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# Helminth Parasites (Trematoda, Cestoda, Nematoda, Acanthocephala) of Herpetofauna from Southeastern Oklahoma: New Host and Geographic Records

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**Abstract:** Between May 2013 and September 2015, two amphibian and eight reptilian species/subspecies were collected from Atoka ( $n = 1$ ) and McCurtain ( $n = 31$ ) counties, Oklahoma, and examined for helminth parasites. Twelve helminths, including a monogenean, six digeneans, a cestode, three nematodes and two acanthocephalans was found to be infecting these hosts. We document nine new host and three new distributional records for these helminths. Although we provide new records, additional surveys are needed for some of the 257 species of amphibians and reptiles of the state, particularly those in the western and panhandle regions who remain to be examined for helminths. ©2015 Oklahoma Academy of Science

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## Introduction

In the last two decades, several papers from our laboratories have appeared in the literature that has helped increase our knowledge of the helminth parasites of Oklahoma's diverse herpetofauna (McAllister and Bursey 2004, 2007, 2012; McAllister et al. 1995, 2002, 2005, 2010, 2011, 2013, 2014a, b, c; Bonett et al. 2011). However, there still remains a lack of information on helminths of some of the 257 species of amphibians and reptiles of the state (Sievert and Sievert 2011). Here, we attempt to augment that void with several new host and distributional records for select herpetofauna from southeastern Oklahoma.

## Methods

Between May 2013 and September 2015, 11 Sequoyah slimy salamander (*Plethodon sequoyah*), nine Blanchard's cricket frog (*Acris blanchardii*), two eastern cooter (*Pseudemys concinna concinna*), two common snapping turtle (*Chelydra serpentina*), two Mississippi mud turtle (*Kinosternon subrubrum hippocrepis*), two western cottonmouth (*Agkistrodon piscivorus leucostoma*), one southern black racer (*Coluber constrictor priapus*), one diamondback watersnake (*Nerodia rhombifer*), one Midland brown snake (*Storeria dekayi wrightorum*), and one western ribbon snake (*Thamnophis proximus proximus*),

were collected by hand or tong from Atoka ( $n = 1$ , *S. d. wrightorum* only) and McCurtain counties. Specimens were placed in collection bags, taken to the laboratory for necropsy within 24 hr and killed by prolonged immersion with a concentrated chloretone® (chlorobutanol) solution (amphibians) or injection of sodium pentobarbital (reptiles). For turtles, a bone saw was used to remove the plastron and expose the viscera which was removed and placed in a Petri dish. For intravascular and ocular trematodes in turtles we followed methods of Snyder and Clopton (2005). The gastrointestinal tract was split lengthwise and examined as well as other organs, including the lungs, liver, and gonads. For other herptiles, a mid-ventral incision was made to expose the viscera and the entire gastrointestinal tract and other organs were examined for helminths. Trematodes and cestodes were fixed in hot tap water without coverslip pressure, stained with acetocarmine, dehydrated in a graded ethanol series, cleared in methyl salicylate (trematodes only) 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. Acanthocephalan cystacanths were placed in 70% ethanol and studied as temporary mounts in glycerol. Helminth voucher specimens were deposited in the Harold W. Manter Laboratory of Parasitology (MWML), Lincoln, Nebraska. If HWML numbers are not listed for a particular parasite, specimens are being retained for future morphological and molecular studies. Host voucher specimens were deposited in the Arkansas State University Museum of Zoology (ASUMZ), Herpetological Collection, State University, Arkansas.

## Results and Discussion

Of the 33 individual amphibians and reptiles examined, ten (30%) harbored at least one helminth. One turtle (*C. serpentina*) from McCurtain County was multiply infected with four helminths (a monogenean, two digeneans, and a nematode), another turtle (*P. c. concinna*) from McCurtain County harbored two helminths (a nematode and an acanthocephalan) and a single snake (*S. d. wrightorum*) from

Atoka County had two helminths (one digenean and one nematode). The helminths found in these Oklahoma herpetofauna are presented below in annotated format.

