Cercarial dermatitis outbreak caused by ruminant parasite with intermediate snail host: schistosome in Chana, South Thailand

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Abstract

A cercarial dermatitis outbreak occurred in Chana district, Songkhla Province, South Thailand, between August and October 2020. A total of 359 cases with cercarial dermatitis were confirmed with three cases of skin biopsy. The species of potential trematodes from infected snails were investigated, and the prevalence of infestation with schistosomes was described. As part of our ongoing studies of trematode diversity in freshwater systems, using morphological traits and sequence data to differentiate species, this study aimed to provide insights into the parasite species that cause cercarial dermatitis in the outbreak area and improve our understanding of parasite species distribution. Snail samples were collected in December 2020 and September and October 2021. Five main areas of outbreaks were investigated, and snails were collected by scooping and examined for infection with cercariae. The cercariae were characterized on the basis of morphological features. We found two species of snails to be infected, viz. Indoplanorbis exustus and Bithynia siamensis siamensis, with infection rates of 2.05% (12/586) and 7.93% (23/290), respectively. Three species of trematodes were found in B. s. siamensis, viz. Gastrothylax crumenifer, Astiotrema monticellii, and Loxogenes liberum. Moreover, three species of trematodes were found in Indoplanorbis exustus, viz. Clinostomum giganticum, Echinostoma spiniferum, and Schistosoma indicum. The latter is a ruminant schistosome, which causes the outbreak of cercarial dermatitis in the area. They were genetically analyzed using the internal transcribed spacer subunit II region to confirm the species identity at generic and infrageneric levels.

Key Words

Schistosoma indicum, snail-borne diseases, trematode infection

Introduction

Human dermatitis may be due to larval stages of trematode parasites penetrating the skin. As an emerging disease, cercarial dermatitis, which is also known as swimmer’s itch or clam-digger’s itch, is due to the larval stage of bird or mammal schistosomes but not human schistosomes. The species of trematodes that are prevalent in a dermatitis
outbreak depend on how humans and birds/mammals come into contact with a particular type of aquatic environment. Cercariae exhibit a chemical reaction through secretions from the skin, and they are not host specific compared with other human-infecting schistosomes. Skin penetration by cercariae causes an allergic reaction to parasites; however, cercariae do not mature into adults, but they often die in the skin of the host. Some studies reported that the hypersensitivity response supervenes with repeated exposure. This reaction occurred 10–15 h after cercarial penetration, and it can be resolved in a week. However, rare, systemic symptoms such as fever, chills, and adenopathy were observed (Hoeffler 1974; Chamot et al. 1998; KolárOVá et al. 2013; Horák et al. 2015).

At present, many diseases are overlooked despite their socio-economic importance, with non-human schistosomiasis being the most ignored schistosome. However, repeated reports of cercarial dermatitis in humans, which has become a vexing problem of some freshwater bathing beaches, have been found. In addition to the traditionally used geographic distribution, additional aspects of contemporary biology, from molecular phylogenetic studies to species diversity and environmental change, should be included to understand schistosome biology.

Based on traditional studies, 20 species of *Schistosoma* have been reported in four species groups, distinguished by the geographic area of origin, snail host, and egg shape. The *S. japonicum* group could be found in Asia, which includes *S. japonicum*, *S. mekongi*, *S. malayensis*, *S. sinensis*, and *S. ovumcatum*. The *S. mansoni* group was originally distributed in Africa; at present, it can be found in South Asia and South America, which includes *S. mansoni*, *S. rodhaini*, *S. hippopotami*, and *S. edwardsiense*. On the contrary, the *S. haematobium* group was almost exclusively distributed in Africa, which includes *S. haematobium*, *S. intercalatum*, *S. bovis*, *S. mattheei*, *S. curassoni*, *S. margrehowiei*, and *S. leiperi*. The *S. indicum* group was reported in Asia, which consists of four species that are transmitted by planorbis or lymnaeid pulmonate gastropods, viz. *S. indicum*, *S. nasale*, *S. spindale*, and *S. incognitum* (Attwood et al. 2007; Webster et al. 2013; Jones et al. 2020).

Ruminant-infecting trematodes, namely, *S. indicum* and *S. spindale*, were reported from mixed infections, and they can cause hepato-intestinal schistosomiasis resulting in reduced milk yield, wasting, and fibrosis because of the granulomas around trapped parasites eggs. Additionally, these species of the *S. indicum* group primarily cause human cercarial dermatitis, which has become an important public health problem for people living in endemic regions. Experimental evidence shows that schistosomula, which is the immature form of a parasitic schistosome after it has entered the blood vessels of its host, can migrate to the lungs or central nervous system in “incompatible” mammalian hosts causing severe pathologies beyond cercarial dermatitis (Agrawal et al. 2000; Horák and KolárOVá 2010; Jones et al. 2020). In South India and Southeast Asia, where *S. indicum* and *S. spindale* were reported to be widespread, these parasites can cause major pathology and mortality to livestock leading to welfare and socio-economic issues, predominantly among poor subsistence farmers and their families. In addition, cercarial dermatitis may represent a devitalized occupational disease among rice farmers (Chamot et al. 1998; Jones et al. 2020). Furthermore, these two species of the *S. indicum* group are closely related to each other based on morphological and molecular phylogenetic studies.

Here we focused on snail parasite species studied after the outbreak of cercarial dermatitis between August and October 2020 in the south of Thailand among the people of the Chana district, Songkhla Province (Fig. 1). The 359 patients were rice farmers, who live in ten villages of the district. Following the short investigation, three cases of patients were confirmed to be cercarial infections by skin biopsy (Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand). Health professionals and citizens were informed about the possibility of a problem with cercarial dermatitis in the areas of outbreak. A surveillance system was implemented to document the time-space distribution of the cases and the extent of the health problem.

The present investigation, which was conducted as part of our ongoing studies of trematode diversity, using morphological traits and sequence data to differentiate species, aimed to support diagnosis by identifying the species of cercariae prevalent in the outbreak areas. The results of this study will provide insights into the parasite species that cause cercarial dermatitis and may improve our understanding of public health problems in the outbreak and agricultural vicinity areas.

### Materials and methods

#### Snail collection and identification

Snails in paddy field from Chana district, Songkhla Province, South Thailand, were collected using stainless-steel scoops. Geographic coordinates (WGS84 datum) of sampling sites were determined using the global positioning system (Garmin PLUS III, Taiwan). Collections were performed on December 2020 and September and October 2021 (Figs 2 and 3 and Table 1). The collected snails were...
Figure 2. Map showed the five locations of Chana district, Songkhla province, south Thailand.

