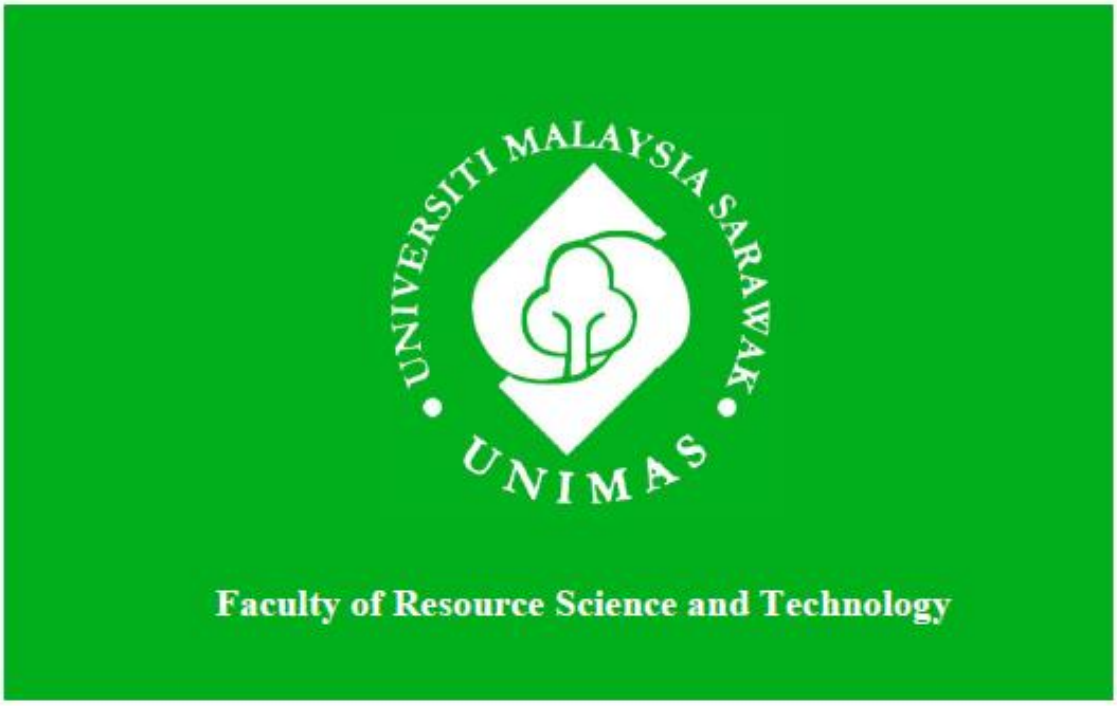

THE DIET OF TWO SPECIES OF PEANUT WORMS (SOFT SUBSTRATE, *SIPUNCULUS NUDUS)* AND (HARD SUBSTRATE, *ANTILLESOMA ANTILLARUM)*

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UNIVERSITI MALAYSIA SARAWAK
2015

Declaration

I hereby declare that no portion of this dissertation has been submitted in support of an application for another degree of qualification of this or any other university or institution of higher learning.

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List of Abbreviations

Cm	Centimeter
G	Gram
GPS	Global Positioning System
%	Percentage
IRI	Index of Relative Importance
%IRI	Percentage Index of Relative Importance
%O	Percentage Frequency of Occurrence
%N	Percentage of Number
%V	Percentage of Volume
SPSS	Statistical Package for Social Science
±	Standard deviation

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Study on the Diet of Two Species of Peanut worms (Soft Substrate, *Sipunculus nudus*) and (Hard Substrate, *Antillesoma antillarum*)

