THE CERCARIA OF *CREPIDOSTOMUM CORNUTUM* (OSBORN)

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THE CERCARIA OF *CREPIDOSTOMUM CORNUTUM* (OSBORN)*

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In the summer of 1936 I undertook to find and identify the apparently hitherto unknown cercaria** of *Crepidostomum cornutum* (Osborn), the progenetic metacercaria having been previously found abundantly here (Stillwater, Oklahoma) in crayfish by Abernathy (1937).

Hopkins (1934) in discussing *C. cornutum* states that "The presence of pigmented eyespots and a stylet in the young metacercaria indicates that the cercaria is of the ophthalmoxiphidiocercaria type." The four known species of *Crepidostomum* cercariae are also of this type (Hopkins, 1934).

In the search for cercariae snails and large clams were collected but especial attention was given to small clams because the four above-mentioned crepidostome cercariae develop in sphaeriids. First efforts to find little bivalves were discouraging. Later they were found in numbers by sifting mud from the bottom of ponds (2 or 3 feet from the edge of the water) through screen wire. The collections were made mostly from "Chandler Lake," a small deep old pond, rich in fauna, near Stillwater.

In one lot of 56 large specimens (the smaller individuals were discarded at the time of collecting) of *Musculium transversum texasense* Sterki 34, or 60 per cent, were infected with an apparently new ophthalmoxiphidiocercaria. To determine whether I had a single species of cercaria (of this type) the infected clams were isolated individually. The cercariae from *each* clam varied somewhat in structure but there were always intermediate forms with regard to any variation and no basis could be found for considering the specimens as being other than conspecific. The new cercaria closely resembles the cercaria of *Crepidostomum cooperi* Hopkins, but appears to differ from it in a number of minor details (see discussion below).

About 108 crayfish, *Cambarus simulans* Faxon were collected from a small pond, apparently free from clams and fish. About one-third of the crayfish were carefully examined immediately for *C. cornutum*, with negative results, another third were saved as controls, and the rest were used in penetration experiments. Best results were obtained with crayfish exposed to fresh cercariae, in sunlight and at a temperature of about 38°C.

* Contribution from the Zoological Laboratory, Oklahoma Agricultural and Mechanical College, prepared under the direction of R. Chester Hughes.

Through the courtesy of Dr. H. B. Ward, I have had the privilege of examining preserved specimens (stained and mounted *in toto*) of *Crepidostomum cornutum* from his private collection.

The clams and crayfish referred to in this paper were respectively identified by Drs. Paul Bartch and Waldo L. Schmitt, both of the United States National Museum.

** Subsequently a brief account of this form, considerably at variance with my findings, has appeared.—Ameel, D. J. 1937. The Life history of *Crepidostomum cornutum* (Osborn). Jour. Parasitol. 23: 218–220.

Of 36 experimental animals 20 became infected, 8 being the maximum number of parasites found per host. The last 5 experimental crayfish were examined 22 days after infection. They yielded 17 small crepidostome metacercariae. No crepidostomes were found in the controls. The experiment was discontinued at the time because the laboratory conditions had become too unfavorable, due probably to heavy chlorination of the city water, to keep crayfish alive.

The experimentally-raised metacercariae (fig. 1) were small crepidostomes with typical oral papillae and diffuse eyespots. In some the stylets were still visible. The larger specimens had fully developed reproductive organs but were not egg-producing. They differ from the metacercaria of *C. cooperi* as described by Hopkins (1934)) in that (1) they are considerably larger in all general measurements, (2) the oral sucker is much larger than the acetabulum, (3) the eyespots have largely disappeared in the larger specimens (they are persistent in *C. cooperi*, even in the adult), (4) the diameter of the pharynx is less than one-third that of the oral sucker, (5) the stylet is smaller and approximately the size given by Hopkins for young metacercariae of *C. cornutum*, (6) its occurrence in crayfish, in which *C. cooperi* has not yet been reported—indeed "a few attempts were made to infest . . . crayfish . . . but without success" by Hopkins (1934). In all respects my specimens conform closely to Hopkins' (1934) description of young metacercariae of *C. cornutum*.

No crepidostomes were found in a number of crayfish, taken from "Chandler Lake" in June, 1936. Later (August), at the time of my experiments, crayfish, *Cambarus nais* Faxon, from the pond were naturally infected with the very young crepidostomes—the incidence being almost 100 per cent. Crayfish were collected and examined from time to time until August, 1937 in order to trace the development of the flukes. The high incidence persisted but the worms never became much larger or more advanced in structure than my experimental specimens. Failure of the flukes to become progenetic may have been due to the fact that the pond was an unfavorable habitat. Progenetic specimens were obtained from the same species of crayfish (*C. nais*) taken from Black Bear Creek near Pawnee, Oklahoma.

