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Zoosyst. Evol. 90 (1) 2014, 57–86 | DOI 10.3897/zse.90.7306

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Trematodes obtained from the thiarid freshwater snail *Melanoides tuberculata* (Müller, 1774) as vector of human infections in Thailand

Duangduen Krailas[1], Suluck Namchote[1], Tunyarut Koonchornboon[2], Wivitchuta Dechruksa[1], Dusit Boonmekam[1]

1 *Department of Biology, Faculty of Science, Silpakorn University, Nakhonpathom 73000, Thailand*

2 *Department of Anatomy, Pramongkhutklao College of Medicine, Bangkok 10400, Thailand*

Corresponding author: Duangduen Krailas (kduang@su.ac.th, kduang@gmail.com)

Received 8 Jan 2014
Accepted 18 Feb 2014
Published 28 March 2014

Academic editor:
Matthias Glaubrecht

Abstract

Larval stages of trematodes obtained from the freshwater snail *Melanoides tuberculata* (Cerithioidea, Thiaridae) as intermediate host were studied by using cercarial emergence and crushing snails. Between December 2004 and September 2009 snails from one hundred twenty locations in Thailand were collected every two months for one year at each sampling site. Counts per unit of time method was used in this study, and the samples of snails were collected every 10 minutes per sampling by five collectors. The cercarial stages were examined using shedding and crushing methods. The infection rate was found to be 18.79%, i.e. 6,019 animals infected in a total of 32,026. Nine different types in eighteen species of cercariae were categorized, viz. are (1) Parapleurophocercous cercariae: *Haplorchis pumilio, Haplorchis taichui*, and *Stictodora tridactyla*; (2) Pleurophocercous cercariae: *Centrocestus formosanus*; (3) Xiphidiocercariae: *Acanthatrium hitaense, Loxogenoides bicolor*, and *Haematoloechus similis*; (4) Megalurous cercariae**:** *Cloacitrema philippinum* and *Philophthalmus* sp.; (5) Furcocercous cercariae: *Cardicola alseae*, *Alaria mustelae*, *Transversotrema laruei, Apatemon gracilis*, and *Mesostephanus appendiculatus*; (6) Echinostome cercariae: *Echinochasmus pelecani*; (7) Amphistome cercariae: *Gastrothylax crumenifer*; (8) Renicolid cercariae: *Cercaria caribbea* LXVIII; and, (9) Cotylomicrocercous cercariae: *Podocotyle* (*Podocotyle*) *lepomis*.

Key Words

Trematoda
Cercariae
human health
shedding
Thiaridae
Cerithioidea

Introduction

The neglected tropical diseases (NTDs) represent the most common parasitic infections affecting the world's poorest people (Hotez et al. 2007). In addition to their detrimental effects on health, NTDs have a chronic debilitating effect by undermining the physical and cognitive development of individuals residing in areas infested with NTDs, especially for children and women of child-bearing age. An especially deleterious effect has been shown on their educational performance and future economic productivity (Hotez et al. 2007, 2009).

It is important to note in this context that trematodes infecting humans, especially liver fluke and intestinal flukes, are highly prevalent in Southeast Asian countries (Wongratanacheewin et al. 2001; Chai et al. 2005). These infections have a major public health impact. It has been reported that the highest degree of infections with trematodes were discovered in the gastrointestinal tract of humans living in the north region of Thailand (Pungpak et al. 1998, Radomyos et al. 1998) and the most metacercarial species were found in cyprinoid fish in the north and northeastern regions (Srisawangwong et al. 1997b, Sukontason et al. 1999). The liver fluke *Opisthorchis viverrini* can cause chalangiocarcinoma, a kind of cancer in gall bladder (Sripa et al. 2010), while the intestinal fluke *Haplorchis taichui* is a possible agent of irritable bowel syndrome-like symptoms (Watthanakulpanich et al. 2010). However, Thai people have considerably underestimated these trematodes by continually eating Thai traditional food prepared from raw freshwater fish (Chuboon et al. 2005). So the prevalence of trematodes in Thailand has been a continual problem until now.

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Life cycle of trematodes

Trematodes need three hosts to complete their life cycles. After their eggs hatch in the water source such as canals and streams, the miracidium, their first larval stage, will swim and find the first intermediate host, namely freshwater snails and terrestrial snails close to water. In particular, snail species of the genus *Bithynia* are known as intermediate hosts of the liver fluke *Opisthorchis viverrini* (Tesana 2002). Some edible mollusks, such as the viviparid *Filopaludina* spp. and the bivalve *Corbicula* spp., are known as the first and second intermediate hosts of echinostome intestinal fluke (Temcharoen 1992a, b, Krailas et al. 2008). Thus, we can call these trematode infections mollusk-transmitted diseases.

In the snails, they will develop from miracidium to sporocyst to redia and finally to cercaria. Cercaria will leave the snails, head to the second intermediate host, e.g. freshwater fish, and develop to be metacercaria, the infective stage. At least 18 species of cyprinoid fish act as the second intermediate hosts. If the vertebrates, like animals and humans, eat the infected raw fish, they will receive the metacercaria, which will not be digested by digestive enzymes. Instead, the trematodes will become adult and lay eggs, which are then emitted to the water again with the hosts'stool.

Survey of freshwater snails as the first intermediate hosts in Thailand

Several studies have been conducted on the fauna of mollusks with focus on their trematode infections (Wegeberg et al. 1999, Abdul-Salam et al. 2004). Not only in Asia but also Africa and Australia, these trematodes have been widely studied (Diaz et al. 2008, Derraik 2008). In Thailand, medically-important freshwater snails have been investigated since 1980 (Upatham et al. 1980, 1981, Nithiuthai et al. 2002, Krailas et al. 2003, Sri-aroon et al. 2005, Ukong et al. 2007, Dechruksa et al. 2007, Krailas et al. 2008). It was found that because of their life cycle and host specificity, the distribution of trematodes depends on the presence of the first and second intermediate hosts, as well as the eating habit of local people (Radomyos et al. 1998).

Freshwater Snails of the Thiaridae in Thailand

Many of the trematode infected freshwater snails are from the Cerithoidea, a large, essentially marine, group of caenogastropods with approximately 200 genera and with mainly pan-tropical distributions, which have been used also as model for evolutionary systematic studies (Glaubrecht 1996, 1999, 2000, 2011, Glaubrecht et al. 2009). To focus on the family Thiaridae, this group represents, as became evident in recent studies (Glaubrecht 1996, 1999, 2011, Lydeard et al. 2002, Strong et al. 2008, 2011), one of the two (or three) independent invasions into and colonizations of freshwater habitats. Mainly distributed in Southeast Asia, they are to be considered, together with the Pachychilidae as most significant intermediate hosts for infections in humans. For instance, the thiarids *Tarebia granifera and Thiara toucheana*, as well as the pachychilid *Brotia asperata*, *Brotia costula episcopalis* and *Brotia c. peninsularis* were found to be the first intermediate hosts for lung flukes (Tang 1940, Tubangui et al. 1950, Davis 1971, Brandt 1974). In addition, the thiarids *Tarebia granifera* and *Melanoides tuberculata* are the first intermediate hosts for intestinal fluke and blood flukes (Malek and Cheng 1974, Pointier and Jourdane 2000). In Thailand, *T. granifera* and *M. tuberculata* have been reported as the first intermediate host for lung and intestinal flukes (Upatham et al. 1995, Ukong et al. 2007, Dechruksa et al. 2007).

Melanoides tuberculata (Müller, 1774)

This taxon is common to freshwaters within its native distributional range that covers much of tropical Africa, Asia and the Oceania. It is now also present in much of the tropical and subtropical New World as a consequence of introductions that started during the last century (Madsen and Frandsen 1989). They were described as alien species around the world. Moreover, the species exhibits considerable polymorphism in shell ornamentation across its geographical range; however, at the same time among sites discrete lineages or 'morphs' of *M. tuberculata* can be separated by shell characters, such as coloration and ornamentation, apparently due to the predominantly parthenogenetic reproduction resulting from negligible intrapopulation variability in these traits (Samadi et al. 1999). For example, on Martinique Islands, each morph of *M. tuberculata* is different in terms of juveniles, growth rate and even parasite infection rate.

M. tuberculata is considered to be of medical significance, as most of the above cited parasites can affect humans. Although there can be considerable seasonal variation in the intensity of parasitism in these snails, the incidence of *M. tuberculata* with trematode parasites has been recorded to be as high as 92% (Derraik 2008). A checklist from 136 scientific published studies revealed that *M. tuberculata* could be host for flukes, identified as belonging to 17 families, 25 genera, and 37 species (Pinto and De Melo 2011). These trematodes are both animals and human parasites. Nevertheless, in Thailand there are only very few reports about *Melanoides* infection in certain specific areas of the country to date. However, investigations around Thailand have never been conducted. So, in the present study we have surveyed now the trematode infections of *M. tuberculata* all over Thailand.

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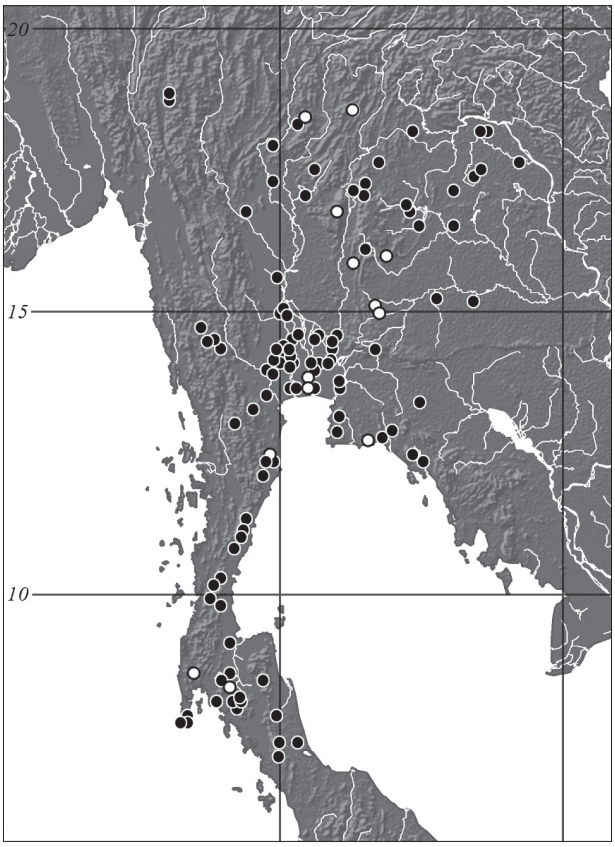

Figure 1. Occurrence of *Melanoides tuberculata* in Thailand. Closed symbol – *M. tuberculata* with infection; open symbol – *M. tuberculata* without infection.

Materials and methods

Sampling sites

One hundred twenty locations in Thailand, which are used by humans as sources of water, were examined (snail collection sites). For the exact data, please refer to the first section of the Result section in this report. The positions of collection sites were recorded by GPS (Garmin PLUS III, Taiwan). The localities of the relevant samples were mapped on a dot-by-dot basis to a digitally reduced version of the drainage pattern map of Thailand, as developed in Dechruksa et al. (2013). This map was created using a Reliefkarte on the basis of the Global 30-Arc-Second Elevation Data (GTOPO30) from the U.S. Geological Survey and a river map from the Map/server Aquarius Geomar, and then compiled using Adobe Photoshop CS3 and Adobe Illustrator.

Collection of snails

Between December 2004 and September 2009, snails were collected every two months for one year from each localities, using the counts per unit of time sampling method (Olivier and Schneiderman 1956). Five researchers collected samples by handpicking and scooping every 10 minutes at each sampling site. The snail samples were then categorized into species according to their shell morphology. They were later examined for trematode infections.

Examination for parasitic infections

Parasitic infections were investigated by using snail shedding and crushing methods. Emerged cercariae were collected in dechlorinated water and observed for their swimming behavior (Krailas et al. 2003). Sporocysts and rediae were examined under a dissecting microscope.

Study of cercarial morphology

The cercariae were studied unstained or vitally stained with 0.5% neutral red and Semichon's acetic carmine & fast green. Descriptions of the morphology and anatomy of cercariae were based on the study of living cercariae that had escaped from the snails. Sample measurements (average size) in micrometers were taken from 20 specimens fixed in 10% formalin. Measurements in micrometers with averages in parentheses were taken from 20 specimens. Details of the cercariae were drawn using a camera lucida, and all their species were identified (Schell 1962, 1970, Nasir 1974, Yamaguti 1975; Ito 1980). For scanning electron microscopy, cercariae were fixed in 2.5% glutaraldehyde phosphate buffer (0.1 mol/l, ph 7.4) at 4 °C for at least 2 hours and post-fixed in 1% osmium tetroxide in the same buffer for 2 hours at 4 °C. They were dehydrated through a graded series of ethanol, and then dried in a critical point dryer using liquid carbon dioxide as a transition medium. The specimens were coated with gold-palladium in an ion-sputtering apparatus (Polaron CPD 7501, UK), and then examined in a scanning electron microscope (Camscan mx 2000, UK or JEOL, JSM-5410 LV, Japan).

Results

Melanoides tuberculata in Thailand

The thiarid *Melanoides tuberculata* was found in and sampled from study sites in five regions in Thailand, as shown in Table 1, Fig. 1. These include the following:

The North: N1 = Sakunotayan Waterfall, Wangtong District, Phitsanulok Province (SUT0109001) (N 16°50'20.6", E 100°32'15.6", Altitude 40 m); N2 = Kaeng Sopha Waterfall, Wangtong District, Phitsanulok Province (SUT0109002) (N 16°52'22.3", E 100°50'29.6", Altitude 398 m); N3 = Thung Salaeng Luang Stream, Wangtong District, Phitsanulok Province (SUT0109003) (N 16°50'50.0", E 100°51'57.2", Altitude 452 m); N4 = Pha Laht Waterfall, Nakhonthai District, Phitsanulok Province (SUT0109004) (N 17°01'69.1", E 100°56'77.8", Altitude 267 m); N5 = Thum Pla Stream, Muang District, Mae Hong Son Province (SUT0109005)

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Table 1. Locations in Thailand with *Melanoides tuberculata* found, the number of collected snails and infection rates.

No. (no. specimen)	Name	GPS	No. of collected snails	No. of Infected snails	Infection rates (%)
North					
N1 (SUT0109001)	Sakunotayan Waterfall, Wangtong District, Phitsanulok	N 16° 50′ 20.6″ E 100° 32′ 15.6″ Altitude 40 m	13	3	23.08
N2 (SUT0109002)	Kaeng Sopha Waterfall, Wangtong District, Phitsanulok	N 16° 52′ 22.3″ E 100° 50′ 29.6″ Altitude 398 m	3	1	33.33
N3 (SUT0109003)	Thung Salaeng Luang Stream, Wangtong District, Phitsanulok	N 16° 50′ 50.0″ E 100° 51′ 57.2″ Altitude 452 m	3	0	0
N4 (SUT0109004)	Pha Laht Waterfall, Nakhonthai District, Phitsanulok	N 17° 01′ 69.1″ E 100° 56′ 77.8″ Altitude 267 m	5	4	80
N5 (SUT0109005)	Thum Pla Stream, Muang District, Mae Hong Son	N 19° 25′ 31.7″ E 97° 59′ 24.9″ Altitude 343 m	60	38	63.33
N6 (SUT0109006)	Huay Sua Thao Stream, Muang District, Mae Hong Son	N 19° 15′ 32.0″ E 97° 54′ 43.7″ Altitude 237 m	51	9	17.65
N7 (SUT0109007)	Klong Nam Lai Waterfall, Klong Lan District, Kam Phaeng Phet	N 16° 11′ 32.7″ E 99° 15′ 51.0″ Altitude 241 m	63	28	44.44
N8 (SUT0109008)	Tad Duen Waterfall, Sri Satchanalai District, Sukhothai	(N 17° 33′ 23.2″ E 99° 29′ 76.8″ Altitude 414 m)	161	38	23.60
N9 (SUT0109009)	Sri Satchanalai Stream, Sri Satchanalai National Park, Sukhothai	N 17° 33′ 5.9″ E 99° 29′ 24.8″ Altitude 182 m	113	24	21.24
N10 (SUT0109010)	Mae Pool Waterfall, Lub Lae District, Uttaradit	N 17° 43′ 45.0″ E 99° 58′ 50.6″ Altitude 164 m	70	9	12.86
N11 (SUT0109011)	Chueng Thong Waterfall, Muang District, Prae	N 18° 01′ 54.2″ E 100° 15′ 52.8″ Altitude 298 m	7	3	42.85
N12 (SUT0109012)	Huay Sa Nien Stream, Muang district, Nan	N 18° 51′ 1.3″ E 100° 39′ 16.2″ Altitude 280 m	60	0	0
N13 (SUT0109013)	Huay Ton Phueng Waterfall, Doi Pu Nang National Park, Chiang Muon District, Phyao	N 18° 55′ 5.3″ E 100° 12′ 15.7″ Altitude 379 m	131	1	0.76
N14 (SUT0109014)	Tansawan Waterfall, Doi Pu Nang National Park, Chiang Muon District, Phyao	N 18° 51′ 22.7″ E 100° 11′ 9.6″ Altitude 420 m	368	16	4.35
N15 (SUT0109015)	Mae Mine Stream, Mae Ta District, Lampang	N 18° 07′ 1.8″ E 99° 37′ 35.1″ Altitude 269 m	201	5	2.49
Northeast					
NE16 (SUT0109016)	Huay Lum Po Dang Stream, Thepsathit District, Chaiyapoom	N 15° 33′ 42.8″ E 101° 24′ 56.9″ Altitude 471 m	311	0	0
NE17 (SUT0109017)	Sai Thong Waterfall, Sai Thong National Park, Nong Bua Ra Hoew District, Chaiyapoom	N 15° 52′ 34.7″ E 101° 30′ 34.7″ Altitude 397 m	275	14	5.09
NE18 (SUT0109018)	Tad Tone Waterfall, Muang District, Chaiyaphom	N 15° 58′ 42.5″ E 102° 02′ 24.9″ Altitude 384 m	70	0	0
NE19 (SUT0109019)	Kongkaew Waterfall, Khao Yai National Park, Pak Chong District, Nakhon Ratchasima	N 14° 26′ 14.8″ E 101° 22′ 37.6″ Altitude 713 m	15	0	0
NE20 (SUT0109020)	Lam Takhong Stream, Khao Yai National Park, Pak Chong district, Nakhon Ratchasima	N 14° 25′ 19.6″ E 101° 23′ 26.3″ Altitude 700 m	344	0	0
NE21 (SUT0109021)	Ban Cha Rut Reservoir, Bua Ched District, Surin	N 14° 25′ 50.4″ E 103° 57′ 47.7″ Altitude 201 m	441	150	34.01
NE22 (SUT0109022)	Nong Bua Rai Reservoir, Khao Panomroong, Pra Kone Chai District, Burirum	N 14° 32′ 51.2″ E 102° 58′ 9.4″ Altitude 202 m	48	22	45.83
NE23 (SUT0109023)	Pla Ba Waterfall, Pu Rua District, Loei	N 17° 23′ 24.3″ E 101° 22′ 27.4″ Altitude 640 m	19	0	0
NE24 (SUT0109024)	Than Thong Waterfall, Sung Kom District, Nong Khai	N 18° 01′ 34.7″ E 102° 22′ 8.7″ Altitude 195 m	678	102	15.04
NE25 (SUT0109025)	Huay Hor Water Gate, Muang District, Nakhon Panom	N 17° 21′ 8.4″ E 104° 47′ 2.1″ Altitude 145 m	215	70	32.56
NE26 (SUT0109026)	Tad Kham Waterfall, Pu Lung Ga National Park, Ban Pang District, Nakhon Panom	N 17° 57′ 1.4″ E 104° 09′ 39.9″ Altitude 148 m	1,257	887	70.56
NE27 (SUT0109027)	Tad Po waterfall, Pu Lung Ga National Park, Ban Pang district, Nakhon Panom	N 17° 59′ 0.9″ E 104° 08′ 34.3″ Altitude 148 m	654	167	25.54

