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Bucephalid (Gasterostome) Cercariae Obtained from Freshwater Clams in Thailand

Duangduen Krailas, Sirilak Chotesaengsri, Namfon Pattaradussadee, Napatsorn Notesiri, Wivitchuta Dechruksa

Parasitology and Medical Malacology Research Unit, Department of Biology, Faculty of Science, Silpakorn University, Nakhon Pathom 73000, Thailand

Abstract

Freshwater clams were collected in 16 sampling localities of 11 provinces in northeast Thailand. The clams were collected every 2 months, between February 2006 and August 2007, using the "counts per unit of time" sampling method. A total of 508 clam samples from 6 species were found–280 *Corbicula* sp, 23 *Pilsbryoconcha* sp, 136 *Scabies crispate*, 6 *Pseudodon mouhoti*, 51 *Limnoperna siamensis*, and 12 *Physunio eximinus*. Bucephalid (Gasterostome) cercariae were found from 3 clams belonging to 2 species, 1 *Corbicula* sp each at Rasi Salai and Lampao dams, and 1 *Limnoperna siamensis* at the Lampao River. The infection rate was 0.59% (3/508). Freshwater clams can serve as the first and second intermediate hosts for trematodes. This is the first study to report cercarial emergence from freshwater bivalves in this geographic region.

Keywords: cercarial emergence, freshwater clams, bivalvia, Bucephalidae

Introduction

Aquatic molluscs have been reported as the first and second intermediate hosts of trematodes. Various studies have been conducted on the fauna of molluscs and their trematode infections [1-3]. Studies on mollusc-transmitted diseases are very important for veterinary and public health. In northeast Thailand, the snail *Bithynia* sp was the intermediate host of the trematode *Opisthorchis viverrini* [4,5]. Some edible molluscs, for example, *Filopaludina* sp (snail) and *Corbicula* sp (clam), were the first and second intermediate hosts of the intestinal fluke echinostomes in Thailand [6].

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

Although parasitic infections of snails have been studied in several locations in Thailand, there has been no comprehensive study of clams as first intermediate hosts. This study examined freshwater clams for both cercarial and metacercarial trematode infections.

Materials and methods

Clams were collected from 16 localities in 11 provinces in northeast Thailand (Fig 1, Table 1); each study site was located by GPS (Garmin PLUS III, Taiwan). The clams were studied every 2 months between February 2006 and August 2007, using the "counts per unit of time" sampling method [7]. For each locality, 5 researchers collected clams by hand and scoop for 10 minutes. The clams were examined for parasitic infections in the laboratory by shedding and crushing

