# การติดเชื้อของตัวอ่อนระยะเมตาเซอร์คาเรียในปูนาจากจังหวัดนนทบุรี

# Infection of Metacercariae in Rice-Field Crab from Nonthaburi Province, Central Thailand

พัชรี ครูขยัน[1*] อารีรัตน์ พิจิตร[1] และ พรเทพ เนียมพิทักษ์[2]
Patcharee Khrukhayan[1*], Areerat Pijit[1] and Pornthep Niamphithak[2]
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## บทคัดย่อ

การสำรวจการติดเชื้อของตัวอ่อนระยะเมตาเซอร์คาเรียในปูนา (*Sayamia bangkokensis*) จากจังหวัดนนทบุรี ซึ่งอยู่ในภาคกลางของประเทศไทย โดยการสุ่มเก็บตัวอย่างปูนาตั้งแต่เดือนมกราคมถึงเมษายน 2551 จำนวน 205 ตัว ตรวจพบตัวอ่อนของพยาธิใบไม้ระยะเมตาเซอร์คาเรียสกุล *Paragonimus* ในปูนาทั้งสิ้น 160 ตัว คิดเป็นค่าความชุกและความหนาแน่นเฉลี่ยของพยาธิเท่ากับ ร้อยละ 78 และ 4.3 ± 3.3 ตัว/ปู 1 ตัว ตามลำดับ ซึ่งบริเวณที่พบจะอยู่ในตับและตับอ่อน (มันปู) เป็นส่วนใหญ่ การติดเชื้อจะพบในปูเพศผู้มากกว่าเพศเมีย โดยมีค่าความชุกเท่ากับ ร้อยละ 81.8 และ 73.3 ตามลำดับ และมีค่าความหนาแน่นเฉลี่ยของพยาธิ เท่ากับ 4.7 ± 3.7 และ 3.8 ± 2.7 ตัว/ปู 1 ตัว ตามลำดับ เมื่อทดสอบทางสถิติแล้วพบว่าไม่มีความแตกต่างกันอย่างมีนัยสำคัญระหว่างเพศ ($p$>0.05) นอกจากนี้ การติดเชื้อของตัวอ่อนระยะเมตาเซอร์คาเรียจะพบในปูที่มีขนาดใหญ่ (ขนาดระหว่าง 4.01-5.00 เซนติเมตร) มากกว่าในปูที่มีขนาดเล็ก (ขนาดระหว่าง 2.00-3.00 เซนติเมตร) เมื่อหาค่าความชุกและความหนาแน่นเฉลี่ยในปูแต่ละขนาด พบว่า มีค่าความชุก เท่ากับ ร้อยละ 33.3, 56.0 และ 82.8 และค่าความหนาแน่นเฉลี่ย เท่ากับ 3.0 ± 0.0, 4.3 ± 2.5 และ 4.3 ± 3.4 ตัว/ปู 1 ตัว ในปูขนาดเล็ก ขนาดกลาง และขนาดใหญ่ ตามลำดับ ทั้งนี้เพื่อความปลอดภัยในการบริโภคปูนาควรปรุงอาหารให้สุกก่อนการบริโภคทุกครั้งเพื่อลดการติดเชื้อของพยาธิใบไม้จากปูนาสู่คน

**คำสำคัญ:** เมตาเซอร์คาเรีย, *Paragonimus*, ปูนา, *Sayamia bangkokensis*, จังหวัดนนทบุรี

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[1] ภาควิชาชีววิทยาประมง คณะประมง มหาวิทยาลัยเกษตรศาสตร์ เขตจตุจักร จังหวัดกรุงเทพมหานคร 10900
[1] Department of Fishery Biology, Faculty of Fisheries, Kasetsart University, Chatuchak, Bangkok 10900, Thailand.
[2] ภาควิชาประมง คณะเกษตรศาสตร์ มหาวิทยาลัยขอนแก่น อำเภอเมือง จังหวัดขอนแก่น 40002
[2] Department of Fisheries, Faculty of Agriculture, Khon Kaen University, Muang, Khon Kaen 40002, Thailand.
[*] Corresponding author, e-mail: ffisprk@ku.ac.th Tel: 0 2942 8701

