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Freshwater pulmonate snails and their potential role as trematode intermediate host in a cercarial dermatitis outbreak in Southern Thailand

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Abstract

This study aimed to investigate the pulmonate snail species in the vicinity of the cercarial dermatitis outbreak area in southern Thailand. In 2020, an outbreak of cercarial dermatitis was reported in Chana district, Songkhla Province, caused by the ruminant schistosome *Schistosoma indicum* and its snail intermediate host *Indoplanorbis exustus*. In the present study, 1,175 pulmonate snails were collected between October 2021 and October 2022 from five provinces covering 34 locations in southern Thailand. Seven pulmonate snail species were identified based on shell morphology, including *Amerianna carinata, Gyraulus bakeri, G. convexiusculus, G. hubendicki, Physella acuta, Indoplanorbis exustus*, and *Radix rubiginosa*. Among these snails, eight species, and five types of cercariae were identified, viz. type (i) Echinostome cercariae consisted of *Echinoparyphium recurvatum, Echinostoma spiniferum*, and *E. revolutum*, type (ii) Brevifurcate-apharyngeate cercariae consisted of *Schistosoma indicum* and *S. spindale*, type (iii) Brevifurcate-pharyngeate cercariae was represented by *Clinostomum giganticum*, type (iv) Longifurcate–pharyngeate cercariae (strigea cercaria) was *Diplostomum baeri eucaliae*, and type (v) Ophthalmoxiphidiocercaria. Among the seven pulmonated snail species, three were found to be infected, viz. *G. convexiusculus, I. exustus*, and *R. rubiginosa*, with infection rates of 1.14% (2/176), 0.25% (2/802), and 4.02% (7/174), respectively.

Key Words

Trematode infection, Cercariae, Snail intermediate host

Introduction

Pulmonate snails are a large and diverse group of snails in Class Gastropoda. They are widely distributed throughout the world in a variety of habitats, including freshwater, terrestrial, and marine environments. Freshwater pulmonates have been reported for their medical and veterinary significance, as they serve as intermediate hosts to various blood, liver and intestinal flukes that affect humans directly or accidentally as parasitic zoonoses (Monzon et al. 1993). The snail hosts recorded for 279 species of flukes indicate that pulmonates play a very important role parasitizing mammals and birds. For example, some species of *Schistosoma* trematodes, also known as blood flukes, use pulmonate snails as intermediate hosts in their life cycle. These parasites cause schistosomiasis, a neglected tropical disease that affects millions of people worldwide. When snail families are considered and

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ranked according to the frequency in which they are involved in the transmission of these flukes, species of the pulmonate Planorbidae and Lymnaeidae are the first two among the top ten (Ewers 1964).

Research on pulmonate snails and their role as intermediate hosts for parasites has focused on understanding the transmission dynamics of these diseases and developing strategies for their control. For example, the factors that influence the transmission of parasites from snails to humans have been investigated, such as the behavior of snails and the environmental conditions that promote parasitic development. Other research on pulmonate snails and snail-borne diseases has focused on identifying and characterizing the genetic and molecular mechanisms that underlie the interactions between snails and parasites. This research has the potential to lead to the development of new treatments or control measures for these diseases. Overall, research on pulmonate snails and their role as intermediate hosts in snail-borne diseases is important for understanding the epidemiology of these diseases and to develop effective strategies for prevention and control.

Many studies in Southeast Asia have reported on medically important snails. The snail-borne parasitic diseases are considered diseases of public health importance in this region. The fauna, ecology, and dynamics of transmission have been reported for the snails in seven families that exist in the region viz. Ampulariidae, Bithyniidae, Viviparidae, Thiaridae, and Pomatiopsidae among the Caenogastropoda as well as Lymnaeidae and Planorbidae among the Pulmonata (Tropmed Technical Group 1986).

Here, we focus on Pulmonate freshwater snails from the families Planorbidae and Lymnaeidae. These snails were observed in the vicinity of the cercarial dermatitis outbreak area in southern Thailand, where they are of significant medical importance as intermediate hosts for trematodes. The aim of this study is to investigate the possibility of the occurrence of cercarial dermatitis and trematodiasis, as well as to gain further knowledge about the potential of freshwater pulmonate snails as intermediate hosts for trematodes. Additionally, we aim to examine the distribution of important parasites affecting both humans and animals in communities near the outbreak area of cercarial dermatitis in Chana district, Songkhla province, Thailand, which occurred in 2020 (Krailas et al. 2022).

Materials and methods

Snail collection and identification

The study was conducted by snail survey, snail collections and investigation of parasites. Snails from paddy fields and streams in irrigation canals were collected from 27 locations in five provinces, viz. Chumphon, Surat Thani, Nakhon Si Thammarat, Phatthalung, and Songkhla. The specimens were collected between October 2021 and October 2022, using the opportunity sampling method, hand picking, and a stainless-steel scoop. The geographic coordinates of the sampling locations were determined with a global positioning system device (Garmin PLUS III, Taiwan). The snails were maintained in aeration tanks and then transferred and studied in the laboratory of the Parasitology and Medical Malacology Research Unit, Department of Biology, Faculty of Science, Silpakorn University, Nakhon Pathom, Thailand. The snail species were identified according to their shell morphology, using taxonomic keys following Brandt (1974) and Upatham et al. (1983).

Trematode infections

Two methods, such as cercarial shedding, and crushing, were employed to investigate the presence of cercariae in the snails. The cercarial shedding method involved placing a snail in a small plastic cup containing dechlorinated water and observing the snails twice a day for 7–10 days to detect the emergence of cercariae. The emergent cercariae were examined under a binocular dissecting microscope and a light microscope, both unstained, and live stained with 0.5% neutral red. Snails that did not shed cercariae during the observed time were crushed to examine for parthenitae (sporocysts/rediae) and cercariae. The morphology of the trematodes was described based on living cercariae that had emerged from the snails. The characteristics of the cercariae were photographed using a differential interference contrast (DIC) microscope (Olympus BX53, Japan) and were drawn and identified based on descriptions from previous studies (see e.g. Krailas et al. 2011, 2014, 2022). Free-swimming cercariae were observed under the dissecting microscope, and measurements of 10 specimens fixed in 10% formalin were taken using an ocular micrometer to determine the average size in micrometers.

Ethics statement

The study adhered to the guidelines set forth by the Institute of Animals for Scientific Purposes Development, Thailand, and all animals were treated with care and respect following the study protocol approved (Approval No. 20/2565) by the Committee of Animal Scientific Researches at Silpakorn University, Thailand.

Results

Snail collection

A total of 1,175 pulmonate snails were collected from 27 locations in five provinces. The snails collected from the water bodies of paddy fields were found on the water surface, as well as in the sand, mud, leaves, and aquatic plants (see map in Fig. 1 and locations in Fig. 2). Table 1 presents the geographic coordinates of the sampling sites and the species of collected and infected snails.



