

# TREMATODE INFECTION IN FRESHWATER SNAILS FROM MAHA SARAOKHAM PROVINCE, THAILAND

Naruemon Bunchom<sup>1</sup>, Warayutt Pilap<sup>1</sup>, Warong Suksavate<sup>2</sup>, Kotchaphon Vaisusuk<sup>3</sup>, Narufumi Sukanuma<sup>4</sup>, Takeshi Agatsuma<sup>4</sup>, Trevor N. Petney<sup>5</sup> and Weerachai Saijuntha<sup>1</sup>

<sup>1</sup>Walai Rukhavej Botanical Research Institute, Biodiversity and Conservation Research Unit, Mahasarakham University, Maha Sarakham; <sup>2</sup>Department of Forest Biology, Faculty of Forestry, Kasetsart University, Bangkok; <sup>3</sup>Department of Veterinary Technology, Faculty of Agricultural Technology, Rajabhat Maha Sarakham University, Maha Sarakham, Thailand; <sup>4</sup>Department of Environmental Medicine, Kochi Medical School, Kochi University, Nankoku, Japan; <sup>5</sup>Department of Paleontology and Evolution, State Museum of Natural History Karlsruhe, Karlsruhe, Germany

**Abstract.** Several trematode species utilize freshwater snails as intermediate hosts. The transmission stage of trematodes from the snail host to their next intermediate or final host involves cercarial and metacercarial stages. Thus, it is important to survey trematode infections in species of freshwater snails in human and/or animal endemic regions. Prevalence and types of trematode cercariae and metacercariae found in common freshwater snails in Maha Sarakham Province, Northeast Thailand were determined. Freshwater snails ( $n = 3,757$ ) collected from canals, ponds and paddy fields at 51 sites belonged to six species, namely, *Bithynia siamensis goniomphalos* (Morelet, 1866) (prevalence = 60.8%, trematode infection = 15.2%), *Filopaludina martensi* (Frauenfeld, 1865) (prevalence = 13.4%, trematode infection = 0.6%), *Indoplaborbis exustus* (Deshayes, 1834) (prevalence = 8.6%, trematode infection = 1.6%), *Melanoides tuberculata* (Müller, 1774) (prevalence = 8.8%, trematode infection = 4.8%), *Pomacea canaliculata* (Lamarck, 1819) (prevalence = 3.3%, trematode infection = 0%), and *Radix rubiginosa* (Michelin, 1831) (prevalence = 5.1%, trematode infection = 6.8%), with nine and eight different types of cercariae and metacercariae respectively. The most common trematodes were xiphidio and longifurcate-pharyngeate (strigea) cercariae, while highest infection rate (5.24%) was with echinostome cercaria. These findings indicate freshwater snails in Maha Sarakham Province act as both first and second intermediate hosts of several trematode species of medical and veterinary importance.

**Keywords:** *Bithynia siamensis goniomphalos* (Morelet, 1866), *Filopaludina martensi* (Frauenfeld, 1865), *Indoplaborbis exustus* (Deshayes, 1834), *Melanoides tuberculata* (Müller, 1774), *Pomacea canaliculata* (Lamarck, 1819), *Radix rubiginosa* (Michelin, 1831), freshwater snail, public health, northeastern Thailand, trematode

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Correspondence: Weerachai Saijuntha, Walai Rukhavej Botanical Research Institute, Mahasarakham University, Maha Sarakham 44150, Thailand.

Tel: +66 (0) 4375 4333, Fax: +66 (0) 4375 4407

E-mail: weerachai.s@msu.ac.th

## INTRODUCTION

Freshwater snails are abundant and constitute a major component of the aquatic food chain (Yeung and Dudgeon, 2014) and have received much attention for their role as intermediate hosts for veterinary and medically important trematodes causing disease in humans and animals. In particular, a carcinogenic liver fluke, *Opisthorchis viverrini*, endemic in Southeast Asia, believed to be associated with bile duct cancer utilizes *Bithynia* snails of family Bithyniidae as first intermediate host (Sithithaworn *et al*, 2012).

