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RESEARCH PAPER

Apicomplexa-Like Parasites in Some Gastropods from Merambong Seagrass Bed, Johor Straits, Malaysia

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Abstract

This study investigates the infestations and prevalence of apicomplexa-like parasites in marine gastropods from Merambong seagrass bed in Johor Straits, Malaysia. The gastropods were collected during spring low tides, when the seagrass bed exposed. Result showed that the digestive gland and digestive duct were the most infected organs, and no parasite was observed in the gills and general tissues. The conchs (*Laevistrombuscanarium* and *Canariumurceus*) displayed highest infestation rates, followed by the volutes, *Cymbiolanobilis* and *Melomelo*, and others (*Nassariuslivescens* and *Notocochlistigrina*). Furthermore, parasite infestations were significantly higher in prey species (conchs), compared with their natural predator (volutes). This indicated that bio-accumulation across the trophic level was not occurred, and does not involved a terminal host. Further studies are greatly needed for better understanding of the histopathology characteristic of parasite infestation at the study area, which is very important for the sustainable management of the gastropod species.

Introduction

The phylum Apicomplexais a large group of spore-forming, single-celled eukaryotes.Most of the Apicomplexa are obligate parasites, and some may cause important human or animal diseases such as malaria, toxoplasmosis, coccidiosis, babesiosis, theileriosis, cryptosporidiosis and others. However, many apicomplexans are not pathogenic to their host or at least not proven to be parasitic in nature. Apicomplexa possess a unique plastid-like organelle (apicoplast) and an apical complex structure involved in penetrating a host's cell (Foth& McFadden, 2003; Lim & McFadden, 2010;Sato, 2011). Members of this phylum include organisms such as Coccidia, gregarines (Gregarinasina), piroplasms (Piroplasmida), haemogregarines (Haemogregarinidae), and plasmodia (Plasmodiidae). They have three transformation stages to complete their life cycle known as gamonts, sporocysts and trophozoites(Rohde, 2005;Sato, 2011).This complex life-cycle involving both asexual and sexual reproduction. After invasion, they divide to produce sporozoites(spores) that enter the host's cells. The cells will eventually burst and releasing merozoites that infect new cells. This may occur several times, until gamontswere produced, forming gametes that fused to create new cysts. However, there are variations in this basic pattern, which may involve a single host (monoxenous) or more (heteroxenous) (Leander, 2003; Mikheev, 2011).

The studies of parasitism relationship between apicomplexa parasites and marine gastropod have widely beenstudied and reported. For examples the coccidian parasite in *Strombusgigas* (Baqueiro-Cárdenas *et al.*, 2007), *Nematopsisgigas* in *Neritaascencionis* (Azevedo&Padovan, 2004), *Pseudoklossiahaliotis* in *Haliotis* spp.(Friedman *et al.*, 1995). Mollusks are important seafood and fishery resource and some of them have been commercially cultured. The study of parasites and diseases affecting mollusks with economic interest is very important both for the management of natural stock and for aquaculture (Boehs *et al.*, 2010; Carnegie *et al.*, 2016). The objective of this study is therefore to investigate the incidence of apicomplexa-like parasites infestation in someimportant gastropods from Merambong Shoal, Johor, Malaysia.

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Materials and Methods

Gastropod Sample Collection

Live gastropod samples were collected from November 2012 to February 2013 at the Merambong Shoal seagrass bed, Johor Straits, Malaysia (1° 19. 55' N, 103° 35. 57' E) (Figure 1). Sampleswere collected by hand and kept in a container with a small volume of seawater, and were brought back to the laboratory for analysis. The seagrass bed contained high diversity of gastropods (Cob *et al*., 2008, 2014), but only the more dominant, conspicuous, and economically important species were sampled. This include theconchs*Laevistrombuscanarium*(Linnaeus, 1758) and *Canariumurceus*(Linnaeus, 1758), the volutes *Cymbiolanobilis*(Lightfoot, 1786) and*Melomelo*(Lightfoot, 1786), the mud snail *Nassariuslivescens*(Philippi, 1849) and the moon snail *Notocochlistigrina* (Roding, 1798). Throughout the sampling period a total of 100 *L. canarium* individuals have been sampled, followed by 75 individuals of *C. urceus*, ten individuals of *C. nobilis*, ten individuals of *N. livescens*, 5 individuals of *M. melo* and 5 individuals of *N. tigrina*.

