New Records of Helminth Parasites (Trematoda, Cestoda, Nematoda) from Fishes in the Arkansas and Red River Drainages, Oklahoma

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Abstract: Between November 2014 and November 2016, 85 individual fishes (13 taxa from seven families) were collected from four sites in the Arkansas River drainage and three sites in Red River drainage of Oklahoma and examined for helminth parasites. Eighteen endoparasites (six trematodes, five cestodes, seven nematodes) were found in 32 of 85 (38%) fish including: Alloglossidium progeneticum in Black Bullheads (Ameiurus melas), Clinostomum marginatum in Slendar Madtoms (Noturus exilis), Crepidostomum sp. in a Plateau Darter (Etheostoma squamosum), *Caecincola* sp. in Slenderhead Darters (*Percina phoxocephala*), *Posthodiplostomum minimum* in a Redspot Chub (Nocomis asper), unknown metacercariae in a Slim Minnow (Pimephales tenellus), Bothriocephalus claviceps in a Banded Sculpin (Uranidea carolinae) and a Cardinal Shiner (Luxilus cardinalis), Essexiella fimbriatum in a Yellow Bullhead (Ameiurus natalis) and in an A. melas, Proteocephalus ambloplitis in a Western Creek Chubsucker (Erimyzon claviformis), Proteocephalus sp. in an E. squamosum and Grass Pickerels (Esox americanus), Schyzocotyle acheilognathi in E. claviformis, Rhabdochona cascadilla in L. cardinalis, Spinitectus micracanthus from largemouth bass (Micropterus salmoides), Dichelyne robusta and Spinitectus macrospinosus in a channel catfish (Ictalurus punctatus), Spiroxys sp. in E. claviformis, Textrema hopkinsi from a M. salmoides, and unknown larval nematodes in an E. americanus. Thirteen new host and six new geographic distributional records are documented. ©2016 Oklahoma Academy of Science

Introduction

Since the seminal publication of Hoffman (1999), which summarized various parasites of

North American fishes, including citing those studies from Oklahoma, more than a decade of neglect occurred in our knowledge of helminth parasites of the state's fish fauna. However, in the past few years, our collaborative community effort has helped us better understand the diversity of parasites in a variety of non-game fishes of the state (McAllister and Bursey 2011, 2013; McAllister et al. 2014b, 2015a, 2015b, 2015c, 2016; Kasl et al. 2015; Fayton et al. 2016). Here, in a similar manner, we continue to expand that information by documenting new host and distributional records for helminth parasites of select fishes from the Arkansas and Red River drainages of Oklahoma.

Methods

Between November 2014 and November 2016, 85 individual fishes (13 taxa from seven families) were examined for helminths including: Catostomidae: 13 Western Creek Chubsuckers (Erimyzon claviformis); Centrarchidae: four Largemouth Bass (Micropterus salmoides); Cyprinidae: two Cardinal Shiners (Luxilus cardinalis), four Redspot Chubs (Nocomis asper), three Slim Minnows (Pimephales tenellus); Cottidae: one Banded Sculpin (Uranidea carolinae); Esocidae: 15 Grass Pickerels (Esox americanus); Ictaluridae:19 Black Bullheads (Ameiurus melas), three Yellow Bullheads (Ameiurus natalis), six Slender Madtoms (Noturus exilis), one Channel Catfish (Ictalurus punctatus); Percidae: seven Plateau Darters (Etheostoma squamosum) and seven

Slenderhead Darters (Percina phoxocephala). They were collected by dipnet, backpack electrofisher, or 3.7 m (1.6 mm mesh) seine from seven sites in four counties of Oklahoma (Fig. 1) including, *Cherokee County*: tributary of the Illinois River off St. Hwy 10 (36° 07' 16.2006"N, 94° 48' 21.3732"W); Delaware County: Flint Creek at Flint (36° 11' 55.734"N, 94° 42' 27.0504"W); Mayes County: Little Saline Creek (36° 17' 4.833"N, 95° 05' 22.74"W) and Snake Creek off St. Hwy 82 (36° 09' 51.8976"N, 95° 09' 25.614"W); McCurtain County: Yanubbee Creek at Currence Road in Broken Bow (34° 02' 45.5568"N, 94° 43' 19.7394"W); Yashau Creek at Airport Road in Broken Bow (34° 01' 7.8996"N, 94° 45' 24.1668"W); Cow Creek Crossing on the Little River (33° 57' 14.3568"N, 94° 35' 37.3446"W). Fish were placed in aerated habitat water, taken to the laboratory for necropsy within 24 hr, killed by prolonged immersion in a concentrated chloretone[®] (chlorobutanol) solution, and measured for total length (TL). A mid-ventral incision was made to expose the viscera and the entire gastrointestinal (GI) tract and other organs were placed in Petri dishes containing 0.6% saline, and examined for helminths. The GI tract was split longitudinally and its contents examined under a stereomicroscope. Trematodes

