การติดเชื้อของตัวอ่อนพยาธิใบไม้ในหอยขม *FILOPALUDINA MARTENSI MARTENSI* จากจังหวัดเชียงใหม่

THE INFECTION OF LARVAL TREMATODES IN POND SNAIL, *FILOPALUDINA MARTENSI MARTENSI* FROM CHIANG MAI PROVINCE

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บทคัดย่อ

การศึกษาในครั้งนี้มีวัตถุประสงค์เพื่อสำรวจความหลากหลายและสถานการณ์การระบาดของตัวอ่อนพยาธิใบไม้ในหอยขม *Filopaludina martensi martensi* โดยทำการเก็บหอยจาก 4 จุดเก็บตัวอย่าง คืออำเภอหางดง (ตำบลหนองแก๋ว และตำบลหารแก้ว) และอำเภอจอมทอง (ตำบลบ้านหลวง และตำบลข่วงเปา) ในจังหวัดเชียงใหม่ระหว่างเดือนกุมภาพันธ์ ถึง เดือนธันวาคม 2563 ผลการศึกษาพบค่าความชุกสูงสุดที่ตำบลหารแก้วมีค่าเท่ากับ 18.52% ความหนาแน่นสูงสุดที่ตำบลบ้านหลวงมีค่าเท่ากับ 38,610.89 และความอุดมสมบูรณ์สูงสุดที่ตำบลหารแก้วมีค่าเท่ากับ 4,373.70 การศึกษาสัณฐานวิทยาด้วยกล้องจุลทรรศน์แบบเลนส์ประกอบ (compound microscope) จัดจำแนกตัวอ่อนพยาธิใบไม้ได้แก่ ระยะสปอโรซีสต์ 1 ชนิด (xiphidiocercaria sporocyst) ระยะเซอร์คาเรีย 4 รูปแบบ คือ xiphidiocercaria, cercariaeum cercaria, echinostome cercaria และ furcocercous cercaria และพบตัวอ่อนระยะเมตาเซอร์คาเรีย 2 ชนิด คือ *Echinostoma revolutum* และ *Thapariella anastomusa* นอกจากนี้ยังพบแนวโน้มที่จะเป็นพยาธิชนิดใหม่ คือ cercariaeum cercaria และพบการระบาดของ striegea cercaria (type furcocercous cercaria)

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เป็นครั้งแรกในอำเภอจอมทอง อย่างไรก็ตามการศึกษาในอนาคตควรมีการใช้วิธีทางสัณฐานวิทยาร่วมกับวิธีทางอณูชีววิทยาเพื่อระบุชนิดของพยาธิใบไม้ในหอยขม *F. martensi martensi* ให้ถูกต้อง การศึกษานี้สามารถนำไปใช้ประโยชน์สำหรับการควบคุม การป้องกัน การติดตามสุขอนามัยของคนและสัตว์ในจังหวัดเชียงใหม่

คำสำคัญ: หอยขม พยาธิใบไม้ เซอร์คาเรีย เมตาเซอร์คาเรีย เชียงใหม่

Abstract

The purpose of this study was to investigate the diversity and epidemic situation of larval trematodes in *Filopaludina martensi martensi*. The snails were collected from four sampling sites in the Hang Dong (Nong Kaeo and Harn Kaeo sub-districts) and Chom Thong districts (Ban Luang and Khuang Pao sub-districts), Chiang Mai province between February and December 2020. The results show that the highest % prevalence was found in the Harn Kaeo sub-district (18.52%), and the highest intensity was found in the Ban Luang sub-district (38,610.89), and the highest abundance was found in the Harn Kaeo sub-district (4,373.7). The morphology of larval trematodes was categorized using a compound microscope; sporocyst (sporocyst of xiphidiocercaria), four types of cercariae (xiphidiocercaria, cercariaeum cercaria, echinostome cercaria, and furcocercous cercaria), and two species of metacercariae (*Echinostoma revolutum* and *Thapariella anastomusa*). In addition, the results show the cercaria that may be new species was cercariaeum cercaria and a new locality of strigea cercaria (furcocercous cercaria type) in the Chiang Mai province. In future research, the combination of traditional and molecular methods could be used to identify the cercariae species in *F. martensi martensi*. The data in this study may be useful for the control, prevention, and further monitoring of human and animal hygiene in the Chiang Mai province.

