
Endoparasites (Apicomplexa, Monogenoidea, Trematoda, Cestoda, Nematoda, Acanthocephala) from Eleven Reptiles (Testudines: Lacertilia: Ophidia) of McCurtain County, Oklahoma, Including the First Report of the Endogenous Stages of *Eimeria robisoni* (Eimeriidae)

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Abstract: Between September 2015 and May 2018, 11 reptiles, including a razor-backed musk turtle (*Sternotherus carinatus*), pallid spiny softshell (*Apalone spinifera pallida*), green anole (*Anolis carolinensis*), broadhead skink (*Plestiodon laticeps*), eastern hognose (*Heterodon platirhinos*), two eastern garter snakes (*Thamnophis sirtalis sirtalis*), prairie kingsnake (*Lampropeltis calligaster calligaster*), southern copperhead (*Agkistrodon contortrix contortrix*), and timber rattlesnake (*Crotalus horridus*) from various sites in McCurtain County, Oklahoma, were examined for endoparasites. Two coccidians (*Eimeria robisoni* and *Choleoeimeria* sp.), an intraerythrocytic hematozoan (*Hepatozoon* sp.), a monogenean (*Polystomoidella oblongum*), two digeneans (*Dasymetra conferta* and *Renifer ellipticus*), two tapeworms (*Testudotaenia testudo* and *Mesocestoides* sp. tetrathyridia), six nematodes (*Capillaria* sp. [ova], *Falcaustra affinis*, *Oswaldocruzia pipiens*, *Serpinema trispinosum*, *Spiroxys amydae* [larvae] and *Kalicephalus inermis coronellae*) and a larval acanthocephalan (*Neoechinorhynchus* sp.) were harbored by these hosts. We document several new host and distributional records for these parasites, including three taxa reported from *L. c. calligaster* for the first time. We also document novel information on the endogenous stages of the coccidian, *E. robisoni*.

Introduction

In the last decade, our parasitological

surveys have attempted to help fill a void in our knowledge of various parasites of Oklahoma's amphibians and reptiles (McAllister et al. 2015, 2016, and references therein). Here, we supplement some of that lack of information by

reporting new host and distributional records for endoparasites from 11 reptiles from the foothills of the southwestern Ouachita Mountains and South Central Plains ecoregions of McCurtain County. In addition, we report on the endogenous stages of the coccidian, *Eimeria robisoni*, for the first time.

Methods

Between September 2015 and May 2018, adult specimens of razor-backed musk turtle (*Sternotherus carinatus*), pallid spiny softshell (*Apalone spinifera pallida*), green anole (*Anolis carolinensis*), broadhead skink (*Plestiodon laticeps*), eastern hognose (*Heterodon platirhinos*), prairie kingsnake (*Lampropeltis calligaster calligaster*), two eastern garter snakes (*Thamnophis sirtalis sirtalis*), southern copperhead (*Agkistrodon contortrix contortrix*), and timber rattlesnake (*Crotalus horridus*) were collected by hand, snake tong, or from dead on the road (DOR) from several sites in McCurtain County (Fig. 1), and examined for endoparasites. Specimens were placed in collection bags in the refrigerator (4°C), and necropsied within 24 hr. They were measured for snout-vent length (SVL) or carapace length (CL), killed by an intraperitoneal injection of sodium pentobarbital (Nembutal®) following accepted guidelines (SIH 2004), and examined for apicomplexan and helminth parasites. A bone saw was used to remove the plastron from turtles to expose the heart and a mid-ventral incision from mouth to cloaca was made to expose the same in other reptiles. Blood was obtained from all reptiles by making a small incision in their heart and taking a sample using ammonium heparinized (75 mm long) capillary tubes. Thin films were smeared onto glass slides, air-dried, fixed for 1 min in absolute methanol, stained for 20–30 min with Wright-Giemsa stain, and rinsed in phosphate buffer (pH = 7.0). Slides were scanned at 100× or 400× and when infected cells were found, photographs were taken and length and width (L × W) measurements were made on gamonts of an intraerythrocytic parasite (n = 20) using a calibrated ocular micrometer under a 1,000× oil immersion lens and are reported in micrometers as means ±1SD followed by the ranges. Feces

