Noteworthy Records of Helminth Parasites (Monogenea, Trematoda, Cestoda, Nematoda, Acanthocephala) from Select Herpetofauna (Anura, Testudines, Ophidia) from McCurtain County, Oklahoma

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Abstract: As part of our continual pursuit to report on the geographic distribution and host records of the helminth parasites of southeastern Oklahoma herpetofauna, we recently had the opportunity to examine select amphibians (two anuran species) and reptiles (two turtle and eight snake species) from the southeastern part of the state. Found were: two monogeneans, two digeneans, three tapeworms, five nematodes, and an acanthocephalan. We document several new host and distributional records for these parasites.

Introduction

For the last several years, our research group has reported biological information in a series of reports on the parasites of amphibians and reptiles in Oklahoma (McAllister and Bursey 2012; McAllister et al. 2014, 2015, 2016, 2018, 2020, and references therein). The present work compliments our aforementioned surveys and provides some new records for helminth parasites of select Oklahoma herpetofauna.

Methods

Between April 2019 and September 2021, two species of amphibians and 10 species of reptiles, including (single specimens except where noted): Cajun chorus frog, *Pseudacris fouquettei*

Lemmon, Lemmon, Collins, and Cannatella, three Coastal Plains leopard frogs, Rana sphenocephala utricularius (Cope), Mississippi mud turtle, Kinosternon subrubrum hippocrepis Grav, eastern river cooter, Pseudemys concinna concinna (LeConte), southern black racer, Coluber constrictor priapus Dunn and Wood, eastern hog-nosed snake, Heterodon platirhinos (Latreille), plain-bellied watersnake, Nerodia erythrogaster (Forster), banded watersnake, Nerodia fasciata confluens diamond-backed (Blanchard), northern watersnake, Nerodia rhombifer rhombifer (Hallowell), flat-headed snake, Tantilla gracilis Baird and Girard, red-sided gartersnake, Thamnophis sirtalis parietalis (Say), and three northern cottonmouths, Agkistrodon piscivorus (Lacépède) were collected by hand, snake tong or dead off the road (DOR) from various sites in McCurtain County and examined for

helminth parasites. Specimens were placed in cloth collection bags, placed in a refrigerator, and necropsied within 24 hr. Turtles were measured for straight-line carapace length (CL) and anurans and snakes for snout-vent length (SVL), killed by an intraperitoneal injection of sodium pentobarbital (Nembutal®) following accepted guidelines (SIH 2004), and examined for 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. For intravascular trematodes in turtles, we followed methods of Snyder and Clopton (2005). Visceral organs, particularly those of the GI tract of all specimens, were examined for helminths by removing and splitting them lengthwise, placing separate organs in a Petri dish with 0.9% saline, and their contents scanned at 20-30× using a stereomicroscope. Trematodes and cestodes were fixed in nearly boiling tap water without coverslip pressure, transferred to 70% (v/v) ethanol, stained with acetocarmine, dehydrated in a graded ethanol series, cleared in methyl salicylate, 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. Encapsulated acanthocephalans were teased from tissues and fixed in 70% (v/v) ethanol and examined as temporary mounts. Further examinations were made at 100 to 400× with an Olympus BX-51 upright research microscope configured for Brightfield (BF) and Differential Interference-Contrast (DIC) microscopy.

We followed the common and scientific names of North American herpetofauna of Crother (2017) except for adopting Yuan et al. (2016) in our usage of *Rana* rather than *Lithobates* for Oklahoma's ranid frogs. Host vouchers are deposited in the Eastern Oklahoma State Vertebrate Collection (EOSC), Idabel, Oklahoma. Genuine vouchers or photovouchers of parasites are deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska. Monogeneans, digeneans, and a nematode species are saved in DNA grade ethanol and being utilized in molecular studies at the University of

North Dakota, Grand Forks (VV Tkach, *pers. comm.*) and as well as *Ophiotaenia* spp. at the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Czech Republic (T Scholz, *pers. comm.*).

