CHAPTER 4

RESULTS

Taxonomic summary of parasite specimens

Haplorchis taichui Witenberg, 1930

Phylum Platyhelminthes

Class Trematoda

Order Digenea

Family Heterophyidae

Genus *Haplorchis*

Species *Haplorchis taichui*

Description:

Body piriform, or pear shaped 230 - 372 (288) μm long, maximum width 140 – 207 (173) μm. Body covered with the scale-like spine. Pre-pharynx very short 5.13 – 20.50 (10) μm. These possess two suckers (the anteriorly located oral sucker and mid-ventrally located the ventral sucker)**.** The bifurcated caeca thick-walled, usually inflated and extend to between mid-level of testis and just beyond posterior border of testis, most commonly to level of posterior border of testis. Ventrogenital sac median 17-31 (23), post-bifurcal comprising, the large ventral chamber, and small dorsal pocket, and processed 13-16 spines arraigned to fan-shaped. Testis globular and quiet large and located on posterior end of body. Ovary spherical and uterus apparently with three loop, located posterior the ovary (Figure 4-1).

Haplorchis pumilio Looss, 1896

Phylum Platyhelminthes

Class Trematoda

Order Digenea

Family Heterophyidae

Genus *Haplorchis*

Species *Haplorchis pumilio*

Description:

Body piriform 152 -181 (173) μm long, maximum width 49 – 61 (52) μm. The body is consist pigment granules in parenchyma and prominent gland cells on either side of pharynx. Pre-pharynx very short 6.4 – 9.1(7) μm. Tegument covered with scale-like spines arranged in regular rows, densely distributed in the fore-body becoming sparse posterior. Oral sucker subterminal. Ventral sucker small, lying in ventrogenital sac 34.2 – 44 (39). Ventral chamber filled by ventral sucker, apex with sclerlize crown of 30–34 skeletal bars, interrupted dorsally between latero-dorsal lobes. Testis single situated at posterior end of body on dorsal side. Cirrus sac absent, pre-testicular, slightly to right of midline. Mehlis' gland small, compact, situated lateral to ovary. Seminal receptacle large. Uterus provided with three loops, fills entire hind body overlapping testis. Mid dorsal lobe tipped with a group of five spines (Figure 4-2).

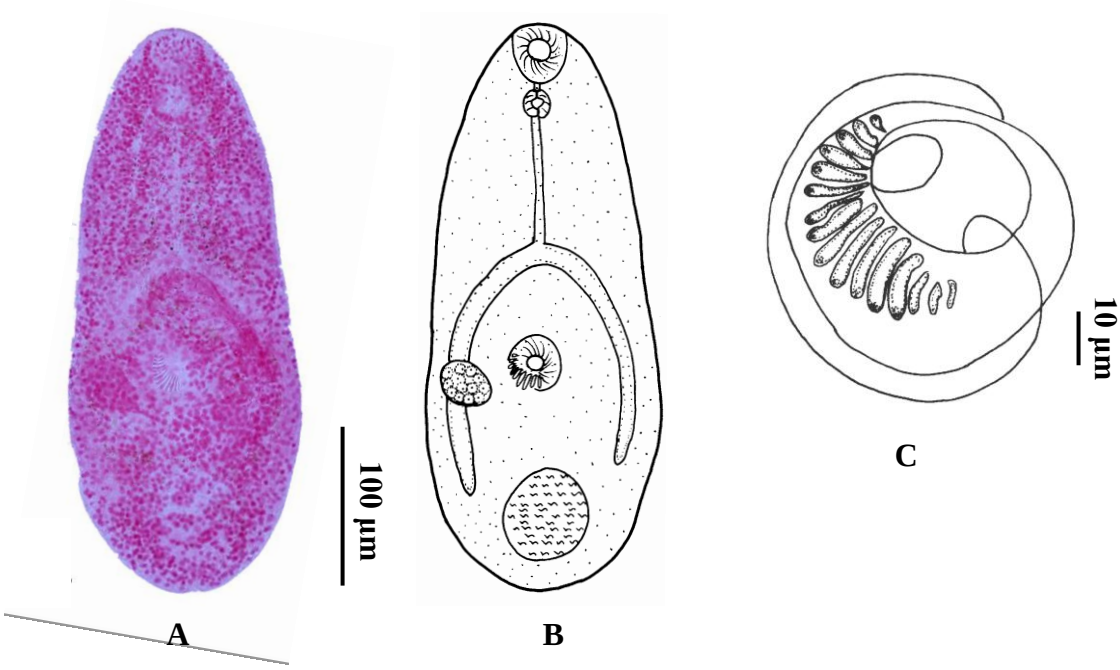


Figure 4-1 Morphological characteristic of *Haplorchis taichui.* A: photograph from permanent slide, B: drawing of body, C: drawing of ventrogenital sac

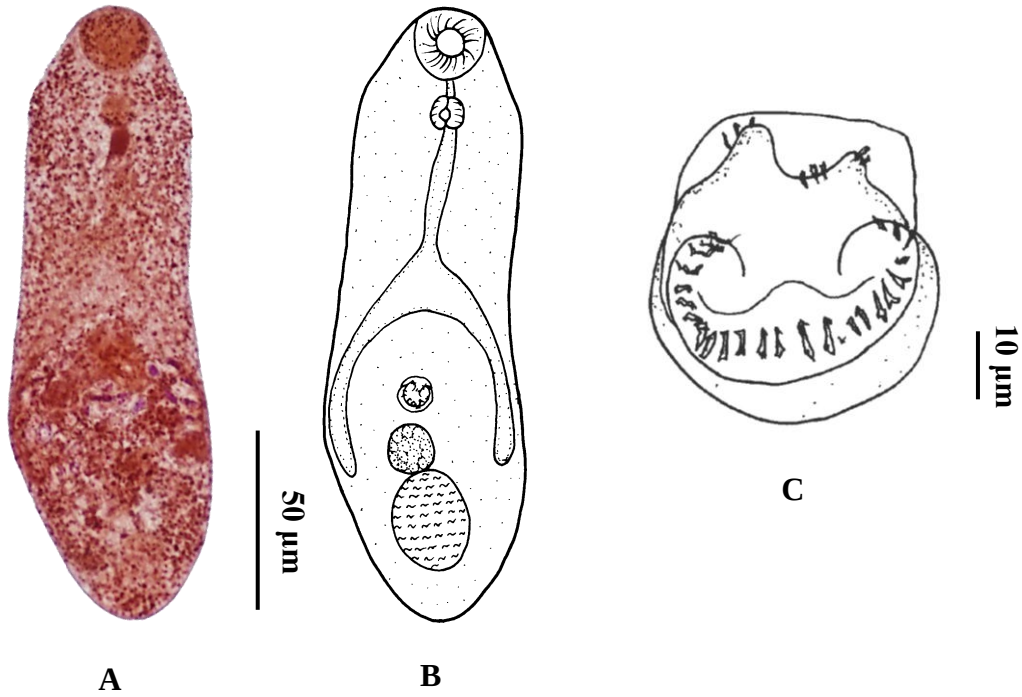


Figure 4-2. Morphological characteristic of *Haplorchis pumilio.* A: photograph from permanent slide, B: drawing of body, C: drawing of ventrogenital sac

Centrocestus caninus Nishigori (1924)

Phylum Platyhelminthes

Class Trematoda

Order Digenea

Family Heterophyidae

Genus *Centrocestus*

Species *Centrocestus caninus*

Description:

Body small 420 -570 (530) μm long, maximum width 137 – 204 (168) μm. The body of this trematode is bottle shaped, covered with scales like spines which decreased in size posteriorly. Oral sucker terminal, encircled with two alternating row of spines of 26-30 circumoral spines. Pharynx well developed. Caeca large, bifurcated about midway between oral and ventral suckers, and terminated slightly in front of ovary. Ventral sucker smaller than oral sucker, and located in the middle of the body. Testis oval, opposite in posterior of body. Ovary oval, located on the right side of the middle of posterior half of the body. Uterus short, coil between ovary and seminal vesicle. Vitelline vesicle large, scattered laterally from posterior end to posterior border of the pharynx and bifurcation region. Excretory bladder x-shaped with short posterior tube. (Figure 4-3).

***Stellantchasmus falcatus* Onji & Nishio, 1916**

Phylum Platyhelminthes

Class Trematoda

Order Digenea

Family Heterophyidae

Genus *Stellantchasmus*

Species *Stellantchasmus falcatus*

Description:

Body small and piriform 420 - 535 (493) μm long, maximum width 188 – 255 (199) μm. Oral sucker sub-terminal. Caeca extended laterally until proceed anterior to testis. Ventral sucker located lateral to caeca, round and composed with the minute spines covered to the muscular protruding or genotyl within the ventrogenital sac, which is the common opening between ventral sucker and genital duct. Testis large, opposite posterior. Seminal vesicle bipartite. Ovary sub-median, slightly to right, behind and separated by uterine coils from ventral sucker. Uterus with three primary loops; first dorsal to left testis, second dorsal to right testis or to bladder, and third ventral to bladder, ascending limb of third loop passes to right of ovary (Figure 4-4).

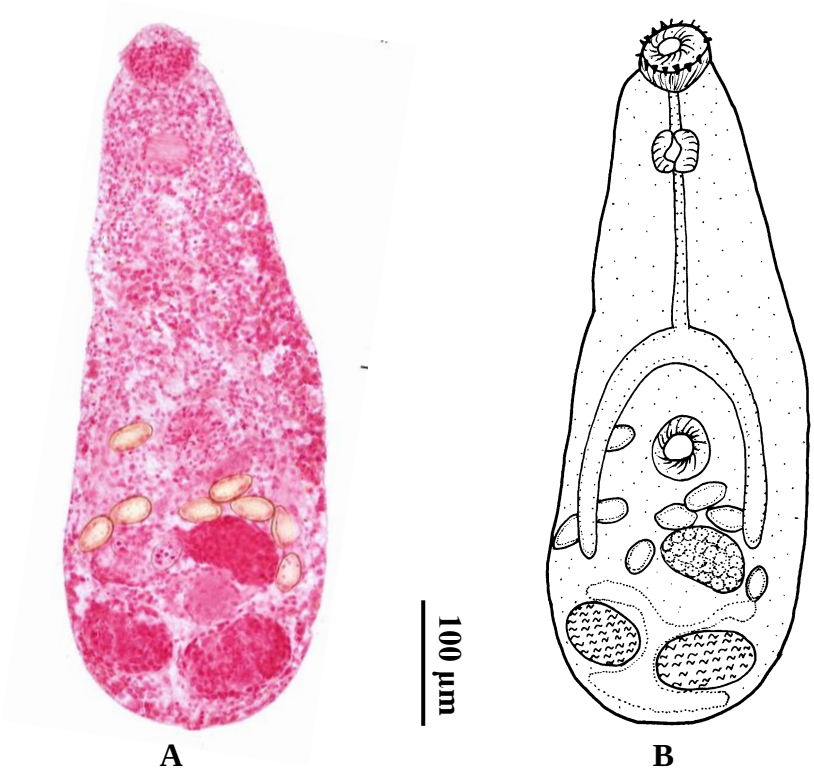


Figure 4-3. Morphological characteristic of *Centrocestus caninus.* A: photograph from permanent slide, B: drawing

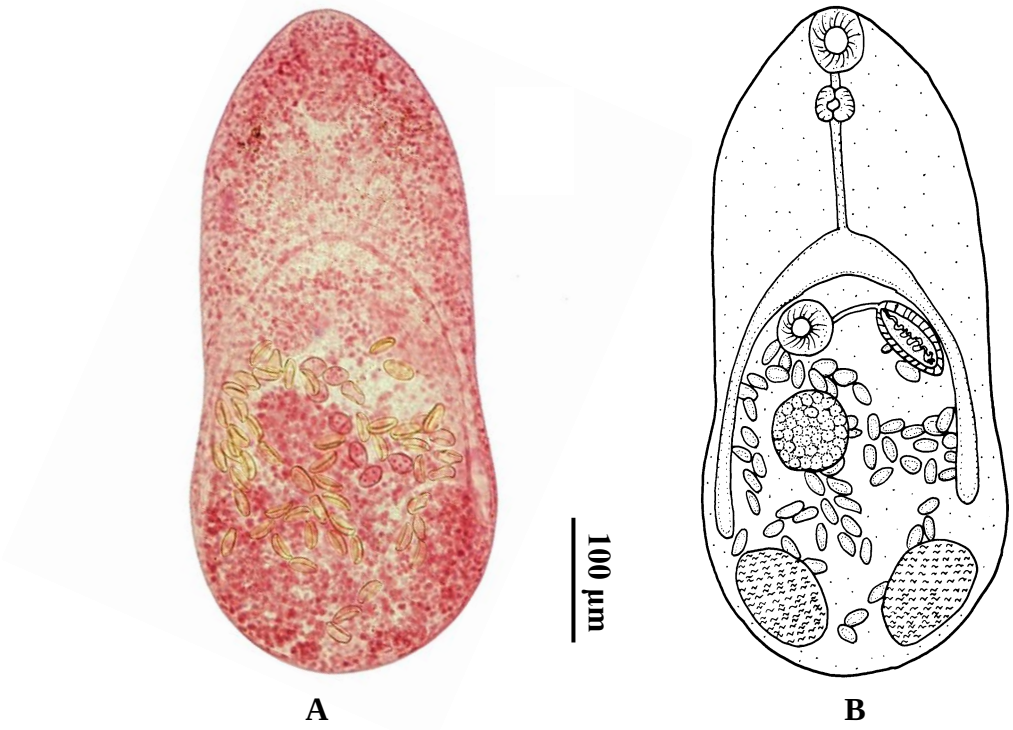


Figure 4-4. Morphological characteristic of *Stellantchasmus falcatus.* A: photograph from permanent slide, B: drawing

Haplorchoides sp. Chen, 1949

Phylum Platyhelminthes

Class Trematoda

Order Digenea

Family Heterophyidae

Genus *Haplorchoides*

Species *Haplorchoides* sp.

Description:

Body elongated 850 -1850 (1490) μm long, maximum width 200 – 375 (257) μm. The body covered with scale like spines. Oral sucker sub-terminal, spherical-shaped. Pre-pharynx very long 261 - 291 (283). Pharynx small 37 – 68 (55). Ventral sucker rudimentary or lacking, enclosed in genital atrium when present. Ovary pre-testicular in median line. Vitellaria rather feebly developed, extending in lateral fields from behind testis to a level between testis and ovary or a little more anteriorly. Uterus occupying most of available space in posterior end of body (Figure 4-5).

Ganeo tigrinus Mehra & Negi, 1928

Phylum Platyhelminthes

Class Trematoda

Order Digenea

Family Lecithodendriidae

Genus *Ganeo*

Species *Ganeo tigrinus*

Description:

Moderate size and flattened 2683 - 3075 (2091) μm long, maximum width 1027 – 1298 (1146) μm. Tegument covered with scale, except the posterior end of the body. Oral sucker sub-terminal. Ventral sucker located anterior half of body. Pharynx spherical. Testis near and before ventral sucker, almost spherical. Cirrus sac elongate claviform, extending to ventral sucker. Genital pore opening lateral, marginal at the level of posterior part of the esophagus. Ovary spherical, postero-lateral to ventral sucker. Uterine coils between ventral sucker and posterior extremely. Vitellaria along lateral side in middle region of the body, over lapping intestinal caeca but leaving free their ends (Figure 4-6).

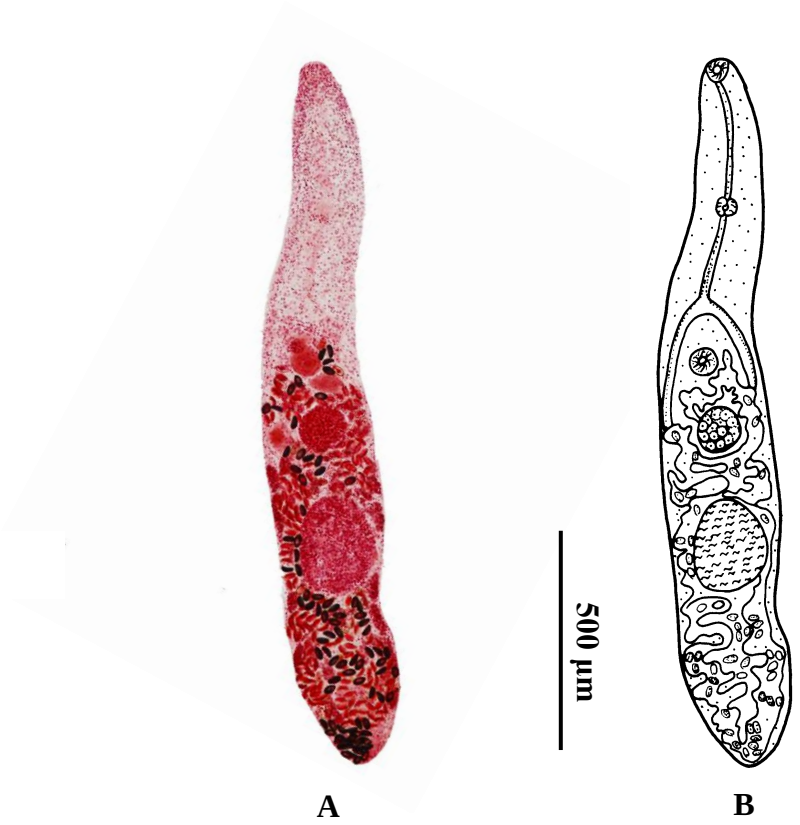


Figure 4-5. Morphological characteristic of *Haplorchoides* sp. A: photograph from permanent slide, B: drawing

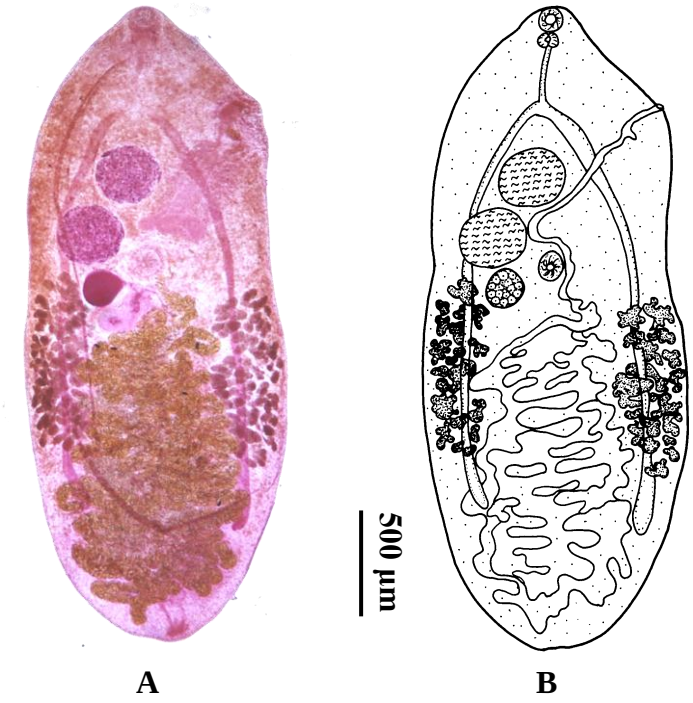


Figure 4-6. Morphological characteristic of *Ganeo tigrinus*. A: photograph from permanent slide, B: drawing

***Prostorchigenes majeedi* Simha & Hakim, 1967**

Phylum Platyhelminthes

Class Trematoda

Order Digenea

Family Lecithodendriidae

Genus *Prostorchigenes*

Species *Prostorchigenes majeedi*

Description:

Moderate size, fusiform and flatten 1938 -3538 (2478) μm long, maximum width 1300 – 1618 (1429) μm. Anterior half of the body covered by minute spines. Oral sucker sub-terminal. Pre-pharynx absent and pharynx small. Caeca reaching posterior extremity or end before it. Ventral sucker small located the middle of body and before ovary. Testis oval, slightly, and obliquely. Ovary spherical at level of ventral sucker. Uterine coils occupying pace between gonads and posterior extremity. Vitelline follicles numerous, small, scattered at caeca bifurcation (Figure 4-7).

Fasciola gigantica Fischoedes, 1940

Phylum Platyhelminthes

Class Trematoda

Order Digenea

Family

Genus *Fasciola*

Species *Fasciola gigantica*

Description:

Large size, flattened and leaf-like shaped 40 – 43.5 (42.2) mm long, maximum width 9.2 – 1.15 (10.7) mm. Head become prominent cephalic cone. Tegument covered with the numerous spines. Oral sucker open sub-terminal. Pharynx short and well developed. Genital pore is at the basal of cephalic cone. Ventral sucker round. Caeca absolutely branched and extended laterally. Testes branching, arranged tandemly and occupied post-ovarian are between vitelline area. Single ovary branched, located sub-median, anterior to testes. Numerous and well developed vitelline follicles and extended laterally. Uterus rosette shaped, situated between ventral sucker and ovary (Figure 4-8).

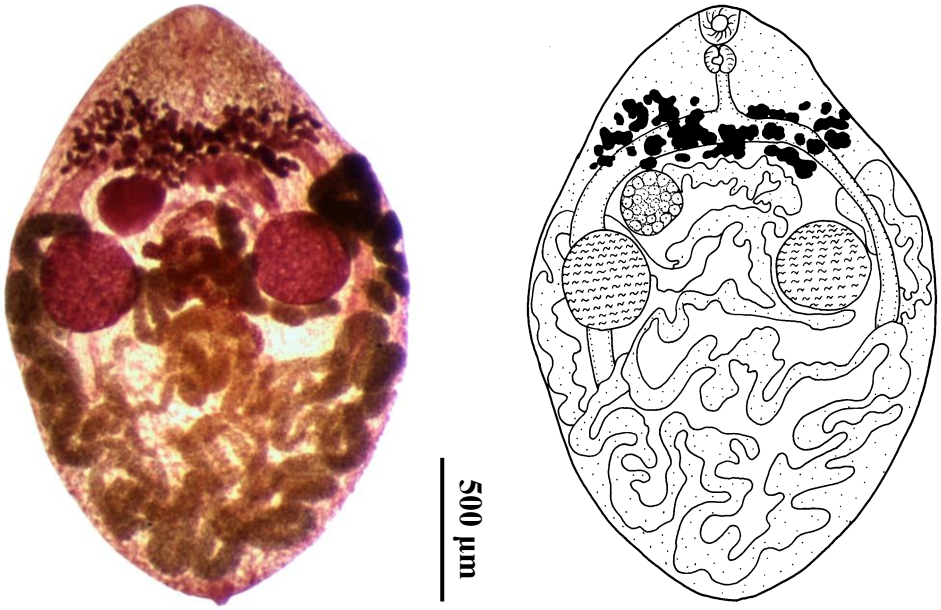


Figure 4-7. Morphological characteristic of *Prostorchigenes majeedi*. A: photograph from permanent slide, B: drawing**.**

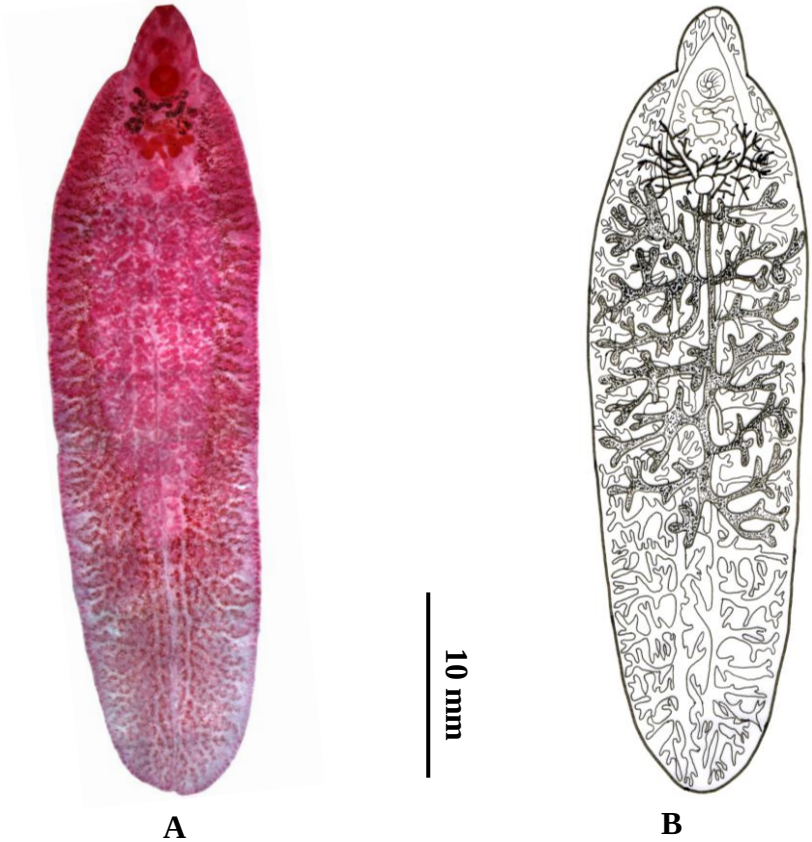


Figure 4-8. Morphological characteristic of *Fasciola gigantica*. A: photograph from permanent slide, B: drawing

Fischoederius elongatus Poires, 1883

Phylum Platyhelminthes

Class Trematoda

Order Digenea

Family Paramphistomidae

Genus *Fischoederius*

Species *Fischoederius elongatus*

Description:

Body elongated and thick tegument without spines 6.81 – 11.00 (8.35) mm long, maximum width 1.88 – 2.88 (2.25) mm. Oral sucker without diverticula. Intestinal caeca extended together nor separate away from each other by ending at the middle part of the body. Two longitudinal tandem of testes and located anterior of the ventral sucker. Genital pore opening into ventral pouch. Ovary inter-testicular. Pre-testicular portion or uterine coils in median dorsal filed from behind genital pore (Figure 4-9).

Orthocoelium streptocoelium Fischoedes, 1901

Phylum Platyhelminthes

Class Trematoda

Order Digenea

Family Paramphistomidae

Genus *Paramphistomun*

Species *Orthocoelium streptocoelium*

Description:

Large size trematode, piriform tegument thick without spines 4.11 – 5.92 (4.64) mm long, maximum width 2.19 – 2.51 (2.31) mm. Oral sucker without diverticula. Ventral sucker located posterior end of the body. Intestinal caeca long, sinuous, reaching to ventral sucker. Testes rounded, tandem or somewhat diagonal. Ovary rounded which is generally somewhat sub-spherical, and located posttesticular in posterior third of body. Uterus winding forward in median filed dorsal to testes. Vitellaria follicle extending in the lateral fields between two sucker (Figure 4-10).

