Myxozoan and Helminth Parasites of the Dwarf American Toad, *Anaxyrus americanus charlesmithi* (Anura: Bufonidae), from Arkansas and Oklahoma

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Abstract: We examined 69 dwarf American toads, *Anaxyrus americanus charlesmithi*, from McCurtain County, Oklahoma (n = 37) and Miller, Nevada and Union counties, Arkansas (n = 32) for myxozoan and helminth parasites. The following endoparasites were found: a myxozoan, *Cystodiscus* sp., a trematode, *Clinostomum marginatum*, two tapeworms, *Cylindrotaenia americana* (Oklahoma only) and *Distoichometra bufonis*, five nematodes, acuariid larvae, *Cosmocercoides variabilis*, *Oswaldocruzia pipiens*, larval *Physaloptera* sp. (Arkansas only), and *Rhabdias americanus* (Arkansas only), and acanthocephalans (Oklahoma only). We document six new host and four new geographic distribution records for these select parasites.

Introduction

The dwarf American toad, *Anaxyrus americanus charlesmithi*, is a small anuran that ranges from southwestern Indiana and southern Illinois south through central Missouri, western Kentucky and Tennessee, and all of Arkansas, to eastern Oklahoma and northeastern Texas (Conant and Collins 1998). It occurs in various habitats, from suburban back yards to mountain wildernesses, where it breeds in temporary pools or ditches or shallow portions of streams. Compared to the eastern American toad, *A. a. americanus* (see Muzzall and Andrus 2014, and refs therein), little is known about its helminth parasites. The following papers report fragmentary information on various helminths of *A. a. charlesmithi* as follows: *Mesocoelium monas* (McAllister et al. 2008), *Cosmocercoides variabilis* (McAllister and Bursey 2012a) and tetrathyridia of *Mesocestoides* sp. (McAllister et al. 2014c) from *A. a. charlesmithi* from Arkansas, and *Clinostomum marginatum* from dwarf American toads from Oklahoma (Cross and Hranitz 2000). In addition, Langford and Janovy (2013) reported *Rhabdias americanus* from *A. a. charlesmithi* from Missouri. Although McAllister and Bursey (2012b) recently provided information on helminth parasites of various herpetofauna from southeastern Oklahoma, we are not aware of any additional reports of helminths from this toad nor has a complete survey of its endoparasites been carried out to date. Here, from a survey on specimens from Arkansas and Oklahoma, we report six new host and

four new distributional records for a myxozoan and helminths of *A. a. charlesmithi*.

Methods

Between August 2012 and October 2014, 69 juvenile and adult *A. a. charlesmithi* (mean ± 1SD snout-vent length [SVL] = 51.3 ± 14.8, range = 28-82 mm) were collected by hand, including 37 from Oklahoma (McCurtain County) at Beavers Bend State Park (*n* = 2) (34.13527ºN, 94.687796ºW), Hochatown (*n* = 34) (34.171155ºN, 94.751834ºW), and Lukfata (*n* = 1) (34.005396ºN, 94.759438ºW), and 32 from Arkansas in Miller at Nix Creek (*n* = 2) (33.433478ºN, 94.027763ºW), Nevada at White Oak Lake (*n* = 1) (33.688228ºN, 93.110322ºW) and Union at Calion Lake (33.330527ºN, 92.528422ºW), El Dorado (33.209011ºN, 92.590186ºW) and Junction City (*n* = 29) (33.01971ºN, 92.7333ºW) counties. Methods for necropsy and examination by light microscopy and processing have been previously described for myxozoans (McAllister and Trauth 2005) and helminths (McAllister and Bursey 2005). For SEM, myxozoan trophozoites were dehydrated in a graded series of increasing ethanol solutions (50-100%), followed by several fluid exchanges in 100% ethanol. An Autosamdri-815 critical point drier (Tousimis Research Corporation, Rockville, MD) was used (31 ºC, 1072 psi, ventilation rate ~100 psi/min) to remove excess ethanol. Samples were then mounted on 25.4 mm aluminum pin stub specimen mounts and coated with gold using a Cressington 108 sputter coater (Cressington Scientific Instruments Ltd, Watford, UK). Samples were analyzed both qualitatively and quantitatively with a Vega TS 5136XM digital scanning electron microscope (Tescan USA Inc., Cranberry Township, PA) at 19.5 kV.

