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Endoparasitic Infection in Threadfin Bream (*Nemipterus hexodon and N. japonicus*) from Department Store in Chiang Mai Province, Thailand

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ABSTRACT

A survey was undertaken to find endoparasites in ornate threadfin bream (*Nemipterus hexodon*) and Japanese threadfin bream (*N. japonicus*) from department store in Chiang Mai province which were originally sent from Chachoengsao province. Fishes were collected from December 2018 to January 2019. A total number of 40 fishes were investigated by using light microscope. Thirty-three fishes were infected by *Anisakis* sp. third-stage larvae (L3) which has distinctive characteristics such as projecting boring tooth and rounded tail bears a mucron. The prevalence of *Anisakis* sp. in *N. hexodon* and *N. japonicus* was 75 and 90%, respectively. Intensity in *N. hexodon* and *N. japonicus* was 4.40 and 5.28 and abundance was 3.30 and 4.75, respectively. Anisakid nematode in third-stage larvae affect a growing number of people in developed countries, which are important endoparasitic nematode causing anisakiasis that make sudden epigastric pain, nausea, vomiting, diarrhea and urticaria in humans who consumed raw or undercooked seafood; for example, sushi and sashimi that are very popular foods in Japan and Thailand. Moreover, it is suspected that *N. japonicus* were infected by *Camallanus* sp. and *Rhadinorhynchus* sp. with 20 and 15% of prevalence that not found in *N. hexodon*. Based on the results of this study with high prevalence of *Anisakis* sp. and other infected parasites, strategies to prevent the potential or allergies risks of anisakiasis to consumers were suggested.

INTRODUCTION

At present, proteins requirement from natural sources especially from the sea fish that provided high protein are increasing. In Thailand, the fisheries industry exported many tons of several fish from the Gulf of Thailand with high economic values according to report by Information and Communication Technology Center (ICTC), Ministry of Commerce. Threadfin bream or Pla-Sai-Dang (*Nemipterus* spp.) in order Perciformes (**Figure 1**) is one of the fishes which has enormous export value, but the amount of fish caught are decreasing. One possible cause is the endoparasitic infection that causing abnormal growth in the fish.

Endoparasite is a parasite that lives in the body of living organisms. One of the most well-known endoparasite is Nematode: an unsegmented roundworm in Phylum Nematoda that has been found in almost every conceivable habitats [1-2]. *Anisakis* spp. is nematode worms of the families Anisakidae that adult stage is cosmopolitan parasites of mammals, marine fish and fish-eating birds. The first intermediate hosts are crustaceans and second intermediate (paratenic hosts) are many species of cephalopods and saltwater fish, that are the source of infection for birds and mammals (human are accidental hosts), which harbor anisakid in third-stage larvae (L3) in the coelomic cavity, viscera or muscles of the secondary intermediate hosts [3]. In Thailand, there are some report on study in some fishes that were infected by *Anisakis* spp., for example; *Johnius carouna* and

Dendrophysa russelli with high prevalence, 31.7% and 87.5%, respectively [4]. However, the study of parasitic infections in threadfin bream in Thailand still low.

Anisakiasis is a human disease caused by the accidental ingestion of third-stage larval of nematodes belonging to the family Anisakidae. Human acquired the infection by eating raw seafood dishes such as sushi and sashimi that are traditional Japanese dishes known worldwide and popular in Thailand, or undercooked fish and squid dishes. The clinical presentation of anisakiasis in human including sudden epigastric pain, nausea, vomiting, diarrhea and urticarial, it can occur 1 to 12 hrs. after the infected meal with most occurring within 6 hrs. but the time and the duration of abdominal pain, nausea and vomiting is about 1 to 5 days [5,6]. The first case in Thailand was reported in 1993, the

Figure 1. Sampling fishes (*Nemipterus hexodon* and *N. japonicus*) from the department store in Chiang Mai province those were sent from Chachoengsao province.

patient showed the symptoms of acute abdominal obstruction and diagnosis was obtained by identification of the parasite in the tissue sections of the resected segment of the small intestine [7].

