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Larval stage of trematodes obtained from brackish water snails in the central and east coast of the gulf of Thailand

Suluck Namchote*, Supatta Sritongtae, Siriphon Butnin, Paporn Wongwain and Duangduen Krailas

Parasitology and Medical Malacology Research Unit, Department of Biology, Faculty of Science, Silpakorn University, Nakhon Pathom 73000 Thailand.

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Brackish water snail is one of the important intermediate hosts of trematode infections. This kind of snail along the Gulf of Thailand were examined for cercariae, the larval stage of trematodes. The aim of this study was to investigate cercarial infections in brackish water snails in the central and eastern side of the Gulf of Thailand. Snails were collected from 45 sampling sites (20 central areas, 25 eastern areas), between August 2013 and February 2014. A total of 8,532 snails were classified into 12 families, 23 genera and 32 species. Cercarial infections were investigated using shedding and crushing methods. The infection rate was 0.27% (23/8,532). The cercariae were categorized into 5 types and 8 species. The first type, Parapleurophocercous cercariae, consisted of *Haplorchis taichui*, *Heterophyes cercaria* I, and *Metorchis intermedius*. The second type, Xiphidiocercariae, consisted of *Ascorhytis charadriformis.* The third type, Furcocercous cercariae, consisted of *Apharyngostrigea pipientis.* The forth type, Cotylomicrocercous cercariae, consisted of *Coitocaecum anaspidis*. The fifth type, Echinostome cercariae, consisted of *Hypoderaeum conoideam* and *Himasthla interrupta*. Seven species of snails were found with trematode infections, comprising of *Assiminea brevicula*, *Cerithidea cingulata*, *Cerithidea alata*, *Cerithidea djadjariensis*, *Cerithidea quadrata*, *Littorinopsis intermedia,* and *Sermyla riqueti*.

Key words: Cercaria, brackish water snails, trematode, the Gulf of Thailand.

INTRODUCTION

Thailand is situated between the Gulf of Siam and the Andaman Sea. Along the coasts line sand beaches, rock beaches, mangrove forests and river tributaries, providing abundant habitats where various aquatic fauna resided, including molluscs. It is well known that both gastropods and bivalves could be the first intermediate hosts of human and animal trematodes. The mollusc-transmitted diseases are very important for the veterinary and public health. Trematodes need two or three hosts to complete their life cycles. Trematode eggs hatch in the water. At

*Corresponding author. E-mail: Namchote01@hotmail.com

the larval stage, they can swim to find the first intermediate hosts, such as freshwater snails, brackish water snails, and terrestrial snails. In the snails, miracidium develops into sporocyst to redia and finally to cercaria. Cercaria leave the snails and head for the second intermediate host, e.g. fish or amphibian. Then they develop into metacercaria. Animals and humans can be infected by eating metacercaria or by the penetration of cercaria. The study of brackish water snails as intermediate hosts of trematode was reported. *Pirenella conica* (Gastropoda: Potamididae), a small sea snail along the coasts of Sinai and Israel were infected with trematode of the family Heterophyidae, Echinostomatidae, Microphallidae, Notocotylidae, Haploporidae, Haplosplanchnidae, Cyathocotylidae and Strigeidae (Taraschewski and Paperna, 1981). *Cerithidea cingulata* of Kuwait Bay found with echinostome cercaria infection was reported as new cercaria species by Salam and Sreelatha (1999). Moreover, the snail, *C. cingulata* of Kuwait Bay were infected with 12 species of trematodes. They belong to the family Cyathocotylidae, Echinostomatidae, Haplosplanchnidae, Heterophidae, Microphallidae, Philophthalmidae, Plagiorchiidae and Schistosomatidae (Al-Kandari et al., 2000). *Zeacumantus subcarinatus* (Prosobranchia: Batillaridae), the southern creeper, was reported as a new echinostome cercaria (Marorelli et al., 2006).

In Thailand, mud whelks in the family Potamididae (*Cerithidea cingulata, C. djadijarensis* and *C. charbonnieri*) could be intermediate host of 3 groups of cercariae. They were separated by morphological characters. The first group was found with a cystogenous gland but with no eye spots and no collar spines. The second group was found with eye spots, 3-4 penetration glands, and a finfold tail. The third group was still undetermined (Sri-aroon et al., 2005). Those cercariae are not known species. And those surveys were limited to only some regions of Thailand (Sri-aroon et al., 2004, 2005, 2010). Therefore, the aim of this study was to investigate and identify trematode infections in brackish water snails in Central and Eastern Thailand. The knowledge of trematode fauna can provide more understanding for veterinary and public health control.

MATERIALS AND METHODS

Sampling sites and collection of snails

Snails were collected from 45 sampling sites (20 central areas, 25 east areas), between August 2013 and February 2014. The counts per unit of time method was used for snail sampling. Five collectors picked the snails by hand and scoop for 10 minutes each station. The sampling sites were mangrove forest, drainage, canal and tributary of river in Trat, Chanthaburi, Rayong, Chon Buri, Chachoengsao, Bangkok, Samut Pakran, Samut Sakhon and Samut Songkhram provinces of Thailand (Figure 1). The collected snails were transferred to the Parasitology and Medical Malacology Research Unit, Department of Biology, Faculty of Science, Silpakorn University, Thailand. Those snails were identified by shell morphology (Brandt, 1974; Upatham et al., 1983).

Examination for trematode infections

Trematode infections were investigated by using snail shedding and crushing methods. Cercariae were collected in dechlorinated water and examined under stereo-microscope and scored the infection rates.

Study of cercarial morphology

Cercarial morphology and anatomy were examined by stained cercariae. They were stained with 0.5% neutral red and identified under light microscope. The cercariae were fixed in 10% formalin, then measured and averaged from 10 specimens. The stained specimens were drawn using *camera lucida*. Scanning electron images of cercariae were taken by Camscan MX 2000 scanning electron microscope (UK). The cercariae were processed by fixed in 2.5% glutaraldehyde phosphate buffer at 4°C for at least 2 h and post-fixed in 1% osmium tetroxide at 4°C for 1 h, dehydrated through a graded series of acetone, then they were air dried. The specimens adhere to carbon stub, were coated with gold-palladium in an ion-sputtering apparatus (Polaron CPD 7501, UK), then examined under SEM. The species of trematodes were identified by their morphology and anatomy following Yamaguti (1975) and Ito (1980).

RESULTS

Snail samples and Cercarial infections

A total of 8,532 brackish water snails were collected from estuaries and mangrove forests along the coast of Central and Eastern parts of Thailand. They were categorized into 32 species of 12 families: Neritidae, Littorinidae, Stenothyridae, Iravadiidae, Assimineidae, Thiaridae, Muricidae, Potamididae, Ellobiiidae, Naticidae, Viviparidae and Cerithiidae. Twenty-three genera and thirty-two species were categorized (Table 1). Seven species were found as intermediate hosts of trematodes, which were *Assiminea brevicula* (Assimineidae), *Cerithidea cingulata*, *C. alata*, *C. djadjariensis*, *C. quadrata* (Potamididae), *Littorinopsis intermedia* (Littorinidae) and *Sermyla riqueti* (Thiaridae)(Figure 2). The infection rates were 0.14% (1/691), 0.09% (1/1,068), 0.13% (1/763), 0.32% (2/623), 4.20% (5/119), 0.61% (7/1,154) and 0.42% (6/1,437), respectively (Table 2). The obtained cercariae were classified into 5 types and 8 species. The first type was Parapleurophocercous cercariae *(Metorchis intermedius, Heterophyes cercaria* I*, Haplorchis taichui*); the second type was Xiphidiocercariae (*Ascorhytis charadriformis*); the third type was Furcocercous cercariae (*Apharyngostrigea pipientis*); the forth type was Cotylogmicrocercous cercariae (*Coitocaecum anaspidis*); and, the fifth type was Echinostome cercariae (*Hypoderaeum conoideam* and *Himasthla interrupta*).

