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PARASITES OF WHITENOSE WHIPRAY (*Pateobatis uarnacoides*) IN TERENGGANU WATERS

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Abstract: This study focuses on the parasites of *Pateobatis uarnacoides*. The commercial value of *P.uarnacoides* creates a need for a comprehensive checklist of its parasites to ensure its safety and quality. This study aims to identify parasites of *P.uarnacoides* and analyse their prevalence and mean intensity in Terengganu waters. In October 2018, 30 *P. uarnacoides* samples were collected from Pulau Kambing Fisheries Complex. A total of 346 individual parasites were retrieved, representing three phyla, three classes, three orders, three families, and one genus. Class Hexanauplia, class Palaeacanthocepha and phylum Nematoda are represented by only a single individual. Ninty-nine percent (343 individuals) of all parasites collected were from the family Gnathiidae. Gnathiids have the most intense infection rate at 19 compared to all other parasites having the least intensity of one each. The mean intensity of parasites infecting *P.uarnacoides* is 5.5. The prevalence of infection of gnathiids is 60%, exceeding the prevalence of other parasites at 3.30% each. The site of attachment of gnathiids is limited to the gills. It was observed that the parasites found did not cause significant physical damage to the host or risk of mortality. However, a heavy infestation of gnathiids may cause difficulty breathing as it congests the gills, thus reducing the general fitness of the host. Overall, the results of this study show that the parasitic fauna of *P.uarnacoides* does not concern with public health and the economy. It also finds that this species is highly susceptible to infection by gnathiids, which was unrecorded in previous studies.

Keywords: South China Sea, Gnathiidae, *Pseudocharopinus* sp., Palaeacanthocepha, Nematoda, prevalence.

Introduction

Terengganu is a coastal state known for its notable fishing industry (Sulaiman & Saat, 2017). It is one of the major fisheries landing states in Peninsula Malaysia (Ahmad *et al*., 2004), largely due to its six fisheries complexes located in Pulau Kambing, Chendering, Dungun, Kuala Besut, Kuala Kemaman, and Marang. Pulau Kambing Fisheries Complex is the largest fish landing port in Kuala Terengganu that lands a steady supply of commercial fish including rays.

Rays make up 56% of all elasmobranch species in Malaysia, with 82 species known to inhabit its waters (Ali *et al*., 2013). They are common in fish markets and widely accepted as table food. Landings of rays in Malaysia experienced an increasing trend from 1985 to 2001. In 1985, rays landed an average of 6000 tonnes, but due to the increase in technology and demand, it rose to 16532 tonnes in 2001 (Ahmad *et al*., 2004). In 2014 alone, 16258.40 tonnes of rays were landed in Malaysia (DOF, 2014). Depending on the landing site, the dominant ray species can vary, but among the widely distributed species is *Pateobatis uarnacoides*.

Pateobatis uarnacoides (Bleeker, 1852), commonly known as Whitenose whip ray (Ali *et al*., 2013) or Bleeker's Whipray (White *et al*., 2016) and locally known as 'Pari pasir', is a species of ray belonging to the order Myliobatiformes, family Dasyatidae. They are inshore stingray that inhabit soft substrate, benthic areas of around 30m depth (White *et al*., 2006). They are a common food fish, and their meat is also salted and dried to be sold commercially. Dried seafood is one of the primary trading exports of Terengganu (Sulaiman & Saat, 2017).

However, seafood has been recorded to harbour many parasites that risk food safety, reduce product quality, and sour public opinion. The impact of parasitic infection cannot be underestimated. For example, in 2017, global prices of salmon soared after an infestation of sea lice rendered the most salmon inedible from farms in Canada, Scotland, Norway, the United States and Chile (The Star Online, 2017).

This study focuses on identifying organisms parasitising Pateobatis uarnacoides and determining the intensity (number of parasites per host) and prevalence (percentage of host infected) of the infection in Terengganu waters. As *Pateobatis uarnacoides* is a commercial fish in Terengganu, it is important to document parasite information and status of infection as it allows for the identification of the parasitic fauna, presence of potentially harmful parasites, and assess the status of infection in Terengganu waters. Thus, ensuring the safety and economic viability of seafood products to the consumers.

