Parasites (Cestoda, Nematoda, Mollusca) of Western Starhead Topminnow, *Fundulus blairae* (Cypriniformes: Fundulidae), from the Red River Drainage, Southwestern Arkansas

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Abstract: Between July 2016 and October 2017, and again during May and September 2018, 64 Western Starhead Topminnows, *Fundulus blairae*, were collected from a single site in the Red River drainage of southwestern Arkansas and examined for gill/fin ectoparasites and coelomic endoparasites. Twenty-nine (45%) were infected, including 8 (13%) with *Proteocephalus* sp. plerocercoids, 1 (2%) with a metacestode, *Valipora minuta*, 23 (36%) with encapsulated larval nematodes, *Spiroxys* sp., 1 (2%) with larval *Eustrongylides* sp., and 1 (2%) with an unknown glochidium. Four (7%) possessed a multiple infection of various parasites. No monogeneans or myxozoans were found on the gills of any fish nor were myxozoans found in the gallbladder. We document five new parasite host records in this contribution which represents only the second published report of any kind of parasite from this host.

Introduction

The Western Starhead Topminnow, *Fundulus blairae* Wiley and Hall, 1975 is a small fundulid that reaches a maximum total length of 80 mm and inhabits heavily-vegetated barrow ditches, oxbow lakes, sloughs, streams, and swampy backwaters (Wiley 1980; Ross 2001). The species is a surface-feeding insectivore (Miller and Robison 2004). Its range includes the Gulf Slope drainages from the Escambia River, Alabama and Florida, west to the Brazos River, Texas, and north into the Red River drainage of southwestern Arkansas and southeastern Oklahoma (Page and Burr 2011). In Arkansas, *F. blairae* has a very restricted distribution, is considered rare in the state (Robison 1977; Robison and Buchanan 1988), and listed as S2 (imperiled) by NatureServe (2018).

Little is known about the parasites of *F. blairae*, and Hoffman (1999) does not provide any taxa from this topminnow. A trematode, *Plagiocirrus loboides* Curran, Overstreet, and Tkach, 2007, has been described from *F. blairae* (as *F. dispar blairae*) from the Pascagoula River, George County, Mississippi (Curran et al. 2007). Here, after surveying 64 *F. blairae* from Arkansas, we document five new parasite host records.

Methods

Between July 2016 and October 2017, and again during May and September 2018, 64 juvenile and adult *F. blairae* (mean ± 1SD total length [TL] = 40.5 ± 7.8, range 32–66 mm) were collected from a small lake (backwater of the Rolling Fork River) in Sevier County and examined for parasites. Because these fish are elusive and difficult to capture, we used a 244 cm long-pole dipnet with a 41 long × 42 cm wide basket to collect specimens. The study site was typical of habitat for *F. blairae* as it was heavily vegetated with duckweed (*Lemna minor*), watermilfoil (*Myriophyllum*), and pondweed (*Potamogeton*). Topminnows were placed in containers with aerated water from their collection site and necropsied within 24 hr. We followed accepted guidelines for processing specimens (Use of Fishes in Research Committee 2014); they were overdosed with a concentrated tricaine methanesulfonate solution, and a mid–ventral incision was made to expose the gastrointestinal tract and internal viscera, which were placed in Petri dishes containing normal saline (0.9%). The stomach, intestinal tract, heart, liver, mesenteries, and urinary bladder were examined under a stereoscopic microscope at 20–30×. The gall bladder was scanned for myxozoans by placing whole tissue under coverslip pressure on a microscopic slide and examining by light microscopy. All 64 fish were preserved in 10% formalin, and their gills sets (left and right gills, 1–4) were examined for monogeneans and myxozoans under a stereomicroscope at 20–30×. Tissues suspected of being infected with helminths were fixed in 10% neutral buffered formalin and processed following standard histological methods (Presnell and Schreibman 1997) with sectioning at 8–10 µm followed by staining with hematoxylin and eosin. Voucher specimens (photovouchers or slides) of parasites were deposited in the Harold W. Manter Laboratory of Parasitology, University of Nebraska, Lincoln, Nebraska. Host voucher specimens were deposited in the Henderson State University Museum (HSU), Arkadelphia, Arkansas as HSU 3644.

