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. 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, cer-
cariae encyst and develop into infective metacercaiae. They infect humans and animals via the consumption of raw fish or improperly cooked fish containing metacercaiae (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 opisthobranch liver fluke *Opisthorchis viverrini* is found in freshwater Bithyniidae, i.e. *Bithynia junctifolia*, *B. siamensis goniomphalos* and *B. siamensis siamensis*, in Thailand, Laos, Cambodia and Vietnam. Small intestinal flukes from Thiariidae 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* (Krrull 1933; sensu Price 1943), *Centrocestus formosanus* (Nishigori 1924; sensu Price 1943) and *Stictodora tridactyla* (Martin & Kuntz, 1955), which are recorded from *Tarebia granifera, Melanoides 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, Thiariidae and Pachychilidae, were collected in a previous study. Parasitic infections were found in snail samples from 13 locations; six thiariid species, viz. *Melanoides tuberculata* (Müller, 1774), *Melanoides jugicostis* (Hanley & Theobald, 1876), *Melanoides scabra* (Müller, 1774), *Sermyla riqueti* (Grateloup, 1840), *Neoradina prasongi* (Brandt, 1974) and *Tarebia granifera* (Lamarck, 1822); and four pachychild species, viz. *Brotia sp. 1, Brotia sp. 2, Brotia wykoffii* (Brandt, 1974) and *Sulcospira housei* (Lea, 1856). Three thiariid species, viz. *M. tuberculata, M. jugicostis* and *N. prasongi*, were infected with two intestinal flukes, viz. *H. taichui* and *H. pumilio* (Kratalas et al. 2011, 2014).

Thiariidae 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* *arthuri* (Brot, 1870), *M. (Stenomelania) aspirans* (Hinds, 1847), *M. (Stenomelania) lutosa* (Gould, 1847), *M. (Stenomelania) plicaria* (Born, 1778) and *M. (Stenomelania) punctata* (Lamark, 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 thiariid snails.

*Stenomelania* is distributed in the Oriental Region, from India to Western Pacific islands (Starmühler 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 shedded juveniles hatch from a subhaemocoeolic 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 shedded juveniles similar to those in other thiariid snails, such as *Melanoides, Tarebia* and *Mięniplotia*. *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 Veeravechesukij 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 MgCl₂ (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 ddH₂O. 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°04'22.70N, 99°47'07.35E Alt. 159 m | 11 (e)                           | 0 (e)               | 0.00       | –        |
| 2   | SUT201910E     | Klong Thapae 1, Thapae District, Satun Province | 06°47'47.70N, 99°57'16.90E Alt. 28 m | 22 (e)                           | 1 (e)               | 4.55      | Haplorchis taichui |
| 3   | SUT201911E     | Klong Thapae 2, Thapae District, Satun Province | 06°48'09.74N, 99°57'50.96E Alt. 28 m | 11 (e)                           | 1 (e)               | 9.1       | Haplorchis taichui |
| 4   | SUT201909E     | Klong La-Ngu 1, La-Ngu District, Satun Province | 06°54'14.74N, 99°48'30.88E Alt. 39 m | 26 (e)                           | 1 (e)               | 3.85      | Haplorchis taichui |
| 5   | SUT201808C     | Klong Mai Phad, Sikao District, Trang Province | 07°33'10.46N, 99°21'01.95E Alt. 11 m | 62 (c)                           | 1 (c)               | 1.61      | Haplorchis taichui |
| 6   | SUT201806C     | Klong La 1, Sikao District, Trang Province | 06°29'35.25N, 099°20'34.25E Alt. 13 m | 111 (c)                          | 1(c)                | 0.90      | 0.90 Haplorchis taichui |
| 7   | SUT201807C     | Klong La 2, Sikao District, Trang Province | 07°29'49.22N, 099°21'28.25E Alt. 28 m | 35 (c)                           | 0 (c)               | 0         | –        |
| 8   | SUT201913E     | Khao Ting Cave, Palian District, Trang Province | 07°09'33.68E, 99°59.54E Alt. 104 m | 50 (e)                           | 3 (e)               | 6         | Loxogenoides bicolor |
| 9   | SUT201804A     | Klong Thanthip 2, Mueang District, Krabi Province | 08°09'37.78N, 98°47'07.51E Alt. 75 m | 304 (a,b,d)                      | 0 (d)               | 0         | 0.32 Procerovum cheni |
| 10  | SUT201801A     | Klong Nong Jik, Mueang District, Krabi Province | 08°13'22.00N, 98°46'24.97E Alt. 39 m | 310 (a,c,d,e)                    | 1 (d)               | 0.32      | Procerovum cheni |
| 11  | SUT201805A     | Klong Yang, Mueang District, Krabi Province | 08°09'57.22N, 98°47'40.3E Alt. 62 m | 151 (a,c,d,e)                    | 0 (e)               | 0         | –        |
| 12  | SUT201802A     | Klong Son 1, Mueang District, Krabi Province | 08°04'15.96N, 98°47'55.06E Alt. 84 m | 399 (a,b,c)                      | 0 (c)               | 0         | –        |
| 13  | SUT201903A     | Klong Son 2, Mueang District, Krabi Province | 08°04'23.68N, 98°48'09.98E Alt. 98 m | 59 (a)                           | 0 (a)               | 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; Veeravechsuksijd 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 striaions, 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.