Keywords: *Filopaludina martensi martensi*, Trematodes, Cercaria, Metacercaria, Chiang Mai

Introduction

Chiang Mai province is located in Northern Thailand. The city is covered with mountains, forests, and water resources that serve as important areas for fisheries, agriculture, and aquaculture (Boonchot & Wongsawad, 2005). Reports of larval trematodes have been recorded in various freshwater snails, including those of the families Bithyniidae, Buccinidae, Bulinidae, Lymnaeidae, Thiaridae, and Viviparidae (Chantima et al., 2013; Chontananarth & Wongsawad, 2013; Chomchoei et al., 2018). *Filopaludina* spp. is a viviparid snail that plays an important role as the first and second intermediate host of trematodes especially, echinostome metacercaria that cause of the Echinostomiasis in animal and human (Toledo and Fried, 2005). These snails should be categorized as ten species, as follows: *F. cambodjensis*, *F. continentalis*, *F. doliaris*, *F. filosa*, *F. maekoki*, *F. martensi*, *F. munensis*, *F. peninsularis*, *F. polygramma* and *F. specisa* (Tarbsripair, 1998). *F. martensi martensi* is a popular food item and is important for the local economy. In addition, *F. martensi martensi* has been harbors several larval trematodes (Brandt, 1974). According to previous reports in Bangkok, Chiang Mai and Chiang Rai province, the four types of cercarial trematodes have been shown to infect *F. martensi martensi*: echinostome cercaria, furcocercous cercaria, mutabile cercaria, and xiphidiocercaria (Wongsawad et al., 2016; Chantima & Rika, 2020; Wiroonpan et al., 2020). Furthermore, metacercaria of some trematodes was found infecting these snails in other areas in Northern Thailand, such as *Thapariella* spp., and echinostomes, namely *Echinostoma revolutum* (Chantima et al., 2013; Noikong & Wongsawad, 2014; Noikong et al., 2014; Chantima et al., 2018; Butboonchoo et al., 2020; Chantima & Rika, 2020).

The purpose of this research is to study the diversity of larval trematodes in the freshwater snail, *F. martensi martensi*, from rivers and agriculturral areas, Hang Dong and Chom Thong districts, Chiang Mai province that some areas in these districts have been reported larval trematodes infections in this snail for a long time (Chontananarth, 2013; Chantima, 2014). However, some areas in Hang Dong and Chom Thong district have never been recorded. So the report of the present study can be used to update the epidemic situation and can be used to estimate the infection rate and epidemic situation of larval trematodes, improve public health management, and serve as a basis for higher research in the parasitology field.

Materials and methods

1. Site sampling

Four hundred and forty-four viviparid snails, *Filopaludina martensi martensi*, were collected between February and December 2020 from two districts in the Chiang Mai province: Chom Thong (Ban Luang and Khuang Pao sub-districts) and Hang Dong (Nong Kaeo and Harn Kaeo sub-districts) (Figure 1). All sampling sites were surrounded by rivers, big trees and agricultural activities. The snails were identified as species based on the key of Brandt (1974). The snails were randomly collected in the canal using the hand-picking method (Chantima et al., 2018). The alive snails were packed in a plastic box with water and holes during transfer to the laboratory. The larval trematode in living snails was investigated by the crushing method in the laboratory. The internal organs of snails were separated and examined larval trematodes under stereo microscopes (Olympus, Japan) and compound microscopes (Olympus, Japan) (Chontananarth & Wongsawad, 2013).

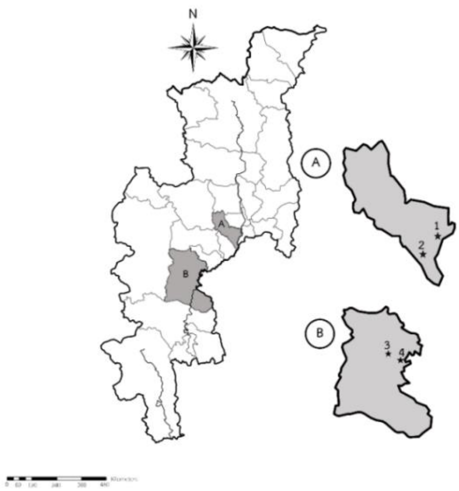

Figure 1 The map showing sampling sites in Chiang Mai province:
A) Hang Dong district B) Chom Thong district
1) Nong Kaeo sub-district 2) Harn Kaeo sub-district
3) Ban Luang sub-district 4) Khuang Pao sub-district

