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The pleurophocercous cercariae infection in snail Family Thiaridae Grey, 1847 Northern, Thailand

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ABSTRACT

Objective: To investigate the prevalence of pleurophocercous cercariae infection in thiarid snails from natural infection in 12 provinces of Northern Thailand.
Methods: The snail specimens were collected and examined for pleurophocercous cercariae infection using crushing method. The morphological characteristics of cercariae, which were identified by a light microscope and illustration were done using a camera lucida.
Results: Based on a sample of 2 075 thiarid snails of four snail species, it was found that *Melanoides tuberculata, Tarebia granifera, Thiara scabra,* and *Adamieta hoesei* served as the first intermediate hosts of heterophyid trematode with an overall prevalence of 14.78%, revealing four different morphological characteristic types. Pleurophocercous cercaria Type I had the highest the prevalence (13.06%), followed by the pleurolophocercous cercaria Type II (0.29%), pleurolophocercous cercaria Type III (0.05%), and pleurophocercous cercaria Type IV (1.4%), respectively.
Conclusions: This research report will serve a preliminary step providing new and fundamental information regarding the current geographical distribution trends of opisthorchiid and/or heterphyid trematodes infection. The findings of this study will be useful to the development of effective control measures for this type of parasitic infection in Northern Thailand.

1. Introduction

Snail-borne zoonotic intestinal trematodes are recognized as an important group of livestock and human pathogens. Several species of trematodes in the family Heterophyidae and Opisthorchiidae have been found minute intestinal and liver parasites of carnivores, piscivorous animals and birds, as well as humans[1]. These trematodes are known to be widely distributed throughout Easthern and Southeastern Asia including Thailand[2-6]. They exist result of a completed life cycle that involves various species of freshwater snails as their first intermediate host. This has led to extensive trematode distribution[7].

The cercaria stage has been found to display varied morphological characteristics. This stage of trematodes exists among various types, and their classification is usually based on their external morphology. Early morphological designations were based on the number and position of the sucker present in the cercarial body, as well as the shape, relative size of the tail, and tail structure. The following types have been recognized in the previous reports[5,8-10].

Various species of trematode Family Heterophyidae and Opisthorchiidae have been described from pleurophocercous cercaria and, display a dorso-ventral finfold[5,11]. Among these, *Opishorchis viverrini, Haplorchis taichui* (*H. taichui*), *Haplorchis pumilio* (*H. pumilio*), *Haplorchoides* sp., *Centrocestus formosanus* (*C. formosanus*), *Procerovum varium* and *Stellantchasmus falcatus* have been recovered in abundant numbers from freshwater snails and fish in northern and north-eastern of Thailand[4,5,12-14]. Of particular interest, thiarid snails have been reported that they played an important role in medical and veterinary parasitology as primary intermediate host of many species of heterophyid and opisthorchid trematodes. For instance, *Melanoides tuberculata* (*M. tuberculata*), *Thiara scabra* (*T. scabra*) and *Tarebia granifera* (*T. granifera*) are the primary intermediate host for *H. taichui*, *H. pumilio*, *C. formosanus*[15-18], while *T. granifera* and *Stenomelania newcombi* are the primary intermediate hosts for *Stellantchasmus falcatus*[19]. In Northern Thailand, there have been a number of

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reports that have dealt with the occurrence of pleurophocercous cercaria (heterophyid trematode) in thiarid snails. Dechruksa *et al.* discovered the parapleurolophocercous cercaria and xiphidiocercaria in thairid snails with an overall prevalence of 0.90% at Phitsanulok Province[20]. Dechruksa *et al.* found various type of the cercariae in *M. tuberculata* with an overall prevalence of 18.79% includes parapleurophocercou, pleurophocercous, xiphidiocercaria, megalurous cercariae, furcocercous cercariae, echinostome cercariae, amphistome cercaria, renicolid cercariae and cotylomicrocercous cercariae[20]. In case of the opisthorchid trematodes especially *Opisthorchis viverini* have been reported in north-east region of Thailand[21].