The apparently recent establishment of crepidostomes in the crayfish of "Chandler Lake" in the summer of 1936 was attributed to the fact that a few large catfish were added to the pond in the spring (1936). Ameiurids are natural hosts of *C. cornutum*. Efforts to find crepidostomes in any fish of the pond were unsuccessful but this may have been due either to a very light infestation or to a loss of infestation in the meantime.

The cercaria was studied both alive and preserved. Most anatomical details were seen to better advantage in living specimens. *Intra-vitam* staining with neutral red brought out the cystogenous glands, penetration

glands, and female genital primordium. Methyl blue was better for the penetration glands. The flame-cells and excretory tubules were very successfully seen in specimens mounted in dilute fresh human urine under considerable cover-glass pressure—a method suggested by West (1935). The integumentary papillae, always difficult to demonstrate, were best observed in live cercariae mounted under supported cover-glass—the papillae being apparently "ironed out" by pressure. On the other hand, the large papillae around the acetabulum show more clearly under pressure. It was impossible to get satisfactory measurements from living specimens, because of the heavy cover-glass pressure necessary to overcome their motility. The use of some anaesthetics was unsuccessful because the animals came to rest in distorted shapes and soon died. For measurements, best results were obtained by staining *in vivo* in neutral red, fixing in hot 70 per cent alcohol, and mounting in glycerine under supported cover-glass. This seemed to produce a minimum of shrinkage and the specimens died fully extended with their various structures in apparently life-like arrangement and proportions. For permanent mounts cercariae were fixed in sublimate-acetic and stained with borax carmine. Only in these were the testes and brain observed.

The Cercaria of *Crepidostomum cornutum* (Osborn)

Description. Ophthalmoxiphidiocercaria of typical crepidostome form and structure. Body proper elongate, slightly flattened dorso-ventrally. Tail longer than body, slender, gradually tapering to sharply pointed posterior end, contains from 30 to 40 nuclei in stained preserved specimens. Cuticle of body proper covered with minute quincuncially arranged papillae, larger and more numerous on ventral and lateral surfaces. Oral sucker ventro-terminal, a little longer than wide, its orifice fringed with papillae slightly larger than and continuous in distribution with those on the general body surface. Acetabulum slightly posterior to middle of body, a little longer than wide, its orifice also fringed with papillae (the "sticky protuberances" of Hopkins, 1934), those around the posterior three-fourths being very large, those of the anterior fourth much smaller (fig. 3). Eyespots very prominent, a little closer to oral sucker than acetabulum. Pharynx slightly anterior to eyespots, prepharynx distinct, oesophagus longer, bifurcation shortly anterior to acetabulum, caeca saccate extending back nearly to mid-acetabular level, sometimes farther. Three pairs of penetration glands with coarsely granular cytoplasm and large hyaline nuclei, situated lateral to acetabulum. Ducts of the antero-medial pair run forward medial to eyespots, the others lateral to eyespots, thense (all) forward dorsal and lateral to oral sucker to pores closely grouped about point of stylet.

Cystogenous glands (fig. 5), 49 to 62 (average 55) in number, often irregular in shape but generally ovoidal, situated near surface of body

everywhere posterior to level midway between eyespots and acetabulum, have large nuclei and marked affinity for neutral red. Female genital primordium, large, very irregular in shape and size, generally somewhat bilobate, postero-dorsal to acetabulum, varying slightly from median in position toward either side. Testes smaller, ventral to urinary bladder, separated by considerable space, approximately median, tandem or slightly oblique.

Urinary bladder completely surrounded by large apparently glandular cells with very coarsely granular cytoplasm and small nuclei. These cells elongate, pyriform, with smaller ends adjacent to lumen of bladder, arranged in single layer upon basement membrane of bladder like the drupelets of a blackberry upon the receptacle. Bladder with this surrounding coat of cells largely fills body posterior to acetabulum. Lumen of bladder; variable in size and shape in response to body movements; three characteristic forms shown in figure 3; most commonly a small transversely elongate space close to base of tail; partially expanded it becomes pyriform, narrow end forward; fully expanded it is longitudinally elongate and nearly uniform in width; occasionally and momentarily it disappears entirely; continuous with median caudal canal which was traced only a little way. Primary collecting excretory tubules connect to bladder antero-laterally. Flame-cell pattern, 2[(2+2+2)+(2+2+2)], the formula established by Hopkins (1934) and confirmed by Abernathy (1937) for both the metacercaria and the adult.

