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Cover Page Footnote

The Arkansas Game and Fish Commission issued a Scientific Collecting Permit to CTM. We thank Drs. Scott L. Gardner and Gabor Racz (HWML) and Renn Tumblison for expert curatorial assistance. We also thank Dr. Gary Tucker (FTN Associates, Ltd., Little Rock) for identification of emergent vegetation.

Helminth Parasites of the Golden Topminnow, *Fundulus chrysotus* (Cyprinodontiformes: Fundulidae) from Desha County, Arkansas

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Running Title: Helminth Parasites of Golden Topminnows

Abstract

During July 2019, 21 Golden Topminnows (*Fundulus chrysotus*) were collected from an oxbow lake in McGehee, Desha County, Arkansas, and examined for parasites. Found were 4 taxa of endohelminths, including 3 digeneans (*Clinostomum marginatum*, *Homalometron* sp., and *Posthodiplostomum minimum*) and a nematode (*Spiroxys contortus*). We document a new host record for *S. contortus* and the first report of parasites in *F. chrysotus* from the lower Mississippi River Drainage.

Introduction

The Golden Topminnow, *Fundulus chrysotus* (Günther, 1866) is a small, compressed species that ranges in the lower Mississippi River basin from Kentucky and Missouri southward through Louisiana, South Carolina, and Florida west to Oklahoma, Arkansas, and Texas (Page and Burr 2011). In Arkansas, *F. chrysotus* occurs in all major drainages of the Coastal Plain lowlands, further extending through the Arkansas River Valley (Robison and Buchanan 2020).

McAllister *et al.* (2020) recently provided information from a comprehensive parasitological survey of the Golden Topminnow (*F. chrysotus*) from 4 river drainages of Arkansas. They reported a coccidian, myxozoan, monogenean, 3 digenetic trematodes, a cestode, nematode, 3 acanthocephalans, and a crustacean from that host and localities. Here, we report a new host record as well as the first parasite survey of *F. chrysotus* from a site in the lower Mississippi River Drainage.

Materials and Methods

On 24 July 2019, 21 *F. chrysotus* (mean $\pm$ 1 SD total length = 59.7 ± 8.9 mm, range 45–79 mm) were collected with a one-man seine and dipnet from an oxbow lake off US 65 in McGehee, Desha County (33° 37'50.58"N, -91°23'42.563"W) (Figs. 1A–B). Specimens were placed in a container of aerated habitat water and processed within 24 h. We followed the methods of McAllister *et al.* (2020) for fish handling, processing, and the examination of their parasites. Tissues suspected of being infected were fixed in 10% neutral-buffered formalin, sectioned at 8 μ m, and further processed following standard histological methods (Presnell and Schreiber 1997).

Parasites were either retained for future molecular studies or deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska-Lincoln, Lincoln, Nebraska. Host voucher specimens are deposited in the Henderson State University Vertebrate Collection (HSU), Arkadelphia, Arkansas. Prevalence, mean intensity $\pm$ 1SD, and range of infection are provided in accordance with terminology given in Bush *et al.* (1997).

Results and Discussion

Found were 4 taxa of parasites, including 3 digeneans (*Clinostomum marginatum*, *Homalometron* sp., and *Posthodiplostomum minimum*) and a nematode (*Spiroxys contortus*). No fish was harboring monogeneans or myxozoans on their gills. The data is presented below in annotated format.

TREMATODA: DIGenea: APOCREADIIDAE

Homalometron sp. – Ten specimens (mean intensity = 2.0 ± 0.6 , range 1–3) of a *Homalometron* sp.

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Figure 1. (A) County map of Arkansas showing approximate collecting locales (●) of McAllister *et al.* (2020) in 8 counties and present collecting site (star) in Desha County. (B) Oxbow lake collecting site in McGehee showing cypress trees and main emergent vegetation, alligator weed (*Alternanthera philoxeroides*). (Photo by H.W. Robison).

(Fig. 2A) were found in the intestinal tract of 5 (24%) *F. chrysotus*. Fayton *et al.* (2016) characterized undocumented diversity of *Homalometron* in freshwater fundulids, describing 2 new species from hosts from Oklahoma and New York, and resolving *Homalometron* specific to fundulids as a highly supported monophyletic group based on a molecular phylogeny of 28S rDNA. Subsequently, McAllister *et al.* (2020) reported *Homalometron* sp. from *F. chrysotus* collected from sites in Pulaski and Union counties, Arkansas, with morphological deviation from the most closely allied species, *Homalometron robisoni* Fayton and McAllister, 2016 and a small number of specimens ($n = 2$, with only a single mature specimen) precluding a specific identification. The specimens

collected in the present study, similar to those of McAllister *et al.* (2020), were morphologically similar to *H. robisoni*, which notably is the only species of *Homalometron* with the vitellarium extending anteriorly into the forebody (*Homalometron frocioneae* Fayton and Andres, 2016, the only other species of *Homalometron* from a freshwater fundulid, has a vitellarium that at most only slightly overlaps the posterior margin of the ventral sucker). Of the 3 specimens of *Homalometron* sp. available for morphological study (Table 1), the vitellarium either overlaps 80% of the length of the ventral sucker or is slightly anterior or posterior to its anterior margin. The

Table 1. Measurements (μm) of *Homalometron* sp. collected from *Fundulus chrysotus* from this study compared with most morphologically similar congeners from fundulid hosts.