### Monogenea: Polystomatidae

#### *Neopolystoma* sp. (Fig. 1A)

Two monogeneans, *Neopolystoma* sp. were found in the conjunctival sacs of an adult (310 mm carapace length [CL]) male *C. serpentina* collected on 15 April 2015 near Holly Creek off Tebo Jones Road, McCurtain County (33.968086°N, 94.816722°W). *Neopolystoma* spp. is found in the cloaca, urinary and accessory bladders, oral, pharyngeal and nasal cavities, as well as in the conjunctival cavities of freshwater chelonians (Pichelin 1995). There is a high degree of site-specificity for *Neopolystoma* spp. (Du Preez and Lim 2000). In the United States, Mexico, and Central America, eight *Neopolystoma* are known, including *Neopolystoma domitalae* (Caballero, 1938) Price, 1939, *Neopolystoma elizabethae* Platt, 2000, *Neopolystoma fentoni* Platt, 2000, *Neopolystoma grossi* Du Preez and Morrison, 2015, *Neopolystoma moleri* Du Preez and Morrison, 2015, *Neopolystoma orbiculare* (Stunkard, 1916) Price, 1939, *Neopolystoma rugosa* (MacCallum, 1918), *Neopolystoma terrapenis* (Harwood, 1932) and an undescribed *Neopolystoma* sp. Héritier, Badets, Du Preez, Aisien, Lixian, Combes and Verneau (Thatcher 1964; Platt 2000a, b; Du Preez and Morrison 2015; Héritier et al. 2015). Of these, only *N. elizabethae* from the western painted turtle, *Chrysemys picta belli* from Indiana, Michigan, and Wisconsin, *N. fentoni* from white-lipped mud turtle, *Kinosternon leucostomum* and painted wood turtle, *Rhinoclemmys pulcherrima* from Costa Rica, *N. moleri* from Florida softshell turtle, *Apalone ferox*, *N. grossi* from Florida cooter, *Pseudemys concinna floridana* from Florida and an unknown species of *Neopolystoma* sp. (C. s.) from *C. serpentina* from Nebraska (O. Verneau, pers. comm.) are known from the conjunctival sac of the eye (Platt 2000a, b; Du Preez and Morrison 2012; Héritier et al. 2015). However, we document a new host as well as the first time a *Neopolystoma* sp. from the eye of a turtle has been reported from Oklahoma.