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):**

| Parameter           | Range            | Average |
|---------------------|------------------|---------|
| 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) |

**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.

**Figure 4.** *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-
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. tortulosa* 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, calculated from 10 cercariae):

### 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 Hsü, 1951 | Haplorchis taichui Veeravecksukij et al. (2018) | Procerovum cheni Hsü (1951) | Procerovum cheni This study | 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 (60–75) × 110 (113–130)  | 69 (63–78) × 91 (79–103)     | 72 (63–88) × 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 (43–50) × 39 (20–55)                      | 27 (22–33) × 27 (23–31)     | n/a                         | 25 (11–35) × 14 (10–25)       | 33 (18–55) × 20 (10–35)                    |
| Pharynx                 | 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                     |

### 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.

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

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

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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):

| Structure            | Size Range       |
|----------------------|------------------|
| 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) |

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 *Melanoïdes* 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...
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. jucicostis*, *T. granifera*, *S. riqueti*, are intermediate hosts of trematodes families 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*, *Heterophyes*, *Heterophyopsis*, *Metagonimus*, *Pygidiosis*, *Procerovum*, *Stellantchasmus* and *Stictodora* (Pearson 1964; Yamaguti 1971; Pearson and Ow-Yang 1982; 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).

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. jucicostis*, *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 haptorchiid species (Upatham et al. 1980, 1981; Kumechoo et al. 2005; Sri-aroon et al. 2005; Ukong et al. 2007; Dechruckska et al.

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### 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                 | cercaria           | Klong La-Ngu 1, Satun | this study |
|                      | SUT172003E  | MT949316                 | metacercaria       | Chachoengsao    | Bualhong et al. (2019) |
|                      | –           | MK415601                 | –                  | –         | –          |
|                      | –           | MK415602                 | –                  | –         | –          |
|                      | –           | MK415603                 | –                  | –         | –          |
|                      | –           | MK415605                 | –                  | –         | –          |
|                      | –           | MK415596                 | –                  | –         | –          |
| *Procerovum sp.*     | –           | GQ176376                 | adult              | Thailand   | Van et al. (2009) |
| *Procerovum cheni*   | SUT172004D  | MT949317                 | cercaria           | Klong Nong Jik, Krabi | Thaenkham et al. (2010) |
|                      | –           | HM004164                 | –                  | –         | –          |
|                      | –           | 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                 | –                  | –         | –          |
Kitja Apiraksena et al.: Trematode infections of snail genus Stenomelania spp. in Thailand

2007; Krailas et al. 2008, 2011, 2014, 2016; Wongsawad et al. 2009; Sritongtae et al. 2015; Veeravechskij 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 (Loos 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 cercarias on cercariae were conducted to confirm the specific identity and prevalence of various infectious trematodes in the collected *S. punctata* from Klong Nong Jik in Trang Province. One *S. 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*, *S. riquetti* and *S. punctata* (Veerasathian 1973; Surin 1993; Umadevi and Madhavi 2000), which were similar to those found in the present study. Heterophid 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 with *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. arcumanum* (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. dautezenbergiana* and *B. wykoffi*) have been reported (Dechruska et al. 2007, 2013; Ukong et al. 2007; Krailas et al. 2011, 2014; Pratumsrikajorn et al. 2017; Veeravechskij 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 parapluerophocercous 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 Khoa 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 confirmation 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 Haplorchini 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 related *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 parapluerophocercous 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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References

Africa CM, Garcia E (1935) Two more new heterophyid trematodes from the Philippines. Philippine Journal of Science 57: 443–450.

