
New Host and Geographic Distributional Records for *Eustrongylides* sp. (Nematoda: Dioctophymatoidea: Dioctophymatidae) from Eight Vertebrates (Osteichthyes, Amphibia, Reptilia) from Arkansas, Oklahoma and Texas

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Abstract: During May 1989 and again between February 2012 and July 2015, the following eight vertebrates (five fishes, one amphibian, two reptiles) from Arkansas, Oklahoma or Texas were collected and found to be infected with larval nematodes, *Eustrongylides* sp. as follows: Ozark Bass (*Ambloplites constellatus*, Arkansas), Grass Carp (*Ctenopharyngodon idella*, Arkansas), Grass Pickerel (*Esox americanus*, Oklahoma), Golden Topminnow (*Fundulus chrysotus*, Arkansas), Green Sunfish (*Lepomis cyanellus*, Oklahoma), western lesser siren (*Siren intermedia nettingi*, Arkansas), Midland water snake (*Nerodia sipedon pleuralis*, Arkansas) and Gulf Coast ribbon snake (*Thamnophis proximus orarius*, Texas). In the life cycle of this nematode, these new hosts represent paratenic or transport hosts. This is the first time *Eustrongylides* sp. has been reported from Oklahoma, and we document seven new host records for the parasite. ©2015 Oklahoma Academy of Science

Introduction

Nematodes of the genus *Eustrongylides* Jägerskiöld, 1909 are pathogenic parasites found as adults in the proventriculus of piscivorous Proc. Okla. Acad. Sci. 95: pp 26 - 32 (2015)

wading birds (Spalding and Forrester 1993) with larvae encysted in the body cavity and musculature of fishes (Hoffman 1999). This parasite causes pathology in piscivorous fish hosts (Mitchell et al. 2009) and renders smaller forage fish more susceptible to predation

(Coyner et al. 2001). There are two valid species of *Eustrongylides* that occur in the New World, *E. ignotus* Jägerskiöld, 1909 and *E. tubifex* (Nitzsch in Rudolphi, 1819) (Measures 1988a).

In the life cycle, early larval development occurs in blood vessels of first intermediate host freshwater tubificine oligochaetes after they ingest infective eggs (Measures 1988b; Anderson 2000), planktivorous and benthivorous fishes are second intermediate hosts (Measures 1988c) and piscivorous birds are generally considered to be the definitive host (Spalding and Forrester 1993; Franson and Custer 1994). However, predatory fish and reptiles and amphibians that ingest infected fish serve as paratenic, or transport, hosts (Xiong et al. 2013). Rarely mammals, including humans have become infected with larval *Eustrongylides* sp. (Abram and Lichtenfels 1974; Guerin et al. 1982; Deardorff and Overstreet 1991; Cole 1999).

To our knowledge, nothing has been published previously on *Eustrongylides* sp. in any host from Oklahoma (Hoffman 1999) and, as far as we know, there are three recent reports (McAllister et al. 2014, 2015, 2016) from Arkansas fishes. Here, we provide new host records for this nematode as well as the first report of the genus from Oklahoma.

Methods

During May 1989 and again between February 2012 and July 2015, the following eight vertebrate species were collected and examined for helminth parasites, including one Ozark Bass (*Ambloplites constellatus*) from Marion County, Arkansas, five Golden Topminnows (*Fundulus chrysotus*) from Lincoln County, Arkansas, two Grass Carp (*Ctenopharyngodon idella*) from Pulaski County, Arkansas, five Green Sunfishes (*Lepomis cyanellus*) from McCurtain County, Oklahoma, five Grass Pickerel (*Esox americanus*) from McCurtain County, Oklahoma, three western lesser sirens (*Siren intermedia nettingi*) from Craighead ($n = 1$) and Sevier ($n = 2$) counties, Arkansas, nine Midland water snakes, (*Nerodia sipedon pleuralis*) from Franklin ($n = 1$), Fulton ($n =$