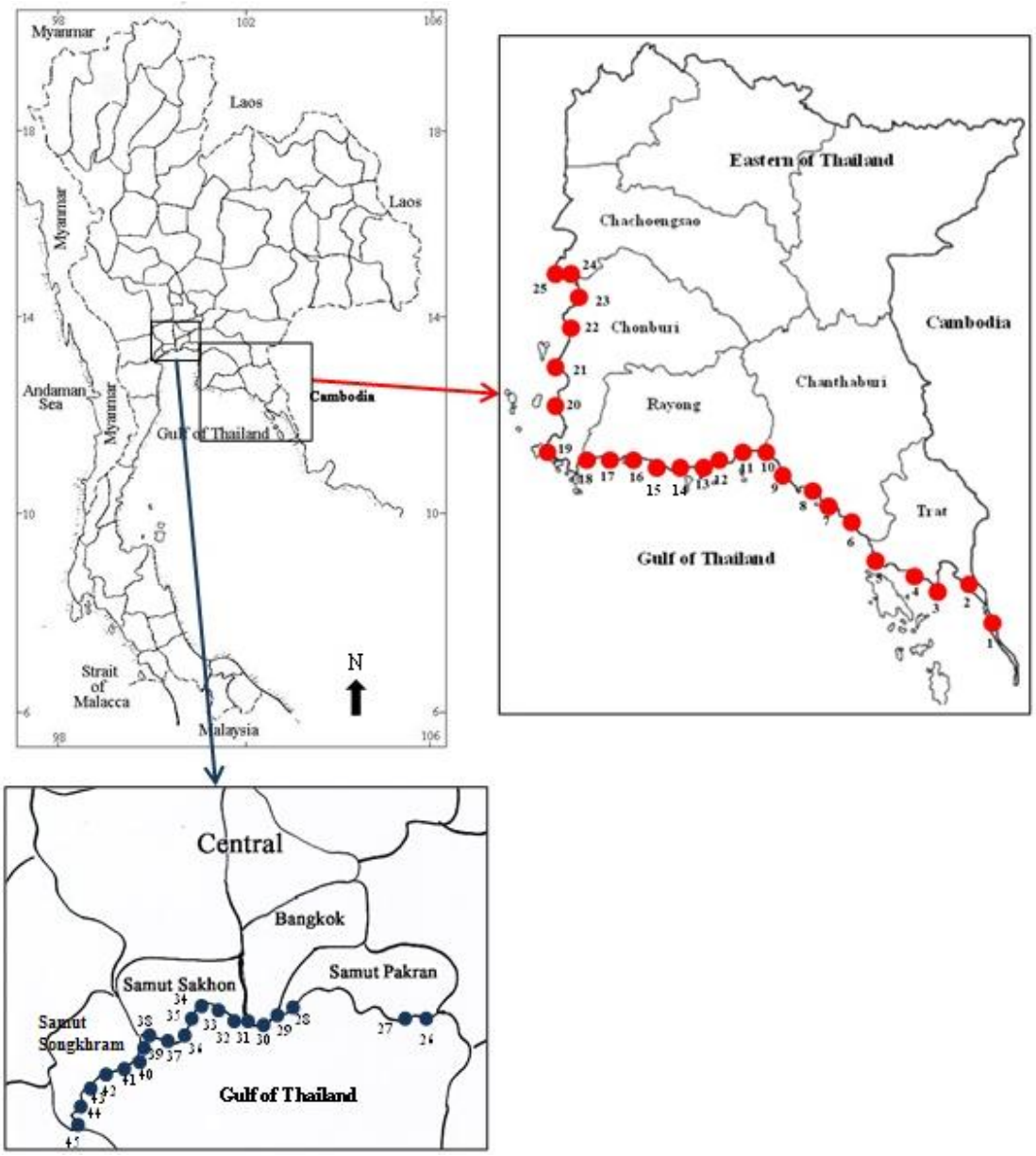

Figure 1. Maps of Thailand showing 5 provinces on the east coast: locations 1-25 (Trat, Chanthaburi, Rayong, Chonburi, and, Chachoengsao); and, 4 provinces in the middle of the Gulf: locations 26-45 (Samut Prakarn, Bangkok, Samut Sakhon, and, Samut Songkhram).

Morphology of cercariae

Characteristics of cercariae were described from fixed cercariae and living cercariae. Size of cercariae were measured for identification of cercarial species.

Type 1. Parapleurophocercous cercariae

Metorchis intermedius Heinemann, 1937

The body was oval in shape and yellowish brown in color. Four rows of spines were found around an oral sucker. There was a long prepharynx. Esophagus and ceca were not found. One pair of eye spots were apparently without lens. Fourteen penetration glands formed a compact median groups of 3:4:4:3 each. The ventral sucker was a prevesicular cell. The excretory vesicle was round, and lined with epithelia. Tail tubule median opened at a distance from the base of tail. The dorsal finfold began at the posterior end of basal swelling and passed over to the ventral finfold which reached the middle of the tail. The cercariae were produced within the redia (Figure 3).

The following figures are size ranges and average sizes

Table 1. Brackish water snalis were collected between August 2013 and February 2014 from the Central and Eastern Coasts of the Gulf of Thailand.

Family	Genus / Species	Province* / no. of collected snails (no. of infected snails)									
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Total**
Assimineidae	1. *Assiminea brevicula*	30	0	29	342	0	0	87(1)	53	150	**691**
Cerithiidae	2.*Clypeomorus moniliferus*	0	0	3	0		0	0	0	0	**3**
Ellobiidae	3.*Cassidula aurisfelis*	16	107	96	44	0	0	6	0	19	**288**
	4. *Cassidula mustelina*	1	1	12	9	0	0	0	3	0	**26**
	5.*Cassidula multiplicata*	0	0	1	1	0	0	0	0	3	**5**
	6. *Ellobium aurisjudae*	0	15	4	24	0	0	2	0	1	**46**
	7. *Laemodonta punctigera*	0	1	0	10	0	0	9	0	26	**46**
	8. *Laemodonta siamensis*	0	0	0	5	0	0	0	0	0	**5**
	9. *Micromelampus siamensis*	0	0	1	0	0	0	0	88	0	**89**
	10. *Melampus nucleolus*	0	0	0	0	0	0	0	0	2	**2**
Iravadiidae	11.*Fairbankia cochinchinensis*	0	57	0	0	0	0	0	0	0	**57**
Littorinidae	12. *Littorinopsis intermedia*	0	1	116	13	0	0	58(3)	240	726(4)	**1154**
	13. *Littorinopsis scabra*	43	29	42	1	0	0	0	5	0	**120**
	14. *Littorinopsis melanostoma*	0	0	2	40	0	0	0	1	3	**46**
Muricidae	15.*Chicoreus capucinus*	10	5	22	0	0	0	0	0	0	**37**
Naticidae	16. *Euspira* sp.	0	0	0	4	0	0	0	23	0	**27**
Neritidae	17. *Clithon peguensis*	339	103	311	181	0	0	0	0	0	**934**
	18. *Dostia violacea*	0	6	3	24	0	2	0	3	58	**96**
	19. *Neritodryas cornea*	0	0	0	0	0	0	0	0	22	**22**
Potamididae	20. *Cerithidea alata*	66(1)	51	40	137	0	0	0	0	0	**294**
	21. *Cerithidea cingulata*	134	125	153	188	0	0	446(1)	5	17	**1068**
	22.*Cerithidea djadjariensis*	107	129	345(2)	42	0	0	0	0	0	**623**
	23.*Cerithidea quadrata*	34	20(1)	35(3)	16(1)	0	0	0	0	0	**105**
	24.*Cerithidea obtusa*	0	0	0	5	0	0	0	0	0	**5**
	25. *Telescopium telescopium*	0	2	0	0	0	0	0	0	0	**2**
	26. *Terebralia palustris*	12	0	1	0	0	0	0	0	0	**13**
	27. *Faunus ater*	0	0	34	0	0	0	0	0	0	**34**
Stenothyridae	28. *Stenothyra* sp.	0	0	0	1	0	188	0	4	0	**193**

Table 1. Contd.