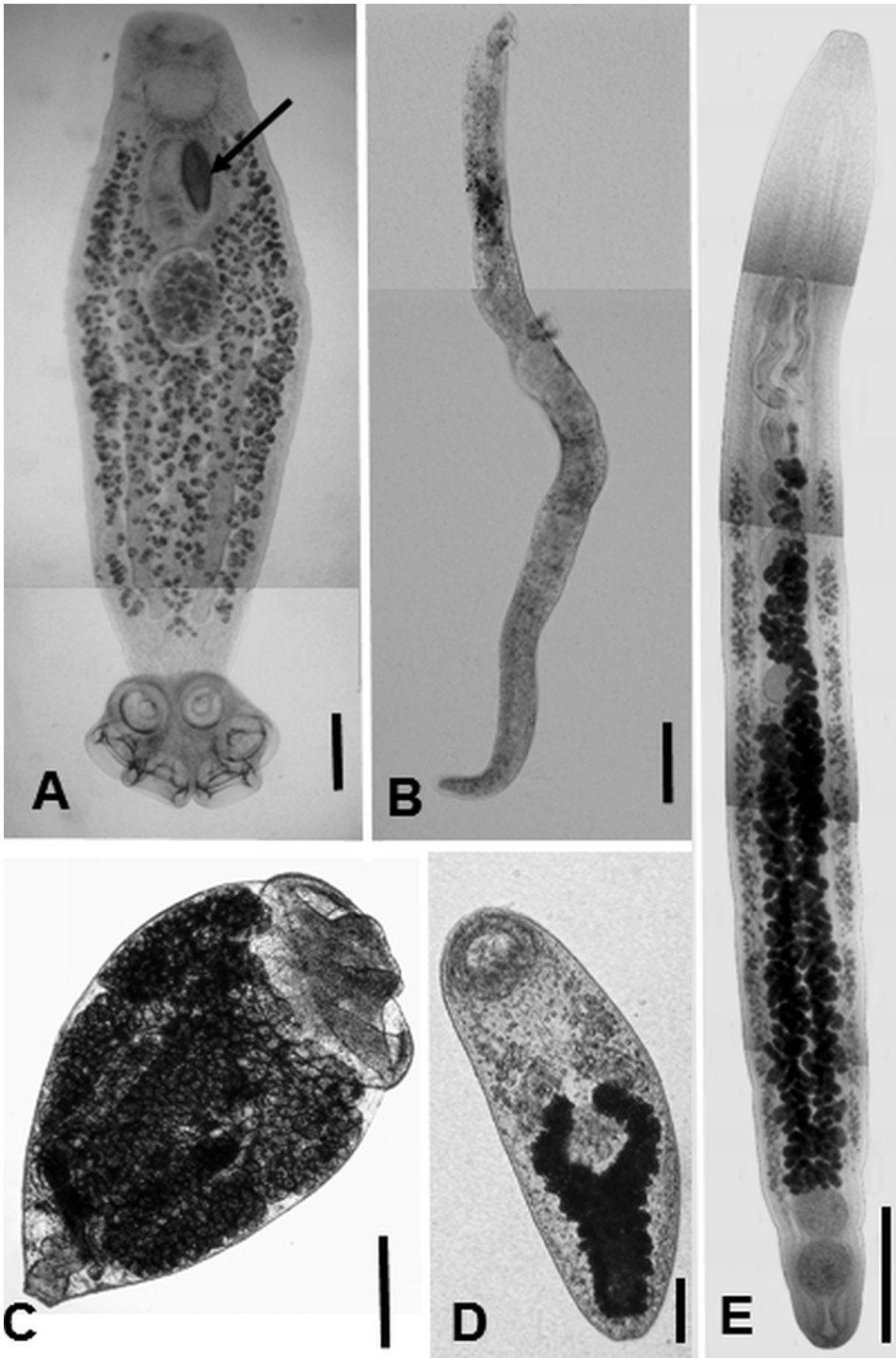


Figure 1. Select helminths of Oklahoma herpetofauna. A. *Neopolystoma* sp. from conjunctival sac of *Chelydra serpentina*. Arrow = egg. Scale bar = 300  $\mu$ m. B. Immature *Hapalorhynchus* from blood vascular system of *C. serpentina*. Scale bar = 100  $\mu$ m. C. *Megalodiscus* sp. from rectum of *Acris blanchardii*. Scale bar = 400  $\mu$ m. D. Metacercariae of *Dasymetra* sp. from esophagus of *Thamnophis proximus proximus*. Note Y-shaped cecum. Scale bar = 50  $\mu$ m. E. *Telorchis corti* from intestinal tract of *Kinosternon subrubrum hippocrepis*. Scale bar = 1 mm.

Our specimens ( $n = 2$ ) of *Neopolystoma* sp. can be morphologically distinguished from *N. moleri* in having a smaller body length (2,968–3,043  $\mu\text{m}$  as opposed to 3,249–7,944  $\mu\text{m}$ ) and possession of 7–8 as opposed to 12–13 genital spines; from *N. grossi* in having intestinal diverticula, a smaller body length (2,968–3,043  $\mu\text{m}$  compared to 3,298–4,873  $\mu\text{m}$ ), a wider pharynx (280–283 vs. 209–246  $\mu\text{m}$ ), a wider genital bulb (67–69  $\mu\text{m}$  as opposed to 58–60  $\mu\text{m}$ ), longer and wider testis (length 378–413  $\mu\text{m}$  compared to 186–293  $\mu\text{m}$ , width 287–367  $\mu\text{m}$  vs. 149–245  $\mu\text{m}$ ), and an ovary that is only 100–108  $\mu\text{m}$  wide compared to 118–135  $\mu\text{m}$  wide; from *N. elizabethae* in having a single egg (see Fig. 1A) that is 280–303  $\mu\text{m}$  in length vs. three eggs that are 322–367  $\mu\text{m}$  long and more narrow haptorial suckers 227–235  $\mu\text{m}$  wide vs. 344–408  $\mu\text{m}$  wide; and from *N. fentoni* in possessing shorter genital spines (9 compared to 11), in having a body length that is 2,968–3,043  $\mu\text{m}$  as opposed to 1,500–2,450  $\mu\text{m}$ , and in having the vitellarium and ceca not extending posteriorly to the haptor. Given these morphological differences with congeners also parasitizing the conjunctival sac of turtles, we suspect that our species is new and are currently in the process of collecting additional specimens for a description.

