TREMATODE INFECTIONS OF THE FRESHWATER SNAIL FAMILY THIARIDAE IN THE KHEK RIVER, THAILAND

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Abstract. The freshwater snail family Thiaridae was studied at five different locations: water sources for the Khek River, Thailand. Snail samples were collected by hand using counts per unit of time sampling method between December 2004 and October 2005. The physico-chemical quality of the water changed with the seasons and affected the sampling areas during both the dry season and the flood season. A total of 9,568 snail samples comprised of 14 species were found. These were 284 *Tarebia granifera*, 24 *Melanoides tuberculata*, 86 *Thiara scabra*, 3,295 *Paracrostoma pseudosulcospira pseudosulcospira*, 736 *P. paludiformis paludiformis*, 3,266 *P. paludiformis dubiosa*, 117 *P. morrisoni*, 304 *Brotia (Brotia) binodosa binodosa*, 1,250 *B. (Brotia) microsculpta*, 146 *B. (Senckenbergia) wykoffi*, 1 *B. (Brotia) pagodula*, 5 *B. (Brotia) binodosa spiralis*, 5 *B. (Brotia) insolita* and 49 *B. (Brotia) manningi*. The cercariae were investigated using shedding and crushing methods where they were categorized into two types and five species. The first type, Parapleurolophocercous cercariae, were comprised of *Haplorchis pumilio* Looss, 1899 and *Centrocestus formosanus* Nishigori, 1924. The second type, Xiphidiocercariae were comprised of *Acanthatrium hitaense* Koga, 1953, *Loxogenoides bicolor* Kaw, 1945 and *Haematoloechus similis* Looss, 1899. The cercarial infection rates in the above 5 species were 0.1% (5:9,568), 0.2% (15:9,568), 0.3% (24:9,568), 0.4% (37:9,568) and 0.1% (5:9,568), respectively. Five species of snails were susceptible to trematode infections. They were *T. granifera*, *M. tuberculata*, *T. scabra*, *P. paludiformis paludiformis* and *B. (Senckenbergia) wykoffi*; infections were found in 26.1% (74:284), 33.3 % (8:24) , 1.2% (1:86), 0.3% (2:736) and 0.7% (1:146), respectively.

INTRODUCTION

This paper details a study of trematodes living in intermediate host snails. Cercariae, the larva stage of flukes, may be found in freshwater and terrestrial gastropods (Ito, 1980). There are reports showing that snails in the family Thiaridae serve as the first intermediate host for trematodes. For instance, *Tarebia granifera*, *Thiara toucheana*, *Brotia asperata*, *B. costula episcopalism* and *B. c. peninsularis* are the first intermediate hosts for lung flukes (Tang, 1940; Tabangui *et al*, 1950; Davis, 1971; Nakagawa, 1971; Brandt, 1974), and *T. granifera* and *Melanoides tuberculata* are the first intermediate hosts for intestinal and blood flukes (Malex, 1962; Pointier and Jourdane, 2000). In Thailand, *T. granifera* and *Melanoides tuberculata* have been reported as the first intermediate hosts for lung and intestinal flukes (Upathum *et al*, 1995; Ukong *et al*, 2007).

The Khek River, located in Phetchabun and Phitsanulok Provinces, has its water

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source in the Phetchabun Mountain Range. On the Phetchabun Mountain Range, there are three of the most famous national parks in Thailand: Thung Salaeng Luang, Phu Hin Rong Kla and Khao Kho the National Parks. The Khek River flows past these three national parks of Phetchabun Mountain Range, joining the Nan River, one of the most important rivers in Northern Thailand.

Thiarid snails in the Khek River were studied between December 2004 and October 2005. They were examined for trematode infection. The objectives of this study were to investigate: 1) the species and distribution of Thiarid snails in the Khek River, 2) the infection rates of trematodes in Thiarid snails, and 3) the types of parasites found in Thiarid snails. Rivers usually support more species and are very important for the conservation of biodiversity. Information from this study can be used in the control and prevention of trematode infections in animals and humans in this community.

MATERIALS AND METHODS

Sampling sites

Five sites in the Phetchabun Mountain Range, which are the sources of water for the Khek River, were used as snail collection sites. The precise positions of the collection sites were obtained by GPS (Garmin PLUS III, Taiwan). They were Sakunotayan Waterfall (N 16º 50′ 20.6″, E 100º 32′ 15.6″, altitude 40 m), Kaeng Sopha Waterfall (N 16º 52 22.3″, E 100º 50′ 29.6″, altitude 398 m), Thung Salaeng Luang Stream (N 16º 50′ 50.0″, E 100º 51′ 57.2″, altitude 452 m), Pha Laht Waterfall (N 17º 01′ 69.1″, E 100º 56′ 77.8″, Altitude 267 m) and Si Dit Waterfall (N 17º 37′ 43.3″ E, 100º 56′ 39.5″, altitude 472) (Fig 1). Microhabitats of the study areas and physico-chemical char-

