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Running Title: Helminths of *Fundulus olivaceus*

The Blackspotted Topminnow, *Fundulus olivaceus* (Storer) is a medium-sized fish that ranges in the Mississippi River basin from eastern Tennessee, western Kentucky, southern Illinois, and central Missouri south through eastern Oklahoma and Texas, Arkansas, Mississippi, and Alabama to the Gulf of Mexico (Page and Burr 2011). In Arkansas it is one of the most common and widespread fishes, occurring in all river drainages with a statewide distribution (Robison and Buchanan 1988). This fish prefers lentic habitats, is typically found in lower order streams, prefers areas near the land/stream margin, and is a surface feeder on insects and small crustaceans (Rice 1942).

Although information is available on the ecology of *F. olivaceus* (see Etnier and Starnes 1993, Pflieger 1997), little is known about its helminth parasites. As far as we can determine, only a single monogene, *Gyrodactylus megacanthus* Wellborn and Rogers, from Alabama, Arkansas and Mississippi and an acanthocephalan, *Neoechinorhychus cylindratus* (Van Cleave) Van Cleave, have been reported from this topminnow (Wellborn and Rogers 1967, Hoffman 1999). Herein, we report four new host records as well as two new geographic locality records for parasites collected from *F. olivaceus*.

Between May 2014 and June 2015, 44 juvenile and adult *F. olivaceus* (mean ± 1SD, range = 56.2 ± 14.0, 31–83 mm total length [TL]) were collected using a backpack electroshocker, a dipnet, or 6.1 m seine (3.2 cm mesh), from the Ouachita River drainage of Middle Fork of Saline River and Bear, Mill and Walnut creeks, Garland County (n = 38), the Caddo River, Clark County (n = 4), and the White River drainage of Crooked Creek, Boone County (n = 2). Specimens were placed in containers with cool aerated water from their collection site and necropsied within 24 hr. We followed accepted guidelines for the use of fish in research (AFS 2004); specimens were overdosed by immersion in a concentrated chloretone solution and a mid–ventral incision was made to expose the gastrointestinal tract and internal viscera. Monogenes were removed with minuten nadlen from the gills of select fish (n = 17) preserved in 10% formalin from Bear Creek, and mounted in Gray and Wess medium stained with Gomori's trichrome. Digeneans from the intestine were fixed in near boiling tap water without coverslip pressure, transferred to 70-95% v/v ethanol, stained with acetocarmine and mounted in Canada balsam. Nematodes were placed on a glass slide in a drop of undiluted glycerol for identification. Voucher specimens of parasites were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, Nebraska. Host voucher specimens were deposited in the Henderson State University (HSU) fish collection, Arkadelphia, Arkansas. Prevalence, mean intensity ± 1SD, and range of infection are provided and are in accordance with terminology given in Bush et al. (1997).

Thirty-one of 44 (70%) of the *F. olivaceus* harbored one of three helminths, including 29 (66%) with the digenean, *Creptotrema* sp. in the intestines, one (2%) with the nematode, *Eustrongylides* sp. in the mesentery, and one (2%) with the nematode, *Rhabdochona cascadilla* in the intestines. In addition, 2 of 17 (12%) from one site in the Ouachitas harbored the monogene, *Salsuginus umbraensis*. Of the sites collected, only Bear and Crooked creeks provided

positive hosts. The following is an annotated list of data as follows: host total length (TL, mean ± 1SD, range), prevalence, intensity (mean ± 1SD), collection site, collection date(s), HWML accession number.

Monogenoidea: Dactylogyrida: Ancyrocephalidae: *Salsuginus umbraensis* (Mizelle, 1938) Murith and Beverley-Burton, 1985 (Fig. 1)

65.0 ± 4.2, 62–68 mm TL, 2/17 (12%), 1.0 ± 0.0, range 1, Bear Creek at Bear, Garland Co. (34.535034°N, 93.286517°W). 30 May 2014, HWML 75372.

Figure 1. *Salsuginus umbraensis* from *Fundulus olivaceus*. Note hamuli. Scale bar = 20 µm.

