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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; Kolářǒvá 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 and salt-water 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. sinensium*, and *S. ovuncatum*. The *S. mansoni* group was originally distributed in Africa; at present, it can be found in Southwest Asia and South America, which includes *S. mansoni*, *S. rodhaini*, *S. hippopotami*, and *S. edwardiense*. 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 planorbid or lymnaeid pulmonated 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 Kolářǒvá 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

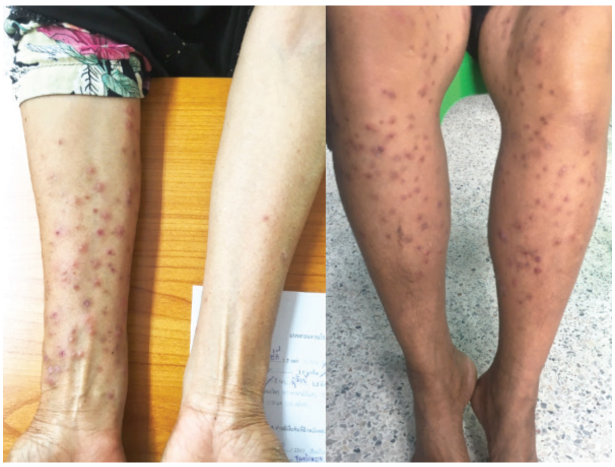

Figure 1. Cercarial dermatitis cases contracted from Chana district in October 2020.

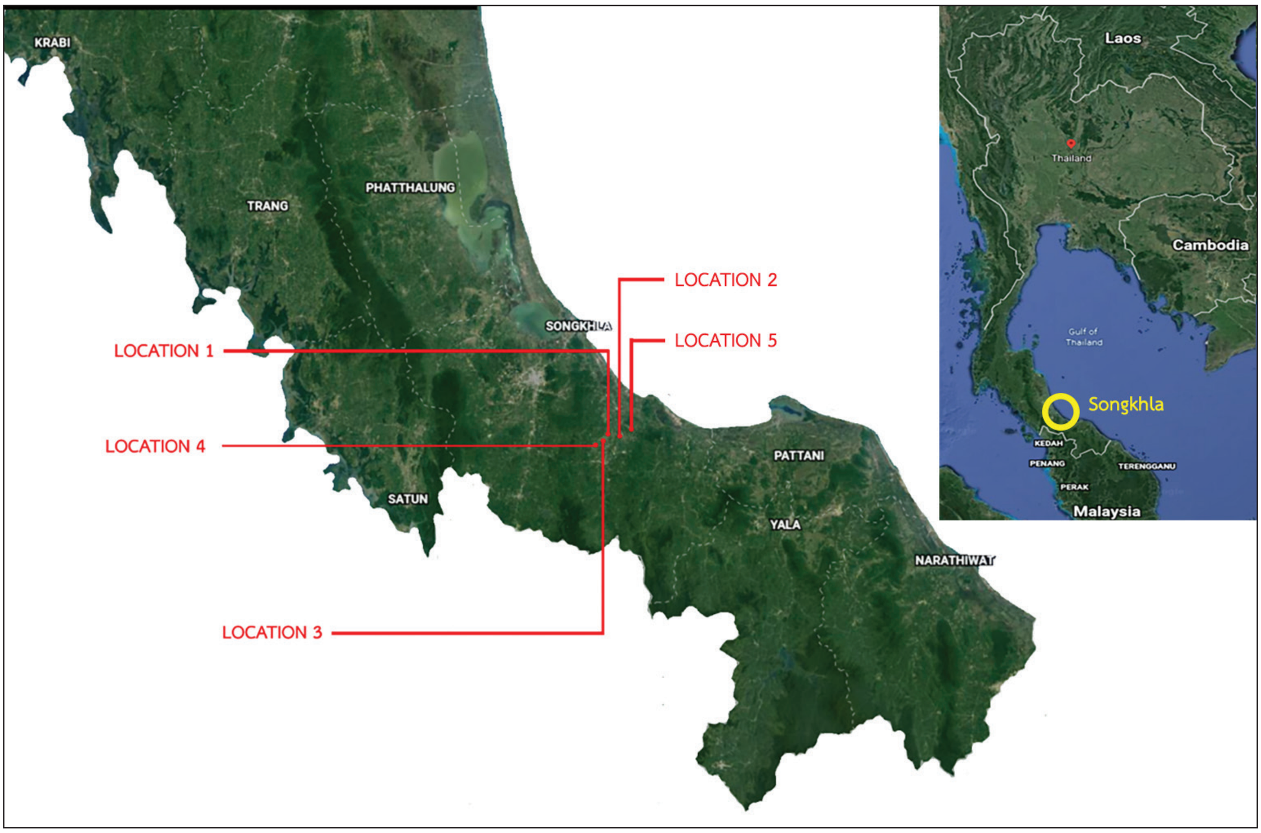

Figure 2. Map showed the five locations of Chana district, Songkhla province, south Thailand.

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)	-	-	-
			Bithynia s. siamensis (54)	2	3.70	*Gastrothylax crumenifer*
				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°48'86.3"N, 100°40'84.0"E Alt. 13 m	*Indoplanorbis exustus* (117)	1	0.85	*Schistosoma indicum* + *Echinostoma spiniferum* (double infection)
				1	0.85	*Schistosoma indicum*
				2 (c)	1.71	*Echinostoma spiniferum*
			Bithynia s. siamensis (112)	1	0.89	*Gastrothylax crumenifer*
				2	1.79	*Astiotrema monticellii*
				10	8.93	*Loxogenes liberum*
			Pomacea canaliculata (34)	-	-	-
			Filopaludina m. cambodjensis (3)	-	-	-
			Filopaludina s. polygramma (1)	-	-	-
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°50'99.1"N, 100°44'11.7"E Alt. 11 m	*Indoplanorbis exustus* (179)	1(c)	0.6	*Clinostomum giganticum*
				3	1.68	*Schistosoma indicum*
			Bithynia s. siamensis (17)	1	5.88	*Astiotrema monticellii*
			Pomacea canaliculata (1)	-	-	-
			Filopaludina s. peninsularis (4)	-	-	-
			Total collected snails (993)	35	3.52	5 species of cercariae

*c = crushing.

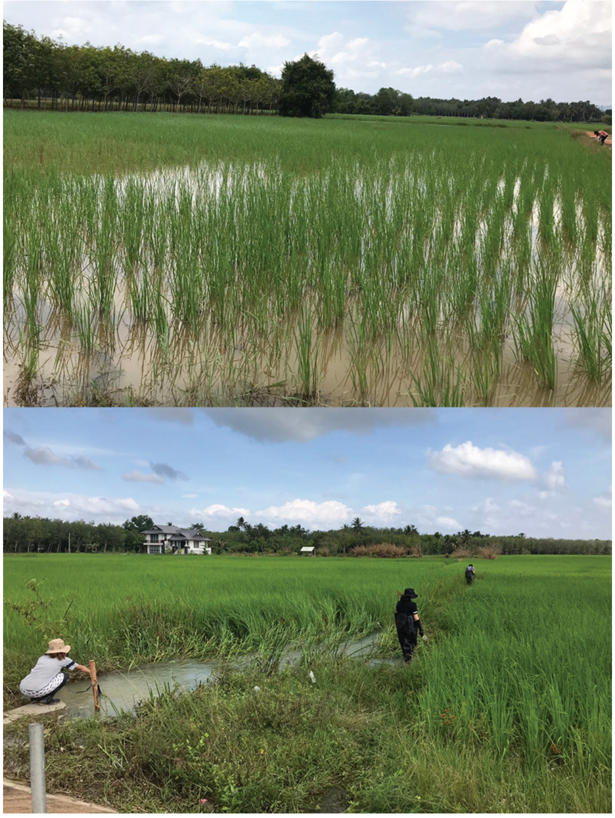

Figure 3. Locations and field study in outbreak areas.

kept in aeration tanks and brought to the Parasitology and Medical Malacology Research Unit, Department of Biology, Faculty of Science, Silpakorn University, Nakhon Pathom. Then, they were identified by their shell morphology based on Brandt (1974) and Upatham et al. (1983).