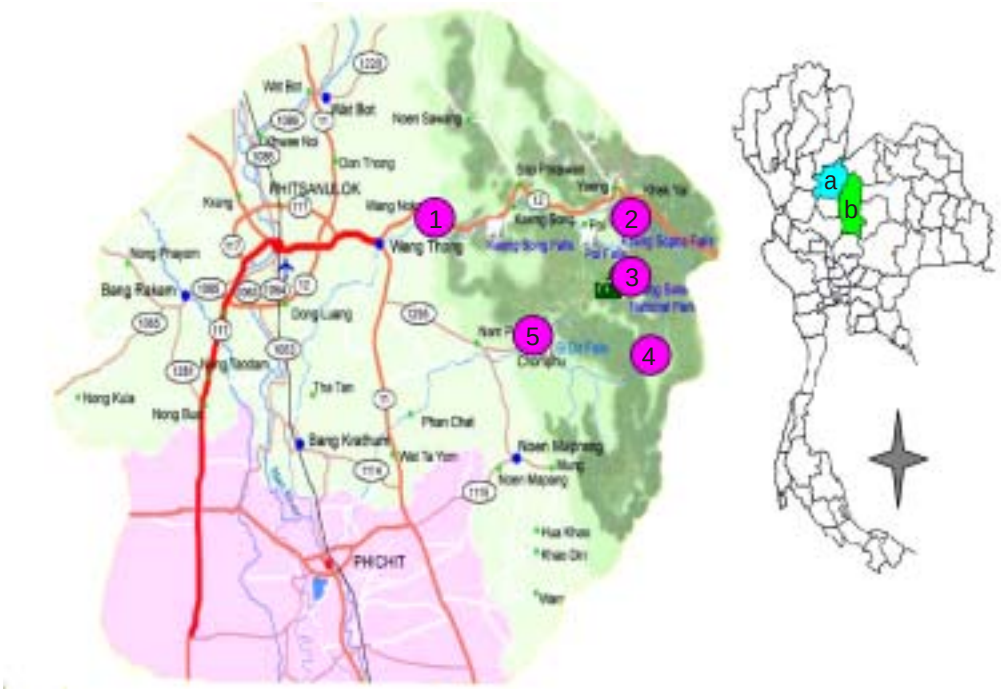

Fig 1–Map showing localities in Khek River at Phisanulok Province[a] and Phetchabun Province[b], Thailand; 1: Sakunotayan Waterfall; 2: Kaeng Sopha Waterfall; 3: Thung Salaeng Luang; [4]Pha Laht Waterfall; 5: Si Dit Waterfall.

acteristics were studied and recorded. The chemical characteristics of the water were measured by a spectrophotometer (Orbeco-Hellige 975 MP the analyst, USA). The water temperature, the pH value of the water, the amount of dissolved oxygen in the water (U10 Horiba, Japan) and the water velocity (Flow-meter model 2030, General Oceanics, USA) were measured.

Collection of snails

The snails were collected every other month from December 2004 to October 2005. The counts per unit of time method, recommended by Olivier and Schneiderman (1956), was used for snail sampling. Five researchers collected snail samples every 10 minutes. The snail samples were classified by conchology and were examined for trematode infections.

Examination for parasitic infections

The parasitic infections with trematode cercariae were studied by using shedding and crushing methods. The cercariae were collected in dechlorinated water and observed for their swimming behavior (Krailas *et al*, 2003). The occurence of sporocysts and/or rediae was examined under a dissecting microscope.

Study of cercarial morphology

The emerged cercariae were studied unstained or vitally stained with 0.5% neutral red, Ehrlich's hematoxylin stain and Semichon's carmine' and fast green. Measurements in micrometers were taken from 20 specimens fixed with 10% formalin. Details of the cercariae were drawn using a camera lucida and identified according to Schell (1962, 1970), Nasir (1974), Yamaguti (1975) and Ito (1980). On scanning electron microscope, cercariae were fixed in 2.5% glutaraldehyde phosphate buffer (0.1 mol/l, pH 7.4) at 4ºC for at least 2 hours and post-fixed in 1% osmium tetroxide in the same buffer for 2 hours at 4ºC. They were dehydrated through a graded series of ethanol, then dried in a critical point dryer using liquid carbon dioxide as a transition medium. The specimens were coated with gold-palladium in an ion-sputtering apparatus (Polaron CPD 7501, UK) then examined in a Camscan mx 2000 scanning electron microscope (UK).

RESULTS

Microhabitat of the sampling sites

All sampling sites were covered with tall or and medium sized trees, and small-to-medium sized rocks lay along the river. The current was somewhat swift in the rainy season. The average light intensity was >100,000 lux at noon. The average water temperature was 25ºC, rising to near 30ºC. The snail samples were found on the rock, rough sand, dried leaves and on aquatic plants. The physico-chemical quality of the water changed with the seasons and affected the sampling areas during both the dry season and flood season, however the collection of samples was done throughout the year (Table 1).

Snail samples

Using conchological characteristics, the 9,568 snail samples were classified into 14 species (Fig 2). They were 284 *Tarebia granifera*, 24 *Melanoides tuberculata*, 86 *Thiara scabra*, 3,295 *Paracrostoma pseudosulcospira pseudosulcospira*, 736 *Paracrostoma paludiformis paludiformis*, 3,266 *Paracrostoma paludiformis dubiosa*, 117 *Paracrostoma morrisoni*, 304 *Brotia (Brotia) binodosa binodosa*, 1,250 *Brotia (Brotia) microsculpta*, 146 *Brotia (Senckenbergia) wykoffi*, 1 *Brotia (Brotia) pagodula*, 5 *Brotia (Brotia) binodosa spiralis*, 5 *Brotia (Brotia) insolita* and 49 *Brotia (Brotia) manningi*. The density of snails was highest at Thung Salaeng Luang Stream and the highest parasitic infection rate was found at Kaeng Sopha Waterfall. Cercarial infections were found in 86 snails; the infection rate was 0.9% (86:9,568) (Table 2). Markedly different intensities of infection were found for different snail species. *M. tubuculata* and *T. granifera*

Table 1
Microhabitats of the study areas and physico-chemical characteristics from the Khek River.