2. Morphological study

The living larval trematodes were fixed in 2% formalin between glass slides and cover slips and washed with distilled water. The larvae specimens were stained with haematoxylin and eosin. Then, specimens were dehydrated in increasing ethanol series, cleared in xylene, and mounted with permount. The permanent slides were observed with a compound microscope (Olympus, Japan) and drawn as figures using a camera lucida (Olympus, Japan). The measurements of larvae were shown in micrometres (μm) and millimetres (mm). Each type of specimen was identified morphologically according to the references (Yamaguti, 1958; Schell, 1970; Frandsen & Christensen, 1984; Noikong & Wongsawad, 2014; Phalee et al., 2018; Butboonchoo et al., 2020).

3. Data analysis

The numbers of larval trematodes were counted in a petri dish using a dropper under a stereo microscope. The counted larvae were separated into another petri dish. The prevalence, intensity, and abundance of infection were analyzed according to Bush et al. (1997).

Results

1. Epidemiological study

Four hundred and forty-four viviparid snails (*Filopaludina martensi martensi*) (Figure 2) were collected from 2 districts in the Chiang Mai province: Hang Dong (Nong Kaeo and Harn Kaeo sub-districts) and Chom Thong (Ban Luang and Khuang Pao sub-districts) between February and December 2020. The epidemic data were detailed in Table 1 and Figure 3. Among the sampling sites, the highest prevalence was found in the Harn Kaeo sub-district, followed by the Nong Kaeo, Khuang Pao, and Ban Luang sub-districts at 18.52%, 12.38%, 9.09%, and 8.18%, respectively. The highest intensity was found in the Ban Luang sub-district (38,610.89), followed by the Nong Kaeo (34,192.69), Khuang Pao (32,658.73), and Harn Kaeo (23,618) sub-districts. The highest abundance was recorded in the Harn Kaeo sub-district (4,373.7), followed by the Nong Kaeo (4,233.38), Ban Luang (3,159.07), and Khuang Pao (2,968.98) sub-districts. The larval trematodes from the present study were categorized based on morphology into one sporocyst, four types of cercariae (xiphidiocercaria, cercariaeum cercaria, echinostome cercaria, and furcocercous cercaria), and two species of metacercaria (*Echinostoma revolutum* and *Thapariella anastomusa*). The morphology of the larvae was described as follows.

Figure 2 The morphology of pond snail, *Filopaludina martensi martensi*

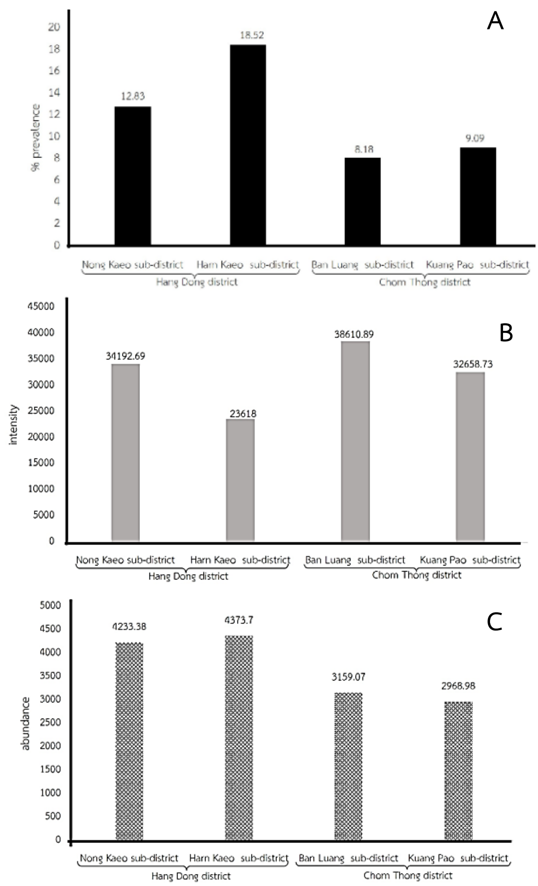

Figure 3 Total prevalence (A), intensity (B), and abundance (C) of larval trematodes infections in pond snail, *Filopaludina martensi martensi* from four sub-districts in Chiang Mai province between February and December 2020