Parasites were deposited in the United States National Parasite Collection (USNPC), Beltsville, Maryland or the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, Nebraska. Host voucher specimens are deposited in the Arkansas State University Herpetological Collection (ASUMZ), State University, Arkansas, or the Henderson State University Herpetological Collection (HSU), Arkadelphia, Arkansas.

Results

Forty-eight of 69 (70%) of the *A. a. charlesmithi*, including 27 (84%) from Arkansas and 21 (57%) from Oklahoma harbored Protista and nine helminths as follows: a myxozoan, a trematode, two tapeworms, five nematodes and an acanthocephalan (Table 1). Nine (24%) of the *A. a. charlesmithi* from Arkansas and nine (28%) from Oklahoma were concurrently infected with myxozoans and one or two helminths or with two or three helminths. The mean number of helminths found in Arkansas toads was 1.3 ± 0.5 and in Oklahoma toads 1.3 ± 0.7. An annotated list of the myxozoans and helminths found and the host data follows.

Protista: Myxosporea: Myxidiidae

Cystodiscus sp. Lutz, 1889 (Fig. 1)

Trophozoites and free spores (HWML photovoucher 75105) of a *Cystodiscus* sp. (syn. *Myxidium*) identified by ribosomal DNA sequencing (C. Whipps, *pers. comm*.) was found in the gall bladder of six toads (47.8 ± 9.6, 36-62 mm SVL) from Union County, Arkansas, and six *A. a. charlesmithi* (53.5 ± 12.3, 36-70 mm SVL) from Hochatown, McCurtain County, Oklahoma. *Cystodiscus serotinus* (=*Myxidium serotinum*) (Kudo and Sprague 1940) Hartigan, Fiala, Dyková, Rose, Phalen, and Šlapeta, 2012 has previously been reported from one of five (20%) *A. a. charlesmithi* from Arkansas (McAllister and Trauth 1995); however, we are not aware of any amphibian myxozoan reported previously from Oklahoma. Other bufonid hosts of *C. serotinus* include the green toad (*Anaxyrus debilis*), Texas toad (*Anaxyrus speciosus*), Woodhouse's toad (*Anaxyrus woodhousii*) and Coastal Plain toad (*Incilius nebulifer*) from Texas (McAllister et al. 1989; McAllister and Trauth 1995) and southern toad (*Anaxyrus terrestris*) from Florida (Kudo 1943).

Table 1. Myxozoa and helminths found during this study in *Anaxyrus americanus charlesmithi* from Arkansas and Oklahoma.

Helminth	State	Prevalence*	Intensity†
Myxosporea			
Cystodiscus sp.	Arkansas	6/32 (19%)	-
	Oklahoma‖	6/37 (16%)	-
Trematoda			
Clinostomum marginatum	Arkansas	1/32 (3%)	1
	Oklahoma	2/37 (5%)	5, 32
Cestoidea			
Cylindrotaenia americana‡	Oklahoma	8/37 (22%)	7.5 ± 10.1, 1-28
Distoichometra bufonis‡	Arkansas‖	2/32 (6%)	5
	Oklahoma	3/37 (8%)	1-5
Nematoda			
Acuariid larvae‡	Arkansas	3/32 (9%)	1-3
	Oklahoma‖	1/37 (3%)	3
Cosmocercoides variabilis	Arkansas	6/32 (19%)	4.7 ± 5.3 (1-15)
	Oklahoma	4/37 (15%)	1, 1, 1, 1
Oswaldocruzia pipiens‡	Arkansas	17/32 (53%)	2.6 ± 1.9 (1-7)
	Oklahoma	8/37 (22%)	2.3 ± 2.3 (1-7)
Physaloptera sp. larvae‡	Arkansas	2/32 (6%)	1, 1
Rhabdias americanus	Arkansas‖	1/32 (3%)	1
Acanthocephala			
Unknown species‡	Oklahoma	2/37 (5%)	1

*Number infected/number examined = %.

