Title

Annotated key to the trematode species infecting Batillaria attramentaria (Prosobranchia : Batillariidae) as first intermediate host

Journal
Parasitology International, 56

ISSN
1383-5769

Author
Hechinger, Ryan F.

Publication Date
2007

DOI
doi:10.1016/j.parint.2007.06.004

Peer reviewed

Annotated key to the trematode species infecting *Batillaria attramentaria* (Prosobranchia: Batillariidae) as first intermediate host

Ryan F. Hechinger
Marine Science Institute and the
Department of Ecology, Evolution, and Marine Biology
University of California
Santa Barbara, CA 93106-6150 USA
Email: hechinger@lifesci.ucsb.edu
805-893-8083
fax: 805-893-8062

note: this is a version of the published article, which can be found at doi:10.1016/j.parint.2007.06.004 or directly requested from the author.

Abstract (250 words max)

In eastern Asia and western North America at least nine morphologically distinguishable species of digenean trematode infect the mud snail, *Batillaria attramentaria* (Sowerby 1855) (=*B. cumingi* (Crosse 1862)), as first intermediate host. Further, molecular and morphological evidence indicates that several of these trematode species comprise complexes of cryptic species. I present an identification key to these nine trematode morphospecies (including four newly reported species). Additionally, I provide an annotated list, which includes further diagnostic information on the larval stages (cercariae, parthenitae, and metacercariae), information on second intermediate host use, links to the previous relevant reports of trematodes infecting *B. attramentaria* and notes on other aspects of the species' biology.

Keywords

Batillaria attramentaria; *Batillaria cumingi;* Digenea; Trematoda

Introduction

In eastern Asia, at least nine morphologically recognized species of digenean trematode infect the mud snail, *Batillaria attramentaria* [see 1, 2-4, 5 and this report]. Additionally, one of these trematode species is common in *B. attramentaria* populations introduced to the west coast of North America [6, 7]. There exists no comprehensive listing of the trematodes of *B. attramentaria*, nor a key to their identification. This absence is an impediment to research on this ensemble (sensu [8]) of larval trematodes.

The snail, *B. attramentaria* (Sowerby 1855) (=*B. cumingi* (Crosse 1862)) ranges widely along the east coast of Asia [9]. Additionally, this snail has been introduced to the west coast of North America [10]. The snails are common in the intertidal on soft and hard bottoms of estuaries and protected outer coast sites [e.g., see 11].

I recently (11 June – 30 June 2003) took part in an ecological study of *B. attramentaria* and its trematode parasites (Torchin et al., unpublished work). During this survey, I examined trematodes from 17 populations of *B. attramentaria* from Osaka, Wakayama, Chiba, and Miyagi prefectures, Honshu, Japan. I recognized, based on morphology, eight species of digenean trematodes infecting *B. attramentaria* as their first intermediate host. To my knowledge, two of these species have been described from this snail in both Japan [1, 2, 4, 5] and eastern Russia [3] and two have been previously reported only from eastern Russia [3]. A ninth trematode species has been described infecting *B. attramentaria* in eastern Russia [3], but this species has never been reported from Japan. Thus, I encountered undescribed larval stages of four species of trematodes that infect *B. attramentaria*. However, they are relatively easily placed in appropriate taxonomic families [following 12, 13], sometimes tentatively to genus, and provisionally recognized as "morphospecies."

Here, I provide a key to, and an annotated listing of, the species of digenean trematodes known to infect *B. attramentaria*. I include species that have been clearly described from *B. attramentaria* (from both Japan and Russia) and the four newly reported species that I encountered during the aforementioned ecological survey. Trematodes are frequently highly specific for their first intermediate host snails [14-18]. In the absence of data demonstrating otherwise, I believe it is prudent to assume trematodes are different species if they infect different snail species, particularly sympatric snails that belong to different genera. Further support for hesitating to assume conspecific identity of trematodes in different snail species is the continual discovery of cryptic trematode species existing even within the *same* host species [19, 20], including some that use *B. attramentaria* [21]. Consequently, I do not include in the key two species previously reported infecting *B. attramentaria*. This is because these species were described from a different genus of snail and we are lacking descriptions of specimens from *B. attramentaria*. However, in the annotations for related species, I alert workers to the possible existence of these additional species and detail morphological characters that might distinguish them from the species in the key. This key should be considered provisional, as the existence of cryptic species has been demonstrated by molecular genetic work and is suggested by morphological evidence. Additionally, although I provide descriptive details and working names for the newly reported species, I am not attaching formal species names or designating type specimens—further work is necessary for complete descriptions. I hope this key facilitates ecological work and highlights needed taxonomic research on this ensemble of larval trematodes.

Materials and Methods

The key primarily uses characters of the cercariae and should be used in conjunction with the figures and species annotations. The line drawings primarily complement the key, and I only included details that appeared to be consistently and readily discernable in live specimens. The annotations have more descriptive detail, as well as information on parthenitae (sporocysts or rediae) and metacercariae. I additionally provide notes on the types of second intermediate host potentially used by the various trematode species. All of these trematodes probably use birds as final hosts (based upon known life cycles of taxonomically related trematodes). I also provide potential links to previous records of trematodes in *B. attramentaria*, as well as notes of additional biological interest.

Except as otherwise indicated, all drawings are based on my observations of live specimens from dissected snails. For each species I encountered, I obtained measurements using an ocular micrometer on haphazardly-picked cercariae and parthenitae, which originated from a single dissected host, were heat-killed, fixed in 10% formalin, stained with Semichon's acetocarmine, and mounted wet in glycerin. If cercarial bodies or tails fixed dorso-ventrally flexed (noted below when this was consistent for a species), I took length measurements in several straight sections along the lateral view. For bilaterally paired features (e.g., eye spots, lateral fin lengths) I alternatingly measured either the left or the right structures for each new individual. For cercarial counts in parthenitae, I attempted to include all embryos larger than 5 µm. Unless otherwise noted, all measurements are in µm ± 1 s.d. Also, I use the term "flame bulb" instead of "flame cell," believing it to more clearly reflect the structure of a protonephridium. To facilitate access, vouchers have been deposited at the U.S. National Parasite Collection.

Results and Discussion

Key to the digenean trematodes known to use Batillaria attramentaria *as first intermediate host*

1 a. Cercariae with eye spots……………………………………………………………. 2

b. Cercariae without eye spots………………………………………………………… 3

2 a. Tail of cercaria with two lateral fins (proximally) and one dorsal-ventral fin (distally)……………………….…………...*Cercaria batillariae* Shimura and Ito 1980

b. Tail of cercaria forked………………………………….........Schistosomatid sp. I

3 a. Tail of cercaria forked……………………….....…….….….Cyathocotylid sp. I

b. Tail of cercaria not forked…………………………………………………...... 4

4 a. Tail of cercaria with parenchymous cells and an invaginated posterior tip… ….……………………………………………..…….…….....(philophthalmids) 5

b. Tail of cercaria without such characters……………………………………... 6

5 a. Mature cercaria translucent (immature stages more opaque, with well-defined cyst glands), with hundreds of obvious thin ducts (from cyst glands) in tegument of ventral surface (potentially misinterpreted as spines); metacercariae may form

flask-shaped cysts in dissection dish……………………………………………………... ………………………………………………………...……...Philophthalmid sp. I

b. Mature and immature cercaria opaque, with many dark, poorly-defined cyst glands throughout body; cercaria without obvious tegumental ducts or spines; metacercariae can form circular cysts in dissection dish…………………………… ……………………………………………………………..Philophthalmid sp. II

6 a. Cercaria with distinctive pinnately-branched excretory system; with no oral stylet; develop in rediae………………………………………….….… (*Acanthoparyphium*) 7

b. Cercaria without a pinnately-branched excretory system; with oral stylet; develop in sporocysts…………………………………………..…………………….. 8

7 a. Cercaria without pronounced cephalic collar; cercarial body longer than ~290 µm… ………..…………………………………..………...… *Acanthoparyphium* sp. I

b. Cercaria with pronounced cephalic collar; cercarial body shorter than 200 µm…….. …………..….*Acanthoparyphium macracanthum* Rybakov and Lukomskaya 1988

8 a. Cercaria with prominent oral stylet (~21 µm long); body translucent, with highly visible penetration ducts and glands; V-shaped excretory bladder extending less than one fifth into the body; ventral sucker not present…….…………………………… …………………………..……….*Cercaria hosouminae* Shimura and Ito 1980

b. Cercaria with small indistinct oral stylet (~9 µm long); most of body filled with opaque cyst glands; penetration glands and ducts not clearly visible; cercaria with

distinctive Y-shaped excretory bladder extending about half way up the body; ventral sucker present…….……………..…………………………*Renicola* sp. I

Species annotations

***Acanthoparyphium* sp. I (Echinostomatidae)—**Fig. 1, Fig. 2

Cercariae develop in active rediae in gonadal region of snail visceral mass. Cercariae positively phototactic, swim by cupping body at ventral sucker, lash tail and wiggle side to side, pivoting along long axis.

