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

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3    **Abstract (250 words max)**

4    In eastern Asia and western North America at least nine morphologically distinguishable species

5    of digenean trematode infect the mud snail, *Batillaria attramentaria* (Sowerby 1855) (=*B.*

6    *cumingi* (Crosse 1862)), as first intermediate host. Further, molecular and morphological

7    evidence indicates that several of these trematode species comprise complexes of cryptic species.

8    I present an identification key to these nine trematode morphospecies (including four newly

9    reported species). Additionally, I provide an annotated list, which includes further diagnostic

10   information on the larval stages (cercariae, parthenitae, and metacercariae), information on

11   second intermediate host use, links to the previous relevant reports of trematodes infecting *B.*

12   *attramentaria* and notes on other aspects of the species' biology.

13

16    **Introduction**

17          In eastern Asia, at least nine morphologically recognized species of digenean trematode

18    infect the mud snail, *Batillaria attramentaria* [see 1, 2-4, 5 and this report]. Additionally, one of

19    these trematode species is common in *B. attramentaria* populations introduced to the west coast

20    of North America [6, 7]. There exists no comprehensive listing of the trematodes of *B.*

21    *attramentaria*, nor a key to their identification. This absence is an impediment to research on this

22    ensemble (sensu [8]) of larval trematodes.

23

24          The snail, *B. attramentaria* (Sowerby 1855) (=*B. cumingi* (Crosse 1862)) ranges widely

25    along the east coast of Asia [9]. Additionally, this snail has been introduced to the west coast of

26    North America [10]. The snails are common in the intertidal on soft and hard bottoms of

27    estuaries and protected outer coast sites [e.g., see 11].

28

29          I recently (11 June – 30 June 2003) took part in an ecological study of *B. attramentaria*

30    and its trematode parasites (Torchin et al., unpublished work). During this survey, I examined

31    trematodes from 17 populations of *B. attramentaria* from Osaka, Wakayama, Chiba, and Miyagi

32    prefectures, Honshu, Japan. I recognized, based on morphology, eight species of digenean

33    trematodes infecting *B. attramentaria* as their first intermediate host. To my knowledge, two of

34    these species have been described from this snail in both Japan [1, 2, 4, 5] and eastern Russia [3]

35    and two have been previously reported only from eastern Russia [3]. A ninth trematode species

36    has been described infecting *B. attramentaria* in eastern Russia [3], but this species has never

37    been reported from Japan. Thus, I encountered undescribed larval stages of four species of

38    trematodes that infect *B. attramentaria*. However, they are relatively easily placed in appropriate

39    taxonomic families [following 12, 13], sometimes tentatively to genus, and provisionally

40    recognized as "morphospecies."

41

42        Here, I provide a key to, and an annotated listing of, the species of digenean trematodes

43    known to infect *B. attramentaria*. I include species that have been clearly described from *B.*

44    *attramentaria* (from both Japan and Russia) and the four newly reported species that I

45    encountered during the aforementioned ecological survey. Trematodes are frequently highly

46    specific for their first intermediate host snails [14-18]. In the absence of data demonstrating

47    otherwise, I believe it is prudent to assume trematodes are different species if they infect

48    different snail species, particularly sympatric snails that belong to different genera. Further

49    support for hesitating to assume conspecific identity of trematodes in different snail species is the

50    continual discovery of cryptic trematode species existing even within the *same* host species [19,

51    20], including some that use *B. attramentaria* [21]. Consequently, I do not include in the key two

52    species previously reported infecting *B. attramentaria*. This is because these species were

53    described from a different genus of snail and we are lacking descriptions of specimens from *B.*

54    *attramentaria*. However, in the annotations for related species, I alert workers to the possible

55    existence of these additional species and detail morphological characters that might distinguish

56    them from the species in the key. This key should be considered provisional, as the existence of

57    cryptic species has been demonstrated by molecular genetic work and is suggested by

58    morphological evidence. Additionally, although I provide descriptive details and working names

59    for the newly reported species, I am not attaching formal species names or designating type

60    specimens—further work is necessary for complete descriptions. I hope this key facilitates

61    ecological work and highlights needed taxonomic research on this ensemble of larval trematodes.

62

63      **Materials and Methods**

64          The key primarily uses characters of the cercariae and should be used in conjunction with

65      the figures and species annotations. The line drawings primarily complement the key, and I only

66      included details that appeared to be consistently and readily discernable in live specimens. The

67      annotations have more descriptive detail, as well as information on parthenitae (sporocysts or

68      rediae) and metacercariae. I additionally provide notes on the types of second intermediate host

69      potentially used by the various trematode species. All of these trematodes probably use birds as

70      final hosts (based upon known life cycles of taxonomically related trematodes). I also provide

71      potential links to previous records of trematodes in *B. attramentaria*, as well as notes of

72      additional biological interest.

73

74          Except as otherwise indicated, all drawings are based on my observations of live

75      specimens from dissected snails. For each species I encountered, I obtained measurements using

76      an ocular micrometer on haphazardly-picked cercariae and parthenitae, which originated from a

77      single dissected host, were heat-killed, fixed in 10% formalin, stained with Semichon's

78      acetocarmine, and mounted wet in glycerin. If cercarial bodies or tails fixed dorso-ventrally

79      flexed (noted below when this was consistent for a species), I took length measurements in

80      several straight sections along the lateral view. For bilaterally paired features (e.g., eye spots,

81      lateral fin lengths) I alternatingly measured either the left or the right structures for each new

82      individual. For cercarial counts in parthenitae, I attempted to include all embryos larger than 5

83      μm. Unless otherwise noted, all measurements are in μm ± 1 s.d. Also, I use the term "flame

84      bulb" instead of "flame cell," believing it to more clearly reflect the structure of a

85  protonephridium. To facilitate access, vouchers have been deposited at the U.S. National Parasite

86  Collection.

87

88  **Results and Discussion**

89  *Key to the digenean trematodes known to use* Batillaria attramentaria *as first intermediate host*
90
91
92  **1 a.    Cercariae with eye spots…………………………………………………. 2**

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

94

95  **2 a.    Tail of cercaria with two lateral fins (proximally) and one dorsal-ventral fin**

96  **(distally)………………..………….....*Cercaria batillariae* Shimura and Ito 1980**

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

98

99  **3 a.    Tail of cercaria forked……………………….....………..………Cyathocotylid sp. I**

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

101

102  **4 a.    Tail of cercaria with parenchymous cells and an invaginated posterior tip…**

103  **….……………………………………..…….……....(philophthalmids) 5**

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

105

106  **5 a.    Mature cercaria translucent (immature stages more opaque, with well-defined cyst**

107  **glands), with hundreds of obvious thin ducts (from cyst glands) in tegument of**

108  **ventral surface (potentially misinterpreted as spines); metacercariae may form**

109         **flask-shaped cysts in dissection dish……………………………………………………...**

110         **…………………………………………………...……...Philophthalmid sp. I**

111     **b.    Mature and immature cercaria opaque, with many dark, poorly-defined cyst glands**