	29. *Melanoides tuberculata*	0	7	0	0	7	0	0	9	3	**26**
Thiaridae	30. *Sermyla riqueti*	0	6	130[(4)]	196[(1)]	0	14	37[(1)]	417	118	**918**
	31. *Tarebia granifera*	0	0	2	0	904	0	0	341	303	**1550**
Viviparidae	32. *Filopaludina martensi*	6	0	0	0	1	0	0	0	0	**7**
	Total	**798**	**665**	**1382**	**1283**	**912**	**204**	**645**	**1192**	**1451**	**8,532**

* Eastern Provinces: 1. Trat 2. Chanthaburi 3. Rayong 4. Chon Buri 5. Chachoengsao Central Provinces: 6. Samut Prakarn 7. Bangkok 8. Samut Sakhon 9. Samut Songkhram.

of 10 cercariae:

Body: 88-103 µm (av. 93 µm) x 180-183 µm (av. 180 µm)
Tail: 25-28 µm (av. 27 µm) x 338-430 µm (av. 373 µm)
Eye spot: 10-20 µm (av. 15 µm) x 8-15 µm (av. 10 µm)
Oral sucker: 28-40 µm (av. 35 µm) x 28-43 µm (av. 35 µm)
Pharynx: 15-23 µm (av. 19 µm) x 10-13 µm (av. 11 µm)
Excretory bladder: 70-88 µm (av. 78 µm) x 45-55 µm (av. 50 µm)
Lateral finfold: 25-28 µm (av. 27 µm) x 175-180 µm (av. 178 µm)
Dorso-ventral finfold : 15-18 µm (av. 16 µm) x 158-163 µm (av. 160 µm)

Heterophyes cercaria I Martin, 1959

The body was oval in shape and yellowish brown in color. The body surface was covered with tiny barbed and sensory hair. Pre-pharynx was found. Pharynx was small and round in shape. Eye spots were round and triangular in shape. Seven pairs of penetration glands were in two longitudinal rows. Their ducts were in 2 bundles. Acetabulum was not observed. Excretory vesicle was thick wall and V-shape. Tail was tubule with finfolds. The characteristics of finfolds were shown; the dorsal fin arose from the tail base, extended to the whole length of the tail, and continued with the ventral fin to the tail tip. The excretory duct opened at the tail tip. The cercariae were produced within the rediae (Figure 4). The following figures are size ranges and average sizes of 10 cercariae:

Body: 98-130 µm (av. 114 µm) x 163-195 µm (av. 179 µm)
Tail: 40-45 µm (av. 43 µm) x 523-580 µm (av. 551 µm)
Eye spot: 10-15 µm (av. 12 µm) x 8-10 µm (av. 9 µm)
Oral sucker: 33-38 µm (av. 35 µm) x 23-33 µm (av. 28 µm)
Pharynx: 13-15 µm (av. 14 µm) x 15-18 µm (av. 16 µm)
Excretory bladder: 23-25 µm (av. 24 µm) x 65-70 µm (av. 68 µm)
Dorso-ventral finfold: 30-40 µm (av. 35 µm) x 250-280 µm (av. 265 µm)

Haplorchis taichui (Nishigori, 1924) Witenberg, 1930

The body was oval in shape and orange or yellow in color. Spines and sensory hairs covered the whole body. An oral sucker was situated in the head region. The mouth aperture had transverse rows of spines. One pair of eye spots and a pharynx were present. Seven pairs of penetration glands extended from the pharynx to the end of the body. There were two longitudinal rows of genital primodia. The excretory bladder had a round shape and was composed of fine pigments. A long tail was attached to the dorsal end of the body, with lateral finfolds and a dorso-ventral finfold.
The cercariae were produced within the rediae (Figure 5). The following are size ranges and average sizes of 10 cercariae:

Body:75-100 µm (av. 83 µm) x 80-113 µm (av. 100 µm)
Tail: 20-35 µm (av. 28 µm) x 325-380 µm (av. 357 µm)
Eye spot: 8-13 µm (av. 10 µm) x 5-10 µm (av. 8 µm)
Oral sucker: 38-45 µm (av. 41 µm) x 35-45 µm (av. 40 µm)
Ventral sucker: 10-13 µm (av. 12 µm) x 8 -13 µm (av. 12 µm)
Pharynx: 10-15 µm (av. 11 µm) x 10-13 µm (av. 11 µm)
Excretory bladder: 48-55 µm (av. 52 µm) x 13-23 µm (av. 16 µm)

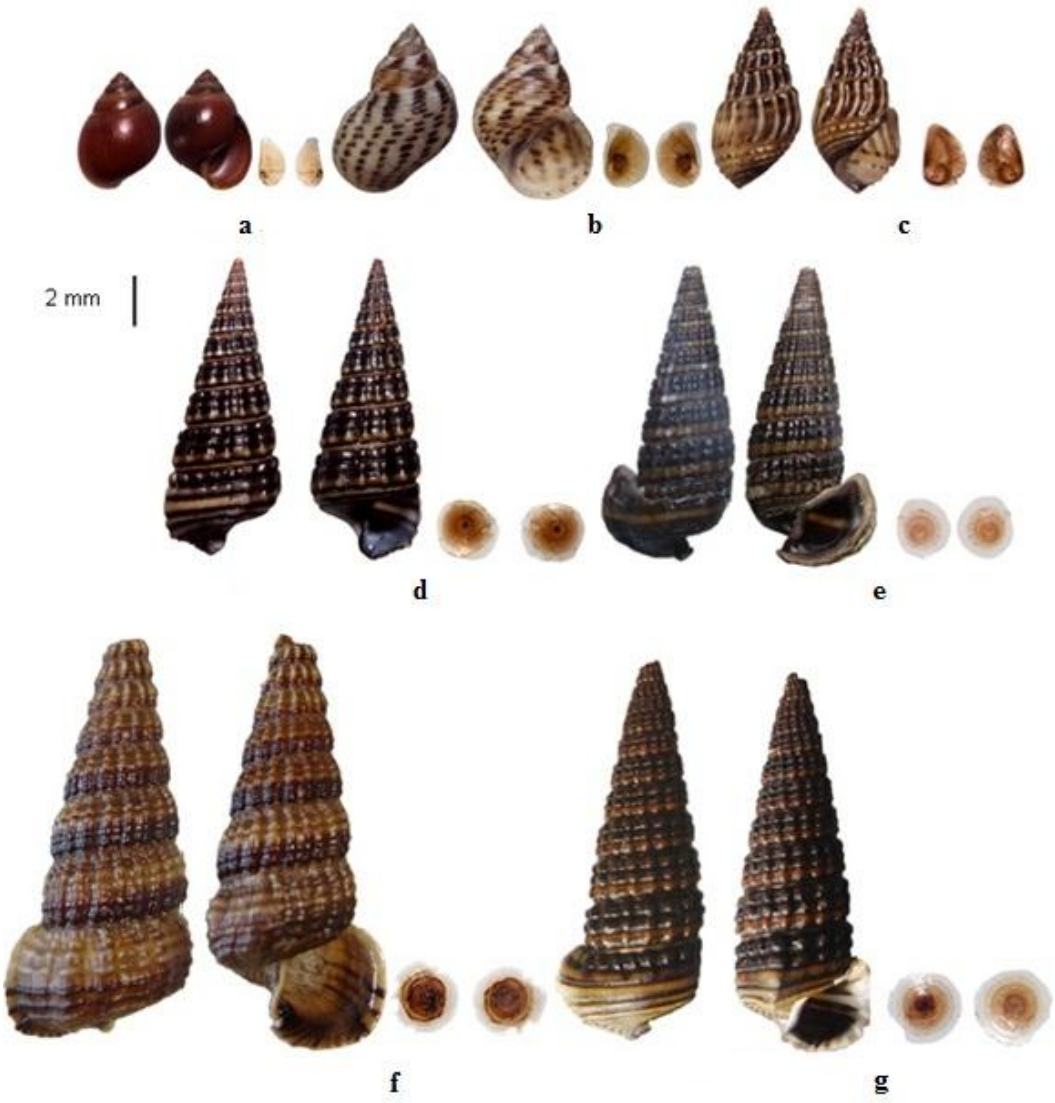

Figure 2. Seven species of brackish water snails of the Central and the Eastern Coasts along the Gulf of Thailand found with cercarial infections (a) *Assiminea brevicula* (b). *Littorinopsis intermedia* (c). *Sermyla riqueti* (d). *Cerithidea cingulata* (e). *C. alata* (f). *C. quadrata* (g). *C. djadjariensis*.