This species has not previously been reported (but see below). I examined specimens from Chiba Prefecture (Obitsu River) and Miyagi Prefecture (Torinoumi), the description being based on an infection from the latter locality (voucher deposited, USNPC No. 099682.00).

Diagnosis: Cercaria non-oculate, pharyngeate, with oral and ventral suckers. Cercaria body oblong to oval in dorsal view, dorsal-ventrally flattened, length 289-350 (318 ± 16, n = 13), max width 130-161 (144 ± 8, n = 12) at or slightly anterior to ventral sucker. Collar not very pronounced, with 23 spines of similar size in single row, interrupted ventrally. Length of terminal collar spine 6.2-8.8 (8.1 ± 0.9, n = 10), width at base 1.5-2.5 (2.2 ± 0.4, n = 10). Tegument otherwise without spines, but with papillae discernable laterally. Tail simple, circular in cross-section, attached subterminally, length 267-363 (322 ± 32, n = 12), width 29-41 (36 ± 4, n = 12) just posterior to base. Cystogenous glands obscure much of internal structure. Oral sucker ± circular, length 41-51 (47 ± 3, n = 13), width 43-46 (44 ± 1, n = 13), with undetermined number of glands, with apparently two pairs of ducts (one pair/side) opening anteriorly. An additional two pairs of ducts (one pair/side) observed from just posterior to pharynx extending anteriorly and opening at sides of oral sucker, their origin (presumably at penetration glands) not observed and obscured by cystogenous glands. Ventral sucker ± circular, about 2/3 body length from anterior end, length 55-73 (65 ± 5, n = 13), width 56-67 (61 ± 3, n = 13), may appear wider than long in live specimens. Prepharynx evident, shorter than pharynx. Pharynx length 24-32 (27 ± 2, n = 12), width

12-18 (15 ± 2, n = 12). Esophagus slender, bifurcating just anterior to ventral sucker. Digestive ceca curve around ventral sucker, continue posteriorly to middle of excretory bladder, almost to posterior of body. Excretory bladder thin-walled, more-or-less square, with short anterior-medial connection receiving main collecting ducts. Main collecting ducts extend anteriorly from connection with bladder, laterally around ventral sucker, anteriorly to oral sucker, with several medially and laterally projecting diverticula between ventral sucker and pharynx. Rest of body excretory ducts and complete flame bulb formula not observed. Caudal excretory duct extends into tail, bifurcating and opening laterally. Mass of primordia (probably genital) evident upon acetocarmine staining, along midline, just posterior to ventral sucker, length 13-18 (15 ± 2, n = 7), width 15-20 (17 ± 2, n = 7). Redia with orange-pigment clusters in tegument. Redia length 501-1065 (799 ± 165, n = 14), width 150-258 (214 ± 29, n = 14). Redia with collar, relative position of collar apex along body 0.10-0.30 (.17 ± .05, n = 12). Redia with posterior pair of appendages, relative position along body 0.66-0.91 (0.77 ± 0.07, n = 11). Redia pharynx length 63-105 (78 ± 14, n = 13), width 47-79 (60 ± 9, n = 12). Rediae with 8-27 (18 ± 6, n = 14) cercariae in various stages of development.

Species of *Acanthoparyphium* use mollusks [e.g., 22, 23, 24] and polychaetes (Hechinger and Smith, unpublished data) as second intermediate hosts. In an unpublished study, Armand Kuris and I found metacercariae of an *Acanthoparyphium* species in the feet of clams (*Venerupis* (=*Ruditapes*) *philippinarum*) only at the locality where we found first intermediate host infections of *Acanthoparyphium* sp. I in *B. attramentaria* (Hechinger and Kuris, unpublished data).

Harada and Suguri [5] reported an *Acanthoparyphium*-like cercaria from *B. attramentaria* in Japan, and identified that cercaria as *Cercaria yamagutii* Ito 1957. However, Ito [1] described and reported *C. yamagutii* from the three snail species, *Cerithidea rhizophorarum, Cerithidea largillierti*, and *Tympanotonus microptera*. As mentioned in the introduction, it seems preferable to assume that trematodes using different first intermediate host species, particularly different genera, are different species unless evidence shows otherwise. Barring further study, I consider the *C. yamagutii* reported from *B. attramentaria* by Harada and Suguri [5] to be the same as *Acanthoparyphium* sp. I, although several of the characters (e.g., size of the body, suckers and collar spines) that I measured for *Acanthoparyphium* sp. I are smaller than those Harada and Suguri reported for *C. yamagutii*.

***Acanthoparyphium macracanthum* Rybakov 1987 (Echinostomatidae)—**Fig. 1

Cercariae develop in rediae, which reside in the gonad and digestive gland of snail [3].

Rybakov [3] described this species infecting *B. attramentaria* from Peter the Great Bay, eastern Russia. I never encountered this species, and all descriptive information is from Rybakov [3] (M.C. and E. Rigby kindly provided the translation and I have slightly modified their language).

Diagnosis (range (average), no n was provided): cercaria body length 152-169 (162), width 61-68 (63), tail length 139-155 (147), tail width 17-20 (19), oral sucker length 21-24 (22), oral sucker width 24-27 (26), pharynx length 11-14 (13), pharynx width 5-8 (7), ventral sucker length 29-34 (32), width 27-32 (30). Cercariae belong to the morphological group Echinostomata. Spiny collar has 23 spines. Tegument on ventral side from spiny collar to ventral sucker has 15 to 18 rows of spines, and dorsal side from spiny collar to level of ventral sucker has wide horizontal creases. Rest of body without armor. Digestive system well developed, cecal branches reach posteriorly to anterior end of urinary bladder. Only two pairs of penetration glands were found. Additionally, in dorsal part of oral sucker, three unique glandular cells were observed. Cystogenous glands are located very close together under tegument over entire body of cercaria. Excretory system is as usual for echinostomatids, flame bulb formula, perhaps, is 2[(3+3+3+3+3) + (3+3+3+3+3)] = 60.

Rybakov and Lukomskaya [25] described the life cycle of this trematode in Peter the Great Bay, finding that the trematode used three bivalve species (including *Ruditapes philippinarum*) as second intermediate hosts, and that they were able to use chickens as experimental final hosts. They also described *A. macracanthum* as a new species in that publication, but it seems that Rybakov [3] was a valid description and thus has priority as author of the species name.

***Cercaria batillariae* Shimura and Ito 1980 (Heterophyidae)—**Fig. 1

Cercariae develop in active rediae, residing primarily in the gonadal region of the snail.

Shimura and Ito [2] described this species from *B. attramentaria* from Kanagawa and Chiba Prefectures, Japan. I examined infections from nine localities on the Pacific coast of Honshu, from Wakayama Prefecture to Miyagi Prefecture. The following description is based on an infection from Waka River (voucher deposited, USNPC No. 099683.00).