Materials and Methods

Sampling Area

Thirty samples were collected during October 2018 (North-East Monsoon season) from Pulau Kambing Fisheries Complex, Kuala Terengganu (Figure 1). The collection was done at dawn (6:00 to 7:00 am) when fishing vessels arrived at the port to land their catch.

Figure 1: Pulau Kambing Fisheries Complex, South of Kuala Terengganu (5°19'18.86"N, 103° 7'57.24"E). The red marker highlights the location and infrastructure of the complex

Sampling Methodology

Pateobatis uarnacoides were identified on-site based on Ali *et al.* (2013), then immediately purchased and transported in an ice-filled cooler. Upon arrival, the rays were measured, weighed, and the information was recorded. The rays were inspected for any external parasites. The parasites found were recorded in the data sheet and then preserved with 70% ethanol in an Eppendorf tube. The specimens were then separated into individually labelled (specimen no, date, etc.) plastic bags and placed in a freezer.

Parasitological Examination

Dissection began with the snout through the nasal cavity and was inspected for parasites. The dissection of its softer ventral side was followed. A horizontal incision was then made below the mouth, followed by a vertical incision from the mouth to the cloaca. The skin was cut away slowly at the abdominal area to reveal the body cavity. An inspection was made of the body cavity and the surface of the visceral organs for parasites. All internal organs were proceeded to be carefully removed. Cuts were made along the gill slits to remove the entire section of the body containing the gills. Inspection of the branchial chamber and removal of each gill were made for further inspection. The skull was sliced, and the brain was scooped out using a spatula. A thorough inspection of each organ was carried out using a dissecting microscope, followed by an in-depth inspection of the organ tissue and any liquids using a compound microscope. Any parasites found were recorded in a data sheet and then preserved with 70% ethanol in a labelled Eppendorf tube. Photographs of it were taken under a compound microscope using a dino eye. Selected parasites will then be photographed using a scanning electron microscope. Identification of the parasites was done by referring to Brusca *et al.* (2001), Arai & Smith (2016), Margolist & Kabata (1989), and Arthur & Shariff (2015).

Results and Discussion

Ray collection data

A total of 30 *Pateobatis urnacoides* samples were examined, comprising ten females and twenty males. Referring to Table 1, the weight of samples varied greatly and ranged from a minimum of 59.69 g to a maximum of 446.55 g. The mean weight of samples is 153.34 g with a standard deviation of 90.91. The disk width and length shared the same minimum length of 13.2 cm, whereas the maximum is 23.5 cm and 48.5 cm, respectively. The range of length is 22.5 cm to 55.4 cm, with a mean of 39.31 cm. The mean disk width for *P. urnacoides* samples is 16.96 cm and the mean disk length is 18.51 cm. Juvenile *P. urnacoides* were around 24.2cm disc width and reached sexual maturity of 76 cm disc width (White *et al*., 2016), the ray samples are fully comprised of juveniles.

Table 1: Total number, minimum, maximum, mean, and standard deviation of weight and length of *P. urnacoides*

Pateobatis urnacoides	N	Min	Max	Mean	Standard Deviation
Weight (g)	30	59.6	446.55	153.34	90.91
Length (cm)	30	22.5	55.4	39.31	7.6
Disk Length (cm)	30	13.2	48.5	18.51	6.87
Disk Width (cm)	30	13.2	23.5	16.96	2.71

Gnathiidae (Leach, 1814)

Phylum	: Arthropoda
Class	: Malacostraca
Order	: Isopoda
Family	: Gnathiidae
Infection Site	: Gills
Prevalence	: 60%
Mean intensity	: 19
Number of individuals	: 323

Diagnostic Remarks:

Gnathiid parasites are immediately recognisable by their highly expanded posterior region of the pereon. Pleon is abruptly narrower than the pereon (Figure 2A). Five free pleonites (including the pleotelson), five pairs of pereopods, and six free pereonites (Figure 2C, D). The eyes of the specimen are well developed (Figure 2B). As all specimens representing this family are juveniles, thus it could not be further identified using physical morphology alone as the taxonomy of this suborder is based solely on adult males (Brusca *et al*., 2001). Of the 346 individual parasites recovered, 90% (343 individuals) are gnathiids. The parasites are all attached to the inter-branchial septum of the gills (Figure 3).