The majority of freshwater snails in Thailand can act as intermediate hosts of trematodes. For example, snails in genus *Filopaludina* act as first intermediate hosts of lung fluke genus *Paragonimus* (Yaemput *et al*, 1994) and genera *Filopaludina*, *Indoplanorbis*, *Pila*, *Pomacea* and *Radix* (syn. *Lymnaea*) as first or second intermediate hosts of small intestinal flukes and echinostomes (Sri-Aroon *et al*, 2005; Chantima *et al*, 2013; Anucherngchai *et al*, 2016). Several snails in genera *Indoplanorbis*, *Melanooides* and *Radix* also are intermediate hosts of blood fluke, furcocercous (Chontanarith *et al*, 2017).

Maha Sarakham Province, located in northeastern Thailand, is largely an agricultural region with rice cultivation and animal farming (Oechaiyaphum *et al*, 2020). Lifestyles and consumer patterns of the local population involve consumption of raw or undercooked cyprinid fish, the second intermediate hosts of the small liver fluke, and of freshwater crabs, the second intermediate hosts of lung fluke (Sithithaworn *et al*, 2012; Chantima *et al*, 2013). Freshwater snails in family Viviparidae constitute second intermediate hosts of echinostomes

(Chantima *et al*, 2013).

Here, trematode infections in six species of freshwater snails in Maha Sarakham Province, Thailand were examined. The information obtained will further extend our understanding of trematode parasites transmitted via freshwater snails infecting animals and humans in this region of the country.

## MATERIALS AND METHODS

### Freshwater snail collection

Freshwater snails were collected by hand picking at the shore of stagnant water, *ie* canals, ponds and paddy fields in 51 localities of four districts (Kantharawichai, Kosum Phisai, Mueang, and Phayakkaphum Phisai), in Maha Sarakham Province, northeastern Thailand, from July 2016 to June 2017 (Fig 1). These four districts are located in Chi River wetland surrounding Mahasarakham University. Snails were transported to the laboratory in plastic bottles with water from their natural habitat.

### Trematode collection

Snails were classified according to species as previously described (Brandt, 1974; Chitramvong, 1992), then individually placed in small plastic cups containing 5 ml of dechlorinated tap water, exposed to light (1,200 lux) for five hours at ambient temperature, and then examined for presence of cercariae under a stereomicroscope (10x magnification). Thereafter, snails were crushed between glass plates and cercariae and metacercariae removed using a needle (Kaewkes, 2003; Kiatsopit *et al*, 2012). For *P. canaliculata*, due to its large size, only visceral organs were removed and processed as described above. Trematodes were classified as

