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Research Note

**Trematode infection in a freshwater snail *Hydrobioides nassa*
 (Gastropoda: Bithyniidae) in Thailand**

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Summary

1,024 individuals of *Hydrobioides nassa* were collected from 12 different localities in eight provinces from north, west, and central regions of Thailand. The infection of parasitic trematodes was investigated using shedding and crushing methods to search for cercariae and metacercariae. Trematode infection was found at a relatively low prevalence of 5.57%. Five different morphological types of cercariae were detected; xiphidio, monostome, mutabile, ophthalmoxiphidio, and microcercous, and three different morphological types of unknown metacercariae were observed. Microcercous cercariae of the lung fluke genus *Paragonimus* is reported here for the first time in a bithyniid snail. Our current finding show that *H. nassa* can serve as intermediate host for a range of parasitic trematodes in Thailand.

Keywords: Bithyniid; parasitic trematode; cercariae; shedding methods; crushing methods

Introduction

Usually, digenetic trematodes require freshwater snails as an intermediate host to complete their life cycles (Saijuntha *et al.*, 2021). The prevalence of parasitic trematodes infection in the final hosts are usually related to the population dynamics of the snail hosts (Chitsulo *et al.*, 2000). Several snails, particularly those of the family Bithyniidae, can serve as hosts for medically important parasitic trematodes endemic in Thailand (Saijuntha *et al.*, 2021). There are currently 10 species/subspecies recognized in Thailand, belonging to four genera; *Bithynia*, *Hydrobioides*, *Gabbia*, and *Watbledia* of the family Bithyniidae (Chitramvong, 1992). Previous data suggests that bithyniid snails, especially those of the genus *Bithynia*, serve as the sole intermediate host for a number of medically important trematodes, such as echinostomes, schistosomes, heterophyid, and the carcinogenic liver fluke *Opisthorchis viverrini* (Saijuntha *et al.*, 2021). Information on trematode infection in other bithyniid snails, including *H. nassa*, is still scarce.

Hydrobioides nassa possibly serves as an intermediate host for parasitic trematodes. It has been reported to be endemic in Myanmar, Thailand, and the Lao People's Democratic Republic (Brandt, 1974). The morphology of *H. nassa* is very similar to the two subspecies of genus *Bithynia*, i.e. *B. s. siamensis* and *B. s. goniomphalos*, leading to confused differentiation between these snails using only morphological characters (Bunchom *et al.*, 2021a). In addition, they co-occur in the same geographic localities in north and central regions of Thailand (Bunchom *et al.*, 2021b). However, mitochondrial cytochrome c oxidase subunit 1 and 16S ribosomal DNA regions have been proven to be potential genetic markers for differentiation of these morphological similar species of snails (Bunchom *et al.*, 2021b; Tantrawatpan *et al.*, 2020). To our knowledge, there is currently no evidence that *H. nassa* serves as an intermediate host for medically important trematodes. Although there have been a few previous studies of trematode infections in snails of the family Bithyniidae, no evidence of trematode cercariae infection in *H. nassa* has yet been found (Kulsantiwong *et*