Other important endoparasites are acanthocephalans or thorny-headed worms in phylum Acanthocephala. The adults are always parasites in the intestine of many vertebrates. In the definitive host, the proboscis – a retractile organ armed with spines – of parasites attached to intestinal mucosa of the hosts causing primarily traumatic and weight loss. In some study found that the parasites can change the behaviour of the intermediate hosts in order to increase the probability of being eaten by the definitive hosts. [8,9]

The purpose of this study is to survey endoparasites in threadfin bream (*Nemipterus hexodon* and *N. japonicus*) from department store in Chiang Mai province based on morphological characteristics by the study using light microscope. The result from this study will be useful to prevent the decrease of threadfin bream or other fish caused by endoparasitic infection as well as preventing parasitic diseases in human.

METHODOLOGY

Fish collections

Threadfin bream (*Nemipterus hexodon* and *N. japonicus*) (n=40, 20 each species) were purchased from a department store in Chiang Mai province, these fish were sent from Chachoengsao province. Samples were transferred under refrigerated conditions (4 ºC) to the laboratory for examination. The fish species were identified according to their morphological characteristics. Standard length (cm) and weight (g) of the sampling fishes were recorded.

Collection of endoparasites

The fishes were examined for endoparasites in the gastrointestinal tract (GI) and the body cavity under stereo microscope. The parasite larvae in the visceral cavity, liver and small intestine, were collected and placed in normal saline (NaCl 0.85%). Morphology of the parasites were studied under light microscope.

Permanent slides and identification of parasites

The nematodes and acanthocephalans were prepared for morphological investigation. Parasites were fixed in 4% formalin and dehydrated with graded alcohol series, cleared with xylene, and mounted in permount. The permanent slides were illustrated using compound microscope with a drawing tube. Measurement were performed using an ocular micrometer and showed in micrometers (µm). The species were identified based on morphology according to journals and textbooks [10-15].

RESULTS

Average length and weight of sampling fishes were showed in **Table 1**. *Nemipterus hexodon* was significant longer and heavier than *N. japonicus* (P = 0.05 for all comparison).

The sampling fishes were infected by nematodes and acanthocephalans, *N. hexodon* were infected by *Anisakis* sp. with 75% of prevalence mean while *N. japonicus* were 90%. All *Anisakis* sp. that were infected both *N. hexodon* and *N. japonicus* were the same morphology including body elongated, an anterior part with a projecting boring tooth, transverse striation of the body cuticle, the proventricular followed by ventriculus, oblique joint between ventriculus and intestine and rounded tail with terminal mucron in **Figure 3**. The distribution of anisakid lavae in the fish body were almost in the body cavity (99.38%) and some in the liver (0.62%).

Intensity and abundance of *Anisakis* sp. in *N. hexodon* was 4.40 and 3.30 while *N. japonicus* was 5.28 and 4.75.

In addition, *N. japonicus* were infected by nematode (*Camallanus* sp.) and acanthocephalan (*Rhadinorhynchus* sp.) with 20% and 15% of prevalence. The morphology of *Camallanus* sp. including body fusiform, mouth aperture slit-shaped, buccal capsule with longitudinal ridges, some incomplete ridges followed by several small dots arranged in longitudinal rows or irregularly scattered, capsule well-developed with orange-brown color typical of the genus. The morphology of *Rhadinorhynchus* sp. including body cylindrical, trunk spines separated by a spinose area into two groups, proboscis long with many of spines, proboscis receptacle long and double-walled, lemnisci long and slender. For *Camallanus* sp. and *Rhadinorhynchus* sp., the distribution was only in the intestine.

DISCUSSION

This study was the first report of endoparasites in threadfin bream (*Nemipterus* spp.) from Chachoengsao province being sold in the department store in Chiang Mai province, northern of Thailand. There

Table 1. Average length and weight of sampling fishes.

Fish species	Mean of fish length (cm)	Mean of fish weight (g)
Nemipterus hexodon	18.97	182.75
N. japonicus	18.22	160.45

Table 2. Prevalence, intensity and abundance of *Anisakis* sp. in *Nemipterus hexodon* and *N. japonicus* from department store in Chiang Mai province.