Results and Discussion

Twenty-nine (45%) *F. blairae* were infected, 8 (13%) with *Proteocephalus* sp. plerocercoids, 1 (2%) with a *Valipora minuta* metacestode, 23 (36%) with encapsulated larval nematodes, *Spiroxys* sp., 1 (2%) with larval *Eustrongylides* sp., and 1 (2%) with an unknown glochidium; 4 (7%) possessed a multiple infection of various parasites. No myxozoans or monogeneans were found on the gills and no myxozoans were found in the gallbladder. Specific information on each parasite follows.

Cestoda: Proteocephalidea: Proteocephalidae
***Proteocephalus* sp. (Figs. 1A–C)**

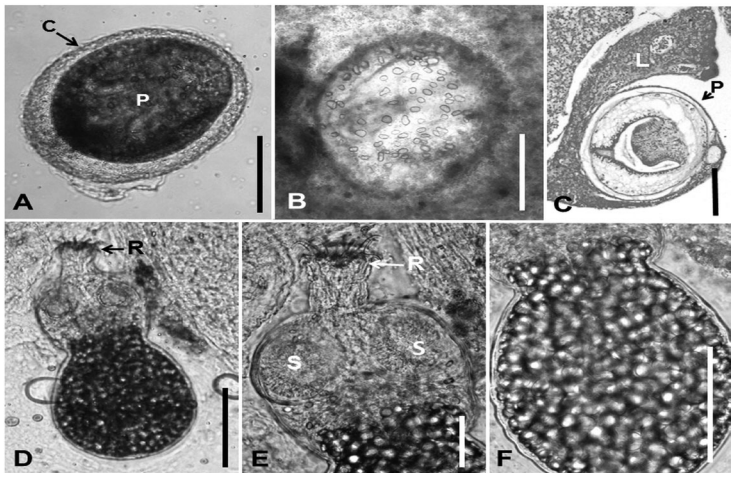
***Hosts and locality*:** 8 *F. blairae* (56.0 ± 3.1, 51–59 mm TL); 5 collected on 22 May 2017, 1 collected on 15 May 2018, and 2 collected on 30 September 2018 from just east of West Otis off St. Hwy 24, Sevier County, Arkansas (33° 57' 14.6088"N, 94° 25' 37.452"W).

Prevalence: 8/64 (13%).

***Intensity*:** Numerous plerocercoids (not counted) in each host.

Site of infection: Mesenteries and liver tissue.

***Other reported fundulid hosts*:** Northern Studfish, *Fundulus catenatus*; Golden Topminnow, *F. chrysotus*; Banded Killifish, *F. diaphanus*; Gulf Killifish, *F. grandis*; Mummichog, *F. heteroclitus*; Blackstripe Topminnow, *F. notatus*; Starhead Topminnow, *F. dispar* (Bangham 1941; Amin 1990; Hoffman 1999; McAllister et al. 2016a, b).



Figures 1A–F. Cestodes of *Fundulus blairae*. A. *Proteocephalus* sp. plerocercoid (P) within capsule (C); scale bar = 250 µm. B. *Proteocephalus* sp. plerocercoid showing numerous calcareous corpuscles; scale bar = 250 µm. C. *Proteocephalus* sp. plerocercoid (P) encapsulated in liver (L) tissue; scale bar = 250 µm. D. *Valipora minuta* larvae from gallbladder showing rostellum (R) with hooks; scale bar = 200 µm. E. Higher magnification of anterior view of same *V. minuta* showing rostellum (R) with hooks and suckers (S); scale bar = 100 µm. F. Posterior view of same *V. minuta*; scale bar = 250 µm.

Geographic range of genus in North America: The genus has been reported in nearly every U.S. state (see Hoffman 1999; McAllister et al. 2016a, b).

