Research Article

Natural Trematode Infections of Freshwater Snail *Melanoides jugicostis* Hanley & Theobald, 1876 (Family Thiaridae), the First Intermediate Host of Animal and Human Parasites in Thailand

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Abstract

Melanoides jugicostis Hanley and Theobald (1876), a freshwater snail was reported as a species with very wide distribution ranging from Burma to Thailand and down to Java island of Indonesia. In Thailand, this species was found to live in a few localities. However they can transmit various kinds of parasites of animal and human trematodes. The aim of this study was to evaluate the natural infections of *M. jugicostis* snail in Thailand. The investigation was done by non systematic randomize collection only for adult snails during February to March in 2012. The collected snails were then examined for trematode infections by shedding and crushing techniques. The comparative analysis on morphology and anatomy of cercariae used for differentiating the species of parasite were done by describing from living and fixed cercariae. A total of 555 snails were collected, 103 were found positive for cercarial infections. The overall infection rates was 18.6% (103/555). Four types and four species of cercariae were categorized. Of these types were Parapleurophocercous cercariae (*Haplorchis pumilio*), Pleurophocercous cercariae (*Centrocestus formosanus*), Xiphidiocercariae (*Loxogenoides bicolor*), and Furcocercous cercariae (*Alaria mustelae*). The overall infection rates of these parasites were 16.04%, 0.54%, 1.08%, 0.72%, and 0.18%, respectively.

***Key Words*:** Cercaria; Intermediate host; *Melanoides jugicostis*; Thiaridae; Trematode infections

Introduction

It has long been known that the snails in the family Thiaridae played an important role as intermediate host of human and animal trematodes. In Thailand, five species of thiarid snails were reported as the first intermediate host of intestinal trematodes (*Haplorchis pumilio, H. taichui, Loxogenoides bicolor, Centrocestus formosanus, Stictodora tridactyla*), and fish blood flukes (*Transversotrema laruei, Apatemon gracilis, Mesostephanus appendicalatus, Cardicola alseae, Alaria mustelae*). Of these snails were *Tarebia granifera, Thiara scabra, Adamietta housei, Melanoides tuberculata* and *M. jugicostis* (Krailas et al., 2006, 2011, 2014). Especially, snails in the genus *Melanoides* are widely distributed and more susceptible to trematode infections than other snails. In Thailand, Two species of *Melanoides*; *M. tuberculata* and *M. jugicostis* were reported (Brandt, 1974). Both of them can also transmit parasites of native birds and fishes (Scholz and Salgado-Maldonado, 2000; Mitchell et al., 2005) including of mammals by acting as the first intermediate host of trematodes of respiratory, intestinal and hepatic systems in human, which represents a serious threat to public health (Mitchell et al., 2005; Dechruksa et al., 2007; Ukong et al., 2007; Krailas et al., 2011; Pointier et al., 2011; Pinto and Melo, 2011; Krailas et al., 2014). The shell of freshwater *M. jugicostis* is slender elongate and

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moderately turriculate, colour light brown to reddish brown, deep sutures with a strong axial ribs. It is transparent and slightly thin shell. Three to five of spiral structures are distinct on the basal of the last whorl. The spire is about 2-3 times higher than the aperture, but often eroded. Aperture is oval in shape and moderately transparent covered with a paucispiral operculum which is always oval in shape and the colour is light to dark brown (Figure 1a, 1b). This snail was found only at a few localities in Thailand, such as at Muok Lek, Saraburi Province; Erawan waterfall, Kanchanaburi Province; Ramon waterfall, Phang Nga Province etc. (Brandt, 1974; Krailas et al., 2006; Ukong et al., 2007). They can live in freshwater and some snails live mostly in stagnant and slowly running water. Usually, they can be found with the other thiarid snails such as *M. tuberculata* and *Thiara scabra*, but rarely sharing habitat with *Tarebia granifera*. The objectives of this study were to investigate type of parasites and the infection rates of trematodes in *M. jugicostis*. Undoubtedly, the results of the investigation will provide new finding benefit to the prevention and/or control the infection that transmit by *M. jugicostis* in the study and related areas. Besides that an ultimate results could be applied for possible controlling of other snail-borne diseases of other trematode infections in Thailand.

