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Survey of *Stenomelania* Fischer 1885 (Cerithioidea, Thiaridae): The potential of trematode infections in a newly-recorded snail genus at the coast of Andaman Sea, South Thailand

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Abstract

Stenomelania snails (Fischer 1885) have been reported from the coastal regions of the Indian Ocean and the Pacific Ocean, spanning India to Australia. Here, the species diversity and distribution of these snails in the south of Thailand are recorded. The snails were also examined for trematode infections in 13 locations in three Provinces, viz. Krabi, Trang and Satun, along the coast of the Andaman Sea. A total of 1,551 snails were in five morphs tentatively identified as *Stenomelania aspirans*, *S. crenulata*, *S. punctata*, *S. torulosa* and the closely-related *Neoradina prasongi*. With 10 infected snails, the trematode infection rate was 0.64%. The cercariae were categorised into three species from two morphologically-distinguishable types, viz. parapleurolophocercous cercariae (*Haplorchis taichui* and *Procerovum cheni*) and xiphidiocercariae (*Loxogenoides bicolor*), through the morphological characterisation of the larval stage. These trematodes were also analysed using the internal transcribed spacer subunit II region to confirm the species identity at generic and infrageneric levels.

Key Words

Cerithioidea, *Haplorchis taichui*, *Loxogenoides bicolor*, parapleurolophocercous cercariae, *Procerovum cheni*, *Stenomelania*, Thiaridae, xiphidiocercariae

Introduction

Trematode infections are major public health problems affecting humans in southeast Asia. At least 70 species of food- and water-borne trematodes, such as blood, intestinal, liver and lung flukes, are commonly found in various animals (Chai et al. 2005; Andrews et al. 2008; Johansen et al. 2010). Trematode infections depend not only on the habit of people, but also on the presence of first and second intermediate host species, resulting in the endemic spread of parasites, such as intestinal and liver flukes in Thailand. Two major agents of fish-borne infections are intestinal flukes belonging to Heterophyidae and liver flukes belonging to Opisthorchiidae. In a complex life cycle, trematode eggs are released by humans and animals. The first larval stage (miracidium) hatches from the egg in water and penetrates snails as the first intermediate host. A miracidium in embryonated eggs infects snails through passive uptake and subsequently hatches within hosts. The miracidium initially develops directly into sporocysts or rediae and then into cercariae that are released in water. In the second intermediate host, cercariae encyst and develop into infective metacercariae. They infect humans and animals via the consumption of raw fish or improperly cooked fish containing metacercariae (Dung et al. 2007; Skov et al. 2009; Tran et al. 2009; De et al. 2012).

In Thailand, medically important freshwater snails acting as the intermediate host of human and animal infections are reported from several taxa. For example, the opisthorchiid liver fluke *Opisthorchis viverrini* is found in freshwater Bithyniidae, i.e. *Bithynia funiculata*, *B. siamensis goniomphalos* and *B. siamensis siamensis*, in Thailand, Laos, Cambodia and Vietnam. Small intestinal flukes from Thiaridae serve as the first intermediate host. Some of them include *Haplorchis pumilio* (Looss, 1896; sensu Looss 1899), *H. taichui* (Nishigori 1924; sensu Witenberg 1930), *Loxogenoides bicolor* (Krull 1933; sensu Kaw 1945), *Centrocestus formosanus* (Nishigori 1924; sensu Price 1932) and *Stictodora tridactyla* (Martin & Kuntz, 1955), which are recorded from *Tarebia granifera*, *Mieniplotia scabra*, *Melanoides tuberculata* and *M. jugicostis*.

In the south of Thailand, *Haplorchis taichui* and *H. pumilio* are small intestinal flukes that are considered important causative agents of food-borne parasitic zoonoses. Two Cerithioidean snail families, namely, Thiaridae and Pachychilidae, were collected in a previous study. Parasitic infections were found in snail samples from 13 locations; six thiarid species, viz. *Melanoides tuberculata* (Müller, 1774), *Melanoides jugicostis* (Hanley & Theobald, 1876), *Mieniplotia scabra* (Müller, 1774), *Sermyla riqueti* (Grateloup, 1840), *Neoradina prasongi* (Brandt, 1974) and *Tarebia granifera* (Lamarck, 1822); and four pachychilid species, viz. *Brotia* sp. 1, *Brotia* sp. 2, *Brotia wykoffi* (Brandt, 1974) and *Sulcospira housei* (Lea, 1856). Three thiarid species, viz. *M. tuberculata*, *M. jugicostis* and *N. prasongi*, were infected with two intestinal flukes, viz. *H. taichui* and *H. pumilio* (Krailas et al. 2011, 2014).

Thiaridae is a group of cerithioidean gastropods, which are widely distributed and thriving in lotic (springs, creeks, rivers and streams) and lentic (lakes and ponds) habitats in tropic and subtropic regions (Glaubrecht 1996; Glaubrecht and Neiber 2019). This family includes snails belonging to *Stenomelania* (Fischer, 1885), whose members have elongated and pointed shells and are found near and in the brackish water environment of estuaries. Dey (2007) used shell morphology to identify four species, viz. *Stenomelania torulosa*, *S. plicaria*, *S. punctata* and *S. aspirans.* Haynes (2001) reported five species, viz. *Melanoides* (*Stenomelania*) *arthurii* (Brot, 1870), *M.* (*Stenomelania*) *aspirans* (Hinds, 1847), *M.* (*Stenomelania*) *lutosa* (Gould, 1847), *M.* (*Stenomelania*) *plicaria* (Born, 1778) and *M.* (*Stenomelania*) *punctata* (Lamarck, 1822), from the tropical Pacific Region. However, this freshwater snail taxon has insufficient data and a contentious taxonomy because its shell morphology is similar to that of other thiarid snails.

Stenomelania is distributed in the Oriental Region, from India to Western Pacific islands (Starmühlner 1976, 1979, 1984, 1993). Its reproductive mode, as taxonomically constituted currently, covers ovoviviparous species, which release numerous offspring as veliger larvae and euviviparous taxa, whose shelled juveniles hatch from a subhaemocoelic brood pouch. Normally, adult *Stenomelania* snails inhabit freshwater and brackish water environments of estuaries, where veliger larvae can be dispersed via marine currents. *S. denisoniensis* (Brot, 1877), which is found in Australia, releases shelled juveniles similar to those in other thiarid snails, such as *Melanoides*, *Tarebia* and *Mieniplotia*. *Stenomelania* species also exist in freshwater resources along the Andaman coast of southern Thailand, although there are only cursory remarks from there yet (Glaubrecht 1996, 2006; Bandel et al. 1997; Glaubrecht et al. 2009; Wiggering et al. 2019).