Table 1. Collected snails and trematode infections from 5 locations of Chana district, Songkhla province, South of Thailand. Collection during December 2020 – October 2021.

| No. | Locations | Coordinates | Collected snails (number) | Infected snails | Cercariae species |
|-----|-----------|-------------|---------------------------|----------------|-----------------|
|     |           |             |                           | Number         | Infection rate (%) | |
| 1   | Khae moo 1, Chana district Songkhla province | 06° 49' 17.40"N, 100° 41’ 85.20"E Alt. 11 m | Indoplanorbis exustus (67) | 2 | 3.70 | Gastrothylax crumenifer |
|     |           |             | Bithynia s. siamensis (54) | 2 | 3.70 | Astiotrema monticellii |
|     |           |             |                            | 3 | 5.56 | Loxogenes liberum |
|     |           |             | Pomacea canaliculata (28) | - | - | - |
|     |           |             | Filopaludina s. peninsularis (3) | - | - | - |
| 2   | Khae moo 5, Chana district Songkhla province | 06° 48’ 36.3"N, 100° 40’ 84.0"E Alt. 13 m | Indoplanorbis exustus (117) | 1 | 0.85 | Schistosoma indicum + Echinostoma spiniferum (double infection) |
|     |           |             | Bithynia s. siamensis (112) | 2 (c) | 1.71 | Schistosoma indicum |
|     |           |             |                            | 1 | 0.89 | Gastrothylax crumenifer |
|     |           |             | Pomacea canaliculata (34) | - | - | - |
|     |           |             | Filopaludina m. cambodjensis (3) | - | - | - |
|     |           |             | Filopaludina s. polygramma (1) | - | - | - |
|     |           |             | Filopaludina s. polygramma (9) | - | - | - |
| 3   | Khu moo 4, Chana district Songkhla province | 06° 50’ 00.50"N, 100° 42’ 37.60"E Alt. 25 m | Indoplanorbis exustus (111) | 3 | 2.70 | Schistosoma indicum |
|     |           |             | Bithynia s. siamensis (74) | 1 | 1.35 | Loxogenes liberum |
|     |           |             | Pomacea canaliculata (11) | - | - | - |
|     |           |             | Filopaludina m. cambodjensis (1) | - | - | - |
|     |           |             | Filopaludina s. polygramma (4) | - | - | - |
|     |           |             | Filopaludina s. peninsularis (9) | - | - | - |
| 4   | Khu moo 5, Chana district Songkhla province | 06° 50’ 00.50"N, 100° 42’ 37.60"E Alt. 568 m | Indoplanorbis exustus (112) | 1 | 0.89 | Schistosoma indicum |
|     |           |             | Bithynia s. siamensis (33) | 1 | 3.03 | Gastrothylax crumenifer |
|     |           |             | Pomacea canaliculata (13) | - | - | - |
|     |           |             | Filopaludina m. cambodjensis (1) | - | - | - |
|     |           |             | Filopaludina s. polygramma (4) | - | - | - |
| 5   | Saphan Mai Kaen Chana district Songkhla province | 06° 50’ 00.50"N, 100° 44’ 11.7"E Alt. 11 m | Indoplanorbis exustus (179) | (c) | 0.6 | Clinostomum giganticum |
|     |           |             | Bithynia s. siamensis (17) | 3 | 1.68 | Schistosoma indicum |
|     |           |             | Pomacea canaliculata (1) | - | - | - |
|     |           |             | Filopaludina s. peninsularis (4) | - | - | - |
|     |           |             | Total collected snails (993) | 35 | 3.52 | 5 species of cercariae |

* c - crushing.
**Trematode infection study**

The collected snails were investigated for trematode infections by shedding and crushing. The snails were placed in individual cup with dechlorinated water to observe the emergence of cercariae. Each cup was screened for the presence of cercariae three times over three consecutive days after sampling under a binocular dissecting microscope. Snails that did not shed cercariae during the observed time were crushed and examined for prepatent infections (sporocysts/rediae). The trematode morphology was described on the basis of living cercariae that had emerged from the snails and had been collected from the snail tissues. The cercariae were studied unstained and vitally stained with 0.5% neutral red. Details of the cercariae were photographed under a trinocular microscope (Nikon eclipse E200, Japan), and the differential interference contrast (Olympus BX53, Japan) was drawn and identified on the basis of Komiya (1961), Schell (1970), Yamaguti (1971, 1975), Ito (1980), Nasir (1984), Kraelas et al. (2011, 2014), and Veeravechsukij et al. (2018). Free-swimming cercariae were observed under a dissecting microscope, and live cercariae were observed before sample measurements (average size in micrometer) were taken using an ocular micrometer from ten specimens fixed in 10% formalin. Then, some cercariae belonging to the identified trematode species were preserved in 95% ethanol for DNA analysis.

**Molecular study of cercariae**

The genomic DNA from preserved cercariae and sporocysts of trematodes was extracted by using PureLink Genomic DNA Kits (Invitrogen, USA). Polymerase chain reaction (PCR) was performed for nuclear internal transcribed spacer 2 region (ITS2) amplification by using the primer combination ITS2-F: (5'-CTT GAACGC ACA TTG CGG CCA TGG G-3') and ITS2-R: (5'-GCG GGT AAT CACGTC TGA GCC GAG G-3') (Sato et al. 2009). Reactions were set up in 50 µL volumes containing 2 µL of DNA (50–100 ng), 0.5 µL of dNTPs (5 mM each), 2.5 µL of MgCl₂ (1.5 mM of MgCl₂), 5 µL of Buffer A (10× Buffer A, Invitrogen by Thermo Fisher Scientific, USA), 2.5 µL of each primer (10 µM), 0.5 µL of Taq DNA polymerase (1.5 U/µL, Invitrogen), and 34.5 µL of ddH₂O. The DNA samples were initially denatured at 94 °C for 4 min followed by 35 cycles (denaturation at 94 °C for 1 min, annealing at 60 °C for 30 s, and elongation at 72 °C for 2 min; Sato et al. 2009) and a final elongation step at 72 °C for 10 min and then kept at 4 °C. Then, the PCR products were loaded onto 1% agarose gels for electrophoresis. The ITS2 PCR products underwent purification and sequencing by Biobasic (Canada).

Forward and reverse strands were assembled as consensus sequences by using MEGA X with Clustal W under the default settings, and a phylogenetic tree was created with neighbor-joining analysis based on p-distances with 3,000 bootstrap replicates.

**Results**

**Sampling sites**

Snails were collected from five locations at the paddy fields around the villages of Chana district, Songkhla Province, South Thailand, based on the cases of cercarial dermatitis reported by the Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Thailand. Information on sampling sites, including geographic coordinates, is shown in Table 1. All sampling sites were rice fields, and the collection period was during the rainy season. The snail samples were found on the water surface or on the sand, mud, leaves, and aquatic plants.

