



Two *Plenosominoides* Metacercariae from Mountain Crabs in Central and Eastern Thailand

Jitra Waikagul, Somchit Pubampen, Surapol Sa-nguankait, Tippayarat Yoonuan

Department of Helminthology, Faculty of Tropical Medicine, Mahidol University, 420/6 Ratchawithi Road, Bangkok 10400, Thailand

Abstract

Mountain or waterfall crabs from Nakhon Nayok, Saraburi, Kanchanaburi, and Chantaburi provinces, were collected and examined for trematode metacercariae. Among the metacercariae recovered, two species of the family Microphallidae were found—*Plenosominoides yangshanensis* and *P. vajrasthira* n comb. The cysts were oval with a thick wall; the metacercariae inside the cyst wall were fully developed—all organs were visible, but no eggs. Their common features were as follows: vitellaria formed into two groups anterior-lateral to each caecum, cirrus pouch incompletely circular, not enclosing a ventral sucker. Little information was known of these trematodes and there has been no report of their public-health importance.

Keywords: *Plenosominoides*, trematode, metacercaria, crab

Introduction

Crab meat is a favorite food for humans. However, crab is well-known as a second intermediate host of the *Paragonimus* lung fluke, particularly mountain or waterfall crabs in the families Potamidae and Parathelphusidae. At least three families of trematodes reported in humans—*Paragonimidae*, *Achillurbainiidae*, and *Microphallidae*—are transmitted via freshwater crabs.

In Thailand, microphallid flukes have not been reported to infect humans, but in areas endemic for lung fluke, the metacercariae of these trematodes have been found infecting the same crabs. Metacercariae of the *Paragonimus*

lung fluke [1] and *Achillurbainia*-like lung fluke have been reported [2]. In this paper we report 2 species of trematode metacercariae in the family Microphallidae.

Materials and methods

Crabs were collected from several waterfalls in previously reported endemic areas of paragonimiasis—Kanchanaburi, Saraburi, Nakhon Nayok, and Chantaburi provinces. Each crab was identified before examination by compression method. The crab flesh and internal organs were pressed between two glass plates and searched for metacercariae under a stereomicroscope. Metacercariae were removed from each crab. Cysts were studied and measured, and excysted metacercariae fixed in warm 10% formalin and stained with acid carmine.

Correspondence:

Jitra Waikagul,

E-mail: <tmjwk@mahidol.ac.th>

Results

The positive rates of the 2 crab species ranged between 13.4-30.0%, and the number of metacercariae in each crab ranged between 1-74, but was <10 in most crabs.

The metacercarial cyst of *Plenosominoides* is large and oval. Cysts of two different sizes present in all localities where the crabs have been collected. The first cyst had a length:width ratio of 3:1; and the second 2:1. Their excysted metacercariae were strikingly different in features and are fully described below. The first morphologically resembled *Plenosominoides yangshanensis* Ke & Liang, 1984, while the second agreed morphologically with the fluke formerly described as *Microphalloides vajrasthira* Waikagul, 1983. After close consideration, the name has been changed to *Plenosominoides vajrasthira* new combination.

Plenosominoides yangshanensis Ke & Liang, 1984

Description (Figs 1a; 2a, b)

The cyst is oval, 1.35 mm long and 0.45 mm wide (Fig 1a). The cyst wall has two layers; the outer layer is relatively thick, and the inner layer thin.

The body of the metacercaria is oval, 1.25 (0.89-1.39) mm in length and 0.59 (0.50-0.76) mm in width (Fig 2). The oral sucker is small; the prepharynx short and narrow; the pharynx is slightly smaller than the oral sucker; the esophagus is long; bifurcated at one third of the body length; the caeca are long and end at the anterior half of the testes. The ventral sucker is small, located posterior to the cirrus sac. The testes are oval, opposite, located posterior to the ovary and anterior to the excretory bladder. The cirrus pouch covers the seminal vesicle and ejaculatory duct; it is incompletely circular and anterior to the ventral sucker (Fig 2). The ovary is triangular and lies between the right testis and ventral sucker. The vitelline follicles are arranged in two groups, one on each side of the mid-body, from the caecal

bifurcation to the ventral sucker. The excretory bladder is V-shaped, with primary branches of each arm, and with excretory pore terminal.

- Host and locality : *Demannieta suanphung* Yeo, Nayonetr & Ng, 1999, Kanchanaburi Province
 : *Larnaudia buesekome* (Blott, 1970), Nakhon Nayok Province
 : *Larnaudia larnaudii* (A Milne Edwards, 1869), Saraburi Province
 : *Potamon smithianum* Kemp, 1923, Chanthaburi Province
- Location : Liver, gills
- Deposition : Department of Helminthology, Faculty of Tropical Medicine, Mahidol University (MUTMHM-2009001.1 & MUTMHM-2009001.2)



Fig 1 Encysted metacercariae of microphallid flukes: **a.** *Plenosominoides yangshanensis*, **b.** *P. vajrasthira*.