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No. (no. specimen)	Name	GPS	No. of collected snails	No. of Infected snails	Infection rates (%)
NE28 (SUT0109028)	Nong Haan, Muang Distrcit, Sakol Nakhon	N 17° 09′ 50.1″ E 104° 09′ 43.7″ Altitude 161 m	68	3	4.41
NE29 (SUT0109029)	Nam Poong Dam, Gud Bak Distrcit, Sakol Nakhon	N 16° 58′ 11.8″ E 103° 59′ 13.4″ Altitude 290 m	260	57	21.92
NE30 (SUT0109030)	Lam Pow Dam, Muang District, Sri Sa Ket	N 16° 36′ 22.3″ E 103° 26′ 27.5″ Altitude 165 m	329	9	2.74
NE31 (SUT0109031)	Ban Nong Wang Wong Reservior, Sri Som Det District, Roi-Et	N 15° 56′ 53.5″ E 103° 31′ 27.8″ Altitude 178 m	1,281	126	9.84
NE32 (SUT0109032)	Bung Toong Sang, Muang District, Khon Kaen	N 16° 26′ 27.8″ E 102° 51′ 28.1″ Altitude 154 m	173	3	1.73
NE33 (SUT0109033)	Bung Kaen Nakhon, Muang District, Khon Kaen	N 16° 24′ 46.6″ E 102° 50′ 21.9″ Altitude 143 m	218	18	8.26
NE34 (SUT0109034)	Nong Sa Ad Bamroong Reservoir, Kosum Pisai District, Mahasarkham	N 16° 18′ 0.5″ E 102° 54′ 38.5″ Altitude 169 m	552	31	5.62
NE35 (SUT0109035)	Tad Tone Waterfall, Nong Soong District, Mukdaharn	N 16° 29′ 34.9″ E 104° 19′ 1.1″ Altitude 219 m	427	81	18.97
East					
E36 (SUT0109036)	Khao Khaew National Park, Sriracha District, Chonburi	N 13° 12′ 45.0″ E 101° 03′ 50.2″ Altitude 128 m	613	93	15.17
E37 (SUT0109037)	Ban Nong Pla Lai, Bang lamung District, Chonburi	N 12° 57′ 54.3″ E 100° 56′ 47.8″ Altitude 17 m	610	80	13.11
E38 (SUT0109038)	Rayong River, Muang District, Rayong	N 12° 39′ 52.6″ E 101° 14′ 48.5″ Altitude 6 m	51	0	0
E39 (SUT0109039)	Hin Khao Canal, Muang District, Rayong	N 12° 36′ 31.7″ E 101° 23′ 22.4″ Altitude 1 m	252	2	0.79
E40 (SUT0109040)	Pung rad Canal, Klang District, Rayong	N 12° 42′ 49.5″ E 101° 46′ 23.4″ Altitude 15 m	827	42	5.08
E41 (SUT0109041)	Chantaburi River, Muang District, Chantaburi	N 12° 36′ 13.8″ E 102° 07′ 11.6″ Altitude 8 m	278	18	6.47
E42 (SUT0109042)	Nam Tok Plew Stream, Plew District, Chantaburi	N 12° 31′ 14.3″ E 102° 10′ 35.4″ Altitude 39 m	223	14	6.28
E43 (SUT0109043)	Pa Tong Canal, Soi Dao District, Chantaburi	N 13° 07′ 5.9″ E 102° 13′ 13.6″ Altitude 231 m	206	20	9.71
E44 (SUT0109044)	Klong Kaew Waterfall, Bo Rai District, Trad	N 12° 37′ 3.0″ E 102° 34′ 52.0″ Altitude 81 m	347	83	23.92
E45 (SUT0109045)	Sra Kaew, Muang District, Sra Kaew	N 13° 49′ 7.0″ E 102° 03′ 37.9″ Altitude 43 m	480	188	39.17
E46 (SUT0109046)	Eto Waterfall, Muang District, Prachinburi	N 14° 08′ 58.9″ E 101° 24′ 45.4″ Altitude 39 m	810	317	39.14
Central					
C47 (SUT0109047)	Dusit Zoo Pond, Dusit, Bangkok	N 13° 46′ 17.4″ E 100° 31′ 14.8″ Altitude 2 m	26	9	34.62
C48 (SUT0109048)	Drainage at Kasetsart University, Bang Khen campus, Bangkok	N 13° 50′ 40.7″ E 100° 34′ 33.5″ Altitude 5 m	199	5	2.51
C49 (SUT0109049)	Pond at Kasetsart University, Bang Khen Campus, Bangkok	N 13° 50′ 22.6″ E 100° 34′ 43.4″ Altitude 1 m	92	1	1.09
C50 (SUT0109050)	Hin Dad Waterfall, Thong Pa Poom District, Kanchanaburi	N 14° 37′ 29.8″ E 98° 43′ 40.2″ Altitude 186 m	13	0	0
C51 (SUT0109051)	Pha Tad Waterfall, Sri Sa wat District, Kanchanaburi	N 14° 38′ 54.9″ E 98° 46′ 41.6″ Altitude 196 m	14	2	14.29
C52 (SUT0109052)	Sai Yok Noi Waterfall, Sai Yok District, Kanchanaburi	N 14° 14′ 27.6″ E 99° 03′ 55.9″ Altitude 166 m	158	35	22.15
C53 (SUT0109053)	Sai Yok Yai Waterfall, Sai Yok District, Kanchanaburi	N 14° 26′ 03.0″ E 98° 51′ 14.7″ Altitude 140 m	91	3	3.30
C54 (SUT0109054)	Wans Soong Canal, Bang Kla District, Chachuengsao	N 13° 39′ 46.2″ E 101° 10′ 48.2″ Altitude 18 m	161	19	11.80

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No. (no. specimen)	Name	GPS	No. of collected snails	No. of Infected snails	Infection rates (%)
C55 (SUT0109055)	Sua Noi Canal, Bnag pa Kong District, Chachuengsao	N 13° 34′ 31.0″ E 100° 57′ 13.8″ Altitude 2 m	239	8	3.35
C56 (SUT0109056)	Bung Sam Pao, Muang District, Chainat	N 15° 16′ 5.9″ E 100° 05′ 11.1″ Altitude 41 m	482	39	8.09
C57 (SUT0109057)	Bird park Pond, Muang District, Chainat	N 15° 12′ 26.5″ E 100° 09′ 21.9″ Altitude 31 m	433	242	55.89
C58 (SUT0109058)	Fish Pond at Bird Park, Muang District, Chainat	N 15° 12′ 18.8″ E 100° 09′ 20.0″ Altitude 39 m	885	41	4.63
C59 (SUT0109059)	Khun Daan Prakarnchon Dam, Muang District, Nakhonnayok	N 14° 18′ 36.5″ E 101° 19′ 14.3″ Altitude 25m	501	130	25.95
C60 (SUT0109060)	Ban mai Phai Chedi, Kampangsaen District , Nakhonpathom	N 14° 02′ 10.5″ E 100° 03′ 27.3″ Altitude 10 m	361	21	5.82
C61 (SUT0109061)	Rice paddy, Banglen District, Nakhonpathom	N 14° 01′ 57.1″ E 100° 10′ 24.0″ Altitude 5m	260	38	14.62
C62 (SUT0109062)	Rice Field at Nong Kra Done, Muang District, Nakhonpathom	N 13° 52′ 41.6″ E 99° 55′ 50.0″ Altitude 14 m	276	22	7.97
C63 (SUT0109063)	Pond at Silpakorn University, Muang District, Nakhonpathom	N 13° 48′ 84.2″ E 100° 02′ 64.5″ Altitude 11 m	272	27	9.93
C64 (SUT0109064)	Bung Bo Ra Ped Lake, Muang District, Nakhon Sawan	N 15° 42′ 3.06″ E 100° 10′ 28.1″ Altitude 17 m	274	23	8.39
C65 (SUT0109065)	Klong bang Ta nai, Pak Kred District, Nontaburi	N 13° 57′ 10.1″ E 100° 29′ 05.4″ Altitude 15 m	380	41	10.79
C66 (SUT0109066)	Ratniyom Canal, Sai Noi District, Nontaburi	N 14° 04′ 17.8″ E 100° 19′ 23.7″ Altitude 9 m	568	139	24.47
C67 (SUT0109067)	Pra Udom Canal, Lad Loom Kaew District, Pathumtani	N 14° 01′ 31.0″ E 100° 22′ 01.1″ Altitude 14 m	49	12	24.49
C68 (SUT0109068)	Na Mai Canal, Lad Loom Kaew District, Pathumtani	N 14° 03′ 32.7″ E 100° 26′ 54.6″ Altitude 22 m	95	9	9.47
C69 (SUT0109069)	Wat Ko Phai, Bang Ban District, Ayutthaya	N 14° 24′ 40.9″ E 100° 26′ 44.6″ Altitude 9 m	4	2	50
C70 (SUT0109070)	Pond at Ban Ta Woong, Ta Woong District, Lopburi	N 14° 50′ 24.4″ E 100° 28′ 21.4″ Altitude 13 m	161	11	6.83
C71 (SUT0109071)	Suan Ma Dua waterfall, Pattana Nikom District, Lopburi	N 14° 55′ 06.0″ E 101° 13′ 09.2″ Altitude 125 m	233	16	6.87
C72 (SUT0109072)	Pasak Chonlasit Reservior, Pattana Nikom District, Lopburi	N 14° 56′ 22.2″ E 101° 04′ 47.4″ Altitude 44 m	15	1	6.67
C73 (SUT0109073)	Tam Ru Canal, Muang District, Samut Prakan	N 13° 30′ 54.7″ E 100° 41′ 12.0″ Altitude 1 m	99	0	0
C74 (SUT0109074)	Prachachomchuen Canal, Ampawa District, Samut Songkram	N 13° 25′ 06.7″ E 99° 57′ 17.8″ Altitude 4 m	29	5	17.24
C75 (SUT0109075)	Don Ko Canal, Ban Phaew District, Samut Sakhon	N 13° 38′ 08.0″ E 100° 05′ 03.0″ Altitude 7 m	42	6	14.29
C76 (SUT0109076)	Ta Pa Canal, Ban Phaew District, Samut Sakhon	N 13° 38′ 07.3″ E 100° 06′ 20.1″ Altitude 30 m	85	1	1.18
C77 (SUT0109077)	Muak Lek Waterfall, Muak Lek District, Saraburi	N 14° 43′ 13.2″ E 101° 11′ 19.4″ Altitude 156 m	199	2	1.01
C78 (SUT0109078)	Dong Phya Yen Waterfall, Muak Lek District, Saraburi	N 14° 44′ 0.6″ E 101° 11′ 44.6″ Altitude 162 m	180	5	2.78
C79 (SUT0109079)	Site1 at Ched Kot Waterfall, Kaeng Koi District, Saraburi	N 14° 28′ 48.5″ E 101° 10′ 22.3″ Altitude 185 m	118	39	33.05
C80 (SUT0109080)	Site2 at Ched Kot waterfall, Kaeng Koi District, Saraburi	N 14° 28′ 34.6″ E 101° 10′ 16.4″ Altitude 157 m	37	12	32.43
C81 (SUT0109081)	Reservoir at Sam Lan Waterfall, Muang District, Saraburi	N 14° 25′ 52.2″ E 100° 57′ 49.6″ Altitude 88 m	315	8	2.54
C82 (SUT0109082)	Muang Moo pond , Muang District, Singhaburi	N 14° 52′ 09.1″ E 100° 24′ 59.1″ Altitude 16 m	282	29	10.28

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No. (no. specimen)	Name	GPS	No. of collected snails	No. of Infected snails	Infection rates (%)
C83 (SUT0109083)	Ban Bang Mae Mai Pond, Bang Pla Ma District, Suphanburi	N 14° 20′ 32.2″ E 100° 09′ 04.9″ Altitude 8 m	349	85	24.36
C84 (SUT0109084)	Wat bang Yai Pond, Bang Pla Ma District, Suphanburi	N 14° 18′ 41.2″ E 100° 09′ 03.7″ Altitude 5 m	956	46	4.81
C85 (SUT0109085)	Bung Cha Wak Pond, Derm Bang Nang Buad District, Supahanburi	N 14° 54′ 04.4″ E 100° 03′ 48.0″ Altitude 26 m	176	30	17.05
C86 (SUT0109086)	Huay Po Canal, Muang District, Angthong	N 14° 36′ 08.3″ E 100° 24′ 12.9″ Altitude 14 m	65	10	15.38
South					
S87 (SUT0109087)	Water Gate km. 19+500, Cha-Am District, Petchaburi	N 12° 51′ 15.1″ E 99° 59′ 48.5″ Altitude 17 m	95	4	4.21
S88 (SUT0109088)	Emergency Water Gate, Cha-Am District, Petchaburi	N 12° 57.4′ 26.0″ E 100° 02′ 07.5″ Altitude 14 m	3	0	0
S89 (SUT0109089)	Petchaburi Dam, Tayang District, Petchaburi	N 12° 54′ 58.6″ E 99° 51′ 34.4″ Altitude 20 m	137	5	3.65
S90 (SUT0109090)	Pond at Silpakorn University Petchaburi Campus, Cha-Am District, Petchaburi	N 11° 26′ 04.6″ E 99° 26′ 56.9″ Altitude 90 m	1,074	1,051	97.86
S91 (SUT0109091)	Huai Yang stream, Huai Yang District, Prachuabkirikhun	N 11° 36′ 47.0″ E 99° 40′ 08.4″ Altitude 18 m	211	30	14.22
S92 (SUT0109092)	Thap sakae stream, Tuap sakae District, Prachuabkirikhun	N 11° 29′ 40.1″ E 99° 36′ 20.3″ Altitude 13 m	124	8	6.45
S93 (SUT0109093)	Kha On waterfall, Bangsapan District, Prachuabkirikhun	N 11° 26′ 04.6″ E 99° 26′ 56.9″ Altitude 90 m	32	4	12.50
S94 (SUT0109094)	Sai Khu waterfall, Bangsapan District, Prachuabkirikhun	N 11° 14′ 21.8″ E 99° 21′ 36.1″ Altitude 83 m	429	25	5.83
S95 (SUT0109095)	Kapoh Waterfall, Ta Sae District, Chumporn	N 10° 44′ 28.7″ E 99° 12′ 53.9″ Altitude 69 m	133	5	3.76
S96 (SUT0109096)	Ra Canal, Lungsuan District, Chumporn	N 09° 59′ 04.3″ E 99° 00′ 59.8″ Altitude 44 m	739	97	13.13
S97 (SUT0109097)	Si Kheed Waterfall, Sichon District, Nakhon Sri Thammarat	N 09° 00′ 40.8″ E 99° 46′ 30.1″ Altitude 45 m	9	1	11.11
S98 (SUT0109098)	Krung Ching Waterfall, Noppitum District, Nakhon Sri Thammarat	N 08° 43′ 14.0″ E 99° 40′ 15.2″ Altitude 45 m	18	5	27.78
S99 (SUT0109099)	Yod Leung Stream, Noppitum District, Nakhon Sri Thammarat	N 08° 38′ 10.5″ E 99° 45′ 11.6″ Altitude 68 m	872	343	0.39
S100 (SUT0109100)	Palian River, Yan Ta Khao District, Trang	N 07° 22′ 11.5″ E 99° 40′ 51.6″ Altitude 12 m	205	22	10.73
S101 (SUT0109101)	Palian Dam, Palien District, Trang	N 07° 19′ 13.2″ E 99° 48′ 28.8″ Altitude 43 m	506	46	9.09
S102 (SUT0109102)	Tone Tok Waterfall, Palien District, Trang	N 07° 16′ 44.2″ E 99° 53′ 10.6″ Altitude 41 m	301	78	25.91
S103 (SUT0109103)	Tone Plew Waterfall, Nayong District, Trang	N 07° 32′ 48.2″ E 99° 47′ 17.0″ Altitude 63 m	1,049	97	0.09
S104 (SUT0109104)	Ang Thong Waterfall, Sikao District, Trang	N 07° 33′ 01.2″ E 99° 24′ 56.9″ Altitude 37 m	311	107	34.41
S105 (SUT0109105)	Falan Waterfall, Srinakarin District, Pattaloong	N 07° 36′ 25.9″ E 99° 54′ 39.0″ Altitude 80 m	1	0	0
S106 (SUT0109106)	Ban Au Rua Stream, Muang District, Ratchaburi	N 13° 31′ 21.9″ E 99° 50′ 57.3″ Altitude 152 m	9	2	22.22
S107 (SUT0109107)	Huay Haeng Stream, Suanphueng District, Ratchaburi	N 13° 31′ 03.9″ E 99° 20′ 29.2″ Altitude 113 m	213	18	8.45
S108 (SUT0109108)	Huay Nuang Stream, Suanphueng District, Ratchaburi	N 13° 31′ 21.9″ E 99° 17′ 36.5″ Altitude 151m	234	23	9.83
S109 (SUT0109109)	Bangborn Stream, Kraburi District, Ranong	N 10° 20′ 10.8″ E 98° 46′ 48.7″ Altitude 18 m	34	2	5.88

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No. (no. specimen)	Name	GPS	No. of collected snails	No. of Infected snails	Infection rates (%)
S110 (SUT0109110)	Na Ca Stream, Wild Life Sancuatuary, Muang District, Ranong	N 9° 27′ 26.6″ E 98° 30′ 36.9″ Altitude 3 m	56	1	1.79
S111 (SUT0109111)	Wiphawadee Waterfall, Donsak District, Surat Thani	N 9° 8′ 9.6″ E 99° 40′ 31.2″ Altitude 10 m	70	2	2.86
S112 (SUT0109112)	Yan Canal, Wipawadee District, Surathani	N 9° 12′ 12.8″ E 98°57′20.3″ Altitude 66 m	292	21	7.19
S113 (SUT0109113)	Ton Sai Waterfall, Tha Lang District, Phuket	N 8° 1′ 32.4″ E 98° 21′58.8″ Altitude 45 m	222	27	12.16
S114 (SUT0109114)	Bang Pae waterfall, Tha Lang District, Phuket	N 8° 2′ 20.5″ E 98° 23′49.3″ Altitude 50 m	76	13	17.11
S115 (SUT0109115)	Kathu Waterfall, Kathu District, Phuket	N 7° 55′ 49.4″ E 98° 19′34″ Altitude 43 m	385	5	1.30
S116 (SUT0109116)	Raman Waterfall, Ta Kua Tung District, Phang Nga	N 8° 27′ 8.5″ E 98° 28′0.9″ Altitude 33 m	3	0	0
S117 (SUT0309117)	Sa Morakot Stream, Klong Tom District, Krabi	N 7° 55′ 14.9″ E 99° 15′47.1″ Altitude 24 m	356	4	1.12
S118 (SUT0109118)	Panan Waterfall, Kuan galung District, Satoon	N 6° 51′ 22.8″ E 100° 9′48.6″ Altitude 47 m	170	6	3.53
S119 (SUT0109119)	Tha Phae Dam, Kuan Done District, Satoon	N 6° 49′ 26″ E 100° 2′2.3″ Altitude 41 m	760	8	1.05
S120 (SUT0109120)	Klong Muang, Kuan Niang District, Songkhla	N 7° 12′ 24.5″ E 100° 22′43.1″ Altitude 13 m	82	9	10.98

(N 19°25’31.7”, E 97°59’24.9”, Altitude 343 m); N6 = Huay Sua Thao Stream, Muang District, Mae Hong Son Province (SUT0109006) (N 19°15’32.0”, E 97°54’43.7”, Altitude 237 m); N7 = Klong Nam Lai Waterfall, Klong Lan District, Kam Phaeng Phet Province (SUT0109007) (N 16°11’32.7”, E 99°15’51.0”, Altitude 241 m); N8 = Tad Duen Waterfall, Sri Satchanalai District, Sukhothai Province (SUT0109008) (N 17°33’23.2”E 99°29’76.8”, Altitude 414 m); N9 = Sri Satchanalai Stream, Sri Satchanalai National Park, Sukhothai Province (SUT0109009) (N 17°33’5.9”, E 99°29’24.8”, Altitude 182 m); N10 = Mae Pool Waterfall, Lub Lae District, Ut-taradit Province (SUT0109010) (N 17°43’45.0”, E 99°58’50.6”, Altitude 164 m); N11 = Chueng Thong Waterfall, Muang District, Prae Province (SUT0109011) (N 18°01’54.2”, E 100°15’52.8”, Altitude 298 m); N12 = Huay Sa Nien Stream, Muang District, Nan Province (SUT0109012) (N 18°51’1.3”, E 100°39’16.2”, Altitude 280 m); N13 = Huay Ton Phueng Waterfall, Doi Pu Nang National Park, Chiang Muon District, Phyao Province (SUT0109013) (N 18°55’5.3”, E 100°12’15.7”, Altitude 379); N14 = Tansawan Waterfall, Doi Pu Nang National Park, Chiang Muon District, Phyao Province (SUT0109014) (N 18°51’22.7”, E 100°11’9.6”, Altitude 420 m); N15 = Mae Mine Stream, Mae Ta District, Lampang Province (SUT0109015) (N 18°07’1.8”, E 99°37’35.1”, Altitude 269 m).

