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Helminths of the Western Mosquitofish (*Gambusia affinis*) in Bayou Traverse, Louisiana, U.S.A.

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ABSTRACT: Helminth parasites of mosquitofish (*Gambusia affinis*) were surveyed in Bayou Traverse, a waterway in the LaBranche wetlands of southeastern Louisiana, U.S.A. In total, 1,255 parasites belonging to 8 species were collected and identified, including *Ascocotyle ampullacea*, *Ascocotyle mcintoshi*, *Ascocotyle tenuicollis*, *Phagicola diminuta*, *Phagicola* sp., *Echinochasmus swartzi*, *Posthodiplostomum minimum*, and *Glossocercus* sp. Adult parasites were obtained by infecting mice (*Mus musculus*), ducks (*Anas platyrhynchos*), and chicks (*Gallus domesticus*) with metacercariae from *G. affinis*. Bayou Traverse is characterized by snails and fish typical of brackish water habitats and is home to a rich piscivorous bird and mammal fauna. The parasites found in this study are consistent with helminths that parasitize birds and mammals as adults. This study is a survey of the trematodes and cestodes from an estuarine habitat.

KEY WORDS: *Gambusia affinis*, bayou, Louisiana, helminth parasites, digenetic trematodes, *Ascocotyle ampullacea*, *Ascocotyle mcintoshi*, *Ascocotyle tenuicollis*, *Phagicola diminuta*, *Phagicola* sp., *Echinochasmus swartzi*, *Posthodiplostomum minimum*, *Glossocercus* sp.

Gambusia affinis, mosquitofish, are members of the live-bearer family Poeciliidae. Members of this family are dominant constituents of the freshwater and brackish fish fauna of the Americas (Ross, 2001). They are characterized by an upward turned mouth, flattened head, and internal fertilization facilitated by a male structure called a gonopodium. *Gambusia affinis* are small fish characteristic of quiet, shallow waters along the edges of streams, ponds, lakes, and estuaries. They consume a variety of phyto- and zooplankton, including organic detritus, microcrustaceans, cladocerans, and mosquito pupae (Ross, 2001). *Gambusia affinis* are tolerant of a broad range of environmental conditions, including large fluctuations in temperature, salinity, and dissolved oxygen (Al-Daham and Bhatti, 1977). Their tolerance, small size, and short generation time make them excellent laboratory models (Meffe and Snelson, 1989). This study is a survey of the helminth parasites of *G. affinis* in Bayou Traverse, Louisiana, U.S.A., and represents the first survey of the helminth fauna of this fish species in North America.

MATERIALS AND METHODS

Located on the southwest corner of Lake Pontchartrain in Louisiana, the LaBranche wetlands encompass 20,000 acres of swamp and marshland. These wetlands are part of the essential infrastructure that protects Louisiana from the ravages of hurricanes as well as providing breeding and nursery grounds for commercially valuable fish and crustacean species. Located within the La Branche wetlands, Bayou Traverse is characterized by sparse stands of bald cypress (*Taxodium distichum*). Cypress knees cover the low-lying banks and extend well into the bayou, which is approximately 20–30 m wide and 0.5–2.5 m deep.

Thirty adult female *G. affinis* (20–30 mm) were collected from Bayou Traverse 0.8 km upstream from the confluence of Bayou Traverse with Bayou LaBranche (30°1.18′N; 90°21.28′W). Live fish were transported to the laboratory in buckets of habitat water and maintained in aquariums with standard filtration and aeration. Fish were anesthetized with TMS-222 (Sigma Chemical Co., St. Louis, Missouri, U.S.A.), placed in a petri dish containing 0.7% saline, and necropsied using standard parasitological techniques (Dailey, 1996) and a Bausch and Lomb 0.7–3× dissecting microscope. Wet mounts of the gills, heart, and viscera were examined under a BH-2 compound light microscope (Olympus). The number and location of all metazoan parasites were recorded, and a preliminary identification of each specimen was made.

Metacercariae were excysted in 0.85% saline containing 0.22 (w/v) sodium bicarbonate, 0.5 (w/v) trypsin, and 0.3 (w/v) pepsin in a 37°C water bath (Irwin, 1983). Excysted metacercariae were viewed with a light microscope. Specimens were fixed using Berland's solution (acetic acid: formaldehyde, 9:1) and alcohol-formalin-acetic acid (AFA) and subsequently stained with Semichon's acetic-carmine, destained in 70% acid ethanol, dehydrated in an alcohol series, cleared with xylene, and mounted on permanent slides with gum damar.

Adult trematodes were obtained by infecting mice (*Mus musculus*), ducks (*Anas platyrhynchos*), and chicks (*Gallus domesticus*) with metacercariae from *G. affinis*. Parasite species were separated into 5-cm glass dishes containing 0.85% NaCl and kept on ice. Excess tissue and viscera were

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removed from around the metacercariae. Mice were anesthetized and intubated using a 5-cc syringe modified with a 1.0-mm-diameter flexible plastic tube containing approximately 200 metacercariae from a single species of trematode. Mice were necropsied 1–14 days postinfection (dpi). Ducks and chicks were fed viscera or gills containing metacercaria. The birds were necropsied 1–3 dpi. The small intestine from each host was divided into 4 parts anterior to posterior and placed in separate 5-cm glass dishes containing 0.85% NaCl and kept on ice. Each piece of the small intestine was opened using a pair of small, sharp scissors and laid flat. A glass slide was used to scrape villi from the intestinal wall that was then examined for parasites.

Avian hosts shed infections fairly quickly (Font et al., 1984a), so the presence of eggs was used to confirm the maturity of adult specimens. Recovered adult specimens were examined under light microscopy and fixed using Berland's and AFA. Specimens were stained using the procedure outlined above. Identifications were made using original descriptions and species lists. Voucher specimens are deposited in the United States National Parasite Collection (USNPC), Beltsville, Maryland, U.S.A. Infection parameters follow Bush et al. (1997).

Measurements are presented in micrometers as range values followed by mean values ± SDs in parentheses. Measurements not reported previously are denoted with a single asterisk (*), and measurements that extend the range or differ from previous reports are denoted with a double asterisk (**).

RESULTS

In total, 1,255 parasites belonging to 8 species were collected and identified from *G. affinis*, including *Ascocotyle ampullacea*, *Ascocotyle mcintoshi*, *Ascocotyle tenuicollis*, *Phagicola diminuta*, *Echinochasmus swartzi*, *Posthodiplostomum minimum*, and *Glossocercus* sp. One species of metacercariae that differed morphologically from descriptions of known species was designated *Phagicola* sp.