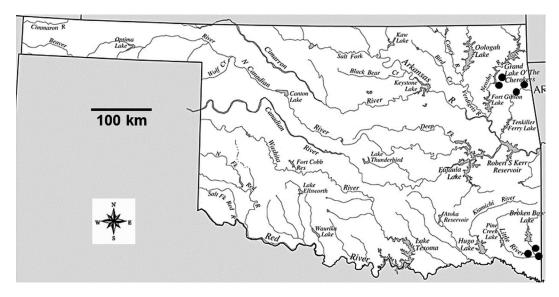


Figure 1. Map of Oklahoma showing major watersheds. Dots represent approximate location of study sites.

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and cestodes were fixed in near boiling tap water without coverslip pressure, stained with acetocarmine, dehydrated in a graded ethanol series, cleared in methyl salicylate or xylene, and mounted in Canada balsam or damar gum. Nematodes were fixed in a similar manner, preserved in 70% (v/v) ethanol, and studied as temporary mounts in glycerol. To examine tissues containing suspected helminths, sections from the liver and mesenteries were fixed in 10% neutral buffered formalin (NBF). We used routine histological techniques to prepare them for light microscopy and employed paraffin embedding methods found in Presnell and Schreibman (1997). Dehydrated tissues were placed in a graded series of increasing ethanol solutions (50-100%, v/v), cleared with xylene, and infiltrated and embedded in paraffin wax for 8 hr. We trimmed paraffin/tissue blocks of excess wax, serially sectioned them into ribbons 6 µm thick using a rotary microtome, and affixed sections to microscope slides using Haupt's adhesive while floating on a 2% NBF solution. Tissues were stained using Harris hematoxylin followed by counterstaining with eosin (H & E). For photomicroscopy, we utilized a Nikon Eclipse 600 epi-fluorescent light microscope with a Nikon DXM 1200C digital camera (Nikon Instruments Inc., Melville, NY).

Voucher specimens of parasites were were either retained for DNA studies or deposited in the Harold W. Manter Laboratory of Parasitology (MWML), Lincoln, Nebraska. Host voucher specimens were deposited in the Henderson State University Collection (HSU), Arkadelphia, Arkansas.

Results and Discussion

Thirty-two of 85 (38%) fish harbored helminths; infected fish came from all six sites in both drainages. The helminths found are presented below in annotated format.

Trematoda: Digenea: Strigeatida:

Diplostomidae

Posthodiplostomum minimum (MacCallum, 1921)

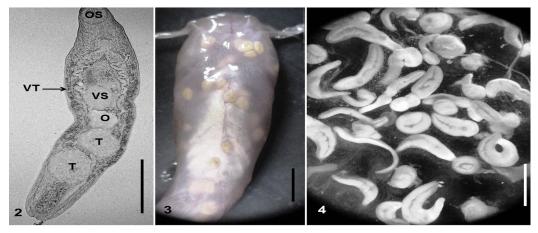
One of four (25%) N. asper (96 mm TL)

collected on 5 June 2015 from a tributary of the Illinois River, Cherokee County harbored *P. minimum* metacercaria. White grub has been reported from various fishes from North America (see Hoffman 1999). Physid snails serve as first intermediate hosts and natural infections of *Posthodiplostomum* have been reported from piscivorous birds of the Orders Ciconiiformes, Charadriiformes, and Pelecaniformes (Bedinger and Meade 1967; Dubois 1970). Metacercariae of strigeiforms that mature in these fish-eating birds are common and widely distributed. We document a new host record for *P. minimum*.