†mean ± 1SD, range (where applicable).

‡New host record.

‖New distributional record.

Platyhelminthes: Trematoda: Digenea: Clinostomidae

Clinostomum marginatum Rudolphi, 1819 (Fig. 2)

We found metacercaria ("yellow grubs") of this digenean (USNPC 107669) in the musculature and viscera of two toads (72 and 82 mm SVL) from the Hochatown site and in one toad (52 mm SVL) from El Dorado. This digenean has been previously reported from 17 of 69 (25%) *A. a. charlesmithi* from Oklahoma (Cross and Hranitz 2000). It has also been reported in a wide variety of amphibians in North America that primarily live, or breed in, lentic habitats (see McAllister et al. 2010). More recently, *C. marginatum* has been reported in *Eurycea* spp. salamanders (Bonett et al. 2011) and Pirate Perches, *Aphredoderus sayanus* (McAllister and Bursey 2013) from Oklahoma, and madtoms, *Noturus* spp. from Arkansas (McAllister et al. 2014b).

Cestoidea: Cyclophyllidea: Cylindrotaeniidae

***Cylindrotaenia americana* Jewell, 1916 (Fig. 3)**

This tapeworm (HWML 75056) was found in the small intestine of eight toads (44.5 ± 8.9, 34-55 mm SVL) from the Hochatown site. One of these *A. a. charlesmithi* (50 mm SVL) collected on 12 August 2014 had a massive infection of *C. americana* that completely filled its intestinal tract from the duodenum to near its rectum (Fig. 3). McAllister et al. (2013b) recently summarized the hosts and Western Hemisphere localities of *C. americana* and several previously reported bufonid hosts from North America have been documented with this cestode, including *A. a. americanus* from Iowa (Ulmer and James 1976). It has also been reported from amphibians in Oklahoma (Trowbridge and Hefley 1934) and Arkansas (McAllister et al. 1993, 2013b). We document a new host for *C. americana*.

Nematotaeniidae

***Distoichometra bufonis* Dickey, 1921 (= *Distoichometra kozloffi* Douglas, 1958)**

Specimens of *D. bufonis* (HWML 64657) were taken from the small intestine of five toads, three (52, 54, 71 mm SVL) from Hochatown and two (49, 60 mm SVL) from El Dorado. This cestode has been previously reported from Oklahoma in Great Plains toads, *Anaxyrus cognatus* (Kuntz 1941) and from Ohio in *A. a. americanus* (Odlaug 1954). It has also been reported from other anurans of the genera *Anaxyrus*, *Pseudacris*, *Rana*, *Scaphiopus*, *Smilisca* and *Spea* in Arizona, California, Georgia, Nebraska, New Mexico, North Carolina, Ohio, Oregon, and Utah, and *Rhinella* in Mexico (Koller and Gaudin 1977; Hardin and Janovy 1988; Goldberg and Bursey 1991; Goldberg et al. 2001; and others). This is the first time *D. bufonis* has been reported from *A. a. charlesmithi* and from Arkansas.

Nematoda: Rhabditida: Rhabdiasidae

***Rhabdias americanus* Baker, 1978**

This nematode (retained in author's collection) was found in the lung of a single toad (40 mm SVL) from El Dorado. The species has been previously reported from *A. a. americanus* (type host) from Canada and the eastern United States (see Baker 1987), Nebraska (Langford and Janovy 2013), Michigan (Muzzall and Andrus 2014) and Wisconsin (Bolek and Coggins 2000, 2003;Yoder and Coggins 2007), and from *A. a. charlesmithi* from Missouri (Langford and Janovy 2013). Other bufonid hosts include *A. alvarius*, *A. cognatus*, *A. debilis*, *A. hemiophrys*, *A. microscaphus*, *A. retiformis*, and *A. woodhousii*. In addition, Kuzmin (2013) recently provided a review of the Rhabdiasidae from the Holarctic. In the life cycle, infective larvae penetrate the host skin and eventually migrate to the body cavity where subadults invade the lungs, mature and produce eggs (Anderson 2000). This is the first time, to our knowledge, that *R. americanus* has been reported from Arkansas.