Figure 1. Survey sites where Pulmonate snails were discovered across five provinces and 27 locations in southern Thailand. The color-coded marks represent the distribution as follows: Red marks: Pulmonate snails were found in 3 locations within Chumphon province. Yellow marks: Pulmonate snails were found in 6 locations within Surat Thani province. Green marks: Pulmonate snails were found in 9 locations within Nakhon Si Thammarat province. Purple marks: Pulmonate snails were found in 4 locations within Phatthalung province. Blue marks: Pulmonate snails were found in 5 locations within Songkhla province. Scale bars: 100 km.

Snail samples and parasitic infections

The pulmonate snails were identified based on their conchological characteristics, revealing the presence of three families, five genera, and seven species. The first family was Lymnaeidae, represented by 174 *Radix rubiginosa* Michelin, 1831. The second family was Physidae, with 5 *Physella acuta* Draparnaud, 1805. The third family was Planorbidae, which included 12 *Amerianna carinata* H. Adams, 1861; 5 *Gyraulus bakeri* Brandt, 1974; 176 *G. convexiusculus* Hutton, 1849; 1 *G. hubendicki* Brandt, 1974; and 802 *Indoplanorbis exustus* Deshayes, 1834 (see Table 1 and Fig. 3).

Among the snails collected, 11 were infected with cercariae, resulting in an overall infection rate of 0.94% (11/1,175). The morphology and organ characteristics of the cercariae allowed their classification into five morphotypes and eight species, including (i) Echinostome cercariae, which comprised *Echinoparyphium recurva*tum Luehe, 1909; *Echinostoma spiniferum* Ahmed, 1959



Figure 2. Images of study areas.

(sensu Našincová 1992), and *Echinostoma revolutum* Looss, 1899; (ii) Brevifurcate-apharyngeate cercariae, which included *Schistosoma indicum* Montgomery, 1906 (Syn. *S. nasalis* Rao, 1933) and *S. spindale* Montgomery, 1906; (iii) Brevifurcate-pharyngeate-Clinostomatoid cercariae, which comprised *Clinostomum giganticum* Agarwal, 1959; (iv) Longifurcate–pharyngeate cercariae (Strigea cercaria), which comprised *Diplostomum baeri eucaliae* Hoffman & Hundley, 1957, and (v) Ophthalmoxiphidiocercaria. Three pulmonate snail species, such as *G. convexiusculus*, *I. exustus*, and *R. rubiginosa*, were infected with cercariae, with infection rates of 1.14% (2/176), 0.25% (2/802), and 4.02% (7/174), respectively.

Morphology of the cercariae

The morphology and organ characteristics of the cercariae were analyzed using living cercariae that had emerged from snail specimens, as well as cercariae fixed in 10% formalin. Images of the cercariae were captured using a DIC microscope, and the sizes of the cercariae were measured to identify the cercarial species. Previous descriptions (e.g., Komiya 1961; Schell 1970; Yamaguti 1975; Ito 1980; Nasir 1984; McCarthy 1989; Krailas et al. 2011, 2014, 2022; Faltýnková et al. 2015; Veeravechsukij et al. 2018) were used as references to categorize the morphology and organ characteristics of the cercariae. Seven dis**Table 1.** Locations, number of collected snails, number of infected snails, and cercariae obtained from collected snails; 27 sampling sites from 5 provinces in southern Thailand.

| No. | Locations | Coordinates | Collected snails (number) | Number of infected snail (Infection rate %) | Type: Cercariae species |
|-----|---------------------------|-------------------|------------------------------|--|-------------------------------------|
| 1 | Khuha Sawan, Mueang | 7°37'41.8"N. | Indoplanorbis exustus (12) | 0 | - |
| - | District. Phatthalung | 100°07'16.1"E. | Radix rubiginosa (6) | - | |
| | Province | Alt. 12 m | Gvraulus bakeri (1) | | |
| 2 | Lam Pam 1. Mueang | 7°37'33.3"N. | Gyraulus bakeri (4) | 0 | - |
| | District, Phatthalung | 100°07'46.2"E. | Indoplanorbis exustus (1) | | |
| | Province | Alt. 12 m | Physella acuta (2) | | |
| 3 | Lam Pam 2, Mueang | 7°38'18.4"N. | Indoplanorbis exustus (22) | 0 | - |
| | District, Phatthalung | 100°08'55.4"E, | 1 | | |
| | Province | Alt. 1.4 m | | | |
| 4 | Thale Noi 1, Khuan | 7°44'26.9"N, | Indoplanorbis exustus (24) | 0 | - |
| | Khanun District, | 100°08'23.4"E, | Gyraulus convexiusculus (9) | | |
| | Phatthalung Province | Alt. 5 m | • | | |
| 5 | Ban Mai, Ranot District, | 7°48'30.9"N, | Indoplanorbis exustus (5) | 0 | - |
| | Songkhla Province | 100°17'09"E, | Radix rubiginosa (2) | | |
| | | Alt. 5 m | | | |
| 6 | Ranot, Ranot District, | 7°46'06.17"N, | Gyraulus convexiusculus (46) | 0 | - |
| | Songkhla Province | 100°18'53.26"E, | | | |
| | | Alt. 8 m | | | |
| 7 | Pak Trae, Ranot District, | 7°46'06.2"N, | Indoplanorbis exustus (4) | 0 | - |
| | Songkhla Province | 100°21'10.2"E, | Gyraulus convexiusculus (4) | | |
| | | Alt. 6 m | | | |
| 8 | Ko Yai, Krasae Sin | 7°34'36.1"N, | Gyraulus hubendicki (1) | 0 | - |
| | District, Songkhla | 100°17'27.4"E, | | | |
| _ | Province | Alt. 43 m | | | |
| 9 | Khlong Ri, Sathing | 7°31'27.3"N, | Indoplanorbis exustus (5) | 0 | - |
| | Phra District, Songkhla | 100°24'23.5"E, | | | |
| 10 | Province | Alt. 8 m | | 0 | |
| 10 | Bo Lo I, Chian Yai | 8°06'52.4"N, | Indoplanorbis exustus (9) | 0 | - |
| | District, Nakhon Si | 100°06'09.3"E, | Radix rubiginosa (12) | | |
| 11 | De Le 2 Chier Vei | AIL O III | Gyraulus convexiusculus (11) | 1 (0.000/) | F |
| 11 | Do Lo 2, Cillan Tal | 0 00 33 IN, | Ruaix rubiginosa (4) | 1 (0.08%) | Providurante anhamingante apreciaci |
| | Thermoret Province | $100\ 00\ 12\ E,$ | | | Schistosoma spindala |
| | Thannharat Tiovince | Alt. 15 III | Indonlanorhis grustus (20) | 0 | Schistosoma spinaute |
| 12 | Thong Lan Thong | 8°08'08 4''N | Gyraulus converiusculus (6) | 0 | |
| 12 | Lamchiak Chian Vai | 100°07'48 9"F | Indonlanorhis erustus (12) | 0 | |
| | District. Nakhon Si | Alt. 9 m | Radir rubiginosa (9) | | |
| | Thammarat Province | | nuun noiginosu ()) | | |
| 13 | Don Jik 1, Thong | 8°08'48"N, | Indoplanorbis exustus (54) | 0 | - |
| | Lamchiak, Chian Yai | 100°07'34"E, | Gvraulus convexiusculus (12) | | |
| | District, Nakhon Si | Alt. 7 m | Radix rubiginosa (1) | | |
| | Thammarat Province | | | | |
| 14 | Don Jik 2, Thong | 8°09'13"N, | Gyraulus convexiusculus (8) | 1 (0.08%) | Echinostome cercariae: |
| | Lamchiak, Chian Yai | 100°06'25"E, | | | Echinoparyphium recurvatum |
| | District, Nakhon Si | Alt. 4 m | Indoplanorbis exustus (5) | 0 | - |
| | Thammarat Province | | | | |
| 15 | Chian Yai, Chian Yai | 8°10'07"N, | Radix rubiginosa (1) | 1 (0.08%) | Echinostome cercariae: Echinostoma |
| | District, Nakhon Si | 100°08'56.8"E, | | | spiniferum |
| | Thammarat Province | Alt. 10 m | Indoplanorbis exustus (27) | 0 | - |
| | | | Gyraulus convexiusculus (3) | 0 | - |
| 16 | Takhanan, Chian Yai | 8°10'02.5"N, | Indoplanorbis exustus (24) | 0 | - |
| | District, Nakhon Si | 100°09'19.7"E, | | | |
| | Thammarat Province | Alt. 6 m | | | |
| 17 | Hu Long, Pak Phanang | 8°17'06.6"N, | Indoplanorbis exustus (17) | 0 | - |
| | District, Nakhon Si | 100°10'00.4"E, | | | |
| | Thammarat Province | Alt. 6 m | | | |
| 18 | Khlong Krabue, Pak | 8°17'08.3"N, | Radix rubiginosa (4) | 0 | - |
| | Phanang District, | 100°09'04.5"E, | Indoplanorbis exustus (175) | | |
| | Naknon Si Thammarat | Alt. 14 m | | | |
| 10 | Thakhoei The Change | 0°12'11 5"N | Indonlanowhis musture () | 0 | |
| 17 | District Surat Thani | 99°10'08 15"E | $\frac{1}{2}$ | U | - |
| | Province | Alt. 20 m | Nuun ruoiginosa (49) | | |