Andrews RH, Sithithawarn P, Petney TN (2008) Asitic infections in humans and animals in southeast Asia. Zoosyst. Evol. 96 (2) 2020, 807–819.

Brandt AM (1974) The non-marine aquatic Mollusca of Thailand. Ar-

Brot A (1874–1879) Die Melaniaceen (Melanidae) in Abbildungen nach der Natur mit Beschreibungen. In Martini F, Chemnitz JH (Eds) Systematisches Conchylien-Cabinett (Vol. 1). Nürnberg Bauer & Raspe.

Chai JY (2017) *Metagonimus*. In: Liu D (Ed.) Laboratory models for foodborne infections. Food Microbiology Series CRC Press. Taylor & Francis Group, Boca Raton, 743–763. https://doi.org/10.1201/9781315120089-46

Chai JY, Murrell KD, Lymberry AJ (2005) Fish-borne parasitic zoonoses: status and issues. International Journal for Parasitology 35: 1233–1254. https://doi.org/10.1016/j.ijpara.2005.07.013

Chai JY, Shin EH, Lee SH, Rum HJ (2009) Foodborne intestinal flukes in Southeast Asia. Korean Journal of Parasitology 47: s69–s102. https://doi.org/10.3347/kjp.2009.47.S.S69

Chen HT (1936) A study of Haplorchiinae (Looss, 1899) Poche, 1926 (Trematoda: Heterophyidae). Parasitology 28: 40–55. https://doi.org/10.1017/S003118200002233X

Chontananarth T, Wongsawad C (2010) Prevalence of *Haplorchis taichui* in field-collected snails: A molecular approach. Korean Journal of Parasitology 48: 343–346. https://doi.org/10.3347/kjp.2010.48.4.343

Christensen BM (1981) A taxonomic review of the genus *Laxogenoides* (Digenea: Lecithodendriidae) with a description of *Laxogenoides loborchis* sp. n. from *Rana catesbeiana* Shaw in Western Kentucky. Proceedings of the Helminthological Society of Washington 48(1): 65–70.

De NV, Le TH, Murrell KD (2012) Prevalence and intensity of fish-borne zoonotic trematodes in cultured freshwater fish from rural and urban areas of Northern Vietnam. Journal of Parasitology 98(5): 1023–1025. https://doi.org/10.1645/G-E-3112.1

Dechruksa W, Kralias D, Glaubrecht M (2013) Evaluating the status and identity of *“Melanoides” jugicostis* Hanley & Theobald, 1876- an enigmatic thiarid being host to human parasites in Thailand (Caenogastropoda, Cerithioidae). Zoosystematics and Evolution 89(2): 293–313. https://doi.org/10.1002/zoos.201300015

Dechruksa W, Kralias D, Ukong S, Inkapatanakul W, Dangprasert T (2007) Trematode infections of freshwater snails family Thiaridae in Khek River. The Southeast Asian Journal of Tropical Medicine and Public Health 38(6): 1016–1028.

Dung DT, De NV, Waikagul J, Dalsgaard A, Chai JY, Sohn WM, Murrell KD (2007) Fishborne zoonotic intestinal trematodes, Vietnam Emerging Infectious Disease 13: 1828–1833. https://doi.org/10.3201/eid1312.070554

Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792–1797. https://doi.org/10.1093/nar/gkh340

Faust EC, Nishigori M (1926) The life cycles of two new species of Heterophyidae, parasitic in mammals and birds. Journal of Parasitology 13: 91–126. https://doi.org/10.2307/3271705

Fisher P (1885) Manuel de conchyliologie et de paléontologie conchyliologique ou historie naturelle des mollusques vivants et fossils suivi d’um appendice sur les brachiopodes. Fascicule (Vol. 9). Librairie F. Savy, Paris.

Glaubrecht M (1996) Evolutionsökologie und Systematik am Beispiel von Süß- und Brackwasserschnecken (Mollusca: Caenogastropoda: Cerithioidae): Ontogenese-Strategien, paliøntologische Befunde und Historische Zoogeographie (Vol. 1). Backhuys Publishers, Leiden.

Glaubrecht M (2006) Independent evolution of reproductive modes in viviparous freshwater Cerithioidae (Gastropoda, Sorboconcha): A brief review. Basteria 69: 23–28.