Fish hosts and parasites	No. of total fishes	No. of infected fishes	No. of worms	Prevalence	Intensity	Abundance
Nemipterus hexodon						
Nematoda						
- *Anisakis* sp.	20	15	66	75%	4.40	3.30
Nemipterus japonicus						
Nematoda						
- *Anisakis* sp.	20	18	95	90%	5.28	4.75
- *Camallanus* sp.	20	4	10	20%	2.50	0.50
Acanthocephala						
- *Rhadinorhynchus* sp.	20	3	6	15%	2.00	0.30

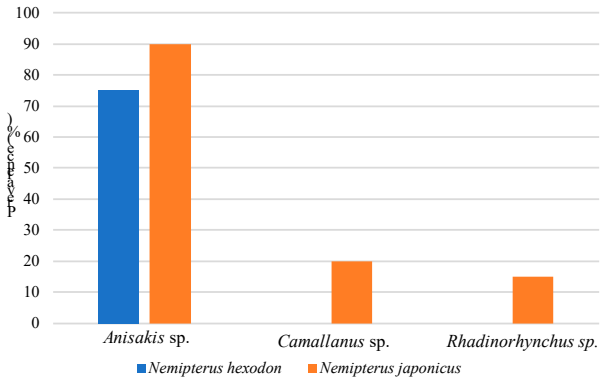

Figure 2. The total prevalence of endoparasites in sampling fishes (*Nemipterus hexodon* and *N. japonicus*) from department store in Chiang Mai province.

were a high prevalence of *Anisakis* sp. in both fish species (*Nemipterus hexodon* and *N. japonicus)* that were similar to a previous report [16] which study in the Perciformes fish collected from the coast of Chonburi province, where is closed to Chachoengsao province, the prevalence of *Johnius carouna* and *Dendrophysa russelli* were 31.7% and 87.5%, respectively.

But the density of recent study were higher than a pervious study (*N. hexodon* = 4.40, *N. japonicus* = 5.28 vs *J. carouna* = 2.4, *D. russelli* = 3.9) [16]. It was suspected that *N. japonicus* were infected more than *N. hexodon* but still need additional study. The morphology of *Anisakis* sp. in the study were identified as *Anisakis simplex* and they were similar to recent study [10-12], possibly *Anisakis* sp. in this study were *Anisakis simplex.* However, due to the third-stage larvae are unclearly identified by using only study of morphology under light microscope, Scanning Electron Microscope (SEM) or molecular techniques may be required.

Almost all Anisakid larvae in the present study were found in the body cavity, and it was higher than a previous report by Abollo *et al*. (2001), they studied 28 species of marine fish from the Galician coast, Spain, and the nematodes is found in body cavity (≥58.9%) and other organs including liver, stomach, mesenteries, gonads and musculature [17]. According to Stromnes *et al*. (1998) who proposed that the distribution of *Anisakis* sp. mainly in the viscera and the muscle of fish, possibly related to the availability of exploitable nutrients and encapsulation [18]. But there is no definite conclusion for the distribution of Anisakids larvae.

In the previous parasitic infection studies in Japanese threadfin bream (*N. japonicus*) reported that they were infected by nematodes and acanthocephalans [19-22]. One of infected nematode that were found is *Camallanus* which is also found in many of fishes and amphibians. Moravec *et al*. (2006) reported *Camallanus carangis* infection from three fish hosts in New Caledonia, in the southwest Pacific Ocean, one of them is *Nemipterus furcosus* or fork-tailed threadfin bream.

The report of *Camallanus* sp. infection in marine fishes were mostly from the order Perciformes in the warmer Indo-Pacific regions but the morphological explanation was incorrect or unusable type specimens [23]. In Thailand, *Camallanus* sp. is found in the marine fishes such as *Johnius coitor* (order Perciformes) from the coastal ecosystem of the Andaman Sea near Tarutao Island, Satun province, the prevalence of infection was higher than the present study (47.5%) [24].

In addition, a report by Amin (2013) reveal the low incidence of infection (15%) of *Rhadinorhynchus* sp. (Acanthocephala: Rhadinorhynchidae) in *N. japonicus* [25]. Smales (2014) report and describe four new species and five other species of the genus *Rhadinorhynchus* from many Perciformes fishes in Australia such as *Caranx mertensii, Trachinotus botla, T. coppingeri, T. bailonii* and *T. coppingeri* [26]. Even in Amin *et al.* (2017) report the new species (*Rhadinorhynchus oligospinosus* n. sp.) from Perciformes fishes include *Scomber japonicus* and *Trachurus murphyi* [27].

In conclusion, the high prevalence of *Anisakis* sp. and some of other helminths in marine fish from the department store in Chiang Mai, Thailand in the present study will be an useful information for responsible government agencies in order to plan to control the distribution of helminths that may cause economic damage by decreasing of fishes or even reducing the risk of parasitic disease in human caused by the consumption of infected fishes.

