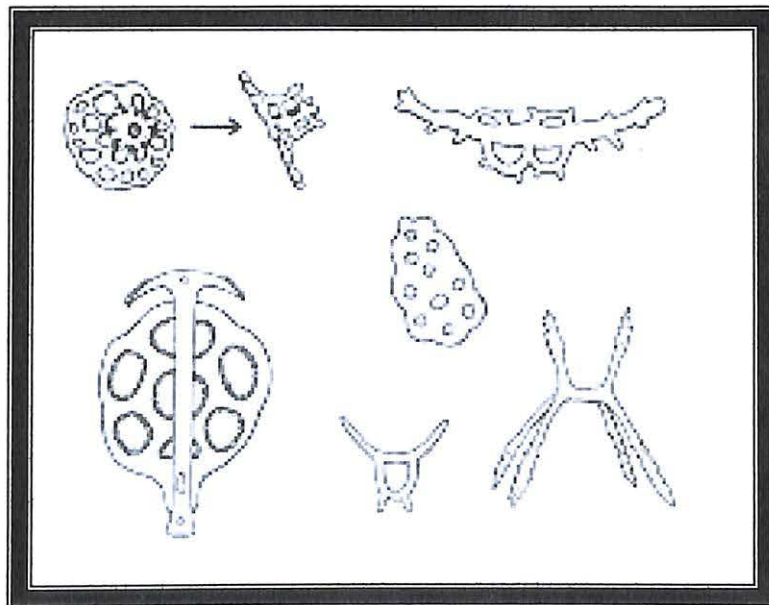


Figure 8: Microscopic ossicle of sea cucumbers. (After Bell)

(Images was scanned, modified and adapted from Invertebrate Zoology, Barnes, 1991)