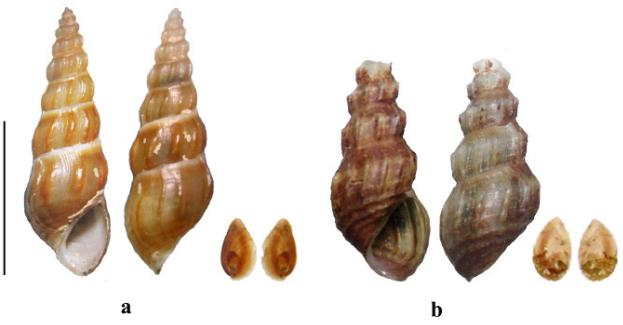

Figure 1 Shells morphology and operculum of *Melanoides jugicostis*. (a) Collected at Erawan waterfall, Kanchanaburi Province; (b) Collected at Klong Bang Bon, Ranong Province. (scale bar = 0.5 mm).

Materials and Methods

Sampling sites

The sites of snail were collected five sampling areas from five provinces were at Erawan waterfall, Sri Sa Wad District, Kanchanaburi Province (14º22'23.9"N 99º08'51.8"E Altitude 81 m); Klong Bang Bon, Kra Buri District, Ranong Province (10º20'10.8"N 98º46'48.7"E Altitude 18 m); Klong Yan, Wipawadee District, Suratthani Province (09º12'12.8"N 98º57'20.3"E Altitude 66 m); Ramon waterfall, Ta Gua Thong District, Phangnga Province (08º27'8.5"N 98º28'0.9"E Altitude 33 m) and at Klong Pa Lien, Yan Ta Khao District, Trang Province (07º22'11.5"N 99º40'51.6"E Altitude 12 m) (Figure 2) and the precise positioning of the collection sites were done by GPS measurements (Garmin Plus III, Taiwan).

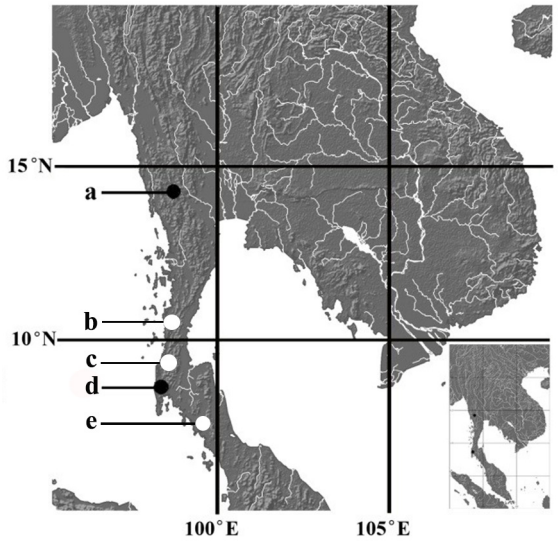

Figure 2 Sampling sites. (a) Erawan waterfall, Kanchanaburi Province; (b) Klong Bang Bon, Ranong Province; (c) Klong Yan, Suratthani Province; (d) Ramon waterfall, Phang Nga Province; (e) Klong Pa Lien, Trang Province. ●: represent snails with trematode infections, ○: represent snails without trematode infections.

Collection of snails

The snails were collected by opportunistic randomized collection for only adult snails by hand and sieving techniques during the month of February to March in 2012.

Parasite infections

All snail samples were examined for trematode infections by shedding and crushing techniques. The occurence of cercariae, sporocysts and rediae were examined under a dissecting microscope. Comparative analysis on morphology and anatomy of cercariae were differentiated from living and fixed cercariae. The newly emerged cercariae were observed for swimming behavior. The examination of cercariae were done from both unstained and vitally stained with 0.5% neutral red. Measurements of an individual cercariae were done in micrometers taken from 20 specimens which were fixed with 10% formalin. Details of the cercariae were drawn by a camera lucida for differentiation of trematodes species. The voucher specimens of cercariae were fixed and deposited in the Parasitology and Medical Malacology Research Unit, Department of Biology, Faculty of Science, Silpakorn University, Thailand.