| No. | Locations | Coordinates | Collected snails (number) | Number of infected | Type: Cercariae species |
|-----|-----------------------|------------------|-------------------------------------|--------------------------|---|
| | | | | snail (Infection rate %) | |
| 20 | Lamet 1, Chaiya | 9°22'15.6"N, | Indoplanorbis exustus (193) | 0 | - |
| | District, Surat Thani | 99°12'28.8"E, | Radix rubiginosa (44) | | |
| | Province | Alt. 9 m | Gyraulus convexiusculus (2) | | |
| 21 | Lamet 2, Chaiya | 9°22'16"N, | Indoplanorbis exustus (65) | 1 (0.08%) | Furcocercous cercariae/Brevifurcate- |
| | District, Surat Thani | 99°12'28"E, Alt. | | | pharyngeate-Clinostomatoid- |
| | Province | 10 m | | | cercariae: Clinostomum giganticum |
| | | | Radix rubiginosa (2) | 0 | - |
| 22 | Lamet 3, Chaiya | 9°22'53"N, | Physella acuta (1) | 0 | - |
| | District, Surat Thani | 99°12'30"E, Alt. | Radix rubiginosa (2) | | |
| | áProvince | 7 m | Indoplanorbis exustus (38) | | |
| 23 | Thung 1, Chaiya | 9°23'23.4"N, | Indoplanorbis exustus (10) | 1 (0.08%) | Furcocercous cercariae/ Brevifurcate- |
| | District, Surat Thani | 99°12'31.1"E, | | | apharyngeate cercariae: Schistosoma |
| | Province | Alt. 11 m | | | indicum |
| | | | Radix rubiginosa (22) | 1 (0.08%) | Echinostome cercariae: |
| | | | | | Echinoparyphium recurvatum |
| | | | <i>Physella acuta</i> (2) | 0 | - |
| | | | <i>Gyraulus convexiusculus</i> (45) | 0 | - |
| 24 | Thung 2, Chaiya | 9°23'28"N, | Indoplanorbis exustus (4) | 0 | - |
| | District, Surat Thani | 99°12'32.1"E, | Amerianna carinata (6) | | |
| 25 | Province | Alt. 13 m | | 0 | |
| 25 | Hadpunkrai I, Mueang | 10°34'40"N, | Indoplanorbis exustus (18) | 0 | - |
| | District, Chumphon | 99-10'00"E, Alt. | Radix rubiginosa (1) | | |
| 26 | | 10 111 | Amerianna carinata (2) | 0 | |
| 26 | Hadpunkrai 2, Mueang | 10°34'39"N, | Radix rubiginosa (2) | 0 | - |
| | District, Chumphon | 99-100/*E, Alt. | Amerianna carinata (4) | | |
| 27 | Province | 10 111 | Indoplanorbis exustus (2) | 2 (0.170/) | |
| 27 | Bang Luk, Mueang | 10°32'33"N, | Radix rubiginosa (13) | 2 (0.17%) | Echinostome cercariae: Echinostoma |
| | District, Chumphon | 99°10'35"E, Alt. | | 1 (0.000/) | revolutum |
| | Province | 15 m | | 1 (0.08%) | Purcocercous cercariae/ Longiturcate- pharyngeate cercariae/ Strigea |
| | | | | | cercaria: Diplostomum baeri eucaliae |
| | | | <i>Gyraulus convexiusculus</i> (30) | 1 (0.08%) | Xiphidiocercaria: |
| | | | Indonlanophia organia (54) | 0 | Opinianioxipindiocercaria |
| | | | Total 1 175 | 11 (0.94%%) | - |



Figure 3. Pulmonata snails were collected from 27 locations across 5 provinces in the southern region of Thailand. A. *Gyraulus bakeri*; B. *Gyraulus hubendicki*; C. *Gyraulus convexiusculus*; D. *Indoplanorbis exustus*; E. *Amerianna carinata*; F. *Physella acuta*; G. *Radix rubiginosa*. Scale bar: 1 mm.

tinct morphological cercarial species were identified and described as follows (refer to Suppl. materials 1–8 for differentiated morphotypes and cercarial species):

Type 1. Echinostome cercariae

Echinostomatidae Looss, 1899

1.1 *Echinoparyphium recurvatum* (Linstow, 1873) Lühe 1909

The cercariae were obtained from one *Gyraulus convexi*usculus and one *Radix rubiginosa*, representing infection rates of 0.085% (1/1,175) and 0.085% (1/1,175), respectively. The body shape of the cercariae is large and ovate or pear-shaped, with a prominent oral sucker larger than the ventral sucker and collar spines surrounding it. Eyespots are absent. A prepharynx is present, and the pharynx is conspicuous. The esophagus is long, and the ceca are bifurcated in the front of the ventral sucker (acetabulum) end at three-quarters of the body. The ventral sucker is located posteriorly. Excretory ducts between the pharynx and ventral sucker are dilated and filled with numerous granules. The tail is longer than the body and lacks a finfold. The cercariae develop within rediae (see Fig. 4 and Suppl. material 1 for reference).

