MORPHOLOGICAL CHARACTERIZATION AND GENETIC IDENTIFICATION OF CERCARIAE FROM INFECTED SNAILS INTERMEDIATE HOST, GENUS *STENOMELANIA* FISCHER, 1885 IN THAILAND.

By
MR. Kitja APIRAKSENA

A Thesis Submitted in Partial Fulfillment of the Requirements
for Doctor of Philosophy BIOLOGY
Department of BIOLOGY
Graduate School, Silpakorn University
Academic Year 2021

ลักษณะทางสัณฐานวิทยาและการจำแนกพันธุกรรมของตัวอ่อนพยาธิระยะเซอร์คาเรีย จากหอยที่เป็นโฮสต์กึ่งกลางสกุล *Stenomelania* Fischer, 1885 ในประเทศไทย

โดย
นายกิจจา อภิรักษ์เสนา

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Title	Morphological characterization and genetic identification of cercariae from infected snails intermediate host, genus *Stenomelania* Fischer, 1885 in Thailand.
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Field of Study	BIOLOGY
Advisor	Professor Duangduen Krailas, Ph.D.

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MR. KITJA APIRAKSENA : MORPHOLOGICAL CHARACTERIZATION AND GENETIC IDENTIFICATION OF CERCARIAE FROM INFECTED SNAILS INTERMEDIATE HOST, GENUS *STENOMELANIA* FISCHER, 1885 IN THAILAND. THESIS ADVISOR : PROFESSOR DUANGDUEN KRAILAS, Ph.D.

Stenomelania (Fischer, 1885) is one of the snail species in the family Thiaridae. It has been realized that the snail in this family played an important role as an intermediate host of human and animal trematodes. The objective of this study was to investigate the trematode infections, identification of cercarial species and distribution of snail genus *Stenomelania* spp. in Thailand. Snail samples were collected from 24 localities between 2017 and 2020 by hand picking and scooping methods. The snails were transferred and studied in the laboratory of the Parasitology and Medical Malacology Research Unit, Silpakorn University, Nakhon Pathom, Thailand. The snails were identified according to their shell morphology and confirmed with molecular genetics by using the CO1 gene marker. A total of 3,026 *Stenomelania* snails were classified into six species consist of *S*. cf. *aspirans*, *S*. cf. *crenulata*, *Stenomelania* sp., *S*. cf. *punctata*, *S*. cf. *torulosa*, and *S*. cf. *denisoniensis*. The shells were conical in shape and varied in length and width. They were found in short and tall shells, as slender and wide shapes, smooth or rib. The collected snails were investigated the trematode infections by shedding and crushing methods. The infection rate was found to be 0.63 %. The emerging cercariae were described as the morphology based on living cercariae which were unstained or vitally stained with 0.5% neutral red. They were categorized into a total of four species from morphologically, viz. *Loxogenoides bicolor*, *Haplorchis taichui*, *Procerovum cheni* and *Acanthotrema tridactyla*. In addition, a phylogenetic marker (ITS2) was employed in generic and infrageneric level classifications of these trematodes. This study represents both morphological characterization and genetic identification of cercariae, which could be recognized as the basis reference of the larval trematode fauna, and could predict their potential to evolve for intermediate snail hosts.

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CHAPTER I
INTRODUCTION

Trematodiasis is still a major concern public health for people in the world, especially, Southeast Asia. There are at least 70 species of food-borne and water-borne trematodes, as well as blood flukes, intestinal flukes, liver flukes and lung flukes, which are shared with a great variety of animals (Andrews et al., 2008; Chai et al., 2005; Chai et al., 2009). The occurrence of trematodes depends on the presence of first and second intermediate host species, as well as eating behavior. It can be result in the epidemic spreading of parasites, for example intestinal and liver flukes; two major agents of fish-borne infections are intestinal flukes (Heterophyidae) and liver flukes (Opisthorchiidae). Their complex life cycles, with a series of sporocyst and redia stages occurring in freshwater snails and developing into cercariae that are eventually released into the water. These cercariae encyst in freshwater fish, where they develop into infective metacercariae. Human and animal infections occur by eating raw fish or improperly cooked fish which contain metacercariae (De et al., 2012; Dung et al., 2007; Skov et al., 2009; Tran et al., 2009).

In Thailand, medically-important freshwater snails have been investigated since 1980 for trematode infections (Dechruksa et al., 2017; Dechruksa et al., 2013; Dechruksa et al., 2007; Krailas et al., 2008; Krailas et al., 2003; Krailas et al., 2014; Sri-aroon et al., 2005; Ukong et al., 2007; Upatham et al., 1980). It has been realized that the snail family, Thiaridae, played an important role as intermediate host of human and animal trematodes. They were reported that various species of thiarid snails are the first intermediate host of intestinal trematodes (*Haplorchis pumilio, H. taichui, Loxogenoides bicolor, Centrocestus formosanus, Stictodora tridactyla*), and fish blood flukes (*Transversotrema laruei, Apatemon gracilis, Mesostephanus appendicalatus, Cardicola alseae, Alaria mustelae*) of these snails were *Tarebia granifera, Mieniplotia scabra, Melanoides tuberculata* and *M. jugicostis* (Krailas et al., 2014; Krailas et al., 2006; Krailas et al., 2011)

Stenomelania Fisher (1885) is belong to Thiaridae family, with elongate, pointed shells, and mostly found in the brackish environment. The distribution of *Stenomelania* was found includes widely the Oriental region from India to the islands of the western Pacific (Starmühlner, 1976; Starmühlner, 1979; Starmühlner, 1984, 1993). *Stenomelania* spp. were reported in the water resource of Andaman coast, southern Thailand, however, there are very few reports about these snails in Thailand (Wiggering et al., 2019). Their morphology is similar to *Melanoides*. The shell character has variation in shape, there were conical body vary from short to tall and broad to slender shape. The color of shells varies from light to dark brown. The pattern of shell surface both horizontally and vertically show grooves between the apex to body whorl with or without rib, the whorls number was 8-13 whorls.

This study represents both morphological characterization and genetically identification of cercariae, larval stage of trematodes that can be found from the snail. The samples were collected in particular from *Stenomelania* spp. in Thailand. This could be recognized as the basis reference of the larval trematode fauna in Thailand, and could predict their potential evolve for intermediate snail host.

Family Thiaridae

The Thiaridae Troschel, 1857 was a member of cerithioidean gastropods, which widely distributed and inhabit limnic environments in the tropics of the world. Thiaridae has estimated 200 species greater than 30 genera (Figure 1). Dechruksa et al. (2007) reported the snail family Thiaridae from five locations at Khek River in Thailand that were categorized as well as *Tarebia granifera*, *Melanoides tuberculata, Thiara scabra* and etc. Since the Thiaridae were suspected to represent a polyphyletic group, and systematics are currently under revision, the snail has a specialized incubatory pouch in the head-foot. The reproductions of gastropods in family Thiaridae have two strategies consist of: eu-viviparous and ovo-viviparous. Normally, we can identify snail by shell morphology. Glaubrecht et al. (2009) reported freshwater snails in Australia can be categorized; (1) ovo-viviparity: *Thiara amarula, Ripalania queenslandica, Stenomelania cf. aspirans, Sermyla riqueti* (2) eu-viviparity: *Thiara australis, Plotiopsis balonnensis, Stenomelania denisoniensis, Sermyla venustula, Melasma onca, Melanoides tuberculata*, *Plotia scabra*. In Thailand, Brandt (1974) reported 8 genera and 15 species of snails in Thiaridae family, they were divided into two subfamilies of Thiarinae and Melanatriinae. The members of Thiarinae were *Thiara* Roding, 1798; *Melanoides* Olivier, 1804; *Tarebia* Adam, 1854; *Sermyla* Adam, 1854; and *Neoradina* Brandt, 1974. The members of Melanatriinae were *Adamietta* Brandt, 1974; *Brotia* H. Adams, 1866; and *Paracrostoma* Cossmann, 1900. Nowadays, the thairidae genera in Thailand were revised into 7 genera, viz. *Thiara*, *Melanoides*, *Tarebia*, *Sermyla Neoradina*, *Mieniplotia* Low & Tan, 2014 and *Stenomelania*

Fischer, 1885 (Boonmekam et al., 2019; Brandt, 1974; Dechruksa et al., 2013; Dechruksa et al., 2007; Glaubrecht, 1996, 2006; Glaubreht & Kőhler, 2004; Krailas et al., 2014; Krailas et al., 2011; Veeravechsukij et al., 2018b; Wiggering et al., 2019). *Stenomelania* was distribution in Asia and Pecific (Table 1).

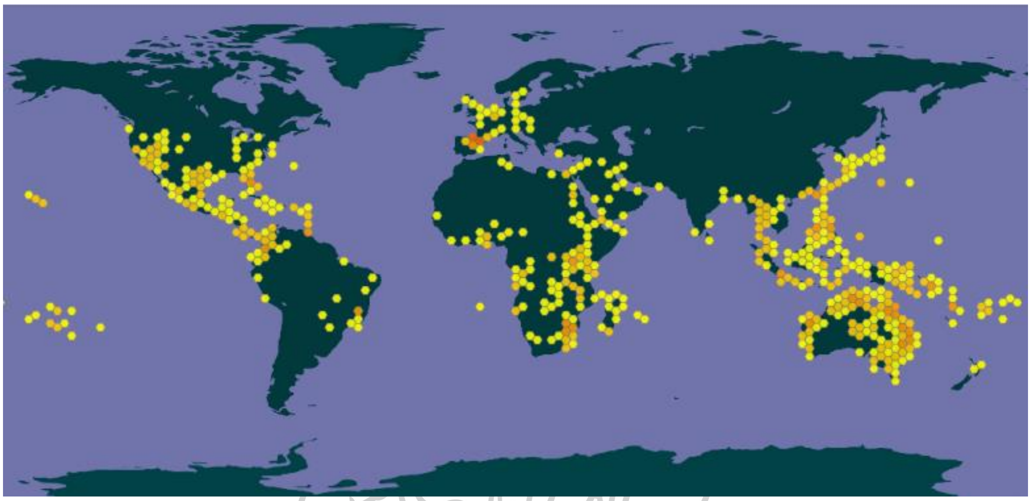

Figure 1. Distribution of snail Thiaridae family in the world.
Thiaridae in GBIF (2019). Accessed march, 2021, available from https://www.gbif.org/species/7063

Table 1. Species and locations of snail genus *Stenomelania*

Species	Location
Stenomelania aspirans (Hinds, 1844)	Bismarck Archipelago, Solomon Island Vanuatu, New Caledonia, Fiji, Samoa, Andaman Island, Nicobar Islands
Stenomelania boninensis (I. Lea, 1856)	Japan (Bonin Island)
Stenomelania costellaris (I. Lea & H. C. Lea, 1851)	Philippines, Japan (Bonin Island)
Stenomelania crenulata (Deshayes, 1838)	Japan (Bonin Island)
Stenomelania denisoniensis (Brot, 1877)	Australia
Stenomelania dollfusi (Jodot, 1928) †	Type locality contain in France (Fossil)
Stenomelania juncea (Lea, 1851)	Japan (Bonin Island)
Stenomelania plicaria (Born, 1780)	South East Asia, Solomon Island, Vanuatu, Malay Archipelago and Andaman Island
Stenomelania waigiensis (Lesson,	Indo-West Pacific

1831)	
Stenomelania torulosa (Bruguiere, 1789)	Fiji, Samoa, Malagasy, South East Asia, Malaysia, Philippines, Taiwan, Solomon Islands, Andaman Island, Nicobar Islands
Stenomelania punctata (Lamarck, 1822)	South East Asia, Solomon Island, Vanuatu, New Guinea, Australia
Stenomelania hustula (Lea, 1850)	Philippines, Taiwan, Indonesia
Stenomelania loebbeckii (Brot, 1877)	Japan (Urado Bay, Kochi)

Snails Genus Stenomelania

Stenomelania Fisher, 1885 is a genus of aquatic gastropod in the Thiaridae family. In the previous studies, the snail was classified as either of "*Melania*" (Hinds, 1844) or "*Melanoides*" (Olivier, 1804)., Haynes (2001) reported five species of *Stenomelania* from the Tropical Pacific region, viz. *Melanoides* (*Stenomelania*) *aspirans* (Hinds, 1847), *M.* (*Stenomelania*) *arthurii* (Brot, 1870), *M.* (*Stenomelania*) *lutosa* (Gould, 1847), *M.* (*Stenomelania*) *plicaria* (Born, 1778), and *M.* (*Stenomelania*) *punctata* (Lamarck, 1822). Four species from India were identified using shell morphology, viz. *S. torulosa*, *S. plicaria*, *S. punctata* and *S. aspirans* (Ramakrishna & Dey, 2007). *Stenomelania aspirans* was the type species of the genus *Stenomelania*, that was descripted for shell morphology (Fisher, 1885).The shell was elongated, slender, dark-brown in color; spire attenuated and pointed, sculpture smooth or with spiral-threaded or rows of beads, columella thin and without callus. In our preliminary work, the snails were collected in the south of Thailand, the pattern of shell surface both horizontally and vertically show grooves between the apex to body whorl and had rib, dark color (Figure 2, Table 2).

Figure 2. Shell morphology of *Stenomelani*a sp.

These freshwater snail data were insufficient and contentious about taxonomy because shell morphology similarity with other snails in the same family. Bandel et al. (1997) reported snail genus *Stenomelania* from a Nipa mangrove on Cebu Island, Philippines by shell characters consist of; *Stenomelania plicaria* and *S. punctata* (planktotrophic species). The two species can easily be differentiated with the protoconch and both of them shares several characters with *Melanoides tuberculata*. That was evident of relationship between *Stenomelania* and *Melanoides*. As the mentioned above, both of *Melanoides*, and *Stenomelania* are very similar, the combination of morphological, reproductive strategy, genetic, and biogeographic methods against the background of phylogenetic systematics to study a taxon’s evolutionary history, is the evaluation of nomenclatural redundancy (Glaubrecht, 2010; Glaubrecht et al., 2009). We know that the evolutionary systematics approaches are especially necessary in groups with high phenotypic plasticity and taxonomic diversity, as regularly a plethora of names have been assigned for potentially only few lineages that deserve recognition as evolutionary entities, as has been exemplified. Moreover, *Neoradina* was report a new genus of thiarid snail in Thailand (Brandt, 1974). In addition to this genus, morphologically almost indistinguishable shells were the basis for the description and the establishment of *Neoradina*. Wiggering et al. (2019) studied whether these shells represent specimens from a distinct evolutionary lineage of three genera from Thailand, using molecular genetics, shell characters via geometric morphometrics, radula morphology, and reproductive biology. The report explained some information that might be distinguished three thiarid snails in the genus *Melanoides*, *Stenomelania* and *Neoradina*. Although there are some differences between the three types of shells. *Stenomelania* and *Melanoides* are currently distinguished by their reproductive mode, *Stenomelania* release the veliger larva (exceptional species, *S. denisoniensis* Brot, 1877, releases shelled juveniles similar to those in other thiarid snails, such as *Melanoides*), while *Melanoides* release shelled juvenile (see Figure 3;(Glaubrecht et al., 2009)). For *Neoradina*, Brandt (1974) reported an oviparous reproductive mode, contradicting his own statement by mentioning that the females were found subhaemocoelic brood-pouch with many small embryonic shells in all stages of development.

Table 2. The characters and habitat of snail genus *Stenomelania*

Species	Shell morphology	Habitat
Stenomelania arthurii	Shell color was olive-brown with many longitudinal red lines on larger whorl, 7-8 whorls, aperture brown and white. Operculum was horn color, oval and eccentric.	Alongside the streams and in ditches on gravel, sand or mud not far from the coastline.
Stenomelania aspirans	Shell was long, grows to 80 mm. high with 11-12 whorl, apex eroded. Color brown with few darker lines on the body whorl. Whorl flat and smooth, aperture white-gray. Operculum was horn color, oval-pointed.	In brackish and fresh water at the coastline.
Stenomelania punctata	Shell was turret shaped with long body whorl and long pointed spire, 12 whorls. Whorl with radial striation, color yellow- brown with many longitudinal red-brown dashes between the striations, aperture white on lip. Operculum was horn-brown color.	On the stones and gravel in the stream and pond.
Stenomelania lutosa	Shell color was olive-brown but encrusted black, 7-8 whorls, smooth with the darker ring	On the stones and gravel in the stream and river.

	around the middle of each whorl. Aperture white- gray and operculum was horn-brown color.	
Stenomelania plicaria	Shell was very long and slender, up to 100 mm. high, whorls with flat sides and many dark red stripes on each whorl. 16-18 whorls tapering to a fine acute apex. Operculum was horn-brown color.	In brackish and fresh water near the river and stream.

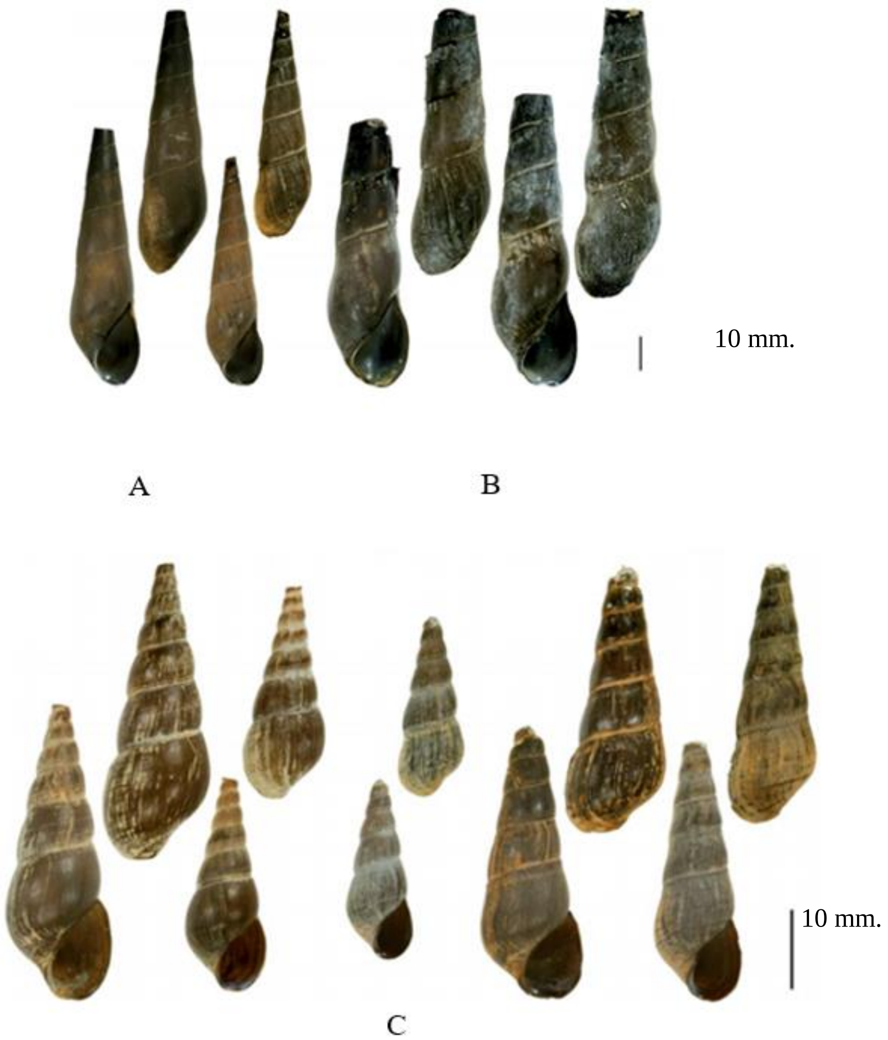

Figure 3. Shell morphology of *Stenomelania* cf. *aspiran* (A, B), *Stenomelania denisoniensis* (C), that found from Australia (Glaubrecht et al., 2009)

The diversity of *Stenomelania* species is still a lot of confusion, because the distinction of shell characters was very similar. However, reproductive mode and radula characters combined with genetic analyses were ambiguous for implications on genus affiliations and did not support the classification of some species as part of *Stenomelania*. However, there were many species of *Stenomelania* in the world, Ng et al. (2016) reported two species from ornamental pet trade in Singapore, viz. *S. offachinsis* and *S. plicaria*. (Figure 4). Miura et al. (2008) studied snails *Stenomelania* spp. in Bonin Islands (Ogasawara Island), which are about 1,000 miles from the mainland, this area never connected to the mainland. From that reason could be evolution of species *S. boninensis* and they confirm the snail was an endemic species on this island. In addition, they found other species of snail genus *Stenomelania* consisting of *S. costellaris, S. rufescens, S. uniformis, S. crenulatus,* and *S. juncea*. Sasaki et al. (2009) reported that *S. boninensis* is an endemic species, which corresponds to previous studies with (Miura et al., 2008). *S. boninensis* was the dominant species in Bonin Island. *S. boninensis.* was not found since the reservoir was built in this area. They found only *M. tuberculata* distributions after 3 months. Fortunately, *S. boninensis* was found 9 months later, so *S. boninensis* needs more time to recover and increase the population. Moreover, *S. crenulata* in Okinawa Island and *S. rufescens* in the main island of Japan (Figure 5), that both types of snails have many characteristics of shell morphology. That was difficult to identify by only shell morphology (Hidaka & Kano, 2014).

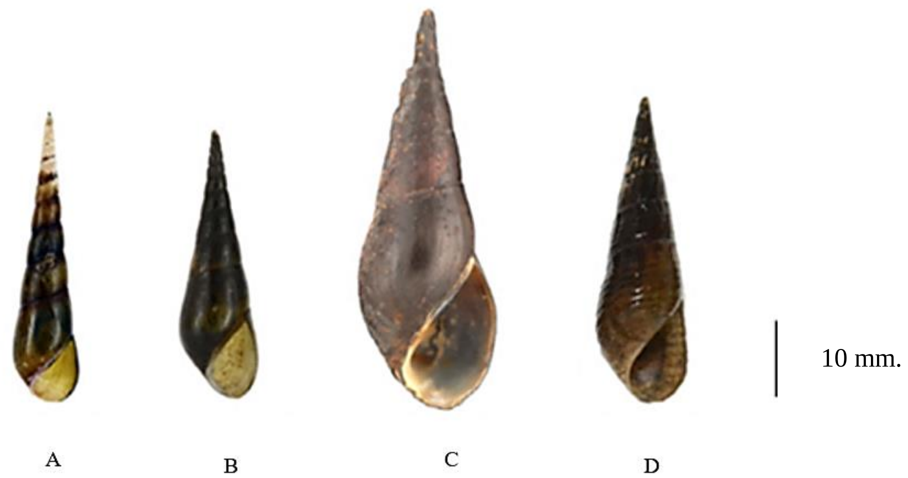

Figure 4. Shell morphology of *Stenomelania offachinsis* (A), *Stenomelania plicaria* (B), *Stenomelania* cf. *plicaria* (C) and *Stenomelania* sp. (D). The snails found at ornamental aquatic pet trade in Singapore. (Ng et al., 2016)

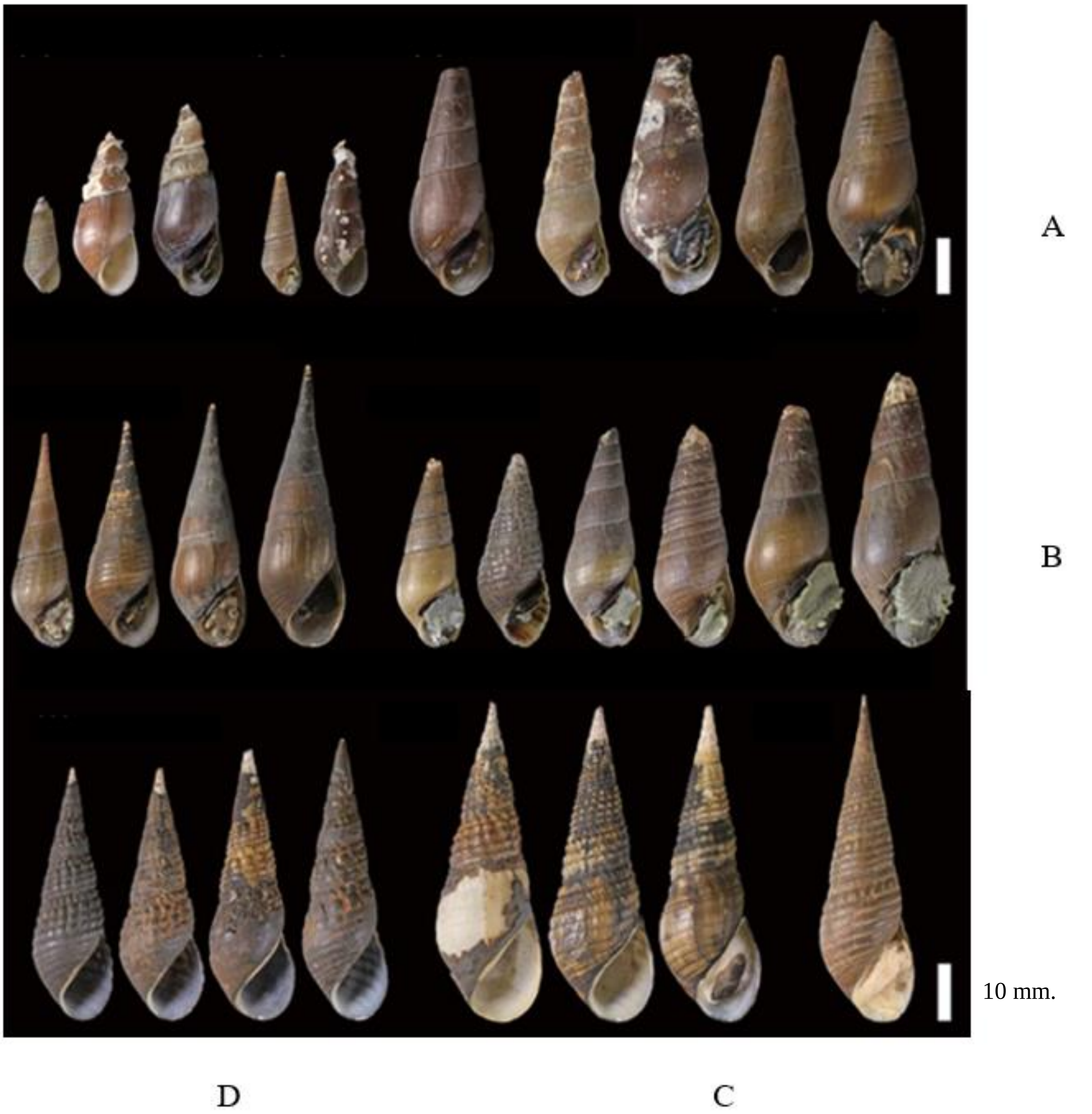

Figure 5. *Stenomelania rufescens* from mainland Japan (A), *S. crenulata* from Okinawa (B), Taiwan (D) and Philippines (C). (Hidaka & Kano, 2014)

Digenetic trematodes

Flukes or trematodes are identified in Phylum Platyhelminthes, Class Trematoda, Subclass Digenea. The trematodes or flukes could be categorized 18,000 to 24,000 species (Littlewood & Bray, 2000), divided into two subclasses. Trematode infection can result in severe liver lung intestinal and blood disease and these diseases are estimated to cause 2 million life years lost to disability and death worldwide every year. Trematodes are flattened oval or worm-like animals. Their most distinctive external feature is the presence of two suckers, one close to the mouth, and the other on the underside. The body surface comprises a tough syncytial tegument, which can help to protect against digestive enzymes in the host's digestive tract. The mouth is located at the anterior end, It has a pharynx that connects with short esophagus, to the ending caeca, which occupies most of the length of the body, bladder opening to the posterior end. It also has a nervous system, the head region has a pair of ganglia, nerve cords located in the ventral, but lack special sense organs. Most of them are hermaphrodites, having both male and female organs. We can categorize trematode by adult morphology in definitive host into 4 types consist of:

1. Intestinal fluke: The adult worms were growing in the small or large intestinal.
2. Liver fluke: The adult worms were growing in the bile duct or liver.
3. Lung fluke: The adult worms were growing in the lung.
4. Blood fluke: The adult worms were growing in a blood vessel (vein).

Flukes were internal parasites of freshwater snails, fishes, mammals, etc. Most trematodes have a complex life cycle with at least two hosts. The intermediate host is usually in a snail. Trematodes are released from the definitive host as eggs mix with feces urine or phlegm. After that miracidium was hatched from the egg into an environment. This is the first infected stage to the intermediate host as freshwater snails, either active or passive transmission and developed to sporocyst, rediae and cercariae inside the first intermediate host. The cercariae are adapted to recognize and penetrate the second intermediate host such as prawn, snail, crab, fishes and aquatic plants. When cercariae are successfully infected in a secondary intermediate host, they will be developed into metacercaria, that is the infective stage. The adult is the fully developed form which infects the definitive host (Figure 6).