The Northeast: NE 16 = Huay Lum Po Dang Stream, Thepsathit District, Chaiyapoom Province (SUT0109016) (N 15°33’42.8”, E 101°24’56.9”, Altitude 471 m); NE 17 = Sai Thong Waterfall, Sai Thong National Park, Nong Bua Ra Hoew District, Chaiyapoom Province (SUT0109017) (N 15°52’34.7”, E 101°30’34.7”, Altitude 397 m); NE18 = Tad Tone Waterfall, Muang District, Chaiyappom Porvince (SUT0109018) (N 15°58’42.5”, E 102°02’24.9”, Altitude 384 m); NE19 = Kongkaew Waterfall, Khao Yai National Park, Pak Chong District, Nakhon Ratchasima Province (SUT0109019) (N 14°26’14.8”, E 101°22’37.6”, Altitude 713 m); NE20 = Lam Takhong Stream, Khao Yai National Park, Pak Chong District, Nakhon Ratchasima Province (SUT0109020) (N 14°25’19.6”, E 101°23’26.3”, Altitude 700 m); NE21 = Ban Cha Rut Reservoir, Bua Ched District, Surin Province (SUT0109021) (N 14°25’50.4”, E 103°57’47.7”, Altitude 201 m); NE22 = Nong Bua Rai Reservoir, Khao Panomroong, Pra Kone Chai District, Burirum Province (SUT0109022) (N 14°32’51.2”, E 102°58’9.4”, Altitude 202 m); NE23 = Pla Ba Waterfall, Pu Rua District, Loei Province (SUT0109023) (N 17°23’24.3”, E 101°22’27.4”, Altitude 640 m); NE24 = Than Thong Waterfall, Sung Kom District, Nong Khai Province (SUT0109024) (N 18°01’34.7”, E 102°22’8.7”, Altitude 195 m); NE25 = Huay Hor Water Gate, Muang District, Nakhon Panom Province (SUT0109025) (N 17°21’8.4”, E 104°47’2.1”, Altitude 145 m); NE26 = Tad Kham Waterfall, Pu Lung Ga National Park, Ban Pang District, Nakhon Panom province (SUT0109026) (N 17°57’1.4”, E 104°09’39.9”, Altitude 148 m); NE27 = Tad Po Waterfall, Pu Lung Ga National Park, Ban Pang District, Nakhon Panom Province (SUT0109027) (N 17°59’0.9”, E 104°08’34.3”, Altitude 148 m); NE28 = Nong Han, Muang Distrcit, Sakol Nakhon Prov-

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ince (SUT0109028) (N 17°09’50.1”, E 104°09’43.7”, Altitude 161 m); NE29 = Nam Poong Dam, Gud Bak Distrcit, Sakol Nakhon Province (SUT0109029) (N 16°58’11.8”, E 103°59’13.4”, Altitude 290 m); NE30 = Lam Pow Dam, Muang District, Sri Sa Ket Province (SUT0109030) (N 16°36’22.3”, E 103°26’27.5”, Altitude 165 m); NE31 = Ban Nong Wang Wong Reservior, Sri Som Det District, Roi-Et Province (SUT0109031) (N 15°56’53.5”, E 103°31’27.8”, Altitude 178 m); NE32 = Bung Toong Sang, Muang District, Khon Kaen Province (SUT0109032) (N 16°26’27.8”, E 102°51’28.1”, Altitude 154 m); NE33 = Bung Kaen Nakhon, Muang District, Khon Kaen Province (SUT0109033) (N 16°24’46.6”, E 102°50’21.9”, Altitude 143 m); NE34 = Nong Sa Ard Bamroong Reservoir, Kosum Pisai District, Mahasarkham Province (SUT0109034) (N 16°18’0.5”, E 102°54’38.5”, Altitude 169 m); NE35 = Tad Tone Waterfall, Nong Soong District, Mukdaharn Province (SUT0109035) (N 16°29’34.9”, E 104°19’1.1”, Altitude 219 m)

The East: E36 = Khao Khaew National Park, Sriracha District, Chonburi Province (SUT0109036) (N 13°12’45.0”, E 101°03’50.2”, Altitude 128 m); E37 = Ban Nong Pla Lai, Bang lamung District, Chonburi Province (SUT0109037) (N 12°57’54.3”, E 100°56’47.8”, Altitude 17 m); E38 = Rayong River, Muang District, Rayong Province (SUT0109038) (N 12°39’52.6”, E 101°14’48.5”, Altitude 6 m); E39 = Hin Khao Canal, Muang District, Rayong Province (SUT0109039) (N 12°36’31.7”, E 101°23’22.4”, Altitude 1 m); E40 = Pung rad Canal, Klang District, Rayong Province (SUT0109040) (N 12°42’49.5”, E 101°46’23.4”, Altitude 15 m); E41 = Chantaburi River, Muang District, Chantaburi Province (SUT0109041) (N 12°36’13.8”, E 102°07’11.6”, Altitude 8 m); E42 = Nam Tok Plew Stream, Plew District, Chantaburi Province (SUT0109042) (N 12°31’14.3”, E 102°10’35.4”, Altitude 39 m); E43 = Pa Tong Canal, Soi Dao District, Chantaburi Province (SUT0109043) (N 13°07’5.9”, E 102°13’13.6”, Altitude 231 m); E44 = Klong Kaew Waterfall, Bo Rai District, Trad Province (SUT0109044) (N 12°37’3.0”, E 102°34’52.0”, Altitude 81 m); E45 = Sra Kaew, Muang District, Srakaew Province (SUT0109045) (N 13°49’7.0”, E 102°03’37.9”, Altitude 43 m); E46 = Eto Waterfall, Muang District, Prachinburi Province (SUT0109046) (N 14°08’58.9”, E 101°24’45.4”,Altitude 39 m).

The Central: C47 = Dusit Zoo Pond, Dusit, Bangkok (SUT0109047) (N 13°46’17.4”, E 100°31’14.8”, Altitude 2 m); C48 = Drainage at Kasetsart University, Bang Khen Campus, Bangkok (SUT0109048) (N 13°50’40.7”, E 100°34’33.5”, Altitude 5 m); C49 = Pond at Kasetsart University, Bang Khen Campus, Bangkok (SUT0109049) (N 13°50’22.6”, E 100°34’43.4”, Altitude 1 m); C50 = Hin Dad Waterfall, Thong Pa Poom District, Kanchanaburi Province (SUT0109050) (N 14°37’29.8”, E 98°43’40.2”, 186 m); C51 = Pha Tad Waterfall, Sri Sa wat District, Kanchanaburi Province

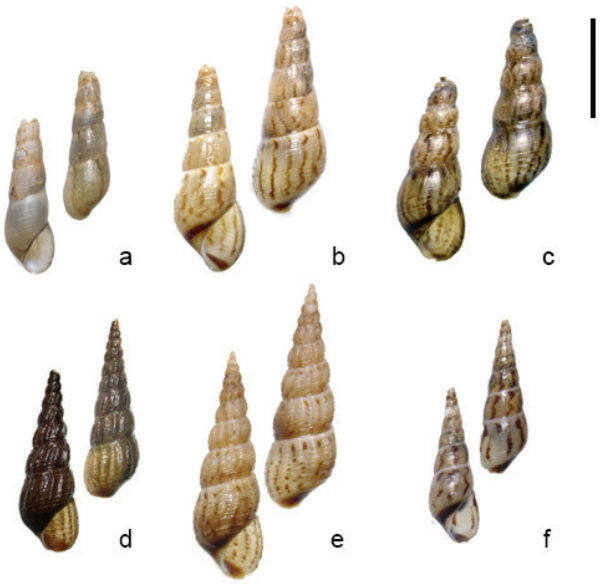

Figure 2. Shell Morphology of *Melanoides tuberculata* in Thailand
a. Sri Satchanalai Stream, Sri Satchanalai National Park, Sri Satchanalai District, Sukhothai Province (SUT0109009)
b. Lam Takhong Stream, Khao Yai National Park, Pak Chong District, Nakhon, Ratchasima Province (SUT0109020)
c. Rice Field at Nong Kra Done, Muang District, Nakhon Pathom Province (SUT0109062)
d. Pond in Silpakorn University, Muang District, Nakhon Pathom Province (SUT0109063)
e. Bang Ta-nai Canal, Pak Kred District, Nontaburi Province (SUT0109065)
f. Huay Nuang Stream, Suanphueng District, Ratchaburi Province (SUT0109108)
Scale bar = 1.0 mm.

(SUT0109051) (N 14°38’54.9”, E 98°46’41.6”, Altitude 196 m); C52 = Sai Yok Noi Waterfall, Sai Yok District, Kanchanaburi Province (SUT0109051) (N 14°14’27.6”, E 99°03’55.9”, Altitude 166 m); C53 = Sai Yok Yai Waterfall, Sai Yok District, Kanchanaburi Province (SUT0109053) (N 14°26’03.0”, E 98°51’14.7”, Altitude 140 m); C54 = Wans Soong Canal, Bang Kla District, Chachuengsao Province (SUT0109054) (N 13°39’46.2”, E 101°10’48.2”, Altitude 18 m); C55 = Sua Noi Canal, Bnag pa Kong District, Chachuengsao Province (SUT0109055) (N 13°34’31.0”, E 100°57’13.8”, Altitude 2 m); C56 = Bung Sam Pao, Muang District, Chainat Province (SUT0109056) (N 15°16’5.9”, E 100°05’11.1”, Altitude 41 m); C57 = Bird Park Pond, Muang District, Chainat Province (SUT0109057) (N 15°12’26.5”, E 100°09’21.9”, Altitude 31 m); C58 = Fish Pond at Bird Park, Muang District, Chainat Province (SUT0109058) (N 15°12’18.8”, E 100°09’20.0”, Altitude 39 m); C59 = Khun Daan Prakarnchon Dam, Muang District, Nakhonnayok Province (SUT0109059) (N 14°18’36.5”, E 101°19’14.3”, Altitude 25m); C60 = Ban mai Phai Chedi, Kampangsaen District, Nakhonpathom Province (SUT0109060) (N 14°02’10.5”, E 100°03’27.3”, Altitude 10 m); C61 = Rice paddy, Ban-

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glen District, Nakhonpathom Province (SUT0109061) (N 14°01’57.1”, E 100°10’24.0”, Altitude 5m); C62 = Rice Field at Nong Kra Done, Muang District, nakhon Pathom Province (SUT0109062) (N 13°52’41.6”, E 99°55’50.0”, Altitude 14 m); C63 = Pond at Silpakorn University, Muang District, Nakhonpathom Province (SUT0109063) (N 13°48’84.2”, E 100°02’64.5”, Altitude 11 m); C64 = Bung Bo Ra Ped Lake, Muang District, Nakhon Sawan province (SUT0109064) (N 15°42’3.06”, E 100°10’28.1”, Altitude 17 m); C65 = Bang Ta nai Canal, Pak Kred District, Nontaburi Province (SUT0109065) (N 13°57’10.1”, E 100°29’05.4”, Altitude 15 m); C66 = Ratniyom Canal, Sai Noi District, Nontaburi Province (SUT0109066) (N 14°04’17.8”, E 100°19’23.7”, Altitude 9 m); C67 = Pra Udom Canal, Lad Loom Kaew District, Pathumtani Province (SUT0109067) (N 14°01’31.0”, E 100°22’01.1”, Altitude 14 m); C68 = Na Mai Canal, Lad Loom Kaew District, Pathumtani Province (SUT0109068) (N 14°03’32.7”, E 100°26’54.6”, Altitude 22 m); C69 = Wat Ko Phai, Bang Ban District, Ayutthaya Province (SUT0109069) (N 14°24’40.9”, E 100°26’44.6”, Altitude 9 m); C70 = Pond at Ban Ta Woong, Ta Woong District, Lopburi Province (SUT0109070) (N 14°50’24.4”, E 100°28’21.4”, Altitude 13 m); C71 = Suan Ma Dua Waterfall, Pattana Nikom District, Lopburi Province (SUT0109071) (N 14°55’06.0”, E 101°13’09.2”, Altitude 125 m); C72 = Pasak Chonlasit Reservior, Pattana Nikom District, Lopburi Province (SUT0109072) (N 14°56’22.2”, E 101°04’47.4”, Altitude 44 m); C73 = Tam Ru Canal, Muang District, Samut Prakan Province (SUT0109073) (N 13°30’54.7”, E 100°41’12.0”, Altitude 1 m); C74 = Prachachomchuen Canal, Ampawa District, Sanut Songkram Province (SUT0109074) (N 13°25’06.7”, E 99°57’17.8”, Altitude 4 m); C75 = Don Ko Canal, Ban Phaew District, Samut Sakhon Province (SUT0109075) (N 13°38’08.0”, E 100°05’03.0”, Altitude 7 m);C76 = Ta Pa Canal, Ban Phaew District, Samut Sakhon Province (SUT0109076) (N 13°38’07.3”, E 100°06’20.1”, Altitude 30 m); C77 = Muak Lek Waterfall, Muak Lek District, Saraburi Province (SUT0109077) (N 14°43’13.2”, E 101°11’19.4”, Altitude 156 m); C78 = Dong Phya Yen Waterfall, Muak Lek District, Saraburi Province (SUT0109078) (N 14°44’0.6”, E 101°11’44.6”, Altitude 162 m); C79 = Site1 at Ched Kot Waterfall, Kaeng Koi District, Saraburi Province (SUT0109079) (N 14°28’48.5”, E 101°10’22.3”, Altitude 185 m); C80 = Site2 at Ched Kot Waterfall, Kaeng Koi District, Saraburi Province (SUT0109080) (N 14°28’34.6”, E 101°10’16.4”, Altitude 157 m); C81 = Reservoir at Sam Lan Waterfall, Muang District, Saraburi Province (SUT0109081) (N 14°25’52.2”, E 100°57’49.6”, Altitude 88 m); C82 = Muang Moo Pond, Muang District, Singhaburi Province (SUT0109082) (N 14°52’09.1”, E 100°24’59.1”, Altitude 16 m); C83 = Ban Bang Mae Mai Pond, Bang Pla Ma District, Suphanburi Province (SUT0109083) (N 14°20’32.2”, E 100°09’04.9”, Altitude 8 m); C84 = Wat bang Yai Pond, Bang Pla Ma District, Suphanburi Province (SUT0109084) (N 14°18’41.2”, E 100°09’03.7”, Altitude 5 m); C85 = Bung Cha Wak, Derm Bang Nang Buad District, Supahanburi Province (SUT0109085) (N 14°54’04.4”, E 100°03’48.0”, Altitude 26 m); C86 = Huay Po Canal, Muang District, Angthong Province (SUT0109086) (N 14°36’08.3”, E 100°24’12.9”, Altitude 14 m).