Results

Microhabitat of the sampling sites

The microhabitat of the selected sampling sites were done mostly at downstream of waterfall, where covered with medium to large sized trees and shrubs grown on the banks and small to medium sized rocks laid all over the creek bed. The average light intensity was less than 100,000 lux at noon. The average of normal water temperature was 26ºC, usually rising up to near 30ºC at noon. The snail samples were found mostly on rock, rough sand, dried leaves and/or on aquatic plants.

Collection of snails

The collections of *Melanoides jugicostis* snails were made according to conchological characteristic for genus *Melanoides* (Brandt, 1974), based on the morphological characteristics to differentiate from *M. tuberculata* using an axial rib and the sculpture with spiral line of the body whorl. *M. tuberculata* has weak axial ribs and have no spiral lines, whereas the sculpture of *M. jugicostis* has strong ribs and the sculpture with spiral lines found only at the base of body whorl (Figure 1a, 1b). A total of 555 samples of *M. jugicostis* were collected from 5 studied areas (222 snails from Erawan waterfall, Kanchanaburi Province; 20 snails from Klong Bang Bon, Ranong Province; 19 snails from Klong Yan, Suratthani Province; 288 snails from Ramon waterfall, Phang Nga province and 6 snails from Klong Pa Lien, Yan Ta Khao District, Trang Province).

Parasite infections

The trematode infections were found only at 2 of the 5 sampling sites, Erawan and Ramon waterfalls. One hundred and three of collected snails were found positive forcercarial infections, thus the overall infection rates was 18.6% (103/555) (Table 1). Of these, 98 infected snails (42.34%) were collected at Erawan waterfall and 5 infected snails (1.74%) were at Ramon waterfall (Table 1). Four types of cercariae were found from the collected snails and could be identified into four different species. The first type was Parapleurophocercous type, the cercariae of *Haplorchis pumilio* Looss, 1899. The second type was Pleurophocercous, the cercariae of *Centrocestus formosanus* Nishigori, 1924. The third type was Xiphidiocercariae, the cercariae that belonged to *Loxogenoides bicolor* Kaw, 1945. And the fourth type was Furcocercous, the cercariae of *Alaria mustelae* osma, 1899. Three species of parasites found at Erawan waterfall were *H. pumilio, C. formosanus* and *L. bicolor* while three species found at Ramon waterfall were *H. pumilio, L. bicolor* and *A. mustelae* (Table 1). Morphology and anatomical descriptions of cercariae of each species were described.

***Haplorchis pumilio* Looss, 1899**

The cercariae of this parasite was found in 89 *M. jugicostis* at two localities (Erawan waterfall and Ramon waterfall), the infection rate was 16.04% (89/555). Body shape is oval with yellowish brown pigment (Figure 3a), covered entirely with fine reverse

spines and sensory hairs on the side of the body. Pigment eyespot and a pharynx are present. There are seven pairs of penetration glands, which are arranged in two longitudinal series with a ventral sucker and primordial genital. The penetration glands are well developed; eight ducts are arranged in two bundles, four of them open through the dorsal wall and four through the ventral wall of the oral sucker in two oblique symmetrical rows. The ventral sucker and genital primordia are prevesicular. The excretory bladder has a rounded shape and is composed of fine pigments. No flame cells were found in the tail stem. Cercaria developed into redia (Figure 3b). A long tail is attached to the dorsal end of the body, with lateral finfolds nearby and a dorso-ventral finfold for the greater distal portion.