Class Trematoda
Heterophyidae
Ascocotyle ampullacea Miller and Harkema, 1962
(Figs. 1, 2)

Metacercaria: From 5 *G. affinis* (n = 15). Cysts spherical 175.5–207.9 (187.92 ± 12.60)**; thick-walled 24.3–48.6 (36.72 ± 9.07)**. Body pyriform, tegument finely spined. Body length 331.5–397.8 (369.7 ± 31.6) **, body width at pharynx 134.0–175.5 (153.9 ± 15.0)*, body width at ventral sucker 116.0–191.1 (153.4 ± 29.6)*, maximum body width 134.0–184.0 (163.3 ± 25.9)*. Oral sucker width 46.8–62.4 (54.6 ± 7.3)**, with appendix, armed with 2 rows of 22–24 spines. Oral spines equal in length 5.4–10.8 (7.7 ± 1.3)**. Prepharynx 42.9–80 (53.9 ± 14.9) and pharynx present, 42.9–70.2 (55.4 ± 12.8)** long by 46.8–62.4 (54.6 ± 7.3)** wide. Esophagus 19.5–28.0 (25.6 ± 3.5)* long. Cecal platelets discoid 8.1–16.2 (12.0 ± 2.9)*. Cecal platelets thin, mostly single, but stacking 2–5 together in some instances. Ventral sucker 38.0–58.5 (45.1 ± 7.8)** long by 42.0–54.6 (46.3 ± 5.2)** wide. Gonotyl 7.8–26.0 (15.5 ± 6.6)* long by 28.0–40.0 (34.8 ± 6.2)* wide. Left testis 28.0–66.0 (50.2 ± 15.7)** long by 39.0–60.0 (47.1 ± 8.4)** wide and right testis 30.0–66.3 (50.1 ± 17.3)* long by 32.0–74.0 (50.1 ± 16.4)* wide. Ovary 10.0–24.0 (19.4 ± 5.7)* long by 30.0–42.9 (37.6 ± 4.9)* wide (Fig. 1).

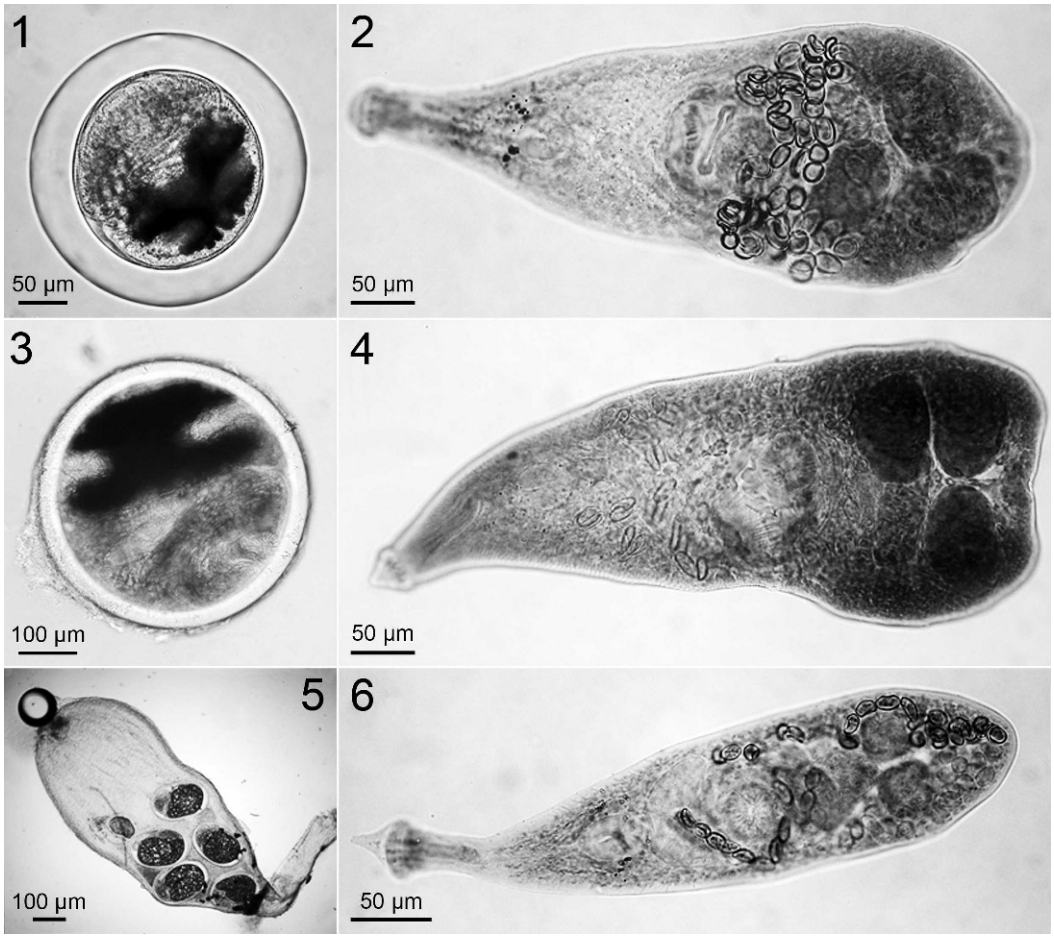
Abundance, prevalence, and range of infection: 9.1, 80%, 1–38.

Sites of infection: Intestinal tract, liver, and fat bodies.

Adults: From 5 chicks 3dpi (n = 15). Body pyriform, tegument finely spined. Body length 347.6–429.0 (388.2 ± 35.3)**, body width at pharynx 74.1–98.0 (81.2 ± 9.6)*, body width at ventral sucker 114.0–154.0 (134.6 ± 14.7)*, maximum body width 130.0–160.0 (148.5 ± 12.8)**. Oral sucker width 27.3–40.0 (31.3 ± 5.1)**, with appendix, armed with 2 rows of 22–24 spines. Oral spines equal in length 5.4–10.8 (7.7 ± 1.3)**. Prepharynx 58.5–100.0 (85.58 ± 16.0)* and pharynx present, 30.0–40.0 (32.9 ± 4.0)** long by 23.4–36 (26.9 ± 5.3)* wide. Esophagus 24.0–46.0 (33.8 ± 10.0)** long. Ventral sucker 39.0–50.0 (42.2 ± 4.5)** long by 44.0–50.7 (47.3 ± 2.9)* wide. Gonotyl 12.0–20.0 (17.1 ± 3.8)* long by 40.0–50.7 (44.1 ± 5.7)* wide and bearing 12–14 refractile bodies. Left testis 34.0–50.0 (43.3 ± 6.0)** long by 40.0–56.0 (49.3 ± 6.5)** wide and right testis 36.0–58.5 (47.7 ± 9.8)** long by 38.0–60.0 (47.7 ± 8.9)** wide. Ovary 36.0–50.0 (42.56 ± 5.7)** long by 40.0–66.3 (49.26 ± 10.9)** wide. Eggs 18.0–24.0 (20.2 ± 2.3)* long by 10.0–14.0 (11.2 ± 1.8)* wide (Fig. 2).

