J Trop Med Parasitol. 2012;35:37-47.

ORIGINAL ARTICLE

Available online at www.ptat.thaigov.net

Species Diversity of Aquatic Mollusks and Their Cercarial Infections; Khao Yai National Park, Thailand

Duangduen Krailas[1], Sirilak Chotesaengsri[1], Wivitchuta Dechruksa[1] Suluck Namchote[1], Chatapat Chuanprasit[1], Nuanpan Veeravechsukij[1] Dusit Boonmekam[1], Tunyarut Koonchornboon[2]

[1] Department of Biology, Faculty of Science, Silpakorn University, Nakhon Pathom;
[2] Department of Anatomy, Phramongkutklao College of Medicine, Bangkok, Thailand

Abstract

Aquatic mollusks were investigated by opportunistic collection in Khao Yai National Park, Thailand, every two months from March 2006 to January 2007. Our study focused on the habitat and species diversity of aquatic mollusks, which can act as intermediate hosts of parasitic trematodes. The study assembled baseline information on parasitic animal and human diseases in this area. Three sampling areas were investigated for mollusk diversity: Kongkaew Waterfall, Lam Takhong Stream, and Heo Suwat Waterfall. The mollusks were collected by hand-pick and scoop methods. Five species of gastropod and bivalve mollusks were found: *Melanoides tuberculata*, *Filopaludina martensi martensi*, *Clea (Anentome) helena*, *Pseudodon mouhoti*, and *Corbicula javanica*. The physicochemical characteristics and water quality of the streams varied slightly from one area to another, and between seasons. The water temperature was around 22-25 °C, and dissolved oxygen 7.1-9.5 mg/l. The cercarial infections of the mollusks collected were investigated by shedding and crushing methods, and were categorized into three species: *Apatemon gracilis*, *Mesostephanus appendiculatus,* and *Loxogenoides bicolor*. *A. gracilis* and *M. appendiculatus* cercariae were found in the snail *C. helena*, in Lam Takhong Stream and Heo Suwat Waterfall. The infection rates were 4.35% (2/46) and 6.52% (3/46), respectively. *L. bicolor* was found in the snail *F. martensi* in Lam Takhong Stream. The infection rate was 2.38% (1/42). *M. tuberculata* was the most densely populated species of aquatic mollusk in this study, whereas *C. helena* was the most important species for parasitic disease.

Keywords: aquatic mollusks, intermediate host, cercariae, trematode infections

Introduction

Khao Yai National Park, the first National Park in Thailand, is both a UNESCO World Heritage Site and an ASEAN Heritage Park. Consisting in the main of evergreen forests, it is 250-1,350 meters above sea level and covers an area of 2,168 square kilometers [1]. In Lam Takhong, the stream flows down to the Pak Chong District plain, on past Sung Noen District, merging with the Mun River in Northeast Thailand. The tropical surroundings and fertility of its waters make Khao Yai an ideal

Correspondence:
Duangduen Krailas,
E-mail: <kduang@su.ac.th>;
<kduang@gmail.com>

habitat and a breeding ground for species diversity.

In our study, aquatic mollusks consisted of gastropods (snails) and bivalves (clams), and were examined for cercarial infection and for species diversity. It is the first time, we believe, these have been systematically recorded in Khao Yai National Park. Aquatic mollusks serve as the first and second intermediate host of trematodes. Diseases transmitted by mollusks are highly significant for veterinary and public health, and various investigations have been carried out on the fauna of mollusks and trematode infection [2-5]. In Thailand, where cercarial infection of freshwater snails has been reported, Lophocercous cercariae were found in *Bithynia siamensis goniomphalus* [6]. Also, *Paludomus petrosus* at Pa la-U Waterfall were infected by 4 types of cercariae: Xiphidiocercariae, Amphistome cercariae, Furcocercous cercariae type I, and Furcocercous cercariae type II [7]. Three types of cercariae were found in Thiarid snails at Erawan Waterfall. The cercariae were categorized into six species: *Haplorchis pumilio*, *Stictodora tridactyla*, *Mesostephanus appendiculatus*, *Transversotrema laruei*, and *Cardicola alseae* [8]. The purpose of this study was to demonstrate the species diversity of aquatic mollusks, and their susceptibility to trematode infections, in Khao Yai National Park.

Materials and methods

Study sites

The study started with a site survey and GPS (Garmin Plus III, Taiwan) measurements of the survey spots. The three sampling areas were Kongkaew Waterfall (N 14° 26′ 14.8″ E 101° 22′