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ABSTRACT

Peanut worm is a type of marine soft bodied and non-segmented coelom worm. Peanut worm usually lives in temperate, subtropical, and tropical regions. In recent years, peanut worm become popular among countries such as Vietnam, Taiwan and China as special food of high nutritional and pharmaceutical value. Besides, peanut worm plays an important role as source of energy for fish, gastropods, cephalopods, carnivorous worms, crabs, starfishes and anemones. In Sarawak, peanut worm is usually use as fish bait among the local people. However, the basic knowledge especially the diet of peanut worm is still poor. Hence, this study was designed (1) to investigate the types of food with the body size of peanut worm (*Sipunculus nudus* and *Antillesoma antillarum*) and (2) to study the relationship between food items in gut content of peanut worm with the sampling locations (Kg. Pugu, Kg. Lebak and Pantai Puteri). The gut of *S. nudus* consist of foraminifera, foraminifera fragments, polychaete and unidentified plant materials while in the gut of *A. antillarum*, only sediments had been found. Besides, result showed that the highest percentage of Index Relative Importance (%IRI) was foraminifera in gut of large *S. nudus* but absence in small individuals. %IRI based on locations proved that foraminifera was the important food items. The gut length (cm) was positively significant correlated with the gut weight (g) in large *S. nudus* ($r = 0.941$, $p = 0.002$, $n = 7$) and gut of *A. antillarum* ($r = 0.710$, $p = 0.000$, $n= 30$). From this research, the data provided knowledge for local people and researchers in Malaysia in order to manage the resource sustainability.

Key words: Peanut worm, gut content, food items, gut length, gut weight

ABSTRAK

Wak-wak merupakan sejenis cacing marin yang bertubuh lembut dan tidak mempunyai segmen coelom. Wak-wak selalunya tinggal di kawasan sederhana, sub-tropika dan tropika. Sejak kebelakangan ini, wak-wak menjadi terkenal dalam kalangan negara-negara seperti Vietnam, Taiwan dan China sebagai makanan khas yang mempunyai nutrisi yang tinggi dan nilai farmaseutikal. Selain itu, wak-wak memainkan peranan penting sebagai sumber tenaga kepada ikan, gastropod, cephalopod, cacing karnivor, ketam, tapak sulaiman dan buran. Di Sarawak, wak-wak selalunya digunakan sebagai umpan oleh penduduk tempatan. Oleh itu, biologi dan dinamik populasi wak-wak mendapat perhatian sejak kebelakangan ini. Namun, pengetahuan asas terutamanya diet tentang wak-wak masih lagi kurang. Oleh itu, kajian ini telah dijalankan untuk (1) mengkaji bahan makanan dengan saiz badan wak-wak (Sipunculus nudus dan Antillesoma antillarum) (2)mengkaji hubung kait antara bahan makanan yang dijumpai di dalam kandungan perut wak-wak dengan tempat kajian (Kg. Pugu, Kg. Lebak dan Pantai Puteri). Kandungan perut S. nudus mengandungi foraminifera, serpihan foraminifera, polychaeta dan bahan tumbuh-tumbuhan yang tidak dapat dikenalpasti manakala di dalam kandungan perut A. antillarum hanya sedimen sahaja yang dijumpai. Selain itu, keputusan menunjukkan foraminifera mempunyai peratusan tertinggi Indeks Kepentingan Relatif (%IRI) di dalam kandungan perut S. nudus besar tetapi serpihan foraminifera tiada di dalam individu kecil. %IRI berdasarkan tempat membuktikan bahawa foraminifera adalah bahan makanan yang penting. Namun begitu, tiada keputusan bahan makanan dijumpai di dalam usus A. antillarum. Panjang usus (cm) mempunyai perkaitan positif dan jelas dengan berat usus (g) dalam S. nudus besar ($r = 0.941$, $p = 0.002$, $n = 7$) dan kandungan perut A. antillarum ($r = 0.710$, $p = 0.000$, $n= 30$). Maklumat di dalam kajian ini memberi pengetahuan kepada penduduk tempatan dan ahli penyelidik di Malaysia bagi mengurus sumber tersebut secara mapan.

Kata kunci: Wak-wak, kandungan usus, bahan makanan, panjang usus, berat usus

1.0 Introduction

Peanut worms are the marine non-segmented coelom worms which usually inhabit in burrows in mud or sand, in empty mollusc shells or in coral crevices (Acik, 2011; Lemer et al., 2015). According to Kedra and Wlodarska-Kowalczuk (2008), peanut worm is a poor phylum of Sipuncula as there are only 150 species and subspecies occur worldwide. They are known as peanut worm because of their bodies will contract into peanut kernel shape when disturbed. Besides, peanut worms also have other shapes such as slender cylinder, spindle and flask shape and almost spherical (Cutler, 1994). These invertebrate worms are all benthic and their habitats are in shallow water from the intertidal to 30 m depth (some records between 100-900 m) and the waters are in temperate, subtropical, and tropical regions (Boyle and Rice, 2014).