Diagnosis: Cercaria bioculate, pleurolophocercous (lateral and dorso-ventral fins), pharyngeate, with oral and ventral suckers. Cercaria body oval to oblong in dorsal view, dorsal-ventrally flattened, body length 142-149 (146 ± 2, n = 12), max width 56-62 (60 ± 2, n = 11) at midbody. Tegument with small spines, arranged in transverse rows, particularly evident over oral sucker. Tail attached in pronounced subterminal socket, tail length 255-295 (282 ± 13, n = 8), width 20-24 (22 ± 2, n = 8) just posterior to base. Tail with two lateral fins and one continuous dorso-ventral fin, extending around the tail tip. Lateral fin length 110-120 (113 ± 3, n = 8), width 10-17 (13 ± 3, n = 4). Dorsal portion of dorso-ventral fin length 159-196 (184 ± 12, n = 8), dorsal origin 6-34 (19 ± 11, n = 6) anterior to insertion of lateral fins, ventral insertion of dorso-ventral fin ~7-11 (9 ± 2, n = 3) posterior to insertion of laterals. Oral sucker very well developed, rounded, length 25-27 (26 ± 1, n = 12), width 20-27 (24 ± 2, n = 11). At least two transverse rows of oral spines present, but number of rows or spines not accurately observed. Ventral sucker not developed. Prepharynx length 22-28 (25 ± 2, n = 11), not very prominent. Pharynx length 6-9 (8 ± 1, n = 11), width 9-11 (10 ± 1, n = 11). Eye spots black, cuboidal to slightly rectangular, length 6.1-7.5 (6.7 ± 0.6, n = 11), width 5.5-7.4 (6.5 ± 0.6, n = 11). Esophagus and ceca not observed. Excretory bladder thick-walled, v-shaped to cordate, appears to empty directly into the subterminal notch, just below tail. Flame bulb formula not observed. Seven pairs of penetration glands (each ~13 diameter), clustered just past midbody, partly surrounding anterior edge of primordial mass (which appears clear, before staining). This mass of primordia (probably genital) evident upon acetocarmine staining, along midline, just posterior to cluster of penetration glands, anterior to excretory bladder, diamond-shaped, length 15-20 (18 ± 1, n = 10) along longest side. Redia sausage-shaped, with no appendages, length 756-877 (809 ± 35, n = 10), width 108-149 (133 ± 12, n = 10). Redia pharynx length 21-27 (24 ± 2, n = 10), width 22-25 (24 ± 1, n = 10). Gut of redia not discernable due to cercariae. Redia packed with ~20-31 (26 ± 3, n = 10) cercariae in various stages of development (cercariae are generally more developed in anterior of redia).

Shimura and Ito [2], and I (unpublished data) have shown experimentally that *C. batillariae* infects fish as second intermediate hosts. I also obtained adults by infecting laboratory mice with metacercariae, but have not succeeded in retrieving adults with the female components of the reproductive system matured (unpublished work).

C. batillariae has been the subject of several ecological and population genetics studies. *C. batillariae* is the single morphospecies of trematode that has invaded the West Coast of North America with its snail host, *B. attramentaria* [6]. Miura et al. [21] recently used molecular genetic methods to demonstrate that *C. batillariae* likely is a complex of eight cryptic species in Japan. Subsequently, Miura et al. [7] determined that at least three of the eight cryptic species are present in the introduced range on the west coast of North America, with only two being common. Additionally, Miura et al. [26] showed that *C. batillariae* strongly affects its host snail's growth and distribution in the intertidal zone.

The above measurements and my description largely fit within the range of those provided in Shimura and Ito's description [2]. But there are exceptions. First, the measurements that Shimura and Ito provided for the prepharynx length (53-66, ave. 60) are more than twice as large as my measurements. However, I believe Shimura and Ito's prepharynx measurements may have been reported in error, for they do not correspond with their figure of the cercarial body (from which I estimate the prepharynx to be ~33 µm—very close to my measurements). The second major difference between our descriptions pertains to the dorso-ventral tail fin. Shimura and Ito report that the dorsal fin originates posterior to the posterior end of the laterals and the ventral inserts anterior to the posterior end of the laterals. The dorso-ventral fins on the specimens that I have examined carefully (dozens from Japan and the United States), have the opposite arrangement (as described above). This difference is either due to a mistake in the original description, or it is real and due to either intraspecific variation or variation across cryptic species. The other exceptions are that my measurements are smaller in tail and lateral fin length and the eyespots of Shimura and Ito's specimens appear to be more rectangular, versus tending to be cuboidal as were the specimens upon which I based the above description (although I have examined many other specimens with more rectangular eyes). These differences may be an artifact of my dealing only with cercariae from dissected snails, as such cercariae are potentially not fully developed. Alternatively, the differences may be due to intraspecific variation, or these differences may reflect variation across the cryptic species of *C. batillariae* uncovered by Miura et al [21].

Rybakov [3] reported and provided a description of *C. batillariae* Shimura and Ito 1980 from *B. attramentaria* in Peter the Great Bay, eastern Russia. The cercariae he described are about 30 µm longer in body length, and over 100 µm longer in tail length. It is possible that the difference for body length is because Rybakov made measurements on live specimens. Additionally, the dorsal tail fin originates very far anterior (near the base of the tail). Either these differences are due to intraspecific variation, or, perhaps more likely, Rybakov described a different cryptic species of *C. batillariae*.

Harada and Suguri [5] reported a "Cercaria sp. 5" from *B. attramentaria*. Their measurements appear to slightly differ from those I provide here, but this is hard to assess since they provided averages with no indication of the dispersion of the data. The oral spine arrangement they reported for Cercaria sp. 5 (6-10, 6-10, 4) differs from that reported by Shimura and Ito [2] in the original description of *C. batillariae* (7-10, 8-9, 5-6). Cercaria sp. 5 is probably a member of the cryptic species complex of *C. batillariae* of Miura et. al [21].

***Cercaria hosoumininae* Shimura and Ito 1980 (Microphallidae)—**Fig. 1

Cercariae develop in inactive sporocysts residing primarily in gonadal region of snail host.

Shimura and Ito [2]described this species infecting *B. attramentaria* from Kanagawa and Chiba Prefectures. I examined infections from Wakayama Prefecture (Uchino River, Yukashi Lagoon, and Hashiguiiwa) and Miyagi Prefecture (Mangoku River and Nagazura River). The following is based on an infection from Uchino River (voucher deposited, USNPC No. 099684.00).

Diagnosis: Cercariae non-oculate, apharyngeate, monostomate (no ventral sucker), with simple tail and oral stylet. Cercaria body oval to oblong to elongate in dorsal view, dorsal-ventrally flattened, usually fixed dorsal-ventrally flexed. Body length (taken in lateral view, to not underestimate because body flexed) 132-155 (140 ± 7, n = 13), max width 37-49 (43 ± 4, n = 13) at or slightly anterior to midbody. Tail attached slightly subterminally, circular in cross-section, with fine tegumental crenulations, length 84-112 (104 ± 8, n = 9), width 10-12 (11 ± 1, n = 9). Oral sucker not very strongly developed, length 32-34 (33 ± 1, n = 13), width difficult to discern and not measured. Oral sucker with well developed stylet. Stylet sclerotized more than half its anterior length, narrow, slightly higher (dorso-ventrally) than wide (left to right), base non-sclerotized and rounded, sometimes forming a bulb. Stylet length 17-23 (20 ± 2, n = 10), width 2.6-5.5 (4.5 ± 0.8, n = 12). Three pairs of pronounced penetration glands sequentially arranged anterior to posterior, restricted roughly to third quarter of body; anterior two pairs filled with coarse granules, posterior pair with fine granules. Penetration gland ducts pronounced, extend anteriorly to anterior of oral sucker where they narrow and turn sharply medially. Penetration gland ducts from anterior pair positioned singly and medial to posterior two pairs, which are positioned more laterally, and are bundled together. Excretory bladder v-shaped. Rest of excretory system not observed. Mass of primordia (putatively ventral sucker primordium) evident upon acetocarmine staining, along midline, filling region posterior to penetration glands and anterior to excretory bladder. Sporocyst simple, thin-walled (~2-6 µm thick), round to oval, length 198-316 (251 ± 34, n = 10), width 159-221 (191 ± 20, n = 10), densely packed with estimated number of cercariae 13-27 (20 ± 4, n = 10) mostly in late stage of development, with few germ balls.

This species probably uses crustaceans as second intermediate hosts, as do most microphallids [see 17].

The description I provide agrees with that provided by Shimura and Ito [2] in their description of *C. hosouminінae* except that the measurements of the cercariae I examined (including those from at least two other sites, data not given here) are apparently smaller in body, tail and stylet length. This may be an artifact of my dealing only with cercariae from dissected snails, as such cercariae are potentially not fully developed, or represent intraspecific variation. Alternatively, the infections I encountered may belong to a different species of microphallid. Indeed, molecular evidence indicates that there are cryptic species of *C. hosoumininae* (O.Miura, pers. comm.). Further study is called for to resolve this issue.

Rybakov [3] reported and described *C. hosoumininae* Shimura and Ito 1980 in *B. attramentaria* from Peter the Great Bay, eastern Russia. His measurements agree with Shimura and Ito's description.