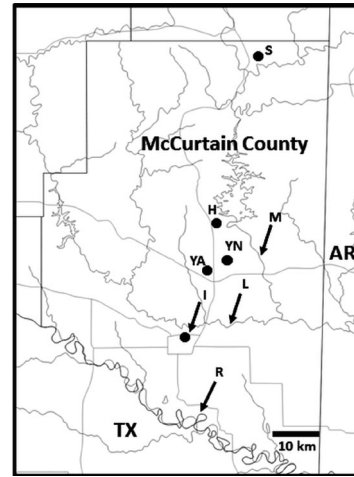


Figure 1. Map showing location of McCurtain County, Oklahoma, and sites (●) where reptilian hosts were collected. Abbreviations: H (Hochatown●); I (Idabel●); L (Little River); M (Mountain Fork River); R (Red River); S (Smithville●); YA (Yashau Creek●); YN (Yanubbee Creek●).

from the rectum was collected from all specimens and examined for coccidia and helminth eggs following the methods of McAllister et al. (2014). Intestinal tissues from *A. carolinensis* were placed in 10% neutral-buffered formalin and processed following Presnell and Schreibmann (1997) for examination of endogenous coccidial stages by light microscopy. Other visceral organs, particularly those of the GI tract from all specimens, were examined for helminths by removing and splitting organs lengthwise, placing separate organs in a Petri dish with 0.9% saline, and their contents scanned at 20–30× using a stereomicroscope. The liver and other suspected infected tissues from the turtle and mesenteries from two eastern garter snakes were also biopsied and specimens processed for examination by light microscopy following Presnell and Schreibman (1997). The conjunctival sac and blood of both turtles were examined for polystomatid monogenes and spirorchiid trematodes, respectively, per Snyder and Clopton (2005). Monogeneans, trematodes and cestodes were fixed in nearly 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. Nematodes were

fixed in hot tap water and studied as temporary mounts on a microscopic slide in a drop of glycerol. Acanthocephalans were placed in a Petri dish with tap water overnight to allow for their proboscides to evert, after which they were transferred to 95% (v/v) DNA grade ethanol. Host vouchers are deposited in the Arkansas State University Museum of Zoology (ASUMZ) Herpetological Collection, State University, Arkansas, or the Henderson State University Herpetological Collection (HSU), Arkadelphia, Arkansas. Actual vouchers or photovouchers of parasites are deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska.

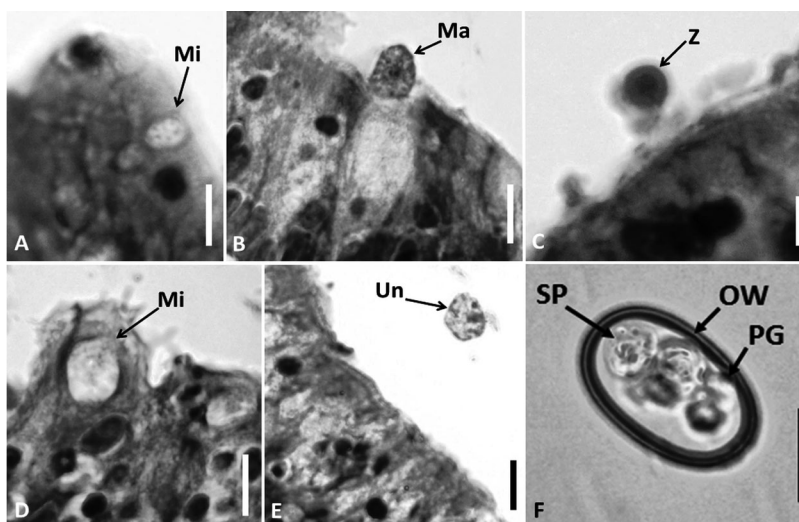
Results and Discussion

Fifteen taxa of endoparasites, including three apicomplexans, one monogenean, two digeneans, two tapeworms, six nematodes, and an acanthocephalan were harbored by 11+ hosts. An annotated list of the parasites found and the host data follows.