Locality	Date	Physical characteristics					Chemical characteristics (mg/l)				
		Velocity (m/sec)	pH	Conduc (mhos/cm)	Turb (NTU)	DO_2 (mg/l)	Cr	NO_3	Mn	PO_4	$CaCO_3$
Sakunotayan	Dec 2004	0.49	7.29	0.066	11	8.13	0.02	3.00	0.30	0.50	54
Waterfall	Feb 2005	0.27	8.64	0.078	19	8.25	0.03	5.00	0.30	1.00	36
	Apr 2005	0.83	7.32	0.097	115	8.04	0.05	3.00	0.10	0.70	54
	Jun 2005	0.91	7.14	0.057	485	9.84	0.02	1.00	0.30	0.50	71
	Aug 2005	1.58	7.50	0.040	170	8.60	0.10	3.00	0.10	0.30	89
	Oct 2005	2.03	8.62	0.049	80	9.18	0.02	3.00	0.10	0.30	71
Kaeng Sopha	Dec 2004	0.69	7.40	0.068	70	8.35	0.10	1.00	0.30	0.30	36
Waterfall	Feb 2005	0.69	7.60	0.083	30	8.52	0.03	1.00	0.10	0.20	36
	Apr 2005	1.26	7.13	0.060	647	9.74	0.02	5.00	0.30	0.20	36
	Jun 2005	0.98	7.90	0.049	521	8.38	0.05	3.00	0.10	0.50	71
	Aug 2005	2.11	7.50	0.040	90	11.7	0.05	3.00	0.50	3.00	36
	Oct 2005	2.97	8.21	0.037	>999	9.25	0.03	3.00	0.10	3.00	54
Thung Salaeng	Dec 2004	0.89	7.91	0.069	11	8.85	0.02	1.00	0.30	0.30	54
Luang Stream	Feb 2005	0.55	7.79	0.095	54	8.47	0.05	3.00	0.50	0.50	107
	Apr 2005	2.10	7.77	0.072	756	10.85	0.05	5.00	0.10	0.20	54
	Jun 2005	1.47	7.57	0.049	435	8.29	0.02	3.00	0.30	0.70	54
	Aug 2005	2.61	8.60	0.068	110	11.10	0.05	3.00	0.50	2.00	36
	Oct 2005	2.34	8.45	0.040	>999	9.22	0.05	3.00	0.30	1.00	71
Pha Laht	Dec 2004	1.02	8.34	0.028	20	8.24	0.02	3.00	0.30	0.50	54
Waterfall	Feb 2005	0.26	8.37	0.048	44	9.32	0.05	3.00	0.10	1.00	71
	Apr 2005	0.52	8.10	0.055	120	8.83	0.02	3.00	0.10	0.20	36
	Jun 2005	1.95	7.92	0.034	260	8.59	0.05	5.00	0.50	0.30	18
	Aug 2005	2.14	7.90	0.010	140	9.60	0.02	3.00	0.30	0.50	36
	Oct 2005	2.48	7.52	0.042	320	8.65	0.02	3.00	0.30	1.00	54
Si Dit Waterfall	Dec 2004	1.00	8.50	0.062	5	9.12	0.03	3.00	0.50	0.50	71
	Feb 2005	0.81	7.89	0.067	12	8.74	0.02	1.00	0.70	0.50	117
	Apr 2005	1.63	8.30	0.076	86	12.25	0.02	5.00	0.10	0.30	54
	Jun 2005	1.77	7.59	0.048	266	8.37	0.05	3.00	0.30	0.30	36
	Aug 2005	2.02	8.00	0.040	110	8.50	0.05	3.00	0.30	0.70	36
	Oct 2005	2.62	7.51	0.041	540	10.14	0.02	3.00	0.50	1.00	71

exhibited a high prevalence of infection. Their infection rates were 33.3% and 26.1%, respectively (Table 3).

Types of cercariae

Two types of cercariae were found in the five species of snail samples. The cercariae were categorized into five species. The first type was Parapleurolophocercous cercariae. Tails provided with dorso-ventral and sometimes lateral finfolds; pigmented eyespots were present; they were excretory vesical bilobed, and thick-walled (Schell, 1970). They were *Haplorchis pumilio* Looss, 1899 (Fig 3) and *Centrocestus formosanus* Nishigori, 1924 (Fig 4). The second type was Xiphidiocercariae; had a stylet in oral sucker; eyespots were absent; the excretory vesicle was

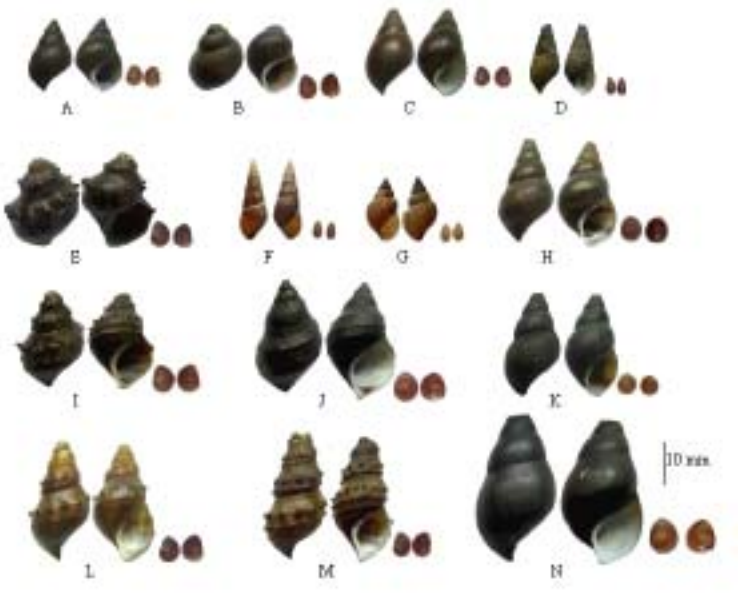

Fig 2–Shell morphology and operculae of snails collected from the Khek River; A: *Brotia (Brotia) microsculpta*; B: *Paracrostoma paludiformis paludiformis*; C: *Paracrostoma pseudosulcospira pseudosulcospira*; D: *Tarebia granifera*; E: *Paracrostoma paludiformis dubiosa*; F: *Melanoides tuberculata*; G: *Thiara scabra*; H: *Brotia (Senckenbergia) wykoffi*; I: *Paracrostoma morrisoni*; J: *Brotia (Brotia) binodusa spiralis*; K: *Brotia (Brotia) insolita*; L: *Brotia (Brotia) pagodula*; M: *(Brotia) binodosa binodosa* and N: *Brotia (Brotia) manningi*.

thin-walled (Schell, 1970); They consisted of *Acanthatrium hitaense* Koga,1953 (Fig 5), *Loxogenoides bicolor* Kaw, 1945 (Fig 6) and *Haematoloechus similis* Looss, 1899 (Fig 7).