Ascocotyle mcintoshi Price, 1936
(Figs. 3, 4)

Metacercaria: From 5 *G. affinis* (n = 15). Cysts spherical 299.6–466.2 (407.7 ± 73.0)**; thick-walled 29.7–33.3 (31.9 ± 2.0)**. Body pyriform, tegument finely spined. Body length 624.1–756.6 (744.14 ± 112.5)**, body width at pharynx 113.1–179.4 (151.3 ± 24.1)*, body width at ventral sucker 152.1–230.1 (199.7 ± 29.5)*, maximum body width 202.8–273.0 (234.0 ± 30.8)*. Oral sucker width 31.2–66.3 (5.8 ± 13.3)**, with appendix, armed with



Figures 1–6. *Ascocotyle* species infecting mosquitofish, *Gambusia affinis*. **1.** *Ascocotyle ampullacea* metacercaria. **2.** *Ascocotyle ampullacea* adult. **3.** *Ascocotyle mcintoshi* metacercaria. **4.** *Ascocotyle mcintoshi* adult. **5.** *Ascocotyle tenuicollis* metacercariae. **6.** *Ascocotyle tenuicollis* adult.

2 rows of 18–20** spines. Anterior row of oral spines longer than posterior; anterior 13.2–16.2 (14.3 ± 1.3) posterior 8.1 (8.1 ± 0)**. Prepharynx 117.0–175.5 (137.3 ± 26.9) and pharynx present, 35.1–66.3 (49.1 ± 11.6)** long by 35.1–46.8 (41.3 ± 4.4)** wide. Esophagus 31.2–58.5 (42.9 ± 14.3)* long. Cecal platelets discoid 8.1 (8.1 ± 0)*. Ventral sucker 46.8–54.6 (49.1 ± 3.5) long by 46.8–54.6 (50.7 ± 2.8)** wide. Gonotyl 39.0–58.5 (46.8 ± 8.3)* long by 78.0–89.7 (82.7 ± 5.1)* wide. Left testis 58.5–85.8 (71.0 ± 11.2) long by 81.9–109.2 (94.4 ± 10.8)** wide and right testis 58.5–78.0 (67.86 ± 8.1)* long by 78.0–105.3 (88.1 ± 10.5)* wide. Ovary 31.2–78.0 (43.7 ± 19.4)** long by 50.7–74.1 (62.4 ± 11.0)** wide (Fig. 3).

Abundance, prevalence, and range of infection: 10.8, 87%, 1–47.

Sites of infection: Intestinal tract, liver, and fat bodies.

Adults: From 5 chicks 3dpi (n = 15). Body pyriform, tegument finely spined. Body length 678.6–906.2 (722.5 ± 31.4)**, body width at pharynx 117.0–148.2 (134.9 ± 12.5)**, body width at ventral sucker 117.0–206.7 (184.1 ± 37.8)*, maximum body width 173.8–234.0 (205.6 ± 22.0). Oral sucker width 39.0–54.6 (46.0 ± 5.8)**, with appendix, armed with 2 rows of 18–20 spines. Anterior row of oral spines longer than posterior; anterior 13.2–16.2 (14.3 ± 1.3) posterior 8.1–8.1 (8.1 ± 0)**. Prepharynx 117.0–175.5 (137.3 ± 26.9) and pharynx present, 46.8–50.7

(49.1 ± 2.1) long by 42.9–46.8 (43.7 ± 1.7) wide. Esophagus 39.0–42.9 (40.6 ± 2.1) long. Cecal platelets discoid 2.7–8.1 (6.1 ± 1.5)*. Ventral sucker 46.8–54.6 (49.1 ± 3.5)** long by 46.8–54.6 (50.7 ± 2.8) wide. Gonotyl 89.7–202.8 (118.6 ± 47.4)* long by 39.0–58.5 (46.8 ± 9.1)* wide. Left testis 62.4–78.0 (71.0 ± 7.0) long by 74.1–85.8 (78.8 ± 4.3) wide and right testis 50.7–81.9 (67.9 ± 11.6)** long by 97.5–105.3 (100.6 ± 4.3) wide. Ovary 46.8–74.1 (63.2 ± 10.1) long by 78.0–85.8 (81.9 ± 3.9) wide. Eggs 19.5 (19.5 ± 0) long by 11.7 (11.7 ± 0) wide (Fig. 4).

Specimens deposited: USNPC 101108, 101109

Ascocotyle tenuicollis Price, 1935 (Figs. 5, 6)

Metacercaria: From 10 *G. affinis* (n = 15). Cysts oval 288.6–399.6 (359.6 ± 45.5) long by 222.2–244.2 (185.4 ± 92.0)**, cyst wall of moderate thickness 5.4–8.1 (7.3 ± 1.2)*. Body pyriform, tegument finely spined. Body length 362.7–448.5 (237.9 ± 45.7)**, body width at pharynx 32.0–84.0 (58.7 ± 26.0)*, body width at ventral sucker 88.0–96.0 (91.3 ± 4.2)*, maximum body width 114.0–124.0 (119.3 ± 50.0)**. Oral sucker width 30.0–40.0 (35.3 ± 5.0)**, with appendix, armed with two rows of 16 spines. Anterior row of oral spines longer than posterior row; anterior 13.5–16.2 (14.7 ± 1.4)**, posterior 8.1–10.8 (8.4 ± 0.9). Prepharynx 100.0–132.6 (118.9 ± 16.9)** and pharynx present, 30.0–32.0 (31.3 ± 1.2)* long by 24.0–30.0 (28.0 ± 3.5)* wide. Cecal platelets discoid 13.5–16.2 (15.3 ± 1.3)*. Cecal platelets thin, mostly single, sometimes stacking side by side. Ventral sucker 36.0–40.0 (38.7 ± 2.3) long by 40.0–50.0 (44.0 ± 5.3)** wide. Gonotyl 10.0 (10.0 ± 0)* long by 18.0 (18.0 ± 0)* wide. Left testis 28.0–30.0 (28.7 ± 1.2)** long by 24.0–30.0 (27.3 ± 3.0)** wide and right testis 24.0–32.0 (28.7 ± 4.2)* long by 20.0–40.0 (30.7 ± 10.1)* wide. Ovary 14.0–20.0 (17.0 ± 4.2) long by 20.0 (20.0 ± 0)* wide (Fig. 5).

Abundance, prevalence, and range of infection: 1.8, 67%, 1–10.

Sites of infection: Conus arteriosus.