## ABSTRACT

A survey on metacercariae infection was conducted with rice field crabs (*Sayamia bangkokensis*) from Nonthaburi province, Central Thailand. Crab samples were collected from January to April 2018. The metacercarial cysts from genus *Paragonimus* were detected from 160 crabs out of 205. Prevalence and mean intensity were 78% and 4.3 ± 3.3 parasites/crab, respectively. Most of the parasites were found in hepatopancreas. The infection was higher in males than females with the prevalence of 81.8% and 73.7%, and mean intensity of 4.7 ± 3.7 and 3.8 ± 2.7 parasites/crab, respectively. However, the prevalence and mean intensity were not significantly different ($p$>0.05) between genders. Moreover, the infection was found higher in large crab (4.01-5.00 cm) than small crab (2.00-3.00 cm). The prevalence rates were 33.3%, 56.0% and 82.8% and mean intensity values were 3.0 ± 0.0, 4.3 ± 2.5 and 4.3 ± 3.4 parasites/crab in small, medium, and large size, respectively. Consequently, it is recommended that the crab should be cooked before they are consumed to prevent infection.


**Key words:** metacercariae, *Paragonimus*, rice field crab, *Sayamia bangkokensis*, Nonthaburi province

## INTRODUCTION

Rice farming area in Sai Noi District, Nonthaburi province is a minor part in urban areas near the capital city, Bangkok about 20 kilometers. Rice farming is usually carried out during May-October and November-April. During rice is grown, farmers will catch crabs out of the fields of their own because they will bite and destroy the stem of rice. Then, these crabs were cooked or sold at the market. The carb is also a source of protein for daily diets of the people for a few seasons and high demand for domestic markets (Panalikul *et al*., 2017). At present, the rice field crabs are consumed much more widely, e.g. jaew bong, fried crab paste and crab salad. Some foods made from cooked crab or raw crab might cause zoonotic diseases since crabs are secondary intermediate host of fluke in the metacercariae stage. Fluke (Trematoda: Digenea) is a group of parasites that are important in medicine and the vet. The parasitic adult stage is found in the digestive tract or internal organs in definitive mammal host including the human. Metacercariae is infection stage that found in rice field crab. It can infect the host by eating especially eating raw crab or consuming an ingredient in papaya salad and crab salad. The metacercariae will develop into an adult worm living in the digestive tract or internal organs of the definitive host. The past, the studies found many species of metacercariae in crab such as genus *Paragonimus*, *Microphallus*, *Microphaloides*, *Achillurbainia* and *Plenosominoides* (Habe *et al*., 1993; Yaemput *et al*., 1994; Waikagul and Yeamput, 1999; Sugiyama *et al*., 2004; Doanh *et al*., 2007; Sugiyama *et al*., 2007; Sugiyama. *et al*., 2009; Waikagul *et al*., 2009; Pina *et al*., 2011; Chamavit and Sahaisook, 2012; Doanh *et al*., 2013; Goswami *et al*., 2013; Habe *et al*., 2013; Sanpool *et al*., 2013; Athokpam and Tandon, 2015; Pachanawan *et al*., 2017; Wongchum *et al*., 2018). These parasites cause lung fluke disease (paragonimiasis) and intestinal fluke disease in humans. In Thailand has reported the paragonimiasis in some areas of the province, Phetchabun, Saraburi, Nakhon Nayok, Chiang Rai, Nan, Loei, Ratchaburi province (Hospital for Tropical Diseases, 2013) and northern Thailand (Pachanawan *et al*., 2017). These diseases are the problems the health of the population in the area directly. If the surveillance of the disease spread or the dissemination of knowledge and information to the public to reduce

infectious disease caused by a fluke in humans. Although the fluke worm in rice field crab in Thailand studied in Kanchanaburi province, Chanthaburi province (Waikagul *et al*., 2009) Nakhon Nayok province (Yaemput *et al.,* 1994 and Waikagul *et al*., 2009), Phra Nakhon Si Ayutthaya province, Bangkok (Yaemput *et al*., 1994) Samut Prakan province (Chamavit and Sahaisook, 2012) Saraburi province (Waikagul *et al*., 2009 and Sanpool *et al*., 2013), Phitsanulok province, Phetchabun province (Sanpool *et al*., 2013), Surat Thani province(Sugiyama *et al*., 2007 and Sanpool *et al*., 2013) Nakhon Phanom province (Pachanawan *et al.*, 2017) and Ubon Ratchathani province (Wongchum *et al*., 2018) but it is only a minority. Moreover, in Nonthaburi province, there were no data, so this study was determined the effect of host size and sex on the incidence of parasitic metacercariae infection to be used as guidelines for control and prevention of outbreak of infectious disease. Crab specimens were collected in the end of rice farming because in this period can easily find crabs.

