

Cotylogaster occidentalis (Aspidogastrea: Aspidogastridae) from Freshwater Drum, *Aplodinotus grunniens* (Actinopterygii: Sciaenidae), in Northeastern Oklahoma

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Running Title: *Cotylogaster occidentalis* in Oklahoma

Introduction

Freshwater Drum, *Aplodinotus grunniens* Rafinesque, 1819 are endemic to freshwater environs of the Americas, and their distributional range extends as far north as the Hudson Bay of Canada and reaches as far south as the Usumacinta River Basin of Guatemala (Fremling 1980). In the United States, eastward distribution includes the southern Great Lakes, eastern Appalachians and the entire Mississippi basin westward as far as Kansas, Oklahoma, and Texas (Page and Burr 2011). This fish appears to have the greatest latitudinal range of any freshwater fish in North America. It feeds mostly on small crustaceans, clams, snails, insect larvae (especially chironomids) and small fish (Miller and Robison 2004). In Oklahoma, *A. grunniens* occurs throughout the state, mainly in the larger lakes and rivers but uncommon to absent in the northwest (Miller and Robison 2004).

Freshwater Drum have been reported to harbor over 66 taxa of parasites, including protists, aspidogastreae, monogeneans, trematodes, cestodes, nematodes, acanthocephalans, leeches, molluscs, and crustaceans (Hoffman 1999). One of these parasites, an aspidogastreae, *Cotylogaster occidentalis* Nickerson, 1902, was originally described from *A. grunniens* in the Minnesota River, Minnesota (Nickerson 1902). To our knowledge, *C. occidentalis* has also been reported from *A. grunniens* from Iowa, Louisiana, Mississippi, Tennessee, and Lake Erie, Canada (Simer 1929; Bangham and Venard 1942; Sogandares-Bernal 1955; Dechtiar 1972; Stromberg 1970; Hoffman 1999), and from freshwater mussels from Iowa (Kelley 1927), Michigan (Fredericksen 1972), North Dakota and Manitoba, Canada (Carney 2015). In addition, a report by Huehner and Etges (1972) describing *Cotylogasteriodes barrowi* from freshwater mussels (*Lampsilis* spp.) from Ohio was found to represent an immature stage of *C. occidentalis* by Fredericksen (1972). The life cycle of *C. occidentalis* normally takes

place in snails but can also involve fishes, including experimental infections of *A. grunniens* (Dickerman 1948).

Nothing is known about *C. occidentalis* in Oklahoma. Here we present data on specimens of *C. occidentalis* obtained from 1 individual *A. grunniens* from the state, including new information on the parasite from scanning electron microscopy.

Materials and Methods

A total of 17 juvenile and adult *A. grunniens* (110 to 475 mm total length) were collected by boat electrofisher or 9.1 m seine from the Mississippi River at Osceola, Mississippi County, Arkansas ($n = 4$), the Red River, Marshall County, Oklahoma ($n = 6$), the Red River at St. Hwy. 37, McCurtain County, Oklahoma ($n = 1$), and the Verdigris River, Wagoner County, Oklahoma ($n = 6$). Specimens were placed on ice and processed within 24 hr for parasites.

Of the 7 aspidogastreae trematodes taken from the posterior intestine of one host; 5 were fixed in hot 10% buffered formalin and stored in 70% ethanol. Two of these specimens were stained with acetocarmine, dehydrated in an ethanol series, cleared in xylene or methyl salicylate, and mounted in Damar gum on slides, as permanent preparations. Three specimens chosen for scanning electron microscopy (SEM) studies were post-fixed in 1.0% osmium tetroxide, dehydrated through a graded ethanol series, infiltrated with hexamethyldisilazane (HMDS), mounted on stubs, and sputter coated with gold. Coated specimens were scanned using a Hitachi TM3030+ scanning electron microscope. Two specimens were fixed in 100% molecular grade ethanol for future molecular studies.

A voucher of *A. grunniens* was deposited in the Henderson State University Vertebrate Collection (HSU), Arkadelphia, Arkansas. A voucher specimen of *C. occidentalis* was deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of

Nebraska, Lincoln, Nebraska as HWML 139990.

Results and Discussion

One of 17 (6%) *A. grunniens* from the Verdigris River was found to harbor 7 specimens of *C. occidentalis*. Worms were found free in the lumen of the posterior one-fourth of the intestine; some worms were intermixed with pelecypod (bivalve) mollusc remains.

