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THE LIFE CYCLE OF *CEPHALOGONIMUS SALAMANDRUS* SP. N. (DIGENEA: CEPHALOGONIMIDAE) FROM *AMBYSTOMA TIGRINUM* (GREEN) FROM EASTERN WASHINGTON*

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ABSTRACT: Mother and daughter sporocysts and xiphidiocercariae develop in *Helisoma trivolvis*. Cercariae penetrate tadpoles of *Rana pretiosa* and small *A. tigrinum* larvae in which they encyst. The adult develops in the anterior small intestine of *A. tigrinum* after ingestion of an infected 2nd intermediate host. The life cycle of *C. salamandrus* is compared with *C. americanus* from *Rana clamitans*. *C. salamandrus* is described as a new species on the basis of cercarial and adult morphology, and the failure to achieve cross-infections in the respective definitive hosts for *C. americanus* and *C. salamandrus*.

Cephalogonimus Poirier, 1886, is the only genus of the family Cephalogonimidae in amphibians. Rai (1961) listed *Cephalogonimus brevicirrus* (Ingles, 1932), *C. amphiumae* (Chandler, 1923), *C. letusus* Dujardin, 1845, and *C. americanus* (Stafford, 1902) from amphibians of North America. Premvati (1969) described *C. sireni* from Florida mud eels. The only life cycle reported is for *C. americanus* from *Rana clamitans* Latreille (Lang, 1968). This parasite has also been reported from *R. catesbeiana* Shaw (Rankin, 1945), and *R. pipiens* Schreber (Brandt, 1936; Najarian, 1955). An undescribed cephalogonimid is a common intestinal parasite of neotenic and adult *Ambystoma tigrinum* (Green) of eastern Washington. Of the other species of *Cephalogonimus*, this parasite most closely resembles *C. americanus*. Comparison of the life cycle stages of this parasite with those of *C. americanus* and the demonstrated specificity of these two parasites to their respective definitive hosts establishes the validity of this new species to which the name *Cephalogonimus salamandrus* is assigned.

MATERIALS AND METHODS

Eggs were teased from adult flukes and stored in filtered spring water at 5 C until used. Fully embryonated eggs were fed to young laboratory-reared *Helisoma trivolvis* (Say). Exposed snails were crushed and examined at intervals after exposure. Naturally infected *H. trivolvis* were collected for cercarial study and laboratory infection of the second intermediate host. Cercariae were studied alive with and without vital stains.

Cercariae were placed with various aquatic invertebrates, small salamander larvae, tadpoles, and small frogs. Penetration and encystment occurred only in amphibian skin. Metacercariae were studied at 2 hr, 1 day, 5 days, 10 days, 12 days, and 21 days after infection. Tadpoles and salamander larvae used as intermediate hosts were laboratory-reared.

Large neotenic *A. tigrinum* for use in laboratory infections were collected from a lake where no trematode or cestode parasites were found in 150 *A. tigrinum* and no *Helisoma* spp. have been found in over 6 years of study. Salamanders were maintained in filtered spring water on a diet of earthworms.

Before exposure 13 neotenic salamanders (20 to 30 cm in length) were starved for 2 weeks. Feeding was resumed the day after exposure. Each of 11 salamanders was permitted to ingest two infected tadpoles, each containing 20 to 30 metacercariae which were 12 days old at exposure. Salamanders were necropsied at 52 hr, 5 days, 10 days, 15 days, 20 days, and 48 days after exposure. Also each of two salamanders was permitted to ingest two infected salamander larvae, each containing 20 to 30 metacercariae which were 12 days old at exposure. These were necropsied at 5 days. Recovered flukes were studied alive, measured, heat-killed, stored in AFA, and stained in Semichon's Carmine.

To determine if *A. tigrinum* and *R. pretiosa* of eastern Washington could be infected with *C. americanus*, hosts were transported to the University of Michigan Biological Station. Methods for handling *C. americanus* and collection of *Cephalogonimus*-free *R. clamitans* have been reported (Lang, 1968). Two salamanders were exposed to 10-day-old metacercariae of *C. americanus*. Each salamander received 50 to 70 metacercariae and was necropsied 10 or 30 days after exposure. During this experiment two *A. tigrinum*