# MATERIALS AND METHODS

## 1. Collection and examination for metacercariae in rice-field crab

In total, 205 specimens of the rice field crab, Sayamia bangkokensis (Naiyanetr, 1982) (syn. Somanniathelphusa bangkokensis) were collected from rice field farmers in Sai Noi District, Nonthaburi province from January to April 2018 by crab traps, 18 cm in diameter and 33 cm long in rice fields and using fish as bait.

Crabs were obtained in a cool box to The Faculty of Fisheries laboratory, Kasetsart University, Bangkok. In the laboratory, the carapace width (Figure 1) was measured by vernier caliper and sex was determined by observing the appearance of the abdomen. In male, the abdomen is narrow and long rectangular while in female, the abdomen is wider and round. During the dissection, various internal organs and hepatopancreas were removed and pressed between two petri dishes for cyst detection. The sample of cysts were observed, photographed and total counted under a stereo microscope.


**Figure 1** Measurement of the carapace width

## 2. Data analysis

Parasites were magnified 40-100x by using a compound microscope and identified as described by Waikagul *et al*. (2009), Pina *et al*. (2011), Goswami *et al*. (2013), Pachanawan *et al*. (2017). The prevalence and mean intensity levels of metacercariae were determined according to Bush *et al*. (1997).

$$\text{Prevalence (\%)} = \frac{\text{Number of crabs infested}}{\text{Number of crab specimens}} \times 100$$

$$\text{Mean intensity (parasites/crab)} = \frac{\text{Total numbers of metacercariae}}{\text{Number of infected crabs}}$$

Standard statistical computation (standard deviation) was carried out using Microsoft Excel (Office 2013). T-test was applied to find significant differences in the mean intensity of the gender of the host. Lastly, One-way ANOVA followed by Tukey's post hoc test were used to test the difference between sizes of crab. The statistical software SPSS (11.5 for windows) was used to analyze the data, with a level of significance of $p<0.05$.

## RESULTS AND DISCUSSION

A total of 205 crabs examined, 160 specimens (78%) were infected for *Paragonimus* sp. metacercariae. The metacercarial cysts were found with an overall prevalence and mean intensity of 78% and 4.3 ± 3.3 parasites/crab, respectively (Table 1). They found in hepatopancreas and some of internal organs of *Sayamia bangkokensis* (Naiyanetr, 1982) (Figure 2). The cyst is a spherical shape and has a thick wall composed of two layers, the external being thinner than the internal cyst wall. The mean diameter of cyst measured 420 µm (403-450) (n=688). The metacercariae were folded in U-shape inside the cyst and were also visible oral sucker, ventral sucker and excretory bladder (Figure 3).