Adults: From 5 chicks 3dpi (n = 15). Body pyriform, tegument finely spined. Body length 358.8–455.1 (399.1 ± 38.0), body width at pharynx 56.0–81.0 (67.0 ± 10.0)*, body width at ventral sucker 74.0–108.0 (89.2 ± 14.0)**, maximum body width 80.0–105.3 (92.7 ± 11.2)**. Preoral lobe 21.6–108.0 (57.2 ± 46.4)*. Oral sucker width 28.0–37.8 (32.6 ± 4.0), with appendix, armed with 2 rows of 16 spines. Anterior row of oral spines longer than posterior row; anterior 10.8–16.2 (13.5 ± 1.9), posterior 8.1–10.8 (8.4 ± 0.9). Prepharynx 109.2–142.0 (121.6 ±12.7)** and pharynx present, 32.0–40.5 (34.5 ± 3.8) long by 26.0–28.0 (27.0 ± 1.0) wide. Ventral sucker 17.0–40.5 (30.7 ± 8.6)** long by 34.0–40.0 (36.23 ± 2.7)** wide. Gonotyl 10.0 (10.0 ± 0)** long by 22.0–30.0 (26.4 ± 3.3)** wide. Left testis 32.0–41.0 (37.1 ± 4.5)** long by 29.7–44.0 (35.1 ± 5.8)** wide and right testis 26.0–40.0 (34.5 ± 5.5)** long by 32.0–48.0 (38.8 ± 5.9) wide. Ovary 21.0–28.0 (24.4 ± 3.0)** long by 16.0–40.5 (24.9 ± 10.2)** wide. Eggs 20.0 (20.0 ± 0.0) long by 10.8 (10.8 ± 0.0) wide (Fig. 6).

Specimens deposited: USNPC 101110, 101111.

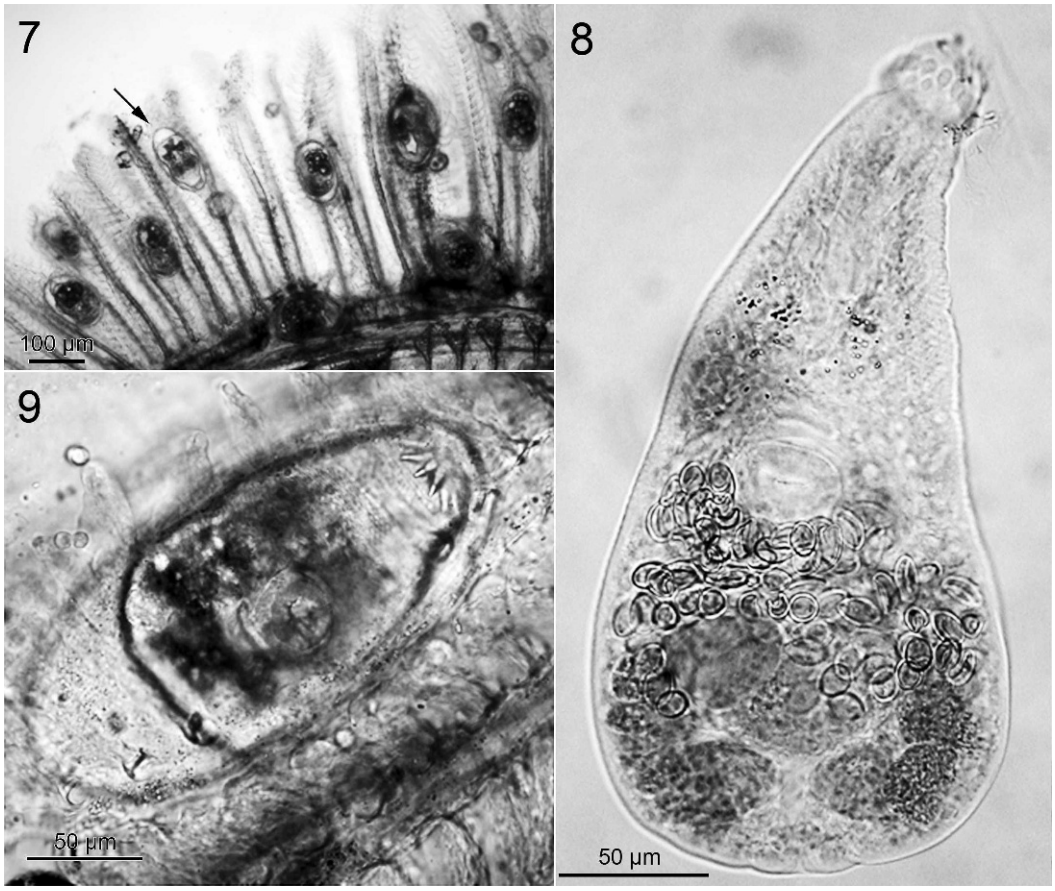
Phagicola diminuta Stunkard and Haviland 1924 (Figs. 7, 8)

Metacercaria: From 5 *G. affinis* (n = 11). Cysts oval, elongate 153.9–197.1(158.8 ± 13.4) long by 94.5–118.8 (100.2 ± 7.9)** wide. Body pyriform, tegument finely spined. Body length 218.7–277.5 (248.9 ± 24.2)**, body width at pharynx 27.0–58.0 (38.7 ± 14.0)*, body width at ventral sucker 56.7–81.0 (71.9 ± 11.3)*, maximum body width 70.2–89.1 (82.8 ± 8.7)**. Oral sucker 22.0–29.7 (27.4 ± 3.7)** wide, with appendix, armed with 1 anterior row of 16 spines and 2 posterior spines. Anterior row of oral spines 8.1–10.8 (10.4 ± 1.0)**, posterior 6.8–8.1 (7.5 ± 0.8). Pharynx present 21.6–27.0 (24.2 ± 2.7)** long by 16.2–20.0 (17.5 ± 2.2)** wide. Cecal platelets discoid 8.1–13.5 (10.2 ± 2.5)*. Ventral sucker 24.0–27.0 (25.3 ± 1.4) long by 20.0–32.4 (27.4 ± 5.4) wide. Gonotyl 8.0–8.0 (8.0 ± 0)* long by 20.0–20.0 (20.0 ± 0)*. Left testis 16.2–20.0 (18.8 ± 1.8)** long by 18.9–24.3 (22.3 ± 2.5)** wide. Right testis 14.0–24.0 (18.3 ± 4.3)* long by 18.9–27.0 (24.0, ± 3.6)* wide. Ovary 12.0–16.2 (13.4 ± 2.0)* long by 10.0–16.2 (13.6 ± 3.1) wide* (Fig. 7).

Abundance, prevalence, and range of infection: 14.2, 97%, 1–10.

Sites of infection: Gill filaments and occasionally in the gill rakers.

Adults: From 2 mice (n = 12), from 1 chick 2 dpi (n = 1), and from 1 chick 3dpi (n = 1). Measurements are given first for all worms combined, and then the mean and SD are given separately for each experimental host. Body pyriform, tegument finely spined. Body length 277.5–421.8 (334.9 ±



Figures 7–9. Species of *Phagicola* infecting the mosquitofish, *Gambusia affinis*. **7.** *Phagicola diminuta*, metacercariae and the metacercaria of an unidentified species of *Phagicola* (arrow) in gill filaments. **8.** *Phagicola diminuta*, adult. **9.** *Phagicola* sp. metacercaria.