Harada and Suguri [5] reported many infections of the microphallid, *Cercaria lanceolata* Holliman 1961, infecting *Cerithidea rhizophorarum* snails and two infections in *B. attramentaria*. Holliman described *C. lanceolata* from *Cerithidea scalariformis* in the Gulf of Mexico [13]. Harada and Suguri assigned the microphallids they encountered to *C. lanceolata* on the basis of the flame bulb pattern of 2[{2+2) + (2+2)]=16 (while the formula for *C. hosoumininae* is 2[(1+1)+(1+1)]=8) and the shared genus of snail host (although that does not explain applying the name to the infections in *B. attramentaria*). In the Gulf of Mexico, *C. lanceolata* was subsequently determined to a species of *Probolocoryphe* [27]. Given the arguments discussed above regarding host specificity, it seems very possible that a species of *Probolocoryphe* infects *C. rhizophorarum*. But, it does not seem likely that the same species would also infect *B. attramentaria*. However, since Harada and Suguri reported two infections of *C. lanceolata* in *B. attramentaria*, workers studying trematodes in *B. attramentaria* should watch for a second microphallid species with 16 flame bulbs. I would refer to such a species as “Microphallid sp. II,” rather than *Probolocoryphe lanceolata*, until further work on adults or late-stage metacercariae is undertaken. If such a second microphallid species infects *B. attramentaria*, I suspect it will differ from *C. hosouminінae* not only in flame bulb formula, but also in other morphological characters, including stylet morphology.

Cyathocotylid sp. I—Fig. 1, Fig. 3

Cercariae develop in very active sporocysts in the gonadal region of the snail visceral mass.

This species has not previously been reported. I examined infections from specimens from Wakayama Prefecture (Yukashi Lagoon and Hashiguiiwa) and Miyagi Prefecture (Nagazura River). The description below is based on an infection from Nagazura River (voucher deposited, USNPC No. 099685.00).

Diagnosis: Cercariae non-oculate, pharyngeate, monostomate (no ventral sucker), longifurcate. Cercaria body oval to pyriform in dorsal view, dorsal-ventrally flattened, length 158-204 (176 ± 19, n = 8), max width 96-123 (110 ± 11, n = 10) at or slightly posterior to midbody. Tail attached to dorsal side of posterior body, width constricted just before attachment. Tail stem laterally flattened, length (to posterior-most part of tail) 412-489 (447 ± 30, n = 11), width at attachment to furcae 40-51 (46 ± 3, n = 11). Tail furcae laterally flattened, length 247-355 (303 ± 29, n = 11), width at base 27-32 (29 ± 1, n = 11), each provided with continuous dorsal-ventral fin-fold. Anterior organ/oral sucker length 31-47 (40 ± 6, n = 8), width 27-39 (32 ± 5, n = 9), with undetermined number of penetration glands. Excretory system collecting ducts follow general pattern typical of cyathocotylid cercariae. Single, blind ducts extend anterio-laterally from juncture of lateral and cross-commissural ducts. Fifteen pairs of protonephridea (flame bulbs, or cells) observed in cercarial body, but capillaries, and thus, flame bulb formula, not observed. Flame bulbs not observed in tail stem. Mass of primordia (probably genital primordia, perhaps with acetabular primordia at anterior) evident upon acetocarmine staining, along midline, in region between the two medial collecting ducts just posterior to point where ducts fuse, length 18-25 (22 ± 2, n = 9), width 12-27 (20 ± 5, n = 9). Sporocysts with transverse annulations, long and thin (can be over 4 mm long), highly intertwined and difficult to separate intact.

This species most likely infects fish as second intermediate hosts, as do most cyathocotylids [17, 28, 29].

Philophthalmid species I ("clear philophthalmid")—Fig. 1, Fig. 4

Cercariae develop in active rediae, primarily in gonadal region of snail visceral mass. Cercariae often swim to top, attach to surface tension, and get an air bubble in their ventral sucker. Cercariae infrequently encyst in dish and form flask-shaped metacercariae (Fig. 1).

This species has not previously been reported. I examined specimens from Chiba Prefecture (Obitsu River) and Miyagi Prefecture (Torinoumi and Ogatsu Bay). The description below is based on an infection from Ogatsu Bay (voucher deposited, USNPC No. 099686.00). I provide measurements, although I had very little fixed material for study.

Diagnosis: Cercaria non-oculate, pharyngeate, with oral and ventral suckers. Cercaria body oval to oblong to slightly spatulate in dorsal view, dorsal-ventrally flattened, length 575-653 (602 ± 44, n = 3), max width 144-155 (149 ± 6, n = 3) slightly anterior to ventral sucker. Tegument apparently without spines, but with prominent thin ducts from cystogenous glands (potentially misinterpreted as spines), particularly over lateral and posterior half of ventrum. Tail simple (except for terminal gland), circular in cross-section, with parenchyma cells, attached slightly subterminally, length 404-417 (411 ± 9, n = 2), width 40-48 (44 ± 4, n = 3) just posterior to base. Tail terminal gland length 116-162 (133 ± 25, n = 3). Cystogenous glands obscure much of internal structure. Oral sucker round, length 59-74 (65 ± 8, n = 3), width 52-56 (55 ± 2, n = 3). Ventral sucker round to slightly oval (may occur more oval in fresh specimens), at beginning of second half of body, length 71-86 (78 ± 8, n = 3), width 66-79 (71 ± 7, n = 3). Prepharynx evident, length 15-32 (25 ± 9, n = 3). Pharynx length 32-41 (36 ± 5, n = 3), width 22-25 (23 ± 2, n = 3). Esophagus bifurcates about half way distance from anterior of cercaria body to ventral sucker, length 39-42 (41 ± 2, n = 2). Digestive ceca diverge laterally, continue posteriorly almost to end of body to around anterior of excretory bladder. Excretory bladder thin-walled, more-or-less square, with short anterior-medial connection receiving main collecting ducts. Main collecting ducts extend anteriorly from connection with bladder, laterally around ventral sucker, or cross over ventral sucker's sides, continue anteriorly to recurve just before oral sucker around level of pharynx. Complete flame bulb formula not observed. Mass of primordia evident upon acetocarmine staining, along midline, about half way between ventral sucker and excretory bladder, sometimes extends anterior as thin strip toward cecal bifurcation. Redia translucent, length 652-1250 (1042 ± 140, n = 16), width 123-218 (184 ± 22, n = 16), with one or two appendages, positioned at ~9/10 redial length, not always prominent. Redia pharynx length 42-55 (49 ± 4, n = 15), pharynx width 44-57 (48 ± 4, n = 15). Redia gut length 245-358 (286 ± 36, n = 9). Cercariae-producing rediae with number of cercariae 3-17 (13 ± 4, n = 16), in all stages of development.

This trematode may be a species of *Philophthalmus* as this genus is known to form flask-shaped cysts [e.g., 30, 31, 32] and infect other species of *Batillaria* [e.g., 31].

Philophthalmid species II ("opaque philophthalmid")—Fig. 1

Cercariae develop in large active rediae, which primarily reside in the gonadal region of the snail visceral mass. Cercariae sometimes encyst in dish and form round to oval metacercariae (Fig. 2).

This species has not previously been reported in Japan, but probably has been reported in eastern Russia (see below). I examined infections from Miyagi Prefecture (Ogatsu Bay and Nagazura River), and the following is based on an infection from the former locality (voucher deposited, USNPC No. 099687.00).