**Snail samples and parasitic infections**

Based on conchological characteristics, 993 snail samples were classified into six species (Fig. 4), including 16 Filopaludina sumatrensis peninsularis, 9 F. sumatrensis polygramma, 5 F. martensi cambodjensis, 386 Indoplanorbis exustus, 290 Bithynia siamensis siamensis,
Pomacea canaliculata. Cercarial infections were found in 35 snails; the overall infection rate was 3.52% (35:993). The intensity of infection was found to be highest for two snail species, viz. B. s. siamensis and I. exustus, which both exhibited a prevalence of infection with infection rates of 2.52% (25:993) and 1.21% (12:993), respectively (Table 1). Based on the morphological and organ characteristics, the cercariae found in this study can be classified into six species with six morphologically distinguishable forms, viz. (i) Amphistome cercariae: Gastrothylax crumenifer Creplin, 1847; (ii) Paraplerolophocercous cercaria: Astiotrema monticellii Stossich, 1904; (iii) Virgulate xiphidiocercariae: Loxogenes liberum Seno, 1907; (iv) Furococercous cercariae/Brevifurcate-apharyngeate cercariae: Schistosoma indicum Montgomery, 1906 (Syn. S. nasalis Rao, 1933); (v) Furococercous cercariae/Brevifurcate-apharyngeate cercariae: Clinostomum giganticum Agarwal, 1959; and (vi) Echinostome cercariae: Echinostoma spiniferum Ahmed, 1959 (sensu Našincová, 1992). In addition, three species of cercariae were collected from B. s. siamensis, viz. G. crumenifer, A. monticellii, and L. liberum, and three species of cercariae were collected from I. exustus, viz. S. indicum, C. gigantica, and E. spiniferum. In this study, double trematode infections were found in one I. exustus snail, which was infected by S. indicum and E. spiniferum.

Morphology of cercariae

The cercariae were categorized by their morphology and organ characteristics based on previous morphological descriptions (e.g., Komiya 1961; Schell 1970; Yamaguti 1971, 1975; Ito 1980; Nasir 1984; Kralas et al. 2011, 2014; Veeravechsukij et al. 2018). The six distinct morphological cercarial types were described as follows.

Type 1. Amphistome cercariae

Family Gastrothylacidae Stiles & Goldberger, 1910
Gastrothylax crumenifer Creplin, 1847

The cercariae were isolated from four Bithynia s. siamensis with an infection rate of 0.4% (4/993) of the total number of the collected snails. The body shape is ovate and large. The eyespots have conical lenses with a black pigment. The end of ceca is at three-quarters of the body. The oral sucker is larger than the ventral sucker. The ventral sucker is located at the posterior end. The swelling of the excretory tube is found near the tip of the tail (Fig. 5 and Table 2).

The cercariae develop within rediae.

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

| Body          | 243–332 µm (av. 286 µm) × 389–566 µm (av. 467 µm) |
| Oral sucker   | 60–91 µm (av. 78 µm) × 46–91 µm (av. 70 µm)     |
| Ventral sucker| 31–47 µm (av. 38 µm) × 32–45 µm (av. 37 µm)    |
| Eyespots      | 18–38 µm (av. 24 µm) × 5–53 µm (av. 41 µm)     |
| Excretory bladder | 17–32 µm (av. 22 µm) × 15–25 µm (av. 19 µm) |

Type 2. Paraplerolophocercous cercariae

Plagiorchiidae Lühe, 1901
Astiotrema monticellii Stossich, 1904

The cercariae were isolated from five Bithynia s. siamensis with an infection rate of 0.5% (5/993) of the total number of the collected snails. The body is pear shaped. Collar spines are located at the oral sucker. One pair of eyespots present with a pigment. The oral sucker is slightly larger than the ventral sucker. Eight to nine pairs of penetration glands were observed at the middle of the body. The excretory bladder is large and thick walled, which is located at the posterior end of the body. The tail is longer than the body with a bilateral finfold and a dorso-ventral finfold. The lateral finfold extends over the whole length of the tail on both sides (Fig. 6 and Table 3).

The cercariae develop within rediae.

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

| Body          | 68–112 µm (av. 86 µm) × 146–229 µm (av. 192 µm) |
| Oral sucker   | 25–31 µm (av. 27 µm) × 22–53 µm (av. 40 µm)     |
| Ventral sucker| 25–43 µm (av. 29 µm) × 23–53 µm (av. 30 µm)    |
| Excretory bladder | 30–67 µm (av. 45 µm) × 23–47 µm (av. 30 µm) |
| Tail          | 55–96 µm (av. 67 µm) × 346–564 µm (av. 442 µm) |
| Lateral finfold | 16–30 µm (av. 24 µm) × 146–213 µm (av. 185 µm) |
Figure 5. Image of Gastrothylax crumenifer Creplin, 1847. a. Images of cercaria stained with 0.5% neutral red (light microscopy). b. Drawing of cercaria structure. c. Images of metacercaria stained with 0.5% neutral red (light microscopy). d. Images of redia stained with 0.5% neutral red (light microscopy). Abbreviations: c: cercaria; eb: excretory bladder; ep: esophagus; es: eyespot; i: intestine; os: oral sucker; p: pharynx; re: redia; sw: swollen tube; ta: tail; tex: transverse excretory; vs: ventral sucker. (Scale bars: 100 µm).
Figure 6. Image of *Astiotrema monticellii* Stossich, 1904 a. Images of cercaria stained with 0.5% neutral red (light microscopy) b. Drawing of cercaria structure c. Body part of cercaria stained with 0.5% neutral red (DIC microscopy) d. Images of rediae stained with 0.5% neutral red (light microscopy) Abbreviations: os: oral sucker, es: eyespot, p: pharynx, pg: penetration gland, eb: excretory bladder, vs: ventral sucker, ta: tail, lf: lateral finfold, op: oral spine, c: cercaria, re: redia. (Scale bars: 100 µm).
Table 2. Some characters of Gastrothylax crumenifer found in this study and the reference sources (measurement in µm, n/a = no data).