Clinostomidae

Clinostomum marginatum Rudolphi, 1819

Three of six (50%) N. exilis (66, 74, 84 mm TL) collected on 2 April 2016 from Flint Creek, Delaware County harbored metacercariae of C. marginatum (yellow grub). One of these N. exilis (66 mm TL) had a hyperinfection of 44 individual worms (Figs. 3-4) but the other two had one and two metacercaria, respectively. Daly et al. (1991) reported hyperinfection of Smallmouth Bass (Micropterus dolomieu) in Arkansas with up to 2,500 individual worms of C. marginatum. In the life cycle, embryonated eggs pass in the feces of the definitive host, which are various piscivorous birds (herons, egrets, herring gulls, and bitterns), and miracidia hatch and penetrate feces of snail first intermediate hosts (Bullard and Overstreet 2008; Caffara et al. 2014). Sporocysts and two generations of rediae develop producing cercariae that eventually encyst as metacercaria (yellow grub) in fishes or amphibian second intermediate hosts (Hopkins 1933; Hunter and Hunter 1933). This digenean is a very common fish trematode that is cosmopolitan in distribution (Lane and Morris 2000); however, this is the first time it has been reported from N. exilis. McAllister et al. (2015c) did not find any C. marginatum in a large sample (n = 43) of N. exilis from Arkansas. In Oklahoma, yellow grub has been reported previously from other non-game fishes, including Pirate Perches, A. sayanus (Hopkins 1933; McAllister and Bursey 2013), Carp, Cyprinus carpio (Spall 1969) and Striped Shiner, Luxilus chrysocephalus (McAllister et al. 2014).



Figures 2–4. 2. Ventral view of adult *Alloglossidium progeneticum* from *Ameiurus melas*. Locations of oral sucker (OS), ovary (O), testes (T), ventral sucker (VS), and vitellaria (VT) are indicated. Scale bar = 400 μ m. 3. Ventral view of *Noturus exilis* with hyperinfection of encysted metacercaria of *Clinostomum marginatum*. Scale bar = 5 mm. 4. Some of the same metacercaria after removal from their cysts. Scale bar = 2 mm.

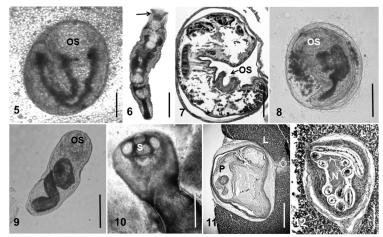
Plagiorchiida: Alloglossiidae

Alloglossidium progeneticum (Sullivan and Heard, 1969) Font and Corkum, 1975

Four of 19 (21%) A. melas (138.5 \pm 38.3, range 100-195 mm TL) collected on 25 June 2015 from Yashau Creek, McCurtain County harbored a total of five A. progeneticum (Fig. 2) in their small intestine. Specimens (Fig. 2) fit the description of those A. progeneticum described from a nonprecocious population (see Kasl et al. 2015). Interestingly, this fluke has a facultative progenetic life cycle in which the worms are able to reach sexual maturity in either a crayfish (2-host) or Ameiurus spp. (3host) final host. In those exhibiting a 2-host life cycle, eggs are produced while still encysted in the antennal gland of crayfishes whereas those utilizing a 3-host life cycle are found as adults in the intestine of fishes (Kasl et al. 2015). Alloglossidium progeneticum has been reported from Yellow Bullheads, Ameiurus natalis in Alabama, Arkansas, Georgia, Louisiana, Oklahoma, and Texas (Kasl et al. 2015). Previous reports of Alloglossidium corti (Lamont) Van Cleave and Mueller from A. melas and A. natalis (Hoffman 1999) most likely represent A. progeneticum. We document a new host record herein for A. progeneticum.

Allocreadiidae *Crepidostomum* sp.

Three specimens of this digenean were found in two (33, 47 mm TL) E. squamosum collected on 5 June 2015 from Little Saline Creek, Mayes County. This is the first time Crepidostomum sp. has been reported from the Plateau Darter. Several *Etheostoma* spp. have been previously reported to harbor Crepidostomum spp. (Hoffman 1999), including Orangebelly Darter, Etheostoma radiosum from the Blue River at Connerville, Johnston County, Oklahoma, with C. cooperi S. H. Hopkins (Scalet 1971). In addition, C. cooperi has also been reported in Oklahoma centrarchid fishes (McDaniel and Bailey 1966). McAllister and Bursey (2013) previously reported C. isostomum S. H. Hopkins from A. sayanus from McCurtain County, Oklahoma. In the life cycle, oculate xiphidiocercaria occur in clams with metacercaria in mayflies and crustaceans (see Hoffman 1999). Crepidostomum has a high number of species parasitizing freshwater fishes of North America among the many trematode genera represented in these hosts in North America (Choudhury et al. 2016). Indeed, undocumented diversity persists in the genus and the specific identity of this form will be examined in a subsequent publication. Nevertheless, E. squamosum is a