This gap in knowledge has hindered our understanding of the prevalence and dynamic transmission of heterophyid and opisthorchid trematodes in this area. Hence, the aim of present the aim of the present study was to investigate the epidemiological situation of pleurophocercous cercaria infection in thiarid snails to the representative 12 provinces of Northern Thailand.

2. Materials and methods

2.1. Collection sites

The 64 collection sites were selected to cover different water bodies and irrigation systems present in 12 provinces of northern, Thailand including Chiang Rai, Chiang Mai, Phayao, Mae Hong Son, Lamphun, Lampang, Phrae, Tak, Sukhothai, Nan, Phisanolok, and Uttaradit Provinces. They were selected on areas of public health problems with trematode infections. The geographical coordinate for each sampling site were recorded by using Global Positioning System (GPS) (Table 1). The snail specimens were collected by stratified sampling method. The specimens were classified with taxonomic key[22], then they were separated into species level.

2.2. Cercarial infection

The prevalence of pleurolophocercous cercaria was investigated for cercarial infections using the crushing method[5]. The alive cercariae were vitally stained with 0.5% neutral red and identified according to morphology described by previous studies[23-26]. In addition, the cercarial specimens were stained with Delafield's haematoxylin or aceto-orcein, dehydrated in an ethyl alcohol series, cleared with xylene, and mounted in permount. Drawings will be done using a camera lucida.

3. Results

A total number of 2075 thiarid snails were collected from 12 provinces in Northern Thailand including *M. tuberculata, T. granifera, T. scabra,* and *Adamieta housei* (*A. housei*). A total number 307 of the 2075 snails were infected with four morphological types of pleurophocercous cercariae with an overall prevalence of 14.78%. From Table 2, the dominant cercarial type displaying high rate of infection was pleurophocercous cercaria Type I, this cercarial type was present in 271 snails followed by pleurophocercous cercaria Type IV (29 specimens), pleurophocercous cercaria Type II (6 specimens), pleurophocercous cercaria Type III (1 specimens), respectively. Moreover, *M. tuberculata* was acknowledged as being the most susceptible for cercarial infection over than the other snail families with a prevalence of 20.67%, *T. granifera* (10.42%), *A. housei* (6.66%) and *T. scabra* (3.15%), respectively.

Table 1
Description of collecting sites where snail collections were performed.