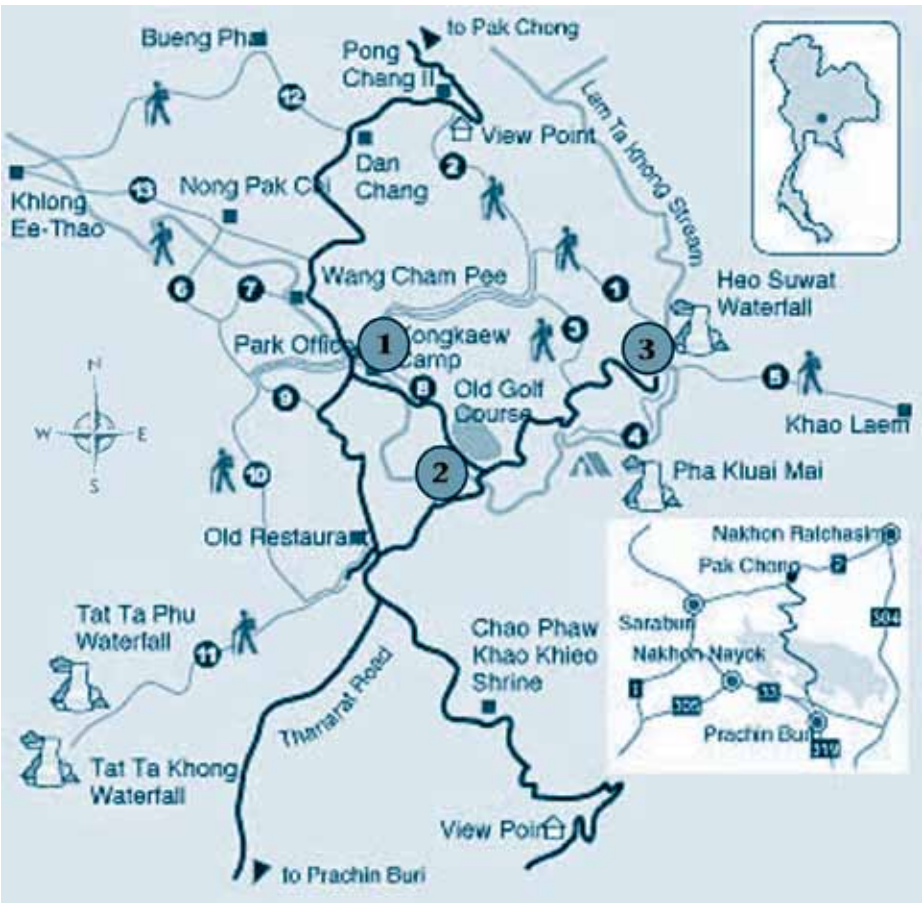

Fig 1 Map of Khao Yai National Park, Nakhon Ratchasima Province: 1) Kongkaew Waterfall; 2) Lam Takhong Waterway; 3) Heo Suwat Waterfall.

37.6'', altitude 713 m); Lam Takhong Stream (N 14° 25' 19.6'' E 101° 23' 26.3'', altitude 700 m), and Heo Suwat Waterfall (N 14° 26' 27.6'' E 101° 24' 53.8'', altitude 625 m) (Fig 1). The microhabitats of the study areas, as well as the physicochemical characteristics of the water, were recorded. The chemical characteristics of the water were analyzed by water analysis kits and by spectrophotometer (Orbeco-Hellige 975 MP Analyst, USA). The water temperature, pH, dissolved oxygen (U10 Horiba, Japan), and velocity (Flowmeter Model 2030, General Oceanics, USA) were all measured.

Collection of aquatic mollusks

Aquatic mollusks were collected every two months between March 2006 and January 2007, using the 'counts per unit of time' method [9]. Five collectors picked the mollusks by hand and scoop; one sample every 10 minutes. The collected mollusks were classified by The Conchology.

Study of trematode infections

The collected aquatic mollusks were investigated for trematode infection by emerging and crushing methods. In the emerging method, mollusks were kept alive in the laboratory, and stored overnight individually in Petri dishes containing room-temperature de-chlorinated water. The collected cercariae were observed carefully as being alive, and then fixed in 10% formalin. Measurements in micrometers with averages in parentheses were taken from 20 specimens. Details of the cercariae were drawn using a camera lucida; all species were then identified [8,10-15].

For scanning electron microscope analysis, cercariae were fixed in 2.5% glutaraldehyde phosphate buffer (0.1 mol/L, pH 7.4) at 4°C for at least 2 hours, and post-fixed in 1% osmium tetroxide in the same buffer for 2 hours at 4°C. They were dehydrated through a graded series of ethanol, and 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 CPD7501, UK) and then examined in a CamScan MX 2000 scanning electron microscope (UK) and JEOL, JSM-5410 LV (Japan). The voucher specimens of cercariae were deposited in the Parasitology and Medical Malacology Research Unit, Department of Biology, Faculty of Science, Silpakorn University, Thailand.

Results

Microhabitat of the study sites

The study sites were covered with medium to large trees, which allowed filtered sunlight to reach the streams; the average light intensity was >10,000 lux at noon. The current was swift in the rainy season, and the water temperature was 21-28°C. Small- to medium-sized rocks were dispersed across all the streams. The collected mollusks were found on the rough sand, on aquatic plants, and on the rocks. The physicochemical quality of the environment and the water changed with the seasons. The study areas were affected by these changes during the dry and flood (wet) seasons.

Table 1 Snails and bivalves found in the 3 sampling areas of Khao Yai National Park.