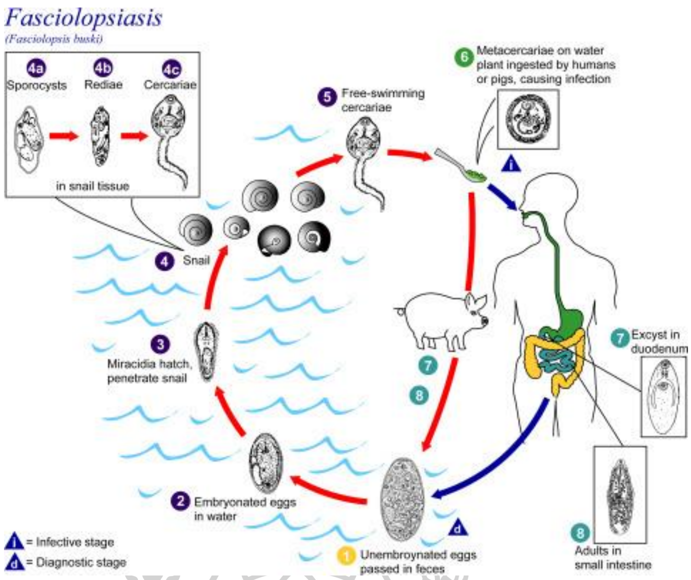

Figure 6. Life cycle of *Fasciolopsis buski* (Alexander & Moser, 2000)

Categorize the cercaria

Cercariae were categorized to family genus and species. The study of morphology, internal organs, behavior, type of motility to family, environmental factor, it would be valuable for identification and epidemics of trematode infections consist of:

1. Temperature: normally cercariae were suitable living between 24-28 degree Celsius, which is optimum temperature for growth and development.
2. Light: the cercaria need light for growing up and shedding from an intermediate host. Cercaria emerge from the host every day and any period of times, some cercaria emerge at night time (Ratanaponglakha et al., 1989; Ratanaponglakha et al., 1988). When cercariae were excyst as free living form, they were continuing to infect in secondary or definitive hosts.
3. Survival period outside host: Free living cercariae can survive up to 12 hours. If they could not infect the specific host or new host within 12 hours, they will be decreased. We can observe cercaria behavior and motility. For example: The cercaria some might arise up and live on the surface, sinking down to the bottom and arise up again in a cyclic pattern, some are not.

Cercariae can be identified and categorized by morphology (Ito, 1980; Komiya, 1961; Krailas et al., 2014; Krailas et al., 2011; NASIR, 1984;

Schell, 1970; Veeravechsukij et al., 2018a; Yamaguti, 1971, 1975). The classification is as follow:

1. Long tail cercaria
   1.1 Furcocercous cercariae:
   - Superfamily Strigeoidea
   - Dominant character was fork tail, normally known as “fork tail cercaria”.
   - Dioecious in adult worms.
   - Body was boat shape, have oral and ventral sucker
   - Divide into two groups consising of: pharynx group present and pharynx group absent.
   - Cercaria developed in sporocyst and changed to metacercaria in secondary intermediate hosts.
   - Definitive host: human, raccoon, owl, cat, dog etc.
   - Example: *Cotylurus communis, Strigea strigis, Diplostomum flexicaudam, Schistosomatium douthitti, S. mansoni* etc.

   1.2 Monostome cercariae
   - Superfamily Notocotyloidea
   - Dominant character was small oral sucker, ventral sucker absent, pharynx present, large body, 2-3 eye spots, tail longer than body, thin excretory bladder, adhesive organs.
   - Cercaria develop well in redia.
   - Definitive host: duck, deer, mammal etc.
   - Example: *Notocotylus seineti, Notocotylus* spp. and etc.

   1.3 Amphistome cercariae
   - Superfamily Paramphistomatoidea
   - Dominant character was small oral sucker, large ventral sucker located near posterior body, large body, pigment present on eye spot, long tail and large caudal fin.
   - Definitive host: ruminant and amphibian.
   - Example: *Paramphistomum* spp., *Diplodiscus* spp., *Gastrodiscus aegyptiacus* etc.

   1.4 Gymnocepharous cercariae
   - Family Fasciolidae
   - Dominant character was round shape, many cystogenous gland, superior oral sucker, ventral sucker located on center of body, finfold absent and adhesive located on posterior tail end.
   - Cercaria developed in redia and changed to metacercaria on aquatic plants, fishes or reptilian.
   - Definitive: ruminant.
   - May cause diseases in humans.

1.5 Echinostome cercariae

- Superfamily Echinostomatoidea
- Dominant character was spine collar around oral sucker, long or short caeca, cylinder shape tail.
- Cercaria developed in redia.
- Definitive host: aves and mammal.
- May cause diseases in humans.
- Example: *Echinostoma revolutum, Echinoparyphium recurvatum*, etc.

1.6 Megalurous cercariae

- Family Philophthalmidae
- Dominant character was adhesive gland on posterior end tail, excretory canal absent, ventral sucker larger than oral sucker.
- Cercaria developed in redia and they will be developed into metacercaria.
- Definitive host: Chicken, bird and duck.
- Example: *Cloacitrema philippinum*, etc.

1.7 Pleurolophocercous cercariae

- Superfamily Opisthorchiodea
- Dominant character was oval shape, lateral finfold absent, penetration gland present, pigment in eyespot, thick wall of excretory bladder.
- Cercaria developed in redia and changed to metacercaria in fishes.
- Definitive host: vertebrates
- Example: *Centrocestus formosanus*

1.8 Parapleurolophocercous cercariae (Figure 7)

- Family Heterophyidae
- Dominant character was lateral finfold present, dorsal-ventral finfold located in tail end, not well-developed ventral sucker, have pigment in eye spot.
- Cercaria growth in redia and developed to metacercaria in fishes.
- Definitive host: human, carnivorous animals.
- Example: *Haplorchis taichui, H. pumilio, stictodora tridactyla,* etc.

1.9 Gasterostome cercariae

- Family Bucephalidae
- Dominant character was mouth located center of body, symmetry fork tail
  similar to a horn.

- Cercaria growth in sporocyst and developed into metacercaria in fishes.
- Definitive host: Fishes
- Example: *Bucephaloid cercariae*

1.10 Xiphidiocercariae

- Superfamily Plagiorchiodae
- Dominant character was stylet on oral sucker, some species found virgula organ on oral sucker.
- Cercaria growth in redia or sporocyst and developed into metacercaria in invertebrates and vertebrates.
- Definitive host: vertebrates
- Example: *Loxogenoides bicolor, Acanthatrium hitaense,* etc.

2. Short tail cercaria

2.1 Microcercous cercariae

- Dominant character was short tail and knob like, cup shape, stylet present, ventral sucker larger than oral sucker.
- cercaria growth in sporocyst and developed into metacercaria in crab or prawn.
- definitive host: Human
- Example: *Paragonimus westermani*

3. Absent tail cercaria

3.1 Cercariaeum cercariae

- Dominant character was found only in the body.
- cercaria developed in sporocyst or redia.
- Example: *Mutabile cercaria*

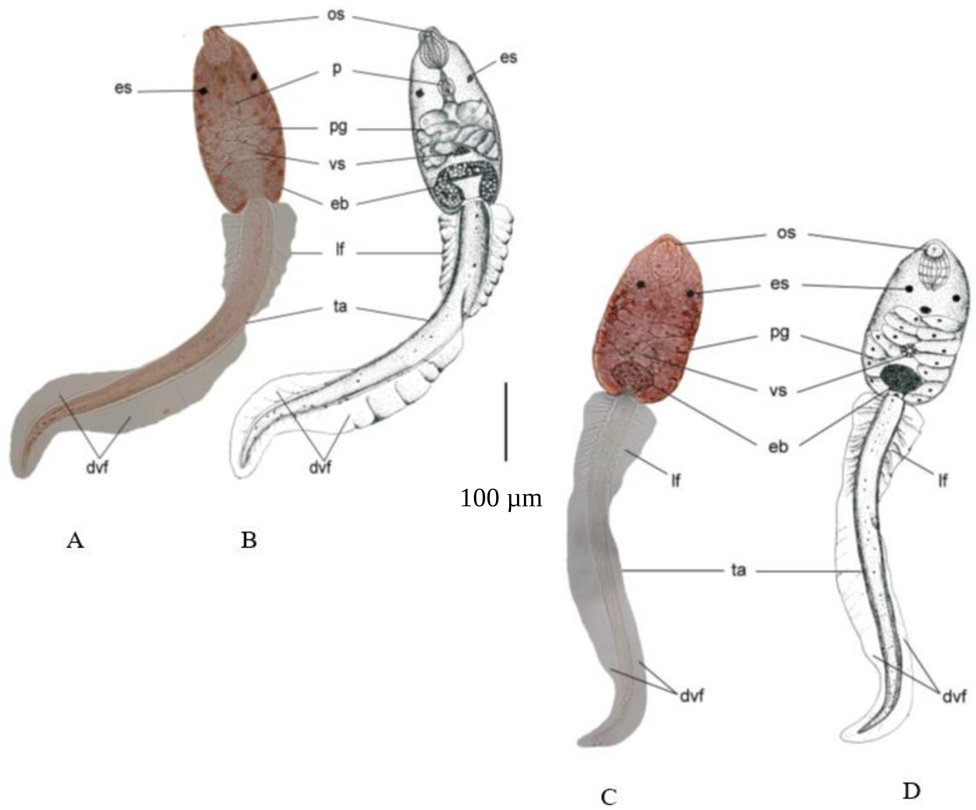

Figure 7. Morphology of Parapleurolophocercous cercariae consist of *stictodora tridactyla*. A. sample stained with 0.5% neutral red and B. drawing of cercaria, *Haplorchis pumilio*. C. sample stained with 0.5% neutral red. And D. drawing of cercaria. Abbreviations – dvf: dorsoventral finfold; eb: excretory bladder; es: eyespot; lf: lateral finfold; os: oral sucker; p: pharynx; pg: penetration gland; ta – tail; vs: ventral sucker (Veeravechsukij et al., 2018a)

In Thailand, the cercarial infections had been reported, e.g. Dechruksa et al. (2007) reported *Tarebia granifera*, *Melanoides tuberculata*, *Thiara scabra* and etc. These are intermediate hosts of trematodes, which are categorised as types and species by using the characteristics of cercariae consist of *Haplorchis pumilio, Centrocestus formosanus, Acanthatrium hitaense, Loxogenoides bicolor* and *Haematoloechus similis*. Krailas et al. (2014) reported *M. tuberculata* is an intermediate host of many species of trematode. The cercariae were categorized into eighteen species by morphology consist of *H. pumili*, *H. taichui*, *Stictodora tridactyla*, *C. formosanus*, *A. histaense, L. bicolor*, *H. similis, Cloacitrema philippinum, Philopthalmus* sp.*, Cardicola alseae, Alaria mustelae, Transversotrema laruei, Apatemon gracilis, Mesostephanus appendiculatus, Echinochasmus pelecani, Gastrothylax crumenifer, Cercaria caribbea* LXVII and *Podocotyle (Podocotyle) lepomis.*

Veeravechsukij et al. (2018a) reported trematode infections of *T. granifera* snails that distributed in Thailand. The cercariae were categorized into 15 species by morphology consist of *L. bicolor*, *L. liberum, A. histaense, Maritreminoides caridinae, M. obstipus, H. pumilio, H. taichui, S. tridactyla*, *C. formosanus, Philophthalmus gralli, C. alseae, A. mustelae, T. laruei*, echinostome-type cercariae, and gymnocephalous-type cercariae.

Molecular markers

Studying the taxonomic of animals or plants when needed to obtain more verifiable information. Taxonomists are able to study on both morphology and genetic traits. The most important markers in molecular systematics and evolution are ITS2, this maker shows significant sequence variability at species level. ITS2 is potentially useful as a standard DNA barcode to identify medicinal plants and animals (Coleman, 2003; Coleman et al., 2009). In addition, CO1 gene (cytochrome c oxidase subunit I) in mitochondrial genome can be used as a barcoding to identify animals, because the gene is highly conserved, it can be copied from unknown organisms (Blaxter et al., 2003; Dayrat, 2005; Hebert et al., 2003).

The internal transcribed spacer (ITS)

The internal transcribed spacer (ITS) is part of the rRNA gene (Figure 8). It is divided into ITS1 and ITS2, which are separated by the gene coding for 5.8S rDNA. It had been assumed that the ITS had no function. The ITS1 region has a greater length variation than the ITS2 region. However, the ITS2 region is more conserved than the ITS1 region. The conserved regions in internal transcribed spacers have been found among diverse eukaryotes that indicate a function of ITS2 in pre-rDNA maturation and ITS1 was more variable than ITS2 (Wang et al., 2015). ITS2 has been successfully to identify many species of trematode, because its sequence is usually conserved within species but more variable among species (Cutmore et al., 2010). Although ITS2 shows a great potential to identify animal species, an extensive evaluation based on a comprehensive sample set is lacking. To validate the potential of using the ITS2 region to identify closely related species of animals (Yao et al., 2010).

Veeravechsukij et al. (2018a) used phylogenetic markers (internal transcribed spacers 2, ITS2) for identifying the types of parasites. They obtained ITS2 sequences of nine species of cercariae from fifteen species. Only data from DNA sequence is not enough to identify cercaria so they combine morphological data and geographical occurrence with phylogeny to analyse (Figure 9).

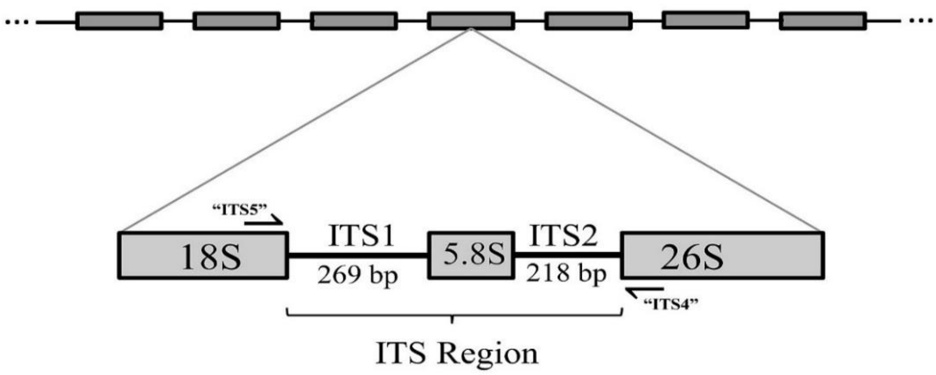

Figure 8. General structure of a nuclear ribosomal RNA Gene ITS1 and ITS2 are internal transcribed spacer regions (Zhang et al., 2007)

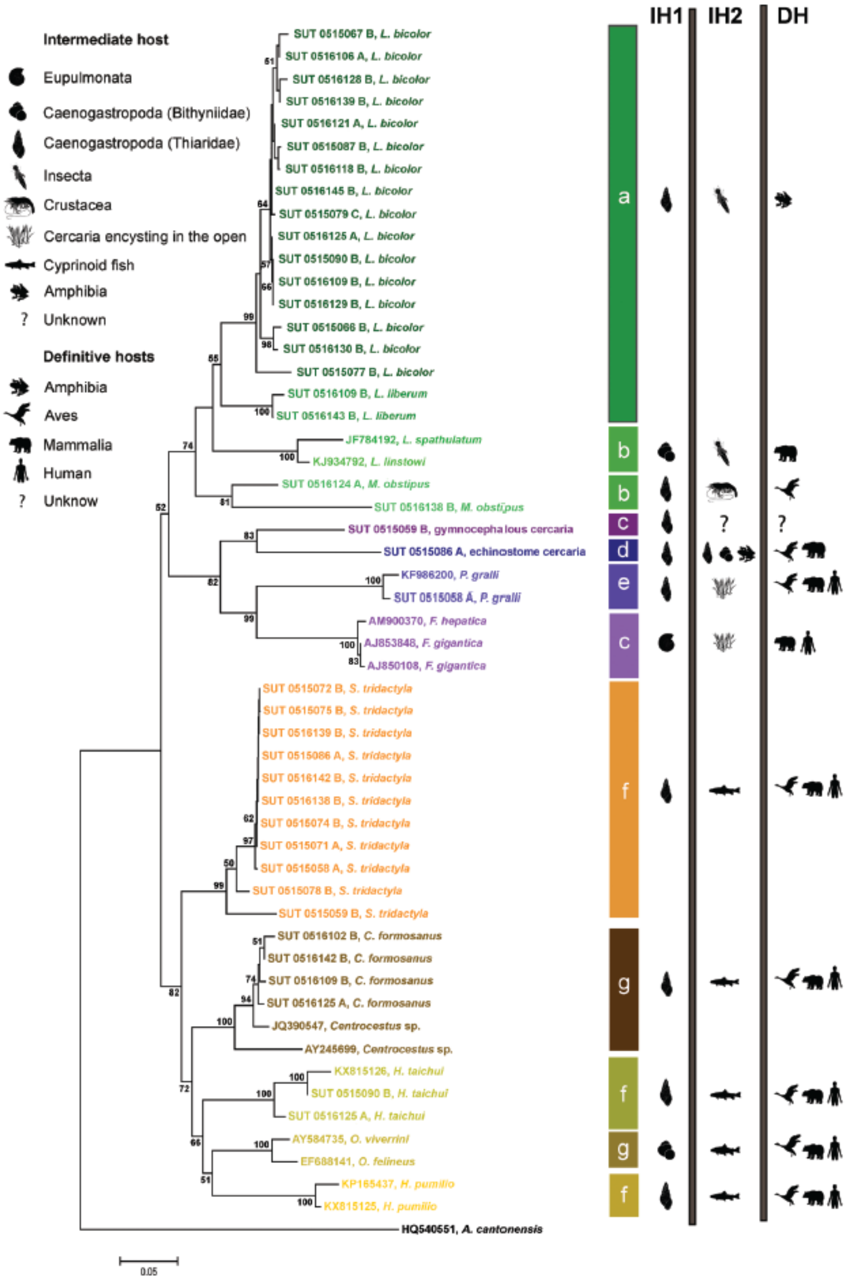

Figure 9. Neighbor-joining tree on the basis of ITS2 sequences of cercarial species obtained from *Tarebia granifera* in Thailand (Veeravechsukij et al., 2018a)

Cytochrome oxidase subunit 1 gene (CO1)

The CO1 gene codes for a protein synthesis that has a role in cellular respiration, which is present in all eukaryotes. This gene is highly conserved across species where energy is generated from mitochondria (Figure 10). For DNA barcoding of animals, this gene can be used to identify individuals belonging to the same species. It was successful to identify animals because the rate of gene sequence changes over time is slow enough so that it's likely to be identical in the same species, but fast enough so that it's different species. However, we can find fake copies of the gene or pseudogenes. The copies of mitochondrial genes are sometimes transferred from mitochondrial DNA to nuclear DNA during evolution.

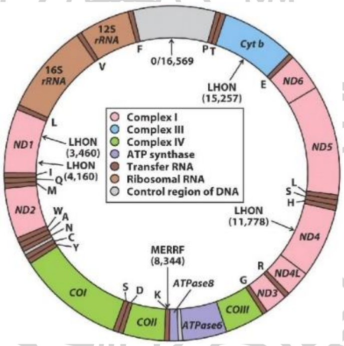

Figure 10. Structure of mitochondrial genome in Mitochondrial gene (Sagen, 1967)

Normally the CO1 gene was used to identify snails in Family Thiaridae and other families. This gene was a strong indication as to the distinction of many genus. Veeravechsukij et al. (2018a) used this gene for evolutionary studis of snail genus *Tarebia* in Thailand. The results show p-distance of CO1 and 16s gene sequences on phylogenetic trees were considered relatively high, hinting potentially at the existence of two genetically distinct species. Ng et al. (2016) reported the DNA barcode which generated from the sequenced region of mitochondrial CO1 and 16s genes and the molecular data were provided for the confirmation of the identification. DNA barcodes were obtained for 50 species from 59 snail species. Krailas (2018) reported CO1 gene maker could be resolved to identify *Stenomelania* separated from *Melanoides*, although two genus were close relationships (Figure 11). Moreover, Wiggering et al. (2019) studied the distinction between nomenclatural multiplicity and biological diversity, using molecular data, shell characters via geometric morphometrics, radula morphology, and reproductive biology. For molecular techniques, mitochondrial 16S rRNA and COI genes were used for the distinction of *Melanoides*, *Stenomelania* and *Neoradina* (Figure 12). The results of genetic analyses were ambiguous for implications on genus affiliations and did not support the classification of some species as part of *Stenomelania*, affecting the taxonomic stability of the current conception of these genera.

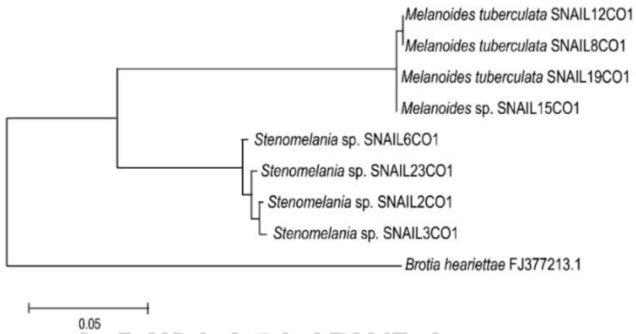

Figure 11. Neighbor-joining tree on the basis of CO1 sequences of *Stenomelania* sp. obtained from south Thailand (Krailas, 2018)

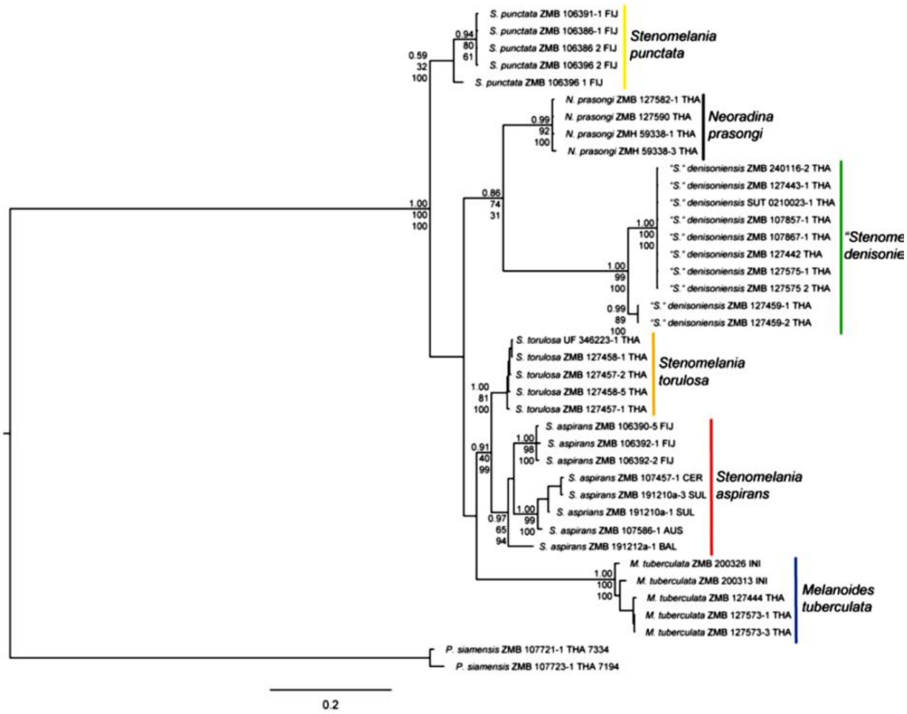

Figure 12. Neighbor-joining tree based on concatenated CO1 and 16s sequences of *Stenomelania* (Wiggering et al., 2019)

CHAPTER II
OBJECTIVES

This research was aimed to understanding the cercaria infection in snails genus *Stenomelania*. The Hypothesis of this research was "The snail genus *Stenomelania* spp. represents as intermediate host of trematode parasites". The objectives are as follows:

1. To investigate the trematode infections of snail genus *Stenomelania* in Thailand.
2. To study the distribution of snail genus *Stenomelania* spp. in Thailand.
3. To identify the cercarial species from infected snail samples by using morphology and genetic techniques.
4. To study the relationship between intermediate host of freshwater snail genus *Stenomelania* and trematodes.

CHAPTER III
MATERIALS AND METHODS

1. Distribution Maps

From the past, we have compiled the data based on all visited localities and material from the field collections with collected snail genus *Stenomelania.* Mapped localities were transferred on a dot-by-dot basis to a digitally reduced version of the master drainage pattern map of Thailand (Figure 13; Table 3).

2. Snail Sampling

Stenomelania snails were collected from streams and rivers near the coastline of the south Thailand (Figure 13). The geographic coordinates (WGS84 datum) of the sampling sites were determined with a global positioning system (Garmin PLUS III, Taiwan). The snails were collected between 2017 and 2020 by hand picking and scooping methods. The snails were transferred and studied in the laboratory of the Parasitology and Medical Malacology Research Unit, Silpakorn University, Nakhon Pathom, Thailand (PaMaSU: code SUT). The snails were identified according to their shell morphology, following essentially (Boonmekam et al., 2019; Brandt, 1974; Glaubrecht, 1996, 2006; Glaubrecht et al., 2009; Haynes, 2001; Krailas et al., 2014; Krailas et al., 2011; Ramakrishna & Dey, 2007; Upatham et al., 1983; Veeravechsukij et al., 2018b; Wiggering et al., 2019)

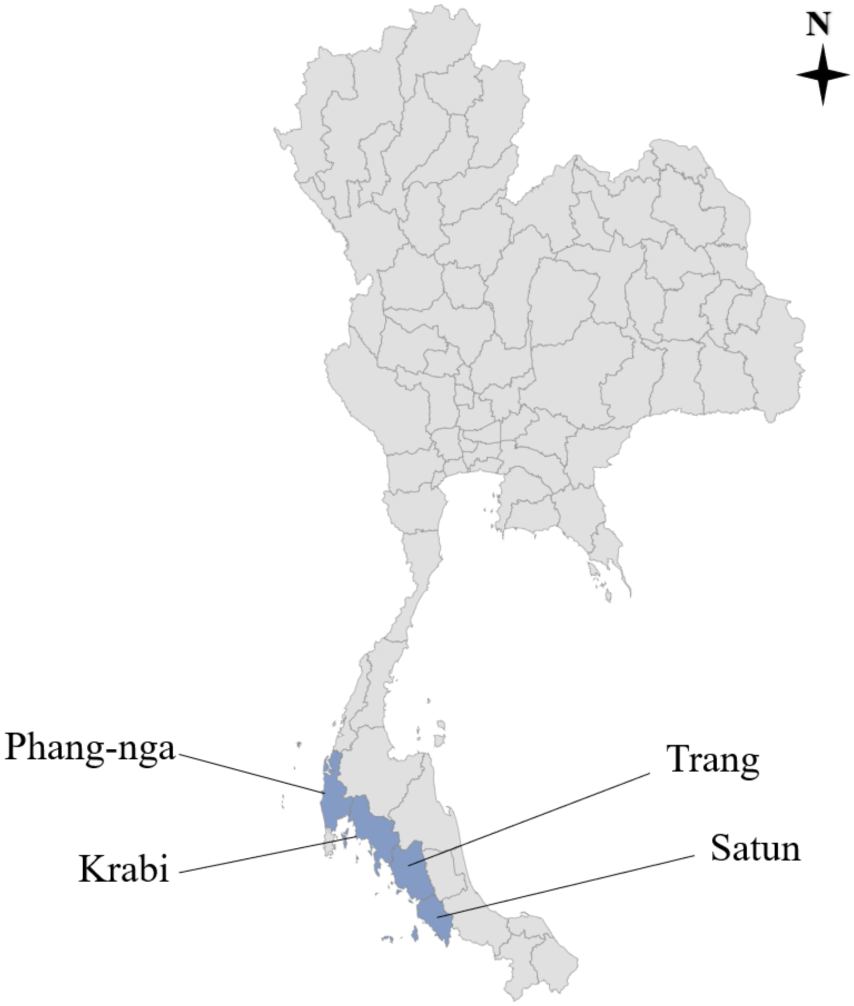

Figure 13. Collected areas near the coastline in the south Thailand, consist of Phang-nga, Krabi, Trang and Satun Provinces.

Table 3. Details of localities were collected snail genus *Stenomelania*.