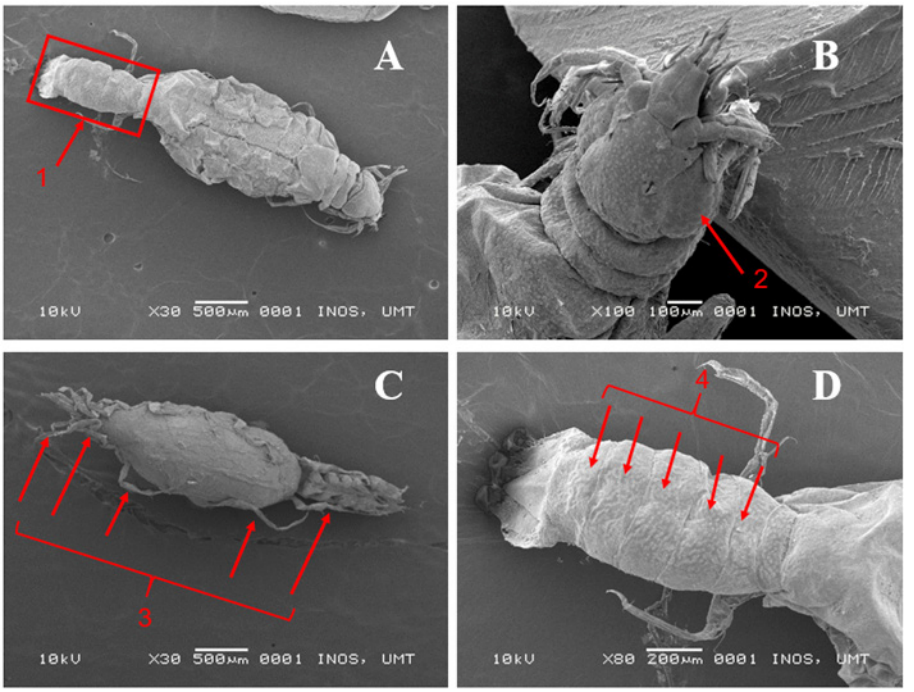

Figure 2: Morphology of Gnathiidae. A) Dorsal view of Gnathiidae; B) Head of Gnathiidae; C) Ventral view of Gnathiidae; D) Pleon of Gnathiidae. 1 - Pleon is abruptly narrower than the pereon; 2 - Well developed eyes; 3 - Five pairs of pereopods; 4 - Five free pleonites (plus the pleotelson)

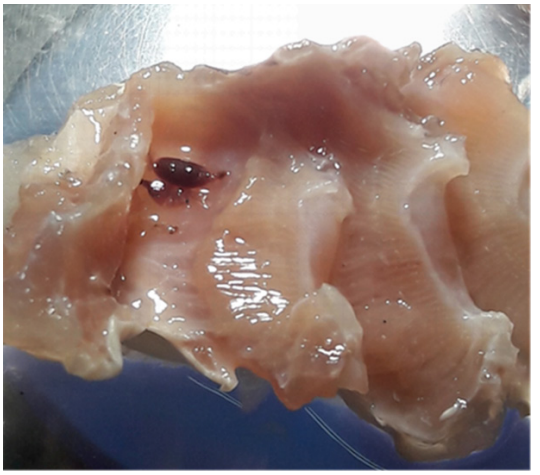

Figure 3: Gnathiids attached to the inter-branchial septum of *P. uarnacoides*

***Pseudocharopinus* sp. (Kabata, 1964)**

Phylum	: Arthropoda
Class	: Hexanauplia
Order	: Siphonostomatoida
Family	: Lernaeopodidae
Genus	: *Pseudocharopinus*
Infection Site	: Gills
Prevalence	: 3.3%
Mean intensity	: 1
Number of individuals	: 1

Diagnostic Remarks:

The specimen is identified as *Pseudocharopinus* sp. by the presence of bulla, posterior trunk, subcylindrical cephalothorax, modified second maxillae, and the absence of legs (Figure 4A). Maxillipeds are near the mouth cone (Figure 4C). Specimen recovered is recognised as female as the second maxillae are altered into arm-like structures fused at the tips. The method of attachment to the host's membrane is by the branching tips of the second maxillae (Figure 4D). The first maxillae possess three setiferous papillae on the endopod and the exopod is placed laterally to the endopod (Figure 4C). *Pseudocharopinus* sp. found was attached to the inter-branchial septum of the host's gills. During the removal process, part of the second maxillae was severed, as seen in Figure 4A. Only one individual representing this genus was able to be recovered.