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), Krailas 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 $MgCl_2$ (1.5 mM of $MgCl_2$), 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 ddH_2O. 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*, 586 *Indoplanorbis exustus*, 290 *Bithynia siamensis siamensis*,

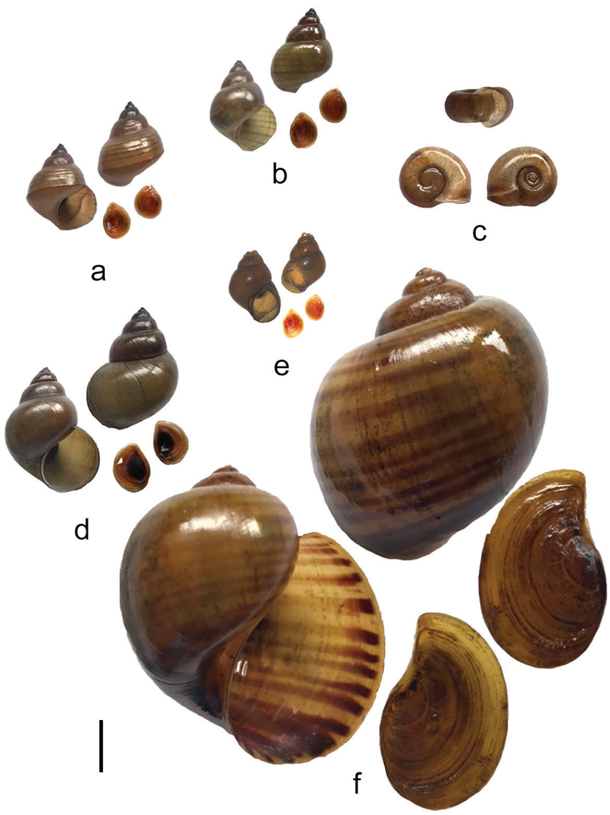

Figure 4. Collected snails from five locations of cercarial dermatitis outbreak area. **a.** *Filopaludina s. peninsularis* **b.** *Filopaludina s. polygramma* **c.** *Indoplanorbis exustus* **d.** *Filopaludina m. cambodjensis* **e.** *Bithynia s. siamensis* **f.** *Pomacea canaliculata* (Scale bar: 1 cm).

and 87 *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-pharyngeate 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. giganticum*, 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; Krailas 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. Parapleurolophocercous 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 finflod 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)

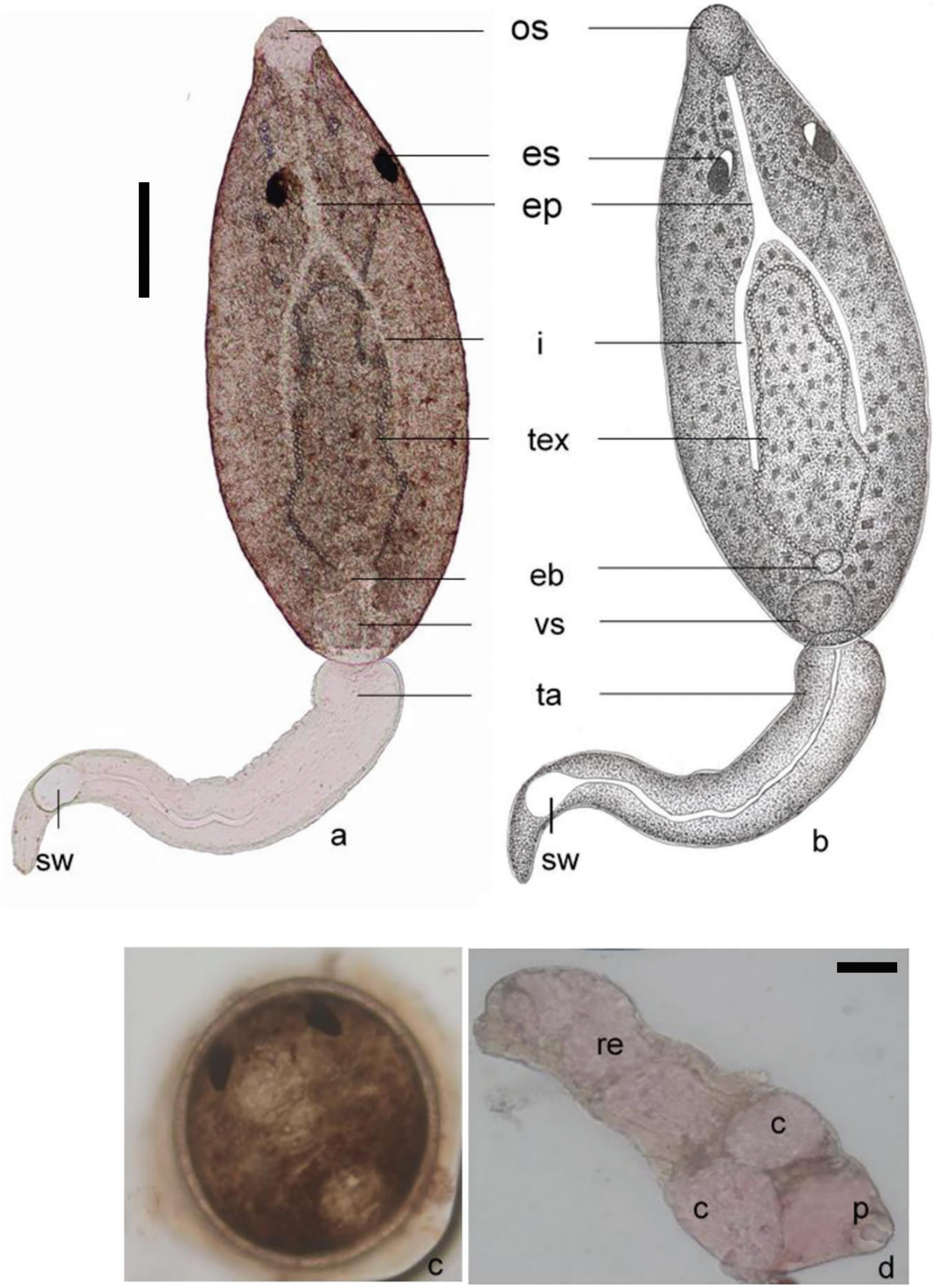

Figure 5. Image of *Gastrothylax crumenifer* Creplin, 1847 **a.** Images of cercaria stained with 0.5% neutral red (light microscopy) **b.** Drawing of cercaria structure **c.** Images of metacercaria stained with 0.5% neutral red (light microscopy) **d.** Images of redia stained with 0.5% neutral red (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).