**Trematoda: Digenea: Brachycoeliidae**  
*Brachycoelium* cf. *salamandrae* (Frölich, 1789) Dujardin, 1845

One of 11 (%) *P. sequoyah* (adult male, 52 mm snout-vent length [SVL]) collected on 15 December 2013 from Beavers Bend State Park, McCurtain County (34.124837°N, 94.670665°W) was found to harbor three *Brachycoelium* cf. *salamandrae* in its small intestine. McAllister and Bursey (2012) previously reported this digenean from central newt, *Notophthalmus viridescens louisianensis* from McCurtain County. In addition, McAllister and Bursey (2004) did not find this trematode in a previous survey of helminths of this endemic salamander.

A single *S. d. wrightorum* (adult female, 140 mm SVL) collected on 8 March 2013 from McGee Creek State Park, Atoka County (34.327533°N, 95.914099°W) was infected

in the small intestine with a single *B.* cf. *salamandrae* (HWML 91938). Harwood (1932) previously reported *B. salamandrae* from Texas brown snake, *S. d. texana* from Houston, Texas. Interestingly, very few snakes have been reported as hosts of this trematode (Bursey et al. 2012).

McAllister et al. (2014b) recently noted they had serious doubts about Old World and New World *B. salamandrae* being conspecific (see summary by Bursey et al. 2012), and suggested caution with their former conclusions (McAllister et al. 2013) until a molecular approach was completed (V.V. Tkach, *pers. comm.*). Regardless of what species is eventually verified, we document two new host records for the genus *Brachycoelium*.

**Telorchidae**

*Telorchis corti* Stunkard, 1915 (Fig. 1E)

A single *K. s. hippocrepis* (adult male, 83 mm CL) collected on 20 June 2015 from the Little River off US 259, McCurtain County (33.942941°N, 94.759119°W) was infected with four *Telorchis corti* (HWML 101025) in the small intestine. This is a common digenean in various turtles and localities including those in Alabama, Arkansas, California, Florida, Idaho, Illinois, Iowa, Louisiana, Nebraska, North Carolina, Texas, Virginia, and Washington, D.C., and British Columbia, Canada (Ernst and Ernst 1977; Macdonald and Brooks 1989). It has also been reported in Oklahoma from *C. serpentina* (Williams 1953), red-eared slider, *Trachemys scripta elegans* (Everhart 1958) and an unknown host from Lake Texoma, Marshall County (Macdonald and Brooks 1989). In addition, *T. corti* was previously reported in an unpublished thesis by McKnight (1958) from *K. s. hippocrepis* from Lake Texoma; however, we document the first published record in this turtle.

**Schistosomatoidea: Spirorchidae**

*Spirorchis haematobius* (Stunkard 1922) Price, 1934

Two (one adult, one immature) blood flukes belonging to *Spirorchis* (HWML 101024) were found in the same *C. serpentina* harboring the *Neopolystoma* sp. Numerous *Spirorchis* spp. have been reported from various turtles (Ernst

and Ernst 1977), including two species from *C. serpentina*: *S. haematobius* (Stunkard 1922) Price, 1934 from Indiana, Iowa, Louisiana, Mississippi, Nebraska, New Jersey, New York, North Carolina, Ohio, Oklahoma, and Tennessee (Stunkard 1923; Byrd 1939; Rausch 1947; Williams 1953; Ulmer 1959; Brooks 1979; Brooks and Mayes 1975) and *S. magnitestis* Byrd, 1939 from Illinois and Tennessee (Byrd 1939; Martin 1973). In Oklahoma, *S. artericola* (Ward, 1921) Stunkard, 1923, *S. elegans* Stunkard, 1923 and *S. scripta* Stunkard, 1923 has been reported from *T. s. elegans* (Harwood 1931; McKnight 1958; Everhart 1975), and *S. innominatus* Ward, 1921 has been documented from *P. concinna* (Harwood 1931). Platt's (1993) key to North American species of *Spirorchis* identifies our specimens as *S. haematobius* given their possession of 10 testes, a testicular field beginning at the midbody, and their large size. This is only the second time in over 75 yr that this blood fluke has been reported from the common snapping turtle from Oklahoma.