In this study, *Stenomelania* snails were investigated in 13 locations in three Provinces, viz. Krabi, Trang and Satun, near the Andaman Sea in the south of Thailand. They were also examined for trematode infections through the morphological characterisation and genetic identification of the snails and the parasitic larval stages of trematodes (cercariae). This study provided basic knowledge about the trematode fauna in Thailand and adjacent countries and the evolutionary potential of these parasites and their prevailing intermediate snail host.

Materials and methods

Sampling sites

Stenomelania snails were collected from streams and rivers near the coastline of the south of Thailand in Krabi, Trang and Satun Provinces. The geographic coordinates (WGS84 datum) of the sampling sites were determined with a global positioning system (Garmin PLUS III, Taiwan).

Collection and determination of snails

Snail specimens were collected between February 2018 and February 2019 via hand picking, scooping and counts per unit of time sampling (Olivier and Schneiderman 1956). The samples were handpicked and scooped by five researchers every 10 min at each sampling site. The snails were then transferred and studied in the laboratory of the Parasitology and Medical Malacology Research Unit, Silpakorn University, Nakhon Pathom, Thailand (PaMaSU: codens SUT). They were identified on the basis of their shell morphology.

Trematode infection analysis

The collected snails were examined for trematode infections by using shedding and crushing methods. The morphological characteristics of the trematodes were described on the basis of living cercariae that emerged from the snails. The studied cercariae were both unstained and vitally stained with 0.5% neutral red. The details of the cercariae were drawn with a *camera lucida* and identified in accordance with the methods described by Komiya (1961), Schell (1970), Yamaguti (1971, 1975), Ito (1980), Krailas et al. (2011, 2014) and Veeravechsukij et al. (2018). The average size of 10 specimens fixed in 10% formalin was measured in micrometres by using an ocular micrometre. Some cercariae belonging to the identified trematode species were preserved in 95% ethanol for further DNA analysis.

Molecular analysis of cercariae

For molecular identification, genomic DNA was extracted from the preserved cercariae by using a DNeasy blood and animal tissue kit (QIAGEN, Germany). The nuclear internal transcribed spacer 2 regions (ITS2) were amplified via a polymerase chain reaction (PCR) with the following primers 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 0.5 µl of dNTPs (5 mM each), 2.5 µl of $MgCl_2$ (1.5 mM), 5 µl of Buffer A (10X Buffer A, Invitrogen, 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 subjected to the following: initial denaturation at 94 °C for 4 min; 35 cycles of 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. Then, the PCR products were loaded on to 1% agarose gels for electrophoresis.

The ITS2 PCR products were sent to Biobasic (Canada) for sequencing analysis. The ITS2 consensus sequences were aligned in MEGA 10 by using MUSCLE (Edgar 2004) under default settings. A phylogenetic tree representing the species groups was constructed with neighbour-joining analysis based on p-distances with 3,000 bootstrap replicates.

Results

Geographical origin of the collected snails

The snails were found at 13 sampling sites in three Provinces, viz. Trang, Krabi and Satun (Fig. 1, Table 1). The collected snails were tentatively categorised into five morphospecies, based on the analysis of the relevant thiarid taxa and comparison with the documented shell morphology. The following morphospecies were identified: morph a, *Stenomelania* cf. *aspirans*; morph b, *S.* cf. *crenulata*; morph c, *Neoradina* aff. *prasongi*; morph d, *S.* cf. *punctata*; and morph e, *S.* cf. *torulosa* (Fig. 2, Table 2).

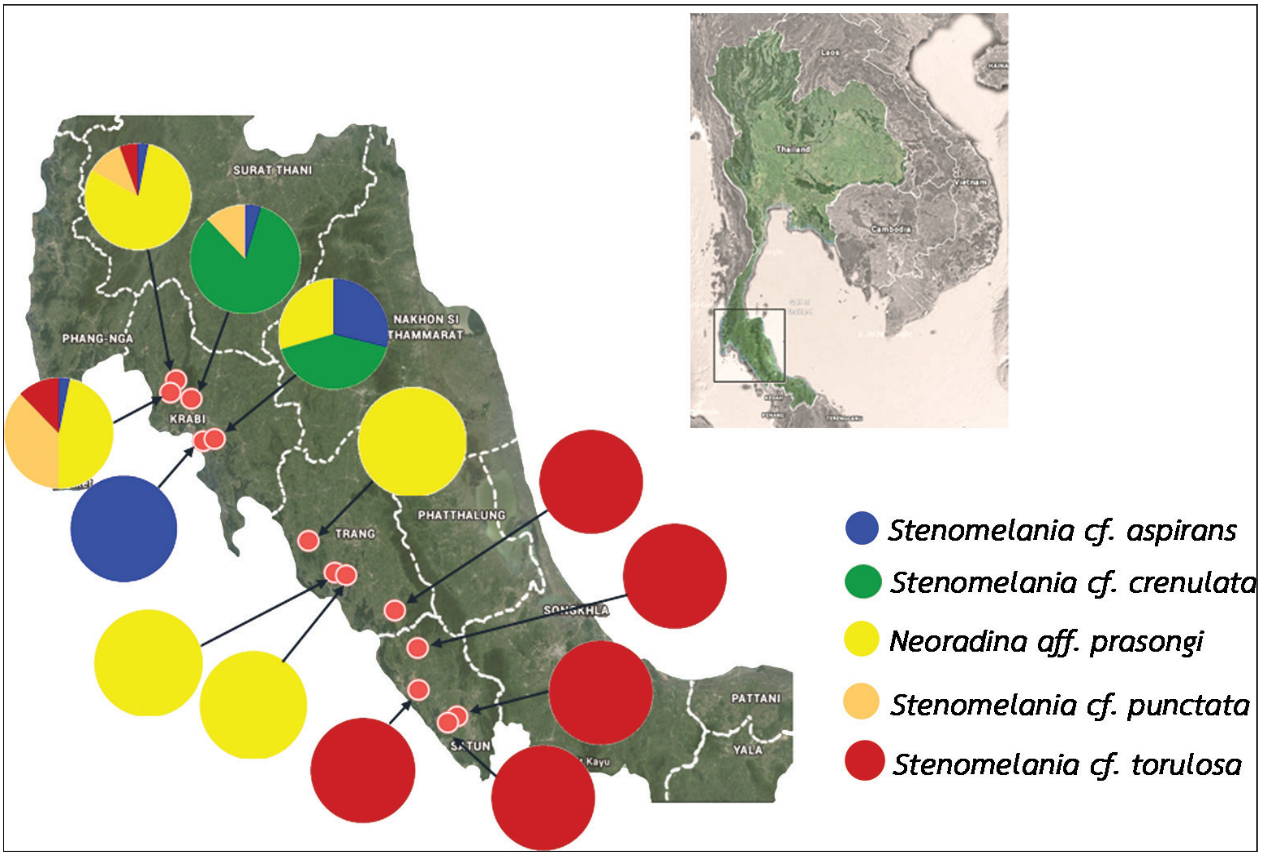

Figure 1. Distribution of collected snails from 13 localities, along the coast of Andaman Sea, south Thailand.