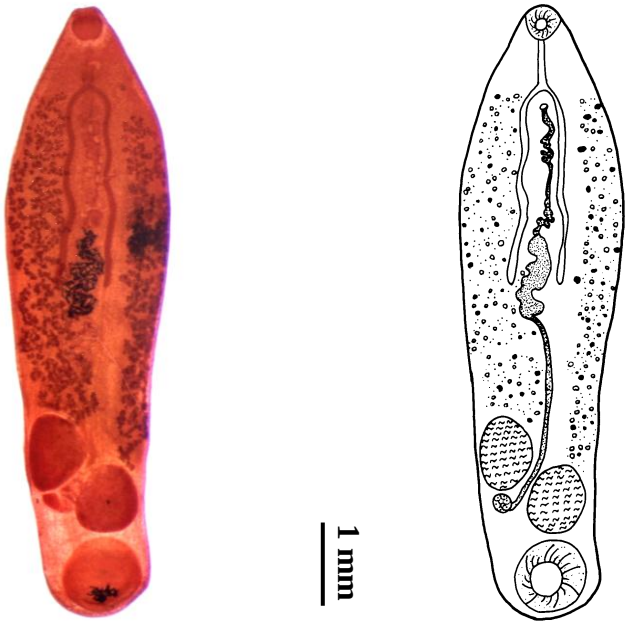


Figure 4-9. Morphological characteristic of *Fischoederius elongatus*. A: photograph from permanent slide, B: drawing

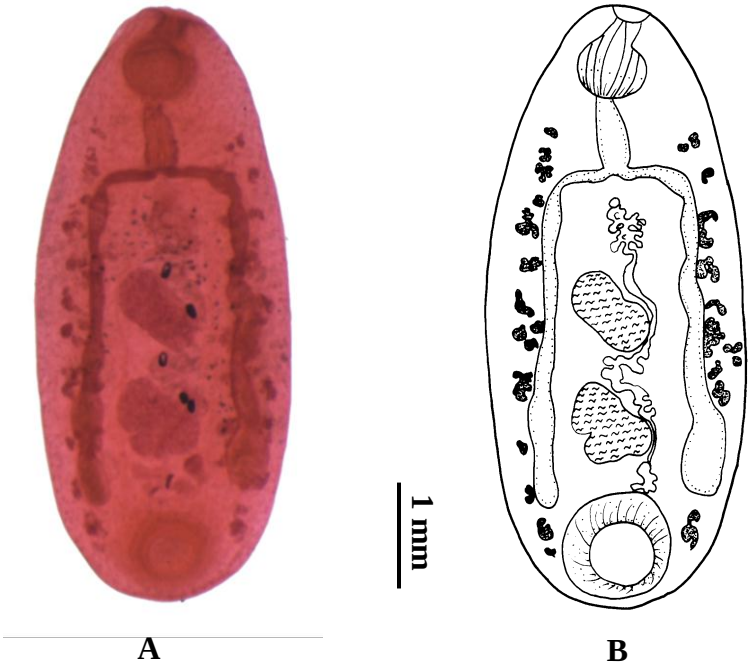


Figure 4-10. Morphological characteristic of *Orthocoelium streptocoelium*. A: photograph from permanent slide, B: drawing

Diversity of intermediate host snails

A total number of 4,533 snail specimens were collected from 12 provinces of northern, Thailand during April 2008 to June 2012. They were classified into 8 families, 15 genera and 21 species/taxa, including 65 *Adamietta housei*, 179 *Bithynia funiculata*, 561 *B. siamensis siamensis*, 7 *Brotia costula costula*, 112 *Br. citrina*, 7 *Br. wykoffi*, 195 *Clea helena*, 38 *Eyriesia eyriesi*, 168 *Filopaludina doliaris*, 562, *F. martensi martensi*, 102 *F. sumatrensis polygramma*, 31 *F. sumatrensis speciosa*, 278 *Indoplanorbis exustus*, 219 *Lymnaea auricularia rubiginosa*, 4 *Makongia swainsoni*, 1,036 *Melanoides tuberculata*, 8 *Paludomus siamensis*, 7 *Pomacea canaliculata*, 756 *Tarebia granifera*, 189 *Thiara scabra*, and 9 *Sinotaia mandahlbarthi*, respectively.

The most widespread snail species were *M. tuberculata* and *T. granifera*, which found in all 12 provinces. Moreover, both of snails species were represented in diverse microhabitats namely, stream, paddy filed, river, irrigation canal, etc. with the highest number of specimens. While, 5 snail species were found rarely which low numbers of snail population including, 4 *Me. Swainsoni* and 9 *S. mandahlbarthi* found only in Phitsanulok province, 8 *P. siamensis* found only in Phrae province, 7 *Po. Canaliculata* and 7 *Br. costula costula* were found only in Chiang Rai province. The number and proportion of those snail collected are shown in Figure 4-11.

Base on surveyed information, the number of each freshwater snails were calculated the species indices for evaluating the biodiversity indices of intermediate host snails abundant in northern Thailand by using Shanon – Weiner Index to investigated the Biodiversity index (*H'*), Diversity value (D) and Evenness (E). The

result shown that, the northern Thailand was indicated the freshwater snail from this area to be a high diversity and distribution.

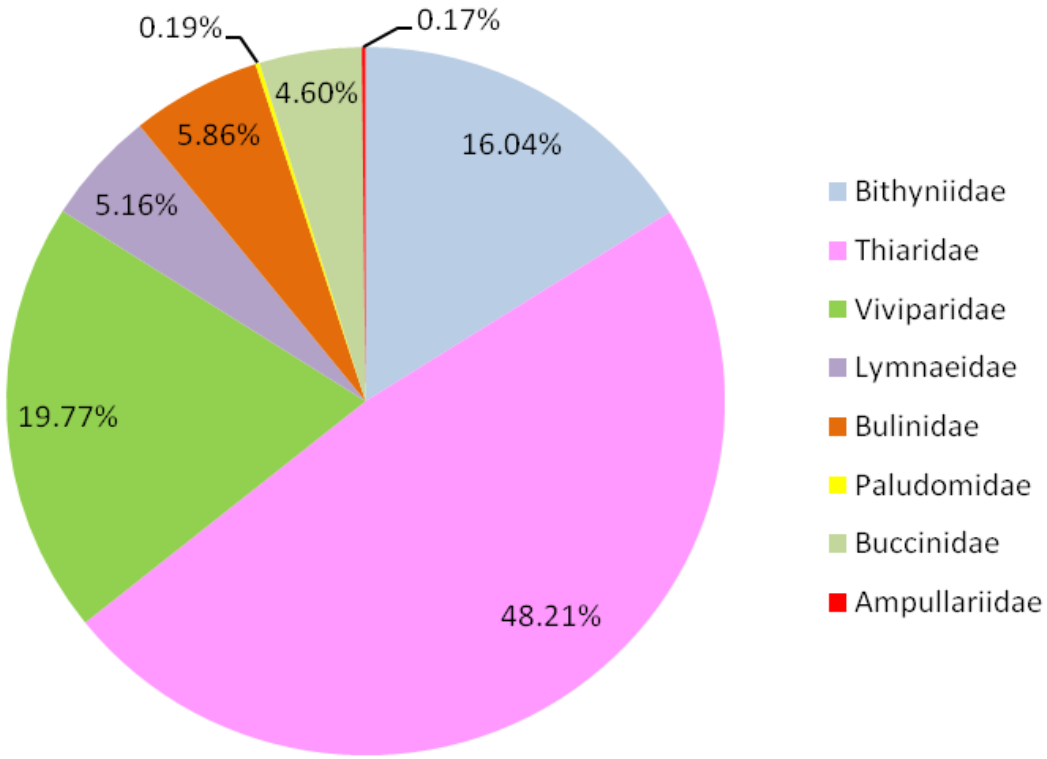


Figure 4-11. The proportion of freshwater snail families were collected from 12 provinces of northern Thailand.

Table 4-1. The total number of each snail species which collecting in 12 provinces of northern Thailand

Snail species	Number of snails												
	CM	**CR**	**HA**	**LA**	**LU**	**NA**	**PY**	**PH**	**PI**	**SU**	**TA**	**UT**	**Total**
Adamietta housei	0	0	0	0	30	0	0	10	0	0	0	25	65
Bithynia siamensis siamensis	197	45	0	109	30	30	12	3	31	0	57	47	561
Bithynia funiculata	54	15	0	80	30	0	0	0	0	0	0	0	179
Brotia costula costula	0	7	0	0	0	0	0	0	0	0	0	0	7
Brotia citrina	60	0	0	0	0	0	0	52	0	0	0	0	112
Brotia wykoffi	5	2	0	0	0	0	0	0	0	0	0	0	7
Clea helena	103	0	0	0	30	0	16	26	0	0	20	0	195
Eyriesia eyriesi	0	8	0	0	30	0	0	0	0	0	0	0	38
Filopaludina doliaris	0	24	0	48	30	0	12	0	47	7	0	0	168
Filopaludina martensi martensi	91	16	10	78	120	47	60	17	53	39	16	15	562
Filopaludina sumatrensis polygramma	16	0	0	0	30	0	5	4	0	47	0	0	102

Remark: CM = Chiang Mai, CR= Chiang Rai, MA= Mae Hong Son, LA= Lampang, LU= Lamphun, NA= Nan, PY= Phayao, PH= Phrae, PI= Phitsanulok, SU= Sukhothai, TA= Tak , UT= Uttaradit

Table 4-1. (Cont.)

Snail species	Number of snails												
	CM	**CR**	**HA**	**LA**	**LU**	**NA**	**PY**	**PH**	**PI**	**SU**	**TA**	**UT**	**Total**
Filopaludina sumatrensis speciosa	0	0	0	0	0	0	0	0	0	0	30	1	31
Indoplanorbis exustus	3	26	54	32	10	30	11	0	52	60	0	0	278
Lymnaea auricularia rubiginosa	90	0	14	0	30	41	30	0	3	0	11	0	219
Makongia swainsoni	0	0	0	0	0	0	0	0	4	0	0	0	4
Melanoides tuberculata	318	57	74	120	60	30	60	52	63	59	60	83	1036
Paludomus siamensis	0	0	0	0	0	0	0	8	0	0	0	0	8
Pomacea canaliculata	0	7	0	0	0	0	0	0	0	0	0	0	7
Tarebia granifera	257	30	76	127	30	60	30	60	0	30	30	26	756
Thiara scabra	118	2	41	0	0	0	0	0	0	0	0	28	189
Sinotaia mandahlbarthi	0	0	0	0	0	0	0	0	9	0	0	0	9
Total	1312	239	269	594	460	238	236	232	262	242	224	225	4533

Remark: CM = Chiang Mai, CR= Chiang Rai, MA= Mae Hong Son, LA= Lampang, LU= Lamphun, NA= Nan, PY= Phayao, PH= Phrae, PI= Phitsanulok, SU= Sukhothai, TA= Tak , UT= Uttaradit

The study of the biodiversity indies, the Shanon-Wiener index containing the species diversity and evenness were used for evaluated the diversity of freshwater snails from northern Thailand in this study. The calculation were performed as detailed in Table 4-2, and it would be concluded as mention following;

Diversity index (*H'*) = $-\sum_{i=1}^{S} (pi\ ln\ pi)$

= 2.360197

Where *H'* = Diversity index

P*i* = Number of each species of freshwater snails by total number of freshwater snails

Diversity value (D) = $e^{H'}$

= **10.593038**

Evenness (J) = H'/H_{max}

= 2.360197 / 3.044522

= **0.775227**

Where H = Shannon - Wiener Index

H_{max} = Maximum Diversity Index where the number of species recorded is equivalent

By H_{max} = *ln* S

Where S = Number of freshwater snails species were found

= *ln* (21)

= **3.044522**

Table 4-2. Biodiversity indices of freshwater snail calculating from northern Thailand

Snail species	No. of specimens	pi	*In* pi	pi *In* pi
A. housei	65	0.014339	-4.244752	-0.060867
B. siamensis siamensis	561	0.123759	-2.089418	-0.258585
B. funiculata	179	0.039488	-3.231753	-0.127616
Br. costula costula	7	0.001544	-6.473229	-0.009996
Br. citrina	112	0.024708	-3.700640	-0.091434
Br. wykoffi	7	0.001544	-6.473229	-0.009996
C. helena	195	0.043018	-3.146140	-0.135340
E. eyriesi	38	0.008383	-4.781553	-0.040084
F. doliaris	168	0.037062	-3.295175	-0.122124
F. martensi martensi	562	0.123980	-2.087637	-0.258825
F. sumatrensis polygramma	102	0.022502	-3.794166	-0.085375
F. sumatrensis speciosa	31	0.006839	-4.985152	-0.034092
I. exustus	278	0.061328	-2.791518	-0.171198
L. auricularia rubiginosa	219	0.048312	-3.030068	-0.146390
Ma. swainsoni	4	0.000882	-7.032845	-0.006206
M. tuberculata	1,036	0.228546	-1.476017	-0.337338
P. siamensis	8	0.001765	-6.339698	-0.011189
Po. ccanaliculata	7	0.001544	-6.473229	-0.009996
T. granifera	756	0.166777	-1.791098	-0.298714
Th. scabra	189	0.041694	-3.177392	-0.132479
S. mandahlbarthi	9	0.001985	-6.221915	-0.012353
Total	4,533	1.000000	-86.636625	-2.360197

Summarized:	Diversity index (H)	=	2.360197
	Diversity value (D)	=	10.593038
	Evenness (J)	=	0.775227

***Adamietta housei* (Lea, 1856)**

Phylum Mollusca

Class Gastropoda

Order Mesogastropoda

Family Thiaridae

Genus *Adamietta*

Adamietta housei

Shell is medium size 320 - 370 (350) mm long, maximum width 95 – 150 (125) mm elongated, turreted, smooth except for the growth lines. The shell color is white-brown. The 12-14 whorls are almost flat or moderately convex, and they increase slowly and regularly in diameter. The body whorl measures about 2/5 of the length of the shell. The aperture of shell is ovate, angled above and well rounded below. Peristome sharp, connected by a thin parietal callus. The operculum is tiny, oval, and paucispiral (Figure 4-12).

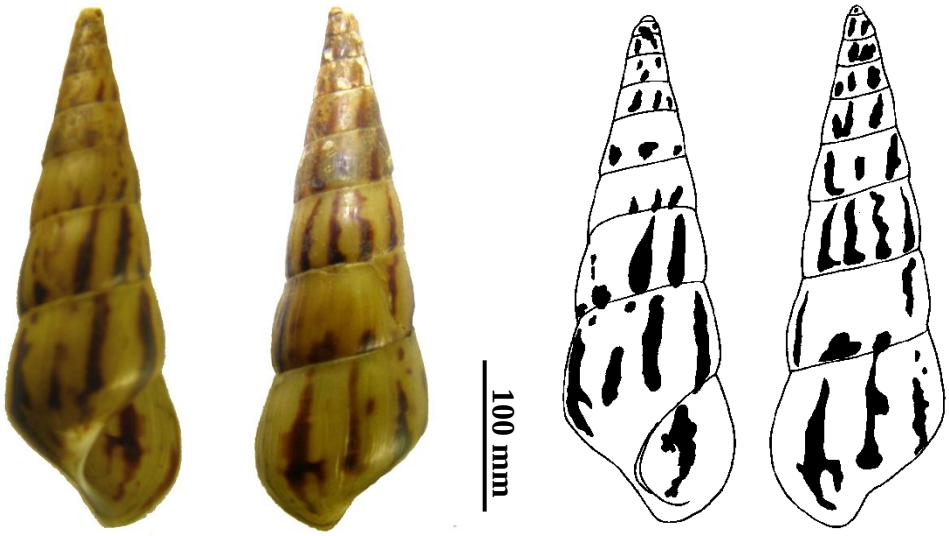


Figure 4-12. Shell characteristics of *Adamietta housei*. A: photograph, B: drawing

***Bithynia siamensis siamensis* (Lea, 1856)**

Phylum Mollusca

Class Gastropoda

Order Mesogastropoda

Family Bithyniidae

Genus *Bithynia*

Bithynia siamensis siamensis

The shell is small 80 - 117 (95) mm long, maximum width 41 – 72 (57) mm, ovate, with rather sharp apex which is generally somewhat eroded with age. The shell color is yellowish-brown or greenish-olive, glossy, with delicate spiral microsculpture. The umbilicus of shell is very narrow. Aperture is ovate-shaped. Operculum is calcareous and with a paucisoiral (Figure 4.-13).

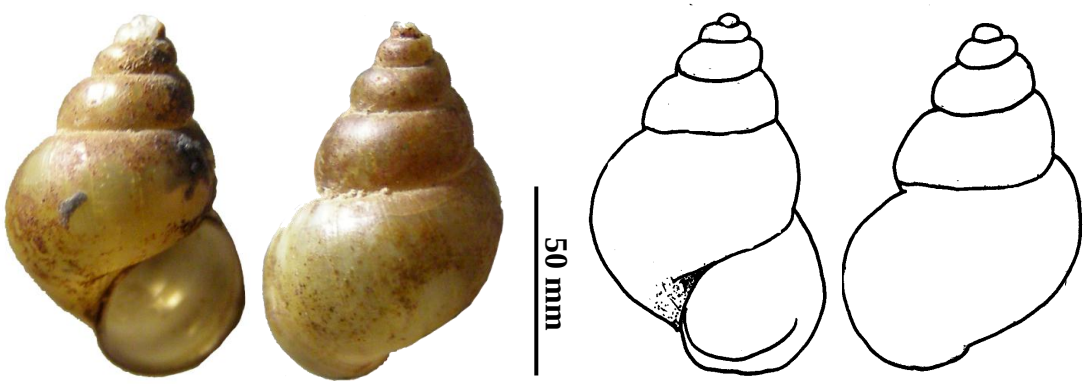


Figure 4-13. Shell characteristics of *Bithynia siamensis siamensis.* A: photograph, B: drawing

***Bithynia funiculata* (Walker, 1927)**

Phylum Mollusca

Class Gastropoda

Order Mesogastropoda

Family Bithyniidae

Genus *Bithynia*

Bithynia funiculata

The shell is large for this genus 95 - 147 (115) mm long, maximum width 59 – 91 (72) mm, conidal-ovate, with short, and conic truncated spire. This species is open funnel-shaped umbilicus which is surrounded by threads-like keel. The body whorl is inflated. It is rather solid, not or barely transparent and near dull. The ground color is olive-brown, but it covered by reddish periderm. Operculum is calcareous and with a paucisoiral (Figure 4-14).

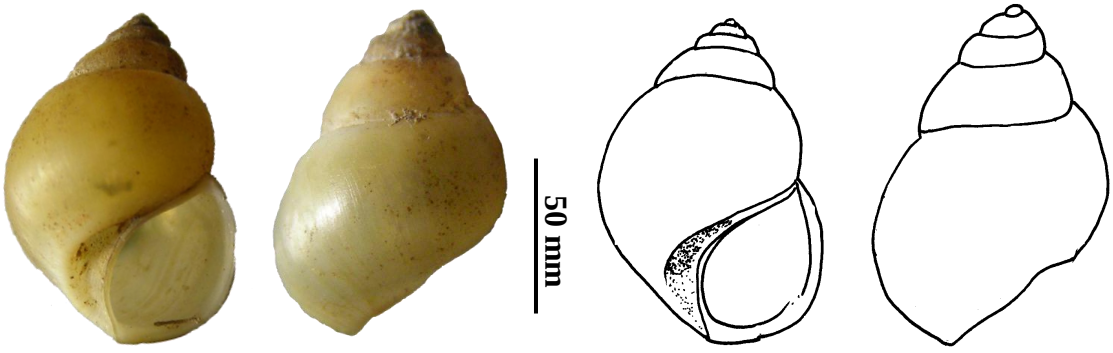


Figure 4-14. Shell characteristics of *Bithynia funiculata.* A: photograph, B: drawing

***Brotia costula costula* (Rafineaque, 1833)**

Phylum Mollusca

Class Gastropoda

Order Mesogastropoda

Family Thiaridae

Genus *Brotia*

Brotia costula costula

In same genus, shell of this snail species is bigger than the others species 270 - 740 (450) mm long, maximum width 95 – 220 (162) mm. The shell is elegantly turreted, solid, very thick, and covered with a dark brownish or olive-brown color. The shell are either unicoloured or show 1-3 brown spiral bands. The sculpture consists of numerous spiral grooves with are weaker on the upper half of the body whorl. The upper spiral ridge may carry nodules which, when well developed, may never attain the suture. Aperture species is large shell is brown or milky-white. Operculum almost circular with 5 whorls (Figure 4-15).

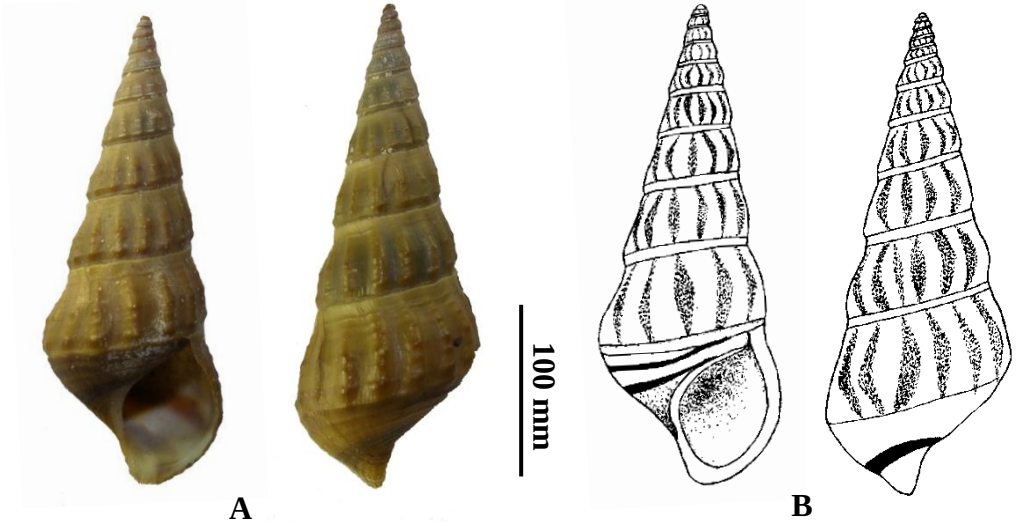


Figure 4-15. Shell characteristics of *Brotia costula costula*. A: photograph, B: drawing

***Brotia citrina* (Brot, 1868)**

Phylum Mollusca

Class Gastropoda

Order Mesogastropoda

Family Thiaridae

Genus *Brotia*

Brotia citrina

The shell is moderate in size 337 - 410 (370) mm long, maximum width 115 – 142 (125) mm, rather solid and not translucent. The shell color is yellowish-brown, olive-green or brownish. The shell cover by a thick black layer of mineral deposit. The apex of the shell is often eroded. There are 9-11 convex whorls which increase slowly and regularly in size. The upper whorls are smooth except for the growth lines. On the base of body whorl there are several weak periomphalic ridges. The aperture of shell is ovate. The operculum almost round, inner surface is glossy, with 4 whorls and almost central nuclear (Figure 4-16).

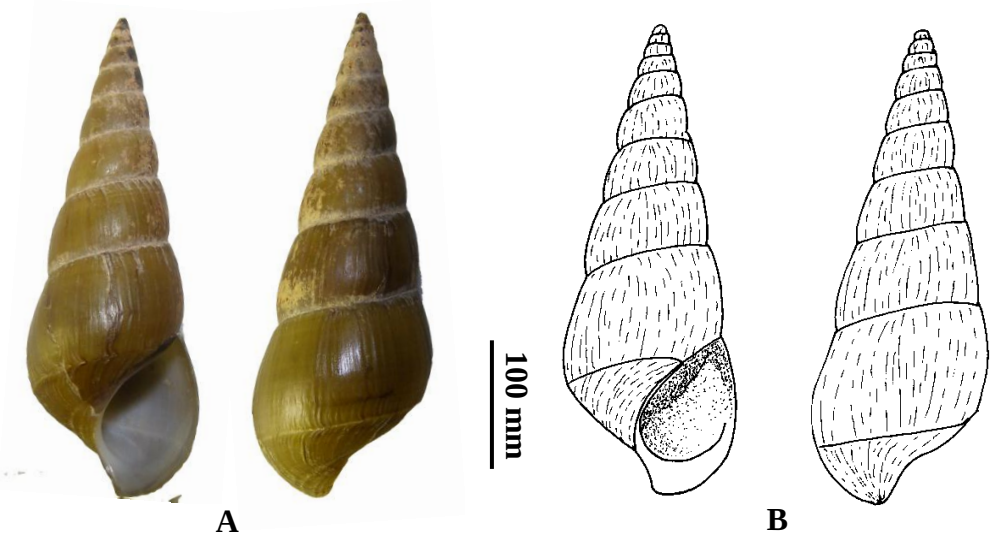


Figure 4-16. Shell and operculate characteristics of *Brotia citrina*. A: photograph, B: drawing

***Brotia wykoffi* (Brandt, 1974)**

Phylum Mollusca

Class Gastropoda

Order Mesogastropoda

Family Thiaridae

Genus *Brotia*

Brotia wykoffi

The shell is medium size 180 - 315 (247) mm long, maximum width 116 – 151 (128) mm, regularly turreted, solid, somewhat translucent, and covered with a reddish-violet periderm and ornate with 3 spiral bands. First below the periphery, second band below the suture and third on the base the body whorl. The growth lines are rather rough. There are several weak spiral ridges on the base of the body whorl. Apex is generally eroded. The aperture of shell is ovate, pointed above and well rounded below and at the columella. Operculum is ovate, corneous, with 4 (1/2) whorl and eccentric nucleus (Figure 4-17).