112         **throughout body; cercaria without obvious tegumental ducts or spines;**

113         **metacercariae can form circular cysts in dissection dish……………………………**

114         **…………………………………………………………..Philophthalmid sp. II**

115

116   **6 a.   Cercaria with distinctive pinnately-branched excretory system; with no oral stylet;**

117         **develop in rediae……………………………………….…… (*Acanthoparyphium*) 7**

118     **b.    Cercaria without a pinnately-branched excretory system; with oral stylet; develop**

119         **in sporocysts……………………………………………...…………………….. 8**

120

121   **7 a.   Cercaria without pronounced cephalic collar; cercarial body longer than ~290 μm…**

122         **…………….…………………………………...………...… *Acanthoparyphium* sp. I**

123     **b.    Cercaria with pronounced cephalic collar; cercarial body shorter than 200 μm……..**

124         **………….…*Acanthoparyphium macracanthum* Rybakov and Lukomskaya 1988**

125

126   **8 a.   Cercaria with prominent oral stylet (~21 μm long); body translucent, with highly**

127         **visible penetration ducts and glands; V-shaped excretory bladder extending less**

128         **than one fifth into the body; ventral sucker not present……………………………**

129         **………………………………...……….*Cercaria hosoumininae* Shimura and Ito 1980**

130     **b.    Cercaria with small indistinct oral stylet (~9 μm long); most of body filled with**

131         **opaque cyst glands; penetration glands and ducts not clearly visible; cercaria with**

132          **distinctive Y-shaped excretory bladder extending about half way up the body;**

133          **ventral sucker present……..……………..………………………………***Renicola* **sp. I**

134

135     *Species annotations*

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

137          Cercariae develop in active rediae in gonadal region of snail visceral mass. Cercariae

138     positively phototactic, swim by cupping body at ventral sucker, lash tail and wiggle side to side,

139     pivoting along long axis.

140

141          This species has not previously been reported (but see below). I examined specimens

142     from Chiba Prefecture (Obitsu River) and Miyagi Prefecture (Torinoumi), the description being

143     based on an infection from the latter locality (voucher deposited, USNPC No. 099682.00).

144

145     *Diagnosis*: Cercaria non-oculate, pharyngeate, with oral and ventral suckers. Cercaria body oblong to oval in

146          dorsal view, dorsal-ventrally flattened, length 289-350 ($318 \pm 16$, n = 13), max width 130-161 ($144 \pm 8$, n = 12)

147          at or slightly anterior to ventral sucker. Collar not very pronounced, with 23 spines of similar size in single row,

148          interrupted ventrally. Length of terminal collar spine 6.2-8.8 ($8.1 \pm 0.9$, n = 10), width at base 1.5-2.5 ($2.2 \pm 0.4$,

149          n = 10). Tegument otherwise without spines, but with papillae discernable laterally. Tail simple, circular in

150          cross-section, attached subterminally, length 267-363 ($322 \pm 32$, n = 12), width 29-41 ($36 \pm 4$, n = 12) just

151          posterior to base. Cystogenous glands obscure much of internal structure. Oral sucker ± circular, length 41-51

152          ($47 \pm 3$, n = 13), width 43-46 ($44 \pm 1$, n = 13), with undetermined number of glands, with apparently two pairs

153          of ducts (one pair/side) opening anteriorly. An additional two pairs of ducts (one pair/side) observed from just

154          posterior to pharynx extending anteriorly and opening at sides of oral sucker, their origin (presumably at

155          penetration glands) not observed and obscured by cystogenous glands. Ventral sucker ± circular, about 2/3 body

156          length from anterior end, length 55-73 ($65 \pm 5$, n = 13), width 56-67 ($61 \pm 3$, n = 13), may appear wider than

157          long in live specimens. Prepharynx evident, shorter than pharynx. Pharynx length 24-32 ($27 \pm 2$, n = 12), width

158     12-18 (15 ± 2, n = 12). Esophagus slender, bifurcating just anterior to ventral sucker. Digestive ceca curve

159     around ventral sucker, continue posteriorly to middle of excretory bladder, almost to posterior of body.

160     Excretory bladder thin-walled, more-or-less square, with short anterior-medial connection receiving main

161     collecting ducts. Main collecting ducts extend anteriorly from connection with bladder, laterally around ventral

162     sucker, anteriorly to oral sucker, with several medially and laterally projecting diverticula between ventral

163     sucker and pharynx. Rest of body excretory ducts and complete flame bulb formula not observed. Caudal

164     excretory duct extends into tail, bifurcating and opening laterally. Mass of primordia (probably genital) evident

165     upon acetocarmine staining, along midline, just posterior to ventral sucker, length 13-18 (15 ± 2, n = 7), width

166     15-20 (17 ± 2, n = 7). Redia with orange-pigment clusters in tegument. Redia length 501-1065 (799 ± 165, n =

167     14), width 150-258 (214 ± 29, n = 14). Redia with collar, relative position of collar apex along body 0.10-0.30

168     (.17 ± .05, n = 12). Redia with posterior pair of appendages, relative position along body 0.66-0.91 (0.77 ± 0.07,

169     n = 11). Redia pharynx length 63-105 (78 ± 14, n = 13), width 47-79 (60 ± 9, n = 12). Rediae with 8-27 (18 ± 6,

170     n = 14) cercariae in various stages of development.

171

172     Species of *Acanthoparyphium* use mollusks [e.g., 22, 23, 24] and polychaetes

173     (Hechinger and Smith, unpublished data) as second intermediate hosts. In an unpublished study,

174     Armand Kuris and I found metacercariae of an *Acanthoparyphium* species in the feet of clams

175     (*Venerupis* (=*Ruditapes*) *philippinarum*) only at the locality where we found first intermediate

176     host infections of *Acanthoparyphium* sp. I in *B. attramentaria* (Hechinger and Kuris,

177     unpublished data).

178

179     Harada and Suguri [5] reported an *Acanthoparyphium*-like cercaria from *B. attramentaria*

180     in Japan, and identified that cercaria as *Cercaria yamagutii* Ito 1957. However, Ito [1] described

181     and reported *C. yamagutii* from the three snail species, *Cerithidea rhizophorarum, Cerithidea*

182     *largillierti,* and *Tympanotonus microptera*. As mentioned in the introduction, it seems preferable

183     to assume that trematodes using different first intermediate host species, particularly different

184    genera, are different species unless evidence shows otherwise. Barring further study, I consider

185    the *C. yamagutii* reported from *B. attramentaria* by Harada and Suguri [5] to be the same as

186    *Acanthoparyphium* sp. I, although several of the characters (e.g., size of the body, suckers and

187    collar spines) that I measured for *Acanthoparyphium* sp. I are smaller than those Harada and

188    Suguri reported for *C. yamagutii*.

189

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

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

192

193           Rybakov [3] described this species infecting *B. attramentaria* from Peter the Great Bay,

194    eastern Russia. I never encountered this species, and all descriptive information is from Rybakov

195    [3] (M.C. and E. Rigby kindly provided the translation and I have slightly modified their

196    language).