Table 1. Localities, number of collected snails, number of infected snails and trematodes obtained from collected snails.

No.	Voucher number	Location	GPS	Number of collected snails (morph)	Number of infected snails (morph)	Infection rate (%)	Cercaria
1	SUT201912E	Klong Saphanwa, Thungwa District, Satun Province	07°04'22.70"N, 99°47'07.35"E Alt.159 m	11 (e)	0	0	–
2	SUT201910E	Klong Thapae 1, Thapae District, Satun Province	06°47'47.70"N, 99°57'16.90"E Alt. 28 m	22 (e)	1 (e)	4.55	*Haplorchis taichui*
3	SUT201911E	Klong Thapae 2, Thapae District, Satun Province	06°48'09.74"N, 99°57'50.96"E, Alt. 28 m	11 (e)	1 (e)	9.1	*Haplorchis taichui*
4	SUT201909E	Klong La-Ngu 1, La-Ngu District, Satun Province	06°54'14.74"N, 99°48'30.88"E, Alt. 39 m	26 (e)	1 (e)	3.85	*Haplorchis taichui*
5	SUT201808C	Klong Mai Phad, Sikao District, Trang Province	07°33'10.46"N, 099°21'01.95"E Alt. 11 m	62 (c)	1 (c)	1.61	*Haplorchis taichui*
6	SUT201806C SUT201906C	Klong La 1, Sikao District, Trang Province	07°29'39.55"N, 099°20'34.42"E Alt. 13 m	111 (c)	1(c) 1(c)	0.90 0.90	*Haplorchis taichui* *Loxogenoides bicolor*
7	SUT201807C SUT201907C	Klong La 2, Sikao District, Trang Province	07°29'49.22"N, 099°21'28.25"E Alt. 7 m	35 (c)	0	0	–
8	SUT201913E	Khao Ting Cave, Palian District, Trang Province	07°09'33.48"N, 99°47'59.54"E Alt.104 m	50 (e)	3 (e)	6	*Loxogenoides bicolor*
9	SUT201804A SUT201804B SUT201904B SUT201904D	Klong Thanthip 2, Mueang District, Krabi Province	08°09'37.78"N, 98°47'07.51"E Alt. 75 m	304 (a,b,d)	0	0	–
10	SUT201801A SUT201801C SUT201801D SUT201801E SUT201901C SUT201901D	Klong Nong Jik, Mueang District, Krabi Province	08°13'22.00"N, 98°46'24.97"E Alt. 39 m	310 (a,c,d,e)	1 (d)	0.32	*Procerovum cheni*
11	SUT201805A SUT201805D SUT201805E SUT201905C SUT201905D	Klong Yang, Mueang District, Krabi Province	08°09'57.2"N, 98°47'40.3"E Alt. 62 m	151 (a,c,d,e)	0	0	–
12	SUT201802A SUT201802B SUT201902A SUT201902B SUT201902C	Klong Son 1, Mueang District, Krabi Province	08°04'15.96"N, 98°47'55.09"E Alt. 84 m	399 (a,b,c)	0	0	–
13	SUT201903A	Klong Son 2, Mueang District, Krabi Province	08°04'23.68"N, 98°48'09.98"E Alt. 98 m	59 (a)	0	0	–
Total				**1,551**	**10**	**0.64**	

morph a: *Stenomelania* cf. *aspirans*, morph b: *Stenomelania* cf. *crenulata*, morph c: *Neoradina* aff. *prasongi*, morph d: *Stenomelania* cf. *punctata* and morph e: *Stenomelania* cf. *torulosa*

Cercarial diversity and infection rates

The infected snails were reported from seven of the above sampling sites. The information on sampling sites, including geographic coordinates and the number of infected snails, is presented in Table 1. A total of 1,551 snails were collected and examined for trematode infections. With 10 parasitised snails, the overall infection rate was 0.64%. The obtained cercariae were classified into three species from two morphologically-distinguishable types: (i) virgulate xiphidiocercariae (*Loxogenoides bicolor*) and (ii) parapleurolophocercous cercariae (*H. taichui* and *Procerovum cheni*).

Morphology of the infecting cercariae

The cercariae were categorised on the basis of their morphological and organ characters in accordance with previously-reported morphological descriptions (Komiya 1961; Schell 1970; Yamaguti 1971, 1975; Ito 1980; Krailas et al. 2011, 2014; Veeravechsukij et al. 2018).

