PARASITES FROM LOUISIANA CRAYFISHES

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Abstract

Two microsporidians, *Thelohania* sp. and *? Plistophora* sp., and eight trematodes, *Crepidostomum cornutum*, *Gorgodera amplicava*, *Microphallus opacus*, *M. progeneticus*, *Maritrema obstipum*, *Macroderoides typicus*, *Ochetosoma* sp., and *Paragonimus kellicotti*, are reported from crayfishes in Louisiana. The presence of the metacercariae of *P. kellicotti* is of particular interest since the crayfishes in which this parasite is found are eaten by humans in Louisiana.

Introduction

Nine species of crayfishes have been examined for parasites during studies of paragonimiasis in Louisiana from 1959-1964.

Method of Procedure

Crayfishes were collected by the most expedient methods and usually transported to my laboratory for examination. The live specimens were usually identified by the late Doctor G. H. Penn, Department of Zoology, Tulane University, or identified with the aid of a key by Penn (1959). The crayfishes were kept in aquaria or fingerbowls until they were decapitated and examined. The muscles and internal organs were separated and carefully teased apart with dissection needles while viewed through a binocular stereoscopic microscope. Most parasites were studied alive under a binocular compound microscope. Specimens not discarded were fixed in Alcohol-Formalin-Acetic solution, stained in Van Cleave's Combination Hematoxylin (Van Cleave, 1953), and mounted in Permount (Fisher Sci. Co., N. Y.). The parish of each locality is mentioned the first time the locality is cited in the text.

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Findings

Protozoa

Microsporidia, Nosematidae

1. *Thelohania* sp.
(Figs. 1-4)

Host: Cambarellus shufeldti (Faxon, 1881)
Location: Body musculature
Localities: Chacahoula (Terrebonne Par.) and near Covington (St. Tammany Par.), Louisiana

Discussion: Sogandares (1962a) reported this *Thelohania* sp. from a *C. shufeldti* collected at Chacahoula, Louisiana. Since that time several additional natural infections have been found in the same host species near Covington, Louisiana. Sogandares (1962a) believed his record to be the first for a microsporidian in North American crayfishes. This belief was in error, since Sprague (1950) named in an abstract, *Thelohania cambari* from *Cambarus bartoni* from streams along the Georgia-North Carolina border. He reported that the sporont (pansporoblast of other authors) of *T. cambari* gave rise to eight spores which averaged 4.6 microns long by 2.2 microns in greatest width, were somewhat oval in shape, being broadly rounded at both extremities, tapered slightly from anterior to posterior, and lacked a persistent sporont membrane.

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The polar filament was reported to be 80-90 microns long.

Thelohania sp. from *Cambarellus shufeldti* had pansporoblasts 6-9 microns in diameter, which as a preterminal product contained eight sporoblasts. Each sporoblast formed one spore, resulting in eight spores surrounded by the pansporoblast membrane. Live spores measured from 3.0 to 3.5 microns long by 1.2 to 1.6 microns wide. Polar filaments about 15 microns long observed in 3 spores were in all probability only partially extruded.

The Louisiana *Thelohania* from *C. shufeldti* probably represents a different species from *T. cambari* since the spores differ in size and the pansporoblast membrane of the former is persistent. *Thelohania contejeani* Henneguy and Thelohan, 1892, the only European species of *Thelohania* from crayfishes, has a spore 2 to 3 microns long. Thus, *T.* sp. from Louisiana has spores which are intermediate in size between more of *T. cambari* and *T. contejeani.* The specific identity of the Louisiana *Thelohania* from *C. shufeldti* awaits information regarding the filament length and life-history, but low incidence of natural infection makes the necessary study difficult to complete.

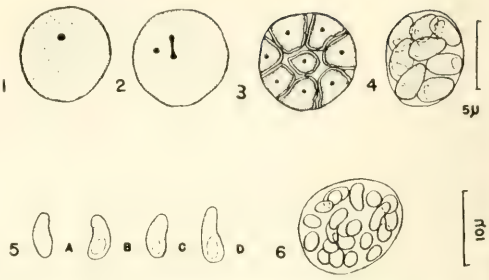
2. ? *Plistophora* sp.
(Figs. 5-6)

Host: Cambarellus puer Hobbs, 1945
Location: Body musculature
Locality: Near Covington, Louisiana
Discussion: Sogandares (1962a) reported this form from one *C. puer.* This species is characterized by 19 to 21 comma-shaped spores, about 6.0 to 9.0 microns long by 4 microns wide, contained in the pansporoblast membrane. I have not collected this species again.