Apicomplexa: Coccidiasina: Eucoccidiorida: Eimeriidae

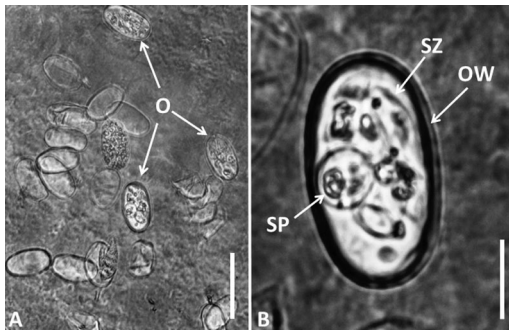
Eimeria robisoni – McAllister, Seville, and

Connior, 2014. – Sporulated oocysts (Fig. 2F) of *E. robisoni* (HWML 139886) were found to be passing in the feces of a single adult (53 mm SVL) *A. carolinensis* collected on 18 September 2016 from Hochatown (34° 09' 55.152"N, 94° 45' 35.8776"W). Four other *A. carolinensis* from the same site collected between March 2013 and April 2014 were not passing oocysts. These oocysts matched the description of *E. robisoni* from Arkansas (see McAllister et al. 2014) quite well. The endogenous stages of *E. robisoni* from the intestine are reported here for the first time (Figs. 2A–E). However, these stages appear to be developing in the area of the brush border of epithelial cells (Figs. 2B–D) pushing out into the lumen. As such, they appear to fit nicely into the genus *Acroeimeria* (rather than *Eimeria*) where localization of endogenous development is reported to occur in the microvillus zone of intestinal epithelial cells of reptiles (see Paperna and Landsberg 1989). Further work will be necessary to resolve this placement including molecular analyses. Nevertheless, this eimerian has now been reported in *A. carolinensis* from Oklahoma and, as such, we suggest that additional populations of this anole in other states may as well be infected with the coccidian.



Figures 2A–F. Endogenous stages from the intestinal epithelium and a sporulated oocyst of *Eimeria robisoni* from feces of *Anolis carolinensis*. A. Mi (Microgamont); scale bar = 10 μ m. B. Ma (Macrogamont); scale bar = 10 μ m. C. Z (zygote); scale bar = 10 μ m. D. Mi (microgamont); scale bar = 10 μ m. E. Un (Unsporulated oocyst); scale bar = 10 μ m. F. Sporulated oocyst, OW (oocyst wall); PG (polar granule); SP (sporocyst); scale bar = 10 μ m.

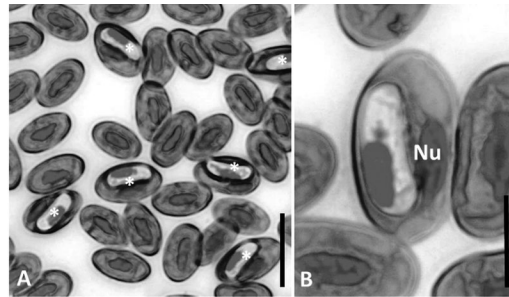
***Choleoimeria* sp.** – Unsporulated and sporulated oocysts (Figs. 3A–B) of an unknown choleoimerian (HWML 139887) were found in the feces of a 550 mm SVL *A. c. contortrix* collected on 21 September 2015 from near Yanubbee Creek, just north of Broken Bow off US 259 (34° 03' 45.9216"N, 94° 44' 17.955"W). Coccidians have not been previously reported from any taxon of copperhead (Duszynski and Upton 2009). Since we do not have endogenous stages from gall bladder tissues of this DOR snake, we are reporting this coccidian here with some hesitation. It could represent a pseudoparasite from a prey item of this viperid (perhaps a skink) that was just passing through the digestive tract of the snake. Additional research will be necessary to determine whether or not this is a true coccidian of *A. c. contortrix*.



Figures 3A–B. Oocysts of a *Choleoimeria* sp. from feces of *Agkistrodon contortrix contortrix*. A. Sporulated oocysts (O, arrows); scale bar = 30 μ m. B. Higher magnification of sporulated oocyst showing OW (oocyst wall), SP (sporocyst), and SZ (sporozoite); scale bar = 10 μ m.