Histological Analyses

The mollusks' digestive gland, digestive duct, gonad, renal organ, proboscis, heart, gill and general tissue were dissected and fixed in Bouin's Solution for 24 hours. The tissues were dehydrated in an ethanol series and embedded in paraffin, sectioned (4-5 µm thick) and stained using a Masson's trichrome staining protocol(Prophet *et al*., 1992). The histological sections were observed undera light microscope.The presence of the Apicomplexaand other parasites was identified following descriptions by Meyers and Burton (2009), Kim *et al.* (2006), Desser *et al.* (1998), and others. Various apicomplexan life cycle stages were identified accordingto Perkins (1991), based on the presence or lack of a thick wall and/or an inner budding.The parasite prevalence (number of gastropod with parasite or pathology per number of gastropod examined), and distributionin the host were evaluated for each snail. The intensity of parasite infestation was evaluated bycounting the number of parasites or pathology per histologic section if possible,or by gross estimation without counting (Kim & Powell, 2006; Boehs *et al*., 2010).

Results

Histological Observations

Microscopic examination of the histologic sections of the gastropod showed that all species studied were infected by the Apicomplexa-like parasite. In general,almost all organs were infected except for the gills and the general tissue. The parasite infestation and pathologies on the respective tissues is presented in Table 1.

Digestive Gland

There are three different types of cells in the digestive gland, that is the vacuolated cells, digestive cells and crypt cells. In *L. canarium* and *C. urceus*, different life cycle stage of Apicomplexa parasite i.e. gamonts, sporocysts and trophozoite were observed in all the three digestive glandcell types, with 100% prevalence (Figure 2a, 2b). The gamonts were characterized by their thin wall, sporocysts by their thick walls, and trophozoite by their apical conoid-like structure. Apart from that,different types of spores and elongated parasite were also observed in the digestive

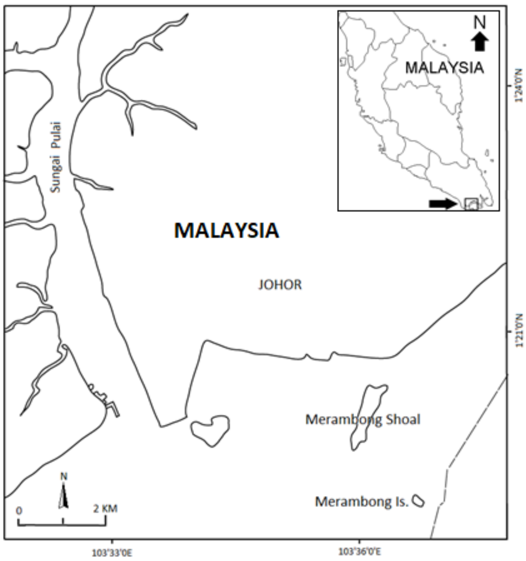

Figure 1: Sampling location, at Merambong Shoal, Johor Straits, Malaysia.

cells of this two conch species. In the predatory volutes, *C nobilis* and *M melo*, sporozoa-like organisms were found in their digestive cells with 100% prevalence and infection intensity (Figure 2c, d). There was no parasite observed in the digestive gland of the scavenging gastropod *N. livescens* and another predator *N. tigrina*.

Digestive Duct

Digestive duct is one of the organs in the digestive gland. There are two sections in the duct that is the primary and secondary duct. The primary duct was bordered with two epithelium structure, i.e. plicate epithelium and flat epithelium. This primary duct contained coarse inclusions and flocculent substance. Histological section showed that the digestive ductwas infected by protozoan parasite, that were also present in the digestive gland described above. The primary duct of *L. canarium* and *C. urceus* were infected by gamonts and sporocyst with high infestation (75%) and prevalence rates (50%) (Figure 3a, b). The primary duct of *C. nobilis* and *M. melo* also showed high infestation and prevalence rates, at 75% and 40% respectively.

