THE NATURAL FIRST INTERMEDIATE HOST OF *PARAGONIMUS SIAMENSIS* (MIYAZAKI AND WYKOFF, 1965) IN THAILAND

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Abstract. The first intermediate host of six-known the *Paragonimus* species in Thailand had not been found until the *Filopaludina* (Siamopaludina) *martensi martensi* snail was discovered to maintain the cercariae of a *Paragonimus* species. An extensive study examined cercarial development through to adult worms by infecting 3 genera of 7 crab species with penetration of cercariae and feeding of snails containing such cercariae. These crabs provided many metacercariae which were fed to cats and bandicoots. The animals gave many *Paragonimus* adult worms which were characterized as *Paragonimus siamensis* by the following criteria: 6-lobed ovary and cuticular spines in groups. It is concluded that the *Filopaludina martensi martensi snail is a susceptable natural first intermediate host of P. siamensis.* Second intermediate hosts *Somanniathelphusa brandti, S. sexpunctatum* and *S. bangkokensis* were experimentally infected; prior to this study only *S. germaini* and *S. dugasti* had ever been naturally infected with metacercariae of this species.

INTRODUCTION

Studies of *Paragonimus* and paragonimiasis have proceeded since 1928 in several areas of northeast, east, north and central parts of Thailand. The first species of *Paragonimus* discovered in Thailand was the adult worm of *Paragonimus westermani.* Kerbert 1878, found in the lungs of two leopards from Chumphon Province in the south (Daengsvang *et al,* 1964) and the second was *P. siamensis* found in cats at Udon Thani Province, in the northeast (Miyazaki and Wykoff, 1965). Vajrasthira (1986) concluded that there are six species of *Paragonimus* in Thailand, two belonging to human hosts (*P. westermani* and *P. heterotremus,* Chen and Hsia, 1964) and four belonging to animal hosts (*P. siamensis, P. bangkokensis,* Miyazaki and Vajrasthira, 1966, *P. harinasuta,* Miyazaki and Vajrasthira, 1968 and *P. macrorchis,* Chen, 1962). Biological investigations of *Paragonimus* have focussed on several stages and host-parasite relationships have been sought for completing their life cycles. Of the 6 species, metacercariae (infective stage) can be found in 2 genera of 6 species of fresh water crabs (second intermediate host), *ie,* mountainous and stream crabs (*Tiwaripotamon beusekomae*), and rice field crabs (*Somanniathelphusa* sp) (Vajrasthira, 1986; Naiyanetr, 1978). Most of the first intermediate hosts of these *Paragonimus* species are not found in natural infections. In other endemic areas than Thailand many intermediate snail hosts are naturally infected with rediae and cercariae of some *Paragonimus* species, *eg, Aroapyrgus alleei* snail with *P. mexicanus* (Lamothe-Argumedo *et al,* 1983) and *Semisulcospira libertina* with *P. westermani* (Tomimura *et al,* 1989). Parasitologists have attempted to do natural first intermediate host surveys. Alternatively, experimental infections have been run in parallel using local snails (*Oncomelania nosophora* and *Melanoides tuberculata*) with *P. heterotremus* miracidium; only *Oncomelania nosophora* showed positive results (Vajrasthira, 1986), also to *P. mexicanus* (Hata *et al,* 1987). *Tricula aperta* (β race) is the experimental first intermediate host of *P. heterotremus* (Yaemput *et al,* 1988). *Aroapyrgus colombiensis,* a laboratory-bred snail, is experimentally infected by miracidia of *P. peruvianus,* they further develop to rediae and cercariae (Malex *et al,* 1985).

In the previous study, rediae and cercariae of a *Paragonimus* species had been detected in the snail *Filopaludina* (Siamopaludina) *martensi martensi* during a first intermediate host survey for *Paragonimus* worms (Yaemput and Waikagul, 1987). The present investigation was undertaken to develop naturally recognized cercaria from this snail species in experimental animals and also to re-examine the results of the first intermediate host survey with several species of snails.