56.8) (327.5 ± 41.0 mice; 277.5 chick 2 dpi; 421.8 chick 3 dpi), body width at pharynx 32.4–94.5 (56.3 ± 25.3)* (54.0 ± 27.8 mice; 40.5 chick 2 dpi; 81.0 chick 3 dpi), body width at ventral sucker 67.5–135.0 (110.3 ± 23.5)* (119.5 ± 13.7 mice; 67.5 chick 2 dpi; 116.1 chick 3 dpi), maximum body width 94.5–163.0 (133.8 ± 25.5) (146.7 ± 17.2 mice; 94.5 chick 2 dpi; 121.5 chick 3 dpi). Oral sucker 21.6–40.5 (28.8 ± 6.3)** (31.1 ± 6.4 mice; 21.6 chick 2 dpi; 27.0 chick 3 dpi) wide, with appendix, armed with 1 anterior row of 16 spines and 2 posterior spines. Anterior row of oral spines 10.8–10.8 (10.8), posterior row 8.1–8.1 (8.1) (anterior and posterior spines same for all infected hosts). Pharynx present 18.9–37.8 (31.1 ± 7.2)** (31.7 ± 8.9 mice; 27.0 chick 2 dpi; 32.4 chick 3 dpi) long by 18.9–27.0 (22.1 ± 4.0)** (20.9 ± 4.1 mice; 21.6 chick 2 dpi; 27.0 chick 3 dpi) wide. Ventral sucker 24.3–43.2 (32.4 ± 6.6) (31.7 ± 3.4 from mice; 24.3 chick 2 dpi; 43.2 chick 3 dpi) long by 32.4–40.5 (38.3 ± 3.2) (39.8 ± 1.4 from mice; 32.4 chick 2 dpi; 37.8 chick 3 dpi) wide. Gonotyl 10.8–54.0 (23.0 ± 18.0)** (26.3 ± 20.5 from mice; 32.4 chick 1–3 dpi) long by 8.1–32.4 (16.2 ± 10.4) (22.3 ± 9.5 from mice; 8.1 chick 3 dpi) wide. Left testis 18.9–48.6 (32.9 ± 10.7)** (28.4 ± 9.0 from mice; 35.1 chick 2 dpi; 48.6 chick 3 dpi) long by 18.9–45.9 (32.4 ± 11.4)** (27.2 ± 10.1 from mice; 39.7 chick 2 dpi; 45.9 chick 3 dpi) wide. Right testis 18.9–43.2 (33.8 ± 8.7)** (31.1 ± 9.2 mice; 35.1 chick 2 dpi; 43.2 chick 3 dpi) long by 32.0–43.2 (36.4 ± 4.8) (34.3 ± 4.1 from mice; 37.8 chick 2 dpi; 43.2 chick 3 dpi) wide. Ovary 24.0–37.8 (31.5 ± 4.8) (24.6 ± 14.2 from mice; 32.4 chick 2 dpi; 37.8 chick 3 dpi. Eggs 16.2–20.3 (15.5 ± 1.5) (18.6 ± 1.7 from mice; 18.9 chick 2 dpi) long by 8.1–10.8 (8.8 ± 1.2)** (8.8 ± 1.4 from mice; 8.1 chick 2 dpi) wide (Fig. 8).

Specimens deposited: USNPC 101106.

Phagicola sp. (Fig. 9)

The metacercaria of *Phagicola* sp. was morphologically distinct from the metacercaria of *P. diminuta* but did not match the description of any heterophyid metacercaria reported previously from the gills of *G. affinis*. Metacercariae of *Phagicola* sp. were relatively rare compared with other heterophyid metacercaria, and adults were not obtained.

Metacercaria: From 10 *G. affinis* ($n = 15$). Cysts oval, strongly elongated 183.6–224.1 (207.2 ± 10.5) long by 81.0–118.8 (100.3 ± 10.2) wide. Body length 345.6 (345.0 ± 0). Prominent anterior row of 16 spines and 2 posterior spines. Anterior row of oral 16.2 (16.2 ± 0) posterior 10.8 (10.8 ± 0). Pharynx 40.5 (40.5 ± 0) long and ventral sucker 54.0 (54.0 ± 0) long (Fig. 9).

Abundance, prevalence, and range of infection: 3.0, 73%, 1–21.

Sites of infection: Gill filaments and occasionally in the gill rakers.

Specimens deposited: USNPC 101107.

Echinostomatidae
Echinochasmus swartzi Price, 1931 (Figs. 10, 11)

Metacercaria: From 10 *G. affinis* ($n = 11$). Body doubled in aspherical cysts 43.2–67.5 (50.32 ± 7.9) long by 35.1–54.0 (34.61 ± 5.8)** wide (Fig. 10).

Abundance, prevalence, and range of infection: 1.2, 43%, 1–2.

Sites of infection: Gill filaments.

Adults: From 1 mouse 1 dpi ($n = 2$). Body spindle shaped with close scale-like spines anteriorly that spread posteriorly and disappear at the posterior margin of the posterior testis. Body length 632.7–899.1 (765.9 ± 188.4)**. Head collar strongly developed, reniform, with single, dorsally interrupted row of 22 spines. Oral spines 46–50 (48 ± 2.8) long and 10–12 (11 ± 1.4)** wide at base. Prepharynx 37.8–113.4 (75.6 ± 53.5)** and pharynx 94.5–105.2 (99.9 ± 7.6) long and 81.0–81.0 (81.0)* wide. Oral sucker 67.5–75.6 (71.55 ± 5.7)* wide. Body width at pharynx 135.0–164.7 (149.9 ± 21.0)**, body width at ventral sucker 102.6–226.8 (164.7 ± 87.8)**, maximum body width 102.6–226.8 (164.7 ± 87.8). Ventral sucker 97.2–102.6 (99.9 ± 3.8)* long and 108.0–113.4 (110.7 ± 3.8)*. Testes tandem; anterior testis 59.4–67.5 (63.45 ± 5.7)** long and 81.0–81.0 (81.0)** wide, posterior testis 32.4–81.0 (56.7 ± 34.4)** long and 67.5–67.5 (67.5)** wide. Ovary 68.0 long and 45.9 wide (Fig. 11).

Specimens deposited: USNPC 101112.

Posthodiplostomum minimum MacCallum 1921 (Fig. 12)

Neascus: From 1 *G. affinis* ($n = 2$). Two distinct body regions; body 777–843.6 (810.3 ± 47.09); forebody 555 (555 ± 0) long by 177.6 (177.6 ± 0)** wide; hindbody conical to spheroidal. Oral sucker 27–29.7 (28.35 ± 1.91)**, prepharynx short, pharynx 24.3 (24.3 ± 0)** long. Ventral sucker 48.6 (48.6 ± 0), tribocytic (= holdfast organ sensu Niewiadomska, 2002) organ 116.1 (116.1 ± 0) in diameter (Fig. 12).

Abundance, prevalence, and range of infection: 0.7, 33%, 1–2.

Sites of infection: Body cavity.

Specimens deposited: USNPC 101113.