| Character                  | Immature cercaria | Liberated from rediae | Cercariae were liberated from the rediae | Cercariae develop within the rediae |
|----------------------------|-------------------|-----------------------|------------------------------------------|-------------------------------------|
| Body                       | 252–569 × 149–275 µm | 550–715× 320–440 µm | Body shape is ovate and large 190–250 µm (av. 220 µm) × 350–415 µm (av. 370 µm) | Oval shape and large, brown 243–332 µm (av. 286 µm) × 389–566 µm (av. 467 µm) |
| Eyespots                   | conical, lensed    | n/a                  | 1 pair, have conical lens with yellow pigment through the body with a smooth surface. | 1 pair, conical lens with black pigment. 18–38 µm (av. 24 µm) × 5–53 µm (av. 41 µm) |
| Pharynx                    | n/a               | 8–10 µm (av. 10 µm) × 8–12 µm (av. 11µm) | n/a                                       | n/a                                 |
| Intestine                  | n/a               | 90–169 µm long       | The ceca ended 0.14–0.17 mm away from the posterior end of the body | The end of ceca is three-quarters of the body. |
| Oral sucker                | 114 × 62 µm       | 95–115 × 70–75 µm    | 45–65 µm (av. 52 µm) × 45–65 µm (av. 52 µm) | 60–91 µm (av. 78 µm) × 46–91 µm (av. 70 µm) |
| Ventral sucker             | 72 × 132 µm       | n/a                  | 48–68 µm (av. 55 µm) × 48–68 µm (av. 55 µm) | Ventral sucker located at the end posterior. 31–47 µm (av. 38 µm) × 32–45 µm (av. 37 µm) |
| Excretory bladder          | n/a               | n/a                  | n/a                                       | 17–32 µm (av. 22 µm) × 15–25 µm (av. 19 µm) |
| Tail                       | 285–630 × 57–89 µm | 528–630 × 110–132 µm | various sizes of vacuole through the tail. 65–95 µm (av. 82 µm) × 328–450 µm (av. 410 µm) | thin wall, 55–96 µm (av. 67 µm) × 346–564 µm (av. 442 µm) |
| 1st IH (snail)             | n/a               | n/a                  | found from 8 snails. Melanoides tuberculata, infection rate of 0.02% (8/32,026) | found from 4 snails Bithynia siamensis siamensis |
| 2nd IH (fish)              | Encysting on pieces of grass blades. cyst dome-shaped, 271 µm diameter | Encysted on the vegetation. cyst 555 µm diameter | Encysted on the plastic container after cercariae shad- ed from snails 3–4 hours. | - |
| DH                         | goat ked, buffalo calf | goat ked           | The cercaria floated on the surface or in the water. It moved by wavering on the surface of the water for around 8–10 seconds, and then rolling up and springing back for about 5–10 seconds. It survived up to 3–4 hours in the water after emergence. The cercariae were photo-sensitive. They shrank rapidly in changing light conditions | The cercaria floated on the surface or in the water. The body sank lower than the tail, for 5–10 seconds. It moved by folding its tail back to the body and moving forward, and moved by swaying the body. It floated and moved forward around 10–15 seconds, and resting for 3–4 seconds. It survived up to 3–4 hours in the water, then encysted. |

Type 3. Virgulate xiphidiocercariae

Family Lecithodendriidae (Lühe 1901) sensu Odhner, 1910

Loxogenes liberum Seno, 1907

The cercariae were found in 14 Bithynia s. siamensis with an infection rate of 1.41% (14/993) of the total number of the collected snails. The body shape is oval. The oral sucker is located at the anterior end of the body, with stylet. The elongated organ is located near the stylet. The ventral sucker is roundish and smaller than the oral sucker. The prepharynx and pharynx were found, whereas the excretory and ceca were not observed. In addition, four pairs of penetration glands were found, which were located near the ventral sucker at the middle of the body; the two anterior pairs have fine granules, and the two posterior pairs have coarse granules. The excretory bladder is V shaped and thin walled. Furthermore, the tail is shorter than the body (Fig. 7 and Table 4).

The cercariae develop within sporocysts.

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

| Character                  | 48–105 µm (av. 97 µm) × 91–152 µm (av. 117 µm) | 2–5 µm (av. 4 µm) × 10–18 µm (av. 14 µm) | 19–27 µm (av. 22 µm) × 15–24 µm (av. 19 µm) | 7–15 µm (av. 9.9 µm) × 7–10 µm (av. 8 µm) |
|----------------------------|------------------------------------------------|----------------------------------------|-----------------------------------------------|--------------------------------------------|
| Body                       | 24–33 µm (av. 29 µm) × 26–36 µm (av. 31 µm) | Body                                   | 41–55 µm (av. 48 µm) × 135–152 µm (av. 142 µm) | 18–37 µm (av. 26 µm) × 17–26 µm (av. 21 µm) |
| Stylet                     | Head organ                                      | Body                                    | Ventral sucker                                | Tail                                       |
| Oral sucker                |                                             |                                            |                                              | 17–36 µm (av. 29 µm) × 225–286 µm (av. 252 µm) | Furcal tail |
| Pharynx                    |                                             |                                            |                                              | 10–21 µm (av. 16 µm) × 80–135 µm (av. 110 µm) | - |
| Ventral sucker             |                                             |                                            |                                              | - |
| Excretory bladder          |                                             |                                            |                                              | - |
| Tail                       |                                             |                                            |                                              | - |

Type 4. Furococercere cercariae/Brevifurcate-apharyngeate cercariae

Family Schistosomatidae Looss, 1899

Schistosoma indicum Montgomery, 1906 (Syn. S. na-salis Rao, 1933)

The cercariae were found in nine Indoplanorbis exustus with an infection rate of 0.91% (9/993) of the total number of the collected snails. The body is elongated oval in shape. The head organ was observed, whereas eyespots and pharynx were not found. The exophagus is long and narrow, and the intestinal caeca are small and saccular. Five pairs of penetration glands are identified: two pairs are transparent, and three pairs are turbid. The excretory bladder is cup shaped and located medially close to the posterior end of the body. The opening of the excretory pores is located at the tip of the furcae. The tail is longer than the body, and it is divided into two furcae. The furcal tail stem is shorter than the tail stem (Fig. 8 and Table 5). The cercariae develop within sporocysts.

Size range and average size (in micrometers, calculated from ten cercariae):
The cercariae were found in one *Indoplanorbis exustus* with an infection rate of 0.1% (1/993) of the total number of the collected snails. The body is elongated oval in shape. The head organ and eyespots are observed. Minute body spines with a delicate dorso-median finfold, extending from the eyespots to the posterior end, are also found. Four pairs of penetration glands are found on each side of the intestine: two pairs are anterior, and two pairs are posterior; their ducts are in one bundle on each side, penetrating the anterior organ to open at its anterior end. A bulbous swelling can be observed at the end of the esophagus, stained with neutral red. The intestine is undivided, and a saccular shape is observed in the middle of body. The excretory bladder is V shaped and thin walled. One pair of excretory cells was observed at the anterior of the tail stem. The tail is longer than the body and divided into two furcae. The fucral tail stem is shorter than the tail stem, with minute spines along the lateral margins. The tip of each fucra is claw shaped (Fig. 9 and Table 6).

The cercariae develop within rediae. It moved by wavering on the surface of the water. It floated and moved for around 6–10 seconds, and rested for 3–4 seconds. It survived up to 3–4 hours in the water, then encysted.