Sampling sites	Locality	Georeference coordinates
CM-01	Baan Pha Lao, Mae Taeng district	19°6' 37.317" N, 99°4'33.439" E
CM-02	Bua Tong waterfall, Mae Taeng	19°5'54.30" N, 99°4'52.87" E
CM-03	Huai Maekajan, Mae Rim district	19°3'05.23" N, 98°9'40.617" E
CM-04	Huai Mae Kuet, Chiang Dao district	19°22'51.51" N, 98°58'2.88" E
CM-05	Chiang Dao district	19°22'57.149" N, 98°58'5.699" E
CM-06	Baan Wan, Hang Dong district	18°42'27.00" N, 98°56'5.68" E
CM-07	Baan Tawai, Hang Dong (irrigation	18°41'10.25" N, 98°56'16.57" E
CM-08	Baan Thung Siao, San Pa Tong district	18°33'59.54"N, 98°52'5.61" E
CM-09	Baan Mai, Chom Thong district	18°27'0.40" N, 98°42'40.99" E
CM-10	Baan Luang, Chom Thong district	18°28'30.72" N, 98°39'3.97" E
CM-11	Tambol Doi LO, Doi Lo district	18°28'36.25" N, 98°47'45.54" E
CM-12	Tambol Yang Noeng, Salaphee district	18°42'18.55" N, 99°2'16.62" E
CM-13	Baan Luang, Chom Thong district	18°27'2.91" N , 98°40'27.99" E
CM-14	Baan Pha Taek, Doi Saket district	18°55'4.56" N, 99°7'44.04" E
CM- 15	BaanLuaen Nuea, Doi Saket district	18°55'31.46" N, 99°8'13.93" E
CM-16	Baan Luang, Mae Ai district	19°54'33.28" N , 99°17'19.23" E
CM-17	Tambol Wiangfang, Fang district	19°55'1.26" N, 99°13'11.29" E
CM-18	Tambol Pongnamron, Fang district	19°57'25.74" N, 99°11'18.68" E
CM-19	Mueang district	18°44'52.47" N, 98°56'57.39" E
CR-01	Mae Suai district	19°38'43.56" N, 99°31'36.52" E
CR-02	Chiang Khong district	20°10'34.37" N, 100°26'14.57" E
CR-03	Tabol Maekhachan, Wiang Pa Pao district	19°11'33.95" N, 99°30'48.43" E
CR-04	Phaya Mengrai district	19°50'58.77" N, 100° 8'59.98" E
CR-05	Huai Pa Daeng, Khun Tan district	19°49'53.74" N, 100°15'31.36" E
MA-01	Pangmapa district	19°31'22.13" N, 98°14'43.73" E
MA-02	Baan Huai Pa, Mueang district	19°25'27.06" N, 97°59'19.84" E
MA-03	Huai Maesuya, Mueang district	19°23'47.09" N, 97°56'43.15" E
MA-04	Baan Wing Tai, Pai district	19°22'45.62" N, 98°25'53.12" E
MA-05	Baan Wian gtai district	19°21'21.37" N, 98°26'50.40" E
LA-01	Tambol Jaeson, Mueang Pan district	18°45'56.20" N, 99°14'20.90" E
LA-02	Huai Maetan, Hang Chat district	18°19'37.24" N, 99°16'51.76" E
LA-03	Tambol Maewa Thoen district	17°29'35.59" N, 99°11'36.93" E
LA-04	Bann Rim nam, Mueang district	18°18'27.15" N, 99°31'9.29" E
LA-05	Nong Lom, Hang Chat district	18°19'13.11" N, 99°20'49.97" E
LA-06	Mae Phrik, Thoen district	17°26'43.03" N, 99°7'40.28" E
LU-01	Mueang district	18°35'3.35" N, 98°59'32.47" E
LU-02	Tambol Makhuea Chae, Mueang district	18°35'31.46" N, 99°4'52.67" E
LU-03	Ban Thi district	18°38'57.15" N, 99°6'33.62" E
LU-04	Pha Sang district	18°32'32.23" N, 98°56'21.32" E
NA-01	Baan Don Tan, Wiang Sa district	18°34'58.88" N, 100°44'53.09" E
NA-02	Tha Wan Pha district	19°7'13.99" N, 100°48'35.27" E
NA-03	Pua district	19°10'42.86" N, 100°52'31.80" E
NA-04	Pua district	19°10'13.71" N, 100°56'2.83" E
PY-01	Gwarnpayao, Mueang district	19°9'46.32" N, 99°54'5.68" E
PY-02	Mueang district	19°11'40.56" N, 99°53'48.02" E
PY-03	Dok KhamTai, Mueang district	19°7'43.54" N, 99°54'31.08" E
PH-01	Huai Kamin reservoir, Mueang district	18°8'27.50" N, 100°13'27.96" E
PH-02	Huai Phakum, Mueang district	18° 9'49.39" N, 100°10'3.41" E
PH-03	Baan Nakuha, Mueang district	18° 7'45.33" N, 100°19'4.37" E
PH-04	Rong Kwang district	18°18'22.01" N, 100°16'28.32" E
PI-01	Tambol Wat Phrik, Mueang district	16°42'29.58" N, 100°14'46.37" E
PI-02	Tambol Wat Bot, Wat Bot district	16°56'23.56" N, 100°20'50.57" E
PI-03	Tambol Baan Krang, Mueang district	16°58'31.44" N, 100°33'30.65" E
PI-04	Tambol Tha Pho, Mueang district	16°45'52.47" N, 100°12'17.44" E
PI-05	Tambol Phrom Phiram, Phrom Phiram district	16°59'10.38" N, 100°11'58.41" E
SU-01	Kong Krailat district	16°57'13.24" N, 99°57'28.83" E
SU-02	Si Samrong district	17°9'59.12" N, 99°51'30.93" E
SU-03	Thung Saliam district	17°18'47.26" N, 99°32'6.46" E
TA-01	Sam Ngao district	17°13'9.72" N, 99°2'35.87" E
TA-02	Mueang district	16°52'31.56" N, 99°7'45.78" E
TA-03	Ban Tak district	17°2'56.46" N, 99°4'37.18" E
UT-01	Huai Pong, Lablae district	17°38'30.14" N, 100°2'18.75" E
UT-02	Baan Ko, Mueang district	17°35'33.54" N, 100°5'58.08" E
UT-03	Tambol Ban Dan Na Kham, Mueang district	17°42'45.81" N, 100°7'36.27" E