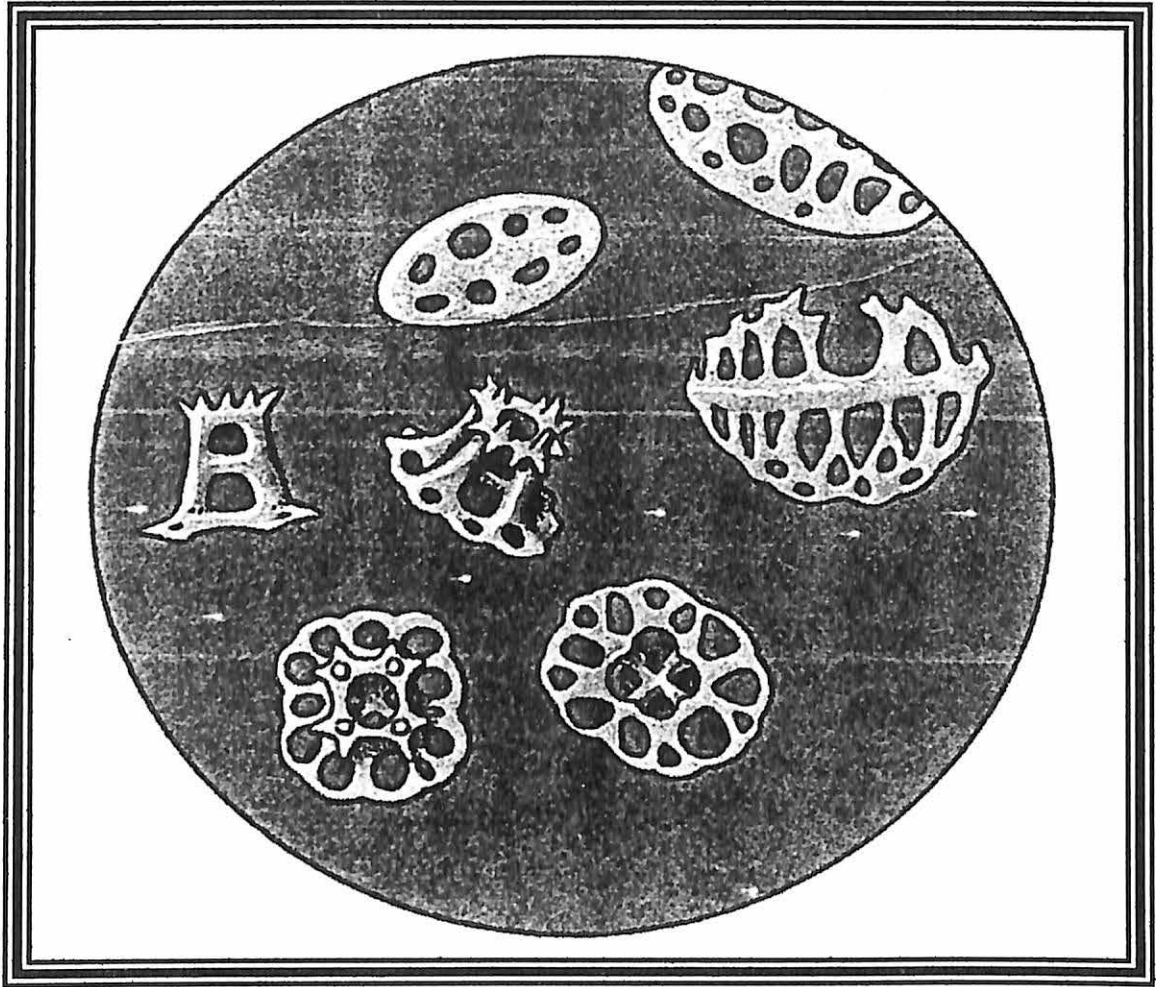


Figure 9: Artist illustration of sea cucumbers ossicle are usually microscopic bodies buried in the thick dermis. They can be extracted from the tissue with commercial bleach and are important taxonomic characteristics. The ossicle shown here, called tables, buttons, and plates, are from the sea cucumber *Holothuria doffcilis*. They illustrate the meshwork (stereo structure observed in ossicles of all echinoderms at some stage in their development

(Images was scanned, modified and adapted from Invertebrate Zoology, Barnes, 1991)

ii. Tentacles

The sea cucumber has oral tentacles around their mouth. The mouth is located in the middle of a buccal membrane at the base of the tentacular crown. These oral tentacles are about 10 to 30, retractile and it is modified of tube feet. Sea cucumbers are chiefly deposit or suspension feeders. They stretch out their branched tentacles and either sweep then over the bottom or hold them out in the seawater. Mobile epibenthic forms such as large *Stichopus* are deposit feeders and they grazing on the bottom with their tentacles.