Size range (average) calculated from 20 cercariae are as follow:

Body: 85-128 µm (108 µm) × 154-257 µm (235 µm)

Tail: 8-30 µm (27 µm) × 421-514 µm (476 µm)

Oral sucker: 30-45 µm (38 µm) × 31-45 µm (37 µm)

Ventral sucker: 12-25 µm (16 µm) × 12 - 25 µm (16 µm)

Pharynx: 8-12 µm (10 µm) × 11-17 µm (14 µm)

Excretory bladder: 25-38 µm (35 µm) × 24-36 µm (33 µm)

Movement behavior

The cercaria moved by rolling up and springing the body backward prior to move forward in a screwing motion for 7-12 sec and then rested for 30-55 sec. It survived up to 2-4 h in the conditioned water after emergence.

***Centrocestus formosanus* Nishigori, 1924**

The cercarial stage of this parasite was found in 3 *M. jugicostis* only at Erawan waterfall, the overall infection rate was 0.54% (3/555). Cercarial body is oval in shape, a pair of eyespots lay at the level of the pharynx (Figure 4a). The oral sucker has two rows of oral spines (four in the anterior and five in the posterior) located on the dorsal wall of the mouth aperture. The parenchymal body is spinulate and yellowish brownish pigment. A large acetabulum is found between the intestinal bifurcation and the excretory vesical. The bladder is a flattened V-shape. Seven pairs of penetration glands lay anterolateral to the acetabulum in front of an inverted V-shape. Cystogenous cells are distributed in the posterior part. The genital primordial part is somewhat elongated and triangular located, between the acetabulum and the excretory vesicle. Cercaria developed into redia (Figure 4b). The tail is slender with a very indistinct dorsal and ventral finfolds, both of which are more conspicuous in the distal half, provided at the tip with a tiny spike.

Size range (average) calculated from 20 cercariaeare as follow:

Body: 42-85 µm (74 µm) × 75-128 µm (115 µm)

Tail: 12-18 µm (16 µm) × 70-98 µm (90 µm)

Oral sucker: 16-29 µm (27 µm) × 17-30 µm (26 µm)

Ventral sucker: 15-21 µm (19 µm) × 16 - 21 µm (18 µm)

Pharynx: 7-9 µm (8 µm) × 7-10 µm (8 µm)

Excretory bladder: 26-32 µm (29 µm) × 32-48 µm (44 µm)

Movement behavior

The cercaria moved by rolling up and springing the body backward prior to move forward in a screwing motion for 6-14 sec and then rested for about 40-45 sec. It survived up to 3-4 h in the conditioned water after emergence.

***Loxogenoides bicolor* Kaw, 1945**

The cercariae of this parasite were found in 6 *M. jugicostis* both at Erawan and Ramon waterfalls, the total infection rate was 1.08% (6/555). Cercarial body is spinose and oval in shape (Figure 5a). Its entire body is dotted with granules. The ventral sucker is smaller than the oral sucker. Virgular organ is located in the region of the oral sucker. A stylet is present. Three pairs of penetration glands exist: two anterior pairs comprises fine granules and at posterior pair filled with coarser granules. The penetration ducts open near the tip of the stylet. There is a C-shaped genital primordium

and a U-shaped excretory bladder. Cercaria developed into sporocyst (Figure 5b). The tail is also spinose, with more slightly longer spines at the tip.

Size range (average) calculated from 20 cercariae are as follow:

Body: 54-82 µm (75 µm) × 85-117 µm (106 µm)

Tail: 18-29µm (26 µm) × 32-77 µm (74 µm)

Oral sucker: 21-28 µm (26 µm) × 20-30 µm (27 µm)

Stylet: 5-9 µm (8 µm) × 12-18 µm (16 µm)

Ventral sucker: 10-17 µm (15 µm) × 11-19 µm (15 µm)

Pharynx: 4-8 µm (7 µm) × 4-8 µm (7 µm)

Excretory bladder: 8-12 µm (10 µm) × 9-26 µm (22 µm)

Table 1 Types of trematodes and infection rates of *Melanoides jugicostis* snails collected at two sampling areas.