The study of cercarial infection indicated that *M. tuberculata* involves many diverse cercarial types (three types), followed by *T. granifera*, *T. scabra* and *A. housei* (two types). Pleurophocercous cercaria Type I and Type IV were found among three snail species.

Each type of cercaria was only successfully identified to be a major type of cercaria, and more specific identification was desirable. The specimens were subsequently described as follows:

Table 2

A summary of thiarid snails species, amount examined, amount infected and prevalence value.

Snail species	No. of examined	No. of infection				Total
		Type 1	Type 2	Type 3	Type 4	
M. tuberculata	987	175 (20.67%)	5 (0.51%)	-	24 (2.43%)	204 (20.67%)
T. granifera	931	94 (10.10%)	1 (0.11%)	-	2	97 (10.42%)
T. scabra	127	2 (1.57%)	-	-	2 (1.57%)	4 (3.15%)
A. housei	30	-	-	1 (3.33%)	1 (3.33%)	2 (6.66%)
Total	2075	271(13.06%)	6 (0.29%)	1 (0.05%)	29 (1.40%)	307 (14.78%)

3.1. Pleurophocercous cercaria Type I: Snail hosts M. tuberculata, T. granifera, and T. scabra

The body of cercarial type is in pear shape, 153–181 (162) μm long, having a maximum width of 52–75 (67.91) μm, and is entirely covered with minute spines. Oral sucker is sub-terminal and the ventral sucker is absent. The two square shape eyespots are located on both side of the body under the pharynx. Seven pairs of penetration glands are situated between the pharynx and distributed to the posterior part of the body, mainly surrounding the genital premordium and arranged in two columns. A tail is attached to the dorsal end of the body, with dorso-ventral finfolds and lateral finfolds.

3.2. Pleurolophocercous cercaria Type II: Snail hosts M. tuberculata and T. granifera

The body of cercaria is ovoid, 191–236 (219) μm long, having a maximum width of 98–112 (104) μm. The tegumental surface is covered with tiny spine. The oral sucker is located at sub-terminal. Pair of eyespots are situated at the anterior third of the body. Intestinal caeca are bifurcate deposit on the pharynx to posterior of excretory vesicle level. Seven pairs of penetration glands are arranged in 2 columns. The tail is slender and the equal body length, which attached to the dorso-ventral and lateral fin folds.