Ascaridida: Cosmocercidae

***Cosmocercoides variabilis* (Harwood, 1930) Travassos, 1931**

This nematode (HWML 64658) was the third most commonly found parasite in *A. a. charlesmithi*, occurring in the rectum of six toads (52 ± 18.7, 34-75 mm SVL) from Calion Lake and El Dorado and four toads (52, 56, 72, 82 mm SVL) from Hochatown. McAllister and Bursey (2012a) previously reported *C. variabilis* from Arkansas *A. a. charlesmithi*; additional amphibians from the state have also been reported to harbor *C. variabilis* (see McAllister et al. 2013a). In Oklahoma, it has been reported in Sequoyah slimy salamanders, *Plethodon sequoyah* (McAllister and Bursey 2004a), American bullfrogs, *Lithobates catesbeianus* (Trowbridge and Hefley 1934) and Hurter's spadefoot, *Scaphiopus hurterii* (McAllister et al. 2005a). *Cosmocercoides variabilis* has been reported from numerous other North American amphibians, including *A. a. americanus*, from at least 24 U.S. states and four provinces of Canada, Baja California Norte, Mexico, Costa Rica, and Panama (see McAllister et al. 2013a).

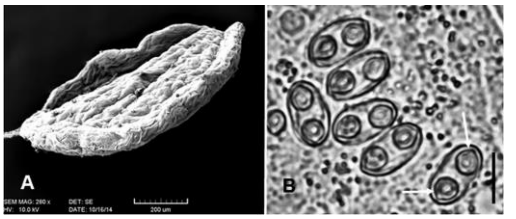

Figure 1. *Cystodiscus* sp. from *Anaxyrus americanus charlesmithi* from Oklahoma. A. Scanning electron micrograph on black background showing trophozoite. B. Light microscopy of spores showing two polar capsules (arrows) per myxospore; scale bar = 10 µm.

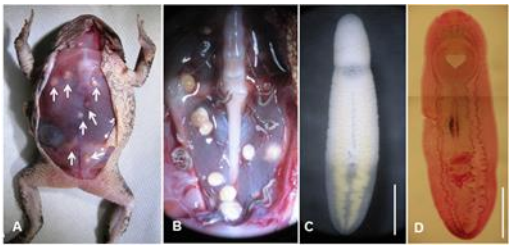

Figure 2. *Clinostomum marginatum* from *Anaxyrus americanus charlesmithi* from Oklahoma. A. View showing venter of frog with numerous encapsulated metacercaria in musculature (arrows). B. Closer view of encapsulated metacercaria deeper in abdominopelvic cavity. C. Unstained metacercaria teased from encapsulation. D. Stained metacercaria. Scale bars = 500 µm.

Figure 3. *Cylindrotaenia americana* from *Anaxyrus americanus charlesmithi* from Oklahoma. A. Massive infection of tapeworms in small intestine. Scale bar = 2 mm. B. Worms removed to Petri dish. Scale bar = 5 mm. C. Individual tapeworm with scolex (arrow) embedded in intestinal mucosa. Scale bar = 1 mm.