Lateral finfold: 10-18 µm (av. 12 µm) x 120-158 µm (av. 134 µm)
Dorso-ventral finfold: 8-18 µm (av. 13 µm) x 145-238 µm (av. 194 µm)

Type 2. Furcocercous cercariae

Apharyngostrigea pipientis (Faust, 1918) Olivier, 1940

The body was oval in shape. The tail was furcae, broad, and flat. There was a distinct pharynx. The esophagus reached half-way from the pharynx to acetabulum. A pair of unpigmented eye spots were anterolateral to acetabulum. There were 4 pairs of penetration glands. The excretory bladder was small. The tail was a tubule branch opening on the dorsal edge of the furcae about halfway down to the furcal tip. There were furcal finfolds (Figure 6). The following are size rangs and average sizes of 10 cercariae:

Body: 87.5-90 µm (av. 87.81 µm) x 125-175 µm(av. 140.94 µm)
Tail: 20-25 µm (av. 23.75 µm) x 150-200 µm (av. 181.25 µm)
Tail fucal: 15-20 µm (av. 19.38 µm) x 62.5-70 µm (av. 65.31 µm)
Eye spot: 3 µm x 3 µm
Oral sucker: 20-25 µm (av. 23.75 µm) x 5-35 µm (av. 29.69 µm)

Table 2. The infection rates of brackish water snails as intermediate host.

Snail	Number of examined snail	Number of infected snail		Infection rate (%)	Cercarial species
		Eastern	Central		
Assiminea brevicula	691	0	1	0.14	*Metorchis intermedius*
Cerithidea cingulata	1,068	0	1	0.09	*Heterophyes cercaria* I
Cerithidea alata	294	1	0	0.34	*Himasthla interrupta*
Cerithidea djadjariensis	623	2	0	0.32	*Ascorhytis charadriformis*
Cerithidea quadrata	105	5	0	4.76	*Hypoderaeum conoideam*
Littorinopsis intermedia	1,154	0	7	0.61	*Ascorhytis charadriformis* (4 infected snails) *Coitocaecum anaspidis* (3 infected snails)
Sermyla riqueti	918	5	1	0.54	*Haplorchis taichui* (4 snails from Eastern, 1 snails from Central) *Apharyngostiger pipientis* (1 snails from Eastern)

Ventral sucker: 12.5-22.5 µm (av. 17.92 µm) x 17.5-25 µm (av. 22.92 µm)
Pharynx: 12.5-17.5 µm (av. 15.36 µm) x 12.5 µm (av. 12.5 µm)
Excretory bladder: 50-62.5 µm (av. 54.69 µm) x 17.5-25 µm (av. 19.69 µm)

Type 3. Xiphidiocercariae

Ascorhytis charadriformis (Young, 1949) Ching, 1965

The body was oval in shape and white in color. There was a long stylet. Four pairs of penetration glands lined near a ventral sucker. The ventral sucker was bigger than the oral sucker. The tail was long and round. The excretory duct opened at the tail-end. The cercariae were produced within the sporocyst (Figure 7). The following are size ranges and average sizes of 10 cercariae:

Body: 38-53 µm (av. 45 µm) x 163-210 µm (av. 190 µm)
Tail: 13-15 µm (av. 13 µm) x 113-150 µm (av. 123 µm)
Oral sucker: 8 µm x 8 µm
Ventral sucker: 10-13 µm (av. 12 µm) x 13 µm
Excretory bladder: 13-20 µm (av. 15 µm) x 10-25 µm (av. 16 µm)
Stylet: 3 µm x 18-20 µm (av. 18 µm)

Type 4. Cotylomicrocercous cercariae

Coitocaecum anaspidis Hickman, 1934

The body was cylindrical in shape with a transparent body. The skin was covered with smaller spines. There was a stylet with double points at the oral sucker. The pre-pharynx was moderately long. The esophagus and intestine did not differ. There were 3 pairs of penetration glands. The excretory bladder was large with thick wall. The tail was cup-shape. There was an adhesive organ at the tail end (Figure 8). The cercariae were produced within the sporocyst. The following are size ranges and average sizes of 10 cercariae:

Body: 60-125 µm (av. 86 µm) x 190-313 µm (av. 230 µm)
Tail: 15-50 µm (av. 15 µm) x 43-50 µm (av. 46 µm)
Oral sucker: 35-50 µm (av. 43 µm) x 38-45 µm (av. 41 µm)
Ventral sucker: 38-50 µm (av. 46 µm) x 40-58 µm (av. 48 µm)
Excretory bladder: 30-58 µm (av. 49 µm) x 10-75 µm (av. 50 µm)
Stylet: 5-8 µm (av. 7 µm) x 8-15 µm (av. 11 µm)

Type 5. Echinostome cercariae

Himasthla interrupta Loos-Frank, 1967

The body was cylindrical in shape and colorless. The skin was covered with spines and sensory hairs. There were collar spines around the oral sucker. There was a long esophagus. The intestines were bifurcated at the ventral sucker, and ending blindly near posterior. The excretory organs were distinct and branching from the anterior to posterior of the body. The excretory bladder was a small thick wall. Both the oral and ventral suckers were large, but the ventral sucker was bigger than the oral sucker. The tail was shorter than the body. Two excretory pores at the lateral sides were about one-fourth of the tail length from its base. The cercariae were produced within the daughter redia (Figure 9). The following are size ranges and average sizes of 10 cercariae:

Body: 150-163 µm (av. 151 µm) x 243-325 µm (av. 270 µm)

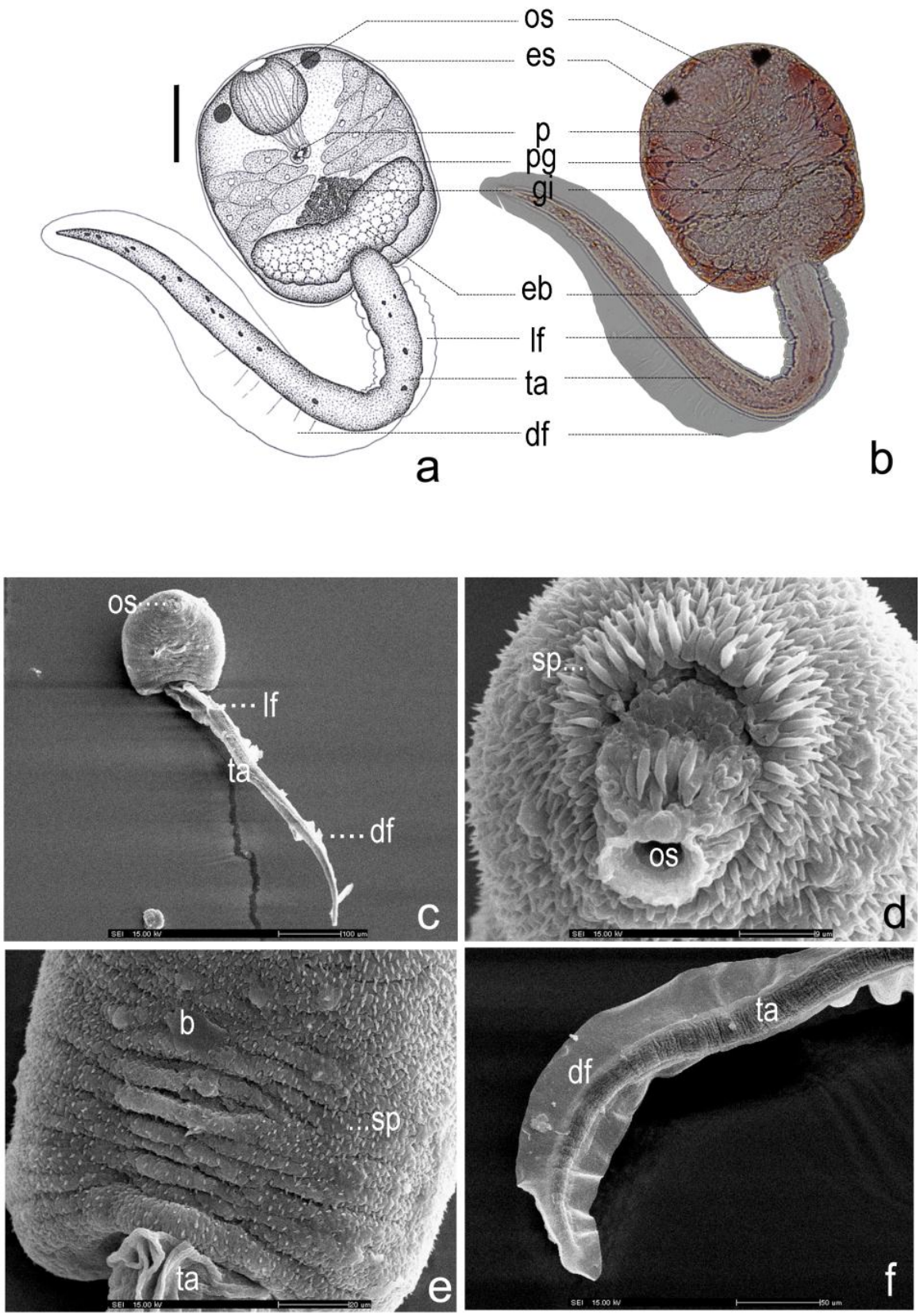

Figure 3. *Metorchis intermedius* cercaria: (a). drawing of cercaria stucture, (b). cercaria stained with 0.5% neutral red, (c-f). SEM micrograph of cercaria. (os = oral sucker, es = eye spot, p = pharynx, pg = penetration gland, lf = lateral finfold, df = dorso-ventral finfold, b = body) (scale bar = 100 µm).