3.3. Pleurolophocercous cercaria Type III: Snail hosts A. housei

The cercaria body is in oval shape, 190–260 (220) μm long, with

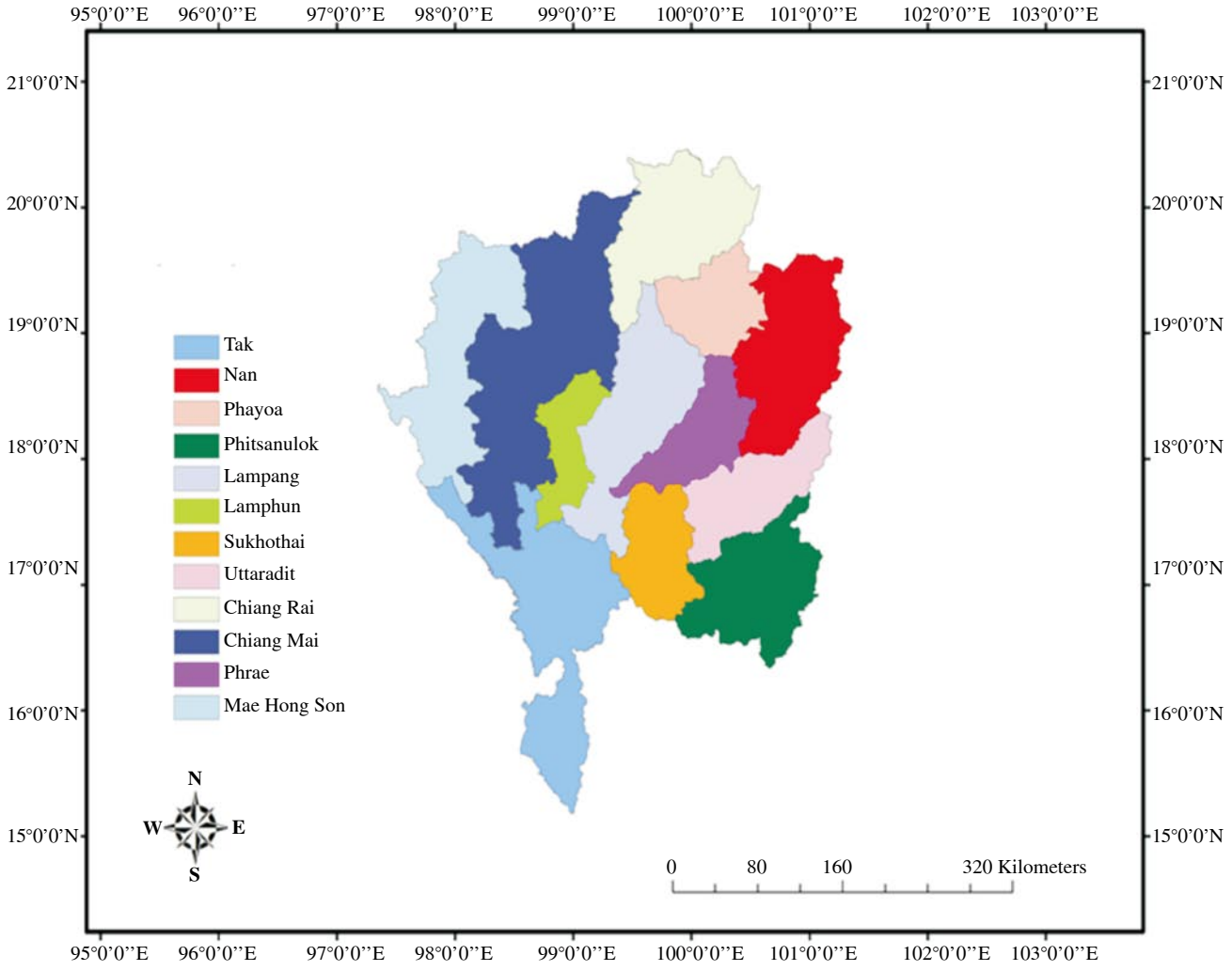

Figure 1. Map of the 12 provinces of Northern Thailand.

a maximum width of 105–135 (120) μm. The tegumental surface is covered with tiny spines. The oral sucker is sub-terminal. Two square shape eyespots are located on both sides under pharynx level (this can be seen in live specimens). Seven pairs of penetration glands are arranged in 2 columns. A long tail [380–520 (440) μm] is attached with dorso-ventral and lateral finfolds. The surface of tail have a longitudinal and fine traverse wrinkles.