Type of trematodes/Locality	Erawan waterfall (n = 222)		Ramon waterfall (n = 288)		Total of infection rates (%)
	Snail infected	Infection rates (%)	Snail infected	Infection rates (%)	
Haplorchis pumilio	88	39.64	1	0.35	16.04 (89/555)
Centrocestus formosanus	3	1.35	0	0	0.54 (3/555)
Loxogenoides bicolor	3	1.35	3	1.04	1.08 (6/555)
Alaria mustelae	0	0	1	0.35	0.18 (1/555)
Total	98	42.34	5	1.74	18.56 (103/555)

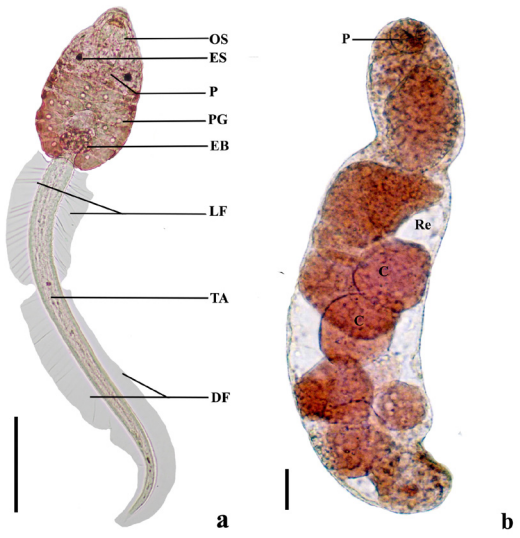

Figure 3 Images of *Haplorchis pumilio*. (a) light micrograph, staining with 0.5% neutral red; (b) redia, stainning with 0.5% neutral red. (OS: oral sucker, ES: eyespot, P: pharynx, PG: penetration gland, EB: excretory bladder, TA: tail, LF: lateral finfold, DF: dorso-ventral finfold, Re: redia, C: cercaria). Scale bar = 100 µm.

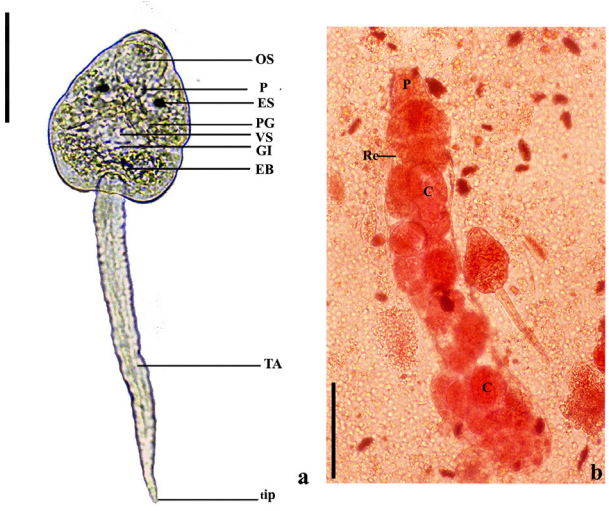

Figure 4 Images of *Centrocestus formosanus*. (a) light micrograph, (b) sporocyst, stainning with 0.5% neutral red. (OS: oral sucker, ES: eyespot, P: pharynx, PG: penetration gland, VS: ventral sucker, GI: genital primordium, EB: excretory bladder, TA: tail, Re: redia, C: cercaria). Scale bar = 100 µm.

Movement behavior

The cercaria 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 sec, and resting for about 6-10 sec prior to move again. It survived up to 2-3 h in the conditioned water after emergence.

***Alaria mustelae* Bosma, 1899**

The cercariae of this parasite was found in 1 *M. jugicostis* only at Ramon waterfall and the overall infection rate was 0.18% (1/555). Cercarial body has a long shape. Unpigmented eyespots lie on the midway between two suckers in lateral fields. The prepharynx is short and the pharynx is small and muscular. The esophagus is rather long. The ceca extend a short distance posterior to acetabulum (Figure 6a). Oral sucker is slightly larger than postequatorial acetabulum. There are two pairs of penetration glands, filled with fine granules and ducts opening on each side of mouth in spineless circumoral area. Body entirely covers with spines and there were two irregular spines around aperture of ventral sucker. Genital primordium is a small mass of cells anterior to excretory vesicle. Cercaria developed into sporocyst (Figure 6b). Tail stem has no spines and furcae is irregularly spinose (long hair like) with no caudal bodies.