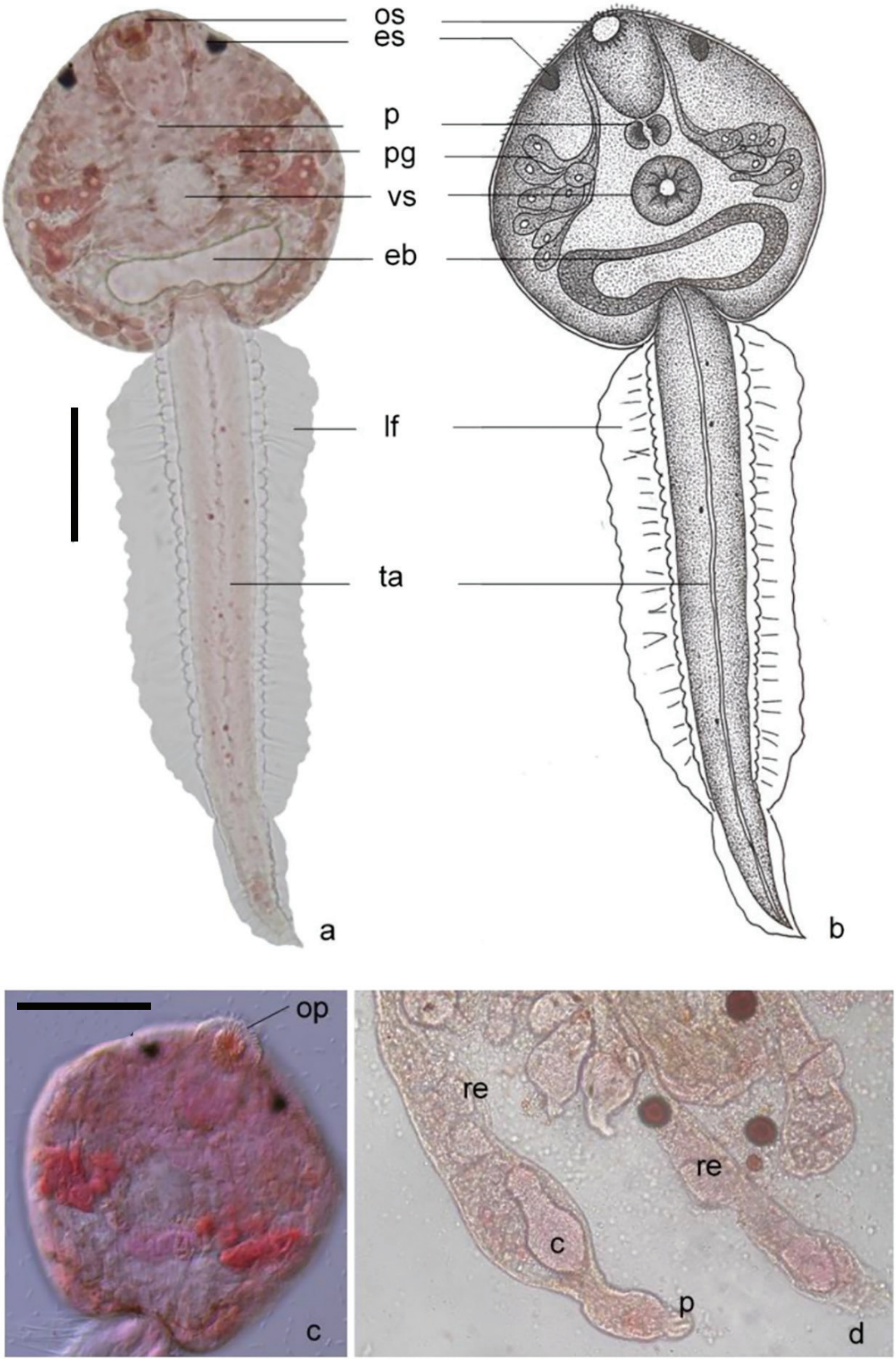

Figure 6. Image of *Astiotrema monticellii* Stossich, 1904 **a.** Images of cercaria stained with 0.5% neutral red (light microscopy) **b.** Drawing of cercaria structure **c.** Body part of cercaria stained with 0.5% neutral red (DIC microscopy) **d.** Images of rediae stained with 0.5% neutral red (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).

	Gastrothylacidae Stiles & Goldberger, 1910		Gastrothylacinae Stiles & Goldberger, 1910	*Gastrothylax crumenifer* Creplin, 1847
	Amphistome cercariae / Cercaria characters			
	Peter and Srivastava (1961)	Tandon (1957)	Krailas et al. (2014)	This study
Immature cercaria	-	Liberated from redia	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, brow 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	n/a	8–10 µm (av. 10 µm) × 8–12 µm (av. 11µm)	n/a
Esophagus	n/a	90–160 µm long	80–140 µm (av. 125 µm) × 80–140 µm (av. 125µm)	n/a
Intestine	n/a	The ceca ended 0.14–0.17 mm away from the posterior end of the body	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 355 µm diameter	-	*Encysted on the plastic container after cercariae shaded from snails 3–4 hours.*
DH	goat kid, buffalo calf	goat kid	-	-
Cercaria behavior	n/a	n/a	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 virgulate 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 esophagus 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):

Body	48–105 µm (av. 97 µm) × 91–152 µm (av. 117 µm)
Stylet	2–5 µm (av. 4 µm) × 10–18 µm (av. 14 µm)
Oral sucker	19–27 µm (av. 22 µm) × 15–24 µm (av. 19 µm)
Pharynx	7–15 µm (av. 9.9 µm) × 7–10 µm (av. 8 µm)
Ventral sucker	8–33 µm (mean: 18 µm) × 13–28 µm (mean: 19 µm)
Excretory bladder	17–32 µm (av. 22 µm) × 15–25 µm (av. 19 µm)
Tail	11–35 µm (av. 22 µm) × 45–67 µm (av. 56 µm)

Type 4. Furococercous cercariae/Brevifurcate-apharyngeate cercariae

Family Schistosomatidae Looss, 1899
Schistosoma indicum Montgomery, 1906 (Syn. *S. nasalis* 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 esophagus 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):

Head organ	23–33 µm (av. 29 µm) × 26–36 µm (av. 31 µm)
Body	41–55 µm (av. 48 µm) × 135–152 µm (av. 142 µm)
Ventral sucker	18–37 µm (av. 26 µm) × 17–26 µm (av. 21 µm)
Tail	17–36 µm (av. 29 µm) × 225–286 µm (av. 252 µm)
Furcal tail	10–21 µm (av. 16 µm) × 80–135 µm (av. 110 µm)

Table 3. Some characters of *Astiotrema monticellii* found in this study and the reference sources (measurement in µm, n/a = no data).

	Plagiorchiidae Lühe, 1901	Astiotrematinae Baer, 1924	*Astiotrema monticellii* Stossich, 1904
	Paraplerolophocercous cercaria / Cercaria characters		
	Shevchenko and Vergin (1960)	Apiraksena (2014)	This study
Immature cercaria	The cercariae develop within rediae.	The cercariae develop within rediae.	The cercariae develop within rediae.
Body	Spherical or pyriform, with extended posterior end: 0.373 mm × 0.09–0.36 mm Contracted: 0.28 mm × 0.27 mm Yellow pigment granules present in subcuticular layer	76–115 µm (av. 96 µm) × 100–175 µm (av. 133 µm)	68–112 µm (av. 86 µm) × 146–229 µm (av. 192 µm)
Eyespots	1 pair At level of posterior end of oral sucker	1 pair with pigment	1 pair with pigment 7–9 µm (av. 7 µm) × 6–9 µm (av. 8 µm)
Prepharynx	very short	short	n/a
Pharynx	18 µm × 22 µm	8–20 µm (av. 14 µm) × 9–23 µm (av. 17 µm)	present
Esophagus	sigmoid	n/a	n/a
Intestine	Bifurcating into short ceca	n/a	n/a
Penetration glands	2 groups (two rows) 9 pairs	9 pairs	8–9 pairs
Oral sucker	50–67 µm × 50–54 µm	35–45 µm (av. 39 µm) × 32–44 µm (av. 38 µm)	25–31 µm (av. 27 µm) × 22–53 µm (av. 40 µm)
Oral spine	Spinose anterior	-	present
Ventral sucker	36–40 µm in diameter (pre-equatorial)	22–28 µm (av. 25 µm) × 22–34 µm (av. 30 µm)	25–43 µm (av. 29 µm) × 23–53 µm (av. 30 µm)
Excretory bladder	Roughly Y shaped	20–35 µm (av. 29 µm) × 55–80 µm (av. 64 µm)	Thick wall 30–67 µm (av. 45 µm) × 23–47 µm (av. 30 µm)
Tail	Tail straight, muscular, 558 µm × 45 µm	28–40 µm (av. 34 µm) × 225–310 µm (av. 254 µm)	55–96 µm (av. 67 µm) × 346–564 µm (av. 442 µm)
Lateral finfold	22 µm wide, extending backward whole length of tail on both sides.	long	16–30 µm (av. 24 µm) × 146–213 µm (av. 185 µm)
Dorsal- ventral finfolds	n/a	present	present
1st IH (snail)	*Bithynia leachi*	*Bithynia siamensis siamensis* 30 (91) 32.98% *Melanoides tuberculata* 17 (37) 45.95% *Tarebia granifera* 16 (33) 48.48%	found from 5 snails *Bithynia siamensis siamensis*
2nd IH	*Pelobates fuscus* (88%) *Rana terrestris* (61.3%) *Hyla arborea* (81.3%) *Rana ridibunda* (8.8%)	n/a	n/a
DH	Reptile: *Natrix natrix* (The grass snake) 97% Karkov region, *Vipera berus* 23%	n/a	n/a
Cercaria behavior	n/a	The cercaria floated on the surface or in the water. It moved by wavering on the surface of the water for around 6–10 seconds,	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.
Metacercaria	n/a	78.5–82.5 µm (av. 79 µm) × 80.5–85.0 µm (av. 82 µm)	n/a