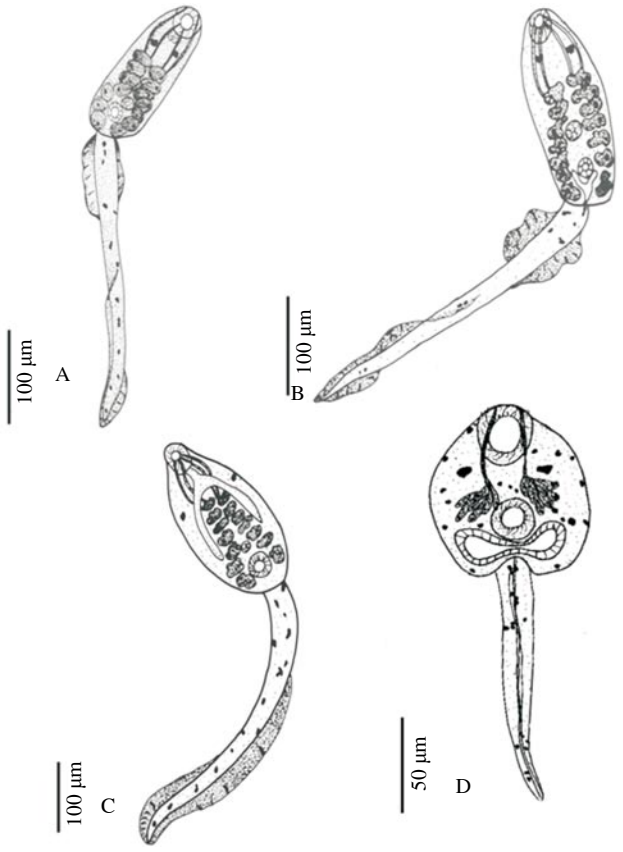

Figure 2. Morphological characteristics of each pleurolophocercous cercariae were investigated among infected thiarid snails (A) pleurolophocercous cercaria Type I, (B) pleurolophocercous cercaria Type II, (C) pleurolophocercous cercaria Type III, (D) pleurolophocercous cercaria Type IV.

3.4. Pleurolophocercous cercariae Type IV: Snail hosts A. housei, M. tuberculata, T. granifera and T. scabra

The body of cercaria is ovoid and 74–105 (91) μm long, with a maximum width of 53–83 (73) μm. The oral sucker is located at sub-terminal. Two square shape eyespots are located on both sides at the end of the anterior third of the body level. Bifurcate ceaca is located on the pharynx to posterior of excretory vesicle level. Seven pairs of penetration glands are arranged into two columns. A long tail is slender and 105–140 (128) μm, and only attached to the dorso-ventral finfolds.

4. Discussions

Of all medically important snails, the thiarid snails play an important role in transmitting heterophyid and opisthorchiid trematodes in Thailand. This study adds new data on this trematode infection among snail intermediate hosts and this is a preliminary step toward better understanding the host-parasite relationship. Form the prevalence of infected snails, the thiarid snails showed a high susceptibility for cercarial infections, which is in agreement with the finding of a previous report by Krailas *et al*., who reported that the thiarid snails especially *M. tuberculata* harbors the larvae of the intestinal trematodes[27]. Furthermore, *M. tuberculata* and *T. granifera* are medically important because they serve as the primary intermediate host for various species of intestinal trematodes species[28].

Regarding the pleurophocercous cercaria Type I was found to be the highest dominant type in this study and the rate of infection of this type in snails was highly prevalence. This morphological characteristic of this cercarial type was discovered to reveal the cercarial stage of the heterophyid trematodes in the genus *Haplorchis*[5,29-31]. In Thailand, four species of snails have been demonstrated as sources of *H. taichui* and *H. pumilio* infection in different geographical habitats; *M. tuberculata*, *T. scabra* and *T. granifera* in central and northern part of Thailand[5,12,16,20,27] and *Neoradina prasongo* in Southern Thailand[29]. Accordingly, a high prevalence of *H. taichui* infection exists among freshwater fish and humans studied from various areas of Northern Thailand. Additionally, Nithikathkul and Wongsawad reported the high prevalence of the metacercarial stage of *Haplorchoides* sp. and *H. taichui* infection in freshwater fish in Family Cyprinidae[32].