Diagnosis: Cercaria non-oculate, pharyngeate, with oral and ventral suckers. Cercaria body oval to oblong to slightly spatulate in dorsal view, dorsal-ventrally flattened, length 352-502 (431 ± 51, n = 8), max width 167-218 (200 ± 16, n = 8) slightly anterior to ventral sucker. Tegument without spines. Tail simple (except for terminal gland), circular in cross-section, with parenchyma cells, attached slightly subterminally, fixed in varying states of contraction, length 145-412 (233 ± 90, n = 8), width 39-74 (56 ± 14, n = 5) just posterior to base. Tail terminal gland 51-86 (65 ± 10, n = 8). Oral sucker round, length 60-69 (66 ± 4, n = 8), width 59-74 (69 ± 6, n = 8). Ventral sucker round, at beginning of posterior half of body, length 71-98 (87 ± 8, n = 8), width 79-105 (96 ± 9, n = 8). Cystogenous glands ~10 diameter, obscure almost all internal structure, few organs being evident even in highly flattened unfixed specimens. Prepharynx present, apparently shorter than pharynx. Esophagus bifurcates about half way between anterior of cercaria body to ventral sucker. Digestive ceca diverge laterally, continue posteriorly at least to ventral sucker, but posterior limits not observed. Excretory bladder thin, saccate, with short medio-anterior duct receiving main collecting ducts. Main collecting ducts extend anteriorly from connection with bladder, path not observed in middle of body, recurve at level of pharynx. Caudal tubule bifurcates a short distance into tail. Redia translucent, length 1002-1225 (1117 ± 92, n = 4), width 277-350 (304 ± 34, n = 4), with one or two appendages, positioned ~9/10 redial length, not always prominent. Redia pharynx length 54-68 (58 ± 7, n = 4), width 49-55 (51 ± 3, n = 4). Redia gut usually obscured by embryos, length 245-466 (356 ± 156, n = 2). Cercariae-producing rediae with number of cercariae 10-14 (12 ± 2, n = 4), in all stages of development.

In a molecular genetic study, Miura et al. [21] showed that Philophthalmid species II is a complex of three cryptic species.

Rybakov [3] reported and described a philophthalmid species (that he tentatively termed "*Cercaria Parorchis* sp.") from *B. attramentaria* from Peter the Great Bay, eastern Russia. I provisionally consider *Cercaria Parorchis* sp. to be the same as Philophthalmid species II, noting that the former is larger in most dimensions. These differences either reflect differences in laboratory technique (Rybakov made measurements on live specimens, and he noted that he had very little material to study), indicate intraspecific variation, or occur because the specimen reported by Rybakov represents one of the cryptic species uncovered by Miura et al. [21]. I do not use Rybakov's name because I believe there is no evidence to assign this philophthalmid to *Parorchis*, versus other philophthalmid genera (e.g., *Cloacitrema*). Indeed, there is evidence against this trematode being a species of *Parorchis*, since it is lacking the strong body spination and the spined collar that characterizes *Parorchis* spp. [12]. Thus, I use a temporary morphospecies name that reflects only the trematode's probable familial affiliation.

Harada [4] and Harada and Suguri [5] reported one infection of a philophthalmid, *Cercaria shikokuensis*, in *B. attramentaria*. However, Harada [4] described *C. shikokuensis* from the snail *Cerithidea rhizophorarum*. As discussed in the introduction, based on general principles and without strong evidence to the contrary, it seems questionable to assume *C. shikokuensis* (described from a species of *Cerithidea*) would also infect *B. attramentaria.* Nevertheless, workers should certainly watch for a *C. shikokuensis*-like cercaria infecting *B. attramentaria*. The most obvious distinguishing trait would be the presence of prominent body spines in *C.*

shikokuensis (and, apparently, *C. shikokuensis* has a longer body, smaller ventral sucker, and a longer esophagus than does Philophthalmid sp. II). If such a cercaria is found, I would refer to it as “Philophthalmid sp. III,” rather than *C. shikokuensis*, barring further study. Perhaps Harada [4] and Harada and Suguri [5] recognized one of the three cryptic species of Philophthalmid Cercaria II reported by Miura et al. [21].

***Renicola* sp. I** (= *Cercaria Renicola* sp. of Rybakov [3])—Fig. 1

Cercariae develop in inactive sporocysts residing in the gonad and sometimes digestive gland regions of the snail.

This species has previously been reported in Japan [33, see below] and eastern Russia [3, see below]. I examined infections from Miyagi Prefecture (Matsushima and Mangoku River), and the below is based on an infection from Matsushima (voucher deposited, USNPC No. 099688.00).

Diagnosis: Cercariae non-oculate, pharyngeate, distomate (oral and ventral suckers), with simple tail and oral stylet. Cercaria body oval to elongate in dorsal view, dorsal-ventrally flattened. Body length 148-233 (174 ± 27, n = 9), width 61-74 (68 ± 4, n = 9) at midbody. Tail attached slightly terminally, circular in cross-section, length 103-123 (114 ± 8, n = 9), width 16-22 (18 ± 2, n = 9). Oral sucker well developed, length 27-35 (30 ± 3, n = 8), width 25-31 (28 ± 2, n = 9). Oral sucker with bullet-shaped stylet anteriorly (and dorsal to mouth), very hard to see in non-compressed fixed specimens, stylet length 8.5-8.6 (8.6 ± 0.1, n = 3), width 2.6-2.9 (2.7 ± 0.2, n = 3). Ventral sucker oval, positioned at mid-body, length 25-33 (28 ± 3, n = 9), width 27-33 (29 ± 2, n = 9). Tegument covered with minute spines, the full distribution of which was not observed. Cystogenous glands fill most of body and obscure internal structures. Pharynx round, just posterior to oral sucker, barely discernable, not measured. Esophagus difficult to see, slender, branching about half way to ventral sucker into two slender cecae, the posterior extent not observed. Excretory bladder prominent and Y-shaped, with lateral arms not extending to anterior of ventral sucker. Rest of excretory system not observed. Sporocyst simple, thick-walled (at least 5-7 thick), round to oval, length 198-472 (313 ± 73, n = 15), width 112-206 (157 ± 24, n = 15), densely packed with estimated number of cercariae 9-16 (11 ± 2, n = 11) in all stages of development.

Renicolid cercariae with stylets are known to encyst in mollusks [34] and polychaete worms (Hechinger and Smith, unpublished data).

Rybakov [3] reported and described this species, from eastern Russia, with the temporary name of "*Cercaria Renicola* sp." His measurements slightly differed from those I present, but these differences could easily reflect differences in laboratory technique (he made measurements on live specimens) or intraspecific variation. I do not use Rybakov's temporary name to maintain consistent naming throughout this manuscript.

Miura and Chiba [33] provide information (using the name "renicolid cercaria I" from a early version of this manuscript) on the distribution of *Renicola* sp. I along an elevational gradient and among host sizes; as well as on the frequency of double infections with *C. batillariae*.

Schistosomatid sp. I—Fig. 1, Fig. 5

Cercariae develop in inactive whitish sporocysts spread throughout the gonad and digestive gland of the snail.

This species has apparently not previously been reported from *B. attramentaria*. I encountered only one infection at one locality in Miyagi Prefecture (Mangoku River) (voucher deposited, USNPC No. 099688.00).