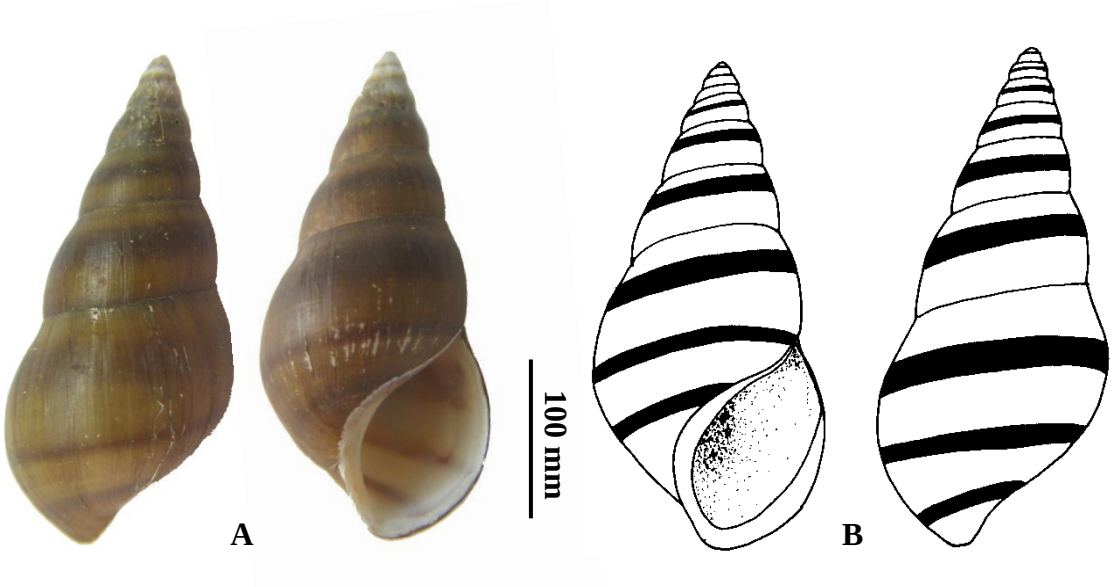


Figure 4-17. Shell characteristics of *Brotia wykoffi*. A: photograph, B: drawing

***Clea helena* (Philippi, 1847)**

Phylum Mollusca

Class Gastropoda

Order Neogastropoda

Family Buccinidae

Genus *Clea*

Clea helena

Shell is medium size 130 - 280 (186) mm long, maximum width 60 – 80 (71) mm, turreted or elongated ovate-conoidal. The shell color is olive-brown, unicolor or with 1-3 dark-brown spiral bands (one below the suture, one at the periphery, and one on the base of the body whorl). The shell is solid, not translucent, with strong axial ribs which are rarely obsolete. There are 14-24 ribs on the last whorl. On the upper half of the whorl are fine spiral lines which become coarser on the base of the body whorl. The 6-8 whorls are somewhat convex or almost flat. Base of the body whorl produced into a broad siphonal process. The operculum is almond-shaped, concentric, with basal nucleus (Figure 4-18).

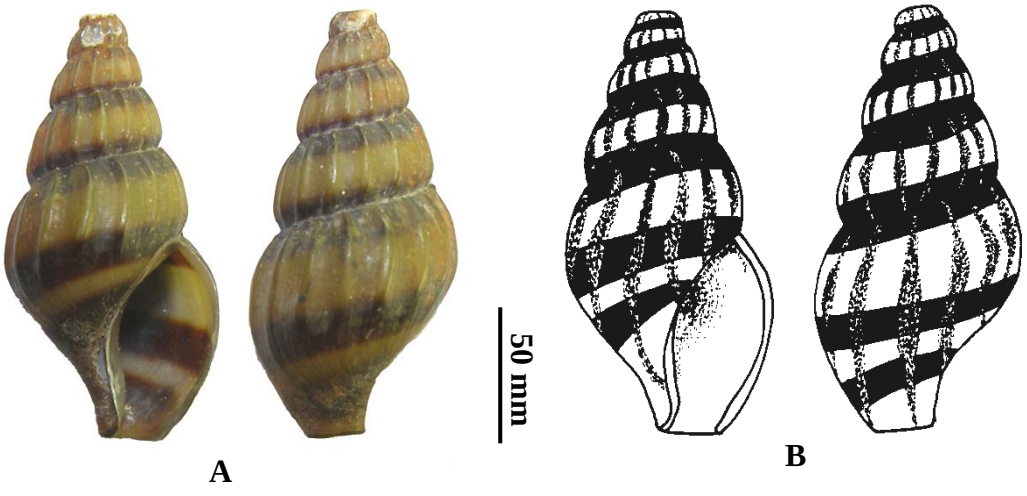


Figure 4-18. Shell characteristics of *Clea helana*. A: photograph, B: drawing

***Eyriesia eyriesi* (Morelet, 1865)**

Phylum Mollusca

Class Gastropoda

Order Mesogastropoda

Family Viviparidae

Subfamily Bellamyinae

Genus *Eyriesia*

Eyriesia eyriesi

The shell is large 115 - 195 (166) mm long, maximum width 120 – 181 (160) mm, rather thin but solid. The shell is depress-conical, with sharp peripheral keel and 1 or 2 spiral ridge between suture and periphery. The greenish periderm shows a delicate spiral microsculpture, particularly so on the base of the body whorl. Aperture of shell is oblique, piriform, bluish-white within. The operculum is tiny, corneous, brittle, copper-brown. The external of face shell is glossy while internal face is puckered (Figure 4-19).

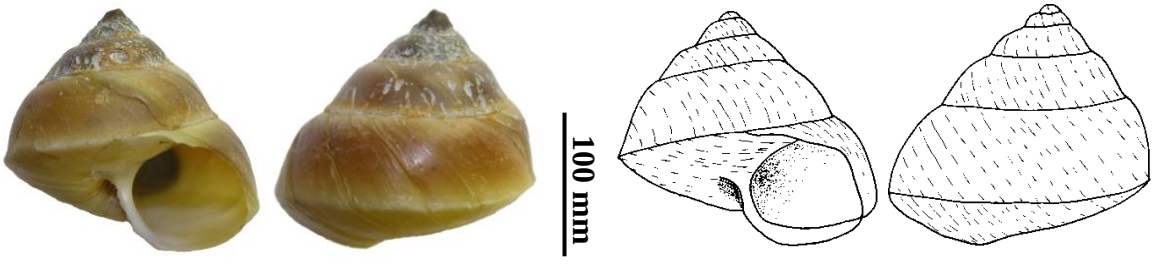


Figure 4-19. Shell characteristics of *Eyriesia eyriesi*. A: photograph, B: drawing

***Filopaludina doliaris* (Grould, 1844)**

Phylum Mollusca

Class Gastropoda

Order Mesogastropoda

Family Viviparidae

Subfamily Bellamyinae

Genus *Filopaludina*

Filopaludina doliaris

Shell is medium size 160 - 250 (195) mm long, maximum width 110 – 134 (116) mm, thinner texture, more inflated body whorl, and open umbilicus. The spire is conic. The color of periderm is greenish and consist with the brown color band. The color bands are prominent on the post-nuclear whorl. There are normally two strong bands between the band on the periphery and the suture. Moreover, it have the four thinner bands on lower half of the body whorl, around and narrow. The aperture is oval, the peristome is connected by very thin, bluish-white callus. Operculum thin, corneous, transparent, concentric, with subcentral, reddish-brown muscle scar (Figure 4-20).

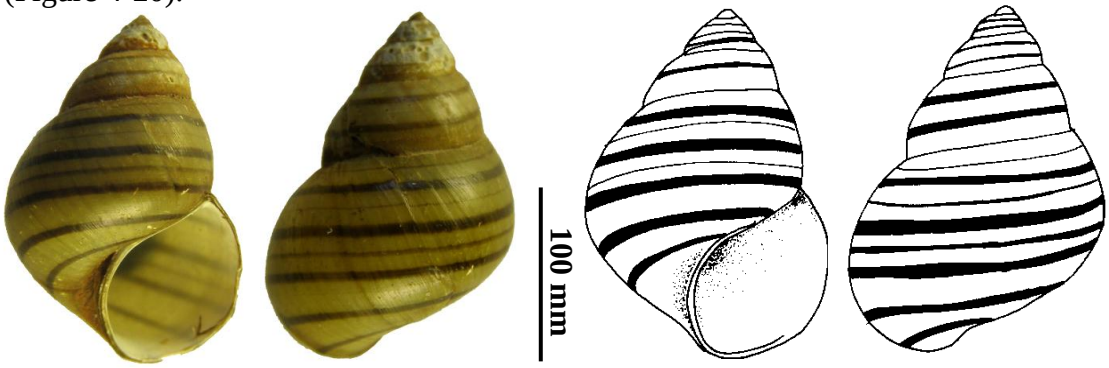


Figure 4-20. Shell characteristics of *Filopaludina doliaris.* A: photograph, B: drawing

***Filopaludina (Siamopaludina) martensi martensi* (Frauenfeld, 1865)**

Phylum Mollusca

Class Gastropoda

Order Mesogastropoda

Family Viviparidae

Subfamily Bellamyinae

Genus *Filopaludina*

Filopaludina martensi martensi

Shell large or at least moderate size 135 - 210 (180) mm long, maximum width 87 – 210 (161) mm, thick, ovate-conic shape. The apex is acute and violet-black in colour. The periderm is olive-green colour which turns brown or blackish with age. Protoconch generally eroded. The shell has 6-7 convex whorl. The shell is smooth with the exception of the delicate spiral lines and without colour bands. The first postnuclear whorl show distinct spiral microsculpture and feeble colour band. The microsculpture is distinct around the umbilicus area and near the peristome. Umbilicus either completely closed or (rarely) somewhat open. Aperture is large, broadly ovate. Operculum broad, and very thick (Figure 4-21).

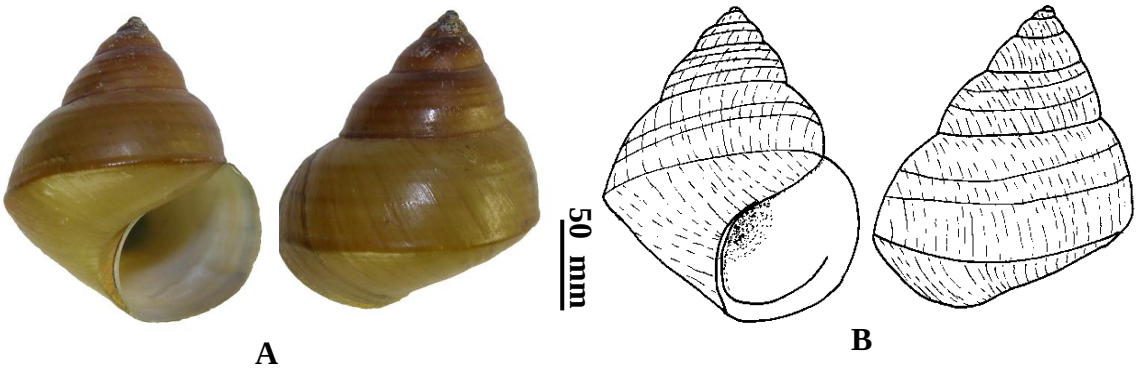


Figure 4-21. Shell characteristics of *Filopaludina martensi martensi*. A: photograph, B: drawing

***Filopaludina sumatrensis polygramma* (Martens, 1860)**

Phylum Mollusca

Class Gastropoda

Order Mesogastropoda

Family Viviparidae

Subfamily Bellamyinae

Genus *Filopaludina*

Filopaludina sumatrensis polygramma

Shell moderate size 90 - 235 (183) mm long, maximum width 75 – 133 (151) mm, slender. The periderm is olive-green colour which spiral bands. There are 4 or 5 band between the suture and periphery and there is no sunsutural shoulder. In addition, it have distinct the colour band on the on lower half of the body whorl. The periphery may be carinated but rarely so in common pletely mature specimen. The aperture is oval, the peristome is connected by very thin, bluish-white callus. Operculum thin, corneous, transparent, and concentric (Figure 4-22).

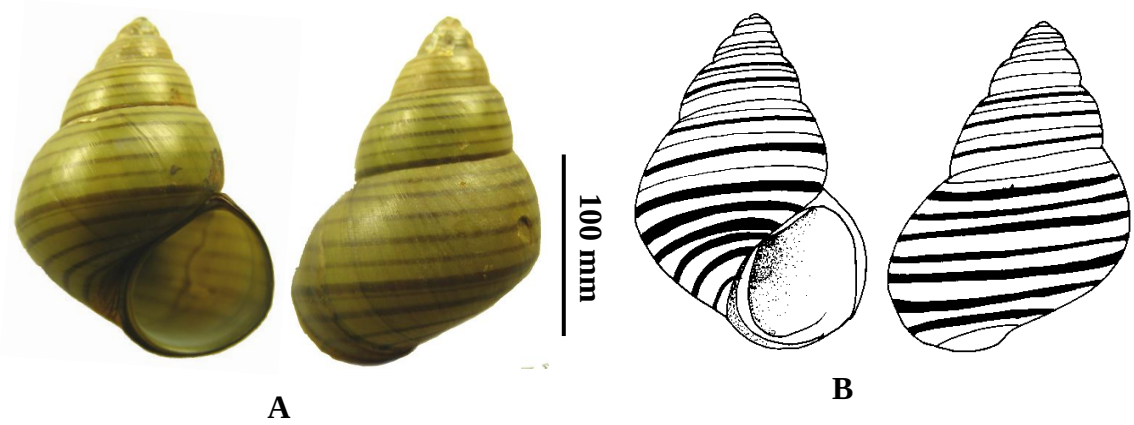


Figure 4-22. Shell characteristics of *Filopaludina sumatrensis polygramma*. A: photograph, B: drawing

***Filopaludina sumatrensis speciosa* (Deshayes, 1876)**

Phylum Mollusca

Class Gastropoda

Order Mesogastropoda

Family Viviparidae

Subfamily Bellamyinae

Genus *Filopaludina*

Filopaludina sumatrensis speciosa

The shell is medium size 185 - 265 (212) mm long, maximum width 127 – 197 (161) mm, conic, which regularly increase whorls. The microstructure is delicate. The periderm color is greenish. The body whorl generally with peripheral carina. The shell is often ornate by four band between the suture and peripheral band and the lower half of body whorl only rarely the spiral band. The operculum is thin, yellowish-brown, inner size is glossy (Figure 4-23).

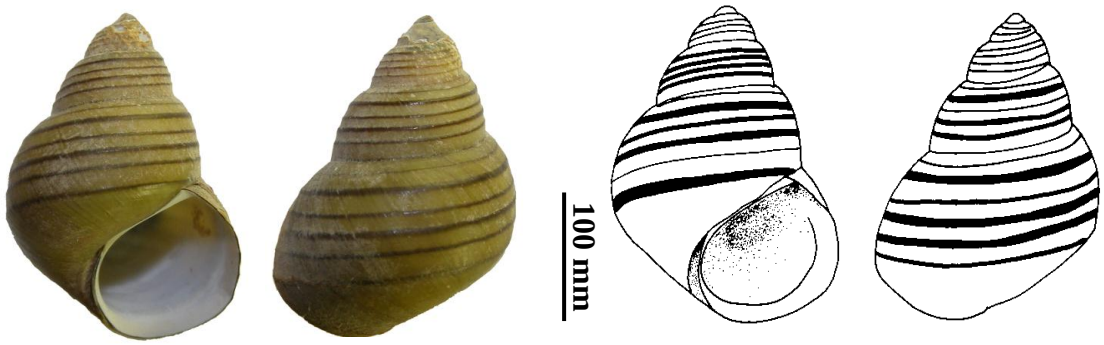


Figure 4-23. Shell characteristics of *Filopaludina sumatrensis speciosa*. A: photograph, B: drawing

***Indoplanorbis exustus* (Deshayes, 1834)**

Phylum Mollusca

Class Gastropoda

Order Basommatophora

Family Bullinidae

Genus *Indoplanorbis*

Indoplanorbis exustus

Shell sinistral and moderate size 73 - 130 (97) mm long, maximum width 71 – 107 (87) mm, discoidal, upper and lower side somewhat concave. The shell color is brownish-yellow or olive. The sculpture is fine, regular, axial rib the 4 rounded whorl increase rapid size. Aperture expanded. Peristome sharp with li. The delicate spiral microsculpture is will visible in young sample (Figure 4-24).

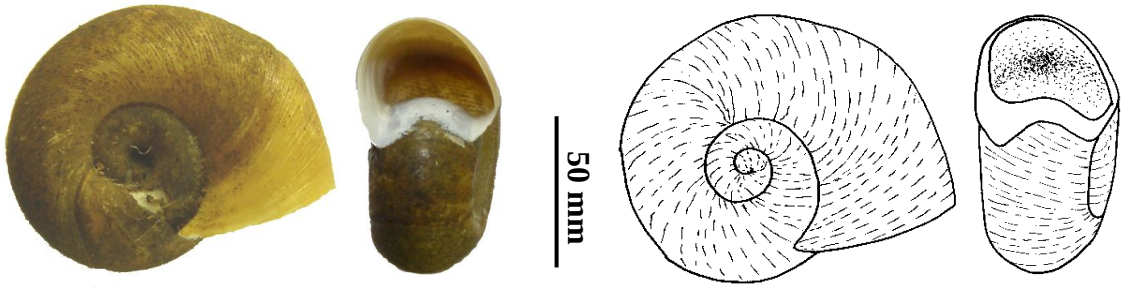


Figure 4-24. Shell characteristics of *Indoplanorbis exustus*. A: photograph, B: drawing

***Lymnaea auricularia rubiginosa* (Michelin, 1831)**

Phylum Mollusca

Class Gastropoda

Order Basommatophora

Family Lymnaeidae

Genus *Lymnaea*

Lymnaea auricularia rubiginosa

Shell thin, small size 117 - 216 (156) mm long, maximum width 63 – 110 (81) mm, translucent, corneous with small short point spire and ridge. The body whorl is oval. The uppermost of the 5(1/2) whorl and almost flat. The penultimate whorl is somewhat convex and the last whorl large and inflated. The slide lines of the spire appear concave because inflated the body whorl. This may be moderately expanded or not, connected by, thin sinuous callus (Figure 4-25).

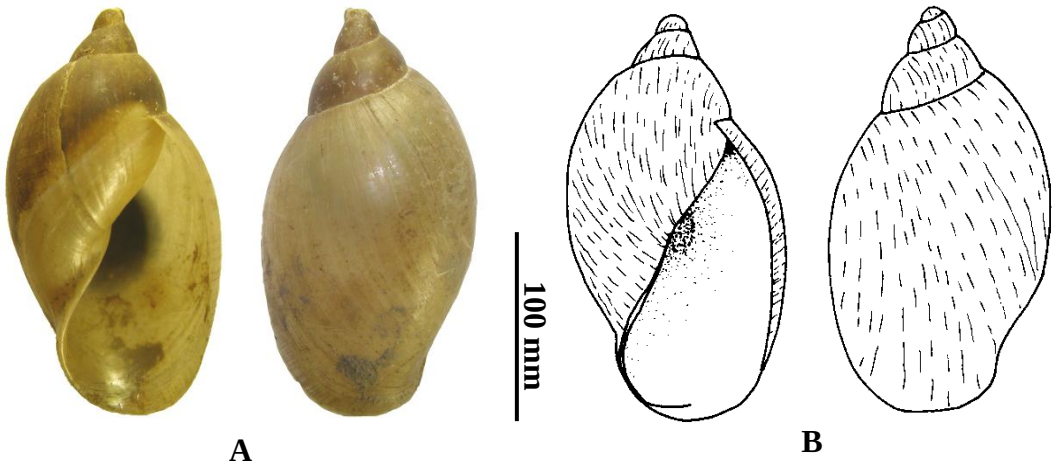


Figure 4-25. Shell characteristics of *Lymnaea auricularia rubiginosa*. A: photograph, B: drawing

***Melanoides tuberculata* (O.F. Müller, 1774)**

Phylum Mollusca

Class Gastropoda

Order Mesogastropoda

Family Thiaridae

Subfamily Thiarinae

Genus *Melanoides*

Melanoides tuberculata

Shell elongate 155 - 300 (220) mm long, maximum width 65 – 110 (81) mm, turreted, with the many whorls. The shell colour is brownish or olive. Moreover, the shell is often ornate by brown frame and spiral band. Apex generally eroded or truncate. The spire is long and consists of many whorls which increase slowly in diameter. The shell is sculpture with more or less strong spiral grooves and axial ribs. The operculum always oval and paucispiral (Figure 4-26).

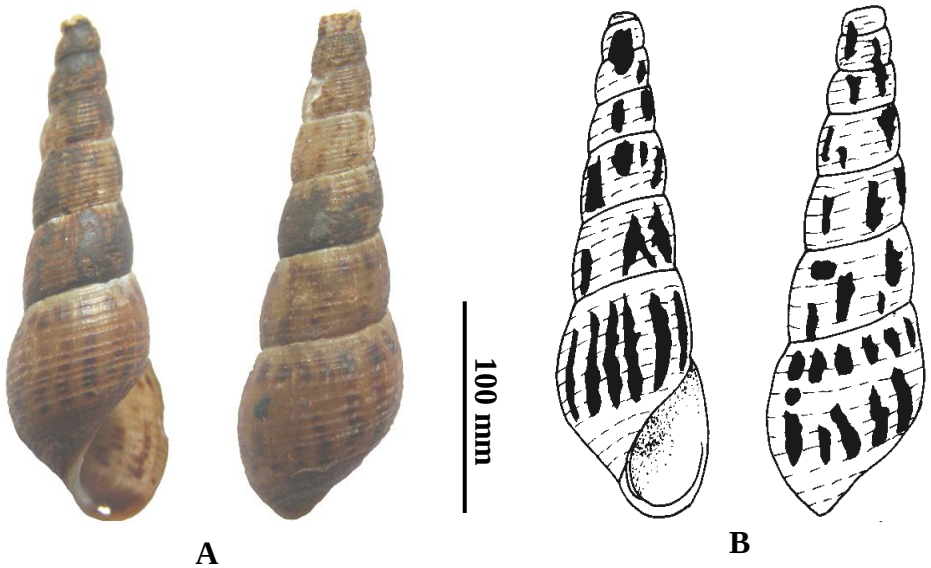


Figure 4-26. Shell characteristics of *Melanoides tuberculata*. A: photograph, B: drawing

***Mekongia swainsoni* (Lea, 1856)**

Phylum Mollusca

Class Gastropoda

Order Mesogastropoda

Family Viviparidae

Subfamily Bellamyinae

Genus *Mekongia*

Species *Mekongia swainsoni*

Shell medium size 142 - 175 (169) mm long, maximum width 94 – 140 (115) mm and subglobose with more or less elevate spire, rather solid, with dark greenish periderm. The surface is either smooth. The apex of shell is flesh-coloured or white, rarely of a pallid violet-brown. Umbilicus is closed or narrow but distinct. Operculum chestnuts-brown. The rachis has a rather straight, not triangular, cutting edge with generally 7-9 small cusps on the either side of the middle cusps (Figure 4-27).

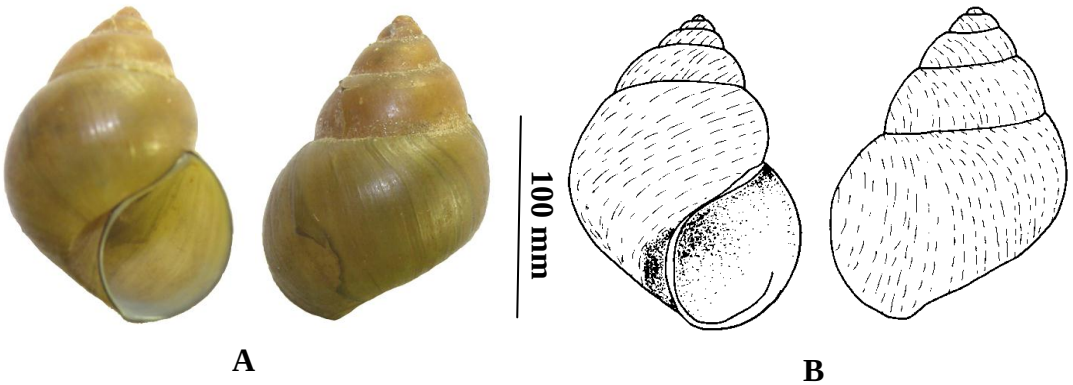


Figure 4-27. Shell characteristics of *Mekongia swainsoni*. A: photograph, B: drawing

***Paludomus siamensis* Blanford, 1903**

Phylum Mollusca

Class Gastropoda

Order Mesogastropoda

Family Paludomidae (Pleuroceridae)

Subfamily Paludominae

Genus *Paludomus*

Species *Paludomus siamensis*

Shell is small size 85 - 137 (109) mm long, maximum width 60 – 85 (67) mm, elongate-conic, rather solid, olive or brownish periderm. This species differs from the proceeding species by it larger average size, higher spire and by its distinct spiral sculpture. Spire often eroded or truncate. The colour is of a chestnut – brown with a tint olive. Body whorl large, rounded, and curved. Generally there are 1-3 distinct spiral bands. Operculum corneous, pauci or multispiral (Figure 4-28).

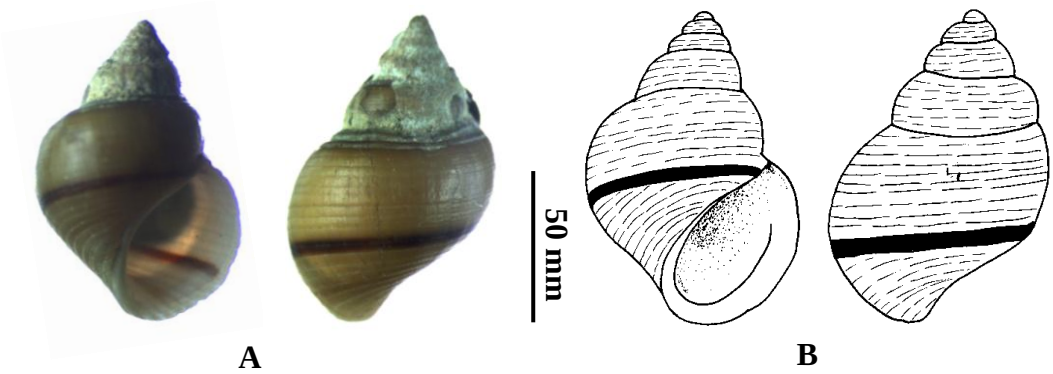


Figure 4-28. Shell characteristics of *Paludomus siamensis.* A: photograph, B: drawing

***Pomacea canaliculata* Lamark, 1819**

Phylum Mollusca

Class Gastropoda

Order Mesogastropoda

Family Ampullariidae

Genus *Pomacea*

Species *Pomacea canaliculata*

The shell of this snail species is rather big 153 - 489 (316) mm long, maximum width 105 – 336 (217) mm, globosely and relatively heavy. The 5 to 6 whorls are separated by a deep, indented suture. Aperture is large and oval to round. The umbilicus is large and deep.. The colour varies completely yellow and green to brown with or without dark spiral bands. The operculum is moderately thick and corneous. The structure is concentric with the nucleus near the centre of the shell. The colour varies light to dark brown. The operculum can be retracted in the aperture (Figure 4-29).