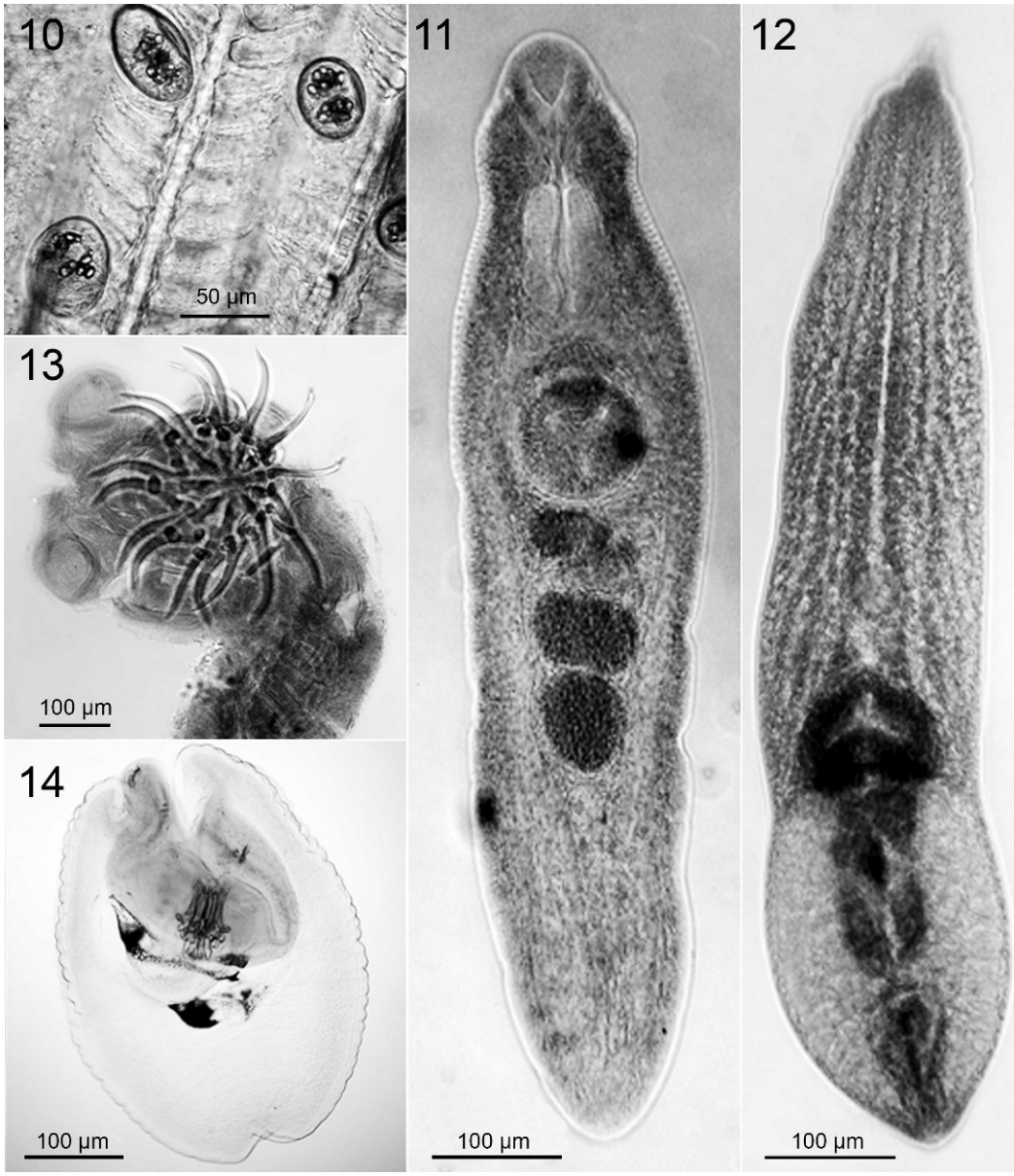
Class Cestoidea
Dilepididae
Glossocercus sp. (Figs. 13, 14)

Metacestode: From 5 *G. affinis* ($n = 5$). Metacestodes very large, with body divided into 2 parts; anterior part with elongate scolex 750–832.5 (791.6 ± 57.9); posterior part larger and wider, tapering posteriorly. Scolex bearing 4 spherical suckers 64.8–155.4 (106.7 ± 33.5) long by 72.9–166.5 (116.1 ± 38.8) wide. Rostellum bearing 2 circles of 20 massive hooks, 10 in each circle, with well-developed amorphous part (epiphyseal thickenings on handle and guard). Distal hooks 210.6 (210.6 ± 0); blade 143.1 (143.1 ± 0); handle 67.5 (67.5 ± 0); blade:handle ratio 2.12. Proximal hooks 175.5 (175.5 ± 0); blade 121.5 (121.5 ± 0); handle 54.0 (54.0 ± 0); blade:handle ratio 2.3 (Figs. 13, 14).

Abundance, prevalence, and range of infection: 0.03, 3%, 1–1.

Sites of infection: Body cavity and intestinal mesenteries.

Specimens deposited: USNPC 101114.



Figures 10–14. Various helminths infecting the mosquitofish, *Gambusia affinis*. **10.** *Echinochasmus swartzi*, metacercaria. **11.** *Echinochasmus swartzi*, adult. **12.** *Posthodiplostomum minimum*, neascus, **13.** *Glossocercus* sp., evaginated metacestode. **14.** *Glossocercus* sp., invaginated metacestode.

DISCUSSION

Numerous trematodes and cestodes have been reported from *G. affinis* in North America, as summarized in Hoffman (1999). Whereas these studies have tended to focus on specific parasite species, this study presents a comprehensive survey of helminth species collected from *G. affinis*. A review of species morphology and life history data from the literature is included because it is used as the criteria for specific identification and provides an ecological context for the relevance of the community of parasites. We believe additional life history and

possibly molecular studies are necessary to establish the phylogenetic relationships among heterophyids. Therefore, our taxonomic treatment of these species does not split genera into smaller taxonomic groups as done by Pérez-Ponce de León et al. (2007).

Ascocotyle ampullacea was described from the small intestine of a raccoon, *Procyon lotor*, as having a double row of 20–24 spines surrounding the oral sucker (Miller and Harkema, 1962). The gonotyl was described as possessing a distinct row of spines. *Ascocotyle ampullacea* is similar to *Ascocotyle angeloi* in that both species have intermediate characteristics between the genera *Ascocotyle* and *Phagicola*. Despite their similarities, *A. ampullacea* has a larger acetabulum, larger oral sucker, shorter ceca, and overall smaller body size than *A. angeloi*. *Pseudascocotyle mollienisicola* also resembles *A. ampullacea* but lacks oral spines altogether, and the gonotyl is penetrated by the uterus (Sogandares-Bernal and Bridgman, 1960). In a survey of raccoon parasites, *A. ampullacea* is listed as being a coastal parasite of birds (Harkema and Miller, 1964).