Results and Discussion

Fourteen taxa of endoparasites, including two monogeneans, three digeneans, three tapeworms, five nematodes, and an acanthocephalan were harbored by 12 host species; two hosts harbored multiple infections. An annotated list of the parasites found and the host data follows.

Platyhelminthes: Trematoda: Monogenea: Polystomatidea: Polystomatidae

Neopolystoma sp. – A single specimen was found in the urinary bladder of an adult (115 mm CL) K. s. hippocrepis collected on 22 May 2021 from off US 259, north of Hochatown (34°10'25.23"N,-94°43'35.2878"W). individual possessed a type III sucker characteristic of Neopolystoma (Du Preez and Theunissen 2021). At present, at least 54 turtle polystome species are known from 55 host species (DuPreez and Van Rooyen 2015). The family Polystomatidae (Platyhelminthes: Monogenea) comprises 25 genera of which three (Polystomoides Ward, 1917, Polystomoidella Price, 1939, and Neopolystoma Price, 1939) infect freshwater turtles (Morrison and DuPreez 2011). Neopolystoma is found in the oral, nasal, and ocular cavities, cloaca, and urinary bladder and has no hamuli. Neopolystoma orbiculare (Stunkard, 1916) Price 1939, has previously been reported from Oklahoma in red-eared slider, Trachemys scripta elegans (Wied) and western painted turtle, Chrysemys picta bellii (Gray) (Harwood 1931; Price 1939; Everhart 1957; McKnight 1959). A Neopolystoma sp. was reported from snapping turtle, Chelydra serpentina (L.) from Oklahoma by McAllister et al. (2015); however, specimens were found in the conjunctival sacs of this host. We document the first report of a *Neopolystoma* sp. from *K. s.* hippocrepis and the first time a member of the genus that inhabits the urinary bladder has been reported in any Oklahoma host.

Polystomoidella oblongum Wright, 1879 - Two individuals were found in the urinary bladder of a 270 mm CL P. c. concinna collected on 29 June 2021 from Broken Bow (34°02' 11.4648"N, -94°45'25.0662"W). Polystomoidella parasitizes the urinary bladder of turtles and possesses a single pair of hamuli. Two Polystomoidella species are known from North America and are P. oblongum Wright, 1879, and Polystomoidella whartoni Wright, 1879. The former was originally described from S. odoratus from Canada (Wright 1879) and has also been reported from C. serpentina, musk Sternotherus razor-backed turtle. carinatus (Gray), and southeastern mud turtle, Kinosternon subrubrum subrubrum (Lacépède) (Stafford 1900; Price 1939). McAllister and Bursey (2012) reported P. whartoni from the urinary bladder of a K. s. hippocrepis from Latimer County, Oklahoma. The eastern river cooter represents a new host for P. oblongum.

Digenea: Schistosomatoidea: Spirorchiidae

Hapalorhynchus sp. – Several spirochiid flukes were found in the blood and body wash of the same K. s. hippocrepis above; it appears to not fit any described species. As currently defined (Platt 2002), the genus Hapalorhynchus Stunkard, 1922, contains 19 species (Smith 1997). Three of the six species of *Hapalorhynchus* previously reported from North America infect various kinosternids, including: H. albertoi Lamothe-Argumento, 1978, from white-lipped mud turtle, Kinosternon leucostomum Duméril and Bibron in Duméril and Duméril, from Tabasco, México, H. reelfooti Byrd, 1939, from eastern musk turtle, Sternotherus odoratus (Latreille, in Sonnini and Latreille) from Tennessee, and H. stunkardi Byrd, 1939, from S. carinatus from Georgia (Byrd 1939; Lamonthe-Argumento 1978; Platt 1988; Platt and Snyder 2002). More recently, however, several species were reported by Roberts et al. (2017) from Alabama and Florida musk turtles, including a new species, Hapalorhynchus conecuhensis Roberts and Bullard, 2017, from innominate musk turtle, Sternotherus cf. minor and loggerhead musk turtle, Sternotherus minor (Agassiz), as well as previously described *H. reelfooti* from *S. minor*,

stripe-necked musk turtle, *Sternotherus peltifer* (Smith and Glass), *S. odoratus*, and *S. cf. minor*, and *Hapalorhynchus* cf. *stunkardi* from *S. minor* and *S. odoratus*. However, this is the first time a *Hapalorhynchus* sp. has been reported from *K. s. hippocrepis*.

