

A NEW RECORD OF *CLINOSTOMUM PHILIPPINENSIS* (VALASQUEZ, 1959) IN *TRICHOGASTER MICROLEPIS* (GÜNTHER, 1861) FROM BUNG BORAPET, NAKHON SAWAN, THAILAND

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Abstract. Thirty-six freshwater fish from Bung Borapet, a large reservoir, were examined for helminths in July 2005. The prevalence of infection was 19.44 (7/36) and the intensity was 4.67 (168/36) per fish. Seven fish were infected with 4 species of helminths: 1 trematode, *Clinostomum philippinensis* (metacercariae); 1 cestode, *Senga Chiangmaiensis*; 1 acanthocephalan, *Pallisentis* sp and 1 nematode, *Camallanus anabantis*. An encysted metacercaria of *Clinostomum philippinensis* was found in the gills of *Trichogaster microlepis*. The present study is a new report of *Clinostomum* metacercariae in the second intermediate host, *T. microlepis*, in Thailand. Future work should investigate the epidemiology of the metacercarial stage in the intermediate host and the adult in the experimental definitive host.

INTRODUCTION

Freshwater fish are an important source of protein for people in many areas, but sometimes being infected with parasites (Srisawangwong *et al*, 1997; Waikagul, 1998; Sukontason *et al*, 1999; Wongsawad *et al*, 2004; Kue-A-Pai and Wiwanitkit, 2005). Eating raw fish dishes is popular among Thai people in some areas, causing the spread of fish-borne parasites that can infect humans (Radomyos *et al*, 1994; 1998; Pungpak *et al*, 1998; Sukontason *et al*, 2005). Bung Borapet a large reservoir is an important source of freshwater fish for food in Nakhon Sawan Province, Thailand. This investigation aimed to study helminthic infections in freshwater fish in Bung Borapet to assess the epidemiological situation of helminthiasis in an endemic area.

MATERIALS AND METHODS

In July 2005, 36 freshwater fish from Bung Borapet reservoir, in Nakhon Sawan Province, were collected identified, and examined for helminths. Each fish was separated into 6 parts: fin rays (all the fins: dorsal, pectoral, pelvic, anal, and caudal), scales, gills, muscles, intestine, and liver. The parasites were observed under a stereomicroscope and numbers recorded. Nematodes were preserved and stored in 70% ethanol, and later cleared in glycerine for further examination. Cestodes and acanthocephalans were fixed in 5% formalin. Digenea, encysted metacercariae of *Clinostomum*

philippinensis were excysted with a needle. Some specimens were stained with hematoxylin, dehydrated in alcohol series, cleared with xylene, and mounted in permount. Some helminths were drawn by drawing tube. Helminth classification was based on Pearse (1933); Yamaguti (1958); Velasquez, 1975; Hanafi (1983); Moravec and Scholz (1991); Sirikanchana (1992); Chung *et al* (1995), and Wongsawad *et al* (1998).

RESULTS

Thirty-six fish were investigated for helminths; 7 were infected with 4 species of helminths. The prevalence of infection was 19.44 (7/36) and the intensity 4.67 (168/36) per fish (Table 1). One species of the infective stage (metacercaria) and three species of adults were found. One species of trematode, *Clinostomum philippinensis* (metacercaria) was found in the gills of *Trichogaster microlepis*. One species of cestode, *Senga Chiangmaiensis* was found in the liver and intestine of *Mastacembelus armatus*. One species of acanthocephalan, *Pallisentis* sp, was found in the intestine of *Anabus tesedineus Chana lucius*, *Cyclohelichthys apogon*, and *Mystus myticetus*. One species of nematode, *Camallanus anabantis* was found in the intestine of *Mystus myticetus* (Table 1).

Description of helminths *Clinostomum philippinensis* (excysted metacercaria) (Fig 1)

The parasite's body is linguliform, 4.33-5.50 x 1.50-3.20 mm. Oral sucker is sub-terminal and slightly oval, 0.35-0.48 x 0.25-0.38 mm. The acetabulum is oval, measured 0.85-0.95 x 0.65-0.93 mm. The testes are slightly lobed and located in the posterior part of the body, 0.31-0.44 x 0.20-0.40 mm. The ovary is oval,

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Table 1
Distribution of helminths in freshwater fish from Bung Borapet, Nakhon Sawan Province.