Gonad

Majority of the gonad samples analyzed were mature gonads of males(spermatozoa) and females (oocyst). An inclusion of a Rickettsia-like organism was observed in the wall of developed ovary of *L. canarium* and *C. urceus*, with moderately high prevalence rate (40% to 46.7%), but low in intensity (1.67% to 1.78%) (Figure 4a &4b).Apart from that,a coccidian infection was also observed in the developingwall of ovary of*C.*

Table 1. Apicomplexa-like parasites stages/species infestation and pathologies of the gastropod tissues and organs. P = Parasite prevalence (number of gastropod with parasite or pathology per number of gastropod examined); I = Infection intensity (number of parasites or pathology per histological section). ND = not detected; number of samples in parenthesis

	Laevistrombuscanarium(100)	*Canariumurceus* (75)	*Cymbiolanobilis* (10)	*Melomelo* (5)	*Nassariuslivescens*(10)	*Notocochlistigrina*(5)
Digestive Gland	Gamonts P = 100; I = 20 Sporocyst P=100; I=33.7 Trophozoite P=100; I =1.0 Elongated parasite P=50; I=1.67 Spore colony P=100; I=75.3	Gamonts P = 100; I = 20.9 Sporocyst P = 100; I = 44.9 Trophozoite P = 100; I = 1.00 Elongated parasite P = 50; I = 3.56 Spore colony P = 100; I = 62.2	Sporozoa-like organisms P = 100; I = 100	Sporozoa-like organisms P = 100; I = 100	ND	ND
Digestive Duct	Sporocyst P = 75; I = 2.0 Gamonts P = 75; I = 15 Spore colony P = 100; I = 29.3	Sporocyst P = 50; I = 22.7 Gamonts P = 50; I = 13.3 Spore colony P = 100; I = 40	Bacteria-like inclusions P = 75; I = 100	Bacteria-like inclusions P = 40; I = 100 Spore colony P = 100; I = 100	ND	ND
Gonad	Rickettsia-like organisms P = 40; I = 1.67	Rickettsia-like organisms P = 46.7; I = 1.78 Coccidian P = 35.6; I = 12.4	ND	ND	ND	ND
Renal Organ (kidney)	Sporocyst P = 75; I = 2.67 Rickettsia-like organisms P = 50, I = 4 Oocyst of *Pseudoklossia* P = 50; I = 1.67 Spore colony P = 50; I = 12.0 Unidentified Coccidia P = 50; I = 20.5	Rickettsia-like organisms P = 50.7; I = 1.33 Spore colony P = 50; I = 16.4	Sporocyst P = 75; I = 100 Spore colony P = 100; I = 100	Spore colony P = 100; I = 100	Bacteria-like inclusions P = 33.3; I = 100	Bacteria-like inclusions P = 66.7; I = 100
Proboscis	Bacteria-like inclusions P = 50; I = 94	Bacteria-like inclusions P = 50; I = 100	ND	ND	ND	ND
Heart	Spore of *Nematopsis* P = 40; I = 1.67 Spore colony P = 50; I = 12.7	Bacteria-like inclusions P = 60; I = 100	Spore colony P = 60; I = 100 Rickettsia-like organisms P = 37.5; I = 10	Spore colony P = 60; I = 100	ND	ND