Sipunculus nudus live in sand and mud and they burrow actively where *Antillesoma antillarum* bore into coralline rock (Murina, 1984). Peanut worms are deposit feeders and they have proboscis with ring of tentacles that extend from the mouth (Karleskint et al., 2013). Organic materials will trap into the mucus which coats the tentacles and the food later drawn into mouth by ciliary action. According to Cutler (1994), *S. nudus* are direct deposit feeders which means they ingested the sand and silt through the location they burrow but *A. antillarum* feed by spread their tentacles over rock surfaces and ingested deposited material.

Guts are the main part to carry out digestion process in the digestion system of peanut worms. Peanut worms usually feed on detritus, diatom, fecal material, bacteria, algae, protozoans and small invertebrates (Maiorova and Adrianov, 2013). There are three main ways in the peanut worms feeding behaviour; filter feeding, ingestion of sediment and scraping and picking up of material from surfaces either rock or sediment (Cutler, 1994).

This study is carrying out because peanut worms are important in bioturbation of sediments and bioerossion of coral reefs (Cutler, 1994; Kędra and Włodarska-Kowalczuk, 2008). Besides, peanut worms play the role as one of the important marine resources for higher trophic level as they consume by fish, gastropods, cephalopods, carnivorous worms, crabs, starfishes, anemones and people (Cutler, 1994; Maiorova and Adrianov, 2013). Furthermore, peanut worms have high commercial value especially in food industry. In Vietnam, peanut worms are a significant source of income for local residents and many poor people are dependent on this resource for their daily life as peanut worms are popular seafood in international and domestic markets and act as special food of high nutritional and pharmaceutical value (Nguyen et al., 2007).

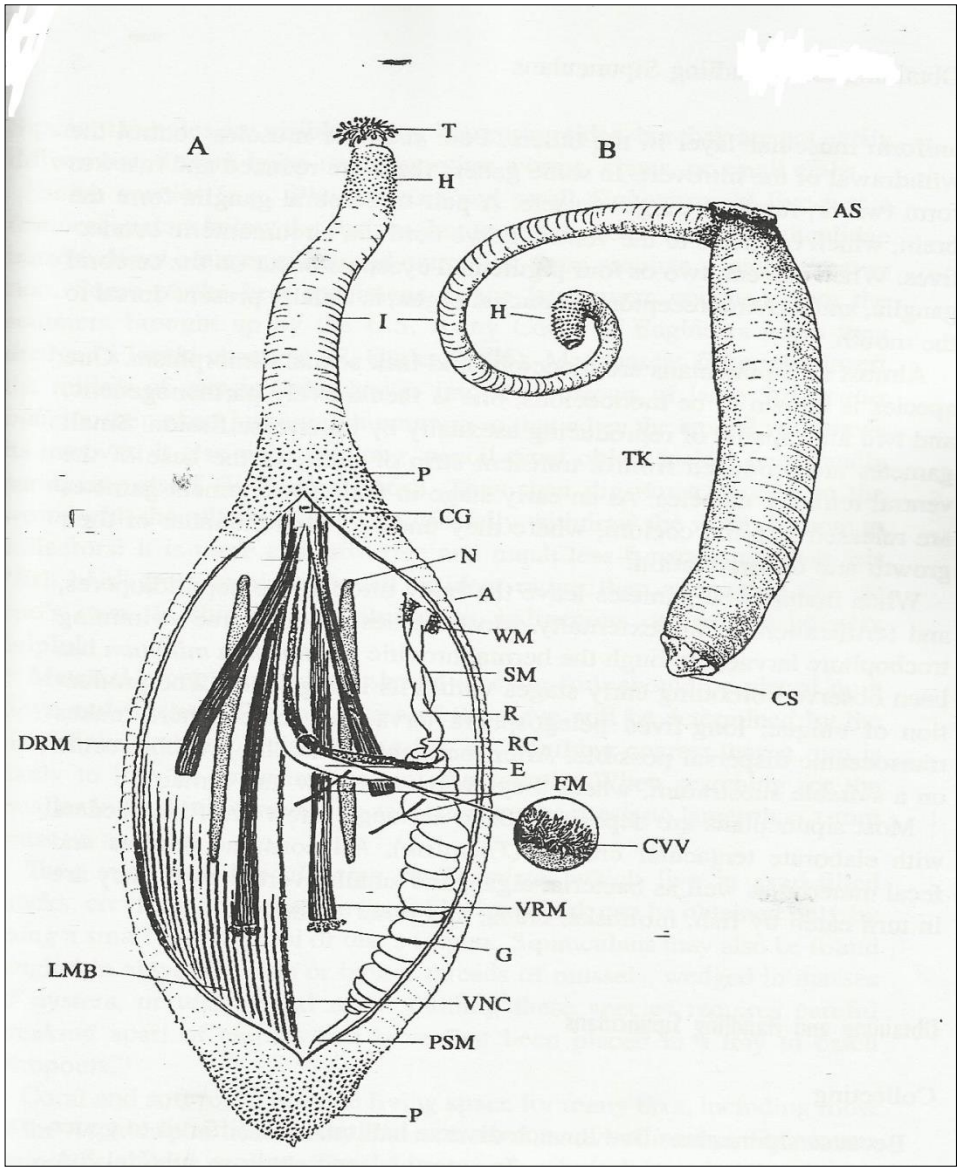
Kawauchi and Giribet (2014) had stated that peanut worm was abundant on the coasts of Atlantic Ocean and Mediterranean Sea. People in Spain used the peanut worm as fish bait while in China and Vietnam, people eat the peanut worm. Besides, peanut worm was important in bioturbation of sediments and act as bioerosion of coral reefs (Hermoso-Salazar et al., 2013). In Malaysia, people usually use the worms as fish bait. Unfortunately, basic knowledge such as diet of peanut worms is still poor for local people in Malaysia. Therefore, study on diet of peanut worm was very important in Malaysia. Hence, this study was designed (1) to investigate the types of food with the body size of peanut worm and (2) to study the relationship between food items in gut content of peanut worm with the sampling locations (Kg. Pugu, Kg. Lebak and Pantai Puteri).