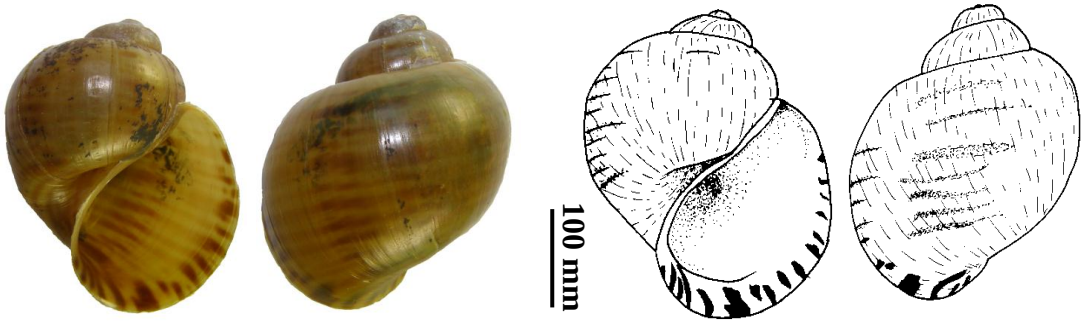


Figure 4-29. Shell characteristics of *Pomacea canaliculata*. A: photograph, B: drawing

Tarebia granifera* (Lamarck, 1822) Syn. *Melania granifera

Phylum Mollusca

Class Gastropoda

Order Mesogastropoda

Family Thiaridae

Genus Tarebia

Tarebia granifera

The shell is medium size 125 - 301 (174) mm long, maximum width 55 – 135 (95) mm, fusiform, and elongated. The shell color is olive or brownish. Apex point but usually eroded. The body whorl is large and measure about half the length of the shell. The sculpture consist the spiral grooves and axial ribs. The latter are crosses by to deep grooves, thus 3 spiral row of tubercle. The aperture of shell is oval with sharp peristome and curved columella. Operculum thin, corneous, and paucispiral with the basal nucleus (Figure 4-30).

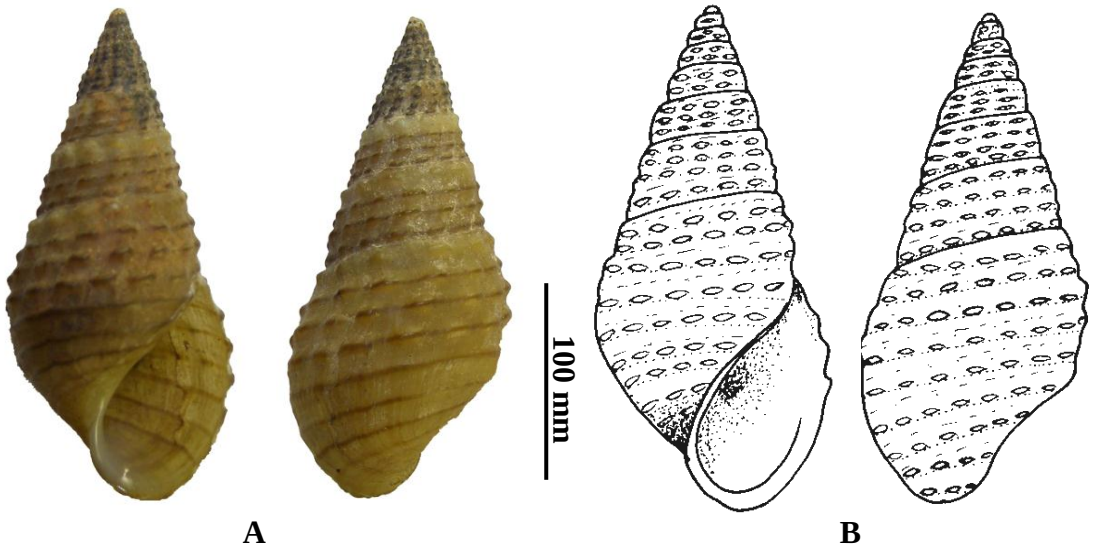


Figure 4-30. Shell characteristics of *Tarebia granifera*. A: photograph, B: drawing

***Thiara scabra* (O.F. Müller, 1774)**

Phylum Mollusca

Class Gastropoda

Order Mesogastropoda

Family Thiaridae

Genus *Thiara*

Thiara scabra

Shell is medium size 104 - 255 (163) mm long, maximum width 44 – 110 (69) mm, ovoid-conical shaped, with large body whorl. The color of shell is olive with the reddish-brown frames and sometime occur 1-3 brown color bands. Sculptured with fine spiral ridge and more or less strong ribs which are absolute on the lower half of the body whorl. These ribs is well develop and often produced into the subsutural spines. The aperture of shell is oval. Operculum thin, corneous, paucispiral with the basal nucleus (Figure 4-31).

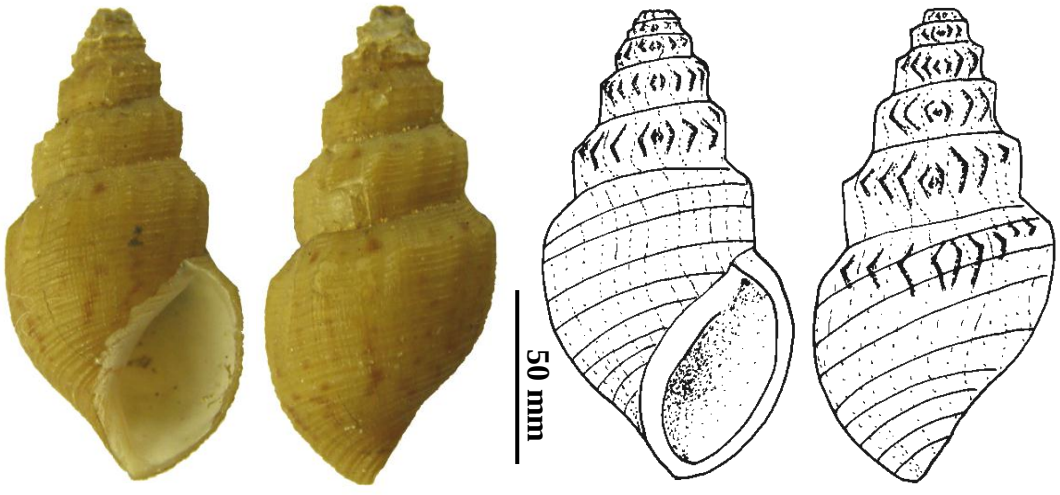


Figure 4-31. Shell characteristics of *Thiara scabra*. A: photograph, B: drawing

***Sinotaia mandahlbarthi* Brandt, 1968**

Phylum Mollusca

Class Gastropoda

Order Mesogastropoda

Family Viviparidae

Subfamily Bellamyinae

Genus *Sinotaia*

Sinotaia mandahlbarthi

Shell medium size 160 - 260 (206) mm long, maximum width 92 – 149 (118) mm, rather solid in old specimen even elongated conoidal, while young specimen are sub-globosely conical. The apex usually eroded, the 6(1/2) whorl are hardly round. Shell are ornate with five strong but obtuse spiral ridge on the periphery. Immature specimen may show a weak carina around the umbilicus. The umbilicus is either completely closed. Aperture milky-white within the peristome. Operculum thin (Figure 4-32).

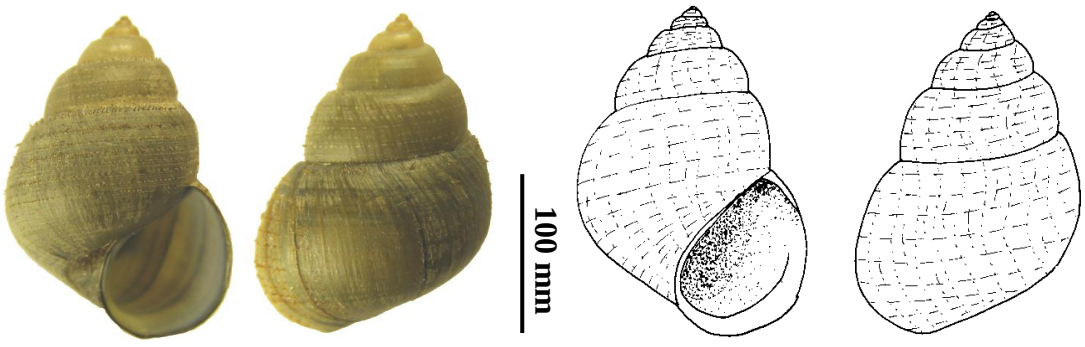


Figure 4-32. Shell characteristics of *Sinotaia mandahlbarthi*. A: photograph, B: drawing

Cercarial infection in intermediate host snails

Laboratory examination revealed that the 5 snail families, 12 snail species were infected by cercariae including, (1) Thiaridae: *Adamietta housei, Brotia citrina, Melanoides tuberculata, Tarebia granifera,* and *Thiara scabra* (2) Bithyniidae: *Bithynia funiculata* and *B. siamensis siamensis,* (3) Viviparidae: *Filopaludina doliaris, F. martensi martensi,* and *F. sumatrensis polygramma,* (4) Lymnaeidae: *Lymnaea auricularia rubiginosa,* and (5) Bullinidae: *Indoplanorbis exustus.* In addition, from the total number of 4,533 snails were collected form 12 provinces of northern Thailand, 664 individuals of 12 snail species in above mention were infected with an overall prevalence was 14.65%. The snail in family Thiaridae was indicated the most susceptible for the cercarial infection then the other snail families with the proportion of prevalence 72.74%. While, other snail families, Bithyniidae (16.87%), Viviparidae (5.27%), Bullinidae (4.81%), and Lymnaeidae (0.31%), respectively (Figure 4-33). Especially, *M. tuberculata, T. granifera, B. siamensis siamensis* and *B. funiculata* were shown a high susceptibility for cercarial infection.

A total of 11 morphology types were found, and divides into 14 separated triads base on morphologically distinguishable differences according to Schell (1970) (internal organ arrangement, place and number of sucker etc.) including distome cercaria, cercariae, gymnocephalous cercaria, megalurous cercaria, monostome cercaria, ophthalmoxiphidiocercaria, parapleurolophocercous cercaria Type I, parapleurolophocercous cercaria Type II, parapleurolophocercous cercaria Type III, pleurolophocercous cercaria, furcocercous cercaria Type I (*Transversotrema* cercaria), furcocercous cercaria Type II (strigea cercaria), xiphidiocercaria, and virgulate cercaria.

The study of cercarial infection in intermediate host snails was indicated the Chiang Mai province has many diverse of cercarial types (13 types), followed by Lamphun, Mae Hong Son and Lampang province (7 types), Uttaradit province (6 types), Chiang Rai, Tak, Phayoa and Phitsanulok province (4 types), Sukhothai and Nan province (3 Types), and Phrae province (2 types), respectively. Furthermore, the highest prevalence of infection was found in Chiang Mai followed by Lampang, Lamphun, Phitsanulok. Uttaradit, Mae Hong Son, Tak, Phrae, Nan, Chiang Rai, Phayao, and Sukhothai province, respectively (Figure 4-34).

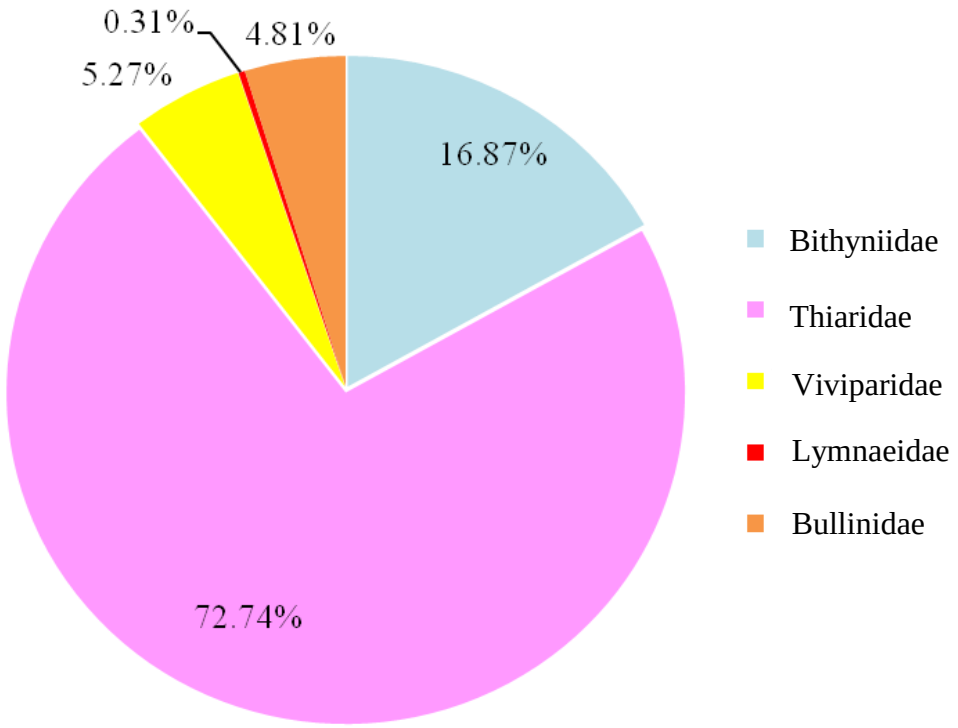


Figure 4-33. The proportion of cercarial infected of each snail families.

The xiphidiocercaria and parapleurolophocercous cercariae Type I were wildly occurred in northern Thailand. Whereas, the parapleurolophocercous cercaria type III was found only in *A. housei* from Lamphun province. As well as, the ophthalmoxiphidiocercaria was found in *I. exustus* from Mae Hong Son province only. Moreover, the three snail species including, *B. funiculata*, *Br. citrina*, and *F. sumatrensis polygramma* were infected by only xiphidiocercaria. The *Br. citrina* and *F. sumatrensis polygramma* were only infected with the xiphidiocercaria. Thus, it seems that these types of cercariae have a high specificity for infection in snail hosts.

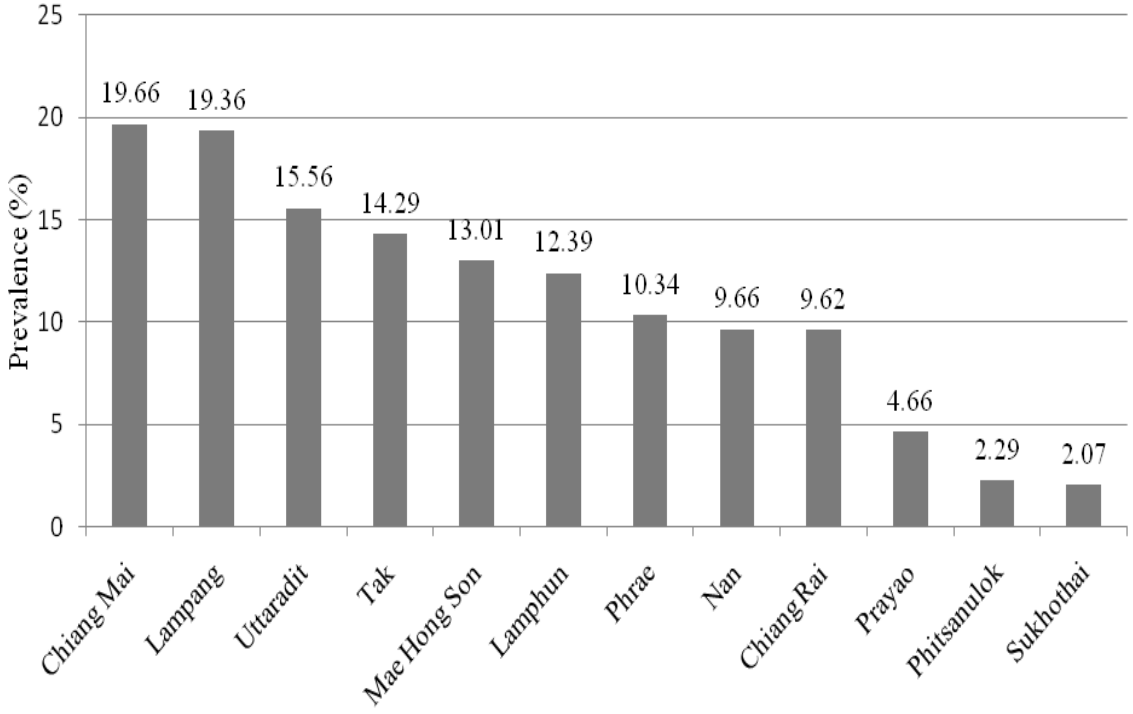


Figure 4-34. Cercarial infections of freshwater snails in northern Thailand.

From the Table 4-3 – Table 4-14, the dominant cercarial type which highly infections was parapleurophocercous cercaria Type I, this cercarial type was infected in 306 snail specimens followed by xiphidiocercaria (146 snails), megalurous cercaria (42 snails), pleurolophocercous cercaria (36 snails), gymnocephalous cercaria (30 snails), virgulate cercaria (28 snails), monostome cercaria (27 snail), and other cercarial type (49 snail), respectively. While seven type of cercarial (distome cercaria, furcocercous cercaria Type II, furcocercous cercaria Type III, parapleurophocercous cercaria Type II, parapleurophocercous cercaria Type III, and ophthalmoxiphidiocercaria) were infected with small number of snails. The concurrent infection of both former cercariae was found only in *B. siamensis siamensis* and *M. tuberculata*.

Bithynia siamensis siamensis were shown to be the suitable intermediate hosts for harboring a wide spectrum of cercarial infection (9 types) followed by the *M. tuberculata* (7 types.), *B. funiculata* (6 types), *T. granifera* (5 types), *Th. scabra* (4 types), *I. exustus* and *F. martensi martensi* (3 types), *F. doliaris* (2 types), and *A. housei*, *Br. citrina*, *F. sumatrensis polygramma* and *L. auricularia rubiginosa* were found only one type of cercaria, respectively.

Each type of cercaria were only identified to major type and more specific identification if possible. They were subsequently described as follows:

Distome cercaria

Snail host: *B. funiculata*

Locality: Chiang Mai and Lampang

The body of this cercaria is oval shape with thick tegument 95 - 108 (100) μm long, maximum width 92 – 102 (90) μm. The oral sucker is sub-terminal and the ventral sucker is close in size of the ventral sucker on the mid of body. The muscular pharynx led to the esophagus and bifurcated in front of the ventral to intestinal caeca which extend to just below the level of the ventral sucker. The body cavity was filled with dense cytogenous glands cell. The genital primordial is composed of a pear-shaped mass of cells localized immediately anterior to the ventral sucker. Simple tail 94 - 108 (97) μm. (Figure 4-35).

Figure 4-35. Morphological characteristic of distome cercaria. A: photograph from permanent slide, B: drawing

Cercariae (mutabile cercaria)

Snail host: *B. siamensis siamensis*

Locality: Chiang Mai

Cercariae is a mutabile cercarium type. The body slender, oval, and without the minute spines on their tegument 410 - 645 (495) μm long, maximum width 110 – 230 (152) μm. This cercaria possess two suckers (the anteriorly located oral sucker and mid-ventrally located the ventral sucker), and equal size of oral and ventral sucker. The digestive system consists of a mouth that lead into thin pre-pharynx which connects with an oval pharynx. Intestinal caeca extend posterior end of the body. This cercarial type is lack tail (Figure 4-36).

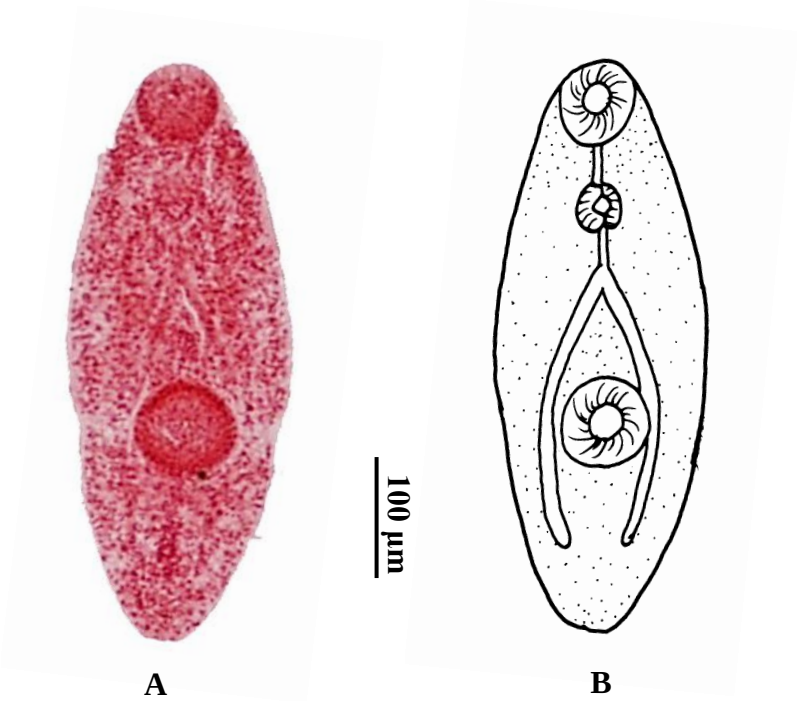


Figure 4-36. Morphological characteristic of cercariae. A: photograph from permanent slide, B: drawing.

Gymnocephalous cercaria: (Fasciolidae)

Snail host: *B. siamensis siamensis*, *I. exustus*, and *F. martensi martensi*

Locality: Chiang Mai, Chiang Rai, Mae Hong Son, Lamphun, Phitsanulok, and Sukhothai

The cercaria is ovoid shaped 200 - 243 (148) μm long, maximum width 104 – 148 (129) μm. Oral sucker sub-terminal. Esophagus bifurcates postero-lateral to ventral sucker and extended close to the posterior quarter of the body. The excretory vesicle is spherically - shaped. The main collecting duct ascends from antero-leteral wall of the vesicle, extending from the area of the pharynx and oral sucker. The excretory ducts contains a large granule. Ventral sucker, which is extremely protractible and larger than the oral sucker and, tends to be slightly posterior from the middle of its body. The tail is strongly developed and its length is almost as longer than body with dorso-ventral finfold 280 - 345 (332) μm (Figure 4-37).

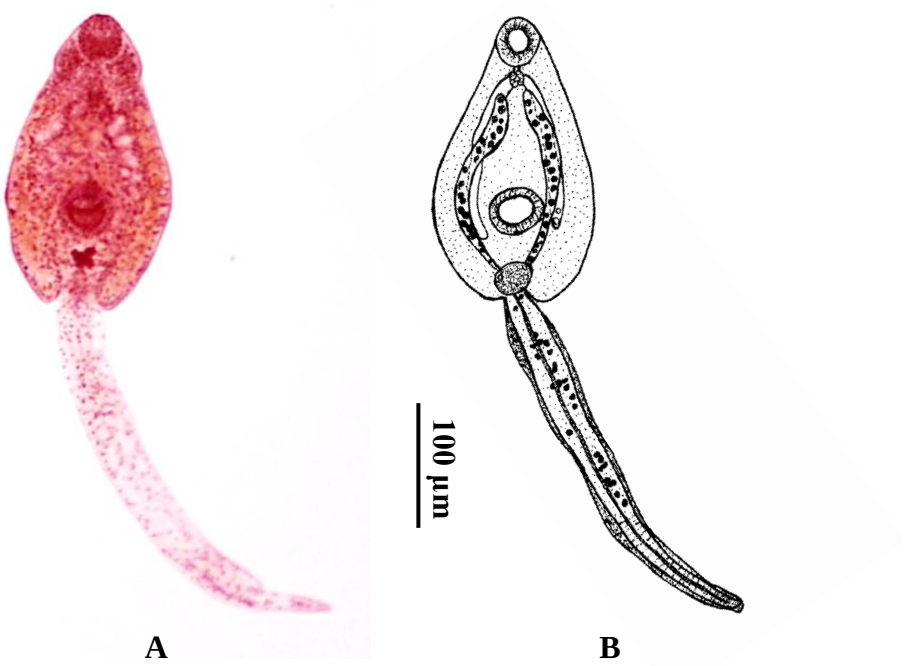


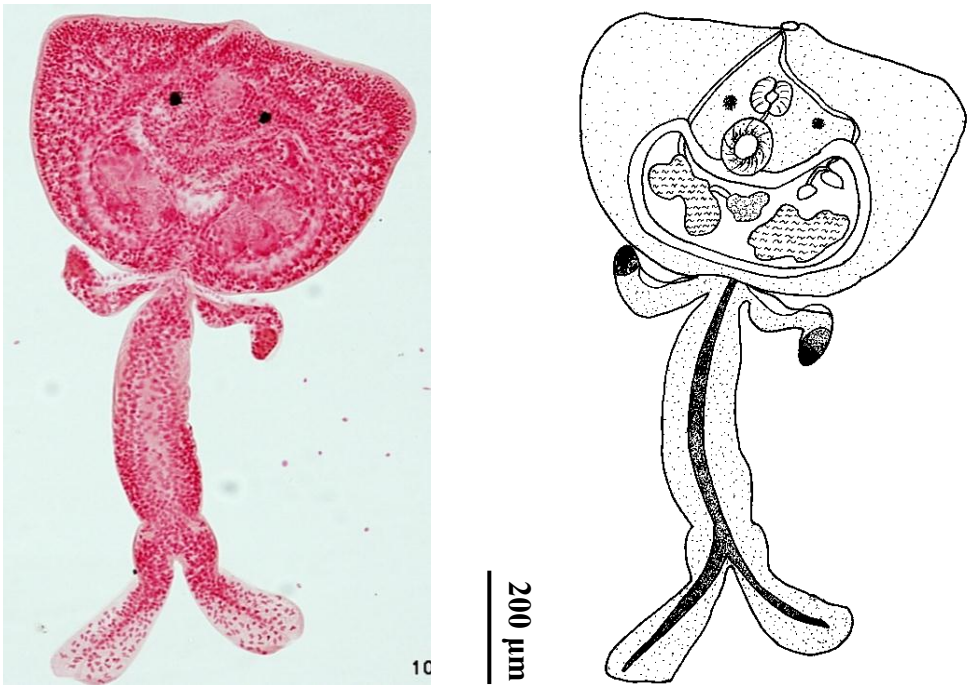
Figure 4-37. Morphological characteristic of gymnocephalous cercaria. A: photograph from permanent slide, B: drawing

Furcocercous cercaria Type I *(Transversotrema* cercaria)

Snail host: *M. tuberculata*

Locality: Chiang Mai, Mae Hong Son, Lamphun, Tak, and Uttaradit

Cercaria body is short 347 - 550 (468) μm long, maximum width 550 – 670 (688) μm, flat, and dish like shaped with a yellowish-brown pigment. The body surface is covered with scale-like spines. Its pharynx is very large, while the esophagus is short and narrow. A pair of eyespots is spherically large and located in the posterior position of the pharynx. An oral sucker is absent while a ventral sucker is present medially on its body. Testes are symmetrical and within the intestinal ring and the genital pore, and are located medially. The tail is longer than the body 600 - 780 (670) μm, and with arm-like processes at the anterior end of the tail-stem (Figure 4-38).