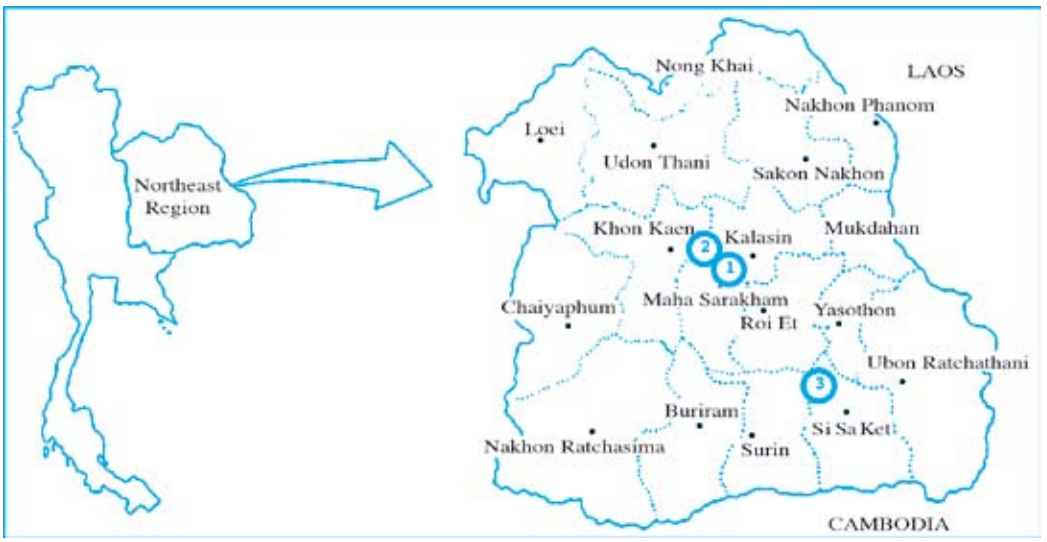

Fig 1 Three positive localities of 11 provinces in northeast Thailand. 1: Lampao river, Kalasin Province, 2: Lampao dam, Kalasin Province, 3: Rasi Salai dam, Si Sa Ket Province.

methods. The expelled cercariae were placed in dechlorinated water and observed for swimming behaviors [8]. The presence of sporocysts was examined under a dissecting microscope.

The emerged cercariae were studied unstained or vitally stained with 0.5% neutral red and Semichon's acetic carmine & fast green. On scanning electron microscope, the cercariae were fixed in 2.5% glutaraldehyde cacodylate buffer (0.1 M, pH 7.4) at 4˚C for at least 2 hours, and post-fixed in 1% osmium tetraoxide 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 CPD 7501, UK) and then examined in a scanning electron microscope (JEOL JSM-5410 LV, Japan).

Results

A total of 508 clam samples belonging to 6 species were found–280 *Corbicula* sp, 23 *Pilsbryoconcha* sp, 136 *Scabies crispate*, 6 *Pseudodon mouhoti*, 51 *Limnoperna siamensis*, and 12 *Physunio eximinus* (Fig 2). One type of digenean was obtained from 3 clam samples–1 *Corbicula* sp at Rasi Salai Dam (N 15° 20′ 27.8″, E 104° 06′ 21.7″, altitude 117 m), 1 *L. siamensis* at Lampao River (N 16° 20′ 20.7″, E 103° 34′ 45.7″, altitude 139 m) and 1 *Corbicula* sp at Lampao Dam (N 16° 36′ 22.6″, E 103° 26′ 27.5″, altitude 162 m). The infection rate was 0.59% (3/508) (Table 1).

The trematode found was a bucephalid (Digenea: Gasterostome) cercariae. The cercariae showed typical bucephalid features: tail present; tail as long as or longer than body; tail not greatly enlarged; body entirely anterior to tail; tail forked; tail stem very short and broad; furcae very long; mouth on midventral surface of body and opening into a saclike intestine; head organ or sucker at anterior end of body (Fig 3, 4). The sporocysts of this trematode were located in the intracellular space connective tissues of the infected clams. The ribbon sporocysts containing the cercariae were also present in gonadal tissues, between the inner and outer mantle epithelium, or in the gill parts (Fig 5). No metacercariae, however, were found in the collected clams.

Discussion

Several researchers have studied trematode infections in mollusc intermediate hosts, where both snails and bivalves were the first and

Table 1 Bivalves collected from sample localities, February 2006-August 2007.

	Locality (Province)	Number of bivalves collected					
		Corbicula sp	*Pilsbryoconcha* sp	*Scabies crispate*	*Pseudodon mouhoti*	*Limnoperna siamensis*	*Physunio eximinus*
1.	Lampao River, Kalasin Province	1	3	12	2	5 (1)*	-
2.	Lampao Dam, Kalasin Province	58 (1)*	-	54	-	30	12
3.	Rasi Salai Dam, Si Sa Ket Province	55 (1)*	-	2	-	14	-
4.	Ban Charat Swamp, Surin Province	-	-	32	-	-	-
5.	Kongkaew Waterfall, Nakhon Ratchasima	-	-	-	1	-	-
6.	Lam Ta Khong Stream, Nakhon Ratchasima	92	-	-	3	-	-
7.	Phimai Dam, Nakhon Ratchasima	11	-	-	-	1	-
8.	Tadton Waterfall, Chaiyaphum Province	1	-	-	-	-	-
9.	Huai Kliang, Yasothon Province	2	-	29	-	1	-
10.	Nong Buarai Swamp, Buri Ram Province	-	-	1	-	-	-
11.	Huai Takua, Buri Ram Province	-	-	2	-	-	-
12.	Huai Ho, Nakhon Phanom Province	14	8	-	-	-	-
13.	Tadkham Waterfall, Nakhon Phanom Province	9	-	-	-	-	-
14.	Nam Pung Dam, Sakon Nakhon Province	26	4	-	-	-	-
15.	Nong Sa-ad Bamrung Swamp, Maha Sarakham Province	5	8	4	-	-	-
16.	Tadton Waterfall, Mukdahan Province	6	-	-	-	-	-
	Total	280 (2)*	23	136	6	51 (1)*	12
	Grand total 508 (3)*						