Measurements in microns of 30 specimens killed in hot 70 per cent alcohol and mounted in glycerine under supported cover-glass. Length×width: body proper, 213 (192–234)×72 (68–85); tail 277 (256–291)×25 (24–26); oral sucker 48 (43–54)×38 (34–42); acetabulum 38 (34–42)×35 (30–40);

EXPLANATION OF PLATE

b, urinary bladder; *c*, cystogenous glands; *g*, apparently glandular cells, with coarsely granular cytoplasm, that surround urinary bladder; *o*, ovary; *p*, genital pores; *t*, testes; *u*, lumen of urinary bladder, represented in three different states of expansion.

All figures concern *Crepidostomum cornutum* (Osborn). Figs. 1, 2, 4, and 5 were drawn with the aid of a camera lucida.

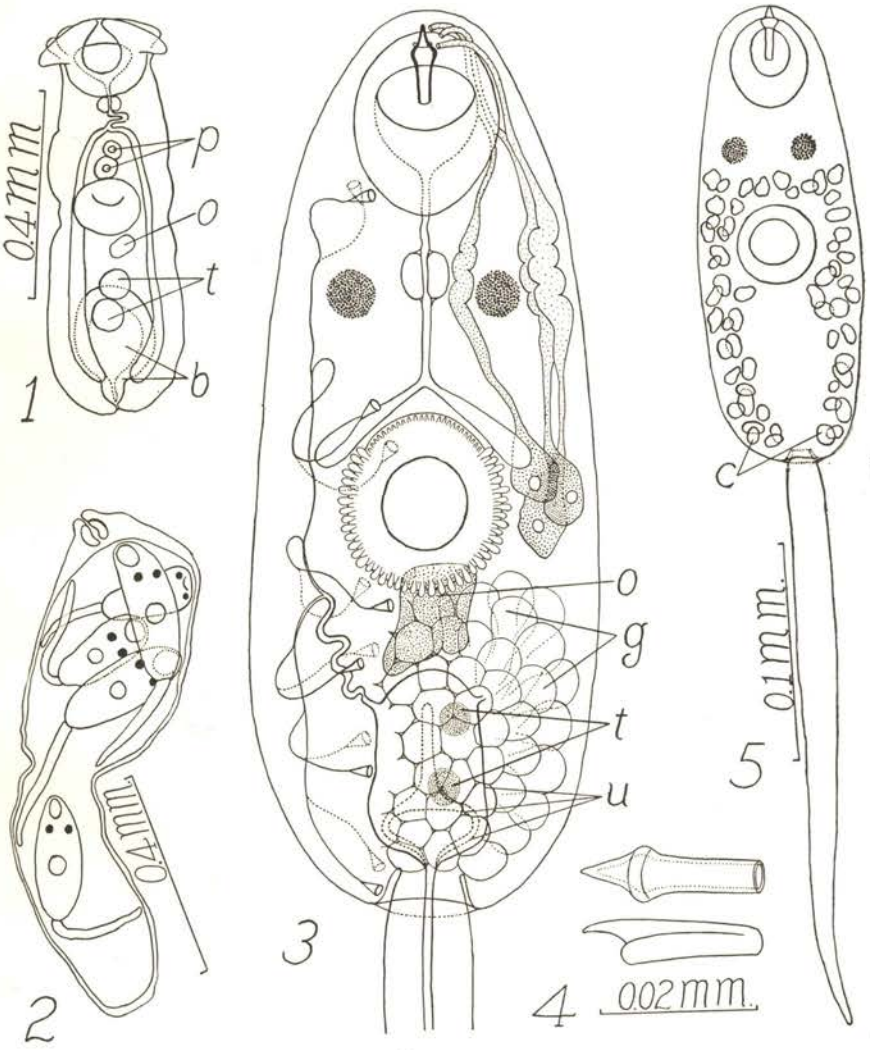
FIG. 1. Ventral view, preserved, experimentally-raised metacercaria, taken from *Cambarus simulans*, 22 days after exposure to cercariae.

FIG. 2. Redia, freshly killed in hot 70 per cent alcohol and mounted in glycerine.

FIG. 3. Cercaria in ventral view. Free-hand drawing based on study of both living and preserved specimens. Flame-cell pattern shown to reader's left, ventral cells in solid lines, dorsal ones in stipples. Penetration glands and glandular cells around urinary bladder shown to right. Papillae around acetabulum as they appear in live specimen, flattened, ventral side up, against a cover-glass.

FIG. 4. Stylet, dorsal and sinistral views.

FIG. 5. Ventral view of cercaria, stained *in vivo* in neutral red, fixed in hot 70 per cent alcohol, and freshly mounted in glycerine.