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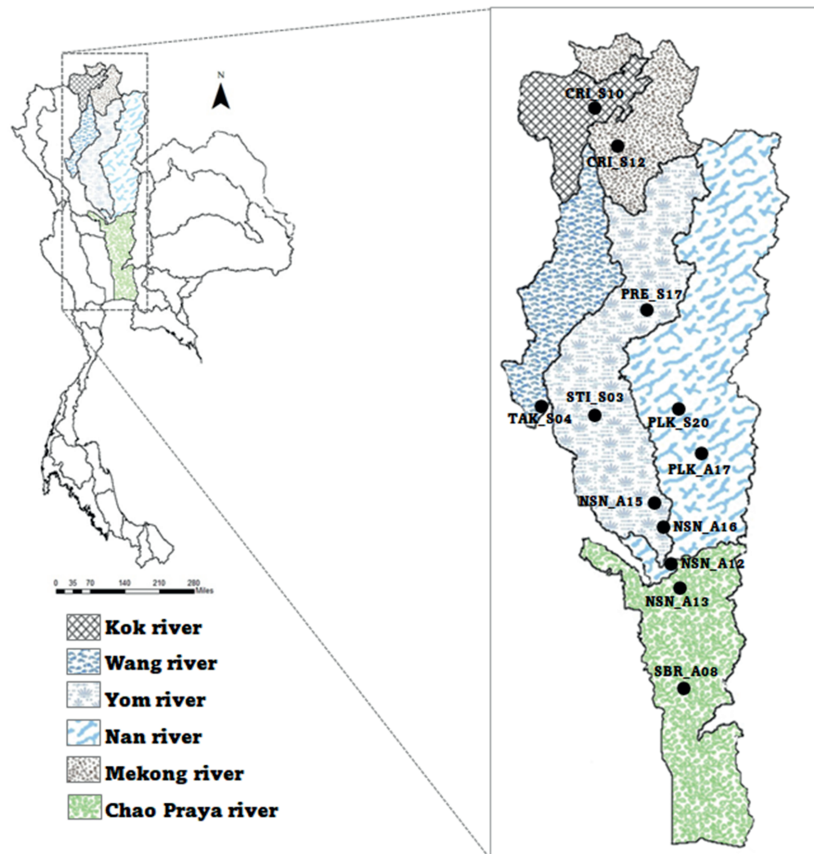


Fig. 1. Map of sample collection sites of *Hydrobioides nassa* from six different catchment systems in north, west, and central regions of Thailand.

al., 2015; Wiroonpan *et al.*, 2020). Thus this study aims to examine trematode cercariae and metacercariae infection in *H. nassa* from different localities and catchment systems in north, west, and central regions of Thailand using shedding and crushing methods.

Materials and Methods

During October and November 2019, snails *Hydrobioides nassa* was collected from natural habitats such as temporary ponds, irrigation canals, rivers, and rice paddy fields in 12 different localities belonging to the six catchment systems, namely the Kok, Wang, Yom, Nan, Mekong, and Chao Phraya catchment systems, in eight provinces of north and central regions of Thailand (Fig. 1, Table 1). Snails were collected by handpicking and scooping net. The collected snails were cleaned several times with tap water, and the water from their shell was absorbed with tissue paper until completely dry, before being packed in newspapers and transported to the laboratory. The collected snails were identified to species by morphology based on previous reports by Brandt (1974), and Chitramvong (1992), prior to shedding and crushing. The snails were then investigated for cercariae and metacercariae of parasitic trematodes using shedding and crushing procedures (Kiatsopit

et al., 2012; Bunchom *et al.*, 2020). The trematode cercariae were classified using a light microscope according to Schell (1970) and Frandsen and Christensen (1984).

After crushing, all infected snails were subjected to molecular analysis, and the additional ten uninfected snail samples from each location (except TAK_S04, NSN_A12, and NSN_A15) were randomly chosen for DNA extraction by using head-foot tissue. Then 492 bp of mitochondrial 16S ribosomal DNA (16S rDNA) were sequenced for molecular confirmation of morphology-based species identification. DNA extraction protocol, primers, and polymerase chain reaction (PCR) condition reported by Bunchom and colleagues (Bunchom *et al.*, 2021a) were employed. All 16S rDNA sequences with a length of 492 bp obtained in this study were blasted in the GenBank database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) for species confirmation. A Microsoft Excel spreadsheet was used to enter the raw data. The finding of prevalence of trematodes infection was assessed as mean and percentage.

Results

Several *H. nassa* samples were randomly selected for 16S rDNA genotyping, and all of those samples showed 99 – 100 % sim-

Table 1. Geographical localities of *Hydrobioides nassa* collection from north, west, and central of Thailand.