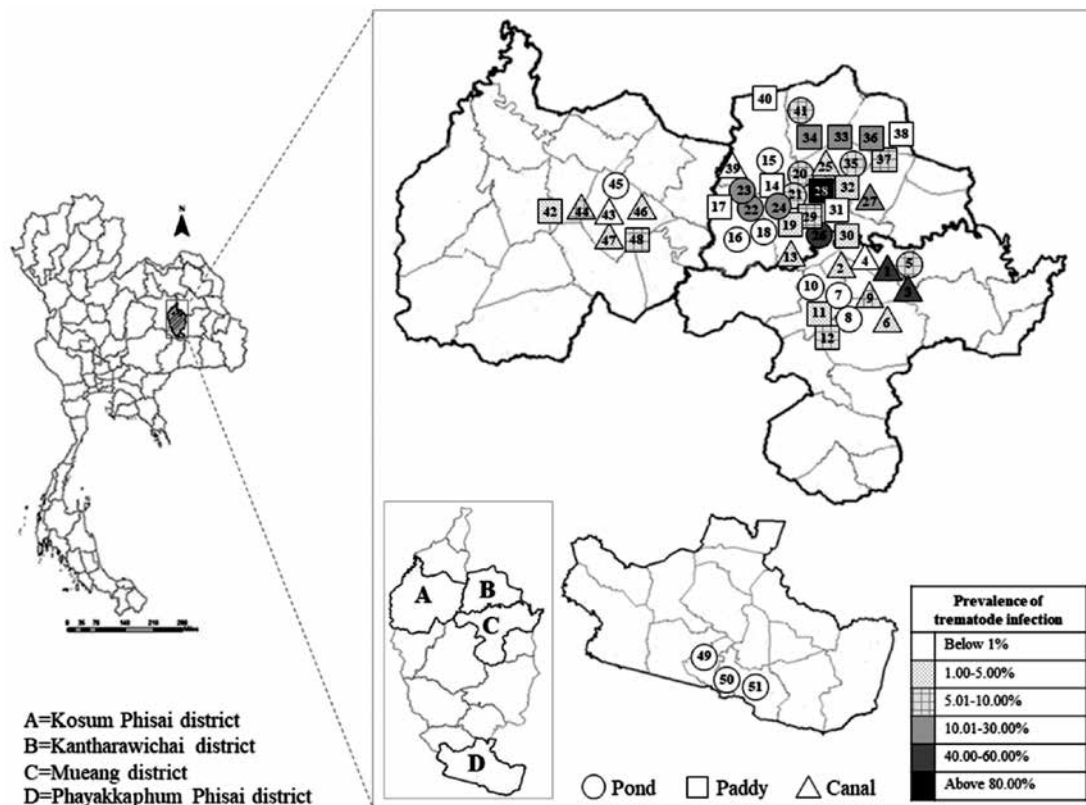


Fig 1-Collection sites and prevalence of trematode infection in freshwater snails in 51 localities from four districts of Maha Sarakham Province, Thailand (July 2016 - June 2017).

described by Schell (1970) and Frandsen and Christensen (1984).

### RESULTS

Of the 3,757 freshwater snails collected, they were identified into six species, namely *Bithynia siamensis goniomphalos* (Morelet, 1866), *Filopaludina martensi* (Frauenfeld, 1865), *Indoplaborbis exustus* (Deshayes, 1834), *Melanoides tuberculata* (Müller, 1774), *Pomacea canaliculata* (Lamarck, 1819), and *Radix rubiginosa* (Michelin, 1831) (Fig 2). The most prevalent among six species identified was *B. s. goniomphalos* (60.8%), followed by *F. martensi* (13.4%), *M. tuberculata* (8.8%), *I. exustus* (8.6%), *R.*

*rubiginosa* (5.1%), and *P. canaliculata* (3.3%), with highest (15%) trematode infection detected in *B. s. goniomphalos* and none in *P. canaliculata* (Table 1). Prevalence of infected snails was highest in canal (12%), followed by paddy field (10%) and pond (7%). The highest infection rate was found in a paddy field (85%; collection site number 28) in Kantharawichai district (Fig 1).

Nine types of cercariae, namely, amphistome, brevifurcate-apharyngeate, cystophorous, echinostome, longifurcate-pharyngeate, monostome, mutabile, ophthalmoxiphidio, and xiphidio were detected by shedding and crushing methods (Fig 3), the former method

Table 1  
Trematode infections in freshwater snails from Maha Sarakham Province, Thailand (July 2016 - June 2017).

Cercaria	Number of infected snails (% infection)							Total (n = 3,757)
	<i>Bithynia siamensis goniophthalos</i> (Morelet, 1866) (n = 2,283)	<i>Filopaludina martensi</i> (Frauenfeld, 1865) (n = 503)	<i>Melanoides tuberculata</i> (Müller, 1774) (n = 125)	<i>Indoplaborbis exustus</i> (Deshayes, 1834) (n = 191)	<i>Radix rubiginosa</i> (Michelin, 1831) (n = 323)	<i>Pomacea camalicutata</i> (Lamarck, 1819) (n = 332)		
Xiphidio	18 (0.8)	2 (0.4)	1 (1)	1 (<1%)	-	-	22 (0.6)	
Amphistome	3 (0.1)	-	-	-	-	-	3 (0.1)	
Monostome	4 (0.2)	-	2 (2)	-	-	-	6 (0.2)	
Mutabile	3 (0.1)	-	-	-	-	-	3 (0.1)	
Ophthalmoxiphidio	5 (0.2)	-	-	-	-	-	5 (0.1)	
Cystophorous	1 (<0.1)	-	-	-	-	-	1 (<0.1)	
Brevifurcate- apharyngeate	-	-	-	-	11 (3)	-	11 (0.3)	
Longifurcate- pharyngeate	4 (0.2)	1 (0.2)	-	2 (1)	5 (2)	-	12 (0.3)	
Echinostome	64 (2.8)	-	-	-	6 (2)	-	70 (1.9)	
Metacercaria								
Unknown-1	19 (0.8)	-	-	-	-	-	19 (0.5)	
Unknown-2	6 (0.3)	-	-	-	-	-	6 (0.2)	
Echinostome	133 (5.8)	-	-	-	-	-	133 (3.5)	
Unknown-3	12 (0.5)	-	-	-	-	-	12 (0.3)	
Unknown-4	29 (1.3)	-	-	-	-	-	29 (0.8)	
Unknown-5	44 (1.9)	-	-	-	-	-	44 (1.2)	
Unknown-6	-	-	3 (2)	-	-	-	3 (0.1)	
Unknown-7	3 (0.1)	-	-	-	-	-	3 (0.1)	
Total	348 (15.2)	3 (0.6)	6 (5)	3 (<2)	22 (7)	0 (0)	382 (10.2)	