PLATE

pharynx 11 (10–12)× (10–12); stylet 21 (17–25)×5. Total length of cercaria 490 (488–525); diameter of eyespots 12 (practically constant); distance between eyespots 23 (20–25); cystogenous glands, long diameter 14 (12–17), short diameter 8 (5–12); distance from center of acetabulum to anterior end of body 112 (102–127), to posterior end 104 (102–110); distance acetabulum may protrude from surface of body 28 (25–34); depth of caudal pocket about base of tail 10 (7–13).

Redia (fig. 2) elongate, roughly ovoidal, thin-walled with distinct oral and slight aboral thickenings; with prominent pharynx and short prepharynx; no caecum or birth pore observed; slightly motile. Rediae contained from 2 to 14 cercariae in varying stages of development but all with eyespots. Measurements in microns of 9 specimens preserved in hot 70 per cent alcohol, length 650 (390–887), width 160 (106–213).

Behavior: Cercaria always very positively phototropic. Swims by very rapid vibration of tail, occasionally coming to rest on bottom of container, ventral side up with tail extended vertically. While in resting position, tail sometimes oscillates slowly, describing figure 8, behind the body with long axis of the "8" transverse and arched concave side forward. After several hours, more and more frequent resting periods occur during which the cercaria often turns over to attach itself by its suckers and vibrate the tail vigorously in apparent attempt to detach it. Next, cercaria starts creeping by use of suckers in leech-like manner, the tail dragged in highly contracted and convoluted condition but occasionally extended and vibrated, sometimes becoming detached, sometimes retained until death. Sunlight and a temperature of about 30 degrees C. seem to cause maximum emigration of cercariae from host.

Host: *Musculium transversum texasense* Sterki.

Locality: Stillwater, Oklahoma.

Museum Specimens: Several slides of cercariae mounted *in toto* in United States National Museum.

Discussion

The present species resembles five other known alocreadiid cercariae, namely *Crepidostomum cooperi* Hopkins, *Crepidostomum farionis* (Müller) *Crepidostomum isostomum* Hopkins, *Megalogonia ictaluri* Surber, and *Cercaria arhopalocerca* Nöller, the first of these being apparently the nearest relative.

The present form differs from the cercaria of *C. cooperi* from *Musculium transversum* (Say) and *Pisidium* sp. as described by Hopkins (1934) in that (1) it is considerably smaller in all measured features (Hopkins' measurements were apparently taken from live specimens which would partly account for the differences in size), (2) it has a larger number of cystogenous glands but they are not distributed so far forward and are not

restricted to dorsal side of body, (3) the stylet is smaller and about the size reported by Hopkins for young metacercariae of *C. cornutum*, (4) the female reproductive fundament is apparently smaller and less complex, (5) the orifice of the acetabulum is only partly surrounded by large "sticky protuberances," (6) the occurrence of minute papillae over the general body surface, (7) the urinary bladder has no distinct posterior muscular bulb, (8) the intestinal caeca are longer, (9) it never becomes negatively phototropic, (10) the tail is not generally quickly detached after the cercaria starts creeping, and (11) it penetrates and becomes established in crayfish.

The cercaria of the European *C. farionis* as described by Brown (1927) differs decidedly from the present species in its much larger size, larger number of flame-cells, much longer caeca, relatively longer prepharynx, and more posteriorly located pharynx. The cercaria of *C. isostomum* found in *Sphaerium notatum* Sterki and briefly described by Hopkins (1934) has a longer stylet, relatively shorter tail, and relatively larger pharynx than the present form. The cercaria of *M. ictaluri* found in *M. transversum* and very briefly described by Hopkins (1934) is much smaller with relatively shorter tail and smaller stylet than my cercaria.

A detailed comparison with the European *Cercaria arhopalocerca* is impossible because the description (Nöller, 1925) is very brief, without measurements, and illustrated only by photomicrographs. In experiments the cercaria penetrated and became established in *Chironomus* and *Corethra* larvae. Later Nöller (1927) suggested, without experimental evidence, that the cercaria was probably the larva of *Crepidostomum metoecus* (Braun).

Crepidostomum cornutum was first described as *Bunodera cornuta* by Osborn (1903) from adult flukes found in various centrarchids and silurids and from metacercariae found in crayfish. The species was changed to the genus *Crepidostomum* by Stafford (1904). Osborn's belief that the adult and larva were conspecific was accepted by Ward (1918) and defended by Hopkins (1934). Abernathy (1937) experimentally developed the adult fluke from the crayfish metacercaria in *Ameiurus melas* (Rafinesque).

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