Name*	Date of collection	Latitude/ Longitude	Catchment	Region
CRI_S10	25-Nov-19	19.787771/99.746522	Kok	North
CRI_S12	5-Nov-19	19.475219/99.752946	Mekong	North
PRE_S17	24-Oct-19	18.076721/100.121770	Yom	North
TAK_S04	26-Nov-19	17.147687/99.117888	Wang	West
NSN_A15	22-Oct-19	15.902935/100.281310	Yom	Central
NSN_A16	23-Oct-19	15.902940/100.281350	Yom	Central
STI_S03	26-Nov-19	17.054422/99.680162	Yom	Central
PLK_S20	26-Nov-19	17.231652/100.252390	Nan	Central
PLK_A17	24-Oct-19	16.847246/100.462520	Nan	Central
NSN_A12	21-Oct-19	15.700322/100.153200	Nan	Central
NSN_A13	22-Oct-19	15.762161/100.082120	Chao Phraya	Central
SBR_A08	19-Oct-19	14.775098/100.451360	Chao Phraya	Central

* The first three letters stand for province name, CRI; Chiang Rai, PRE; Phrae, TAK; Tak, NSN; Nakhon Sawan, STI; Sukhothai, PLK; Phisanulok, SBR; Sing Buri

ilarity to the *H. nassa* 16S rDNA sequence accession number MK629067 deposited in the GenBank database. We found that *H. nassa* was infected by trematode cercariae and metacercariae from two and six different localities, respectively. The prevalence of trematode infection in *H. nassa* was 5.57 % includes cercariae and metacercariae. The trematode parasites found in this study were composed of five and three morphological types of cercariae and metacercariae. *H. nassa* was infected with five different cercariae types, namely xiphidio, monostome, mutabile, ophthalmoxiphidio, and microcercous, with prevalence of 0.10 %, 0.78 %, 0.10 %, 0.10 %, and 0.10 %, respectively. Of these, ophthalmoxiphidocercariae were detected by the crushing method inside of redia. However, three different types of unknown metacercariae type 1, type 2, and type 3 (Fig. 2) were detected, with prevalence of 2.83 %, 1.07 %, and 0.49 %, respectively. In this study, we were unable to specify which snail organ harbored cercariae, redia, and

metacercariae.

Trematode parasites examined by the shedding method showed that locality NSN_A12 had the highest prevalence of infection with 8.47 %, infected by three types of cercariae, namely monostome (6.77 %), mutabile (0.85 %), and microcercous (0.85 %). Xiphidocercariae were detected in a snail from CRI_S10. Ophthalmoxiphidocercariae were observed in a snail from NSN_A16 by the crushing method (Table 2). The crushing method revealed that trematode infection with unknown metacercaria type F was present in *H. nassa* from the localities CRI_S10 and PRE_S17 with incidences of 2.00 % and 2.50 %, respectively. Unknown metacercaria type G was detected in *H. nassa* from the localities TAK_S04 and SBR_A08 with prevalence of 25.00 % and 9.30 %, respectively. The unknown metacercaria type H was detected in *H. nassa* from the localities NSN_A15 and PLK_S20 with prevalence of 14.06 % and 1.49 %, respectively (Table 2).