MATERIALS AND METHODS

Host snails : *Filopaludina* (Siamopaludina) *martensi martensi, Bithynia* sp, *Indoplanorbis exustus* and *Lymnea* sp were obtained from several areas of rice fields in Nakhon Nayok Province, central Thailand, which had been a positive area for the natural first intermediate host of *Paragonimus* cercariae (Yaemput and Waikagul, 1987). Each snail species was cleaned by dechlorinated tap water (kept for 7 days before use). The examinations for certain cercariae of *Paragonimus* were done by either shedding or crushing techniques.

Shedding examination : Two to five snails were distributed in a round plastic container (5 cm diameter × 4.5 cm height) depending on size of the snails and maintained with a proper volume of dechlorinated water. The cercarial shedding time was 4 hours at room temperature and the examination was done under a stereomicroscope after transferring the snails to a new container. If negative for cercariae, examination was carried out by the same procedure at least three times. If positive, each snail would be examined separately as above.

Crushing examination : Non cercarial-shedding snails were crushed between 2 glass plates (40 × 60 × 2 mm) for thin shelled snails and broken for thick shelled snails. The bodies of the snails were dissected apart (*eg,* foot plate, digestive organ) search for rediae and cercariae under the stereomicroscope.

Three genera of 7 crabs species were used experimentally as second intermediate hosts: rock crabs *Siamthelphusa faxoni.* mountainous and stream crabs *Tiwaripotamon beusekomae* and rice field crabs *Somanniathelphusa germaini, S. dugasti, S. brandti, S. bangkokensis* and *S. sexpunctatum.* Fifty of 250 mountainous and stream crabs were used after 200 crabs had been examined to confirm non-natural infection with *Paragonimus* metacercariae. These crabs did not encounter such infection so 50 crabs of the remainder were used.

Fifty rice field crabs were collected from an experimental rice field of Kasetsart University, Bangkok; they were *Somanniathelphusa bangkokensis.* One hundred of each species, *S. germaini, S. brandti* and *S.* (juliae) *sexpunctatum* at Ayutthaya Province and *S. dugasti* at Nakhon Nayok Province were collected in non-endemic areas of paragonimiasis: no metacercariae of *Paragonimus* were found in crab samplings.

Two categories of cercariae infection in crabs were carried out: penetration of cercariae and feeding on cercarial-infected snails. All crabs were divided into group 1 and group 2 following such assessment. Of group 1, each species was exposed to a number of shedding cercariae in a plastic container. The infection was stopped when the number of cercariae had decreased visibly. Group 2 crabs were fed with infected snails containing cercariae after a crushing procedure. All crabs were reared in a laboratory aquarium for 90 days and then examined for metacercariae.

Experimental animal hosts were 2 cats and 5 bandicoots (*Bandicota indica*), obtained from Bangkok Metropolitan Authority and the laboratory animal house, respectively. To ensure use of helminth-free animals, their feces were examined by simple smear technique. Each cat was fed with 35 metacercariae passed through a polyethylene tube (diameter 1 mm) connected to a syringe; 10 metacercariae were fed to each bandicoot. The animals were reared in the laboratory animal house for at least for 60 days, in view of the unknown length of the incubation period. During this period, fecal examination for *Paragonimus* eggs was carried out by simple smear technique on day 45. A search for adult worms in the lungs was done after the stool examination became positive. The morphology of adult worms was studied using fresh specimens prepared on permanent slides stained with acid-carmine.

Since 2 crabs (*S. bangkokensis*) of group 1 died on day 45, they were examined for metacercariae at that time. These crabs contained 90 metacercariae, of which 40 were fed to another 2 bandicoots to study their ability for further development.

RESULTS

From the snail examinations, 3 out of 4, *Bithynia* sp, *Indoplanorbis exustus* and *Lymnea* sp, were not infected by *Paragonimus* cercariae, although *Bithynia* sp contained *Xyphidia* cercariae and *Indoplanorbis exustus* contained fork-tailed cercariae. *Filopaludina martensi martensi* snails (Fig 1) shed a number of cercariae which had the same morphology as previously described. Results of the snail examinations are given in Table 1.

Fig 1—Snail intermediate host, *Filopaludina martensi martensi* (Frauenfeld), a common local fresh water snail or "Hoi Kum"

Both rediae and of cercariae were of elongated-ovoidal shape. Rediae obtained about 1-8 cercariae and their body length averaged 258 μm and width 190 μm. The average morphology of 50 fixed cercariae in 10% formalin was as follows, body length 308 × 88 μm, a round oral sucker with spines (10 μm) and a short tail about 33 × 33 μm (Fig 2).