Snail	No. snail samples (No. infected snails)		
	Kongkaew	Lam Takhong	Heo Suwat
1. *Melanoides tuberculata*	4	355	-
2. *Filopaludina m. martensi*	13	26 (1)	3
3. *Clea (Anentome) helena*	23	15 (2)	8 (3)
4. *Pseudodon mouhoti*	-	6	1
5. *Corbicula javanica*	-	94	1

Collected mollusks

The 549 aquatic mollusks collected were classified by conchological characteristics into 5 species, and divided into 3 snails and 2 clams (Table 1, Fig 2). The snails consisted of: 359 *Melanoides tuberculata* (Family Thiaridae); 42 *Filopaludina (Siamopaludina) martensi martensi* (Family Viviparidae); and 46 *Clea (Anentome) helena* (Buccinidae). The clams consisted of 7 *Pseudodon mouhoti* (Family Amblemidae), and 95 *Corbicula javanica* (Family Corbiculidae).

Parasitic infections

Two types of cercariae were found in two species of collected snails. These cercariae were categorized into 3 different species. The first were

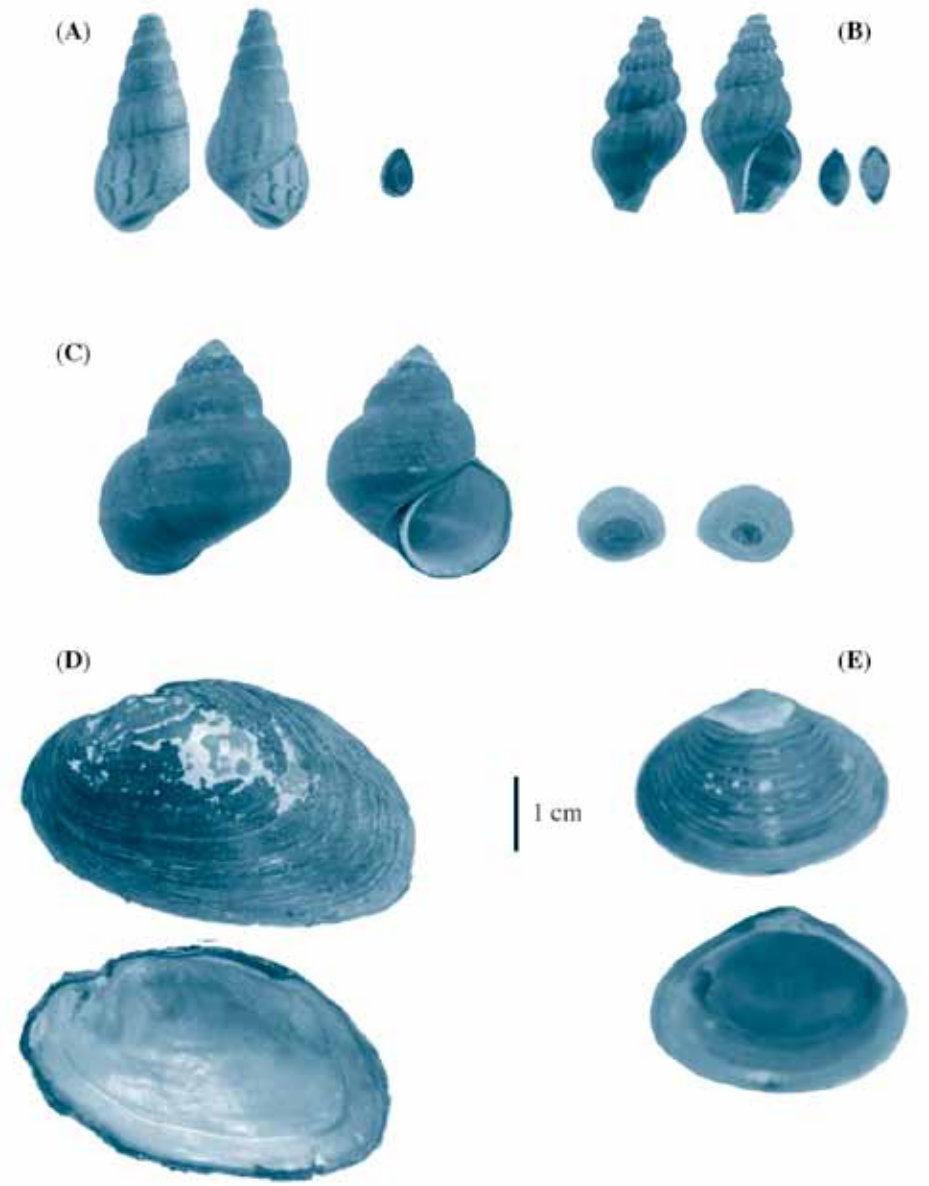

Fig 2 Aquatic mollusks collected in Khao Yai National Park: (A) *Melanoides tuberculata*; (B) *Clea (Anentome) helena*; (C) *Filopaludina m. martensi*; (D) *Pseudodon mouhoti*; (E) *Corbicula javanica*.