2.0 Literature Review

2.1 Peanut worm

Peanut worm is a worm-like marine invertebrate with soft-bodied and unsegmented coelomate. According to Hyllerberg (1994), most peanut worms especially *S. nudus* are cylindrical to subcylindrical body shape. Some species like *A. antillarum* are nearly globular. The colour of peanut worm bodies is white, grey or brownish colour of bodies (Hylleberg, 1994; Cutler, 1994). The size of peanut worm is depending on the species. Fox (2006) said that some species are large (about 10 cm in length) but usually the range of this invertebrate size are from 2 mm to 70 cm. Peanut worm has thin body wall in some species until the coelomic contents and intestine may be seen through the skin. However according to Cutler (1994), the colour of the body and transparency are depend on the environmental factor and the size of peanut worm.

Peanut worm has two main regions: barrel-like trunk and long eversible introvert (Figure 1). The anterior most introverts or heads bears a terminal tentacular apparatus (Maiorova and Arianov, 2013). Ciliary tentacles which associated with the mouth of peanut worm are used in gas exchange and in feeding. The hook or spine of peanut worm is helped in collecting food. The gut of peanut worm is coiled in the J-shaped with dorsal anterior anus. Peanut worm usually live in tropical, temperate and polar seas from the intertidal region of shore to the floor of the oceans (Karleskint et al., 2013). Some species of peanut worms inhibit in the soft sediments or sandy mud. *S. nudus* usually burrow down a meter in coarse or silt sands (Maiorova and Arianov, 2013). For *A. antillarum* species, it can be found in coral and soft rock (Cutler, 1994).



A	Anus	E	Esophagus	N	Nephridia	T	Tentacles
As	Anal shield	FM	Fixing muscle	P	Papillae	TK	Trunk
CG	Cerebral ganglion	G	Gonad	PSM	Posterior spindle muscle	VNC	Ventral nerve cord
CS	Caudal shield	H	Hooks (Scattered on A, in ring on B)	R	Rectum	VRM	Ventral retractor muscle
CVV	Contractile vessel villi	I	Introvert	RC	Rectal caecum	WM	Wing muscle
DRM	Dorsal retractor muscle	LMB	Longitudinal muscle bands	SM	Spindle muscle		

Figure1. Generalized peanut worm morphology (A) Internal amalgam, (B) Aspidosiphonid (Cutler, 1994).