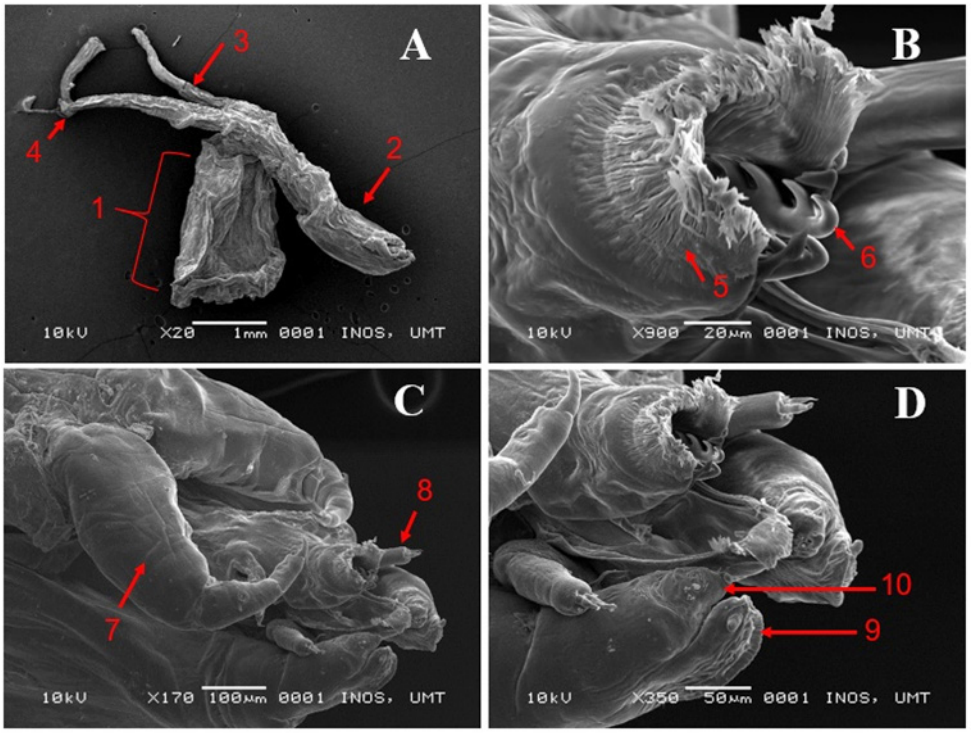

Figure 4: Morphology of Pseudocharopinus sp. A) Full body of Pseudocharopinus sp. B) Mouth of Pseudocharopinus sp. C) First maxillae of Pseudocharopinus sp. D) Buccal region of Pseudocharopinus sp. 1 – trunk; 2 – cephalothorax; 3 - second maxillae; 4 – tip of second maxillae; 5 - branching tips of second maxillae; 6 – mandible; 7 – maxilliped; 8 - first antenna; 9 – exopod; 10 – endopod

Phylum Nematoda

Phylum	: Nematoda
Infection Site	: Liver
Prevalence	: 3.3%
Mean intensity	: 1
Number of individuals	: 1
Diagnostic Remarks	:

The specimen is identified to belong to the Phylum Nematoda by its long, tubular, unsegmented body with triradiate symmetry. The mouth of the specimen is located at the anterior end, while the anus is near the posterior end. Both the anterior and posterior ends are tapered. Nematode specimen is likely female as the tail lacks a curve or broad, fan-shaped bursa. A single individual only represents the taxon. This specimen was recovered on the surface of the liver, with liver tissue partially covering the nematode. This parasite cannot be further identified past phylum level as the stiff and coiled condition, coupled with the loss of the specimen during the identification process, and the lack of SEM photograph of the mouth of the specimen prevented further identification.