**Type 5. Furocercous cercariae/Brevifurcate-pharyngeate cercariae**

*Clinostomidae Lühe, 1901*

*Clinostomum giganticum* Agarwal, 1959

The cercariae were found in one *Indoplanorbis exustus* with an infection rate of 0.1% (1/993) of the total number of the collected snails. The body is elongated oval in shape. The head organ and eyespots are observed. Minute body spines with a delicate dorso-median finfold, extending from the eyespots to the posterior end, are also found. Four pairs of penetration glands are found on each side of the intestine: two pairs are anterior, and two pairs are posterior; their ducts are in one bundle on each side, penetrating the anterior organ to open at its anterior end. A bulbous swelling can be observed at the end of the esophagus, stained with neutral red. The intestine is undivided, and a saccular shape is observed in the middle of body. The excretory bladder is V shaped and thin walled. One pair of excretory cells was observed at the anterior of the tail stem. The tail is longer than the body and divided into two furcae. The fucral tail stem is shorter than the tail stem, with minute spines along the lateral margins. The tip of each fucra is claw shaped (Fig. 9 and Table 6).

The cercariae develop within rediae.

**Type 6. Echinostome cercariae**

*Echinostomatidae Looss, 1902*

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

The cercariae were isolated from three *Indoplanorbis exustus* with an infection rate of 0.3% (3/993) of the total number of the collected snails. The body is elongated and pear shaped. Collar spines can be observed around the
Figure 7. Image of *Loxogenes liberum* Seno, 1907 a. Images of cercaria stained with 0.5% neutral red (light microscopy) b. Drawing of cercaria structure c. Images of cercaria stained with 0.5% neutral red (DIC microscopy) d. Images of sporocyst stained with 0.5% neutral red (light microscopy) Abbreviations: os: oral sucker, s: stylet, vi: virgula gland, p: pharynx, pg: penetration gland, eb: excretory bladder, vs: ventral sucker, ta: tail, c: cercaria, sp: sporocyst. (Scale bars: 100 µm).
oral sucker, whereas eyespots are not found. The prepharynx and esophagus are long. The pharynx is large. The bifurcated caeca reach to the posterior end of the body. The relatively large ventral sucker is located approximately at three-fourth of the body length measured from the front. Penetration glands are clearly present, and they lay along the esophagus in the middle of the body. The excretory bladder is large and sac like, and its two main collecting tubes start at the level of the esophagus. The excretory duct is Y shaped, and two excretory pores open at the anterior of the tail stem. The tail is tubular in shape and almost of the same length as the body. The tail finfold is present along the tail stem (Fig. 10 and Table 7).

The cercariae develop within rediae.
Size range and average size (in micrometers, calculated from ten cercariae):

| Component          | Size Range          | Average Size       |
|--------------------|---------------------|--------------------|
| Body               | 110–205 µm (av. 139 µm) × 139–293 µm (av. 217 µm) |
| Oral sucker        | 33–43 µm (av. 39 µm) × 34–45 µm (av. 41 µm)        |
| Pharynx            | 8–14 µm (av. 10 µm) × 9–12 µm (av. 10 µm)         |
| Ventral sucker     | 45–54 µm (av. 49 µm) × 38–56 µm (av. 49 µm)       |
| Excretory bladder   | 30–38 µm (av. 34 µm) × 26–32 µm (av. 29 µm)       |
| Tail               | 44–61 µm (av. 50 µm) × 307–412 µm (av. 367 µm)    |

Cercarial molecular analysis

In this study, we focused on cercariae that cause cercarial dermatitis. The furcocercous cercariae in *Schistosoma indicum* and *S. spindale* (not shown here) were studied using ITS2 sequences. In particular, *S. indicum* identified by morphology and organ characteristics was found within the outbreak areas. The ITS2 gene sequences of *S. indicum* were approximately 300–340

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**Figure 9.** Image of *Clinostomum giganticum* Agarwal, 1959 a., c., d. Images of cercaria stained with 0.5% neutral red (light microscopy) b. Drawing of cercaria structure f. Images of redia stained with 0.5% neutral red (light microscopy) Abbreviations: h: head organ, es: eyespot, i: intestine, pg: penetration gland, fi: dorso-median finfold, eb: excretory bladder, ta: tail, fu: furca, c: cercaria, re: redia. (Scale bars: 100 µm).
Figure 10. Image of *Echinostoma spiniferum* Ahmed, 1959 (sensu Našincová, 1992) a. Images of cercaria stained with 0.5% neutral red (light microscopy) b. Drawing of cercaria structure c. Images of redia stained with 0.5% neutral red (light microscopy) Abbreviations: os: oral sucker, cs: collar spines, pp: prepharynx, p: pharynx, ep: esophagus, i: intestine, pg: penetration gland, eb: excretory bladder, vs: ventral sucker, exp: excretory pore, ta: tail, fi: finfold, c: cercaria, re: redia. (Scale bars: 100 µm).
### Table 4. Some characters of *Loxogenes liberum* found in this study and the reference sources (measurement in µm, n/a = no data).

| Character | Immature cercaria | Cercaria in sporocysts | The cercariae develop within sporocysts | The cercariae develop within sporocysts |
|-----------|-------------------|------------------------|----------------------------------------|----------------------------------------|
| Body      | Cuticle spinose. Extended: 84–170 µm × 50–84 µm, up to 224 × 98 µm Fixed: 90–99 µm × 63–66 µm | oval 143–203 µm × 66–90 µm | oval 65–93 µm (av. 81 µm) × 95–120 µm (av. 108 µm) | oval 48–105 µm (av. 97 µm) × 91–152 µm (av. 117 µm) |
| Stylet    | 17–19 µm × 3–4 µm | 15–23 µm × 5–6 µm | 3–3 µm (av. 3.3 µm) × 10–23 µm (av. 16 µm) | 2–5 µm (av. 4.4 µm) × 10–18 µm (av. 14 µm) |
| Oral sucker | 25–34 µm × 29–39 µm (24–27 µm in mount) | 38–45 µm in diameter | 13–30 µm (av. 24 µm) × 10–28 µm (mean: 20 µm) | 19–27 µm (av. 22 µm) × 15–24 µm (av. 19 µm) |
| Pharynx | - | 15 ×12 µm | very small 5–15 µm (av. 10 µm) × 8–10 µm (av. 8 µm) | 7–15 µm (av. 9.9 µm) × 7–10 µm (av. 8 µm) |
| Prepharynx | - | - | - | - |
| Virgulate gland | 30–40 µm wide | - | - | - |
| Penetration glands | 4 pairs | 4 pairs | - | 4 pairs |
| Ventral sucker | 17–22 µm in diameter (15 µm in mount) | 27–30 µm | 8–33 µm (av. 18 µm) × 13–28 µm (av. 19 µm) | 9–27 µm (av. 20 µm) × 12–26 µm (av. 22 µm) |
| Excretory bladder | V-shaped | V-shaped | V-shaped 13–35 µm (av. 27 µm) × 13–48 µm (av. 37 µm) | V-shaped, thin wall 17–32 µm (av. 22 µm) × 15–25 µm (av. 19 µm) |
| Tail | 50–112 µm × 14–17 µm | 90–180 µm × 20–30 µm | Tail shorter than body, rather slender and spinose at its tip 15–25 µm (av. 20 µm) × 40–90 µm (av. 72 µm) | Tail shorter than body, 11–35 µm (av. 22 µm) × 45–67 µm (av. 56 µm) |
| Flame cell formula | 2[(2+2)+2] = 12 | - | - | - |
| 1st IH snail | Bulinus stramineus japonicus | Bulinus kiochuenis | found from 23 snails Tarebia granifera | found from 14 snails *Bithynia siamensis siamensis* |
| 2nd IH | nymphs of *Orthetrum albistylum* (dragonfly) | nymphs of *Orthetrum albistylum* (dragonfly) | - | - |
| DH | *Rana nigromaculata* | *Rana nigromaculata* (small intestine) | - | - |
| Infection rate | - | 0.15% (23/15,076) | 5.05% (14/277) | - |
| Cercaria behavior | - | - | The cercariae floated on the surface or in the water. They rest by vertical position, the body sank lower than the tail, for 30–40 seconds. Then moved by folding, the tail bend to the body, and moved by swaying forward around 10–15 seconds. They can survive up to 4–5 hours in the water. | - |