Strongylida: Molineidae

***Oswaldocruzia pipiens* Walton, 1929**

The most common endoparasite of *A. a. charlesmithi* was *O. pipiens* (HWML 64659) found in the small intestine of 17 toads (49.2 ± 17.3, 28-78 mm SVL) from Arkansas (Calion Lake, El Dorado, Junction City, Nix Creek and White Oak Lake) and eight toads (64.8 ± 11.9, 29-82 mm SVL) from Oklahoma (Hochatown). This nematode has been previously reported from *A. woodhousii*, *L. catesbeianus*, southern leopard frog, *Lithobates sphenocephalus utricularius* and *S. hurterii* from Oklahoma (Trowbridge and Hefley 1934; Kuntz 1941; Kuntz and Self 1944; McAllister et al. 2005a) and cave salamander, *Eurycea lucifuga*, bird-voiced treefrog, *Hyla avivoca*, pickerel frog, *Lithobates palustris* and wood frog, *Lithobates sylvaticus* from Arkansas (McAllister et al. 1993, 1995a, b; McAllister and Bursey 2004b). *Oswaldocruzia pipiens* is also a common helminth of *A. a. americanus* (Coggins and Sajdak 1982; Bolek and Coggins 2000, 2003; Yoder and Coggins 2007). In addition, it is obvious that there is no host specificity in *O. pipiens* as this strongylid has also been reported in various North American reptiles, including the ground skink, *Scincella lateralis* from Arkansas (see McAllister et al. 2014a). We document a new host for *O. pipiens*.

Spirurida: Physalopteridae

***Physaloptera* sp. Rudolphi, 1819 (third-stage larvae)**

This nematode (HWML 64661), which has a direct life cycle (Anderson 2000), was found as third-stage larvae in the stomach lumen of two toads, one from El Dorado (52 mm SVL) and one from White Oak Lake (72 mm SVL). Physalopterans have been reported in *A. a. americanus* from Ohio (Ashton and Rabalais 1978). McAllister et al. (2013a) recently reported this nematode from the Cajun chorus frog, *Pseudacris fouquettei* from Arkansas. The dwarf American toad is a new host of *Physaloptera* sp.

Acuarioidea: Acuariidae

Acuariid larvae

Larval acuariids (HWML 64661) were found encapsulated in stomach tissue in a single toad (37 mm SVL) from Hochatown and three toads (36, 40, 51 mm SVL) from El

Dorado. Acuariids typically mature in aquatic birds and require an arthropod intermediate host while anurans may serve as paratenic hosts (Anderson 2000). The occurrence of acuariids in amphibians and reptiles was summarized by Goldberg et al. (2007) and McAllister et al. (2013a) updated the host list. In addition, McAllister et al (2014a) recently reported acuariid larvae from *S. lateralis* from Arkansas and Oklahoma. We document a new host for acuariid larvae.

Acanthocephala

Unknown genus and species

Two female acanthocephalans (retained in author's collection) were found in the stomach and encapsulated on the serosal surface of the stomach of two toads (47, 52 mm SVL) from Hochatown. Unfortunately, because a male was not present, it is not possible to provide an identification. There are several reports of acanthocephalans in anurans, most being noted as unidentified cystacanths (Odlaug 1954) or *Centrorhynchus* sp. cystacanths (Brandt 1936; Campbell 1968; Hollis 1972). However, we report an acanthocephalan in *A. a. charlesmithi* for the first time

Discussion

In summary, we provide the first complete survey on myxozoans and helminths of *A. a. charlesmithi* from Arkansas and Oklahoma. Although its parasite fauna is depauperate, like most of those reported in North American anurans (see Aho 1990), we document six new host and four new distributional records. Also, when our data on *A. a. charlesmithi* and that of McAllister et al. (2014c) are compared to surveys of *A. a. americanus* from Michigan (Muzzall and Andrus 2014), Ohio (Odlaug 1954; Ashton and Rabalais 1978) and Wisconsin (Coggins and Sajdak 1982; Bolek and Coggins 2000, 2003; Yoder and Coggins 2007), six helminths (*D. bufonis*, *Mesocestoides* sp., *C. variabilis*, *O. pipiens*, *Physaloptera* sp., *R. americanus*) are shared by these subspecies. Additional surveys in other parts of its range where *A. a. charlesmithi* has not yet been examined (i.e., Kentucky, Tennessee, Texas) could potentially report additional new host and geographic records for its parasites.

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