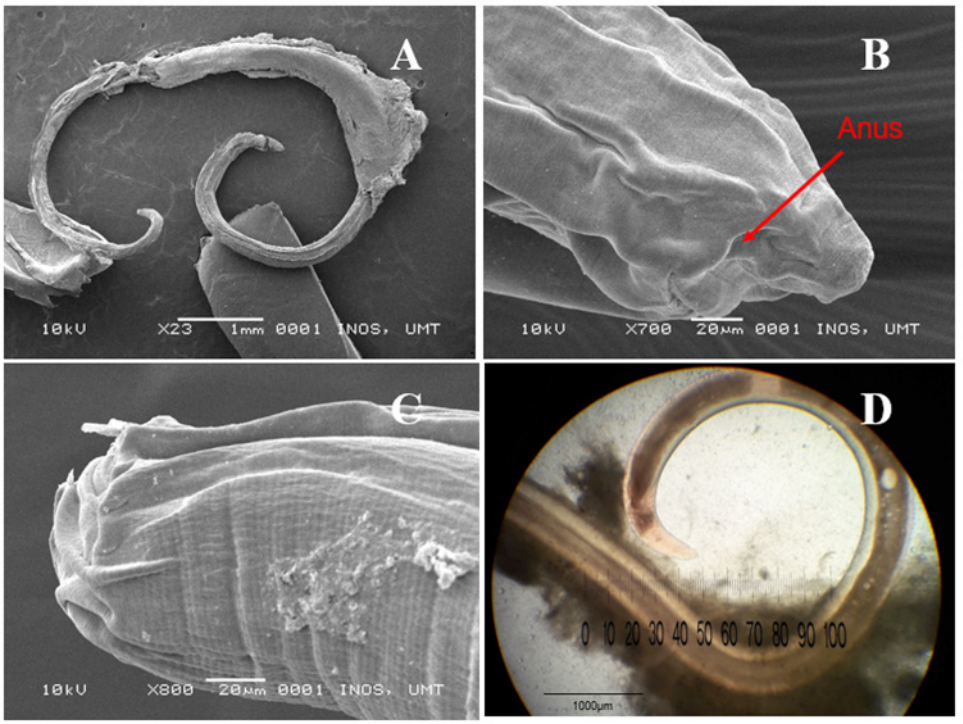

Figure 5: Morphology of Nematoda. A) Full body of Nematoda. B) Posterior region of Nematoda. C) Anterior region of Nematoda. D) Fresh Nematoda under dissecting microscope

Pomphorhynchidae (Yamaguti, 1939)

Phylum	: Acanthocephala
Class	: Palaeacanthocephala
Order	: Echinorhynchida
Family	: Pomphorhynchidae
Infection Site	: Intestine
Prevalence	: 3.3%
Mean intensity	: 1
Number of individuals	: 1

Diagnostic Remarks:

The specimen is worm-like and un-segmented throughout the main body axis. Pomphorhynchidae is distinguished by the appearance of a spiny proboscis followed by a long double-walled proboscis receptacle located at its base (Figure 6C). The proboscis receptacle lacks a nuclear pouch on the outer wall. The specimen neck is also long and cylindrical without bulbous swelling. The main longitudinal lacunar is placed laterally in the body. The specimen is identified as a female due to female genitalia (refer to Figure 6B). Parasite of the family Pomphorhynchidae is common in marine fishes (Amin, 1988). Only one individual representing this family is recovered suspended in the intestinal fluid of *P. uarnacoides* host. As the specimen was exceedingly small, there was much difficulty in transferring and preparing the sample to be photographed using a SEM. This resulted in the loss of specimens and a lack of SEM photographs.