Province	Locality	GPS	Altitude (m.)
Phang-nga	Raman Waterfall Park	E 98° 26’ 50.76” N 08° 27’ 05.28”	94
	Raman Waterfall Stream	E 98° 27’ 09.27” N 08° 27’ 12.34”	5
	Klong Tam Bridge	E 98° 28’ 44.92” N 08° 27’ 57.10”	2
	Klong Phang-nga	E 98° 32’ 12.41” N 08° 27’ 44.24”	18
	Taotong Waterfall	E 98° 35’ 07.8” N 08° 29’ 08.0”	14
	Klong Bo Sean	E 98° 35’ 41.68” N 08° 28’ 45.06”	11
	Khlong Saimat	E 98° 38’ 56.25” N 08° 29’ 40.31”	1
	Klong Marui	E 98° 39’ 09.2” N 08° 29’ 49.8”	1
	Klong Marui Weir	E 98° 39’ 11.01” N 08° 29’ 40.73”	1
Krabi	Klong Thanthip 2	E 98° 47' 07.21" N 08° 09' 37.78"	5
	Klong Thanthip	E 98° 47' 07.51" N 08° 09' 37.78"	75
	Klong Nong Jik	E 98° 46' 24.97" N 08° 13' 22.00"	39

	Klong Son 1	E 98° 47' 55.09" N 08° 04' 15.96"	84
	Klong Son 2	E 98° 48' 09.98" N 08° 04' 23.68"	9
	Klong Yang	E 99° 47' 36.17" N 08° 40' 15.70"	76
	Klong Chilat	E 98° 52' 35.17" N 08° 05' 11.28"	8
	Klong Sai Khao	E 98° 47' 16.31" N 08° 03' 54.36"	5
	Klong Klang	E 98° 43’ 09.91” N 08° 22’ 24.15”	2
	Klong Chong Lom	E 98° 43’ 15.98” N 08° 22’ 13.67”	14
	Ao Luek Paradise Resort	E 98° 43’ 58.97” N 08° 22’ 18.67”	3
	Klong Ao Luek Noi	E 98° 44’ 43.93” N 08° 18’ 09.46”	5
	Klong Nayaw	E 98° 44’ 43.55” N 08° 18’ 15.97”	16
	Klong Tung Yeepeng	E 99° 04’ 16.55” N 07° 35’ 42.62”	2
	Klong Ban Rabieng	E 99° 02’ 47.91” N 07° 32’ 42.76”	2
	Klong Bang Nam Chuet	E 99° 03’ 29.00” N 07° 31’ 16.16”	2
	Klong Bang Nam	E 099° 03’ 57.42”	2

	Chuet Weir	N 07° 31’ 28.36”	
	Klong Pagasai	E 99◦ 02' 08.3″ N 08 03' 13.0″	1
	Klong Kok Hai	E 99◦02' 23.4″ N 08◦03' 23.00″	2
	Klong Phela	E 99◦08' 00.56″ N 07◦56' 00.00″	3
	Klong Thom Park	E 99◦08' 00.40″ N 07◦56' 00.10″	3
	Klong Khuan Juan	E 99°05’15.55” N 07°31’10.85”	3
	Klong Chak (point 1)	E 99°05’11.83” N 07°29’41.90”	2
	Klong Chak (point 2)	E 99°05’07.63” N 07°29’27.92”	7
	Klong Chak (point 3)	E 99°05’03.86” N 07°29’17.63”	4
	Klong Rud	E 98°48’24.50” N 08°05’39.27”	8
	Pak Nam Klong Son	E 98°47’07.21” N 08°09’37.78”	7
Trang	Klong Mai Phad	E 99° 21’01.95” N 07° 33’ 10.46”	11
	Klong La 1	E 99° 20’ 34.42” N 07° 29’ 39.55”	13
	Klong La 2	E 99° 21’28.25” N 07° 29’ 49.22”	7

	Khaoting Cave	E 99° 47' 59.54" N 07° 09' 33.48"	104
	Klong Pom	E 99° 36' 15.52" N 07° 36' 31.10"	55
Satun	Klong Saphanwa	E 99° 47' 07.35" N 07° 04' 22.70"	159
	Klong Tha Phae 1	E 99° 57' 16.90" N 06° 47' 47.70"	28
	Klong Tha Phae 2	E 99° 57' 50.96" N 06° 48' 09.74"	28
	Klong Tha Phae 3	E 99° 58’ 30.68” N 06° 48’ 44.21”	35
	Klong La-ngu 1	E 99° 48' 30.88" N 06° 54' 14.74"	39
	Klong La-ngu 2	E 99° 48’ 45.49” N 06° 54’ 29.73”	40
	Klong Chalung 1	E 100° 03’ 44.78” N 06° 43’ 37.28”	50
	Klong Chalung 2	E 100° 03’ 51.53” N 06° 42’ 58.75”	50
	Khlong Ka Ne	E 100° 04’ 17.60” N 06° 42’ 23.85”	54
	Klong Nam Thun	E 99° 48’ 12.01” N 07° 05’ 52.36”	83

3. Examination of Trematode Infections and Cercarial Study

The collected snails were investigated for the trematode infections by shedding and crushing methods. The emerging cercariae were described as the morphology based on living cercariae which unstained or vitally stained with 0.5% neutral red. Measurements in micrometers were taken from specimens fixed with 10% formalin. Details of the cercariae were draw (Figure 14) and identified according to (Ito, 1980; Komiya, 1961; Krailas et al., 2014; Krailas et al., 2011; NASIR, 1984; Schell, 1970; Veeravechsukij et al., 2018a; Yamaguti, 1971, 1975).

Sample measurements in micrometers were taken, using an ocular micrometer, from 10 specimens fixed in 10% formalin. Some cercariae belonging to identified trematode species were then preserved in 95% ethanol for DNA analysis.

The statistical analysis of the cercariae was performed using the SPSS for Windows (version 15) by Oneway Anova testing and Post-hoc testing using the Duncan and LSD test.

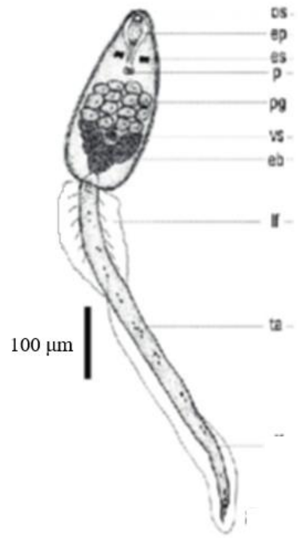

Figure 14. Morphological image of Cercaria (Krailas et al., 2014). Abbreviations – df: dorso-ventral finfold; eb: excretory bladder; ep: esophagus; es: eyespot; lf: lateral finfold; os: oral sucker; p: pharynx; pg: penetration gland; ta: tail; vs: ventral sucker.

4. Examination of Shell Morphology

Standard shell measurements were essentially carried out under a digital caliper. Shells were cleaned with tap water. The biometrical parameters of the adult shells were studied including height of shell (h), width of shell (w), length of aperture (la), width of aperture (wa), height of body whorl (hbw) and height of last three whorls (h3w) and number of whorls (nw). The shells were sketched and photographed; the images were edited with Adobe Photoshop CC 2020 (Figure 15). The statistical analysis of the shell was performed using the SPSS for Windows (version 15) by Oneway Anova testing and Post-hoc testing using the LSD test.

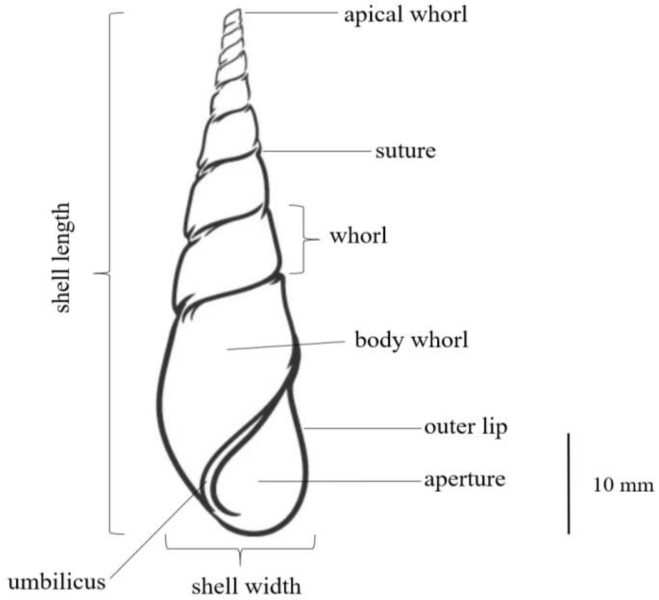

Figure 15. The image of shape and structure of shell.

5. Molecular Study of Snails

DNA was obtained from foot tissues and Genomic DNA was extracted by using the DNeasy blood and animal tissue kit (QIAGEN). For Phylogenetic analyses, DNA fragment of the mitochondrial cytochrome c oxidase subunit 1 (*cox1*; 600-900 bp) region using the primers LCO1490 (5´- GGT CAA ATC ATA AAG ATA TTG G - 3´,(Folmer et al., 1994)) and HCO2198var (5´- TAW ACT TCT GGG TGK CCA AAR AAT - 3´.(Rintelen et al., 2004)) and the 16 S rRNA (16S; 710 bp) gene using the primers 16SF (5´- CTT YCG CAC TGA

TAG CTA G - 3´, (Gimnich, 2015)) and 16SR (5´- CCG GTY TGA ACT CAG ATC ATG T - 3´, (Wilson et al., 2004)). The PCR amplification was performed in a total volume of 50 µl containing; 2 µl of DNA extract, 2 µl of each forward and reverse primers, 35.60 µl of ddH_2O, 5 µl of buffer, 1 µl of 200 mM of dNTPs, 2 µl of 1.5 mM $MgCl_2$ and 0.40 µl of 1-2.5 U Taq polymerase. After initial denaturation step 94 °C at 3 min, 35 cycles of denaturation 94 °C 30 sec, annealing 50 °C 45 sec, extension 72 °C 1 min was performed, follow by final extension 72 °C 10 min. Then, the PCR products were loaded on to 1% agarose gels for electrophoresis. The CO1 PCR products were sent to ATI biotech (Singapore) for sequencing analysis.

The CO1 consensus sequences were aligned in MEGA 10.1.7 by using MUSCLE (Edgar, 2004) under default settings. A phylogenetic tree representing the species groups was constructed with neighbour-joining analysis (Tamura et al., 2013).

6. Molecular Study of Cercariae

The emerging cercariae were studied for molecular techniques. The sizes of the polymerase chain reaction (PCR) products were used to identify the types of parasites. Genomic DNA from the cercariae were extracted by using the DNeasy blood and animal tissue kit (QIAGEN). For Phylogenetic analyses fragment of the internal transcribed spacer 2 regions (ITS2) gene using primers ITS2-F (5'-CTT GAA CGC ACA TTG CGG CCA TGG G-3') and ITS2-R (5'-GCG GGT AAT CAC GTC 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 sec 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 10.1.7 by using MUSCLE (Edgar, 2004) under default settings. A phylogenetic tree representing the species groups was constructed with neighbour-joining analysis (Tamura et al., 2013).

7. Equipment
   - Autoclave
   - Auto-pipette
   - DNA electrophoresis (MJ 105-s Mini Horizontal Electrophoresis system, Germany)
   - Gel documentation (Fujifilm, Thermal Imaging system FTI-500, Japan)
   - GPS plus III (GARMIN eTrex 30, Taiwan)
   - Light microscope (Olympus CH 30, Japan)
   - Micro-centrifuge tube
   - Micro-pipette tip
   - PCR-Thermocycle (Flexcycle Block, Twinblock 48, USA)
   - Rubber gloves
   - Stereomicroscope (Olympus SZ61, Japan)
   - Thermometer
   - UV transilluminator (VILBER LOURMAT, Ecx-15.M, France)
   - Vernier caliper
   - Vial/ glass bottle
   - Water quality checker (U10 Horiba, Japan)

8. Chemical
   - absolute ethanol (97 – 100%)
   - Agarose
   - DNeasy blood and animal tissue kit (QIAGEN)
   - dechlorinated tap water
   - dNTP
   - double distilled water
   - Novel Juice supplied in 6X Loading buffer
   - PCR purification kit (QIAGEN, Germany)
   - Proteinase K
   - Primer CO1 (reverse and forward)
   - Primer ITS2 (reverse and forward)
   - standard marker (1 Kb Plus DNA Ladder)
   - taq DNA polymerase
   - 0.5% neutral red
   - 10x buffer
   - 50X TAE
   - 95% ethanol

CHAPTER IV
RESULTS

Sampling Sites and Environmental Data

Phang-nga province is located on the west coastline of Thailand, in the Andaman Sea and Phang-nga Bay to the south and neighboring provinces are Ranong, Surat Thani and Krabi. We explored this province consists of 3 localities in Mueang Phang-nga district (1. Raman Waterfall, 2. Raman Stream and 3. Klong Tam Bridge) and 6 localities in Tubpud district (4. Klong Phang-nga, 5. Taotong Waterfall, 6. Klong Bo Saen, 7. Klong Saimat, 8. Klong Marui and 9. Klong Marui Weir). These areas are water terrain as well as waterfall and creek. The ground of water is consistent with soil and sand. Surrounding with trees, shrubs and aquatic plants found near shorelines. Temperature of the water was 21-29 ^{o}C and air temperature was 25-29 ^{o}C. pH of all habitats was 7.16-8.50. The dissolved oxygen is 7.21-9.35 mg/l. The depth was 10-100 centimeters and salinity was 0.0 – 0.3 ppt (Figure 16, 20; Table 4).

Krabi province is located on shoreline in the Andaman Sea, there are both land and islands and neighboring provinces are Phang-nga, Surat Thani, Nakhon Si Thammarat and Trang. We explored 28 localities for the collected snails consist of 1. Klong Thanthip, 2. Klong Thanthip 2, 3. Klong Nong Thale, 4. Klong Nong Jik, 5. Klong Son 1, 6. Klong Son 2, 7. Klong Yang, 8. Klong Chilat, 9. Klong Sai khao, 10. Klong Klang, 11. Klong Chong Lom, 12. Ao Luek Paradise Resort, 13. Klong Ao Luek Noi, 14. Klong Nayaw, 15. Tung Yeepeng, 16. Klong Ban Rabieng, 17. Klong Bang Nam Chuet, 18. Klong Bang Nam Chuet Weir, 19. Klong Pagasai, 20. Klong Kok Hai, 21. Klong Phela, 22. Klong Thom Park, 23. Klong Khuan Juan, 24. Klong Chak 1, 25. Klong Chak 2, 26. Klong Chak 3, 27. Klong Rud and 28. Pak Nam Klong Son. Sampling areas were cover from Mueang, Ko Lanta, Klong Thom Nuea, Klong and Ao Luek districts. Most areas are streams, the water is running to the forest. The habitats are some rubber plantations and community houses. The ground of streams is consistent with soil and sand or mud. Surrounding are trees, bush, grass and or aquatic plants near shorelines. Temperature of the water was 24.03 - 30.55 ^{o}C and air temperature was 21 - 30 ^{o}C. pH of all habitats was 6.01 - 9.45. The dissolved oxygen was 2.70 - 17.11 mg/l. The depth was 6 - 150 centimeters and salinity was 0.0 – 0.9 ppt (Figure 17, 21, 22, 23; Table 5).

Trang province is located on the west side of the Malay Peninsula and neighboring provinces are Krabi, Nakhon Si Thammarat, Phatthalung and Satun. The snails were collected from 6 localities, they are 1. Klong Mai Phad, 2. Klong La 1, 3. Klong La 2, 4. Khaoting Cave, 5. Klong Pom, and 6. Klong Tanaw. The habitats of snails are creeks, the water is running into the forest. The streams were almost shallow and slowly water flows. The water was turbid. The ground of streams is consistent with sand or mud. The areas were surrounded by trees, grass and also mangrove. Temperature of the water was 22.25 – 26.70 °C and air temperature was 24 – 29 °C. pH of all habitats was 5.52 - 9.23. The dissolved oxygen was 5.40 – 12.35 mg/l. The depth was 31- 53 centimeters and salinity was 0.1 – 0.3 ppt (Figure 18, 24; Table 6).

Satun province is located on the coastline of the Andaman Sea, located near Perlis state in Malaysia. The snails were collected from 10 localities, they are 1. Klong Saphanwa, 2. Klong Tha Phae 1, 3. Klong Tha Phae 2, 4. Klong Tha Phae 3, 5. Klong La-ngu 1, 6. Klong La-ngu 2, 7. Klong Chalung 1, 8. Klong Chalung 2, 9. Klong Ka Ne and 10. Klong Nam Thun. The habitats are canals, which can be found in both slow and fast water. These areas can be found in both turbid and clear water depending on the tide of water. The ground of streams is consistent with soil with sand or mud. The areas were surrounded by trees or shrubbery. Temperature of the water was 25.02 – 28.40 °C and air temperature was 25 - 31 °C. pH of all habitats was 4.90 – 6.15. The dissolved oxygen was 6.33 – 8.51 mg/l. The depth was 20 - 70 centimeters and salinity was 0.1 – 0.2 ppt (Figure 19, 25; Table 7).

In this study, the survey was done between 2017 and 2020. We found the snail genus *Stenomelania* only 24 localities from 53 sampling localities in 4 provinces as following:

1. Phang-nga province one site: Klong Bo Saen (Figure 15).
2. Krabi province fourteen sites: Klong Thanthip 2, Klong Nong Jik, Klong Son 1, Klong Son 2, Klong Yang, Klong Klang, Klong Chong Lom, Ao Luek paradise resort, Klong Ao Luek Noi, Klong Nayaw, Klong Bang Nam Chuet, Klong Bang Nam Chuet Weir, Klong Chak 1 and Klong Chak 2 (Figure 16).
3. Trang province four sites: Klong Mai Phad, Klong La 1, Klong La 2 and Khaoting cave (Figure 17).
4. Satun province five sites: Klong Sapanwa, Klong Tha Phae 1, Klong Tha Phae 2, Klong La-ngu 1 and Klong Chalung 1 (Figure 18).

In Phang-nga, only one location was found *Stenomelania*. The water physical characteristics were recorded, viz. pH 7.81, dissolved oxygen 8.33 mg/l, salinity 0.3 ppt and conductivity 0.584 ms/cm. The water physical characteristics of fourteen locations in Krabi were pH

6.59-9.78, dissolved oxygen 6.89-11.53 mg/l, salinity 0.1-0.6 ppt and conductivity 0.388-1.12 ms/cm. The four locations in of Trang were recored, pH 5.52-6.73, dissolved oxygen 7.96-12.35 mg/l, salinity 0.1-0.2 ppt and conductivity 0.148-0.319 ms/cm. Five locations in Satun were recorded, pH 4.90-6.15, dissolved oxygen 6.43-8.19 mg/l, salinity 0.1-0.2 ppt and conductivity 0.123-0.418 ms/cm.

Figure 16. Map distribution of collected snails in Phang-nga province 1) Raman Waterfall, 2) Raman Stream, 3) Klong Tam Bridge, 4) Klong Phang-nga, 5) Taotong Waterfall, 6) Klong Bo Saen, 7) Klong Saimat, 8) Klong Marui and 9) Klong Marui Weir. (yellow spot: found *Stenomelania* and white spot: not found *Stenomelania*).

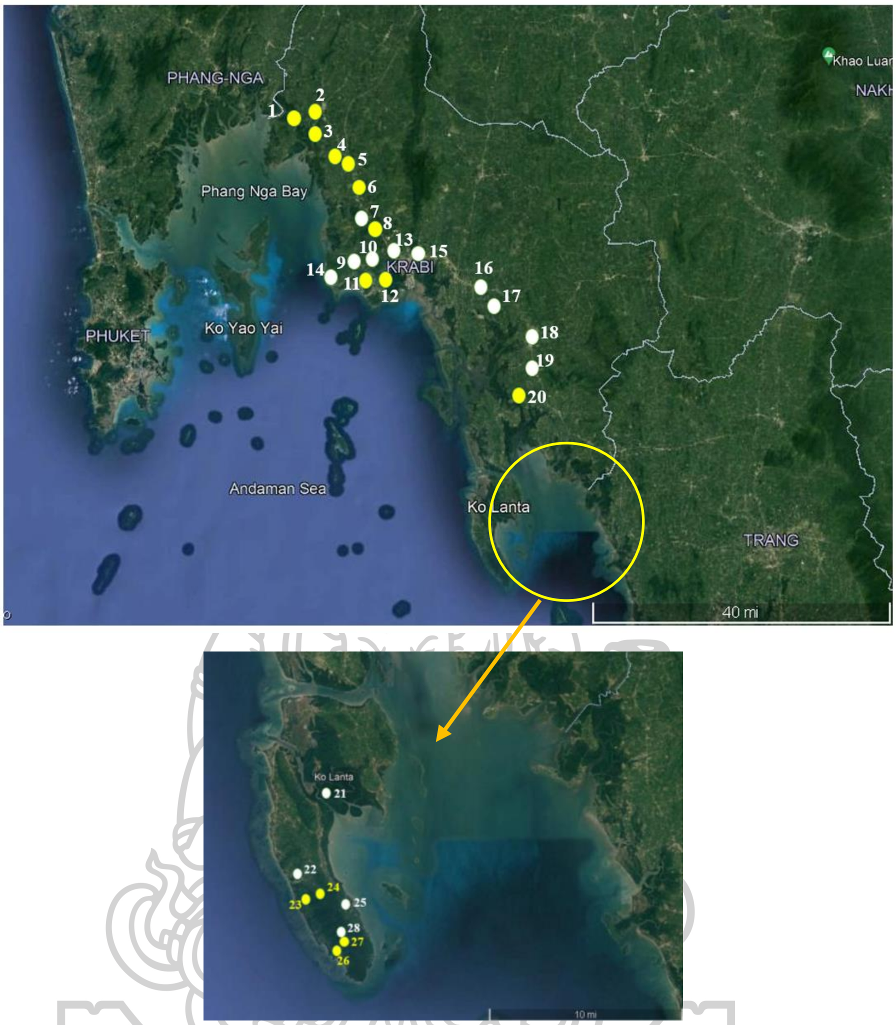

Figure 17. Map distribution of collected snails in Krabi province. 1) Klong Klang, 2) Klong Chong Lom, 3) Ao Luek Paradise Resort, 4) Klong Nayaw, 5) Klong Ao Luek Noi, 6) Klong Nong Jik, 7) Klong Thanthip, 8) Klong Thanthip 2, 9) Pak Nam Klong Son, 10) Klong Rud, 11) Klong Nong Thale, 12) Klong Son 1, 13) Klong Son 2, 14) Klong Sai Khao, 15) Klong Chilat, 16) Klong Pagasai, 17) Klong Kok Hai, 18) Klong Phela, 19) Klong Thom Park, 20) Klong Yang, 21) Tung Yeepeng, 22) Klong Ban Rabieng, 23) Klong Bang Nam Chuet, 24) Klong Bang Nam Chuet Weir, 25) Klong Khuan Juan, 26) Klong Chak (point 1), 27) Klong Chak (point 2) and 28) Klong Chak (point 3). (yellow spot: found *Stenomelania* and white spot: not found S*tenomelania*)

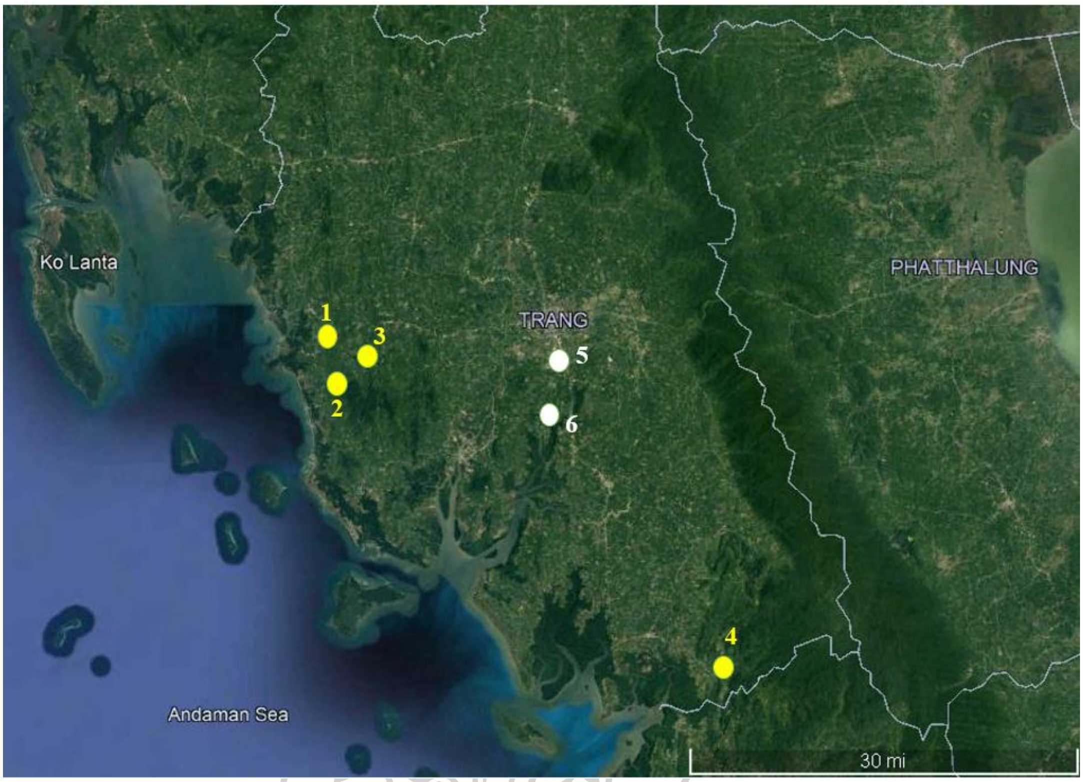

Figure 18. Map distribution of collected snails in Trang Province. 1) Klong Mai Phad, 2) Klong La 1, 3) Klong La 2, 4) Khaoting Cave, 5) Klong Pom and 6) Klong Tanaw. (yellow spot: found *Stenomelania* and white spot: not found *Stenomelania*)

Figure 19. Map distribution of collected snails in Satun province. 1) Klong Saphanwa, 2) Klong Tha Phae 1, 3) Klong Tha Phae 2, 4) Klong Tha Phae 3, 5) Klong La-ngu 1, 6) Klong La-ngu 2,7) Klong Chalung 1, 8) Klong Chalung 2, 9) Klong Ka Ne and 10) Klong Nam Thun. (Yellow spot: found *Stenomelania* and white spot: not found *Stenomelania*)

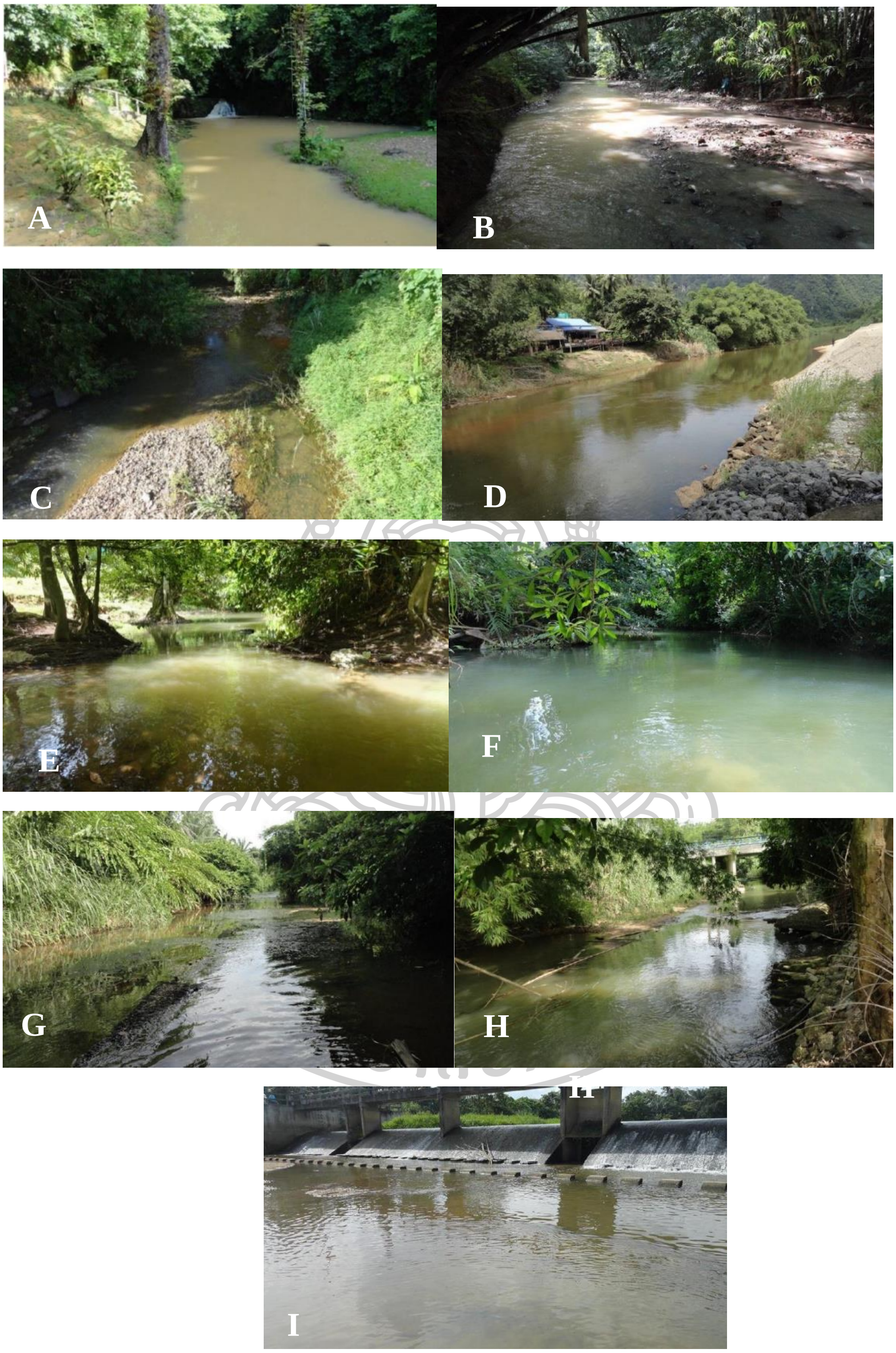

Figure 20. Sampling sites of snail collection in Phang-nga province. A: Raman Waterfall, B: Raman Stream, C: Klong Tam Bridge, D: Klong Phang-nga, E: Taotong Waterfall, F: Klong Bo Saen, G: Klong Saimat, H: Klong Marui and I: Klong Marui Weir.