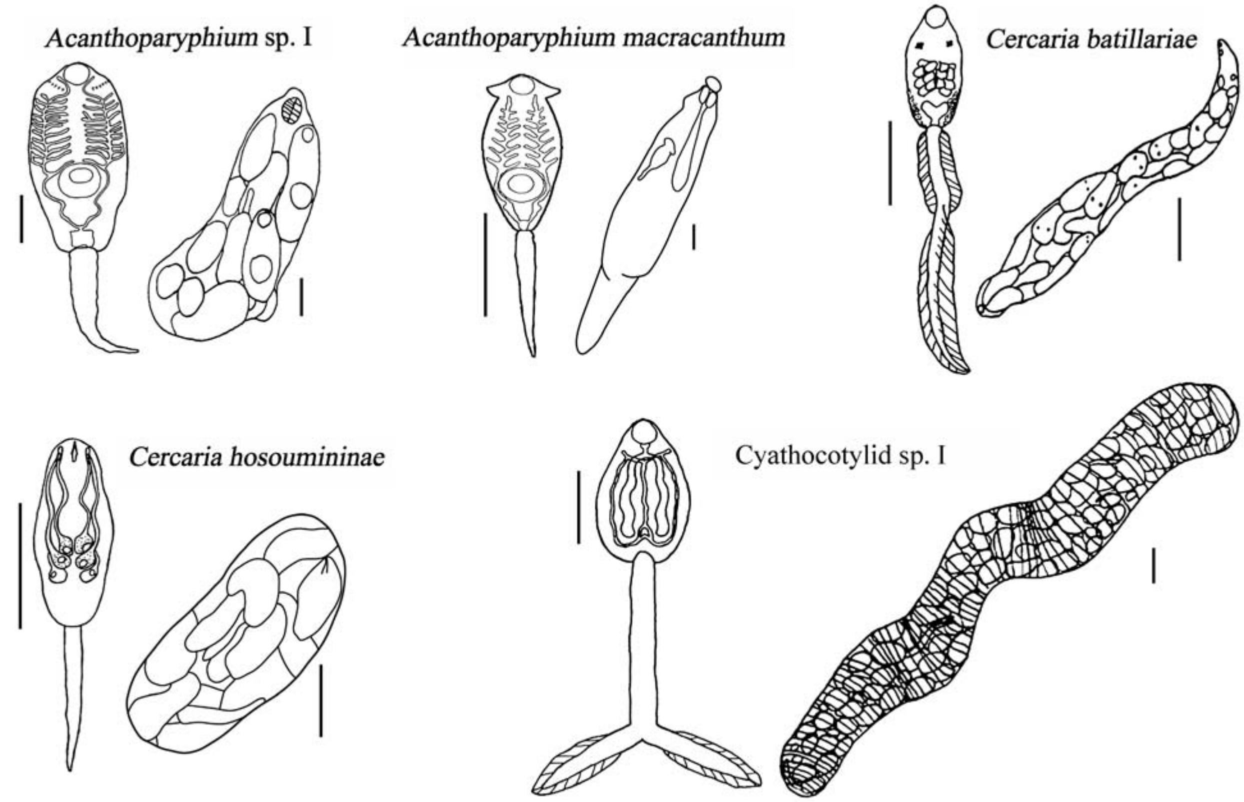
Diagnosis: Cercariae oculate, apharyngeate, distomate (with oral and ventral sucker), brevifurcate. Unless otherwise indicated, n = 12). Cercaria body ~pyriform in dorsal view, dorsal-ventrally flattened, length 197-252 (215 ± 18), width 74-91 (82 ± 5) at or slightly posterior to mid-body. Tail attached terminally. Tail stem length (from insertion to anterior base of furcae) 211-238 (226 ± 8), width at bulge just posterior to insertion 32-42 (38 ± 2), width at furcal attachment 7-21 (15 ± 5). Tail furca with no fins, length 98-130 (118 ± 8), width at base 10-21 (14 ± 3). Anterior organ length 69-93 (80 ± 9), width 54-61 (57 ± 2, n = 11), with several types of glands. Mouth slightly subterminal. Ventral sucker located just posterior to midbody, length 20-27 (24 ± 2, n = 8), width 24-34 (29 ± 3, n = 8). Eyespots black, circular, diameter 10-12 (11.5 ± 0.9). Daughter sporocyst sausage-shaped, with concavity (~30 diameter) observed on one end, potentially marking outside of birth pore. Daughter sporocyst length 412-1311 (765 ± 255, n = 14), width 127-247 (179 ± 36, n = 14), number of cercariae 4-20 (14 ± 4, n = 13), in all stages of development.

These cercariae most likely directly infect bird final hosts as do many other schistosomatids in marine snails [17, 35].

Acknowledgements

For hosting and logistics in Japan, I am grateful to S. Chiba, T. Koga, A. Kogima, A. Wada, and O. Miura. I thank my compadres, A. Kuris, M. Torchin, and E. Dunham, with whom I worked on the ecological survey. A. Kuris, K. Lafferty, J. Shaw, and two anonymous reviewers provided helpful comments on the ms. For translation of Russian, I am very grateful to M. and E. Rigby. I thank O. Miura for translation of Japanese, comments, and sharing information concerning the biology of these trematodes. I also thank J. McLean at the Los Angeles County Natural History Museum for helping sort out the nomenclature of *B. attramentaria*. This manuscript benefited from financial support of a grant from the National Science Foundation through the NIH/NSF Ecology of Infectious Disease Program (DEB-0224565) to A. Kuris and K. Lafferty.

Figure 1. General appearance of the cercariae and parthenitae of digenean trematode species known to infect *Batillaria attramentaria* as first intermediate host. Metacercariae are included for the philophthalmids. The drawing of *Acanthoparyphium macracanthum* is modified from [25]. All scale bars are 100µm.


Acanthoparyphium sp. I
Acanthoparyphium macracanthum
Cercaria batillariae
Cercaria hosoumininae
Cyathocotylid sp. I

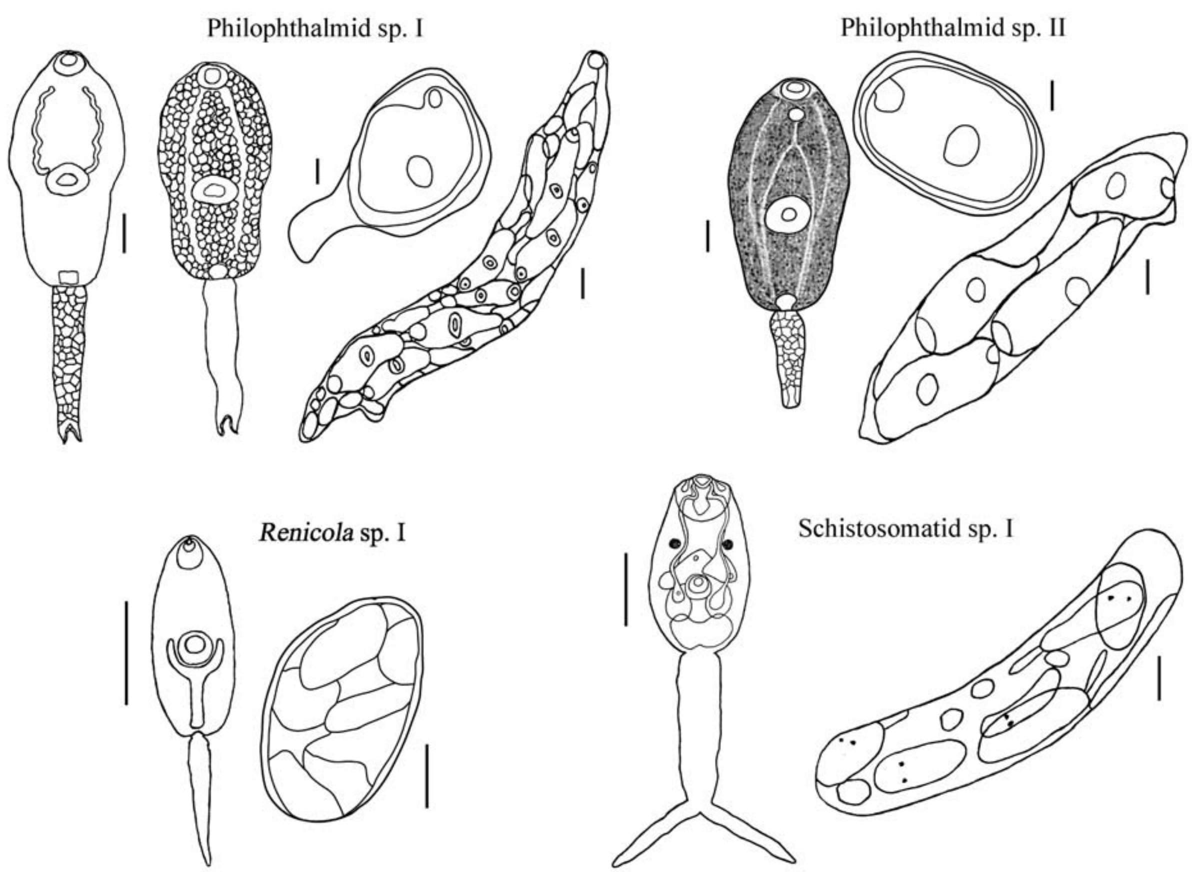

Philophthalmid sp. I
Philophthalmid sp. II
Renicola sp. I
Schistosomatid sp. I

Figure 2. Photograph of cercaria of *Acanthoparyphium* sp. I. Specimen was alive and under some cover-slip pressure. Scale bar = 100µm.