The South: S87 = Water Gate km. 19+500, Cha-Am District, Petchaburi Province (SUT0109087) (N 12°51’15.1”, E 99°59’48.5”, Altitude 17 m); S88 = Emergency Water Gate, Cha-Am District, Petchaburi Province (SUT0109088) (N 12°57.4’26.0”, E 100°02’07.5”, Altitude 14 m); S89 = Petchaburi Dam, Tayang District, Petchaburi Province (SUT0109089) (N 12°54’58.6”, E 99°51’34.4”, Altitude 20 m); S90 = Pond at Silpakorn University Petchaburi Campus, Cha-Am district, Petchaburi Province (SUT0109090) (N 11°26’04.6”, E 99°26’56.9”, Altitude 90 m); S91 = Huai Yang stream, Huai Yang District, Prachuabkirikhun Province (SUT0109091) (N 11°36’47.0”, E 99°40’08.4”, Altitude 18 m); S92 = Thap Sakae Stream, Tuap Sakae District, Prachuabkirikhun Province (SUT0109092) (N 11°29’40.1”, E 99°36’20.3”, Altitude 13 m); S93 = Kha On Waterfall, Bangsapan District, Prachuabkirikhun Province (SUT0109093) (N 11°26’04.6”E 99°26’56.9”, Altitude 90 m); S94 = Sai Khu Waterfall, Bangsapan District, Prachuabkirikhun Province (SUT0109094) (N 11°14’21.8”, E 99°21’36.1”, Altitude 83 m); S95 = Kapoh Waterfall, Ta Sae District, Chumporn Province (SUT0109095) (N 10°44’28.7”, E 99°12’53.9”, Altitude 69 m); S96 = Ra Canal, Lungsuan District, Chumporn Province (SUT0109096) (N 09°59’04.3”, E 99°00’59.8”, Altitude 44 m); S97 = Si Kheed Waterfall, Sichon District, Nakhon Sri Thammarat Province (SUT0109097) (N 09°00’40.8”, E 99°46’30.1”, Altitude 45 m); S98 = Krung Ching Waterfall, Noppitum District, Nakhon Sri Thammarat Province (SUT0109098) (N 08°43’14.0”, E 99°40’15.2”, Altitude 45 m); S99 = Yod Leung Stream, Noppitum District, Nakhon Sri Thammarat Province (SUT0109099) (N 08°38’10.5”, E 99°45’11.6”, Altitude 68 m); S100 = Palian River, Yan Ta Khao District, Trang Province (SUT0109100) (N 07°22’11.5”, E 99°40’51.6”, Altitude 12 m); S101 = Palian Dam, Palien District, Trang Province (SUT0109101) (N 07°19’13.2”, E 99°48’28.8”, Altitude 43 m); S102 = Tone Tok Waterfall, Palien District, Trang Province (SUT0109102) (N 07°16’44.2”E 99°53’10.6”, Altitude 41 m); S103 = Tone Plew Waterfall, Nayong District, Trang Province (SUT0109103) (N 07°32’48.2”E 99°47’17.0”, Altitude 63 m); S104 = Ang Thong Waterfall, Sikao District, Trang Province (SUT0109104) (N 07°33’01.2”, E 99°24’56.9”, Altitude 37 m); S105 = Falan Waterfall, Srinakarin District, Pattaloong Province (SUT0109105) (N 07°36’25.9”, E 99°54’39.0”, Altitude 80 m); S106 = Ban Au Rua Stream, Muang District, Ratchaburi Province (SUT0109106) (N

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13°31’21.9”, E 99°50’57.3”, Altitude 152 m); S107 = Huay Haeng Stream, Suanphueng District, Ratchaburi Province (SUT0109107) (N 13°31’03.9”, E 99°20’29.2”, Altitude 113 m); S108 = Huay Nuang Stream, Suanphueng District, Ratchaburi Province (SUT0109108) (N 13°31’21.9”, E 99°17’36.5”, Altitude 151m); S109 = Bangborn Stream, Kraburi District, Ranong Province (SUT0109109) (N 10°20’10.8”, E 98°46’48.7”, Altitude 18 m); S110 = Na Ca Stream, Wild Life Sancuatuary, Muang District, Ranong Province (SUT0109110) (N 9°27’26.6”E 98°30’36.9”, Altitude 3 m); S111 = Wiphawadee Waterfall, Donsak District, Surat Thani Province (SUT0109111) (N 9°8’9.6”, E 99°40’31.2”, Altitude 10 m); S112 = Yan Canal, Wipawadee District, Surathani Province (SUT0109112) (N 9°12’12.8”, E 98°57’20.3”Altitude 66 m); S113 = Ton Sai Waterfall, Tha Lang District, Phuket Province (SUT0109113) (N 8°1’32.4”, E 98°21’58.8”, Altitude 45 m); S114 = Bang Pae Waterfall, Tha Lang District, Phuket Province (SUT0109114) (N 8°2’20.5”, E 98°23’49.3”, Altitude 50 m); S115 = Kathu Waterfall, Kathu District, Phuket Province (SUT0109115) (N 7°55’49.4”, E 98°19’34”, Altitude 43 m); S116 = Raman Waterfall, Ta Kua Tung District, Phang Nga Province (SUT0109116) (N 8°27’8.5”, E 98°28’0.9”, Altitude 33 m) ; S117 = Sa Morakot Stream, Klong Tom District, Krabi Province (SUT0309117) (N 7°55’14.9”, E 99°15’47.1”, Altitude 24 m); S118 = Panan Waterfall, Kuan galung District, Satoon Province (SUT0109118) (N 6°51’22.8”, E 100°9’48.6”, Altitude 47 m); S119 = Tha Phae Dam, Kuan Done District, Satoon Province (SUT0109119) (N 6°49’26”, E 100°2’2.3”, Altitude 41 m); S120 = Klong Muang, Kuan Niang District, Songkhla Province (SUT0109120) (N 7°12’24.5”, E 100°22’43.1”, Altitude 13 m).

Habitat at the study sites in Thailand

The study sites were usually found to be covered with big and medium trees that allow the sunlight to pass through to the stream. The average light intensity was >10,000 lux at noon. The current was swift in the rainy season, and water temperature was 21–28 °C. There were small to medium sized rocks all over the streams. The collected snails were found on the rocks, rough sand, and on aquatic plants. The physico-chemical quality of the environment and the water changed with the seasons and affected the study areas during the dry and flood season.

Table 2. Distribution of cercariae obtained from Melanoides tuberculata (32,026 snails) in Thailand. Abbreviations: N - North; NE - Northeast; E - East; C - Central; S - South.

Cercaria species	No. infected snails distribution					Total	Infection rates (%)
	N = 15	NE = 20	E = 11	C = 39	S = 35		
Type 1. Parapleurophocercous cercariae:							
1. *Haplorchis pumilio*	23	0	58	265	25	**371**	**1.16**
2. *Haplorchis taichui*	1	2	0	0	89	**92**	**0.29**
3. *Stictodora tridactyla*	0	582	75	210	1,315	**2,182**	**6.81**
Type 2. Pleurophocercous cercariae:							
4. *Centrocestus formosanus*	55	0	3	10	6	**74**	**0.23**
Type 3. Xiphidiocercariae:							
5. *Acanthatrium hitaense*	9	14	54	1	10	**88**	**0.27**
6. *Loxogenoides bicolor*	29	802	485	573	484	**2,373**	**7.41**
7. *Haematoloechus similis*	53	314	92	1	8	**468**	**1.46**
Type 4. Megalurous cercariae:							
8. *Cloacitrema philippinum*	1	0	11	0	0	**12**	**0.04**
9. *Philophthalmus* sp.	0	0	0	52	5	**57**	**0.18**
Type 5. Furcocercous cercariae:							
10. *Cardicola alseae*	0	2	0	2	22	**33**	**0.1**
11. *Alaria mustelae*	0	1	2	22	22	**47**	**0.15**
12. *Transversotrema laruei*	8	0	21	2	58	**89**	**0.28**
13. *Apatemon gracilis*	0	20	0	4	31	**55**	**0.17**
14. *Mesostephanus appendiculatus*	0	3	0	0	0	**3**	**0.009**
Type 6. Echinostome cercariae:							
15. *Echinochasmus pelecani*	0	0	19	0	0	**19**	**0.06**
Type 7. Amphistome cercariae:							
16. *Gastrothylax crumenifer*	0	0	4	0	4	**8**	**0.02**
Type 8. Renicolid cercariae:							
17. *Cercaria caribbea* LXVIII	0	0	33	12	0	**45**	**0.14**
Type 9. Cotylomicrocercous cercariae:							
18. *Podocotyle* (*Podocotyle*) *lepomis*	0	0	0	3	0	**3**	**0.009**
No. infected snails	**179**	**1,740**	**857**	**1,164**	**2,079**	**6,019**	**18.79**
No. of cercaria species	**8**	**9**	**12**	**13**	**13**	**18**	

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Table 3. Size range and average size (in micrometers, calculated from 20 cercariae) of eighteen species of cercariae were measured and obtained from *Melanoides tuberculata* in Thailand.

	Trematodes species																	
	H. pumilio	***H. taichui***	***S. tridactyla***	***C. formosanus***	***A. hitaense***	***L. bicolor***	***H. similis***	***C. philippinum***	***Philophthalmus sp.***	***C. alseae***	***A. mustelae***	***T. laruei***	***A. gracilis***	***M. appendiculatus***	***E. pelecani***	***G. crumenifer***	***C. caribbea LXVIII***	***P (Podocotyle) lepomis***
Body	85-128 (av. 108) ×168-295 (av. 257)	93-135 (av. 132) ×156-276 (av. 242)	69-149 (av. 112) ×255-309 (av. 275)	45-72 (av. 64) ×82-120 (av. 117)	53-92 (av. 78) ×80-110 (av. 100)	54-82 (av. 75) ×90-120 (av. 110)	87-104 (av. 95) ×130-164 (av. 148)	122-184 (av. 169) ×280-450 (av. 396)	120-125 (av. 122) ×525-595 (av. 570)	18-39 (av. 28) ×72-110 (av. 95)	105-154 (av. 138) ×185-280 (av. 255)	425-670 (av. 574) ×280-510 (av. 370)	41.5-90.0 (av. 72) ×115-140 (av. 126)	92-120 (av. 109) ×160-250 (av. 225)	68-87 (av. 85) ×100-125 (av. 118)	190-250 (av. 220) × 350-415 (av. 370)	128-140 (av. 129) ×390-435 (av. 420)	65-93 (av. 73) ×103-145 (av. 123)
Genital pore	-	-	-	-	-	-	-	-	-	-	-	12 –18 (av. 15) ×12-18 (av. 15)	-	-	-	-	-	-
Oral sucker	27-48 (av. 36) ×27-48 (av. 36)	24-45 (av. 38) ×27-45 (av. 38)	36-48 (av. 38) ×33-52 (av. 41)	16-26 (av. 24) ×17-28 (av. 25)	25-32 (av. 30) ×34-40 (av. 37)	24-30 (av. 28) ×24-30 (av. 28)	34-42 (av. 38) ×37-46 (av. 42)	50-60 (av. 56) ×50-60 (av. 56)	50-57 (av. 55) ×55-62.5 (av. 60)	-	28-40 (av. 36) ×28-40 (av. 36)	-	15-25 (av.21.45) ×20-30 (av. 23)	20-45 (av. 35) ×35-48 (av. 39)	21-30 (av. 23) ×24-30 (av. 25)	45-65 (av. 52) ×45- 65 (av. 52)	28-33 (av. 26.) ×28-33 (av. 26)	30-38 (av. 32) ×25-38 (av. 30)
Anterior organ	-	-	-	-	-	-	-	-	-	11-15 (av. 13) ×14-20 (av. 17)	-	-	-	-	-	-	-	-
Stylet	-	-	-	-	8-13 (av. 10) ×11-13 (av. 12)	5-8 (av. 7) ×14-20 (av. 18)	20-34 (av. 30) ×20-34 (av. 32)	-	-	-	-	-	-	-	-	-	-	4-6 (av. 5) ×6-10 (av. 8)
Eye spots	-	-	6-12 (av. 8) ×12-17 (av. 14)	-	-	-	-	-	-	-	-	14-20 (av. 18) ×14-20 (av. 18)	-	-	-	-	-	-
Pharynx	8-10 (av. 9) ×12-19 (av. 15)	8-10 (av. 10) ×12-18 (av. 15)	11-19 (av. 16) ×14-21 (av. 17)	7-9 (av. 8) ×8-10 (av. 9)	10-15 (av. 13) ×12-24 (av. 20)	4-6 (av. 5) ×4-10 (av. 8)	11-13 (av. 12) ×15-17 (av. 16)	23-38 (av. 35) ×25-40 (av. 35)	20-25 (av. 23) ×20-25 (av. 23)	-	10-15 (av. 13) ×14-17 (av. 15)	25 – 57 (av. 574) ×280-510 (av. 370)	6-10 (av. 8) ×7-13 (av. 9)	8-10 (av. 9) ×11-18 (av. 15)	8-15 (av. 12) ×9-15 (av. 12)	8-10 (av. 10) ×8-12 (av. 11)	8-14 (av. 12) ×10-14 (av. 12)	13-18 (av. 14) ×10-13 (av. 11)
Ventral sucker	14-24 (av. 18) ×14-24 (av. 18)	15-25 (av. 20) ×15-25 (av. 20)	10-25 (av. 19) ×10-25 (av. 19)	12-16 (av. 14) ×12-16 (av. 14)	15-16 (av. 16) ×15-18 (av. 17)	12-18 (av. 15) ×13-20 (av. 17)	10-20 (av. 16) ×12-20 (av. 18)	60-70 (av. 68) ×60-75 (av. 70)	62.5-67.5 (av. 64) ×55-70 (av. 64)	-	15-30 (av. 22) ×15-30 (av. 22)	85 -105 (av. 95) ×85–105 (av. 95)	16-25 (av. 23) ×20-30 (av. 25)	15-24 (av. 20) ×15-24 (av. 20)	20-31 (av. 25) ×22-30 (av. 25)	48-68 (av. 55) ×48-68 (av. 55)	29-36 (av. 30) ×29-36 (av. 30)	28-40 (av. 35) ×28-40 (av. 35)
Excre-tory bladder	28-40 (av. 34) ×28-40 (av. 34)	30-42 (av. 37) ×30-42 (av. 37)	87-119 (av. 98) ×52-98 (av. 76)	24-30 (av. 28) ×38-52 (av. 45)	8-12 (av. 10) ×20-46 (av. 38)	8-10 (av. 9) ×10-30 (av. 25)	20-22 (av. 21) ×20-24 (av. 22)	15-20 (av. 18) ×14-21 (av. 18)	37.5-45 (av. 41) ×35-50 (av. 41)	3-8 (av. 5) ×10-35 (av. 21)	-	11-23 (av. 15) ×11-21 (av. 15)	15-25 (av. 18) ×15-30 (av. 20)	20-40 (av. 35) ×20-40 (av. 35)	21-35 (av. 33) ×23-36 (av. 33)	80-140 (av. 125) ×80- 140 (av. 125)	15-20 (av. 17) ×15-20 (av. 17)	20-30 (av. 26) ×18-25 (av. 21)

	Trematodes species																
	H. pumilio	*H. taichui*	*S. tridactyla*	*C. formosanus*	*A. hitaense*	*L. bicolor*	*H. similis*	*C. philippinum*	*Philophthalmus sp.*	*C. alseae*	*A. mustelae*	*T. laruei*	*A. gracilis*	*M. appendiculatus*	*E. pelecani*	*G. crumenifer*	*C. caribbea LXVIII*	*P (Podocotyle) lepomis*
Penetration gland	.	.	20-30 (av. 26) ×25-30 (av. 26)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Tail	10-36 (av. 30) ×465-528 (av. 490)	15-42 (av. 35) ×378-514 (av. 485)	37-55 (av. 45) ×486-595 (av. 546)	14-17 (av. 15) ×69-92 (av. 82)	19-25 (av. 23) ×26-75 (av. 68)	20-30 (av. 27) ×35-80 (av. 75)	27-34 (av. 30) ×90-120 (av. 115)	25-38 (av. 34) ×230-547 (av. 480)	30-32.5 (av. 31) ×425-512.5 (av. 454)	.	.	.	.	.	23-38 (av. 35) ×95-130 (av. 115)	65-95 (av. 82) ×328-450 (av. 410)	34-38 (av. 35) ×395-480 (av. 450)	43-65 (av. 52) ×50-75 (av. 61)
Tail stem	.	.	.	.	.	.	.	.	.	15-30 (av. 26) ×154-197 (av. 185)	48-60 (av. 55) ×220-300 (av. 260)	50 -185 (av. 98) ×254-570 (av. 360)	30-45 (av. 39) ×240-312 (av. 286)	25-40 (av. 35) ×425-525 (av. 495)	.	.	.	.
Tail furcal	.	.	.	.	.	.	.	.	.	7-11 (av. 9) ×28-54 (av. 51)	38-65 (av. 60) ×245-320 (av. 280)	45-110 (av. 66) ×145-310 (av. 204)	10-25 (av. 18) ×130-160 (av. 146)	15-25 (av. 20) ×160-170 (av. 165)	.	.	.	.
Lateral finfold	.	.	9-15 (av. 13) ×88-100 (av. 95)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Dorso-median finfold	.	.	.	.	.	.	.	.	.	5-12 (av. 8)	.	.	.	.	.	.	.	.
Appendages	.	.	.	.	.	.	.	.	.	.	.	18 -72 (av. 47) ×120-250 (av. 160)	.	.	.	.	.	.

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Parasitic infections

A total of 32,026 *Melanoides tuberculata* were collected and examined for trematode infections (Fig. 2). The cercarial infections were examined using shedding and crushing methods. The infection rate was 18.79% (6,019/32,026). Nine types and eighteen species of cercariae were categorized. They were (1) Parapleurophocercous cercariae: *Haplorchis pumilio, Haplorchis taichui* and *Stictodora tridactyla*; (2) Pleurophocercous cercariae: *Centrocestus formosanus*; (3) Xiphidiocercariae: *Acanthatrium hitaense, Loxogenoides bicolor* and *Haematoloechus similis*; (4) Megalurous cercariae: *Cloacitrema philippinum* and *Philophthalmus* sp.; (5) Furcocercous cercariae: *Cardicola alseae*, *Alaria mustelae*, *Transversotrema laruei, Apatemon gracilis* and *Mesostephanus appendiculatus*; (6) Echinostome cercariae: *Echinochasmus pelecani*; (7) Amphistome cercariae: *Gastrothylax crumenifer*; (8) Renicolid cercariae: *Cercaria caribbea* LXVIII; and, (9) Cotylomicrocercous cercariae: *Podocotyle* (*Podocotyle*) *lepomis* (Table 2).

Characteristics of cercariae were described from living cercariae, fixed cercariae and cercarial images from scanning microscope. Sizes of cercariae were measured for identification of cercarial species (Table 3). The behavior of cercariae was studied and reported for the physiological data of trematodes.

Type 1. Parapleurophocercous cercariae

1. *Haplorchis pumilio* Looss, 1899 (Yamaguti, 1975)

Haplorchis pumilio (Fig. 3) were found from 371 *M. tuberculata*. The infection rate was 1.16% (371/32,026) (Table 2). The body shape is oval, and its surface is covered with fine spines and sensory hairs. The pigment eyespots and pharynx are present. There are seven pairs of penetration glands, which are arranged in two longitudinal series with a ventral sucker and genital primordia. Their ducts are arranged in two bundles. Four of them were open through the dorsal wall, and four through the ventral wall of the oral sucker in two oblique symmetrical rows. The mouth aperture has transverse rows of spines. The ventral sucker and genital primordia are prevesicular. The excretory bladder has a rounded shape and is composed of fine pigments. No flame cells were found in the tail stem. The tail is long, attached to the dorsal end of the body, with lateral finfolds nearby and a dorso-ventral finfold for the greater distal portion. Cercariae were produced within rediae.

Size range and average size (in micrometers, calculated from 20 cercariae):

Body:	85-128 μm (av. 108 μm) × 168-295 μm (av. 257 μm)
Oral sucker:	27-48 μm (av. 36 μm) × 27-48 μm (av. 36 μm)
Ventral sucker:	14-24 μm (av. 18 μm) × 14-24 μm (av. 18 μm)
Pharynx:	8-10 μm (av. 9 μm) × 12-19 μm (av. 15 μm)
Excretory bladder:	28-40 μm (av. 34 μm) × 28-40 μm (av. 34 μm)
Tail:	10-36 μm (av. 30 μm) × 465-528 μm (av. 490 μm)

Movement behavior: The cercaria moved by rolling up and springing the body back to move forward in a screwing motion for 2–4 seconds and then rested for 15–20 seconds on the surface of water. It survived up to 2–3 hours in the water after emergence.

