Research Note

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

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Article info

Summary

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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: Bithyniida; 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 Watteildea of the family Bithyniidae (Chitramvong, 1992). Previous data suggests that bithynid 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 bithynid 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 (Bunchorn 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 (Bunchorn 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
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-
Similarity 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 xiphidiocercaria, monostome, mutabile, ophthalmoxiphidiocercaria, and microcercous, with prevalence of 0.10%, 0.78%, 0.10%, 0.10%, and 0.10%, respectively. Of these, ophthalmoxiphidiocercariae 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%). Ophthalmoxiphidiocercariae were detected in a snail from CRI_S10. Ophthalmoxiphidiocercariae 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).

![Image](image.png)

**Fig. 2.** Different morphological types of cercariae and metacercariae: A; xiphidiocercaria, B; monostome cercaria, C; mutabile cercariae, D; ophthalmoxiphidiocercariae, 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 | No. of infection |
|        |     | A   | B   | C   | E   | Prevalence | D   | F   | G   | H   | Prevalence |
| CRI_S10| 100 | 1   | 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   | 1.07       | 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, Lecithodendridae, Philophthalmidae, Cyathocotylidae, Aporocotylidae, Transversotrema-tididae, Hemiuriidae, Schistosomatidae, and Echinostomatidae are present in mainland Southeast Asia, according to previous studies (Sri-Aroon et al., 2007; Kiatsopt et al., 2012, 2016; Kul sanctionwong 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; Kiatsopt 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; Kiatsopt et al., 2016; Bunchom et al., 2021b). Except for micro cercous 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; Kiatsopt et al., 2012, 2016; Kulsan- tiwong et al., 2015; Namsanor et al., 2015; Kopolrat et al., 2020; Wiroonpan et al., 2021). Microcercous cercaria, on the other hand, have been found in paludomids and/or thiarids, including Paludomus species, Melanoides 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 intermediate 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 (Kiatsopt 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 O. 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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