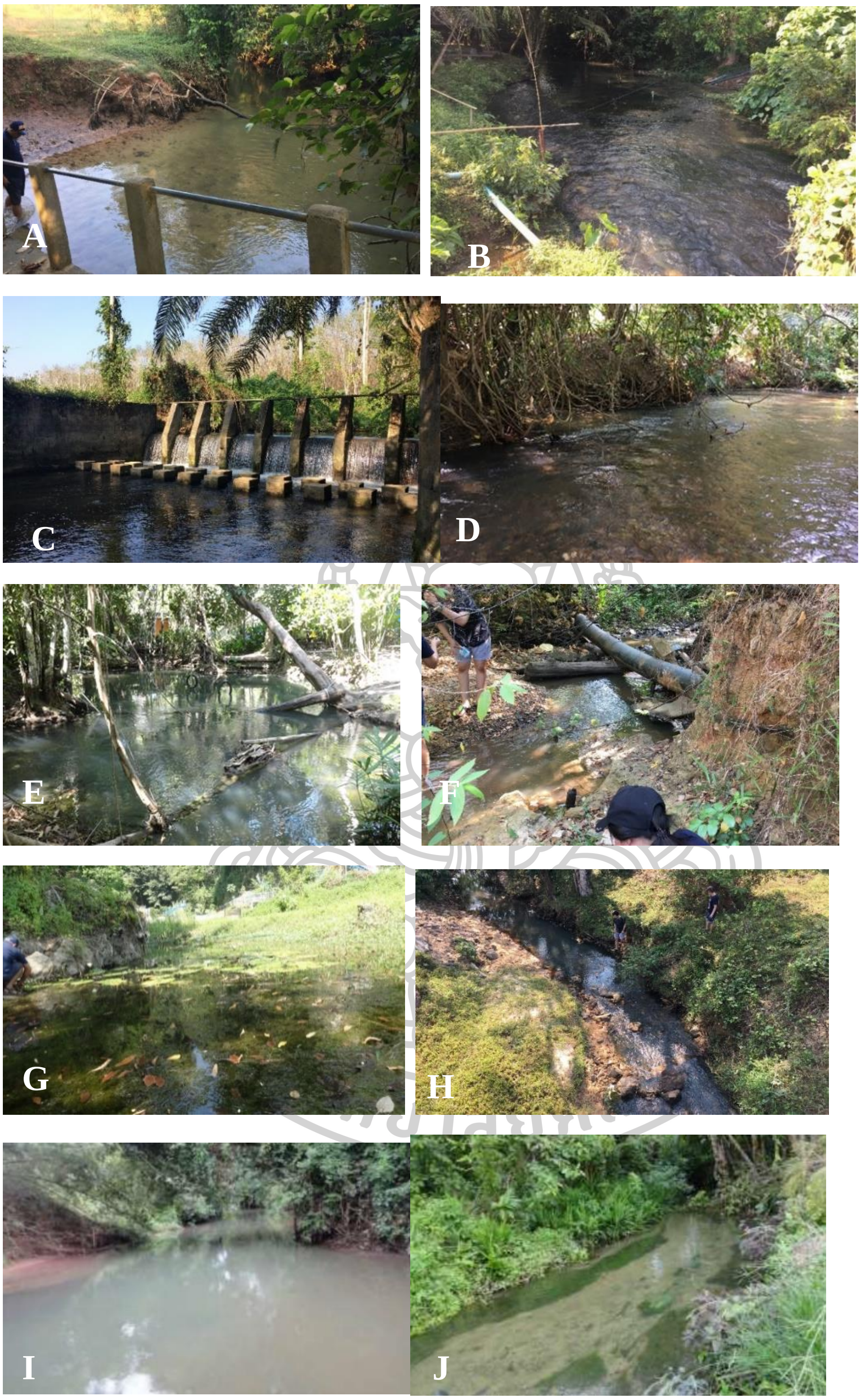

Figure 21. Sampling sites of snail collection in Krabi province. A: Klong Thanthip 2, B: Klong Nong Thale, C: Klong Nong Jik and D: Klong Son 1, E: Klong Son 2, F: Klong Yang, G: Klong Chilat, H: Klong Sai Khao, I: Klong Klang, J: Klong Chong Lom

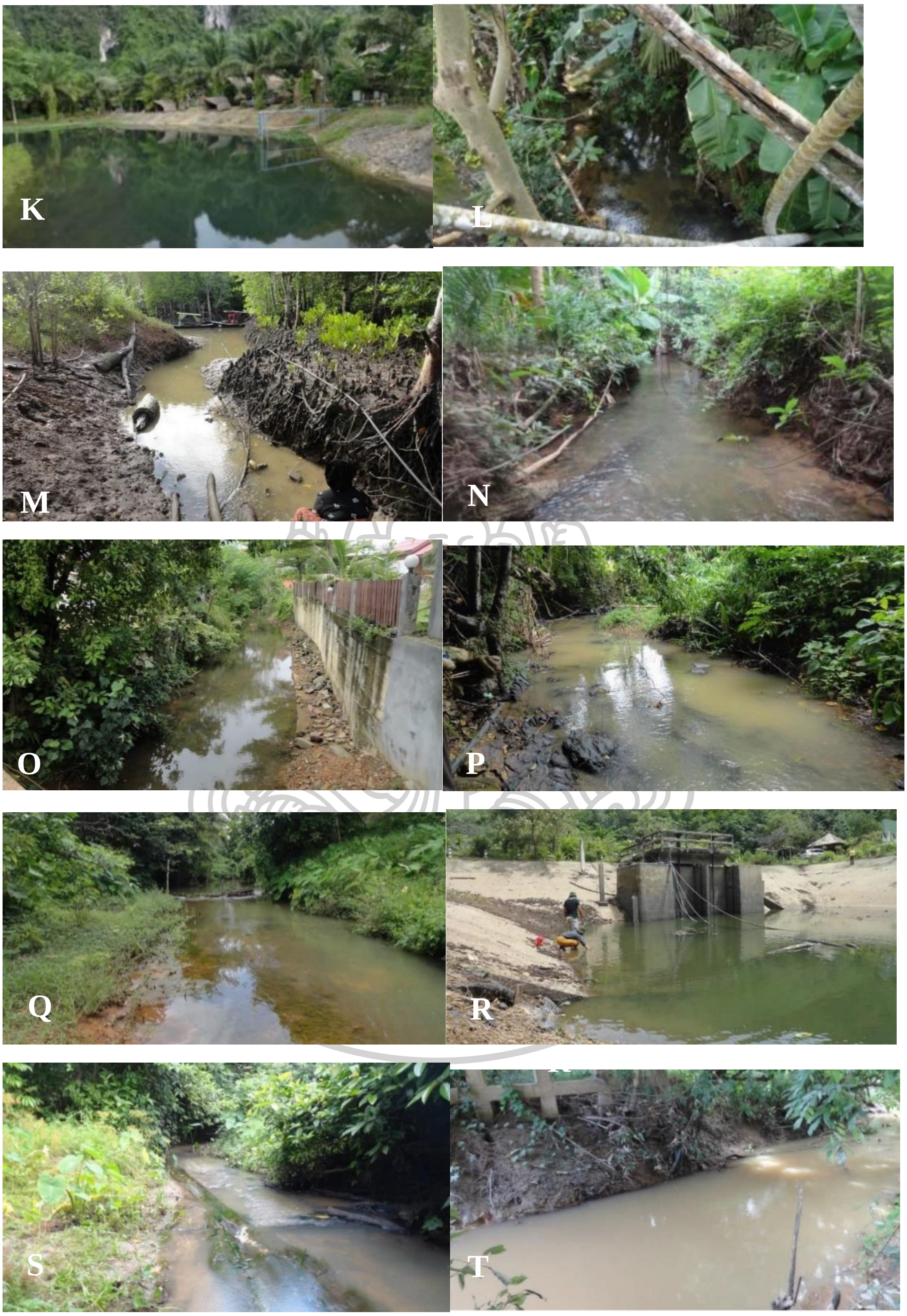

Figure 22. Sampling sites of snail collection in Krabi province. K: Ao Luek Paradise Resort and L: Klong Ao Luek Noi, M: Tung Yee Peng, N: Klong Nayaw, O: Klong Ban Rabieng, P: Klong Bang Nam Chuet, Q: Klong Pagasai, R: Klong Bang Nam Chuet Weir, S: Klong Kok Hai and T: Klong Phela.

Figure 23.Sampling sites of snail collection in Krabi province. U: Klong Kuan Juan, V: Klong Thom Park W: Klong Chak 1, X: Klong Chak 2, Y: Klong Chak 3, Z: Pak Nam Klong Son.

Figure 24. Sampling sites of snail collection in Trang province. A: Klong Mai Phad, B: Klong La 1, C: Klong La 2, D: Khaoting Cave, E: Klong Pom and F: Klong Tanaw.

Figure 25. Sampling sites of snail collection in Satun province. A: Klong Saphanwa, B: Klong Tha Phae 1, C: Klong Tha Phae 2, D: Klong Tha Phae 3, E: Klong La-ngu 1, F: Klong La-ngu 2, G: Klong Chalung 1 and H: Klong Chalung 2. I: Klong Ka Ne and J: Klong Nam Thun.

Table 4. Water physical characteristics of 9 sampling sites in Phang-nga province (June, 2019; * = found snail: abbreviation- pH: Positive potential of the Hydrogen ions, DO: Dissolved oxygen, Tub: Turbidity).

Sampling sites	Depth	pH	Conductivity (mS/cm)	DO (mg/l)	Tub. (NTU)	TDS (g/l)	Salinity (ppt)	Water Temp.	Air Temp
1. Raman Waterfall	50	7.8	0.046	8.53	183	0.031	0.0	21	25.11
2. Raman Stream	20	7.16	0.052	8.05	132	0.035	0.0	28	25.18
3. Klong Tam Bridge	70	7.83	0.105	7.21	19.6	0.071	0.0	25	26.86
4. Klong Phang-nga	100	7.43	0.069	8.33	8.6	0.047	0.0	28	29.03
5. Taotong Waterfall	50	8.52	0.429	9.26	27.8	0.291	0.2	28	26.46
6. Klong Bo Saen*	100	7.81	0.584	8.33	4.0	0.374	0.3	29	26.50
7. Klong Saimat	50	7.70	0.198	9.28	8.4	0.134	0.1	30	28.92
8. Klong Marui	50	7.76	0.001	9.35	168	0.001	0.0	28	28.00
9. Klong Marui Weir	10	7.62	0.158	8.17	16.5	0.108	0.1	29	28.61

Table 5. Water physical characteristics of 28 sampling sites in Krabi province (June, 2019 and June, 2020: * = found snail; n/a = no data: abbreviation- pH: Positive potential of the Hydrogen ions, DO: Dissolved oxygen, Tub: Turbidity).

Sampling sites	Depth	pH	Conductivity (mS/cm)	DO (mg/l)	Tub. (NTU)	TDS (g/l)	Salinity (ppt)	Water Temp.	Air Temp
1. Klong Thanthip	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	a/a
2. Klong Thanthip 2*	46.67	8.38	0.603	10.06	4.6	0.386	0.3	26.62	26
3. Klong Nong Thale	27	7.63	0.423	17.11	23.2	0.260	0.2	24.03	n/a
4. Klong Nong Jik*	19.67	8.73	0.474	9.37	8.8	0.312	0.2	26.05	28
5. Klong Son 1*	20.33	8.92	0.922	11.17	2.4	0.566	0.4	26.27	26
6. Klong Son 2*	56.67	6.01	0.527	7.11	1.7	0.355	0.3	26.95	30
7. Klong Yang*	13.1	8.00	0.388	11.53	4.3	0.237	0.2	26.37	24
8. Klong Chilat	15	7.50	0.386	13.83	5.7	0.249	0.2	30.55	28
9. Klong Sai Khao	22	7.95	0.073	16.15	56.7	0.041	0.0	28.12	29
10. Klong Klang*	150	8.73	1.12	6.89	0.9	0.717	0.6	28.56	27
11. Klong Chong Lom*	50	9.78	1.12	7.25	0.0	0.717	0.6	26.86	28

12. Ao Luek Paradise Resort*	50	6.56	0.826	8.20	14.9	0.550	0.4	27.50	27
13. Klong Ao Luek Noi*	50	7.48	0.649	8.56	102	0.432	0.3	27.08	29
14. Klong Nayaw*	50	7.58	0.718	9.04	0.0	0.460	0.3	26.68	21
15. Tung Yeepeng	7	8.92	17.4	6.86	79.2	108	0.2	26.64	27
16. Klong Ban Rabieng	9	9.45	1.18	2.70	12.8	0.795	0.6	27.22	28
17. Klong Bang Nam Chuet*	6	7.68	0.250	6.61	57.9	0.170	0.1	26.82	27
18. Klong Bang Nam Chuet Weir*	20	7.54	0.309	8.52	37.1	0.210	0.1	27.14	30
19. Klong Pagasai	50	7.40	0.312	8.85	29.8	0.203	0.1	27.45	28
20. Klong Kok Hai	10	7.36	0.001	9.12	16.6	0.001	0.0	28.88	28
21. Klong Phela	55	7.7	0.214	8.83	14.3	0.128	0.1	26.1	28
22. Klong Thom Park	n/a	8.84	1.68	4.74	15.3	1.12	0.9	26.61	28
23. Klong Khuan Juan	20	7.11	0.095	7.20	77.4	0.065	0.1	27.07	29
24. Klong Chak 1*	80	7.32	0.402	7.48	9.7	0.273	0.2	27.22	29
25. Klong Chak 2*	50	7.33	0.402	8.30	29.0	0.284	0.2	28	29

26. Klong Chak 3	40	7.17	0.364	6.52	12.6	0.248	0.2	27.59	28
27. Klong Rud	67	7.95	0.524	9.01	2.4	0.331	0.3	28.21	29
28. Pak Nam Klong Son	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

Table 6. Water physical characteristics of 6 sampling sites in Trang province (February, 2019; * = found snail: abbreviation- pH: Positive potential of the Hydrogen ions, DO: Dissolved oxygen, Tub: Turbidity).

Sampling sites	Depth	pH	Conductivity (mS/cm)	DO (mg/l)	Tub. (NTU)	TDS (g/l)	Salinity (ppt)	water Temp.	air Temp
1. Klong Mai Phad*	53	6.73	0.148	9.93	14.4	0.093	0.1	25.98	26
2. Klong La 1*	44	6.61	0.168	12.35	22.6	0.108	0.1	26.7	29
3. Klong La 2*	31	6.47	0.191	11.78	4.3	0.124	0.1	22.25	26
4. Khaoting Cave*	40	5.52	0.319	7.96	6.0	0.27	0.2	25.38	29
5. Klong Pom	44	6.23	0.547	5.40	14.0	0.350	0.3	25	27
6. Klong Tanaw	46	9.23	4.80	7.40	0.8	3.07	0.2	25.41	24

Table 7. Water physical characteristics of 10 sampling sites in Satun province (February, 2020; * = found snail: abbreviation- pH: Positive potential of the Hydrogen ions, DO: Dissolved oxygen, Tub: Turbidity).

Sampling sites	Depth	pH	Conductivity (mS/cm)	DO (mg/l)	Tub. (NTU)	TDS (g/l)	Salinity (ppt)	Water Temp.	Air Temp
1. Klong Saphanwa*	30	4.90	0.418	6.43	9.5	0.284	0.2	29	25.41
2. Klong Tha Phae 1*	65	6.04	0.251	6.78	10.6	0.171	0.1	29	27.01
3. Klong Tha Phae 2*	40	5.99	0.189	7.10	29.9	0.131	0.1	31	26.96
4. Klong Tha Phae 3	35	6.32	0.309	8.25	25.3	0.206	0.1	31	27.27
5. Klong La-ngu 1*	30	5.93	0.201	8.19	10.4	0.137	0.1	25	25.02
6. Klong La-ngu 2	70	6.08	0.226	8.51	4.6	0.153	0.1	26	26.33
7. Klong Chalung 1*	30	6.15	0.123	8.09	17.6	0.083	0.1	31	27.17
8. Klong Chalung 2	30	6.08	0.127	7.60	14.5	0.08	0.1	30	27.16
9. Klong Ka Ne	20	6.00	0.140	6.33	16.3	0.006	0.1	32	28.4
10. Klong Nam Thun	30	5.18	0.462	7.56	6.4	0.316	0.2	30	25.27

Biogeography and Species Diversity

The distributional range of *Stenomelania* extends from mainland Southeast Asia, India, to the island of Taiwan, The Philippines, Japan, Nicobar Island, Fiji, New Caledonia, Samoa, etc. In Thailand, this species occurs in lentic and lotic water bodies ranging throughout the various regions, canal and rivers system, that of all near the Andaman coastlines. Occasionally, this species is found together with other thiarid snails, such as *Melanoides tuberculata* and *Tarebia granifera.*

A total of 3,026 snails of *Stenomelania* were collected from 24 localities. The snails were identified by shell morphology into 6 species consist of *Stenomelania* cf. *aspirans*, *S.* cf. *crenulata*, *Stenomelania* sp. (*Neoradina* aff. *prasongi*:(Wiggering et al., 2019)), *S*. cf. *punctata*, *S*. cf. *torulosa* and *S.*cf. *denisoniensis*. *Stenomelania* cf. *torulosa* was the only one species that found in Phang-nga province (Klong Bo Sean), five species were found in Krabi province (Klong Nong Jik and Klong Son 1) (Figure 28); they were *S.* cf. *aspirans*, *S.* cf. *crenulata*, *Stenomelania* sp., *S.* cf. *punctata* and *S.* cf. *torulosa.* *Stenomelania* sp. and *S*. cf. *torulosa* were recorded in Trang province while *S*. cf. *torulosa* found mainly in Satun province (Figure 26, 27; Table 8).

In this study, *S*. cf. *torulosa* was typically found in sampling sites and *S. denisoniensis* was rarely found. *S. denisoniensis* was found only in Satun province and not found in another province along the Andaman coastal. In addition, all of *Stenomelania* snails were collected, we found in sampling sites at canal near the Andaman coastline with not over than 0.6 ppt for salinity.

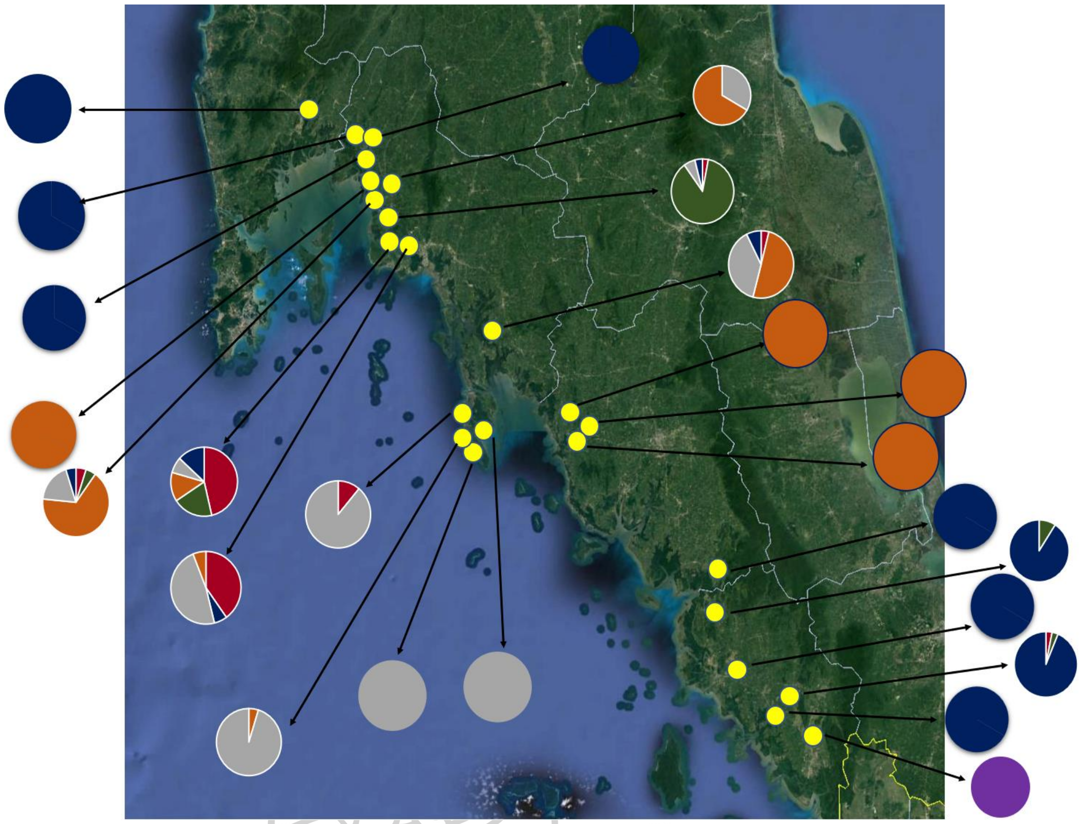

Figure 26. Distribution of 24 localities, along the Coast of Andaman Sea, South Thailand and demonstration of *Stenomelania* snails in each locality. (red color: *Stenomelania* cf. *aspians*, green color: *S.* cf. *crenulata*, orange color: *Stenomelania* sp. (*Neoradina* aff. *prasongi*), grey color: *S.* cf. *punctata*, blue color: *S.* cf. *torulosa* and purple color: *S.* cf. *denisoniensis*).

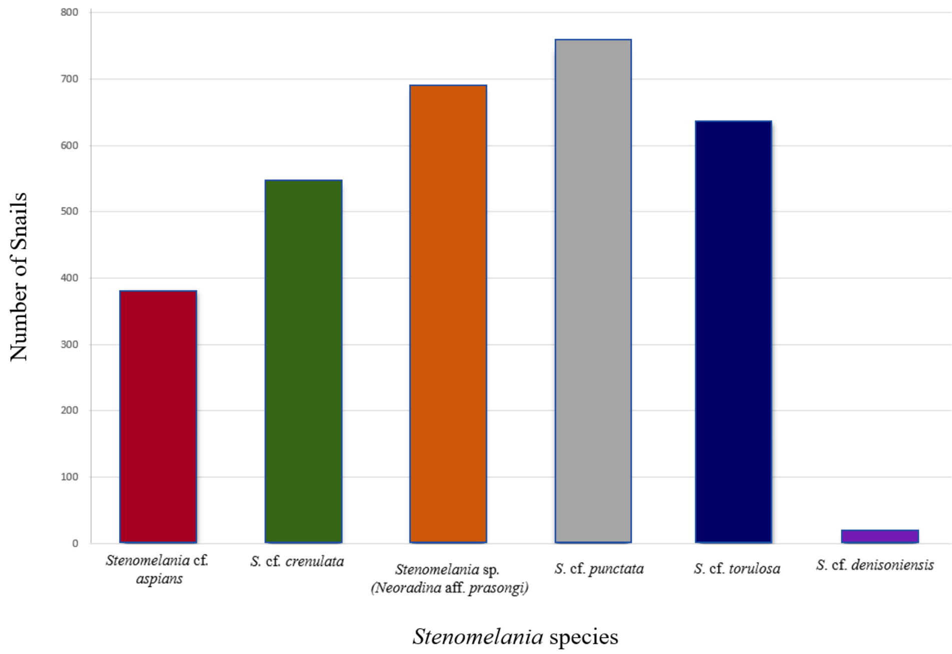

Figure 27. The populations of six *Stenomelania* species were collected from all sampling sites.

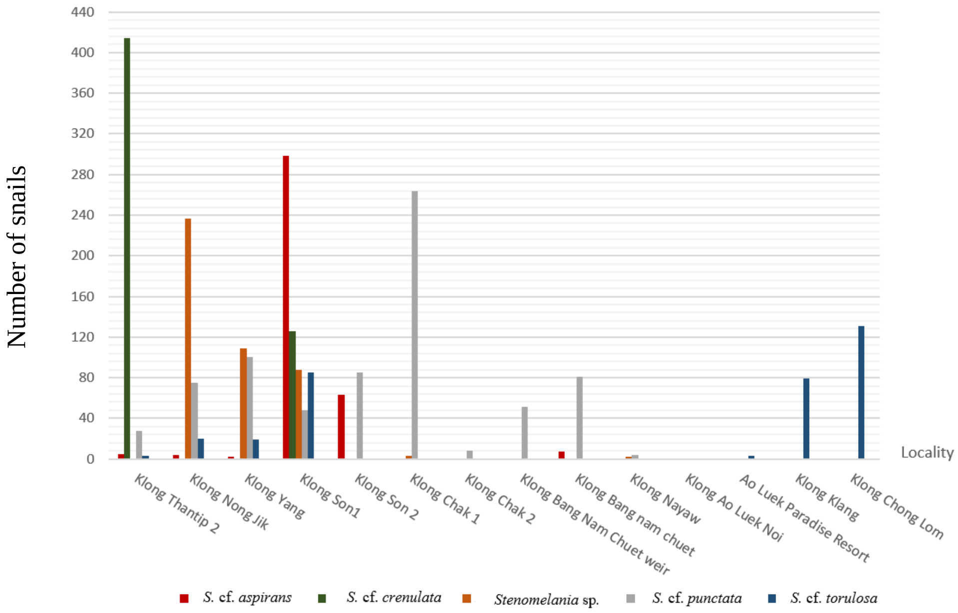

Figure 28. The populations and distribution of *Stenomelania* snail in Krabi province.

Table 8. The number of collected snails, sampling sites and collection voucher number; A = *Stenomelania* cf. *aspirans*, B = *S*. cf. *crenulata*, C = *Stenomelania* sp., D = *S*. cf. *punctata*, E =*S*. cf. *torulosa*

Sampling Sites	Voucher Number	Number of collected snails						
		Stenomelania cf. *aspirans*	*Stenomelania* cf. *crenulata*	*Stenomelania* sp.	*Stenomelania* cf. *punctata*	*Stenomelania* cf. *torulosa*	*Stenomelania* cf. *denisoniensis*	Total
Klong Bosean, Phang-nga	-	-	-	-	-	89	-	89
Klong Thantip 2, Krabi	SUT201804A SUT201804B SUT201904B SUT201904D	5	413	-	28	3	-	449
Klong Nong Jik, Krabi	SUT201801A SUT201801C SUT201801D SUT201901C SUT201901E SUT201901D	4	1	273	75	20	-	373
Klong Yang, Krabi	SUT201805A SUT201805D SUT201805E SUT201905C SUT201905D	2	-	104	100	19	-	225

Klong Son1, Krabi	SUT201802A SUT201802B SUT201902A SUT201902B SUT201902C	298	126	88	48	85	-	645
Klong Son 2, Krabi	SUT201903A	63	-	1	85	1	-	150
Klong Chak 1, Krabi	-	-	-	3	261	-	-	264
Klong Chak 2, Krabi	-	-	-	-	8	-	-	8
Klong Bang Nam Chuet Weir, Krabi	-	-	-	-	51	-	-	51
Klong Bang Nam Chuet, Krabi	-	7	-	-	81	-	-	88
Klong Nayaw, Krabi	-	-	-	2	4	-	-	6
Klong Ao Luek Noi, Krabi	-	-	-	1	-	-	-	1
Ao Luek Paradise Resort, Krabi	-	-	-	-	-	3	-	3

Klong Klang, Krabi	-	-	-	-	-	79	-	79
Klong Chong Lom, Krabi	-	-	-	-	-	131	-	131
Klong Mai Phad, Trang	SUT201808C	-	-	62	-	-	-	62
Klong La 1, Trang	SUT201806C SUT201906C	-	-	111	-	-	-	111
Klong La 2, Trang	SUT201807C SUT201907C	-	-	39	-	-	-	39
Khaoting Cave, Trang	SUT201913E	-	-	-	-	50	-	50
Klong Saphanwa, Satun	SUT201912E	-	2	-	-	27	-	29
Klong La-ngu 1, Satun	SUT201909E	-	-	-	-	33	-	33
Klong Tha Phae 1, Satun	SUT201910E	-	-	-	-	65	-	65

Klong Tha Phae 2, Satun	SUT201911E	1	-	2	-	56	-	59
Klong Chalung 1, Satun	-	-	-	-	-	-	16	16
Total		380	542	686	741	661	16	3,026

Systematics Classification and Shell Morphology
Class gastropoda
Subclass Caenogastropoda
Order Cerithiomorpha
Superfamily Cerithioidea
Family Thiaridae
Genus *Stenomelania* (Fischer, 1885)
Type species: *Melania aspirans* (Hinds, 1844)

In the past, *Stenomelania* was considered as subgenus of *Melanoides* (Morrison, 1954 and Smith 1992). The most *Stenomelania* snails characteristics were elongated shell, slender, many whorls, flat; spire attenuated and pointed, highly turreted and smooth shell or with spiral threaded, thin columella and callus. Mantle margin with 10-14 papillae. Dey and Ramakrishna (2007) reported species identifications of *Stenomelania* to 4 species consisting of *S. torulosa*, *S, plicaria*, *S. punctata* and *S. aspirans*.