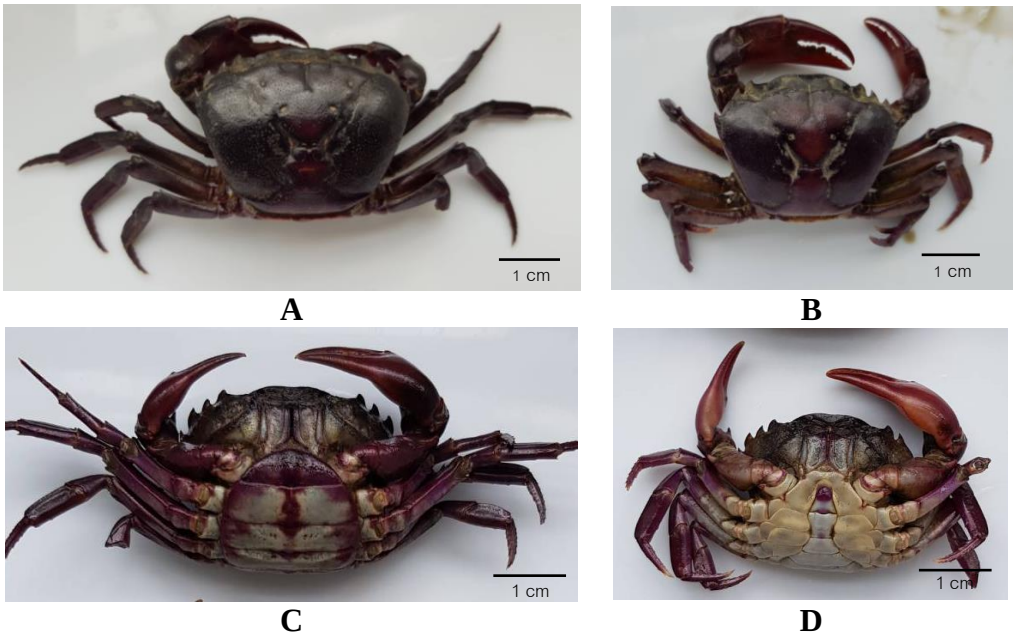


**Figure 2** Morphology of rice-field crab (*Sayamia bangkokensis* (Naiyanetr, 1982)). (A) Dorsal view of female and (B) male rice field crab. (C) Ventral view of female and (D) male rice field crab.

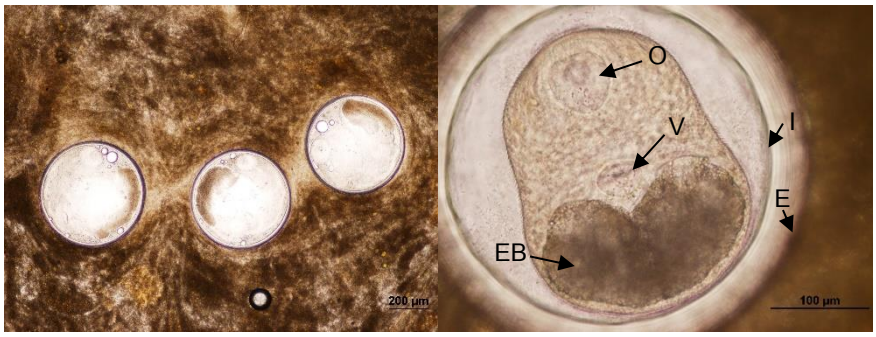


**Figure 3** Photomicrographs of fresh encysted metacercariae of *Paragonimus* sp. O - oral sucker; V - ventral sucker; I - internal cyst wall; E - external cyst wall; EB - excretory bladder.

The prevalence and mean intensity levels of parasites shown that both sexes were high rate and no significant different ($p$>0.05). The prevalence rates were more than 70% in both genders of host, i.e. 81.8% and 73.7% and the mean intensity levels were 4.7 ± 3.7 parasites/crab and 3.8 ± 2.7 parasites/crab in male and female, respectively. Crabs were classed into 3 length classes: small size (2.00-3.00 cm), medium size (3.01-4.00 cm) and large size (4.01-5.00 cm.). The highest prevalence rate was found in the large size (82.8%) followed by the medium size (56.0%) and the lowest rate in the small size (33.3%). The mean intensity values of three sizes of host were 3.0 ± 0.0, 4.3 ± 2.5 and 4.3 ± 3.4 parasites/crab in small size, medium size and large size, respectively and no significant different ($p$>0.05) between size (Table 1).

**Table 1** Prevalence (P) and mean intensity (MI) of *Paragonimus* metacercariae of rice filed crab collected in relation to sex and their carapace width from Nonthaburi province.

| | **No. crab infected/ No. crab examined** | **P (%)** | **MI ± SE (parasites/crab)** |
|---|---|---|---|
| **Gender** | | | |
| Male | 90/110 | 81.8 | 4.7 ± 3.7 |
| female | 70/95 | 73.7 | 3.8 ± 2.7 |
| **Carapace width group (cm)** | | | |
| Small size (2.00-3.00) | 2/6 | 33.3 | 3.0 ± 0.0 |
| Medium size (3.01-4.00) | 14/25 | 56.0 | 4.3 ± 2.5 |
| Large size (4.01-5.00) | 144/174 | 82.8 | 4.3 ± 3.4 |
| **Total** | **160/205** | **78.0** | **4.3 ± 3.3** |