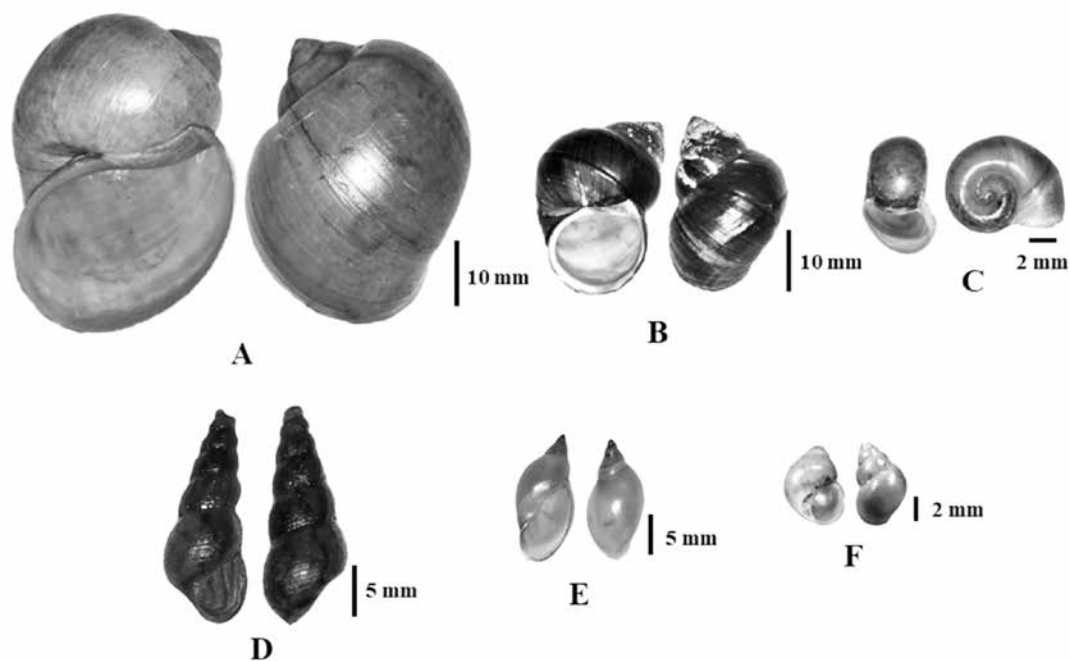


Fig 2-Freshwater snails from Maha Sarakham Province, Thailand.

(A) *Pomacea canaliculate* (Lamarck, 1819), (B) *Filopaludina martensi* (Frauenfeld, 1865), (C) *Indoplanorbis exustus*, (D) *Melanoides tuberculata* Müller, 1774), (E) *Radix rubiginosa* (Michelin, 1831), (F) *Bithynia siamensis goniomphalos* (Morelet, 1866).

yielding an average infection rate of 2% and the latter added an average additional infection rate of 8% (data not shown). The most common cercariae detected were xiphidio and longifurcate-pharyngeate, present in *B. s. goniomphalos*, *F. martensi*, *I. exustus*, *M. tuberculata*, and *R. rubiginosa* (Table 1). The highest prevalence of cercariae among the snails collected was echinostome (1.9%), followed by xiphidio (0.6%) and longifurcate-pharyngeate (0.3%) (Table 1). The crushing method yielded eight types of metacercariae, all unknown except echinostome (Fig 3), latter most common in *B. s. goniomphalos* (Table 1).

## DISCUSSION

The survey in Maha Sarakham

Province, Thailand reveals five species of freshwater snails, namely *B. s. goniomphalos*, *F. martensi*, *I. exustus*, *M. tuberculata*, and *R. rubiginosa*, as the first intermediate host of trematodes, together with nine types of cercariae. Other reports also showed a high diversity of cercariae species in Thai freshwater snails (Sri-Aroon *et al*, 2005; Ngern-Klun *et al*, 2006; Kiatsopit *et al*, 2012; Namchote *et al*, 2015; Anucherngchai *et al*, 2016; Laoprom *et al*, 2016; Kulsantiwong *et al*, 2017), and also in snails from Cambodia (Boonmekam *et al*, 2017) and from Lao PDR (Kiatsopit *et al*, 2012; Kiatsopit *et al*, 2014; Vonghachack *et al*, 2017), Vietnam (Dao *et al*, 2017).

