Research Article

Cercarial Infections of Freshwater Snail Genus *Brotia* in Thailand

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Abstract

Freshwater snail genus *Brotia* is susceptible to trematode infections. In this study, *Brotia* spp. were collected from 61 localities in Thailand during 2004-2009 and 2013-2015 (to collect those in all localities distributed in Thailand, they have to be collected in two time frames). The samples were collected by hand picking and scooping methods based on counts per unit of time. A total of 13,394 snails were collected and identified into 16 species. They were *B. armata*, *B. binodosa*, *B. citrina*, *B. costula*, *B. dautzenbergiana*, *B. henriettae*, *B. insolita*, *B. manningi*, *B. microsculpta*, *B. pagodula*, *B. paludiformis*, *B. peninsularis*, *B. pseudosulcospira*, *B. solemiana*, *B. subgloriosa*, and *B. wykoffi*. Cercariae were investigated using shedding and crushing methods. Three species of *Brotia* had found the cercarial infections, they were *B. costula*, *B. dautzenbergiana*, and *B. wykoffi*. The overall infection rate was 0.20% (27/13,394). The cercariae were categorized into two types and three species. The first type was Xiphidiocercariae with one species, *Loxogenoides bicolor* Kaw, 1945. It was found in those three species of infected snails. The infection rate was 0.18% (24/13,394). The second type was Paraplue rophocercus cercariae with two species. The first species was *Stictodora tridactyla* Martin & Kuntz, 1955. The infection rate was 0.007% (1/13,394). It was found in *B. costula*. The second species was *H. pumilio* Looss, 1899. The infection rate was 0.014% (2/13,394). It was found in *B. costula* and *B. dautzenbergiana*.

***Key Words*:** Cercarial infection; Freshwater snail; *Brotia* spp.

Introduction

The digenean trematodes have the complex life cycle. They require the freshwater snails as the first intermediate host (Hechinger & Lafferrty, 2005). The larval stages, sporocyst, redia and cercaria, are developed in the snails (Elseshecikha et al., 2008). Those parasites cause the loss in public health and livestock. The various freshwater snails can be the first intermediate host of trematodes especially Cerithioidea group viz. Family Thiaridae, Family Paludomiidae and Family Pachychilidae (Krailas et al., 2003, 2011). However, until now the trematode infections of Pachychilid snails have not been studied well as Thiarid snails.

Pachychilid snails distribute in America, Australia, Africa, and Asia including Thailand (Glaubrecht, 1996; Lydeard et al., 2002; Köhler et al., 2004; Köhler & Glaubrecht, 2007). In Thailand, there were only 2 genera, *Brotia* and *Sulcospira (Adamietta)* (Köhler & Dame, 2009). In the past, freshwater snails in family Pachychilidae were belonging to family Thiaridae as subfamily Melatriininae (Brandt, 1974). Over a decade, the syste matic of this subfamily was revised and change to be the family Pachychilidae based on the

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molecular results (Köhler et al., 2004). Interestingly, some species of *Brotia* could be the first intermediate host of human trematodes. For example, *Brotia costula episcopalis* and *B. costula peninsularis* were reported about the cercariae of lung fluke, *Paragonimus westermani* (Brandt, 1974; Tubangui et al., 1950). Until now, there were only a few reports about trematode infections of *Brotia* spp. (Krailas et al., 2011). Hence, the objective of this study was to study the cercarial infections of *Brotia* spp. in Thailand.

Materials and Methods

Snail sampling and parasitic infections

Snails were collected by hand picking and scooping methods from 61 localities during 2004-2009 and 2013-2015 (Figure 1), using counts per unit of time method (Olivier & Schniderman, 1956). The snails were identified based on shell morphology following Brandt (1974) and Köhler & Glaubrecht (2006). Parasitic infections were investigated using snail shedding and crushing methods (Krailas et al., 2003)

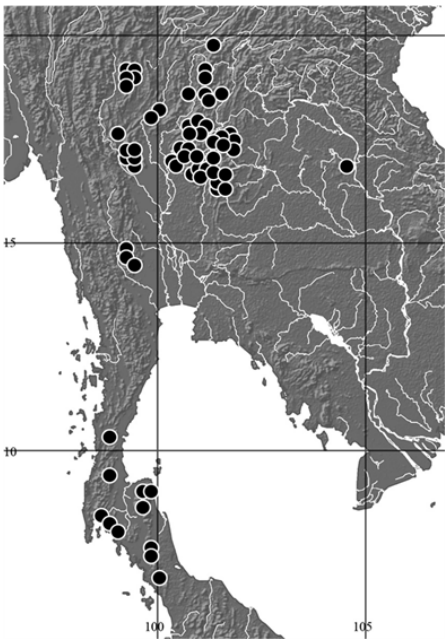

Figure 1 Distribution of freshwater snail genus *Brotia* in Thailand

Study of cercarial morphology

The cercariae were studied unstained or vitally stained with 0.5% neutral red. Descriptions of the morphology and anatomy of cercariae were based on the study of living cercariae that had escaped from the snails. Details of the cercarial morphology were drawn using a *camera lucida*, and all their species were identified (Schell, 1962; 1970; Nasir, 1974; Yamaguti 1975; Ito, 1980).