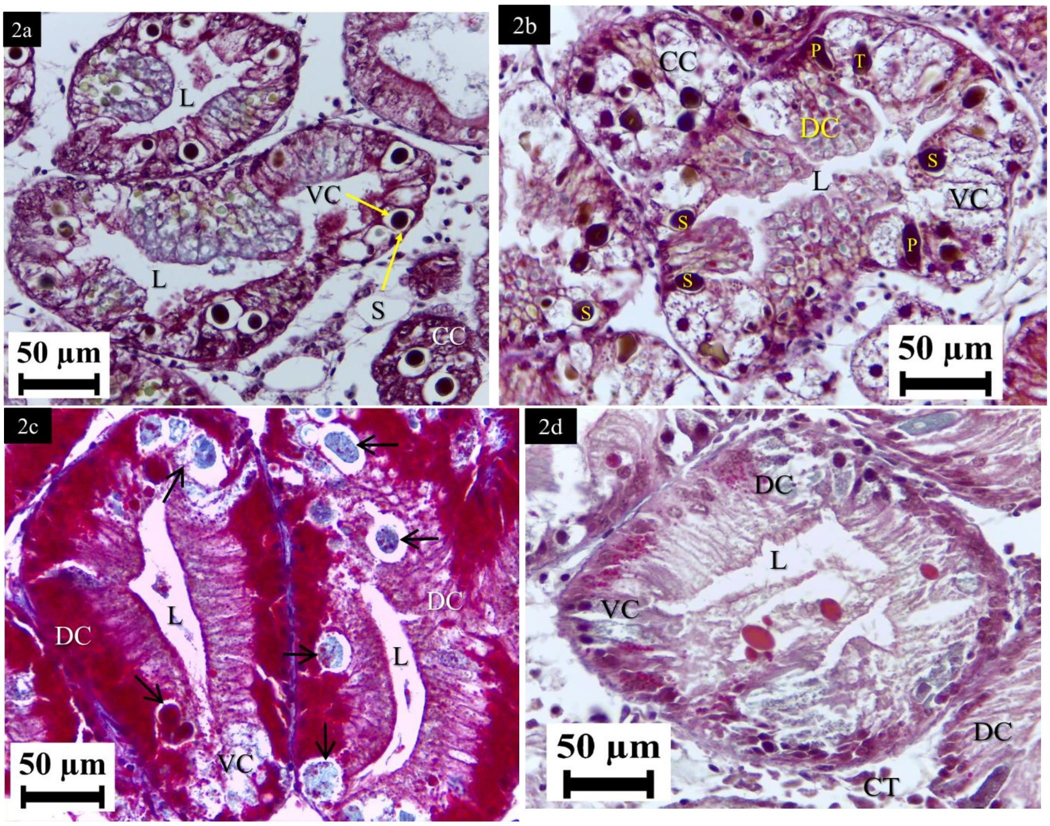

Figure 2. Trophozoite (T) and sporocyst (S) in the vacuolated cells (VC) and digestive cells (DC) of the digestive gland (DG) of *Laevistrombuscanarium* (**2a**) and *Canariumurceus*(**2b**). Sporozoa-like organisms in the vacuolated cells of digestive gland of *Cymbiolanobilis* (**2c**) and *Melomelo* (**2d**). L = Lumen of the tubule; CC = Crypt cells.

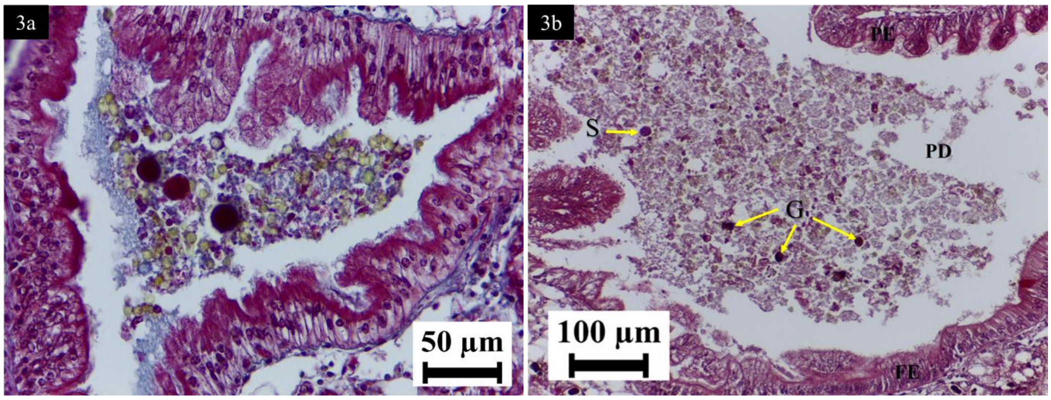

Figure 3. Sporocyst (S) and gamont (G) in the primary duct (PD) of the digestive duct of *Laevistrombuscanarium* (**3a**) and *Canariumurceus* (**3b**). PD is bounded by two epithelium structure such as a plicate epithelium (PE) and flat epithelium (FE).

urceus, but with low intensity 12.4% and 35.6% prevalence (Figure 4b).