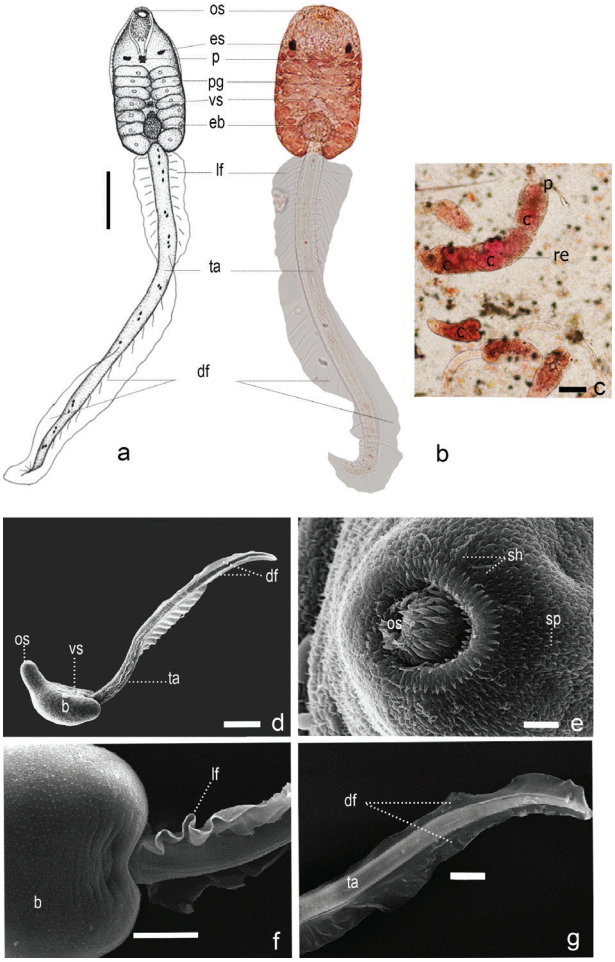

Figure 3. Image of *Haplorchis pumilio*;
a. Drawing of cercaria structure
b. Cercaria stained with 0.5% neutral red
c. Redia stained with 0.5 % neutral red
d.- g. Images of Scanning Electronmicroscope
Abbreviations: os - oral sucker, es - eye spot, p - pharynx, pg - penetration gland, vs - ventral sucker, eb - excretory bladder, lf - lateral finfold, ta - tail, df - dorsal finfold, re - redia, c - cercaria, b - body, sp - spine, sh - sensory hair (scale a, b = 100 μm, c = 10 μm).

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2. *Haplorchis taichui* Nishigori, 1924 (Yamaguti, 1975)

Haplorchis taichui (Fig. 4) were found from 92 *M. tuberculata*, the infection rate was 0.29% (92/32,026) (Table 2). Cercarial body is oval in shape, colored with orange yellow, and entirely covered with minute spines and sensory hairs. The oral sucker is situated ventrally in the head region. There are transverse rows of spines at the mouth aperture. The pigment eyespots and a pharynx are present. Seven pairs of penetration glands extend from the pharynx to the end of the body. There are two longitudinal rows with a ventral sucker and genital primordia; their ducts open on the anterior end of the body. The excretory bladder has a round shape and was composed of fine pigments. A long tail is attached to the dorsal end of the body, with lateral finfolds nearby and a dorso-ventral finfold for the greater distal portion. No flame cells are found in the tail stem. Cercariae were produced within the rediae.

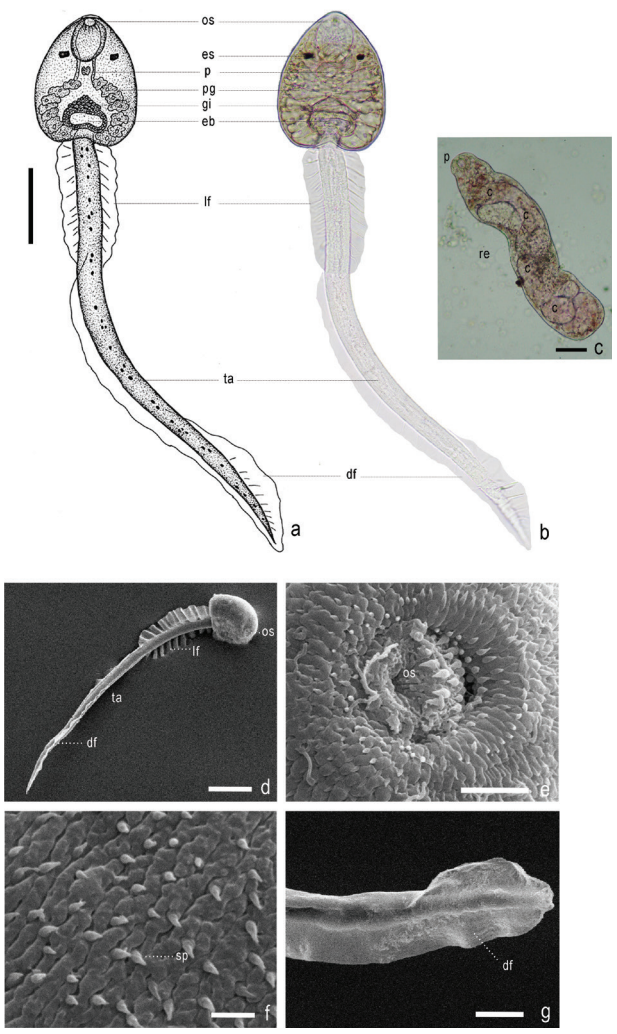

Figure 4. Image of *Haplorchis taichui*;
a. Drawing of cercaria structure
b. Cercaria without staining
c. Redia without staining
d.- g. Images of Scanning Electronmicroscope
Abbreviations: os - oral sucker, es - eye spot, p - pharynx, pg - penetration gland, gi - genital primordial, eb - excretory bladder, lf - lateral finfold, ta - tail, df - dorsal finfold, re - redia, c - cercaria, sp - spine (scale a, b = 100 μm, c = 20 μm).

Size range and average size (in micrometers, calculated from 20 cercariae):

Body:	93-135 μm (av. 132 μm) × 156-276 μm (av. 242 μm)
Oral sucker:	24-45 μm (av. 38 μm) × 27-45 μm (av. 38 μm)
Ventral sucker:	15-25 μm (av. 20 μm) × 15-25 μm (av. 20 μm)
Pharynx:	8-10 μm (av. 10 μm) × 12-18 μm (av. 15 μm)
Excretory bladder:	30-42 μm (av. 37 μm) × 30-42 μm (av. 37 μm)
Tail:	15-42 μm (av. 35 μm) × 378-514 μm (av. 485 μm)

Movement behavior: Cercariae were escaped from the rediae. In the water, they floated on the surface or in the middle. The body part sank lower than the tail. The movement rolled around fast on the water, about 8 to 12 seconds, then rested for 5-10 seconds. It survived up to 2-3 hours in the water after emergence.

3. *Stictodora tridactyla* Martin & Kuntz, 1955 (Yamaguti, 1975)

Stictodora tridactyla (Fig. 5) were found from 2,182 *M. tuberculata*. The infection rate was 6.81% (2,182/32,026) (Table 2). The body is oval in shape and yellowish brown in color. There are 3 rows of oral spines (4-6, 12-14, 22-24), and 7 pairs of penetration glands in 4 groups of 3:4:4:3. The penetration ducts are open near the oral sucker. The eight ducts are arranged in two bundles, four open through the dorsal wall, and four through the ventral wall. The ventral sucker is small. There is one pair of eye spots with coarse granules, with a small globular pharynx between the eye spots. The excretory bladder in the flattened V-shaped is situated at the end of the body. The tail is longer than the body with a bilaterial finfold and a dorso-ventral finfold. Both the dorsal and ventral finfolds arose at a short distance from the anterior and the posterior end of the lateral finfold. There is no flame cell, but 3-5 groups of pigment, an opening duct of the excretory bladder at the tip of the tail. Cercariae were produced within the rediae.

Size range and average size (in micrometers, calculated from 20 cercariae):

Body:	69-149 μm (av. 112 μm) × 255-309 μm (av. 275μm)
Oral sucker:	36-48 μm (av. 38 μm) × 33-52 μm (av. 41 μm)
Eye spots:	6-12 μm (av. 8 μm) × 12-17 μm (av. 14 μm)

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Pharynx:	11-19 µm (av. 16 µm) × 14-21 µm (av. 17 µm)
Ventral sucker:	10-25 µm (av. 19 µm) × 10-25 µm (av. 19 µm)
Excretory bladder:	87-119 µm (av. 98 µm) × 52-98 µm (av. 76 µm)
Penetration gland:	20-30 µm (av. 26 µm) × 25-30 µm (av. 26 µm)
Tail:	37-55 µm (av. 45 µm) × 486-595 µm (av. 546 µm)
Lateral finfold:	9-15 µm (av. 13 µm) × 88-100 µm (av. 95)

Movement behavior: The cercaria floated on the surface or in the middle of the water.

The body sank lower than the tail. The body moves by turning over left and right. The rolling movement is fast on the water, about 5-7 seconds, and rests for about 25-27 seconds. The body sinks on the surface of the water and then moves upside down.

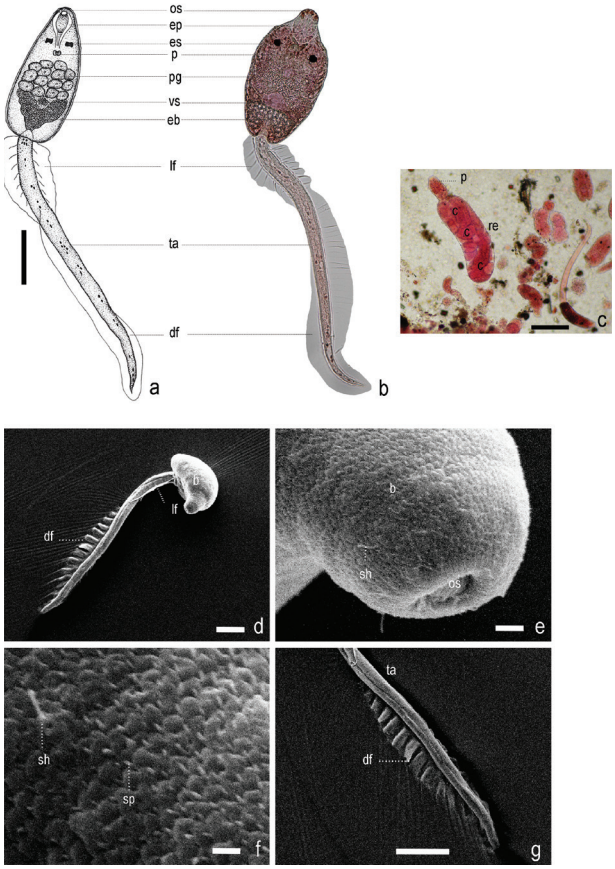

Figure 5. Image of *Stictodora tridactyla*;
a. Drawing of cercaria structure
b. Cercaria stained with 0.5% neutral red
c. Redia stained with 0.5 % neutral red
d.- g. Images of Scanning Electronmicroscope
Abbreviations: os - oral sucker, ep - esophagus, es - eye spot, p - pharynx, pg - penetration gland, vs - ventral sucker, eb - excretory bladder, lf - lateral finfold, ta - tail, df - dorsal finfold, re - redia, c - cercaria, b - body, sp - spine, sh - sensory hair (scale a, b = 100 µm, c = 10 µm).

Type 2. Pleurophocercous cercariae

4. *Centrocestus formosanus* Nishigori, 1924 (Yamaguti, 1975)

This parasite (Fig. 6) was found in 74 *M. tuberculata*. The infection rate was 0.23% (74/32,026) (Table 2). Cercarial body is oval in shape. A pair of eyespots lay at the level of the pharynx. The oral sucker has two rows of oral spines similar to hooks of the tapeworm (rostellar hooks, four in the anterior and five in the posterior) on the dorsal wall of the mouth aperture. Short esophagus, the parenchymal body is spinulate, and yellowish brown in color. Acetabulum is found between the intestinal bifurcation and the excretory vesical. The bladder is a flattened V-shape. Seven pairs of penetration glands lay anterolateral to the acetabulum in front of an inverted V-shape. Cystogenous cells are distributed in the posterior part. The genital primordial part is somewhat elongated and triangular, between the acetabulum and the excretory vesicle. The tail is slender, with a very indistinct dorsal and ventral finfolds, both of which are more conspicuous in the distal half, with a tiny spike on the tip. Cercariae were produced within the rediae.

Size range and average size (in micrometers, calculated from 20 cercariae):

Body:	45-72 µm (av. 64 µm) × 82-120 µm (av. 117 µm)
Oral sucker:	16-26 µm (av. 24 µm) × 17-28 µm (av. 25 µm)
Ventral sucker:	12-16 µm (av. 14 µm) × 12-16 µm (av. 14 µm)
Pharynx:	7-9 µm (av. 8 µm) × 8-10 µm (av. 9 µm)
Excretory bladder:	24-30 µm (av. 28 µm) × 38-52 µm (av. 45 µm)
Tail:	14-17 µm (av. 15 µm) × 69-92 µm (av. 82 µm)

Movement behavior: The cercaria moved by rolling up and springing the body back to move forward in a screwing motion for 8-10 seconds and then rested for about 45-50 seconds. It survived up to 3-4 hours in the water after emergence.

Type 3. Xiphidiocercariae

5. *Acanthatrium hitaense* Koga, 1953 (Yamaguti, 1975)

This parasite (Fig. 7) was found in 88 *M. tuberculata*. The infection rate was 0.27% (88/32,026) (Table 2). The parasite is virgulate xiphidiocercaria. The body is oval in shape and white in color. There are stylet and virgulate glands in the oral sucker, and 2 pairs of penetration glands in each side of the body. The pharynx is round and short, the ventral sucker is smaller than the oral sucker, and, the small excretory bladder is located at the end of the body. The tail

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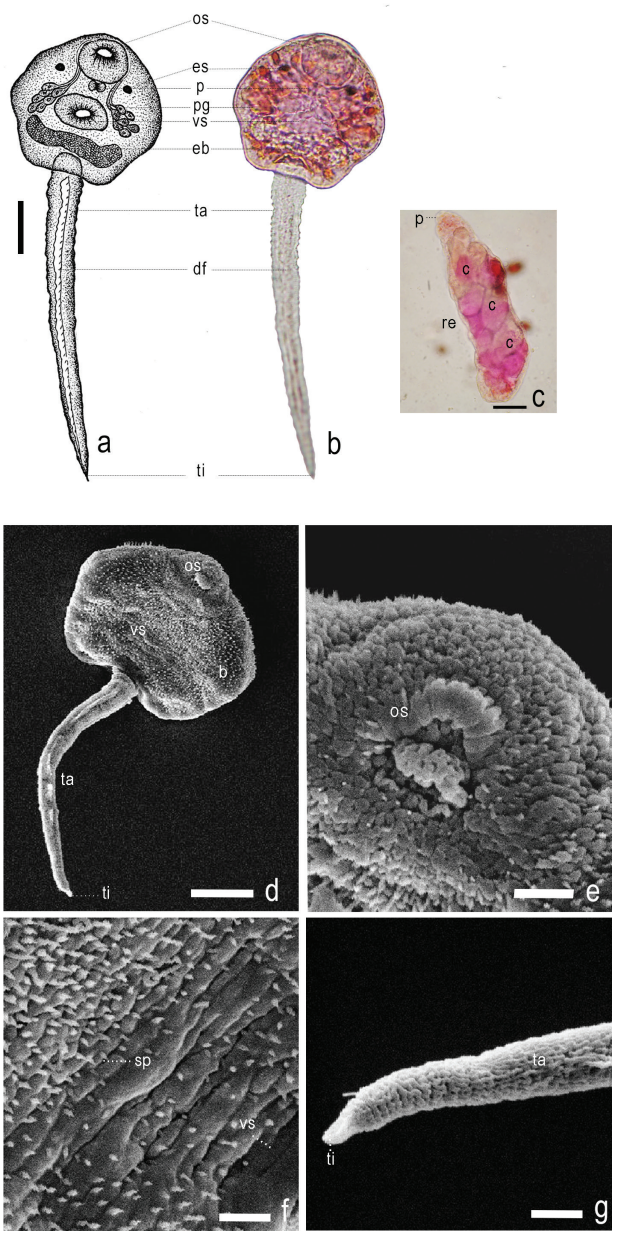

Figure 6. Image of *Centrocestus formosanus*;
a. Drawing of cercaria structure
b. Cercaria stained with 0.5% neutral red
c. Redia stained with 0.5 % neutral red
d.- g. Images of Scanning Electronmicroscope
Abbreviations: os - oral sucker, es - eye spot, p - pharynx, pg - penetration gland, vs - ventral sucker, eb - excretory bladder, ta - tail, df - dorsal finfold, ti - tip, re - redia, c - cercaria, b - body, sp - spine (scale a, b = 100 µm, c = 10 µm).

is shorter than the body, inserted to the posterior end of the body. Cercariae were produced within the sporocyst.

Size range and average size (in micrometers, calculated from 20 cercariae):

Body:	53-92 µm (av. 78 µm) × 80-110 µm (av. 100 µm)
Oral sucker:	25-32 µm (av. 30 µm) × 34-40 µm (av. 37 µm)
Stylet:	8-13 µm (av. 10 µm) × 11-13 µm (av. 12 µm)
Ventral sucker:	15-16 µm (av. 16 µm) × 15-18 µm (av. 17 µm)
Pharynx:	10-15 µm (av. 13 µm) × 12-24 µm (av. 20 µm)
Excretory bladder:	8-12 µm (av. 10 µm) × 20-46 µm (av. 38 µm)
Tail:	19-25 µm (av. 23 µm) × 26-75 µm (av. 68 µm)

Movement behavior: The cercaria floated on the surface or in the middle of the water. They moved by rolling up and springing the body back to move forward in a screwing motion for 45-60 seconds, and then rested for 2-5 seconds at the water surface. Some cercariae were stuck on the surface of the container, and moved by the oral sucker and ventral sucker. It survived up to 2-4 hours in the water after emergence.

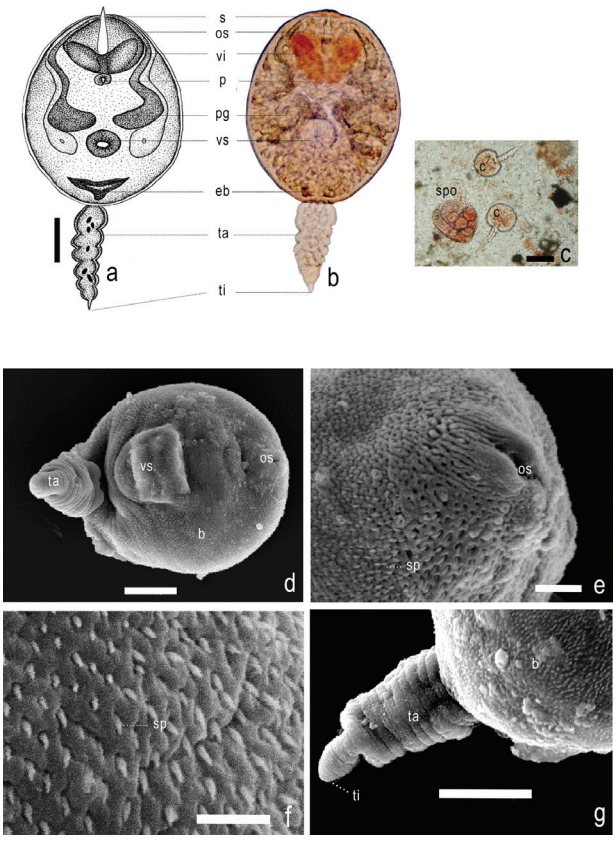

Figure 7. Image of *Acanthatrium hitaense*;
a. Drawing of cercaria structure
b. Cercaria stained with 0.5% neutral red
c. Sporocyst stained with 0.5 % neutral red
d.- g. Images of Scanning Electronmicroscope
Abbreviations: s - stylet, os - oral sucker, vi - vigulate gland, p - pharynx, pg - penetration gland, vs - ventral sucker, eb - excretory bladder, ta - tail, ti - tip, spo : sporocyst, c - cercaria, b - body, sp - spine (scale a, b = 25 µm, c = 50 µm).