1), Independence ($n = 5$), Marion ($n = 1$) and Montgomery ($n = 1$) counties, Arkansas, and one Gulf Coast ribbon snake, *Thamnophis proximus orarius* from Harris County, Texas. Fishes and sirens were collected with a dipnet or 3.7 m (1.6 mm mesh) seine and snakes by hand or tong. Fish and sirens were placed in containers with cool aerated habitat water and snakes in cloth bags on ice; all were necropsied within 24 hr. We followed accepted guidelines for the use of fish (AFS 2004) and amphibians and reptiles in research (HACC 2004); specimens were overdosed with a concentrated Chloretone solution and a mid-ventral incision was made to expose the gastrointestinal tract and internal viscera. Nematodes were studied as temporary mounts in glycerol. Voucher specimens of *Eustrongylides* sp. were deposited in the United States National Parasite Collection (USNPC), Beltsville, Maryland, and Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska. Host voucher specimens were deposited in the Arkansas State University Museum of Zoology Herpetology Collection (ASUMZ), State University, Arkansas or Henderson State University Museum (HSU), Arkadelphia, Arkansas. Prevalence, mean intensity, and range of infection are provided in accordance with terminology given in Bush et al. (1997).

Results

Overall, eight of 31 (26%) vertebrate specimens collected, including one each of *A. constellatus*, *C. idella*, *E. americanus*, *F. chrysotus*, *L. cyanellus*, *S. i. nettingi*, *N. s. pleuralis* and *T. p. orarius* harbored nematodes fitting the description of *Eustrongylides* sp. Watersheds herein where *Eustrongylides* were found in hosts include the Arkansas, Ouachita, St. Francis, and White river systems in Arkansas, the Red River drainage in Oklahoma and the Trinity/San Jacinto river basin in Texas. A summary of host data is as follows:

Nematoda: Dioctophymatoidea: Dioctophymatidae *Eustrongylides* sp. (larvae) (Fig. 1)

Hosts and localities: *A. constellatus*
Proc. Okla. Acad. Sci. 95: pp 27 - 32 (2015)

collected on 23 July 2014 from Crooked Creek at Kelly's Slab, Marion County, Arkansas (36°15'09.9"N, 94°26'25.8"W); *C. idella* collected on 17 October 2014 from Freeze Fish Farm at Keo, Pulaski County, Arkansas (34° 37' 11.6688"N, 92° 01' 28.1922"W); *E. americanus* collected on 29 July 2015 from Yashau Creek, McCurtain County, Oklahoma (34° 00' 41.115"N, 94° 44' 59.7258"W); *F. chrysotus* collected on 29 June 2014 from Cane Creek Lake, Lincoln County, Arkansas (33° 55' 0.3102"N, 91° 45' 56.9592"W); *L. cyanellus* collected on 23 November 2014 from Yashau Creek, McCurtain County, Oklahoma (34° 00' 41.115"N, 94° 44' 59.7258"W); *S. i. nettingi* (ASUMZ 31985) collected on 23 February 2012 from 0.4 km E of South Culberhouse Road off county road 412, Craighead County, Arkansas (35° 45' 46.8648"N, 90° 42' 27.1614"W); *N. s. pleuralis* collected on 24 July 2014 from Crooked Creek at Kelly's Slab, Marion County, Arkansas (36°15'09.9"N, 94°26'25.8"W); *T. p. orarius* collected on 11 May 1989 from San Jacinto Street, Harris County, Texas (29° 44' 35.0262"N, 95° 22' 24.114"W).

Prevalence: 8/31 (26%) overall; 1/1 (100%) *A. constellatus*; 1/5 (20%) *E. americanus*; 1/5 (20%) *F. chrysotus*; 1/2 (50%) *C. idella*; 1/5 (20%) *L. cyanellus*; 1/3 (33%) *S. i. nettingi*; 1/9 (11%) *N. s. pleuralis*; 1/1 (100%) *T. p. orarius*.

Intensity: Most hosts harbored a single larval worm except *S. i. nettingi* which was infected with 12 advanced fourth-stage larval *Eustrongylides* sp.

Site of infection: Encapsulated (all alive) in fat body and mesentery. From fishes, these conspicuous red-colored coiled nematodes when teased from their capsules measured 130.0 ± 23.3 , range 90–143 mm in total length, similar to lengths of third-stage larval *Eustrongylides* (see Lichtenfels and Piliitt 1986).