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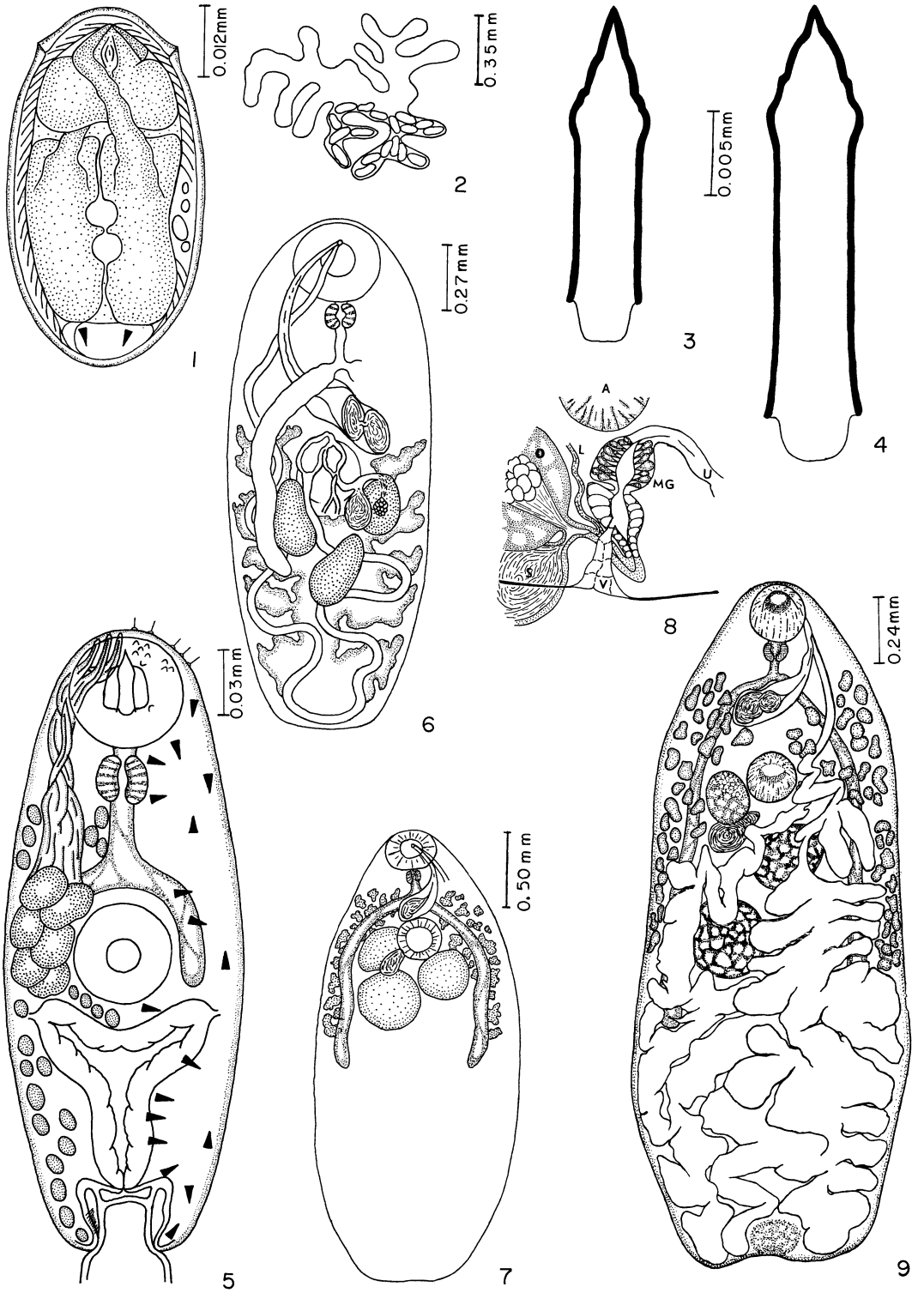

from Michigan were each given 20 to 30 11-day-old metacercariae of *C. americanus*. These were necropsied 4 and 7 days after exposure. Nine *R. clamitans* were also infected with 10-day-old metacercariae of *C. americanus* to serve as controls. Each was given 30 to 40 metacercariae. Four were necropsied at 10 days and five at 30 days after exposure. To determine if *R. clamitans* of Michigan could be infected with *C. salamandrus*, four were exposed to 50 to 60 metacercariae using laboratory-reared *Bufo fowleri* Hinckley and *R. pretiosa* tadpoles as second intermediate hosts. Frogs were necropsied 10, 20, 30, and 60 days after exposure. Four salamanders from Washington were similarly exposed and were necropsied at the same intervals to serve as controls.

All measurements are in microns unless otherwise indicated, the mean followed by the range in parentheses.

RESULTS

Fifty-seven of 64 *A. tigrinum* from Turnbull National Wildlife Refuge in eastern Washington were infected with *Cephalogonimus salamandrus*.

Tadpoles of *R. pretiosa*, small *R. pretiosa*, and young *A. tigrinum* larvae serve as second intermediate hosts for *C. salamandrus*. Apparently, neotenic larvae and adult *A. tigrinum* feed on these hosts during the spring. It is not known if salamanders in nature ingest their own shed skin containing metacercariae as reported for *C. americanus* (Lang, 1969). In both natural and laboratory infections, mature flukes locate near the common opening of the bile and pancreatic ducts.