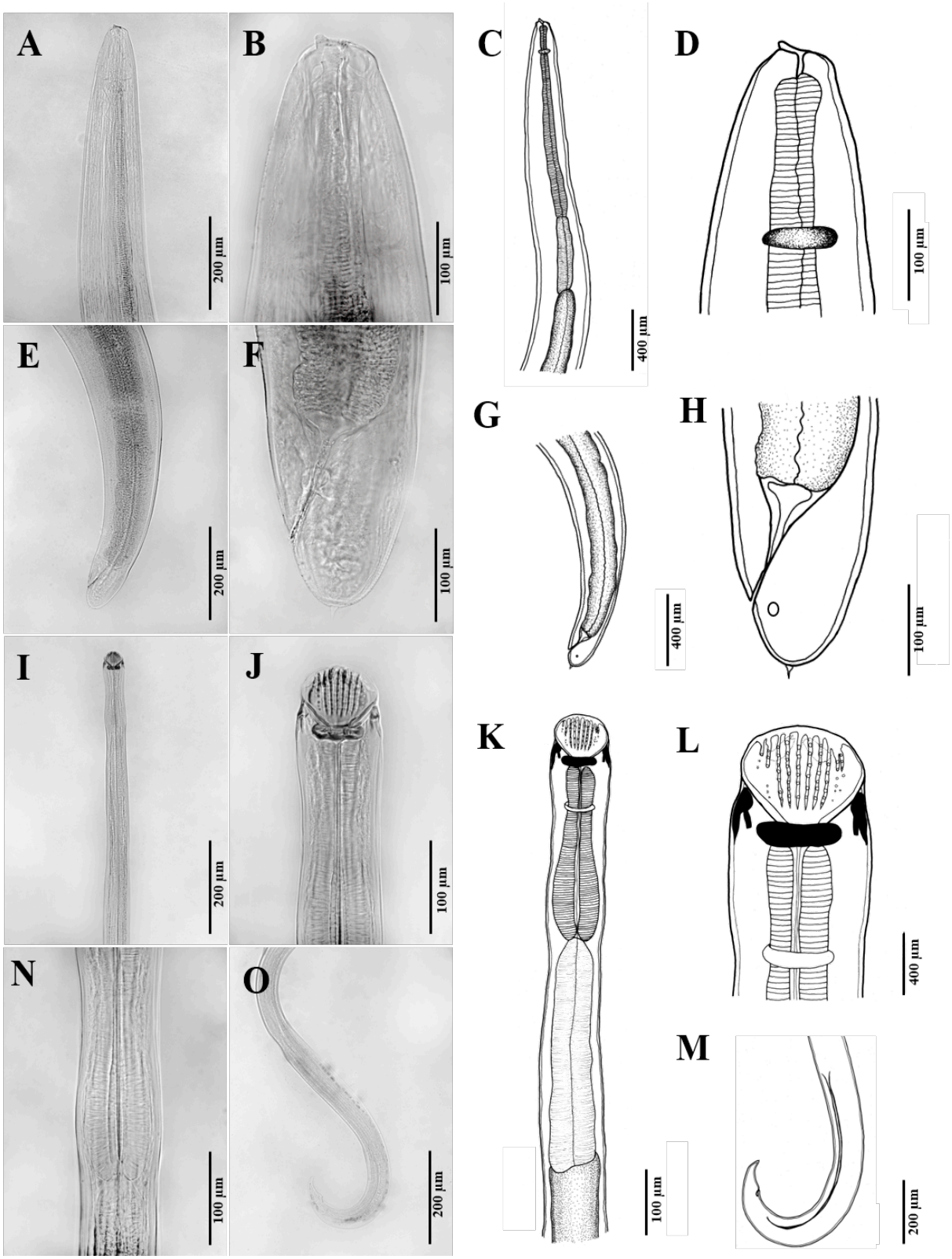

Figure 3. Anterior part (A, B) and posterior part (E, F) of *Anisakis* sp. under light microscope. Drawing of anterior part with a projecting boring tooth (C, D) and posterior part with terminal mucron (G, H). Anterior part (I, J), esophagus bulb (N) and posterior part of male *Camallanus* sp. (O) under light microscope. Drawing of anterior part with cephalic extremity, nerve ring, esophagus and intestine (K), buccal capsule, lateral view (L) and posterior part with spicules (M).