A **B**

Figure 4-38. Morphological characteristic of *Transversotrema* cercaria. A: photograph from permanent slide, B: drawing.

Furcocercous cercaria Type II (Strigea cercaria)

Snail host: *B. siamensis*, *B. funiculata*, *Lymnaea auricularia rubiginosa*, and *F. martensi martensi*

Locality: Chiang Mai, Lampang, Lamphun, and Phayao

The body is ovoid 300 - 400 (350) μm long, maximum width 160 – 220 (188) μm. The body surface is covered with minute spines and is shorter than the tail stem. Furcae is long-shape with a dorsal and ventral finfold. The pharynx is very small and circular. The esophagus bifurcates the ventral sucker. Genital primordial is spherical and located on posterior end of the body. Two pairs of penetration glands are locates between the ceaca posterior to ventral sucker. The tail is longer than the body 520 - 660 (570) μm, (Figure 4-39).

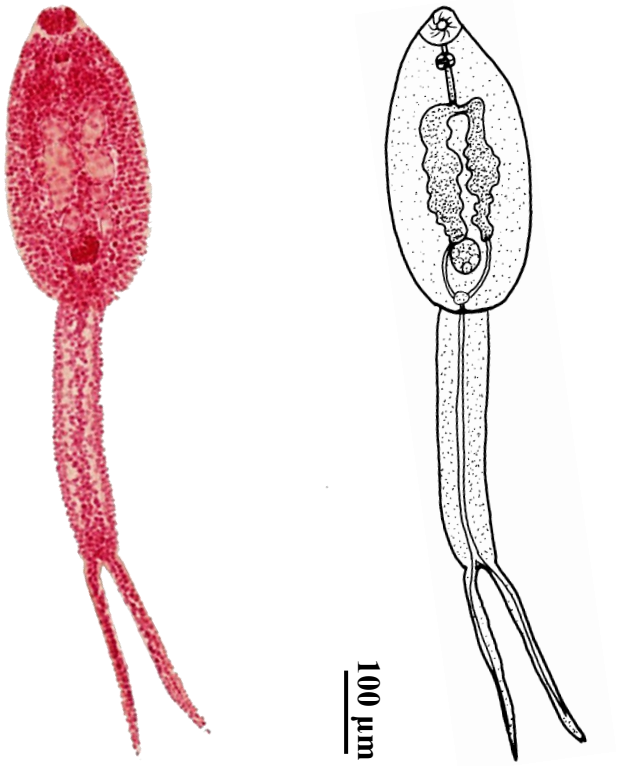


Figure 4-39. Morphological characteristic of strigea cercaria. A: photograph from permanent slide, B: drawing

Megalurous cercaria (Philophthalmidae)

Snail host: *A. housei, M. tuberculata, T. granifera,* and *Th. scabra*

Locality: Chiang Mai, Mae Hon Son, Lampang, Tak, and Uttaradit

The body is elongated with yellowish-brown granules 430 - 550 (483) μm long, maximum width 135 – 157 (142) μm. The width of the body is as the one-third from the body length. The sub-terminal oral sucker has the complex muscular apparatus. The bifurcated esophagus is located in the middle of the pharynx and ventral sucker, and the intestines ended blindly near the posterior end of body. The ventral sucker is slightly larger than the oral sucker and is, located medially on its body. The tail is elastic and slender 250 - 350 (300) μm. The tip of the long tail contains numerous adhesive gland cells and lacks an excretory canal at its tail base. Within an hour, these cercariae encyst on surface of the bottom of the container (Figure 4-40).

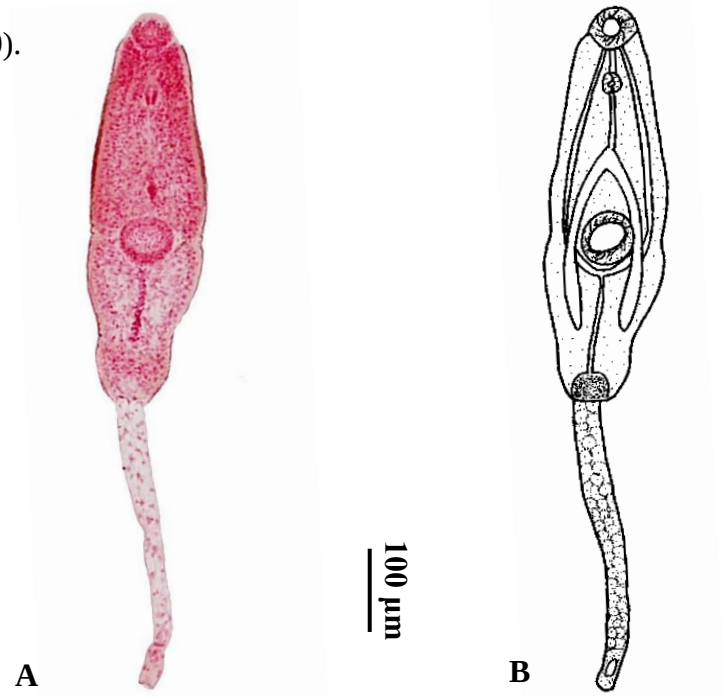


Figure 4-40. Morphological characteristic of megalurous cercaria. A: photograph from permanent slide, B: drawing

Monostome cercaria (Notocotylidae)

Snail hosts: *Bithynia siamensis siamensis*, *B. funiculata*, and *F. doliaris*

Locality: Chiang Mai, Lamphun, Lampang

The cercaria body is in contractile and oval when being relax 193 - 221 (207) μm long, maximum width 109 – 138 (132) μm. The whole body is transparent and the anterior quarter is deeply pigmented, particularly around the eyespots. The sub-terminal oral sucker is conspicuous while the ventral sucker is absent. There is a pair of black-pigmented eyespots in the front of the triangle in the anterior dorsal area of the mature cercaria. The genital primordial is composed of a pear-shaped mass cells localizes posterior end of the body. The tail has almost the same body length and slender 175 - 200 (185) μm (Figure 4-41).

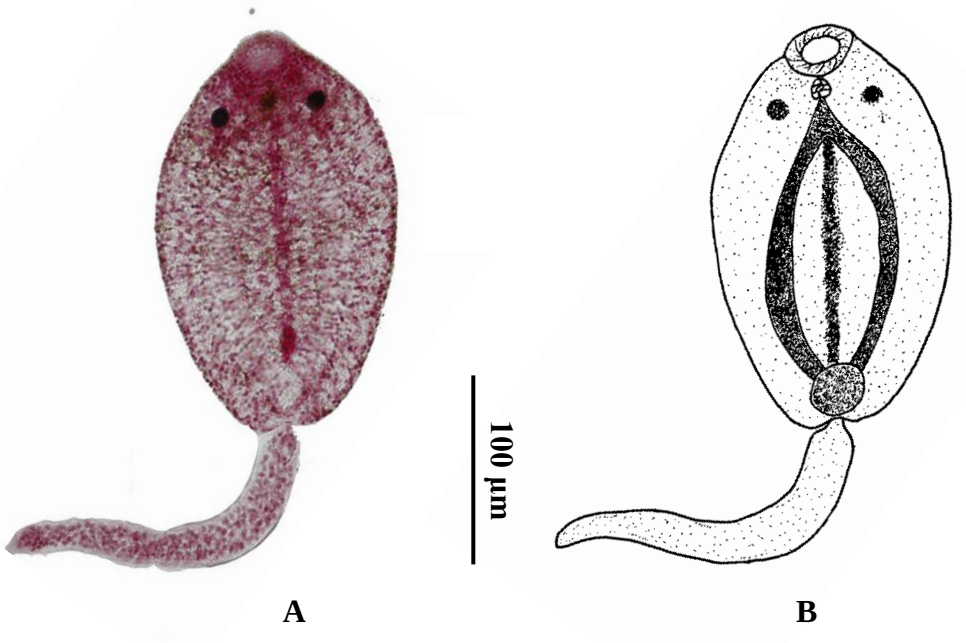


Figure 4-41. Morphological characteristic of monostome cercaria. A: photograph from permanent slide, B: drawing

Ophthalmoxiphidiocercaria

Snail hosts: *I. exustus*

Locality: Mae Hong Son

The body is oval 351 - 422 (401) μm long, maximum width 286 – 325 (310) μm. This cercarial type possess the stylet on the oral sucker and huge pigmented eye spots present. The large ventral sucker located on the posterior end of body. Pharynx near the oral sucker and lead bifurcated intestinal caeca. Tail simple, slender and length of tail equal the length of body 243 - 250 (265) μm (Figure 4-42).

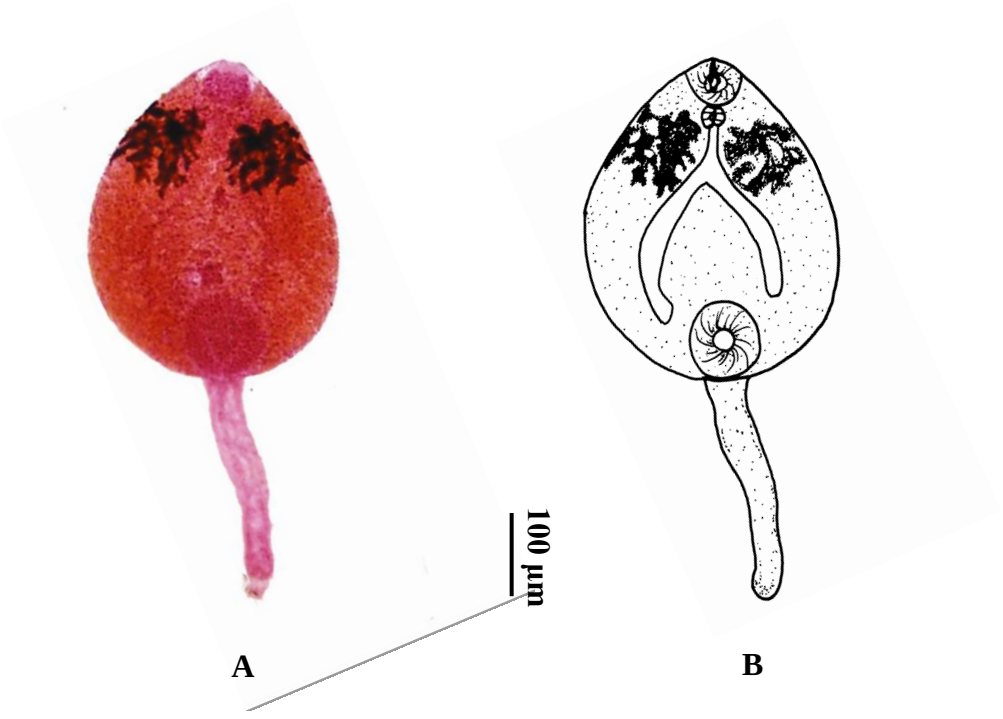


Figure 4-42. Morphological characteristic of Ophthalmoxiphidiocercaria. A: photograph from permanent slide, B: drawing

Parapleurolophocercous cercaria Type I

Snail hosts: *B. siamensis siamensis*, *M. tuberculata*, *T. granifera*, and *Th. scabra*

Locality: Chiang Mai, Chiang Rai, Mae Hon Son, Lampang, Lamphun, Nan, Phayoa, Phrae, Phitsanulok, Sukhothai, Tak, and Uttaradit

The cercaria body is in pear-shaped 114 - 155 (130) μm long, maximum width 50 – 65 (55) μm. Circular oral sucker is sub-terminal. The small pharynx lies just behind the oral sucker. Two eyespots are square in shape and located on each side below the pharynx. Seven pairs of oval penetration glands are located between the pharynx and the posterior part of body, mainly surrounding the genital primordial and arranged in two columns with the ventral sucker. Excretory vesicles are elongated. The long tail is attached to the dorsal end of the body 245 - 295 (265) μm, with lateral finfolds nearby and dorso-ventral finfolds extending along the posterior two thirds (Figure 4-43).

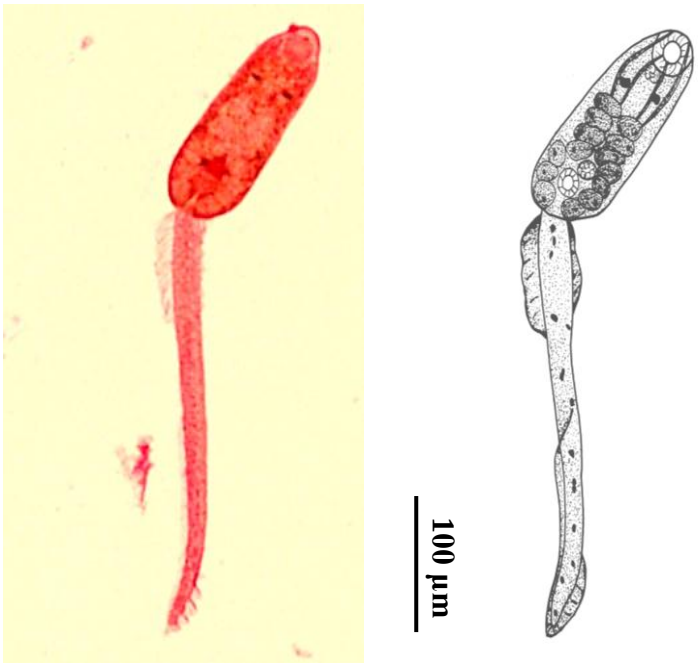


Figure 4-43. Morphological characteristic of parapleurolophocercous cercaria Type I. A: photograph from permanent slide, B: drawing

Parapleurolophocercous cercaria Type II

Snail hosts: *M. tuberculata*

Locality: Chiang Mai, and Uttaradit

This cercarial type is similar to the parapleurolophocercous cercaria Type I 175 - 250 (208) μm long, maximum width 66 – 89 (83) μm, except its excretory bladder is y-shaped. Two eyespot pigments are square in shape and located on each side at level of the pharynx. Seven pairs of lobe penetration glands are located between the pharynx and the posterior part of body, mainly surrounding the genital primordial and arranged in two columns. The long tail is attached to the dorsal end of the body 285 - 420 (340) μm., with lateral finfolds and dorso-ventral finfolds extending along the posterior two thirds (Figure 4-44).

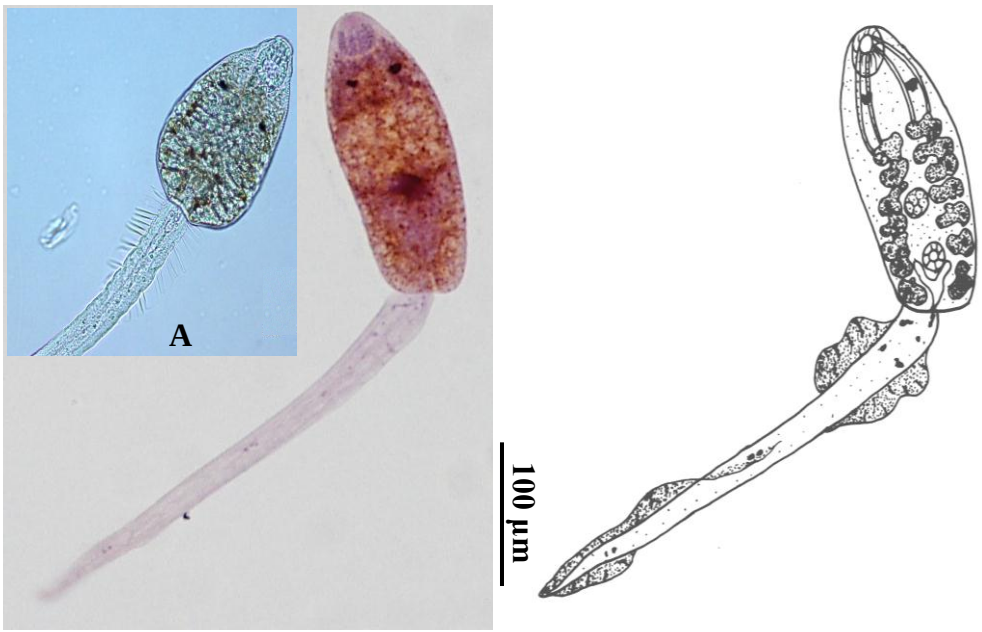


Figure 4-44. Morphological characteristic of parapleurolophocercous cercaria Type II. A: Alive specimen, B: photograph from permanent slide, C: drawing

Parapleurolophocercous cercaria Type III

Snail hosts: *A. housei*

Locality: Lamphun

. The cercaria body is in oval-shaped 190 - 260 (220) μm long, maximum width 105 – 135 (120) μm, which is entirely covered with minute spines. Circular oral sucker is sub-terminal. The small pharynx lies just behind the oral sucker. Two eyespots are square in shape and located on each side below the pharynx (It can see in alive specimens). Seven pairs of penetration glands are located between the pharynx and the posterior part of body, mainly surrounding the genital primordial and arranged in two columns with the ventral sucker. The long tail is attached to the dorsal end of the body 380 - 520 (440) μm, with lateral finfolds nearby and dorso-ventral finfolds extending along the posterior two thirds. The tail surface is covered with longitudinal and fine traverse wrinkles (Figure 4-45).

A **B**

Figure4-45. Morphological characteristic of parapleurolophocercous cercaria Type III. A: photograph from permanent slide, B: drawing

Pleurolophocercous cercariae: (Heterophyidae)

Snail hosts: *M. tuberculata,* and *Tarebia granifera*

Locality: Chiang Mai, Chiang Rai, Phayao, and Uttaradit

The cercaria is ovoid shaped 74 - 105 (91) μm long, maximum width 53 – 83 (73) μm. The oral sucker is sub-terminal. A pair of rectangular conspicuous eyespots is found at the end of the anterior third of the body. The pharynx lies just behind the oral sucker. Intestinal ceaca bifurcate and extend posterior to the excretory vesicle level. Seven pairs of penetration glands are arranged in 2 bundles and are antero-lateral to the genital primordial which is a relatively large triangular mass of cells that is located just anterior to the excretory vesicle. The cytogenous glands are numerous and lie on the later site of the body. The tail is slender 105 - 140 (128) μm, and usually attached to the dorso-ventral finfolds. A brownish pigment is dispersed throughout the body except in the oral sucker (Figure 4-46).

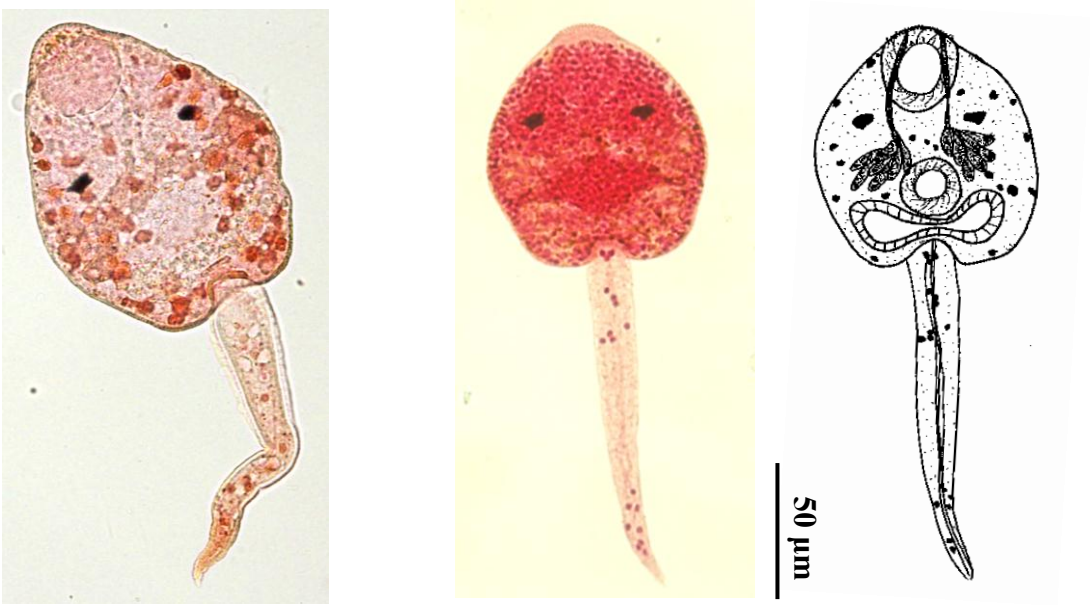


Figure 4-46. Morphological characteristic of pleurolophocercous cercaria. A: alive specimen stained with neutral red, B: photograph from permanent slide, C: drawing

Xiphidiocercaria (Plagiorchiidea)

Snail hosts: *A. housei, M. tuberculata, T. granifera, Th. scabra B. funiculata, B. siamensis siamensis, F. doliaris, F. martensi martensi, F. sumatrensis polygramma, Br. citrina,* and *I. exustus.*

Locality: Chiang Mai, Chiang Rai, Mae Hon Son, Lampang, Lamphun, Nan, Phayoa, Phrae, Phitsanulok, Sukhothai, Tak, and Uttaradit

The body of this cercaria is oval-shaped 96 - 134 (106) μm long, maximum width 48 – 73 (58) μm, colorless and spinose. The oral sucker is circular and its stylet is inserted into the roof of the oral sucker. The ventral sucker is post-equatorial, rounded and smaller than the oral sucker. Pre pharynx is very short. Genital primordial is globular. The tail is slender 112- 140 (119) μm (Figure 4-47).

Figure 4-47. Morphological characteristic of xiphidiocercaria. A: alive specimen, B: photograph from permanent slide, C: drawing

Virgulate cercaria (Lecithodendriidae)

Snail host: *T. granifera, Th. scabra B. funiculata,* and *B. siamensis siamensis*

Locality: Chiang Mai, Mae Hong Son, Lampang, Nan, and Phitsanulok

The cercaria is small in size with a prominent stylet developing in sporocysts 95 - 116 (103) μm long, maximum width 67 – 82 (73) μm. The oral sucker is in the back, close to the pharynx. The esophagus bifurcates into the anterior part of the ventral sucker and encircles the intestinal caeca. The ventral sucker is terminated postacetabularly. The ventral sucker is smaller than the oral sucker. The virgulate organ is butterfly-shaped and, situated below the stylet. Additionally, here are 3 pairs of penetration glands and two anterior pairs with fine granules. A posterior pair with coarser granules is positioned medially on the body. The tail is shorter than its body 72 - 97 (83) μm (Figure 4-48).

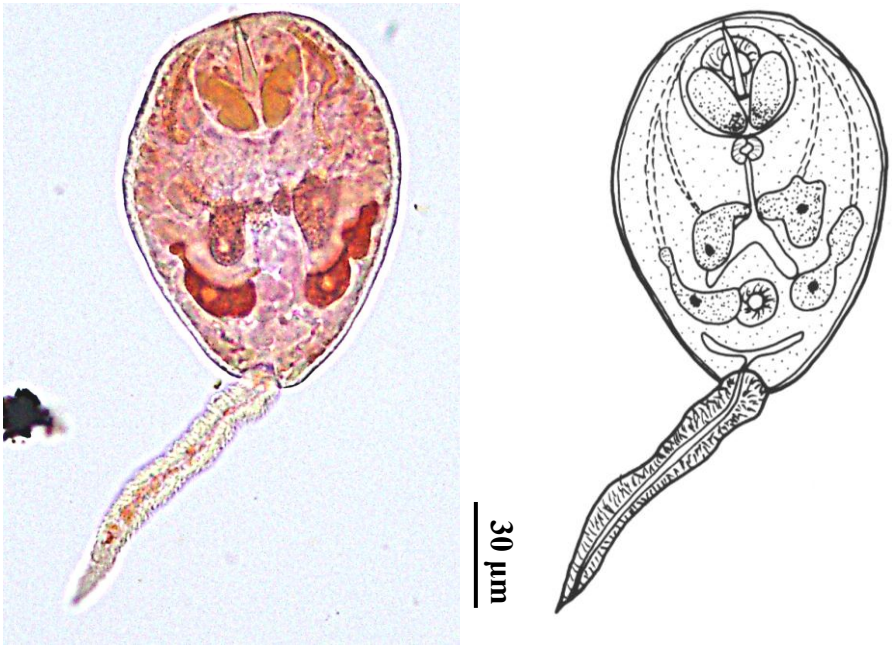


Figure 4-48. Morphological characteristic of virgulate cercaria. A: photograph from alive specimen stained with neutral red, B: drawing

Table 4-3. Composition of the snail species and number of cercarial infections in Chiang Mai province.