Field collections indicated that flukes apparently overwinter in the definitive host. When salamanders become active in the early spring the flukes begin to release fully embryonated eggs. Eggs ingested by *H. trivolvis* hatch in the intestine posterior to the stomach within a few minutes after ingestion. In this process the miracidium within the egg contracts and expands repeatedly, until released. It swims in a spiral pattern until contact with the lining of the intestine is made. Then, the anterior cilia stop beating while the posterior cilia remain active, maintaining contact with the intestine while penetration begins. The miracidium penetrates the intestine, becoming the mother sporocyst. The mother sporocyst produces numerous daughter sporocysts which begin releasing cercariae approximately 50 days after initial exposure. Cercariae leave the snail host, penetrate, and encyst as metacercariae in tadpoles, small frogs, and small salamander larvae.

Egg and miracidium (Fig. 1)

Fifty fully embryonated eggs 58 (56 to 60) long at collection. Miracidium, except at extreme anterior end, with cilia 4.3 (3.8 to 6.2) long; ciliated plates not observed. Two large penetration glands in posterior half of body with ducts emptying at anterior end, and a single large gland in anterior half with duct opening at anterior end. Two flame cells and numerous germ balls in posterior end, eyespots absent. Released miracidia 79 (76 to 81) in length. Extreme anterior end retractable with 3 ducts opening at its tip.

Sporocyst generations (Fig. 2)

Three of 12 snails crushed 24 days after exposure were infected with *C. salamandrus*. Mother sporocysts 1,100 (900 to 1,300) in length highly branched, containing developing daughter sporocysts with germ balls. Mother sporocysts located posterior to stomach on outside of intestine.

Mature daughter sporocysts saclike, located throughout hepatopancreas of snail and containing 3 or 4 fully developed cercariae. Mature daughter sporocysts 250 (150 to 300) by 90 (80 to 100), encapsulated in well-developed paletot.

Cercariae (Figs. 4, 5)

Thirty living cercariae from natural infections, extended 320 (300 to 325) by 60 (55 to 68); contracted 132 (130 to 140) by 90 (80 to 100). Suckers subspherical. Oral sucker 48 (40 to 50) in diameter. Acetabulum 38 (36 to 41) in diameter. Tail straight, slender, 164 (160 to 180) long, no finfold. Ceca extend to or slightly past posterior margin of acetabulum. Stylet: length 26 (25 to 27), width at wing 10 (9 to 12), width at base 10 (9 to 11), width at narrowest point on shaft 6 (5 to 6) (Fig. 4). Refractile globules 1 to 8 in diameter, 25 to 50 per side, throughout parenchyma in lateral fields. Cystogenous glands 20 to 30 per side, extend posterior to level of pharynx. Six or 7 pairs of penetration glands in

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FIGURES 1–9. Stages in the life cycle of *Cephalogonimus salamandrus* including a stylet and a drawing of the adult of *C. americanus*. **1**. Fully embryonated egg. **2**. 24-day-old mother sporocyst demonstrating some daughter sporocysts. **3**. Stylet of *C. americanus*. **4**. Stylet of *C. salamandrus*. **5**. Ventral view of body of cercaria. **6**. 48-day-old fluke, dorsal view. **7**. Adult *C. americanus*, ventral view. **8**. Ootype region of paratype specimen. **9**. Type specimen of *C. salamandrus*, ventral view.

acetabular zone with ducts emptying at tip of stylet. Ten papillae with bristles on lateral margin and dorsal surface of anterior end; 20 papillae on oral sucker, 4 located at corners of mouth and 16 at anterior margin (Fig. 5). Excretory bladder Y-shaped, primary excretory tubules entering posterior to tips of arms, arms of bladder extending to posterior edge of acetabulum, 18 pairs of flame cells arranged in groups of 3, flame cell formula 2 $[(3+3+3)+(3+3+3)]$. Excretory pore at anterior margin of caudal insertion pocket bearing spines on each side.

Cercariae active swimmers, activity increasing greatly on contact with tadpoles or salamander larvae. Cercariae penetrate and encyst in 20 to 30 min. No apparent light response.

Metacercariae

Metacercariae reach maximum size of 132 (116 to 146) by 128 (112 to 140) 12 days after infection.

Immature fluke

A total of 103 immature flukes were recovered from 10 laboratory-infected salamanders utilizing tadpoles as a second intermediate host. Twenty-three 5-day-old flukes were also recovered from two salamanders infected utilizing small salamander larvae as the second intermediate host. Flukes recovered from salamanders infected by either method were identical at that age. Testes, ovary, and seminal receptacle were visible in 52-hr-old flukes and spermatozoa were first present in 10-day-old flukes.

Mature flukes

Forty-eight-day-old flukes (Fig. 6): One fluke was recovered from a salamander 48 days after infection. Fluke 2,100 by 600 relaxed, containing numerous eggs.