The descriptions of the morphology and anatomy of the cercariae were based on a study of living cercariae that had escaped from snail samples. Measurements (average size) in micrometers were taken from 20 fixed cercariae (Table 4).

H. pumilio were found from 5 *M. tuberculata*, which is equivalent to an infection rate of 20.8% of the total number of the *Melanoides* snails (Table 3). The sampling sites in which this parasite was found were Sakunotayan Waterfall and Pha Laht Waterfall. The cercaria body was oval in shape, colored with yellowish brown pigment, entirely covered with fine reverse spines and sensory hairs on the side of the body. The mouth opening had transverse rows of spines (Fig 3C). Pigment eyespots and a pharynx were present. Seven pairs of penetration glands were arranged in two longitudinal series with a ventral sucker and primordial genitalia. The excretory bladder had a round shape and was composed of fine pigments. A long tail was attached to the dorsal end of the body, with lateral finfolds nearby and a dorso-ventral finfold for the greater distal portion (Fig 3D-E). No flame cells were found in the tail stem. The average sizes (in micrometers) measured were body: 90-140 (av 124) x 168-295 (av 257); tail: 10-36 (av 30) x 465-528 (av 490); oral sucker: 27-48 (av 36) x 27-48 (av 36); ventral sucker: 14-24 (av 18) x 14 - 24 (av18); pharynx: 8-10 (av 9) x 12-19 (av 15); and excretory bladder: 28-40 (av 34) x 28-40 (av 34).

Movement behavior. The cercaria floated on the surface or in the middle of the water. The body sank lower than the tail. It moved by rolling up and springing the body back to move forward in a screwing motion for 2-4 seconds and then rested for 15-20 seconds. It survived up to 2-3 hours in the water after emergence.

C. formosanus were found from 15 *T. granifera*, which is equivalent to an infection rate of 5.3% of the total number of *Tarebia* snails (Table 3). This parasite was found at Kaeng Sopha Waterfall. A pair of eyespots lay at the level of the pharynx. The oral sucker had two rows of oral spines (4 in the anterior

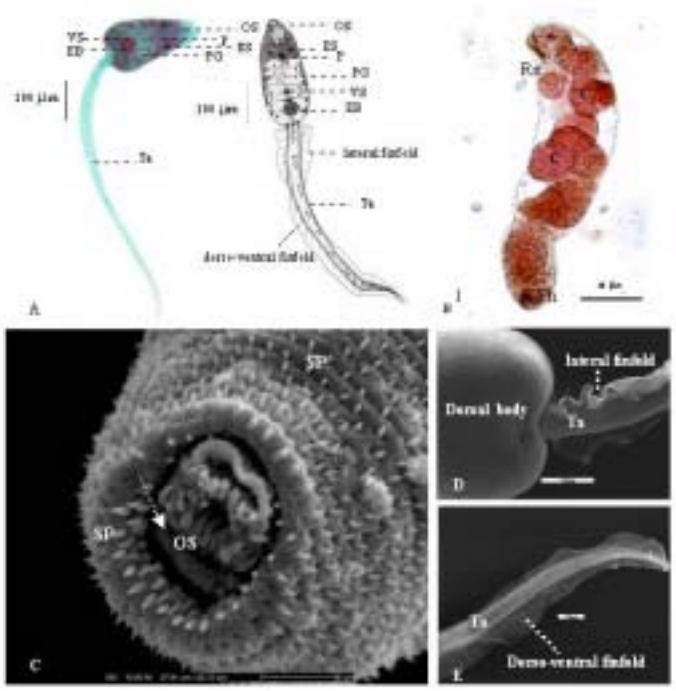

Fig 3–Image of *Haplorchis pumilio* cercaria; A: light micrograph and drawing structure (OS = Oral sucker, ES = eye spot, P = pharynx, PG = penetration gland, VS = ventral sucker, Ta = tail, EB = excretory bladder). B: redia, staining with 0.5% neutral red (Re = redia, Ph = pharynx, C = cercaria). C: SEM micrograph, anterior part showing the spines around the mouth (SP = spine). D-E: SEM micrograph, showing lateral finfold and dorso-ventral finfold.

row and 5 in the posterior row) on the dorsal wall of the mouth aperture (Fig 4C). The parenchymal body had a yellowish brownish pigment and was spinulate (Fig 4D). A large acetabulum was found between the intestinal bifurcation and the excretory vesical. The bladder was a flattened V-shape. Seven pairs of penetration glands lay anterolateral to the acetabulum in front of an inverted V-shape. Cystogenous cells were distributed in the posterior part. The genital primordial part was somewhat elongated and triangular, between the acetabulum and the excretory vesicle. The tail was slender, with a very indistinct dorsal and ventral finfolds, both of which were more conspicuous in the distal half, provided at the tip with a tiny spike (Fig 4E).

The average sizes (in micrometers) measured: body: 45-72 (av 64) x 82 - 120 (av 117); tail: 14-17 (av 15) x 69-92 (av 82); oral sucker: 16-26 (av 24) x 17-28 (av 25); ventral sucker: 12-16 (av 14) x 12-16 (av 14); pharynx: 7-9 (av 8) x 8-10 (av 9); and excretory bladder: 24-30 (av 28) x 38-52 (av 45).

Movement behavior. The cercaria floated on the surface or in the middle of the water. The body sank lower than the tail. It moved by rolling up and springing the body back to move forward in a screwing motion for 8-10 seconds and then rested for about 45-50 seconds. It survived up to 3-4 hours in the water after emergence.