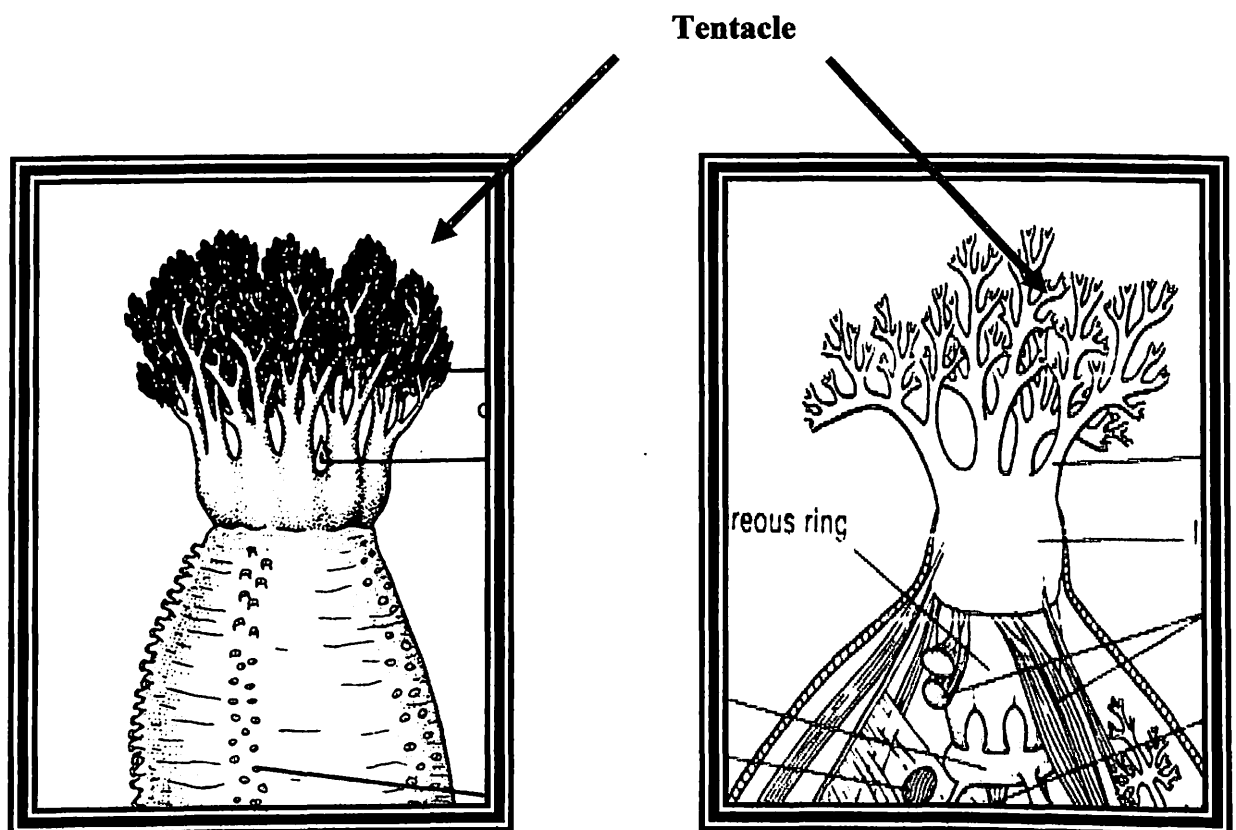


Figure 10: Picture show the tentacles of sea cucumber

iii. Gonad system.

Stichopodidae gonads have two tuft of tubules located on either side of mesentry. The tubuleas branch out distally and are joined at the base in a sac, which bulges out from the dorsal mesentry. Length and diameter of gonad tubules used to describe *S. chloronotus* maturity stages by comparing them with macroscopic gonad characteristics such as color, morphology and consistency and by using other descriptive methods, such as gonad indices, microscopic observations and histological sections. The lengths of gonads tubules were measured from the gonads base to the distal tip to within 5 mm (Hoareau T & Conand C, 2001)