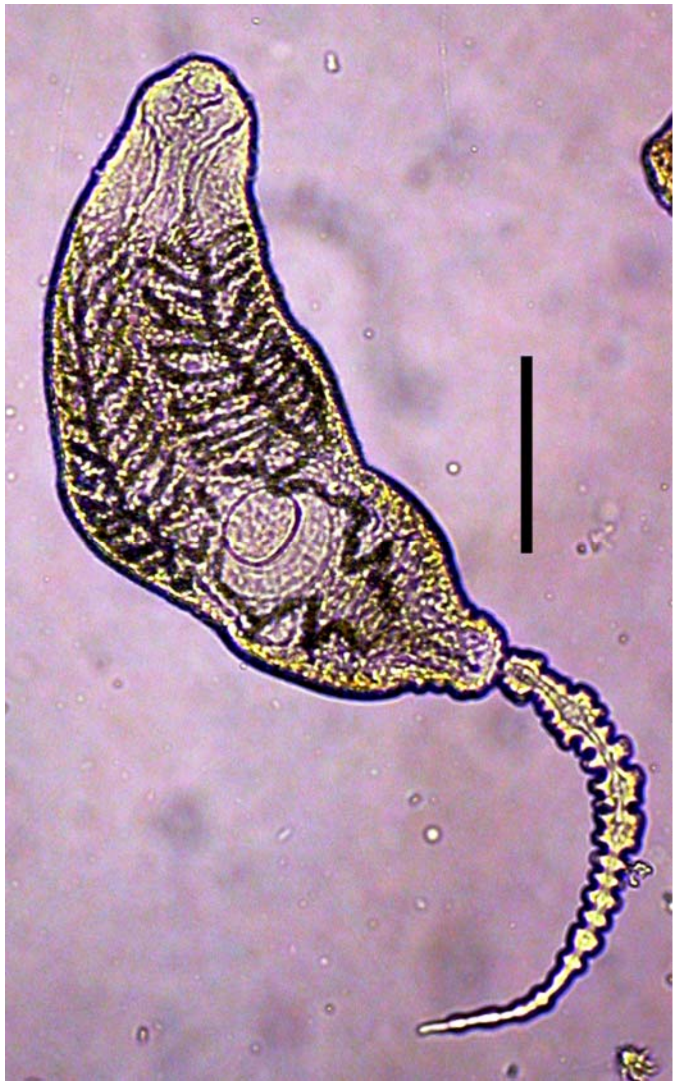


Figure 3. Photograph of cercaria of Cyathocotylid sp. I. Specimen was formalin-fixed and acetocarmine stained. Scale bar = 100µm.

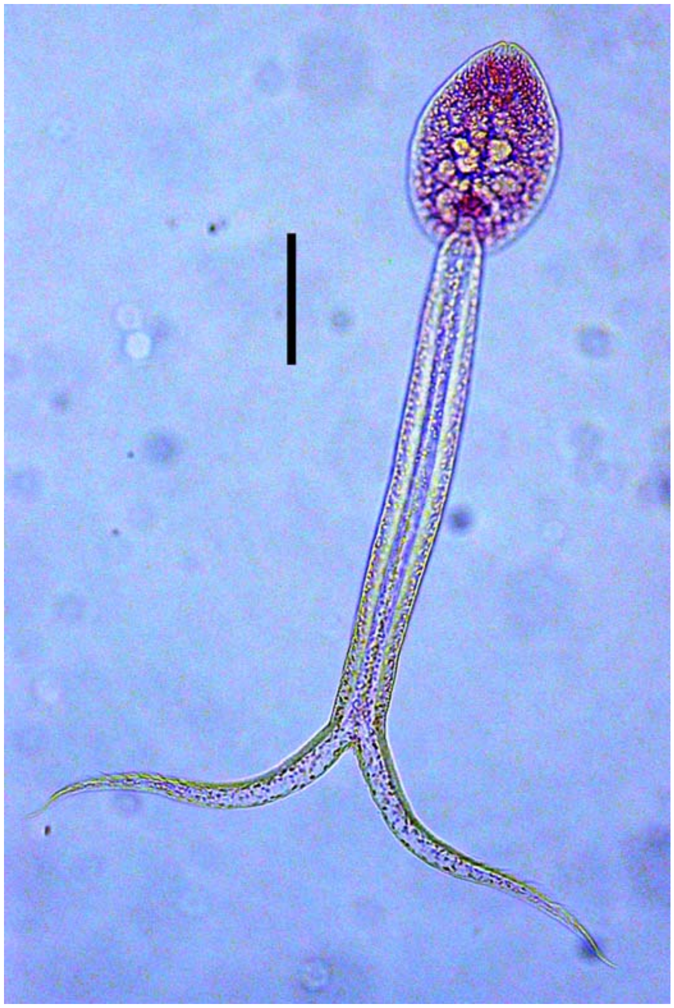


Figure 4. Photograph of cercaria of Philophthalmid sp. I. Specimen was alive and under heavy cover-slip pressure. Scale bar = 100µm.

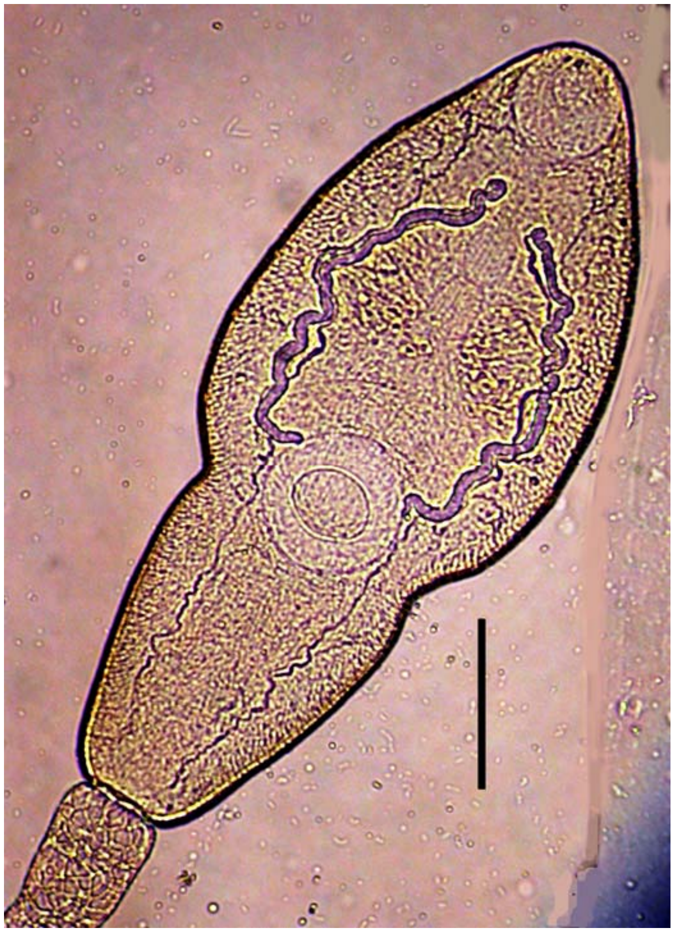
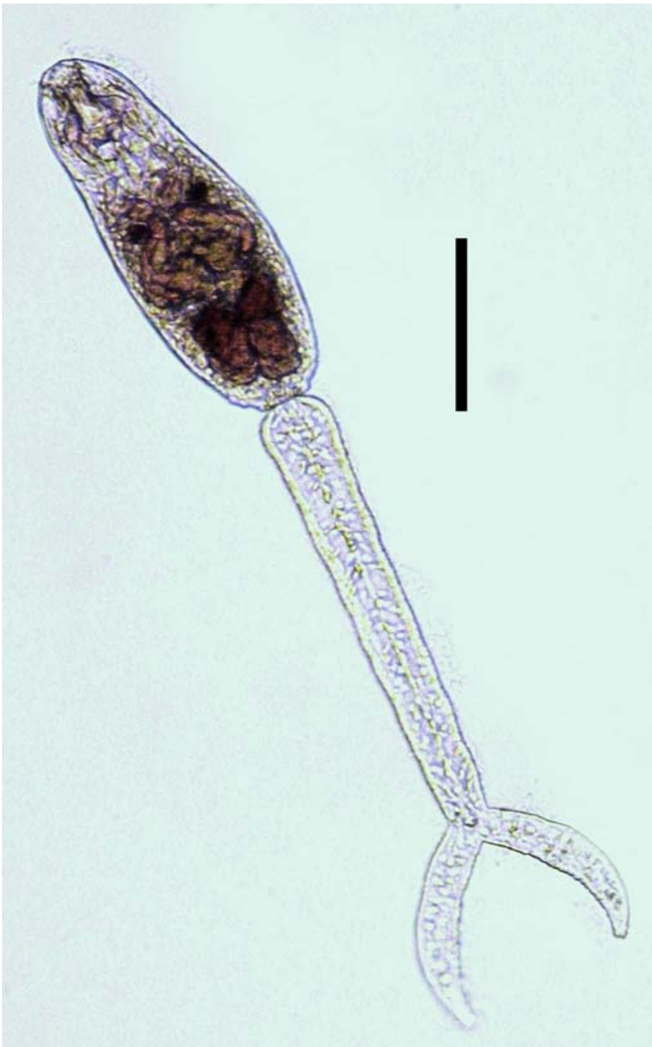


Figure 5. Photograph of cercaria of Schistosomatid sp. I. Specimen was formalin-fixed and acetocarmine stained. Scale bar = 100μm.