Sites	Snail species	No. of snails	Cercariae		Prevalence (%)
			Type	No. of infection	
CM-01	*M. tuberculata*	30	Xiphidiocercaria	1	3.33
(19° 6' 37.317"N 99°4' 33.439"E)			Megalurous	2	6.66
			Parapleurolophocercous Type I	10	33.33
			Parapleurolophocercous Type II	1	3.33
			Pleurolophocercous	2	6.66
			Xiphidio + pleurophocercous	1	3.33
	L. a. rubiginosa	48	-	-	-
	T. granifera	4	-	-	-
	B. s. siamensis	14	Monostome	2	14.29
	Th. scabra	30	Megalurous	5	16.67
CM-02	*T. granifera*	30	Parapleurolophocercous Type I	2	6.66
(19° 5'54.30"N 99°4'52.87"E)			Xiphidiocercaria	2	6.66
	M. tuberculata	4	Parapleurolophocercous Type I	4	100.00
	Th. scabra	2	parapleurolophocercous Type I	2	100.00
CM-03	*Br. citrina*	30	-	-	-
(19° 3'05.23"N 98°9'40.617"E)	*M. tuberculata*	19	Parapleurolophocercous Type I	9	47.37
			Pleurolophocercous	2	10.53
	Br. wykoffi	5	-	-	-
CM-04	*Br. citrina*	30	-	-	-
(19°22'51.51"N 98°58'2.88"E)	*M. tuberculata*	25	Xiphidiocercaria	1	4.00
			Parapleurolophocercous Type I	3	12.00
			Megalurous	4	16.00
			Furcocercous Type I	1	4.00

Table 4-3. (Cont.)

Sites	Snail species	No. of snails	Cercariae		Prevalence (%)
			Type	No. of infection	
CM-05	*M. tuberculata*	30	-	-	-
(19° 22'57.149"N 98°58'5.699"E)	*T. granifera*	39	Parapleurolophocercous Type I	4	10.26
			Pleurolophocercous	1	2.56
			Megalurous	1	2.56
			Xiphidiocercaria	1	2.56
CM-06	*T. granifera*	25	Parapleurolophocercous Type I	9	36.00
(18°42'27.00"N 98°56'5.68"E)			Xiphidiocercaria	2	8.00
	C. helena	6	-	-	-
	F. m. martensi	2	-	-	-
	M. tuberculata	13	Parapleurolophocercous Type I	1	7.69
	F. m. martensi	2	-	-	-
	B. s. siamensis	30	-	-	-
	B. funiculata	30	Xiphidiocercaria	7	23.33
			Furcocercous Type II	1	3.33
			Distome	4	13.33
			Virgulate	1	3.33
CM-07	*M. tuberculata*	5	Parapleurolophocercous Type I	2	40.00
(18°41'10.25"N 98°56'16.57"E)	*F. m. martensi*	1	-	1	100.00
	T. granifera	30	Parapleurolophocercous Type I	1	3.33
			Xiphidiocercaria	2	6.66
			Virgulate	4	13.33
			Megalurous	1	3.33
	Th. scabra	30	Megalurous	2	6.66

Table 4-3. (Cont.)

Sites	Snail species	No. of snails	Cercariae		Prevalence (%)
			Type	No. of infection	
CM-08	*B. s. siamensis*	30	Parapleurolophocercous Type I	2	6.66
(18°33'59.54"N 98°52'5.61"E)			Furcocercous Type II	1	3.33
			Cercariae	3	33.33
	T. granifera	30	Parapleurolophocercous Type I	14	46.67
	L. a. rubiginosa	3	-	-	-
	F. m. martensi	6	-	-	-
	M. tuberculata	30	Parapleurolophocercous Type I	13	43.3
			Furcocercous Type I	1	3.33
			Parapleu Type I + Furco Type I	1	3.33
	B. funiculata	6	Xiphidiocercaria	2	33.33
	Th. scabra	8	-	-	-
CM-09	*B. funiculata*	6	Furcocercous Type II	1	16.67
(18°27'0.40"N 98°42'40.99"E)	*B. s. siamensis*	21	Xiphidiocercaria	1	4.76
			Furcocercous Type II	1	4.76
CM-10	*Th. scabra*	16	-	-	6.2
(18°28'30.72"N 98°39'3.97"E)	*T. granifera*	9	-	-	-
CM-11	*B. s. siamensis*	30	Xiphidiocercaria	4	13.33
(18°28'36.25"N 98°47'45.54"E)	*F. m. martensi*	4	-	-	-
	C. helena	30	-	-	-

Table 4-3. (Cont.)

Sites	Snail species	No. of snails	Cercariae		Prevalence (%)
			Type	No. of infection	
CM-12	*F. s. polygramma*	16	-	-	-
(18°42'18.55"N 99° 2'16.62"E)	*F. m. martensi*	8	-	-	-
	B. s. siamensis	16	-	-	-
	M. tuberculata	29	Parapleurolophocercous Type I	11	37.39
			Pleurolophocercous	4	13.79
			Xiphidiocercaria	2	6.90
	C. helena	30	-	-	-
CM-13	*F. m. martensi*	8	-	-	-
(18°27'2.91"N 98°40'27.99"E)	*B. s. siamensis*	16	-	-	-
	M. tuberculata	29	Pleurolophocercous	3	10.34
			Parapleurolophocercous Type I	12	41.38
			Xiphidiocercaria	2	6.90
	C. helena	30	-	-	-
CM-14	*M. tuberculata*	30	Parapleurolophocercous Type I	13	43.33
(18°55'4.56"N 99° 7'44.04"E)			Xiphidiocercaria	3	10.00
			Parapleu Type I + xiphidio	1	3.33
	T. granifera	30	Parapleurolophocercous Type I	4	13.33
	C. helena	7	-	-	-
CM-15	*F. m. martensi*	30	-	-	-
(18°55'31.46"N 99° 8'13.93"E)	*B. s. siamensis*	10	Xiphidiocercaria	2	20.00
			Parapleu Type I + xiphidio	1	10.00
			Gymnocephalous	4	40.00
	M. tuberculata	8	-	-	-

Table 4-3. (Cont.)

Sites	Snail species	No. of snails	Cercariae		Prevalence (%)
			Type	No. of infection	
CM-16	*T. granifera*	30	Parapleurolophocercous Type I	9	30.00
(19°54'33.28"N 99°17'19.23"E)	*Th. scabra*	30	-	-	-
	L. a. rubiginosa	30	-	-	-
CM-17	*L. a. rubiginosa*	9	-	-	-
(19°55'1.26"N 99°13'11.29"E)	*M. tuberculata*	30	Parapleurolophocercous Type I	2	6.66
	I. exustus	3	-	-	-
CM-18	*T. granifera*	30	Parapleurolophocercous Type I	24	80.00
(19°57'25.74"N 99°11'18.68"E)			Xiphidiocercaria	2	6.66
			Megalurous	1	3.33
	M. tuberculata	30	Parapleurolophocercous Type I	8	26.67
			Pleurolophocercous	1	3.33
	Th. scabra	2	-	-	-
CM-19	*B. s. siamensis*	30	Cercariae	3	10.00
(18°44'52.47"N 98°56'57.39"E)	*F. m. martensi*	30	-	-	-
	M. tuberculata	6	Pleurolophocercous	2	33.33
			Megalurous	1	16.67
	B. funiculata	12	-	-	-

Table 4-4. Composition of the snail species and number of cercarial infections in Chiang Rai province.

Sites	Snail species	No. of snails	Cercariae		Prevalence (%)
			Type	No. of infection	
CR-01	*M. tuberculata*	27	-	-	-
(19°38'43.56"N 99°31'36.52"E)	*T. granifera*	30	Pleurolophocercous	6	13.33
	Th. scabra	2	Parapleurolophocercous Type I	2	100.00
	F. doliaris	3	-	-	-
	Po. canaliculata	1	-	-	-
CR-02	*F. doliaris*	21	-		-
(20°10'34.37"N 100°26'14.57"E)	*E. eyriesi*	2	-	-	-
	B. s. siamensis	5	-	-	-
CR-03	*M. tuberculata*	30	-	-	-
(19°11'33.95"N 99°30'48.43"E)	*I. exustus*	26	Gymnocephalous	6	27.27
	B. funiculata	15	-	-	-
	P. canaliculata	6	-	-	-
	F. m. martensi	3	-	-	-
	B. s. siamensis	10	Xiphidiocercaria	5	50.00
CR-04	*B. s. siamensis*	30	Xiphidiocercaria	4	13.33
(19°50'58.77"N 100° 8'59.98"E)	*F. m. martensi*	13	-	-	-
CR-05	*Br. wykoffi*	2	-	-	-
(19°49'53.74"N 100°15'31.36"E)	*Br. costula costula*	7	-	-	-
	E. eyriesi	6	-	-	-

Table 4-5. Composition of the snail species and number of cercarial infections in Mae Hong Son province.

Sites	Snail species	No. of snails	Cercariae		Prevalence (%)
			Type	No. of infection	
MA-01	*T. granifera*	30	Virgulate	8	26.67
(19°31'22.13"N 98°14'43.73"E)					
MA-02	*I. exustus*	21	Xiphidiocercaria	1	4.76
(19°25'27.06"N 97°59'19.84"E)	*M. tuberculata*	30	Megalurous	3	10.00
	Th. scabra	2	-	-	-
	T. granifera	30	Virgulate	1	3.33
MA-03	*I. exustus*	3	Gymnocephalous	2	66.67
(19°23'47.09"N 97°56'43.15"E)	*M. tuberculata*	14	-	-	-
	T. granifera	12	Virgulate	4	33.33
	Th. scabra	9	Furcocercous Type I	3	33.33
	F. m. martensi	8	-	-	-
MA-04	*I. exustus*	30	Ophthalmoxiphidiocercaria	5	16.67
(19°22'45.62"N 98°25'53.12"E)	*L. a. rubiginosa*	14	-	-	-
MA-05	*T. granifera*	4	Parapleurolophocercous Type I	8	25.00
(19°21'21.37"N 98°26'50.40"E)	*M. tuberculata*	30	-	-	-
	Th. scabra	30	-	-	-
	F. m. martensi	2	-	-	-

Table 4-6. Composition of the snail species and number of cercarial infections in Lampang province.

Sites	Snail species	No. of snails	Cercariae		Prevalence (%)
			Type	No. of infection	
LA-01	*B. s. siamensis*	26	Monostome	2	7.69
(18°45'56.20"N 99°14'20.90"E)	*B. funiculata*	30	Furcocercous Type II	1	3.33
	M. tuberculata	30	Parapleurolophocercous Type I	15	50.00
			Virgulate	2	6.66
LA-02	*B. s. siamensis*	23	Distome	4	17.39
(18°19'37.24"N 99°16'51.76"E)	*B. funiculata*	20	Xiphidiocercaria	8	30.00
	F. doliaris	3	Monostome	3	100.00
	F. m. martensi	3	-	-	-
	I. exustus	2	-	-	-
	B. funiculata	30	Monostome	13	43.33
			Megalurous	1	3.33
	T. granifera	30	Xiphidiocercaria	2	6.66
LA-03	*M. tuberculata*	30	-	-	-
(17°29'35.59"N 99°11'36.93"E)	*T. granifera*	30	Virgulate	4	13.33
	F. m. martensi	30	Xiphidiocercaria	15	50.00
	F. doliaris	15	Xiphidiocercaria	6	40.00

Table 4-6. (Cont.)

Sites	Snail species	No. of snails	Cercariae		Prevalence (%)
			Type	No. of infection	
LA-04	*B. s. siamensis*	30	Monostome	6	20.00
(18°18'27.15"N 99°31'9.29"E)	*F. m. martensi*	8	-	-	-
	M. tuberculata	30	Parapleurolophocercous Type I	11	36.67
			Megalurous	3	10.00
	T. granifera	30	Xiphidiocercaria	2	6.66
LA-05	*F. m. martensi*	7	-	-	-
(18°19'13.11"N 99°20'49.97"E)	*M. tuberculata*	30	Pleurolophocercous	10	33.33
			Xiphidiocercaria	1	3.33
	T. granifera	30	Parapleurolophocercous Type I	4	13.33
	F. doliaris	30	-	-	
LA-06	*B. s. siamensis*	30	Monostome	1	3.33
(17°26'43.03"N 99° 7'40.28"E)	*F. m. martensi*	30	-	-	-
	T. granifera	7	Parapleurolophocercous Type I	1	14.29
	I. exustus	30	-	-	-

Table 4-7. Composition of the snail species and number of cercarial infections in Lamphun province.

Sites	Snail species	No. of snails	Cercariae		Prevalence
			Type	No. of infection	
LU-01	*A. housei*	30	Parapleurolophocercous Type III	1	-
(18°35'3.35"N 98°59'32.47"E)	*F. m. martensi*	30	Furcocercous Type II	2	6.67
LU-02	*C. helena*	30	-	-	-
(18°35'31.46"N 99° 4'52.67"E)	*M. tuberculata*	30	Parapleurolophocercous Type I	11	36.67
			Xiphidiocercaria	3	10.00
			Furcocercous Type I	1	3.33
	F. m. martensi	30	Gymnocephalous	2	3.33
LU-03	*F. m. martensi*	30	-	-	-
(18°38'57.15" N 99° 6'33.62"E)	*I. exustus*	10	-	-	-
	B. s. siamensis	30	Monostome	1	3.33
			Xiphidiocercaria	8	26.67
	B. funiculata	30	Xiphidiocercaria	6	20.00
LU-04	*E. eyriesi*	30	-	-	-
(18°32'32.23"N 98°56'21.32"E)	*F. doliaris*	30	-	-	-
	F. m. martensi	30	Xiphidiocercaria	1	3.33
	F. s. polygramma	30	-	-	-
	L. a. rubiginosa	30	-	-	-
	M. tuberculata	30	Parapleurolophocercous Type I	6	20.00
	T. granifera	30	Parapleurolophocercous Type I	15	50.00

Table 4-8. Composition of the snail species and number of cercarial infections in Nan province.

Sites	Snail species	No. of snails	Cercariae		Prevalence
			Type	No. of infection	
NA-01	*B. s. siamensis*	30	Virgulate	2	6.67
(18°34'58.88"N 100°44'53.09"E)	*L. a. rubiginosa*	2	-	-	-
	F. m. martensi	29	-	-	-
NA-02	*M. tuberculata*	30	Parapleurolophocercous Type I	9	30.00
(19° 7'13.99"N 100°48'35.27"E)			Xiphidiocercaria	3	10.00
	F. m. martensi	18	-	-	-
NA-03	*T. granifera*	30	Parapleurolophocercous Type I	5	16.67
(19°10'42.86"N 100°52'31.80"E)	*L. a. rubiginosa*	39	-	-	-
NA-04	*I. exustus*	30	Xiphidiocercaria	3	10.00
(19°10'13.71"N 100°56'2.83"E)	*T. granifera*	30	Parapleurolophocercous Type I	1	3.33

Table 4-9. Composition of the snail species and number of cercarial infections in Phayao province.

Sites	Snail species	No. of snails	Cercariae		Prevalence (%)
			Type	No. of infection	
PY-01	*T. granifera*	30	-	-	-
(19° 9'46.32"N 99°54'5.68"E)	*C. helena*	16	-	-	-
	F. doliaris	7	-	-	-
	F. s. polygramma	5	-	-	-
	F. m. martensi	30	-	-	-
PY-02	*B. s. siamensis*	2	-	-	-
(19°11'40.56"N 99°53'48.02"E)	*I. exustus*	3	-	-	-
	M. tuberculata	30	Parapleurolophocercous Type I	4	13.33
	F. m. martensi	30	-	-	-
PY-03	*M. tuberculata*	30	Pleurolophocercous	1	3.33
(19° 7'43.54"N 99°54'31.08"E)			xiphidiocercaria	4	13.33
	L. a. rubiginosa	30	Furcocercous Type II	2	6.67
	I. exustus	8	-	-	-
	F. doliaris	5	-	-	-
	B. s. siamensis	10	-	-	-

Table 4-10. Composition of the snail species and number of cercarial infections in Phrae province.

Sites	Snail species	No. of snails	Cercariae		Prevalence (%)
			Type	No. of infection	
PH-01	*F. m. martensi*	17	-	-	-
(18° 8'27.50"N 100°13'27.96"E)	*Br. citrina*	22	Xiphidiocercaria	4	18.18
	M. tuberculata	6	Xiphidiocercaria	5	83.34
PH-02	*Br. citrina*	30	-	-	-
(18° 9'49.39"N 100°10'3.41"E)	*C. helena*	9	-	-	-
	T. granifera	30	-	-	-
PH-03	*C. helena*	17	-	-	-
(18° 7'45.33"N 100°19'4.37"E)	*A .housei*	10	-	-	-
	M. tuberculata	16	-	-	-
	F. s. polygramma	4	-	-	-
	Pa. siamensis	8	-	-	-
	T. granifera	30	Xiphidiocercaria	10	33.33
PH-04	*M. tuberculata*	30	Parapleurolophocercous Type I	4	13.33
(18°18'22.01"N 100°16'28.32"E)	*B. s. siamensis*	3	Xiphidiocercaria	1	33.33

Table 4-11. Composition of the snail species and number of cercarial infections in Phitsanulok province.

Sites	Snail species	No. of snails	Cercariae		Prevalence (%)
			Type	No. of infection	
PI-01	*I. exustus*	30	-	-	
(16°42'29.58"N 100°14'46.37"E)	*B. s. siamensis*	13	Xiphidiocercaria	1	7.69
PI-02	*I. exustus*	22	Gymnocephalous	13	59.09
(16°56'23.56"N 100°20'50.57"E)	*F. doliaris*	17	-	-	-
	L. a. rubiginosa	3	-	-	-
	F. m. martensi	7	Gymnocephalous	1	14.29
	Me. swaisoni	4	-	-	-
PI-03	*F. m. martensi*	30	-	-	-
(16°58'31.44"N 100°33'30.65"E)	*B. s. siamensis*	18	Virgulate	2	11.11
	M. tuberculata	3	Parapleurolophocercous Type I	1	33.33
PI-04	*S. mandahlbarthi*	9	-	-	-
(16°45'52.47"N 100°12'17.44"E)	*M. tuberculata*	30	Parapleurolophocercous Type I	1	3.33
	F. m. martensi	9	Xiphidiocercaria	1	11.11
PI-05	*F. doliaris*	30	Xiphidiocercaria	2	6.66
(16°59'10.38"N 100°11'58.41"E)	*M. tuberculata*	30	Parapleurolophocercous Type I	24	80.00
	F. m. martensis	7	-	-	-

Table 4-12. Composition of the snail species and number of cercarial infections in Sukhothai province.

Sites	Snail species	No. of snails	Cercariae		Prevalence (%)
			Type	No. of infection	
SU-01	*I. exustus*	30	Gymnocephalous	1	3.33
(16°57'13.24"N 99°57'28.83"E)	*F. s. polygramma*	30	Xiphidiocercaria	1	3.33
	M. tuberculata	30	-	-	-
SU-02	*F. doliaris*	7	-	-	-
(17° 9'59.12"N 99°51'30.93"E)	*M. tuberculata*	29	Parapleurolophocercous Type I	1	3.45
	T. granifera	30	Xiphidiocercaria	1	3.33
	F. m. martensi	9	-	-	-
SU-03	*F. m. martensi*	30	-	-	-
(17°18'47.26"N 99°32'6.46"E)	*F. s. polygramma*	17	-	-	-
	I. exustus	30	Gymnocephalous	1	3.33

Table 4-13. Composition of the snail species and number of cercarial infections in Tak province.

Sites	Snail species	No. of snails	Cercariae		Prevalence (%)
			Type	No. of infection	
TA-01	*T. granifera*	30	Parapleurolophocercous Type I	11	36.67
(17°13'9.72"N 99° 2'35.87"E)	*B. s. siamensis*	27	Xiphidiocercaria	4	14.81
	M. tuberculata	30	Parapleurolophocercous Type I	7	23.33
			Furcocercous Type I	1	3.33
			Parapleu Type I + Furco TypeI	1	3.33
TA-02	*F. m. martensis*	16	-	-	-
(16°52'31.56"N 99° 7'45.78"E)	*B. s. siamensis*	30	Xiphidiocercaria	2	6.67
	C. helena	8	-	-	-
	F. s. speciosa	30	-	-	-
TA-03	*C. helena*	12	-	-	-
(17° 2'56.46"N 99° 4'37.18"E)	*L. a. rubiginosa*	11	-	-	-
	M. tuberculata	30	Megalurous	6	20.00

Table 4-14. Composition of the snail species and number of cercarial infections in Uttaradit province.

Sites	Snail species	No. of snails	Cercariae		Prevalence (%)
			Type	No. of infection	
UT-01	*M. tuberculata*	30	Parapleurolophocercous Type I	4	13.33
(17°38'30.14"N 100° 2'18.75"E)			Parapleurolophocercous Type II	1	3.33
	Th. scabra	28	Megalurous	5	17.85
UT-02	*M. tuberculata*	23	Pleurolophocercous	3	13.04
(17°35'33.54"N 100° 5'58.08"E)			Parapleurolophocercous Type I	5	21.74
			Xiphidiocercaria	7	30.43
	A. housei	25	-	0	0.00
	B. s. siamensis	30	-	0	0.00
	F. s. speciosa	1	-	0	0.00
	F. m. martensi	15	-	0	0.00
UT-03	*T. granifera*	26	Pleurolophocercous	1	3.85
(17°42'45.81"N 100° 7'36.27"E)			Megalurous	3	11.54
			Xiphidiocercaria	1	3.85
	M. tuberculata	30	Furcocercous Type I	5	16.67
	B. s. siamensis	17	-	0	0.00

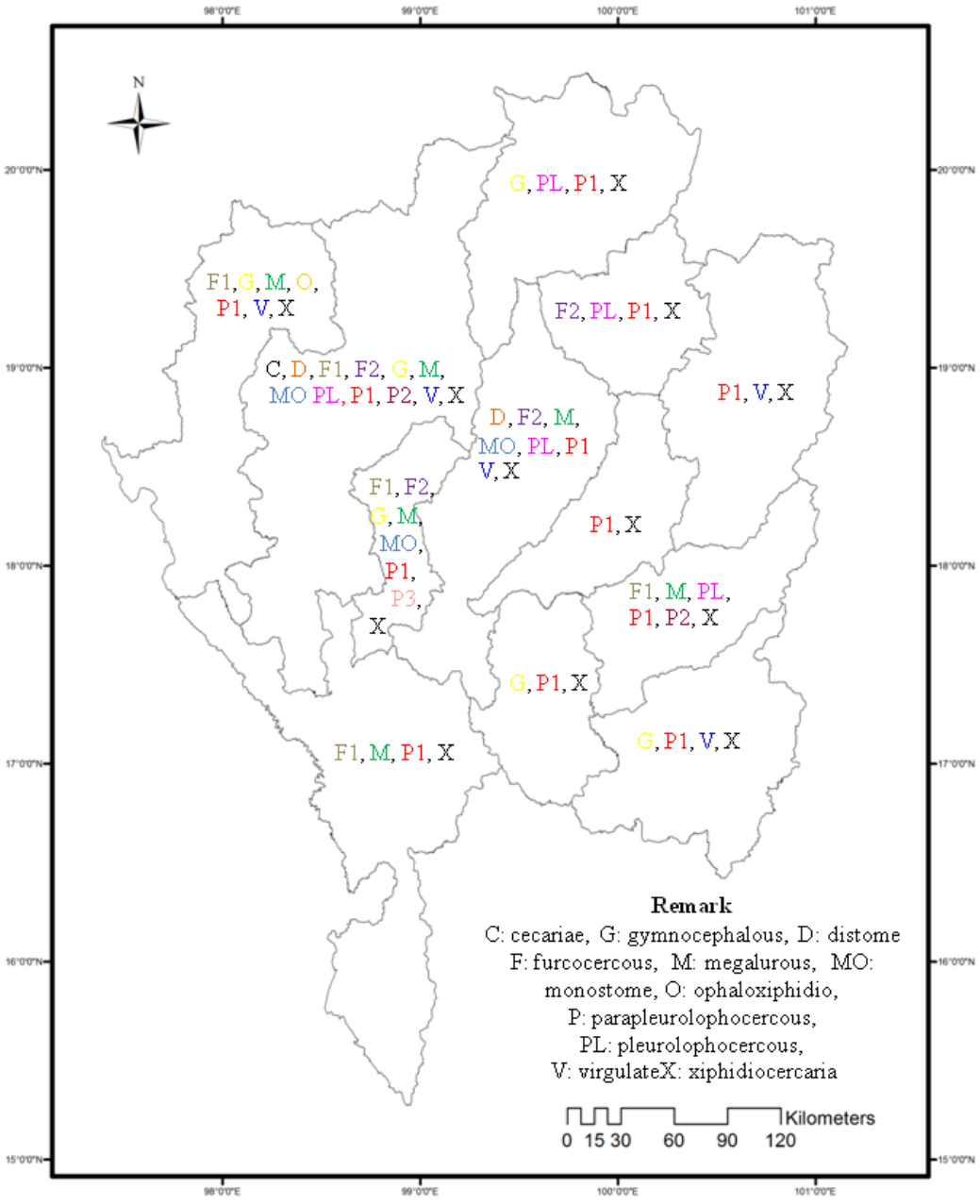


Figure 4-49. Overview of distribution of cercariae infected in freshwater snails in 12 provinces of northern Thailand.

Distome cercaria

Cercariae

Gymnocephalous cercaria

Furcocercous cercaria Type I

Figure 4-50. Distribution of each type of cercaria, which infected in freshwater snails in 12 provinces of northern Thailand. (The geographic coordinates as described in Table 3-1 and information of cercarial infection in Table 4-3 – Table 4-14).

Furcocercous cercaria Type II

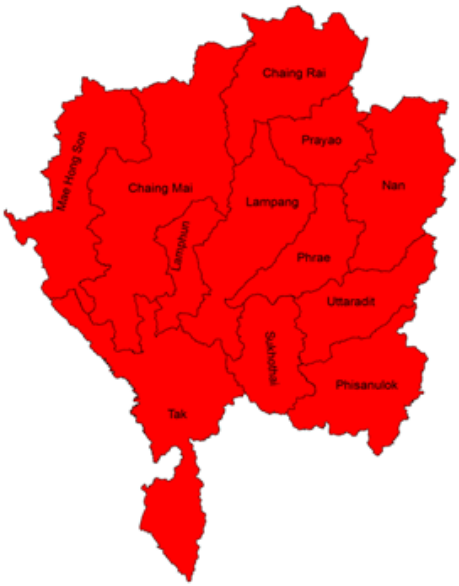
Megalurous cercaria

Monostome cercaria

Ophthalmoxiphidiocercaria

Figure 4-50. (Cont.)