197

198    *Diagnosis* (range (average), no n was provided): cercaria body length 152-169 (162), width 61-68 (63), tail

199           length 139-155 (147), tail width 17-20 (19), oral sucker length 21-24 (22), oral sucker width 24-27 (26),

200           pharynx length 11-14 (13), pharynx width 5-8 (7), ventral sucker length 29-34 (32), width 27-32 (30). Cercariae

201           belong to the morphological group Echinostomata. Spiny collar has 23 spines. Tegument on ventral side from

202           spiny collar to ventral sucker has 15 to 18 rows of spines, and dorsal side from spiny collar to level of ventral

203           sucker has wide horizontal creases. Rest of body without armor. Digestive system well developed, cecal

204           branches reach posteriorly to anterior end of urinary bladder.  Only two pairs of penetration glands were found.

205           Additionally, in dorsal part of oral sucker, three unique glandular cells were observed.  Cystogenous glands are

206           located very close together under tegument over entire body of cercaria.  Excretory system is as usual for

207           echinostomatids, flame bulb formula, perhaps, is $2[(3+3+3+3+3) + (3+3+3+3+3)] = 60$.

208

209        Rybakov and Lukomskaya [25] described the life cycle of this trematode in Peter the

210   Great Bay, finding that the trematode used three bivalve species (including *Ruditapes*

211   *philippinarum*) as second intermediate hosts, and that they were able to use chickens as

212   experimental final hosts.  They also described *A. macracanthum* as a new species in that

213   publication, but it seems that Rybakov [3] was a valid description and thus has priority as author

214   of the species name.

215

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

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

218

219        Shimura and Ito [2] described this species from *B. attramentaria* from Kanagawa and

220   Chiba Prefectures, Japan. I examined infections from nine localities on the Pacific coast of

221   Honshu, from Wakayama Prefecture to Miyagi Prefecture. The following description is based on

222   an infection from Waka River (voucher deposited, USNPC No. 099683.00).

223

224   *Diagnosis*: Cercaria bioculate, pleurolophocercous (lateral and dorso-ventral fins), pharyngeate, with oral and

225       ventral suckers. Cercaria body oval to oblong in dorsal view, dorsal-ventrally flattened, body length 142-149

226       $(146 \pm 2, n = 12)$, max width 56-62 $(60 \pm 2, n = 11)$ at midbody. Tegument with small spines, arranged in

227       transverse rows, particularly evident over oral sucker. Tail attached in pronounced subterminal socket, tail

228       length 255-295 $(282 \pm 13, n = 8)$, width 20-24 $(22 \pm 2, n = 8)$ just posterior to base. Tail with two lateral fins

229       and one continuous dorso-ventral fin, extending around the tail tip. Lateral fin length 110-120 $(113 \pm 3, n = 8)$,

230       width 10-17 $(13 \pm 3, n = 4)$. Dorsal portion of dorso-ventral fin length 159-196 $(184 \pm 12, n = 8)$, dorsal origin

231       6-34 $(19 \pm 11, n = 6)$ anterior to insertion of lateral fins, ventral insertion of dorso-ventral fin ~7-11 $(9 \pm 2, n =$

232       $3)$ posterior to insertion of laterals. Oral sucker very well developed, rounded, length 25-27 $(26 \pm 1, n = 12)$,

233       width 20-27 $(24 \pm 2, n = 11)$. At least two transverse rows of oral spines present, but number of rows or spines

234      not accurately observed. Ventral sucker not developed. Prepharynx length 22-28 (25 ± 2, n = 11), not very

235      prominent. Pharynx length 6-9 (8 ± 1, n = 11), width 9-11 (10 ± 1, n = 11). Eye spots black, cuboidal to slightly

236      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

237      observed. Excretory bladder thick-walled, v-shaped to cordate, appears to empty directly into the subterminal

238      notch, just below tail. Flame bulb formula not observed. Seven pairs of penetration glands (each ~13 diameter),

239      clustered just past midbody, partly surrounding anterior edge of primordial mass (which appears clear, before

240      staining). This mass of primordia (probably genital) evident upon acetocarmine staining, along midline, just

241      posterior to cluster of penetration glands, anterior to excretory bladder, diamond-shaped, length 15-20 (18 ± 1, n

242      = 10) along longest side. Redia sausage-shaped, with no appendages, length 756-877 (809 ± 35, n = 10), width

243      108-149 (133 ± 12, n = 10). Redia pharynx length 21-27 (24 ± 2, n = 10), width 22-25 (24 ± 1, n = 10). Gut of

244      redia not discernable due to cercariae. Redia packed with ~20-31 (26 ± 3, n = 10) cercariae in various stages of

245      development (cercariae are generally more developed in anterior of redia).

246

247         Shimura and Ito [2], and I (unpublished data) have shown experimentally that *C.*

248 *batillariae* infects fish as second intermediate hosts. I also obtained adults by infecting laboratory

249 mice with metacercariae, but have not succeeded in retrieving adults with the female components

250 of the reproductive system matured (unpublished work).

251

252         *C. batillariae* has been the subject of several ecological and population genetics studies.

253 *C. batillariae* is the single morphospecies of trematode that has invaded the West Coast of North

254 America with its snail host, *B. attramentaria* [6]. Miura et al. [21] recently used molecular

255 genetic methods to demonstrate that *C. batillariae* likely is a complex of eight cryptic species in

256 Japan. Subsequently, Miura et al. [7] determined that at least three of the eight cryptic species are

257 present in the introduced range on the west coast of North America, with only two being

258    common. Additionally, Miura et al. [26] showed that *C. batillariae* strongly affects its host

259    snail's growth and distribution in the intertidal zone.

260

261        The above measurements and my description largely fit within the range of those

262    provided in Shimura and Ito's description [2]. But there are exceptions. First, the measurements

263    that Shimura and Ito provided for the prepharynx length (53-66, ave. 60) are more than twice as

264    large as my measurements. However, I believe Shimura and Ito's prepharynx measurements may

265    have been reported in error, for they do not correspond with their figure of the cercarial body

266    (from which I estimate the prepharynx to be ~33 μm—very close to my measurements). The

267    second major difference between our descriptions pertains to the dorso-ventral tail fin. Shimura

268    and Ito report that the dorsal fin originates posterior to the posterior end of the laterals and the

269    ventral inserts anterior to the posterior end of the laterals. The dorso-ventral fins on the

270    specimens that I have examined carefully (dozens from Japan and the United States), have the

271    opposite arrangement (as described above). This difference is either due to a mistake in the

272    original description, or it is real and due to either intraspecific variation or variation across

273    cryptic species. The other exceptions are that my measurements are smaller in tail and lateral fin

274    length and the eyespots of Shimura and Ito's specimens appear to be more rectangular, versus

275    tending to be cuboidal as were the specimens upon which I based the above description

276    (although I have examined many other specimens with more rectangular eyes). These differences

277    may be an artifact of my dealing only with cercariae from dissected snails, as such cercariae are

278    potentially not fully developed. Alternatively, the differences may be due to intraspecific

279    variation, or these differences may reflect variation across the cryptic species of *C. batillariae*

280    uncovered by Miura et al [21].