Glaubrecht M (2009) On “Darwinian Mysteries” or molluscs as models in evolutionary biology: From local speciation to global radiation. American Malacological Bulletin 27: 3–23. https://doi.org/10.4003/006.027.0202

https://doi.org/10.1201/9781315120089-46
Kitja Apirakse et al.: Trematode infections of snail genus Stenomelania spp. in Thailand

Glaubrecht M, Brinkmann N, Poppe J (2009) Diversity and disparity ‘down under’: Systematics, biogeography and reproductive modes of the ‘marsupial’ freshwater Thiariidae (Caenogastropoda, Cerithiidae) in Australia. Zoosystematics and Evolution 85(2): 199–275. https://doi.org/10.1002/zoos.200900004

Glaubrecht M, Neiber MT (2019) Thiariidae Gill, 1873 (1823). In: Lydeard Glaubrecht M, Brinkmann N, Poppe J (2009) Diversity and disparity of aquatic mollusks and their cercarial infections at Pasak Cholasid Reservoir, Thailand. Acta Tropica 156: 79–86. https://doi.org/10.1016/j.actatropica.2016.01.007

Krailas D, Veeravechskujik N, Chuanprasit C, Boonmekam D, Namchote S (2016) Prevalence of fish-borne trematodes of the family Heterophyidae at Pasak Cholasid Reservoir, Thailand. Acta Tropica 156: 79–86. https://doi.org/10.1016/j.actatropica.2016.01.007

Krailas D, Namchote S, Rattanathai P (2011) Human intestinal flukes Haplorchis taichui and Haplorchis pumilio in their intermediate hosts, freshwater snails of the families Thiariidae and Pachychilidae, in southern Thailand. Zoosystematics and Evolution 87(2): 349–360. https://doi.org/10.1002/zoos.201100012

Krailas D, Veeravechskujik N, Chuanprasit C, Boonmekam D, Namchote S (2016) Prevalence of fish-borne trematodes of the family Heterophyidae at Pasak Cholasid Reservoir, Thailand. Acta Tropica 156: 79–86. https://doi.org/10.1016/j.actatropica.2016.01.007

Krull WH (1933) Loxogenses bicolor, a new pigmented fluke from the frog, Rana clamitans. Transactions of the American Micropalaeontological Society 52(1): 47–50. https://doi.org/10.2307/3222226

Kumchoo K, Wongawad C, Chai JY, Yanittanakorn P, Rojanapaiul A (2003) Recovery and growth of Haplorchis taichui (Trematoda: Heterophyidae) in chicks. The Southeast Asian Journal of Tropical Medicine and Public Health 34: 718–722.

Lamarck JBM de (1822). Histoire naturelle des animaux sans vert _ébres_ (Vol. 6). Paris, L’Auteur, Au Jardin Du Roe.

Lea J, Lea HC (1851) Description of a new genus of the family Melania, and of many new species of the genus Melania, chiefly collected by H. Cuming, Esq., during his zoological voyage in the east, and now first described. Proceedings of the Zoological Society of London 18: 179–197.

Loos A (1899) Weitere Beiträge zur Kenntniss der Trematodafau- na Aegyptens, zugleich Versuch einer natürlichen Gliederung des Genus Distomum Retzius. Zoologische Jahrbücher 12: 521–784. https://doi.org/10.5962/bhl.part.2037

Manning GS, Lertprasert P, Watanasirmkit K, Chetty C (1971) A description of a newly discovered intestinal parasites endemic to northem Thailand. Journal of the Medical Association of Thailand 54: 466–474.

Miura O, Mori H, Nakai S, Satake K, Sasaki T, Chiba S (2008) Molecular evidence of the evolutionary origin of a Bonin Islands endem- ic, _Stenomelania boninensis_. Journal of Molluscan Studies 74(2): 199–202. https://doi.org/10.1093/mollus/eyn003

Müller OF (1774) Vermium terrestrium et fluvialium, seuanimalium infusoriorum, helminthicorum, et testaceorum, non marinorum, succincta historia. Volumen alterum. Havniae & Lipsiae, Heineck & Faber.