Size range and average size (in micrometers, calculated from 10 cercariae):

| Body | 187–631 μm (avg. 380 μm) × 159–330 μm (avg. 212 μm) |
|-------------------|--|
| Pharynx | 12–35 μm (avg. 23 μm) × 17–37 μm (avg. 24 μm) |
| Oral sucker | 45–89 μ m (avg. 58 μ m) × 43–96 μ m (avg. 57 μ m) |
| Ventral sucker | 54–75 μ m (avg. 64 μ m) × 59–102 μ m (avg. 71 μ m) |
| Excretory bladder | 14–30 μ m (avg. 20 μ m) × 25–86 μ m (avg. 56 μ m) |
| Tail | 203–504 μ m (avg. 481 μ m) × 42–65 μ m (avg. 56 μ m) |

1.2 *Echinostoma spiniferum* Ahmed, 1959 (sensu Našincová 1992)

One *Radix rubiginosa* released cercariae, resulting in an infection rate of 0.085% (1/1,175) among all collected snails. The cercariae have an elongated, pear-shaped body with collar spines around a circular oral sucker. There are no eyespots, and the prepharynx, and esophagus are long. The pharynx is large. Bifurcated intestinal caeca reach to the posterior end of the body. The ventral sucker is rather

large and locate approximately three-fourths of the way down the body, and there are penetration glands along the esophagus in the middle of the body. The excretory bladder is large, sac-like, and locate at the posterior end of the body. The tail is longer than the body and tubular in shape, with a finfold along the tail stem. The cercariae develop within rediae (see Fig. 5 and Suppl. material 2 for reference).

Size range and average size (in micrometers, calculated from 10 cercariae):

| Body | 197–280 μm (avg. 234 μm) × 163–207 μm (avg. 185 μm) |
|-------------------|---|
| Oral sucker | 37–44 μm (avg. 41 μm) × 35–42 μm (avg. 40 μm) |
| Pharynx | 11–15 μm (avg. 13 μm) × 13–15 μm (avg. 14 μm) |
| Ventral sucker | 49–59 μm (avg. 55 μm) × 48–57 μm (avg. 53 μm) |
| Excretory bladder | 23–33 μm (avg. 29 μm) × 29–36 μm (avg. 32 μm) |
| Tail | 328–422 μm (avg. 387 μm) × 48–69 μm (avg. 61 μm) |
| Finfolds | 21–29 μm (avg. 25 μm) × 9–14 μm (avg. 11 μm) |

1.3 Echinostoma revolutum (Froelich, 1802) Looss 1899

The cercariae were obtained from two *Radix rubiginosa*, resulting in an infection rate of 0.17% (2/1,175). The cercarial body have an elongated pear-shaped form. The oral sucker is circular and adorned with collar spines. The prepharynx is short, and the pharynx is large. The esophagus is bifurcated into two intestinal caeca between the pharynx and ventral sucker, extending almost to the posterior end of the body. The relatively large ventral sucker is positioned approximately one-third to three-fourths of the body length from the anterior end. Four pairs of small penetration glands are present, and the flame cell pattern is not determined. The excretory bladder is small and oval-shaped. The tail is slender, longer than the body, and lack a distinct dorsal finfold. An opening to the excretory duct is at the tip of the tail, which is Y-shaped when view invert. The cercariae develop within rediae (see Fig. 6 and Suppl. material 3 for reference).

Size range and average size (in micrometers, calculated from 10 cercariae):

| Body | 276–420 μm (avg. 348 μm) × 199–259 μm (avg. 227 μm) |
|-------------------|--|
| Oral sucker | 55–62 μm (avg. 59 μm) × 54–63 μm (avg. 60 μm) |
| Pharynx | $11-15 \ \mu m (avg. 13 \ \mu m) \times 13-15 \ \mu m (avg. 14 \ \mu m)$ |
| Ventral sucker | 70–79 μm (avg. 76 μm) × 69–80 μm (avg. 75 μm) |
| Excretory bladder | 14–22 μm (avg. 19 μm) × 48–63 μm (avg. 58 μm) |
| Tail | 370–393 μm (avg. 384 μm) × 51–57 μm (avg. 55 μm) |



Figure 4. Image of *Echinoparyphium recurvatum* (Linstow, 1873) Luche, 1909. **A.** Images of cercaria stained with 0.5% neutral red (DIC microscopy) and drawing of cercarial structure; **B.** The anterior body of cercaria stained with 0.5% neutral red (DIC microscopy); **C.** Body part of cercaria stained with 0.5% neutral red (DIC microscopy). Abbreviations: cs: collar spines, eb: excretory bladder, ep: esophagus, i: intestine, mct: main collecting tube, os: oral sucker, p: pharynx, pg: penetration gland, ta: tail, vs: ventral sucker. Scale bars: 100 μm.

Type 2. Brevifurcate-apharyngeate cercariae

Schistosomatidae Looss, 1899

2.1 *Schistosoma indicum* Montgomery, 1906 (Syn. *S. nasalis* Rao, 1933)

The cercariae were found in one *Indoplanorbis exustus*. The infection rate was 0.085% (1/1,175). The body is

elongate-oval in shape and has small spines surrounding it. A head organ is present. Nevertheless, eyespots and pharynx are absent. The esophagus is narrow and long. The intestinal caeca are small and saccular. Five pairs of penetration glands are located near the ventral sucker like a sac and are stacked from the ventral sucker to the end of the body. The opening of the excretory pores is at the tip of the tail furcae. The tail is longer than the body and divide into two furcae. There is a spine from the anterior



Figure 5. Image of *Echinostoma spiniferum* Ahmed, 1959. **A.** Images of cercaria stained with 0.5% Methylene blue (DIC microscopy) and drawing of cercarial structure; **B.** The tail tip of a cercaria stained with 0.5% neutral red (DIC microscopy); **C.** Unstained body part of cercaria (DIC microscopy). Abbreviations: cs: collar spines, eb: excretory bladder, ep: esophagus, fi: finfold, i: intestine, mct: main collecting tube, os: oral sucker, p: pharynx, pp: prepharynx, pg: penetration gland, ta: tail, vs: ventral sucker. Scale bars: 100 mm.

end of the tail stem to the posterior of the furcae. The cercariae develop within sporocysts (see Fig. 7 and Suppl. material 4 for reference).