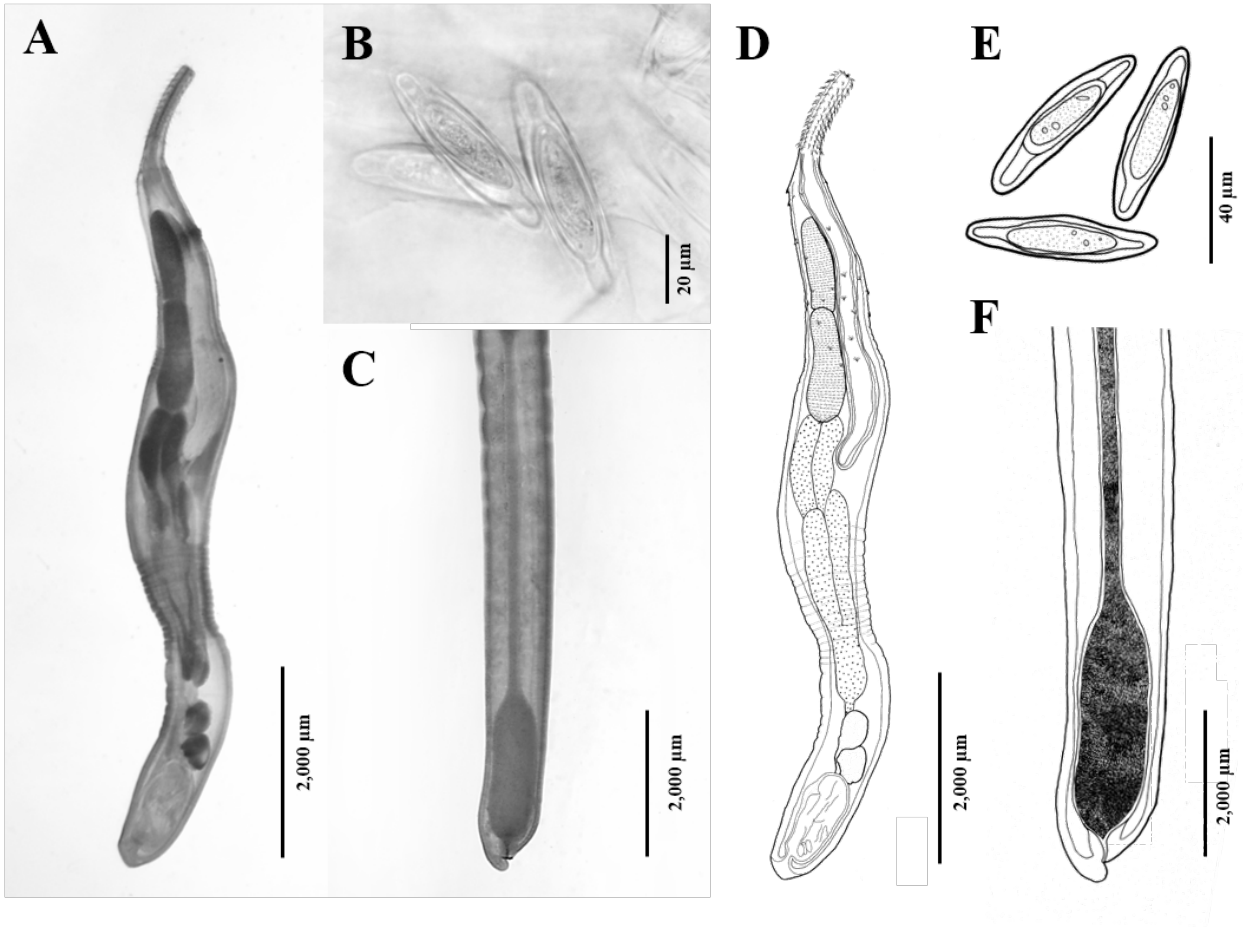

Figure 4. Male (A), eggs (B) and posterior part of female *Rhadinorhynchus* sp. (C) under light microscope. Drawing of male (D), eggs (E) and posterior part of female *Rhadinorhynchus* sp. (F).

CONFLICT OF INTEREST

We have no conflict of interest related to this study.

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