In this study, the snails were categorized into 6 species of *Stenomelania*, based on the analysis of the relevant thiaridae taxa and comparison with shell morphology consist of *Stenomelania* cf. *aspirans*, *S.* cf. *crenulata*, *S.* cf. *punctata*, *S.* cf. *torulosa*, *S. denisoniensis* and *Stenomelania* sp. (*Neoradina* aff. *prasongi)*. In this study, *Neoradina* was named as *Stenomelania* probabilities related to conchology, reproductive biology and embryo development. The shell morphology was described as following;

1) *Stenomelania aspirans* (Hinds, 1987)
Synonyms:
1874 *Melania aspirans* – Hinds, *Ann. Mag. Nat. Hist*., 8.
1908 Melania *charon* - Preston, *Rec. Indian Mus.,* 2: 196, p. 15, fig 22.
1938 *Melanoides (Stenomelania) aspirans* – Wenz, *Handb. Palo. zool*., 1: 714.
1976 *Melanoides (Stenomelania) aspirans* – Stramuehlner, *Ann. Naturhist. Mus. Wien.*, 80: 577, fig 180 a-b, p 17, fig 195, 196.
1989 *Thiara (Stenomelania) aspirans* – Rao, *Handbook of freshwater Molluscs of India*, p 99, fig 206.

Shell is elongated in shape, turreted, solid and slender, dark brown color. The shell is larger than 30 mm., with 10-16 whorls. Shell sculpture has axial rib. The body world is large, smooth, with subsutural ridge, sutures not deep aperture point above and round below, paucispiral operculum, pear or oval shape with eccentric nucleus. (Figure 29).

A B C

Figure 29. A: *Stenomelania* cf. *aspirans*(SUT201802A); Klong Son 1, B: *S. aspirans*; Syntypes, BMNH 1844.9.23.32-31; Fiji Islands and C: *S. aspirans* (ZMB 106344); Mowbray River, Australia(B and C; Glaubrecht et al., 2009)

2) *Stenomelania crenulata* (Deshayes, 1838)

Synonyms:
1838 *Melania crenulata* – Deshayes
1838 *Melania (Stenomelania) crenulata* – Deshayes
1860 *Stenomelania rufescens* – Martens

Shell is elongated conic, dark brown or black color. The shell is larger than 30 mm and has 12-14 whorls. The shell surface sculpture looks like a net, has spiral grooves and axial ribs. Five spiral grooves are found on each whorl, but the body whorl has more than 5 grooves, and deep suture. Aperture oval shape with paucispiral operculum. (Figure 30).

A B

Figure 30. *Stenomelania* cf. *crenulata* (SUT201804B); A: Klong Thanthip 2, Krabi, B: Klong Tha Phae 2, Satun.

3. *Stenomelania* sp. (*Neoradina* aff. *prasongi)* (Brandt, 1974)

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, rounded whorls with deep sutures (Figure 31).

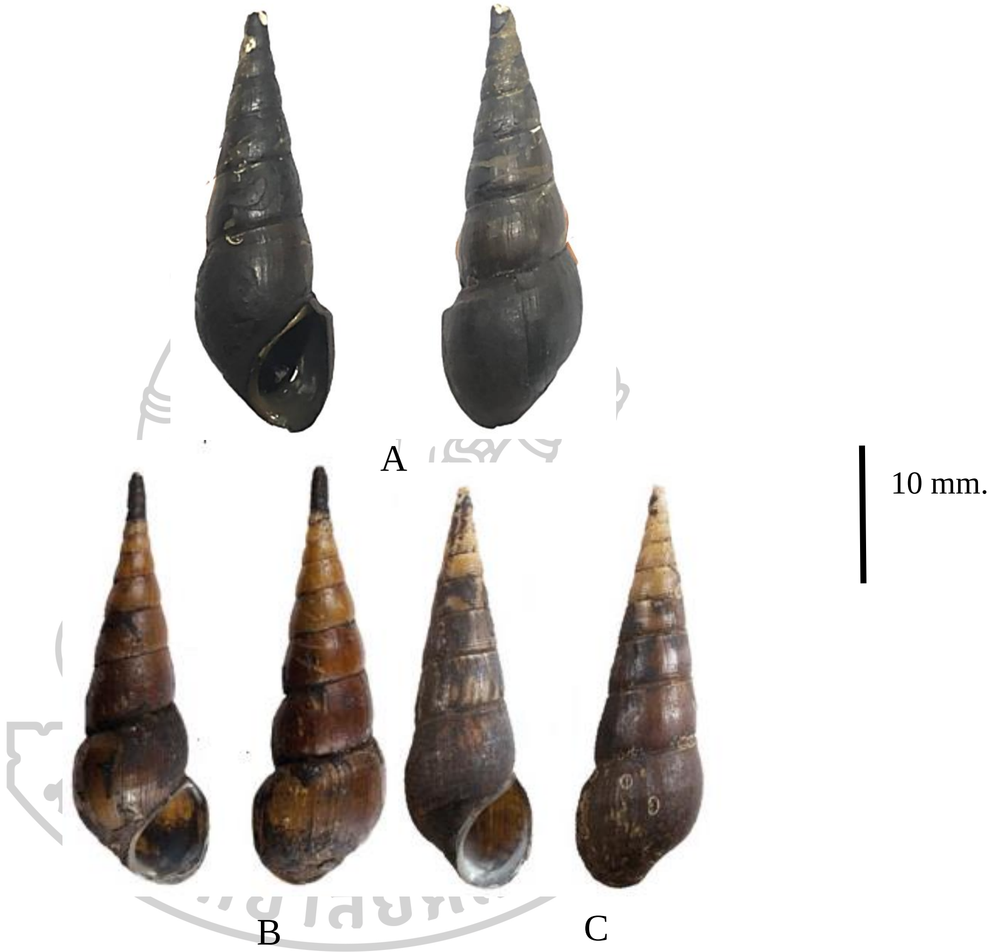

Figure 31. A: *Stenomelania* sp. (SUT201905C); Klong Yang, Krabi, B: *N. prasongi* (paratypes, SMF 215934/7-2;7), C: *N. prasongi* (ZMH 59338-1); stream 5 km from Krabi to Khao Thong. (B, C; (Wiggering et al., 2019))

4. *Stenomelania punctata* (Lamarck, 1822)
Synonyms:

1822 *Melania punctata* – Lamarck, *Hist. Nat. Anim. Sans. Vert*., 6 (2): 165.
1915 *Thiara (radian) clavus* – Preston, *Fauna. Brit. India*. Mollusca (Freshwater Gastropoda and Pelecypoda), p 12.
1976 *Melanoides (Stenomelania) punctata* – Stramuehlner, *Ann. Naturhist. Mus. Wien.,* 80: 586, Fig 98-108, p 17, fig 197, 201.
1989 *Thiara (Stenomelania) punctata* - Rao, *Handbook of freshwater Molluscs of India*, p 9, fig 210-211.

Shell is turret-elongated in shape, body whorl broad and smooth, whorls with radial striations, dark brown color. 12 -14 whorls, long pointed spire with sculpture (some found truncate), smooth and flat, rectangular increasing in size, deep suture, aperture pointed and rounded. Operculum horny, pear to oval shaped with eccentric nucleus situated at the lower left corner (Figure 32).

Figure 32. A: *Stenomelania* cf. *punctata*(SUT201801D); Klong Nong Jik, Krabi, B: *S. punctata* (ZMB 106396-2); Sovi River, Malevu, Coral Coast, Fiji, C: *S. punctata* (ZMB 106386-3); Sovi River, East of Malevu, Coral Coast, Fiji.(B and C; (Wiggering et al., 2019))

5. *Stenomelania torulosa* (Bruguiere, 1789)
Synonyms:
1789 *Bulimus torulosus* – Bruguiere, *Encycl. Meth. Vers*., 1: 332.

1915 Tiara (Radina) crenulate – Preston, *Preston, Fauna. Brit. India. Mollusca (Freshwater Gastropoda and Pelecypoda),* p 11.
1973 *Thiara (Stenomelania) torulosa* – Pace, *Mal. Review. Supplement.,* 1: 66.
1989 *Thiara (Stenomelania) torulosa* - Rao, *Handbook of freshwater Molluscs of India,* p 100, fig 202.
2005 *Stenomelania torulosa* – Dey and Ramakrishna, *Zool. Surv. India. (Fauna of Andhra Pradesh),* 5, p 199.

Shell is turret-elongated conic, dark brown or black color, solid with sculpture. The shell is larger than 30 mm and has 8-12 whorls. The spire often is truncate. Five spiral ridges are found on each whorl, but the body whorl has more than 5 ridges, deep suture. Aperture is pyriform shape, pointed above and rounded below, columellar side twisted and curved to the right (Figure 33).

Figure 33. *Stenomelania* cf. *torulosa*(SUT201905D); Klong Yang, Krabi.

6. *Stenomelania denisoniensis* (Brot, 1877)
Synonyms:
1877 *Melania denisoniensis* - Brot, *Martini & Chemnitz, Conch. Cab*. 1 (24):

234.
1943 *Stenomelania denisoniensis tacita* - Iredale, *Aust. Zool*. 10: 208
1943 *Stenomelania denisoniensis ultra* - Iredale, *Aust. Zool*. 10: 209
1992 *Melanoides (Stenomelania) denisoniensis* –Smith, *Zool. Cat. Aus*. 8.: 76.

The shell is elongate and slender up to 8 whorls. Whorls are corroded only in the upper regions, simple suture, smooth shell surface, only a few spiral lines are visible. The color is from light brown to yellowish green or dark brown. The aperture is wide oval and rarely flaring on the basis (Figure 34).

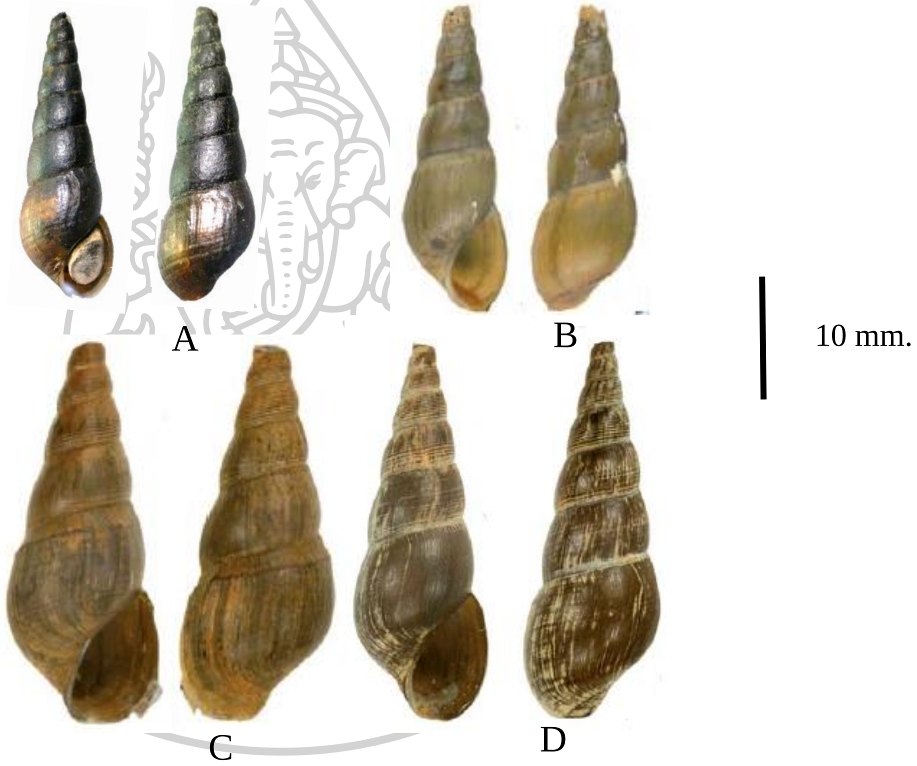

Figure 34. A: *Stenomelania* cf. *denisoniensis*; Klong Chalung 1, Satun, B: *S. denisoniensis*; Syntypes (MHNG); Queensland, Port Denison, C: *S. denisoniensis* (ZMB 106341); QLD, Meelele River, D: *S. denisoniensis* (ZMB 106342); QLD, Woobadda River. (B, C and D; (Glaubrecht et al., 2009))

Comparison of Shell Morphology

All of 6 shell morphological of *Stenomelania* were collected from Phang-nga, Krabi, Trang and Satun, the mean of 6 parameters, height, width, height/width of aperture, three whorl height and body whorl height were plotted in the bar chart (see Figure 35). The results showed the variation of *Stenomelania* in term of size (Table 9). The most height, width, aperture height/width, body whorl height and three whorl height (46.82±6.56, 13.21±1.72, 16.35±2.95/8.92±1.91, 24.18±4.80, 35.29±7.05, respectively) were found in *Stenomelania* cf. *punctata*, the smallest was *S.* cf. *denisoniensis* (26.55±5.15, 8.27±1.34, 7.72±1.69/ 5.46±1.28, 8.52±1.51, 16.37±2.94, respectively). The statistical analysis parameters showed (see appendices), the heights and width of *S.* cf. *puunctata* and *S.* cf. *denisoniensis* were different from the other species with statistically significant (p-value < 0.05). *Stenomelania* cf. *aspirans*, *S.* cf. *crenulata*, *S*. cf. *torulosa* and *Stenomelania* sp. were not different with statistically significant (p-value > 0.05). The height aperture of *S.* cf. *punctata* was different from the other species with statistically significant (p-value < 0.05) but the width aperture was not different, except *Stenomelania* cf. *aspirans* and *S.* cf. *denisoniensis*. The three whorl height of *S.* cf. *puunctata* and *S.* cf. *denisoniensis* were different from the other species with statistically significant (p-value < 0.05) but *S.* cf. *aspirans*, *S.* cf. *crenulata*, and *Stenomelania* sp. (*Neoradina* aff. *prasongi)* were not different. Finally, the body whorl of *S.* cf. *punctata*, *S.* cf. *torulosa* and *S.* cf. *denisoniensis* were different from another species with statistically significant (p-value < 0.05).

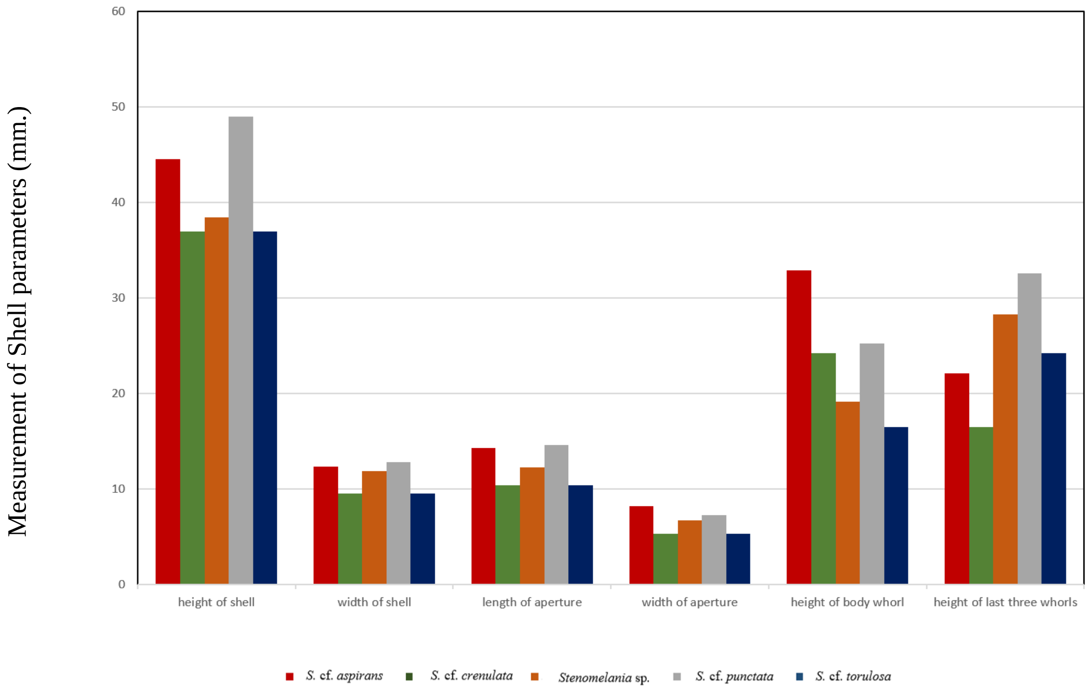

Figure 35. The mean of parameters, height, width, height/width of aperture, three whorl height and body whorl height of snail genus *Stenomelania*.

Table 9. Shell parameters of *Stenomelania*.

Species	Number of whorls	height of shell (mm.)	width of shell (mm.)	length of aperture (mm.)	width of aperture (mm.)	height of body whorl (mm.)	height of last three whorls (mm.)	angle
Stenomelania cf. *aspiran*	10-12	42.12±3.99	10.48±1.68	11.53±1.43	7.95±1.88	16.22±2.82	26.58±3.25	12.9±1.9
Stenomelania cf. *crenulata*	10-12	40.14±1.15	10.07±0.74	11.39±1.40	5.95±1.03	17.53±2.05	26.95±1.78	17.4±0.52
Stenomelania sp.	8-12	37.75±1.08	11.94±0.64	11.01±0.40	6.86±0.53	18.56±0.71	27.55±1.20	21.0±0.70
Stenomelania cf. *punctata*	12-14	46.82±6.56	13.21±1.72	16.35±2.95	8.92±1.91	24.18±4.80	35.29±7.05	22.2±7.15
Stenomelania cf. *torulosa*	10-12	38.13±2.47	10.31±0.70	8.30±0.44	5.39±0.45	12.53±0.86	22.42±1.53	14.5±0.70
Stenomelania cf. *denisoniensis*	6-12	26.55±5.15	8.27±1.34	7.72±1.69	5.46±1.28	8.52±1.51	16.37±2.94	17.5±2.63
Total	-	38.60±7.28	10.71±1.95	11.21±3.24	6.76±1.84	16.26±5.49	25.86±6.69	17.7±3.67

Molecular Study of Collected Snails

Snails genus *Stenomelania* were studied by using the CO1 gene sequences (Figure 36; Table 10). 6 species were categorized on the basis of their morphology. *Stenomelania* sp. (*Neoradina* aff. *prasongi*: species name from Apiraksena et al., 2020; SUT201806C and SUT201807C) were the only one species that could be amplified, approximately 650 bp in length. The phylogenetic tree obtained from neighbour-joining analysis was rooted with the *Bithynia siamensis siamensis* (GenBank accession number: MW832467).

Unfortunately, the *Stenomelania* cf. *aspirans*, *S.* cf. *crenulata*, *S*. cf. *Punctate*, *S*. cf. *torulos*, and *S.* cf. *denisoniensis*, it could not amplify. Specimens of *Stenomelania* sp. were grouped together with highly relative support that was found in the same province. In addition, references sequence from *Melanoides tuberculata* and *Tarebia granifera*, their clade was separated with *Stenomelania*, although *Melanoides* was a closed relationship. *Stenomelania* sp. and *Neoradina prasongi* (Wiggering et al., 2019) were included in the same major clade with *Stenomelania*.

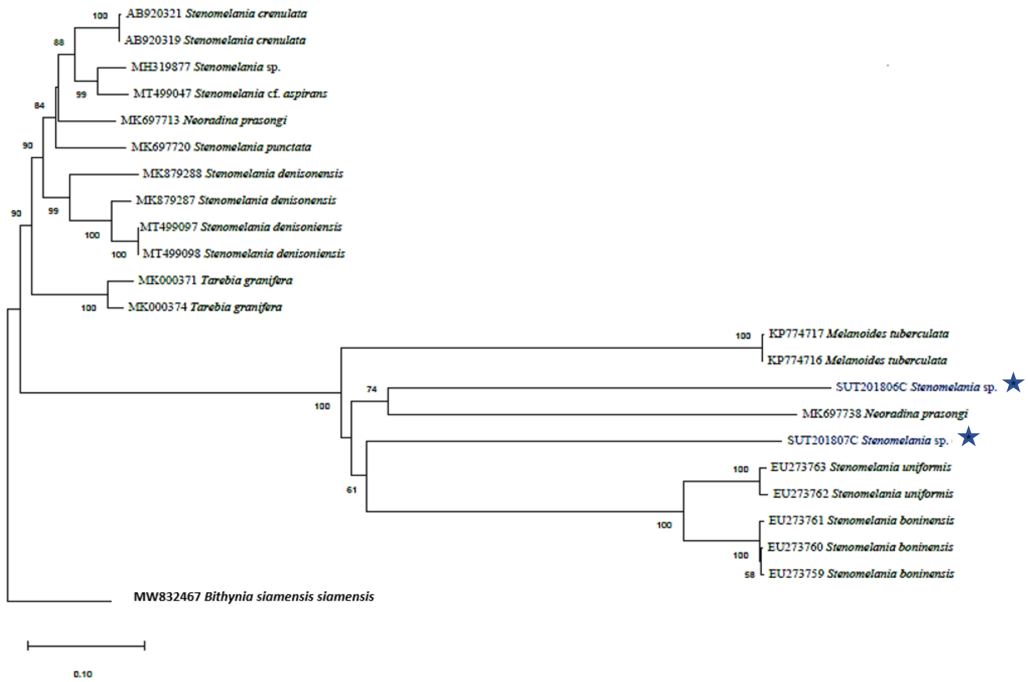

Figure 36. Phylogenetic relationship of *Stenomelania* snailwas constructed using CO1 sequences based on neighbor-joining analysis (1,000 bootstrap replications) and the other published DNA sequences obtained from GenBank. Nodes are annotated with bootstrap support value ≥ 50. Taxon names and voucher or GenBank accession numbers are provided at the tips of the tree (Blue star: this study) (see also Table 10).

Table 10. Sequences used for the phylogenetic analysis.For SUT numbers, see the material lists in the main part of the text.

Species	Voucher code	GenBank accession number	location	reference
Stenomelania sp.	SUT201806C	-	Klong La 1, Trang	this study
	SUT201807C	-	Klong La 2, Trang	this study
Neoradina prasongi	-	MK697713	Klong Chalung, Satun	Wiggering et al. (2019)
	-	MK697738	Pak Meng River,Trang	
Stenomelania crenulata	-	AB920321	Kagoshima, Japan	Hidaka and Kano (2014)
	-	AB920319	Niyado River, Koshi, Japan	
Stenomelania cf. *aspirans*	-	MT499047	Mowbray, Queensland, Australia	Lentge-Maass et al. (2020)
Stenomelania punctata	-	MK697720	Fiji	Wiggering et al. (2019)
Stenomelania denisonensis	-	MK879288	Australia	Boonmekam et al. (2019)
	-	MK879287	Australia	
	-	MT499097	Northern Territory, Australia	Lentge-Maass et al. (2020)
	-	MT499098		
Stenomelania boninensis	-	EU273761	Bonin Island, Japan	Miura et al. (2008)

	-	EU273760		
	-	EU273759		
Stenomelania uniformis	-	EU273763	Bonin Island, Japan	Miura et al. (2008)
	-	EU273762		
Melanoides tuberculata	-	KP774717	Lake Kyoga. Uganda	Van Bocxlaer et al. (2015)
	-	KP774716	Victoria Nile at Jinja, Uganda	
Tarebia granifera	-	MK000371	Krung Ching waterfall	Veeravechsukij et al. (2018b)
	-	MK000374	Surat Thani province: Tha Chang district, Klong Tha Sai	
Bithynia siamensis siamensis	-	MW832467	Thailand	Bunchom et al. (2021)

Cercarial Diversity and Infection Rates

Specimens of *Stenomelania* spp. were found from 24 sampling sites in 4 provinces of Thailand. During the sampling period (2017–2020), infected snails were reported from 8 sampling sites (Figure 37). For information on sampling sites including the number of infected snails were presented in detail (Table 11). A total of 3,026 snails of *Stenomelania* spp. were collected and examined for trematode infections. With 19 parasitized snails the overall infection rate was found to be 0.63 %. The obtained cercariae were classified into a total of four species from morphologically distinguishable types representing at least two distinct trematode families, viz. (i) virgulate xiphidiocercariae (*Loxogenoides bicolor)* (ii) parapleurolophocercous cercariae (*Haplorchis taichui*, *Procerovum cheni* and *Acanthotrema tridactyla*). The parapleurolophocercous cercariae were the dominant cercarial type infecting snails from 7 localities.

Moreover, several species exhibit a more restricted distribution. For example, *Acanthotrema tridactyla* was only detected in *S.* cf. *denisoniensis* samples from the Klong Chalung 1, Satun province. Cercaria of *Loxogenoides bicolor* was detected in *S*. cf. *torulosa* and *Stenomelania* sp. sample from Kaoting Cave and Klong La 1 in Trang province. *L. bicolor* was an only one species that found in Trang province. *Haplorchis taichui* cercaria widely infected in *S*. cf. *punctata, Stenomelania* sp., *S*. cf. *torulosa and S.* cf. *denisoniensis* population from Krabi, Trang and Satun province. *Procerovum cheni* was detected in *S*. cf. *punctata*, that could be found in Klong Nong Jik, Krabi province. In this study, we did not find trematode infection in *S.* cf. *crenulata* and *S.* cf. *aspirans.* In addition, neither double trematode infections nor triple trematode infections of collected *Stenomelania* snails were not found among Thiarid snails.