Whole mounts of 2 stained specimens were compared to the detailed redescription of *C. occidentalis* by Fredericksen (1972) and agreed with it in all essential details. SEM studies showed the retracticle (telescopic) neck (Figs. 1 A–B), pentalobate oral disc, elongate ventral adhesive disc with transverse ridges and marginal alveoli (Figs. 1 A–C), and a dorsal cone (Fig. 1C), all characteristic of this species.

SEM observations in this study add new information on the morphology of this parasite. The mouth opening is round, simple, and surrounded by small papillae (Figs. 2 A–B) and the anterior lobe of the oral disc bears an apical slit-like pit (Fig. 2C). The gonopore, was visible on the telescopic neck of one fully extended specimen (Figs 1B, 2D); it is a sunken ovoidal opening surrounded a plicate tegument and in the specimen examined partially occluded by what appears to be a clump of sperm (Fig 2D). These features were not discussed in a previous SEM study of *C. occidentalis* (Ip *et al.* 1982) likely because the specimens used in that study were contracted and the oral disc was also retracted. As described by Ip *et al.* (1982), the marginal organs bordering the marginal loculi end in ducts that are either inverted/retracted into pore-like cavities (Figs. 3 A–B) or everted (Fig. 3C). The margins and ventral surface of the adhesive disc are covered with small papillae (Figs. 3A–B). A protrusible pharynx reported by Ip *et al.* (1982) was not observed in our study.

Cotylogaster occidentalis appears to be the only bonafide fish parasitic aspidogastreaean in the Nearctic region (Hoffman 1999; Choudhury *et al.* 2016) and the Freshwater Drum is its principal fish host. However, there is a relationship between the diet of *A. grunniens* and infections with *C. occidentalis*; the aspidogastreaean is usually found in larger specimens that are capable of feeding on molluscs (Fredericksen, 1972). The infected fish in this study also had a considerable amount of crushed bivalve shells in its intestine and several worms were found intermixed with these remains.