Results

Snail sampling and cercarial infections

A total of 13,394 snails of *Brotia* spp. were into 16 species, they were *B. armata*, *B. binodosa*, *B. citrina*, *B. costula*, *B. dautzenbergiana*, *B. henriettae*, *B. insolita*, *B. manningi*, *B. microsculpta*, *B. pagodula*, *B. paludiformis*, *B. peninsularis*, *B. pseudosulcospira*, *B. solemiana*, *B. subgloriosa*, and *B. wykoffi* (Figure 2) (Table 1).

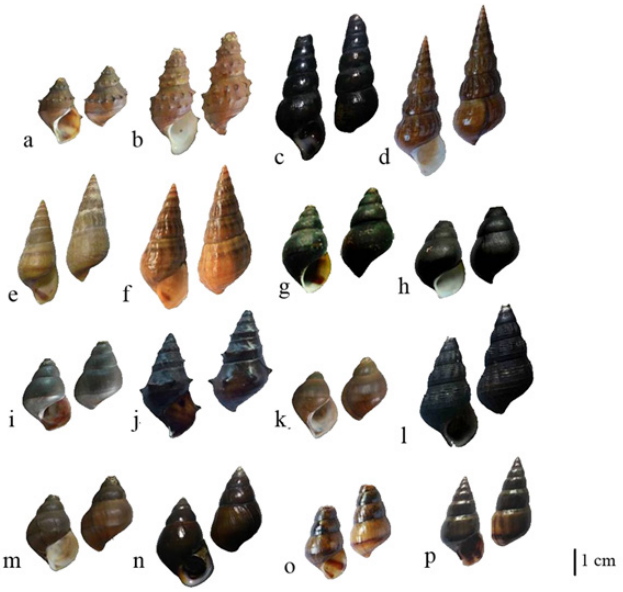

Figure 2 Images of collected snails genus *Brotia* in Thailand a. *B. armata*, b. *B. binodosa*, c. *B. citrina*, d. *B. costula*, e. *B. dautzenbergiana*, f. *B. henriettae*, g. *B. insolita*, h. *B. manningi*, i. *B. microsculpta*, j. *B. pagodula*, k. *B.paludiformis*, l. *B. peninsularis*, m. *B. pseudosulcospira*, n. *B. solemiana*, o. *B. Subgloriosa* and, p. *B. wykoffi*

Table 1 Number of collected freshwater snails genus *Brotia* in Thailand

Snails	Region distribution in Thailand	No. of collected snails
Brotia armata	North	356
Brotia binodosa	North	430
Brotia citrina	Northeast	34
Brotia costula	North, Central and South	4,318
Brotia dautzenbergiana	North	1,908
Brotia henriettae	North	641
Brotia insolita	North	73
Brotia manningi	North and Northeast	427
Brotia microsculpta	North and Northeast	1,544
Brotia pagodula	North	54
Brotia paludiformis	North	336
Brotia peninsularis	South	60
Brotia pseudosulcospira	North and Northeast	389
Brotia solemiana	North	42
Brotia subgloriosa	North and Northeast	131
Brotia wykoffi	North, Central and South	2,651
Total		13,394

Three species of collected snails were found cercarial infections, they were *B. costula*, *B. dautzenbergiana*, and *B. wykoffi*. Two types of cercariae with three species were Parapleurophocercous (*Haplorchis pumilio* and *Stictodora tridactyla*) and Xiphidiocercaria (*Loxogenoides bicolor*). The overall infection rate was 0.20% (27/13,394). The infection rate of *L. bicolor* was 0.18% (24/13,394). This species of cercariae was found in *B. costula* (14 snails), *B. dautzenbergiana* (9 snails) and *B. wykoffi* (1 snail). The infection rate of *S. tridactyla* was 0.007% (1/13,394). The infected snail was *B. costula*. The infection rate of *H. pumilio* was 0.014% (2/13,394). The larvae of *H. pumilio* were found in *B. costula* (1 snail), *B. dautzenbergiana* (1 snail).