Renal Organ

Renal organ of all gastropods studied were found infected by the apicomplexa-like parasite and sporozoa-like organisms. Inclusion of this parasites and sporozoaoccupying most of the intracellular space of the epithelial tissues. In *L. canarium*, different development stages of matured oocysts of *Pseudoklossia* was found in the epithelium cells of the renal organ(Figure 5a). There were also mild inclusions of coccidian detected in renal cells of *L. canarium* with 50% prevalence. Colony of spores were present in renal organ of *L. canarium*, *C. urceus*, *C. nobilis* and *M. melo*, with highest intensity in the renal cells of *C. nobilis* and *M.melo*(Fig. 5b). Bacteria-like inclusions were observed with in the renal organ of *N. livescens* and *N. tigrina*, with 33.3% and 66.7% prevalence respectively.

Proboscis

All the muscles of the proboscis, including the head sections that contains a terminal buccal mass were analyzed. The histological analysis showed a bacterial-like inclusions in the proboscis of *L. canarium* and *C. urceus* with 50% prevalence and up to 100% infection intensity (Figure 6a & b). The bacterial-like organism were rod and spherical shaped. The proboscis of *C. nobilis*, *Melomelo*, *N. livescens* and *N. tigrina* were not infected with any parasites.

Heart

The heart of four species, *L. canarium*, *C. urceus*, *C. nobiilis* and *M. melo* were found infected by parasites with relatively high infestation rates. The *Nematopsis* spores have infected the heart of *L. canarium* with 40% prevalence, but at low intensity of 1.67% (Figure 7a), while bacterial-like inclusions were observed in *C. urceus* with 60% prevalence and 100% infection intensity (Figure 7b). The heart of *C. nobilis* and *Melomelo*were infected by spore colonies with 60% prevalence and 100% intensity (Figure 7c and 7d). Moreover, Rickettsia-like organisms were also observed in the heart of *C. nobilis*, but only with mild prevalence and intensity values.

Gill and Tissue

In this study the gill and tissue was also analyzed but no parasite infestation nor pathology were observed in the six species of gastropods studied.

Discussions

Information on parasite infestations in marine resources are not only important for the management of the species, but also for the people as direct consumer of the resources. The studies of parasitism relationship between apicomplexa parasites and their marine gastropod hostsare very limited throughout the Southeast Asian region. Although Johor Straits is a rapidly developing coastal area where many scientific studies have been conducted over the years, informationregarding parasites in gastropods is currently not available.Study by Nurfauzana, Mazlan and Cob (2014) on bivalves from the same area found high incidence of parasite infestations in the species studied.

This study showed that the digestive gland, digestive duct and renal organ werethe most infected, followed by the gonad, proboscis and heart tissue.