### Table 5. Some characters of *Schistosoma indicum* found in this study and the reference sources (measurement in µm, n/a = no data).

| Character | Immature cercaria | Cercaria in sporocysts | This study |
|-----------|-------------------|------------------------|------------|
| Body      | Cuticle spinose. Extended: 117–156 µm × 39–52 µm | 117–156 µm × 39–52 µm | 41–55 µm (av. 48 µm) × 135–152 µm (av. 142 µm) |
| Tail stem | Cuticle spined | 17–36 µm (av. 29 µm) × 225–280 µm (av. 252 µm) |
| Furcal tail | 30–104 µm | 10–21 µm (av. 16 µm) × 80–135 µm (av. 110 µm) |
| 1st IH | *Lymnaea lateralis*, *Indoplanorbis exustus* | *Indoplanorbis exustus* found from 9 *Indoplanorbis exustus* snails, |
| 2nd IH | *Bithynia siamensis siamensis* | *Bithynia siamensis siamensis* (sensu Odhner 1910) | - |
| DH | Kid, goat, sheep | Kid lamb | Cattle, Goat (believed) |

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The phylogenetic tree obtained from neighboring analysis was rooted with Angiostrongylus cantonensis (MT135083). Three S. indicum cercariae (ON417732–ON417734) were grouped with S. indicum from GenBank (ON597438, KF425714), and S. spindale (ON417736) were grouped with S. spindale from GenBank (ON597444–ON597446), showing their close relationships in the indicum group and distinct difference from S. haematobium (L03656), which is a human Schistosoma (Fig. 11 and Table 8).

Thus, the trematode infection in the study area was confirmed by morphological and molecular observations. Therefore, the cercarial dermatitis outbreak was due to ruminant parasites, viz. Schistosoma indicum, with Indoplanorbis exustus being its intermediate snail host.
Table 7. Some characters of *Echinostoma spiniferum* found in this study and the reference sources (measurement in µm, n/a = no data).

| Echinostomatidae Looss, 1902 | Echinostomatinae Looss, 1899 | Echinoparyphium spiniferum Ahmed, 1959 | Echinostoma spiniferum sensu Nasirivçav, 1992 |
|-----------------------------|-----------------------------|---------------------------------|----------------------------------|
| *Echinostoma revolutum* Froelich, 1882 | *Echinostoma cercariae* | *Echinoparyphium spiniferum* Ahmed, 1959 | *Echinostoma spiniferum* sensu Nasirivçav, 1992 |
| Tubangui (1932) | Beaver (1937) | Johnson and Angel (1941) | Voetsevchokui et al. (2018) | Ahmed (1959) | Falťynková (2005) | This study |

- **Collar spines**
  - Cephalic collar 90–125 µm and 37 spines (10.4–12.5 × 2–3 µm) in to alternate rows.
  - 37 collar spines, 5 are on each ventral lappet, 6 each laterally, 15 dorsally, in 2 alternate rows.
  - 37 collar spines, 5 ventral, 6 laterials, 15 dorsals.

- **Pharynx**
  - 22–27 µm, long
  - Prepharynx is long

- **Esophagus**
  - 95–145 µm
  - Esophagus is shorter than the prepharynx

- **Intestine**
  - Bifurcated in front of acetabulum, ceca extending to near posterior extremity.

- **Body**
  - Body spine 330–520 × 150–250 µm
  - Body spine 95 × (fix specimen).

- **Oral sucker**
  - 54–62 × 50–62 µm

- **Ventral sucker**
  - 58–75 × 62–83 µm

- **Penetration glands**
  - 6 pairs
  - Two rows of 4 each, in region of prepharynx; their narrow ducts opening at anterior end of body.

- **Excretory bladder**
  - Excretory vesicle divided into a small anterior and a large posterior compartment.

- **Tail**
  - 400–480 × 37–50 µm
  - 450 µm with a distinct dorsal finfold, Tail tubule inverted T-shaped.

- **Pharynx**
  - 25–30 × 20–27 µm
  - Large, 13–18 µm (av. 14 µm) × 20–30 µm (av. 24 µm)

- **Esophagus**
  - 95–145 µm
  - Esophagus is shorter than the prepharynx

- **Intestine**
  - Bifurcated in front of acetabulum, ceca extending to near posterior extremity.

- **Body**
  - Body spine 390–450 × 150–230 µm
  - Body spine 95 × (fix in formalin).

- **Oral sucker**
  - 51–56 × 45–60 µm

- **Ventral sucker**
  - 58–75 × 62–83 µm

- **Penetration glands**
  - 6 pairs
  - Two rows of 4 each, in region of prepharynx; their narrow ducts opening at anterior end of body.

- **Excretory bladder**
  - Excretory vesicle divided into a small anterior and a large posterior compartment.

- **Tail**
  - 400–480 × 37–50 µm
  - 450 µm with a distinct dorsal finfold, Tail tubule inverted T-shaped.

- **Pharynx**
  - 22–27 µm, long
  - Prepharynx is long

- **Esophagus**
  - 95–145 µm
  - Esophagus is shorter than the prepharynx

- **Intestine**
  - Bifurcated in front of acetabulum, ceca extending to near posterior extremity.

- **Body**
  - Body spine 330–520 × 150–250 µm
  - Body spine 95 × (fix specimen).

- **Oral sucker**
  - 54–62 × 50–62 µm

- **Ventral sucker**
  - 58–75 × 62–83 µm

- **Penetration glands**
  - 6 pairs
  - Two rows of 4 each, in region of prepharynx; their narrow ducts opening at anterior end of body.

- **Excretory bladder**
  - Excretory vesicle divided into a small anterior and a large posterior compartment.

- **Tail**
  - 400–480 × 37–50 µm
  - 450 µm with a distinct dorsal finfold, Tail tubule inverted T-shaped.