281

282        Rybakov [3] reported and provided a description of *C. batillariae* Shimura and Ito 1980

283    from *B. attramentaria* in Peter the Great Bay, eastern Russia. The cercariae he described are

284    about 30 μm longer in body length, and over 100 μm longer in tail length. It is possible that the

285    difference for body length is because Rybakov made measurements on live specimens.

286    Additionally, the dorsal tail fin originates very far anterior (near the base of the tail). Either these

287    differences are due to intraspecific variation, or, perhaps more likely, Rybakov described a

288    different cryptic species of *C. batillariae.*

289

290        Harada and Suguri [5] reported a "Cercaria sp. 5" from *B. attramentaria*. Their

291    measurements appear to slightly differ from those I provide here, but this is hard to assess since

292    they provided averages with no indication of the dispersion of the data. The oral spine

293    arrangement they reported for Cercaria sp. 5 (6-10, 6-10, 4) differs from that reported by

294    Shimura and Ito [2] in the original description of *C. batillariae* (7-10, 8-9, 5-6). Cercaria sp. 5 is

295    probably a member of the cryptic species complex of *C. batillariae* of Miura et. al [21].

296

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

298        Cercariae develop in inactive sporocysts residing primarily in gonadal region of snail

299    host.

300

301        Shimura and Ito [2]described this species infecting *B. attramentaria* from Kanagawa and

302    Chiba Prefectures. I examined infections from Wakayama Prefecture (Uchino River, Yukashi

303    Lagoon, and Hashiguiiwa) and Miyagi Prefecture (Mangoku River and Nagazura River). The

304    following is based on an infection from Uchino River (voucher deposited, USNPC No.

305    099684.00).

306

307    *Diagnosis*: Cercariae non-oculate, apharyngeate, monostomate (no ventral sucker), with simple tail and oral

308        stylet. Cercaria body oval to oblong to elongate in dorsal view, dorsal-ventrally flattened, usually fixed dorsal-

309        ventrally flexed. Body length (taken in lateral view, to not underestimate because body flexed) 132-155 (140 ±

310        7, n = 13), max width 37-49 (43 ± 4, n = 13) at or slightly anterior to midbody. Tail attached slightly

311        subterminally, circular in cross-section, with fine tegumental crenulations, length 84-112 (104 ± 8, n = 9), width

312        10-12 (11 ± 1, n = 9). Oral sucker not very strongly developed, length 32-34 (33 ± 1, n = 13), width difficult to

313        discern and not measured. Oral sucker with well developed stylet. Stylet sclerotized more than half its anterior

314        length, narrow, slightly higher (dorso-ventrally) than wide (left to right), base non-sclerotized and rounded,

315        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

316        pronounced penetration glands sequentially arranged anterior to posterior, restricted roughly to third quarter of

317        body; anterior two pairs filled with coarse granules, posterior pair with fine granules. Penetration gland ducts

318        pronounced, extend anteriorly to anterior of oral sucker where they narrow and turn sharply medially.

319        Penetration gland ducts from anterior pair positioned singly and medial to posterior two pairs, which are

320        positioned more laterally, and are bundled together. Excretory bladder v-shaped. Rest of excretory system not

321        observed. Mass of primordia (putatively ventral sucker primordium) evident upon acetocarmine staining, along

322        midline, filling region posterior to penetration glands and anterior to excretory bladder. Sporocyst simple, thin-

323        walled (~2-6 μm thick), round to oval, length 198-316 (251 ± 34, n = 10), width 159-221 (191 ± 20, n = 10),

324        densely packed with estimated number of cercariae 13-27 (20 ± 4, n = 10) mostly in late stage of development,

325        with few germ balls.

326

327        This species probably uses crustaceans as second intermediate hosts, as do most

328    microphallids [see 17].

329

330    The description I provide agrees with that provided by Shimura and Ito [2] in their

331    description of *C. hosoumininae* except that the measurements of the cercariae I examined

332    (including those from at least two other sites, data not given here) are apparently smaller in body,

333    tail and stylet length. This may be an artifact of my dealing only with cercariae from dissected

334    snails, as such cercariae are potentially not fully developed, or represent intraspecific variation.

335    Alternatively, the infections I encountered may belong to a different species of microphallid.

336    Indeed, molecular evidence indicates that there are cryptic species of *C. hosoumininae* (O.Miura,

337    pers. comm.). Further study is called for to resolve this issue.

338

339    Rybakov [3] reported and described *C. hosoumininae* Shimura and Ito 1980 in *B.*

340    *attramentaria* from Peter the Great Bay, eastern Russia. His measurements agree with Shimura

341    and Ito's description.

342

343    Harada and Suguri [5] reported many infections of the microphallid, *Cercaria lanceolata*

344    Holliman 1961, infecting *Cerithidea rhizophorarum* snails and two infections in *B.*

345    *attramentaria*. Holliman described *C. lanceolata* from *Cerithidea scalariformis* in the Gulf of

346    Mexico [13]. Harada and Suguri assigned the microphallids they encountered to *C. lanceolata* on

347    the basis of the flame bulb pattern of 2[{2+2) + (2+2)]=16  (while the formula for *C.*

348    *hosoumininae* is 2[(1+1)+(1+1)]=8) and the shared genus of snail host (although that does not

349    explain applying the name to the infections in *B. attramentaria*). In the Gulf of Mexico, *C.*

350    *lanceolata* was subsequently determined to a species of *Probolocoryphe* [27]. Given the

351    arguments discussed above regarding host specificity, it seems very possible that a species of

352    *Probolocoryphe* infects *C. rhizophorarum*. But, it does not seem likely that the same species

353    would also infect *B. attramentaria*. However, since Harada and Suguri reported two infections of

354    *C. lanceolata* in *B. attramentaria*, workers studying trematodes in *B. attramentaria* should watch

355    for a second microphallid species with 16 flame bulbs. I would refer to such a species as

356    "Microphallid sp. II," rather than *Probolocoryphe lanceolata*, until further work on adults or

357    late-stage metacercariae is undertaken. If such a second microphallid species infects *B.*

358    *attramentaria*, I suspect it will differ from *C. hosoumininae* not only in flame bulb formula, but

359    also in other morphological characters, including stylet morphology.

360

361    **Cyathocotylid sp. I**—Fig. 1, Fig. 3

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

363    mass.

364

365            This species has not previously been reported. I examined infections from specimens

366    from Wakayama Prefecture (Yukashi Lagoon and Hashiguiiwa) and Miyagi Prefecture

367    (Nagazura River). The description below is based on an infection from Nagazura River (voucher

368    deposited, USNPC No. 099685.00).