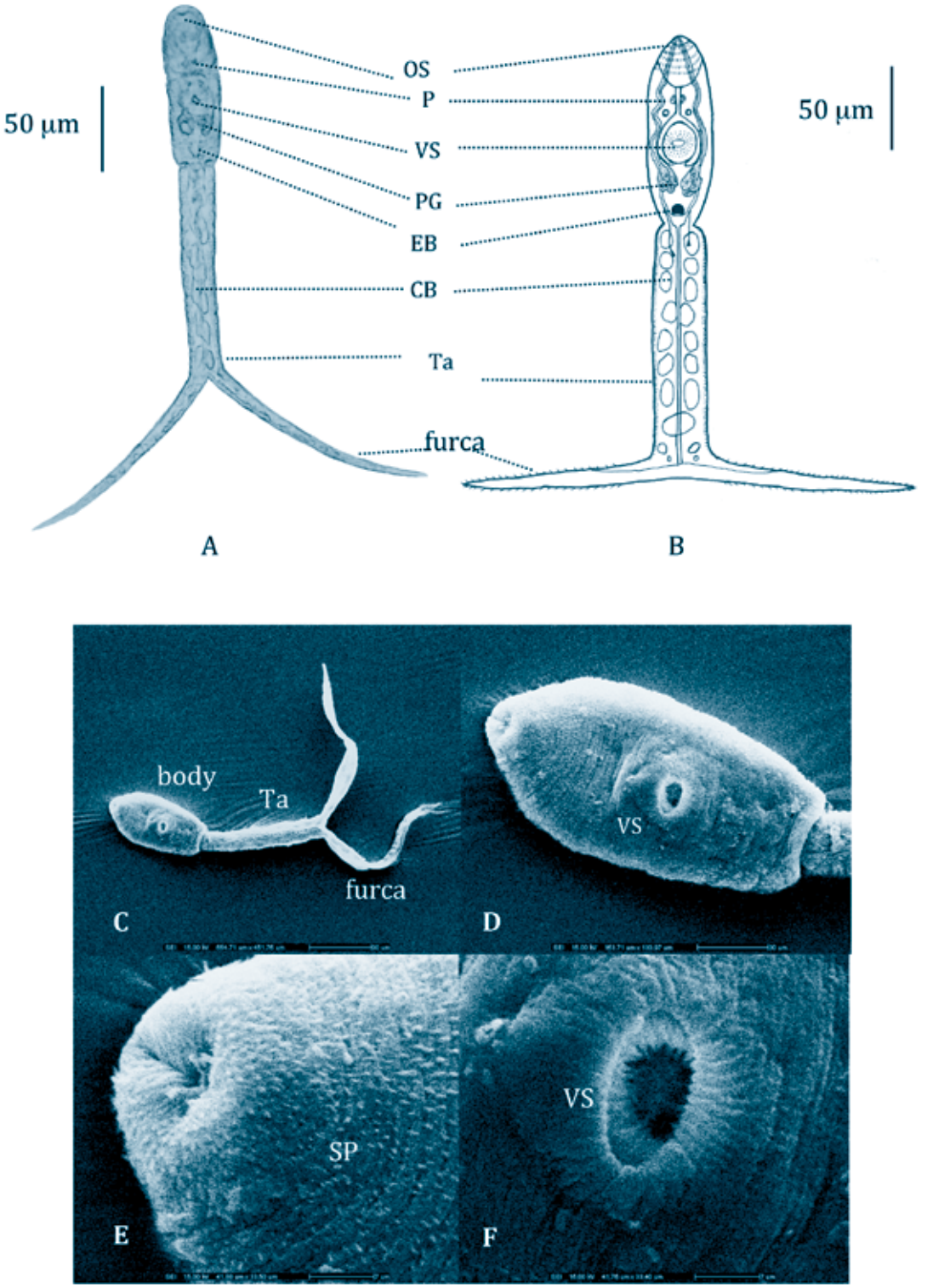

Fig 3 Images of *Apatemon gracilis*: (A) light micrograph; (B) drawing of structure; and (C-F) SEM micrograph (OS = oral sucker; P = pharynx; VS = ventral sucker; PG = penetration gland; EB = excretory bladder; CB = caudal body; Ta = tail; SP = spine).

Furcocercous cercariae, consisting of *Apatemon gracilis* Szidat, 1928 (Fig 3) and *Mesostephanus appendiculatus* Ciurea, 1961 (Fig 4). The second type was Xiphidio cercariae, consisting of *Loxogenoides bicolor* Kaw, 1945 (Fig 5). Cercarial morphology and anatomy were described from living cercariae, fixed cercariae, and cercarial images taken from scanning microscopes (Table 2).

Apatemon gracilis

A. gracilis were found in two *C. helena*, which is equivalent to an infection rate of 4.35% of the total number of *C. helena* collected. The sampling sites were at Lam Takhong Stream. The cercarial body is oval in shape, the prepharynx is practically absent, and the anterior region has a cuticle which is densely spined, like a file. It has un-pigmented eyespots, and the tail stem contains approximately 16 caudal bodies (Fig 3). There are 4 pairs of penetration glands, which lie between the acetabulum and genital primordial.

Average size (in micrometers, calculated from 20 cercariae)

Body:	41.5-90.0 (av 72) x 115-140 (av 126)
Oral sucker:	15-25 (av 21.45) x 20-30 (av 23.25)
Ventral sucker:	16-25 (av 23) x 20-30 (av 25)
Excretory bladder:	15-25 (av 18) x 15-30 (av 20)
Pharynx:	6-10 (av 8) x 7-13 (av 9)
Tail stem:	30-45 (av 39) x 240-312 (av 286)
Tail furca:	10-25 (av 18) x 130-160 (av 146)

Movement behavior

The cercariae floated in the water and on its surface. The body sank lower than the spreading forked tail. It moved by rolling up and springing the body back, and moved forward swiftly in a semi-circular motion. It rested by floating with its head on top for approximately 12-15 seconds, moving quickly for about 3-6 seconds, then floating/resting again.