Pleurolophocercous cercaria



Parapleurolophocercous cercaria Type I

Parapleurolophocercous cercaria Type II

Parapleurolophocercous cercaria Type III

Figure 4-50. (Cont.)

Xiphidiocercaria

Virgulate cercaria

Figure 4-50. (Cont.)

Development of DNA specific primer of *Haplorchis taichui* and *H. pumilio*

Ten species of trematodes were used in this study including *Haplorchis taichui*, *H. pumilio*, *Centrocestus caninus*, *Stellantchasmus falcatus*, *Haplorchoides* sp., *Ganeo tigrinus*, *Prostorchigenes majeedi*, *Orthocoelium streptocoelium*, *Fischoederius elongatus*, and *Fasciola gigantica*. The mtCOI fragment were amplified. Certain of mtCOI nucleotide amplification would reveal only the 470 bp fragment in all trematode species (Figure 4-51), and were obtained and submitted to Genbank.

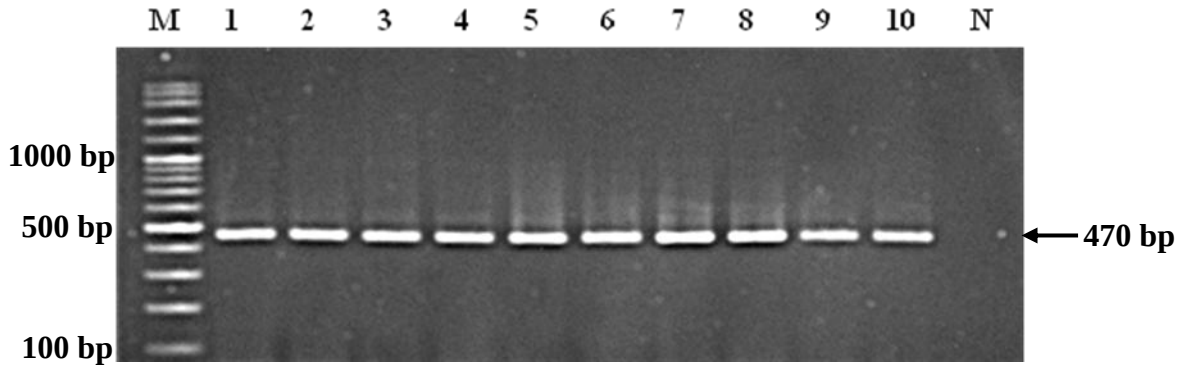


Figure 4-51. The mtCOI product of ten species trematodes. M: 100 bp DNA marker, 1: *Haplorchis taichui*, 2: *H. pumilio*, 3: *Centrocestus caninus*, 4: *Stellantchasmus falcatus*, 5: *Haplorchoides* sp., 6: *Ganeo tigrinus*, 7: *Prostorchigenes majeedi*, 8: *Fasciola gigantica*, 9: *Fischoederius elongatus*, 10: *Orthocoelium streptocoelium*, N: negative control

The specific DNA fragment of mtCOI was sequenced and analyzed data by BLAST (Basic Local Alignment Search Tool) program in the NCBI (National Center for Biotechnology Information) database. This sequence data revealed definitive identity matches in the range of 98%–99% for consensus sequences of *H. taichui* with 49 accession numbers of *H. taichui* that evadible on the NCBI databases as

demonstrated in Table 4-15. While, mtCOI sequence of *H. pumilio* indicated definitive identity matches in the range only 83.% with *H. taichui* (Table 4-16). Therefore, from this study, new sequence data of mtCOI of *H. pumilio* was submitted on NCBI databases.

Table 4-15. List of the sequences of mtCOI producing significant data of *H. taichui* from NCBI databases (http://www.ncbi.nlm.nih.gov/).

Accession number	Description	Max Ident
EF055885.1	*Haplorchis taichui*, coxI gene, partial cds; mitochondrial	99%
JN809867.1	*Haplorchis taichui* isolate HG11 (cox1) gene mitochondrial	99%
JN809866.1	*Haplorchis taichui* isolate HG12 (cox1) gene mitochondrial	99%
JN809865.1	*Haplorchis taichui* isolate HG15 (cox1) gene mitochondrial	99%
JN809864.1	*Haplorchis taichui* isolate HG9 (cox1) gene mitochondrial	99%
JN809863.1	*Haplorchis taichui* isolate HG14 (cox1) gene mitochondrial	99%
JN809862.1	*Haplorchis taichui* isolate HG6 (cox1) gene mitochondrial	99%
JN809861.1	*Haplorchis taichui* isolate HG4 (cox1) gene mitochondrial	99%
JN809894.1	*Haplorchis taichui* isolate QT17 (cox1) gene mitochondrial	99%
JN809893.1	*Haplorchis taichui* isolate QT19 (cox1) gene mitochondrial	99%
JN809892.1	*Haplorchis taichui* isolate QT18 (cox1) gene mitochondrial	99%
JN809891.1	*Haplorchis taichui* isolate QT9 (cox1) gene mitochondrial	99%

Table 4-15. (Cont.)

Accession number	Description	Max Ident
JN809890.1	*Haplorchis taichui* isolate QT4 (cox1) gene mitochondrial	99%
JN809889.1	*Haplorchis taichui* isolate QT13 (cox1) gene mitochondrial	99%
JN809888.1	*Haplorchis taichui* isolate QT5 (cox1) gene mitochondrial	99%
JN809887.1	*Haplorchis taichui* isolate QT15 (cox1) gene mitochondrial	99%
JN809875.1	*Haplorchis taichui* isolate HG10 (cox1) gene mitochondrial	99%
JN809874.1	*Haplorchis taichui* isolate HG1 (cox1) gene mitochondrial	99%
JN809873.1	*Haplorchis taichui* isolate HG8 (cox1) gene mitochondrial	99%
JN809872.1	*Haplorchis taichui* isolate HG3 (cox1) gene mitochondrial	99%
JN809870.1	*Haplorchis taichui* isolate HG7 (cox1) gene mitochondrial	99%
JN809869.1	*Haplorchis taichui* isolate HG13 (cox1) gene mitochondrial	99%
JN809868.1	*Haplorchis taichui* isolate HG2 (cox1) gene mitochondrial	99%
JN809886.1	*Haplorchis taichui* isolate QT6 (cox1) gene mitochondrial	99%
JN809879.1	*Haplorchis taichui* isolate QT8 (cox1) gene mitochondrial	99%
JN809878.1	*Haplorchis taichui* isolate QT12 (cox1) gene mitochondrial	99%
JN809877.1	*Haplorchis taichui* isolate QT2 (cox1) gene mitochondrial	99%
JN809876.1	*Haplorchis taichui* isolate QT11 (cox1) gene mitochondrial	99%
JN809871.1	*Haplorchis taichui* isolate HG5 (cox1) gene mitochondrial	99%
JN809883.1	*Haplorchis taichui* isolate QT16 (cox1) gene mitochondrial	99%
JN809882.1	*Haplorchis taichui* isolate QT7 (cox1) gene mitochondrial	99%
JN809881.1	*Haplorchis taichui* isolate QT14 (cox1) gene mitochondrial	99%

Table 4-15. (Cont.)

Accession number	Description	Max Ident
JN809880.1	*Haplorchis taichui* isolate QT1 (cox1) gene mitochondrial	99%
JN809909.1	*Haplorchis taichui* isolate TH1 (cox1) gene mitochondrial	99%
JN809908.1	*Haplorchis taichui* isolate TH11 (cox1) gene mitochondrial	99%
JN809885.1	*Haplorchis taichui* isolate QT10 (cox1) gene mitochondrial	98%
JN809884.1	*Haplorchis taichui* isolate QT3 (cox1) gene mitochondrial	98%
JN809907.1	*Haplorchis taichui* isolate TH3 (cox1) gene mitochondrial	98%
JN809902.1	*Haplorchis taichui* isolate TH10 (cox1) gene mitochondrial	98%
JN809901.1	*Haplorchis taichui* isolate TH13 (cox1) gene mitochondrial	98%
JN809900.1	*Haplorchis taichui* isolate TH15 (cox1) gene mitochondrial	98%
JN809899.1	*Haplorchis taichui* isolate TH2 (cox1) gene mitochondrial	98%
JN809898.1	*Haplorchis taichui* isolate TH4 (cox1) gene mitochondrial	98%
JN809897.1	*Haplorchis taichui* isolate TH7 (cox1) gene mitochondrial	98%
JN809896.1	*Haplorchis taichui* isolate TH6 (cox1) gene mitochondrial	98%
JN809895.1	*Haplorchis taichui* isolate TH9 (cox1) gene mitochondrial	98%
JN809906.1	*Haplorchis taichui* isolate TH8 (cox1) gene mitochondrial	98%
JN809905.1	*Haplorchis taichui* isolate TH12 (cox1) gene mitochondrial	98%
JN809904.1	*Haplorchis taichui* isolate TH14 (cox1) gene mitochondrial	98%

Table 4-16. List of the sequences of mtCOI producing significant data of *H. pumilio* from NCBI databases (http://www.ncbi.nlm.nih.gov/).

Accession number	Description	Max Ident
JN809867.1	*Haplorchis taichui* isolate HG11 (cox1) gene mitochondrial	83%
JN809866.1	*Haplorchis taichui* isolate HG12 (cox1) gene mitochondrial	83%
JN809865.1	*Haplorchis taichui* isolate HG15 (cox1) gene mitochondrial	83%
JN809864.1	*Haplorchis taichui* isolate HG9 (cox1) gene mitochondrial	83%
JN809863.1	*Haplorchis taichui* isolate HG14 (cox1) gene mitochondrial	83%
JN809862.1	*Haplorchis taichui* isolate HG6 (cox1) gene mitochondrial	83%
JN809861.1	*Haplorchis taichui* isolate HG4 (cox1) gene mitochondrial	83%
EF055885.1	*Haplorchis taichui* (cox1) gene mitochondrial	83%
JN809894.1	*Haplorchis taichui* isolate QT17 (cox1) gene mitochondrial	83%
JN809893.1	*Haplorchis taichui* isolate QT19 (cox1) gene mitochondrial	83%
JN809892.1	*Haplorchis taichui* isolate QT18 (cox1) gene mitochondrial	83%
JN809891.1	*Haplorchis taichui* isolate QT9 (cox1) gene mitochondrial	83%
JN809890.1	*Haplorchis taichui* isolate QT4 (cox1) gene mitochondrial	83%
JN809889.1	*Haplorchis taichui* isolate QT13 (cox1) gene mitochondrial	83%
JN809888.1	*Haplorchis taichui* isolate QT5 (cox1) gene mitochondrial	83%
JN809887.1	*Haplorchis taichui* isolate QT15 (cox1) gene mitochondrial	83%
JN809886.1	*Haplorchis taichui* isolate QT6 (cox1) gene mitochondrial	83%

The specific primer of *H. taichui* and *H. pumilio* were designed from each sequence of them. Base on the alignment of the mtCOI sequence of heterophyid trematodes, there are only 1 polymorphic region between the 10 species of heterophyid trematodes, which species-specific primer sets have been designed (Figure 4-52). The primers were designed to produce amplicons of difference size for accurate discrimination between PCR product on the ethidium bromide-stained agarose gels. Detail and specific information of this specific primer of both trematode species were shown in Table. 4-17.

Table 4-17. Specification of the *H. taichui* and *H. pumilio* specific primers designed based on the partial sequence of mtCOI.

Species	**Primers**	**Sequence 5'-3'**	**Length**	**Tm (°C)**	**Product (bp)**
H. taichui	HT-F	GTT-TGG-TTA-TGG-GGG-TTT-AGT-TCT-T	25	59.7	160
	HT-R	AAC-CTT-TAT-ACC-TGT-GGG-GAC-T	22	58.4	
H. pumilio	HP-F	GGA-TGT-AAA-GAC-GGC-TGT-GTT-CTT-C	25	63.3	125
	HP-R	TAG-GAT-CTC-AAA-ATC-GTC-TA	23	55.3	

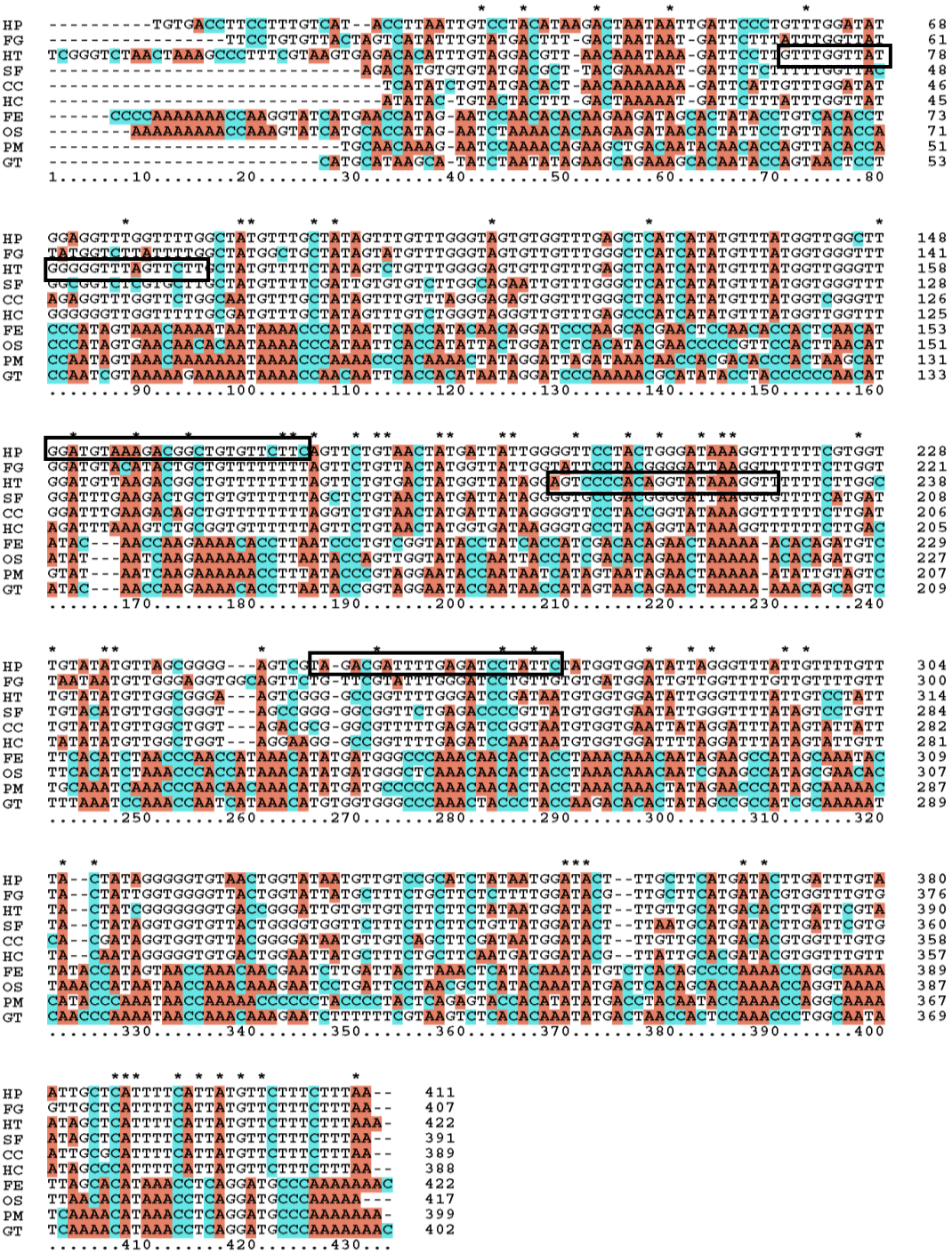


Figure 4-52. The multiple alignment of ten species of trematodes. black box: the region of designed the DNA specific primers). HT: *H. taichui*, HP: *H. pumilio*, CC: *C .caninus*, ST: *S. falcatus*, HC: *Haplorchoides* sp., GT: *G. tigrinus*, PM: *P. majeedi*, FG: *F. gigantica*, FE: *F. elongatus*, OS: *O. streptocoelium*

Optimization of PCR condition

The optimization of PCR conditions for amplified mtCOI fragment of *H. taichui* and *H. pumilio* at varied annealing temperature of 50-60 ^{o}C using HT-F and HT-R primers for *H. taichui* and HP-F and HP-R primers for *H. pumilio*. The result shown that, the optimal temperature for annealing the *H. taichui* and *H. pumilio* was 55 ^{o}C (Figure 4-53). The PCR product, 160 bp fragment of *H. taichui* and 125 bp fragment of *H. pumilio* were sequenced again to confirm identity.

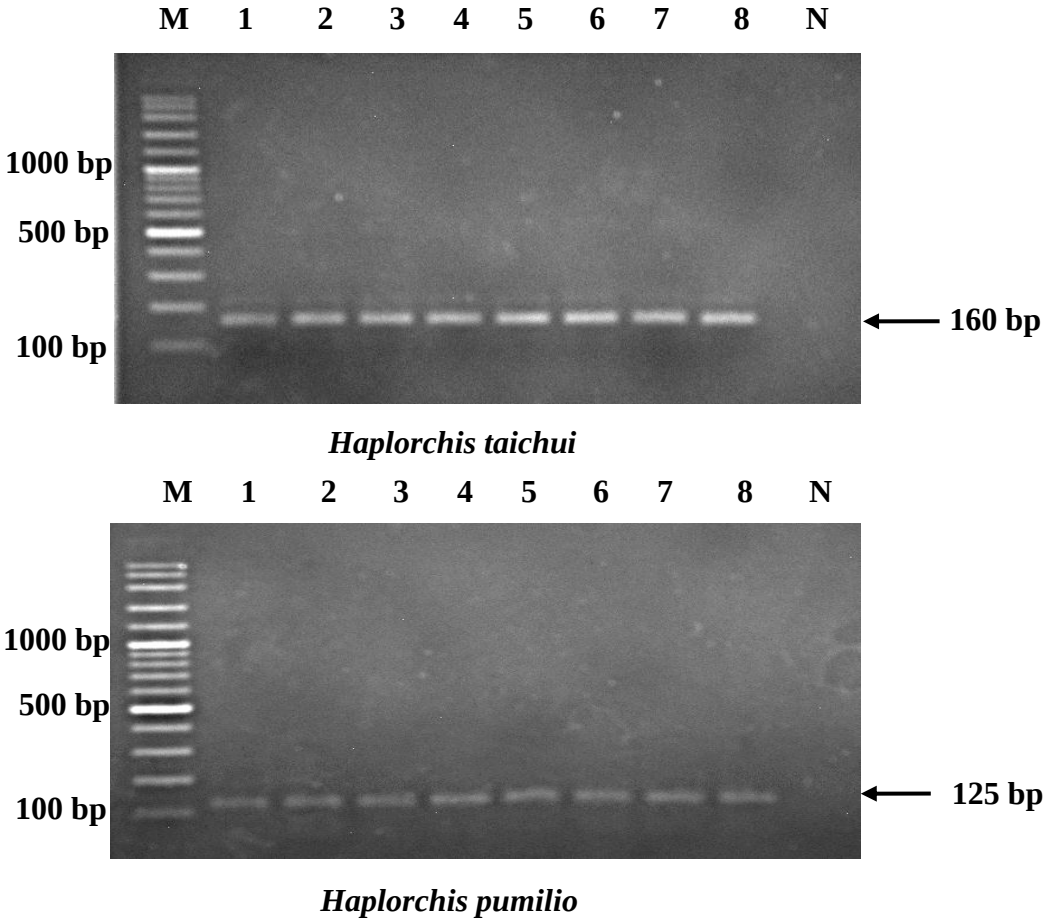
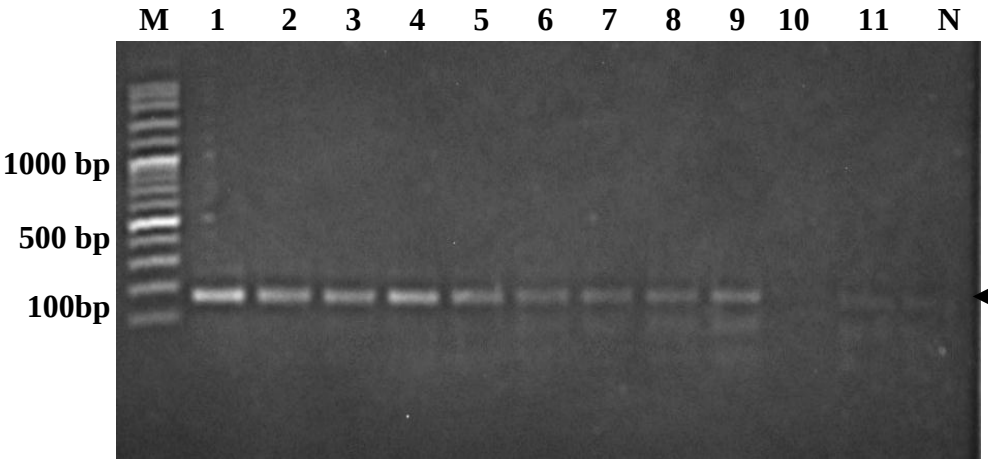


Figure 4-53. PCR product of *H. taichui* and *H. pumilio* specific primers by various annealing temperatures. M: 100 bp DNA marker, 1: 50.0 ◦C, 2: 50.7 ◦C, 3: 51.9 ◦C, 4: 53.7 ◦C, 5: 56.1 ◦C, 6: 58.0 ◦C, 7: 59.2 ◦C, 8: 60.0 ◦C, N: negative control

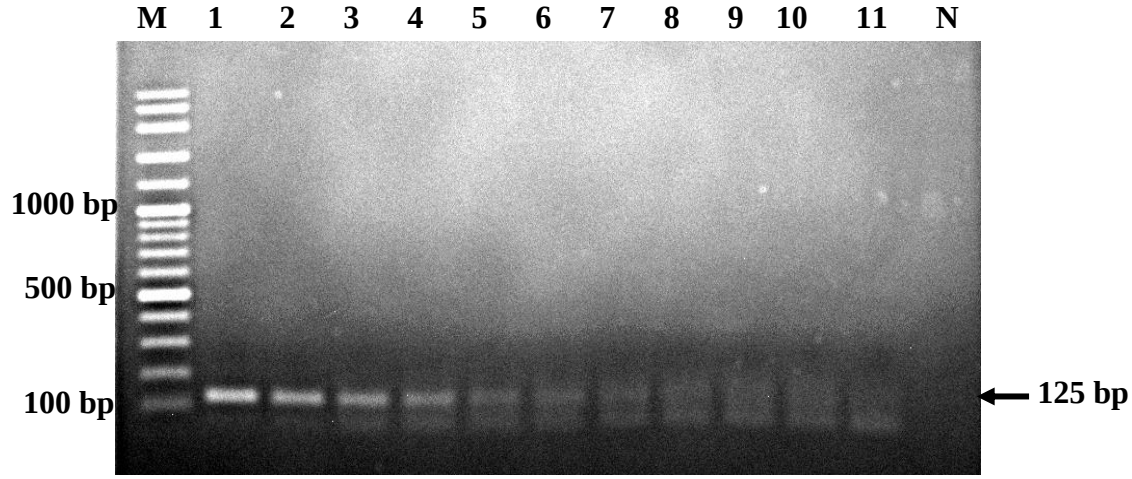
2.1.1 Determination of sensitivity and specificity of specific primers

PCR reaction assays shown the sensitivity in amplified the *H. taichui* and *H. pumilio* by two folds serial diluted genomic DNA. The initial concentration of genomic DNA template was 100 ng/µl which has concentrated between 100 - 0.20 ng/µl. The specific fragment of both trematode species were generated in all concentration although the intensity of band was progressively decreased when the low concentration of DNA templates. The lowest DNA concentration of *H. taichui* was about at 7.5 ng/µl while lowest the concentration of DNA template of *H. pumilio* was 12.5 ng/µl. (Figure 4-54).

For the specificity tests, the *H. taichui* and *H. pumilio* specific primers were tested for species specific to perform in the molecular identification by attempting to amplify them with all 10 adult trematode species and snail tissue. These mentioned worms are widely distribute and high prevalence of infection in Chiang Mai province, Thailand as same as they have been reported frequently in a past decade. It was found that, both of specific primers shown high specificities which no cross reaction with the other trematode species and snail tissue. Only the *H. taichui* and *H. pumilio* were generate the PCR produce (Figure 4-55 and Figure 4-56).