Among cercariae in the second intermediate hosts, mountainous and stream crabs, rock crabs and rice field crabs, of group 1 by cercarial penetration, only five species were of *Somanniathelphusa* presenting metacercariae. There were no metacercariae in the rock crabs (*Siamthelphusa faxoni*) and mountainous and stream crabs (*Tiwaripotamon beusekomae*). Positive crabs with metacercariae in group 2 gave similar results to group 1. Metacercariae were encysted in heart vessels of all *Somanniathelphusa* sp (Fig 3A). Metacercariae were found in a period of 90 days to be fully developed with an average size of 442-530 × 312-402 μm, morphologically round in shape, with 2 cyst walls; the thick and transparent outer region sometimes presented 1 pole and the thin inner region another (Fig 3B). The development of 45-day metacercariae obtained from the two dead crabs had the same morphology as 90-day metacercariae (Fig 4A-B).

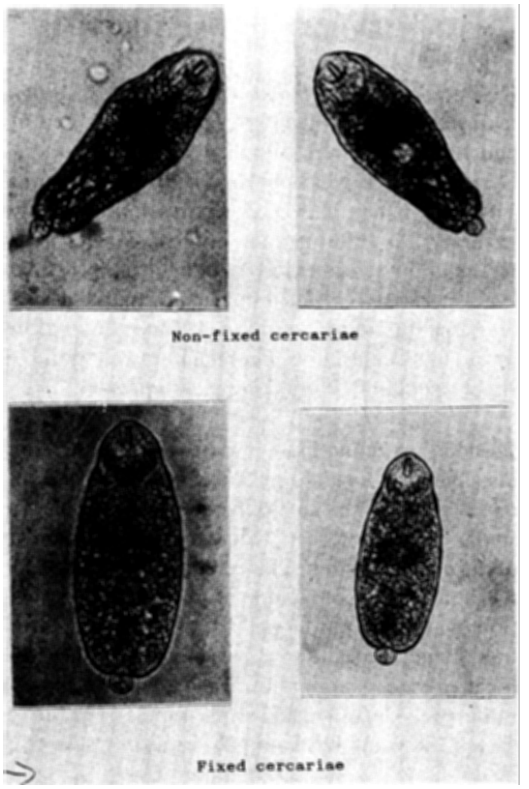

Fig 2—The non-fixed and 10% formalin-fixed cercariae of such *Paragonimus* species showed oral sucker and ventral suckers and small-round tail.

Table 1

Show percentage of infected snails with rediae and/or cercariae by examinations of shedding and crushing.

Snails	No. of snails	No. of positive snails	%	Cercarial types
Filopaludina martensi martensi	1,631	43	2.6	Paragonimus
Bithynia sp	2,449	804	32.8	Xyphidia
Indoplanorbis exutus	1,081	5	0.5	Fork-tailed
Lymnea sp	38	0	0	-

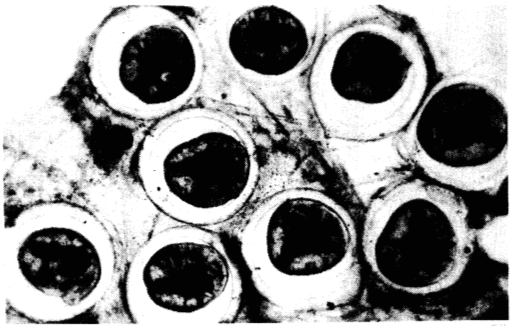

Fig 3A—The 90-day metacercariae connected with heart vessels of rice field crab *Somanniathelphusa* at their poles.