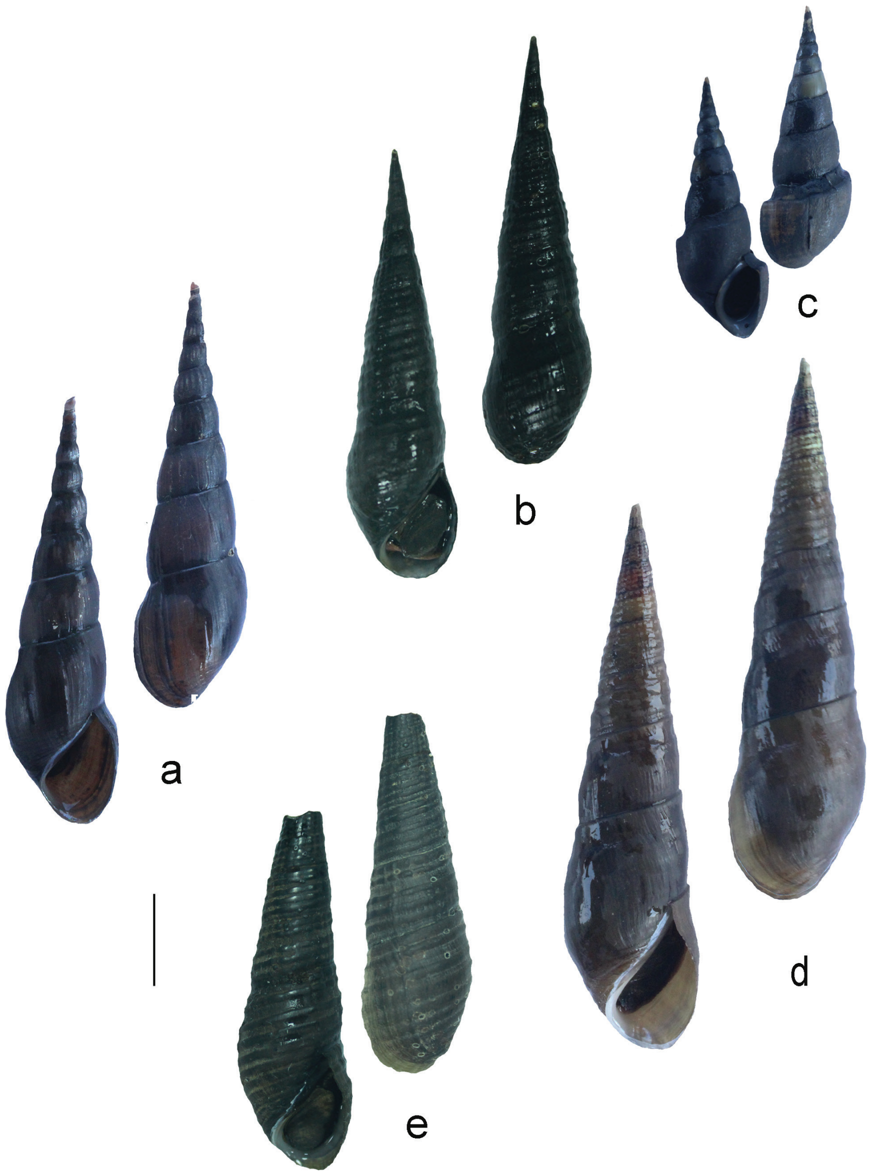

Figure 2. Shells of *Stenomelania* sp. (Fischer 1885) from south of Thailand. **a.** Morph 1: *S.* cf. *aspirans*, Krabi Province; **b.** Morph 2: *S.* cf. *crenulata*, Krabi Province; **c.** Morph 3: *Neoradina* aff. *prasongi*, Krabi and Trang Provinces; **d.** Morph 4: *S.* cf. *punctata*, Krabi and Trang Provinces; **e.** Morph 5: *S.* cf. *torulosa*, Krabi, Trang and Satun Provinces. Scale bar: 10 mm.

Table 2. Shell morphology characters of snail samples.

Morph of snail samples	Species	Shell morphology	references
Morph a	*Stenomelania* cf. *aspirans*	Shell is turriform, solid and slender, smooth, sculptured without strong spiral ridges, apical whorl with some vertical ridges, attenuated spine, whorl of spire not folded, shell colour is black with a tendency to appear greyish or bluish.	Glaubrecht et al. (2009) Haynes (2001) Ramakrishna and Dey (2007)
Morph b	*Stenomelania* cf. *crenulata*	Shell elongated with 12–14 whorls, sculpture with spiral grooves, axial ribs less frequently, aperture longitudinally elongated, colour black or dark grey	Hidaka and Kano (2014)
Morph c	*Neoradina* aff. *prasongi*	Shell elongated turreted with 10–14 whorls, spire pointed, darkish-brown or darkish-green to black, last whorl with more or less pronounced keel at upper third of periphery, whorls rounded with deep sutures.	Wiggering et al. (2019)
Morph d	*Stenomelania* cf. *punctata*	Shell turret shaped with 8–12 whorls, suture deep, body whorl is smooth, long pointed spire with sculpture, whorls with radial striations, dark brown colour.	Bendel et al (1997) Haynes (2001)
Morph e	*Stenomelania* cf. *torulosa*	Shell sculptured with strong spiral ridges, 8–12 whorls, the shell is always eroded, aperture ovate.	Ramakrishna and Dey (2007)

They were described as two distinct morphological cercarial types known and found to date and attributable to at least two distinct trematode families.

Type 1. Virgulate xiphidiocercariae cercariae

Lecithodendriidae Lühe, 1901 (sensu Odhner 1910)

1.1 *Loxogenoides bicolor* (Krull, 1933; Kaw 1945; Fig. 3)

The body of this species was oval and covered with small spines. Brown granules were found underneath the skin of its body. Its oral sucker was globular and clearly observed with one stylet. The virgulate gland was presented in the anterior part of the body. The pharynx was round and small; however, the oesophagus was not found. Three pairs of penetration glands were located at two-thirds of the body and they had two anterior pairs with fine granules and a posterior pair with coarse granules. The ventral sucker was smaller than the oral sucker. The excretory bladder was U shaped and thick walled. The tail was flexible in length, but it was shorter than the body. Spines were observed on the body and excretory ducts opened at the end of the tail. The cercariae developed within sporocysts.

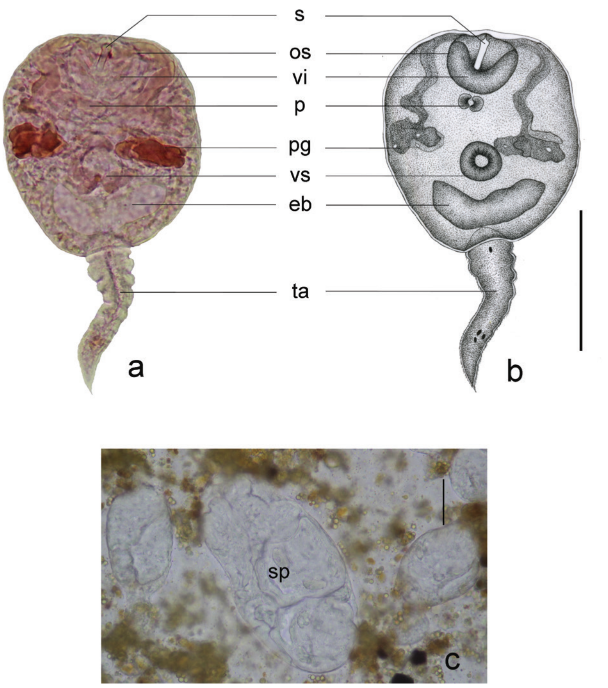

Figure 3. Images of *Loxogenoides bicolor* (Krull, 1933) Kaw 1945. **a.** Specimen stained with 0.5% neutral red; **b.** Drawing image; **c.** Sporocyst stained with 0.5% neutral red. Abbreviations – eb: excretory bladder; p: pharynx; pg: penetration gland; os: oral sucker; s: stylet; sp: sporocyst; ta: tail; vi: virgulate organ; vs: ventral sucker. Scale bars: 100 µm.

Four collected snails were infected with *L. bicolor*: one in *N.* aff. *prasongi* from Klong La 1 and three in *S.* cf. *torulosa* from Khao Ting Cave. The infection rate was 0.26% (4/1,551; Tables 1, 3).

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

table 5.