369

370    *Diagnosis*: Cercariae non-oculate, pharyngeate, monostome (no ventral sucker), longifurcate. Cercaria body

371    oval to pyriform in dorsal view, dorsal-ventrally flattened, length 158-204 (176 ± 19, n = 8), max width 96-123

372    (110 ± 11, n = 10) at or slightly posterior to midbody. Tail attached to dorsal side of posterior body, width

373    constricted just before attachment. Tail stem laterally flattened, length (to posterior-most part of tail) 412-489

374    (447 ± 30, n = 11), width at attachment to furcae 40-51 (46 ± 3, n = 11). Tail furcae laterally flattened, length

375    247-355 (303 ± 29, n = 11), width at base 27-32 (29 ± 1, n = 11), each provided with continuous dorsal-ventral

376    fin-fold. Anterior organ/oral sucker length 31-47 (40 ± 6, n = 8), width 27-39 (32 ± 5, n = 9), with undetermined

377    number of penetration glands. Excretory system collecting ducts follow general pattern typical of cyathocotylid

378     cercariae. Single, blind ducts extend anterio-laterally from juncture of lateral and cross-commissural ducts.

379     Fifteen pairs of protonephridea (flame bulbs, or cells) observed in cercarial body, but capillaries, and thus,

380     flame bulb formula, not observed. Flame bulbs not observed in tail stem. Mass of primordia (probably genital

381     primordia, perhaps with acetabular primordia at anterior) evident upon acetocarmine staining, along midline, in

382     region between the two medial collecting ducts just posterior to point where ducts fuse, length 18-25 ($22 \pm 2$, n

383     = 9), width 12-27 ($20 \pm 5$, n = 9). Sporocysts with transverse annulations, long and thin (can be over 4 mm

384     long), highly intertwined and difficult to separate intact.

385

386          This species most likely infects fish as second intermediate hosts, as do most

387     cyathocotylids [17, 28, 29].

388

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

390          Cercariae develop in active rediae, primarily in gonadal region of snail visceral mass.

391     Cercariae often swim to top, attach to surface tension, and get an air bubble in their ventral

392     sucker. Cercariae infrequently encyst in dish and form flask-shaped metacercariae (Fig. 1).

393

394          This species has not previously been reported. I examined specimens from Chiba

395     Prefecture (Obitsu River) and Miyagi Prefecture (Torinoumi and Ogatsu Bay). The description

396     below is based on an infection from Ogatsu Bay (voucher deposited, USNPC No. 099686.00). I

397     provide measurements, although I had very little fixed material for study.

398

399     *Diagnosis*: Cercaria non-oculate, pharyngeate, with oral and ventral suckers. Cercaria body oval to oblong to

400     slightly spatulate in dorsal view, dorsal-ventrally flattened, length 575-653 ($602 \pm 44$, n = 3), max width 144-

401     155 ($149 \pm 6$, n = 3) slightly anterior to ventral sucker. Tegument apparently without spines, but with prominent

402     thin ducts from cystogenous glands (potentially misinterpreted as spines), particularly over lateral and posterior

403    half of ventrum. Tail simple (except for terminal gland), circular in cross-section, with parenchyma cells,

404    attached slightly subterminally, length 404-417 (411 ± 9, n = 2), width 40-48 (44 ± 4, n = 3) just posterior to

405    base. Tail terminal gland length 116-162 (133 ± 25, n = 3). Cystogenous glands obscure much of internal

406    structure. Oral sucker round, length 59-74 (65 ± 8, n = 3), width 52-56 (55 ± 2, n = 3). Ventral sucker round to

407    slightly oval (may occur more oval in fresh specimens), at beginning of second half of body, length 71-86 (78 ±

408    8, n = 3), width 66-79 (71 ± 7, n = 3). Prepharynx evident, length 15-32 (25 ± 9, n = 3). Pharynx length 32-41

409    (36 ± 5, n = 3), width 22-25 (23 ± 2, n = 3). Esophagus bifurcates about half way distance from anterior of

410    cercaria body to ventral sucker, length 39-42 (41 ± 2, n = 2). Digestive ceca diverge laterally, continue

411    posteriorly almost to end of body to around anterior of excretory bladder. Excretory bladder thin-walled, more-

412    or-less square, with short anterior-medial connection receiving main collecting ducts. Main collecting ducts

413    extend anteriorly from connection with bladder, laterally around ventral sucker, or cross over ventral sucker's

414    sides, continue anteriorly to recurve just before oral sucker around level of pharynx. Complete flame bulb

415    formula not observed. Mass of primordia evident upon acetocarmine staining, along midline, about half way

416    between ventral sucker and excretory bladder, sometimes extends anterior as thin strip toward cecal bifurcation.

417    Redia translucent, length 652-1250 (1042 ± 140, n = 16), width 123-218 (184 ± 22, n = 16), with one or two

418    appendages, positioned at ~9/10 redial length, not always prominent. Redia pharynx length 42-55 (49 ± 4, n =

419    15), pharynx width 44-57 (48 ± 4, n = 15). Redia gut length 245-358 (286 ± 36, n = 9). Cercariae-producing

420    rediae with number of cercariae 3-17 (13 ± 4, n = 16), in all stages of development.

421

422    This trematode may be a species of *Philophthalmus* as this genus is known to form flask-

423    shaped cysts [e.g., 30, 31, 32] and infect other species of *Batillaria* [e.g., 31].

424

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

426    Cercariae develop in large active rediae, which primarily reside in the gonadal region of

427    the snail visceral mass. Cercariae sometimes encyst in dish and form round to oval metacercariae

428    (Fig. 2).

429

430        This species has not previously been reported in Japan, but probably has been reported in

431   eastern Russia (see below). I examined infections from Miyagi Prefecture (Ogatsu Bay and

432   Nagazura River), and the following is based on an infection from the former locality (voucher

433   deposited, USNPC No. 099687.00).

434

435        *Diagnosis*: Cercaria non-oculate, pharyngeate, with oral and ventral suckers. Cercaria body oval to oblong to

436        slightly spatulate in dorsal view, dorsal-ventrally flattened, length 352-502 ($431 \pm 51$, n = 8), max width 167-

437        218 ($200 \pm 16$, n = 8) slightly anterior to ventral sucker. Tegument without spines. Tail simple (except for

438        terminal gland), circular in cross-section, with parenchyma cells, attached slightly subterminally, fixed in

439        varying states of contraction, length 145-412 ($233 \pm 90$, n = 8), width 39-74 ($56 \pm 14$, n = 5) just posterior to

440        base. Tail terminal gland 51-86 ($65 \pm 10$, n = 8). Oral sucker round, length 60-69 ($66 \pm 4$, n = 8), width 59-74

441        ($69 \pm 6$, n = 8). Ventral sucker round, at beginning of posterior half of body, length 71-98 ($87 \pm 8$, n = 8), width

442        79-105 ($96 \pm 9$, n = 8). Cystogenous glands ~10 diameter, obscure almost all internal structure, few organs

443        being evident even in highly flattened unfixed specimens. Prepharynx present, apparently shorter than pharynx.