Pleurophocercous cercaria Type II was found in *M. tuberculata* and *T. granifera*, while pleurophocercous cercaria Type III was found to have only infected *A. housei*. Hence, both cercarial types are believed to bevery specific in terms of infection in snail hosts, which might be due to the challenges related to distribution during their complete life cycle. They were classified as being in the family Heterophyidae because the tail has lateral folded fin. They also have one pair of eyespots. Nevertheless, they could not be identified at the genus level.

Pleurolophocercous cercaria Type IV was discovered in all thiarid snails. The morphological characteristics of these cercarial reassembles those of the other reports[20]. Consequently, this cercaria was identified as the heterophyid trematode, *Centrocestus formosanus*[33]. This cercaria was found for first time in *T. granifera*, whereas several studies have reported identifying it only in *M. tuberculata*[34]. Moreover, this cercarial type was originally reported as being found in Kanchanaburi, which is lacated in central Thailand[35]. Regarding the overall prevalence of cercarial infection, the prevalence in this study was found to increase by 14.78%[20,35-38]. The north of Thailand presents complex and diverse freshwater ecosystems with its high prevalence of cercarial infection. Suitable condition may promote the trematode cercariae to be able to develop in snails and fish which can lead to the effective maturate and development of their life cycle. Furthermore, common structure of the larval stage in the investigated snails might be anthropogenic influenced bydefinite fauna diversity in the investigated areas, comprising various organisms that could be the possible for intermediate

hosts of the larvae found in the freshwater snails[5]. Moreover, the difference in an overall prevalence of cercarial infection may be due to the involvement of many other factors, including differences in localities, collection times, seasons, *etc*.

From the study, it was determined that the thiarid snails have displayed a high susceptibility for the cercarial infection. The pleurophocercous cercaria infected various snail species. There is the existence of a very quiet compatibility and specificity for infection in intermediate hosts. Various reports have indicated the presence of pleurophocercous cercaria at the cercarial stage of the trematodes This type of cercarial are wildly distributed, may result in an increase in the heterophyid trematode infection among second intermediate hosts and/or definitive hosts. Regarding of this relationship, the recent surveys have revealed that the metacercarial stage of heterophyid trematodes are mostly occurred in Mae Taeng District, Chiang Mai province[39,40]. However, this research was studied only the morphological characteristic of them not enough to specific identification at species level. Therefore, the further investigation will be applied the PCR based approach used for accurate identification.

Hence, pleurophocercous cercariae have displayed a wide range of host specificity because they are able to infect various snail species. Snail-borne trematodes can be assessed by the prevalence of infection. Most areas of northern Thailand shown that the high prevalence of trematodes infection was present among primary and secondary intermediate hosts. It is possible that this is because this area comprised of diverse ecosystem and involves farm forestry containing rice paddies, dams, irrigation canals and rivers, all of which result a high level of biodiversity that is suitable for the life cycle of trematodes for both human and animal infection. Moreover, Traditional Thai food, such as 'Plasom', pickled fish, are still be consumed commonly. Therefore, the study of the cercarial stage of infection is very important. The essential control procedural, namely for those species that are important to human and the organism health, require the identification of numerous stages of the trematode life cycle, the data on their life cycle, and the way to discriminate between the different parasites which infect the similar host. Consequently, there is a need to identify the cercarial stage of all the trematodes, even of those that are not considered as very important, due to the opportunity of competition for the similar intermediate hosts.

Finally, the research shown the current status opisthorchiid and/or heterophyid trematode, which was reported as important various species of intestinal and liver trematode in wild animals including human. Therefore, the data can be applied for planning the effective control approach. Moreover, this study can indicate important information understanding and confirmation of trematode life cycle and intermediate host of them.

Conflict of interest statement

We declare that we have no conflict of interest.

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