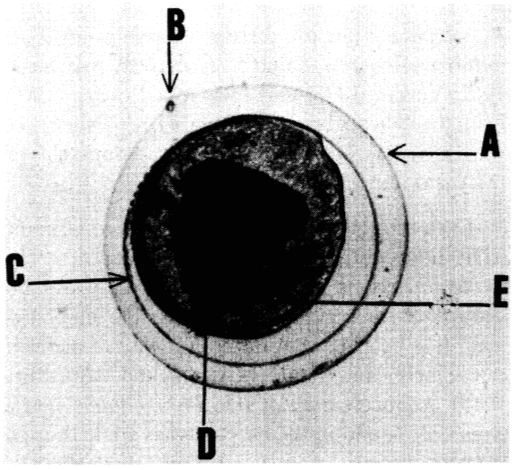

Fig 3B—A 90-day metacercaria showed outer cyst wall (A) pole (B) inner cyst wall (C) excretory bladder (D) and intestine (E).

Fig 4A—The 45-day metacercariae were in heart vessels of rice field crab.

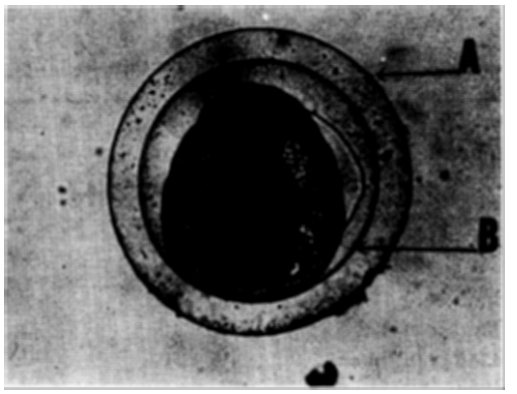

Fig 4B—A 45-day metacercaria showed outer cyst wall (A) and inner cyst wall (B).

After 45 days of rearing these experimental animals, stool examination for *Paragonimus* eggs was done; positive ones were observed in bandicoots on day 58 and in cats on day 74. The animals were sacrified and searched for mature worms in the lungs and tissue surrounding the pleural cavity. Ninety mature worms were found in lungs and pleural cavities of cats and bandicoots (Table 2). Most of the metacercariae were found in the lungs, frequently in a lung cyst.

Average measurements and morphology of 12 adult worms were: body length about 8.5 mm (7-10) × 4.6 mm (4.0-5.2) and body surface completely covered with cuticular spines in groups (Fig 5). Oral and ventral suckers had diameters about 59.2 μm and 71.1 μm, respectively. A six-lobed ovary (Fig 6A) was situated at the right side near the median line of the body. Simple intestinal ceca ran from the short esophagus almost to the posterior end. Vitellarian glands reached from the oral sucker to the end of body. Two simple-lobed testes were at each side and below the midline of body (Fig 6B). Acid carmine-stained worms are shown in Fig 7 and details of all stages are summarized in Table 3.

DISCUSSION

After finding the natural first intermediate host of a *Paragonimus* species, the developments of cercariae through adult worms were observed to show characteristics of *Paragonimus siamensis* presenting cuticular spines in groups, 6-lobed ovaries and the ventral sucker bigger than the oral

Table 2

Discoveries and distributions of *Paragonimus* mature worms in cats and bandicoots.

No. of animals	No. of metacercariae for feeding	Total No. of mature worms	Distributions	
			Lungs	Pleural cavity
Cat 1	35	26	26	-
Cat 2	35	31	31	-
Bandicoot 1	10	7	6	1
Bandicoot 2	10	5	5	-
Bandicoot 3	10	6	6	-
Bandicoot 4	10	7	7	-
Bandicoot 5	10	8	8	-

sucker. The 6-lobed ovary arrangement of these specimens is similar to that of *P. westermani* described by Miyazaki and Wykoff (1965) but spines in groups and single spines showed differences between the 2 species. On their entire body surface, the cuticular spines are arranged in groups like in *P. compactus* but the distinguishable point is the number of ovaries, 6 and 5 respectively (Miyazaki, 1962; Vevers, 1923) and their lobed ovaries differ from the delicate branching ovary of *P. bangkokensis* which has cuticular spines in groups as well (Miyazaki and Vajrasthira, 1967).