Table 1 The number of examined snails, infected snails, and epidemic data of larval trematodes in *Filopaludina martensi martensi* collected from four sub-districts in Chiang Mai province between February and December 2020

Site sampling	Larval Trematodes	No. of snail examined	No. of snail infected	No. of larvae	%P	I	A
Hang Dong district (Nong Kaew sub-district)	Xiphidiocercaria (sporocyst)	105	10	439,554	9.52	43,955.4	4,186.23
	Xiphidiocercaria (cercaria)	105	10	4,944	9.52	494.4	47.09
	Cercariaeum cercaria	105	1	5	0.95	5.0	0.05
	Echinostoma revolutum metacercaria	105	2	2	1.90	1.0	0.02
Hang Dong district (Harn Kaeo sub-district)	Xiphidiocercaria (sporocyst)	108	15	463,623	13.89	30,908.2	4,292.81
	Xiphidiocercaria (cercaria)	108	15	8,322	13.89	554.8	77.06
	Echinostome cercaria	108	1	395	0.93	395.0	3.66
	Thapariella anastomusa metacercaria	108	4	20	3.70	5.0	0.19
Chom Thong district (Ban Luang sub-district)	Xiphidiocercaria (sporocyst)	110	7	344,016	6.36	49,145.14	3,127.42
	Xiphidiocercaria (cercaria)	110	7	3,273	6.36	467.57	29.75
	Furcocercous cercaria (vivax cercaria)	110	1	205	0.91	205.0	1.86
	Thapariella anastomusa metacercaria	110	1	4	0.91	4.0	0.04

Table 1 The number of examined snails, infected snails, and epidemic data of larval trematodes in *Filopaludina martensi martensi* collected from four sub-districts in Chiang Mai province between February and December 2020 (cont.)

Remark %P= % prevalence; I= intensity; A= abundance

Site sampling	Larval Trematodes	No. of snail examined	No. of snail infected	No. of larvae	%P	I	A
Chom Thong district (Khuang Pao sub-district)	Xiphidiocercaria (sporocyst)	121	8	354,556	6.61	44,319.5	2,930.21
	Xiphidiocercaria (cercaria)	121	8	4,393	6.61	549.13	36.31
	Furcocercous cercaria (strigea cercaria)	121	1	289	0.83	289.0	2.39
	Thapariella anastomusa metacercaria	121	2	8	1.65	4.0	0.07

2. Morphological study

Sporocyst

The shape of the sporocyst was an elongated oval. The surface was thin with no spines. The size was about 146.5–165.7 x 317.28–350.1 µm. The matured sporocyst contained developed xiphidiocercaria; the number of xiphidiocercaria in each sporocyst was 1–6. The stylet was observed at the subterminal end of the oral sucker (Figure 4A).

Cercaria consisted of four types:

1. Xiphidiocercaria

The body shape of this cercaria was elongated oval. The body size was 117.5–138.3 µm in length and 48.3–52.5 µm at its widest point. The body surface has tiny spines. The well-developed oral sucker was located subterminal, 21.2–25.1 µm in diameter. The stylet was observed at the oral sucker. The stylet shape was lancet-like. The prepharynx was short, 3.9–4.9 µm in length. The pharynx was small, globular, and 4.7–6.0 µm in diameter. The well-developed acetabulum was 42.5–46.3 µm in diameter. The acetabulum was similar in size to the oral sucker. There were two pairs of penetration glands observed in the middle of the body. Each of the glands was connected with the penetration duct, which runs forward laterally and opens in the oral sucker area. The genital primordial was developed early at the posterior of the acetabulum. The excretory bladder was V-shaped. It was connected with the excretory canal of the tail at the tail extremity. The tail was simple, 57.5–80.1 µm in length, and 15.0–17.1 µm in width (Figure 4B).

2. Cercariaeum cercaria

The body was fusiform in shape. The length of the body was 545.1–574.24 µm with 165.1–175.8 µm at its widest point. The body surface has no spines. The oral sucker was located at the subterminal of the anterior part of the body , 56.0–59.1 x 68.7–78.8 µm in size. The prepharynx was present, 42.5–47.5 µm in length, followed by the muscular pharynx. The pharynx was connected by the esophagus, 35.2–37.9 x 38.8–43.9 µm in size. The esophagus was 52.0–58.0 µm in length. The intestine was furcated and laterally extended to the posterior part of the body. The well-developed globular acetabulum is 86.4–89.4 µm in diameter, found in the middle of the body, and slightly bigger than the oral sucker. The excretory bladder was Y-shaped, thin-walled, and open at the posterior extremity. The five pairs of penetration glands were situated in the anterior part of the body; their ducts were open at the part of the oral sucker. The tail was absent (Figure 4C).