Platyhelminthes
Trematoda, Digenea
Allocreadiidae

3. *Crepidostomum cornutum* (Osborn, 1903) Stafford, 1904
(Fig. 7)

Hosts: Cambarellus puer Hobbs, 1945; *C. shufeldti* (Faxon, 1881); *Orconectes lancifer* (Hagen, 1870); *Procambarus clarkii* (Girard, 1852); *P. blandingi acutus* (Girard, 1852); *P. penni* Hobbs, 1951
Location: Hepatopancreas, heart, pericardial membranes and musculature of cephalothorax.



Figures 1-6. *Microsporidians.* **1-3.** *Thelohania* sp., developing pansporoblasts; **4.** same, pansporoblast membrane surrounding spores; **5A, B, C, D,** *?Plistophora* sp. variation in shape and size of spores; **6.** same, pansporoblast membrane surrounding spores. [Courtesy Journal of Parasitology 48(3): 493]

Localities: C. puer, C. shufeldti, and *O. lancifer* from Gibson (Terrebonne Par.) and Maringouin (Iberville Par.); *P. clarkii* from Gibson, Ama, Bonnet Carre Spillway (St. Charles Par.), Sarpy (St. Charles Par.), Amite River on U. S. Hwy. 190 (East Baton Rouge Par.), Maringouin, Bayou close to Rosedale on La. Hwy. 76 (West Baton Rouge Par.), 1.7 mi. N. Junction La. Hwy. 20 on La. Hwy. 309 (Terrebonne Par.); *P. blandingi acutus* from Amite River on U. S. Hwy. 190; *P. penni* from near Pineville (Rapides Par.); all Loiusiana localities.

Discussion: C. cornutum is without doubt one of the most common and widespread trematodes found encysted in Louisiana crayfishes. Hopkins (1934) reported this species from crayfishes (listed as *Cambarus* sp. from Baton Rouge, *Amphiuma means* Cuvier, "catfish," and *Ictalurus melas* (Rafinesque) (=*Ameiurus melas*) in Louisiana. Stafford (1931) reported this species from *Cambarus* spp. in the neighboring state of Mississippi. The crayfishes reported by Hopkins (1931) and Stafford (1931) cannot be identified because at that time most North American crayfishes were assigned to the genus *Cambarus.*

Hopkins (1934) and Ameel (1937) both observed that encysted metacercariae of *C. cornutum* produced eggs. I observed such progenesis frequently in my specimen of *C. cornutum* from Louisiana. It is really not known if these eggs are the result of parthenogenesis or self-matings, or if the re-

sultant developmental stages are capable of continuing with their normal biological functions of reproduction.

One first intermediate host of *C. cornutum* in Louisiana is a sphaeriid clam of the genus *Musculium.* Naturally infected sphaeriids have been collected at Gibson and at a bayou near Rosedale at the junction of La. Hwys. 76 and 413. Cercariae from clams of both localities readily encysted on the surface of the heart and hepatopancreas of laboratory reared *P. clarkii* and *C. shufeldti,* lightly encysted matacercariae being found as early as 16 hours postexposure.

Natural definitive hosts of *C. cornutum* found in this study were *Amia calva* Linn. and *Lepomis macrochirus* (Rafinesque). Other hosts in Louisiana have been cited above.

Gorgoderidae

4. *Gorgodera amplicava* Looss, 1899

(Figs. 8-10)

Hosts: Orconectes palmeri creolanus (Creaser, 1933), *Procambarus clarkii* (Girard, 1852)

Location: On lower quadrant of stomach wall, usually at level of gastric mill

Localities: O. palmeri creolanus from stream drainage into Bayou Sarah, about 1 mile S. Mississippi State Line (West Feliciana Par.); *P. clarkii* from Ama, Bonnet Carre Spillway, Sarpy, Maringouin, Edgard (Lafourche Par.), Buras (Plaquemines Par.), Pierre Pass (Assumption Par.), Venice (Plaquemines Par.), and 1.7 mi. N. Jct. La. Hwy. 20 on La. Hwy. 309.