A. hitaense were found in 24 *T. granifera*, which is equivalent to an infection rate of 8.5% of the total number of *T. granifera* (Table 3). This parasite was found at the Kaeng Sopha Waterfall. This parasite was classified into virgulate Xiphidiocercaria. The virgula organ was located in the region of the oral sucker; the ventral sucker was smaller than the oral sucker; a stylet was present in the oral sucker; the body was spinulate (Fig 5C-D); two pairs of penetration glands, an anterior pair with fine granules, and a posterior pair with coarser granules were present, and all ducts opened near the tip of the stylet; the genital primordial part was spherical; the excretory bladder was U-shaped; the tail shorter than the body and attached to the posterior part of the body. The average sizes (in micrometers) were measured: body: 53-92 (av 78) x 80-110 (av 100); tail: 19-25 (av 23) x 26-75 (av 68); oral sucker: 25-32 (av 30) x 34-40 (av 37); stylet: 8-13 (av 10) x 11-13 (av 12);

Table 2
Seasonal distribution of cercariae in the Khek River.

Locality	Date	Number of examined snails	Number of infected snails	Percentage
Sakunotayan Waterfall	Dec 2004	429	0	0
	Feb 2005	567	1	0.20
	Apr 2005	187	0	0
	Jun 2005	778	0	0
	Aug 2005	417	0	0
	Oct 2005	113	2	1.80
Kaeng Sopha Waterfall	Dec 2004	149	3	2.00
	Feb 2005	343	47	13.80
	Apr 2005	161	1	0.60
	Jun 2005	442	10	2.30
	Aug 2005	307	11	3.60
	Oct 2005	218	5	2.30
Thung Salaeng Luang	Dec 2004	427	0	0
	Feb 2005	581	0	0
	Apr 2005	844	0	0
	Jun 2005	1,403	0	0
	Aug 2005	422	0	0
	Oct 2005	200	0	0
Pha Laht Waterfall	Dec 2004	91	0	0
	Feb 2005	62	3	4.80
	Apr 2005	173	0	0
	Jun 2005	157	3	1.90
	Aug 2005	34	0	0
	Oct 2005	42	0	0
Si Dit Waterfall	Dec 2004	208	0	0
	Feb 2005	259	0	0
	Apr 2005	275	0	0
	Jun 2005	203	0	0
	Aug 2005	53	0	0
	Oct 2005	23	0	0
Total		9,568	86	0.9

ventral sucker: 15-16 (av 16) x 15-18 (av 17); pharynx: 10-15 (av 13) x 12-24 (av 20); excretory bladder: 8-12 (av 10) x 20-46 (av 38).

Movement behavior. The cercaria floated on the surface or in the middle of water. The body sank lower than the tail. It moved by folding its tail back to the body and turning its body to roll from left to right quickly, darting forward for about 45-60 seconds, and resting for about 2-5 seconds. It survived up to 2-4 hours in the water after emergence.

L. bicolor were found in 3 *M. tuberculata*, 33 *T. granifera* and 1 *T. scabra.* The infection rates were 12.5% (3/24), 11.6% (33/284) and 1.2% (1/86), respectively (Table 3). This parasite was found at Sakunotayan Waterfall, Kaeng Sopha Waterfall and Pha Laht Waterfall. The cercarial body was oval in shape. The entire body was covered with spines (Fig 6C) and dotted by granules. The ventral sucker

Table 3
Collected snails from the Khek River and infection rates of cercarial species (Dec 2004-Oct 2005).

Snail host	Number examined	Number infected	% infection[a]	Cercarial species
Melanoides tuberculata	24	8	33.3	*Haplorchis pumilio* Looss, 1899 (5/24=20.8%) *Loxogenoides bicolor* Kaw, 1945 (3/24=12.5%)
Tarebia granifera	284	74	26.1	*Acanthatrium hitaense* Koga, 1953 (24/284=8.5%) *Loxogenoides bicolor* Kaw, 1945 (33/284=11.6%) *Centrocestus formosanus* Nishigori, 1924 (15/284=5.3%) *Haematoloechus similis* Looss, 1899 (2/284=0.7%)
Paracrostoma paludiformis paludiformis	736	2	0.3	*Haematoloechus similis* Looss, 1899 (2/736=0.3%)
Thiara scabra	86	1	1.2	*Loxogenoides bicolor* Kaw, 1945 (1/86=1.2%)
Brotia (Senckenbergia) wykoffi	146	1	0.7	*Haematoloechus similis* Looss, 1899 (1/146=0.7%)
Paracrostoma pseudosulcospira pseudosulcospira	3,295	0	0	-
Paracrostoma paludiformis dubiosa	3,266	0	0	-
Paracrostoma morrisoni	117	0	0	-
Brotia (Brotia) binodosa binodosa	304	0	0	-
Brotia (Brotia) microsculpta	1,250	0	0	-
Brotia (Brotia) pagodula	1	0	0	-
Brotia (Brotia) binodosa spiralis	5	0	0	-
Brotia (Brotia) insolita	5	0	0	-
Brotia (Brotia) manningi	49	0	0	-

[a] (no. infected snails/no. collected snails = % infection)

was smaller than the oral sucker. The virgula organ was located in the region of the oral sucker. A stylet was present in the oral sucker. There were three pairs of penetration glands, two anterior pairs with fine granules and a posterior pair with coarser granules. All ducts opened near the tip of the stylet. The genital primordial was C-shaped. The excretory bladder was U-shaped. The tail was shorter than the body and attached to the posterior part of the body. The tail was spinose, with slight spines at the tip (Fig 6D). Average sizes (in micrometers) were measured; body; 54-82 (av 75) x 90-120 (av 110); tail: 20-30 (av 27) x 35-80 (av 75); oral sucker: 24-30 (av 28) x 24-30 (av 28); stylet: 5-8 (av 7) x 14-20 (av 18); ventral sucker: 12-18 (av 15) x 13-20 (av 17); pharynx: 4-6 (av 5) x 4-10 (av 8); and excretory bladder: 8-10 (av 9) x 10-30 (av 25).