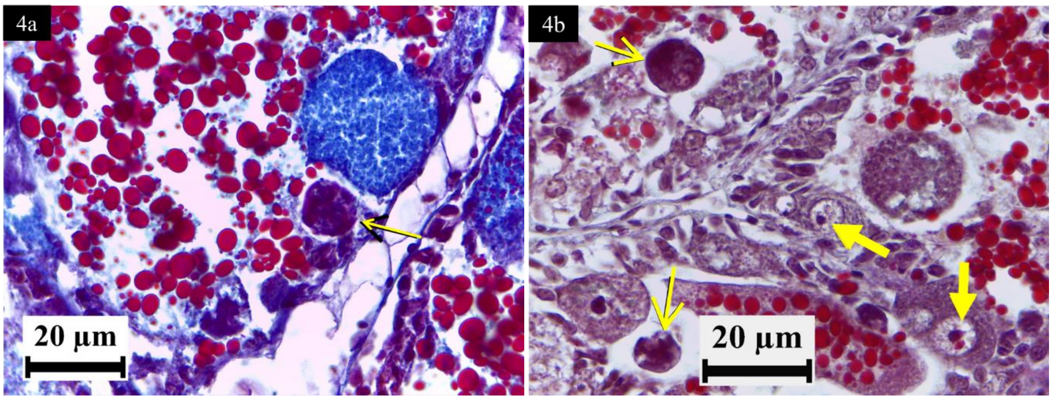

Figure 4. Infestation of Rickettsia-like organism (thin arrow head) and coccidian (thick arrow head) in the developed wall of ovaries of *Laevistrombuscanarium* (4a) and *Canariumurceus* (4b).

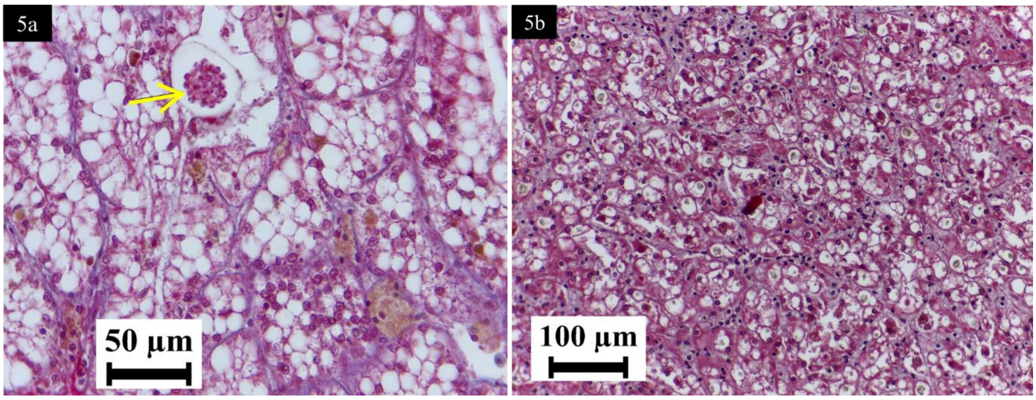

Figure 5. Different level of oocyst of *Pseudoklossia* (arrow) and sporozoa-like organisms in the renal cells of the renal organs of *Laevistrombuscanarium* (**5a**); and spore colony with highest infection in renal cells of *Melomelo* (**5b**).

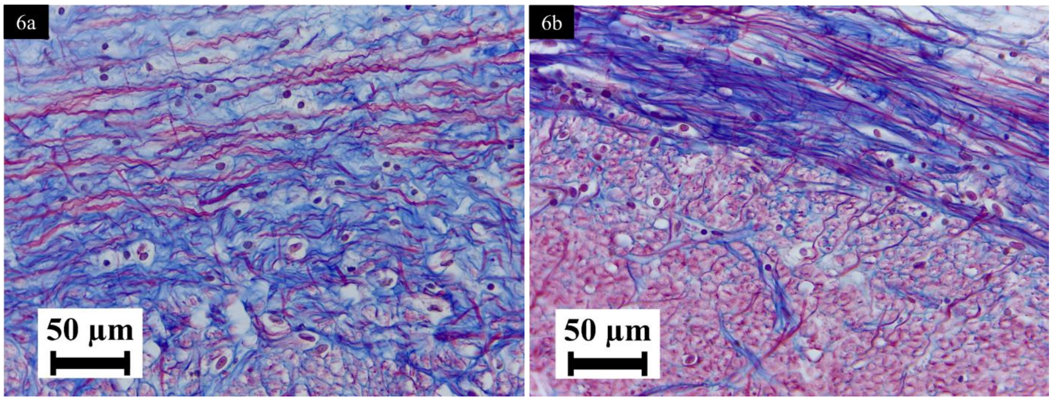

Figure 6. Inclusion of rod and spherical shaped colony of bacteria-like organisms in the infected proboscis of *Laevistrombus canarium* (**6a**) and *Canarium urceus* (**6b**).