Adeleorina: Hepatozoidae

***Hepatozoon* sp. Miller, 1908** – About 40% of the red blood cells (rbc's) of a *L. c. calligaster* (adult male, 550 mm SVL), collected on 5 May 2018 from Smithville (34° 28' 0.4794"N, 94° 38' 37.6794"W) contained an intraerythrocytic hematozoan (HWML 139888) thought to belong to the genus *Hepatozoon* (Figs. 4A–B). Measurements (L \times W) of bean-shaped gamonts were 16.5 \times 6.1 (14–19 \times 5–7) μ m. In nearly every microscopic field scanned at 400 \times , infected rbc's could be seen (Fig. 4A). Hematozoans have been previously reported from two other snakes from



Figures 4A–B. Photomicrographs of hematozoans from *Lampropeltis calligaster calligaster*. A. Six gamonts (*) in a single microscopic field at 400 \times showing parasite intensity; scale bar = 20 μ m. B. Single gamont showing nucleus (Nu) of host red blood cell; scale bar = 10 μ m.

Oklahoma, the western rat snake, *Pantherophis obsoletus* and *C. horridus* (McAllister 2015). All hemogregarines of snakes are considered to be members of the genus *Hepatozoon* even when life-cycle data is not available, as in our case (Smith 1996; Smith and Dessler 1997). We therefore document this hematozoan from *L. c. calligaster* to represent a *Hepatozoon* sp. This is the first time any haemogregarine has been reported from a prairie kingsnake.

Monogenoidea: Polystomatidae: Polystomatidae

***Polystomoidella oblongum* (Wright, 1879) Price, 1939.** – Five polystomes (specimens retained for further work) (Fig. 5) matching the description of *P. oblongum* (Price 1939) were found in the urinary bladder of a 115 mm CL *S. carinatus* collected on 9 October 2016 from Yanubbee Creek off Currence Road at Broken Bow (34° 02' 45.75"N, 94° 43' 19.66"W). Two *Polystomoidella* are known from North America, *P. oblongum* and *P. whartoni* (Wright, 1879) Price, 1939 (Du Preez and Morrison 2012). Both species have been reported from various kinosternid turtles (Ernst and Ernst 1977), and *P. oblongum* was reported previously from *S. carinatus* from Texas (Price 1939). It has also been reported from Oklahoma in common snapping turtle, *Chelydra serpentina* (Williams 1953) but has not, to date, been documented from *S. carinatus* in the state. Here, we report *P. oblongum* in a razor-backed musk turtle from Oklahoma for the first time.

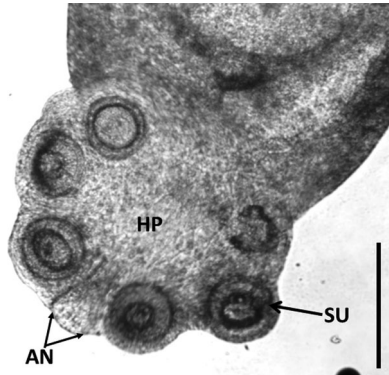


Figure 5. *Polystomoidella oblongum* from urinary bladder of *Sternotherus carinatus*; scale bar = 500 μ m. Abbreviations: AN (paired anchors); HP (haptor), SU (sucker).

Trematoda: Digenea: Ochetosomatidae

Dasymetra conferta Nicoll, 1911 – Thirty-five *D. conferta* (specimens retained) were found in the esophagus of an adult (650 mm SVL) *H. platirhinos* collected on 15 October 2017 from the same Hochatown site herein. McAllister and Bursey (2012) and McAllister et al. (2016) previously reported *D. conferta* from diamondback watersnake (*N. rhombifer*) from Yashau Creek (McCurtain County) and from blotched watersnake (*Nerodia erythrogaster transversa*) from Hochatown, respectively. The eastern hognose is a new host record for *D. conferta*.

Renifer ellipticus Pratt, 1903 – Three *R. ellipticus* (HWML 110494) were taken from the oral cavity and esophagus of an adult (505 mm SVL) *T. s. sirtalis* collected on 11 May 2018 from the same Hochatown site above. In addition, the same *L. c. calligaster* noted herein harbored two *R. ellipticus* (specimens retained) in its esophagus. This digenean was recently documented from a black racer (*Coluber constrictor priapus*) from the identical Hochatown site (McAllister et al. 2016). This digenean shows little host specificity as *R. ellipticus* has also been reported previously from *H. platirhinos* (locality not given), blue racer, *Coluber constrictor foxi* from Illinois (Dyer and Ballard 1989) as well as other colubrid snakes in North America, including *Coniophanes* sp., indigo snake, *Drymarchion corais*, northern speckled racer, *Drymobius margaritiferus*,