Diet of peanut worm is depending on its habitat and species. *S. nudus* eat algae, protista, meiofauna, diatoms, detritus and small invertebrates but for *A. antillarum*, it usually scrapes the sediments and epifaunal organisms in surrounding rock surface (Cutler, 1994). Some study stated that smaller peanut worm usually has smaller meiofauna in the intestines but the statement is not significant after five year (Cutler, 1978). Moreover, peanut worm is important in trophic level and food web because it act as food for other animals such as fish and gastropods. Peanut worm also consumed by the anemones, crabs, cephalopod, carnivorous worms, starfish and other predators (Cutler, 1994; Maiorova and Adrianov, 2013). Human also use the peanut worm as fish bait. The current status about peanut worm is it has been used for food industry. There are some research in Vietnam had stated that the peanut worm has being exploited every day in many tidal flats along the coast in Vietnam. Peanut worm become very popular seafood either in international or domestic market as it content high nutritional and pharmaceutical value (Nguyen et al, 2007).

Poor people in Vietnam take advantages to make the peanut worm a significant resource of income as peanut worm get the high reservation from time to time. This situation affects the peanut worm population because it was being overexploited to satisfy the market demand. Besides, peanut worm is threaten by human activities such as urbanization, pollution by solid and liquid wastes from adjacent areas, from aquaculture activities, and destructive fishing methods (using dynamite, toxic chemicals, and electric shock) (Nguyen et al., 2007). The size of peanut worm decreases due to daily exploitation. Fortunately, the management of the exploitation of peanut worm is hindered by the lack of scientific knowledge on their distribution in relation with living conditions. The understanding of peanut worms living conditions, especially geo environmental factors,

will provide a basic background for sustainable coastal planning and natural resources management for long-term period.

In Malaysia, peanut worm is only use as fish bait for local people. There is no research or information about making the peanut worm as a food. Therefore, the population of peanut worm in Malaysia is still high. However, if the importance of peanut worm can be discover in future, the overexploitation situation also may happen same as in Vietnam.

2.2 Feeding Habits

In the gut of peanut worm usually contained sand, mud, small particles of coral or limestone rock, fragments of algae and sea grasses, pieces of molluscan shells, echinoderm exoskeleton and skeletal parts of diatoms and foraminiferans (Karleskint et al., 2013). All these types of food materials have make the peanut worm known as detritus feeders or deposit feeders as it ate whatever it ingested. Cutler (1994) had stated that the tentacles are very sensitive to small particles of food and the anterior region of introvert is sensitive with foreign food materials.

For peanut worm that inhibited in the rock like *A. antillarum*, there were two types of feeding behavior (Rice, 1976 as cited in Cutler, 1994). The species of peanut worms with long, extensible introverts and short digiform tentacles feed by extending the introvert from the mouth of the burrow and grazing on the surface of rock surrounding them (Cutler, 1994). However, for the species with small hooks of introvert will scraped off the rock directly to the tentacles. The larger sand-dwelling species like *S. nudus* usually engulf some sediment to tunnel through the sand (Karleskint et al., 2013). Once the burrow process is complete, peanut worm will stay in it for a long period of time. For peanut worm that live in burrow, the upper part is about more or less vertical. Peanut worm use its

tentacular crown which located near the opening of burrow and collect everything that fall into the burrow during daytime. The introvert moves through the sediments a few millimetre (mm) below the surface.

2.3 Digestive System

Peanut worm has a unique J-shaped of gut which forms a long coiled loop between the mouth and anus. The gut usually twisted into the double helix. The mouth is in the center of the oral disc. Food is collected with short tentacles and convey to mouth. The food particles move into buccal cavity which located in a line with ciliated cells. Peanut worm has short pharynx which surrounded by fused dorsal and ventral retractors (Akesson, 1958 as cited in Cutler, 1994). A straight esophagus is attached to the ventral retractors for half of the length by numerous musculo-connective strands. A simple contractile vessel is attached to the esophagus for entire length. Sphincter and blind termination of contractile vessel is formed for a sharp transition between esophagus and anterior descending intestine. The esophagus epithelium is lined with abundant ciliated cells.