- **Pharynx**
  - 25–30 × 20–27 µm
  - Large, 13–18 µm (av. 14 µm) × 20–30 µm (av. 24 µm)

- **Discussion**

Although our study focused on identifying the parasitic species that cause cercarial dermatitis in the outbreak areas, the results of this study show several important findings. Based on data of average annual rainfall for 2020 and 2021 of Songkhla Province from the Southern-East Coast Meteorological Center (Thai Meteorological Department, Ministry of Digital Economy and Society), the outbreak falls during the rainy season and rice cultivation in the study area. Between August and October 2020, the amount of rainfall was 245.1 mm in August 2020, 146.8 mm in September 2020, and 285.6 mm in October 2020. During the collection time in December 2020, September 2021, and October 2021, the rainfall was 624.9, 148.1, and 95.8 mm, respectively.

Cercarial dermatitis occurs as an emerging and re-emerging infectious disease, normally found in people engaged in water activities such as farmers, fishermen, and agricultural workers (Chamot et al. 1998; Verbrugge et al. 2004; Bauri et al. 2015). In addition, during this time of the season, many migratory birds were observed in the outbreak areas. Therefore, cercarial dermatitis could have been due to avian and mammalian blood flukes. The incidence of bird schistosomes and cercarial dermatitis is considered as an emerging disease worldwide, particu-
larly in cases involving the cercariae of *Trichobilharzia*. Furthermore, snails of Lymnaeidae, Physidae, and Planorbidae are often found to be the intermediate host of bird schistosomes (Horák et al. 1999, 2010, 2015; Brant and Loker 2009; Marszewsk et al. 2018). Although no reports of human cercarial dermatitis caused by avian schistosomes have been found in Thailand, infected ducks occurred in a pond in Kalasin Province with infected *Radix (Lymnaea) rubiginosa* (Kruatrachue et al. 1968). In addition, six *R. (L.) rubiginosa* from freshwater reservoirs in Phayao Province, north Thailand, were infected with *T. regenti* (Japa et al. 2021).

Moreover, three species of mammalian blood flukes were reported from the northeast and north of Thailand, first in 1967, where infected ducks occurred in a pond in Kalasin Province with infected *Radix (Lymnaea) rubiginosa* (Kruatrachue et al. 1968). In addition, six *R. (L.) rubiginosa* from freshwater reservoirs in Phayao Province, north Thailand, were infected with *T. regenti* (Japa et al. 2021).

Moreover, three species of mammalian blood flukes were reported from the northeast and north of Thailand, first in 1967, where infected ducks occurred in a pond in Kalasin Province with infected *Radix (Lymnaea) rubiginosa* (Kruatrachue et al. 1968). In addition, six *R. (L.) rubiginosa* from freshwater reservoirs in Phayao Province, north Thailand, were infected with *T. regenti* (Japa et al. 2021).
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The case of one woman discovered with schistosome infection in a subcutaneous nodule of her jaw. They reported three species of Schistosoma, S. japonicum, and S. indicum infections of wild rats in Thailand. The other rodent schistosome that was reported in Thailand is S. sinensium, with Tricula bollingi being the snail intermediate host (Baidkul et al. 1984). Furthermore, many reports are found in adjacent counties of Thailand, indicating the outbreak of cercarial dermatitis in humans in Malaysia, with six species of schistosome: two belong to birds (Trichobilharzia brevis and Psuedobilharziella longchurae), three belong to mammals (Schistosoma spindale, S. nasale, and S. incognitum), and one belongs to humans (S. malayensis) (Buckley 1938; Basch 1966; Fischthal and Kuntz 1973; Lee et al. 1986; Greer et al. 1988; Krishnasamy et al. 2003). For ruminant schistosomes, Schistosoma spindale, S. indicum, S. nasalis, and S. japonicum are the most prevalent causes of visceral schistosomiasis among bovines. Cercariae of S. spindale can cause not only pathology in animals but also dermatitis in humans in Asia, with freshwater snail Indoplanorbis exustus being the major source of infection. In addition, rodents were proven to be susceptible to infections with S. spindale (Kruatrachue and Harinasuta 1963, 1964; Kruatrachue et al. 1964; Bunnag et al. 1986; Inder Singh et al. 1997; Nithiuthai et al. 2004; Lakshmanan et al. 2016).

In our present study, two of the six collected snail species were infected with six trematode species, with Indoplanorbis exustus and Bithynia s. siamensis being the most abundant snails in the study areas. In the former pulmonate bulinine snail, three species of cercariae were found on the basis of morphological identification, viz. Schistosoma indicum, Clinostomum giganticum, and Echinostoma spiniferum. In this study, we suggested that S. indicum was a ruminant parasite that caused the outbreak of cercarial dermatitis. Furthermore, we reported on the discovery of two more trematode species in I. exustus, viz. C. giganticum and E. spiniferum, which infected birds and were found in the oral cavity and intestine of the host. Clinostomum (Digenea, Clinostomidae) is a cosmopolitan genus of digenetic trematode, with its life cycle requiring two intermediate hosts (snail and fish or frog) and one definitive host (bird). Adult flukes live in the digestive tract, esophagus, pharynx, and/or mouth of fish-eating birds (Osborn 1911, 1912; McAllister et al. 2010; Calhoun et al. 2019). Helisoma, a freshwater air-breathing snail, or pulmonate gastropod of Planorbidae and Lymnaeidae (Radix) were commonly reported as hosts.

In freshwater fish as the secondary intermediate host, metacercariae of Clinostomum cause “yellow-spot disease/yellow grubs.” Such spots result from encystment below the integumentary tissue, causing visible nodular swelling. They are common in the caudal, dorsal, and pectoral fins; on the inside surface of the operculum; and in the flesh. The metacercariae can live within the host for several years until eaten by a bird host. Humans may be infected with the parasite when eating raw or undercooked fish meat carrying the metacercarial stage. Therefore, this trematode must be considered not only for its losses in production and discards of fish, but also for its zoonotic potential (Hunter and Hunter 1935; Esch et al. 2001; Wang et al. 2017; Rossier et al. 2018; Sohn et al. 2019; de Souza et al. 2020; Won et al. 2020).

Cercariae of the intestinal fluke Echinostoma spiniferum isolated from planorbid snails in our present report may be introduced to the area in question because of a diverse spectrum of migrating birds, as bird hosts generally serve as the main source of infection for snails (Chai et al. 2011; Fältýnková et al. 2015). Našincová (1992) described E. spiniferum flukes armed with 37 collar spines on their head collar as Echinostoma revolutum, being a large group of collar-spined species Echinostomatidae that naturally infect birds in Europe and Asia. In the present study, given the double infection of S. indicum and E. spiniferum found in I. exustus, we reported a new record for Thailand for the first time.