Mesostephanus appendiculatus

M. appendiculatus were found in three *C. helena*, equivalent to an infection rate of 6.52% of the total number of *Clea* sp (Table 1). The sampling sites were at Heo Suwat Waterfall. The cercarial body is spinose and oval in shape (Fig 4D). It carries spines across its surface and on the oral sucker (Fig 4E). Coarse granules and cytogenous glands are scattered throughout the inside of the body. The pharynx is small and round. The prepharynx and esophagus are almost as long as the pharynx. A large intestine, composed of two caeca, terminates near a small excretory bladder. A vestigial ventral sucker is made up as a collection of small groups. The tail is forked and is longer than the overall size of the body, and is covered with many spines (Fig 4E, 4F). The tail stem is longer than the furca. The tail tubule opens at the tip of each furca; no flame cells are to be found.

Average size (in micrometers, calculated from 20 cercariae)

Body:	92-120 (av 109) x 160-250 (av 225)
Oral sucker:	20-45 (av 35) x 35-48 (av 39)
Ventral sucker:	15-24 (av 20) x 15-24 (av 20)
Excretory bladder:	20-40 (av 35) x 20-40 (av 35)
Pharynx:	8-10 (av 9) x 11-18 (av 15)
Tail stem:	25-40 (av 35) x 425-525 (av 495)
Tail furca:	15-25 (av 20) x 160-170 (av 165)

Movement behavior

The cercariae floated in the water and on its surface, and the body sank lower than the spreading forked tail. It moved by rolling up and springing the body back, and moved forward swiftly in a semi-circular motion for approximately 2-4 seconds. It then rested by floating with its head on top of the tail, slowly rotating its body to the bottom while lifting up its spreading forked tail. It floated for about 20-30 seconds.

Loxogenoides bicolor

L. bicolor were found in one *F. martensi*, equivalent to an infection rate of 2.38% of the total number of *Filopaludina* sp (Table 1). The sampling sites were at Lam Takhong Stream. The cercarial body is spinose and oval in shape. Its entire body is dotted with granules. The ventral