#### **Haplorhynchus sp. (Fig. 1B)**

Ten immature *Haplorhynchus* sp. (HWML 101023) was found in the blood vascular system of the same *C. serpentina* above. These blood digeneans are commonly found in turtles (Ernst and Ernst 1977), including at least four species in *C. serpentina*: *H. brooksi* Platt, 1988 and *H. foliorchis* Brooks and Mayes, 1975 from Nebraska (Brooks and Mayes 1975; Platt 1988), *H. gracilis* Stunkard, 1922 from Indiana (Stunkard 1922; Platt 1988) and Wisconsin (Guilford 1959), and *H. stunkardi* Byrd, 1939 from Nebraska (Brooks and Mayes 1976). To our knowledge, there are no previous reports of *Haplorhynchus* spp. from Oklahoma so we document a new distribution record for this blood fluke in the state. Unfortunately, the immature nature of our specimens prevents any specific identification.

#### **Ochetosomatidae**

##### ***Dasymetra* sp. (Fig. 1C)**

Over 100 immature flukes (metacercaria) thought to represent *Dasymetra* sp. was found in the esophagus of an adult (270 mm SVL) *T. p. proximus* collected on 7 September 2013 from Lukfata, McCurtain County (34.006658°N,

94.764592°W). The only previously reported trematode from this host is *Paralechiorchis megacystis* (Ochetosomatidae) from Kansas (Stewart 1960). *Dasymetra longicirrus* (Odlaug, 1938) Denton, 1938, originally described from banded water snake (*Nerodia sipedon*) from Louisiana, has been reported previously from the related eastern garter snake, *Thamnophis sirtalis* (Ernst and Ernst 2006). In addition, McAllister and Bursey (2012) reported *D. conferta* Nicoll, 1991 from *N. rhombifer* from McCurtain County. We document a new host record for *Dasymetra* sp.

#### **Echinostomatiformes: Diplodiscidae**

##### ***Megalodiscus* sp. (Fig. 1D)**

One of nine (11%) *A. blanchardi* (male, 25 mm SVL) collected on 13 October 2013 from Hochatown, McCurtain County (34.171077°N, 94.75184°W) harbored a single *Megalodiscus* sp. in its rectum. *Megalodiscus temperatus* (Stafford, 1905) Harwood, 1932 is a common trematode of North American anurans (see Bolek and Janovy 2008). In Oklahoma, *M. temperatus* has been previously reported from southern leopard frog, *Lithobates sphenoccephalus utricularius* (Trowbridge and Hefley 1934; Kuntz and Self 1944; Vhora and Bolek 2015). We were not, however, able to identify this helminth to species until a molecular analysis can be completed. However, this is the first time *Megalodiscus* sp. has been reported in *A. blanchardi*.

#### **Cestoda: Eucestoda: Bothriocephalidea:**

##### **Proteocephalidae**

##### ***Ophiotaenia marenzelleri* (Barrois, 1898)**

##### **Railliet, 1899**

A single *O. marenzelleri* (HWML 101840) was removed from the small intestine of a juvenile (335 mm SVL) *A. p. leucostoma* collected on 8 October 2014 from Lukfata, McCurtain County (34.01445°N, 94.774386°W). This tapeworm has been reported previously from *A. piscivorus* from Alabama (Detterline et al. 1984), Louisiana (Brooks 1978; Fontenot and Font 1996), North Carolina (Collins 1969), and Texas (Harwood 1933) and an unknown locality in the Catskill Mountains (Fantham and Porter 1954). This is the first time *O. marenzelleri* has been reported from Oklahoma.

**Nematoda: Spirurida:****Gnathostomatidae*****Spiroxys contorta* (Rudolphi, 1819) Hedrick, 1935**

Nematodes matching the description of *S. contorta* (HWML 91949) were found in the stomach of an adult male (745 mm SVL) *N. rhombifer* collected on 6 August 2014 from Yashau Creek, McCurtain County (33.987161°N, 94.743637°W). This nematode has been reported previously in Oklahoma from eastern river cooter, *Pseudemys concinna concinna* (Harwood 1931) and *T. s. elegans* (Everhart 1958) from Oklahoma. This is the first time this nematode has been reported from *N. rhombifer*. However, it is possible the snake obtained the infection from eating prey (fishes, amphibians, turtles) normally associated with the *S. contorta* life cycle (Hedrick 1935).