Ng TH, Tan SK, Wong WH, Meier R, Chan SY, Tan HH, Yeo DCJ (2016) Molluscs for sale: assessment of freshwater gastropods and bivalves in the ornamental pet trade. PLoS ONE 11(8): 1–23. https://doi.org/10.1371/journal.pone.0161130

Nishigori M (1924) Two new trematodes of the family Heterophyidae, found in Formosa. Taiwan Igakkai Zasshi 14: 439–442.

Olivier GA (1804) Voyage dans l’Empire Othoman, à l’Égypte et la Perse, fait par ordre du gouvernement, pendant les six premières années de la République (Vol. 2). Paris, Agasse.

Olivier LC, Schneiderman M (1956) Method for estimating the density of aquatic snail population. Experimental Parasitology 5: 109–117. https://doi.org/10.1016/0014-4894(56)90008-X

Ouji Y, Nishio T (1916) A review of new intestinal flukes. Igaku Chuo Zasshi 14: 439–442.
Pearson JC (1964) A revision of the subfamily Haplorchiinae Looss, 1899 (Trematoda: Heterophyidae). 1. The Haplorchis group. Parasitology 54: 601–676. https://doi.org/10.1017/S0031182000008269X

Pearson JC, Ow-Yang CK (1982) New species of Haplorchis from Southeast Asia, together with keys to the Haplorchis-group of heterophyid trematodes of the region. The Southeast Asian Journal of Tropical Medicine and Public Health 13: 35–60.

Pratumtrakorn P, Namchote S, Boonmekam D, Koonchornboon T, Glaubrecht M, Kraillas D (2017) Cercarial Infections of Freshwater Snail Genus Brotia in Thailand. Silpakorn University Science and Technology Journal 11(2): 9–15.

Price EW (1940). A review of the heterophyid trematodes, with special reference to those parasitic in man. International Congress for Microbiology, 446–447.

Ramakrishna, Dey A (2007) Handbook on Indian Freshwater Molluscs. Zoological Survey of India, Kolkata, India, 399 pp.

Sasaki T, Satake K, Tsuchiya K (2009) Distributions of an alien snail, Melanoides tuberculata and an endemic snail Stenomelania boninensis in the Ogasawara (Bonin) Islands with special reference to the effects of stream bank construction on the thiarid snails. Japanese Journal of Limnology 70(1): 31–38. https://doi.org/10.3739/rikusui.70.31

Sato M, Thaengkham U, Dekumyoy P, Waikagul J (2009) Discrimination of O. viverrini, C. sinensis, H. pumilio and H. taichui using nuclear DNA-based PCR targeting ribosomal DNA ITS regions. Acta Tropica 109: 81–83. https://doi.org/10.1016/j.actatropica.2008.09.015

Skov J, Kania PW, Dalsgaard A, Jorgensen TR, Buchmann K (2009) Life cycle stages of heterophyid trematodes in Vietnamese freshwater fishes traced by molecular and morphometric methods. Veterinary Parasitology 160: 66–75. https://doi.org/10.1016/j.vetpar.2008.10.088

Schell SC (1970) How to know the Trematode. W. C. Brown Publishers, Iowa.

Sri-aroon P, Lohachit C, Harada M (2005) Brackish-water Mollusks of Southeast Asia, together with keys to the Haplorchis-group of heterophyid trematodes. Zoosystematics and Evolution 94(2): 245–255.

Starmühler F (1976) Beiträge zur Kenntnis der Süßwasser Gasteropoden pazifischer Inseln. Annalen der Naturhistorischen Museums in Wien 80: 473–466.

Starmühler F (1979) Distribution of freshwater molluscs in mountain streams of tropical Indo-Pacific islands (Madagascar, Ceylon, New Caledonia). Malacologia 18: 245–255.

Starmühler F (1984) Results of the Austrian-Indian hydrobiological mission 1976 to the Andaman-Islands: Part IV: The freshwater gastropods of the Andaman-Islands. Annalen der Naturhistorischen Museums in Wien 86: 145–204.

Starmühler F (1993) Ergebnisse der österreichischen Tonga-Samoa Expedition 1985 des Instituts für Zoologie der Universität Wien: Beiträge zur Kenntnis der Süß- und Brackwasser-Gastropoden der Tonga- und Samoa-Inseln (SW-Pazifik). Annalen der Naturhistorischen Museums in Wien 94/95: 217–306.

Surin J (1993) A description of a pleurolophocercous cercaria of Procerovum sp. from the Haplorchis group of heterophyid trematodes. The Southeast Asian Journal of Tropical Medicine and Public Health 24(4): 692–696.