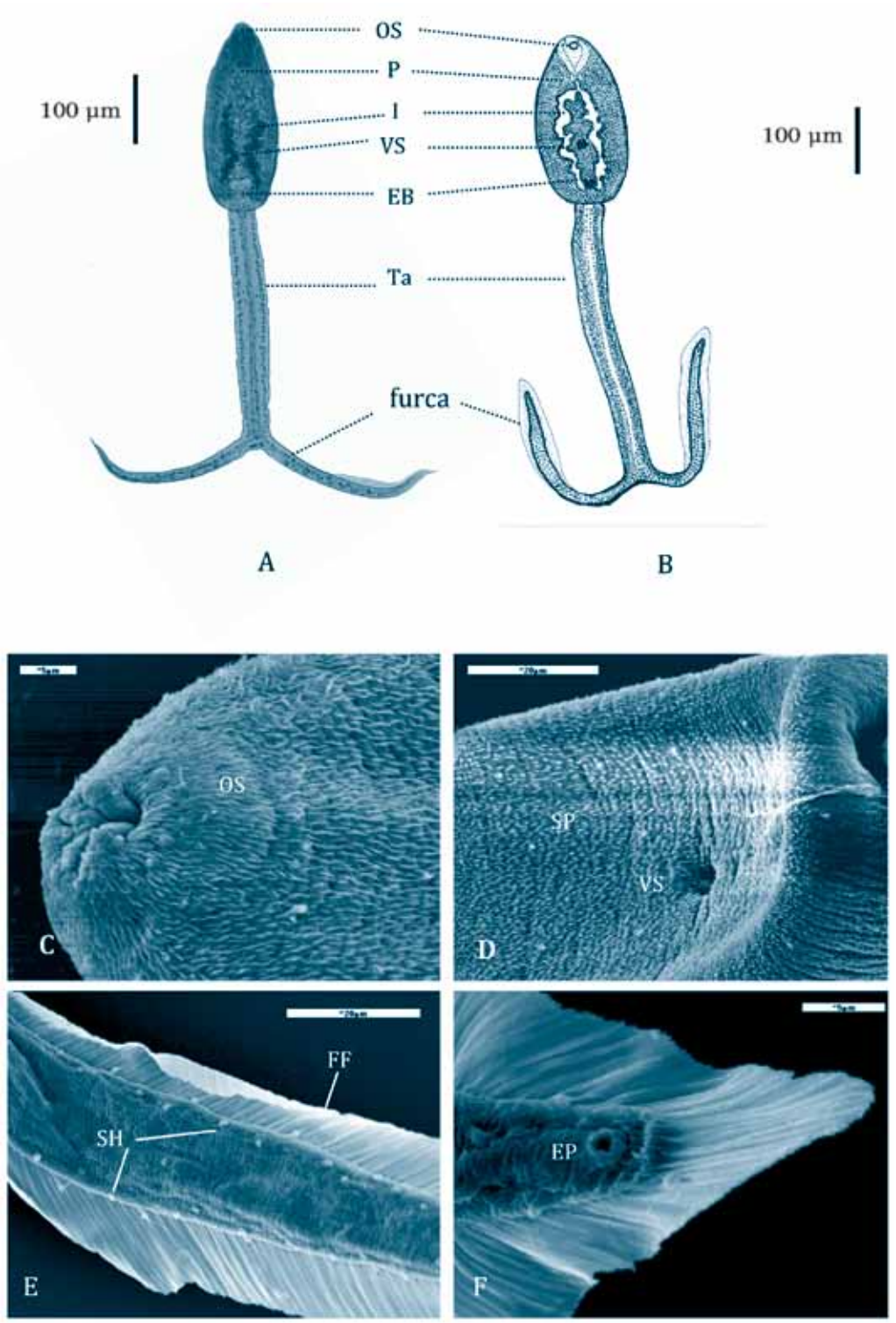

Fig 4 Images of *Mesostephanus appendiculatus*: (A) light micrograph; (B) drawing of structure; and (C-F) SEM micrographs (OS = oral sucker; P = pharynx; I = intestine; VS = ventral sucker; EB = excretory bladder; EP = excretory pore; SH = sensory hairs; FF = furcal finfold; Ta = tail; SP = spine).

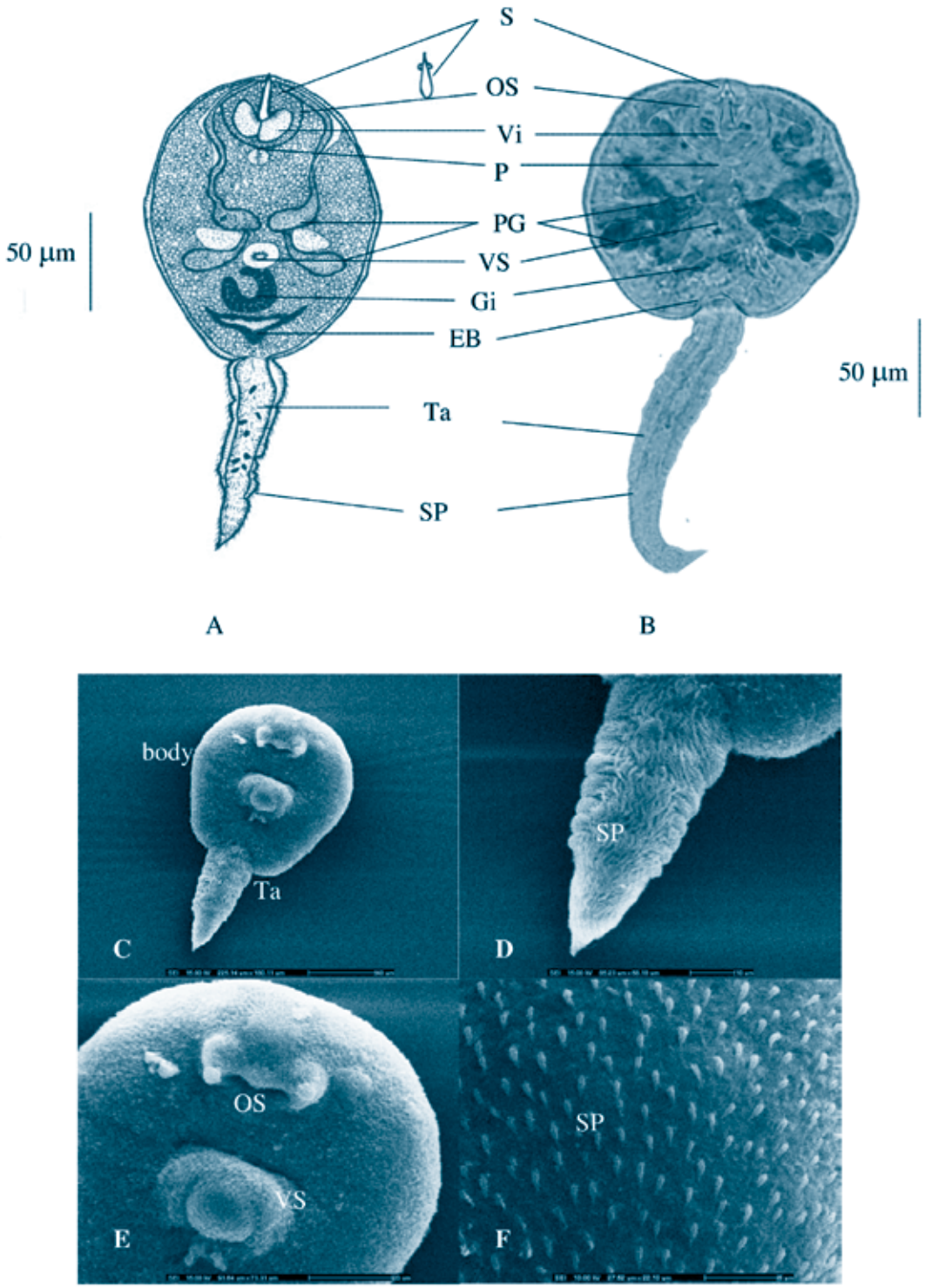

Fig 5 Images of *Loxogenoides bicolor*: (A) drawing structure; (B) light micrograph; and (C-F) SEM micrographs (S = stylet; OS = oral sucker; Vi = virgulate gland; P = pharynx; PG = penetration gland; VS = ventral sucker; Gi = genital primordial; EB = excretory bladder; Ta = tail; SP = spine).