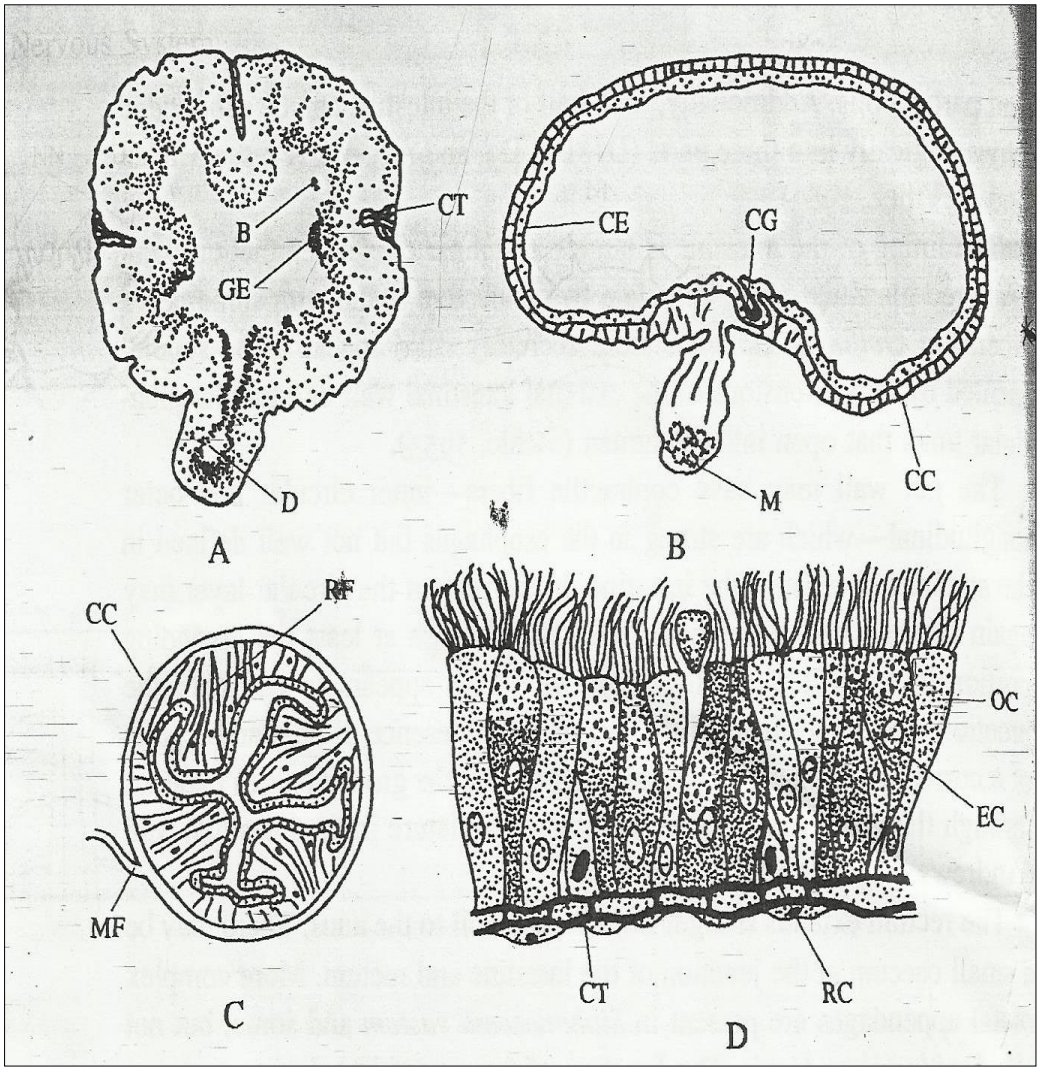
Fox (2006) stated that the intestine of peanut worm is divided into two; descending and ascending. Descending intestine is long and coiled. It exits the stomach and extends far posterior in the trunk. Ascending intestine is the reverses direction and its climbs back into anterior trunk. The descending and ascending intestine are coiled around each other and form intestinal loop. The intestine is yellow in colour as the chloragogie cells are dominant on the surface (Cutler, 1994). The lining of intestine has large columnar ciliated epithelium with scattered glandular cells which underlain by connective tissue with some nerve fibers. The gut wall has contractile fibers called inner circular and outer longitudinal (Cutler, 1994; Fox, 2006). Peanut worm also has straight caecum that joins the body wall on

anterior and dorsal midline in anus. The tiny and bulbous caecum will turn to rectum at a point in the intestines. Rectum usually attached to the body wall by a small mesentery.