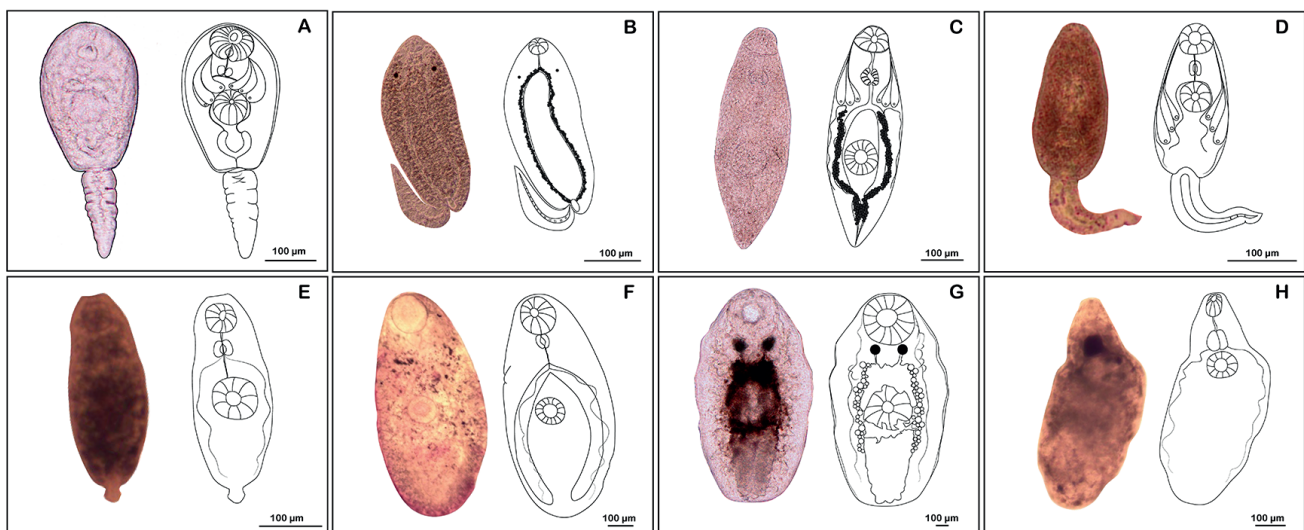


Fig. 2. Different morphological types of cercariae and metacercariae: A; xiphidocercaria, B; monostome cercaria, C; mutabile cercariae, D; ophthalmoxiphidocercariae, E; microcercous cercariae, F, G, and H; three different morphological types of unknown metacercariae named as type 1, type 2, and type 3, respectively.

Table 2. The prevalence of trematode cercariae and metacercariae infection in *Hydrobioides nassa*.

Code	N*	Shedding method					Crushing method						
		No. of infection	A	B	C	E	Prevalence	No. of infection	D	F	G	H	Prevalence
CRI_S10	100	1	-	-	-	-	1.00	2	-	2	-	-	2.00
CRI_S12	62	-	-	-	-	-	0.00	-	-	-	-	-	0.00
TAK_S04	100	-	-	-	-	-	0.00	25	-	-	25	-	25.00
PRE_S17	120	-	-	-	-	-	0.00	3	-	3	-	-	2.50
NSN_A15	64	-	-	-	-	-	0.00	9	-	-	-	9	14.06
NSN_A16	65	-	-	-	-	-	0.00	1	1**	-	-	-	1.54
STI_S03	34	-	-	-	-	-	0.00	-	-	-	-	-	0.00
PLK_S20	134	-	-	-	-	-	0.00	2	-	-	-	2	1.49
PLK_A17	79	-	-	-	-	-	0.00	-	-	-	-	-	0.00
NSN_A12	118	10	-	8	1	1	8.47	-	-	-	-	-	0.00
NSN_A13	105	-	-	-	-	-	0.00	-	-	-	-	-	0.00
SBR_A08	43	-	-	-	-	-	0.00	4	-	-	4	-	9.30
Total	1,024	11	1	8	1	1	1.07	46	1	5	29	11	4.49

* Number of *H. nassa* examined for trematodes infection, ** cercariae detected inside of redia.

Different morphological types of cercariae and metacercariae: A; xiphidiocercariae; B; monostome cercariae; C; mutabile cercariae; D; ophthalmoxiphidiocercariae; E; microcercous cercariae; F, G, and H; unknown metacercariae type 1, type 2, and type 3, respectively (see Fig. 2).