Additional Arkansas records in fishes: see McAllister et al. (2016a).

Specimens deposited**:** HWML 139402 (slide).

Remarks**:** Because these were plerocercoids, specific identification was not possible. This is the initial report of *Proteocephalus* sp. in *F. blairae*.

Cyclophyllidea: Gryporhynchidae
***Valipora minuta* (Coil, 1950) Baer and Bona, 1960 (Figs. 1D–F)**

Host and locality**:** 1 *F. blairae* (54 mm TL) collected on 30 September 2018 from same locale noted herein.

Prevalence**:** 1/64 (2%).

Intensity: A single metacestode.

Site of infection: Gall bladder epithelium.

Other reported fundulid hosts**:** None.

Other reported fish hosts: The larval form (metacestode) of the family infects more than 100 freshwater fish species and the adults, as well, are intestinal tapeworm parasites of fish-eating birds, mainly those in the Palearctic realm (Scholz et al. 2004). *Valipora minuta* occurs in a wide-variety of fishes of the families Centrarchidae, Heptateridae, and Poeciliidae, thus exhibiting a euryxenous type of host specificity (Scholz et al. 2004).

Geographic range of genus in fishes of North America**: USA:** Arkansas (Hoffman 1999; Scholz et al. 2004); Texas (Davis and Huffman 1975). **México:** Guerrero, Quintana Roo, Yucatán (Scholz et al. 2004). Coil (1950) described *V. minuta* from a green heron (*Butorides virescens*) from Indiana.

Additional Arkansas records in fishes: Spotted Bass, *Micropterus punctulatus*, Largemouth Bass, *M. salmoides* (Hoffman 1999).

Specimens deposited: HWML 139884 (photovoucher).

***Remarks*:** This specimen is tentatively identified as *V. minuta* based on its delicate hooks and shape of the smaller hooks (see fig. 2E, length of larger hooks ~35–40 µm). Identification of gryporhynchid larvae is almost always based on the number, shape, and size of rostellar hooks, which are situated in one layer. However, our specimen was not processed properly and future specimens should be prepared according to the GAP method of Malmberg (1957). We nevertheless document a new host record for this cestode.

Nematoda: Spirurida: Gnathostomidae
***Spiroxys* sp. (larvae) (Figs. 2A–E)**

***Hosts and locality*:** 23 *F. blairae* (48.0 ± 5.7, 39–54 mm TL); 2 collected on 9 July 2016, 1 collected on 21 August 2016, 1 collected on 22 May 2017; 7 collected on 8 October 2017; 4 collected on 15 May 2018; and 8 collected on 30 September 2018 from same locale noted herein.

Prevalence: 23/64 (36%).

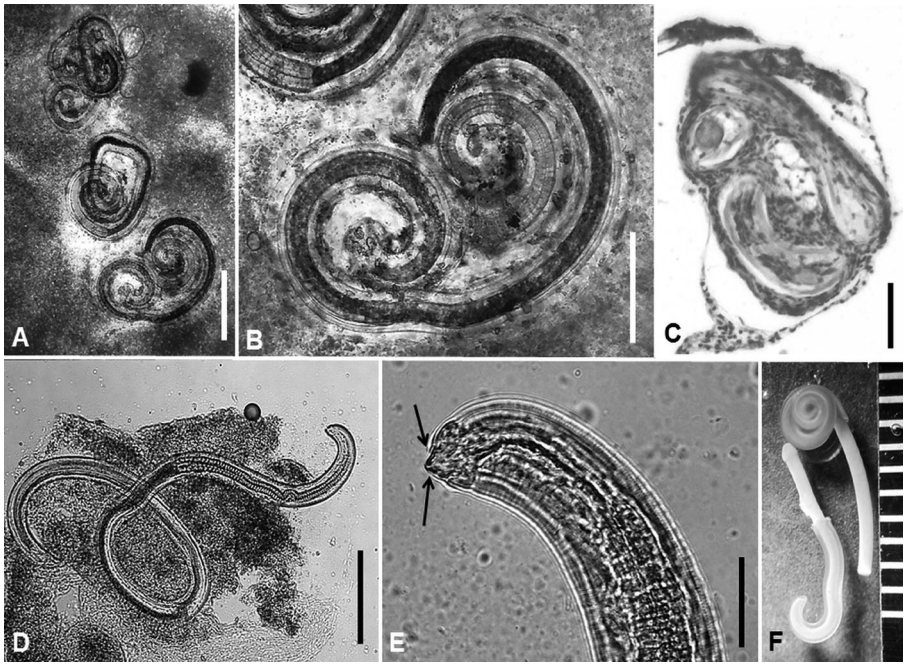
Intensity: Numerous larval worms (not counted) in each host.