Two species of freshwater snail belonging to the family Bithyniidae, viz. Bithynia funiculara and B. siamensis, have been reported to serve as the first intermediate host of human liver fluke (Opisthorchis viverrine), particularly two subspecies, namely, B. s. siamensis and B. s. goniomphalos (Wykoff et al. 1965; Harinasuta et al. 1984; Brockelman et al. 1986; Waikagul 1998; Sri-aroon et al. 2005). Additionally, ten other snail species of the Bithyniidae were recorded during October 2008 to July 2009 from various regions in Thailand: B. funiculara and Gabbia pygmaea in the north; B. s. goniomphalos, Wattenbladia siamensis, and W. crosseana in the northeast; B. s. siamensis, Hydrobioides nassa, and G. wykoffi in the central; and W. baschi and G. erawan in the south and at Erawan waterfall (Kanchanaburi). For B. s. goniomphalos, seven types of cercariae were reported, viz. amartae xiphidiocercariae, virgulate xiphidiocercariae, xiphidiocercariae, amphistome cercariae, furcocercariae, monostome cercariae, and parapleurolophocercous cercariae, whereas B. s. siamensis have been infected with monostome cercariae and virgulate xiphidiocercariae (Adam et al. 1993; Nithiuthai et al. 2002; Sri-aroon et al. 2005; Kul-sanitiwong et al. 2015). In our study, B. s. siamensis were found to be infected with three species of trematodes, viz. amphistome cercariae (Gastrothylax crumenifer), parapleurolophocercous cercariae (Astistrema monticelli), and virgulate xiphidiocercariae (Loxogenes liberum). G. crumenifer is a blood-sucking ruminant parasite, belonging to the family Paramphistomatidae; the infection causes anemia and accidental death of ruminant animals, which is a major health problem of domestic animals.

Furthermore, in Thailand, three snail species have been infected with trematode, viz. one bithyniid snail (B. s. siamensis) and two thiarid snails (Melanoides tuberculata and Tarebia granifera) (Shevcheko and Vergin 1960; Apiraksena 2014). M. tuberculata has been reported as the intermediate host of this amphistome trematode in Thailand (Krailas et al. 2014). Astistrema monticelli is a parapleurolophocercous cercariae that was recorded from...
Bithynia leachi as the first intermediate host in Russia. The genus Astotrema, which belongs to the Plagiocchiidae, represent trematodes infecting a wide range of fishes, amphibians, and reptilians. Many reports have indicated that Astotrema (sensu stricto) is widely spread throughout Asia, Europe, and Africa, particularly in northeastern Africa (Egypt, Sudan), eastern Asia (China, southern Korea), southern Asia (India, Pakistan), southeastern Asia (Myanmar), northern Asia (Russia), and central Europe (Poland) (Karar et al. 2021). However, several authors were unclear as to the diagnostic features of Astotrema used, which are considered to be highly variable. They are either not clear in some cases or poorly described in others, leading to confusion whether these features characterize specimens of the same species or closely related species. In this study, the cercariae were classified whether or not they belong to A. monticellii based on the cercarial morphology. The last species represented in this study, Loxogenes liberum, was categorized by morphological characteristics of cercariae, which emerged from B. s. siamensis. Virgulate xiphidiocercariae belongs to Lecithohordidiidae, for which many reports have indicated Bulinus striatutus japonicus, B. kiushuensis, and thiarid Tarebia granifera being the first intermediate host. Arthropods (insects/crustacea) serve as the second intermediate host, whereas amphibians, birds, and mammals serve as the final host (Okabe 1937; Yamaguti 1937, 1938; Veeravechsukij et al. 2018).

Molecular phylogenetics

Our molecular analysis revealed the presence of the emerging cercariae of Schistosoma indicum and S. spindale (for the latter specimens from Surat Thani Province in south Thailand, unpublish to date) based on the largest similarity to ITS2 ribosomal DNA sequences to those of S. indicum and S. spindale from GenBank and clear distinction from S. haematobium (Michot et al. 1993) and S. japonicum (Bowles et al. 1993). Sequence analysis of ITS2 rDNA among S. indicum cercariae (ON417732–ON417734) includes clustering with S. indicum (ON597438, Anisuzzaman and Hasan 2022; KF425714, Bindu et al. 2013) and S. spindale (ON417736, in this study; ON597444–ON597446, Anisuzzaman and Hasan 2022) sequenced from GenBank. Most ribosomal DNA sequence information of S. indicum and S. spindale revealed similarity of up to 93%–95% among species of conserved regions. In the present study, both schistosome species from Thailand cluster with freshwater mammalian schistosomes, although the ITS2 gene is an informative DNA marker utilized for population genetic and phylogeographic studies in animals (Littlewood et al. 2006; Horák et al. 2015). Thus, the indicum group is hardly separated from each other by only ribosomal DNA analysis. Our study presents the first molecular evidence of S. indicum ITS2 rDNA sequences. In addition, the sequence data generated here are the first S. indicum DNA sequences from Thailand, which will be useful for further genetic study of the other blood flukes in this region.

A phylogenetic tree was constructed to assess the genetic relationship between S. indicum from Songkhla and S. spindale from Surat Thani (not show here). We identified S. indicum and S. spindale by morphology and compared with S. spindale and S. indicum from Bangladesh and S. indicum from India, based on ITS rDNA sequence. This study demonstrated that the detected Schistosoma cercariae were closely related to S. spindale, which are often found in outbreaks of cercarial dermatitis caused by schistosomula that die in the human skin. This occurrence of S. indicum and S. spindale implies the spread of cattle blood fluke cercariae in aquatic environments. The study of intermediate host and definitive host in the outbreak area are important for the control program of snail-borne disease. In addition, the populations of snails fluctuate on the basis of rainfall, with the snail populations potentially spreading and surviving after flooding. Notwithstanding, the snail population may decline as a result of heavy rains in the rainy season, which cause flushing of snail habitats. However, they can resettle and subsequently migrate and begin to reproduce, reaching a carrying capacity of the new environment within a few months. Furthermore, many factors are involved in the trematode infection of snail hosts, with prevalence usually varying among different geographical localities, density of snail population, capacity of reservoir and human hosts, water quality, temperature, and rainfall (Upatham et al. 1983; Nithiuthai et al. 2002; Sri-aroon et al. 2005).

Conclusion and outlook

The results of our study provide insights into the infection and distribution of snails involved in disease outbreaks, which can be used in introducing future control strategies. These studies of snail-borne infections are based on long-term efforts in surveying the malacoafauna, for example, in Thailand, combined with systematically screening for cercariae of infectious trematodes and other parasites. Snail-borne schistosomiasis remains a serious debilitating disease affecting humans and animals in many regions of the world. Thus, comprehensive understanding on the basic biology, biodiversity, host-parasite relationship, and evolutionary associations of parasitic trematodes is necessary. Although our study provides new insight into the occurrence of Schistosoma species in the respective outbreak areas in Thailand, integrating all available knowledge on the status of intermediate hosts, definitive hosts, and epizootiology of human and animal schistosomiasis in the context of any control efforts is necessary.

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