Morphology of Cercariae

Xiphidiocercariae

Loxogenoides bicolor Kaw, 1945

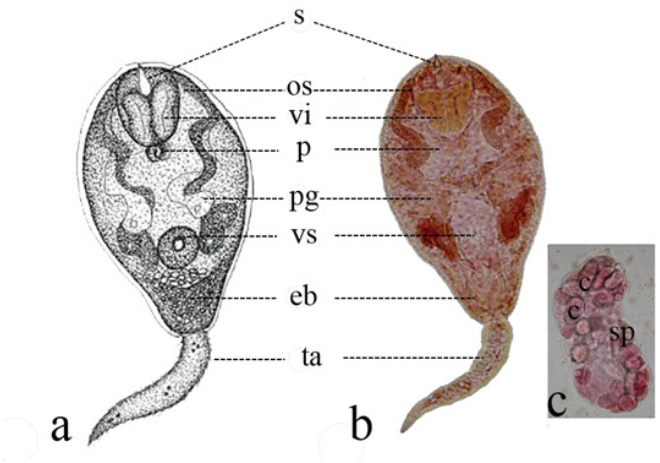

Figure 3 Images of Loxogenoides bicolor cercaria and redia. a. cerceria drawing; b. staining with 0.5% neutral red; c. redia, staining with 0.5% neutral red. (s: stylet, os: oral sucker, vi: virgulate gland, p: pharynx, pg: penetration gland, vs: ventral sucker, eb: excretory bladder, ta: tail, sp: sporocyst, c: cercaria)

Snail hosts: *Brotia costula*, *Brotia dautzenbergiana*, *Brotia wykoffi*

Definitive host: frog

Morphology

Sprocyst: round or oval

Cercaria

Body was oval shape. There were minute spines covered the whole body. Brown granule distributed underneath skin of the body. The stylet and virgulate gland were presented, they laid near the oral sucker. Three pairs of penetration glands were observed. Pharynx was round and small. Ventral sucker was smaller than oral sucker. The excretory bladder was small and located at the end of body. Tail had flexible length, but shorter than the body. Spines were also observed as well as body. Excretory ducts opened at the end of tail.

Parapleurophocercouscercariae

Stictodora tridactyla Martin & Kuntz, 1955

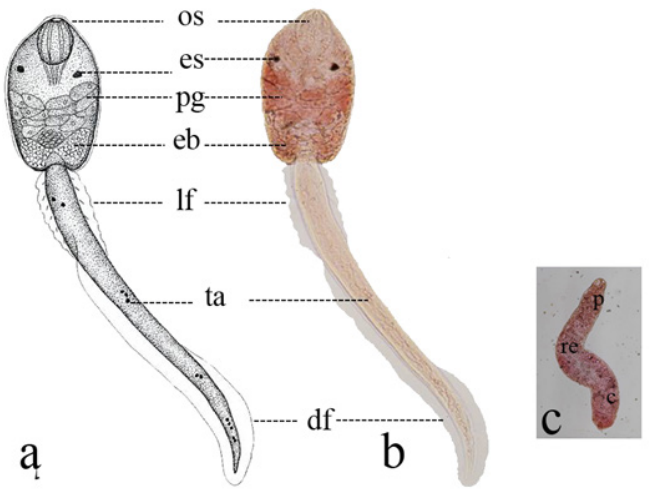

Figure 4 Images of *Stictodora tridactyla* cercaria and redia. a. cerceria drawing; b. staining with 0.5% neutral red; c. redia, staining with 0.5% neutral red. (os: oral sucker, es: eye spot, pg: penetration gland, eb: excretory bladder, lf: lateral finfold, df: dorsal finfold, ta: tail, p: pharynx, re: redia, c: cercaria)

Snail host: *Brotia costula*

Definitive host: bird, mammal and human

Morphology

Sporocyst: slender with brownish.

Cercaria

Body was oval shape, yellowish to brownish in color. Minute spines on the body were observed. There were three rows of spines with number 4-6, 12-14 and 22-24. Four pairs of penetration duct were opened between the eye spots. Eye spots were square shape. 7 pairs of penetration glands were observed between pharynx and excretory bladder. Penetration glands divided into 4 rows (3:4:4:3). Ventral sucker was small. The wall of excretory bladder was thick. Tail was long with lateral finfold and dorso-ventral finfold, there was not flame cell. Excretory ducts opened at the end of tail.