Body:	63–78 µm (avg. 69 µm) × 79–103 µm (avg. 91 µm)
Stylet:	2–5 µm (avg. 3 µm) × 11–17 µm (avg. 15 µm)
Oral sucker:	11–24 µm (avg. 19 µm) × 11–16 µm (avg. 11 µm)
Ventral sucker:	8–17 µm (avg. 12 µm) × 9–15 µm (avg. 11 µm)
Pharynx:	4–8 µm (avg. 6 µm) × 5–9 µm (avg. 7 µm)
Excretory bladder:	11–35 µm (avg. 25 µm) × 10–25 µm (avg. 14 µm)
Tail:	15–22 µm (avg. 18 µm) × 64–115 µm (avg. 95 µm)

Type 2. Parapleurolophocercous cercariae

Heterophyidae (Leiper 1909; sensu Odhner 1914)

2.1 *Haplorchis taichui* (Nishigori, 1924; Chen 1936; Fig. 4)

The body of this species was oval and brownish. Its mouth aperture was found at the oral sucker and covered with two rows of spines. The first row had six spines and the second row had five spines. Sensory hairs were observed on the ven-

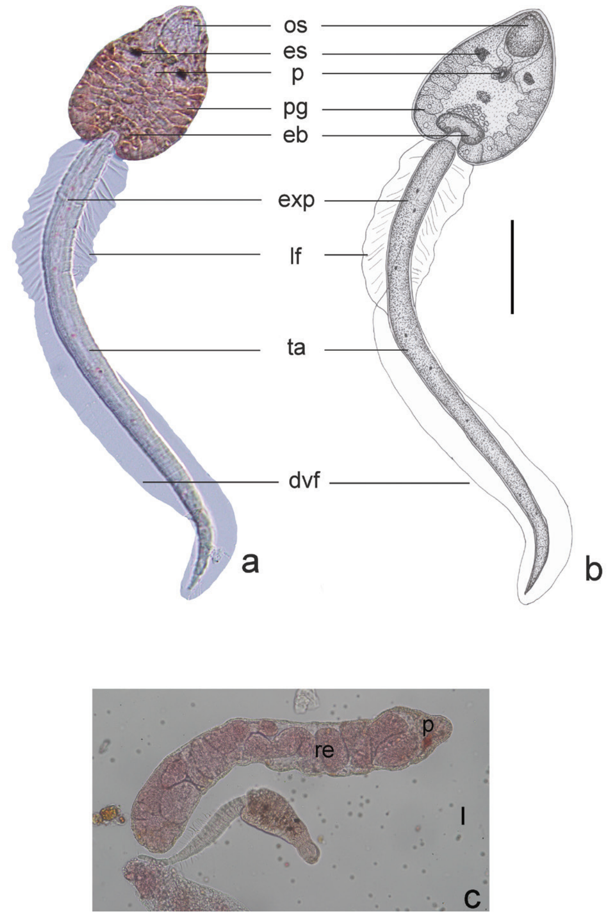

Figure 4. *Haplorchis taichui* (Nishigori, 1924) Chen 1936. **a.** Specimen stained with 0.5% neutral red; **b.** Drawing image; **c.** Redia stained with 0.5% neutral red. Abbreviations – dvf: dorso-ventral finfold; eb: excretory bladder; exp: excretory pore; es: eyespot; lf: lateral finfold; os: oral sucker; p: pharynx; pg: penetration gland; re: redia; ta: tail. Scale bar: 100 µm.

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

Species source	*Haplorchis taichui* This study	*Haplorchis taichui* Veeravecksukij et al. (2018)	*Procerovum cheni* This study	*Procerovum cheni* Hsü (1951)	*Loxogenoides bicolor* This study	*Loxogenoides bicolor* Veeravecksukij et al. (2018)
Body	91 (78–116) × 124 (101–151)	99 (80–118) × 202 (168–207)	74 (64–85) × 142 (109–176)	69 (60–73) × 110 (113–130)	69 (63–78) × 91 (79–103)	72 (53–88) × 117 105–138)
Oral sucker	32 (29–40) × 32 (25–40)	34 (28–38) × 41 (30–50)	25 (21–31) × 28 (24–35)	n/a	19 11–24) × 11 (10–16)	33 (23–40) × 29 (23–33)
Ventral sucker	17 (13–20) × 16 (13–19)	23 (13–35) × 27 (15–45)	n/a	n/a	12 (8–17) ×11 (9–15)	18 (13–25) ×16 (8–20)
Excretory bladder	40 (37–42) × 26 (24–30)	64 (43–90) × 39 (20–55)	27 (22–33) × 27 (23–31)	n/a	25 (11–35) × 14 (10–25)	33 (18–55) × 20 (10–35)
Stylet	Not found	Not found	Not found	Not found	3(2–5) × 15 (11–17)	6(5–8) × 30(20–40)
Eyespot	9 (7–10) × 11 (9–13)	9 (5–15) × 9 (5–15)	9 (8–11) × 6 (4–7)	n/a	Not found	Not found
Tail	24 (20–27) × 384 (352–413)	18 (20–33) × 558 (405–495)	23 (19–28) × 357 (270–398)	n/a × 378 (301–390)	15 (18–22) × 95 (64–115)	21 (10–28) × 44 (25–88)
Lateral finfold	20 (15–25) × 116 (96–127)	18 (10–25) × 108 (74–148)	11 (7–14) × 102 (84–117)	n/a	Not found	Not found
Dorso-ventral finfold	24 (18–28) × 289 (265–306)	n/a	12 (6–22) × 277 (220–349)	n/a	Not found	Not found

tral surface of the body. A pair of eyespots, prepharynx and pharynx were presented. Seven pairs of penetration glands extended from the pharynx to the posterior end of the body. Fourteen ducts of penetration glands opened at the anterior end of the body. A small ventral sucker was found at the middle of the body. The excretory bladder was round and thick walled. The tail was longer than the body and the end of the tail was always bent. The lateral and dorso-ventral finfolds were observed. The cercariae developed within rediae.

Five collected snails found at five locations were infected with *H. taichui*, viz. four *S.* cf. *torulosa* from Klong Thapae 1, Klong Thapae 2, Klong La-Ngu 1 and Klong Mai Phad and one *N.* aff. *prasongi* from Klong La 1. The infection rate was 0.32% (5/1,551; Tables 1, 3).

Size range and average size (in micrometres, calculat-

table 6.

Body:	78–116 µm (avg. 91 µm) × 101–151 µm (avg. 124 µm)
Oral sucker:	29–40 µm (avg. 32 µm) × 25–40 µm (avg. 32 µm)
Ventral sucker:	13–20 µm (avg. 17 µm) × 13–19 µm (avg. 16 µm)
Eyespot:	7–10 µm (avg. 9 µm) × 9–13 µm (avg. 11 µm)
Pharynx:	10–12 µm (avg. 11 µm) × 7–15 µm (avg. 12 µm)
Excretory bladder:	37–42 µm (avg. 40 µm) × 24–30 µm (avg. 26 µm)
Tail:	20–27 µm (avg. 24 µm) × 352–413 µm (avg. 384 µm)
Lateral finfold:	15–25 µm (avg. 20 µm) × 96–127 µm (avg. 116 µm)
Dorso-ventral finfold:	18–28 µm (avg. 24 µm) × 265–306 µm (avg. 289 µm)

ed from 10 cercariae):

2.2 *Procerovum cheni* Hsü, 1951 (Fig. 5)