Movement behavior. The cercaria floated on the surface or in the middle of the water. The body sank lower than the tail. It moved

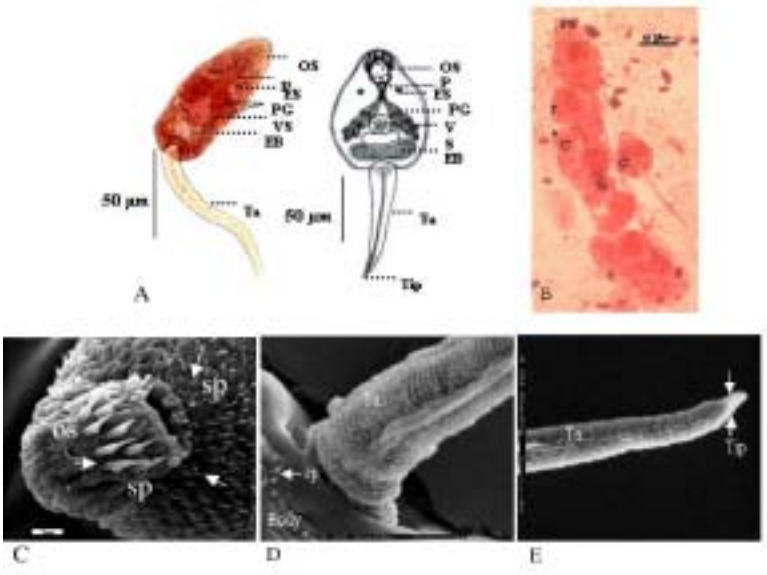

Fig 4–Image of *Centrocestus formosanus* cercaria; A: light micrograph and drawing structure. (OS = Oral sucker, ES = eye spot, P = pharynx, PG = penetration gland,VS = ventral sucker, Ta = tail, EB = excretory bladder). B: redia, staining with 0.5% neutral red. (Re = redia, Ph = pharynx, C = cercaria). C: Oral sucker had two rows of oral spines (4 in anterior row and 5 in posterior row). D-E: SEM micrograph (sp = spine,Ta = tail).

by folding its tail back to the body and turning its body to roll from left to right quickly, darting forward for about 60-75 seconds, and resting for about 2-5 seconds. It survived up to 2-3 hours in the water after emergence.

H. similis was found in 2 *T. granifera*, 2 *P. paludiformis paludiformis* and 1 *B. wykoffi*. The infection rates were 0.7% (2/284), 0.3% (2/736) and 0.7% (1/146), respectively (Table 3). The sampling sites in which this parasite could be found were Kaeng Sopha Waterfall and Pha Laht Waterfall. This parasite was classified into simple-tailed Xiphidiocercaria. The body surface was covered with spines (Fig 7C). The acetabulum was equatorial. Cystogenous cells were not observed, though refractile globules were scattered in the body and the tail. The stylet was long. Six pairs of penetration glands of irregular shape were present, extending from the pre-acetabular level to near the posterior end of body, each with large nuclei and fine granules. Their ducts were bundled, one on each side, opening near the tip of stylet. The prepharynx was short; the pharynx poorly differentiated. The esophagus, ceca and genitalia were not developed. The excretory vesicle was Y-shaped. The tail tubule was obliterated; the tail was without finfolds (Fig 7D). The average sizes (in micrometers) were measured: body: 87-104 (av 95) x 130-164 (av 148); tail: 27-34 (av 30) x 90-120 (av 115); oral sucker: 34-42 (av 38) x 37-46 (av 42); stylet: 20-34 (av 30) x 20-34 (av 32); ventral sucker: 10-20 (av 16) x 12-20 (av 18); pharynx: 11-13 (av 12) x 15-17 (av 16); and excretory bladder: 20-22 (av 21) x 20-24 (av 22).

Movement behavior. The cercaria floated on the surface or in the middle of the water. The body sank lower than the tail. It moved by folding its tail back to the body and turning its body to roll from left to right quickly, darting forward for about 15-20 seconds, and resting for about 10-15 seconds. It survived up to 1-2 hours in the water after emergence.

Five species of the snails were infected with trematode infections: *T. granifera*, *M. tuberculata*, *T. scabra*, *P. p. paludiformis* and *B. wykoffi*. The rates of infection were 26.1% (74:284), 33.3% (8:24), 1.2% (1:86), 0.3% (2:736) and 0.7% (1:146), respectively. In terms of the rate of cercarial infections, *L. bicolor* cercariae were found more frequently than other cercariae. They were found in 33 *T. granifera*, 1 *T. scabra* and 3 *M. tuberculata* (Table 3).

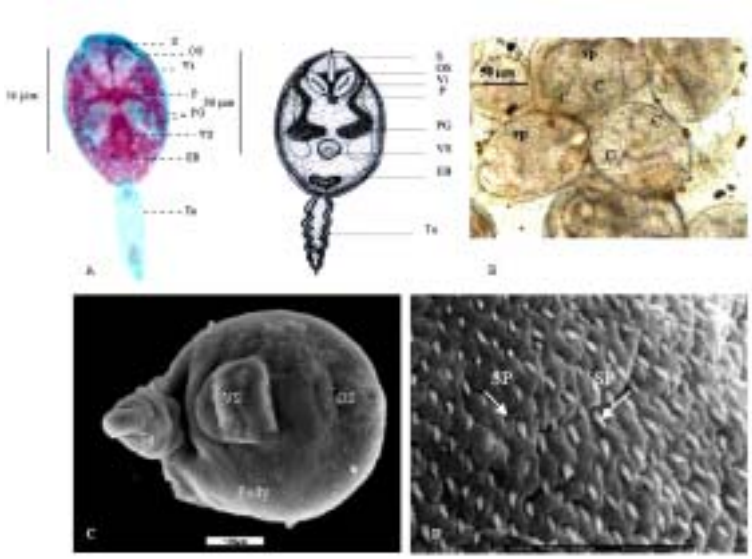

Fig 5–Image of *Acanthatrium hitaense* cercaria; A: light micrograph and drawing structure (OS = Oral sucker, S = stylet, Vi = virgulate gland, P= pharynx, PG = penetration gland,VS = ventral sucker, Ta = tail, EB = excretory bladder). B: sporocyst, staining with 0.5% neutral red. (sp = sporocyst, C = cercaria). C-D: SEM micrograph (OS = Oral sucker, VS = ventral sucker, Ta = tail, SP = spine).