Haplorchis pumilio Looss, 1899

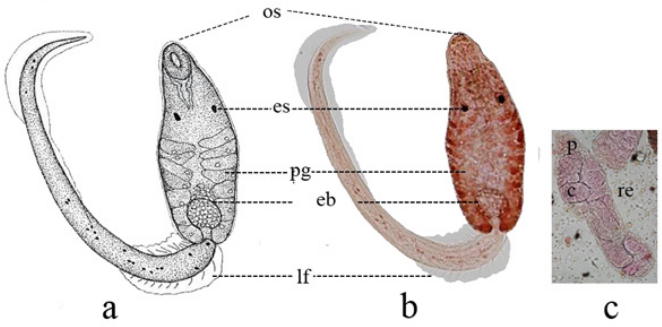

Figure 5 Images of *Haplorchis pumilio* cercaria and redia. a. cerceria drawing; b. staining with 0.5% neutral red; c. redia, staining with 0.5% neutral red. (os: oral sucker, es: eye spot, pg: penetration gland, eb: excretory bladder, lf: lateral finfold, p: pharynx, re: redia, c: cercaria)

Snail hosts: *Brotia costula*, *Brotia dautzenbergiana*

Definitive host: bird, human

Morphology

Redia: elongate shape, the cercariae were inside of redia.

Cercaria

Body was oval shape, brownish in color. Minute spines laid along oral sucker. Sensory hairs on the ventral surface of body were observed. At the oral sucker, there were 8 ducts of penetration glands with 4 in the dorsal part and 4 in the ventral part of the body. One pair of eye spots, pharynx was round and small. The penetration glands were very big. Fourteen of penetration glands were observeds. Ventral sucker was at the middle of body. Genital primary laid under the penetration glands. Excretory bladder was round and black in color. The cystogenous glands were on the edge and posterior part of body. Tail was longer than body length, the tip of the tail always bend. Lateral finfold and dorso-ventral finfold were observed. There were groups of pigment in the tail, no flame cell was observed. Excretory ducts opened at the end of tail.

Discussion

Brotia sp. is a freshwater snail in family Pachichylidae. It distributes in Southeast Asia from foothill of Himalayas mountain in Northeast of India and Bangaladesh to Myanmar, Thailand, The Malay Peninsula, Sumatra, Java and Borneo (Köhler & Glaubrecht, 2006). In this study, we had collected *Brotia* from creek and stream in the North, the South and the West of Thailand. We did not find any snail in the East; however we got the snail in one location of the Northeast region. Although the systematic revision of this snail was done quite clear, but the knowledge about parasitic infection is still lacking. Dechruksa et al. (2007) undertook the malacological and parasitological survey of *Brotia* from Kaek River. Only 2 species from 10 species (*B. wykoffi* and *B. paludiformis*) were the first intermediate host of Xiphidiocercariae and Parapleurophocercus cercariae. But at that time, the identification of snails was still confusing due to the using of old syste matic. This study is the first report of trematode infection of all *Brotia* in Thailand with the new syste matic. Only 2 types of cercariae were found, Xiphidocercariae and Parapleurophocercus cercariae. The cercariae of lung fluke, *Paragonimus westermani*, was not observed in this study. The season of collecting snail may be the answer (Zhou et al., 2010). Or perhaps the misidentification of snail in the previous study might be possible as well. For example, *Brotia asperat*a in Philippines was the intermediate host of *P. westermani*. Later, it was changed to be *Jagora asperata* (Köhler & Glaubrecth, 2003). For Xiphidiocercariae, only one species was detected, *Loxogenoides bicolor*. It is an amphibian trematode, this parasite is common in Thailand due to the broad range of intermediate host. Not only pachychilids, but thiarids also can be the first intermediate host (Krailas et al., 2014). Interestingly, the 2 species of Parapleurophocercus cercariae obtained from *Brotia* are the human intestinal fluke. The first one is *Stictodora tridactyla*. This species was reported about human infection in Korea (Chai et al., 1988). The second species is *Haplorchis pumilio*. This trematode has wide distribution in Thailand as well as *L. bicolor*. It could be found in all species of thiarids in Thailand. *Haplorchis pumilio* has been recorded human infections in Asia (Chung et al., 2011; Radomyos et al., 1998). As same as *S. tridactyla*, humans can ingest metacercariae of *H. pumilio* when consuming raw or lightly cooked fish (Díaz et al., 2008). With the heavy infection, *Haplorchis* spp. were believed that cause the ulceration and gastrointestinal disturbance in the patients (Chung et al., 2011).

However, the infection rates of those parasites in *Brotia* were not high as in *Melanoides tuberculata* or other thiarids. But, at least, this study showed the check lists of trematodes in freshwater snail genus *Brotia* based on the new systematization. This study is valuable for the parasitology framework in term of host and parasite interaction.

Acknowledgements

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