Plagiorchiida: Plagiorchiidae

Styphlodora magna Byrd and Denton, 1938 – Eight S. magna (Fig. 1) was found in the gallbladder of an adult (480 mm SVL) A. piscivorus collected on 1 May 2021 from Hochatown (34°10'12.4926"N, -94°45'01.1442"W). McAllister et al. (2020) previously reported S. magna from western cottonmouths from the same locale. Byrd and Denton (1938) described S. magna from the gallbladder of northern watersnake, Nerodia sipedon sipedon (L.) from Georgia and Mississippi. It has been previously reported from A. piscivorus from North Carolina (Collins 1969). We document the second report of S. magna from A. piscivorus in Oklahoma.

Ochetosomatidae

Renifer ancistrodontis (MacCallum, 1921) Talbot, 1934 – Six specimens were found in the oral cavity of the same A. p. leucostoma above. The type host and locality of R. ancistrodontis is a captive specimen of the copperhead, Agkistrodon contortrix (L) from the New York Zoological Gardens, New York (MacCallum 1921). Since then, this digenean has been previously reported from northern cottonmouths from Alabama (Detterline et al. 1984) and Texas (McAllister et al. 2008). However, this is the first time this parasite has been reported from Oklahoma. The genus is badly in need of revision which should embrace molecular analyses.

Cestoda: Eucestoda: Bothriocephalidea: Proteocephalidae

Ophiotaenia grandis La Rue, 1911 – Several specimens (Fig. 2) were taken from the intestine of two *A. piscivorus* (437 mm SVL) collected on 23 June 2019 and 17 August 2019 in Hochatown and from off US 259 at US 259A junction (34°07'10.3038"N, -94°44'23.3016"W), respectively. La Rue (1911) originally described



Figures 1–3. Macrophotographs of some of the helminth parasites reported herein. (1) Several Syphlodora magna from intestine of Agkistrodon piscivorus; scale bar = 1 mm. (2) Numerous Ophiotaenia grandis from intestine of A. piscivorus. (3) Two Rhabdias cf. joaquinensis from lungs of Rana sphenocephala utricularius; scale bar = 1 mm.

O. grandis from A. piscivorus from a captive specimen at the National Zoological Park, Washington, D.C. (exact collection locality unknown). It has been previously reported from A. piscivorus, Mississippi green watersnake, Nerodia cyclopion (Duméril, Bibron and Duméril), Florida green watersnake, Nerodia floridana (Goff), northwestern gartersnake, Thamnophis ordinoides (Baird and Girard), and T. sirtalis from Florida, Louisiana, Mississippi, Oklahoma, and Texas, and Sasketchewan, Canada (see Ernst and Ernst 2006).

Ophiotaenia perspicua La Rue, 1911 - Several individuals were collected from the intestine of an adult (755 mm SVL) N. rhombifer collected on 10 June 2019 from 4.8 km N of Idabel from an oxbow lake off the Little River at the Turner Ranch (33°55'58.3278"N. -94°43'42.6642"W). This tapeworm originally described by La Rue (1911) from N. rhombifer from the Illinois River at Havana, Illinois; additional specimens came from an unknown locality in Oklahoma from the same host species. Detterline et al. (1984) reported it from N. rhombifer, N. sipedon, and A. piscivorus from Alabama, Fontenot and Font (1996) reported it from N. cyclopion, N. f. confluens, N. rhombifer, and A. piscivorus from Louisiana, and McAllister et al. (2012) reported O. perspicua from prairie ringneck snake, Diadophis punctatus arnyi Kennicott from Oklahoma. The geographic range of this cestode includes Alabama, Florida, Idaho, Illinois, Louisiana, Mississippi, North Carolina, Ohio, Oklahoma, Texas, and Québec, Canada, and Hidalgo and Veracruz, México (Brooks 1978; Detterline et al. 1984; Fontenot and Font 1996; Ernst and Ernst 2006; Goldberg et al. 2012; McAllister et al. 2012). These specimens are being retained for molecular analyses (T Scholz, pers. comm.).