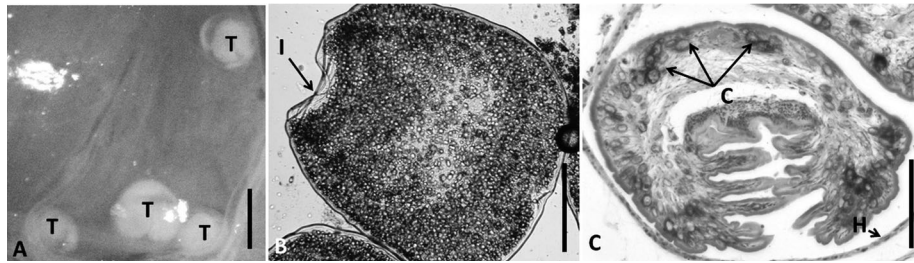
common kingsnake, *Lampropeltis getula*, northern cat-eyed snake, *Leptodeira septentrionalis*, *Micrurus* sp., *N. rhombifer*, blackneck garter snake, *Thamnophis cyrtopsis*, and eastern ribbonsnake, *Thamnophis sauritis* from Arizona, New Mexico, South Dakota, and Tabasco, México (see Ernst and Ernst 2006). We document two new host records for *R. ellipticus*.

Cestoda: Eucestoda: Proteocephalidea: Proteocephalidae

Testudotaenia testudo (Magath, 1924) de Chambrier, Coquille, Mariaux, and Tkach, 2009 – Several gravid *T. testudo* were taken from the small intestine of an adult (360 mm CL) *A. s. pallida* collected on 23 September 2017 from Yashau Creek at Broken Bow (34° 02' 27.0018"N, 94° 45' 21.8046"W). Although this tapeworm has been reported previously from Oklahoma in red-eared sliders (*Trachemys scripta elegans*) and map turtles (*Graptemys geographica*), it was in an unpublished dissertation by McKnight (1959). This cestode was originally described by Magath (1924) from eastern spiny softshell (*A. spinifera spinifera*) from Minnesota. It has also been reported from *A. spinifera* (most likely Gulf Coast spiny softshell, *A. s. aspera*) from Louisiana (Acholonu 1970) and redescribed from eastern spiny softshells and bowfins (*Amia calva*) from Tennessee (de Chambrier et al. 2009). In addition, Brooks (1978) reported *Proteocephalus* (= *T.*) *testudo* from *T. s. elegans* from Nebraska but de Chambrier et al. (2009) noted his material “may represent another species” and we concur. We document the first published report of *T. testudo* from Oklahoma as well as a new host record for the tapeworm in the subspecies, *A. s. pallida*. Specimens are being retained for molecular studies (T Scholz, pers. comm.).

Cyclophyllidea: Mesocestoididae

Mesocestoides sp. – Tetrathyridia of *Mesocestoides* sp. (Figs. 6A–C) were found in two *T. s. sirtalis* (505, 550 mm SVL) collected on 8 October 2016 and 12 May 2018, both from the same Hochatown site herein. This enigmatic cestode, for whom no complete life cycle is known, has been previously reported from *T. s. sirtalis* from Arkansas (McAllister et



Figures 6A–C. Tetrathyridia of *Mesocestoides* sp. from *Thamnophis sirtalis sirtalis*. A. Low power macroscopic view showing encapsulated tetrathyridia (T) in mesenteries; scale bar = 1.0 mm. B. Unstained whole mount of free tetrathyridium with invaginated scolex (I); scale bar = 250 μ m. C. Brightfield microscopic view of stained histological section of tetrathyridium showing calcareous corpuscles (C) in a host-derived fibrotic capsule (H); scale bar = 250 μ m.

al. 2014b); it has also been reported previously from Oklahoma in Sequoyah slimy salamander (*Plethodon sequoyah*), Hurter's spadefoot (*Scaphiopus hurterii*), plains spadefoot, (*Spea bombifrons*), American bullfrog (*Rana catesbeiana*), and ground skink (*Scincella lateralis*) (see McAllister et al. 2017c, 2018). It is a cosmopolitan genus that has been documented from a variety of amphibians and reptiles from the Asian, Australo-Papuan, Ethiopian, Nearctic, Neotropical, and Palearctic regions (Bursey et al. 2012; McAllister et al. 2014b). We report *Mesocestoides* sp. in Oklahoma specimens of *T. s. sirtalis* for the first time. These specimens are being processed further for molecular analysis of the genus *Mesocestoides* in reptiles (VV Tkach, pers. comm.).