Size range (average) calculated from 20 cercariae are as follow:

Body: 105-154 µm (138 µm) × 185-280 µm (255 µm)

Oral sucker: 28-40 µm (36 µm) × 28-40 µm (36 µm)

Ventral sucker: 15-30 µm (22 µm) × 15-30 µm (22 µm)

Pharynx: 10-15 µm (13 µm) × 14-17 µm (15 µm)

Tail stem: 48-60 µm (55 µm) × 220-300 µm (260 µm)

Tail furcal: 38-65 µm (60 µm) × 245-320 µm (280 µm)

Movement behavior

The cercariae moved by rolling up and springing back the body to swiftly move forward quickly in a semicircular motion. It then rested by floating with its head on top for a long period of time about 20-30 sec then moved quickly about 7-15 sec and rest by floating again. The movement cycle continued until or otherwise die. The life spand of the cercariae is rather short only 1 up to 5 h in the conditioned water after emergence.

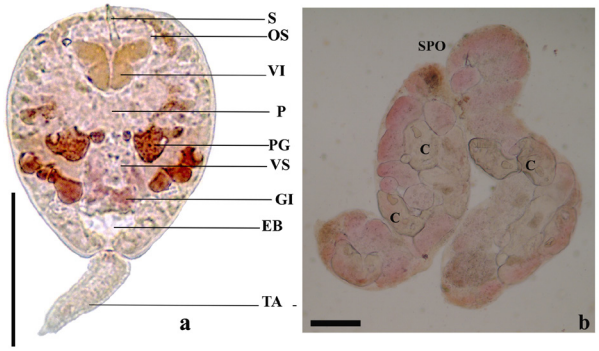

Figure 5 Images of *Loxogenoides bicolor*. (a) light micrograph, staining with 0.5% neutral red; (b) sporocyst, stainning with 0.5% neutral red. (S: stylet, OS: oral sucker, VI: virgulate gland, P: pharynx, PG: penetration gland, VS: ventral sucker, GI: genital primordium, EB: excretory bladder, TA: tail, SPO: sporocyst, C: cercaria). Scale bar = 100 µm.

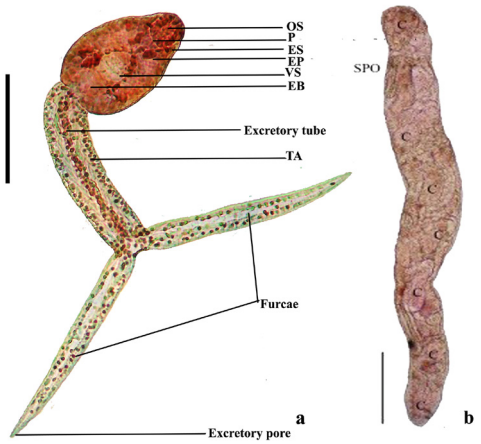

Figure 6 Images of *Alaria mustelae*. (a) light micrograph, staining with 0.5% neutral red, (b) sporocyst, staining with 0.5% neutral red. (OS: oral sucker, P: pharynx, EP: esophagous, ES: eyespot, VS: ventral sucker, EB: excretory bladder, TA: tail, SPO: sporocyst, C: cercaria). Scale bar = 100 µm.

Discussion

Freshwater snail *M. jugicostis* was studied in 5 sampling areas from five provinces of Thailand. The trematode infections were found only Erawan and Ramon waterfalls. No trematode infections were found at 3 sampling areas, Klong Bang Bon (20 snails), Klong Yan (19 snails) and Klong Pa Lien (6 snails). These might have been due to the number of snails used for study were too small and/or the infectivity rate of parasite was very low or none. The cercariae were classified into four types which its belong to four different species. In this study, *H. pumilio* had the highest infection rate which was 16.04% (89/555).