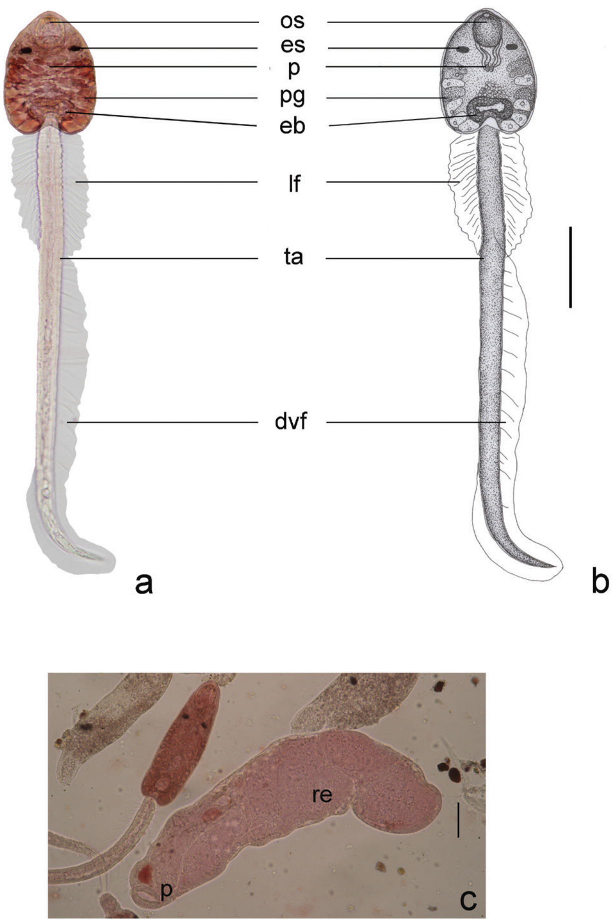

Figure 5. Images of *Procerovum cheni* Hsü, 1951. **a.** Specimen stained with 0.5% neutral red; **b.** Drawing of image; **c.** Sporocyst stained with 0.5% neutral red. Abbreviations – dvf: dorso-ventral finfold eb: excretory bladder; es: eyespot; lf: lateral finfold; os: oral sucker; p: pharynx; pg: penetration gland; re: redia; ta: tail. Scale bar: 100 µm.

The cercaria was oval. Its oral sucker was located at the anterior of the body and its mouth aperture was covered with three transverse rows of spines. The first row had four spines, the second row had five spines and the third row had six spines (4:5:6). A pair of pigmented eyespots was conspicuous from the anterior end and the pharynx was presented. Seven pairs of penetration glands extended from the pharynx to the posterior end of the body. Numerous cystogenous glands in the cell were arranged in the middle third of the body and extended to the lateral fields of the body. The excretory system was mesostomate, the excretory bladder was saccular and thick walled and the tail was longer than the body. The lateral finfold was found at one-third of the tail trunk and the dorso-ventral finfold was located at the distal portion. The cercariae developed within rediae.

Only one *S.* cf. *punctata* from Klong Nong Jik was infected. The infection rate was 0.06% (1/1,551; Tables 1, 3).

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

table 7.

Body:	64–85 µm (avg. 74 µm) × 109–176 µm (avg. 142 µm)
Oral sucker:	21–31 µm (avg. 25 µm) × 24–35 µm (avg. 28 µm)
Eyespot:	8–11 µm (avg. 9 µm) × 4–7 µm (avg. 6 µm)
Pharynx:	17–20 µm (avg. 18 µm) × 16–19 µm (avg. 18 µm)
Penetration gland:	19–28 µm (avg. 24 µm) × 11–15 µm (avg. 13 µm)
Excretory bladder:	22–33 µm (avg. 27 µm) × 23–31 µm (avg. 27 µm)
Tail:	19–28 µm (avg. 23 µm) × 270–398 µm (avg. 357 µm)
Lateral finfold:	7–14 µm (avg. 11 µm) × 84–117 µm (avg. 102 µm)
Dorso-ventral finfold:	6–22 µm (avg. 12 µm) × 220–349 µm (avg. 277 µm)

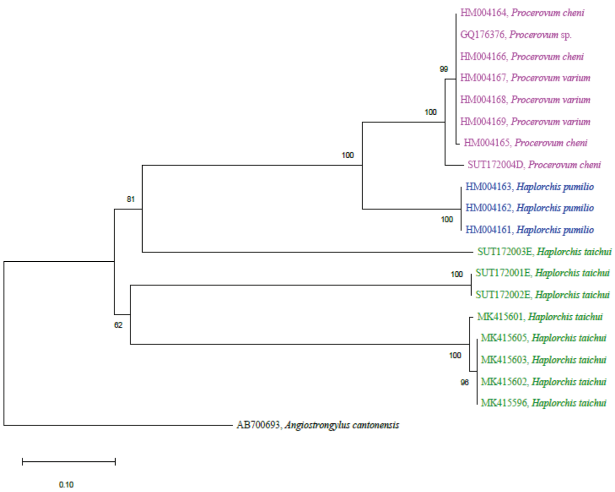

Figure 6. The phylogenetic relationship of trematodes was constructed using ITS2 sequences, based on neighbour-joining analysis (3,000 bootstrap replications) and the other published DNA sequences obtained from GenBank. Nodes are annotated with bootstrap support value ≥ 50. Taxon names and voucher or GenBank accession numbers are provided at the tips of the tree (see also Table 4).

Molecular analysis

The cercariae were studied using the ITS2 sequences (Fig. 6 and Table 4). Three trematode species were categorised on the basis of their morphological and organ characters from 10 collected snails. The heterophyid trematodes consist of *H. taichui* and *P. cheni*. The ITS2 gene sequences of *H. taichui* and *P. cheni* were approximately 310–330 and 255 bp in length, respectively. The phylogenetic tree obtained from neighbour-joining analysis was rooted with the nematode *Angiostrongylus cantonensis* (GenBank accession number: AB700693). Unfortunately, *L. bicolor*, the virgulate xiphidiocercariae cercariae of Lecithodendriidae, could not be amplified. However, this trematode species was distinguished through morphological identification.

In the phylogenetic tree, the two *H. taichui* SUT172001E and SUT172002E clustered together, whereas *H. taichui* SUT172003E was more distant. These *H. taichui* samples, which grouped together with a relatively-high support, were collected from the same snail intermediate host, viz. *S.* cf. *torulosa*, but from different locations in Satun Province. The second heterophyid cercaria species, *P. cheni* (SUT172004D), grouped together with *Procerovum* sp. (GQ176376), *P. cheni* (HM004164, HM004165 and HM004164) and *P. varium* (HM004167, HM004168 and HM004169; Van et al. 2009; Thaenkham et al. 2010), with a high support. *P. cheni* and *P. varium* grouped together and could not be resolved unequivocally. Therefore, *P. cheni* (Hsü 1951) was confirmed morphologically on the basis of previously-published data.