Size range and average size (in micrometers, calculated from 10 cercariae):

| Head organ | 14–26 μm (avg. 17 μm) × 7–22 μm (avg. 14 μm) |
|----------------|--|
| Body | 131–195 μm (avg. 157 μm) × 56–75 μm (avg. 62 μm) |
| Ventral sucker | 20–23 μm (avg. 21 μm) × 21–26 μm (avg. 23 μm) |
| Tail | 233–279 μm (avg. 251 μm) × 20–38 μm (avg. 26 μm) |
| Furcal tail | 96–117 μm (avg. 105 μm) × 12–22 μm (avg. 17 μm) |



Figure 6. Image of *Echinostoma revolutum* (Froelich, 1802) Looss, 1899. **A.** Images of cercaria stained with 0.5% neutral red (DIC microscopy) and drawing of cercarial structure; **B.** Unstained body part of cercaria (DIC microscopy); **C.** Body part of cercaria stained with 0.5% neutral red (DIC microscopy). Abbreviations: cs: collar spines, eb: excretory bladder, ep: esophagus, i: intestine, mct: main collecting tube, os: oral sucker, p: pharynx, pp: prepharynx, pg: penetration gland, ta: tail, vs: ventral sucker. Scale bars: 100 µm.

2.2 Schistosoma spindale Montgomery, 1906

The infection rate of cercariae in one *Radix rubigino*sa was 0.085% (1/1,175), and the cercariae are elongate-oval in shape with a thick wall and spiny surface. The head organ is present and reveals a narrow and long esophagus, but eyespots and pharynx are absent. The intestine bifurcates into two short caeca, which stain with neutral red. Five pairs of penetration glands are located lateral to the ventral sucker, with ducts opening near large apical papillae at the anterior end of the body. These glands align in a long line on the side of the body, which differs from the sac-like position of Schistosoma indicum. The ventral sucker, which appears round and is covered with small spines, is rather posterior to the body, with slight elongation.



Figure 7. Image of *Schistosoma indicum* Montgomery, 1906 (Syn. *S. nasalis* Rao, 1933). **A.** Images of unstained cercaria (light microscopy) and drawing of cercarial structure; **B.** Unstained head organ of cercaria (DIC microscopy); **C.** Body part of cercaria stained with 0.5% neutral red (DIC microscopy); **D.** Unstained body part of cercaria (DIC microscopy). Abbreviations: ep: esophagus, fu: furca, h: head organ, i: intestine, pg: penetration gland, ta: tail, vs: ventral sucker. Scale bars: 100 μm.

The excretory bladder is small, thin-walled, and is located at the rear of the body. The tail is longer than the body and cylindrical, with two fucae shorter than the tail stem and an opening for the excretory duct at the tip. The cercariae develop within sporocysts (see Fig. 8 and Suppl. material 5 for reference).

Size range and average size (in micrometers, calculated from 10 cercariae):

| Head organ | 42–68 μm (avg. 59 μm) × 22–54 μm (avg. 37 μm) |
|----------------|---|
| Body | 116–187 μm (avg. 164 μm) × 42–109 μm (avg. 74 μm) |
| Ventral sucker | 15–29 μm (avg. 23 μm) × 12–28 μm (avg. 22 μm) |
| Tail | 100–114 μm (avg. 107 μm) × 25–53 μm (avg. 39 μm) |
| Furcal tail | 52–131 μm (avg. 92 μm) × 10–23 μm (avg. 17 μm) |

Type 3. Brevifurcate-pharyngeate-clinostomatoid-cercariae

Clinostomidae Lühe, 1901

Clinostomum giganticum Agarwal (1959)

Two snail species, *Indoplanorbis exustus*, and *Radix rubiginosa*, contained cercariae with an overall infection rate of 0.17% (2/1,175) among the specimens collected. The cercariae are elongated and oval in shape with minute body spines and a delicate dorso-median finfold extending from the eyespots to the posterior end. The head organ and eyespots are observable, and four pairs of penetration glands are present on each side of the intestines, with two pairs located anteriorly and two pairs located posterior-ly. The ducts of the glands are bundled on each side and penetrate the anterior organ to open at its anterior end. A





Figure 8. Image of *Schistosoma spindale* Montgomery, 1906. **A.** Unstained images of cercaria (DIC microscopy) and drawing of cercarial structure; **B.** Unstained body part of cercaria (DIC microscopy); **C.** Body part of cercaria stained with 0.5% neutral red (DIC microscopy); **D.** Unstained tail tip of a cercaria (DIC microscopy). Abbreviations: ep: esophagus, exp: excretory pore, fu: furca, h: head organ, i: intestine, pg: penetration gland, sp: spines, ta: tail, vs: ventral sucker. Scale bars: 100 µm.

bulbous swelling is present at the end of the esophagus, which stains with neutral red. The intestines are undivided, with a saccular shape in the middle of the body. The excretory bladder is V-shaped and thin-walled, located medially, and close to the posterior end of the body. The tail is longer than the body and is divided into two furcae, with the furcal tail stem shorter than the tail stem and minute spines present along the lateral margins. The tip of each furca is claw-shaped. The cercariae develop within rediae (see Fig. 9 and Suppl. material 6 for reference).

Size range and average size (in micrometers, calculated from 10 cercariae):

| Head organ | 34–70 μm (avg. 49 μm) × 16–37 μm (avg. 25 μm) |
|-------------|---|
| Body | 135–177 μ m (avg. 160 μ m) × 35–60 μ m (avg. 45 μ m) |
| Eyespots | 10–19 μm (avg. 14 μm) × 6–14 μm (avg. 9 μm) |
| Tail | 320–331 μm (avg. 328 μm) × 23–33 μm (avg. 28 μm) |
| Furcal tail | 77–117 μm (avg. 99 μm) \times 16–19 μm (avg. 17 μm) |



Figure 9. Image of *Clinostomum giganticum* Agarwal (1960). **A.** Images of cercaria stained with 0.5% neutral red (DIC microscopy) and drawing of cercarial structure; **B.** Body part of cercaria stained with 0.5% neutral red (DIC microscopy); **C.** Unstained tail tip of a cercaria (DIC microscopy). Abbreviations: eb: excretory bladder, es: eyespots, exp: excretory pore, fi: dorso-median finfold, fu: furca, h: head organ, i: intestine, pg: penetration gland, sp: spines, ta: tail. Scale bars: 100 µm.

Type 4. Longifurcate-pharyngeate cercariae (Strigea cercaria)

Diplostomidae Poirier, 1886

Diplostomum baeri eucaliae Hoffman & Hundley, 1957

Cercariae were detected in one *Radix rubiginosa* snail, with an infection rate of 0.085% (1/1,175) among the snails collected. The body of the cercariae is elongat-

ed-oval in shape. The anterior organ appears pyriform with an oral sucker. No eyespots are detected. The upper part of the body is covered with numerous spines. The pharynx is fully developed, and the esophagus is short and bifurcated approximately midway between the oral and ventral suckers. The ventral sucker is located in the middle of the body, while the excretory bladder is small and thin-walled. Two pairs of penetration glands are located after the acetabulum. The tail is as long as the body and is cylindrical and features long hair-like structures on each side of the tail stem, exhibiting nucleated caudal bodies. The spinous furcae are as long as the tail stem. Movement is achieved by rolling up and springing back the body, resulting in non-directional, spiraling motion for forward movement. The largest number of cercariae emerge during the late morning, and their development occurs within sporocysts (see Fig. 10 and Suppl. material 7 for reference).