Discussion: Krull (1935) described the life cycle of this species. The partial life-cycle of *G. amplicava* has been experimentally established in this laboratory in young *Rana clamitans* Latreille and adult *Amphiuma means* Cuvier. *Rana catesbeiana* Shaw is one definitive natural host of *G. amplicava* in Louisiana, and has been shown by Penn (1950) to feed on crayfishes. *Hyla cinerea* (Schneider) and *Chaenobryttus gulosus* (Cuv. & Val.) were refractory to infection. Judging from the site of encystment, I suspect that crayfishes become infected by ingesting the cercariae. Almost all crayfishes collected in Sarpy, an oil field near Norco, Louisiana, were infected with *G. amplicava.* The species has been previously reported from crayfishes by Krull (1936).

Microphallidae

5. *Microphallus opacus* (Ward, 1894) Ward, 1901

(Figs. 11-13)

Host: Cambarellus puer Hobbs, 1945 and *Procambarus clarkii* (Girard, 1852)

Location: Hepatopancreas

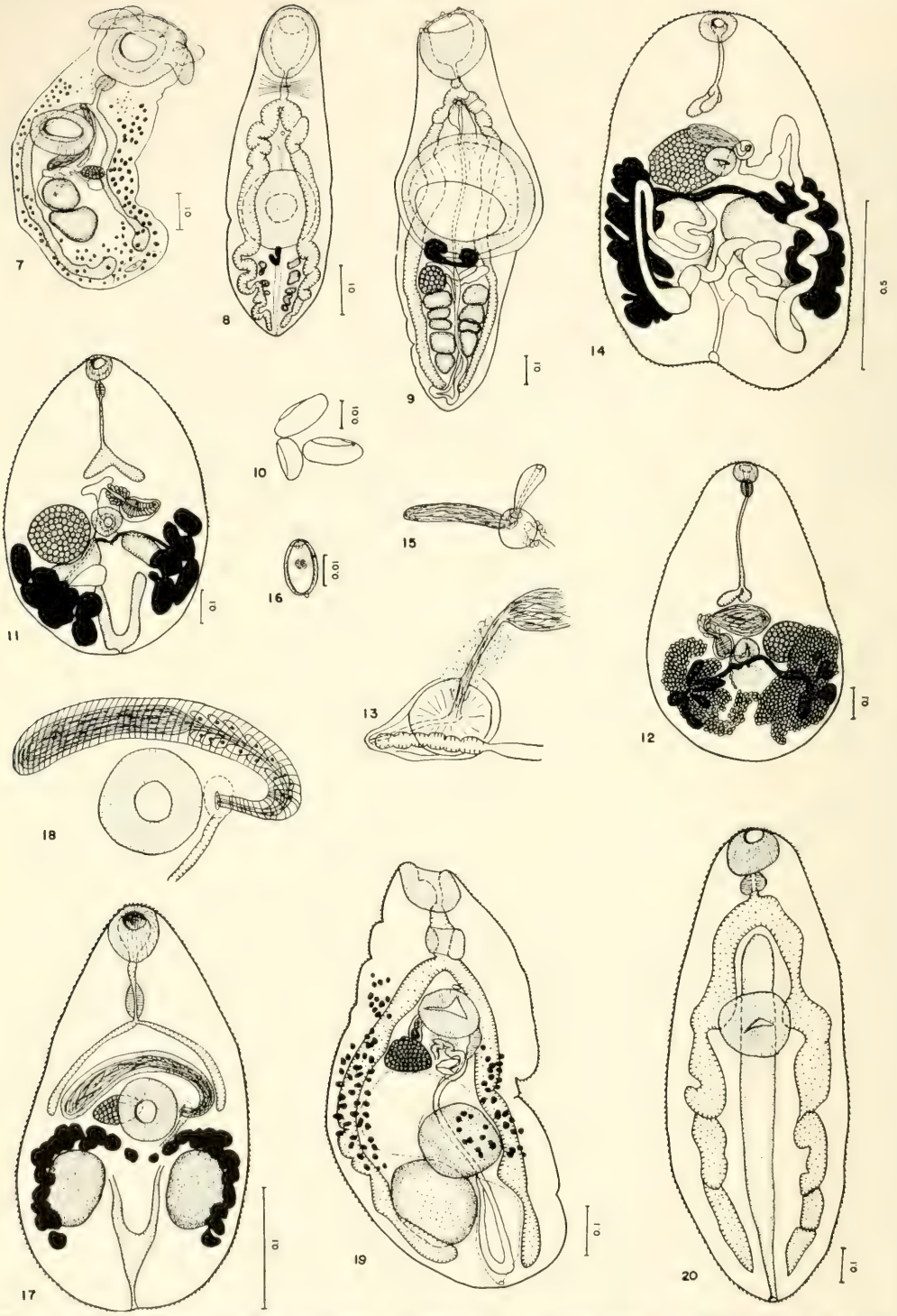
Locality: Bayou close to Rosedale on La. Hwy. 76.