Discussion

Stenomelania is widespread in the Oriental Region, ranging from India to the south-western Pacific and Australia (Bandel et al. 1997; Glaubrecht et al. 2009). Wiggering et al. (2019) studied thiarid snails reported from Thailand and focused on *N. prasongi* (Brandt 1974) *Stenomelania*-like freshwater snail in comparison with *Melanoides* and *Stenomelania* species. In the present study, the thiarids resembling *Stenomelania* in south Thailand were examined to explore the occurrence of these snails and their infections with trematodes. Here, a parasitological approach, based on the morphological characteristics of the cercarial stages, was combined with a molecular approach and a preliminary phylogenetic analysis of the parasites obtained from the collected snails in Thailand was performed.

In this study, a total of 1,551 collected snails from 13 localities in the coastal of Andaman Sea were identified into five species: (1) *S.* cf. *aspirans*, (2) *S.* cf. *crenulata*, (3) *N.* aff. *prasongi*, (4) *S.* cf. *punctata* and (5) *S.* cf. *torulosa*. Interestingly, the distribution of the snail species exhibited a distinct pattern. In Satun Province, only *S.* cf. *torulosa* was found, whereas *N.* aff. *prasongi* was collected only in Trang Province. By contrast, all the taxa were

Table 4. List of ITS2 sequences used for the phylogenetic analysis.

Species of trematode	Voucher code	Genbank accession number	Stage of trematode	Location	References
Haplorchis taichui	SUT172001E	MT949314	cercaria	Klong Thapae 1, Satun	this study
	SUT172002E	MT949315		Klong La-Ngu 1, Satun	this study
	SUT172003E	MT949316		Klong Thapae 2, Satun	this study
	–	MK415601	metacercaria	Chachoengsao	Buathong et al. (2019)
	–	MK415602			
	–	MK415603			
	–	MK415605			
	–	MK415596			
Procerovum sp.	–	GQ176376	adult	Thailand	Van et al. (2009)
Procerovum cheni	SUT172004D	MT949317	cercaria	Klong Nong Jik, Krabi	this study
	–	HM004164	adult	Chachoengsao	Thaenkham et al. (2010)
	–	HM004165			
	–	HM004166			
Procerovum varium	–	HM004167	adult	Nakhon Pathom	Thaenkham et al. (2010)
	–	HM004168			
	–	HM004169			
Haplochis pumilio	–	HM004163	adult	Nakhon Pathom	Thaenkham et al. (2010)
	–	HM004162			
	–	HM004161			

observed in Krabi Province. Therefore, the presence of these species might be correlated with the circulation of sea currents. The flow of water along the Andaman coast is affected by the monsoon season, i.e. between January and May with a clockwise flow direction (northeast monsoon season) and between August and October with an anticlockwise direction (southwest monsoon season; Department of Marine and Coastal Resources, Thailand). *Stenomelania* produces veliger larvae and may represent a transitional stage in the invasion of freshwater habitats (Glaubrecht 1996, 2004; Bandel et al. 1997). Veligers move from one habitat to another via ocean currents.

Previous studies in Thailand found that thiarid snails, such as *M. tuberculata*, *M. jugicostis*, *T. granifera*, *M. scabra* and *S. riqueti*, are intermediate hosts of trematodes, which are categorised as types and species by using the characteristics of cercariae, viz. (i) parapleurolophocercous cercariae: *H. taichui*, *H. pumilio* and *Stictodora tridactyla*; (ii) pleurolophocercous cercariae: *Centrocestus formosanus*; (iii) virgulate xiphidiocercariae: *Loxogenoides bicolor*, *Loxogenes liberum* and *Acanthatrium histaense*; (iv) armatae xiphidiocercariae cercariae: *Maritreminoides caridinae* and *M. obstipus*; (v) furcocercous cercariae: *Haematoloechus similes*, *Transversotrema laruei*, *Cardicola alseae*, *Alaria mustelae*, *Apatemon gracilis* and *Mesostephanus appendicalatus*; (vi) megarulous cercariae: *Cloacitrema philippinum* and *Philophthalmus gralli*; (vii) echinostome-type cercariae: *Echinochasmus pelecani*; (viii) amphistome cercariae: *Gastrothylax crumenifer*; (ix) renicolid cercariae: *Cercaria caribbea* LXVIII; (x) cotylomicrocercous cercariae: *Podocotyle* (*Podocotyle*) *lepomis* and (xi) gymnocephalous-type cercariae (Dechruksa et al. 2007; Ukong et al. 2007; Krailas et al. 2011, 2014; Sritongtae et al. 2015; Veeravechsukij et al. 2018).

In this study, three trematodes species infecting snails at seven localities were reported: *N.* aff. *prasongi* in Trang, *S.* cf. *punctata* in Krabi and *S.* cf. *torulosa* in Trang and Satun Provinces. The three species from two trematode families were identified on the basis of the morphological characteristics of the emerged cercariae. The parthenitae at the larval stage (sporocysts or rediae) that produced the cercariae were observed. The two families were Heterophyidae (*H. taichui* and *Procerovum cheni*) and Lecithodendriidae (*L. bicolor*). The heterophyid trematode causes one of the fish-borne zoonoses which infect vertebrate animals, including humans and birds. Human infections are scattered and the major endemic areas are located in southeast Asia, including Thailand. Humans are infected by 13 genera, viz. *Acanthotrema*, *Apophallus*, *Ascocotyle*, *Centrocestus*, *Cryptocotyle*, *Haplorchis*, *Heterophyopsis*, *Heterophyes*, *Metagonimus*, *Pygidiopsis*, *Procerovum*, *Stellantchasmus* and *Stictodora* (Pearson 1964; Yamaguti 1971; Pearson and Ow-Yang 1982; Chai and Jung 2017).

In Thailand, *H. taichui* was first reported in 1971 from autopsy cases at Udonthani Provincial Hospital in the northeast region (Manning et al. 1971). Even though *H. taichui* is a small intestinal fluke, usually less than 5 mm in length, it can cause intestinal histopathology of hosts by mechanical and chemical irritations. It also induces chemical irritation by producing some substances that can act as antigens and toxins in the host's body (Chai and Jung 2017). Moreover, this fluke can elicit inflammatory reactions, together with ulcers and superficial necrosis of the intestinal mucosa. Some reported cases in humans were from Chiang Mai in northern Thailand (Kliks and Tantachamrun 1974; Sukontason et al. 2005).

Since 1980, thiarid snails have been reported as medically important gastropods, especially *H. taichui* and their snail hosts *M. tuberculata*, *M. jugicostis*, *M. scabra*, *T. granifera* and *S. riqueti*. *H. taichui* is one of the most frequently-reported species in southeast Asia, including Thailand. The prevalence of *H. taichui* has been observed in every region in Thailand, where it is found more frequently in the southern part than other haplorchiinid species (Upatham et al. 1980, 1981; Kumchoo et al. 2005; Sri-aroon et al. 2005; Ukong et al. 2007; Dechruksa et al.