References

1. Ito, J. Studies on the brackish water cercariae in Japan III. Three new echinostome cercariae in Tokyo Bay, with a list of Japanese echinostome cercariae (Trematoda). Japanese Journal of Medical Science and Biology 1957; 10: 439-453.

2. Shimura, S. & Ito, J. Two new species of marine cercariae from the Japanese intertidal gastropod, *Batillaria cumingii* (Crosse). Japanese Journal of Parasitology 1980; 29: 369-376.

3. Rybakov, A. V. Helminth fauna of *Batillaria cumingii* (Gastropoda: Potamididae) in the Peter the Great Bay from the Sea of Japan. in ed. Mamaev, Y. L. Gel'-minty i vyzyvaemye imi zabolevaniya: sbornik nauchnykh trudov [Helminths and diseases caused by them]. Vladivostok: Dal'nevostochnii Nauchnii Tsentr [Far East Science Center], 1987: pp. 77-87.

4. Harada, M. A new cercaria, *Cercaria shikokuensis* n.s. (Trematoda), from littoral gastropods in Kagawa Prefecture, Shikoku, Japan. Japanese Journal of Parasitology 1989; 38: 135-138.

5. Harada, M. & Suguri, S. Surveys on cercariae in brackish water snails in Kagawa Prefecture, Shikoku, Japan. Japanese Journal of Parasitology 1989; 38: 388-391.

6. Torchin, M. E., Byers, J. E. & Huspeni, T. C. Differential parasitism of native and introduced snails: replacement of a parasite fauna. Biological Invasions 2005; 7: 885-894.

7. Miura, O., Torchin, M. E., Kuris, A. M., Hechinger, R. F. & Chiba, S. Introduced cryptic species of parasite exhibit different invasion pathways. Proceedings of the National Academy of Sciences 2006; 103: 19818-19823.

8. Fauth, J. E., Bernardo, J., Camara, M., Resetarits, W. J., VanBuskirk, J. & McCollum, S. A. Simplifying the jargon of community ecology: A conceptual approach. American Naturalist 1996; 147: 282-286.

9. Hasegawa, K. Batillariidae. in ed. Okutani, T. Marine mollusks in Japan. Tokyo: Tokai University Press, 2000: pp. 130-133.

10. Byers, J. E. The distribution of an introduced mollusc and its role in the long-term demise of a native confamilial species. Biological Invasions 1999; 1: 339-352.

11. Adachi, N. & Wada, K. Distribution of *Batillaria cumingii* (Gastropoda, Potamididae) in Tanabe Bay, middle Japan. Nankiseibutu 1997; 39: 33-38.

12. Cable, R. M. Marine cercariae of Puerto Rico. in ed. Miner, R. W. Scientific survey of Porto Rico and the Virgin Islands. New York: New York Academy of Sciences, Vol. XVI-Part 4, 1956: pp. 491-577.

13. Holliman, R. B. Larval trematodes from the Apalachee Bay area, Florida, with a checklist of known marine cercariae arranged in a key to their superfamilies. Tulane Studies in Zoology 1961; 9: 2-74.

14. Dawes, B. The Trematoda, with special reference to British and other European forms, Cambridge University Press: Cambridge, 1946.

15. Ewers, W. H. Analysis of molluscan hosts of trematodes of birds and mammals and some speculations on host-specificity. Parasitology 1964; 54: 571-578.

16. Ginetsinskaya, T. A. Trematodes, their life cycles, biology and evolution (translated from Russian, 1988), Amerind Publishing Co: New Delhi, 1968.

17. Yamaguti, S. A synoptical review of life histories of digenetic trematodes of vertebrates with special reference to the morphology of their larval forms, Keigaku Publishing Company: Tokyo, 1975.

18. Gibson, D. I. & Bray, R. A. The evolutionary expansion and host-parasite relationships of the Digenea. International Journal for Parasitology 1994; 24: 1213-1226.

19. Donald, K. M., Kennedy, M., Poulin, R. & Spencer, H. G. Host specificity and molecular phylogeny of larval Digenea isolated from New Zealand and Australian topshells (Gastropoda : Trochidae). International Journal for Parasitology 2004; 34: 557-568.

20. Lucas, T., O'Brien, E. K., Cribb, T. & Degnan, B. M. Digenean trematodes infecting the tropical abalone *Haliotis asinina* have species-specific cercarial emergence patterns that follow daily or semilunar spawning cycles. Marine Biology 2005; 148: 285-292.

21. Miura, O., Kuris, A. M., Torchin, M. E., Hechinger, R. F., Dunham, E. J. & Chiba, S. Molecular-genetic analyses reveal cryptic species of trematodes in the intertidal gastropod, *Batillaria cumingi* (Crosse). International Journal for Parasitology 2005; 35: 793-801.

22. Bearup, A. J. Life history of *Acanthoparyphium spinulosum* Johnston, 1917 (Trematoda: Echinostomatidae). Australian Journal of Zoology 1960; 8: 217-225.

23. Kim, Y. G., Yu, J. E., Chung, E. Y. & Chung, P. R. *Acanthoparyphium tyosenense* (Digenea : Echinostomatidae): Experimental confirmation of the cercaria and its complete life history in Korea. Journal of Parasitology 2004; 90: 97-102.

24. Martorelli, S. R., Poulin, R. & Mouritsen, K. N. A new cercaria and metacercaria of *Acanthoparyphium* (Echinostomatidae) found in an intertidal snail *Zeacumantus subcarinatus* (Batillaridae) from New Zealand. Parasitology International 2006; 55: 163-167.

25. Rybakov, A. V. & Lukomskaya, O. G. On the life cycle of *Acanthoparyphium macracanthum* sp. n. (Trematoda, Echinostomatidae). Parazitologiya (St. Petersburg) 1988; 22: 224-229.

26. Miura, O., Kuris, A. M., Torchin, M. E., Hechinger, R. F. & Chiba, S. Parasites alter host phenotype and create a new ecological niche for snail hosts. Proceedings of the Royal Society of London - Series B: Biological Sciences 2006; 273: 1323-1328.

27. Heard, R. W., Jr. Microphallid trematode metacercariae in fiddler crabs of the genus *Uca* Leach, 1814 from the northern Gulf of Mexico. unpublished Ph.D. thesis, University of Southern Mississippi, 1976.

28. Combes, C., Albaret, J.-L., Arvy, L., Bartoli, P., Bayssade-Dufour, C., Deblock, S., Durette-Desset, M. C., Gabrion, C., Jourdane, J., Lambert, A., Leger, N., Maillard, C., Matricon, M., Nassi, H., Prevot, G., Richard, J. & Theron, A. Atlas mondial des cercaires. Mémoirs du Muséum National D'Histoire Naturelle, Nouvelle Série; Série A, Zoologie 1980; 115.

29. Schell, S. C. Handbook of trematodes of North America: North of Mexico, University Press of Idaho: Moscow, Idaho, 1985.

30. Thakur, A. S. & Cheng, T. C. Formation structure and histochemistry of metacercarial cyst of *Philophthalmus gralli* Mathis & Léger. Parasitology 1968; 58: 605-618.

31. Fried, B. & Grigo, K. L. Observations on the cercaria and metacercaria of *Philophthalmus hegeneri* (Trematoda). Transactions of the American Microscopical Society 1976; 95: 86-91.

32. Abdul-Salam, J., Sreelatha, B. S. & Ashkanani, H. The eye fluke *Philophthalmus hegeneri* (Digenea: Philophthalmidae) in Kuwait Bay. Kuwait Journal of Science and Engineering 2004; 31: 119.

33. Miura, O. & Chiba, S. Effects of trematode double infection on the shell size and distribution of snail hosts. Parasitology International 2007; 56: 19-22.

34. Stunkard, H. W. Studies on the trematode genus *Renicola*: observations on the life-history, specificity, and systematic position. Biological Bulletin 1964; 126: 467-489.

35. Blair, D., Davis, G. M. & Wu, B. Evolutionary relationships between trematodes and snails emphasizing schistosomes and paragonimids. Parasitology 2001; 123: S229-S243.