3. Echinostome cercaria (*Echinostoma revolutum*)

The cercaria body has an elongated shape. The body was 425.5–470.1 µm in length and 167.5–215.1 µm at the widest point. The body was covered with tiny spines. The globular oral sucker was located subterminal, 44.5–45.9 µm in diameter, and surrounded by the unique characteristic of *Echinostoma revolutum*, 37 collar spines. The prepharynx was short, 27.5–31.3 µm in length, followed by the globular pharynx. The pharynx was 15.2–18.5 x 19.5–27.5 µm in size. The esophagus was narrow, long (107.5–147.5 µm in length), and extends to the excretory bladder. The circular acetabulum was located at the post-equatorial of the body and was 66.3–67.5 µm in diameter. The early development of the reproductive system was found above and below the acetabulum. The main collecting tube was observed between the pharynx level and laterally extended to the excretory bladder. The excretory bladder was connected with the excretory tube of the tail. The tube was bifurcated at the anterior part of the tail. The tail was flattened and shorter than the body, 310.2–370.5 µm in length and 53.8–65.4 µm in width (Figure 4D).

4. Furcocercous cercaria

4.1 Subtype strigea cercaria

The body was elongated oval-shaped, 197.5–270.1 µm in length and 80.4–95.2 µm in width. The surface was smooth. The oral sucker was circular and situated at the subterminal of the anterior part of the body, 35.5–47.5 µm in diameter, and surrounded by a single row of 12–14 spines. The prepharynx was short and followed by the muscular pharynx. The prepharynx was 11.9–15.2 µm in length. The pharynx was globular and 20.3–23.1 µm in diameter. The cecum was short and divided into two intestinal caeca. The acetabulum was situated behind the mid body. The diameter of the acetabulum was 36.3-42.5 µm, almost equal in size to the oral sucker. The two pairs of penetration glands were observed near the blind of the intestinal caeca, between the pharynx and the acetabulum. The tail was divided into two furca in the middle of the tail. The tail stem was 65.1–74.5 µm in length and 21.9–28.9 µm in width. The furca tail was 52.5–65.4 µm in length and 7.1–7.9 µm in width. The triangular-shaped excretory bladder was located at the posterior of the body, followed by a canal that opens at the side of the furca tail (Figure 4E).

4.2 Subtype vivax cercaria

The body was pyriform in shape, 410–490 µm in length, and 310–390 µm in width. The oral sucker was oval and 50.4–65.4 x 65.5–72.5 µm in size. The acetabulum was absent. The prepharynx is 44.1–45.6 µm in length and followed by the small pharynx. The pharynx is 34.39–42.2 µm in diameter. The esophagus was divided into the large bifurcate cecum. The cecum was divided at the pre-equatorial of the body and extended near the excretory bladder. The length of the tail stem was 88.8–103.5 x 425.2–460.1 µm in size; it was divided into two furca at 1/3 from the posterior end. The furca tails were 41.9–57.6 x 240.4–320.4 µm in size. The fin folds were at the tail. The two pairs of penetration glands were present in the middle of the body, external to the caeca. The small excretory bladder was located at the end of the body and connected with the excretory duct, which opened at the tail furca (Figure 4F).

Metacercaria consisted of two species:

1. *Echinostoma revolutum*

The body shape of the excysted metacercaria was elongated oval, 640.5–646.2 µm in length and 117.6–125.8 µm in width. The oral sucker was globular in shape and 51.0–60.7 µm in diameter. The head crown was well developed, surrounded by 37 collar spines. The prepharynx was short, 40.0–42.5 µm in length, followed by the muscular pharynx. The pharynx was 26.3–28.8 x 33.8–36.5 µm in size. The esophagus was long distance and furcated at the anterior of the acetabulum. The large acetabulum was 72.7–81.8 µm in diameter, located at the post equatorial part of the body. Two early developed genital primordial were observed at the acetabulum level (cirrus pouch) and posterior extremity (testis) (Figure 4G).