Figures 5-12. 5. *Caecincola* sp. metacercaria from *Percina phoxocephala*. Oral sucker (OS). Scale bar = 50 μ m. 6. Same *Caecincola* sp. removed from cyst. Arrow shows characteristic cup-shaped oral sucker. Scale bar = 100 μ m. 7. *Caecincola* sp. metacercaria from mesenteries of *P. phoxocephala* showing OS. Scale bar = 50 μ m. 8. Unknown metacercaria in cyst from *Pimephales tenellus* showing OS. Scale bar = 50 μ m. 9. Same metacercaria removed from cyst showing OS. Scale bar = 50 μ m. 9. Same metacercaria from *Esox americanus* showing suckers (S). Scale bar = 500 μ m. 11. Plerocercoid (P) from *E. americanus* in liver (L) tissue. Scale bar = 500 μ m. 12. Unknown larval nematodes (N) in cysts of *E. americanus* liver (L). Scale bar = 500 μ m.

new host record for this helminth.

Opisthorchiida: Cryptogonimidae *Caecincola* sp.

Four of seven (57%) P. phoxocephala (53.5 \pm 6.6, 48–63 mm TL) collected on 10 October 2015 from Cow Creek Crossing on the Little River, McCurtain County, harbored immature Caecincola sp. in the liver and mesenteries (Figs. 5–7). This is the initial helminth parasite reported from P. phoxocephala and the first time Caecincola sp. has been reported from any Percidae as well as, in so far as we know, the first report from Oklahoma. The geographic range of the genus now includes Arkansas, Florida, Georgia, Illinois, Louisiana, Michigan, Mississippi, New York, Oklahoma, Tennessee, Texas, Wisconsin, and Ontario and Lake Huron, Canada (McAllister et al. 2015c; this report). Other fish hosts include several species of centrarchids and ictalurids (McAllister et al. 2015c). In the life cycle, pleurolophocercous cercaria are found in amnicolid snails with metacercaria in Lepomis spp. (Greer and Corkum 1980).

Textrema hopkinsi Dronen, Underwood, and Suderman, 1977

A single *T. hopkinsi* was found in the intestine of one of four (25%) *M. salmoides* (103 mm TL) collected on 18 September 2016 from Yanubbee Creek, McCurtain County. This trematode has been previously reported from various centrarchids from Louisiana and Texas, including *M. salmoides* (Dronen et al. 1977; Greer and Corkum 1979, 1980; Underwood and Dronen 1984). We document a new geographic record for *T. hopkinsi* in Oklahoma as well as adding to a molecular database on cryptogonimids (A. Choudhury, *pers. comm.*).

Unknown metacercariae

One of three (33%) *P. tenellus* (64 mm TL) collected on 10 October 2015 from Cow Creek Crossing on the Little River, McCurtain County, was infected with unknown metacercariae encapsulated in various tissues (Figs. 8–9). This fish species has not been reported previously to harbor any parasite so we document the initial report of a digenean from this host.

Cestoda: Bothriocephalidea: Bothriocephalidae

Bothriocephalus claviceps (Goeze, 1782) Rudolphi, 1810

A single (130 mm) U. carolinae collected on 6 June 2015 from Snake Creek, Mayes County harbored a single B. claviceps (HWML 99886) in its intestine. In addition, one L. cardinalis collected on 5 June 2015 from a tributary of the Illinois River, Cherokee County was also infected with a single B. claviceps (HWML 99887). Both fish represent new host records for B. claviceps as well as the first records of this tapeworm from the Cottidae and Cyprinidae. More importantly, this is the initial parasite reported from L. cardinalis. McDaniel (1963) previously reported immature B. claviceps from bluegill (Lepomis macrochirus) and longear sunfish (Lepomis megalotis) from Lake Texoma, Oklahoma. This cestode is distributed in six Canadian provinces as well as eight US states (Scholz 1997) with the life cycle typical of the genus (Jarecka 1959). Interestingly, we surveyed (McAllister et al. 2014a) a large sample of U. (syn. Cottus) carolinae from Arkansas (some from Flint Creek, Benton County) and did not find any to be infected with *B. claviceps*.