In a survey of brackish water fish parasites of Florida (Stein, 1968), the metacercarial cysts of *A. ampullacea* from *G. affinis* were described as being "of the 'thick wall' type" and, except for their smaller size, closely resembling *A. mcintoshi*. *Ascocotyle ampullacea* metacercaria from the liver of *G. affinis*, were infective to chicks and recovered, mature helminths were identical to the description of Miller and Harkema (1962). *Ascocotyle ampullacea* were found in multiple sites of different species of fish from Mexico, including muscles (*Gambusia yucatana*), intestinal wall (*Poecilia sphenops*), mesenteries (*Poecilia* sp.), and gonads (*Belonesox belizanus*). The original description of *A. ampullacea* was corrected by describing the gonotyl as possessing 12–14 refractile bodies rather than spines (Scholz et al., 2001).

Ascocotyle ampullacea collected from *G. affinis* in Bayou Traverse were consistent with the original description (Harkema and Miller, 1964), and the cysts were thick-walled as described by Stein (1968). Metacercariae of *A. ampullacea* were abundant in the intestinal tract, liver, and fat bodies of *G. affinis* and often were found together with large numbers of *A. mcintoshi* metacercaria. Adult *A. ampullacea* were recovered only from chicks.

Ascocotyle mcintoshi was described from the small intestine of the white ibis, *Guara alba*, in Florida, U.S.A. *Ascocotyle mcintoshi* differs from other species by the length of the intestinal ceca, which extends to the level of the testes rather than terminating before the posterior edge of the ovary. A double row of 18–20 circumoral spines is present (Price, 1936). The life history of *A. mcintoshi* was described by Leigh (1974). The rediae and cercaria of *A. mcintoshi* were obtained from infected brackish water prosobranch snails, *Littoridinops monoroensis*. Larger size, more robust appearance, and lack of a fin fold on the tail distinguished *A. mcintoshi* from other heterophyid cercariae. Unlike typical heterophyid cercariae that penetrate the gills, skin, and musculature of the fish intermediate host, *A. mcintoshi* becomes entrapped in the fish's respiratory currents, are taken into the mouth, and swallowed. Metacercaria were recovered from the intestinal mesenteries, attached to the visceral peritoneum, liver, gall bladder, and fat bodies. Heavy infections had masses of cysts attached to viscera or fat bodies. *Ascocotyle mcintoshi* metacercariae were reported in the mesenteries of *G. a. holbrooki*, *Mollienesia latipinna*, *Xiphophorus helleri*, *Xiphophorus* sp., and *Poecilia mexicana* (Leigh, 1956; Scholz et al., 2001). Individual worms were reported with equal numbers of spines in each circumoral row but with variation among individuals possessing 19, 20, and 21 spines per row (Scholz et al., 2001). Mature *A. mcintoshi* were reported from experimentally infected day-old, unfed chicks (Leigh, 1974; Scholz et al., 2001). *Ascocotyle mcintoshi* have been reported from the Roseate spoonbill (*Ajaia ajaja*), little blue heron (*Egretta caerula*), and the great egret (*Ardea albus*) (Sepúlveda et al., 1994, 1996, 1999).

Ascocotyle mcintoshi were recovered in large numbers from mesenteries around the intestinal tract, liver, and fat bodies of *G. affinis* in Bayou Traverse. Metacercariae were frequently associated with concurrent infections by *A. ampullacea*. Heavy infections were as described by Leigh (1974), with large masses of cysts embedded in mesenteries and viscera. Adult trematodes were obtained only from experimentally infected chicks and matched the original description by Price (1936). The number of circumoral spines varies slightly among individuals in this species and our results were consistent with Price (1936) of 18–20 spines per circumoral row.

Ascocotyle tenuicollis metacercaria were described by Scholz et al. (1997) from the heart of a naturally infected *Astyanax fasciatus* and from the gills of *Poecilia petenensis* that were experimentally infected with cercariae from the hydrobiid snail *Pyrgophorus coronatus*. The presence of *A. tenuicollis* metacercaria in the gills and gill musculature is considered a

departure from the typical site specificity demonstrated by most species of *Ascocotyle* metacercaria (Font et al., 1984a, b). Because *A. tenuicollis* penetrates the epithelium of the gills and migrates to the heart via the circulatory system, the encystment of some parasites in the gills cannot be excluded (Scholz et al., 1997). Leigh (1956) reports *A. tenuicollis* metacercaria from *Chaenobryttus coronarius* and fishes in the genera *Gambusia* and *Mollienesia*. Ostrowski de Núñez (1976) reports *A. tenuicollis* from the freshwater fishes *Cnesterodon decemmaculatus* and *Phalloceros caudimaculatus*. *Ascocotyle tenuicollis* has been frequently reported in the hearts and occasionally the gill arches of numerous species of Characidae, Poeciliidae, Cichlidae, and Synbrachidae in Mexico (Aguirre-Macedo and García-Magaña, 1994; Scholz et al., 1995, 1997, 2001; Salgado-Maldonado et al., 2004, 2005).

Ascocotyle tenuicollis was described from adult worms in the small intestine of an American bittern (*Botaurus lentiginosus*) in Texas, U.S.A. (Price, 1935). Adult *A. tenuicollis* were found in the little blue heron, the tricolor heron (*Hydranassa tricolor ruficollis*), and experimentally in chicks (Leigh, 1956; Yamaguti, 1971). Herons (*Casmerodius albus*), eagles (*Buteogallus anthracinus*), great blue herons (*Ardea herodias*), and cormorants (*Phalacrocorax olivaceus*) have been reported as hosts (Aguirre-Macedo and García-Magaña, 1994; Scholz et al., 2001). The ultrastructure of the metacercarial cysts of *A. tenuicollis* from California was examined by transmission electron microscope (TEM) (Armitage, 2000). In a study of the seasonal dynamics of the helminths of bluegill sunfish (*Lepomis macrochirus*), microhabitat was found to significantly influence the seasonal prevalence of *A. tenuicollis*. Although some parasite recruitment occurred year-round, transmission of *A. tenuicollis* peaked in the summer and declined in the fall (Steinauer and Font, 2003).

Ascocotyle tenuicollis was commonly encountered in the conus arteriosus of *G. affinis* from Bayou Traverse but not in large numbers. Adult specimens were recovered from experimentally infected chicks.

Ascocotyle (*Parascocotyle*) *diminuta* was described from the intestine of a rat in New York (Stunkard and Haviland, 1924). This description included only a single row of 16 oral spines. Experimental infections of rats, mice, hamsters, herring gull (*Larus argentatus*), and black-crowned night heron (*Nycticorax nycticorax*) yielded adult *P. diminuta*. Chicks proved refractory to infection. Based on the adult specimens obtained from experimental infections, the description of *P. diminuta* was expanded and emended to include the row of 16 oral spines plus an additional 2 spines in a second row (Stunkard and Uzmann, 1955).