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6. Loxogenoides bicolor Kaw, 1945 (Yamaguti, 1975)

Loxogenoides bicolor (Fig. 8) was found from 2,373 *M. tuberculata*. The infection rate was 7.41% (2,373/32,026) (Table 2). The body of cercaria is spinose and oval in shape. Its entire body is dotted with granules. The ventral sucker is smaller than the oral sucker. A virgular organ is located in the region of the oral sucker. A stylet is present. Three pairs of penetration glands exist: two anterior pairs and a posterior pair. The penetration glands had granules and ducts. The ducts opened near the tip of the stylet. There is a C-shaped genital primordium and a U-shaped excretory bladder. The tail is spinose, with slightly longer spines at the tip. Cercariae were produced within the sporocyst.

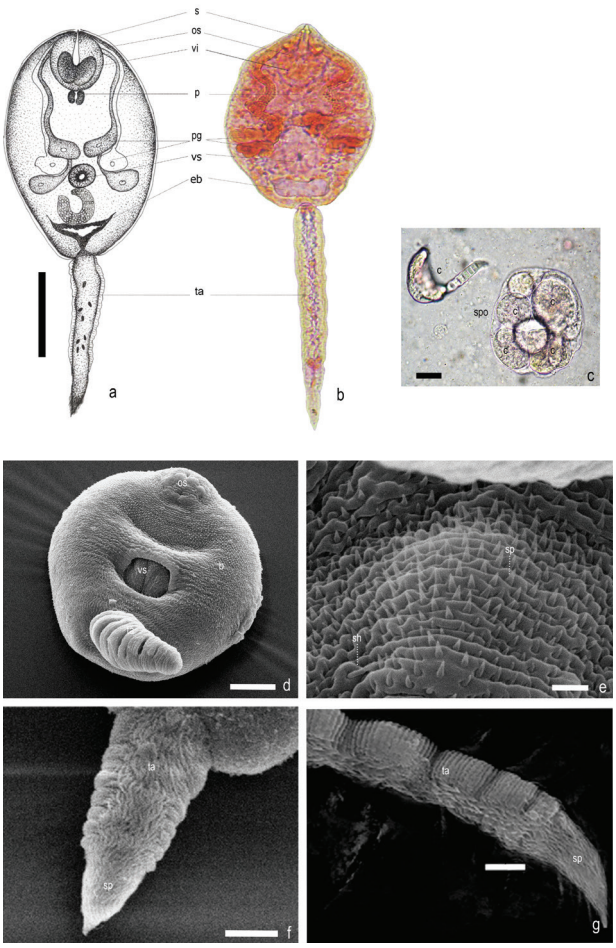

Figure 8. Image of *Loxogenoides bicolor*;
a. Drawing of cercaria structure
b. Cercaria stained with 0.5% neutral red
c. Sporocyst stained with 0.5 % neutral red
d.- g. Images of Scanning Electronmicroscope
Abbreviations: s - stylet, os - oral sucker, vi - vigulate gland, p - pharynx, pg - penetration gland, vs - ventral sucker, eb - excretory bladder, ta - tail, spo : sporocyst, c - cercaria, b - body, sp - spine (scale a, b = 50 μm, c = 20 μm).

Size range and average size (in micrometers, calculated from 20 cercariae):

Body:	54-82 μm (av. 75 μm) × 90-120 μm (av. 110 μm)
Oral sucker:	24-30 μm (av. 28 μm) × 24-30 μm (av. 28 μm)
Stylet:	5-8 μm (av. 7 μm) × 14-20 μm (av. 18 μm)
Ventral sucker:	12-18 μm (av. 15 μm) × 13-20 μm (av. 17 μm)
Pharynx:	4-6 μm (av. 5 μm) × 4-10 μm (av. 8 μm)
Excretory bladder:	8-10 μm (av. 9 μm) × 10-30 μm (av. 25 μm)
Tail:	20-30 μm (av. 27 μm) × 35-80 μm (av. 75 μm)

Movement behavior: The cercaria moved by folding its tail and rolling up the body and moved from left to right quickly. In resting position, they floated on the surface or in the middle of the water. The body sank lower than the tail. The cercaria moved about 60-75 seconds, and rested for about 2-5 seconds. It survived up to 2-3 hours in the water after emergence.

7. *Haematoloechus similis* Looss, 1899 (Yamaguti, 1975)

Haematoloechus similis (Fig. 9) was found in 468 *M. tuberculata*. The infection rate was 1.46% (468/32,026) (Table 2). This parasite was classified into Xiphidiocercariae. The body is ovate, and the surface is covered with spines. Cytogenous cell is not observed. A stylet is 30-32 μm long, with no virgulate gland. Six pairs of penetration glands of irregular shape are present, extending from the middle of the body to near the posterior end of body, each with large nuclei and fine granules. Their ducts are bundled, one on each side, opening near the tip of the stylet. Prepharynx is short, and pharynx poorly differentiated. Esophagus, ceca and genitalia are not developed. The excretory vesicle is Y shape. The flame cell formula is 2[(3+3+3)+(3+3+3)]=36. The tail is not finflod. Cercariae were produced within the sporocyst.

Size range and average size (in micrometers, calculated from 20 cercariae):

Body:	87-104 μm (av. 95 μm) × 130-164 μm (av. 148 μm)
Oral sucker:	34-42 μm (av. 38 μm) × 37-46 μm (av. 42 μm)
Stylet:	20-34 μm (av. 30 μm) × 20-34 μm (av. 32 μm)
Ventral sucker:	10-20 μm (av. 16 μm) × 12-20 μm (av. 18 μm)
Pharynx:	11-13 μm (av. 12 μm) × 15-17 μm (av. 16 μm)
Excretory bladder:	20-22 μm (av. 21 μm) × 20-24 μm (av. 22 μm)

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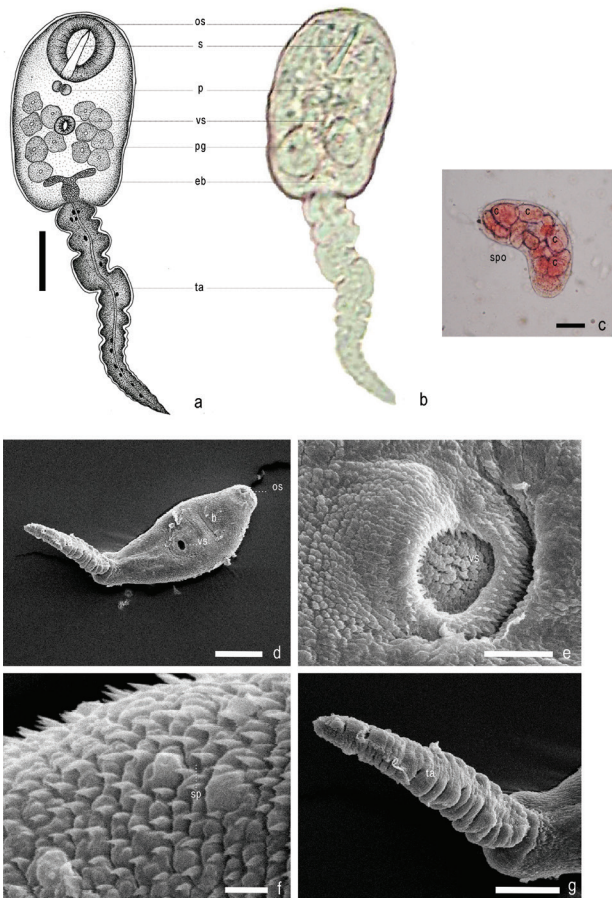

Figure 9. Image of *Haematoloechus similis*;
a. Drawing of cercaria structure
b. Cercaria without staining
c. Sporocyst stained with 0.5 % neutral red
d.- g. Images of Scanning Electronmicroscope
Abbreviations: s - stylet, os - oral sucker, p - pharynx, pg - penetration gland, vs - ventral sucker, eb - excretory bladder, ta - tail, spo : sporocyst, c - cercaria, b - body, sp - spine (scale a, b = 50 μm, c = 50 μm).

Tail: 27-34 μm (av. 30 μm) × 90-120 μm (av. 115 μm)

Movement behavior: The cercaria floated on the surface or in the middle of the water. The body sank lower than the tail. It moved by folding its tail back to the body and turning its body to roll quickly from left to right, darting forward for about 15-20 seconds, and resting for about 10-15 seconds. It survived up to 1-2 hours in the water after emergence.

Type 4. Megalurous cercariae

8. *Cloacitrema philippinum* Velasquez, 1969 (Yamaguti, 1975)

Cloacitrema philippinum (Fig. 10) was found in 12 *M. tuberculata*. The infection rate was 0.04% (12/32,026) (Table 2). The body is elongate and muscular. There is no eye spot, and no spine on the body surface. There are long prepharynx and pharynx. The long ceca runs almost to the end of the body. There are numerous sensory papillae on the surface of body. The oral sucker has 12 opening ducts. The cystogenous cells were found all over the body. The thin wall of the excretory vesicle extends when moving, and the ventral sucker is bigger than the oral sucker. The long tail is inserted to the posterior end of the body. The clear vacuoles distributed along the tail, containing granules. There is an adhesive organ at the tip of the tail, with no lateral finfolds. Cercariae were produced within the rediae.

Size range and average size (in micrometers, calculated from 20 cercariae):
Body: 122-184μm (av. 169 μm) × 280-450 μm (av. 396 μm)
Oral sucker: 50-60 μm (av. 56 μm) × 50-60 μm (av. 56 μm)

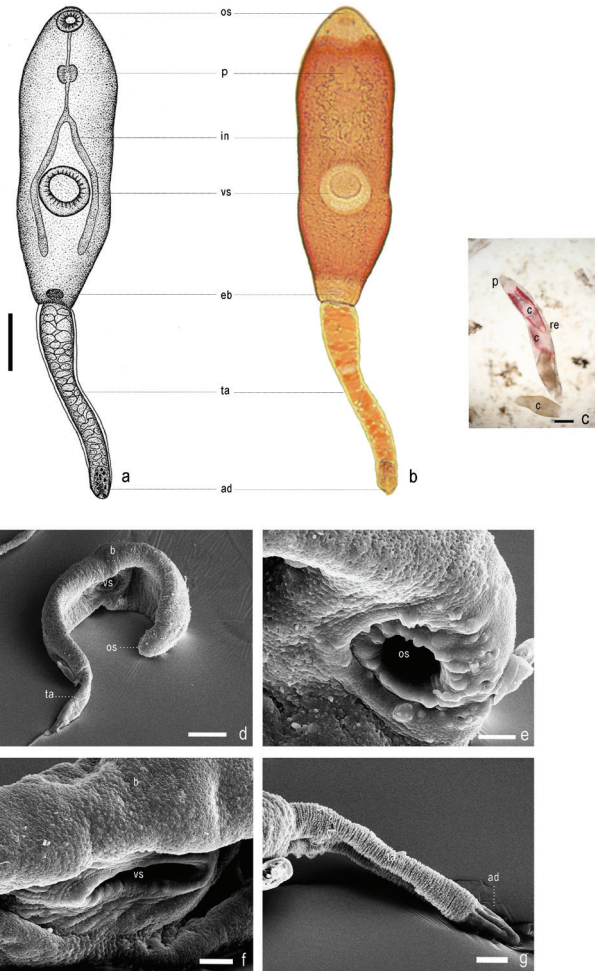

Figure 10. Image of *Cloacitrema philippinum*;
a. Drawing of cercaria structure
b. Cercaria stained with 0.5% neutral red
c. Redia stained with 0.5 % neutral red
d.- g. Images of Scanning Electronmicroscope
Abbreviations: os - oral sucker, p - pharynx, in - intestine, vs - ventral sucker, eb - excretory bladder, ta - tail, ad - adhesive gland, re - redia, c - cercaria, b - body(scale a, b = 100 μm, c = 50 μm).

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Ventral sucker: 60-70 µm (av. 68 µm) × 60-75 µm (av. 70 µm)
Pharynx: 23-38 µm (av. 35 µm) × 25-40 µm (av. 35 µm)
Excretory bladder: 15-20 µm (av. 18 µm) × 14-21 µm (av. 18 µm)
Tail: 25-38 µm (av. 34 µm) × 230-547 µm (av. 480 µm)

Movement behavior: The cercaria floated on the surface or in the middle of the water. It moved by stretching the body and rolling on the water surface around 20-28 seconds and resting for about 2-4 seconds. The adhesive gland anchored on the container surface. It survived up to 2-4 hours in the water after emergence.

9. *Philophthalmus sp.* Looss, 1899 (Urabe, 2005)

Philophthalmus sp. (Fig. 11) was found in 57 *M. tuberculata.* The infection rate was 0.18% (57/32,026) (Table 2).

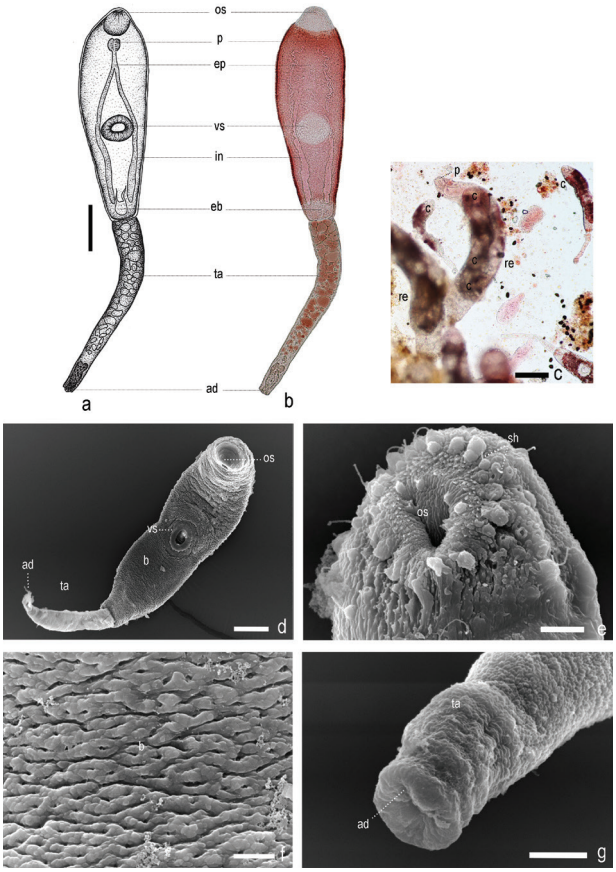

Figure 11. Image of *Philopthalmus* sp.;
a. Drawing of cercaria structure
b. Cercaria stained with 0.5% neutral red
c. Redia stained with 0.5 % neutral red
d.- g. Images of Scanning Electronmicroscope
Abbreviations: os - oral sucker, p - pharynx, ep - esophagus, vs - ventral sucker, in - intestine, eb - excretory bladder, ta - tail, ad - adhesive gland, re - redia, c - cercaria, b - body (scale a, b = 100 µm, c = 50 µm).

The body is elongated and white in color, with numerous minute spines on the half body at the posterior end. It has a muscular body, no eye spot, long prepharynx and pharynx, Y-shaped esophagus, and a large ventral sucker. The intestine runs almost to the end of the body. There are plenty of cyst glands along their bodies. The tail is the same length as the body but flexible, with various sizes of vacuole and granules along the tail. The adhesive gland cells were found. Cercariae were produced within the rediae.

Size range and average size (in micrometers, calculated from 20 cercariae):
Body: 120-125 µm (av. 122 µm) × 525-595 µm (av. 570 µm)
Oral sucker: 50-57µm (av. 55 µm) × 55-62.5 µm (av. 60 µm)
Ventral sucker: 62.5-67.5 µm (av. 64 µm) × 55-70 µm (av. 64 µm)
Pharynx: 20-25 µm (av. 23 µm) × 20-25 µm (av. 23 µm)
Excretory bladder: 37.5-45 µm (av. 41 µm) × 35-50 µm (av. 41 µm)
Tail: 30-32.5 µm (av. 31 µm) × 425-512.5 µm (av. 454 µm)

Movement behavior: The cercaria floated on the surface or in the middle of the water. It moved by stretching and floating on the surface of the water, and moved forward. Adhesive gland are stuck on the container for cercarial moving. The cercariae became metacercarial cyst immediately in the container.

Type 5. Furcocercous cercariae

10. *Cardicola alseae* Meade & Pratt, 1965 (Yamaguti, 1975)

Cardicola alseae (Fig. 12) were found from 47 *M. tuberculata* which is equivalent to an infection rate of 0.1% (33/32,026) of the total number of the collected snails (Table 2). The small hook-liked body had an anterior organ, and was covered with minute spines. The longer spines were found in some parts of the body, the dorsal and ventral of the posterior end. The dorso-median finfold was observed in the middle part of the body. Many large granules were observed, with a penetration gland located at the middle part of the body. The excretory bladder was small. The tail is furcocercous; its furcae are shorter than the beginning of the tail, and sharp like animal crawls. Minute spines and sensory hairs were observed. A caudal body, longitudinal muscle, no flame cell, and furcal finfold were observed, with the opening duct of excretory bladder at the fork tail tip. Cercariae were produced within the sporocyst.

Size range and average size (in micrometers, calculated from 20 cercariae):
Body: 18-39 µm (av. 28 µm) × 72-110 µm (av. 95 µm)

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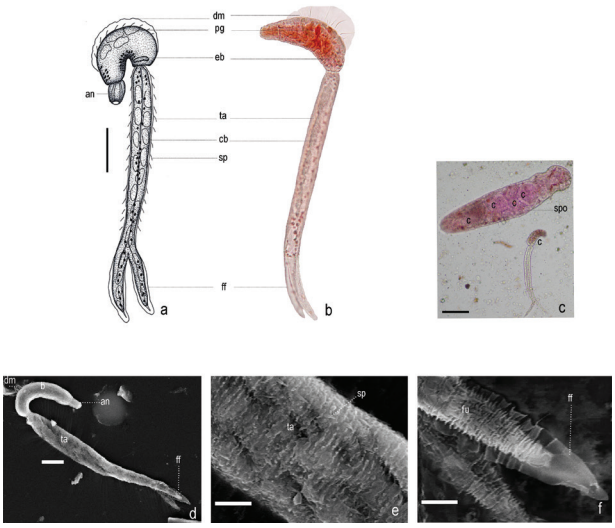

Figure 12. Image of *Cardicola alseae*;
a. Drawing of cercaria structure
b. Cercaria stained with Semichon's acetic carmine & fast green
c. Sporocyst stained with 0.5 % neutral red
d.- f. Images of Scanning Electronmicroscope
Abbreviations: dm - dorso-median finfold, pg - penetration gland, eb - excretory bladder, an - anterior organ, ta - tail, cb - caudal body, sp - spine, fu - furca, spo : sporocyst, c - cercaria (scale a, b = 50 µm, c = 20 µm).

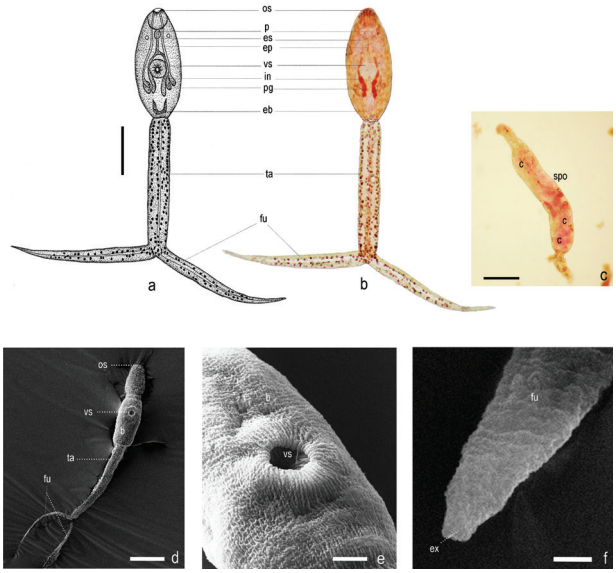

Figure 13. Image of *Alaria mustelae*;
a. Drawing of cercaria structure
b. Cercaria stained with 0.5% neutral red
c. Sporocyst stained with 0.5 % neutral red
d.- f. Images of Scanning Electronmicroscope
Abbreviations: os - oral sucker, p - pharynx, es - eye spot, ep - esophagus, vs - ventral sucker, in - intestine, pg - penetration gland, eb - excretory bladder, ta - tail, fu - furca, spo : sporocyst, c- cercaria (scale a, b = 50 µm, c = 10 µm).