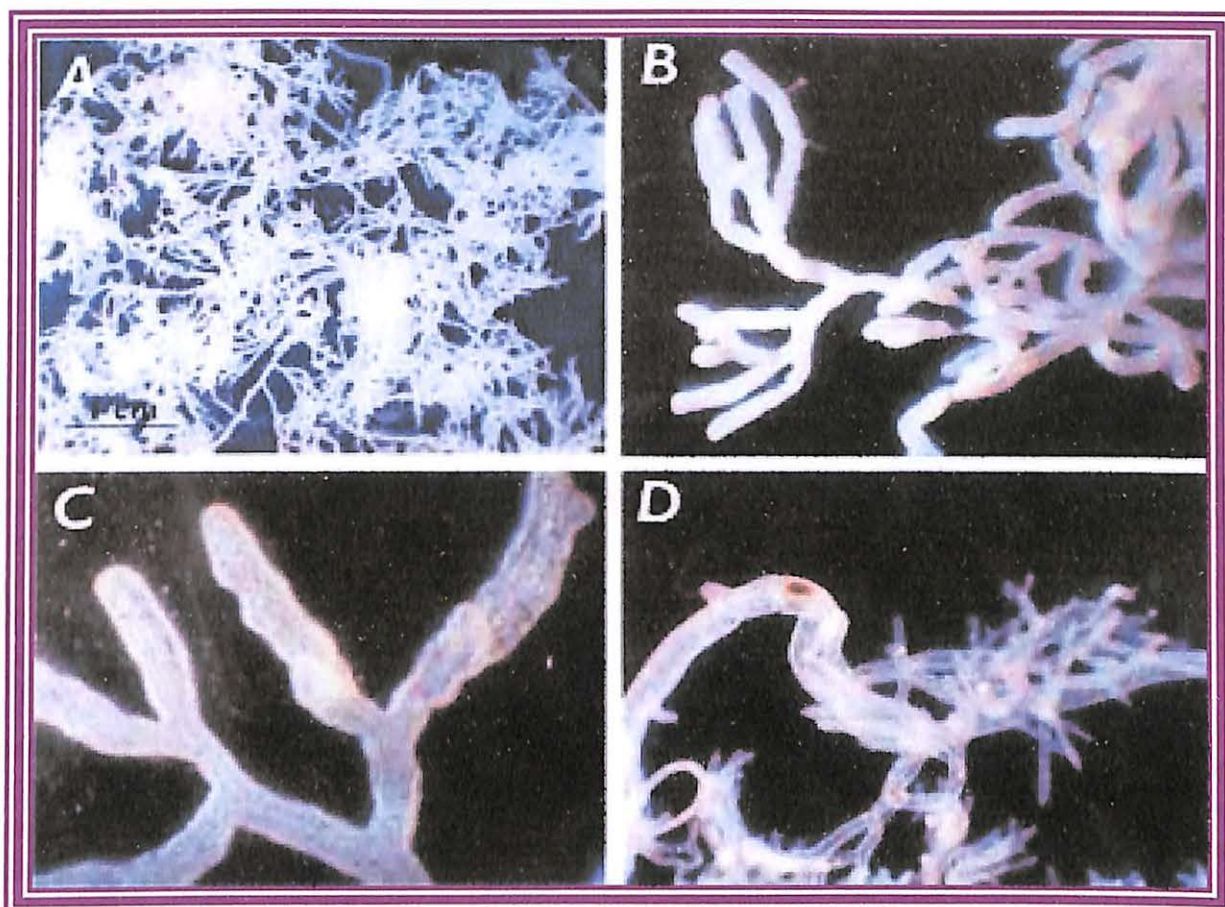


Figure 11: Macroscopic gonad characteristics in *S. chloronotus*. A. Testis. B. Mature testicular tubule C. Mature ovarian tubule. D. Post-spawning testis showing atresia.

(Image was *adapted and taken from*
<http://www.spc.org.nc/coastfish/News/BDM/15/BDM15-02.htm>)

1.2.4 Nervous tissue

The main components of holothurian nervous system are a circumoral nerve ring and the five radial nerve cords (Inoue, *et al.*, 2002). The circumoral nerve ring lies in the buccal membrane near the base of the tentacle. The ring supplies nerves to the tentacles and to the pharynx. The five radial nerves, on leaving the ring, pass through the notch in the radial plates of the calcareous ring and run the length of the ambulacra in the coelomic side of the dermis. (Barnes, 1991)

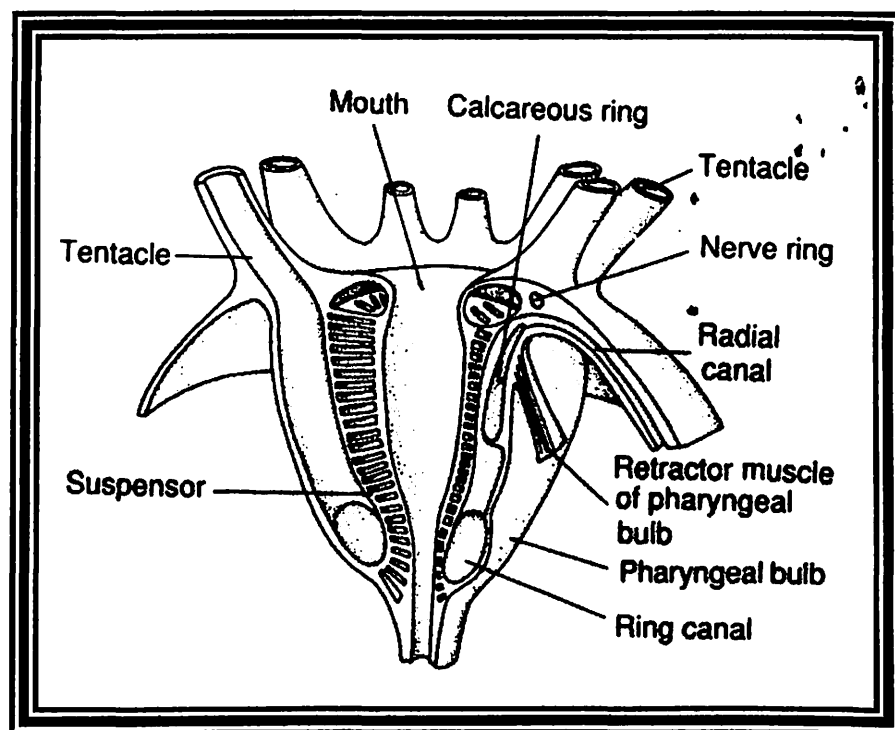


Figure 12: Section through anterior end of *Ocnus planci*.

(Image was scanned, modified and adapted from Invertebrate Zoology, Barnes, 1991)