Descriptions of the redia, cercaria, metacercaria, and adult stages of *P. diminuta* led to its resurrection from synonymy with *A. angrense* (Sogandares-Bernal and Lumsden, 1963; Ostrowski de Núñez, 1993). The life cycle was reproduced experimentally using naturally infected *Littoridina castellanosae* and *L. parchappei* (Hydrobiidae); experimentally exposed *Cnesterodon decemmaculatus*; and naturally infected fish, including *Gambusia affinis*, *Jenynsia lineate*, and *Cyclasoma fascetum*. Ostrowski de Núñez (1993) recovered adults from experimentally infected chicks and mice and a naturally infected egret (*Egretta thula*).

Metacercaria of *P. diminuta* have been reported from *Fundulus heteroclitus* and *F. majalis* in Connecticut, U.S.A. (Stunkard and Uzmann, 1955); *Poecilia petenensis*, *P. latipunctata*, and *P. velifera* in Mexico (Scholz et al., 1995); *Fundulus similis* in Florida, U.S.A. (Hutton and Sogandares-Bernal, 1960b); *Cyprinodon variegatus*, *F. grandis*, *F. jenkinsi*, *Lucania parva*, rainwater fish (Cypinodontidae), and *Mollienisia latipinna* in Louisiana (Sogandares-Bernal and Bridgman, 1960); and *Belonesox belizanus*, *F. similis*, *F. grandis*, and *Floridichthys carpio* in Mexico (Scholz et al., 1997). Although poecilliids were successfully infected with cercaria released from the hydrobiid snail *P. coronatus*, experimental infections with the cichlid *Cichlasoma meeki* failed (Scholz et al., 1997). The green heron (*Butorides virescens*), cormorant (*Phalacrocorax auritus floridanus*), tricolor heron, raccoon, great egret (*Casmerodius albus*), and great blue heron (*Ardea herodias*) are natural, definitive hosts (Price, 1932; Hutton and Sogandares-Bernal, 1960a; Harkema and Miller, 1962, 1964; Scholz et al., 1997, 2001; Sepúlveda et al., 1999). Adult *P. diminuta* were reported from experimental infections of hamsters with the gills of *F. similis* in Florida (Hutton and Sogandares-Bernal, 1960b). The ultrastructure of the metacercarial cysts of *P. diminuta* from California, U.S.A., was examined by TEM (Armitage, 2000).

Phagicola diminuta was the most frequently encountered helminth parasite of *G. affinis* in Bayou Traverse. Specimens were found throughout the gill filaments and occasionally in the gill rakers. Adults were recovered from the small intestines of experimentally infected mice and chicks.

Echinochasmus swartzi (Price, 1931) was described from the small intestine of a muskrat (*Ondatra zibethica*) and a dog (*Canis familiaris*). Metacercariae were found encysted in the gills of *F. heteroclitus* and yielded adult worms when fed to rats, mice, guinea pigs, pigeons, and dogs (Lillis and Nigrelli, 1956).

Metacercaria of *E. swartzi* were found exclusively in the gill filaments of *G. affinis* from Bayou Traverse. Adult worms were recovered from experimentally infected mice. Although *G. affinis* from other sites in Louisiana frequently are heavily infected with *E. swartzi*, fish from Bayou Traverse have relatively light infections with only 1 or 2 metacercaria per fish (Brock and Font, personal observations).

Posthodiplostomum minimum MacCallum (1921) is a ubiquitous parasite in aquatic environments; it has a high rate of infection per fish, parasitizes a great many species of fish, and has a large geographic distribution. *Posthodiplostomum minimum* demonstrates a high degree of morphological variability both within and between fish hosts (Haderlie, 1953). A valuable checklist of hosts, including more than 75 species of fish, is provided by Pérez-Ponce de León (2007). The adult is found in the small intestines of herons. Eggs pass with the feces and miracidia hatch in freshwater. The miracidia of *P. minimum* infect snails in the genus *Physa*, among others (Hoffman, 1958). Sporocysts develop in the snail, and daughter sporocysts have been documented from natural infections (Miller, 1954). Cercariae emerge from the snail and penetrate a fish, developing into almost sexually mature metacercaria in the kidney, liver, pericardium, and spleen. This allows *P. minimum* to emerge quickly and begin producing eggs rapidly when ingested by a heron (Hoffman, 1958).

Posthodiplostomum minimum has a long taxonomic history complicated by multiple names for different life stages. Two cercariae were described, *Cercaria multicellulata* (Miller, 1923, 1925) and *Cercaria louisiana* (Miller, 1936), both from *Physa gyrina*. *Cercaria multicellulata* from *Ph. gyrina*, *Ph. acuta*, and *Ph. heterostropha* was used to infect fish with *P. minimum* (Ferguson, 1936, 1938; Hunter, 1936; Ferguson, 1937; unpublished thesis abstract, University of Illinois, Champaign-Urbana, Illinois, U.S.A.). The description of the cercariae *C. louisiana* was considered erroneous by Hoffman (1958). The metacercaria of *P. minimum* encysted in the liver of a centrarchid was initially misidentified by Leidy (1856) as *Diplostomum cuticola* von Nordmann, 1832. Agerborg (1926) described a metacercaria, *Diplostomum vancleavei*, that later proved identical to the metacercaria described by Leidy (1856). The metacercaria was placed in the larval group *Neascus* by Hughes (1928) and renamed *Neascus vancleavei*. Adult *P. minimum* was described from a heron in the New York Zoo, New York, U.S.A. (MacCallum, 1921). Adult specimens of *P. minimum* were mistakenly described as *Neodiplostomum orchilongum* (Noble, 1936). When the genus *Posthodiplostomum* was created by Dubois (1936), *Diplostomum minimum* was reduced to synonomy with *Posthodiplostomum minimum*.

There are many species of *Neascus* that are morphologically similar when immature so adult parasites are needed for accurate identification. Because we were unable to obtain adult *P. minimum* from experimental infections, this is a provisional identification. *Posthodiplostomum minimum* was found in the body cavity of roughly a third of the *G. affinis* sampled from Bayou Traverse.

A single metacestode of *Glossocercus* sp. was obtained in this study. We can only assign this larva to the genus *Glossocercus* until adult worms are recovered. The life cycle of *Glossocercus* is currently unknown, but it probably uses a copepod as the first intermediate host; freshwater or brackish fish as the second intermediate host; and reaches sexual maturity in piscivorous birds, such as herons and cormorants (Bona, 1975, 1994; Scholz et al., 2001, 2002).

The LaBranche wetlands are dominated by fish typical of brackish to freshwater, including Lepisosteidae, Clupeidae, Fundulidae, Poeciliidae, and Centrarchidae (Hastings et al., 1987; Neill and Turner, 1987; Moore, 1992). The wetlands are also home to a rich piscivorous bird and mammal fauna, including egrets, herons, spoonbills, ducks, raccoons, opossums, minks, and nutria rats. The parasites found in this study are consistent with helminths that parasitize birds and mammals as adults.

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