Type 5. Furococercous 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 furcal tail stem is shorter than the tail stem, with minute spines along the lateral margins. The tip of each furca is claw shaped (Fig. 9 and Table 6).

The cercariae develop within rediae.

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

Head organ	18–47 µm (av. 27 µm) × 33–59 µm (av. 45 µm)
Body	34–88 µm (av. 45 µm) × 129–203 µm (av. 159 µm)
Eyespot	6–12 µm (av. 9 µm) × 7–10 µm (av. 8 µm)
Excretory bladder	11–27 µm (av. 19 µm) × 12–32 µm (av. 20 µm)
Tail	15–52 µm (av. 38 µm) × 157–339 µm (av. 242 µm)
Furcal tail	11–35 µm (av. 16 µm) × 85–214 µm (av. 115 µm)

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

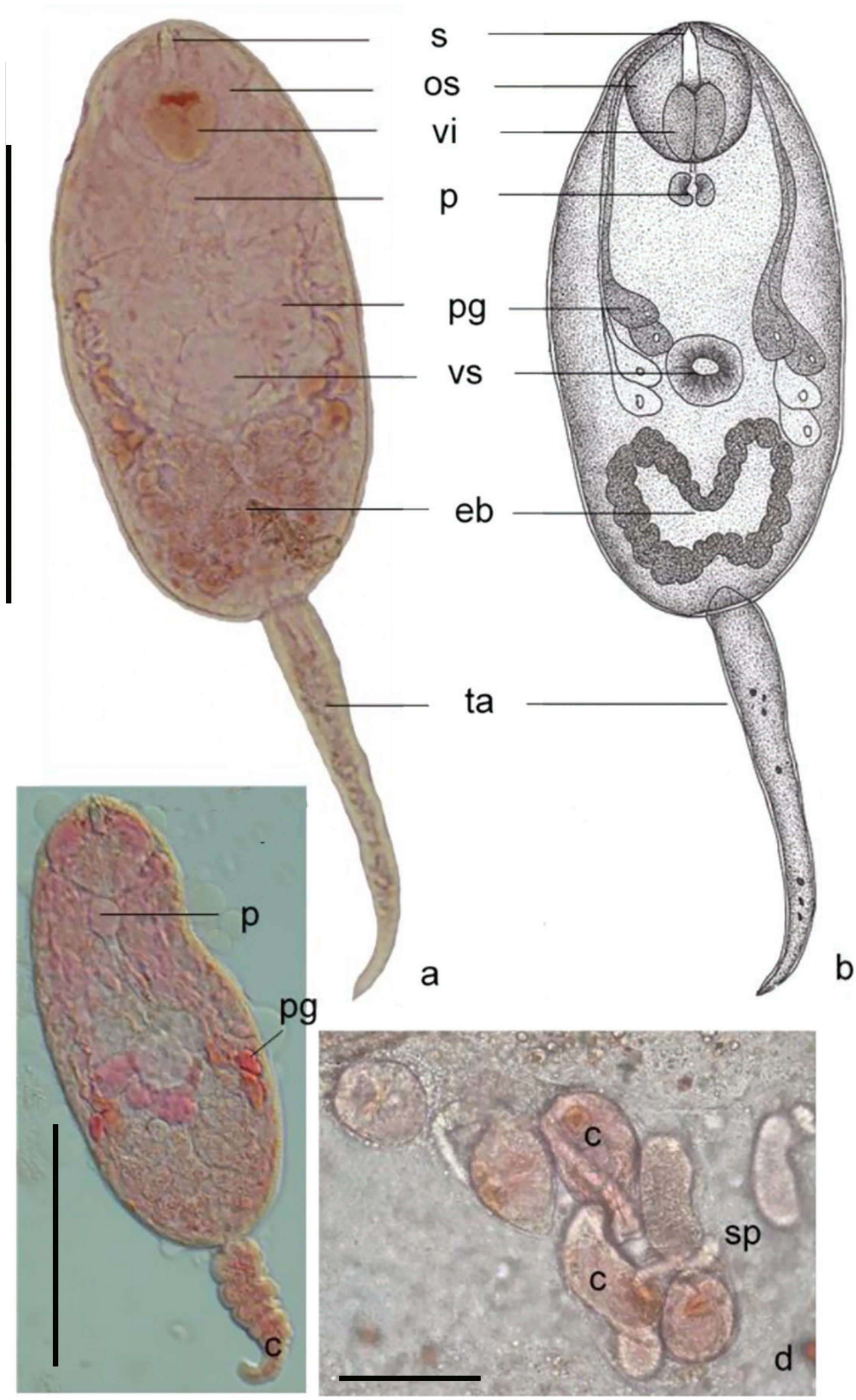

Figure 7. Image of *Loxogenes liberum* Seno, 1907 **a.** Images of cercaria stained with 0.5% neutral red (light microscopy) **b.** Drawing of cercaria structure **c.** Images of cercaria stained with 0.5% neutral red (DIC microscopy) **d.** Images of sporocyst stained with 0.5% neutral red (light microscopy) 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).