DISCUSSION

Fourteen species of Thiarid snails were found in this study. The two species of snails with highest frequency were *Paracrostoma pseudosulcospira pseudosulcospira* (34.4%) and *Paracrostoma paludiformis dubiosa* (34.1%). In previous reports, *Paracrostoma* was the only genus of the Thiarid snail that was recorded in the Khek River, whereas other snails were reported in other areas in Thailand (Brandt, 1974; Klinhom, 1989). In this study, *Paracrostoma* was the dominant species in the Khek River; however, other genera of Thiarid snails were also found in this area.

Five species of snails shedded cercariae. Two types and five species of trematodes were categorized. The first type, Pleurolophocercous cercariae, consisted of *Haplorchis pumilio* and *Centrocestus formosanus*. This type of cercariae is said to have a median dorso-ventral finfold and can be subdivided into Parapleurolophocercous cercariae (Schell, 1970). Pleurolophocercous cercariae are the cercariae in the superfamily Opisthorchioidea, and Parapleurolophocercous cercariae developed in rediae in operculate snails and they have been reported in the family Heterophyidae. The second type, Xiphidiocercariae, consisted of *Acanthatrium hitaense*, *Loxogenoides bicolor* and *Haematoloechus similis*. This type of cercariae has an oral sucker armed with a piercing stylet for penetration of host tissues. The body has penetration and cystogenous glands and, in some cases, a virgula organ. Cercariae develop in sporocysts, which in some species are enclosed in a cellular envelope or paletot. The excretory system is of the mesostomate type. Cercariae are poor swimmers, creep on the substrate, and develop in rediae and encyst in a variety of vertebrate and invertebrate animals (Schell, 1970).

Two species of cercariae were found in *Melanoides tuberculata: H. pumilio* and *L. bicolor*. *Tarebia granifera* shed four species of cercariae: *C. formosanus*, *A. hitaensis*, *L. bicolor* and *H. similis*. *Paracrostoma paludiformis paludiformis* shed only one species of trematode: *H. similis*. *Thiara scabra* sheded *L.bicolor* and *Brotia (Senckenbergia) wykoffi* shed *H. similis*. Host specificity in mollusc-trematode relationships have been described by many malacologists. The attraction of miracidia to a specific mollusc is influenced by physico-chemical factors present in the environment (Upatham, 1972; Sturrock and Upatham, 1973). The nature of the environment is known to determine whether the rela-

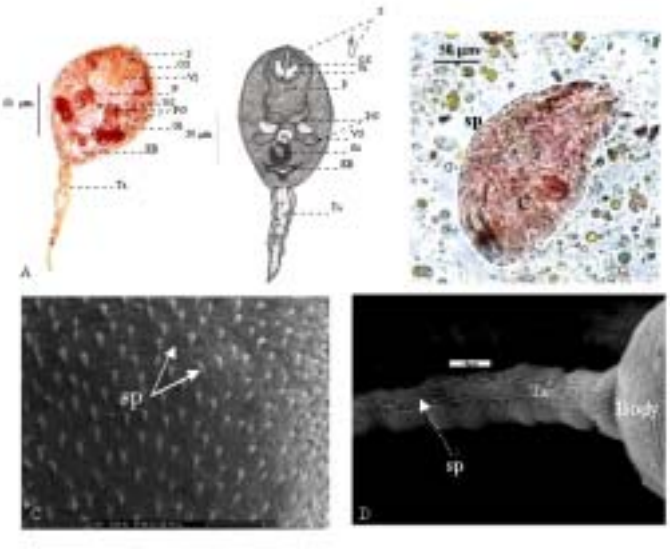

Fig 6–Image of *Loxogenoides bicolor* cercaria; A: light micrograph and drawing structure (OS = Oral sucker, S = stylet, Vi = virgulate gland, P= pharynx, PG = penetration gland, VS = ventral sucker, Ta = tail, EB = excretory bladder, Gi = genital primordium). B: sporocyst, staining with 0.5% neutral red. (sp = sporocyst, C = cercaria). C-D: SEM micrograph (Ta = tail, sp = spine).

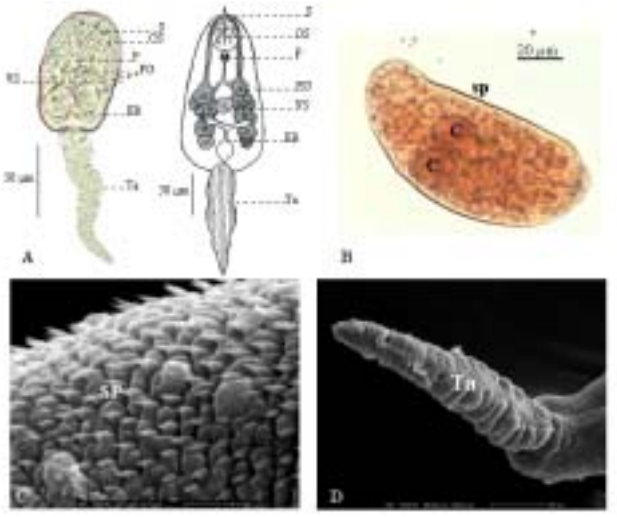

Fig 7–Image of *Haematoloechus similis* cercaria; A: light micrograph and drawing structure (OS = Oral sucker, S = stylet, P= pharynx, PG = penetration gland, VS = ventral sucker, Ta = tail, EB = excretory bladder). B: sporocyst, staining with 0.5% neutral red. (sp = sporocyst, C = cercaria). C-D: SEM micrograph (Ta = tail, sp = spine).

tionship between mollusc and miracidium can be productive. The lung fluke, *Paragonimus ohirai*, infects only one of three species of brackish-water snails of the genus *Assiminea* (*A. parasitologica*, *A. japonica* and *A. latericea*). Investigators demonstrated the influence of the molluscs' attractants can be masked by ambient factors and that attraction of miracidia to the mollusc does not mean successful subsequent development (Malek and Cheng, 1974).