Haplorchis taichui



Haplorchis pumilio

Figure 4-54. Sensitivity tests of *Haplorchis taichui* and *H. pumilio* specific primers. M: 100 bp marker**,** 1**:** 100 ng/µl, 2: 50 ng/µl, 3: 25 ng/µl, 4: 12.5 ng/µl, 5: 7.25 ng/µl, 6: 3.62 ng/µl, 7: 1.81 ng/µl, 8: 0.90 ng/µl, 9: 0.45 ng/µl, 10: 0.22 ng/µl, N: negative control

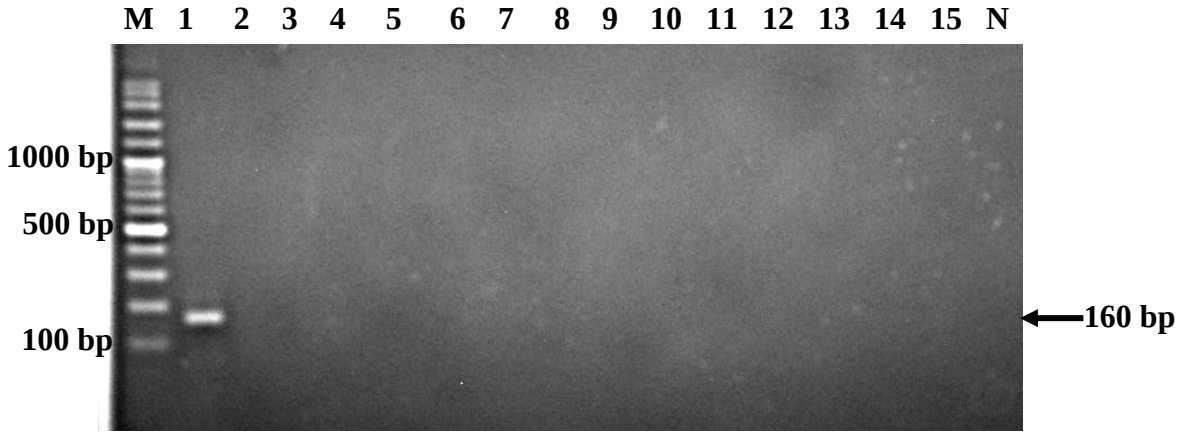


Figure 4-55. Specificity tests of *H. taichui* specific primer by various species of trematode and freshwater snails. M: 100 bp DNA marker, 1: *Haplorchis taichui*, 2: *H. pumilio*, 3: *Centrocestus caninus*, 4: *Stellantchasmus falcatus*, 5: *Haplorchoides* sp., 6: *Ganeo tigrinus*, 7: *Prostorchigenes majeedi*, 8: *Fasciola gigantica*, 9: *Fischoederius elongatus*, 10: *Orthocoelium streptocoelium*, 11: *Melanoides tuberculata*, 12: *Tarebia granifera*, 13: *Thiara scabra*, 14: *Adamietta housei* 15: *Bithynia siamensis siamensis*, N: negative control

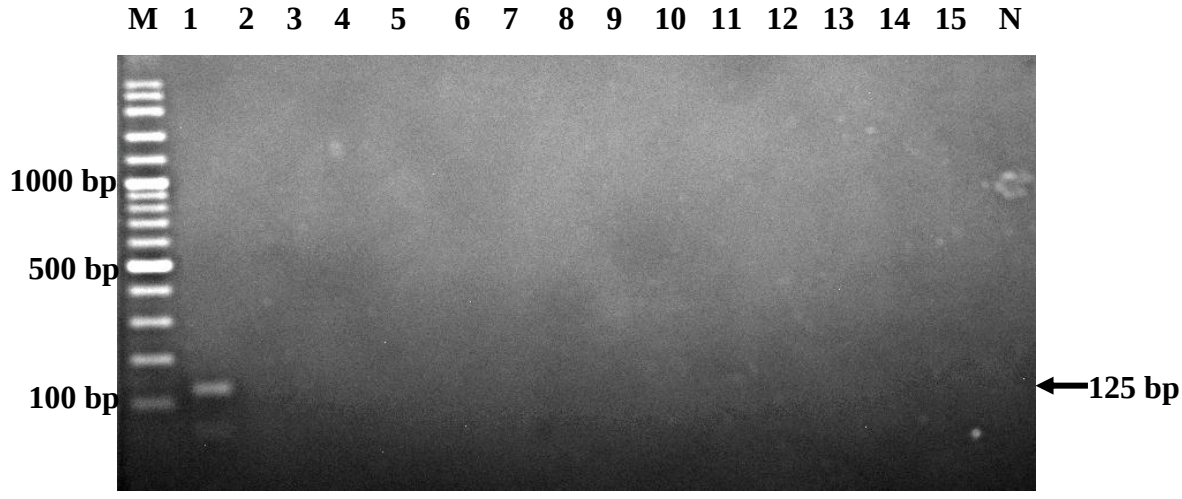


Figure 4-56. Specificity tests of *H. pumilio* specific primer by various species of trematode and freshwater snails. M: 100 bp DNA marker, 1: *Haplorchis pumilio*, 2: *H. taichui*, 3: *Centrocestus caninus*, 4: *Stellantchasmus falcatus*, 5: *Haplorchoides* sp., 6: *Ganeo tigrinus*, 7: *Prostorchigenes majeedi*, 8: *Fasciola gigantica*, 9: *Fischoederius elongatus*, 10: *Orthocoelium streptocoelium*, 11: *Melanoides tuberculata*, 12: *Tarebia granifera*, 13: *Thiara scabra*, 14: *Adamietta housei* 15: *Bithynia siamensis siamensis*, N: negative control

Development of multiplex PCR

Both of designed primers of *Haplorchis taichui* and *H. pumilio* were investigated for optimal PCR condition, sensitivity, and specificity with genomic DNA template of 10 trematode species. The multiplex PCR for detection of *H. taichui* and *H. pumilio* was also developed. The multiplex PCR conditions were uses as same as those of species-specific identification PCR, and optimizing PCR combination until two specific fragments size were obviously appeared together. The mix of both DNA specific primer were generated a strong PCR product fragment of 160 and 125 bp for mix DNA templates of *H. taichui* and *H. pumilio*, and no cross-reaction with the other trematodes and snail tissues as shown in Figure 4-57.

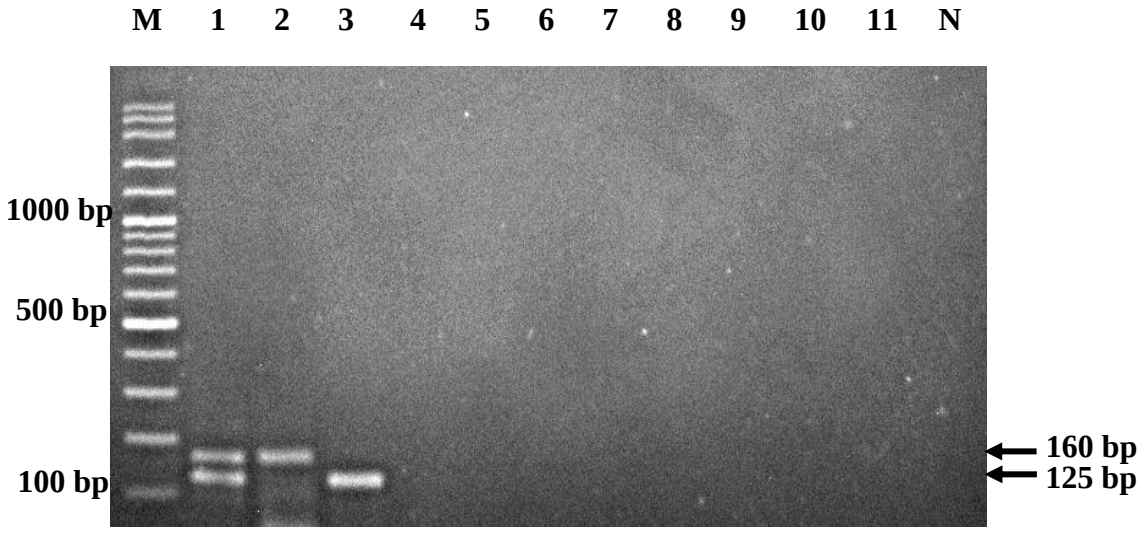
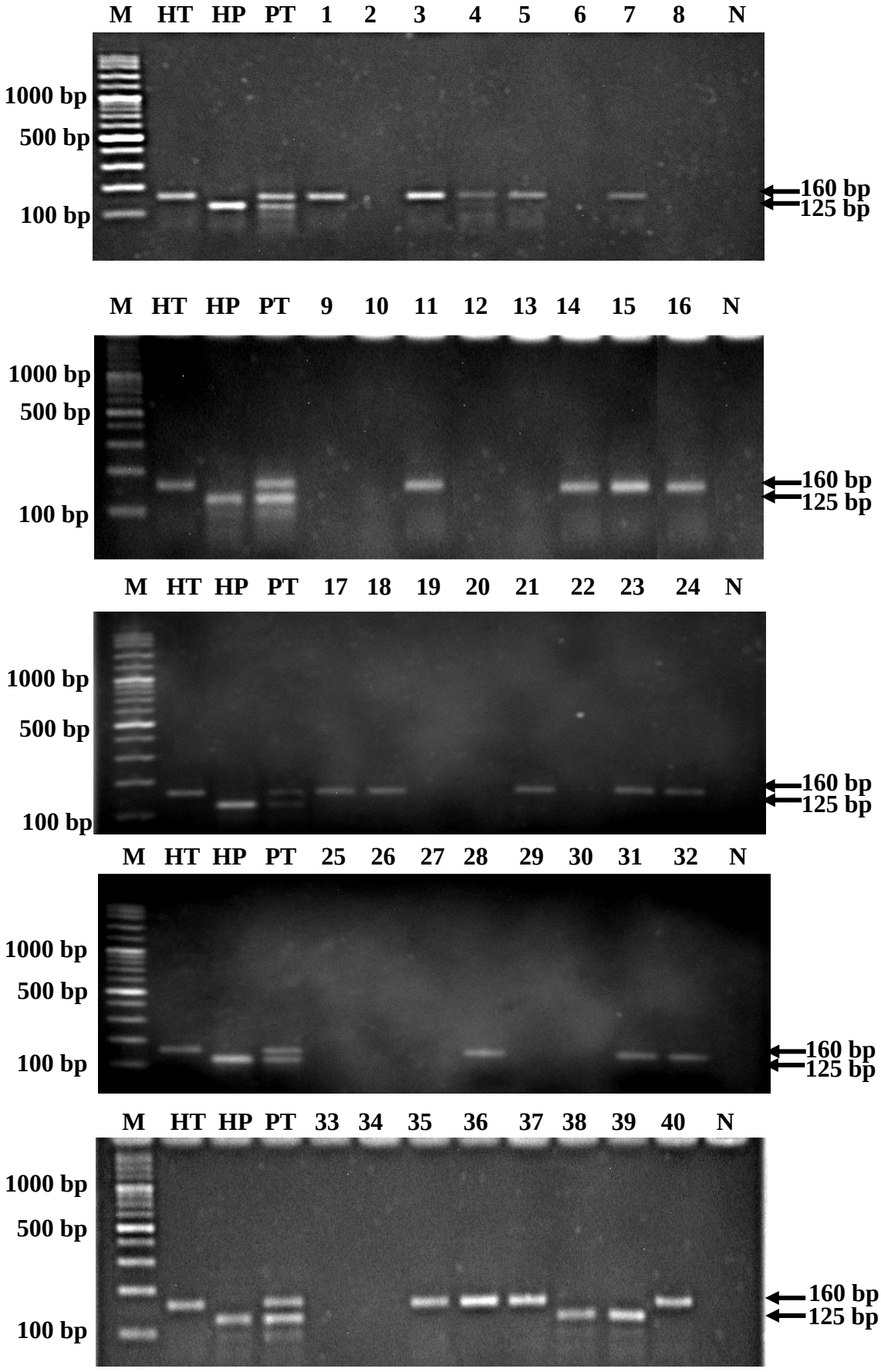


Figure 4-57. Multiplex PCR detection the *H. taichui* and *H. pumilio.* M: 100 bp DNA marker, 1: Mix DNA template of *H. taichui* and *H. pumilio*, 2: *H. taichui*, 3: *H. pumilio*, 4: *Centrocestus caninus*, 5: *Stellantchasmus falcatus*, 6: *Haplorchoides* sp., 7: *M. tuberculata*, 8: *T. granifera*, 9: *Th. scabra*, 10: *A. housei*, 11: *B. siamensis siamensis*, N: negative control

Molecular identification of cercarial stage of *Haplorchis taichui* and *H. pumilio* for investigated geographic distribution

For the detection of *H. taichui* and *H. pumilio* in freshwater snails from northern Thailand, the specific fragment were generated in difference in parapleurolophocercous cercariae. Mix genomic DNA of both of adult stage trematodes were used as a positive control, and also gave an amplification product of 160 and 125 bp (Figure 4-58).

The results shown that, three snail species in Family Thiaridae namely, *Melanoides tuberculata*, *Tarebia granifera* and Thiara *scabra*, are the intermediate hosts of *H. taichui*. While, the *H. pumilio* were infected in two snail species including *M. tuberculata* and *T. granifera*. This result provided the effective evidence by revealing that not only of *H. taichui* and *H. pumilio* that was developed from parapleurolophocercous cercariae, while there were other trematode species which were generated from the same cercarial type like *H. taichui* and *H. pumilio*.


M HT HP PT 1 2 3 4 5 6 7 8 N
1000 bp
500 bp
100 bp
160 bp
125 bp
M HT HP PT 9 10 11 12 13 14 15 16 N
1000 bp
500 bp
100 bp
160 bp
125 bp
M HT HP PT 17 18 19 20 21 22 23 24 N
1000 bp
500 bp
100 bp
160 bp
125 bp
M HT HP PT 25 26 27 28 29 30 31 32 N
1000 bp
500 bp
100 bp
160 bp
125 bp
M HT HP PT 33 34 35 36 37 38 39 40 N
1000 bp
500 bp
100 bp
160 bp
125 bp

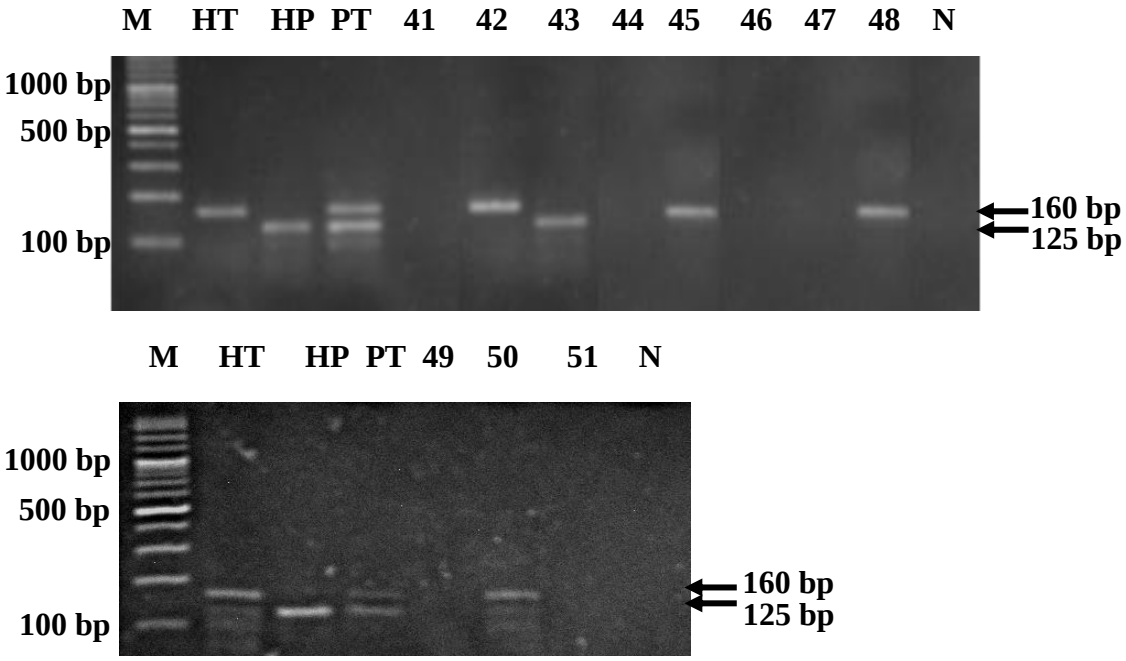


Figure 4-58. Molecular identification by multiplex PCR reaction. M: 100 bp DNA marker, HT: *H. taichui*, HP: *H. pumilio*, PT: Mix DNA template of *H. taichui* and *H. pumilio*, Lane 1-51 of each gel: parapleurolophocercous cercaria from each collecting sites N: negative control (the descriptions of lane 1 -51 explained in Table 4-18)

Table 4-18. Summaries of positive results for the molecular identification using multiplex PCR.

Lane	Sites		Type of cercariae	Snail species	Positive	
					HT	HP
1	19°6' 37.317"N	99°4'33.439"E	Parapleu Type I	*M. tuberculata*	✓	
2	19°6' 37.317"N	99°4'33.439"E	Parapleu Type II	*M. tuberculata*		
3	19°5'54.30"N	99°4'52.87"E	Parapleu Type I	*T. granifera*	✓	
4	19°5'54.30"N	99°4'52.87"E	Parapleu Type I	*M. tuberculata*	✓	
5	19°5'54.30"N	99°4'52.87"E	Parapleu Type I	*Th. scabra*	✓	
6	19°3'05.23"N	98°9'40.617"E	Parapleu Type I	*M. tuberculata*		
7	19°22'51.51"N	98°58'2.88"E	Parapleu Type I	*M. tuberculata*	✓	
8	19°22'57.149"N	98° 58'5.699"E	Parapleu Type I	*T. granifera*		
9	18°42'27.00"N	98°56'5.68"E	Parapleu Type I	*T. granifera*		
10	18°42'27.00"N	98°56'5.68"E	Parapleu Type I	*M. tuberculata*		
11	18°41'10.25"N	98°56'16.57"E	Parapleu Type I	*M. tuberculata*	✓	
12	18°41'10.25"N	98°56'16.57"E	Parapleu Type I	*T. granifera*		
13	18°33'59.54"N	98°52'5.61"E	Parapleu Type I	*B. s. siamensis*		
14	18°33'59.54"N	98°52'5.61"E	Parapleu Type I	*T. granifera*	✓	
15	18°33'59.54"N	98°52'5.61"E	Parapleu Type I	*M. tuberculata*	✓	
16	18°33'59.54"N	98°52'5.61"E	Co-infection	*M. tuberculata*	✓	
17	18°42'18.55"N	99° 2'16.62"E	Parapleu Type I	*M. tuberculata*	✓	
18	18°27'2.91"N	98°40'27.99"E	Parapleu Type I	*M. tuberculata*	✓	

Table 4-6 (Cont.).

Lane	Sites		Type of cercariae	Snail species	Positive	
					HT	HP
19	18°55'4.56"N	99° 7'44.04"E	Parapleu Type I	*M. tuberculata*		
20	18°55'4.56"N	99° 7'44.04"E	Co-infection	*M. tuberculata*		
21	18°55'4.56"N	99° 7'44.04"E	Parapleu Type I	*T. granifera*	✓	
22	18°55'31.46"N	99° 8'13.93"E	Co-infection	*B. s. siamensis*		
23	19°54'33.28"N	99°17'19.23"E	Parapleu Type I	*T. granifera*	✓	
24	19°55'1.26"N	99°13'11.29"E	Parapleu Type I	*M. tuberculata*	✓	
25	19°57'25.74"N	99°11'18.68"E	Parapleu Type I	*T. granifera*		
26	19°57'25.74"N	99°11'18.68"E	Parapleu Type I	*M. tuberculata*		
27	19°38'43.56"N	99°31'36.52"E	Parapleu Type I	*Th. scabra*		
28	19°21'21.37"N	98°26'50.40"E	Parapleu Type I	*T. granifera*	✓	
29	18°45'56.20"N	99°14'20.90"E	Parapleu Type I	*M. tuberculata*		
30	18°35'3.35"N	98°59'32.47"E	Parapleu Type II	*A. housei*		
31	18°35'31.46"N	99° 4'52.67"E	Parapleu Type I	*M. tuberculata*	✓	
32	18°32'32.23"N	98°56'21.32"E	Parapleu Type I	*M. tuberculata*	✓	
33	18°32'32.23"N	98°56'21.32"E	Parapleu Type II	*T. granifera*		
34	19° 7'13.99"N	100°48'35.27"E	Parapleu Type I	*M. tuberculata*		

Table 4-6 (Cont.).

Lane	Sites		Type of cercariae	Snail species	Positive	
					HT	HP
35	19°10'42.86"N	100°52'31.80"E	Parapleu Type I	*T. granifera*	✓	
36	19°10'13.71"N	100°56'2.83"E	Parapleu Type I	*T. granifera*	✓	
37	19°11'40.56"N	99°53'48.02"E	Parapleu Type I	*M. tuberculata*	✓	
38	16°58'31.44"N	100°33'30.65"E	Parapleu Type I	*M. tuberculata*		✓
39	16°45'52.47"N	100°12'17.44"E	Parapleu Type I	*M. tuberculata*		✓
40	16°59'10.38"N	100°11'58.41"E	Parapleu Type I	*M. tuberculata*	✓	
41	17° 9'59.12"N	99°51'30.93"E	Parapleu Type I	*M. tuberculata*		
42	17°13'9.72"N	99° 2'35.87"E	Parapleu Type I	*T. granifera*	✓	
43	17°13'9.72"N	99° 2'35.87"E	Parapleu Type I	*M. tuberculata*		✓
44	17°13'9.72"N	99° 2'35.87"E	Co-infection	*M. tuberculata*		
45	17°38'30.14"N	100° 2'18.75"E	Parapleu Type I	*M. tuberculata*	✓	
46	17°38'30.14"N	100° 2'18.75"E	Parapleu Type II	*M. tuberculata*		
47	17°35'33.54"N	100° 5'58.08"E	Parapleu Type I	*M. tuberculata*		
48	18°18'22.01"N	100°16'28.32"E	Parapleu Type I	*M. tuberculata*	✓	
49	18°18'27.15"N	99°31'9.29"E	Parapleu Type I	*M. tuberculata*		
50	18°19'13.11"N	99°20'49.97"E	Parapleu Type I	*T. granifera*	✓	
51	17°26'43.03"N	99° 7'40.28"E	Parapleu Type I	*T. granifera*		

For the molecular identification by multiplex PCR, the parapleurolophocercous cercaria Type I only are *H. taichui* and *H. pumilio.* This result indicated that the *H. taichui* are widely distribution in northern Thailand including Chiang Rai, Chiang Mai, Lamphun, Phayao, Mae Hong Son, Phrae, Nan, Tak, Uttaradit, and Phitsanulok provinces (Figure 4-58). Whereas, *H. pumilio* were found only in Tak and Phitsanulok provinces.

For the distribution study, the prevalence of cercarial stage of *H. taichui* infection in snails from northern Thailand was higher than the *H. pumilio*.

Phylogenetic relationship of heterophyid trematodes

The partial mtCOI nucleotide sequences of heterophyid trematode samples were used to understand their phylogenetic relationships. The length of partial mtCOI nucleotide sequence data was 393-425 bp. Heterophyid trematodes appear to be monophyletic tree.

Phylogenetic trees were by analyzed for the mtCOI (Figure 4-59) sequences data using neighbor joining method (NJ). The phylogram form these result are separated into three clade including clade A: heterophyid group and clade B: *Metagonimus* group. Five distinct sub-clade which consist of heterophyid clade (*Haplorchis taichui* lineages, *H. pumilio* lineages, *Centrocestus caninus* lineages, *Haplorchoides* sp. lineages and *Stellantchasmus falcatus* lineages) and Clade C: other trematodes (*P. maladies*, *O. streptocoelium*, *F. elongatus*, *G. tigrinus* and *F. gigantica*). The *H. taichui* and *H. pumilio* are more related than to the heterophyid

species group. While, *Metagonimus* species are in the same *Metagonimus* group (Figure 4-59).

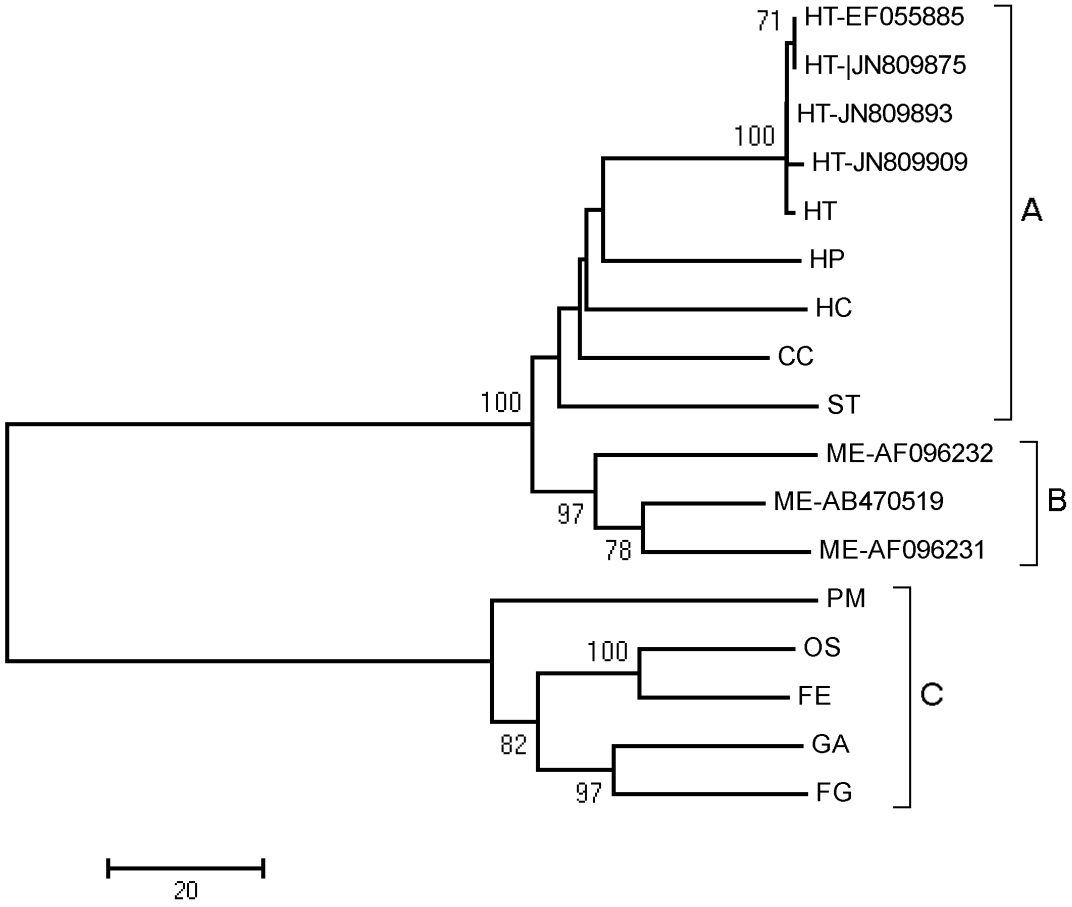


Figure 4-59. The rooted phylogeny form partial mtCOI sequences of 17 trematode species using NJ method base on the Kimura two–parameter model. Bootstrap values were computed independently for 1,000 resembling. A: heterophyid group, B: *Metagonimus* group, C: other trematodes, HT: *H. taichui*, HP: *H. pumilio*, HC: *Haplorchoides* sp., CC: *C. caninus*, ST: *S. falcatus*, ME: *Metagonimus* spp., PM : *P. majeedi*, OS: *O. streptocoelium*, FE: *F. elongatus*, GA: *G. tigrinus*, FG: *F. gigantica*