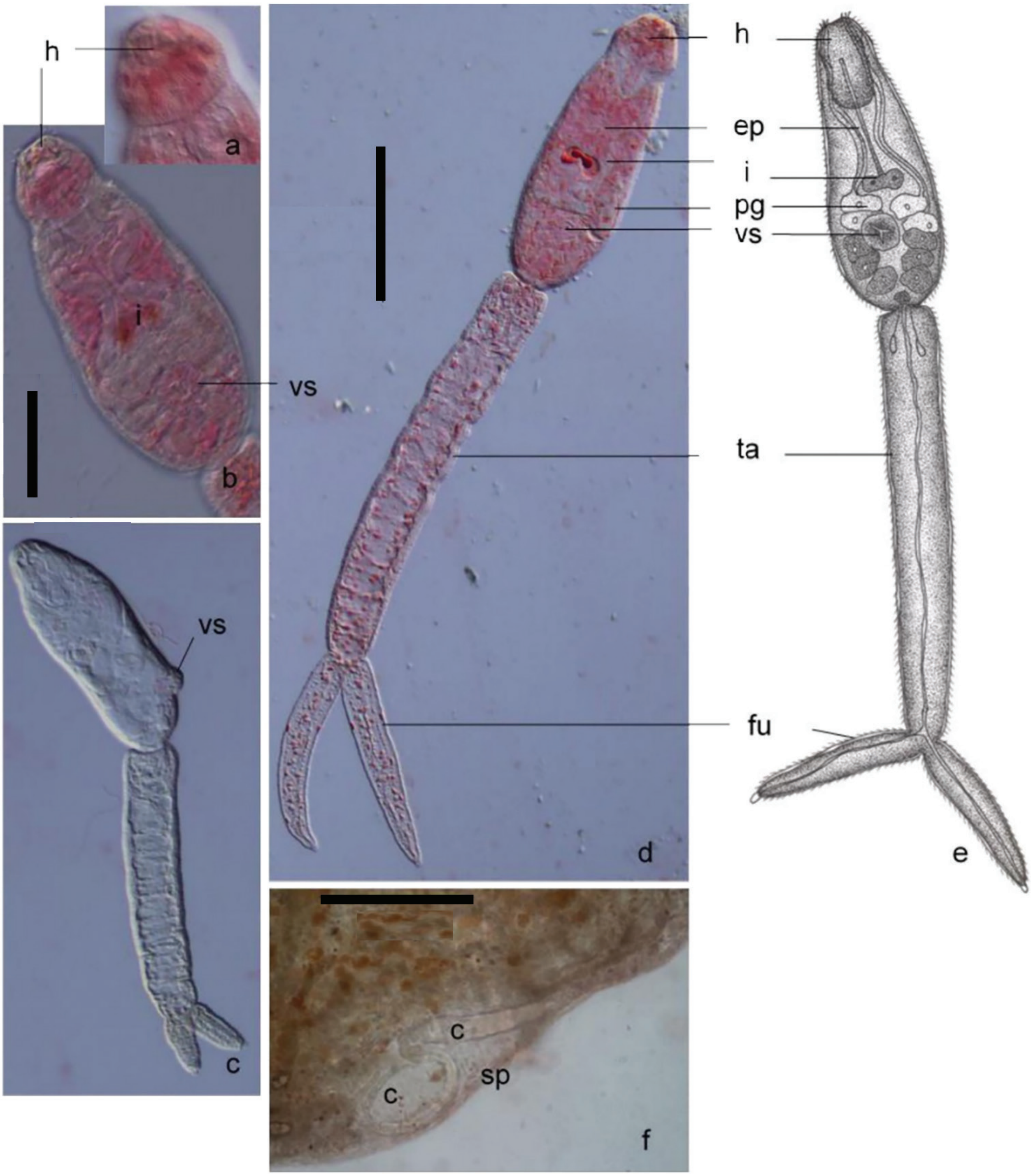

Figure 8. Image of *Schistosoma indicum* Montgomery, 1906 (Syn. *S. nasalis* Rao, 1933) **a.** Head organ of cercaria stained with 0.5% neutral red (DIC microscopy) **b.** Body part of cercaria stained with 0.5% neutral red (DIC microscopy) **c.** Image of unstained cercaria (DIC microscopy) **d.** Images of cercaria stained with 0.5% neutral red (DIC microscopy) **e.** Drawing of cercaria structure **f.** Images of sporocyst stained with 0.5% neutral red (light microscopy) Abbreviations: c: cercaria, eb: excretory bladder, ep: esophagus, fu: furca, h: head organ, i: intestine, pg: penetration gland, sp: sporocyst, ta: tail, vs: ventral sucker.. (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):

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

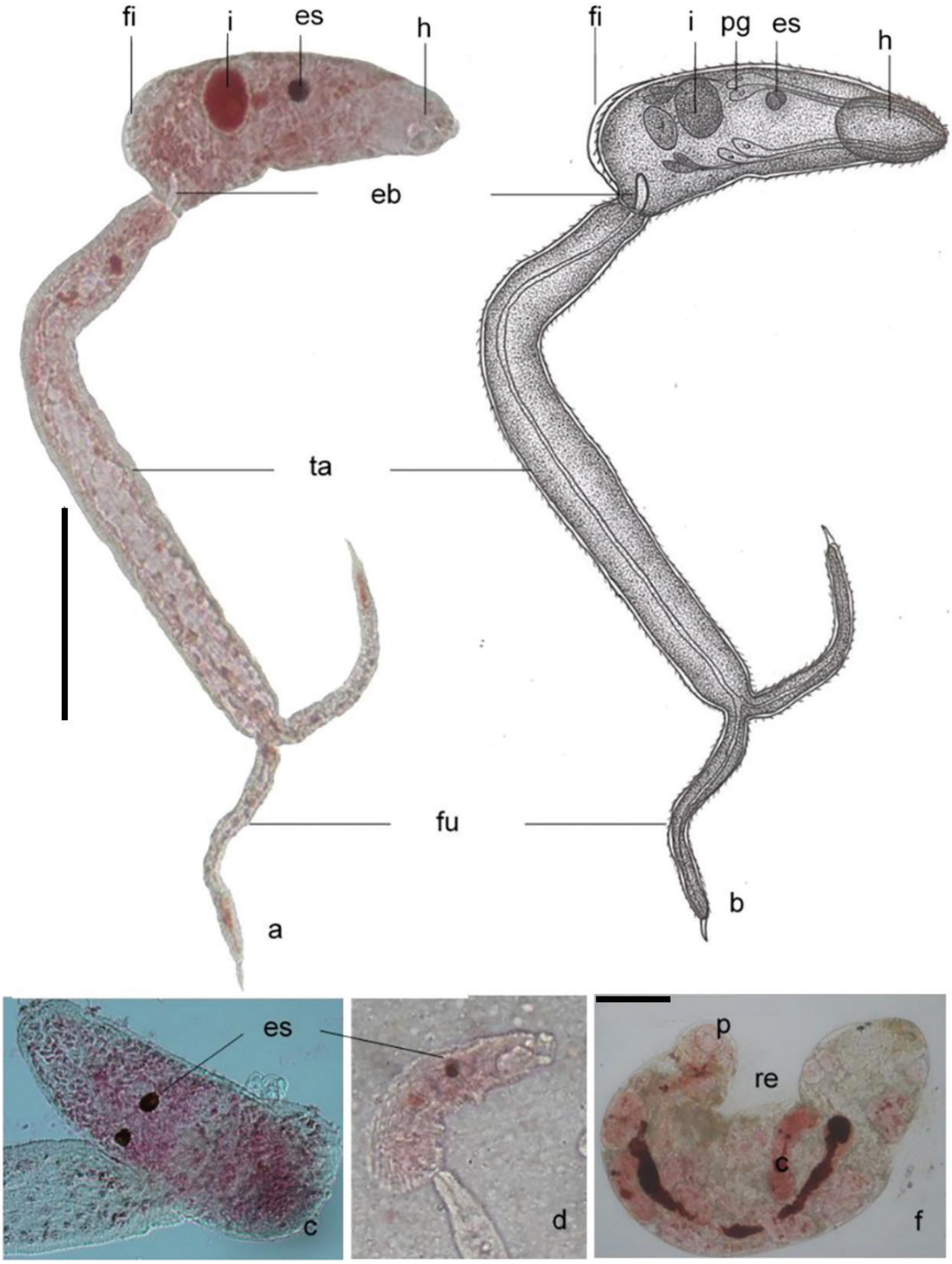

Figure 9. Image of *Clinostomum giganticum* Agarwal, 1959 **a., c., d.** Images of cercaria stained with 0.5% neutral red (light microscopy) **b.** Drawing of cercaria structure **f.** Images of redia stained with 0.5% neutral red (light microscopy) Abbreviations: h: head organ, es: eyespot, i: intestine, pg: penetration gland, fi: dorso-median finfold, eb: excretory bladder, ta: tail, fu: furca, c: cercaria, re: redia. (Scale bars: 100 µm).