Discussion: M. opacus was the most common metacercaria in *P. clarkii* at the Rosedale locality, being found in about 98% of those examined. *Amnicola,* probably *integra,* from the same locality released three different types of microphallid cercariae, probably corresponding with the 3 microphallid tremadoes encysted in crayfishes from the same bayou.

Encysted specimens of *M. opacus* were injected under the cephalothorax of *P. clarkii* and *C. puer* from uninfected localities, but these worms did not produce eggs, even after three weeks, though they did remain alive for this period of time. CF_1 white mice were fed cysts of *M. opacus* and gravid specimens were recovered after 24 hours. The reservoirs for the adults of *M. opacus* in the Rosedale locality are unknown, though the species will develop to maturity in a number of vertebrates (Rausch, 1947).

In this laboratory *M. opacus* would produce eggs in 0.85% NaCl-1:20,000 Streptomycin sulfate solution in 12 to 36 hours at 30 C. Metacercariae were artificially excysted by vigorously shaking and incubating the entire infected crayfish hepatopancreas in a pepsin-HCl solution (100 ml. 0.3% HCl and 40 ml. 0.5% pepsin N.F.) at 40 C for 30 minutes, then incubating the washed cysts (0.85% NaCl) in a trypsin solution (0.5% trypsin in 0.85% NaCl, adjusted to pH 7.8 with K_2HPO_4) held at 40 C for 10-12 min. The excysted metacercariae were then washed in 30 C 0.85% NaCl-1:20,000 Streptomycin sulfate solution and later incubated in the same but clean solution. Thousands of clean excysted metacercariae could be obtained by following the above method.

Rausch (1947) observed that when metacercariae of *M. opacus* were left in crayfish hepatopancreas extract overnight at room temperatures they excysted, found partners and began to mate. Ward (1900) also observed worms from natural infections *in*



Figures 7-20. *Digenetic Trematodes.* **7.** *Crepidostomum cornutum,* excysted metacercaria; **8.** *Gorgodera amplicava,* excysted metacercaria; **9.** same, adult from experimental infection of *Amphiuma;* **10.** same, eggs; **11.** *Microphallus opacus;* excysted metacercaria; **12.** same, adult from experimental infection of CF_1 mouse; **13.** same, dorsal view of terminal genitalia; **14.** *Microphallus progeneticus,* adult from *Cambarellus puer;* **15.** same, terminal genitalia; **16.** same, egg containing miracidium; **17.** *Maritrema obstipum,* excysted metacercaria; **18.** same, terminal genitalia; **19.** *Macroderoides typicus,* excysted metacercaria; **20.** *Paragonimus kellicotti,* excysted metacercaria. The projected scales have their values indicated in millimeters. All figures accompanied by a projected scale were drawn with the aid of a Leitz camera lucida for inclined microscopes. Those figures lacking a scale are sketches.