Schyzocotyle acheilognathi (Yamaguti, 1934) Brabec, Waeschenbach, Scholz, Littlewood, and Kuchta, 2015

А larval S. (syn. *Bothriocephalus*) acheilognathi was collected from one of 13 (8%) E. claviformis (99 mm TL) taken on 30 October 2016 from Yanubbee Creek, McCurtain County. The Asian fish tapeworm has been reported from western mosquitofish, Gambusia affinis from McCurtain County (McAllister et al. 2015b). This is the only the second time, to our knowledge, it has been reported from any fish of the family Catostomidae (Minytrema melanops, see McAllister et al. 2015b), and the first report from E. claviformis. In the life cycle, procercoids occur in copepods, with smaller fishes acting as "carriers", and the adult is found in the intestine of teleost fishes (see Hoffman 1999). This tapeworm is a known pathogen having a potential negative impact on many fishes, particularly cyprinids (Salgado-Maldonado and Pineda-Lopez 2003); however, the effect of *S. acheilognathi* on fishes in Oklahoma is unknown.

Proteocephalidea: Proteocephalidae *Essexiella fimbriatum* (Essex, 1927) Scholz, de Chambrier, Mariaux, and Kuchta, 2011

Scholz et al. (2011) erected a new genus Essexiella for Corallobothrium fimbriatum. A single E. fimbriatum (HWML 103024) was found in the small intestine of one of three (33%) A. natalis (153 mm TL) collected on 10 October 2015 from Cow Creek Crossing at the Little River, McCurtain County. In addition, eight specimens of E. fimbriatum (HWML 99888, 103025) were taken from the intestinal tract of A. melas collected on 5 August 2016 from Yashau Creek, McCurtain County. Adult E. fimbriatum occur in various members of the Ictaluridae with the procercoid in Cyclops and plerocercoid in minnows (Notropis spp.) and other fishes (Hoffman 1999). Although E. fimbriatum has been previously reported from both bullheads (Hoffman 1999) and from Oklahoma in Flathead Catfish (Pylodictis olivaris) and I. punctatus (Spall 1968), this is the first time this tapeworm has been reported from Oklahoma non-game fishes.

Proteocephalus ambloplitis (Leidy, 1887) Benedict, 1900

A plerocercoid of P. ambloplitis was collected from one of 13 (8%) E. claviformis (99 mm TL) taken on 30 October 2016 from Yanubbee Creek, McCurtain County. The "bass tapeworm" is a common helminth of many fishes and has been reported from several US states and southern Canada (Hoffman 1999). McDaniel and Bailey (1974) reported P. ambloplitis in the Little River (Cleveland County) and Lake Texoma, Oklahoma. This tapeworm has also been reported from the related Lake Chubsucker (Erimyzon sucetta) from Wisconsin (Amin 1990). In the life cycle, procercoids are found in the hemocoel of copepods, plerocercoids occur in the viscera of small fishes and basses mainly serve as definitive hosts (Hunter and Hunter 1929). It can be a major concern in fish culture because plerocercoids can cause fibrosis in gonads of bass, sometimes rendering them sterile (Hoffman 1999). We report *P. ambloplitis* for the first time from *E. claviformis*.

Proteocephalus sp.

A single plerocercoid of *Proteocephalus* sp. (HWML 99889) was found in the intestinal tract of one of seven (14%) *E. squamosum* (55 mm TL) collected on 6 June 2015 from Little Saline Creek, Mayes County. No cestodes have been previously reported from this darter (Hoffman 1999), so we document a new host record here. However, this genus of tapeworm has been commonly reported from various fishes, including several from Oklahoma (see McAllister et al. 2014).

Three of 15 (14%) E. americanus (135, 148, 190 mm TL) collected on 20 November 2014, 13 February, and 17 July 2015 from Yashau Creek, McCurtain County, had Proteocephalus plerocercoids in their liver (Figs. 10–11). Three species of Proteocephalus, as adults, have been reported previously from E. americanus, including P. pearsei La Rue, P. perplexus La Rue and P. pinguis La Rue, although none of these have been reported to date from any fish in Oklahoma. Proteocephalus ambloplitis (Leidy) Benedict plerocercoids have been documented from centrarchid fishes from the state (Spall 1969) as well as E. claviformis herein. This is the first time encapsulated plerocercoids have been reported from E. americanus.