* Number of clams infected with bucephalid cercariae.

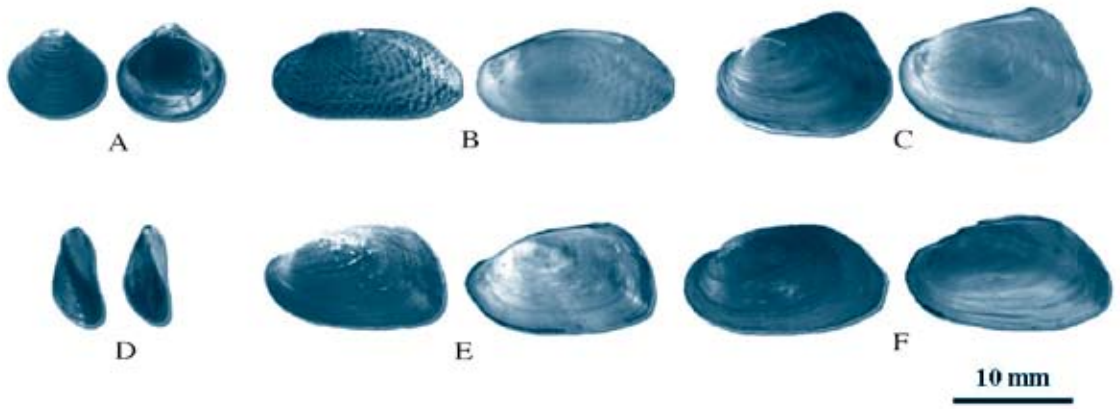

Fig 2 Clam samples were collected from the sampling areas. (A) *Corbicula* sp, (B) *Scabies crispate*, (C) *Physunio eximinus*, (D) *Limnoperna siamensis*, (E) *Pilsbryoconcha* sp, (F) *Pseudodon mouhoti*.