Anterior organ:	11-15 µm (av. 13 µm) × 14-20 µm (av. 17 µm)
Excretory bladder:	3-8 µm (av. 5 µm) × 10-35 µm (av. 21 µm)
Tail stem:	15-30 µm (av. 26 µm) × 154-197 µm (av. 185 µm)
Tail furcal:	7-11 µm (av. 9 µm) × 28-54 µm (av. 51 µm)
Dorso-median finfold:	5-12 µm (av. 8 µm)

Movement behavior: The cercaria floated on the surface of the water. The body and tail hanged rolling. It moved by folding its tail back to the body, moving forward around 6-10 seconds, and resting for 3-4 seconds. When at rest the body hangs upwards and rolls back slowly downwards with the tail stem and furcae moved upwards. It survived up to 2-3 hours in the water after emergence.

11. *Alaria mustelae* Bosma, 1899 (Yamaguti, 1975)

Alaria mustelae (Fig. 13) were found from 47 *M. tuberculata* which is equivalent to an infection rate of 0.15% (47/32,026) of the total number of the collected snails (Table 2). Cercarial body has a long shape. Unpigmented eyespots lay on the midway between two suckers in lateral fields, prepharynx short, pharynx small and muscular, esophagus rather long, ceca extending a short distance posterior to acetabulum. The oral sucker is slightly larger than the postequatorial acetabulum. There are two pairs of penetration glands, filled with fine granules. The duct openings on each side of the mouth are in spineless circumoral area. The body is covered entirely with spines and two irregular spines around the aperture of the ventral sucker. Genital primordium is a small mass of cells anterior to the excretory vesicle. Cercaria developed within the sporocyst. The tail stem was without spines; the furcae was irregularly spinose (long hair like), with no caudal bodies. The excretory pore was found at the fork tail tip.

Size range and average size (in micrometers, calculated from 20 cercariae):

Body:	105-154 µm (av. 138 µm) × 185-280 µm (av. 255 µm)
Oral sucker:	28-40 µm (av. 36 µm) × 28-40 µm (av. 36 µm)
Ventral sucker:	15-30 µm (av. 22 µm) × 15-30 µm (av. 22 µm)
Pharynx:	10-15 µm (av. 13 µm) × 14-17 µm (av. 15 µm)
Tail stem:	48-60 µm (av. 55 µm) × 220-300 µm (av. 260 µm)
Tail furcae:	38-65 µm (av. 60 µm) × 245-320 µm (av. 280 µm)

Movement behavior: The cercariae moved by rolling up and springing back the body to swiftly move forward

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in a quick semi-circular motion. It then rested by floating with its head on top for a long time about 20-30 seconds then moved quickly about 7-15 seconds and rested by floating again. It survived up to 2-3 hours in the water after emergence.

12. *Transversotrema laruei* Velasguez, 1958 (Yamaguti, 1975)

Transversotrema laruei (Fig. 14) were found from 89 *M. tuberculata,* which is equivalent to an infection rate of 0.28% (89/32,026) of the total number of the collected snails (Table 2). The body is in a bowl-liked shape and light brownish in color, with numerous spines like fish scales on the body surface. Many granules are observed. The genital pore of the seminal vesicle is at the anterior end of the body. There are very big round eye spots, and the ventral sucker is globular. There is a mouth on the ventral sucker. The esophagus is narrow and long, attached to the intestine. There are 1 pair of testes, with an ovary on the left side of the testes. A small excretory bladder is at the posterior end. The tail is longer than the body length with a fork tip, and one pair of appendage at the base of the tail. An adhesive pad was observed at the end of the tail. The furcal tails are spatulate. There are 4 flame cells, and an opening duct of the excretory bladder is on the furcal tail. Cercariae were produced within the rediae.

Size range and average size (in micrometers, calculated from 20 cercariae):

Body:	425-670 µm (av. 574 µm) × 280-510 µm (av. 370 µm)
Genital pore:	12 - 18 µm (av. 15 µm) × 12 - 18 µm (av. 15 µm)
Pharynx:	25 – 57 µm (av. 574 µm) × 280- 510 µm (av. 370 µm)
Eye spot:	14 –20 µm (av. 18 µm) × 14 - 20 µm (av. 18 µm)
Excretory bladder:	11-23 µm (av. 15 µm) × 11-21 µm (av. 15 µm)
Ventral sucker:	85 -105 µm (av. 95 µm) × 85 - 105 µm (av. 95 µm)
Tail stem:	50 -185 µm (av. 98 µm) × 254-570 µm (av. 360 µm)
Tail furcae:	45-110 µm (av. 66 µm) × 145-310 µm (av. 204 µm)
Appendages:	18 -72 µm (av. 47 µm) × 120-250 µm (av. 160 µm)

Movement behavior: The cercaria floated on the surface or in the middle of the water. The body sank lower than the tail. It moved very fast by turning from left to right, and moving forward in a screwing motion for about 5-8 seconds, and resting about 2-3 minutes by floating with its head and tail folded together. The furcae floated upwards, moving by wavering on the container surface.

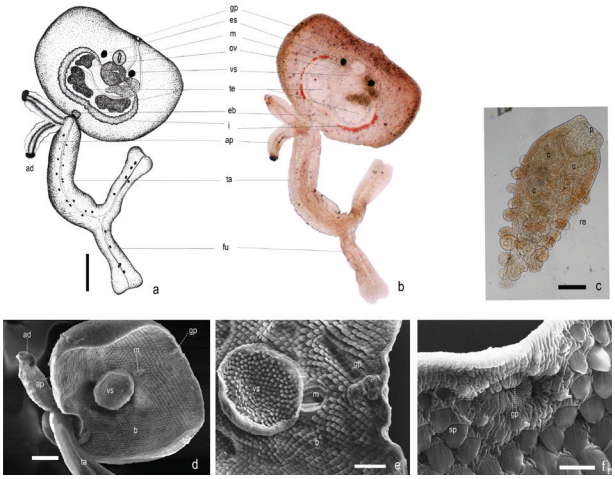

Figure 14. Image of *Transversotrema laruei*;
a. Drawing of cercaria structure
b. Cercaria stained with 0.5% neutral red
c. Redia stained with 0.5 % neutral red
d.- f. Images of Scanning Electronmicroscope
Abbreviations: gp - genital pore, es - eye spot, m - mouth, ov - ovary, vs - ventral sucker, te - testes, eb - excretory bladder, i - intestine, ap - appendages, ta - tail, fu - furca, p - pharynx, re - redia, c - cercaria (scale a, b = 100 µm, c = 20 µm).

13. *Apatemon gracilis* Szidat, 1928 (Yamaguti, 1975)

Apatemon gracilis (Fig. 15) were found from 55 *M. tuberculata,* which is equivalent to an infection rate of 0.17% (55/32,026) of the total number of the collected snails (Table 2). The cercarial body is oval in shape, and the prepharynx is practically absent. The anterior region has a cuticle spine. It has unpigmented eyespots. The tail stem contains about 16 caudal bodies. There are 4 pairs of penetration glands which lie between the acetabulum and genital primordia. The excretory bladder is very small. Cercariae were produced within the sporocyst.

Size range and average size (in micrometers, calculated from 20 cercariae):

Body:	41.5-90.0 µm (av. 72 µm) × 115-140 µm (av. 126 µm)
Oral sucker:	15-25 µm (av. 21.45 µm) × 20-30 µm (av. 23.25 µm)
Ventral sucker:	16-25 µm (av. 23 µm) × 20-30 µm (av. 25µm)
Excretory bladder:	15-25 µm (av. 18 µm) × 15-30 µm (av. 20 µm)
Pharynx:	6-10 µm (av. 8 µm) × 7-13 µm (av. 9 µm)
Tail stem:	30-45µm (av. 39 µm) × 240-312 µm (av. 286 µm)
Tail furcae:	10-25µm (av. 18 µm) × 130-160 µm (av. 146 µm)

Movement behavior: The cercariae floated on the surface or in the water. The body sank lower than the spread-

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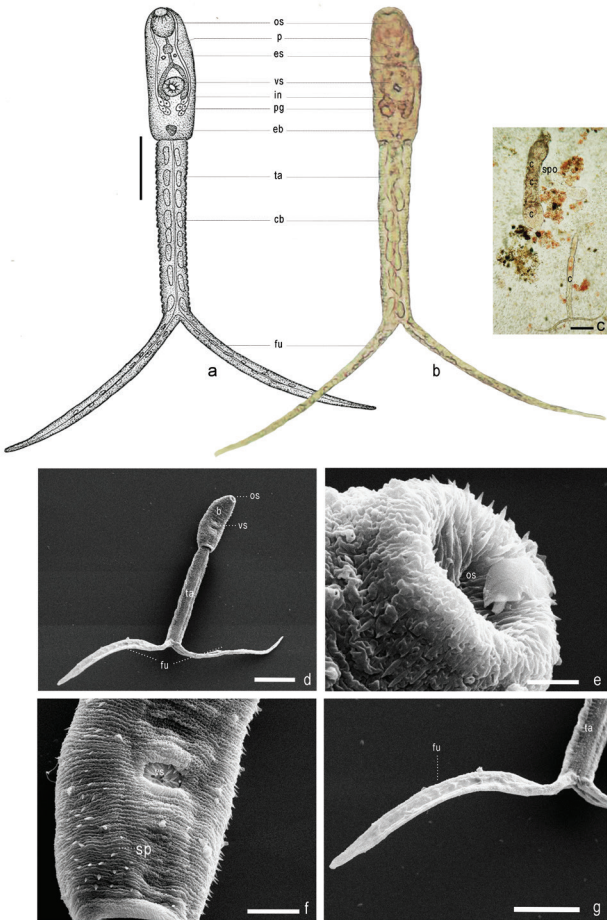

Figure 15. Image of *Apatemon gracilis*;
a. Drawing of cercaria structure
b. Cercaria stained with 0.5% neutral red
c. Sporocyst stained with 0.5 % neutral red
d.- g. Images of Scanning Electronmicroscope
Abbreviations: os - oral sucker, p - pharynx, es - eye spot, vs - ventral sucker, in - intestine, pg - penetration gland, eb - excretory bladder, ta - tail, cb - caudal body, fu - furca, b - body, sp - spine, spo - sporocyst, c - cercaria (scale a, b = 100 μm, c = 20 μm).

ing fork tail. It moved by rolling up and springing back the body to swiftly move forward in a semi-circular motion. It then rested by floating with its head on top for about 12-15 seconds, then moved quickly about 3-6 seconds and rested by floating again.

14. *Mesostephanus appendiculatus* (Cicrea, 1916) Lutz, 1935 (Yamaguti, 1975)

Mesostephanus appendiculatus (Fig. 16) were found from 3 *M. tuberculata*, which is equivalent to an infection rate of 0.009% (3/32,026) of the total number of the collected snails (Table 2). The cercarial body is oval in shape and spinose. There are many spines on the surface and the oral sucker. Coarse granules and cytogenous gland are scattered inside the body. The pharynx is small and round. The prepharynx and esophagous are almost as long as the pharynx. A large intestine, composed of two cecae, is terminated near a small excretory bladder. A ventral sucker vestigial is found to be in small groups. The tail is forked and longer than the body and the tail surface is covered with many spines. The tail stem is longer than the furca. The tail tubule opens at the tip of each tail furca in which no flame cell is found. Cercariae were produced within the sporocyst.

Size range and average size (in micrometers, calculated from 20 cercariae):

Body:	92-120 μm (av. 109 μm) × 160-250 μm (av. 225 μm)
Oral sucker:	20-45 μm (av. 35 μm) × 35-48 μm (av. 39 μm)
Ventral sucker:	15-24 μm (av. 20 μm) × 15-24 μm (av. 20μm)
Excretory bladder:	20-40 μm (av. 35 μm) × 20-40 μm (av. 35 μm)
Pharynx:	8-10 μm (av. 9 μm) × 11-18 μm (av. 15 μm)

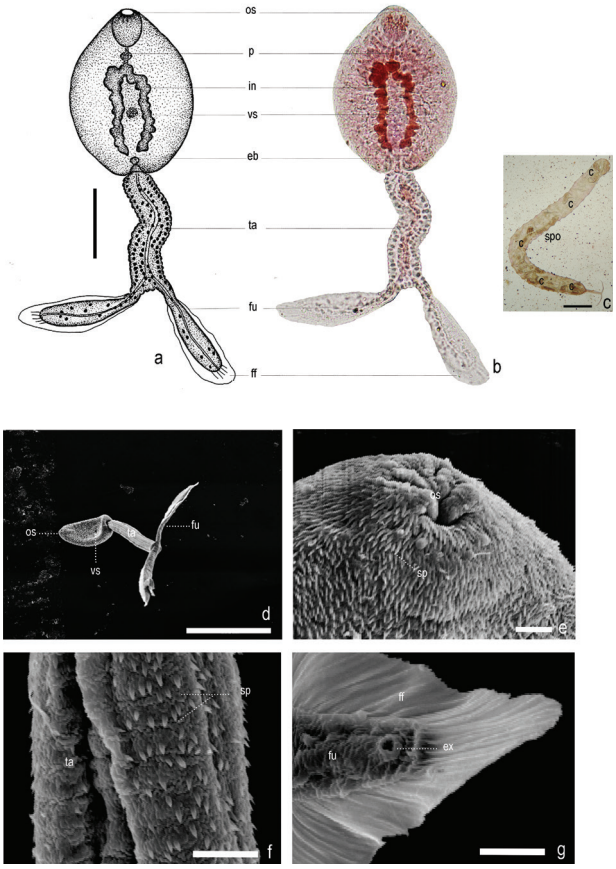

Figure 16. Image of *Mesostephanus appendiculatus*;
a. Drawing of cercaria structure
b. Cercaria stained with 0.5% neutral red
c. Sporocyst stained with 0.5 % neutral red
d.- g. Images of Scanning Electronmicroscope
Abbreviations: os - oral sucker, p - pharynx, in - intestine, vs - ventral sucker, eb - excretory bladder, ta - tail, fu - furca, ff - furcal finfold b - body, sp - spine, ex - excretory pore, spo - sporocyst, c - cercaria (scale a, b = 100 μm, c = 20 μm).

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Tail stem: 25-40 µm (av. 35µm) × 425-525 µm (av. 495µm)
Tail furcae: 15-25 µm (av. 20 µm) × 160-170 µm (av. 165 µm)

Movement behavior: The cercaria floated on the surface or in the water. The body sank lower than the spreading fork tail. It moved by rolling up and springing back the body to swiftly move forward in a semi-circular motion for about 2-4 seconds. It then rested by floating with its head on top of the tail and slowly rotated its body to the bottom while lifting up its spreading fork tail. It rested for about 20-30 seconds and survived up to 2-3 hours in the water.

Type 6. Echinostome cercariae

15. *Echinochasmus pelecani* Johnston & Simpson, 1944 (Yamaguti, 1975)

Echinochasmus pelecani (Fig. 17) were found from 19 *M. tuberculata* which is equivalent to an infection rate of 0.06% (19/32,026) of the total number of the collected snails (Table 2). Cercarial body is elongate, white in color, no eye spot, oral sucker with 3 opening of duct (1 median, 2 submediant), cystogenous cell containing rhabdites. Collar spine is not appearance. Esophagus was between pharynx and ventral sucker, ceca reaching to bladder, two main excretory tubes meet together before entering bladder, Genital primordia are two mass behind ventral sucker, no penetration gland, flame cell pattern not determine. Tail is the same length as body, flexible, vacuole appearance along the tail. Cercariae were produced within rediae.

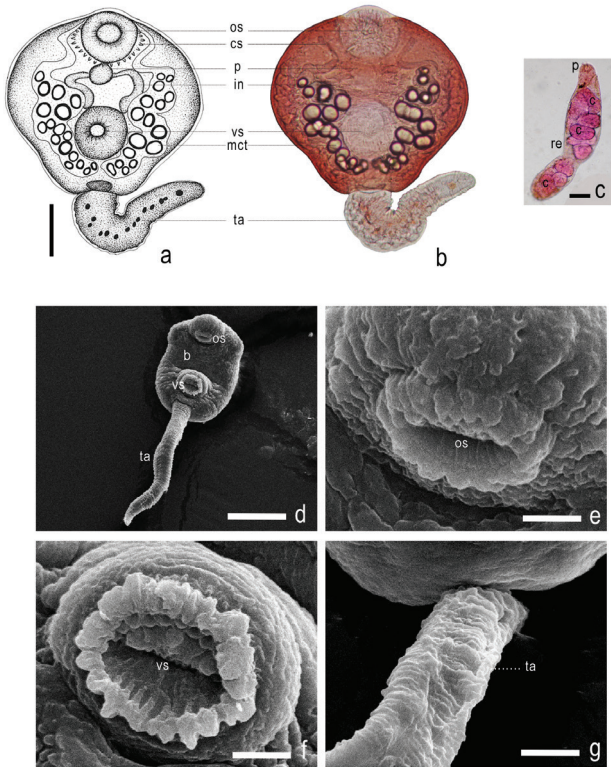

Figure 17. Image of *Echinochasmus pelecani*;
a. Drawing of cercaria structure
b. Cercaria stained with 0.5% neutral red
c. Redia stained with 0.5 % neutral red
d.- g. Images of Scanning Electronmicroscope
Abbreviations: os - oral sucker, cs - collar spine, p - pharynx, in - intestine, vs - ventral sucker, mct - main collecting tube, ta - tail, re - redia, c - cercaria (scale a, b = 50 µm, c = 10 µm).

Size range and average size (in micrometers, calculated from 20 cercariae):
Body: 68-87 µm (av. 85 µm) × 100-125 µm (av. 118 µm)
Oral sucker: 21-30 µm (av. 23 µm) × 24-30 µm (av. 25 µm)
Ventral sucker: 20-31 µm (av. 25 µm) × 22-30 µm (av. 25 µm)
Pharynx: 8-15 µm (av. 12 µm) × 9-15 µm (av. 12 µm)
Excretory bladder: 21-35 µm (av. 33 µm) × 23-36µm (av. 33 µm)
Tail: 23-38 µm (av. 35 µm) × 95-130 µm (av. 115 µm)

Movement behavior: The cercaria floated on the surface or in the water. It moved very fast by rolling up and springing back about 5-10 seconds. It survived up to 3-4 hours in the water after emergence.

Type 7. *Amphistome cercariae*

16. *Gastrothylax crumenifer* (Creplin, 1847) Otto, 1896 (Yamaguti, 1975)

Gastrothylax crumenifer (Fig. 18) were found from 8 *M. tuberculata* which is equivalent to an infection rate of 0.02% (8/32,026) of the total number of the collected snails (Table 2). Cercariae were liberated from the rediae. The body shape is ovate and large. The eye spots have conical lens with yellow pigment through the body with a smooth surface. The ceca ended 0.14-0.17 mm away from the posterior end of the body, with symmetrical testes at the level of the ceca end. The oral sucker is equal to the ventral sucker. The tail inserted to the posterior end of the body. There are various sizes of vacuole through the tail.