Based on the size and shape of the hamuli (Fig. 1) (dorsal and ventral hamuli 18–19 and 19–21 µm long, respectively; angle of superficial/deep root notch about 60º; deep root forming a distinct thumb-like projection; blades long and thin, smoothly curved, thinner in dorsal hamulus), our specimens closely resemble the description of *S. umbraensis* provided by Mizelle (1938) and Murith and Beverley-Burton (1985) from Blackstripe Topminnow, *Fundulus notatus*. In addition, *S. umbraensis* has been reported from *F. notatus* from Illinois (Mizelle 1938, Murith and Beverley-Burton 1985), Louisiana (Duobinis-Gray and Corkum 1985) and Kentucky (Kozel and Whitaker 1985). This is the first report of *S. umbraensis* from *F. olivaceus* and Arkansas.

In spite of the high host specificity of species of *Salsuginus* (Murith and Beverley-Burton 1985), the very close phylogenetic relationship of *F. notatus* and *F. olivaceus* (Cashner et al. 1992) may enhance the ability of *S. umbraensis* to parasitize both species of hosts. Alternatively, the two specimens observed in this study may represent a cryptic undescribed species warranting further study with additional specimens.

Digenea: Plagiorchiida: Allocreadiidae: ***Creptotrema* sp. (Fig. 2)**

53.5 ± 11.8, 33–73 mm TL, 29/44 (66%) overall, 10.1 ± 14.3, range 1–68, 29/36 (81%) Bear Creek at Bear, Garland Co. (34.535034°N, 93.286517°W). 21 May, 2 Jul., 6 Sept., 16 Oct., 25 Nov., 2014, 20 May, 8 Jun., 2015, HWML 75384-86.

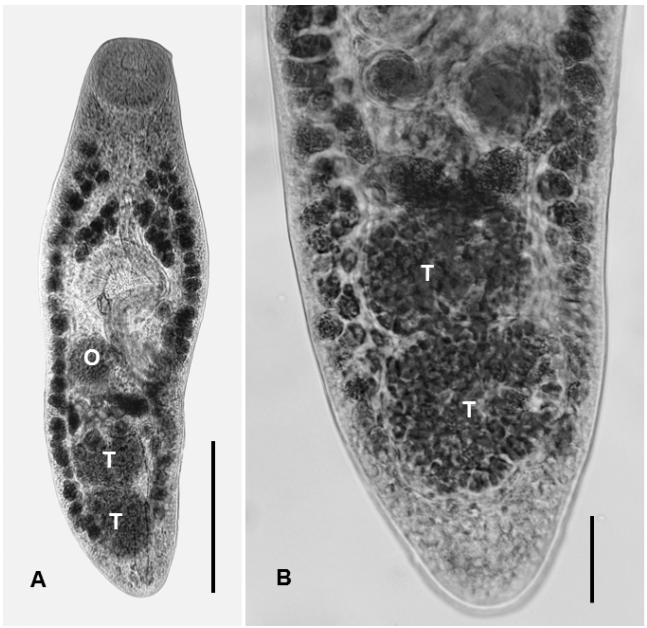

Figure 2. *Creptotrema* sp. from *Fundulus olivaceus*. A. Entire specimen showing ovary (O) and testes (T). Scale bar = 300 µm. B. Higher magnification of another specimen showing irregular margins on testes (T). Scale bar = 100 µm.

Interestingly, the highest intensity of infection of *Creptotrema* sp. was 68 adult and immature worms observed in a 63 mm TL specimen of *F. olivaceus* collected on 8 June 2015 from our Bear Creek site. In addition, one of the smallest juvenile *F. olivaceus* (33 mm TL) examined harbored 15 worms, also from Bear Creek.

Species of *Creptotrema* have been reported from esocids, fundulids, gasterosteids and percids in Arkansas, Florida, Mississippi, New York and Ohio, USA, and Manitoba, Nova Scotia and Ontario, Canada (Hoffman 1999, Curran et al. 2012, McAllister et al. 2016). Recently in Arkansas, McAllister et al. (2016) reported *Creptotrema* sp. from Northern Studfish, *Fundulus catenatus* from a stream within the Bear Creek watershed that cannot be morphologically