Discussion

Several medically important trematodes in the family Opisthorchiidae, Fasciolidae, Heterophyidae, Strigeidae, Lecithodendriidae, Philophthalmidae, Cyathocotylidae, Aporocotylidae, Transverso-trematidae, Hemiuridae, Schistosomatidae, and Echinostomidae are present in mainland Southeast Asia, according to previous studies (Sri-Aroon *et al.*, 2007; Kiatsopit *et al.*, 2012, 2016; Kulsantiwong *et al.*, 2015; Namsanor *et al.*, 2015; Kopolrat *et al.*, 2020; Wiroonpan *et al.*, 2021). The life cycle of these trematodes is highly intricate, with freshwater snails serving as the first intermediate host. Our current study revealed that *H. nassa* served as the first intermediate host of various trematodes in Thailand. We detected five types of cercariae in *H. nassa*, while the other reports showed a high diversity of cercariae found in bithyniid snails in Thailand (Sri-Aroon *et al.*, 2007; Kiatsopit *et al.*, 2012, 2016; Kulsantiwong *et al.*, 2015; Namsanor *et al.*, 2015; Kopolrat *et al.*, 2020; Wiroonpan *et al.*, 2021). Monostome cercariae were determined to be the most common type of cercaria found in *H. nassa* in this investigation, despite previous studies indicating that xiphidio cercariae were mostly found in the other bithyniid snails (Kulsantiwong *et al.*, 2015; Kiatsopit *et al.*, 2016; Bunchom *et al.*, 2021b).

Except for microcercous cercaria, which are cercariae of trematodes in the genus *Paragonimus* of the family Paragonimidae, all morphological types of cercariae recently discovered in *H. nassa* had previously been documented in other bithyniid snails (Sri-Aroon *et al.*, 2007; Kiatsopit *et al.*, 2012, 2016; Kulsantiwong *et al.*, 2015; Namsanor *et al.*, 2015; Kopolrat *et al.*, 2020; Wiroonpan *et al.*, 2021). Microcercous cercariae, on the other hand, have been found in paludomids and/or thiarids, including *Paludomus* species, *Melanooides tuberculata*, and *Tarebia granifera*, which were snail intermediate hosts of the lung fluke *Paragonimus* (Saijuntha *et al.*, 2021). Furthermore, the identification of *Paragonimus* cercariae in our study occurred in a paragonimiasis endemic area in the central region of Thailand (Nawa & Doanh, 2009). As a result, our latest discovery strongly suggests that *H. nassa* is a potential intermediate host of *Paragonimus* in Thailand. Moreover, three unidentified metacercariae have been discovered in *H. nassa*, indicating that this snail can serve as a second intermediate host for a variety of trematodes.

Trematode cercariae and metacercariae found in this study commonly require the freshwater snails as their first and/or second intermediate host to complete their life cycle. Such as the liver fluke *O. viverrini* needs the bithyniid snails as their first intermediate hosts, namely *B. siamensis* and *B. funiculata*, then the second and final hosts will be consequently infected to complete the cycle. These bithyniid snails have been shown to harbor *O. viverrini* in endemic areas in the central and northeast regions of Thailand (Kiatsopit *et al.*, 2012, 2016; Namsanor *et al.*, 2015; Kopolrat *et al.*, 2020). While the current study found no cercariae of *O. viverrini* detected in *H. nassa*. This observation is probably due either to the fact that the studied areas in this study are not

the endemic areas of opisthorchiasis or that *H. nassa* is not susceptible to *O. viverrini* infection. However, due to the low number of cercariae types found, we only examined trematodes infection in *H. nassa* in a few localities in Thailand. As a result, more research is needed to expand the study areas to include more of the distribution range of *H. nassa*, as well as different habitat types and environments. Also, molecular genotyping of each cercariae and metacercariae type isolated from *H. nassa* should be investigated in order to more precisely identify the genus and species of these trematodes.

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Ethical statement

The ethics of animal use and biosafety for this research were approved by the Animal Care and Use Committee (permission number 008/2561) and the Institute Biosafety Committee (permission number 044/2561) of Thammasat University, respectively.

Conflict of interest

We have no conflicts of interest to disclose.

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