Other reported North American hosts: Larval *Eustrongylides* spp. have been reported from a number of fish within 14 orders (see Xiong et al. 2013); in amphibians, including three-toed amphiuma, *Amphiuma tridactylum* ([experimental infection] von Brand 1944;

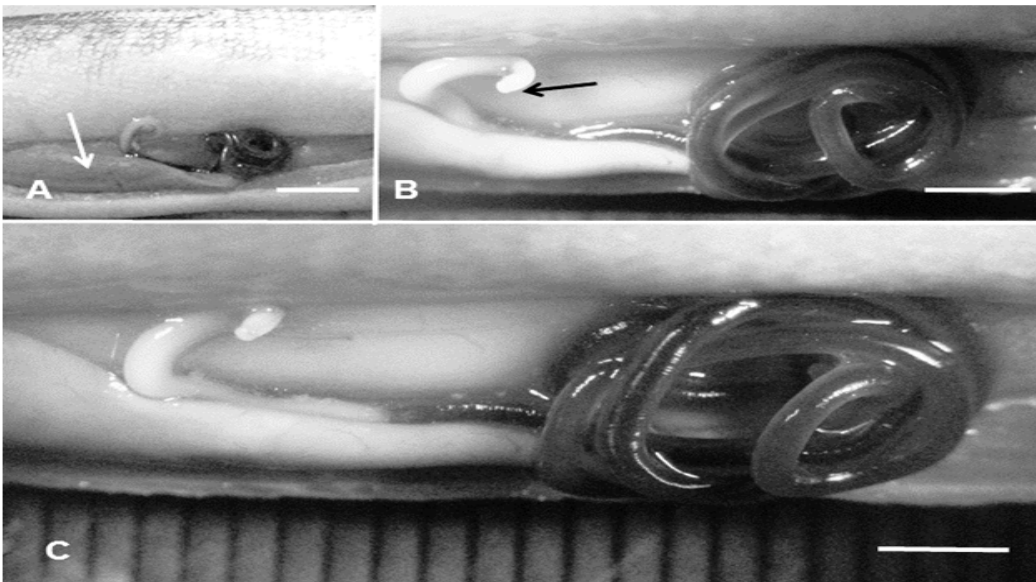


Figure 1. *Eustrongylides* sp. infection in *Esox americanus* from Yashau Creek, McCurtain County, Oklahoma. **A.** Free worm in coelomic cavity near liver (arrow); scale bar = 10 mm. **B.** Closer view of infection showing anterior end of worm (arrow); scale bar = 5 mm. **C.** Higher magnification showing infection; scale bar = 2 mm.

Paneser and Beaver 1979), mudpuppy, *Necturus maculosus* ([experimental infection, von Brand 1944), Blanchard's cricket frog, *Acris blanchardi* (Muzzell and Sonntag 2012), leopard frog, *Lithobates pipiens* ([experimental infection], von Brand 1944; Walton 1949), African clawed frog, *Xenopus laevis* (introduced population, Kuperman et al. 2004), American bullfrog, *Lithobates catesbeianus* (von Brand 1944; [experimental infection], Modzelewski and Culley 1974); in reptiles, including common snapping turtle, *Chelydra serpentina serpentina* (Rausch 1947), painted turtle, *Chrysemys picta* ([experimental infection], von Brand 1944), American alligator, *Alligator mississippiensis* ([experimental infection], von Brand 1944), copperhead, *Agkistrodon contortrix*, eastern racer, *Coluber constrictor*, coachwhip, *Coluber flagellum*, indigo snake, *Drymarchon corais*, northern water snake, *Nerodia sipedon sipedon*, gopher snake, *Pituophis catenifer*; eastern pine snake, *Pituophis melanoleucus*, Mexican garter snake, *Thamnophis eques*, eastern garter snake, *Thamnophis sirtalis sirtalis* and red-sided garter snake, *Thamnophis sirtalis parietalis* ([experimental infection], Lichtenfels and Lavies 1976) (see Winsor 1948; Ernst and Ernst 2006).

Other Western Hemisphere hosts: Cuban treefrog, *Hyla septentrionalis*, Cuba (Walton 1940); Paraguayan caiman, *Caiman yacare*, Paraguay (Goldberg et al. 1991).

Geographic range of genus outside of North America: North and South America, Caribbean, Europe, eastern Africa, China, and Turkey (Xiong et al. 2013).

Geographic range of genus in North America: **U.S.A.:** Arkansas (McAllister et al. 2014, 2015, 2016), California, Florida, Louisiana, Maine, Maryland, Massachusetts, Mississippi, Montana, Nevada, New York, Ohio, Oklahoma (this report), Oregon, Pennsylvania (Philadelphia Zoo captives), Tennessee, Texas (also this report), Utah, Virginia, Washington (DC), Wyoming (Hoffman 1999). **Canada:** British Columbia, Manitoba, Ontario (Hoffman 1999). **Mexico:** Campeche, Guanajuato, Guerrero, Hidalgo, Jalisco, Morelos, Oaxaca, San Luis

Potosí, Veracruz, Yucatán (Salgado-Maldonado 2006), Michoacán (Hoffman 1999; Ernst and Ernst 2006; Salgado-Maldonado 2006).