Species	<i>Homalometron</i> sp. $n = 3$ (This study)	<i>Homalometron</i> sp. $n = 1$ McAllister <i>et al.</i> (2020)	<i>Homalometron robisoni</i> $n = 8$ Fayton <i>et al.</i> (2016)
BL	1,398–1,504	1,970	1,380–1,879
BW	326–386	544	414–467
BL to BW ratio	1:0.23–0.26	1:0.28	1:0.22–0.34
OSL as % BL	11	11	9–11*
OSW as % BW	42–48	40	34–44*
VSL as % BL	11–12	12	10–12*
VSW as % BW	47–51	43	36–46*
OS to VSW ratio	1:1.04–1.18	1:1.07	1:0.96–1.2
PL as % BL	6–7	7	5–6*
PW as % BW	25–26	25	18–24*
OL as % BL	3–6	5	4–6*

Table 1 *continued*

IB as %	22–27	24	24–29*
PS as % BL	13–17	8	12–18
F as % BL	31–33	28	27–31
ATL as % BL	8–9	8	6–9*
PTL as % BL	8–11	10	8–12*
PTS as % BL	20–22	23	20–25
SVL as % BL	7–11	8	7–10*
GP as % BL	28–31	26	26–30*
OL as % BL	7–9	8	7–9*
EL	100–114	99–107	91–111
EW	48–66	54–68	49–58
AEV as % BL	32–34	29	24–30*
EV as % BL	18–20	19	14–21

*Unpublished measurements of type material from Fayton *et al.* (2016). Abbreviations: BL = body length; BW = body width; OSL = oral sucker length; OSW = oral sucker width; VSL = ventral sucker length; VSW = ventral sucker width; PL = pharynx length; PW = pharynx width; OL = oesophagus length; IB = position of intestinal bifurcation; PS = postcaecal space; F = forebody; ATL = anterior testis length; PTL = posterior testis length; PTS = post-testicular space; SVL = seminal vesicle length; GP = genital pore position; OL = ovary length; EL = egg length; EW = egg width; AEV = anterior extent of vitellarium; EV = excretory vesicle length.

position of the anterior extent of the vitellarium is slightly more posterior compared to that of *H. robisoni* when expressed as a percent of body length. Our specimens also deviated from the type material of *H. robisoni* as follows: (1) smaller body width despite overlapping body lengths, and (2) larger ventral sucker and pharynx width expressed as a percent of body width. As to whether these 2 morphological deviations represent intraspecific or interspecific differences will have to be elucidated through molecular analysis. The site of collection for *Homalometron* from this study is

notably ~ 370 km (230 mi.) east from the type locality of *H. robisoni* and in the lower Mississippi River drainage of Arkansas vs. the Red River drainage of Oklahoma.

To date, the only known first intermediate hosts for *Homalometron* spp. are mud snails belonging to the family Hydrobiidae. On the other hand, metacercariae are found in a much broader assemblage of second intermediate hosts including snails (Hydrobiidae and Viviparidae), veneroid bivalves (Sphaeriidae, Unionidae, and Veneridae), oligochaetes (Naididae), and captive polychaetes (Stunkard 1964; Fayton *et al.* 2016).

Voucher specimens of the *Homalometron* sp. are being retained for future DNA analyses; a photovoucher is deposited as HWML 216340.

STRIGEOIDEA: STRIGIFORMES: DIPLOSTOMIDAE

***Posthodiplostomum minimum* (MacCallum, 1921) Dubois, 1936.** – Nine (43%) *F. chrysotus* harbored a total of 38 individual (4.2 ± 2.9 , range 1–10) metacercariae of *P. minimum* (HWML 216341) in their coelomic cavity and mesenteries. McAllister *et al.* (2020) previously reported specimens of *P. minimum* from *F. chrysotus* collected from sites in Howard, Lincoln, and Pulaski counties. Hoffman (1999) also previously reported *P. minimum* from *F. chrysotus*. In addition, McAllister *et al.* (2016b) reported this digenean from Northern Studfish, *Fundulus catenatus* from Arkansas. The host list and geographic range of this North American trematode (also called “white grub”) includes other fundulid fishes in North America, Canada, and México (Hoffman 1999). In the life cycle, the parasite reproduces asexually in bladder snails (Physidae, *Physella gyrina* and *P. acuta*) that serve as the first intermediate host, metacercariae are found in a wide range of fishes, and fish-eating ardeid birds (bitterns, herons, and egrets) serve as definitive hosts that defecate eggs into streams (Miller 1954; Palmieri 1976).