**Camallanidae*****Serpinema trispinosus* (Leidy, 1852) Yeh, 1960**

Two female *S. trispinosus* (HWML 91939) were found in the small intestine of *P. c. concinna* collected on 10 September 2015 from off US 259 N of Broken Bow (34.094728°N, 94.739301°W). In addition, 43 *S. trispinosus* were taken from the small intestine of the same *C. serpentina* noted herein multiply infected with *Neopolystoma* and blood flukes. To our knowledge, this nematode has not been previously reported in the literature from Oklahoma. It was, however, noted in the unpublished thesis of McKnight (1959) where he reported the synonym *Camallanus trispinosus* from four species of turtles. In addition, *S.* (= *Camallanus*) *microcephalus* (Dujardin, 1845) Yeh, 1960 has been reported from the state and elsewhere in turtles (Harwood 1931; Williams 1953; Everhart 1958); however, this parasite is an Asian and European species of turtles (Baker 1987). Various Nearctic turtles have been reported as hosts of *S. trispinosus*, including *C. serpentina* and *P. concinna* (Baker 1987) and commonly (98%) in *T. s. elegans* from neighboring Arkansas (Rosen and Marquardt 1978)

**Ascaridida: Cosmocercidae*****Cosmocercoides variabilis* (Harwood, 1930)****Travassos, 1931**

A single *S. d. wrightorum*, the same host of *B. cf. salamandrae* above, was infected with an immature female *C. variabilis* (HWML 91950). Harwood (1930, 1932) previously reported *C. variabilis* from *S. d. texana* from Houston, Texas, and Rau and Gordon (1980) reported this nematode from 8% northern brown snakes, *S. d. dekayi* from Montreal, Quebec, Canada. There are several other snakes reported as hosts and the parasite has a vast range in the Neotropical and Nearctic regions (see Bursey et al. 2012). In Oklahoma, *C. variabilis* has been reported from *P. sequoyah* (McAllister and Bursey 2004), American bullfrog, *Lithobates catesbeianus* (Trowbridge and Hefley 1934), Hurter's spadefoot, *Scaphiopus hurterii* (McAllister et al. 2005) and dwarf American toad, *Anaxyrus americanus charlesmithi* (McAllister et al. 2014a). We document a new host record for this nematode.

**Centrorhynchidae gen. sp. Van Cleave, 1916****Undetermined cystacanth**

Two acanthocephalan cystacanth (HWML 91951) were found in the coelomic cavity of an adult male (810 mm SVL) *C. constrictor priapus* collected on 28 May 2013 from the Eastern Oklahoma State College Campus-Idabel, McCurtain County (33.920565°N, 94.77717°W). The only acanthocephalans (Oligacanthorhynchidae) reported previously from the species were from a northern black racer (*C. constrictor constrictor*) and include *Macracanthorhynchus ingens* (Linstow, 1879) Meyer, 1932 from Louisiana and Pennsylvania (Elkins and Nickol 1983; Bolette 1998) and a *Macracanthorhynchus* sp. from New York (see Ernst and Ernst 2006). Juvenile stages of centrorhynchid acanthocephalans have been found in several amphibians and reptiles (see summary in Bursey et al. 2012) and these are considered paratenic hosts. We document the first report of a centrorhynchid cystacanth from this host and the initial record, to our knowledge, for any snake from the Nearctic zoogeographical region (Bursey et al. 2012).

**Neoechinorhynchidae**

*Neoechinorhynchus* sp.

A single adult male *P. c. concinna* (270 mm CL) collected on 10 September 2015 from off US 259 N of Broken Bow (34.094728°N, 94.739301°W) harbored 12 acanthocephalans (HWML 91960) in its small intestine belonging to the genus *Neoechinorhynchus*. However, because no fully developed eggs were found, identification to species is problematic. Based on the posterior end of worms, specific identity is thought to be *N. emyditoides* Fisher, 1960 or *N. pseudemydis* Cable and Hopp, 1954 (M. Barger, pers. comm.). Both of these *Neoechinorhynchus* have been previously reported from the state, *N. emyditoides* from McIntosh County and Lake Talawanda, Pittsburg County, and *N. pseudemydis* from an unspecified locality in Oklahoma (Barger 2004). However, either species represents a new host record for *P. concinna* (Barger 2004).

In summary, we have documented additional new host and distributional records for some amphibians and reptiles of the state. Future work should target those species for which we know very little about, many of which occur in the western portion and Panhandle of Oklahoma where the ecoregions (Central Great Plains and High Plains) differ considerably from those in the current study of McCurtain County.

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