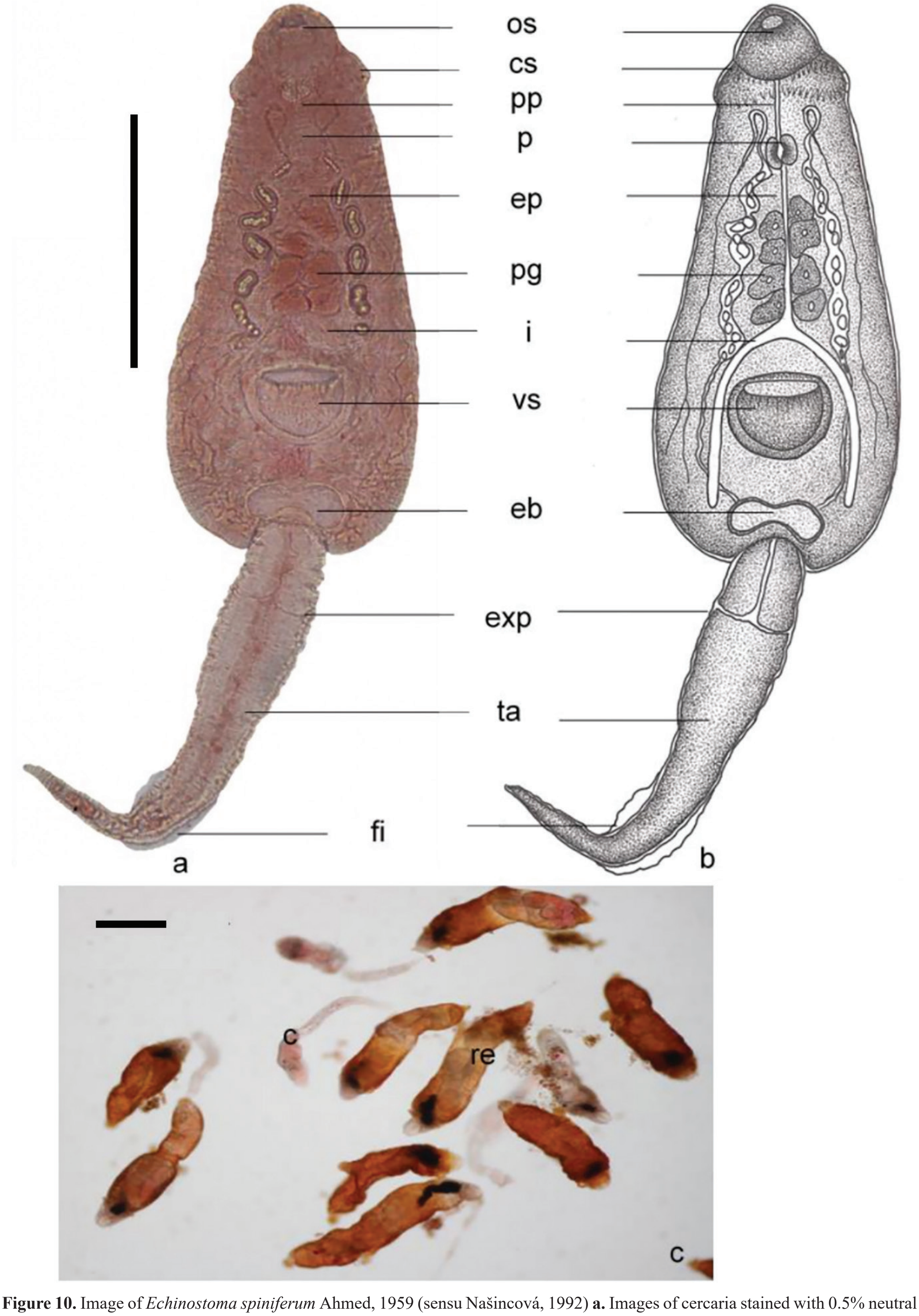

Figure 10. Image of *Echinostoma spiniferum* Ahmed, 1959 (sensu Našincová, 1992) **a.** Images of cercaria stained with 0.5% neutral red (light microscopy) **b.** Drawing of cercaria structure **c.** Images of redia stained with 0.5% neutral red (light microscopy) Abbreviations: os: oral sucker, cs: 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).

Lecithodendriidae Lühe, 1901 (sensu Odhner 1910)		Pleurogeninae Looss, 1899		*Loxogenes liberum* Seno, 1907
		Virgulate xiphidiocercariae/Cercaria characters		
	Yamaguti (1937, 1938)	Okabe (1937)	Veeravechsukij et al. (2018)	This study
Immature cercaria		Cercaria in sporocyst	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 µm) × 10–23 µm (av. 16 µm)	2–5 µm (av. 4 µm) × 10–18 µm (av. 14 µm)
Oral sucker	25 – 34 µm × 25 – 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)
Finfold	-	without	without	without
Flame cell formula	2|(2+2)+2| = 12	-	-	-
1st IH snail	*Bulinus striatutus japonicus*	*Bulinus kiushuensis*	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).

	Family Schistosomatidae Looss, 1899	Subfamily Schistosomatinae Stiles & Hassall, 1898	*Schistosoma indicum* Montgomery, 1906 (Syn. *S. nasalis* Rao, 1933)
		Furococercous cercariae/ Brevifurcate -apharyngeate cercariae	
	Singh (1958) *Cercaria indicae* XXX	Srivastava and Dutt (1951)	This study
Eyespots	no	n/a	no
Head organ	Head gland three fourths as long as head organ, situated dorsally in center of head organ, 27 ×16.3 µm	29–35 µm	23–33 µm (av. 29 µm) × 26–36 µm (av. 31 µm)
Pharynx	no	n/a	no
Esophagus	Narrow, long		Long
Intestine	Small, saccular		Small, saccular
Penetration glands	5 pairs Two anterior pair coarsely granular oxyphilic. Three posterior pairs finely granular basophilic	n/a	5 pairs Transparent 2 pairs, Turbid 3 pairs.
Ventral acetabulum	15–21 µm in diameter, anterior part of last quarter of body, with two rows of spines larger than body spines in triradiate lumen.	n/a	18–37 µm (av. 26 µm) × 17–26 µm (av. 21 µm)
Genital primodia	Represented by a postacetabular cluster of primordia represented by a postacetabular cluster of small cells.		
Excretory vesicle	Small cup shaped vesicle, primary collecting tubules curving backward behind caecal pouches.	n/a	cup shaped 7–32 µm (av. 22 µm) × 15–25 µm (av. 19 µm)
Flame cell	2|(1+1)+1+1+(1)| = 10	n/a	n/a
Excretory duct	Tail tubule Bifurcating a little short of distal end of tail stem, each branch opening at tip of furcae	n/a	Excretory pore opening at the tip of furcae
Body	Cuticle spined Extended: 182× 39 µm Contracted 104× 65 µm	117–156 µm × 39–52 µm	41–55 µm (av. 48 µm) × 135–152 µm (av. 142 µm)
Tail stem	Cuticle spined 182–234 µm	n/a	17–36 µm (av. 29 µm) × 225–286 µm (av. 252 µm)
Furcal tail	30–104 µm	n/a	10–21 µm (av. 16 µm) × 80–135 µm (av. 110 µm)
1st IH	*Lymnaea luteola, Indoplanorbis exustus*	*Indoplanorbis exustus*	found from 9 *Indoplanorbis exustus* snails,
2nd IH	no	no	no
DH	Kid, goat, sheep	Kid lamb	Cattle, Goat (believed)
Cercarial behavior	When at rest the body hangs downwards, with tail stem upward and furcae at right angles to tail stem, rising with wriggling motion, creeping in inch-worm manner by alternate use of suckers, living for 24–30 hours in tap water and 36 hours in pond water.	Cercarial emergence occurred during the morning hours.	When at rest the body hangs downwards, with tail stem upward. It moved by rolling up and springing back the body to spiral move forward, non-direction for about 30–48 seconds.

Table 6. Some characters of *Clinostomum giganticum* found in this study and the reference sources (measurement in µm, n/a = no data).