Size range and average size (in micrometers, calculated from 10 cercariae):

| Body | 117–255 μm (avg. 208 μm) × 44–85 μm (avg. 65 μm) |
|----------------|---|
| Oral sucker | 31–60 μm (avg. 49 μm) × 23–41 μm (avg. 34 μm) |
| Pharynx | 8–19 μm (avg. 13 μm) × 6–12 μm (avg. 10 μm) |
| Ventral sucker | 26–40 μm (avg. 34 μm) × 16–42 μm (avg. 36 μm) |
| Tail | 200–209 μm (avg. 205 μm) × 25–27 μm (avg. 26 μm) |
| Furcal tail | 154–207 μm (avg. 183 $\mu m) \times$ 11–13 μm (avg. 14 $\mu m)$ |



Figure 10. Image of *Diplostomum baeri eucaliae* Hoffman & Hundley, 1957. **A.** Unstained image of cercaria (DIC microscopy) and drawing of cercarial structure; **B**, **C.** Unstained body part of cercaria (DIC microscopy); **D.** Unstained tail with hairs of cercaria (DIC microscopy); **E.** Unstained head organ with spine of cercaria (DIC microscopy); **F.** Unstained furca with spine of cercaria (DIC microscopy); **E.** Unstained head organ with spine of cercaria (DIC microscopy); **F.** Unstained furca with spine of cercaria (DIC microscopy). Abbreviations: c: caecum, cb: caudal body, fu: furca, h: head organ, ha: hair, p: pharynx, pg: penetration gland, sp: spines, ta: tail, vs: ventral sucker. Scale bars: 100 μm.

Type 5. Ophthalmoxiphidiocercaria

Allocreadiidae

Cercariae were found in one *Gyraulus convexiusculus* snail, representing an infection rate of 0.085% (1/1,175) of the total number of snails collected. These cercariae have an elongated-oval body shape covered in small spines. A large oral sucker with oral spines and a stylet is present at the anterior end. A pair of eyespots with black pigment inside is present at approximately one-fourth of the body length. The pharynx is immature, but a fully developed

ventral sucker is located on the midventral surface of the body. The esophagus has a narrow opening extending straight down, and the body has two intestinal ceca. Penetration glands lie above the acetabulum, but their number is unclear. Cystogenous cells appear bunched, sorted down to the middle of the body, and stain dark with neutral red. The posterior end of the body has a thick-walled, epithelial excretory bladder. The tail is cylindrical and is as long as the body. The cercariae develop within rediae (see Fig. 11 and Suppl. material 8 for reference).

Size range and average size (in micrometers, calculated from 10 cercariae):

| Oral sucker | 73–82 μm (avg. 76 μm) × 83–86 μm (avg. 85 μm) |
|----------------|---|
| Stylet | 12–17 μm (avg. 14 μm) |
| Body | 420–604 μm (avg. 512 μm) × 218–222 μm (avg. 220 μm) |
| Eyespots | 10–15 μm (avg. 12 μm) × 15–21 μm (avg. 17 μm) |
| Ventral sucker | 57–64 μm (avg. 60 μm) × 60–71 μm (avg. 64 μm) |
| Tail | 516–591 μm (avg. 554 μm) × 52–72 μm (avg. 62 μm) |



Figure 11. Image of Ophthalmoxiphidiocercaria (Family Allocreadiidae Looss, 1902). Show images of cercaria stained with 0.5% neutral red (DIC microscopy) and drawing of cercarial structure. Abbreviations: cg: cystogenous glands, eb: excretory bladder, es: eyespots, exp: excretory pore, os: oral sucker, p: pharynx, pg: penetration gland, s: stylet, ta: tail, vs: ventral sucker. Scale bar: 100 µm.

Discussion

Snail-borne parasitic diseases have been reported in the Southeast Asian region for more than 50 years. In particular, liver fluke opisthorchiasis, blood fluke schistosomiasis, intestinal fluke echinostomiasis, and fasciolopsiasis are public health issues that affect socioeconomic development in this region.

Freshwater snails that serve as intermediate hosts, are commonly found in various water resources, and Thailand is home to one of the world's richest freshwater and brackish water snail faunas, with over 170 freshwater species and 96 brackish water species recorded across 75 genera and 23 families. Among these non-marine aquatic Mollusca of Thailand, according to Brandt (1974), there are three superfamilies of snails within the Pulmonata, such as Ancyloidea Rafinesque 1815, Planorboidae Rafinesque 1815, and Lymnaeoidea Rafinesque 1815, all of which belong to Basommatophora Keferstein 1864 (Bouchet and Rocroi 2005).

The SEAMEO-TROPMED organization held a technical meeting on "Snails of Medical Importance in Southeast Asia" in 1985 to update and review the species distribution of medically important snails in the region. The aim was to provide guidance for controlling these snails. The pulmonate snails of Thailand were listed according to their geographical location, viz. Lymnaeidae consisting of Austropeplea ollula, Radix viridis (reported to Radix (Austropeplea) ollula), R. auricularia rubiginosa and R. a. swinhoei and Planorbidae consisting of Hippeutis umbilicalis, Indoplanorbis exustus, Gyraulus convexiusculus, Segmentina hemisphaerula, and S. trochoideus (Brandt 1974; Tropmed Technical Group 1986). These snails are widely distributed throughout Thailand and are commonly found in freshwater environments, serving as intermediate hosts for human and animal trematodes. Besides the medically important snails, there have been numerous regional surveys, species lists, and reviews of pulmonate snail species significant for public health and veterinary purposes, particularly in the vicinity of the cercarial dermatitis outbreak area in southern Thailand (Krailas et al. 2022).

Radix rubiginosa (Lymnaeidae) serves as an intermediate host for the liver fluke *Fasciola gigantica* and the intestinal flukes *Echinostoma malayanum*, *E. revolutum*, and *Hypoderaeum conoideum*. This snail has been reported throughout the water resources of Thailand, except for the northernmost provinces. *Radix swinhoei* is an intermediate host for *F. gigantica* and many species of intestinal flukes in Echinostomatidae, and has been reported in the northernmost provinces. *Austropeplea ollula* serves as an intermediate host for *F. gigantica* and echinostomes and is distributed in central and northern Thailand.