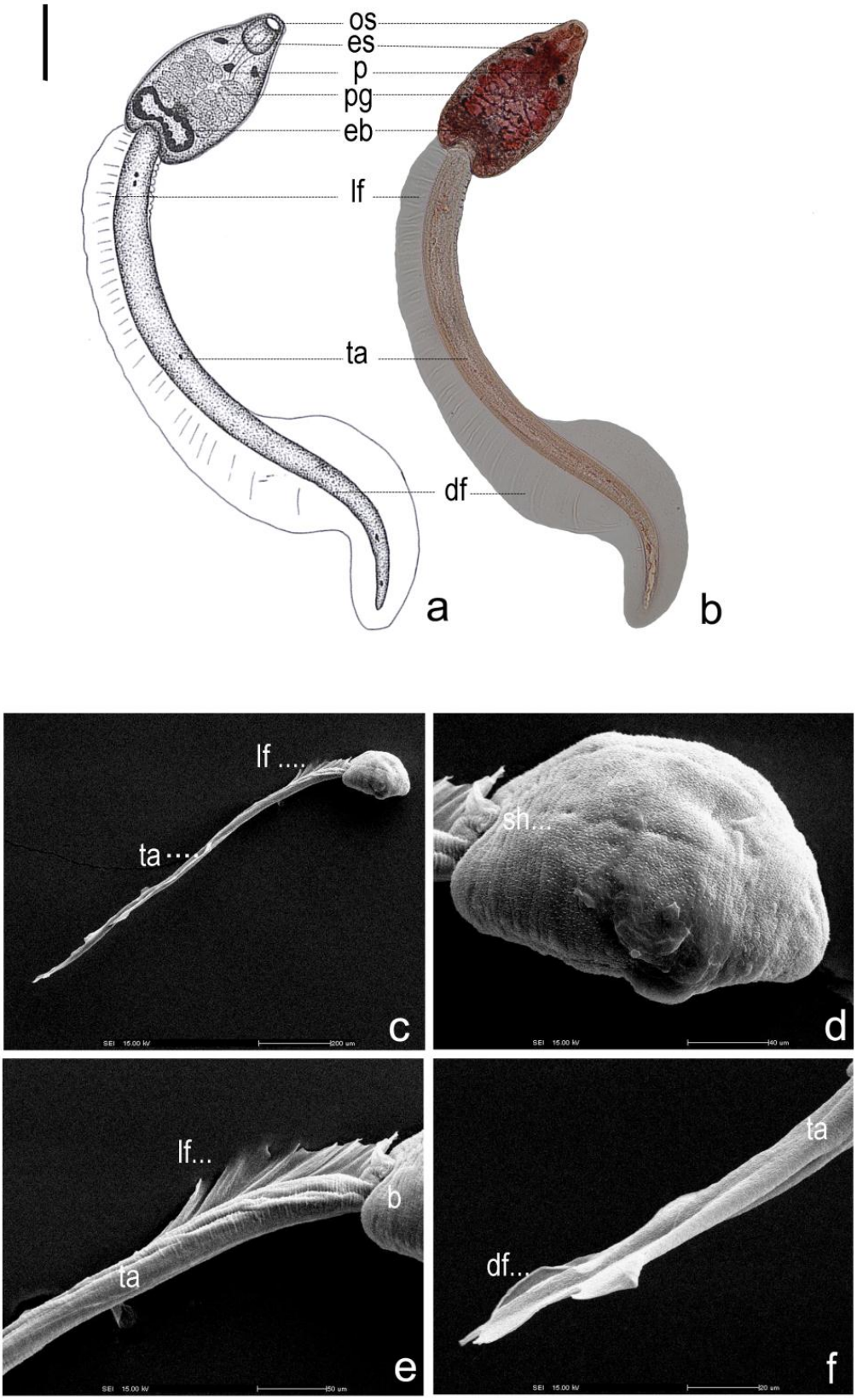

Figure 4. *Heterophyes cercaria* l cercaria: a. drawing of cercaria stucture, (b). cercaria stained with 0.5% neutral red, c-f. SEM micrograph of cercaria. (os = oral sucker, es = eye spot, p = pharynx, pg = penetration gland, eb = excretory bladder, ta = tail, lf = lateral finfold, df = dorso-ventral finfold, b = body, sh = sensory hair) (scale bar = 100 µm).

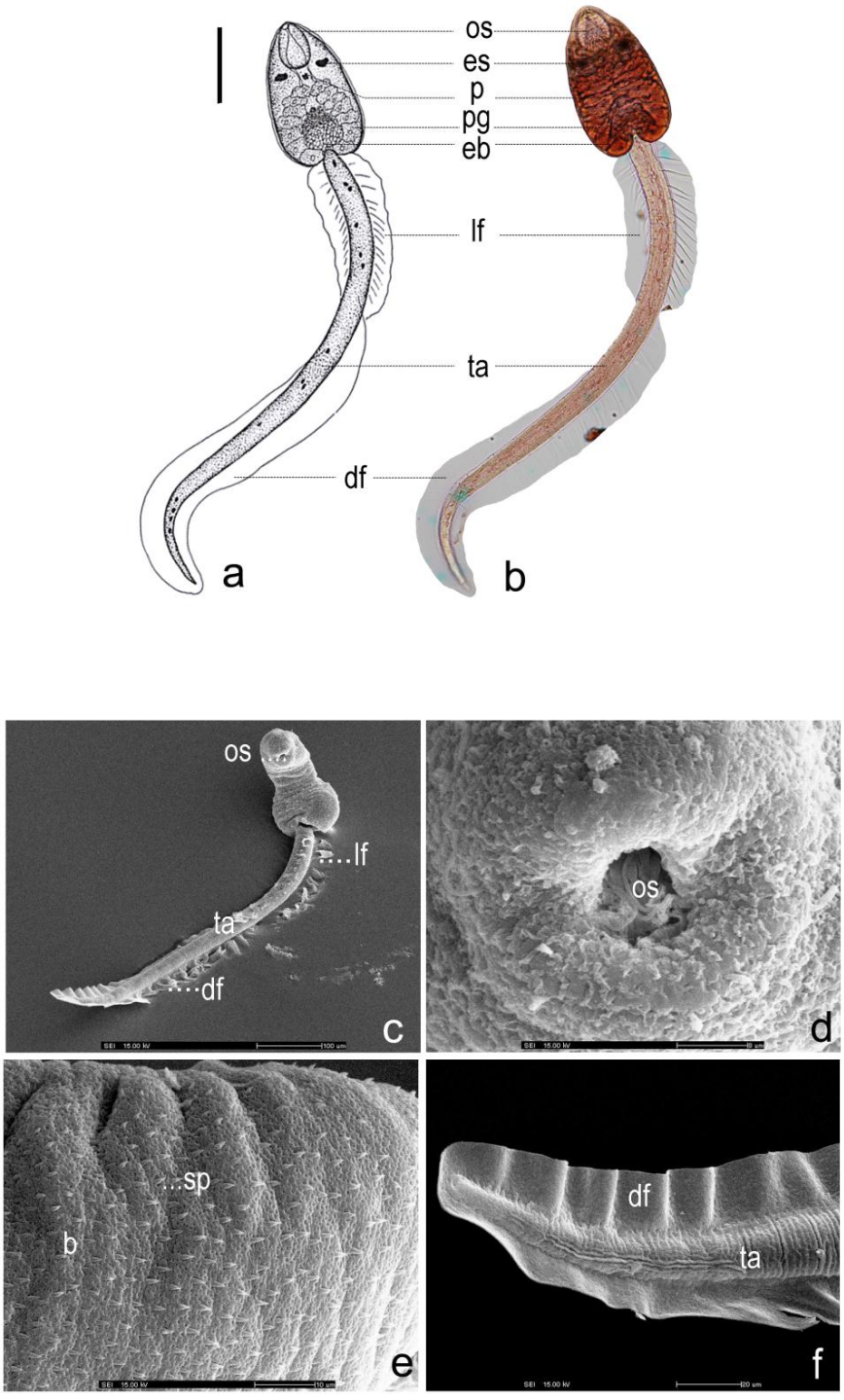

Figure 5. *Haplochis taichui* cercaria: (a). drawing of cercaria stucture, (b). cercaria stained with 0.5% neutral red, (c-f). SEM micrograph of cercaria. (os = oral sucker, es = eye spot, p = pharynx, pg = penetration gland, eb = excretory bladder, sp = spine, ta = tail, b = body, lf = lateral finfold, df = dorso-ventral finfold) (scale bar = 100 µm).