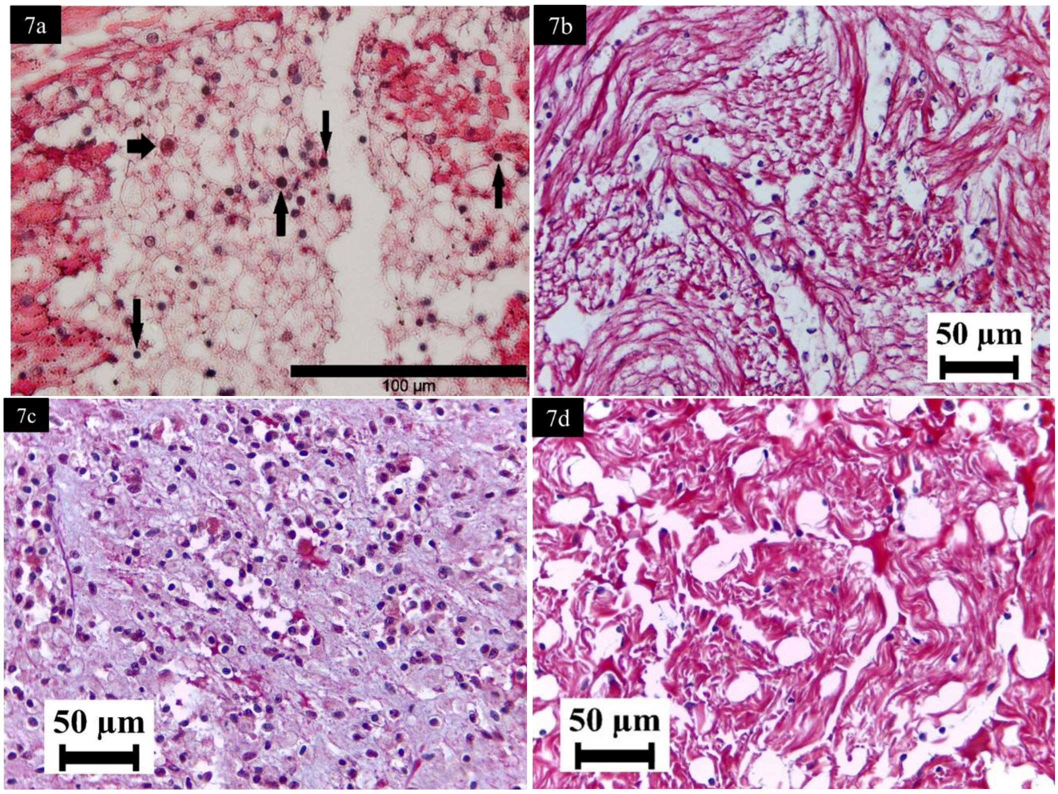

Figure 7.*Nematopsis* spore (thick arrow) and spore colony (thin arrow) in the infected heart of *Laevistrombuscanarium* (7a); infestation of bacterial-like inclusion in the infected heart of *Canariumurceus* (7b); spore colony in the heart cells of *Cymbiolanobilis* (7c) and *Melomelo* (7d).

Parasiteinfestation was however not detected in the gills andthe general tissues. Different apicomplexa development stages were observed, which include thegamonts, sporocysts and trophozoites, and most of them were particularlyabundant in the digestive gland and digestive duct. Different types of apicomplexa such as the oocyst of *Pseudoklossia* and coccidian were also observed. Apart from that,other infections and pathological condition were also observed such as infestation by spores, bacterial, Sporozoa-like organisms, Rickettsia and others.