Echinostome and seven unknown metacercariae were detected in *B. s. goniomphalos* and *M. tuberculata*,

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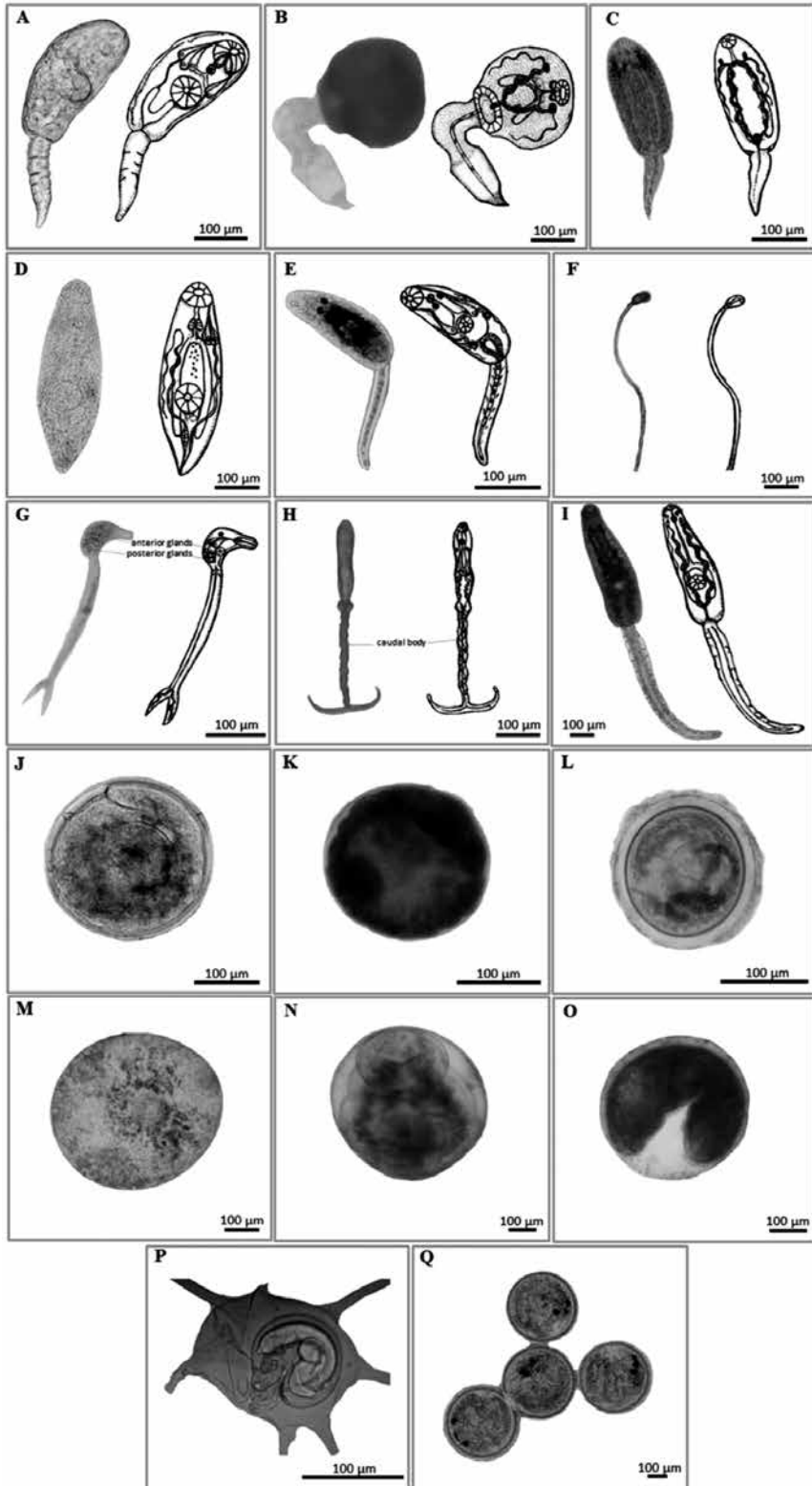


Fig 3-Cercariae and metacercariae from infected freshwater snails collected in 51 localities from four districts of Maha Sarakham Province, Thailand (July 2016 - June 2017).