444        Esophagus bifurcates about half way between anterior of cercaria body to ventral sucker. Digestive ceca diverge

445        laterally, continue posteriorly at least to ventral sucker, but posterior limits not observed. Excretory bladder

446        thin, saccate, with short medio-anterior duct receiving main collecting ducts. Main collecting ducts extend

447        anteriorly from connection with bladder, path not observed in middle of body, recurve at level of pharynx.

448        Caudal tubule bifurcates a short distance into tail. Redia translucent, length 1002-1225 ($1117 \pm 92$, n = 4), width

449        277-350 ($304 \pm 34$, n = 4), with one or two appendages, positioned ~9/10 redial length, not always prominent.

450        Redia pharynx length 54-68 ($58 \pm 7$, n = 4), width 49-55 ($51 \pm 3$, n = 4). Redia gut usually obscured by

451        embryos, length 245-466 ($356 \pm 156$, n = 2). Cercariae-producing rediae with number of cercariae 10-14 ($12 \pm$

452        2, n = 4), in all stages of development.

453

454        In a molecular genetic study, Miura et al. [21] showed that Philophthalmid species II is a

455    complex of three cryptic species.

456

457        Rybakov [3] reported and described a philophthalmid species (that he tentatively termed

458    "*Cercaria Parorchis* sp.") from *B. attramentaria* from Peter the Great Bay, eastern Russia. I

459    provisionally consider *Cercaria Parorchis* sp. to be the same as Philophthalmid species II,

460    noting that the former is larger in most dimensions. These differences either reflect differences in

461    laboratory technique (Rybakov made measurements on live specimens, and he noted that he had

462    very little material to study), indicate intraspecific variation, or occur because the specimen

463    reported by Rybakov represents one of the cryptic species uncovered by Miura et al. [21]. I do

464    not use Rybakov's name because I believe there is no evidence to assign this philophthalmid to

465    *Parorchis*, versus other philophthalmid genera (e.g., *Cloacitrema*). Indeed, there is evidence

466    against this trematode being a species of *Parorchis*, since it is lacking the strong body spination

467    and the spined collar that characterizes *Parorchis* spp. [12]. Thus, I use a temporary

468    morphospecies name that reflects only the trematode's probable familial affiliation.

469

470        Harada [4] and Harada and Suguri [5] reported one infection of a philophthalmid,

471    *Cercaria shikokuensis*, in *B. attramentaria*. However, Harada [4] described *C. shikokuensis* from

472    the snail *Cerithidea rhizophorarum*. As discussed in the introduction, based on general principles

473    and without strong evidence to the contrary, it seems questionable to assume *C. shikokuensis*

474    (described from a species of *Cerithidea*) would also infect *B. attramentaria*. Nevertheless,

475    workers should certainly watch for a *C. shikokuensis*-like cercaria infecting *B. attramentaria*.

476    The most obvious distinguishing trait would be the presence of prominent body spines in *C.*

477   *shikokuensis* (and, apparently, *C. shikokuensis* has a longer body, smaller ventral sucker, and a

478   longer esophagus than does Philophthalmid sp. II). If such a cercaria is found, I would refer to it

479   as "Philophthalmid sp. III," rather than *C. shikokuensis*, barring further study. Perhaps Harada

480   [4] and Harada and Suguri [5] recognized one of the three cryptic species of Philophthalmid

481   Cercaria II reported by Miura et al. [21].

482

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

484          Cercariae develop in inactive sporocysts residing in the gonad and sometimes digestive

485   gland regions of the snail.

486

487          This species has previously been reported in Japan [33, see below] and eastern Russia [3,

488   see below]. I examined infections from Miyagi Prefecture (Matsushima and Mangoku River),

489   and the below is based on an infection from Matsushima (voucher deposited, USNPC No.

490   099688.00).

491

492   *Diagnosis*: Cercariae non-oculate, pharyngeate, distomate (oral and ventral suckers), with simple tail and oral

493          stylet. Cercaria body oval to elongate in dorsal view, dorsal-ventrally flattened. Body length 148-233 (174 ± 27,

494          n = 9), width 61-74 (68 ± 4, n = 9) at midbody. Tail attached slightly terminally, circular in cross-section, length

495          103-123 (114 ± 8, n = 9), width 16-22 (18 ± 2, n = 9). Oral sucker well developed, length 27-35 (30 ± 3, n = 8),

496          width 25-31 (28 ± 2, n = 9). Oral sucker with bullet-shaped stylet anteriorly (and dorsal to mouth), very hard to

497          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).

498          Ventral sucker oval, positioned at mid-body, length 25-33 (28 ± 3, n = 9), width 27-33 (29 ± 2, n = 9).

499          Tegument covered with minute spines, the full distribution of which was not observed. Cystogenous glands fill

500          most of body and obscure internal structures. Pharynx round, just posterior to oral sucker, barely discernable,

501          not measured. Esophagus difficult to see, slender, branching about half way to ventral sucker into two slender

502    cecae, the posterior extent not observed. Excretory bladder prominent and Y-shaped, with lateral arms not

503    extending to anterior of ventral sucker. Rest of excretory system not observed. Sporocyst simple, thick-walled

504    (at least 5-7 thick), round to oval, length 198-472 ($313 \pm 73$, n = 15), width 112-206 ($157 \pm 24$, n = 15), densely

505    packed with estimated number of cercariae 9-16 ($11 \pm 2$, n = 11) in all stages of development.

506

507          Renicolid cercariae with stylets are known to encyst in mollusks [34] and polychaete

508    worms (Hechinger and Smith, unpublished data).

509

510          Rybakov  [3] reported and described this species, from eastern Russia, with the

511    temporary name of "*Cercaria Renicola* sp." His measurements slightly differed from those I

512    present, but these differences could easily reflect differences in laboratory technique (he made

513    measurements on live specimens) or intraspecific variation. I do not use Rybakov's temporary

514    name to maintain consistent naming throughout this manuscript.

515

516          Miura and Chiba [33] provide information (using the name "renicolid cercaria I" from a

517    early version of this manuscript) on the distribution of *Renicola* sp. I along an elevational

518    gradient and among host sizes; as well as on the frequency of double infections with *C.*

519    *batillariae*.

520

521    **Schistosomatid sp. I**—Fig. 1, Fig. 5

522          Cercariae develop in inactive whitish sporocysts spread throughout the gonad and

523    digestive gland of the snail.

524

525      This species has apparently not previously been reported from *B. attramentaria*. I

526   encountered only one infection at one locality in Miyagi Prefecture (Mangoku River) (voucher

527   deposited, USNPC No. 099688.00).

528

529   *Diagnosis*: Cercariae oculate, apharyngeate, distomate (with oral and ventral sucker), brevifurcate. Unless

530      otherwise indicated, n = 12). Cercaria body ~pyriform in dorsal view, dorsal-ventrally flattened, length 197-252

531      (215 ± 18), width 74-91 (82 ± 5) at or slightly posterior to mid-body. Tail attached terminally. Tail stem length

532      (from insertion to anterior base of furcae) 211-238 (226 ± 8), width at bulge just posterior to insertion 32-42 (38

533      ± 2), width at furcal attachment 7-21 (15 ± 5). Tail furca with no fins, length 98-130 (118 ± 8), width at base 10-

534      21 (14 ± 3). Anterior organ length 69-93 (80 ± 9), width 54-61 (57 ± 2, n = 11), with several types of glands.