2.4 Physiology of Peanut worm

Once the food and inorganic material are ingested, digestion begins in descending intestine (Brown et al., 1979). Esophagus is not glandular but epithelium of ill-defined stomach has sparsely distributed gland cells (Figure 2). This region secretes weakly acidic mucus and neutral mucopolysaccharides via exocytosis in merocrine manner but not the enzyme. The pH in descending intestine is about 7.8. The rough endoplasmic reticulum (RER) produces zymogen granules and the granular materials become an apocrine secretion from cells with apical microvilli and cilia (Fox, 2006).

Enzyme becomes active towards end of descending coil and at beginning of ascending coil. Ventral ciliated gutter in ascending intestine appears to produce necessary enzyme activator. Past study shows that all absorption takes place in ascending intestine. The energy loss between anterior gut and mid gut was 70% while loss between mid-gut and hindgut was only 19% (Cutler, 1994). The caecum can degenerate or reabsorbed partially in some worms. Peanut worm has no liver and digestible glands but there are report of bulging, clavate chloragogue cells on outer peritoneum of esophagus and intestine (Stehle, 1953, as cited in Cutler, 1994). The peanut worm morphology and yellow granular contents suggest a role in storage and fats metabolism of the peanut worm.



B	Bleb	CT	Connective tissue	GC	Glandular epithelium	OC	Ordinary intestinal cell
CC	Chloraguogue cells	D	Diverticulum	M	Muscle	RF	Replacement cells
CE	Ciliated epithelium	EC	Enzymatic cell	M F	Mesentarial fibers	RC	Radial fibers.
CG	Ciliary glandular gutter						

Figure 2. Digestion components of peanut worm. Cross sections of (A) descending and (B) ascending intestine (Michel and de Villez, 1984), (C) Cross section of stomach showing four ridges of radiating fibers that reduce the lumen to an X shape (Andrew, 1890), (D) Glandular epithelium of peanut worm intestine (Stehle, 1953) (Cutler, 1994).