Site of infection: Encapsulated in mesenteries and liver tissue (Figs. 2A–D).

Other reported fundulid hosts: *F. diaphanus* (Amin 1984); *F. notatus* (McAllister et al. 2016a); Bayou Topminnow, *F. notti* (Hoffman 1999).

***Geographic range of genus in fishes of North America*: USA:** Alabama, Arkansas, California, Georgia, New York, North Dakota, Ohio, Oklahoma, Pennsylvania, Texas, West Virginia, Wisconsin. **Canada:** British Columbia, Ontario. **México:** Campeche, Durango, Guanajuato, Hidalgo, Jalisco, Michoacán, Oaxaca, Querétaro, Quintana Roo, San Luis Potosi, State of México, Tabasco, Veracruz, Yucatán (see Salgado-Maldonado 2006; McAllister et al. 2016a).

Additional Arkansas records in fundulids:



Figures 2A–E. Nematodes of *Fundulus blairae*. A. Three encapsulated *Spiroxys* sp. in liver tissue; scale bar = 250 µm. B. Higher magnification of single encapsulated *Spiroxys* sp. in liver tissue; scale bar = 250 µm. C. Cross section of *Spiroxys* sp. encapsulated in mesenteries; scale bar = 25 µm. D. Single *Spiroxys* sp. teased from encapsulation. Scale bar = 125 µm. E. Anterior end of *Spiroxys* sp. showing characteristic lips (arrows); scale bar = 25 µm. F. *Eustrongylides* sp. nematode removed from cyst in coelomic cavity; scale = each bar on ruler is 1.0 mm.

F. notatus (McAllister et al. 2016a).

Specimens deposited: HWML 139373 (photovoucher).

***Remarks*:** Because these were larval specimens, we were unable to assign them to species. Adult worms are found in the intestinal tract of amphibians and the stomach of turtles; the first intermediate host is the copepod *Cyclops* (see Hedrick 1935).

Dioctophymatoidea: Dioctophymatidae
***Eustrongylides* sp. (larvae) (Fig. 2F)**

Host and locality: 1 *F. blairae* (54 mm TL) collected on 30 September 2018 from same site noted herein.

Prevalence: 1/64 (2%).

Intensity: 1 worm.

Site of infection: Encapsulated in coelomic cavity.

Other reported fundulid hosts: Summarized by McAllister et al. (2016a).

Geographic range of genus in North America: **USA:** Arkansas, Florida, Maine, Maryland, Massachusetts, Montana, Nevada, New York, Ohio, Oklahoma, Oregon, Tennessee, Texas, Utah, Wyoming. **Canada:** British Columbia, Ontario. **México:** Campeche, Guanajuato, Hidalgo, Jalisco, Michoacán, Morelos, Oaxaca, San Luis Potosi, Veracruz, Yucatán (see McAllister et al. 2016a).

Additional Arkansas records in fish: Pirate Perch, *Apheroderus sayanus*, Ozark Bass, *Ambloplites constellatus*, Grass Carp, *Ctenopharyngodon idella*, Northern Studfish, *F. catenatus*, Golden Topminnow, *F. chrysotus*, and Blackspotted Topminnow, *F. olivaceus* (McAllister et al. 2015, 2016a, b).

Specimens deposited: HWML 139885 (photovoucher).