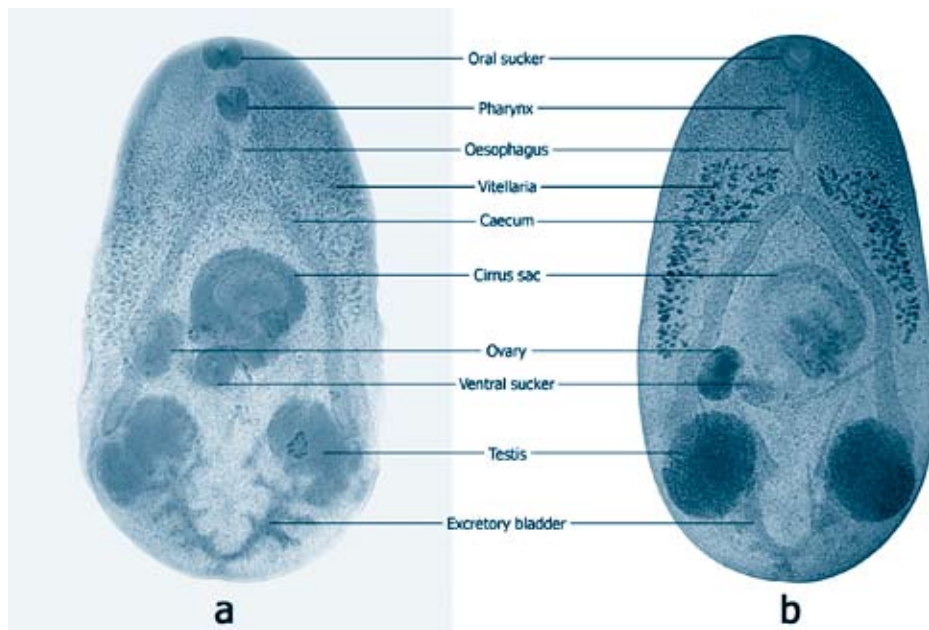


Fig 2 Excysted metacercariae of *Plenosominoides yangshanensis*: a. stained with acid carmine, b. stained with Haemalum (revealing vitelline follicles).

***Plenosominoides vajrasthira* n comb (Waikagul, 1983)**

Description (Figs 1b, 3)

The cyst is oval, 1.05 mm long and 0.55 mm wide (Fig 1b). The cyst wall has two layers; the outer layer is relatively thick and the inner layer thin. During encystation, both lateral sides of the metacercarial body fold ventrally.

The metacercarial body is oval, 755 (680-825) µm in length and 725 (680-770) µm in width (Fig 3). The body is covered with minute scales. The mouth is terminally surrounded by an oral sucker, which is small, about 80 (70-90) µm; the prepharynx is short and narrow; the pharynx is about 55 (45-65) µm long and 50 (45-55) µm wide; the esophagus is about 90 (65-120) µm long and bifurcated at one third of the body length; the caeca are long, terminating at the anterior half of the testes. The ventral sucker is small, about 100 (90-110) µm, slightly modified, and located posterior to the cirrus sac. The testes are opposite, oval, located posterior to the ovary and anterior to the excretory bladder; the right testis is about 190 (175-210) µm long and 110 (90-130)

µm wide; the left testis is about 180 (175-200) µm long and 125 (110-140) µm wide. The cirrus pouch covers the seminal vesicle and ejaculatory duct, is incompletely circular and anterior to the ventral sucker (Fig 3). The ovary is oval, 120 (110-130) µm long and 100 (90-120) µm wide, and lies anterior to the right testis. The uterus has two loops, one on each side of the body, extended anterior to the caeca to the excretory bladder. The vitelline follicles are arranged in two groups, one on each side of the body from posterior, to pharynx, to cirrus sac. The excretory bladder is V-shaped, with primary branches on the anterior and posterior walls of each arm, and with excretory pore terminal.

Host and locality : *Demannieta suanphung* Yeo, Nayonetr & Ng, 1999, Kanchanaburi Province
 : *Larnaudia buesekome* (Blott, 1970), Nakhon Nayok Province
 : *Larnaudia larnaudii* (A Milne Edwards, 1869), Saraburi Province