copula. In several experiments in this laboratory, encysted metacercaries from post-pepsin-HCl treatment were individually isolated and incubated in trypsin-saline solution. The resulting excysted worms, which had not been able to mate with a partner, were individually isolated in 4 x 40 mm tubes containing NaCl-Streptomycin solution and kept at room temperature. Approximately 11% of the single worms treated as indicated above produced eggs of unascertained viability. By following almost the same procedure in another experiment, 50 pairs of excysted *M. opacus* metacercariae were placed in identical tubes (2 worms per tube) and solutions. Eighty-four *percent* of these worms produced eggs, the viability of which was not determined. Further experimentation on this aspect has not been continued since a severe drought in 1963 decimated the snail and crayfish populations. At present it is not known if mating is always necessary for the production of viable eggs, nor is it known if temperature or possible increase in titer of materials released by the mated pairs was responsible for egg production. Ching (1963a), for example, has indicated that increased (30 to 40 C) temperature stimulated egg production in *Levenseniella charadriformis* Young, 1949. In another experiment (Ching, 1963b), she did not observe egg production of another microphallid, *Maritrema laricola* Ching, 1963, when held at 30 to 40 C. The viability of the eggs of the former species was not tested. Experiments of this type are necessary to answer certain questions of interest to trematode specialists. For example: Are eggs from isolated worms a result of parthenogenesis or self-fertilization? Are the developmental stages of eggs produced by "self-matings" viable throughout their life-cycles? If self-matings are possible, what is the statistical significance of such self-matings on the species population structure?

6. *Microphallus progeneticus* Sogandares, 1962

(Figs. 14-16)

Host: Cambarellus puer Hobbs, 1945, *Procambarus clarkii* (Girard, 1852)

Location: Cephalothoracic cavity

Locality: Gibson, Maringouin, Bayou close to Rosedale on La. Hwy. 76, Louisiana.

Discussion: This species, first found by Dr. Joseph Fitzpatrick, then a graduate student and assistant in my laboratory, was described by Sogandares (1962b). It is unique in lacking a complete pharynx and may be gravid when unencysted and wandering over the organs of the cephalothorax of the affected hosts. Eggs *in utero* contain actively moving miracidia.

Live specimens of *Microphallus progeneticus* were intubated *per ora* into several CF_1 laboratory mice, but none were recovered upon necropsy after 24 hours. The worms presumably were passed or digested by the host mice. *M. progeneticus,* except for the lack of a pharynx, is close to *M. opacus,* and may represent a sibling species of the latter. However, *M. opacus* is encysted and *M. progeneticus* is free in the crayfish hosts. Even if *M. opacus* could excyst in a crayfish host under certain conditions, which seems unlikely, excysted specimens experimentally introduced into the hemocoels of crayfishes failed to mature in three weeks, (see under *M. opacus*), though maturation took place in CF_1 laboratory mice and *in vitro.* It seems unlikely that gene flow between the two forms could occur even if both were ingested by a single vertebrate host.

7. *Maritrema obstipum* (Van Cleave and Mueller, 1932) Mueller, 1934

(Figs. 17-18)

Host: Cambarellus shufeldti (Faxon, 1881) and *Procambarus clarkii* (Girard, 1852)

Location: Central shaft of gill filaments and hepatopancreas

Locality: Bayou close to Rosedale on La. Hwy. 76, Louisiana

Discussion: This species was first observed by me in the gill filaments of its hosts. Later, great numbers were easily recovered from the hepatopancreas of affected hosts with the excystment procedure described under *Microphallus opacus.* Etges (1953) studied the life-history of *Maritrema obstipum* and found it encysted in the isopod *Asellus communis.* His identification of *Maritrema obstipum* seems doubtful at present, and completion of the life-history of the forms from crayfishes may elucidate the identity of his species. The first intermediate host reported by Etges (1953) was *Amnicola pilsbryi.* One of the three microphallid cercariae from *Amnicola,* reported under

Microphallus opacus, is probably the larva of this species. Stafford (1931) reported a *Maritrema* sp. from *Cambarus* in Mississippi.

Plagiorchiidae

8. *Macroderoides typicus* (Winfield, 1929) Van Cleave and Mueller, 1932

(Fig. 19)

Hosts: Procambarus blandingi acutus (Girard, 1852) *P. clarkii* (Girard, 1952), and *Orconectes lancifer* (Hagen, 1870)

Location: Cephalothoracic and antennal musculature

Localties: P. blandingi acutus and *P. clarkii* from Edgard, *P. clarkii* from Bayou near junction of La. Hwys. 413 and 76 (West Baton Rouge Par.), and *O. lancifer* from Gibson, Louisiana.