535      Mouth slightly subterminal. Ventral sucker located just posterior to midbody, length 20-27 (24 ± 2, n = 8),

536      width 24-34 (29 ± 3, n = 8). Eyespots black, circular, diameter 10-12 (11.5 ± 0.9). Daughter sporocyst sausage-

537      shaped, with concavity (~30 diameter) observed on one end, potentially marking outside of birth pore. Daughter

538      sporocyst length 412-1311 (765 ± 255, n = 14), width 127-247 (179 ± 36, n = 14), number of cercariae 4-20 (14

539      ± 4, n = 13), in all stages of development.

540

541      These cercariae most likely directly infect bird final hosts as do many other

542   schistosomatids in marine snails [17, 35].

543

544   **Acknowledgements**

554    **Figure 1**. General appearance of the cercariae and parthenitae of digenean trematode species

555    known to infect *Batillaria attramentaria* as first intermediate host. Metacercariae are included

556    for the philophthalmids. The drawing of *Acanthoparyphium macracanthum* is modified from

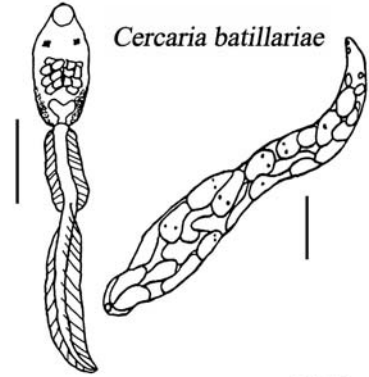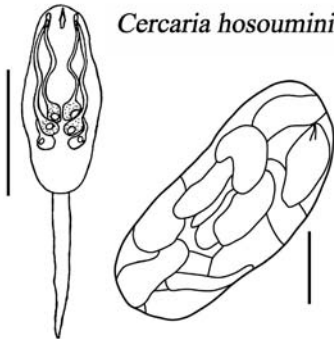557    [25]. All scale bars are 100µm.
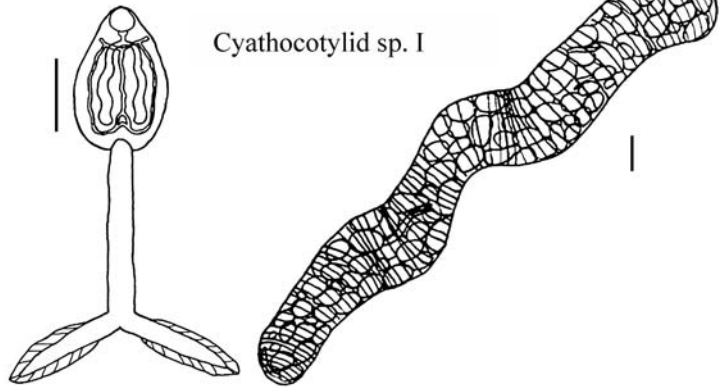
*Acanthoparyphium* sp. I
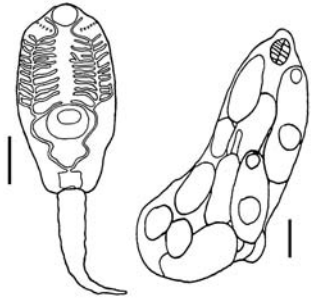
*Acanthoparyphium macracanthum*
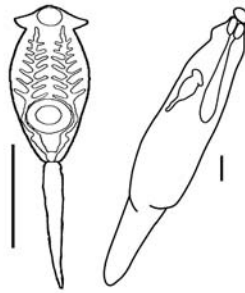
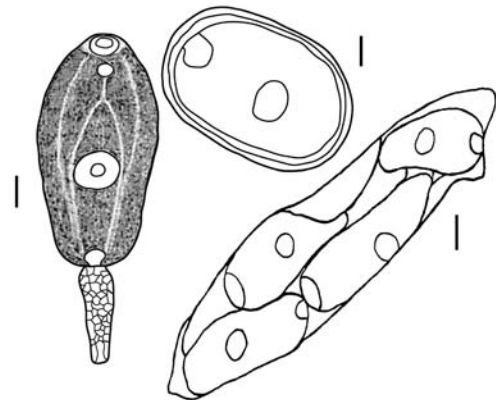*Cercaria batillariae*