Tail: 28-40 µm (av. 34 µm) x 195-313 µm (av. 240 µm)
Oral sucker: 38-48 µm (av. 44 µm) x 38-48 µm (av. 44 µm)
Ventral sucker: 40-73 µm (av. 62 µm) x 55-63 µm (av. 60 µm)
Pharynx: 13-18 µm (av. 14 µm) x 20-30 µm (av. 24 µm)

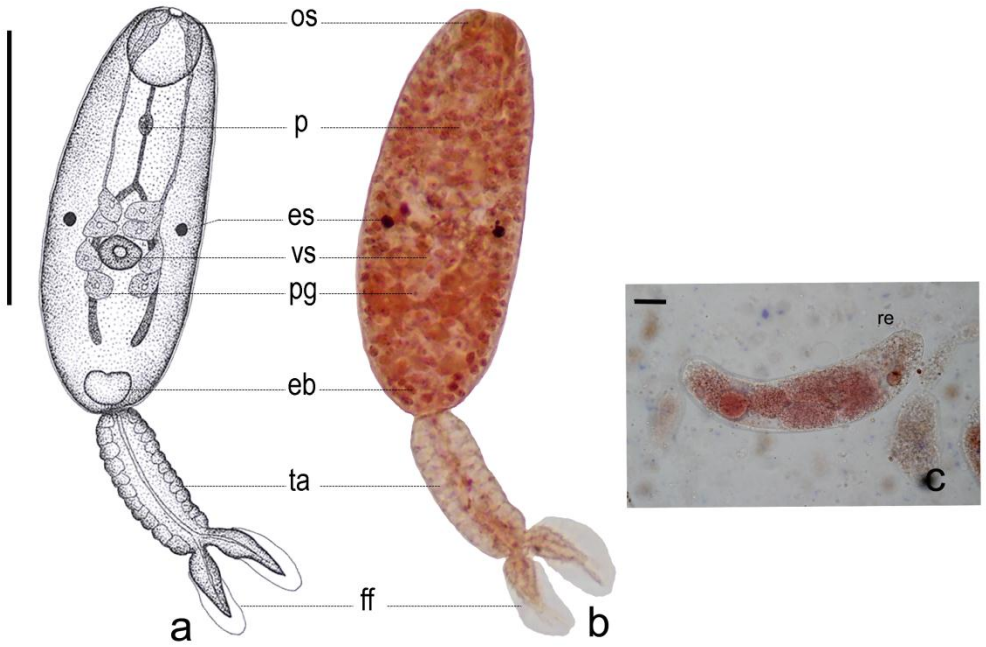

Figure 6. *Apharyngostiger pipientis* cercaria: a. drawing of cercaria stucture, (b-c). cercaria and redia stained with 0.5% neutral red. (os = oral sucker, es = eye spot, p = pharynx, pg = penetration gland, vs = ventral sucker, eb = excretory bladder, ta = tail, ff = furcal finfold, re = redia) (scale bar = 100 µm).

Excretory bladder:18-55 µm (av. 38 µm) x 18-55 µm (av. 33 µm)

Hypoderaeum conoideam (Bloch, 1982) Dietz, 1909

The body was round and colorless. There was no eye spot. There were two rows of collar spines. The first row had 15-20 spines, and the second row had 25-30 spines. There was a round pharynx. The esophagus was long; the bifurcate ceca terminated at the posterior end of the body. The ventral sucker was about two-third of the body length. The excretory vesicle was a simple sac. There was a Y-shaped excretory tube at the tail; the tube opened at the tail lateral, around one-fourth of the tail length from its base. The cercariae were produced within the rediae (Figure 10). The following are size ranges and average sizes of 10 cercariae:

Body: 270-350 µm (av. 323 µm) x 470-530 µm (av. 494 µm)
Tail: 70-90 µm (av. 79 µm) x 950-1180 µm (av. 1100 µm)
Oral sucker: 70-80 µm (av. 73 µm) x 50-70 µm (av. 65 µm)
Ventral sucker: 60-100 µm (av. 84 µm) x 60-80 µm (av. 74 µm)
Pharynx: 40-50 µm (av. 42 µm) x 40-50 µm (av. 43 µm)
Excretory bladder: 70-100 µm (av. 81 µm) x 40-60 µm (av. 50 µm)

DISCUSSION

The collected snails were separated into brackish and fresh water snails. They were collected from the river estuary and mangrove forests. Some fresh water snails found in this study are, for example, *Melanoides tuberculata*, *Tarebia granifera* and *Filopaludina martensi*. Normally, the fresh water snails were reported as the intermediate host of several trematodes, but we did not find trematode infections in this study. However, the present study confirmed the medical and veterinary importance of brackish water snails recorded in previous studies (Taraschewski & Paperna, 1981; Marorelli et al., 2006; Sri-aroon et al., 2010). In this study, either more species of snail or more species of trematodes were reported.

In terms of the species diversity of snails, we compared the species diversity of snails from the Eastern Coast and the Central Coast. We found that the Eastern Coast was more species diverse. Of the total thirty-two species found, six species of snails dispersed in the East Coast, the Central Coast, and the South of the Gulf of Thailand. These were *Assiminea brevicula*, *Littorinopsis intermedia*, *Dostia violacea*, *Cerithidea cingulata*, *Sermyla riqueti* and *Tarebia granifera*. They were common species generally found in the Gulf of Thailand (Sritongtae et al., 2015). More species of the Potamididae and Ellobiidae snails were found than others. Particularly, the family Potamididae were the most variation of snails found