Ophiotaenia cf. perspicua La Rue, 1911 – Several individuals that differ from typical O. perspicua were found in the intestinal tract of an adult (425 mm SVL) N. f. confluens collected on 29 June 2019 from Broken Bow (34°00'41.994"N, -94°44'58.9662"W). Ophiotaenia perspicua has been previously reported from N. fasciata from Louisiana (Fontenot and Font 1996). Additional material from two adult colubrid snakes from Hochatown, including N. erythrogaster (735 mm SVL) collected on 26 August 2020 and a T. s. parietalis (700 mm SVL) collected on 30 April 2021, were identified as O. cf. perspicua. Ophiotaenia perspicua has been previously reported from *T. sirtalis* (Ernst and Ernst 2006). However, no tapeworms, to our knowledge, have been previously reported from *N. erythrogaster* (Detterline et al. 1984; Ernst and Ernst 2006) so we here document the first report of an *Ophiotaenia* in this host.

Nematoda: Rhabditoidea: Rhadiasidae

Rhabdias cf. **joaquinensis** Ingles, 1935 – A total of nine (3.0 ± 1.4) , range 2–5, Fig. 3) specimens that differed from typical *R. joaquinensis* were taken from the lungs of three (49, 75, 80 mm SVL) *R. s. utricularius* collected on 20 July 2020, 9 May 2021, and 25 July 2021 from the Hochatown site. The type host

and locality of *R. joaquinensis* is the northern red-legged frog, *Rana aurora* Baird and Girard from California (Ingles 1935). Since then, the species has been reported in various ranid frogs, including *R. s. utricularius* and the American toad, *Anaxyrus americanus* (Holbrook) from Arkansas, Iowa, Kansas, Mississippi, Nebraska, Oklahoma, and Tennessee (Kuzmin et al. 2003; Vhora and Bolek 2015). We document the first report of *R.* cf. *joaquinensis* in an Oklahoma host.

Enoplida: Trichuroidea: Capillaridae

Capillaria sp. – Ova of an unknown species of Capillaria (Fig. 4) was found in the feces of A. piscivorus collected on 1 May 2021 from Hochatown. Capillaria heterodontis Harwood, 1932, has been previously reported from A. piscivorus from Texas (Harwood 1932) and Louisiana (Fontenot and Font 1996); a Capillaria sp. and Capillaria colubra Pence, 1970, was reported from A. piscivorus from North Carolina (Collins 1969; Davis et al. 2016). In Oklahoma, McAllister et al. (2018) reported a Capillaria sp. egg from the feces of a timber rattlesnake, Crotalus horridus L. This nematode belongs to the only known trichuroid genus that infects reptiles and which possesses a direct life cycle. The presence of thick-shelled ova with polar plugs at both ends of the egg (Fig. 4) allows for generic diagnosis. Although we are unable to provide a specific identity, we document the genus from an A. piscivorus from Oklahoma for the first time.

Spirurida: Physalopteridae

Physaloptera abjecta Leidy, 1856 – Three specimens (2 males, 1 female) were found in the stomach of a *H. platirhinos* (600 mm SVL) collected from Hochatown on 15 April 2019. Physaloptera abjecta was originally described by Leidy (1856) from Coluber (=Masticophis) flagellum Shaw (host identification is questionable) from Pennsylvania. This nematode has been previously reported by Mawson (1956) as Physaloptera variegata Reiber, Byrd, and Parker, 1940, from *H. platirhinos* from Georgia. It has also been documented in various colubrid

