2014

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Recommended Citation

McAllister, C. T.; Connior, M. B.; and Trauth, S. E. (2014) "New Host Records for *Mesocetoides* sp. Tetrathyridia (Cestoidea: Cyclophyllidea) in Anurans (Bufonidae, Ranidae) from Arkansas, with a Summary of North American Amphibian Hosts," *Journal of the Arkansas Academy of Science*: Vol. 68 , Article 29.

DOI: <https://doi.org/10.54119/jaas.2014.6824>

Available at: <https://scholarworks.uark.edu/jaas/vol68/iss1/29>

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New Host Records for *Mesocestoides* sp. Tetrathyridia (Cestoidea: Cyclophyllidea) in Anurans (Bufonidae, Ranidae) from Arkansas, with a Summary of North American Amphibian Hosts

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Running Title: New Host Records for *Mesocestoides*

Adult *Mesocestoides* (Cestoidea) are cosmopolitan parasites of placental mammals and birds and rarely humans (Padgett et al. 2012). The tetrathyridial stage is often found in the body cavity and encapsulated in tissues of vertebrate second intermediate hosts such as amphibians, reptiles, and rodents. Although there have been numerous attempts to transmit tetrathyridia to definitive hosts, none have been successful. Indeed, this is one of the few tapeworms for which the life cycle has yet to be conclusively demonstrated. In addition, there are no morphological characters that allow assignment of tetrathyridia to a given species. Over the last three decades in Arkansas, *Mesocestoides* sp. tetrathyridia have been previously reported by McAllister and associates from numerous amphibians and reptiles (see McAllister et al. 2014). The purpose of this report is to document three new host records for *Mesocestoides* sp. in common anurans of the state. We also provide a summation of the North American amphibians known to harbor *Mesocestoides*.

Between September 2013 and February 2014, 99 frogs and toads including 29 dwarf American toads, *Anaxyrus americanus charlesmithi*, 6 Fowler's toads, *Anaxyrus fowleri* and 64 southern leopard frogs, *Lithobates sphenoccephalus utricularius* were collected by hand from various sites in Union County. Specimens were euthanized with a concentrated Chloretone solution and a mid-ventral incision was made and the viscera and body cavity examined for *Mesocestoides* sp. When suspected encapsulated tapeworms were observed, they were excised with a bit of tissue and preserved in 10% v/v neutral buffered formalin. Free cestodes were collected and preserved in 70% v/v ethanol, stained with Semichon's acetocarmine and mounted in Kleermount®. For light microscopy of plastic-embedded tissues, we followed the methods of Bozzola and Russell (1999). Following

fixation, tissues were dehydrated in a graded series of increasing ethanol solutions (70–100% v/v), placed in a 50/50 % v/v acetone/plastic mixture for overnight infiltration, and then embedded in Mollenhauer's Epon-Araldite #2 (Dawes 1988). For thick sectioning (approximately 1 µm in thickness) and staining, we used glass knives on an LKB Ultratome (Type 4801A) with Ladd® multiple stain (LMS), respectively. For photomicroscopy, we used a Nikon Eclipse 600 epifluorescent light microscope with a Nikon DXM 1200C digital camera (Nikon Instruments Inc, Melville, NY). Voucher specimens of hosts were deposited in the Arkansas State University Herpetological Museum (ASUMZ), State University, Arkansas, U.S.A. or the Henderson State University (HSU) Collection, Arkadelphia, Arkansas. Voucher specimens of *Mesocestoides* sp. were deposited in the United States National Parasite Collection (USNPC 107667-107668), Beltsville, Maryland.

Four of 29 (14%) *A. a. charlesmithi* (54-70 mm SVL males and females) collected from Calion Lake ($n = 3$) and Grady Bell Road ($n = 1$) were found to harbor free tetrathyridia of *Mesocestoides* in their body cavity (Fig. 1A). One of 6 (17%) *A. fowleri* (62 mm SVL female) collected from 9.5 km S of El Dorado and 1.5 km N of jct. of Jackson Street and US 167 had tetrathyridia in its liver (Fig. 2). In addition, 6 of 64 (9%) *L. s. utricularius* (54 mm SVL female, 5 males 52-67 mm SVL) from Calion Lake ($n = 1$) Grady Bell Road ($n = 1$), 19th Street ($n = 1$), and 19th Street/Champagnolle Road ($n = 3$) had tetrathyridia in their mesenteries (Fig. 1B). All tetrathyridia from all hosts possessed characteristic individual features of a single invaginated scolex, a generally deep invagination canal (Fig. 1A), a prominent single excretory pore at the end opposite the scolex, and a solid hindbody. No tetrathyridium possessed a divided scolex, somatic bud, or any tegumental or excretory

New Host Records for *Mesocestoides*

anomalies such as those reported rarely from tetrathyridia in some aberrant acephalic tetrathyridia from other host species (see recent review by Conn et al. 2011).

Figure 1. Tetrathyridia (unstained whole mounts) from anurans. A. Free tetrathyridium from *Anaxyrus americanus charlesmithi*. Bar = 250 μ m. B. Free tetrathyridium from *Lithobates sphencephalus utricularius* showing invagination canal (arrow). Bar = 250 μ m.

Figure 2. *Mesocestoides* sp. tetrathyridia from liver of *Anaxyrus fowleri*. A. Four tetrathyridia encapsulated in tissue; scale bar = 50 μ m. B. Closer view of two tetrathyridia in encapsulation; scale bar = 25 μ m.