The adults of *Haplorchis pumilio* live in small intestine of birds, mammals and humans (Krailas et al., 2011, 2014). In previous reports, *Haplorchis pumilio* cercariae were found in rediae from *Melania reiniana* var. *Hidachiensis* and *Melanoides tuberculata* chinensis (Yamaguti, 1975). In Thailand, four thiarid snails (*Melanoides tuberculata, Tarebia granifera, Thiara scabra* and *Sermyla riqueti)* and two Pachychilid snails *(Brotia costula* and *B. citrina*) were reported as the first intermediate host of *H. pumilio*, and the second intermediate host were found encysted parasite in pectoral fins and the base of the tail fin of fishes, *Carassius* and *Cyprinus* (Yamaguti, 1975; Dechruksa et al., 2007; Ukong et al., 2007; Krailas et al., 2011). Human infection with heterophyid flukes were confined to the subfamily Haplorchinae, viz. *Haplorchis pumilio, H. taichui, H. yokogawai, Stellantchasmus falcatus* and *Centrocestus caninus* (Dechruksa et al., 2007; Ukong et al., 2007; Krailas et al., 2011, 2014). The prevalence of intestinal flukes was reported from sixteen provinces in the Northeast, sixteen provinces in the North and eight provinces in the South of Thailand (Krailas et al., 2011). Moreover, *Melanoides jugicostis* has been reported in Thailand, as the first intermediate host of *Haplorchis taichui, Stictodora tridactyla, Centrocestus formosanus, Loxogenoides bicolor, Haematoloechus similis, Alaria mustelae* and *Mesostephanus appendicalatus* (Ukong et al., 2007; Krailas et al., 2011, 2014). In a previous study, *Melanoides jugicostis* was reported that it can be infected by three species of trematodes; they were *Haplorchis pumilio, Mesostephanus appendicalatus* and *Stictodora tridactyla,* the snail was the first intermediate host of trematodes that were found at Erawan waterfall (Ukong et al., 2007). While, the present work was found trematodes infections of *Melanoides jugicostis* for two different types of cercariae at Erawan waterfall; they were *Centrocestus formosanus* and *Loxogenoides bicolor.* Only one species of blood flukes, *Alaria mustelae* was found at Ramon waterfall could be claimed to be first report as a new record of blood trematode that transmitted by *Melanoides jugicostis*. *Centrocestus formosanus* cercariae developed from rediae in snails *Melanoides tuberculata, Stenomelania newcombi, Tarebia granifera, Thiara scabra* and *Neoradina prasongi*, encysting in gills, the buccopharyngeal cavity, gastro-intestinal wall, muscle, heart, liver, kidney, peritoneum and adipose tissues of cyprinoid fish (Yamaguti, 1975; Dechruksa et al., 2007). *Loxogenoides bicolor* cercariae developed from sporocysts in snails *Gonibasis depygis, Tarebia granifera, Thiara scabra, Melanoides tuberculata, M. jugicostis, Brotia* spp., and adult flukes found in frogs (Yamaguti, 1975; Dechruksa et al., 2007; Ukong et al., 2007). In addition, *Loxogenoides bicolor* can be found from various freshwater snails in Thiarid snails (Krailas et al., 2006, 2014). *Alaria mustelae* cercariae of blood fluke was found to develop from sporocysts in snails *Planorbula armigera, Tarabia granifera, Melanoides tuberculata and M. jugicostis*, whereas the other stages of development were reported that the mesocercaria stage was found in tadpole and frogs, and the metacercaria stage was found in rats *Peromyscus leucopus noveboracensis, Mustela vison, Procyon lotor* and adult worm was found in mongoose, cat and dog (Yamaguti, 1975; Ukong et al., 2007). Therefore, this work can help to elucidate new knowledge about the larval trematode fauna in Thailand in relation to *M. jugicostis* snail intermediate host.

Conclusion

The overall infection rates was 18.6% (103/555). Trematode of the freshwater snail *M. jugicostis* was infected by *Haplorchis pumilio*, *Centrocestus formosanus, Loxogenoides bicolor* and *Alaria mustelae*. The percentages of infection rates of these parasites were 16.04%, 0.54%, 1.08%, 0.72%, and 0.18%, respectively.

Acknowledgments

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