Among the gastropod species studied, the conch snails,*L.canarium* and *C. urceus*recorded the highest prevalence and intensity values,which ranged between 50% to 100%, and 1% to 75% respectively. Furthermore, both species showed similar pattern of infection where highest prevalence and intensitieswere recorded in the digestive gland and digestive duct. Conchs are highly commercial species and some have been listed in CITES due to overexploitation (Acosta, 2006). Studies on other conchs, particularly in the Caribbean region, have reported apicomplexa-like parasitesinfestation in species such as *Strombusgigas* (Baqueiro-Cárdenas *et al.*, 2005, 2007;Gros *et al.*, 2009), *Strombusgallus* (Volland *et al.*, 2008), *Strombusraninus* (Volland *et al.*, 2008), *Strombuscostatus*and *Strombuspugilis* (Volland *et al.*, 2009). These above studies also reported similar observations where different apicomplexa life stages were present in a single host. Furthermore,similar pattern of parasite infectionwas reported where the digestive gland and digestive ductwere the most infected.

Apart from the digestive gland and digestive duct, the gonad, proboscis and heart of *L. canarium* and *C. urceus* were also infected. The gonad and proboscis were infected by bacteria-like inclusions and Rickettsia-like organisms. The heart of *L. canarium* was infected by*Nematopsis* and spore colony, while the heart tissue of*C. urceus*was infected by bacteria-like inclusion.Previous studies on parasite infections in gastropods and other mollusks were focusedmore on the digestive gland, as it is the most likely organ to be infected (Gros *et al*., 2009;Volland *et al*., 2008, 2009; Aldana Aranda *et al*., 2011;Baqueiro-Cárdenas *et al*., 2012). If these organs become highly infected, then some parasites may secondarily invade adjacent organs such as gonad and others (Cheng & Cooperman, 1964; Pan, 1965). The low infection rates recorded in gonad, proboscis and other tissues indicate that parasite infectionswere not in deleterious state, and no mortality or serious pathological respond was observed. Nevertheless, the present of bacteria-like inclusions and Rickettsia-like organisms should be monitored as they have been associated with diseases and mortalityin some mollusks (Antonio *et al*., 2000; Moore *et al*., 2000, Hine &Diggles, 2002; Hine *et al*., 2002;Azevedo *et al*., 2005, 2006).

The gastropod species selected for this study were from different trophic levels. The conchs (*L.canarium* and *C.urceus*) are surface grazers, the mud snail (*N. livescens*) is a scavenger, while the moon snail (*N. tigrina*) and the volutes (*C. nobilis* and *M. melo*) are predators. The moon snail preyingmostly on bivalves, while the volutes are more voracious predators, preying on other mollusks, particularly the conchs. There were significantly higher parasite infestation rates in the conchs compared with their natural predator, the volutes. This indicated that there was no trophic transfer involved, and the volutes are not functioning as terminal host, which is a common trait for many other parasites. Furthermore, the apicomplexa-like parasites wereable to complete their life cycle in single host (monoexious), as showed by the histological results where various stages were found in a single host. Thus it was suggested that the main route of apicomplexa infection probably from the sediment, during the conch feeding (grazing) on the surface sediment. Coccidian parasites are transmitted between hosts by the ingestion of food contaminated with oocysts, followed by the release of infectious sporozoites and invasion of cells and tissues. Since parasite in the sediment, in the water and the surroundings was not sampled, further studies are needed to confirm this hypothesis.

Conclusion

The present study is very important as a pioneer researchthatdescribed the presence of protozoan parasites in gastropods from Malaysian coastal waters. Currently, contamination of molluscs by pathogenic protozoa has been presented as an emerging aspect with implications for public health (Cardozo *et al*., 2013). Although there has not been reports of protozoan infections outbreaks related to the ingestion of seafood, the occurrence of Apicomplexa-like parasites has been reported in many bivalves and gastropods worldwide (Leal & Franco, 2008;Gros *et al*., 2009; Cardozo *et al*., 2013; Nur-Fauzana *et al*., 2014). Thus, there is potential risks of acquiring protozoosis by ingestion of raw or undercooked shellfish (Rose *et al*., 2002). This study also showed highest infestations in the conchs, which were harvested and consumed by the locals, thus need more detail examination. Information on health profile of tradedspecies are important not only to the research community, but also to the general public and the local authorities. Further studies are therefore suggested, and should probably be using more advanced approaches such as the molecular techniques, PCR-nested methods, the use of Ray's Fluid Thioglycollate Medium (RFTM) and in-vitro method.

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