Thekhaen U, Dekumyoy P, Konalamisra C, Sato M, Dung DT, Waikagul J (2010) Systematics of the subfamily Haplorchiinae (Trematoda: Heterophyidae), based on nuclear ribosomal DNA genes and ITS2 region. Parasitology International 59: 460–465. https://doi.org/10.1016/j.parint.2010.06.009

Tran TK, Murrell KD, Madsen H, Nguyen VK, Dalsgaard A (2009) Fishborne zoonotic trematodes in raw fish dishes served in restaurants in Nam Dinh Province and Hanoi, Vietnam. Journal of Food Protection 72: 2394–2399. https://doi.org/10.4315/0362-028X-72.11.2394

Ungk S, Kraillas D, Danprasert T, Channgarm P (2007) Studies on the morphology of cercariae obtained from freshwater snails at Erawan Waterfall, Erawan National Park, Thailand. The Southeast Asian Journal of Tropical Medicine and Public Health 38 (2): 302–312.

Umadevi K, Madhavi R (2000) Observations on the morphology and life-cycle of Procerovum varium (Onji & Nishio, 1916) (Trematoda: Heterophyidae). Systematic Parasitology 46: 215–225. https://doi.org/10.1023/A:1006398205390

Upatham ES, Sornmai S, Thirachandra S, Sitaputra P (1980) Field studies on the biometrics of alpha and gamma races of Tricula aperta in the Mekong River at Khemmarat, Ubol Ratthathani Province, Thailand. In: Bruce JI, Sornmai S, Asch HL, Crawford KA (Eds) The Mekong Schistosome. Malacological Review suppl 2: 239–261.

Upatham ES, Koura M, Ahmad MD, Awad AH (1981) Studies on the transmission of Schistosoma haematobium and the biometrics of Bulinus (Ph.) abyssinicus in the Somali Democratic Republic. Annals of Tropical Medicine and Parasitology 75: 63–69. https://doi.org/10.1080/00034983.1981.11687409

Van KV, Dalsgaard A, Blair D, Le TH (2009) Haplorchis pumilio and H. taichui in Vietnam discriminated using ITS-2 DNA sequence data from adult and larvae. Experimental Parasitology 123: 146–151. https://doi.org/10.1016/j.exppara.2009.06.011

Velasquez CC (1973) Life cycle of Procerovum calderoni (Africa and Garcia, 1935) Price, 1940 (Trematoda: Digenea: Heterophyidae). Journal of Parasitology 59: 813–816. https://doi.org/10.2307/3278413

Veeravichakij N, Namchote S, Neiber NM, Glaubrecht M, Kraillas D (2018) Exploring the evolutionary potential of parasites: Larval stages of pathogen digenic trematodes in their thiarid snail host Tarebia granifera in Thailand. Zoosystematics and Evolution 94(2): 425–460. https://doi.org/10.3897/zse.94.28793

Waikagul J, Thekhaem U (2014) Approaches to Research on the Systematics of Fish-Borne Trematodes (1st edn). Academic Press, Cambridge, 130 pp. https://doi.org/10.1016/B978-0-12-407720-1.00001-7

Wiggering B, Neiber TM, Kraillas D, Glaubrecht M (2019) Biological diversity or nomenclatural multiplicity: the Thai freshwater snail Neo-radina prosangi Brandt, 1974 (Gastropoda: Thiaridae). Systematics and Biodiversity 17(3): 260–276. https://doi.org/10.1111/azo.12192

Witenberg G (1929) Studies on the trematode family Heterophyidae. Systematic Parasitology 46: 215–225. https://doi.org/10.1016/j.parint.2010.06.009

Wongsawad C, Wongsawad P, Chubon S, Anuntalabhochai S (2009) Co-pro-diagnosis of Haplorchis taichui infection using sedimentation and PCR-based methods. The Southeast Asian Journal of Tropical Medicine and Public Health 40: 924–928. https://doi.org/10.1016/j.exppara.2009.06.016

Yamaguti S (1971) Synopsis of Digeneric Trematodes of Vertebrates (Vol. 1). Keigaku Publishing Co., Tokyo, 1074 pp.

Yamaguti S (1975) A synoptical Review of life Histories of Digeneric Trematodes of Vertebrates. Keigaku Publishing Co., Tokyo, 590 pp. [219 pls]