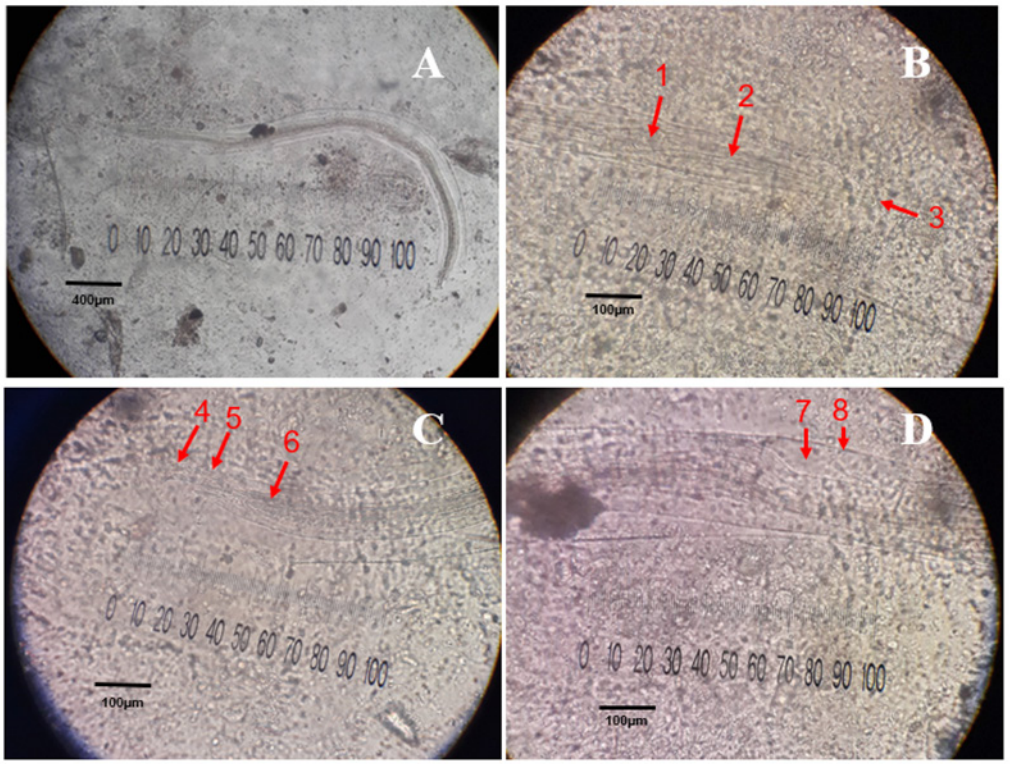

Figure 6: Morphology of Pomphorhynchidae. A) Full body of Pomphorhynchidae. B) Posterior region of Pomphorhynchidae. C) Anterior region of Pomphorhynchidae. D) Trunk of Pomphorhynchidae. 1 – uterine bell; 2 – uterus; 3 – genital pore; 4 – spiny proboscis; 5 - lemnisci; 7 – body cavity; 8 – body wall.

Discussion

Known Parasitic Assemblage of P. uarnacoides

Most samples in the studies of parasites infecting *P. uarnacoides* are collected from east Malaysia and Borneo. As listed by Pollerspöck (2018), a study by Srivastav and Capoor (1980) shows the presence of *Hexacanalis sasoonensis* infecting *P. uarnacoides* in Bombay waters, India. As the source journal could not be recovered, it is not listed in the reference section. In reference to table 2, parasites of *P. uarnacoides* comprise 15 genus and 26 species. In a study of elasmobranch parasites in Terengganu waters by Ihwan *et al*. in 2013, it was discovered that parasitic infections were mainly dominated by digenean and gnathiid parasites. However, the sample species selection did not include stingrays of the family Dasyatidae. All parasites retrieved in this study are common elasmobranch parasites, yet they have not been specifically recorded to infect *P. uarnacoides* (refer to table 2).