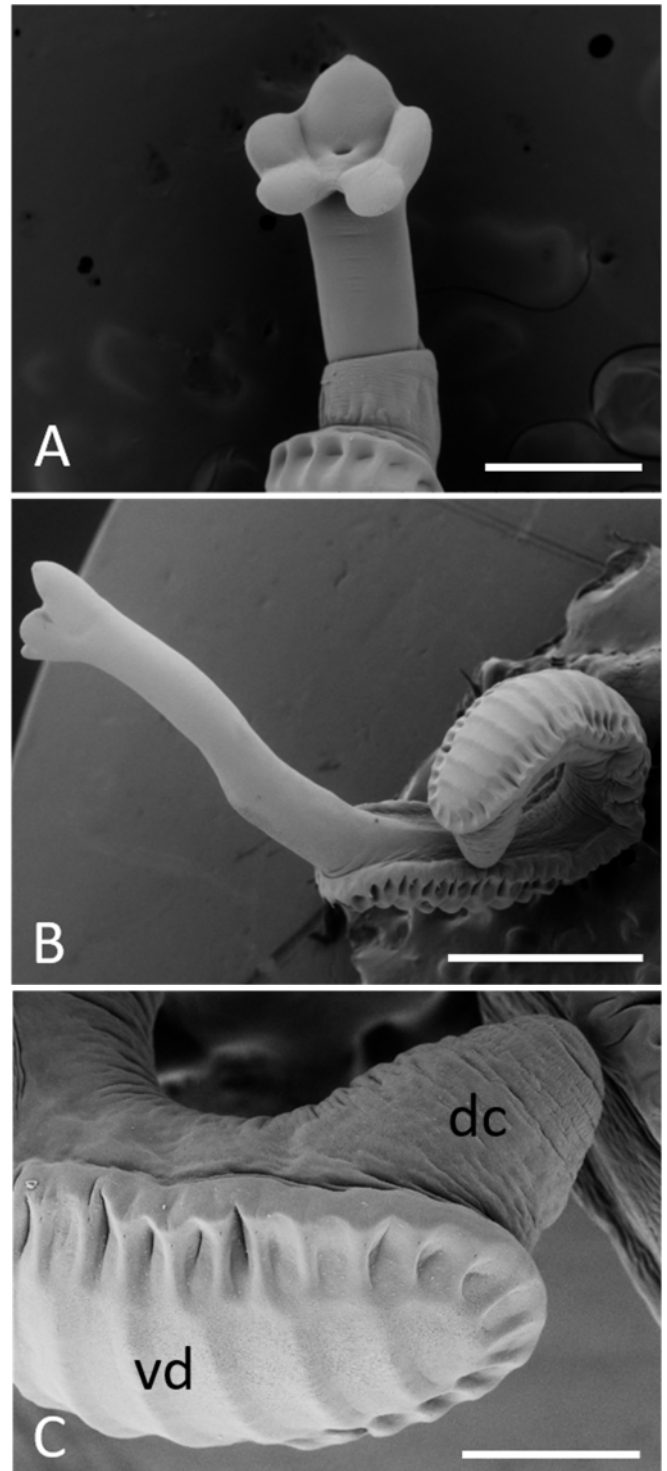


Figure 1. Scanning Electron Microscope (SEM) images of *C. occidentalis*. (A) Anterior end with partially retracted neck and expanded pentalobate oral disc; scale bar = 500 μm. (B) Whole mount with fully extended neck and partially opened oral disc; scale bar = 1 mm. (C) Terminal dorsal body cone (dc) and posterior end of ventral adhesive disc (vd); scale bar = 250 μm.