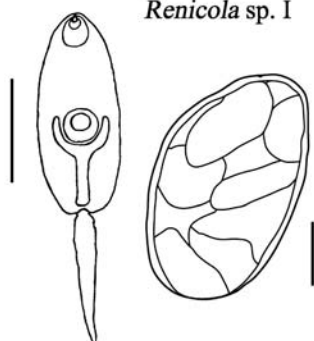*Cercaria hosoumininae*

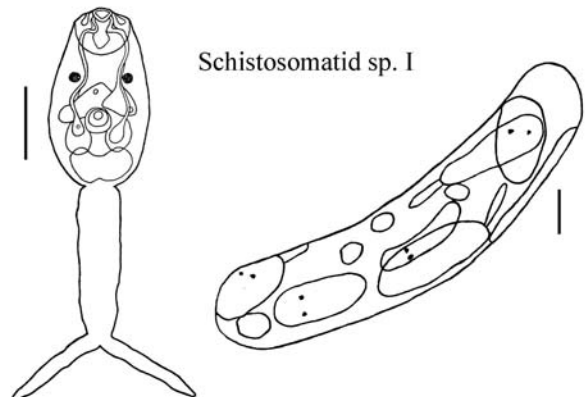Cyathocotylid sp. I

558

Philophthalmid sp. I
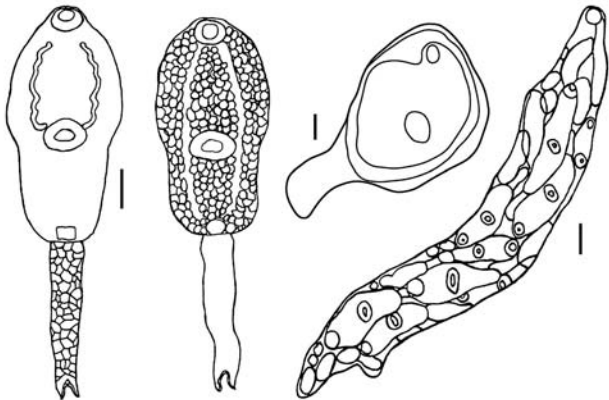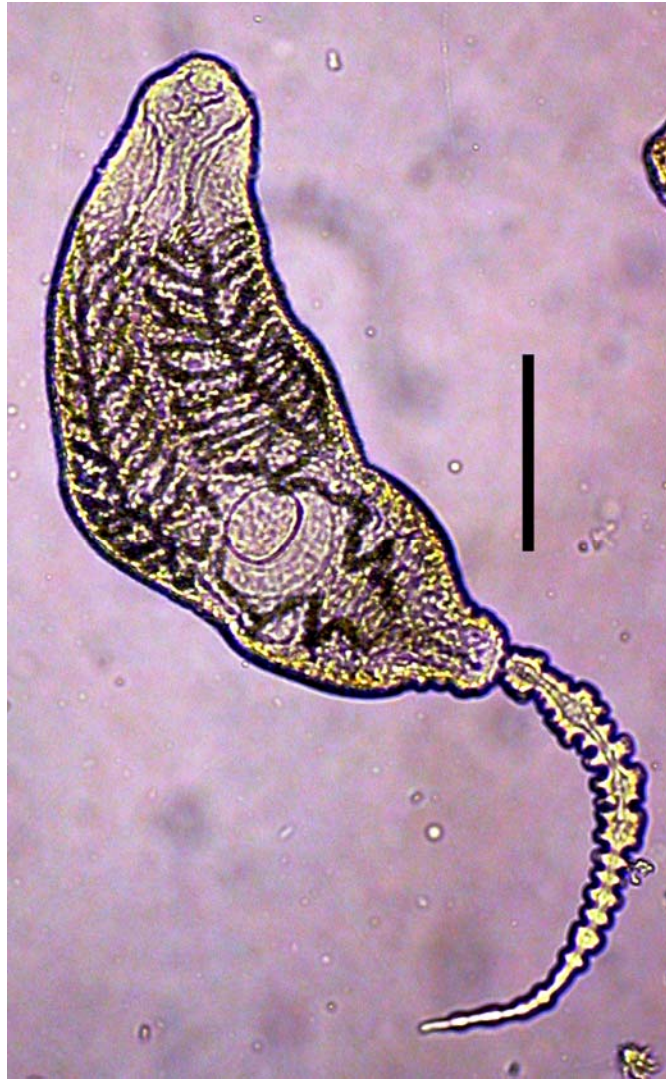
Philophthalmid sp. II

*Renicola* sp. I

Schistosomatid sp. I

559

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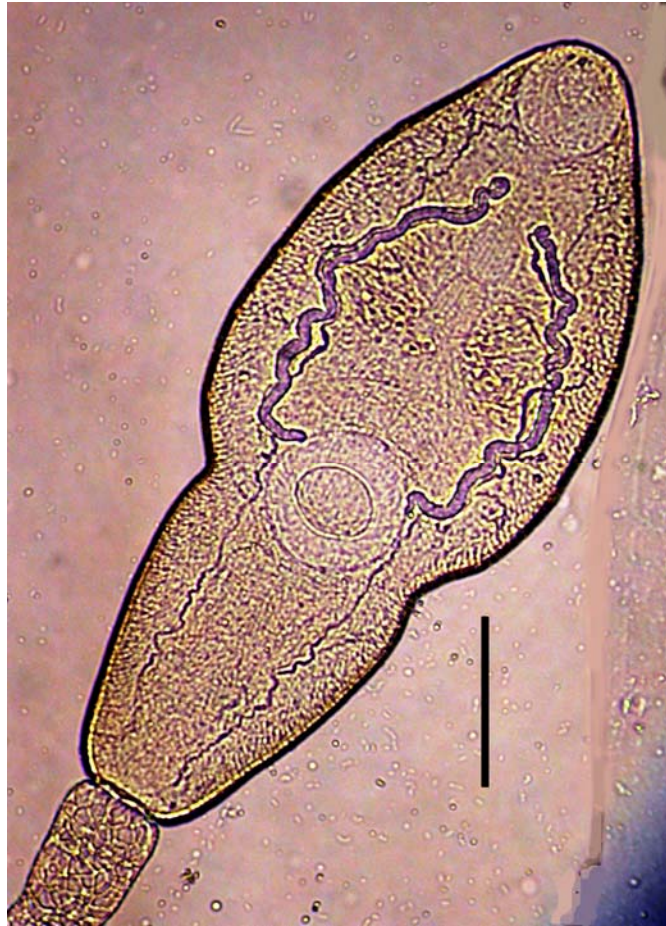
561      some cover-slip pressure. Scale bar = 100µm.

562

563    **Figure 3.** Photograph of cercaria of Cyathocotylid sp. I. Specimen was formalin-fixed and

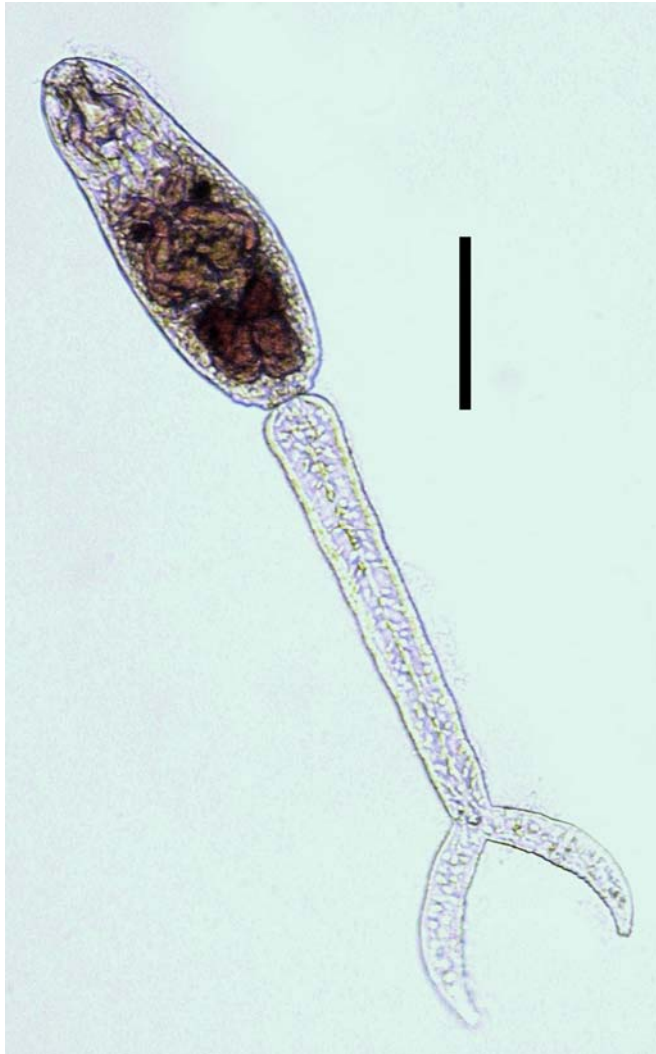564    acetocarmine stained. Scale bar = 100µm.



565

566     **Figure 4.** Photograph of cercaria of Philophthalmid sp. I. Specimen was alive and under heavy

567     cover-slip pressure. Scale bar = 100µm.



568

569     **Figure 5.** Photograph of cercaria of Schistosomatid sp. I. Specimen was formalin-fixed and

570     acetocarmine stained. Scale bar = 100µm.



571

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