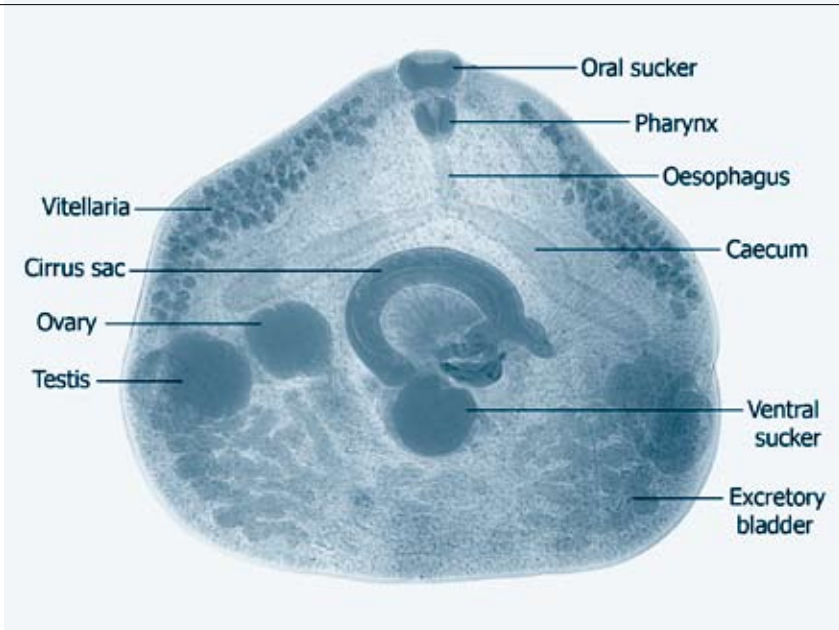


Fig 3 Excysted metacercaria of *Plenosominoides vajrasthiraе* stained with acid carmine.

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|------------|--|
| | : <i>Potamon smithianum</i> Kemp, 1923, Chanthaburi Province |
| Location | : Liver, gills |
| Deposition | : Department of Helminthology, Faculty of Tropical Medicine, Mahidol University (MUTMHM-2009002) |

Discussion

Ke and Liang [3] suggested a new genus *Plenosominoides* in the Family Microphallidae for an adult trematode recovered from a puppy 40 hours after experimental infection with metacercariae dissected from the gonad and liver of crabs of the species *Somanniathelphusa sinensis sinensis*, collected in Yangshan County, Guangdong, PR China. The new genus closely resembles two related genera, *Plenosoma* Ching, 1960 and *Microphalloides* Yoshida, 1938. The specific features of *Plenosominoides* are vitelline follicles located lateral to the anterior two-thirds of each caecum, uterus extending anterior to the caeca, and cirrus pouch not enclosing the ventral sucker. In *Plenosoma*, the cirrus pouch has no surface muscular fibers and the vitelline follicles are situated lateral to the entire length of the caeca. For *Microphalloides*, the uterus occupies only the

hind body space and the vitelline follicles are located just anterior-laterally to each caecum, and ventral sucker is enclosed within the cirrus pouch [3]. A trematode recovered from a cat from Nakhon Nayok Province was classified as a new species, *Microphalloides vajrasthiraе*, but the uterus of the fluke extends to the anterior caeca, the vitelline follicles are located in the anterior two-thirds of each caecum, and the ventral sucker is not enclosed in the arch of the cirrus pouch [4]. According to Ke and Liang [3], the characteristics of *M. vajrasthiraе* did not fit the genus *Microphalloides*, but did fit the genus *Plenosominoides*. In this report, *M. vajrasthiraе* was removed from *Microphalloides* for inclusion in the genus *Plenosominoides*, as a new combination *P. vajrasthiraе*.

The natural definitive host of *Plenosominoides yangshanensis* was unknown, but for *P. vajrasthiraе* it was the cat [3,4]. The metacercariae of both species found varied in degrees of development, and possessed well-developed reproductive organs. This observation agreed with the report of Ke and Liang [3] on the metacercaria of *P. yangshanensis*. Adult worms of *P. yangshanensis* were found in experimental dogs after 40 hours' infection; in the culture medium, eggs were well developed after 142 hours of cultivation [3]. It seemed that

microphallid metacercariae developed easily into adults *in vivo* and *in vitro*. Fujino *et al* [5] reported that metacercariae of *Microphalloides japonicus* (Osborn, 1919) started to produce eggs at 12 hour post-infection and reached maximum production after 3 days in a mouse. *In vitro*, *M. japonicus* metacercariae produced eggs (lower numbers than in a mouse) with 3-5 days in Eagle's MEM and NCTC 109 culture media with serum. Metacercariae of *M. similis* (Jagerskiold, 1900) started producing eggs on day 2 post-infection in a mouse, and *in-vitro* egg production started on day 3 onwards; some flukes survived up to 30 days when serum was present in the culture medium [6]. Egg production *in vitro* has also been reported for *Gynaecotyle adunca* (Linton, 1905) [7].

The prevalence of these two species of metacercariae has not been closely observed, but they were present in all localities the crabs were collected. Since they have no known public-health significance, limited information is available. Further study of their life cycle is still needed to confirm that they are really no danger to the health of humans or livestock.

References

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