2007; Krailas et al. 2008, 2011, 2014, 2016; Wongsawad et al. 2009; Sritongtae et al. 2015; Veeravechsukij et al. 2018). In the present study, *H. taichui* infections were detected in *S.* cf. *torulosa* and *N.* aff. *prasongi* from four locations in Satun and one location in Trang Provinces. For the first time, *H. taichui* infections were observed in *Stenomelania* in Thailand.

Procerovum cheni, with *P. varium* as the type species, is a small fluke that belongs to the same subfamily Haplorchiinae (Looss 1899). Three species have been described: *P. calderoni* (Africa and Garcia 1935; Price 1940), *P. varium* (Onji and Nishio 1916) and *P. cheni* (Hsü 1950). *P. calderoni* was first reported in dogs, cats and two humans in the Philippines, whilst *P. varium* was described in the adult stage from experimental dogs infected with metacercariae from mullet fish in Japan (Price 1940; Onji and Nishio 1916). *Procerovum* differs from *Haplorchis* in terms of the structure of the ventro-genital complex that presents an expulsor and a gonotyle with numerous spines. As such, some species, previously included in *Haplorchis*, have been transferred to *Procerovum*, based on these differentiating characters. The occurrence of metacercariae in fishes and the development of adults from experimental hosts have been used to categorise trematodes under *Procerovum* (Hsü 1950a, b, 1951; Umadevi and Madhavi 2000). Here, morphological and molecular studies on cercariae were conducted to confirm the specific identity and prevalence of various infectious trematodes in the collected *S.* cf. *punctata* from Klong Nong Jik in Trang Province. One *S.* cf. *punctata* was infected with *P. cheni*, with a prevalence of 0.32% (1/310; Table 1) at this location. In previous reports, the first intermediate host of *Procerovum* was found to be either freshwater or brackish water thiarid snails, viz. *M. tuberculata*, *Sermyla riquetti* and *Stenomelania denisoniensis* (Velasquez 1973; Surin 1993; Umadevi and Madhavi 2000), which were similar to those found in the present study. Heterophyid flukes, including *Haplorchis* and *Procerovum*, cause erratic extra-intestinal parasitism, such as ocular parasitosis, in humans. The ocular infection of *Procerovum* was first reported in the Philippines. In South India, an ocular granuloma in a single patient was attributed to *P. varium* infection. Later, 42 children with ocular granulomatous inflammation were infected with this trematode and all of them were exposed to snail-infested water, for example, ponds and rivers. Molecular analysis was performed to identify the species causing granulomas and 13 of the 42 samples tested positive for *P. varium* (Arya et al. 2016). In our study, only one snail was infected were *Procerovum.* However, this trematode has not been reported in other thiarid snails in Thailand. This finding indicated that the resulting parasitic diseases are still largely neglected in tropical medicine, so further studies should be performed on the prevalence of various trematode-borne diseases in locations with snail occurrences in Thailand.

Stafford (1905) classified *L. bicolor* as a trematode belonging to Lecithodendriidae when he reviewed *Loxogenes* and compared *L. bicolor* with *L. arcanum* (Kaw 1945). Yamaguti (1971) subsequently transferred it from Heterophyidae to Lecithodendriidae. This parasite is found in the terminal portion of the bile duct of frogs. It is regarded as an accidental parasite of the herring gull, which probably ingests an infected frog (Christensen 1981). Although *Loxogenoides* was first described in North America, it was studied in its adult form from a definitive and accidental avian host. In Thailand, *L. bicolor* from its snail intermediate host has been widely reported. Here, thiarid snails, such as *M. tuberculata*, *M. jugicostis*, *M. scabra*, *S. riquetti* and *N. prasongi*, act as the first intermediate hosts. Snails belonging to cerithioidean Pachychilidae are also infected with *L. bicolor* and three species (viz. *Brotia costula*, *B. dautzenbergiana* and *B. wykoffi*) have been reported (Dechruksa et al. 2007, 2013; Ukong et al. 2007; Krailas et al. 2011, 2014; Pratumsrikajorn et al. 2017; Veeravechsukij et al. 2018). Moreover, *L. bicolor* has the highest infection rate in infected thiarid snails. It also doubles or even triples the infection in their snail hosts when other trematodes are present. For example, *L. bicolor* infections doubled when it was combined with *Stictodora tridactyla* in *M. tuberculata* and *L. bicolor* was detected with *S. tridactyla* and *Cardicola alseae* in triple infections. *S. tridactyla* is a small intestinal fluke of the paraplurolophocercous cercaria type and *C. alseae* is a blood-dwelling trematode of the furcocercous cercariae type. In the present study, two locations in Trang Province had *L. bicolor* infections: one with *N.* aff. *prasongi* at Klong La 1 and three with *S.* cf. *torulosa* at Khao Ting Cave (Table 1).

Molecular analysis was conducted to confirm the results of cercarial identification, based on morphology, as this study aimed to combine classical morphology with molecular genetics, resulting in the conformation of cercarial infections by two distinct trematode families. As a noteworthy result, the nucleotide sequences of *Haplorchis* and *Procerovum* were found to be closely related. For phylogenetic analysis, some GenBank data, based on different parasite stages, such as metacercarial or adult stage (Van et al. 2009; Thaenkham et al. 2010; Buathong et al. 2019), were used. However, a similar phylogenetic pattern was observed and the relationships within the molecular clades of *H. taichui* could not be resolved clearly. All the samples originated not only from the locations in Satun Province, but also collected from the same snail species, viz. *S.* cf. *torulosa*. In a previous molecular genetic study, Van et al. (2009) found that *Procerovum* and *Haplorchis* are monophyletic. Thaenkham et al. (2010) reported a phylogeny of six species from Haplorchiinae by using the ITS2 region and other molecular markers (18S rDNA and 28S rDNA). They revealed the same topology of the phylogenetic tree. In our study, *P. cheni* was difficult to be clearly separated from the very closely relationed *P. varium* through molecular genetics. Furthermore, the sequences of *H. taichui* and *P. cheni*, obtained from *Stenomelania*, did not group together, although they were both of parapleurolophocercous cercaria type.

Conclusion and outlook

Stenomelania is considered a widely-distributed thiarid snail inhabiting freshwater and brackish environments in the tropical region of southeast Asia. Here, it is established as an intermediate host of trematode parasites along the Andaman coast in south Thailand. Information on the susceptibility of *Stenomelania* snails to food-borne zoonotic infections provides knowledge on public health in this region. Thus, the biodiversity and biology of thiarid snails should be further understood by studying their geographical distribution, morphological characteristics, molecular phylogenies and evolutionary associations with parasitic trematodes. Further in-depth evolutionary systematic analyses that involve the combination of data on reproductive biology, geographical distribution, morphology and molecular phylogenies of *Stenomelania* will enhance our understanding of the details of the host-parasite relationships of these snails as the first intermediate host populations in Thailand. Such analyses will also determine the role of parasitic infections in humans and animals in southeast Asia.

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