Family Clinostomidae Lühe, 1901		**Subfamily Clinostominae Pratt, 1902**	***Clinostomum giganticum* Agarwal, 1959**	
Furococercous cercariae/ Brevifurcate -pharyngeate cercariae				
	***C. marginatum* Rudolphi, 1809**		***C. giganticum* Agarwal, 1959**	
	Hunter and Hunter (1934, 1935)	Krull (1934)	Agarwal (1959) 7 living, 15 fixed specimens	This study
Brevifurcate pharyngeate	present	Oculate, pharyngeate, furcocercous, lophocercous.	n/a	Oculate, pharyngeate, furcocercous.
Body	126 µm, Dorsal surface finfold is present (extend from eyespots to posterior)	120–138 × 30–32 µm. Dorsal convex, ventral concave, 9 transverse rows of alternately staggered hairy spine. Delicate dorsal median finfold 7–8 µm wide in living specimens (extend from eyespots to posterior).	Live 112–160× 40–60 µm (fixed 76–112×28–44 µm) Minute body spines Numerous small nucleated cell readily staining with neutral red, scattered in parenchyma.	Minute body spines with delicate dorso-median finfold, extend from eyespots to posterior end. 34–88 µm (av. 45 µm) × 129–203 µm (av. 159 µm)
Eyespots	1 pair	Pigmented, crescentic in optical section.	1 pair	1 pair, under head organ and near penetration gland. 6–12 µm (av. 9 µm) × 7–10 µm (av. 8 µm)
Head organ	n/a	Elongate, with ventral mouth.	Anterior organ 28–44 × 20–24 µm Mouth opening ventrally at level of posterior end of anterior organ	Elongate, 18–47 µm (av. 27 µm) × 33–59 µm (av. 45 µm)
Prepharynx	short prepharynx	Conspicuously long	Tubular prepharynx (esophagus after Krull, 1934)	n/a
Pharynx	n/a	large	Bulbous, at posterior end of prepharynx	n/a
Esophagus	n/a	Half as long as pharynx.	n/a	n/a
Intestine	rod shape	Undivided, sac-like.	Undivided, saccular 80–100 µm	n/a
Penetration glands	3–4 pairs	4 pairs Situated in pharyngo-intestinal region.	4 pairs, on each side of intestine, 2 anterior, 2 posterior to caecum	4 pairs, found on each side of intestine.
Penetration duct	n/a	Bundled in one, opening at anterior tip of head organ.	One bundle on each side, Open at anterior end by small papillae	Open at the anterior end around head organ.
Ventral acetabular anlage	Present	Present, Anlage represented by a mass of cells lying posteroventral to cecum.	Not well defined	n/a
Genital primodium	Behind acetabulum	Anlage postero-dorsal to acetabulum near posterior end of body.	As a compact mass of cells posterior to caecum.	n/a
Excretory vesicle	n/a	small	V shaped. Giving rise at its posterior end to the tail tubule, each arm produced forward into main collecting vessel which turns back on itself before dividing at level of caecum into an anterior and posterior tubule.	V shaped. 11–27 µm (av. 19 µm) × 12–32 µm (av. 20 µm).
Flame cell	n/a	5 pairs, last pair at base of tail stem.	flame cell formula 2[(1+1) + (1+1) + 1] = 10	n/a
Excretory duct	-	Opening at tip of each furca.	Excretory pore open at the base of furcal spines.	Excretory pore open at the base of furcal spines.
Tail stem	272 µm	250–285 µm, spine	Long, slender, more than twice as long as body, with spines bent backward at their tips. Live 224–340×24–32 µm (fixed 184–320×20–30 µm)	Tail spinose, 15–52 µm (av. 38 µm) × 157–339 µm (av. 242 µm)
Furcae	Furcae spinose Small finfold, 90–91 µm	Small fins at their tips 75–98 µm, spine 6 pairs of long hairs, one pair on the furcae.	Furcae, tail tubule with nucleated cells on either side. Live 80–116× 8–20 µm (fixed 68–132× 3–20 µm Long hair absent, furcae shorter than tail stem, with minute spines along lateral margins).	Tip at the end of furcae. 11–35 µm (av. 16 µm) × 85–214 µm (av. 115 µm)
1st IH (snail)	n/a	*Helisoma antrosa*	*Lymnaea acuminata, L. luteola*	One *Indoplanorbis exustus,* found cercariae in tissues by crushing.
2nd IH (fish)	*Eupomotis gibbosus* (Linnaeus, 1758) sunfish	n/a	*Ophiocephalus punctatus* spotted snake-head fish	n/a
Cercaria behavior	n/a	Short-lived, infective for only few hours, suspended in water for a short time, with the anterior end curved ventrad and hanging down, tail furcae held relative close together, then sink slowly to the bottom.	The cercariae emerged during daytime, suspended in the water with the body downward and bent at a characteristic angle. The tail stem and furcae straight up, remaining stationary at a point for some time, movements by vibrations of the tail and furcae with undiscernible swiftness.	n/a

base pairs. The phylogenetic tree obtained from neighbor-joining 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 revolutum* Froelich, 1802**	**Echinostome cercariae**		***Echinoparyphium spiniferum* Ahmed, 1959**		***Echinostoma spiniferum* sensu Našincová, 1992**	
	Tubangui (1932)	Beaver (1937)	Johnston and Angel (1941)	Veeravechsukij et al. (2018)	Ahmed (1959)	Faltýnková (2005)	This study
Collar spines	cephalic collar 90–125 μm 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 two alternate rows.	37 collar spines, 5 ventrals, 6 laterals, 15 dorsals.	present	n/a	present	present
Prepharynx	very short.	n/a	22–27 μm, long	prepharynx is long	n/a	prepharynx is long	prepharynx is long
Pharynx	25–30 × 20–27 μm	17 × 21 μm	29–33 × 16–24 μm	large, 13–18 μm (av. 14 μm) × 20–30 μm (av. 24 μm)	n/a	large	large, 8–14 μm (av. 10 μm) × 9–12 μm (av. 10 μm)
Esophagus	95–145 μm	n/a	131–164 × 12 μm	esophagus is shorter than the prepharynx	n/a	esophagus is long	esophagus is long
Intestine	Bifurcated in front of acetabulum, ceca extending to near posterior extremity.	n/a	n/a	Bifurcating into two intestinal caeca that almost reach to the posterior end of the body.	n/a	Bifurcated in front of acetabulum	Bifurcating into two intestinal caeca that almost reach to the posterior end of the body.
Body	Body spinose 330–520 × 150–250 μm	323 × 95 μm (fix specimen)	284–350 × 92–109 μm (fix in formalin)	elongate pear-shaped 150–163 μm (av. 151 μm) × 243–325 μm (av. 270 μm)	n/a	elongate pear-shaped	elongate pear-shaped 110–205 μm (av. 139 μm) × 139–293 μm (av. 217 μm)
Oral sucker	54–62 × 50–62 μm	41 × 46 μm	44 × 37 μm	38–48 μm (av. 44 μm) × 38–48 μm (av. 44 μm)	n/a	n/a	33–43 μm (av. 39 μm) × 34–45 μm (av. 41 μm)
Ventral sucker	58–75 × 62–83 μm	58 μm (diameter)	48 μm (in diameter)	large ventral sucker, 40–73 μm (av. 62 μm) × 55–63 μm (av. 60 μm)	n/a	large ventral sucker	large ventral sucker, 45–54 μm (av. 49 μm) × 38–56 μm (av. 49 μm)
Penetration glands	n/a	6 pairs	Two rows of 4 each, in region of prepharynx; their narrow ducts opening at anterior end of body.	absent	n/a	absent	4 pairs
Excretory bladder	n/a	Excretory vesicle divided into a small anterior and a large posterior compartment.	n/a	small and triangular in shape, 18–55 μm (mean: 38 μm) × 18–55 μm (mean: 33 μm)	n/a	Sac like, collecting vessels arising at anterior end of bladder.	Sac like, collecting vessels arising at anterior end of bladder, 30–38 μm (av. 34 μm) × 26–32 μm (av. 29 μm)
Tail	400–480 × 37–50 μm	450 μm with a distinct dorsal finfold. Tail tubule inverted T-shaped.	284–384 × 38 μm A definite finfold is present on the dorsal side of the tail.	slender and almost of the same length as the body, 28–40 μm (mean: 34 μm) × 195–313 μm (mean: 240 μm)	n/a	finfold present.	44–61 μm (av. 50 μm) × 307–412 μm (av. 367 μm), with finfold.
1st IH	*Stagnicola palustris, Helisoma trivolvis (=Planorbella trivolvis), physa gyrina*	*Physa occidentalis* (experiment)	*Lymnaea pervia*	*Tarebia granifera* The infection rate was 0.07% (10/15,076)	*Planorbis corneus* (natural & experiment)	*Planorbarius corneus*	Found from 3 snails *Indoplanorbis exustus*
2nd IH	*Fossaria modicella, F. obrussa, Spaerium* sp.	*Lymnaea attenuate* (experiment)	n/a	n/a	Tadpoles of *Rana esculenta* (experiment)	n/a	n/a
DH	n/a	guinea pig, rat (experiment)	n/a	n/a	Peking duck (experiment)	n/a	n/a
Cercaria behavior	The cercariae develop within redia	The cercariae develop within redia.	The cercariae develop within redia. Emerging before noon.	The cercariae develop within redia.	n/a	The cercariae develop within redia.	The cercariae develop within redia.