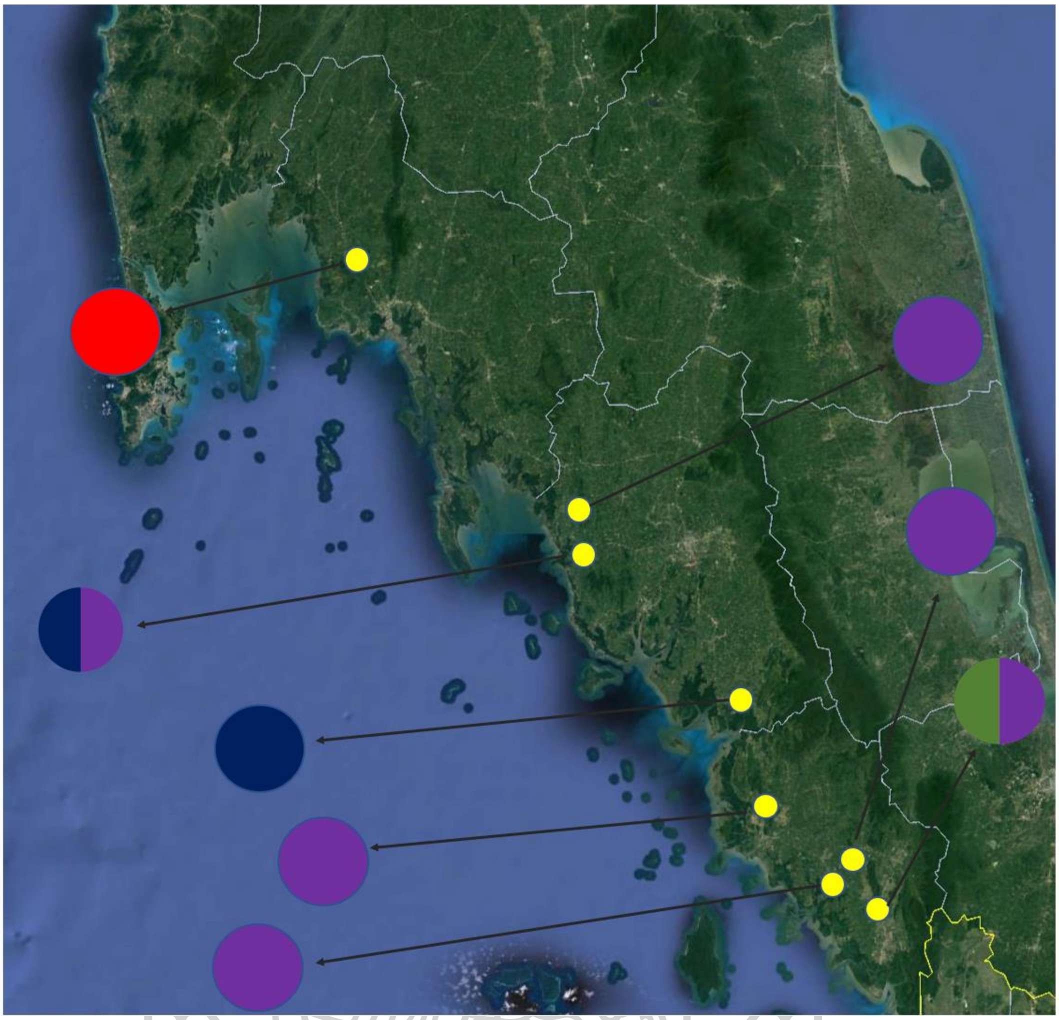

Figure 37. Map distribution of trematode infections at Krabi, Trang and Satun province during 2017-2019.(yellow spot: localities) red color: *Procerovum cheni*, purple color: *Haplorchis taichui*, blue color: *Loxogenoides bicolor* and green color: *Acanthotrema tridactyla*)

Table 11. Demonstration of infection in different localities, number of infected snails and trematodes obtained from collected snails.

No.	Location	Snail number	Infected snails	Infection rate (%)	Cercaria	Intermediated Host
1	Klong Nong Jik, Krabi Province	373	1	0.27	*Procerovum*	*S.* cf. *punctata*
2	Klong Mai Phad, Sikao District, Trang Province	62	1	1.61	*Haplorchis taichui*	*Stenomelania* sp.
3	Klong La 1, Sikao District, Trang Province	111	1 1	0.9 0.9	*Haplorchis taichui* *Loxogenoides bicolor*	*Stenomelania* sp.
4	Khaoting Cave, Palian District, Trang Province	50	3	6.00	*Loxogenoides bicolor*	*S.* cf. *torulosa*
5	Klong Tha Phae 1, Tha Phae District, Satun Province	44	1	2.27	*Haplorchis taichui*	*S.* cf. *torulosa*
6	Klong Tha Phae 2, Tha Phae District,	56	1	1.79	*Haplorchis taichui*	*S.* cf. *torulosa*

	Satun Province					
7	Klong La-ngu 1, La-ngu District, Satun Province	33	1	3.70	*Haplorchis taichui*	*S.* cf. *torulosa*
8	Klong Chalung 1, Mueang District, Satun Province	16	5 3	31.25 18.75	*Haplorchis taichui* *Acanthotrema tridactyla*	*S.* cf. *denisoniensis*

Morphology of the Infecting Cercariae

The cercariae were categorized on their morphological and organ characters in accordance with previously-reported morphological descriptions (Ito, 1980; Komiya, 1961; Krailas et al., 2014; Krailas et al., 2011; NASIR, 1984; Schell, 1970; Veeravechsukij et al., 2018a; Yamaguti, 1971, 1975). 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; Figure 38)

The body was oval and covered with tiny spines. Granules were brown and located underneath the skin. Oral sucker was round shaped and bigger than ventral sucker, found one stylet in oral sucker. The virgulate gland was shown in the anterior part of the body, pharynx round and absent esophagus. Three pairs of penetration glands were located at two-thirds of the body and they had two anterior pairs and one posterior pair. 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.

Movement behavior: The cercaria floated on the upper or in the middle of the water. The body sank lower than the tail. It moved, folding its tail back to the body and turning its body to roll from left to right, rapid mobility forward for about 15 seconds, resting on the lower water for about 2-3 seconds.

Type 2. Parapleurolophocercous cercariae

Heterophyidae (Leiper 1909; sensu Odhner 1914)

2.1 *Haplorchis taichui* (Nishigori, 1924; Chen 1936; Figure 39)

The body was oval shaped and brownish. The 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 located on the ventral of the body surface. It had one pair of eyespots, and a pharynx was presented. The seven pairs of penetration glands spread from the pharynx to the posterior end of the body. The penetration glands were fourteen ducts and opened on the anterior end of the body. A ventral sucker located in the middle of the body. The excretory bladder was round and thick walled. The tail was longer than the body. The end of the tail was bent. The lateral and dorso-ventral finfolds were presented. The cercariae developed within rediae.

Movement behavior: The cercaria floated on the upper or the middle of the water. It moved folding its tail back to the body, rapid swim forward for about 2-3 seconds, resting on the upper water for about 10-20 seconds, the body sank lower than the tail.

2.2 *Procerovum cheni* (Hsǜ, 1951; Figure 40)

The cercaria was oval. The 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.

Movement behavior: The cercaria floated in the middle of the water. It swims forward for about 2-4 seconds and not definite directions, resting on the upper water for about 9-15 seconds.

2.3 *Acanthotrema tridactyla (*Martin & Kuntz, 1955; Figure 41)

The body is oval-shaped. The oral sucker was located at the anterior end of the body and ventral sucker was poorly developed. It had three transverse rows of oral spines and a pharynx was presented. It had seven pairs of penetration glands in four groups of 3:4:4:3, situated between the pharynx to the excretory bladder. A pair of pigmented eyespots presented. The excretory bladder is V-shaped and thick-walled. The tail was longer than the body. The bilateral finfold and a dorso-ventral finfold were on their tail. The cercariae develop within rediae.

Movement behavior: The cercaria floated on the upper and middle of the water. It moves by folding its tail upside down to the body, it swims very fast forward for about 2 seconds and not definite directions, resting on the upper water for about 5-8 seconds. After resting, the tail was spinning and the body fell to the ground water.

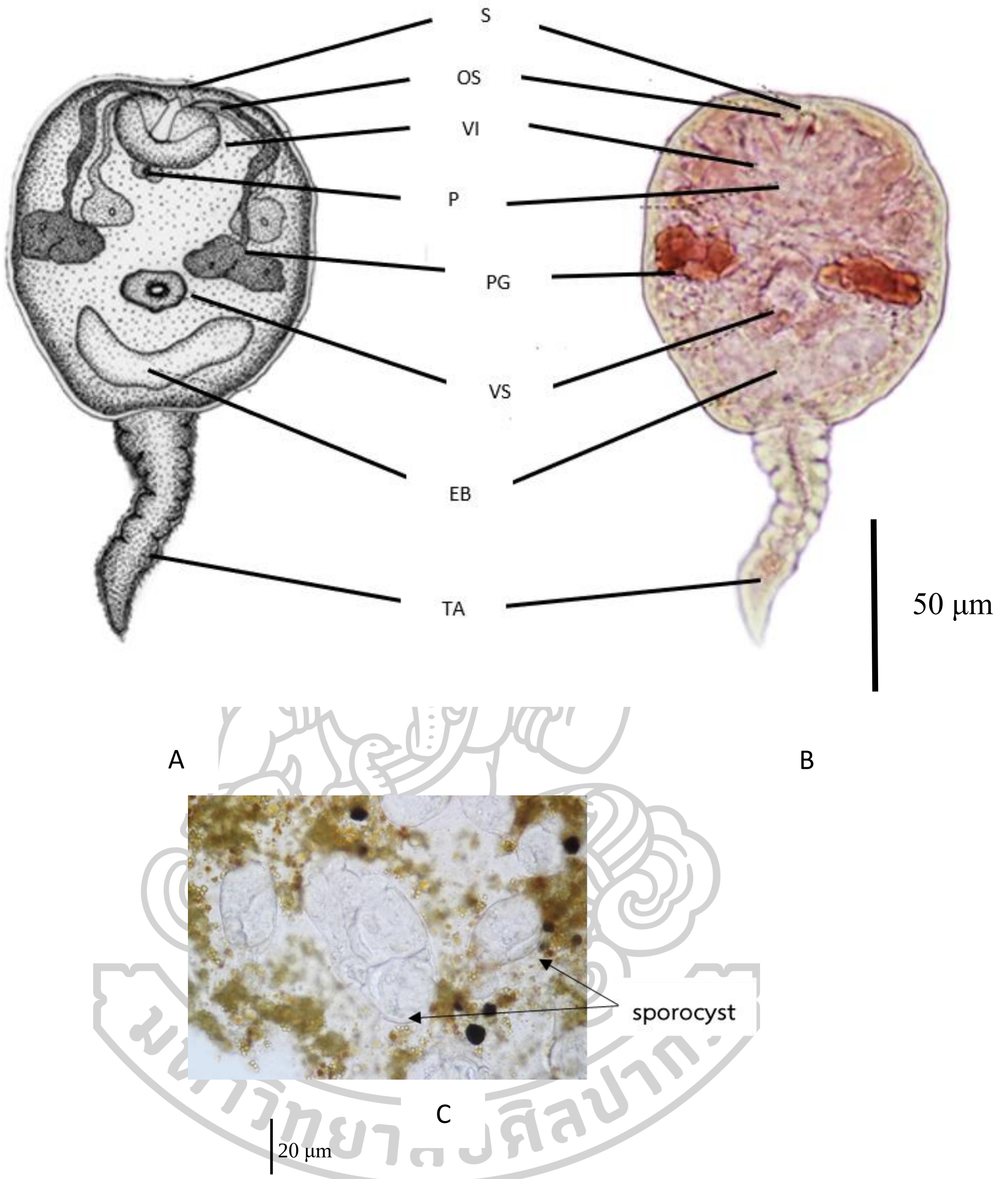

Figure 38. *Loxogenoides bicolor*, A. Drawing image; B. Specimen stained with 0.5% neutral red; C. Sporocyst stained with 0.5% neutral red. Abbreviations – EB: excretory bladder; OS: oral sucker; P: pharynx; PG: penetration gland; S: stylet; SP: sporocyst; TA: tail; VI: virgulate organ; VS: ventral sucker.

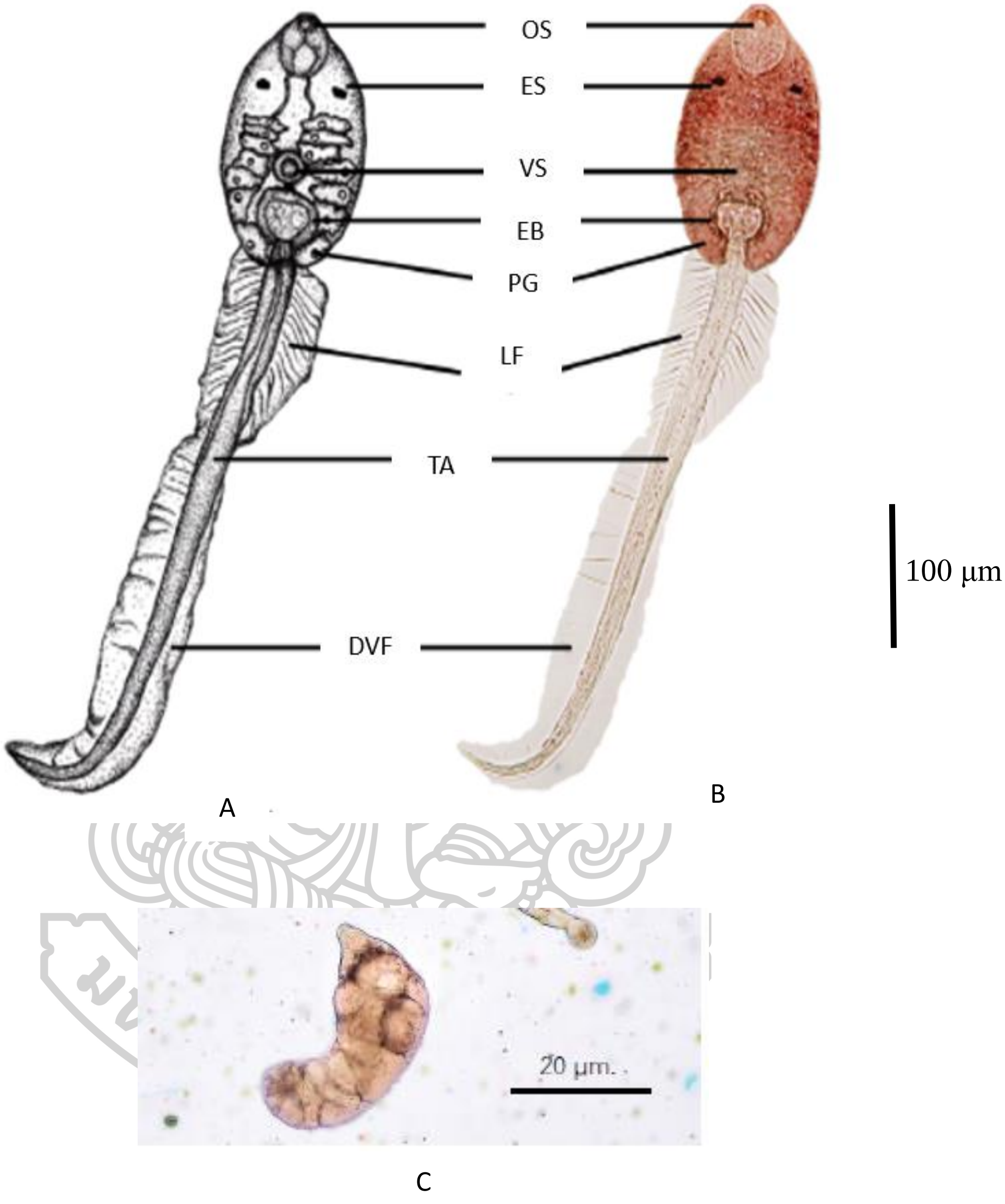

Figure 39. *Haplorchis taichui* (Nishigori, 1924; Chen 1936). A. Drawing image; B. Specimen stained with 0.5% neutral red; C. Redia stained with 0.5% neutral red. Abbreviations – DVF: dorso-ventral finfold; EB: excretory bladder; ES: eyespot; IF: lateral finfold; OS: oral sucker; P: pharynx; PG: penetration gland; TA: tail.

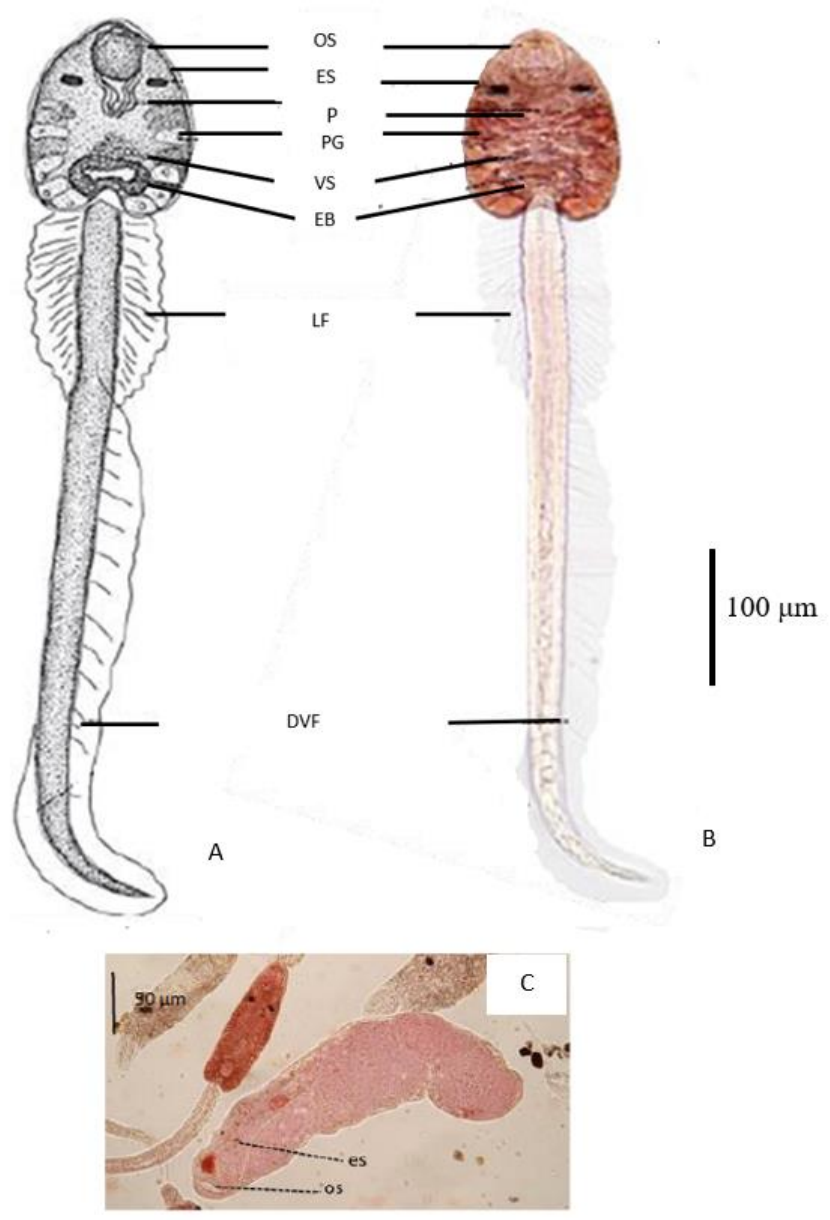

Figure 40. *Procerovum cheni* Hsü, 1951. A. Drawing image; B. Specimen stain with 0.5% neutral red; C. Redia staind with 0.5% neutral red. Abbreviations- DVF: dorso-ventral finfold; EB: excretory bladder; ES: eyespot; IF: lateral finfold; OS: oral sucker; P: pharynx; PG: penetration gland; TA: tail.

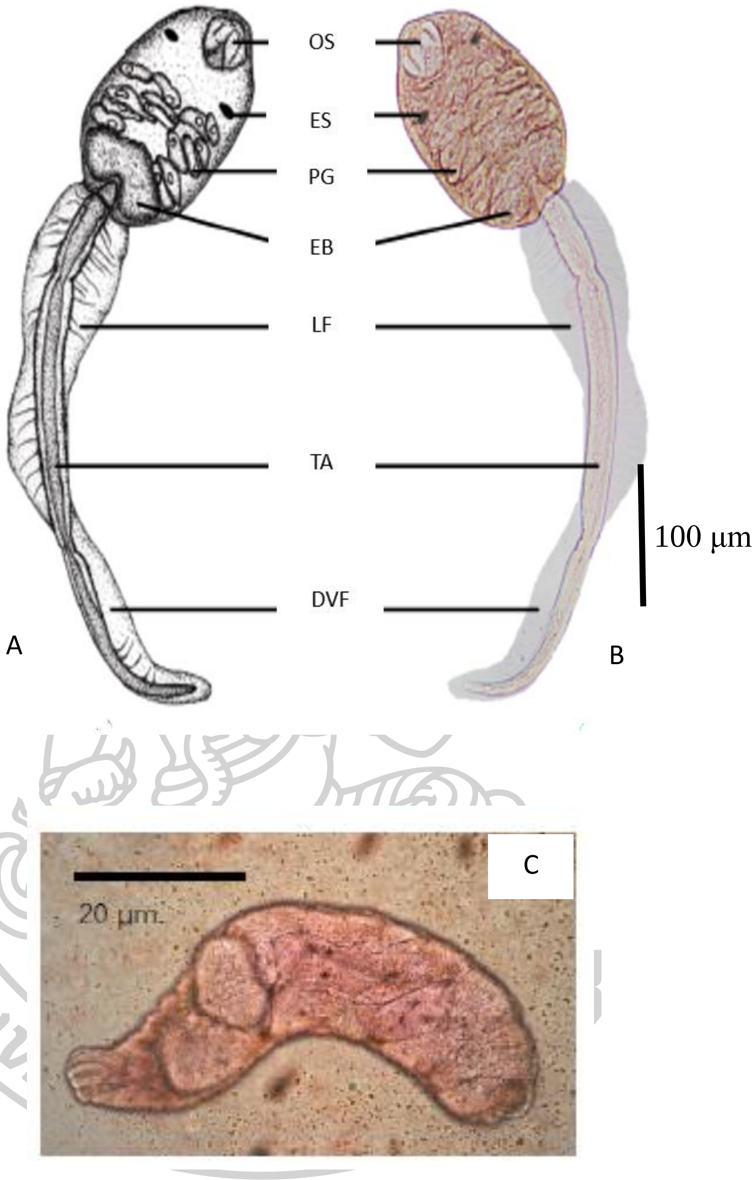

Figure 41. *Acanthotrema tridactyla* A. Drawing image; B. Specimen stain with 0.5% neutral red; C. Redia staind with 0.5% neutral red. Abbreviations- DVF: dorso-ventral finfold; EB: excretory bladder; ES: eyespot; IF: lateral finfold; OS: oral sucker; P: pharynx; PG: penetration gland; TA: tail.

Comparison of the Cercarial Morphologies

Ten cercarial, each species consisting of *Haplorchis taichui*, *Procerovum cheni*, *Acanthotrema tridactyla* and *Loxogenoides bicolor* were measured. We measured size of body, tail, oral sucker, ventral sucker, lateral finfold, dorso-ventral finflod, excretory bladder, eyespot and stylet (Table 12, 13, 14, 15). In this study, we focused on comparison among parapleurolophocercous compared to references from previous reported; e.g., Veeravechsukij et al. (2018), Dechruksa et al. (2017), Krailas et al. (2014), Umadevi et al. (2000) and Hsu (1951). We analyzed only the body and tail (length and width) with a significant proportion of variances (p - value < 0.05) by one-way Anova testing, Post-hoc testing using the Duncan and LSD test was conducted to find significant differences among groups. The results within the group (Figure 42), the body of *H. taichui, P. cheni, A. tridactyla* were different (p value < 0.05), also tail length. The tail width of *A. tridactyla* was different from *P. cheni* but not different from *H. taichui.*

In addition, comparison of *H. taichui* with the references that were reported form Veeravechsukij et al. (2018a) and Krailas et al. (2014). For the result, the body, tail, oral sucker, ventral sucker, excretory bladder length and width of *H. taichui* were found that smaller than other samples from previous reported *P. cheni* were found, its body length was bigger than Hsu (1951) reported and *P. varium* (Umadevi & Madhavi, 2000), the tail length longer than Hsu (1951)reported but shorter than *P. varium*. *A. tridactyla* were found that body, tail, oral sucker, ventral sucker, excretory bladder length and width were smaller than previous reported from Veeravechsukij et al. (2018b) and Krailas et al. (2014). Finally, *L. bicolor* were found that body, tail, oral sucker, ventral sucker, stylet length and width were smaller than previous reported from Veeravechsukij et al. (2018b) and Dechruksa et al. (2017) (Figure 42, 43, 44, 45, 46).

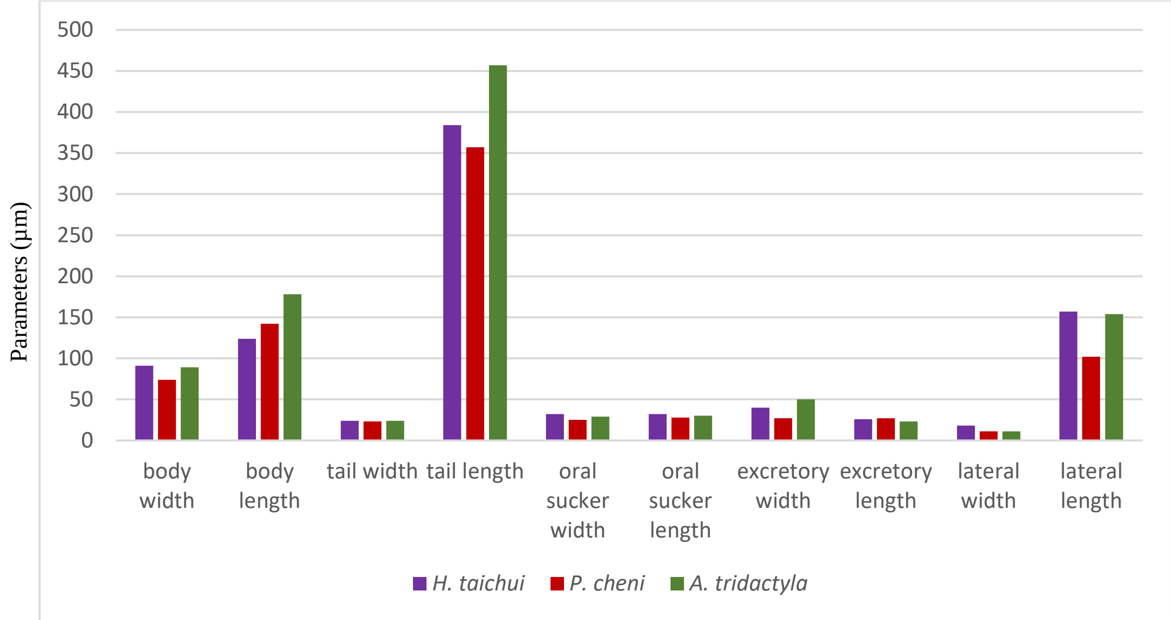

Figure 42. Comparison of the morphological character of the *Haplorchis taichui* (blue chart), *Procerovum cheni* (orange chart) and *Acanthotrema tridactyla* (grey chart), in this study.

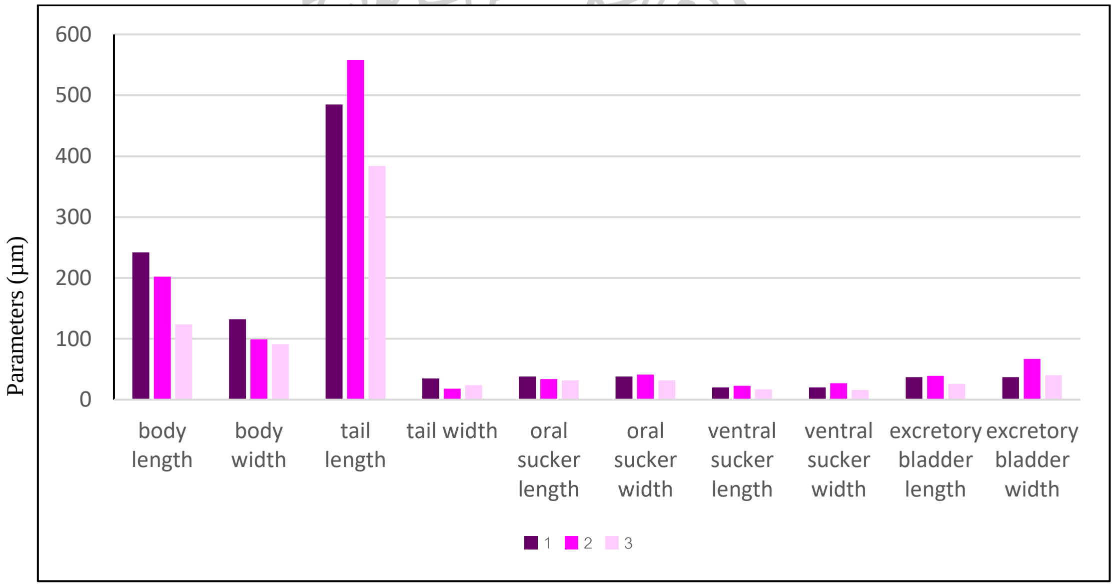

Figure 43. Comparison of the morphological character of the *Haplorchis taichui*.1) Krailas et al. (2014), 2) Veeravechsukij et al. (2018a), 3) this study.