2. *Thapariella anastomusa*

The excysted metacercaria was a large linguiform shape, 4.5–5.2 mm in length, and 1.9–2.2 mm in length. The surface was smooth. The oral sucker was situated at the subterminal, 454.7–471.7 x 528.4–545.1 µm in size, directly followed by the globular pharynx. There was no prepharynx. The pharynx was 263.7–279.5 x 247.1–249.9 µm in size. The cecum was divided immediately from the pharynx, and it extends to the posterior of the body. The well-developed acetabulum was situated at one-third of the anterior part of the body length. The size of the acetabulum was 823.5–867.7 µm in diameter. The posterior part of the body was occupied by the reproductive system. The testes were oval and 191.2–235.3 x 308.8–382.4 µm in size. The ovary was circular, situated between the testes and the excretory bladder. The size of the ovary was 205.9–220.6 µm in diameter. There were six to seven vitelline follicles. The excretory bladder was observed at the posterior of the body and was almost circular (Figure 4H).

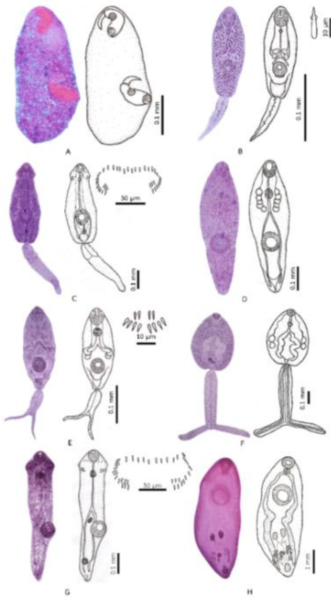

Figure 4 The illustrations of larval trematodes infected in pond snail, *F. martensi martensi* between February-December 2020.
A) Sporocyst of xiphidiocercaria B) Xiphidiocercaria C) Cercariaeum cercariae D) Echinostome cercaria E) Strigea cercaria F) Vivax cercaria
G) *Echinostoma revolutum* metacercaria
H) *Thapariella anastumosa* metacercaria

Discussion

Filopaludina martensi martensi act as intermediate hosts of several larval trematodes (Brandt, 1974; Butboonchoo et al., 2020; Wiroonpan et al., 2020). This study represents the epidemic analysis and diversity of its larval trematodes in Chom Thong (Ban Luang and Khuang Pao sub-districts) and Hang Dong (Nong Kaeo and Harn Kaeo sub-districts) of Chiang Mai province during February to December 2020. Seven larval trematodes were indicated, including: sporocyst, cercaria (xiphidiocercaria, cercariaeum cercaria, echinostome cercaria, and furcocercous cercaria), and metacercaria (*Echinostoma revolutum* and *Thapariella anastomusa*). Some of these trematodes can cause diseases in the animal and humans for example, Echinostomiasis (Toledo & Fried, 2005)

As shown by the results, the highest prevalence of infection was found in the Harn Kaeo sub-district (18.52%), followed by the Nong Kaeo sub-district (12.38%). The two sampling sites are small and low flow streams, which consist of trees, rocks, and aquatic plants. Low prevalence was found in the Khuang Pao sub-district (9.1%) and was similar to that in the Ban Luang sub-district (8.18%), which is the high flow stream with few rocks or aquatic plants. This can be explained by the reason that, the flow stream can increase or decrease the occurrence of miracidium contact with the intermediate host (Luka & Mbaya, 2015). The highest intensity of infection was recorded in the Ban Luang sub-district (38,610.89), followed by the Nong Kaeo (34,192.69), Khuang Pao (32,658.73), and Harn Kaeo (23,618) sub-districts. The intensity represented the number of larval trematodes in a single infected host in each locality; when infection intensity is high, the parasite could impact the health of the host by influencing body condition, behavior, and physiology (Margolis et al., 1982; Turgeon et al., 2018). The intensity of this study is higher than that of a previous report (Ahn, 1986). For the abundance of infection, the highest was observed in the Harn Kaeo sub-district (4,373.7), followed by the Nong Kaeo (4,233.38), Ban Luang (3,159.07), and Khuang Pao (2,968.98) sub-districts. This parameter shows the density and spread of larval trematodes in the examined host population in the sampling site (Margolis et al., 1982; Bush et al., 1997). However, an abundance of infections has never been reported from this snail, *Filopaludina martensi martensi*. But, when comparing the abundance in the present study with Chung et al. (2001) in *Austropeplea ollula* snail revealed that the result in this study is higher this study. According to previous *F. martensi martensi* observations, the intensity and abundance of cercarial infections have not been reported (Krailas et al., 2012; Chontananarth & Wongsawad, 2013; Wongsawad et al., 2016; Dunghungzin & Chontananarth, 2020). Here, we have indicated the intensity and abundance of cercarial infection in *F. martensi martensi* for the first time in Thailand. Therefore, these parameters, prevalence, intensity, and abundance are important for clearly estimating the outbreak and severity of larval trematodes in *F. martensi martensi*. Moreover, the present study was found the type of the cercaria higher than previous report in Ban Luang sub-district (Chontananarth, 2013).