differentiated from our specimens from *F. olivaceus*. Comparison of our specimens of *Creptotrema* sp. (*n* = 10) to the description of *Creptotrema funduli* Mueller, 1934 from Banded Killifish, *Fundulus diaphanus* from Oneida Lake, New York, show significant morphological differences as follows: (1) the testes in our specimens (Fig. 2A) have irregular margins versus those of Mueller's which have smooth margins; (2) *Creptotrema* sp. from Arkansas attain a greater body length (up to 1,300 µm as opposed to a maximum length of 1,000 µm in *C. funduli*); and (3) our specimens possess a longer cirrus sac representing 47–54% of body length, BL (cirrus sac measured along middle of sac throughout its length) with more pronounced coiling than that of *C. funduli* from New York that measures in length only 28% BL (Mueller's line drawing) (Mueller 1934). Our specimens most closely resemble those of the *C. funduli* reported by Curran et al. (2012) from *F. notatus* from Mississippi that also have similar testes and a long cirrus sac (42% of BL in line drawing of Curran et al [2012]). However, *Creptotrema* sp. from Arkansas can be readily differentiated from both *C. funduli* from Mississippi and *C. funduli* from New York in having oral sucker-to-ventral sucker-width ratios ranging from 1:1.0–1.1 as opposed to those of *C. funduli* from Mississippi and New York that respectively range from 1:1.2–1.4 and from 1:1.2–1.5 (Curran et al. 2012). Therefore, we think that our *Creptotrema* sp. is new and will describe it in a forthcoming publication that will include comparison of rDNA sequences and tegument ultrastructure (through SEM) with congeners from North America. This digenean is reported for the second time from Arkansas and *F. olivaceus* is a new host.

Nematoda: Dioctophymatoidea: Dioctophymatidae: *Eustrongylides* sp. (4th stage larvae) (Fig. 3)

60 mm TL, 1/44 (2%) overall, 1/2 (50%) Boone Co., one worm, Crooked Creek at Harmon, Boone Co. (36.233894°N, 92.922276°W), 23 Jul., 2014, HWML 64766.

Eustrongylides spp. are found as adults in the proventriculus of piscivorous wading birds, with larvae encysted in the body cavity and musculature of fishes (Hoffman 1999). Specific identification of *Eustrongylides* requires rearing larvae in an avian host or DNA sequencing and our study did not include these techniques. This large, red-colored nematode (130 mm TL) was found encapsulated in the mesentery (Fig. 3A). McAllister et al. (2016) previously reported *Eustrongylides* from *F. catenatus* from Crooked Creek, Marion County. Therefore, we report, for the first time, *Eustrongylides* sp. from *F. olivaceus*.

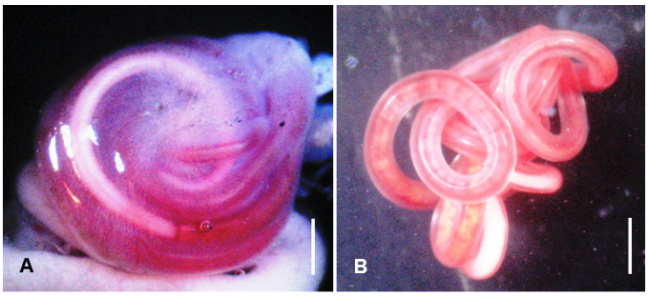

Figure 3. *Eustrongylides* sp. from *Fundulus olivaceus*. A. Encapsulated larvae from mesenteries. B. Individual worm teased from encapsulation. Scale bars = 2.5 mm.

Spirurida: Rhabdochonidae: *Rhabdochona cascadilla* Wigdor, 1918

55 mm TL, 1/44 (2%) overall, 1/2 (50%) Boone Co., two (one male, one female) worms, Crooked Creek at Harmon, Boone Co., (36.233894°N, 92.922276°W), 23 July 2014, HWML 64767.

In Arkansas, Cloutman (1976) reported *R. cascadilla* from Stonerollers, *Campostoma* spp., from the White River, Washington Co., and, more recently, McAllister et al. (2016) found this nematode commonly in *F. catenatus*, from Crooked Creek, Marion Co. Intermediate hosts of *Rhabdochona* spp. are primarily mayflies but caddisflies and stoneflies also serve (Gustafson 1939, Barger and Janovy 1994, Moravec 1995). This is the second time *R. cascadilla* has been reported from any member of the family Fundulidae, and *F. olivaceus* is a new host. This nematode shows little host specificity as it has been previously reported from at least 38 genera within 13 families of freshwater fishes in Canada and the USA (see Hoffman 1999, Moravec 2007, 2010).

In conclusion, the information provided herein serves to supplement the known information regarding parasites of non-game fishes in Arkansas. With additional surveys, more new host and distributional records will be expected as well as description of new species.

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