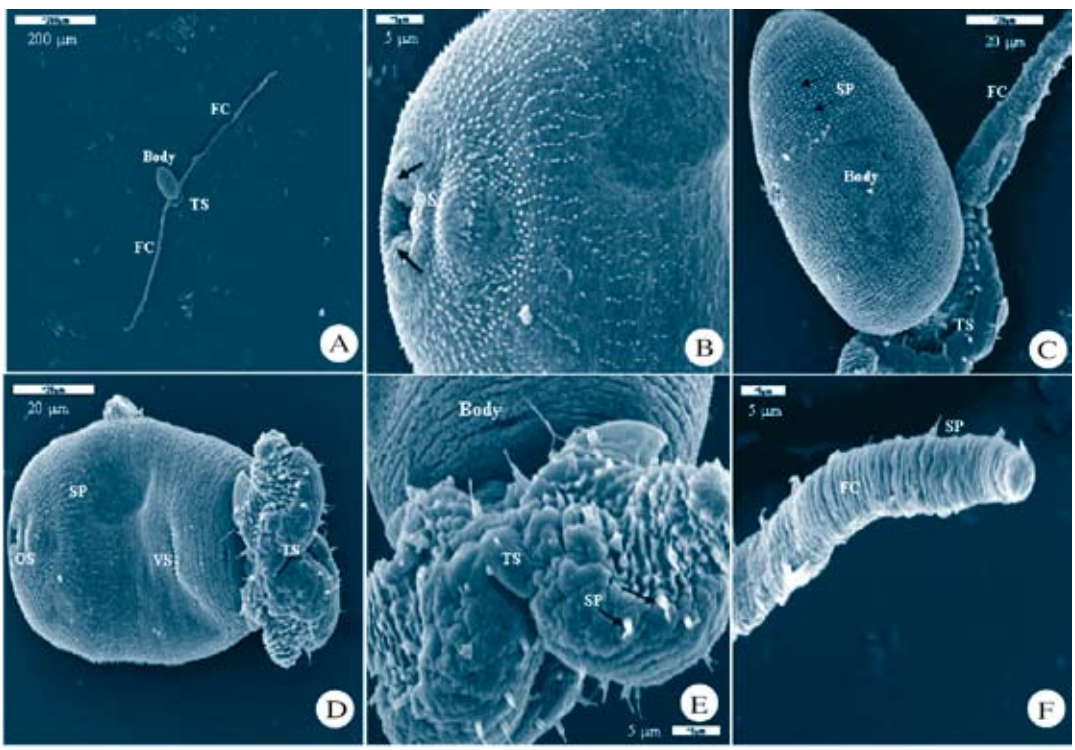

Fig 3 Scanning electron micrographs of bucephalid cercariae. A: extended long furcae, B: mouth and oral sucker, C: anterior of dorsal body with longitudinal spinous surface, D: anterior of ventral body with spinous surface, E: broad tail stem having some papillae in the upper view and papillae with sensory hair, F: papillae present on edges of furcae, the center absent, no found furcae tip and pore at the end. (OS: oral sucker, VS: ventral sucker, SP: spine, TS: tail stem, FC: furcae).

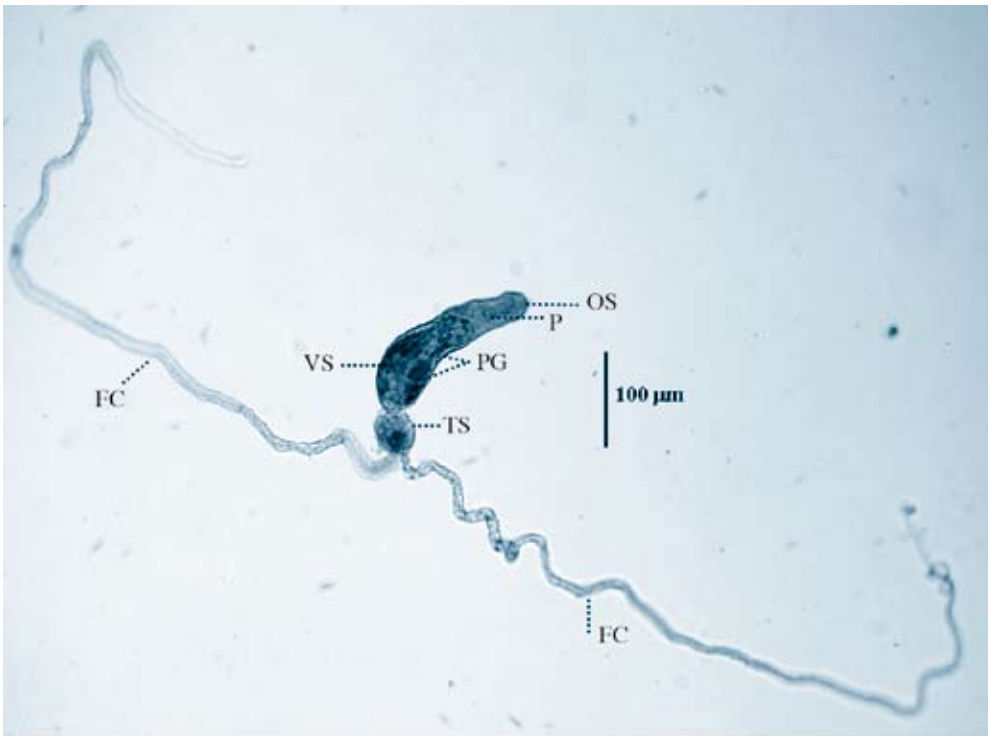

Fig 4 Light microscope photograph of bucephalid cercariae. (OS: oral sucker, VS: ventral sucker, P: pharynx, SP: spine, TS: tail stem, FC: furcae, PG: penetration gland).