A summary of North American anuran hosts of *Mesocestoides* sp. was provided nearly 25 years ago by McAllister and Conn (1990) and we update that list herein and add caudate hosts to it (Table 1). Interestingly this tapeworm remains to be reported from a number of U.S. states, and as far as we can determine, it has only been reported to date from amphibians from 11 (22%), states including Arkansas, California, Iowa, Kansas, Michigan, Nebraska, New York, Oklahoma, South Dakota, Texas, and Wisconsin (Table 1). In all, *Mesocestoides* sp. has been reported from six species of salamanders of the world and 18 species/subspecies of frogs and toads from North America. There are numerous states east of the Mississippi River that support a great diversity of amphibians (i.e., Kentucky, North Carolina, Tennessee) and we predict that examination of several of those species could result in new geographic distribution records in those states and perhaps new host records for *Mesocestoides* sp.

In conclusion, we document 3 new host records for *Mesocestoides* sp. in anurans from Arkansas. This parasite has now been reported from nine of 59 (15%) amphibians of the state (Trauth et al. 2004).

We thank P.R. Pilitt (USNPC) and Dr. R. Tumilson (HSU) for expert curatorial assistance. The Arkansas Game & Fish Commission provided Scientific Collecting Permits to CTM and MBC.

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New Host Records for *Mesocestoides*

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Table 1. Summary of reports of *Mesocestoides* sp. in North American amphibians.

Host	State	Prevalence*	Reference
Caudata			
Ambystomatidae			
<i>Ambystoma maculatum</i>	Arkansas	1/25 (4%)	McAllister et al. (2014)
Plethodontidae			
<i>Batrachoseps pacificus</i>	California	1/174 (1%)	Goldberg et al. (2000a)
<i>Desmognathus brimleyorum</i>	Arkansas	8/41 (20%)	McAllister et al. (1995c)
<i>D. conanti</i>	Arkansas	1/26 (4%)	McAllister et al. (2013)
<i>Plethodon albagula</i>	Arkansas	1/17 (6%)	McAllister et al. (2014)
<i>P. sequoyah</i>	Oklahoma	2/25 (8%)	McAllister and Bursey (2004)
Anura			
Bufonidae			
<i>Anaxyrus americanus americanus</i>	Iowa, South Dakota	11/415 (3%)	James and Ulmer (1967)
	Iowa	4/101 (4%)	Ulmer and James (1976)
	Michigan**	10/71 (15%)	Muzzall and Andrus (2014)
	Wisconsin	6/47 (13%)	Bolek and Coggins (2000)
		5/30 (17%)	Bolek and Coggins (2003)
		3/39 (8%)	Yoder and Coggins (2007)
<i>A. a. charlesmithi</i> †	Arkansas	4/29 (14%)	This report
<i>A. cognatus</i>	Iowa, South Dakota	1/17 (6%)	James and Ulmer (1967)
<i>A. fowleri</i> †	Arkansas	1/6 (17%)	This report
	Michigan**	6/31 (19%)	Muzzall and Andrus (2014)
<i>A. houstonensis</i>	Texas	2/17 (12%)	Thomas et al. (1984)
<i>Incilius nebulifer</i>	Texas	3/23 (13%)	McAllister et al. (1989)
Hylidae			
<i>Hyla chrysoscelis</i>	Wisconsin	8/65 (12%)	Bolek and Coggins (1998)
<i>Pseudacris crucifer</i>	Wisconsin	2/79 (3%)	Yoder and Coggins (2007)
<i>P. streckeri</i>	Texas	3/42 (7%)	McAllister (1987)

Table 1. *continued.* Summary of reports of *Mesocestoides* sp. in North American amphibians.

Host	State	Prevalence	Reference
Ranidae			
<i>Lithobates berlandieri</i>	Texas	1/2 (50%)	McAllister and Conn (1990)
<i>L. blairi</i>	Iowa	4/16 (25%)	Goldberg et al. (2000b)
	Kansas	1/18 (6%)	Goldberg et al. (2000b)
	Nebraska	5/55 (9%)	Goldberg et al. (2000b)
<i>L. clamitans</i>	Michigan	49/239 (21%)	Muzzall et al. (2001)
	Wisconsin	6/412 (2%)	Williams and Taft (1980)
<i>L. palustris</i>	Arkansas	1/26 (4%)	McAllister et al. (1995a)
<i>L. pipiens</i>	Iowa, South Dakota	27/1568	James and Ulmer (1967)
	Iowa	10/491 (2%)	Ulmer and James (1976)
	Michigan	4/43 (9%) [‡]	Gilliland and Muzzall (1999)
	New York	1/34 (3%)	McAllister and Conn (1990)
	Wisconsin	1/22 (5%)	Williams and Taft (1980)
		13/31 (42%)	Bolek and Coggins (2003)
<i>L. sphenoccephalus utricularius</i> [†]	Arkansas	6/64 (9%)	This report
<i>L. sylvaticus</i>	Arkansas	3/42 (7%)	McAllister et al. (1995b)
	Wisconsin	9/78 (12%)	Yoder and Coggins (2007)
Scaphiropodidae			
<i>Scaphiopus bombifrons</i>	Oklahoma	2/3 (67%)	McAllister et al. (2005)
<i>S. hurterii</i>	Oklahoma	3/14 (21%)	McAllister et al. (2005)

*Prevalence = number infected/number examined (%).

[†]New host record.

[‡]froglings < five mo. old only.

**Note added in proof: After this paper went to press, Muzzall and Andrus (2014) reported *Mesocestoides* sp. in *Anaxyrus a. americanus* and *Anaxyrus fowleri* from western Michigan.