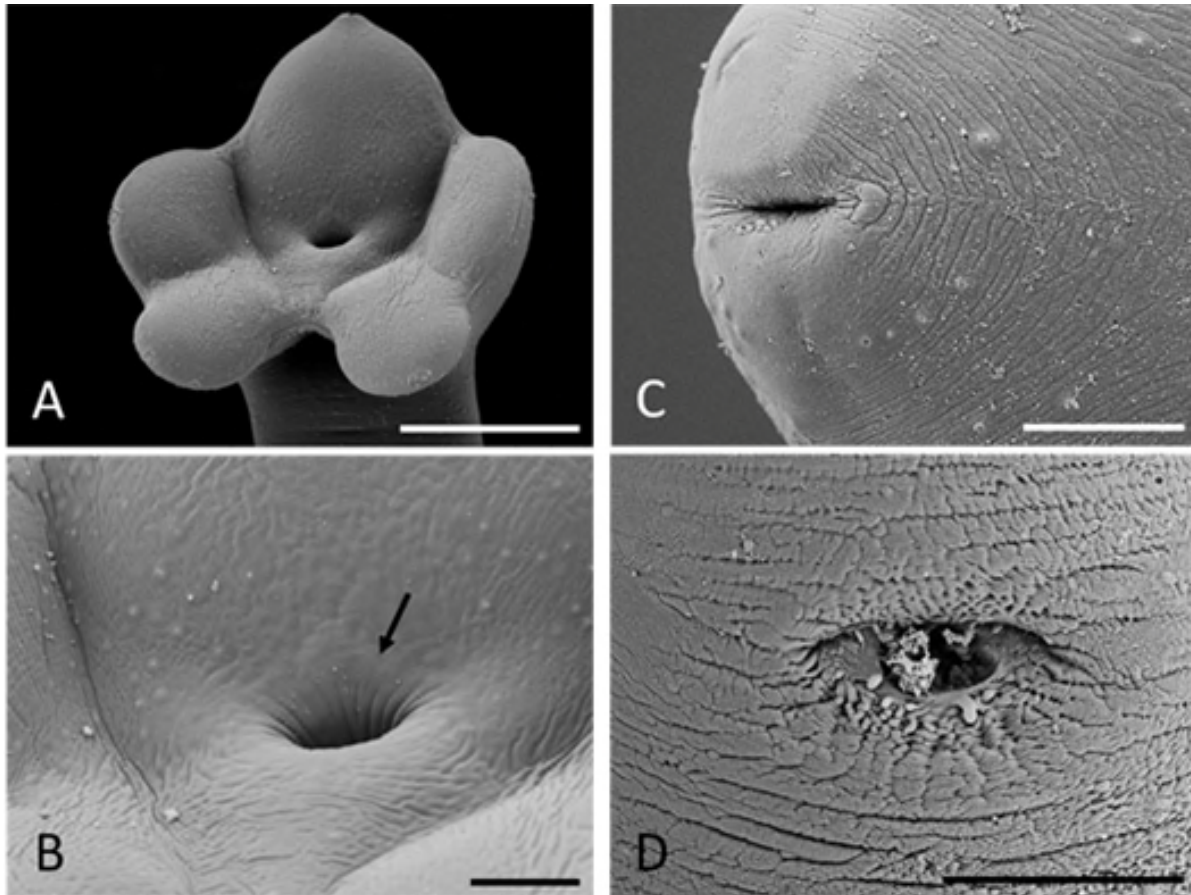


Figure 2. *C. occidentalis* (A) Close-up view of expanded pentalobate oral disc; scale bar = 250 μ m. (B) Mouth (arrow) with papillae on either side; scale bar = 50 μ m. (C) Terminal slit-like apical pore on anterior lobe of oral disc; scale bar = 25 μ m. (D) Sunken gonopore surrounded by plicate tegument and opening containing a clump of sperm; scale bar = 50 μ m.

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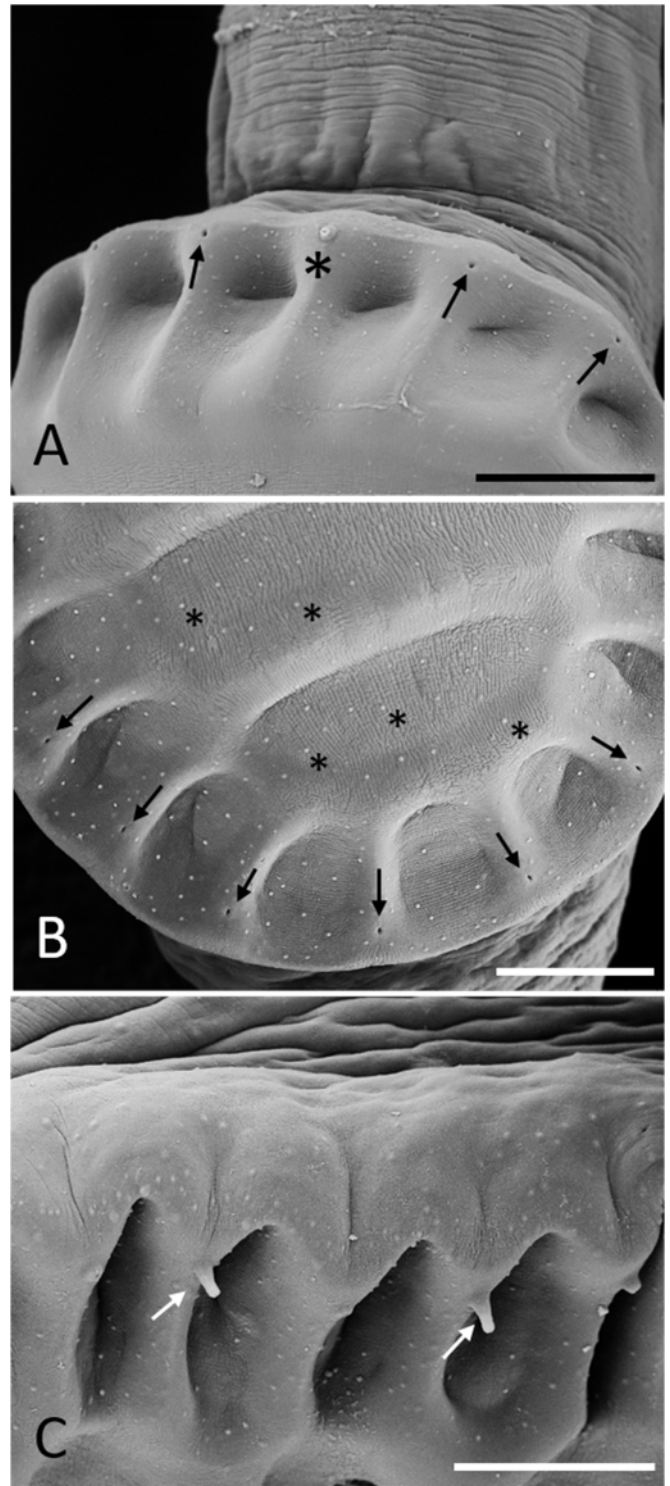


Figure 3. *C. occidentalis*. (A) Pore like cavities (arrows) of the marginal organs with one partially everted terminal duct (asterisk); scale bar = 150 μ m. (B) Surface of adhesive disc with papillae (asterisks beside some papillae) and pores of the marginal organs (arrows); scale bar = 100 μ m. (C) Everted ducts (arrows) of the marginal organs; scale bar = 100 μ m.