In a previous study, *Haplorchis pumilio* cercariae were found in rediae from *Melania reiniana* var. *hidachiensis* and *Melanoides tuberculata chinensis*, and were encysted in pectoral fins and the base of the tail fin of fishes, *Carassius* and *Cyprinus* (Yamaguti, 1975). *Centrocestus formosanus* cercariae developed from rediae in *Melania* spp, *Melania (Melanoides) tuberculata chinensis*, *Stenomelania newcombi* and *Tarebia granifera*, encysting in gills, the buccopharyngeal cavity, gastro-intestinal wall, muscle, heart, liver, kidney, peritoneum and adipose tissues of cyprinoid fish (Yamaguti, 1975). For *Acanthatrium hitaensis*, their sporocysts had been found in the snail genus *Semisulcospira liberlina*. Their second intermediate host (2nd host) is an aquatic insect larva (stone fly, firefly, *Chironomus*, etc), and the final host is *Rattus norvegicus* (Ito,1980). For *Loxogenoides bicolor*, their sporocysts had been found in the snail genus *Goniobasis depygis*. The cercariae penetrated and encysted in aquatic naiads of Odonata and Epheme-

Table 4
Characteristics of cercariae in the Khek River (measurements in μm).

	Trematodes species				
	Haplorchis pumilio	*Centrocestus formosanus*	*Acanthatrium hitaense*	*Loxogenoides bicolor*	*Haematoloechus similis*
Body shape	Oval	Oval	Oval	Oval	Oval
Body size	90-140 (av 124) x 168 - 295 (av 257)	45-72 (av 64) x 82 - 120 (av 117)	53-92 (av 78) x 80 - 110 (av 100)	54-82 (av 75) x 90-120 (av 110)	87-104 (av 95) x 130-164 (av 148)
Color	brownish-yellow	light brown	light brown	light brown	light brown
Stylet	-	-	8-13 (av 10) x 11-13 (av 12)	5-8 (av 7) x 14-20 (av 18)	20-34 (av 30) x 20-34 (av 32)
Eyespot	1 pair	1 pair	-	-	-
Pharynx	8-10 (av 9) x 12-19 (av 15)	7-9 (av 8) x 8-10 (av 9)	10-15 (av 13) x 12-24 (av 20)	4-6 (av 5) x 4-10 (av 8)	11-13 (av 12) x 15-17 (av 16)
Oral sucker	27-48 (av 36) x 27-48 (av 36)	16-26 (av 24) x 17-28 (av 25)	25-32 (av 30) x 34-40 (av 37)	24-30 (av 28) x 24-30 (av 28)	34-42 (av 38) x 37-46 (av 42)
Ventral sucker	14-24 (av 18) x 14-24 (av18)	12-16 (av 14) x 12-16 (av 14)	15-16 (av 16) x 15-18 (av 17)	12-18 (av 15) x 13-20 (av 17)	10-20 (av 16) x 12-20 (av 18)
Penetration glands	7 pairs	7 pairs	2 pairs	3 pairs	6 pairs
Excretory bladder	28-40 (av 34) x 28-40 (av 34)	24-30 (av 28) x 38–52 (av 45)	8-12 (av 10) x 20-46 (av 38)	8-10 (av 9) x 10-30 (av 25)	20-22 (av 21) x 20-24 (av 22)
Tail length	465-528 (av 490)	69-92 (av 82)	26-75 (av 68)	35-80 (av 75)	90-120 (av 115)
Tail width	10-36 (av 30)	14-17 (av 15)	19-25 (av 23)	20-30 (av 27)	27-34 (av 30)
Lateral finfold	anterior 1/3 of tail	-	-	-	-
Dorsal-ventral finfold	posterior 2/3 of tail	1/2 of tail	-	-	-

roptera (Yamaguti, 1975). For *Haematoloechus similis*, their sporocysts had been found in the snail genus *Planorbis planorbis*, and cercariae creeped onto dragonfly nymphs in search of a suitable place to enter, usually penetrating them at the base of the head or appendage, more rarely at the base of the head or appendage, even more rarely at the abdominal inter-segments (Yamaguti, 1975). In Thailand, four species of thiarid snails at Erawan Waterfall, Kanchanaburi Province were *T. granifera*, *M. jugicostis*, *M. tuberculata* and *T. scabra*. They shed Pleurolophocercous cercariae (*Haplorchis pumilio* and *Stictodora tridactyla*), Xiphidiocercariae (*Loxogenoides bicolor*) and Furcocercous cercariae (*Mesostephanus appendicalatus*, *Transversotrema laruei* and *Cardicola alseae*) (Ukong *et al*, 2007).

This study is a progressive report of thiarid snail distribution and diversity of trematodes in Thailand. Four Thiarid snails were intermediate hosts for five trematodes. *Haplorchis pumilio* was found in *Melanoides tuberculata*; *Centrocestus formosanus* and *Acanthatrium hitaensis* from *Tarebia granifera*; *Loxogenoides bicolor* from *Tarebia granifera*, *Thiara scabra* and *Melanoides tuberculata*; and finally *Haematoloechus similis* from *Paracrostoma paludiformis paludiformis* and *Brotia (senckenbergia) wykoffi*. Thus, it seems that *Loxogenoides bicolor* can be found in various snails, whearas snails of the genus *Tarebia granifera* are more susceptible snail hosts than other snails.

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