Nematoda: Ascaridida: Cucullanidae *Dichelyne robusta* (Van Cleave and Mueller, 1932) Petter, 1974

A single *D. robusta* was found in the intestine of *I. punctatus* (345 mm TL) collected on 30 October 2016 from Yanubbee Creek, McCurtain County. This nematode has been previously reported from several ictalurids (including *I. punctatus*) from Arkansas, Kansas, New York, North Dakota, Ohio, Tennessee, Texas, and Wisconsin (Hoffman 1999; Dutton and Barger 2015). To our knowledge, the life cycle has yet to be determined. We document the first report of *D. robusta* from Oklahoma.

Spirurida: Gnathostomatidae

Spiroxys sp. (larvae)

Larval *Spiroxys* sp. (1 or 2 each) were found in the mesenteries of five of 13 (38%) *E. claviformis* (96.8 \pm 14.2, range 71–111 mm TL) taken on 30 October, 20 November, and 27 November 2016 from Yanubbee Creek, McCurtain County. This nematode has been reported from at least 23 genera of freshwater fishes from numerous states and Canada (Hoffman 1999). The adult of *Spiroxys* is found in the stomach of turtles and intestines of amphibians and the experimental life cycle has shown the first intermediate host is *Cyclops* (Hedrick 1935). This is the first time *Spiroxys* sp. has been reported from *E. claviformis*.

Cystidicolidae

Spinitectus macrospinosus Choudhury and Perryman, 2003

Seven female S. macrospinosus were collected from the large intestine of an I. punctatus (345 mm TL) taken on 30 October 2016 from Yanubbee Creek, McCurtain County. This nematode was originally described from I. punctatus from the Assiniboine and Red rivers, Winnipeg, Manitoba, Canada, and also reported from Blue Catfish (Ictalurus furcatus) from Kentucky Lake, Tennessee (Choudhury and Perryman 2003). The authors also noted that S. macrospinosus was taken from an "I. *lacustris*" (= *I. punctatus*) from Lake Texoma, Oklahoma and Texas. As the middle of the reservoir is roughly situated on the southern and northern borders of both states and, more importantly, a specific collection locale was not given, we provide the first definitive report of S. macrospinosus from Oklahoma.

Spinitectus micracanthus Christian, 1972

Four *S. micracanthus* were collected from the intestine of two of four (50%) *M. salmoides* (82, 103 mm mm TL) taken on 18 September 2016 from Yanubbee Creek, McCurtain County. Previous records of *S. micracanthus* are primarily from centrarchids, including *Lepomis* spp. and *Micropterus* spp. from Alabama, Indiana, Ohio, Pennsylvania, and Texas (Hoffman 1999). In the life cycle, larval *Spinitectus* use larval mayflies as intermediate hosts (Gustafson 1939). This is the first report of *S. micracanthus* from Oklahoma.

Rhabdochonidae

Rhabdochona cascadilla Wigdor, 1918

Two *L. cardinalis* (not measured) collected on 5 June 2015 from a tributary of the Illinois River, Cherokee County harbored 12 (2 male, 10 female) and 14 (1 male, 13 female) *R. cascadilla* (HWML 99890), respectively. This nematode shows little host specificity as it has been previously reported from more than 38 genera and 12 families of North American freshwater fishes (Hoffman 1999; Moravec 2007, 2010; Moravec and Muzzall 2007). In the life cycle, larval members of the genus develop in mayflies and the adult is parasitic in the intestine of freshwater fishes (Byrne 1992; Moravec 2010). We document a new host as well as a new distributional record for *R. cascadilla*.

Unknown larval nematodes

Unidentified larval nematodes were found encapsulated in the liver of one of 15 (7%) *E. americanus* (190 mm TL) collected on 13 February 2015 from Yashau Creek, McCurtain County (Fig. 12). There are numerous nematodes reported from *E. americanus* (Hoffman 1999). However, this is the first time larval nematodes have been documented from tissue of a Grass Pickerel.

In summary, we have provided several new host and geographic distributional records for fishes from Oklahoma. As noted by Scholz and Choudhury (2013) and reiterated here, there is still considerable work to be done to have a better understanding of the diversity of fish helminths of the state. More exploratory and opportunistic surveys on fishes are urgently needed throughout Oklahoma from other hydrologic units, including those in the western part and panhandle where we know very little about their helminth parasites.

Acknowledgments

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