In addition, the data of this study revealed several larval trematodes that can cause of disease of the hosts. So, the consumption, especially pond snails should be cook at high temperature for killing of these larval trematodes that can decrease a risk of trematodes infection in humans in Chiang Mai province. The animal husbandry should be conducted under the closed and cleaned farm for prevent the transmission of larval stage to animal. So, the risk of trematodes disease should be reduced.

In this study, the sporocyst was shown to have high intensity from every site sampling, with the highest in the Ban Luang sub-district (49,145.14). The highest abundance was in the Harn Kaeo sub-district (4,292.81), which was not related to the % prevalence of infection (table 1). The sporocysts generally have a sacciform hollow structure with a thin layer, no pharynx, intestine, and spines on the surface (Ito, 1980). The sporocyst from this study contained the non-virgulate xiphidiocercaria. The organs of the xiphidiocercaria were not developed. In a previous study, this sporocyst has been reported from *Melanoides tuberculata* and *Tarebia granifera* and morphologically identified as *Haematoloechus similis*, *Maritreminoides caridinae*, and *M. obstipus* (Krailas et al., 2014; Veeravechsukij et al., 2018). However, the intensity and abundance of infection of the sporocyst have not been reported in the Chiang Mai province.

In the present study, the xiphidiocercaria was distributed in every region of the sites studied. The data are shown in Table 1. The morphology of the present xiphidiocercaria closely resembles the cercaria of *Paralecithodendrium chilostomum* in the snail, *Viviparus viviparus*, from Ukraine by the spines on the surface of the body, two pairs of penetration glands, the position of acetabulum (located at the middle of the body), and shape of the excretory bladder (V-shaped) (Kudlai et al., 2015). The interesting stylet shape from this study was similar to that of the *Lecithodendrium* sp. from *Bithynia tentaculata* in Lithuania (Kudlai et al., 2015). These trematodes are members of the family Lecithodendriidae, which are parasites of bats (Kudlai et al., 2015; Horvat et al., 2017). These morphological differences may indicate that the xiphidiocercaria in this study can belong to other species of the family Lecithodendriidae.

The cercariaeum cercaria was found in the Nong Kaeo sub-district with the lowest prevalence of infection (0.1%). The cercariaeum cercaria has been morphologically characterized by the absence of a tail (Schell, 1970). These larvae belong to the families: Cyclocoelidae (trematodes in the respiratory tract of birds) and Monorchiidae (intestinal trematodes of fish) (Frandsen & Christensen, 1984). The cercariaeum cercaria was found in the same snails from Bangkok and identified as mutable cercaria (Wiroonpan et al., 2020). The comparison of cercariaeum cercaria in the present work with the description given by Anucherngchai et al. (2016) revealed similar morphology, such as the long distance of the prepharynx and long caeca reaching the posterior end of the body. Mutabile cercaria described by Wiroonpan et al. (2020) do not have an observable prepharynx and the cecum extends to 2/3 of the length of the body. Therefore, the present cercariaeum cercaria from this study may be considered the appearance of a new species. However, molecular approach especially sequencing technology of nuclear DNA and mitochondrial DNA should be used to identify this cercaria in the future (Kudlai et al., 2015; Sakda et al., 2018).

The echinostome cercaria from this study was found only in the Harn Kaeo sub-district with a prevalence, intensity, and abundance of infection of 0.93%, 395, and 3.66, respectively. The prevalence is quite similar to that previously recorded in the central part of Thailand (Dunghungzin & Chontananarth, 2020). In addition, in Thailand, the echinostome cercaria has been found in five snail families including *F. martensi martensi* (Wiroonpan et al., 2020). The morphological characteristics of the present echinostome cercaria are similar to that of *Echinostoma revolutum* regarding the number and arrangement of the 37 collar spines (Noikong & Wongsawad, 2014; Butboonchoo et al., 2020). As mentioned above, the cercarial stage of *E. revolutum* was found in the Harn Kaeo sub-district, while the metacercaria of *E. revolutum* was found in Nong Kaeo sub-district. The result elucidated that *F. martensi martensi* can act as a first and second intermediate host of *E. revolutum* (Chantima et al., 2013; Chantima et al., 2018; Dunghungzin & Chontananarth, 2020).