Size range and average size (in micrometers, calculated from 20 cercariae):
Body: 190-250 µm (av. 220 µm) × 350-415 µm (av. 370 µm)
Oral sucker: 45-65 µm (av. 52 µm) × 45-65 µm (av. 52µm)
Ventral sucker: 48-68 µm (av. 55 µm) × 48-68 µm (av. 55µm)
Pharynx: 8-10 µm (av. 10 µm) × 8-12 µm (av. 11µm)

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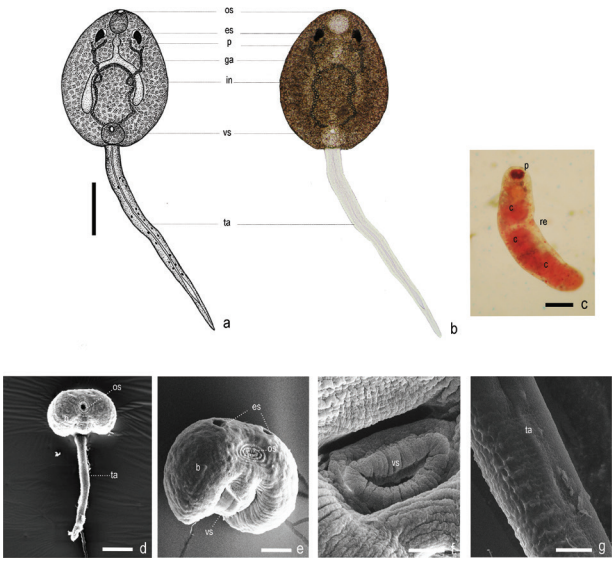

Figure 18. Image of *Gastrothylax crumenifer*;
a. Drawing of cercaria structure
b. Cercaria stained with 0.5% neutral red
c. Redia stained with 0.5 % neutral red
d.- g. Images of Scanning Electronmicroscope
Abbreviations: os - oral sucker, p - pharynx, es - eye spot, vs - ventral sucker, ga - genital atrium, in - intestine, vs - ventral sucker, ta - tail, re - redia, c - cercaria (scale a, b = 100 µm, c = 10 µm).

Esophagous:	80-140 µm (av. 125 µm) × 80-140 µm (av. 125µm)
Tail:	65-95 µm (av. 82 µm) × 328-450 µm (av. 410 µm)

Movement behavior: The cercaria floated on the surface or in the water. It moved by wavering on the surface of the water for around 8-10 seconds, and then rolling up and springing back for about 5-10 seconds. It survived up to 3-4 hours in the water after emergence. The cercariae were photo-sensitive. They shrank rapidly in changing light conditions.

Type 8. *Renicolid cercariae*

17. *Cercaria caribbea* LXVIII (Cable, 1963) (Yamaguti, 1975)

Cercaria caribbea LXVIII (Fig. 19) were found from 45 *M. tuberculata* which is equivalent to an infection rate of 0.14% (45/32,026) of the total number of the collected snails (Table 2). Cercariae developed in the sporocyst. Its body is flat with yellow pigment and numerous minute spines on the surface of the body, with no eye spot, a small sucker of 28-36 µm, a short prepharynx, a pharynx of 12-14 µm, plenty of small cephalic glands in the middle of the body, a short excretory vesicle split into two since the upper of the acetabulum. The tail is straight, and is longer than the length of the body, with no lateral finfold, and no flame cells.

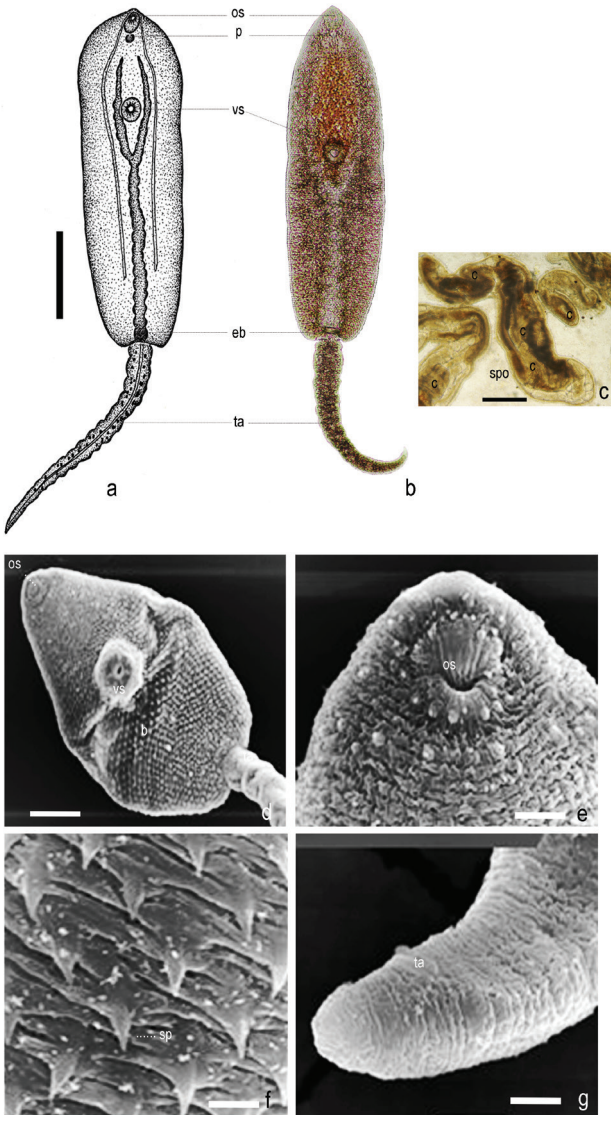

Figure 19. Image of *Cercaria caribbea* LXVIII;
a. Drawing of cercaria structure
b. Cercaria stained with 0.5% neutral red
c. Sporocyst stained with 0.5 % neutral red
d.- g. Images of Scanning Electronmicroscope
Abbreviations: os - oral sucker, p - pharynx, vs - ventral sucker eb - excretory bladder, ta - tail, spo - sporocyst (scale a, b = 100 µm, c = 10 µm).

Size range and average size (in micrometers, calculated from 20 cercariae):

Body:	128-140 µm (av. 129 µm) × 390-435 µm (av. 420 µm)
Oral sucker:	28-33 µm (av. 26 µm) × 28-33 µm (av. 26 µm)
Ventral sucker:	29-36 µm (av. 30 µm) × 29-36 µm (av. 30 µm)
Pharynx:	8-14 µm (av. 12 µm) × 10-14 µm (av. 12 µm)
Excretory bladder:	15-20 µm (av. 17 µm) × 15-20 µm (av. 17 µm)

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Tail: 34-38 µm (av. 35 µm) × 395-480 µm (av. 450 µm)

Movement behavior: The cercaria moved slowly on the bottom of the container, and swam continuously. It survived up to 2-3 hours in the water after emergence.

Type 9. *Cotylomicrocercous cercariae*

18. *Podocotyle* (*Podocotyle*) *lepomis* Dobrovolny, 1939 (Yamaguti, 1975)

Podocotyle lepomis (Fig. 20) were found from 3 *M. tuberculata* which is equivalent to an infection rate of 0.009% (3/32,026) of the total number of the collected snails (Table 2). Cercariae developed in the sporocyst. The body is cylindrical in shape, clear white in color, with no sensory hair, spine, 6 papillae on the head, and rough granules present on the body. The stylet is present in oral sucker. There were 2 rows of sensory papillae around the oral sucker, with a long prepharynx. Pharynx is round. The ceca extended to the posterior end, with 5 pairs of penetration glands. 2 of them were not stained with 0.5% neutral red while 3 of them were stained with 0.5% neutral red. Excretory vesicle had a thick wall and open dorsally at the tail, the flame cell formula is 2[(2+2)+(2+2)]. The tail is short, only half the length of the body, cup-shaped, with an adhesive gland present at the end of the tail for attaching.

Size range and average size (in micrometers, calculated from 20 cercariae):

Body: 65-93 µm (av. 73 µm) × 103-145 µm (av. 123 µm)

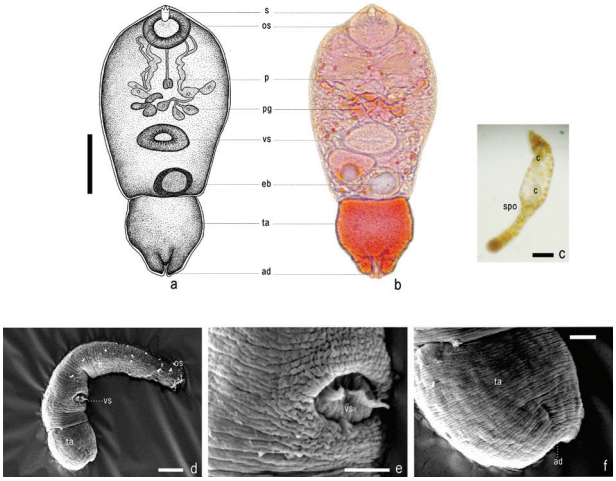

Figure 20. Image of *Podocotyle* (*Podocotyle*) *lepomis*;
a. Drawing of cercaria structure
b. Cercaria stained with 0.5% neutral red
c. Sporocyst stained with 0.5 % neutral red
d.- f. Images of Scanning Electronmicroscope
Abbreviations: s - stylet, os - oral sucker, p - pharynx, penetration gland, vs - ventral sucker, eb - excretory bladder, ta - tail, ad - adhesive organ, spo - sporocyst, c - cercaria (scale a, b = 100 µm, c = 10 µm).

Stylet: 4-6 µm (av. 5 µm) × 6-10 µm (av. 8 µm)
Oral sucker: 30-38 µm (av. 32 µm) × 25-38 µm (av. 30 µm)
Ventral sucker: 28-40 µm (av. 35 µm) × 28-40 µm (av. 35 µm)
Pharynx: 13-18 µm (av. 14 µm) × 10-13 µm (av. 11 µm)
Excretory bladder: 20-30 µm (av. 26 µm) × 18-25 µm (av. 21 µm)
Tail: 43-65 µm (av. 52 µm) × 50-75 µm (av. 61 µm)

Movement behavior: The cercaria floated with the ventral upside. It moved by floating with its head and tail folded together, and then sprang up. Normally it creeps on the surface of the container using the oral and ventral sucker. The cercaria floated for about 15-20 seconds, and rested for about 10-15 seconds. It survived up to 2-3 hours in the water.

Discussion

Melanoides tuberculata Müller, 1774 is a benthic freshwater thiarid native to Africa and Asia. Its original native range seemed uncertain but nonetheless wide, including parts of Africa, the Mediterranean, Asia and the Pacific Islands (Pace 1973, Clench 1969). Glaubrecht (1996) hypothesized that its origin lies rather in Asia than Africa, given its natural occurrences and the history of Thiaridae; see also Glaubrecht et al. (2009) and Glaubrecht (2011). It had also become established in several other countries. (Facon et al. 2004, Derraik 2008). Aquarium plants exchange by humans caused the brake down of natural dispersal barriers to these snails (Kolar and Lodge 2001). Moreover, *Melanoides* had also adapted well to new environments. For example, on Martinique Island *M. tuberculata* was surveyed in 1979 and 2003. While there were only two morphs found in 1979, in 2003 there were nine morphs. Population dynamics and distribution of *M. tuberculata* were studied in many areas, where they dominated the streams, ponds, and lakes (e.g. Supian and Ikhwanuddin 2002, Eldblom and Kristensen 2003, Facon et al. 2003).

Melanoides tuberculata was found to be intermediate host for a number of trematode parasites (e.g. Pinto and De Melo 2011, Ukong et al. 2007). As a consequence, the introduction of *M. tuberculata* leads to new parasitic cycles in humans in the invaded area. However, there are also reports that show the efficacy of *M. tuberculata* as a biocontrol agent against the schistosome (blood fluke) vector snails *Biomphalaria glabrata* (Pointier and Jourdane 2000).

In Thailand, Brandt (1974) reported that thiarid snails were found in lakes, ponds, marshes, canals, streams, rivers, and other sources of river such as waterfalls. In the present work, we did a smaller scale of investigation than Brandt's. More than thirty thousand of *M. tubercu-*

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lata snails were collected from rice paddies, drainages, ponds, canals, water reservoirs, marshes, streams, waterfalls, and rivers in Thailand. The classification of *M. tuberculata* was performed as reported by Brandt (1974). It was quite clear to distinguish *M. tuberculata* from other thiarids, although among these snails there were quite different shell morphologies in terms of ribs, color, pigmentation and even size. Although, based on the shell, the destinction of discrete several morphs was possible (see e.g. Pointier 1989, 1993, Samadi et al. 1999), all of these morphs were still considered to be conspecific within *M. tuberculata*. It is hoped that molecular techniques will help us to eventually solve this question of intraspecific versus intraspecific variation.

In this study, the recovery of adult trematode stages are not completely recorded, but the morphological distinction of cercariae are quite clear from the unstained, stained and electronmicroscopic images, allowing to classify the eighteen species of cercariae from this thiarid snail species into nine types of cercarial morphology, as compiled in the Result section.

In the present study, we also found human trematodes, viz. *Haplorchis taichui, Haplorchis pumilio, Centrocestus formosanus* and *Cercaria caribbea* LXVIII. Especially the *H. pumilio* human minute intestinal fluke (371/6,019 = 6.16%) showed a high level of prevalence in Thailand. As they complete their complex life cycle not only in humans but also in other vertebrates, it is difficult to control their infection. *H. taichui* is another important minute intestinal fluke. Three cases of humans were reported with mucosal ulceration, mucosal and submucosal haemorrhages, fusion and shortening villi, chronic inflammation and fibrosis of submucosa; in addition, there was a report of the pathology in the small intestine of patients caused by *H. taichui* (Sukontason et al. 2005). In earlier reports, *Haplorchis* spp. were found to be of high prevalence of infection in the north of Thailand (Chontananarth and Wongsawad 2010). In the present report, we now found *Haplorchis* infections in every region of Thailand.

The minute intestinal flukes were reported not only in Asia but also in South America. The life cycle of *H. pumilio* was studied from redia to adult under natural and experimental conditions in the digestive gland of *Melanoides tuberculata*, collected from Agasanta, Venezuela (Diaz et al. 2008). It seems that *M. tuberculata* was one of the important intermediate host snails of humans and animal trematodes in the world.

For animal parasites, the cercariae with the highest prevalence were *L. bicolor* (2,373/6,019 = 39.43%), an amphibian trematode. However, the other animal parasites were also very important for public health. For example, the eye fluke *Philophthalmus* spp. are parasites of birds, using a snail intermediate host and birds as the definitive host. However, they also occurred in human and other animals. Human infection by these eye flukes occur via direct contact with the eye by cercariae in the water or by ingestion of cercariae in contaminated water (Alicata 1962, Waikagul et al. 2006, Derraik 2008).

One of heterophyid trematodes, *Stictodora tridactyla* was reported that they occasionally infested brackish water and marine snails, while metacercariae encyst in fish, with the definitive hosts being birds and mammals including humans (Chai et al. 1988, Abdul-Salam et al. 2000). *S. tridactyla* also infected *M. tuberculata* with other cercariae as well. The infection rate of *S. tridactyla* is 6.81% (2,182/6,019). In our previous study, we found *S. tridactyla* in *M. tuberculata* and even more in *M. jugicostis* (Ukong et al. 2007).

The highest infection rate of parasite is 7.41% (2,373/6,019) with *Loxogenoides bicolor* being the most common parasite found in the present study. This parasite is one of the Xiphidiocercariae, being produced by trematodes from the superfamily Plagiorchioidea (Schell 1962, Malek and Cheng 1974). They were found in other thiarid snails, such as *Thiara* (*i.e. Plotia*) *scraba* at Erawan Waterfall, Kanchanaburi, Thailand (Ukong et al. 2007).

Interestingly, it can cause double infection or even triple infection in *M. tuberculata* together with other trematodes. We found a total of 326 double infections and 13 triple infections in *M. tuberculata* (Table 4). *S. tridactyla* and *L. bicolor* were found to be common in double infections, while *S. tridactyla*, *L. bicolor* and *C. alseae* were commonly found triple infections. *C. alseae* is a blood-dwelling trematode.

Table 4. Double trematode infections and triple trematode infections of collected *Melanoides tuberculata.*

Infections	Type	No. of infected snails
Double	*Loxogenoides bicolor* + *Stictodora tridactyla*	135
	Cardicola alseae + *Stictodora tridactyla*	162
	Apatemon gracilis + *Stictodora tridactyla*	3
	Loxogenoides bicolor + *Cercaria caribbea* LXVIII	3
	Loxogenoides bicolor + *Philophthalmus* sp.	7
	Loxogenoides bicolor + *Haplorchis pumilio*	10
	Alaria mustelae + *Haplorchis pumilio*	1
	Haplorchis taichui + *Alaria mustelae*	1
	Haplorchis taichui + *Loxogenoides bicolor*	2
	Haplorchis taichui + *Acanthatrium hitaense*	2
Triple	*Loxogenoides bicolor* + *Stictodora tridactyla* + *Podocotyle lepomis*	1
	Loxogenoides bicolor + *Stictodora tridactyla* + *Cardicola alseae*	12
Total		339

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This parasite is furcocercous cercariae. It is produced by trematodes from the family Sanguinicolidae. Found in freshwater fishes they were reported to have sporocysts that developed in the visceral mass of the snail *Oxytrema silicula* from Alsae River, Oregon, USA (Meade 1967). In Thailand, *C. alseae* was also found in the thiarid snail *Tarebia granifera* at Erawan Waterfall (Ukong et al. 2007). The others furcocercous cercariae *Alaria mustelae*, *Transversotrema laruei, Apatemon gracilis*, and *Mesostephanus appendiculatus*, can also be found in *M. tuberculata*. These parasites, such as *Transversotrema laruei and Apatemon gracilis*, were found with metacercariae in brackish and freshwater fishes. The adult stages of these flukes inhibit the small intestine of their bird hosts (Smith and Hickman 1983).

Although the counts per unit of time method (Olivier and Schneiderman 1956) used to measure the density of the snail population in the marked areas does not represent the total population, our observations were performed all year round. That way we were able to document seasonal variation in the intensity of parasitism in *M. tuberculata*. Thus, we consider our data of cercariae infection rates from this research to be reliable. Since most of the above cited intestinal flukes affect humans and animals alike (Derraik 2008), in conclusion we can state that the thiarid snail *Melanoides tuberculata* can doubtlessly be considered as of considerable medical significance in Thailand and elsewhere.

Acknowledgements

This research was supported by the Research and Development Institute, Silpakorn University, Thailand. We also thank Department of Biology, and Faculty of Science, Silpakorn University. We would like to especially thank Assist. Prof. Dr. Chalermsri Chantasingh and PD. Dr. Matthias Glaubrecht for valuable suggestions and editing the manuscript.

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Digitale Literatur/Digital Literature

Zeitschrift/Journal: Zoosystematics and Evolution

Jahr/Year: 2014

Band/Volume: 90

Autor(en)/Author(s): Krailas Duangduen, Namchote Suluck, Koonchornboon Tunyarut, Dechruksa Wivitchuta, Boonmekam Dusit

Artikel/Article: Trematodes obtained from the thiarid freshwater snail Melanoides tuberculata (Müller, 1774) as vector of human infections in Thailand 57-86