More than 25 species of *Posthodiplostomum* have been described worldwide (Ritossa *et al.* 2013; López-Hernández *et al.* 2018). Recent ecological and molecular studies (Locke *et al.* 2010; Lane *et al.* 2015) have suggested the possibility of cryptic diversity within the subspecies, *P. minimum centrarchi*, a parasite of centrarchid fishes. Whether or not the same phenomenon exists in *P. m. minimum* is not presently known.

PLAGIORCHIIDA: CLINOSTOMIDAE

***Clinostomum marginatum* (Rudolphi, 1819).** – A

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total of 28 individual (4.0 ± 4.6 , range 1–15) *C. marginatum* metacercariae (HWML 216344) were found encapsulated in the dermis, musculature, and liver of 7 (33%) *F. chrysotus*. McAllister *et al.* (2020) previously reported *C. marginatum* from *F. chrysotus* collected from sites in Lincoln and Pulaski counties; all of their specimens were found in dermal tissues. However, in the present study, numerous metacercariae were found encapsulated in liver tissue (Figs. 2B,C). This digenean has been previously reported from other fundulids (Hoffman 1999), including *F. catenatus* from Arkansas (McAllister *et al.* 2016b). Hoffman (1999) reported it is likely capable of infecting any species of freshwater fish. The first and second intermediate hosts of this trematode are planorbid snails and mainly fishes, respectively, with piscivorous (ciconiiform) birds serving as the definitive host, usually herons or egrets.

Caffara *et al.* (2011), using morphological and molecular data to differentiate adult and metacercarial stages of *C. complanatum* (Rudolphi, 1814) and *C. marginatum*, concluded that *C. complanatum* is the “European” species and is not present in hosts of the Americas. Therefore, previous reports of *C. complanatum* from Western Hemisphere native fishes (Hoffman 1999) are not valid and represent *C. marginatum*.

NEMATODA: SPIRURIDA: GNATHOSTOMATIDAE

***Spiroxya contortus* (Rudolphi, 1819) Schneider, 1866.** – Eleven (2.8 ± 1.5 , range 1–5) third-stage larval specimens of *S. contortus* (HWML 216342) were found in the intestinal tract of 4 (19%) *F. chrysotus*. Amin (1984) reported this nematode from Bayou Topminnow, *Fundulus notti* from Wisconsin. However, this is the third time *S. contortus* has been

reported from any fundulid fish in Arkansas as McAllister *et al.* (2016a, 2018) reported it previously from Blackstripe Topminnow (*Fundulus notatus*) and Western Starhead Topminnow (*Fundulus blairae*), respectively. As such, *F. chrysotus* is a new host record for this nematode. This roundworm is distributed widely in various vertebrate hosts (primarily turtles) in Palearctic Eurasia, North Africa, North and South America, and the Caribbean (Ernst and Ernst 1977; Baker 1987). The life cycle involves copepods as intermediate hosts in which the third-stage larvae develop (Hedrick 1935). Some potential paratenic hosts include mollusks, dragonfly nymphs, fish, and anurans (Mascarenhas and Müller 2015).

In conclusion, various populations of the Golden Topminnow have now been surveyed for parasites from all the major river drainages of the state (McAllister *et al.* 2020, this study). As has been shown when data from the present study is compared to that reported by McAllister *et al.* (2020), *F. chrysotus* from various Arkansas watersheds share some of the same helminth parasites. It is interesting to note, however, that none in the present study harbored myxozoans or monogeneans since McAllister *et al.* (2020) reported 8% and 18% of the Golden Topminnows they surveyed harbored *Myxobolus* sp. and *Salsuginus* sp., respectively. In addition, several watersheds within those drainages that support this species (Robison and Buchanan 2020) have not been surveyed to date (i.e., western Arkansas River Valley and along the tier of counties of extreme southern Arkansas), and additional collection efforts within these sites could reveal more information on the parasitological fauna of *F. chrysotus*, including that on the enigmatic *Homalometron* sp.

Acknowledgments

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Fig. 2. Trematodes from *Fundulus chrysotus*. (A) *Homalometron* sp. Scale bar = 200 µm. (B) Several metacercariae of *Clinostomum marginatum* teased from liver. Scale bar = 1 mm. (C) Higher magnification of single *C. marginatum* metacercaria. Scale bar = 500 µm.

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