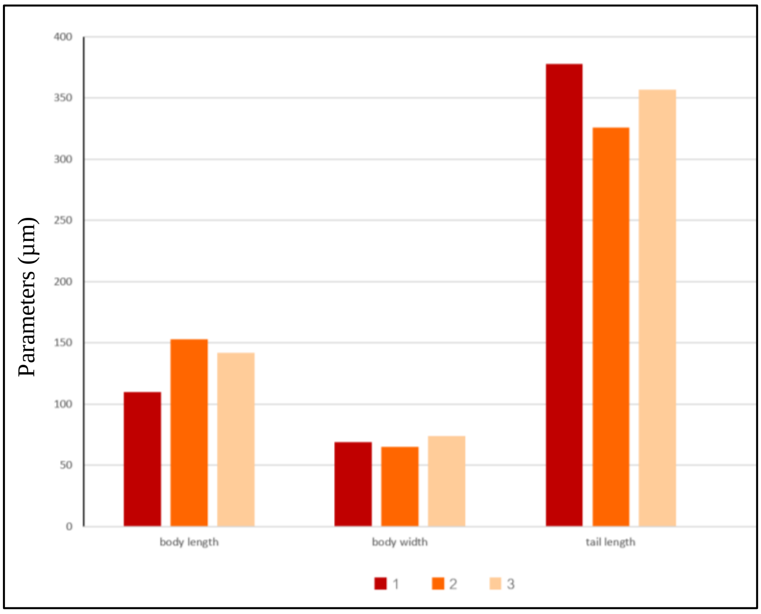

Figure 44. Comparison of the morphological character of the 1) *P. cheni* Hsu (1951), 2) *P. varium* Umadevi and Madhavi (2000), 3) *P. cheni* (this study).

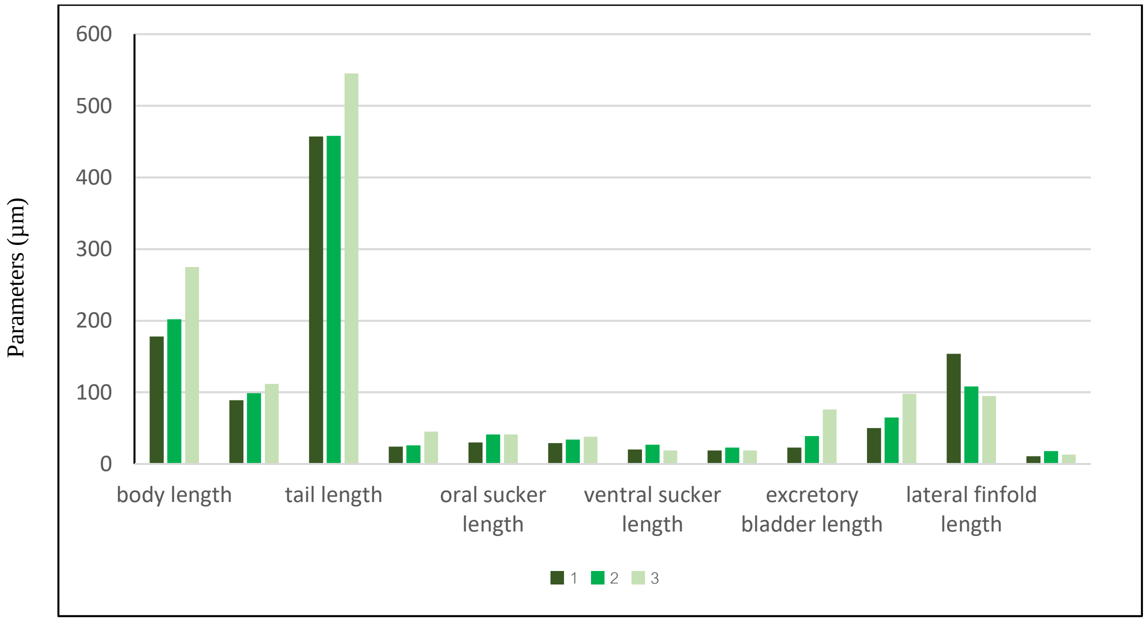

Figure 45. comparison of the morphological character of the *Acanthotrema tridactyla*. 1) Krailas et al. (2014), 2) Veeravechsukij et al. (2018a), 3) this study.

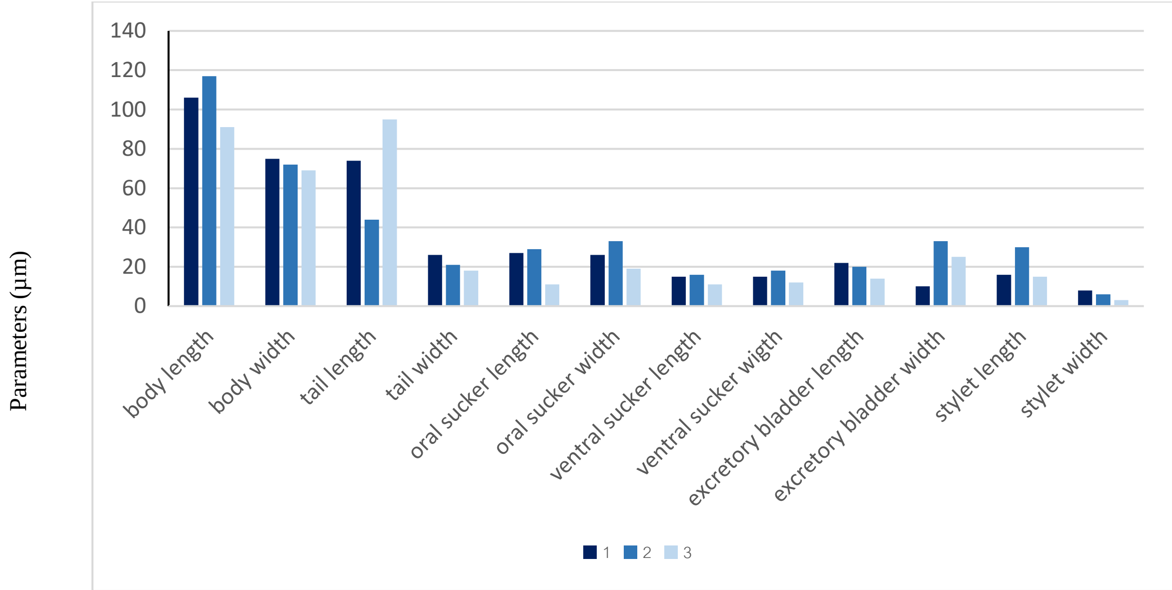

Figure 46. Comparison of the morphological character of the *Loxogenoides bicolor*1) Dechruksa et al. (2017), 2) Veeravechsukij et al. (2018a), 3) this study.

Table 12. Some characters of *Haplorchis taichui*found in this study and the reference sources (measurement in µm, calculated from 10 cercariae, n/a = no data)

	Haplorchis taichui Krailas et al. (2014)	*Haplorchis taichui* Veeravechsukij et al. (2018a)	*Haplorchis taichui* This study
Body	132 (93-135) x 242 (156-276)	99 (80-118) x 202 (168-207)	91 (78 – 116) x 124 (101 – 151)
Tail	35 (15-42) x 485 (378-514)	18 (20 - 33) x 558 (405 – 495)	24 (20 – 27) x 384 (352 – 413)
Oral sucker	38 (24-45) x 38 (27-45)	34 (28-38) x41 (30-50)	32 (29 – 40) x 32 (25 – 40)
Ventral sucker	20 (15-25) x 20 (15-25)	23 (13-35) x 27 (15 -45)	17 (13 – 20) x 16 (13 – 19)
Excretory bladder	37 (30-42) x 37 (30-42)	64 (43 – 90) x 39 (20 – 55)	40 (37 – 42) x 26 (24 – 30)
Stylet	Not found	Not found	Not found
Eyespot	n/a	9 (5-15) x 9 (5-15)	9 (7 – 10) x 11 (9 – 13)
Lateral finfold	n/a	13 (8-15) x 103 (75-125)	18 (13-23) x 157 (102-135)
Dorso-ventral finfold	n/a	n/a	24 (18–28) × 289 (265–306)

Table 13. Some characters of *Procerovum cheni*found in this study and the reference sources (measurement in µm, calculated from 10 cercariae, n/a = no data)

	Procerovum cheni Hsu (1951)	*Procerovum verium* Umadevi and Madhavi (2000)	*Procerovum cheni* This study
Body	69 (60 - 73) x 110 (113 - 130)	65 (60-72) x 153 (140-168)	74 (64 – 85) × 142 (109 - 176)
Tail	n/a x 378 (301–390)	n/a x 326 (316-340)	23 (19 – 28) × 357 (270 – 398)
Oral sucker	n/a	n/a	25 (21 – 31) × 28 (24 – 35)
Ventral sucker	n/a	n/a	n/a
Excretory bladder	n/a	n/a	27 (22 – 33) × 27 (23 – 31)
Stylet	Not found	Not found	Not found
Eye spot	n/a	n/a	9 (8 – 11) × 6 (4 – 7)
Lateral finfold	n/a	n/a	11 (7-14) x 102 (85-117)
Dorso-ventral finfold	n/a	n/a	12 (6–22) × 277 (220–349)

Table 14. Some characters of *Acanthotrema tridactyla* found in this study and the reference sources (synonym: Stictodora tridactyla). (measurement in µm, calculated from 10 cercariae, n/a = no data)

	Stictodora tridactyla Krailas et al. (2014)	*Stictodora tridactyla* Veeravechsukij et al. (2018a)	*Acanthotrema tridactyla* This study
Body	112 (69–149) × 275 (255–309)	99 (80–118) × 202 (168– 207)	89 (58 – 103) x 178 (158 – 195)
Tail	45 (37–55) × 545 (486–595)	26 (20–33) × 458 (405–495)	24 (23 – 28) x 457 (400 – 575)
Oral sucker	38 (36–48) × 41 (33–52)	34 (28–38) × 41 (30–50)	29 (20 – 38) x 30 (25 – 38)
Ventral sucker	19 (10–25) × 19 (10–25)	23 (13–35) × 27 (15–45)	19 (10 – 28) x 20 (15 – 28)
Excretory bladder	98 (87–119) × 76 (52–98)	65 (43–90) × 39 (20–55)	50 (40 – 58) x 23 (18 – 28)
Stylet	Not found	Not found	Not found
Eye spot	8 (6–12) × 14 (12–17)	9 (5–15) × 9 (5–15)	n/a
Lateral finfold	13 (9–15) × 95 (88–100)	18 (10–25) × 108 (74–148)	11 (8– 15) x 154 (125 – 200)

Table 15. Some characters of *Loxogenoides bicolor* found in this study and the reference sources. (measurement in µm, calculated from 10 cercariae, n/a = no data).

	Loxogenoides bicolor Dechruksa et al. (2017)	*Loxogenoides bicolor* Veeravechsukij et al. (2018a)	*Loxogenoides bicolor* This study
Body	75 (54-82) × 106 (85-117)	72 (53–88) × 117 (105–138)	69 (63 - 78) × 91 (79 - 103)
Tail	26 (18-29) × 74 (32-77)	21 (10–28) × 44 (25–88)	18 (18 – 22) × 95 (64 – 115)
Oral sucker	26 (21-28) × 27 (20-30)	33 (23–40) × 29 (23–33)	19 (11 – 24) × 11 (10 – 16)
Ventral sucker	15 (10-17) × 15 (11-19)	18 (13–25) × 16 (8–20)	12 (8 – 17) × 11 (9 – 15)
Excretory bladder	10 (8-12) × 22 (9-26)	33 (18–55) × 20 (10–35)	25 (11- 35) × 14 (10 – 25)
Stylet	8 (5-9) × 16 (12-18)	6 (5–8) × 30 (20–40)	3 (2 – 5) × 15 (11 – 17)
Eye spot	Not found	Not found	Not found

Molecular Study of Emerging Cercariae

The emerging cercariae were studied by using the ITS2 gene sequences (Figure 47; Table 16). Four trematode species were categorized on the basis of their morphological and organ characters from 10 collected samples. The heterophyid trematodes were categorized, they were *Haplochis taichui*, *Acanthotrema tridactyla* and *Procerovum cheni*. The ITS2 gene sequences of *H. taichui*, *A. tridactyla* and *P. cheni* were approximately 310–330, 320 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). Specimens of *H. taichui* which all isolated were group together with relatively high supported. *A. tridactyla* was group together with *A. tridactyla*, that infected in *Tarebia granifer* from Phattalung, Nakhon Si Thammarat (Veeravechsukij et al., 2018a). The intermediate host of *P. cheni* was *S.* cf. *punctata* and *S.* cf. *torulosa*, they were grouped together with *P. cheni* and *P. varium* (Buathong et al., 2019). Unfortunately, the virgulate xiphidiocercariae cercariae of Lecithodendriidae *(L. bicolor*), it could not amplified. However, this trematode species was distinguished through morphological identification.

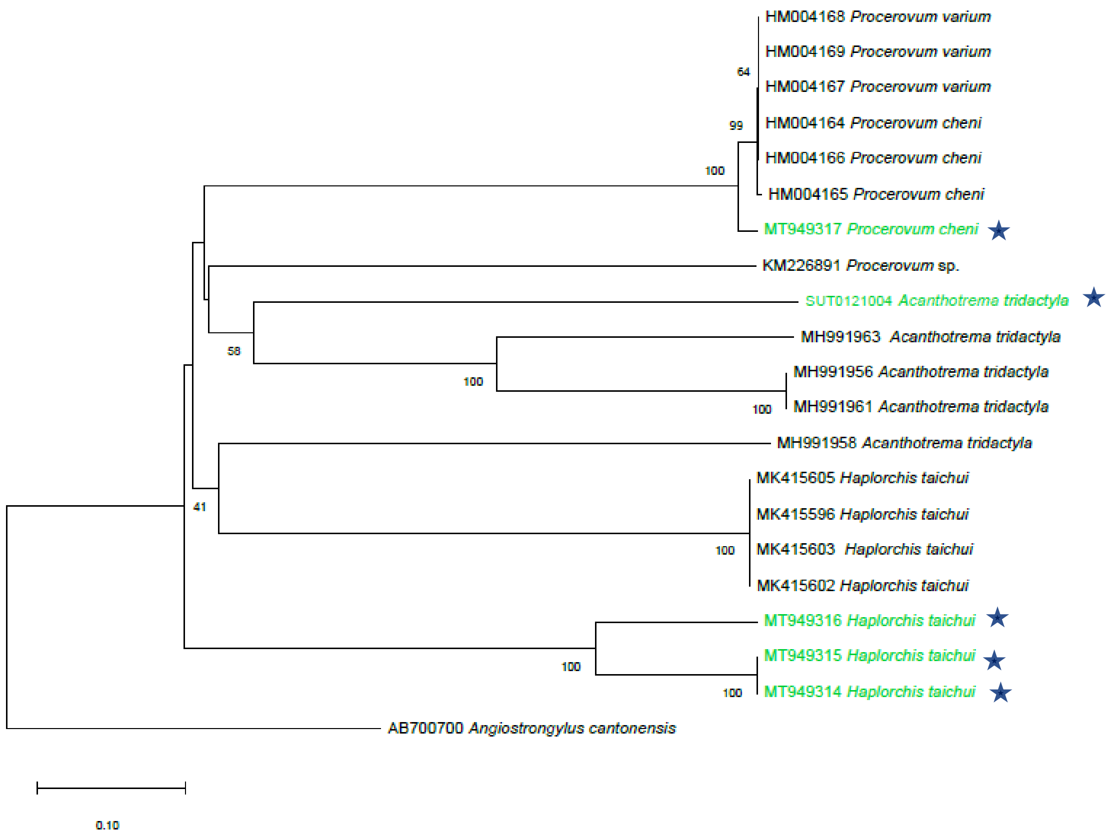

Figure 47. Phylogenetic relationship of trematodes was constructed using ITS2 sequences based on neighbor-joining analysis (3,000 bootstrap replications) and the other published DNA sequences obtained from GenBank. Nodes are annotated with bootstrap support value ≥ 50. Taxon names and voucher or GenBank accession numbers are provided at the tips of the tree (Blue star: this study) (see also Table 16)

Table 16. Sequences used for the phylogenetic analysis.For SUT numbers (Silpakorn University, Nakhon Pathom, Thailand), see the material lists in the main part of the text.

Species of trematode	Voucher code	GenBank accession number	stage of trematode	location	references
Haplorchis taichui	SUT172001E	MT949314	cercaria	Klong Tha Phae 1, Satun	this study
	SUT172002E	MT949315		Klong La-ngu 1, Satun	this study
	SUT172003E	MT949316		Klong Tha Phae 2, Satun	this study
	-	MK415605	metacercaria	Chachoengsao	Buathong et al. (2019)
	-	MK415596			
	-	MK415603			
	-	MK415602			
Acanthotrema tridactyla	SUT0121004	-	cercaria	Klong Chalung 1, Satun	this study

	-	MH991963		Mae Hong Son	Veeravechsukij et al. (2018b)
	-	MH991956		Nakhon Si Thammarat	
	-	MH991961		Phattalung	
	-	MH991958		Ratchaburi	
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			
Procerovum sp.	-	KM226891	cercaria	Tamil Nadu, India	Arya et al. (2016)

CHAPTER V
DISCUSSION

Sampling Sites and Environmental Data

Snail genus *Stenomelania* is distributed in the Asia-Pacific consist of India, South East Asia, Japan, Philippines and South Pacific Island (Bandel et al., 1997; Glaubrecht et al., 2009; Hidaka & Kano, 2014; Miura et al., 2008).Typically, thiarid snails are widespread in the canal or river far from the coastlines, but *Stenomelania* snail are distributed in the stream or canal near the coastlines. Bandel et al. (1997) reported *Stenomelani*a sp. was also observed in the river or creek near the mangrove system. In this study, the localities could be found *Stenomelania* with slightly salinity, range 0.1-0.6 ppt, pH 4.90-9.78, conductivity 0.123-1.12 ms/cm, DO 6.43-12.35 and TDS 0.083-0.717 mg/L. *Stenomelania* were tolerant with acidic water. According to previous study, the snails can live in either freshwater and tidal zones around mangrove and coastlines. As for the physical characteristics of the habitat, there were some variations depending on the area and topography, but the water value was not much different. It was found that the environment is generally suitable for the growth of mollusks and other food chain organisms. Normally, the water quality parameters include chemical, physical, and biological properties which can be tested or monitored based on the desired water parameters of concern. Parameters that are frequently sampled or monitored for water quality include temperature, dissolved oxygen, pH, conductivity, turbidity, and salinity. In this study, we concern the salinity for amphidromous animals like *Stenomelania* snail. It was reported *Stenomelania*'s habitat and their reproductive biology (Bandel et al., 1997; Haynes, 2001; Sasaki et al., 2009). However, there were the reports of the snail habitats e.g., the snails did not find in the waters with low conductivity (0.025 ms/cm) because the low of conductivity was significantly reduce the survival and growth (Herbst et al., 2008). The researcher commented that attributed to the ability of snails to tolerate a wide range of water hardness. The water quality standards that suit for organism growth in the environment, should generally have dissolved oxygen around 6.5-8.0 mg/L (Berrie, 1970; Horne & Goldman, 1994; Malek, 1958). In this study, the collected snails were dominantly found only in provinces near Andaman coastline consisting of Phang-nga, Krabi, Trang and Satun. All of the localities are stream to the Andaman Sea and these localities were not rivers. Moreover, we cannot find these snails in the localities that are far away from coastlines. We reported that *Stenomelania* were not widespread like other Thiarid snails such as *Tarebia granifera* and *Melanoides tuberculata*, they are widely spread in Thailand. *Tarebia, Melanoides* and *Stenomelania* can be found in both natural and created water bodies, however *Stenomelania* were normally found in natural water resource limit in the stream located near the tidal zone. Ng et al. (2016) reported that *Stenomelania* spp. were accidentally found in Singapore ornamental pet trade, it was patched on aquarium plants. From the information of the snail's habitat, *Stenomelania* should be found from the other provinces on Andaman coastline such as Phuket and Ranong provinces, besides of the Andaman Sea's distribution, there is also the possibility that the snail distribution may be found in the Gulf of Thailand to the South China Sea.

Distribution of *Stenomelania* in Thailand

In this study, a total of 3,026 collected snails from 24 localities from the coastal of Andaman Sea were identified into 6 species: *S.* cf. *aspirans*, *S.* cf. *crenulata*, *S.* cf. *punctata*, *S.* cf. *torulosa*, *S.* cf. *denisoniensis* and *Stenomelania* sp., the sixth species is a reported species named *Neoradina prasongi* of Wiggering et al. (2019). It is believed that the sixth species belongs to the genus *Stenomelania*. According to the reproductive strategy, *Stenomelania* spp. release the veliger larvae from the brood chamber of the female snails, while *Neoradina* release shelled juvenile (Brandt, 1974). Interestingly the distribution of the snail species exhibited a distinct pattern. *Stenomelania* cf. *torulosa* was found in Phang-nga Province, and *S.* cf. *denisoniensis* was found in Satun Province. For comparison, five taxa were observed in Krabi Province except *S.* cf. *denisoniensis.* 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. The veliger will move from one habitat to different habitat by ocean currents (Bandel et al., 1997; Glaubrecht, 1996; Glaubreht & Kőhler, 2004).

Shell Morphological Study

Ramakrishna and Dey (2007) categorized *Stenomelania* from India by using shell characters. The snails were categorized into four species; they were *S. torulosa*, *S. plicaria*, *S. punctata* and *S. aspirans*. The type species of the genus *Stenomelania* was descripted for shell morphology; using *S. aspirans* (Fisher, 1885). In this study, the shell morphology of *Stenomelania* sp., were conchological variability within the genus. Wiggering et al. (2019) reported, shell shapes of *Stenomelania* are significantly different from *N. prasongi* species but are indistinguishable from *M. tuberculata*. In this study, the snails were recognized to be the species of genus *Stenomelania*; they are six types of shell morphology. It is believed that at least six species of *Stenomelania* distributed in Thailand. Four provinces were the collected areas; they are Phang-nga, Krabi, Trang and Satun. Although the six species of snails can be remarked by interspecific variations in shell size, shell shape, shell coloration and shell sculpture, the mean value of shell parameters in the same species and each other localities did not different. However, *S.* cf. *denisoniensis*, was smaller than other species of the collected *Stenomelania* snail, *S.* cf. *punctata* was bigger than other species. Obviously, all of *Stenomelania* species were found to have variations of those shells. Shell of *S.* cf. *crenulata* and *S.* cf. *torulosa* were thicker than another species. Schilthuizen (2003) gave some mention that the land snails can be used interspecific variations in shell coloration, banding pattern and ornamentation for the purpose of taxonomy. In the sense of variation of shell morphology, many snails such as *Lymnaea* spp. were tried to convey the variation in shell characters by application of subspecific categories of different rank, most often ‘morph’ or ‘variety’. The ranks were used rather inconsistently and usually (but not always) were applied to subspecific groups distinguishable on the basis of diverse phenotypic traits (shell size, shell proportions, surface coloration, morphological abnormality and so on) (Vinarski, 2014). Moreover, for some land snails, shell diversification is often mentioned as related species usually differ in the settings for the major shell-developmental parameters. They can produce a variety of shell shapes that often may be correlated with differences in the environmental factors. Conchological diversification is always represented in terms of ornamentation. These include ribs, spines and lamellae on the shell surface, and flaps and flanges at the aperture. In some cases, such ornamentation can be shown to be a response to structural demands from the environment, including camouflage and defense against predators and parasites (Coomans, 1973; Goodfriend, 1984; Schilthuizen, 2003; Vermeij, 1993). Although, in our study we recognize the species of snails by morphological and genetic studies, we still do not exactly confirm the species of *Stenomelania* that are reported in Thailand. The research of species diversity and their distribution should have been more studied in the future.

Molecular Analysis of Snail Samples

Based on molecular genetics, the phylogenetic tree was including the same clade. In this study, only *Stenomelania* sp. (*Neoradina* aff. *prasongi*; SUT201806C and SUT201807C) from Klong La 1 in Trang province was only one species could be amplified. The specimens of *Stenomelania* sp. were group together with relatively high support. They were in the same clade with *N. prasongi* (MK697738; (Wiggering et al., 2019)), that was found in same Trang province. Noteworthy from phylogenetic tree, references sequence from *Melanoides tuberculata*, *Tarebia granifera* and another species of *Stenomelania* were group together but not *N. prasongi*. In addition, references sequence from *M. tuberculata* and *T. granifera,* their clade was separated with *Stenomelania,* although *Melanoides* was closed relationship. *Stenomelania* sp. (*N.* aff. *prasongi*; SUT201806C and SUT201807C) and *N. prasongi* (Wiggering et al., 2019) were included in the same major clade with *Stenomelania*, typically, the different genus was not group in the same clade. However, the snail samples still need to be distinguished through shell morphology, anatomy and reproductive biology.

Trematode Infections

Thiarid snails, that transmit parasites of native birds, fishes or mammals, have frequently been reported as first intermediate hosts and *Stenomelania* is Thiarid snail. In this study, *Stenomelania* from the south Thailand were examined to explore the occurrence of these snails and their infections with trematodes. We were categorized based on the morphological characteristics of the cercarial stages and combined with a molecular approach.

Previous studies in Thailand found that snails of Thiaridae family, such as *Malanoides tuberculata*, *M. jugicostis*, *Tarebia granifera*, *Mieniplotia scabra* and *Sermyla riqueti,* are intermediate hosts of trematodes, which are categorized as types and species by using the characteristics of cercariae as following;

1) paraplurolophocercous cercariae: *Haplochis taichui*, *H. pumilio* and

Stictodora tridactyla, 2) pleurolophocercous cercariae: *Centrocestus formosanus*, 3) virgulate xiphidiocercariae: *Loxogenoides bicolor, Loxogenes liberum* and *Acanthatrium histaensa,* 4) armatae xiphidiocercariae cercariae: *Maritreminoides caridinae* and *M. obstipus*, 5) furcocercous cercariae: *Haematoloechus similes, Transversotrema laruei,Cardicola alseae, Apatemon gracilis* and *Mesostephanus appendic alatus,* 6) megarulous cercariae: *Cloacitrema philippinum* and *Philophthalmus gralli,* 7) echinostome-type cercariae: *Echinochasmus pelecani,* 8) amphistome cercariae: *Gastrothylax crumenifer*, 9) renicolid cercariae: *Cercaria caribbea* LXVIII, 10) Cotylomicrocercous cercariae: *Podocotyle (Podocotyle) lepomis,* 11) gymnocephalous- type cercariae (Dechruksa et al., 2017; Krailas et al., 2014; Veeravechsukij et al., 2018a).

In this study, four trematode species infecting snails at eight localities were reported in three provinces; Krabi, Trang, and Satun. The four species from two trematode families were identified on the basis of the morphological characteristics of the emerging cercariae. The two families were Heterophyidae and Lecithodendriidae. Parapleurolophocercous cercariae was reported to be commonly found also in other freshwater snails in Thailand, such as *M. tuberculata* and *T. granifera* (Krailas et al., 2014; Veeravechsukij et al., 2018a). Three species of parapleurolophocercous cercariae were found in *Stenomelania*. Interestingly, *Acanthotrema tridactyla* was found only in Satun province. The heterophyid trematode causes one of the fish-borne zoonoses which infect vertebrate animals, including humans and birds. Humans are infected by 13 genera consist of *Acanthotrema, Haplorchis, Heterophyopsis, Heterophyes, Procerovum* and etc. (Chai & Jung, 2017; Pearson, 1964; Yamaguti, 1971), there are infected by eating raw fishes of the brackish or fresh water fish such as the shad, perch, mullet and goby (Chai & Lee, 2002).

H. taichui is a small intestinal fluke, 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 & 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 province, Thailand (Kliks & 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* and etc. They are the most frequently-reported species in southeast Asia. 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 (Dechruksa et al., 2007; Krailas et al., 2008; Krailas et al., 2014; Krailas et al., 2011; Kumchoo et al., 2003; Sri-aroon et al., 2005; Sritongtae et al., 2015; Ukong et al., 2007; Upatham et al., 1980; Upatham et al., 1981; Veeravechsukij et al., 2018a; Wongsawad et al., 2009). In the present study, *H. taichui* infections were detected in *S.* cf. *torulosa*, *Stenomelania* sp. and *S.* cf. *denisoniensis*. For the first time, *H. taichui* infections were observed in *Stenomelania* in Thailand (Apiraksena et al., 2020).

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 & Garcia, 1935; Price, 1940), *P. varium* (Onji & Nishio, 1916) and *P. cheni* (Hsü, 1950a). *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 metacercaria from mullet fish in Japan (Onji & Nishio, 1916; Price, 1940). *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 metacercaria in fishes and the development of adults from experimental hosts have been used to categories trematodes under *Procerovum* (Hsu, 1951; Hsü, 1950a; Hsü, 1950b; Umadevi & Madhavi, 2000). In this study, 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 Krabi Province. 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. denisoniensis*, which were similar to those found in the present study (Surin, 1993; Umadevi & Madhavi, 2000; Velasquez, 1973). 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 this study, two snails (one: *S.* cf. *punctata* and one: *S.* cf. *torulosa*) were infected with *Procerovum* (see Table 11). 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.