(A) Xiphidio cercaria, (B) Amphistome cercaria, (C) Monostome cercaria, (D) Mutabile cercaria, (E) Ophthalmoxiphidio cercaria, (F) Cystophorous cercaria, (G) Brevifurcate-apharyngeate cercaria, (H) Longifurcate-pharyngeate (strigea) cercaria, (I) Echinostome cercaria, (J) Unknown-1 metacercaria, (K) Unknown-2 metacercaria, (L) Echinostome metacercaria, (M) Unknown-3 metacercaria, (N) Unknown-4 metacercaria, (O) Unknown-5 metacercaria, (P) Unknown-6 metacercaria, (Q) Unknown-7 metacercaria.

demonstrating these snails were the second intermediate host of trematodes, in agreement with other reports (Chantima *et al*, 2013; Kiatsopit *et al*, 2016). Echinostome is the most common intestinal trematode infecting duck as free-grazing ducks usually feed on aquatic animals including *Bithynia* and *Melanoides* snails in rice paddy, pond or canal, leading to a high prevalence of echinostome infection (Saijuntha *et al*, 2013). It is worth noting that other still unidentified metacercariae recovered from these snails as previously reported (Kulsantiwong *et al*, 2015), are likely to be trematodes infecting other taxa of snail-eating animals. These unknown trematodes can be characterized genetically as well as morphologically (Anucherngchai *et al*, 2016; Kulsantiwong *et al*, 2017).

Infected snails were found in all habitat types surveyed, paddy, pond and canal, but prevalence of infection was not correlated with habitat type and mostly related to the surrounding environment. For example, site no. 28, where the highest prevalence of infection was found, is paddy surrounded by free-ranging farm animals, such as ducks. Similarly, site nos. 1, 3 and 26, where snails were infected by echinostome cercariae, are canals and paddies located distant from villages and where fauna and migratory fish-eating birds were present. Thus, these areas are at high risk of zoonotic echinostome

infection/transmission.

No *O. viverrini* cercaria was detected in any *B. s. goniomphalos*, which may be due to its very low infection rate in this species (Kiatsopit *et al*, 2012; Kiatsopit *et al*, 2014; Laoprom *et al*, 2016; Vonghachack *et al*, 2017). However, *B. s. goniomphalos* had the highest infection rate for several cercariae and metacercariae, especially echinostome. High rates of echinostome infection have been reported in *I. exustus* and *R. rubiginosa* (Anucherngchai *et al*, 2016), and *Lymnaea* or *Radix* species are the most important hosts worldwide (Devkota *et al*, 2015; Gauffre-Autelin *et al*, 2017). Lymnaeid snails act as intermediate hosts of *Fasciola gigantica* in Thailand (Kaset *et al*, 2010). The findings demonstrate *B. s. goniomphalos* plays a significant role as intermediate host of trematode parasites, echinostomes, in Maha Sarakham Province, Thailand.

Animal blood fluke, *eg* brevifurcate-apharyngeate and longifurcate-pharyngeate, were also commonly found. These are blood fluke of birds, reptiles, fish, and mammals (Mard-Arhin *et al*, 2001; Anucherngchai *et al*, 2016; Kiatsopit *et al*, 2016). These are causative agents of animal disease and human dermatitis (Ohmae *et al*, 2004; Savioli *et al*, 2017) and constitute a major health problem for farmers and agriculturists working in paddy fields in Southeast Asia (Ohmae *et al*, 2004; Savioli *et al*, 2017). However, schistosomiasis is

rare in Thailand (Gordon *et al*, 2019).

In summary, of six freshwater snails examined in this study we found that *B. s. goniomphalos* has a highest infection rate with a wide variety of cercarial trematodes. Echinostomes are the most common trematode found in freshwater snails in Maha Sarakham Province. Therefore *B. s. goniomphalos* play an important role as an intermediate of zoonotic trematodes endemic in Maha Sarakham province. Snail-eating animals and livestock, as well as humans in these areas are at risk of zoonotic trematode infection. To control and prevent zoonotic trematode infections, eating live or partially cooked snails in these areas should be avoided.

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