Fish species	Number of fish infected / examined	Helminths species	Number of helminthes in each part						Total
			Fins	Scales	Gills	Muscle	Intestine	Liver	
<i>Anabas testudineus</i>	1/3	<i>Pallisentis</i> sp	0	0	0	0	2	0	2
<i>Channa lucius</i>	1/2	<i>Pallisentis</i> sp	0	0	0	0	103	0	103
<i>Cyclocheilichthys apogon</i>	1/2	<i>Pallisentis</i> sp	0	0	0	0	2	0	2
<i>Mastacembelus armatus</i>	2/4	<i>Senga chiangmaiensis</i>	0	0	0	0	50	3	53
<i>Mystus myticetus</i>	1/5	<i>Pallisentis</i> sp	0	0	0	0	2	0	2
		<i>Camallanus anabantis</i>	0	0	0	0	2	0	2
<i>Osteuchilus hasselti</i>	0/2								
<i>Oxyeleotris marmoratus</i>	0/2								
<i>Pristolepis fasciatus</i>	0/6								
<i>Rasbora tornier</i>	0/5								
<i>Trichogaster microlepis</i>	1/5	<i>Clinostomum philippinensis</i>	0	0	4	0	0	0	4
Total	7/36		0	0	4	0	161	3	168

intertesticular, 0.15-0.20 x 0.08-0.12 mm. The cirrus pouch is located anterior to the ovary (Fig 1).

Senga chiangmaiensis (Fig 2)

The scolex is large, pear-shaped, 0.75-1.54 x 0.50-1.20 mm. Four large, elongated, bothria occupy a major portion of the scolex. The rostellum is large and located on the anterior end of the scolex. It consists of 28 hooks placed in 4 quadrants, in a circle. The ovary is bilobed, butterfly-shaped, and situated in the posterior third of the segment. The testes are ovoid, distributed in 2 fields in the central medulla, 0.03-0.05 mm diameter. Eggs are ovoid and round, 0.04-0.05 mm.

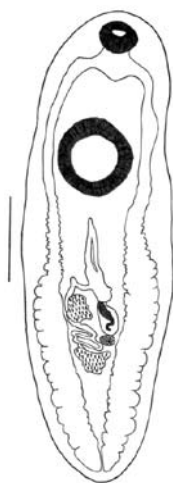


Fig 1- Excysted metacercaria of *Clinostomum philippinensis* (scale bar 0.5 mm).

Pallisentis sp (Fig 3)

The parasite is elongate; males measure 2.5-5.7 x 1.0-3.0 mm, with 2 ovoid testes, situated in the posterior part. Females are 2.5-5.7 x 1.0-3.0 mm. The proboscis consist of 4 rows of hooks; each row has 12 hooks.

Camallanus anabantis (Fig 4)

The worm is small- to medium-sized. The buccal capsule consists of 2 identical lateral valves, each valve containing 9 beaded longitudinal ridges. The tail of both sexes has finger-shaped mucrons located at the tip. Males are 3.85-5.83 x 0.10-0.18 mm. Caudal papillae consist of 5 pairs of pre-anal papillae, 2 pairs of cloacal papillae, and 5 pairs of post-anal papillae. Two spicules are similar in shape, but unequal in size. Females are 12.30-19.50 x 0.25-0.50 mm.

DISCUSSION

Fish-borne helminthic infections are common in Thailand. Many kinds of freshwater fish have been reported as the intermediate host of parasites (Sukontason *et al*, 1999; Wongsawad *et al*, 2004; Kue-A-Pai and Wiwanitkit, 2005). Humans acquire the parasite from eating raw or insufficiently cooked fish containing infective-stage parasites. Our study included 3 species of adult worms that do not infect mammals, *ie Senga chiangmaiensis*, *Pallisentis* sp, and *Camallanus anabantis*. *Senga chiangmaiensis* has been reported in the intestine of *Mastacembelus armatus* (Wongsawad *et al*, 1998). This study found it in the intestine and some worms were recovered from the liver of

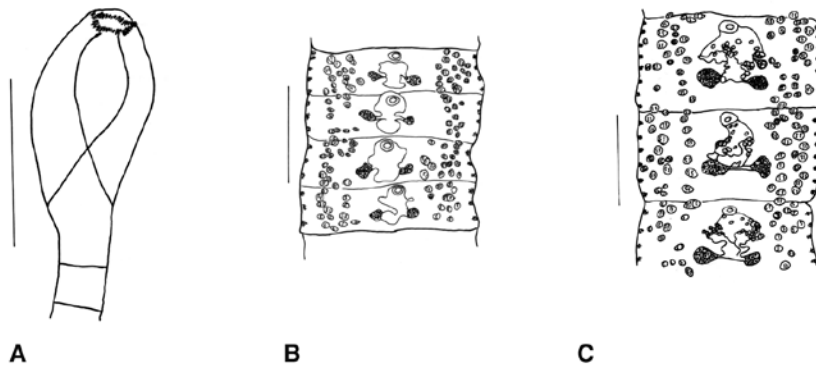


Fig 2- Adult *Senga chiangmaiensis* (scale bars 0.5 mm). A. Scolex of worm. B. Mature segments. C. Gravid segments.