The planorbid snails serve as intermediate hosts for human schistosomes, but in Thailand, they mediate echinostomes, *Fasciolopsis buski*, and non-human schistosomes. *Gyraulus convexiusculus* is an intermediate host for *E. malayanum* and *E. ilocanum*, while *Indoplanorbis exustus* is an intermediate host for the intestinal flukes *E. malayanum*, *E. revolutum*, *H. conoideum*, *Schistosoma spindale*, and *S. indicum*. *Segmentina* spp. is intermediate hosts for the largest intestinal fluke *F. buski*, which is found throughout Thailand, particularly in the central region. (Kruatrachue and Harinasuta 1963, 1964; Harinasuta et al. 1965; Manning 1969; Manning et al. 1969; Sornmani 1969a, b; Brandt 1974; Schneider et al. 1974; Ratanaponglakha et al. 1988, 1989; Burch and Upatham 1989; Kullavanijaya and Wongwaisayawan 1993; Krailas et al. 2022).

As reported here, we collected pulmonate snails from five provinces in southern Thailand, categorized into three families based on the morphological characters of their shells, including Lymnaeidae, Physidae, and Planorbidae. Originally, Physidae was native to Holarctic, but extending its distribution now into Central and South America, and has invaded freshwater lentic habitats nearly worldwide, except those in Antarctica (Burch 1988; Wethington and Lydeard 2007; Bousset et al. 2014). Among these *Physella acuta* (syn. *Physa acuta*) is an invasive species in Thailand, having previously been collected from tanks within a research facility in Bangkok and subsequently spread throughout the country (Rico et al. 2014; Ng et al. 2018).

In this study, seven pulmonate species were collected, including Amerianna carinata, Gyraulus bakeri, G. convexiusculus, G. hubendicki, Physella acuta, Indoplanorbis exustus, and Radix rubiginosa. Naturally infected snails were found in six of 27 locations that were in the vicinity of the cercarial dermatitis outbreak (Krailas et al. 2022). We related the occurrence and distribution of these pulmonate snails to the cercarial dermatitis outbreak of 2020 in this region, as we found that the snail act as intermediate hosts to several trematode species of medical importance. In summary of our study, trematode infections were found in eight species of five cercarial types from three snail species, including G. convexiusculus, I. exustus, and R. rubiginosa. The infection rates were 1.14% (2/176), 0.25% (2/802), and 4.02% (7/174), respectively. Although the infection rate of parasites is low, we have found something interesting in the ability of snails to serve as intermediate hosts for the trematode parasites encountered in this study, which has not been reported extensively before. This is a report that should be further investigated in the future. Next, we would like to discuss the importance of pulmonate snails and their significance in serving as intermediate hosts for parasitic diseases that are of public health and veterinary importance.

Gyraulus convexiusculus is a widespread snail species that has been collected from ponds, lakes, canals, and rice fields in tropical Asia and surrounding regions (Van Damme 2014). It is an intermediate host for many trematode parasites, including *Artyfechinostomum malayanum* (Leiper, 1911) Mendheim 1943 (syn. *Echinostoma malayanum* Leiper, 1911; *Euparyphium malayanum* Odhner, 1913), which was first described in a human in Malaysia and has since been found in other countries, such as Singapore, Thailand, Indonesia, India, and the Philippines (Premvati and Pande 1974; Maji et al. 1993;

Radomyos et al. 1998; Belizario et al. 2007; Chai et al. 2009). G. convexiusculus is the first intermediate host of the intestinal fluke Fasciolopsis buski in South Kalimantan Indonesia and amphistomataous parasites of domestic animals in India. These parasites cause significant losses due to poor health and heavy mortality of humans and commercially important animals (Djajasasmita 1989; Kishore and Shoeb 2017). During our survey, we collected G. convexiusculus snails from 11 locations across five provinces. However, infected snails were only found in two locations located in Chian Yai District of Nakhon Si Thammarat Province and Mueang District of Chumphon Province. We identified two trematode species in these snails, such as Echinoparyphium recurvatum, and Ophthalmoxiphidiocercaria. The genus Echinoparyphium is an important taxon in the family Echinostomatidae, as species in this genus are involved in intestinal foodborne trematodiases in Southeast Asia. E. recurvatum is an intestinal fluke of wild fowl and mammals and is a cosmopolitan species. Many species of snails in the families Lymnaeidae and Planorbidae are susceptible intermediate hosts for E. recurvatum, including Lymnaea pervia, L. limosa, L. palustris, L. auricularia rufescens, Planorbis, Planorbarius dufouri, and Indoplanorbis exustus. Although E. recurvatum infections have mostly been reported in birds, human infections have been reported in Taiwan, Indonesia, Korea, and Egypt (Sohn 1998; Chai et al. 2009; Huffman and Fried 2012), but have never been reported in Thailand to date. In particular, a natural infection was detected in G. convexiusculus. We also report a natural infection of G. convexiusculus with an Ophthalmoxiphidiocercaria trematode in the family Allocreadiidae. The cercariae of this parasite develop in freshwater bivalves of the family Sphaeriidae, but not in the subfamily Crepidostominae, and have also been reported in L. peregra, a freshwater gastropod in the Lymnaeidae (Henderson 1938; Schell 1970). Interestingly, researchers have suggested that trematode cercariae, such as those from Echinoparyphium recurvatum can be distinguished into lymnaeidarum and planorbidarum types. The planorbidarum type, which refers to Echinoparyphium cercariae shed from planorbid snails (Odening 1964; Grabda-Kazubska and Kiseliene 1989; McCarthy 1990).

The planorbid snail, *Indoplanorbis exustus* was the most abundant of the snails collected in this study. Moreover, *I. exustus* was collected from 25 locations and are widely distributed in Thailand, India, Sri Lanka, Indonesia, the Philippines, Japan, and Hawaii. The freshwater bulinine planorbid snail *I. exustus* is the sole intermediate host of the *Schistosoma indicum* species group, which causes severe outbreaks of cattle schistosomiasis and human cercarial dermatitis in India and Southeast Asia (Burch and Upatham 1989; Agrawal et al. 2000; Gauffre-Autelin et al. 2017). The medically important digenetic trematode *Schistosoma* includes four prominent species groups, including the *S. japonicum* species group in Asia, which are *S. japonicum*, *S. mekongi*, *S. sinensium*, *S. malayensis*, and *S. ovuncatum*; the *S. mansonia* species