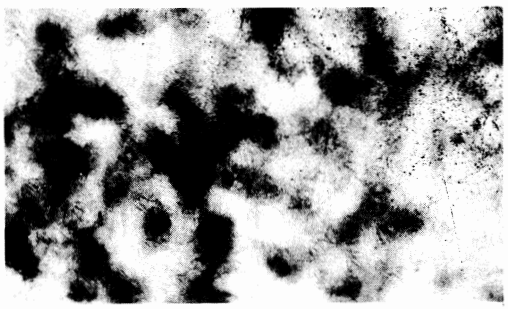

Fig 5—Demonstration of the cuticular spine groupings on the body surface of detected *Paragonimus* sp.

Even though metacercariae seemed to be somewhat different from those of *P. siamensis* described by Miyazaki and Wykoff (1965), they did not show the outer cyst wall close to the inner cyst wall. It is believed that outer cyst wall of metacercariae is thin and fragile (Miyazaki and Vajrasthira, 1967). Metacercariae of this *Paragonimus* species were only found in the heart vessels while those of *P. siamensis* were found in blood vessels near the heart and also rarely outside the heart, whereas *P. westermani* were mainly in gill vessels and muscles (Miyazaki and Wykoff, 1965).

By the feeding method, cercariae or rediae containing mature cercariae could be grown and become metacercariae just as by penetration. The

Fig 6A—Demonstration of 6-lobed ovary of adult worm.

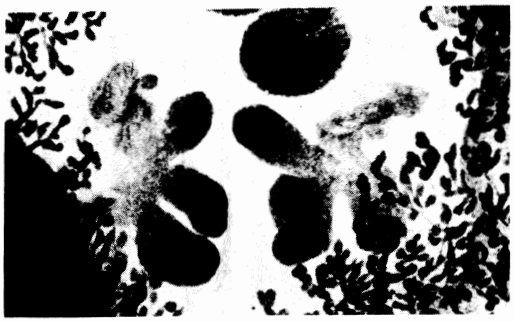

Fig 6B—Demonstration of simple testes of adult worm.

Fig 7—Demonstration of the adult worm stained by acid carmine.

Table 3

Summary of details and characteristics of detected *Paragonimus* sp.

Stage	Characteristic
Redia	
shape	elongated-ovoidal
size	average 558 × 190 μm
Cercaria	
shape	elongated-ovoidal
size	average 308 × 88 μm
oral sucker	cup-shaped structure with a 10 μm long stylet
tail	short, average 33 × 33 μm
Metacercaria	
shape	round or less ovoidal
size	average 442-530 × 312-402 μm
cyst wall	thin and transparent outer cyst wall and thicker inner cyst wall
Adult	
shape	flattened ventral side and ovoidal
size	average 7.0-10.0 × 4.0-5.2 mm
body surface	entirely covers with cuticular spines in groups
ovary	6 lobes
testes	simple-lobed testes
oral sucker	average diameter 59.2 μm
ventral sucker	average diameter 71.1 μm

penetration of cercariae into crabs appears to occur for *P. kellicolti* and *P. iloktsuenensis* but for *P. westermani,* the crabs eat the parasites because the cercariae of the latter rarely escape from the snail host under the natural conditions (Ameel, 1934; Chen, 1940; Yokogawa, 1952). An unexpected result was that *P. siamensis* cercariae could develop completely to metacercariae in certain crab hosts by day 45 or before.

On re-examination of the first intermediate host survey, *Filopaludina martensi martensi* served naturally this host-parasite relationship of *P. siamensis.* The snail has the local name "Hoi Kum", and generally resides in fresh water. *Bithynia* sp, *Indoplanorbis exustus* and *Lymnea* sp snails did not show such a relationship.

In the study of the second intermediate host, 7 species were experimentally used; 5 species of rice field crabs harbored metacercariae as reported by Vajrasthira (1986): *Somaniathelphusa germaini* and *S. dugasti* were infected naturally by *P. siamensis* metacercariae; the other three were experimental second intermediate hosts. In support of our study about the harboring of *P. siamensis* metacercaria, *ie* Miyazaki and Wykoff (1965) pointed out that only *S. germini* contain this *Paragonimus* species in their survey. Vajrasthira's conclusion (1986) was that *Tiwaripotamon smithianus (Potamon smithianus)* very rarely harbored *P. siamensis* metacercaria; our study used 200 *T. beusekomae* for examination of free-metacercaria infection and 50 for processing of experimental infection and there was no cercarial development in these crabs.

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