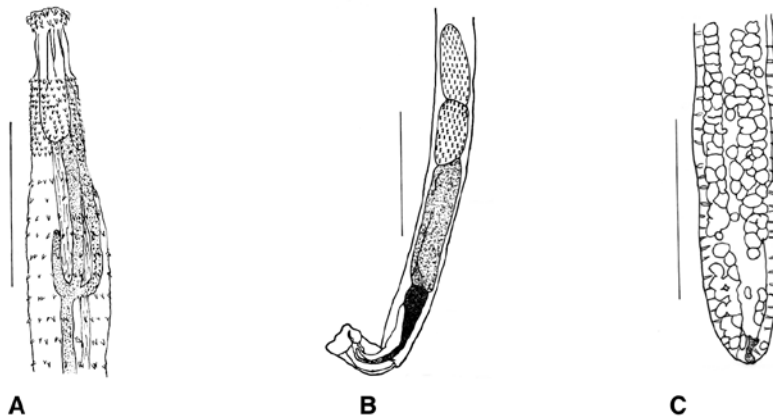


Fig 3- Adult *Pallisentis* sp (scale bars 0.5 mm). A. The anterior part of a male. B. The posterior part of a male. C. The posterior part of a female.

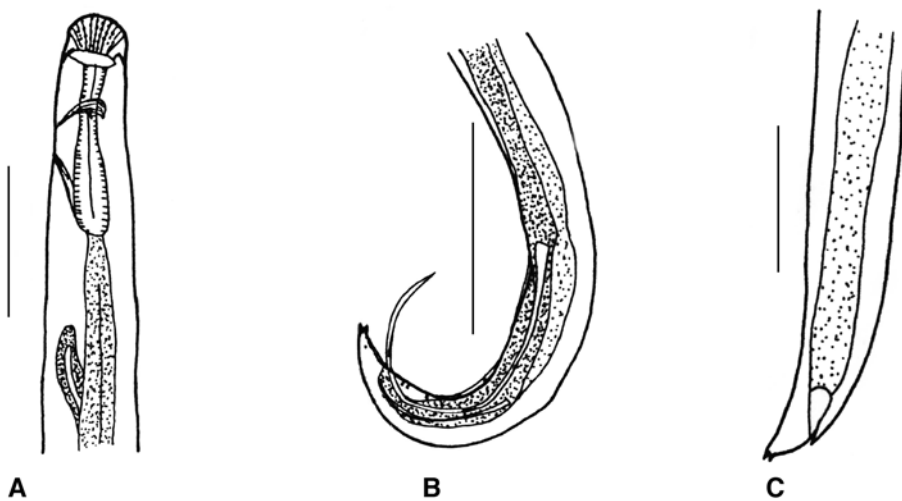


Fig 4- Adult *Camallanus anabantis* (scale bars 0.5 mm). A. The anterior part of a female. B. The posterior part of a male. C. The posterior part of a female.

Mastacembellus armatus. *Pallisentis* sp can be found in some freshwater fish, which agrees with this study (Luadee, 1998; Wongsawad *et al*, 2004). *Camallanus anabantis* infects many species of freshwater fish (Pearse, 1933; Sirikanchana, 1998; Kumchoo *et al*, 1999; Wongsawad *et al*, 2004). One metacercaria of *Clinostomum philippinensis* was found in the gills of *Trichogaster microlepis*. Adult flukes have been found in the buccal cavity and esophagus of birds, while metacercariae can encyst in fish, frogs, salamanders, and land snails (Yamaguti, 1958). In the Philippines, *Clinostomum philippinensis* Valasquez, 1959 was the first record of this species in *Ophiocephalus striatus*. The worm encysted in the tissues outside the eyeball and linings of the pericardial and opercular cavities and tissues under the pectoral fins of fish (Velasquez, 1975). Anyhow, the fluke in the genus *Clinostomum* have been recovered from human such as *Clinostomum complanatum* has been reported in humans from Japan and Korea (Yamashita, 1938; Chung *et al*, 1995). In Thailand, *C. philippinensis* has been reported from the visceral cavity of *Trichogaster trichopterus* (Pallas) from fresh water in Ayutthaya Province (Hanafi, 1983). Freshwater fish in the genus *Trichogaster* are cooked in the form of Plah-ra (fermented fish) and salted fish, a traditional Thai dish in some areas. Metacercarial cysts can survive in salted fish for 7 days (Pica *et al*, 2003). Eating raw and/or undercooked fish is believed to be the source of metacercarial infections of humans. This study determined the source of human infection using an investigation on the intermediate host. To confirm adult infection, *Clinostomum philippinensis* will be studied on experimental hosts in the future.

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