Remarks: As adults, nematodes of the genus *Eustrongylides* are found in the proventriculus of piscivorous wading birds, with larvae encysted in the body cavity and musculature of fishes (Hoffman 1999). In the life cycle, early larval development occurs in the blood vessels of first intermediate host (freshwater oligochaetes) after they ingest infective eggs (Measures, 1988a), fishes are second intermediate hosts (Measures 1988b) and piscivorous birds are generally considered to be the definitive host (Spaulding and Forrester 1993; Franson and Custer 1994). However, predatory fish that ingest infected fish serve as paratenic, or transport, hosts (Xiong et al. 2013). Specific identification of *Eustrongylides* requires rearing larvae in an avian host, and our study did not include this experimental transmission. However, we report, for the first time, *Eustrongylides* sp., from *F. blairae*.

Mollusca: Bivalvia
Glochidia (Figs. 3A–B)

***Host and locality*:** 1 *F. blairae* (60 mm TL) collected on 15 May 2018 from same locale noted herein.

Prevalence: 1/64 (2%).

Intensity: Three glochidia.

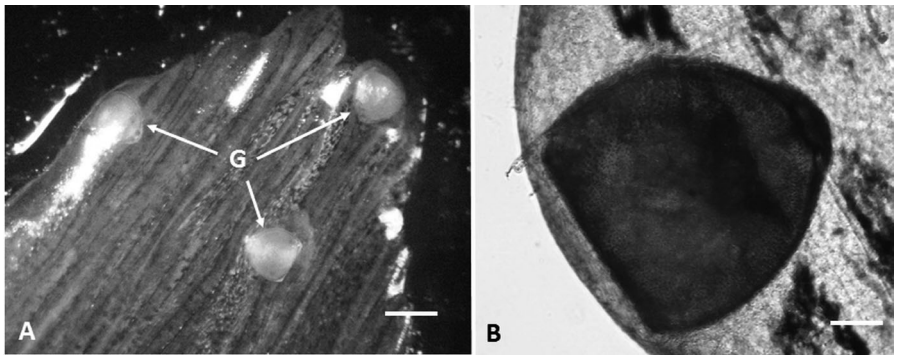
Site of infection: Encapsulated in caudal fin (Figs. 3A–B).

***Other reported fundulid hosts*:** *F. diaphanus*, Canada (Margolis and Arthur 1979).

Additional Arkansas records in fundulids: None.

Specimens deposited: HWML 139852 (photovoucher).

***Remarks*:** Larvae of most freshwater clams go through an obligate parasitic stage on the gills or fins of various fishes (Coker et al. 1921). We made no attempt to collect mussels at the study locale and, although it is not possible to identify the parent species of this glochidium, we document a new host record.



Figures 3A–B. Glochidia from *Fundulus blairae*. A. Three glochidia (G, arrows) in caudal fin; scale bar = 1.0 mm. B. Higher magnification of single glochidium in caudal fin; scale bar = 200 µm.

In summary, we examined a large sample of *F. blairae* from a single location in southwestern Arkansas and its parasite fauna is depauperate. This parasite fauna included five taxa, and four were represented by larval specimens, indicating these small topminnows are forage species that, as part of the life cycle, pass their parasites onto larger fish, reptilian, or bird definitive hosts. Additional populations from a variety of aquatic sites should be surveyed in an attempt to find additional parasites in this rarely studied fish.

Acknowledgments

We thank Drs. Scott L. Gardner and Gabor Racz (HWML) and Renn Tumlison (HSU) for expert curatorial assistance. We also thank the Arkansas Game and Fish Commission for Scientific Collecting Permits issued to CTM and HWR, and James T. McAllister, III (University of Arkansas-Fayetteville, AR) and Nikolas H. McAllister (Lukfata Elementary School, Broken Bow, OK) for assistance with collecting. Dr. Tomáš Scholz (Institute of Parasitology, České Budějovice) provided valuable information on gryporhynchid cestodes.

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Submitted October 10, 2018 Accepted November 18, 2018