Table 2 Characteristics of the cercariae at Khao Yai National Park (measurements in µm).

Characteristics	*Apatemon gracilis*	*Mesostephanus appendiculatus*	*Loxogenoides bicolor*
Body shape	Oval	Oval	Oval
Size of body	41.5-90 x 115-140 (av 72 x 126)	98-137 x 141-240 (av 116 x 187)	52-80 x 88-120 (av 74 x 105)
Color	light brown	brownish-yellow	light brown
Stylet	-	-	5-8 x 15-20 (av 7 x 18)
Eyespot	1 pair	-	-
Pharynx	6-10 x 7-13 (av 8 x 9)	3-5 x 7-9 (av 4 x 8)	4-6 x 7-10 (av 5 x 8)
Oral sucker	15-25 x 20-30 (av 21 x 23)	32-43 x 39-45 (av 38 x 42)	24-30 x 24-30 (av 28 x 28)
Ventral sucker	16-25 x 20-30 (av 23 x 25)	10-14 x 10-14 (av 12 x 12)	12-17 x 15-20 (av 15 x 18)
Penetration glands	4 pairs	-	3 pairs
Excretory bladder	15-25 x 15-30 (av 18 x 20)	17-28 x 24–64 (av 23 x 42)	8-10 x 17-30 (av 9 x 25)
Tail length	240-312 (av 286)	410-474 (av 447)	37-82 (av 78)
Tail width	30-45 (av 39)	37-64 (av 49)	23-29 (av 26)
Fork length	130-160 (av 146)	306-375 (av 336)	-
Fork width	10-25 (av 18)	27-41 (av 37)	-

sucker is smaller than the oral sucker. A virgula organ is located in the same region as the oral sucker. A stylet is present (Fig 5). Three pairs of penetration glands exist: two anterior pairs with fine granules, and a posterior pair with coarser granules staining darker with neutral red. All ducts opened near the tip of the stylet. There is a C-shaped genital primordium and a U-shaped excretory bladder. The tail is also spinose, with slightly longer spines at the tip.

Movement behavior

The cercariae floats on the surface or in the water. The body sank lower than the tail. It moves by folding its tail back to the body, then quickly turning its body to roll from left to right. It darts forward for 60-77 seconds, resting for about 2-5 seconds. It survives for 2-3 hours in water after emergence.

Two species of collected snails were susceptible to trematode infection. They were *C. helena* and *F. martensi*.

Discussion

Most aquatic mollusks can become intermediate hosts for trematode infections. Both snails and bivalves are utilized as the first and second intermediate hosts of trematodes [3,6,16,17]. Among the diverse mollusks in Lam Takhong Reservoir, *C. helena*, *M.tuberculata,* and *Corbicula* sp, have been reported as the dominant snail species; however, they have not been found to have trematode infections [18]. In this study, 2 of 5 species of aquatic mollusks were susceptible to cercarial infections – *C. helena* and *F. martensi*; the infection rates were 0.18% (1/549) and 0.9% (5/549), respectively. In a total of 549 aquatic mollusks, *M. tuberculata*, a thiarid snail, had the highest species density among the collected snails. None of the 359 *M. tuberculata* in this study had cercarial infections. Normally, they are more susceptible to host infections by parasitic trematodes than other snails. They have been reported as first intermediate hosts of lung and intestinal flukes, such as *Haplorchis pumilio*, *H. taichui*, *Loxogenoides bicolor*, *Centrocestus formosanus*, *Acanthatrium hitaense*, *Haematoloechus similis*, *Cloacitrema philippinum*, *Transversotrema laruei*, *Stictodora tridactyla*, *Apatemon gracilis*, *Mesostephanus appendiculatus*, *Cardicola alseae,* and *Alaria mustelae*. These are important parasites of humans and some domestic animals in Thailand [8,15,19]. Three thiarid snails – *M. tuberculata*, *M. jugicostis* and *Neoradina prasongi* – were reported as intermediate hosts of *H. taichui*, but *H. pumilio* was only found in *M. tuberculata* in the South of Thailand [19]. Moreover, *H. pumilio* was reported from *M. jugicostis* in Erawan Waterfall and *M. tuberculata* in the Khek River [8,15]. The intermediate host of the human blood fluke was not found. We found only bird blood fluke of two species of Furcocercous cercariae from *C. helena*; they were *A. gracilis* and *M. appendiculatus*. The Furcocercous cercariae (fork-tailed cercariae) were subdivided into several groups: Lophocercous apharyngeate, Brevifurcate apharyngeate, Brevifurcate pharyngeate and Longifucate pharyngeate cercariae. Both *A. gracilis* and *M. appendiculatus* were the longifurcate pharyngeate cercariae. *C. helena* showed very few recent records of trematode infections of this species and the first report of blood flukes in Thailand. One parasite, *L. bicolor*, was the most common species of trematode found from *F. martensi* in this study. *L. bicolor* was reported in adult worms in frogs and cercariae in *Goniobasis depygis* snails [13,14]. *L. bicolor* cercariae was reported from thiarid snails (*Tarebia granifera*, *Thiara scabra*, *M. tuberculata*, *M. jugicostis*, *N. prasongi*, *Adamietta housei*), and paludomidae snails (*Paludomus siamensis*, *P. petrosus*) [8,15,19,20]. Given that aquatic mollusks are widespread in Thailand and participate in the life cycles of trematodes, the impact of introducing and spreading these snail intermediate hosts around the country must be better evaluated, to prevent future cases of human and animal disease.