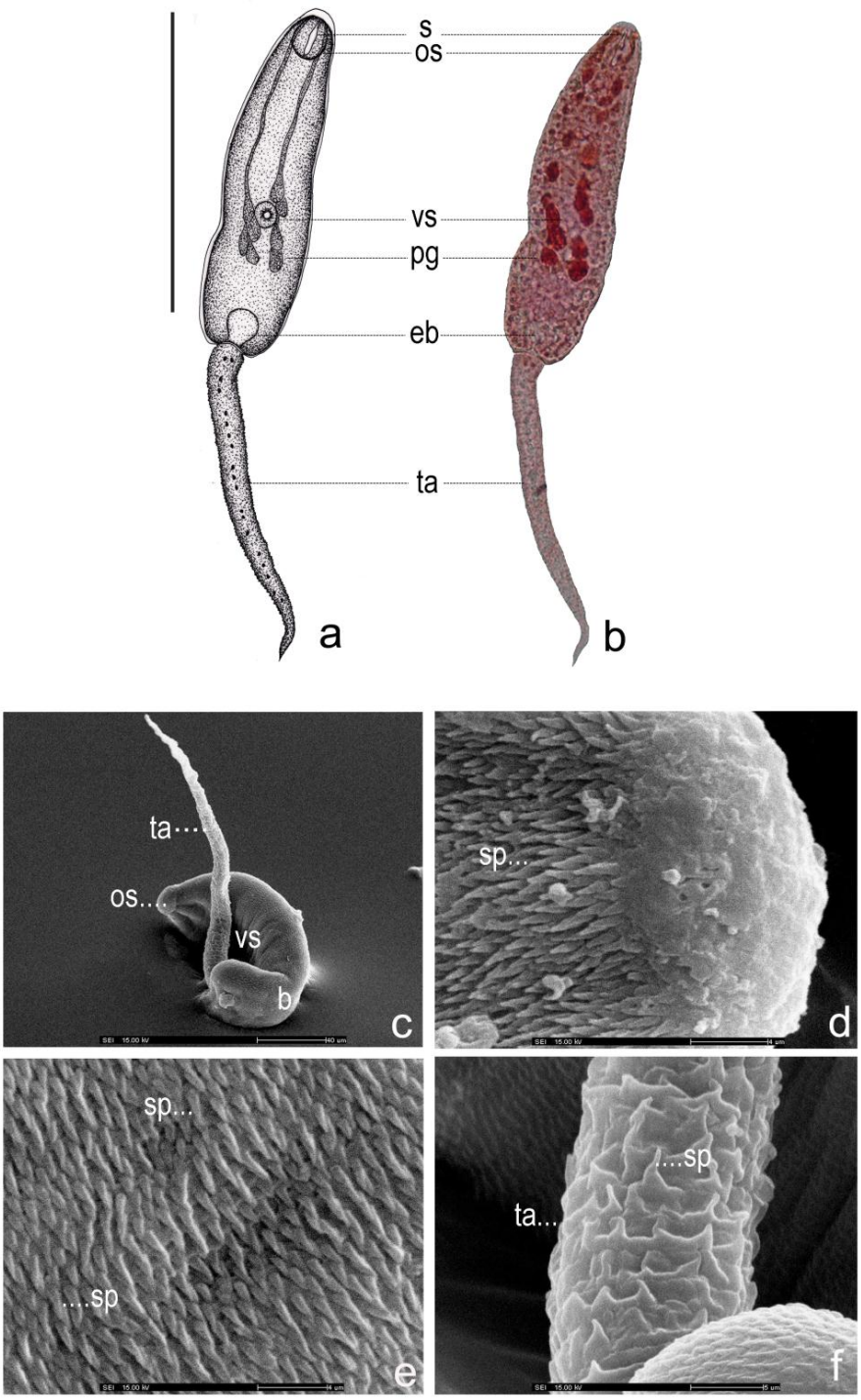

Figure 7. *Ascorhytis charadriformis* cercaria: (a). drawing of cercaria stucture, (b). cercaria stained with 0.5% neutral red, (c-f). SEM micrograph of cercaria. (s = stylet, os = oral sucker, vs = ventral sucker, pg = penetration gland, eb = excretory bladder, b = body, ta = tail, sp = spine) (scale bar = 100 µm).

infected with trematodes, as previously reported (Sri-aroon et al., 2010; Sritongtae et al., 2015). *C. cingulata, C. djadjariensis* and *C. quadrata* were known to be a host of human, bird and amphibian digenea. But this study showed that *C. alata* could be the intermediate host of bird trematode as well. Moreover, *Littorinopsis intermedia* was not reported with trematode infection in the past. Only *L. scabra* was mentioned. But only in this study *L.*

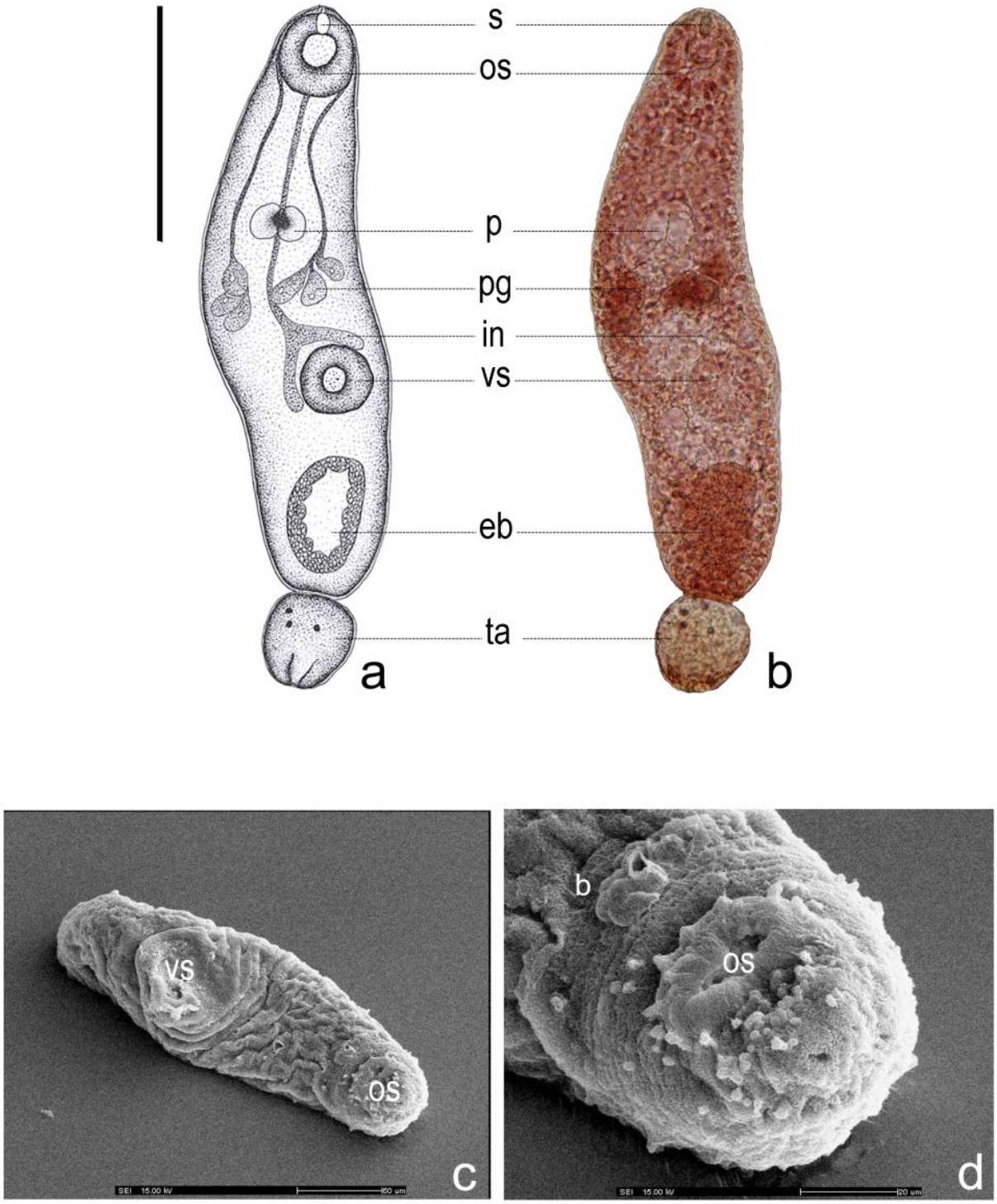

Figure 8. *Coitocaecum anaspidis* cercaria: (a). drawing of cercaria stucture, (b). cercaria stained with 0.5% neutral red, c-d. SEM micrograph of cercaria. (s= stylet, os = oral sucker, p = pharynx, vs = ventral sucker, pg = penetration gland, in = intestine, eb = excretory bladder, ta = tail, b = body) (scale bar = 100 µm).

intermedia was found infected with *Ascorhytis charadriformis* (amphibian trematode), while *L. scabra* was not. Thiarid snails, *Sermyla riqueti* were infected with *Apharyngostiger pipentis* (fish trematode) and *Haplorchis taichui* (human minute intestinal fluke), causing a major public health problem in Thailand and the other countries