**Nematoda: Enoplida: Trichuroidea:
Capillariidae**

Capillaria sp. – Ova of an unknown *Capillaria* sp. (HWML 139889, Fig. 7) was found to be passing in feces of a 1,000 mm SVL *C. horridus* collected on 10 May 2017 from the campus of Eastern Oklahoma State College, Idabel (33° 55' 16.0572" N, 94° 46' 35.1084" W). *Capillaria* spp. has been reported previously from *C. horridus* from Virginia (Soloman 1974). *Capillaria* is the only known trichurid genera affecting reptiles and they have a direct life cycle; diagnosis is based on the presence of thick-shelled eggs with polar plugs at both ends (Fig. 7). We document this nematode from an Oklahoma *C. horridus* for the first time.

Ascaridida: Kathlaniidae

Falcaustra affinis (Leidy, 1856) Harwood, 1932 – Twenty-three *F. affinis* (HWML 110495) were found in the rectum of the same *A. s. pallida* noted herein. Mackin (1936) reported *F. affinis* from the eastern river cooter, *Pseudemys concinna concinna* from Oklahoma. A similar species, *F. chelydrae* (Harwood, 1932) has been reported to occur in *A. spinifera* in Oklahoma by McKnight (1959) but that was in his unpublished dissertation. It is obvious that *F. affinis* is a common and widely distributed parasite of turtles (and anurans) of North America, including several species in the families Chelydridae and Emydidae from Arkansas, Florida, Georgia, Illinois, Indiana, Maryland, Ohio, Oklahoma, Oregon, Texas, and Wisconsin, Ontario, Canada, and México (Baker 1986, 1987). We report *F. affinis* from *A. s. pallida* for the first time.



Figure 7. *Capillaria* ova from feces of *Crotalus horridus* showing the characteristic bipolar plugs; scale bar = 30 μ m.

Spirurida: Camallanata: Camallanidae

***Serpinema trispinosum* (Leidy, 1852) Yeh, 1960** –Thirteen *S. trispinosum* (HWML 110496) were found in the small intestine of the same *A. s. pallida* noted herein. This nematode has previously been reported from *P. c. concinna* and *C. serpentina* (McAllister et al. 2015), both from McCurtain County. It has also been reported from at least 18 species of Nearctic turtles ranging from the Canadian border to Texas (Baker 1987; Wiles and Bolek 2015), including an older report from Oklahoma (Harwood 1931). In the life cycle, copepods serve as intermediate hosts, and paratenic hosts include lymnaeid snails, damselflies, anurans, and fish (Moravec and Vargas-Vázquez 1998). Prey items of *A. spinifera* recorded from other parts of its range are larval and adult aquatic insects, crayfish, and fish (Lagler 1943; Ernst and Barbour 1972; Cochran and McConville 1983). To our knowledge, this nematode has not been previously reported from *A. s. pallida*, so we document a new host record for *S. trispinosum* here.

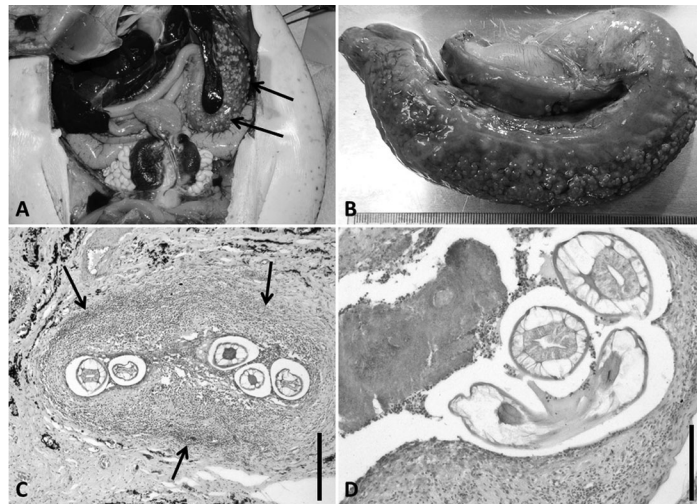
Spirurata: Gnathostomatidae

***Spiroxys amydae* Cobb, 1929 (Figs. 8A–D)** – Nematodes of *S. amydae* (HWML 139893)

were found encysted in the stomach, small intestine, and other visceral organs of the same *A. s. pallida* noted above. This nematode has been reported previously in encysted tissues of *A. s. aspera* from Mississippi (Cobb 1929; Hedrick 1935) and México (Peña-Rivera et al. 1994), and from *A. s. pallida* from Texas (Harwood 1932). Similar eroding lesions were reported from Florida softshell (*Apalone ferox*) from Florida (Foster et al. 1998). We report *S. amydae* from Oklahoma for the first time.