Additional Arkansas records: Pirate Perch, *Aphredoderus sayanus* (McAllister et al. 2014) collected on 24 October 2013 from the Rolling Fork River, Sevier County (34.064667N, 94.380023W); Blackspotted Topminnow, *Fundulus olivaceus* (McAllister et al. 2015) collected on 23 July 2014 from Crooked Creek at Harmon, Boone County (36° 14' 02.0328"N, 92° 55' 20.2224"W); Northern Studfish, *Fundulus catenatus* (McAllister et al. 2016) collected on 23 July 2014 from Crooked Creek at Kelly's Slab in Yellville, Marion County (36°15'9.9"N, 94°26'25.8"W).

Additional Oklahoma records: None.

Additional Texas records: Rock Bass, *Ambloplites rupestris*, Warmouth, *Lepomis gulosus*, Largemouth Bass, *Micropterus salmoides*, upper San Marcos River, Hays County (Underwood and Dronen 1984).

Specimens deposited: USNPC 105449, 107686, HWML 64707-09 (in EtOH).

Discussion

Specific identification of *Eustrongylides* requires rearing larvae in an avian host and our study did not include this experimental transmission. We did not observe any noticeable pathological effect on these hosts; however, Kaur et al. (2013) reported that fecundity of fish could be reduced with a decline in fish populations, while Mir et al. (2012) noted that fish had a profound decrease in luteinizing hormone with accompanying abnormal histology of their ovaries and testes. When these larvae migrate under the skin and musculature of fishes, it can cause extensive inflammation and necrosis with severe pathologic changes in adjacent tissues (Paperna 1974; Xiong et al. 2009). In one of the largest surveys to date (Weisberg et al. 1986), over 11,000 fishes (20 species) were examined for *Eustrongylides* infection from the Chesapeake Bay region of

Maryland, and only three species of *Fundulus* were found to be harboring these worms. In addition, epizootics are known in various piscivorous birds which can lead to large-scale mortality, particularly among nestlings (Wiese et al. 1977; Roffe 1988; Xiong et al. 2009).

Interestingly, a previous helminth survey on *S. i. nettingi* in Arkansas (McAllister et al. 1994) did not report *Eustrongylides* sp. from this amphibian. And, to our knowledge, this nematode has also not been previously reported from *A. constellatus*, *C. idella*, *E. americanus*, *F. chrysotus*, *L. cyanellus*, *N. s. pleuralis* or *T. p. orarius* (Hoffman 1999; Ernst and Ernst 2006). No *Eustrongylides* sp. were found in 31 and 20 northern water snakes, *N. s. sipedon* from North Carolina and Ohio by Collins (1969) and Gibson and Rabalais (1973), respectively; however, Winsor (1948) was the first to find *Eustrongylides* sp. in *N. s. sipedon* from captive specimens at the Fairmount Park Aquarium in Pennsylvania, and Bursley (1986) later reported *Eustrongylides* sp. from a natural population of *N. s. sipedon* from Virginia. In addition, no *Eustrongylides* sp. was reported from a comprehensive survey on 46 and over 200 water snakes from Alabama and Louisiana by Dettlerline et al. (1984) and Fontenot and Font (1996), respectively. As more and more surveys on helminth parasites of vertebrates of Arkansas, Oklahoma and Texas are conducted, we expect additional hosts of *Eustrongylides* sp. to be reported, particularly new fish hosts.

Acknowledgments

We thank Patricia R. Pilitt (USNPC) and Drs. Scott L. Gardner (HWML) and Renn Tumblison (HSU) for expert curatorial assistance. We also thank Mike Freeze (Keo Fish Farm, Inc., Keo, AR) for providing the Grass Carp, and Paul S. Freed (Scotts Mills, OR) for collecting the *T. p. orarius*. Nikolas H. McAllister (Lukfata Elementary, Broken Bow, OK) assisted the senior author with collecting at Yashau Creek. The Arkansas Game and Fish Commission and Oklahoma Department of Wildlife Conservation provided Scientific Collecting Permits to CTM and HWR.

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Submitted July 30, 2015 Accepted October 26, 2015