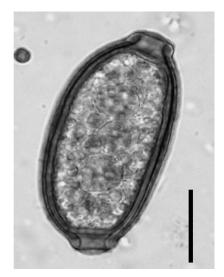


Figure 4. Light microscopy of an egg of a *Capillaria* sp. from feces of *Agkistrodon piscivorus* showing the characteristic bipolar plugs; scale bar = $30 \mu m$.

and viperid snakes from at least 10 U.S. states, and Québec and Saskatchewan, Canada (see McAllister et al. 2008). McAllister and Bursey (2012) previously reported *P. abjecta* from a *C. c. priapus* from Oklahoma. This is the first report of *P. abjecta* from an eastern hog-nosed snake in Oklahoma.

Trichostrongyloidea: Molineidae

Oswaldocruzia pipiens Walton, 1929 – A single male specimen was collected from an adult (31 mm SVL) P. fouquettei collected on 21 February 2019 from Hochatown. Trowbridge and Hefley (1934) were the first to report O. pipiens from Oklahoma in Rana spp., and Woodhouse's toad, Anaxyrus woodhousii and Kuntz and Self (1944) reported an Oswaldocruzia sp. from an unspecified anuran host. This nematode was earlier reported from P. fouquettei (as P. nigrita triseriata, P. triseriata or P. feriarum) in Oklahoma in an unpublished thesis by Kuntz (1940). Bouchard (1953), in an unpublished dissertation, reported O. pipiens from P. fouquettei (as P. triseriata) from Oklahoma. In addition, McAllister et al. (2015) previously reported O. pipiens from Fowler's toad, Anaxyrus fowleri (Hinckley) from Hochatown. Other hosts in Oklahoma

include A. a. americanus, Rocky Mountain toad (A. woodhousii woodhousii), L. s. utricularius, and Hurter's spadefoot (Scaphiopus hurterii) (see McAllister et al. 2014a). This nematode has been reported from various amphibians and reptiles from at least 12 U.S. states and México (see McAllister et al. 2014a for summary). McAllister et al. (2013, 2015) previously reported the similar Oswaldocruzia leidyi Steiner, 1924 from P. fouquettei from Arkansas and Texas, and Oklahoma, respectively. This is the first definitive published host record of O. pipiens from P. fouquettei.

Ascaridida: Cosmocercidae

Cosmocercoides sp. - A single female specimen was taken from the lower intestine of an adult male (130 mm SVL) Tantilla gracilis collected on 26 September 2021 from the Hochatown site. Unfortunately, without a male, it is not possible to determine the specific identity of this nematode. Cosmocercoides variabilis (Harwood, 1930) Travassos, 1931 was reported previously from Dekay's brownsnake, Storeria dekayi (Holbrook) from Oklahoma by McAllister et al. (2015). The life cycle involves terrestrial gastropods as intermediate hosts and amphibians and reptiles as definitive hosts (Anderson 2000). This is the first report of any helminth parasite from *T. gracilis*.

Acanthocephala: Giganthorhynchidea: Oligacanthorhynchidae

Oligacanthorhynchid cystacanths – Four cystacanths were encysted in subdermal tissues and muscle fascia of a gravid female (960 mm SVL) *C. c. priapus* collected DOR on 3 June 2021 from the vicinity of the Eastern Oklahoma State College Campus in Idabel (33°55′10.3584″N, -94°46′30.6336″W). Snakes serve as paratenic (transport) hosts of oligacanthorhynchids and, as adults, they are parasitic in terrestrial birds and mammals. The eventual development of a cystacanth to an adult occurs when a paratenic host is ingested by an appropriate definitive host. This is the first time acanthocephalan cystacanths have been reported in this host.

In summary, a number of new host and geographic distribution records are reported herein for some parasites of select herpetofauna from southeastern Oklahoma. Future surveys should include amphibians and reptiles from other parts of the state where they have been rarely examined for parasites in general. Therefore, the prospect of additional records as well as the likelihood of discovery of novel species is promising, especially with inclusion of molecular analyses.

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