160 (78%) of 205 rice filed crab (*Sayamia bangkokensis* (Naiyanetr, 1982)) from Sai noi district, Nonthaburi province, were found *Paragonimus* sp., that causes paragonimiasis in human. The parasite was found spread in Asia such as Thailand, Lao (Habe *et al*., 2013), Vietnam (Doanh *et al*., 2013), Malaysia (Habe *et al*., 1993), Japan (Habe, 1979; Habe and Terasaki, 1982; Sugiyama *et al*., 2004), Korea (Kim *et al*., 2009) and China (Wang *et al*., 2002). In Thailand, paragomiasis in human cause by *Paragonimus heterotremus* and *P. westermani* and in animals (cats, dogs and monkeys) cause by *P. bangkokensis*, *P. harinasutai*, *P. macrochis* and *P. siamensis*

(Miyazaki, 1974). The outbreak has been found in Phetchabun province, Saraburi province, Nakhon Nayok provice, Chiang Rai province, Nan provice, Loei province, Ratchaburi province and some provinces in upper of northern Thailand (Radomyos *et al*., 1998; Hospital for Tropical Diseases, 2013; Pachanawan *et al*., 2017). When the parasites infected, patients will have pneumonia, chest pain and chronic cough. Maybe the parasite can live on other organs such as liver, intestine, muscle, diaphragm and brain cause of abnormal symptoms of those organs (Hospital for Tropical Diseases, 2013). Although Nonthaburi province is a non-epidemic area of Paragonimus, this study is a preliminary data to know the distribution of parasites for to serve as a guideline for preventing infection of helminth species to humans. The prevalence of metacercarial parasite showed a high risk of infection as 78%, while the mean intensity value was low level as 4.3 ± 3.3 (range 1-12) parasites/crab. Despite the number of samples in each size varies greatly. The trend to find that mostly the parasites were found highest prevalence in biggest crab size (82.8%). Conversely, Pachanawan *et al*. (2017) reported Nakhon Phanom province, northeast Thailand, found the highest mean intensity of *Microphaloides* spp. metacercariae was 200.38 parasites/crab in Phon Sawan district which is more than Nonthaburi province about 50 times. It causes affect the high risk of infection in humans. Moreover, Wongchum *et al*. (2018) also reported the prevalence and intensity of *Paragonimus* metacercariae were 42.96% and 11.53 parasites/crab, respectively in Ubon Ratchathani province, northeast Thailand. There was a lower prevalence but higher intensity than Nonthaburi province. From the information previously shown that *Paragonimus* is a common species (high prevalence value) which found in crabs. Thus, raw crab-eating has a risk of parasitic infection. The infection of the parasites was found in both males and females crab with similar rate, according to Wongchum *et al*. (2018). Causes may be due to the crab (both sexes) has lived in the same environment and food source, thus the infection rate is not different. In addition, they were found in larger than small size crab. Crab has to molt for growth and increase size. Therefore, the size of the crab will increase with age. It is possible that the larger crab feed more food than smaller crab, so the larger one has a chance to get more infection, as well as Abu-Madi *et al*. (2001), Treml *et al*. (2012) and Haas *et al*. (2012) reported that the prevalence values of the parasite were related with age and size of the host. Most of the metacercariae were found in hepatopancreas because this organ was absorption, storage of nutrients and vitellogenesis, during growth and ovarian development (Wang *et al*., 2014) therefore suitable for the metacercariae infected. Due to the hepatopancreas is the most delicious part of the crab thus people who eat raw crabs will have a chance to get more parasitic infected. Then, the rice field crab food should be cooked before consumption.

## CONCLUSION

This study of metacercariae survey in rice field crab (*Sayamia bangkokensis*) from Nonthaburi province from January to April 2018. About 78% (160 of 205 examination crabs) was infected with *Paragonimus* sp. and found 4.3 ± 3.3 parasites/crab. Most of the parasites were found in hepatopancreas. The larger crab size (4.01-5.00 cm) was infected higher than smaller ones (2.00-3.00 cm). This study is only one study in Sai Noi district, Nonthaburi province. If there were more studies in the provinces close to Nonthaburi, will allow us to know the spread of this parasite and to be able to predict the results that will happen in the future. Therefore, the consumption of rice field crab should be cooked. Do not feed pets such as dogs and cats with raw crab

because they are susceptible to definition hosts.

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