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-

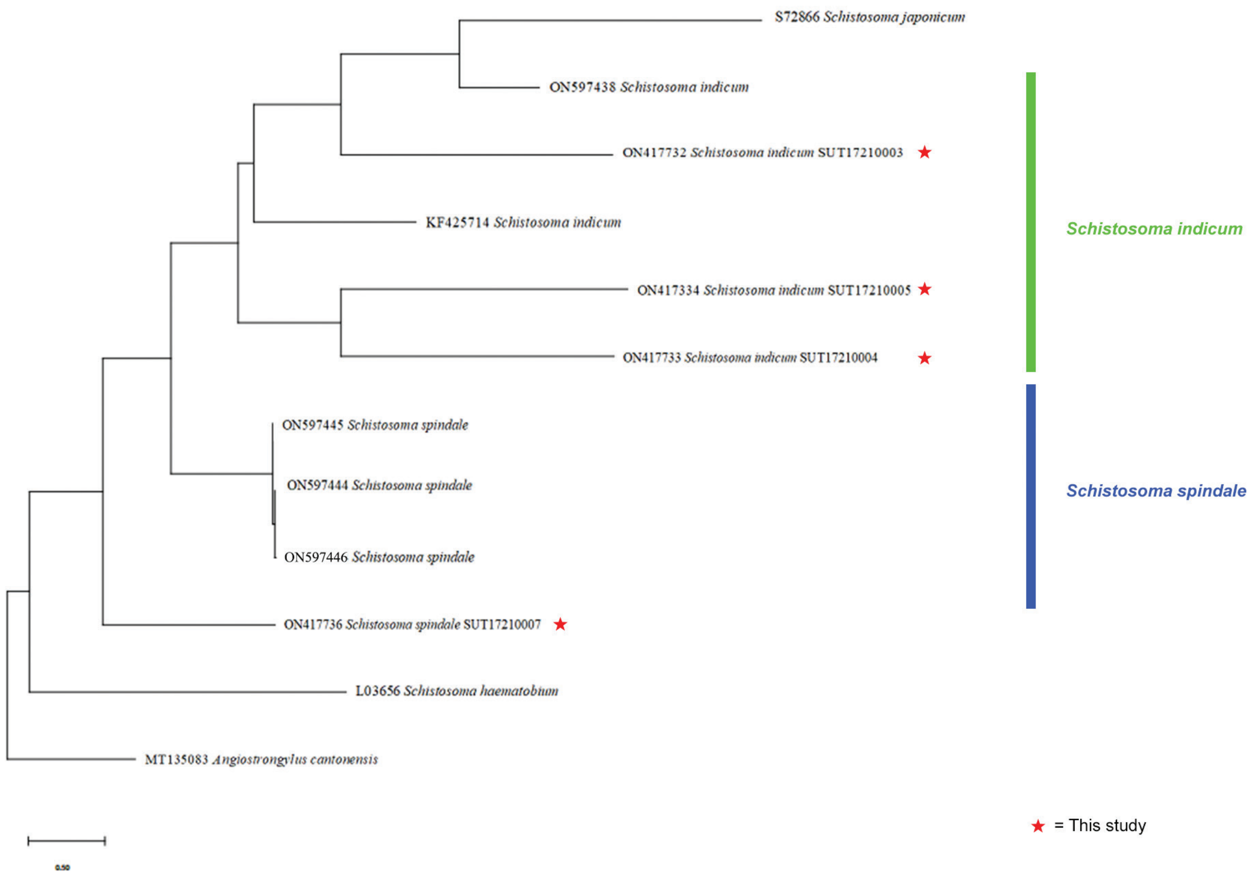

Figure 11. The phylogenetic relationship of trematodes was constructed using *ITS2* sequences, based on neighbour-joining analysis (3,000 bootstrap replications) and the other published DNA sequences obtained from GenBank. Taxon names and voucher or GenBank accession numbers are provided at the tips of the tree (see also Table 8).

Table 8. List of *ITS2* sequences used for the phylogenetic analysis.

Species of trematode	Voucher code	Genbank accession number	Stages of trematode	Locations	References
Schistosoma indicum	SUT17210001	-	cercaria	Khae moo 5, Chana district, Songkhla province	This study
	SUT17210002	-	sporocyst		
	SUT17210003	ON417732	cercaria	Sapan Mhai Kaen, Chana district, Songkhla province	This study
	SUT17210004	ON417733		Sapan Mhai Kaen Chana district, Songkhla province	This study
	SUT17210005	ON417734		Sapan Mhai Kaen, Chana district, Songkhla province	This study
	-	ON597438	-	Bangladesh	Anisuzzaman and Hasan (2022)
	-	KF425714	-	India	Bindu et al. (2013)
Schistosoma spindale	SUT17210006	ON417735	cercaria	Chaiya district, Surat Thani province	unpublished
	SUT17210007	ON417736			unpublished
	-	ON597444	-	Bangladesh	Anisuzzaman and Hasan (2022)
	-	ON597445			
	-	ON597446			
Schistosoma japonicum	-	S72866	-	China	Bowles et al. (1993)
Schistosoma haematobium	-	L03656	-	-	Michot et al. (1993)
Angiostrongylus cantonensis	-	MT135083	-	-	Chan et al. (2020)

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, furcocercous cercariae of *Trichobilharzia maegraithi* 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 in central, south, and northeast Thailand, *viz. Schistosoma japonicum*, *S. spindale*, and *Orientobilharzia harinasutai* (Harinasuta and Kruatrachue 1962; Harinasuta et al. 1965; Kruatrachue et al. 1965; Kullavanijaya and Wongwaisayawan 1993). Lee and Wykoff (1966) followed 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. incognitum* 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 (Baidikul 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 lonchurae*), 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 *Lymnaea* (*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 encystation 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; Rosser 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; Faltýnková et al. 2015). Našincová (1992) descripted *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 funiculata* 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. funiculata* and *Gabbia pygmaea* in the north; *B. s. goniomphalos*, *Wattebledia siamensis*, and *W. crosseana* in the northeast; *B. s. siamensis*, *Hydrobioides nassa*, and *G. wykoffi* in the central; and *W. baschi* and *G. erawanensis* 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; Kulsantiwong 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 (*Astiotrema monticellii*), 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). *Astiotrema monticellii* is a parapleurolophocercous cercariae that was recorded from

Bithynia leachi as the first intermediate host in Russia. The genus *Astiotrema*, which belongs to the Plagiorchiidae, represent trematodes infecting a wide range of fishes, amphibians, and reptilians. Many reports have indicated that *Astiotrema* (*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 *Astiotrema* 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 Lecithodendriidae, 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 occurance 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 malacofauna, 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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