Strongylida: Diaphanocephaloidea: Diaphanocephalidae

***Kalicephalus inermis coronellae* (Ortlepp, 1923) Lichtenfels, 1980** – Two (male, female) *K. i. coronellae* (HWML 110417) were taken from the intestine of the same *L. c. calligaster* noted herein. This nematode has been reported from various colubrid and viperid snakes from the United States (Colorado, Florida, Georgia, Louisiana, Massachusetts, North Carolina, and Texas), Québec, Canada, and Guerrero, Michoacán, and Vera Cruz, México (Schad 1962; Baker 1987). This is the first time *K. i. coronellae* has been documented from a prairie kingsnake and from Oklahoma, and more importantly, only the third helminth reported



Figures 8A–D. *Spiroxys amydae* infection in *Apalone spinifera pallida*. A. Macroscopic view of infection in stomach and intestinal tract (arrows). B. Closer view of infection showing white lesions in sectioned stomach. C. Cross sectional microscopic view of infection in stomach showing five *S. amydae* producing a host granulomatous reaction (arrows); scale bar = 2.0 mm. D. Higher power microscopic view of cross section showing three *S. amydae*; scale bar = 1.0 mm.

from this host (McAllister et al. 2008; this study).

Trichostrongyloidea: Molineidae

***Oswaldocruzia pipiens* Walton, 1929** – An adult *P. laticeps* (102 mm SVL) collected on 20 August 2017 from Hochatown harbored eleven (9 male, 2 female) *O. pipiens* (HWML 110502) in its small intestine. This nematode shows little host specificity as it has been reported from various amphibians and reptiles, including salamanders, frogs and toads, skinks (*Scincella lateralis* and *Plestiodon fasciatus*) and other reptilian hosts from Arizona, Arkansas, California, Illinois, Indiana, Louisiana, Maryland, Ohio, Oklahoma, Texas, Washington, and México (see McAllister et al. 2014 for summary). We report a new host record for *O. pipiens* in *P. laticeps*.

Acanthocephala: Eoacanthocephala: Neoechinorhynchida: Neoechinorhynchidae

***Neoechinorhynchus* sp.** – Five juvenile (2 male, 3 female) *Neoechinorhynchus* sp. (HWML 139853–139854) were found in the intestinal tract of the same *A. s. pallida* noted herein. Since these worms were juveniles it was not possible to determine their specific identity. However, *N. chrysemydis* Cable and Hopp, *N. emyditoides* Fisher, and *Neoechinorhynchus* sp. have been reported from *A. spinifera* (subspecies not specified but most likely *A. s. aspera*, see Powell et al. [2016]) from southeastern Louisiana (Acholonu 1966, 1969). We therefore document acanthocephalans from the pallid spiny softshell for the first time.

In summary, we report some new host and geographic records for these parasites, and, more importantly, several for a colubrid snake that has rarely been reported to be harboring any parasite. Indeed, although much is known about the ecology of *L. c. calligaster* (Blaney 1979), information on its parasites is mostly lacking. Coccidian parasites (Apicomplexa) have been reported from this snake in Arkansas (McAllister et al. 1995, 2017b), Illinois (Anderson et al. 1968), and Texas (McAllister et al. 2017a). A single nematode, *Physaloptera abjecta* was documented from *L. c. calligaster* from Arkansas (McAllister et al. 2008). However, the current

study is the initial report of an intraerythrocytic hematozoan from *L. c. calligaster* as well as the second and third helminth ever reported from this host. With the diverse reptilian fauna in Oklahoma (Sievert and Sievert 2011), we expect additional new host and geographic distribution records to be reported with extensive surveys, including the possibility of discovering new taxa.

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