Table 2: List of known parasites of *P. uarnacoides*

Class	Species	Area of infection	References
Monogenea	*Merizocotyle rhadinopeos*	Nasal Tissue	Chisholm & Whittington, 2012
Monogenea	*Empruthotrema dasyatidis*	Nasal Tissue	Arthur & Shariff, 2015
Rhabditophora	*Acanthobothrium foulki*	Spiral Intestine	Arthur & Shariff, 2015
Rhabditophora	*Acanthobothrium dighaensis*	Spiral Intestine	Srivastava & Capoor, 1980
Rhabditophora	*Acanthobothrium gnomus*	Spiral Intestine	Arthur & Shariff, 2015
Rhabditophora	*Acanthobothrium larsoni*	Spiral Intestine	Arthur & Shariff, 2015
Rhabditophora	*Acanthobothrium lepidum*	Spiral Intestine	Arthur & Shariff, 2015
Rhabditophora	*Acanthobothrium tetabuanenese*	Spiral Intestine	Arthur & Shariff, 2015
Rhabditophora	*Acanthocephalum* sp.	Spiral Intestine	Arthur & Shariff, 2015
Rhabditophora	*Diphyllidea* sp.	Spiral Intestine	Arthur & Shariff, 2015
Rhabditophora	*Lecanicephalidea* sp.	Spiral Intestine	Arthur & Shariff, 2015
Rhabditophora	*Rhinebothrium* sp.	Spiral Intestine	Arthur & Shariff, 2015
Rhabditophora	*Trypanorhyncha* sp.	Musculature, Spiral Intestine	Arthur & Shariff, 2015
Rhabditophora	*Zygorhychus borneensis*	Spiral Intestine	Arthur & Shariff, 2015
Rhabditophora	*Anthocephalum decrisantisorum*	Spiral Intestine	Ruhnke *et al*., 2015
Rhabditophora	*Dollfusiella angustiformis*	Spiral Intestine	Schaeffner & Beveridge, 2013
Rhabditophora	*Dollfusiella hemispinosa*	Spiral Intestine	Schaeffner & Beveridge, 2013
Rhabditophora	*Dollfusiella spinulifera*	Spiral Intestine	Schaeffner & Beveridge, 2013
Rhabditophora	*Kotorella pronosoma*	Spiral Intestine	Schaeffner & Beveridge, 2014
Rhabditophora	*Prochristianella aciculata*	Spiral Intestine	Schaeffner & Beveridge, 2012
Rhabditophora	*Prochristianella monomegacantha*	Spiral Intestine	Schaeffner & Beveridge, 2014
Rhabditophora	*Prochristianella clarkeae*	Spiral Intestine	Schaeffner & Beveridge, 2012
Rhabditophora	*Prochristianella indonesiensis*	Spiral Intestine	Schaeffner & Beveridge, 2014
Rhabditophora	*Prochristianella baverstocki*	Spiral Intestine	Schaeffner & Beveridge, 2014
Rhabditophora	*Pterobothrium platycephalum*	Spiral Intestine	Schaeffner & Beveridge, 2012
Rhabditophora	*Halysiorhynchus macrocephalus*	Spiral Intestine	Schaeffner & Beveridge, 2014

Gnathiidae parasites of P. uarnacoides in Terengganu waters

According to the results of this study, 60% of all *P. uarnacoides* samples are infected by gnathiids and the mean number of individuals per host is 19. The heavy infection of gnathiids is consistent with findings from a study of parasitic infections of elasmobranch on the east coast of peninsular Malaysia by Ihwan (2013). This indicates that gnathiid parasites are common and occur in high numbers within Terengganu waters. Although the infection of gnathiid is highly prevalent and intense in *P. urnacoides*, consumers are not likely to encounter the parasite due to common preparation methods. As only the pectoral fins of rays would be consumed and the main body disposed of, there is very little need for consumers to view the gills, ergo consumers are not likely to come across gill parasites such as *Pseudocharopinus* sp. and gnathiids. Gnathiids are also frequently mistaken for blood clots due to the colour and shape of their bodies when engorged with blood.

Gnathiids are the parasitic larval stage of certain isopods (Stachowitsch, 1992). Gnathiids are common in marine and estuarine habitats (Smith *et al*., 2004). These isopods are not limited to one host throughout their parasitic larval stage. After feeding on the blood or other bodily fluid of the host, it will swim down to the benthos to moult and then infect a new host (Smith *et al*., 2004). The high infection rate in Terengganu waters is most likely due to their voracious nature, high mobility, low host specificity and ability to continue living apart from the host for significant periods. *P. urnacoides* are especially susceptible to infection as it also inhabits the same benthic environment as gnathiids. Moreover, *P. urnacoides* are also constantly gliding on the seafloor. The dorsal side of the body containing the gill slits is exposed and accessible to gnathiids exchanging hosts after moulting. Currently, there have not been any reports indicating the occurrence of disease or mortality of chondrichthyan directly caused by this parasite (Smith *et al*., 2004). In this study, the maximum number of gnathiid to infect a single host was 82 individuals. The gills of that host was heavily congested and the host would have difficulty breathing as the parasite obstructed the gill slit opening and filled the buccal cavity.

Pseudocharopinus* sp. *parasites of P. uarnacoides in Terengganu waters

Pseudocharopinus is a genus of marine parasite consists of 11 species, which are *Pseudocharopinus bicaudatus, Pseudocharopinus concavus, Pseudocharopinus dasyaticus, Pseudocharopinus dentatus, Pseudocharopinus malleus, Pseudocharopinus markewitschi, Pseudocharopinus narcinae, Pseudocharopinus pillaii, Pseudocharopinus pteromylaei, Pseudocharopinus pteroplateae,* and *Pseudocharopinus squali* (Kabata, 1988). The range of distribution of this genus is unknown, however, it is believed that some of the 11 species are cosmopolitan (Kabata, 1988).