group in Africa, Southwest Asia, and South America, which are S. mansoni, S. rodhaini, S. hippopotami, and S. edwardiense; the S. haematobium species group in Africa, which are S. haematobium, S. intercalatum, S. bovis, S. mattheei, S. curassoni, S. margrehowiei, and S. leiperi, and the S. indicum species group in Asia, which consists of S. indicum, S. nasale, S. spindale, and S. incognitum (Attwood et al. 2007; Webster et al. 2013; Devkota et al. 2015; Jones et al. 2020). In our previous study, we reported that I. exustus is an intermediate host of S. indicum, a ruminant parasite that caused the outbreak of cercarial dermatitis in Chana district, Songkhla Province, South Thailand (Krailas et al. 2022), In this study, S. indicum was detected in one snail of the same species. We also found another type of trematode infection, Furcocercous cercariae/Brevifurcate-pharyngeate-clinostomatoid-cercariae, specifically Clinostomum giganticum Agarwal, 1959, in one I. exustus specimen from a different location. The genus Clinostomum Leidy, 1856 is a cosmopolitan parasite found in the oral cavity, pharynx, and esophagus of fish-eating birds. Its life cycle requires two intermediate hosts (snails and fish or frogs) and one avian definitive host (Osborn 1911, 1912; Kanev et al. 2002; McAllister et al. 2010; Calhoun et al. 2019). The pulmonate gastropods in the families Planorbidae and Lymnaeidae are commonly reported as first intermediate hosts. In this study, both snail families were infected by C. giganticum. Including a previous study, the Clinostomum distribution tended to coincide with the distributions of Planorbidae and Lymnaeidae snails in the same area. Clinostomum is associated with "yellow-spot disease/yellow grubs" in freshwater fish as a secondary intermediate host. The metacercaria larval stage encysts below the integumentary tissue, causing visible swelling of the nodules. The parasites remain encysted and alive within the host for several years until eaten by a bird host. Humans can also become infected by eating raw or undercooked fish meat carrying the metacercarial stage. Therefore, this trematode must be considered not only for its losses in production and fish farming waste but also for its potential zoonotic impact on humans (Hunter and Hunter 1934, 1935; Esch et al. 2001; Wang et al. 2017; Rosser et al. 2018; Sohn et al. 2019; de Souza et al. 2020; Won et al. 2020; Krailas et al. 2022).

Only one species, *Radix rubiginosa*, was collected from the family Lymnaeidae at 16 of 27 locations. We found trematode infections in seven snails, with four types, and six species of cercariae being identified, viz. (i) Echinostome cercariae consisted of *Echinoparyphium recurvatum*, *Echinostoma spiniferum*, and *Echinostoma revolutum*, type (ii) Brevifurcate-apharyngeate cercariae which was *Schistosoma spindale*, type (iii) Brevifurcate-pharyngeate-clinostomatoid-cercariae which was *Clinostomum giganticum*, and type (iv) Longifurcate–pharyngeate cercariae (Strigea cercaria) which was *Diplostomum baeri eucaliae*. *R. rubiginosa* is a common freshwater snail that is widely distributed throughout Thailand. It is an abundant species in Lymnaeidae and serves as an intermediate host of the intestinal flukes of the Echinostomatidae. It has also been recorded in Laos, Cambodia, Vietnam, Malaysia, and Indonesia. In the present study, three species of Echinostomatidae were collected, including E. recurvatum, E. spiniferum, and E. revolutum. This is a new record for R. rubiginosa infected with E. recurvatum and E. spiniferum in Thailand. In our previous study of parasites that caused an outbreak of cercarial dermatitis, a double infection of S. indicum and E. spiniferum was found in I. exustus (Krailas et al. 2022). Now, in the present study, E. spiniferum was found in R. rubiginosa. Furthermore, we here report the discovery of three more cercarial types from R. rubiginosa viz. Brevifurcate-apharyngeate cercariae, such as S. spindale, Brevifurcate-pharyngeate-clinostomatoid-cercariae: C. giganticum, and Longifurcate-pharyngeate cercariae (Strigea cercaria): Diplostomum baeri eucaliae. Trematodes in the Family Diplostomatidae are commonly observed in freshwater environments. They have a complex life cycle that involves multiple hosts, including snails, fish, and birds. Many studies on the life histories of members of the genus Diplostomum have been reported, which was initially discovered in Europe, Africa, and the Americas (Palmieri et al. 1976). It was only decades later that the metacercarial characters were resolved causing a disease in fish known as diplostomatosis or eye fluke disease.

The schistosome species, S. spindale causes cercarial dermatitis in the same areas where I. exustus is distributed. The different distribution areas that we reported in this study indicate the prevalence of S. spindale in Nakhon Si Thammarat Province, while S. indicum was detected in Chaiya District, Surat Thani Province. Although R. rubiginosa was common in the Nakhon Si Thammarat in Southern Thailand, they are the intermediate host of S. japonicum, the human blood fluke, and Orientobilharzia harinasutai, the cattle blood fluke, in this area. The freshwater snail I. exustus is susceptible to infection by S. spindale, but here we found S. spindale from R. rubiginosa (Harinasuta and Kruatrachue 1962; Kruatrachue and Harinasuta 1963, 1964; Kruatrachue et al. 1964, 1965, 1968; Bunnag et al. 1986; Inder Singh et al. 1997; Lakshmanan et al. 2016).

Accordingly, the results we present here provide additional information on the potential of pulmonate snails as intermediate hosts for trematodes in Thailand. We emphasize that the distribution of these snail intermediate hosts plays a significant role in the transmission of parasites, which holds great medical, and veterinary significance.

Conclusion

Snail-borne diseases pose significant health risks to animals and humans, and trematodiasis remains a serious problem in Southeast Asia. In particular, cercarial dermatitis affects paddy workers, agricultural laborers, and fishermen. While surveys on larval trematodes in freshwater snails have been conducted, comprehensive data on gastropod infections are lacking. Therefore, this study aimed to determine the prevalence and types of cercarial infections in snails. A review of previous studies on pulmonate snails provided valuable insight into the diversity of parasites within their intermediate host populations. Both Lymnaeidae and Planorbidae snails play important roles as intermediate hosts for various digenean parasites, including schistosomes, echinostomes, clinostomes, and siplostomes. Effective control of trematode infections requires a holistic approach that focuses on managing the overall ecosystem and reducing exposure to the infective stages. This may involve maintaining good water quality, controlling the snail populations, and limiting bird access to fish habitats (as Clinostomum and Diplostomum infections primarily affect fish populations, with no direct transmission to humans). The presence of trematodes in snails presents a significant health concern in southern Thailand. Therefore, further studies are warranted to characterize these infections in other regions and expand our understanding of the extent and impact of snail-borne diseases.

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Supplementary material 1

Some characters of *Echinoparyphium recurvatum* found in this study and the reference sources

Author: Duangduen Krailas

Data type: docx

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Link: https://doi.org/10.3897/evolsyst.7.107847.suppl1

Supplementary material 2

Some characters of *Echinostoma spiniferum* found in this study and the reference sources

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Data type: docx

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Supplementary material 3

Some characters of *Echinostoma revolutum* found in this study and the reference sources

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Data type: docx

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Supplementary material 4

Some characters of *Schistosoma indicum* found in this study and the reference sources

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Supplementary material 5

Some characters of *Schistosoma spindale* found in this study and the reference sources

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Supplementary material 6

Some characters of *Clinostomum giganticum* found in this study and the reference sources

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Supplementary material 7

Some characters of *Diplostomum baeri eucaliae* cercaria found in this study and the reference sources

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Supplementary material 8

Some characters of Ophthalmoxiphidiocercaria (Family Allocreadiidae) found in this study and the reference sources

Author: Duangduen Krailas

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