Cephalogonimus salamandrus *sp. n.* (Figs. 8, 9): Two hundred fifty stained and mounted specimens: 2,936 (2,455 to 3,454) by 936 (714 to 1,292), widest in posttesticular region, spines on anterior half. Oral sucker 176 (156 to 240) by 195 (164 to 240), acetabulum 169 (144 to 200) by 180 (140 to 244) in anterior third of body. Mouth subterminal, prepharynx short, pharynx 56 (45 to 80), followed by short esophagus. Ceca extending to level of posterior testis. Elongate cirrus sac enclosing bipartite seminal vesicle, may overlap acetabulum, base of sac median or dextral. Genital pore terminal, above oral sucker on antero-dorsal surface. Testes in middle third of body, slightly diagonal, anterior testis slightly sinistral, 189 (127 to 232) by 197 (149 to 271), posterior testis slightly dextral, 200 (164 to 272) by 213 (163 to 268), immediately posterior to anterior testis. Ovary generally dextral, posterolateral to acetabulum, 150 (128 to 201) by 140 (104 to 180). Seminal receptacle (81 to 206) in diameter, postero-acetabular, dextral, dorsal to ovary. Vitellaria follicular, in lateral fields from level of pharynx to level of posterior testis. Uterus with ascending and descending loops, occupying most of hindbody. Eggs numerous, 56 (49 to 62). Excretory bladder in posterior half of body, Y-shaped, with lateral outpocketings of arms and stem, pore terminal.

Host: *Ambystoma tigrinum.*

Site: Upper small intestine.

Type locality: Findley Lake, Spokane County, Washington.

Type specimens: USNM Helm. Coll. No. 72599.

Cephalogonimus americanus would stay in *A. tigrinum* from Washington for 10 days when 25 flukes were recovered from the posterior half of the intestine. None was found at 30 days.

Similarly a *R. pretiosa* necropsied 10 days after exposure harbored 34 *C. americanus* in the middle third of the small intestine. These flukes had large concretion globules and were not nearly as well developed as in the controls. A second *R. pretiosa* necropsied at 30 days after exposure was negative. Two *A. tigrinum* from Michigan were necropsied, one at 4 days and the second at 7 days after exposure to *C. americanus.* These were both negative. All nine *R. clamitans* controls were infected and a total of 128 *C. americanus* recovered from the usual host location. At 10 days the lengths of flukes from *A. tigrinum*, *R. pretiosa*, and *R. clamitans* averaged 206.5, 162.5, and 295.0, respectively.

It was also found that *C. salamandrus* would stay in *R. clamitans* for 10 days but flukes were much smaller than in *A. tigrinum* controls and were displaced down the intestine. Six small *C. salamandrus* were recovered from the middle third of the small intestine of a *R. clamitans* necropsied 10 days after exposure. *R. clamitans* necropsied at 20, 30, and 60 days were negative. All *A. tigrinum* controls were infected and *C. salamandrus* were recovered from the proper location in the intestine. Sixty-day flukes from controls contained eggs and all flukes 20 days and older contained sperm.

DISCUSSION

Adults of *C. americanus* and *C. salamandrus* are very similar. The testes of *C. salamandrus* are more oblique and extend into the posterior half of the body, whereas they are always confined to the anterior half of the body in *C.*

americanus. Also the ovary in *C. salamandrus* is more posterior to the acetabulum and the seminal receptacle is dorsal rather than ventral.

Fully embryonated eggs of *C. salamandrus* are 58 long while those of *C. americanus* are 46. The miracidia of *C. salamandrus* have cilia that are 4.3 long compared to 1.5 for the miracidia of *C. americanus*.

Most intrasnail stages of these two parasites are similar. There is a tendency for more extensive branching in the mother sporocysts of *C. salamandrus*.

Cercariae of *C. salamandrus* are larger (320 long extended and 132 contracted) than those of *C. americanus* (175 long extended and 75 contracted). Also, the structure and size of the stylet of the cercariae of *C. salamandrus* differs from that found in *C. americanus* (Fig. 3). The stylet of the former is 26 long and 6 wide at the narrowest point on the shaft, while in the latter it is 17 long and 3.5 wide at the narrowest point on the shaft. The stylet has been shown to be a good indicator in the determination of a species (Lang, 1963).

The authors feel that cercarial morphology can be a more dependable basis for taxonomy of digenetic trematodes than adult morphology. This has been demonstrated by Lang (1963), Cable (1965), Anderson and Anderson (1967), and Martin (1969). It is possible that use of adult morphology alone can be misleading and that before a new species is described, all stages of the life cycle should be evaluated. The morphological differences between these two parasites, and the failure to establish cross-infections, validate *Cephalogonimus salamandrus* as a new species.

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