Loxogenoides bicolor is belong to Lecithodendriidae (Stafford, 1905). This parasite was found in the terminal portion of the bile duct of frogs. It is regarded as 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. 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., 2013; Dechruksa et al., 2007; Krailas et al., 2014; Krailas et al., 2011; Pratumsrikajorn et al., 2017; Ukong et al., 2007; Veeravechsukij et al., 2018a). 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. trudactyla* and *Cadicola alseae* in tripled infection. In this study, morphological studies on cercariae were conducted to confirm the specific identity and prevalence of various infectious trematodes in the collected *S.* cf. *torulosa* and *Stenomelania* sp. in Trang Province.

Acanthotrema tridactyla or *Stictodora tridactyla* (Martin & Kuntz, 1955) is a small intestinal fluke of the paraplurolophocercous cercariae type. This trematode was first reported in Egypt, *Pirenella conica* snail was living in brackish or saline water since the collecting site is near the connection of the lake with the Mediterranean Sea. The adult worms obtained by feeding naturally and experiment fishes to chicks proved to be different from any heterophyids describe hence they are assigned to a new species provisionally placed. *A. tridactyla* most closely resembles as *A. acanthotrema* and *A. felis*, but different from them in the shape of the sclerites. Three species also different in terms of the presence and position of minute spines on the sclerites, which are the tips of the 3 sclerites in *A. tridactyla*, are near the basal portions of the 3 sclerites in *A. felis*, but near the tips of only the 2 sclerites *in A. acanthotrema*. The first intermediated host was found in Thiarid snail and etc. In Thailand, *A. tridactyla* could be infected in *Melanoides tuberculata, Sermyla riqueti, Tarebia granifera, Thiara scabra, M. jugicostis, Adamietta housei* and *Neoradina prasong*i (Rattanathai, 2010; Ukong et al., 2007). The second intermediate hosts are fish such as *Aphanius fasciatus,* were found metacercaria (Yamaguti, 1975). It is widely range of definitive hosts has been reported for *Acanthotrema* species as Kittens and another mammal (Abdul-Salam et al., 2000; Velasquez, 1973). In this study, we found infected in *S.* cf. *denisoniensis*.

Cercarial Size Comparison

In this study, we focused on parapleurolophocercous cercariae, we were compared size of body, tail and organs of *Haplorchis taichui*, *Procerovum cheni* and *Acanthotrema tridactyla*, they were significantly different. In additional, we compared with previous report by each species, the cercaria size may vary different. All of cercariae in this study were smaller than previous report (Dechruksa et al., 2017; Krailas et al., 2014; Veeravechsukij et al., 2018a). Assumed, the intermediated host and habitat may affect to the size of cercaria such as *H. taichui* infected in *M. tuberculata* is bigger than infected in *S.* cf. *torulosa*. However, to clarify cercaria species distinguish, the size of cercaria was selected with genetic data and morphology.

Molecular Analysis of Cercariae

Molecular analysis was conducted to confirm the results of cercarial identification. The aims of this study were combined classical morphological identification with molecular genetics identification, resulting in the conformation of cercarial infections by two distinct trematode families. As a noteworthy result, the nucleotide sequences of *Haplorchis taichui, Procerovum cheni* and *Acanthotrema tridactyla* were found to be closely related. For phylogenetic analysis, GenBank data, based on different parasite stages, such as metacercarial or adult stage (Arya et al., 2016; Buathong et al., 2019; Thaenkham et al., 2010; Veeravechsukij et al., 2018a), were used. The similar phylogenetic pattern was observed and the relationships within the molecular clades of *H. taichui* could be resolved clearly. All the *H. taichui* samples originated not only from the locations in Satun Province and collected from the different snail species as *S.* cf. *torulosa* and *S.* cf. *denisoniensis*. *A. tridactyla* samples originated only from Satun Province and the molecular clades in phylogenetic pattern could be resolved clearly when compared with GenBank data (Veeravechsukij et al., 2018a). In a previous molecular genetic study, Van et al. (2009) reported the *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 unclearly separated from *P. varium* through molecular genetics. Furthermore, the sequences of *H. taichui*, *P. cheni* and *A. tridactyla* obtained from *Stenomelania*, did not group together, although they were both of parapleurolophocercous cercaria type.

CHAPTER VI
CONCLUSION

These studies found variation morphology of *Stenomelania* spp. in Southern Thailand. The snails were identified by shell morphology into 6 species consist of *Stenomelania* cf. aspirans, *S.* cf. crenulata, *Stenomelania* sp., *S.* cf. *punctata*, *S.* cf. *torulosa* and *S.*cf. *denisoniensis*. According to the reproductive strategy, Stenomelania spp. release the veliger larvae from the brood chamber of the female snails except *S.*cf. *denisoniensis*. We recognized the species of snails by morphological and genetic studies, but we still do not exactly confirm the species of *Stenomelania*. For the epidemiology of cercarial stage in *Stenomelania* spp. were found to have an infection rate to be 0.63%, which infected four species of cercariae from two types, viz. (i) virgulate xiphidiocercariae (*Loxogenoides* (ii) parapleurolophocercous cercariae (*Haplorchis taichui*, *Procerovum cheni* and *Acanthotrema tridactyla*). The parapleurolophocercous cercariae were the dominant cercarial type infecting these snails. This study represents both morphological characterization and genetic identification of cercariae. This could be recognized as the basis reference of the larval trematode fauna in Thailand, and could predict their potential to evolve for intermediate hosts.

APPENDICES

Appendix A: Reagents for DNA extraction

- 1 M Tris-HCl

Tris base	121.1 g
HCl	42.0 ml

Add distilled water to a final volume of 1000 ml and autoclave before used.

- Proteinase solution (K 20 mg/ml)

Proteinase	0.2 mg
Distilled water	10 µl

Aliquots before used and stored at -20 ºC

TE buffer

10 mM Tris-HCl (pH 8.0)	1.0 ml of 1 M
1 mM EDTA (pH 8.0)	200 µl of 0.5 M

Add distilled water to a final volume of 100 ml and autoclave before used.

Appendix B: Reagents for DNA electrophoresis and staining solution

- 50X TBE buffer solution
- 1X TBE buffer

50X TBE buffer	100 ml
Distilled water	900 ml

- 1 % Agarose gel

Agarose gel	0.8 g
1X TBE buffer	100 ml

- 6X loading buffer (novel juice)

Appendix C: Shell statistic data

		Sum of Squares	df	Mean Square	F	Sig.
hight	Between Groups	2284.474	5	456.895	29.140	.000
	Within Groups	846.670	54	15.679		
	Total	3131.144	59			
width	Between Groups	143.482	5	28.696	19.180	.000
	Within Groups	80.793	54	1.496		
	Total	224.274	59			
opeL	Between Groups	477.667	5	95.533	35.985	.000
	Within Groups	143.361	54	2.655		
	Total	621.028	59			
opeW	Between Groups	103.059	5	20.612	11.834	.000
	Within Groups	94.051	54	1.742		
	Total	197.109	59			
threewhor	Between Groups	1953.060	5	390.612	30.851	.000
	Within Groups	683.707	54	12.661		
	Total	2636.766	59			
whol	Between Groups	1433.559	5	286.712	44.369	.000
	Within Groups	348.950	54	6.462		
	Total	1782.510	59			
angle	Between Groups	680.933	5	136.187	64.284	.000
	Within Groups	114.400	54	2.119		

Multiple Comparisons

LSD

Dependent Variable	(I) 1=As, 2=Cr, 3=Ne, 4=Pu, 5=To, 6=De	(J) 1=As, 2=Cr, 3=Ne, 4=Pu, 5=To, 6=De	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
hight	1.00	2.00	1.98500	1.77082	.267	-1.5653	5.5353
		3.00	4.37600*	1.77082	.017	.8257	7.9263
		4.00	-4.70000*	1.77082	.010	-8.2503	-1.1497
		5.00	3.90600*	1.77082	.032	.3557	7.4563
		6.00	15.57200*	1.77082	.000	12.0217	19.1223
	2.00	1.00	-1.98500	1.77082	.267	-5.5353	1.5653
		3.00	2.39100	1.77082	.183	-1.1593	5.9413
		4.00	-6.68500*	1.77082	.000	-10.2353	-3.1347
		5.00	1.92100	1.77082	.283	-1.6293	5.4713
		6.00	13.58700*	1.77082	.000	10.0367	17.1373
	3.00	1.00	-4.37600*	1.77082	.017	-7.9263	-.8257
		2.00	-2.39100	1.77082	.183	-5.9413	1.1593
		4.00	-9.07600*	1.77082	.000	-12.6263	-5.5257
		5.00	-.47000	1.77082	.792	-4.0203	3.0803
		6.00	11.19600*	1.77082	.000	7.6457	14.7463
	4.00	1.00	4.70000*	1.77082	.010	1.1497	8.2503

		2.00	6.68500*	1.77082	.000	3.1347	10.2353
		3.00	9.07600*	1.77082	.000	5.5257	12.6263
		5.00	8.60600*	1.77082	.000	5.0557	12.1563
		6.00	20.27200*	1.77082	.000	16.7217	23.8223
	5.00	1.00	-3.90600*	1.77082	.032	-7.4563	-.3557
		2.00	-1.92100	1.77082	.283	-5.4713	1.6293
		3.00	.47000	1.77082	.792	-3.0803	4.0203
		4.00	-8.60600*	1.77082	.000	-12.1563	-5.0557
		6.00	11.66600*	1.77082	.000	8.1157	15.2163
	6.00	1.00	-15.57200*	1.77082	.000	-19.1223	-12.0217
		2.00	-13.58700*	1.77082	.000	-17.1373	-10.0367
		3.00	-11.19600*	1.77082	.000	-14.7463	-7.6457
		4.00	-20.27200*	1.77082	.000	-23.8223	-16.7217
		5.00	-11.66600*	1.77082	.000	-15.2163	-8.1157
width	1.00	2.00	.40400	.54702	.463	-.6927	1.5007
		3.00	-1.46300*	.54702	.010	-2.5597	-.3663
		4.00	-2.73500*	.54702	.000	-3.8317	-1.6383
		5.00	.16300	.54702	.767	-.9337	1.2597
		6.00	2.20800*	.54702	.000	1.1113	3.3047
	2.00	1.00	-.40400	.54702	.463	-1.5007	.6927
		3.00	-1.86700*	.54702	.001	-2.9637	-.7703
		4.00	-3.13900*	.54702	.000	-4.2357	-2.0423

		5.00	-.24100	.54702	.661	-1.3377	.8557
		6.00	1.80400*	.54702	.002	.7073	2.9007
	3.00	1.00	1.46300*	.54702	.010	.3663	2.5597
		2.00	1.86700*	.54702	.001	.7703	2.9637
		4.00	-1.27200*	.54702	.024	-2.3687	-.1753
		5.00	1.62600*	.54702	.004	.5293	2.7227
		6.00	3.67100*	.54702	.000	2.5743	4.7677
	4.00	1.00	2.73500*	.54702	.000	1.6383	3.8317
		2.00	3.13900*	.54702	.000	2.0423	4.2357
		3.00	1.27200*	.54702	.024	.1753	2.3687
		5.00	2.89800*	.54702	.000	1.8013	3.9947
		6.00	4.94300*	.54702	.000	3.8463	6.0397
	5.00	1.00	-.16300	.54702	.767	-1.2597	.9337
		2.00	.24100	.54702	.661	-.8557	1.3377
		3.00	-1.62600*	.54702	.004	-2.7227	-.5293
		4.00	-2.89800*	.54702	.000	-3.9947	-1.8013
		6.00	2.04500*	.54702	.000	.9483	3.1417
	6.00	1.00	-2.20800*	.54702	.000	-3.3047	-1.1113
		2.00	-1.80400*	.54702	.002	-2.9007	-.7073
		3.00	-3.67100*	.54702	.000	-4.7677	-2.5743
		4.00	-4.94300*	.54702	.000	-6.0397	-3.8463
		5.00	-2.04500*	.54702	.000	-3.1417	-.9483
opeL	1.00	2.00	.14400	.72867	.844	-1.3169	1.6049

	3.00	-.47200	.72867	.520	-1.9329	.9889
	4.00	-4.81400*	.72867	.000	-6.2749	-3.3531
	5.00	3.23000*	.72867	.000	1.7691	4.6909
	6.00	3.81000*	.72867	.000	2.3491	5.2709
2.00	1.00	-.14400	.72867	.844	-1.6049	1.3169
	3.00	-.61600	.72867	.402	-2.0769	.8449
	4.00	-4.95800*	.72867	.000	-6.4189	-3.4971
	5.00	3.08600*	.72867	.000	1.6251	4.5469
	6.00	3.66600*	.72867	.000	2.2051	5.1269
3.00	1.00	.47200	.72867	.520	-.9889	1.9329
	2.00	.61600	.72867	.402	-.8449	2.0769
	4.00	-4.34200*	.72867	.000	-5.8029	-2.8811
	5.00	3.70200*	.72867	.000	2.2411	5.1629
	6.00	4.28200*	.72867	.000	2.8211	5.7429
4.00	1.00	4.81400*	.72867	.000	3.3531	6.2749
	2.00	4.95800*	.72867	.000	3.4971	6.4189
	3.00	4.34200*	.72867	.000	2.8811	5.8029
	5.00	8.04400*	.72867	.000	6.5831	9.5049
	6.00	8.62400*	.72867	.000	7.1631	10.0849
5.00	1.00	-3.23000*	.72867	.000	-4.6909	-1.7691
	2.00	-3.08600*	.72867	.000	-4.5469	-1.6251
	3.00	-3.70200*	.72867	.000	-5.1629	-2.2411
	4.00	-8.04400*	.72867	.000	-9.5049	-6.5831

		6.00	.58000	.72867	.430	-.8809	2.0409
	6.00	1.00	-3.81000*	.72867	.000	-5.2709	-2.3491
		2.00	-3.66600*	.72867	.000	-5.1269	-2.2051
		3.00	-4.28200*	.72867	.000	-5.7429	-2.8211
		4.00	-8.62400*	.72867	.000	-10.0849	-7.1631
		5.00	-.58000	.72867	.430	-2.0409	.8809
opeW	1.00	2.00	2.00300*	.59020	.001	.8197	3.1863
		3.00	1.09400	.59020	.069	-.0893	2.2773
		4.00	-.96700	.59020	.107	-2.1503	.2163
		5.00	2.56200*	.59020	.000	1.3787	3.7453
		6.00	2.48800*	.59020	.000	1.3047	3.6713
	2.00	1.00	-2.00300*	.59020	.001	-3.1863	-.8197
		3.00	-.90900	.59020	.129	-2.0923	.2743
		4.00	-2.97000*	.59020	.000	-4.1533	-1.7867
		5.00	.55900	.59020	.348	-.6243	1.7423
		6.00	.48500	.59020	.415	-.6983	1.6683
	3.00	1.00	-1.09400	.59020	.069	-2.2773	.0893
		2.00	.90900	.59020	.129	-.2743	2.0923
		4.00	-2.06100*	.59020	.001	-3.2443	-.8777
		5.00	1.46800*	.59020	.016	.2847	2.6513
		6.00	1.39400*	.59020	.022	.2107	2.5773
	4.00	1.00	.96700	.59020	.107	-.2163	2.1503
		2.00	2.97000*	.59020	.000	1.7867	4.1533

		3.00	2.06100*	.59020	.001	.8777	3.2443
		5.00	3.52900*	.59020	.000	2.3457	4.7123
		6.00	3.45500*	.59020	.000	2.2717	4.6383
	5.00	1.00	-2.56200*	.59020	.000	-3.7453	-1.3787
		2.00	-.55900	.59020	.348	-1.7423	.6243
		3.00	-1.46800*	.59020	.016	-2.6513	-.2847
		4.00	-3.52900*	.59020	.000	-4.7123	-2.3457
		6.00	-.07400	.59020	.901	-1.2573	1.1093
	6.00	1.00	-2.48800*	.59020	.000	-3.6713	-1.3047
		2.00	-.48500	.59020	.415	-1.6683	.6983
		3.00	-1.39400*	.59020	.022	-2.5773	-.2107
		4.00	-3.45500*	.59020	.000	-4.6383	-2.2717
		5.00	.07400	.59020	.901	-1.1093	1.2573
threewhor	1.00	2.00	-.37700	1.59130	.814	-3.5674	2.8134
		3.00	-.97600	1.59130	.542	-4.1664	2.2144
		4.00	-8.71400*	1.59130	.000	-11.9044	-5.5236
		5.00	4.15500*	1.59130	.012	.9646	7.3454
		6.00	10.20200*	1.59130	.000	7.0116	13.3924
	2.00	1.00	.37700	1.59130	.814	-2.8134	3.5674
		3.00	-.59900	1.59130	.708	-3.7894	2.5914
		4.00	-8.33700*	1.59130	.000	-11.5274	-5.1466
		5.00	4.53200*	1.59130	.006	1.3416	7.7224
		6.00	10.57900*	1.59130	.000	7.3886	13.7694

	3.00	1.00	.97600	1.59130	.542	-2.2144	4.1664
		2.00	.59900	1.59130	.708	-2.5914	3.7894
		4.00	-7.73800*	1.59130	.000	-10.9284	-4.5476
		5.00	5.13100*	1.59130	.002	1.9406	8.3214
		6.00	11.17800*	1.59130	.000	7.9876	14.3684
	4.00	1.00	8.71400*	1.59130	.000	5.5236	11.9044
		2.00	8.33700*	1.59130	.000	5.1466	11.5274
		3.00	7.73800*	1.59130	.000	4.5476	10.9284
		5.00	12.86900*	1.59130	.000	9.6786	16.0594
		6.00	18.91600*	1.59130	.000	15.7256	22.1064
	5.00	1.00	-4.15500*	1.59130	.012	-7.3454	-.9646
		2.00	-4.53200*	1.59130	.006	-7.7224	-1.3416
		3.00	-5.13100*	1.59130	.002	-8.3214	-1.9406
		4.00	-12.86900*	1.59130	.000	-16.0594	-9.6786
		6.00	6.04700*	1.59130	.000	2.8566	9.2374
	6.00	1.00	-10.20200*	1.59130	.000	-13.3924	-7.0116
		2.00	-10.57900*	1.59130	.000	-13.7694	-7.3886
		3.00	-11.17800*	1.59130	.000	-14.3684	-7.9876
		4.00	-18.91600*	1.59130	.000	-22.1064	-15.7256
		5.00	-6.04700*	1.59130	.000	-9.2374	-2.8566
whol	1.00	2.00	-1.31300	1.13684	.253	-3.5922	.9662
		3.00	-2.34300*	1.13684	.044	-4.6222	-.0638
		4.00	-7.95700*	1.13684	.000	-10.2362	-5.6778

	5.00	3.68900*	1.13684	.002	1.4098	5.9682
	6.00	7.69600*	1.13684	.000	5.4168	9.9752
2.00	1.00	1.31300	1.13684	.253	-.9662	3.5922
	3.00	-1.03000	1.13684	.369	-3.3092	1.2492
	4.00	-6.64400*	1.13684	.000	-8.9232	-4.3648
	5.00	5.00200*	1.13684	.000	2.7228	7.2812
	6.00	9.00900*	1.13684	.000	6.7298	11.2882
3.00	1.00	2.34300*	1.13684	.044	.0638	4.6222
	2.00	1.03000	1.13684	.369	-1.2492	3.3092
	4.00	-5.61400*	1.13684	.000	-7.8932	-3.3348
	5.00	6.03200*	1.13684	.000	3.7528	8.3112
	6.00	10.03900*	1.13684	.000	7.7598	12.3182
4.00	1.00	7.95700*	1.13684	.000	5.6778	10.2362
	2.00	6.64400*	1.13684	.000	4.3648	8.9232
	3.00	5.61400*	1.13684	.000	3.3348	7.8932
	5.00	11.64600*	1.13684	.000	9.3668	13.9252
	6.00	15.65300*	1.13684	.000	13.3738	17.9322
5.00	1.00	-3.68900*	1.13684	.002	-5.9682	-1.4098
	2.00	-5.00200*	1.13684	.000	-7.2812	-2.7228
	3.00	-6.03200*	1.13684	.000	-8.3112	-3.7528
	4.00	-11.64600*	1.13684	.000	-13.9252	-9.3668
	6.00	4.00700*	1.13684	.001	1.7278	6.2862
6.00	1.00	-7.69600*	1.13684	.000	-9.9752	-5.4168

		2.00	-9.00900*	1.13684	.000	-11.2882	-6.7298
		3.00	-10.03900*	1.13684	.000	-12.3182	-7.7598
		4.00	-15.65300*	1.13684	.000	-17.9322	-13.3738
		5.00	-4.00700*	1.13684	.001	-6.2862	-1.7278
angle	1.00	2.00	-4.50000*	.65093	.000	-5.8050	-3.1950
		3.00	-8.60000*	.65093	.000	-9.9050	-7.2950
		4.00	-9.30000*	.65093	.000	-10.6050	-7.9950
		5.00	-1.60000*	.65093	.017	-2.9050	-.2950
		6.00	-4.60000*	.65093	.000	-5.9050	-3.2950
	2.00	1.00	4.50000*	.65093	.000	3.1950	5.8050
		3.00	-4.10000*	.65093	.000	-5.4050	-2.7950
		4.00	-4.80000*	.65093	.000	-6.1050	-3.4950
		5.00	2.90000*	.65093	.000	1.5950	4.2050
		6.00	-.10000	.65093	.878	-1.4050	1.2050
	3.00	1.00	8.60000*	.65093	.000	7.2950	9.9050
		2.00	4.10000*	.65093	.000	2.7950	5.4050
		4.00	-.70000	.65093	.287	-2.0050	.6050
		5.00	7.00000*	.65093	.000	5.6950	8.3050
		6.00	4.00000*	.65093	.000	2.6950	5.3050
	4.00	1.00	9.30000*	.65093	.000	7.9950	10.6050
		2.00	4.80000*	.65093	.000	3.4950	6.1050
		3.00	.70000	.65093	.287	-.6050	2.0050
		5.00	7.70000*	.65093	.000	6.3950	9.0050

	6.00	4.70000*	.65093	.000	3.3950	6.0050
5.00	1.00	1.60000*	.65093	.017	.2950	2.9050
	2.00	-2.90000*	.65093	.000	-4.2050	-1.5950
	3.00	-7.00000*	.65093	.000	-8.3050	-5.6950
	4.00	-7.70000*	.65093	.000	-9.0050	-6.3950
	6.00	-3.00000*	.65093	.000	-4.3050	-1.6950
6.00	1.00	4.60000*	.65093	.000	3.2950	5.9050

Appendix D: Cercaria statistic data

		Sum of Squares	df	Mean Square	F	Sig.
Body W	Between Groups	1198.532	2	599.266	5.642	.009
	Within Groups	2867.940	27	106.220		
	Total	4066.472	29			
Body L	Between Groups	7763.154	2	3881.577	15.356	.000
	Within Groups	6824.965	27	252.776		
	Total	14588.119	29			
Tail W	Between Groups	20.418	2	10.209	1.176	.324
	Within Groups	234.310	27	8.678		
	Total	254.728	29			
Tail L	Between Groups	50395.082	2	25197.541	11.572	.000
	Within Groups	58789.053	27	2177.372		
	Total	109184.135	29			
Oral W	Between Groups	193.637	2	96.819	7.086	.003
	Within Groups	368.929	27	13.664		
	Total	562.566	29			
Oral L	Between Groups	5.370	2	2.685	.115	.892
	Within Groups	629.662	27	23.321		
	Total	635.032	29			
Lateral fin W	Between Groups	353.803	2	176.902	21.979	.000
	Within Groups	217.315	27	8.049		
	Total	571.118	29			
Lateral fin L	Between Groups	18914.596	2	9457.298	10.634	.000
	Within Groups	24012.083	27	889.336		
	Total	42926.679	29			
Excretory W	Between Groups	3032.302	2	1516.151	61.632	.000
	Within Groups	664.205	27	24.600		
	Total	3696.507	29			
Excretory L	Between Groups	72.260	2	36.130	2.592	.093
	Within Groups	376.341	27	13.939		
	Total	448.601	29			

Dependent Variable	(I) 1=Haplorchis, 2=Procerovum, 3= Acanthotrema	(J) 1=Haplorchis, 2=Procerovum, 3= Acanthotrema	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Body W	1.00	2.00	11.51800*	4.60912	.019	2.0609	20.9751
		3.00	-3.20100	4.60912	.493	-12.6581	6.2561
	2.00	1.00	-11.51800*	4.60912	.019	-20.9751	-2.0609
		3.00	-14.71900*	4.60912	.004	-24.1761	-5.2619
	3.00	1.00	3.20100	4.60912	.493	-6.2561	12.6581
		2.00	14.71900*	4.60912	.004	5.2619	24.1761
Body L	1.00	2.00	2.85000	7.11022	.692	-11.7390	17.4390
		3.00	-32.61000*	7.11022	.000	-47.1990	-18.0210
	2.00	1.00	-2.85000	7.11022	.692	-17.4390	11.7390
		3.00	-35.46000*	7.11022	.000	-50.0490	-20.8710
	3.00	1.00	32.61000*	7.11022	.000	18.0210	47.1990
		2.00	35.46000*	7.11022	.000	20.8710	50.0490
Tail W	1.00	2.00	-1.65600	1.31743	.220	-4.3591	1.0471
		3.00	-1.83100	1.31743	.176	-4.5341	.8721
	2.00	1.00	1.65600	1.31743	.220	-1.0471	4.3591
		3.00	-.17500	1.31743	.895	-2.8781	2.5281
	3.00	1.00	1.83100	1.31743	.176	-.8721	4.5341
		2.00	.17500	1.31743	.895	-2.5281	2.8781
Tail L	1.00	2.00	39.41000	20.86802	.070	-3.4077	82.2277
		3.00	-60.26000*	20.86802	.008	-103.0777	-17.4423
	2.00	1.00	-39.41000	20.86802	.070	-82.2277	3.4077

		3.00	-99.67000*	20.86802	.000	-142.4877	-56.8523
	3.00	1.00	60.26000*	20.86802	.008	17.4423	103.0777
		2.00	99.67000*	20.86802	.000	56.8523	142.4877
Oral W	1.00	2.00	-.66000	1.65312	.693	-4.0519	2.7319
		3.00	-5.68900*	1.65312	.002	-9.0809	-2.2971
	2.00	1.00	.66000	1.65312	.693	-2.7319	4.0519
		3.00	-5.02900*	1.65312	.005	-8.4209	-1.6371
	3.00	1.00	5.68900*	1.65312	.002	2.2971	9.0809
		2.00	5.02900*	1.65312	.005	1.6371	8.4209
Oral L	1.00	2.00	.99800	2.15967	.648	-3.4333	5.4293
		3.00	.25700	2.15967	.906	-4.1743	4.6883
	2.00	1.00	-.99800	2.15967	.648	-5.4293	3.4333
		3.00	-.74100	2.15967	.734	-5.1723	3.6903
	3.00	1.00	-.25700	2.15967	.906	-4.6883	4.1743
		2.00	.74100	2.15967	.734	-3.6903	5.1723
lateral fin W	1.00	2.00	7.47400*	1.26876	.000	4.8707	10.0773
		3.00	7.07990*	1.26876	.000	4.4766	9.6832
	2.00	1.00	-7.47400*	1.26876	.000	-10.0773	-4.8707
		3.00	-.39410	1.26876	.758	-2.9974	2.2092
	3.00	1.00	-7.07990*	1.26876	.000	-9.6832	-4.4766
		2.00	.39410	1.26876	.758	-2.2092	2.9974
Lateral fin L	1.00	2.00	55.17600*	13.33669	.000	27.8114	82.5406
		3.00	4.05300	13.33669	.764	-23.3116	31.4176
	2.00	1.00	-55.17600*	13.33669	.000	-82.5406	-27.8114
		3.00	-51.12300*	13.33669	.001	-78.4876	-23.7584
	3.00	1.00	-4.05300	13.33669	.764	-31.4176	23.3116
		2.00	51.12300*	13.33669	.001	23.7584	78.4876
Excretory	1.00	2.00	2.54400	2.21812	.261	-2.0072	7.0952

W		3.00	-19.94100*	2.21812	.000	-24.4922	-15.3898
	2.00	1.00	-2.54400	2.21812	.261	-7.0952	2.0072
		3.00	-22.48500*	2.21812	.000	-27.0362	-17.9338
	3.00	1.00	19.94100*	2.21812	.000	15.3898	24.4922
		2.00	22.48500*	2.21812	.000	17.9338	27.0362
Excretory L	1.00	2.00	-2.99200	1.66964	.084	-6.4178	.4338
		3.00	.53500	1.66964	.751	-2.8908	3.9608
	2.00	1.00	2.99200	1.66964	.084	-.4338	6.4178
		3.00	3.52700*	1.66964	.044	.1012	6.9528
	3.00	1.00	-.53500	1.66964	.751	-3.9608	2.8908
		2.00	-3.52700*	1.66964	.044	-6.9528	-.1012

*. The mean difference is significant at the 0.05 level.

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