Acknowledgements

This work was supported by the Research and Development Institute, Silpakorn University and the Department of Biology, Faculty of Science, Silpakorn University, Thailand.

References

1. Tourism Authority of Thailand. Khao Yai Dong Phaya Yen Forest range. 1st ed. Bangkok: Amarin Printing and Publishing Public Company Limited: 2000.
2. Ismail NS. Two new furcocercariae from *Melanopsis praemorsa* in Jordan. Helminthologia. 1989;261:15-20.
3. Wegeberg AM, Montaudouin XD, Jensen KT. Effect of intermediate host size

(*Cerastoderma edule*) on infectivity of cercariae of three *Himasthla* species (Echinostomatidae, Trematoda). J Exp Mar Bio Ecol. 1999;238: 259-69.

4. Abdul-Salam J, Sreelatha BS, Ashkanani H. The eye fluke *Philophthalmus hegeneri* (Digenea: Philophthalmidae) in Kuwait Bay. Kuwait J Sci Eng. 2004;31(1):119-33.
5. Farahnak A, Vafaie-Darian R, Mobedi I. A faunistic survey of cercariae from freshwater snails: *Melanopsis* spp. and their role in disease transmission. Iranian J Public Health. 2006;35(4):70-4.
6. Adam R, Arnold H, Pipitgool V, Sithithaworn P, Hinz E, Storch V. Studies on Lophocercous cercariae from *Bithynia siamensis goniomphalus* (Prosobranchia: Bithyniidae). Southeast Asian J Trop Med Public Health. 1993;24(4): 697-700.
7. Krailas D, Dechruksa W, Ukong S, Janecharut T. Cercarial infection in *Paludomus petrosus*, Freshwater snail in Pa La-U Waterfall. Southeast Asian J Trop Med Public Health. 2003;34:286-90.
8. Ukong S, Krailas D, Dangprasert T, Channgarm P. Studies on the morphology of cercariae obtained from freshwater snails at Erawan Waterfall, Erawan National Park, Thailand. Southeast Asian J Trop Med Public Health. 2007;38(2):302-12.
9. Oliver LC, Schneiderman M. A method for estimating the density of aquatic snail population. Exp Parasitol.1956;5:109-17.
10. Schell SC. Parasitology laboratory manual. 1st ed. New York: John Wiley and Sons; 1962.
11. Schell SC. How to know the trematode. 1st ed. Iowa: Wm C Brown Publishers; 1970.
12. Nasir P. British freshwater cercariae. 1st ed. London: The British Museum; 1974.
13. Yamaguti S. A synoptical review of life histories of digenetic trematodes of vertebrates. 1st ed. Kyoto: Keigaku Publishing; 1975.
14. Ito J. Studies on cercariae in Japan. Japan: Shizuoka University; 1980.
15. Dechruksa W, Krailas D, Ukong S, Inkapatanakul W, Koonchornboon T. Trematode infections of the freshwater snail family Thiaridae in the Khek river, Thailand. Southeast Asian J Trop Med Public Health. 2007;38(6):1016-28.
16. Urabe M. Cercariae of a species of Philophthalmus detected in a freshwater snail, *Semisulcospira libertina*, in Japan. Parasitol Int. 2005;54:55-7.
17. Martorelli SR, Poulin R, Mouritsen KN. A new cercaria and metacercaria of Acanthoparyphium (Echinostomatidae) found in an intertidal snail *Zeacumantus subcarinatus* (Batillaridae) from New Zealand. Parasitol Int. 2006;55:163-7.
18. Tesana S. Diversity of mollusks in the Lam Ta Khong reservoir, Nakhon Ratchasima, Thailand. Southeast Asian J Trop Med Pub Health. 2002;33(4):733-8.
19. Krailas D, Namchot S, Rattanathai P. Human intestinal flukes *Haplorchis taichui* and *Haplorchis pumilio* in their intermediate hosts, freshwater snails of the families Thiaridae and Pachychilidae, in Southern Thailand. Zoosyst Evol. 2011;87(2):349-60.
20. Krailas D, Janecharat T, Ukong S, Notesiri N, Ratanathai P. Preliminary report on freshwater snails in Toa Dum Forest, Saiyok District, Kanchanaburi. Silpakorn Univ Int J. 2003;3 (1-2):194-205.