Pseudocharopinus sp. is generally known to infect elasmobranchs in all regions of the world (Kabata, 1988). Thus it is highly probable that it would infect *P. urnacoides* in Terengganu waters. The impact this genus has on its host parasites is similar to gnathiids as it may cause skin and gill lesions that can lead to secondary infections, blood loss, osmotic imbalance or other deleterious metabolic demands (Kabata, 1988). However, it is unlikely to be the case in this study as the mean intensity and prevalence of this parasite are incredibly low. Although *Pseudocharopinus* is highly specific to elasmobranch, not much else is known about this parasite.

Nematode parasites of P. uarnacoides in Terengganu waters

Nematodes are invertebrate roundworms that inhabit marine, freshwater, and terrestrial areas. They can be found throughout the globe and parasitise a wide range of hosts, including humans and animals, primarily fishes. There are ten families of nematodes, however only one family (Acanthocheilidae) of nematodes are restricted to parasitise chondrichthyans

(Benz, 2004). In this study, only 1 unidentified species of nematode was recovered from the intestine of *P. uarnacoides*. The lifecycle of nematodes that infect chondrichthyans is poorly understood, however few studies have suggested that nematodes use chondrichthyans as definitive, intermediate, and paratenic hosts, in which the transmission between hosts is the result of predation (Benz, 2004). A study by Love and Moser (1983) found that 70% of 17 nematode species (representing six families) were restricted to chondrichthyans and 30% infected other fishes including chondrichthyans.

It is possible that the nematode recovered in this study could be of the family Acanthocheilidae as members of this family are only restricted to chondrichthyans (Benz, 2004). Attachment of the nematode parasite to the liver could pose a health risk to *P. uarnacoides* as the feeding and tissue migration caused by the nematode can weaken the liver. Not only can it inhibit liver functions, but it can also disrupt depth regulation and buoyancy of *P. uarnacoides*.

Pomphorhynchidae parasites of P. uarnacoides in Terengganu waters

Pomphorhynchidae is a family of parasite worms under the phylum Acanthocephala, commonly known as thorny-headed or spiny-headed worms. The family Pomphorhynchidae consists of five genera: *Longicollum*, *Paralongicollum*, *Pomphorhynchus*, *Pyriproboscis*, *Tenuiproboscis*. Most family representatives inhabit the marine environment except *Pyriproboscis* inhabiting freshwater areas (Amin *et al*., 2003). Based on the result of this study, only one individual from the family Pomphorhynchidae from the phylum Acanthocephala was found in *P. uarnacoides* intestine.

The occurrence of this parasite could be due to the life cycle of Acanthocephala as the parasite is known to parasitise crustacean during their development phase (Amin *et al*., 2003). The diet of *P. uarnacoides* consists of benthic organisms such as small crustaceans (Amin *et al.,* 2003) and evidence from this study has confirmed that marine prawn was found in the stomach of *P. uarnacoides*. Thus, the predation of infected marine shrimp could contribute to the presence of Acanthocephala (Family: Pomphorhynchidae) in *P. uarnacoides.*

Conclusion

From 30 *P. urnacoides* samples, 346 individual parasites were successfully recovered. All parasites that were retrieved consisted of three phyla (Arthropoda, Nematoda and Acanthocephala), three classes (Malacostraca, Hexanauplia and Palaeacanthocepha), three orders (Isopoda, Siphonostomatoida and Echinorhynchid), three families (Gnathiidae, Lernaeopodidae, and Pomphorhynchidae), and one genus (Pseudocharopinus), have not been explicitly recorded to infect *P. uarnacoides* (refer to table 2).

However, they are common elasmobranch parasites thus, it was probable for them to be found in *P. uarnacoides*. *Pseudocharopinus* sp. and gnathiids are not known to be zoonotic and thus pose no health risk to the public. Typically, the infection of almost one or no parasite per infected fish is not significant enough to cause stress or mortality to the fish, especially in the natural environment.

Fortunately, *Anisakis* sp. has not been recorded to infect *P. uarnacoides* (refer to Table 2). Assuming the parasite found is from the family Acanthocheilidae, the specimen would be very host specific and would not be able to infect humans. Therefore, proper preparation and the low mean intensity and prevalence of the parasites suggest that parasites of *P. uarnacoides* are generally not concerning to human health and do not pose any problems economically.

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