3.0 Materials and Methods

3.1. Source of peanut worms

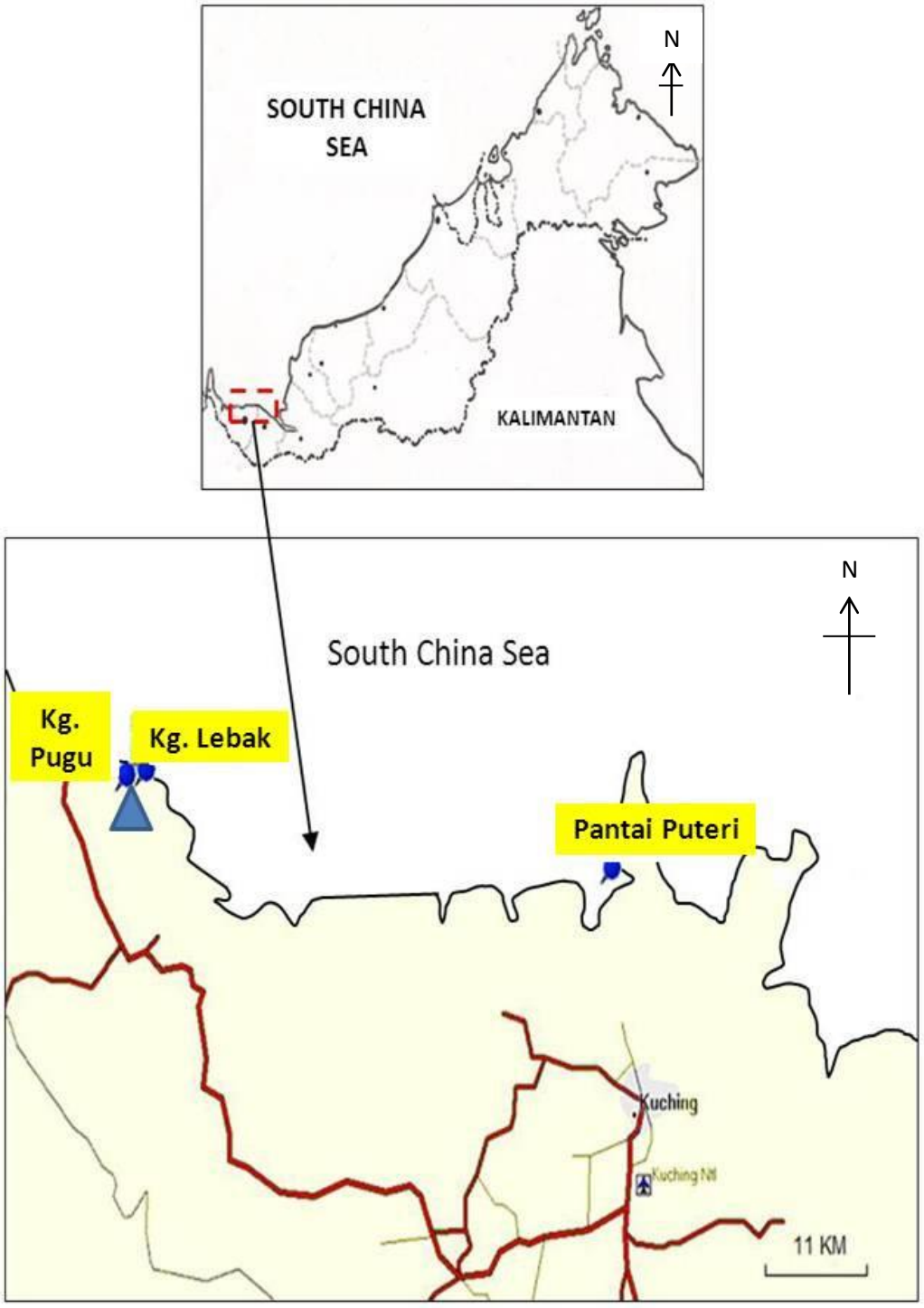


Figure 3. Map of sampling locations (Kg. Lebak, Kg. Pugu and Pantai Puteri).

A total of 12 individuals *Sipunculus nudus* which 7 were large (> 10 cm) and 5 were small *S. nudus* (<5cm) collected randomly in soft substrate by digging out the sediment using shovel. Besides, 30 individuals of *Antillesoma antillarum* had been collected randomly in hard substrate by scraping off the rock oyster bed using hammer and chisel. The sampling in Kg. Lebak, Kg. Pugu and Pantai Puteri were done during low tide of spring and sunny weather. The date and coordinates of each sampling site at Kuching Division (Table 1) were recorded using Global Positioning System (Garmin GPSmap 60CSx).

Table 1. Date and coordinates of sampling locations (Kg. Lebak, Kg. Pugu and Puteri Beach) and the species of peanut worms (*Sipunculus nudus* and *Antillesoma antillarum*).

Date	**Species of Peanut worms**	**Locations**	**Coordinates**
23 December 2014	*A. antillarum*	Pantai Puteri,	N01°43’39.0”,
	S. nudus (small)	Santubong	E110°18’50.1”
2 February 2015	*S. nudus* (large)	Kg.Pugu, Lundu	N01°47’58.6”,
			E109°48’29.6”
3 February 2015		Kg. Lebak, Lundu	N01°47’81.8”,
			E109°49’50.6”

All specimens were measured using ruler to the nearest 0.1 cm. To get the actual length of peanut worm; the measurements were taken starting from the tentacles until caudal shield (Figure 4, 5 and 6). Then, the *S. nudus* specimen were injected with pure (100%) formalin through their mouth part before immersing them into 10% formalin. For *A. antillarum,* the specimens were preserved with 10% formalin. All specimen bottles were labeled (date, location and time) before bringing them back to laboratory for further analyses.