Fig 5 Light microscope photograph of bucephalid sporocyst. (Spo: sporocyst).

second intermediate hosts, respectively [2,9-13]. In Thailand, several species of molluscs serve as intermediate hosts for parasites. A survey of freshwater clams found *L. siamensis* to be the most common species in Sirikit dam reservoir, in Uttaradit Province, northern Thailand [6]. Tesana [4] found *Corbicula* sp to be the dominant mollusk in Lam Ta Khong reservoir, Nakhon Ratchasima Province, northeast Thailand. Metacercariae of trematode parasites were found in freshwater clams that served as second intermediate hosts of intestinal fluke echinostomes [14].

Bucephalid digeneans have been reported from molluscs and fishes. For instance, cercarial infections were found in the bivalve *Anodonta anatina*, a freshwater clam in Lake Saravesi, Finland [12]. Within the sporocysts, the cercariae were typically in a wide range of developmental stages. Cercariae of the family Bucephalidae developed in branched, tubular sporocysts in bivalve mollusks, and encysted in fishes [15]. It was reported that the cercariae of *Rhipidocotyle campanula* and *R. fennica* developed in bivalve *Anodonta piscinalis* and metacercariae occurred in the skin and fins of the freshwater fish *Rutilus rutilus* (roach) [16]. Taskinen *et al* [6] reported that the sporocyst ribbons of *R. campanula* and *R. fennica* were present in the gonadal tissues of the clam *A. piscinalis*. Similarly, the trematodes *Rhipidocotyle* sp were found infecting the gonadal tissues of the freshwater clam *Lampsilis rafinesqueana* [17]. Laruelle *et al* [18] reported sporocysts of *Bucephalus polymorphus* in connective-tissue spaces, *ie* gonadal tissues, mantle epithelium, and gills. Several studies have also reported the impact of trematode parasitism. When the freshwater clams *A. pincinalis* were infected with *R. fennica*, an inverse correlation between the number of eggs produced by the clams and the number of trematode infections, was observed [13]. The trematode *Bucephalus* destroyed the gonads of pearl oysters and the heavily infected oysters could not initiate gametogenesis [19]. Ngo and Choi [11] suggested that high levels of trematode parasites could disturb the reproductive processes of the host organism, possibly retarding gonadal development or destroying gametes. Shiver [17] reported that the presence of trematodes (Bucephalidae) may impact the host response to environmental factors. This has also been documented for *R. fennica* infection in the unionid clam *A. piscinalis* [13]. Parasitized clams had high mortality rates in low-oxygen conditions [20].

Freshwater clams reportedly serve as first and second intermediate hosts for trematodes. However, cercariae in the clams of Thailand have not been reported. This is therefore the first study to report cercarial emergence from freshwater bivalves in Thailand. The cercariae were identified as bucephalids (Gasterostomes) and were found in 2 clam species, *Corbicula* sp and *L. siamensis*. The cercariae could be a species belonging to the *Bucephalus*, *Rhipidocotyle* or *Prosorhynchoides*. Unfortunately, the data were insufficient to identify the cercariae precisely. We thus hope to pursue further investigations.

The study of trematode infections in bivalves should be of concern, because trematode parasitism can affect bivalve growth; *eg Rhipidicotyle* infection decreased the growth of the bivalve host [21]. This study has significant findings for parasite control in both veterinary and medical Parasitology, as well as economic value to the freshwater clam industry.

Acknowledgements

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