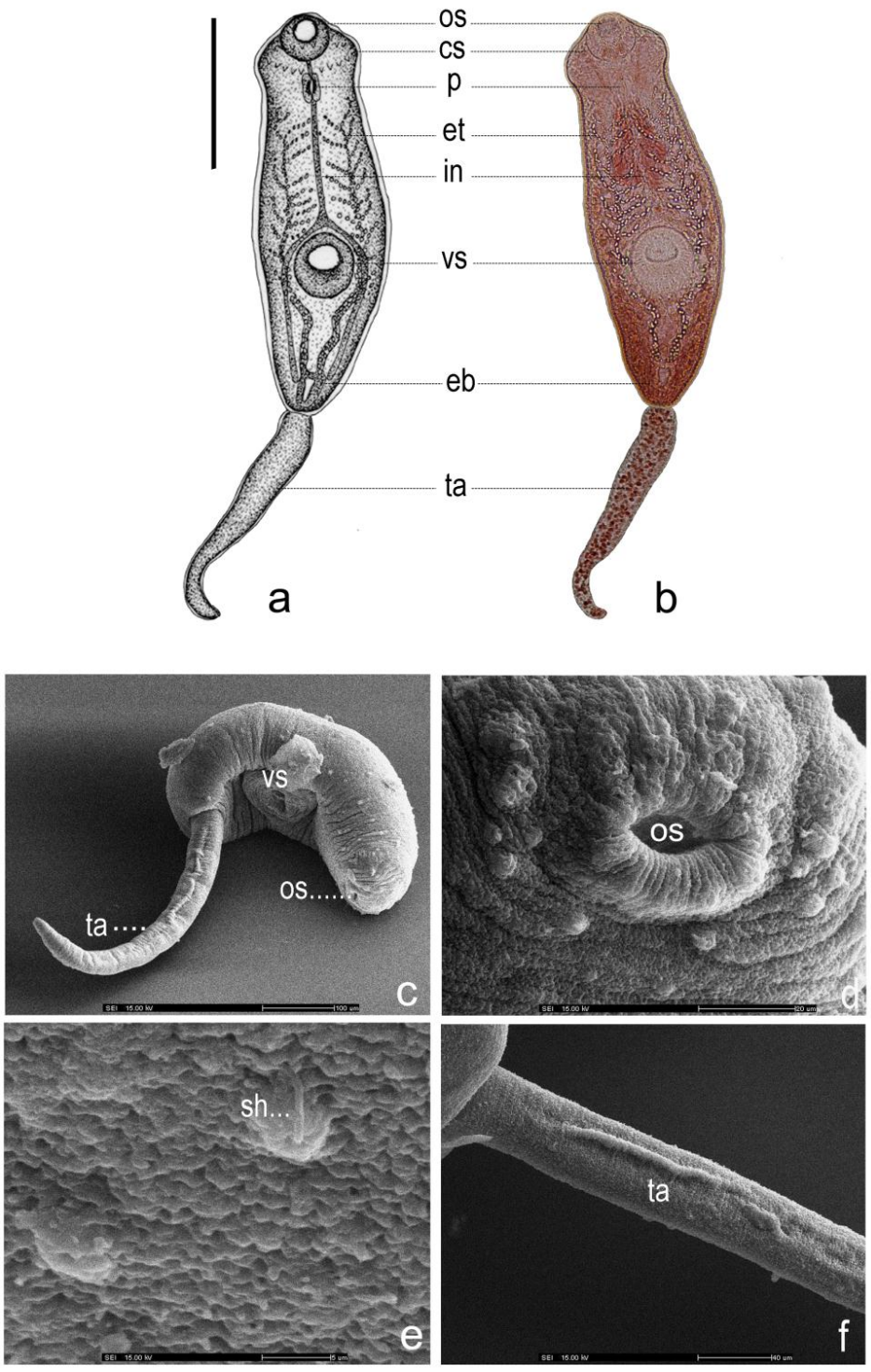

Figure 9. *Himasthla interrupta* cercaria: (a). drawing of cercaria stucture, (b). cercaria stained with 0.5% neutral red, c-f. SEM micrograph of cercaria. (os = oral sucker, cs = collar spines, p = pharynx, in = intestine, vs = ventral sucker, eb = excretory bladder, et = excretory tube, ta = tail, sh = sensory hair) (scale bar = 100 µm).

in South East Asia (Chai et al., 2005; Krailas et al, 2011). *S. riqueti* was reported with the highest infection rate in a previous study of cercarial infections of brackish water snails on the east coast of southern Thailand (Sritongtae

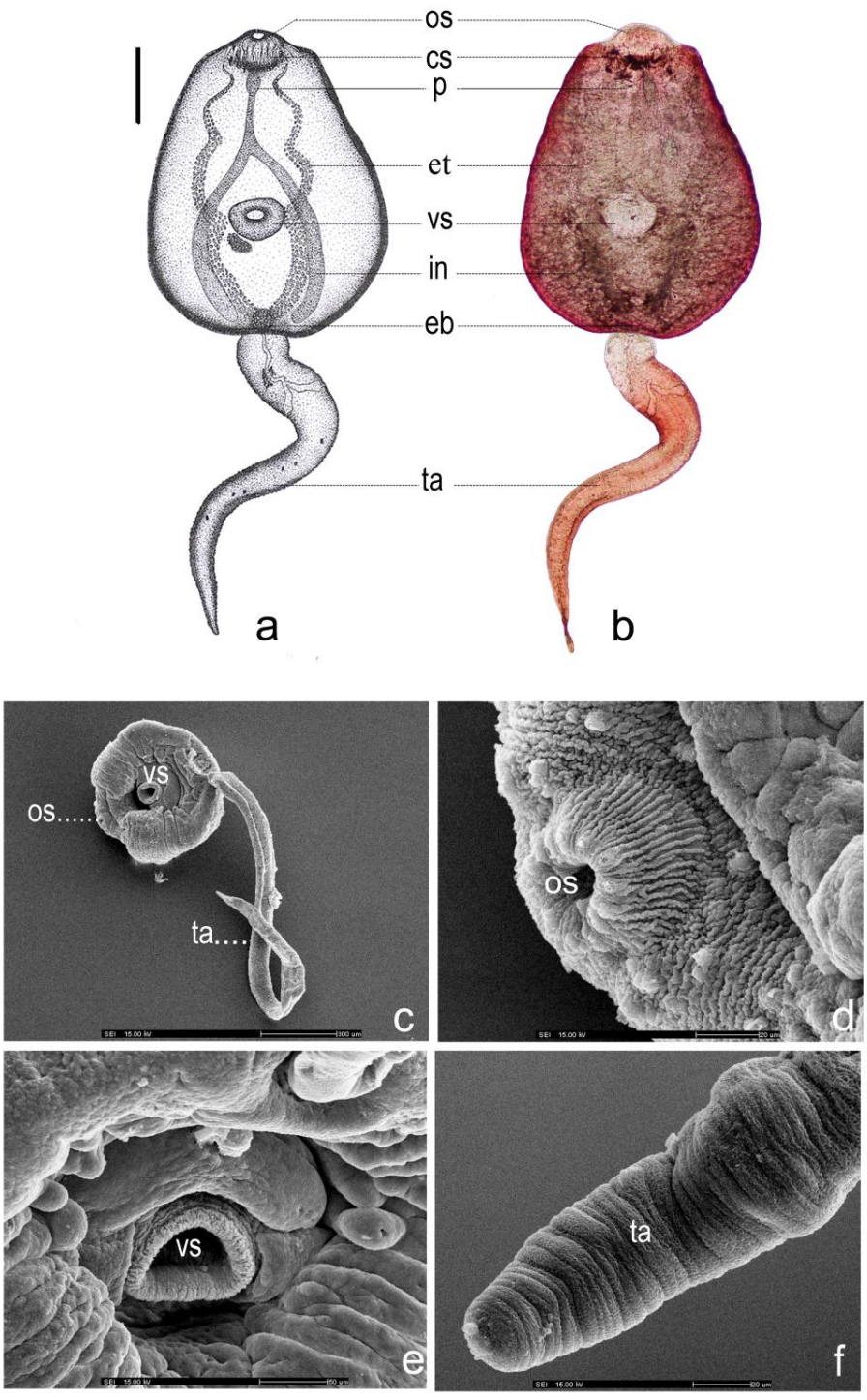

Figure 10. *Hypoderaeum conoideam* cercaria: a. drawing of cercaria stucture, b. cercaria stained with 0.5% neutral redia, c-f. SEM micrograph of cercaria. (os = oral sucker, cs = collar spines, p = pharynx, in = intestine, vs = ventral sucker, eb = excretory bladder, et = excretory tube, ta = tail) (scale bar = 100 µm).

et al., 2015). In the present study, *C. quadrata* had the highest infection rate with *Hypoderaeum conoideam* (human echinostome fluke).It should also be noted here that identification into groups might not be sufficient. This study showed that identification at the species level was very useful to determine the exact species of trematodes

and their intermediate hosts.

Conflict of Interest

The authors have not declared any conflict of interest.

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