Discussion: Macroderoides typicus also utilizes tadpoles as second intermediate hosts (McMullen, 1935). The cercaria emerges from *Helisoma trivolvis lentum* in Louisiana. Adults of *M. typicus* have been found in *Amia calva* Linn. from Louisiana. Exposure of various species of sunfishes to cysts of *M. typicus* produced negative results.

9. *Ochetosoma* sp.

Host: P. clarkii (Girard, 1852)

Location: Abdominal musculature

Locality: Bayou close to Rosedale on La. Hwy. 76, Louisiana

Discussion: Adult hosts of *Ochetosoma* in Louisiana are usually snakes of the genera *Natrix* and *Agkistrodon.* Certain species of Louisiana *Ochetosoma* are known to utilize physid snails and tadpoles as intermediate hosts (Byrd, 1935). Physids, tadpoles, and watersnakes were present in the locality in which the crayfishes were found infected with *Ochetosoma.* Penn (1950) has indicated that certain watersnakes feed heavily on crayfishes. Ninety-seven of 100 crayfishes from the locality were found infected with *Ochetosoma.*

Troglotrematidae

10. *Paragonimus kellicotti* Ward, 1908

(Fig. 20)

Hosts: Procambarus blandingi acutus (Girard, 1852) and *P. clarkii* (Girard, 1852)

Location: Heart and surrounding membranes

Locality: Amite River on U. S. Hwy. 190

Discussion: My colleague, Dr. E. A. Malek, and I first collected this species in crayfishes. Experimental infections have been established in his and my laboratories, and a separate paper describing our results is in preparation.

Summary

Parasites found in Louisiana crayfishes during this study are listed in Table 1. All localities visited for collection of crayfishes are indicated in Figure 21. Localities from which infected crayfishes were taken are indicated, under the specific parasite being considered, in the text. Where possible, local, experimental, and natural intermediate and definitive hosts of parasites discussed are cited in the text.

The parasites found inhabiting crayfishes in Louisiana are represented by two microsporidians of the Nosematidae, and 8 digenetic trematodes by one species of Allocreadiidae, one species of Gorgoderidae, three species of Microphallidae, two species of Plagiorchiidae, and one species of Troglotrematidae.

The presence of *Paragonimus kellicotti,* the North American mammalian lung fluke, is of particular interest since the specific hosts in which this parasite was found are utilized as food by humans in Louisiana.

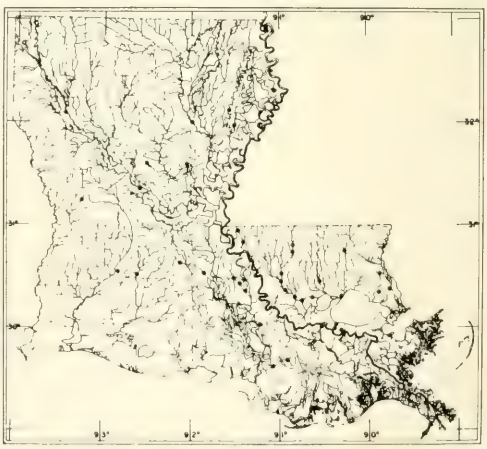


Figure 21. Map showing collecting localities in Louisiana. Areas visited are indicated by dark circles.

Host-Parasite Index

Cambarellus puer
- *Crepidostomum cornutum*
- *Microphallus opacus*
- *Microphallus progeneticus*
- ?*Plistophora* sp.

Cambarellus shufeldti
- *Crepidostamum cornutum*
- *Maritrema obstipum*
- *Thelohania* sp.

Orconectes clypeatus (Hay, 1899)
- Negative

Orconectes lancifer
- *Crepidostomum cornutum*
- *Macroderoides typicus*

Orconectes palmeri creolanus
- *Gorgodera ampuicava*

Procambarus clarkii
- *Crepidostomum cornutum*
- *Gorgodera amplicava*
- *Macroderoides typicus*
- *Maritrema obstipum*
- *Microphallus opacus*
- *Microphallus progeneticus*
- *Ochetosoma* sp.
- *Paragonimus kellicotti*

Procambarus blandingi acutus
- *Crepidostomum cornutum*
- *Macroderoides typicus*
- *Paragonimus kellicotti*

Procambarus penni
- *Crepidostomum cornutum*

Procambarus vioscai (Penn, 1946)
- Negative

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