Two types of furcocercous cercaria were identified in this study. The first type is the strigea cercaria and the second type is the vivax cercaria. The strigea cercaria found in the Khuang Pao sub-district had a prevalence, intensity, and abundance of infection of 0.83%, 289, and 2.39, respectively. This cercaria is produced by trematodes of the Strigeidae and Diplostomadidae families and infects intestinal parasites of birds and mammals (Frandsen & Christensen, 1984). Interestingly, the morphology of this strigea cercaria has distinct spines surrounding the oral sucker. With its unique spines on the oral sucker, the present strigea cercaria is slightly similar to the strigea cercaria described by Moema et al. (2008) in *Bulinus tropicus* from South Africa. However, the present strigea cercaria has a bigger body size (about 2 times larger) and only two penetration glands. Previously, the strigea cercaria has been recorded from the Chiang Mai province in the Mae Taeng district (Chontananarth & Wongsawad, 2013). So our results noted a new locality of strigea cercaria infection in *F. martensi martensi* in Chiang Mai province. For the vivax cercaria occurred only in the Ban Luang sub-district in this study with a 0.91% prevalence, intensity of 205, and an abundance of infection of 1.86. This fluke belongs to the family Cyathocotylidae and is parasitic as an adult in fish, reptiles, birds, and mammals, including humans (Frandsen & Christensen, 1984; Chai, 2019). This cercaria is morphologically distinguished among larval trematodes in the present study by the absence of the acetabulum. Based on the morphology, this vivax cercaria is similar to cercaria in different snails from the Republic of Korea (Han et al., 2012) and Thailand (Wiroonpan et al., 2020). Therefore, the present vivax cercaria may be the same species, but future classical or molecular method studies are required to obtain more information.

This study found two metacercaria: *Echinostoma revolutum* and *Thapariella anastomusa*. The metacercaria of *E. revolutum* was found in the Nong Kaeo sub-district. The prevalence and intensity of this study were lower than that of previous reports in the Chiang Mai province (Chantima et al., 2013; Butboonchoo et al., 2020). This sampling site was not found the *E. revolutum* cercaria could relate to the maturation or change in the richness of intermediate and definitive host of the trematodes (Kanev, 1994; Chontananarth & Wongsawad, 2013).

Thapariella anastomusa metacercaria. The prevalence and intensity of infection from this study are higher than that of *T. anastomusa* reported by Chantima and Rika (2020) from the Chiang Rai province. However, the present epidemic data is lower than that reported in the Phitsanulok province by Phalee et al. (2018). In addition, metacercaria of *T. anastomusa* have been found in other viviparid snails, for example, *F. sumatrensis polygramma* and *F. doliaris* (Phalee et al., 2018; Chantima & Rika, 2020). The excysted metacercaria was found during observation under a stereo-microscope. So, this characteristic of *T. anastomusa* is interesting for study in the future.

Conclusion

In the present study, *Filopaludina martensi martensi* were collected from 4 sampling sites in two districts in the Chiang Mai province. The present study revealed the diversity and epidemic status of infection of larval trematodes infecting *F. martensi martensi* in the Chiang Mai province, including one sporocyst (sporocyst of xiphidiocercaria), four types of cercariae (xiphidiocercaria, cercariaeum cercaria, echinostome cercaria, and furcocercous cercaria), and two species of metacercariae (*Echinostoma revolutum* and *Thapariella anastomusa*). This study may have found a new species of cercariaeum cercaria and a new locality of strigea cercaria (furcocercous cercaria type) in the Chiang Mai province. Additionally, our results show the role of *F. martensi martensi* as the first and second intermediate host of *Echinostoma revolutum*. However, further studies are required on the classical and molecular methods to describe and identify these cercariae species in *F. martensi martensi*. The data can be useful for the prevention, control, and further monitoring of the health of humans and animals in the area. Additionally, the consumption of cooked snails should be decreasing the rate of trematode infections.

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