
First Report of *Mesocestoides* sp. Tetrathyridia (Cestoda: Cyclophyllidea) from the American Bullfrog, *Rana catesbeiana* (Anura: Ranidae)

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Abstract: A single adult American bullfrog, *Rana* (= *Lithobates*) *catesbeiana* collected from McCurtain County, Oklahoma, was found to harbor tetrathyridia of *Mesocestoides* sp. Specimens representing the non-proliferating type of *Mesocestoides* occurred in the mesenteries as well as encapsulated in the liver, spleen, and gastrointestinal tract. This discovery is rare and, most interesting, because many surveys of large numbers of *R. catesbeiana* have failed to report this cestode in American Bullfrogs from throughout its range in North America. Although seven other species of ranid frogs from Arkansas, Iowa, Kansas, Michigan, Nebraska, New York, South Dakota, Texas, and Wisconsin have been reported to harbor *Mesocestoides* sp., we report it for the first time from the largest species of North American frog.

Introduction

Over 25 yr ago, McAllister and Conn (1990) were the first to provide a summary of North American anuran hosts of *Mesocestoides* sp. Noticeably absent from that host list was the largest North American frog, the American Bullfrog, *Rana* (= *Lithobates*) *catesbeiana* Shaw, 1802. More recently, McAllister et al. (2014) updated that list to include all North American amphibian hosts of this tapeworm. In their list, seven species of frogs of the family Ranidae were reported as hosts of *Mesocestoides* sp. collected from Arkansas, Iowa, Kansas, Michigan, Nebraska, New York, South Dakota, Texas, and Wisconsin. Again, *R. catesbeiana* was not on this list. The reason for this absence is an enigma

as its ecology is very similar to other ranid frogs (Stebbins et al. 1995; Casper and Hendricks 2005), including northern and southern leopard frogs (*Rana pipiens* and *R. sphenoccephalus*), who have previously been commonly reported as hosts (McAllister et al. 2014). Indeed, a summary of parasites of *R. catesbeiana* was provided by Andrews et al. (1992) and Mata-López et al. (2010), and, although they collectively list at least 10 species of cestodes from native and non-native populations of this host, neither lists *Mesocestoides* sp. Many surveys utilizing hundreds of specimens of *R. catesbeiana* have been reported from numerous North American localities; however, none, to date (Harwood 1932; Trowbridge and Hefley 1934; Brandt 1936; Odlaug 1954; Najarian 1955; Campbell 1968; Lank 1971; Hollis 1972; Brooks 1976; Lemke et al. 1982; Muzzall 1991;

Andrews et al. 1992; McAlpine 1997; Yoder and Gomez 1997; Goldberg et al., 1998; McAlpine and Burt 1998; Goldberg and Bursey 2002; Conn et al. 2002; Mata-López et al. 2010; and others) have reported this frog as a host of this tapeworm. Here, we document the first report of *Mesocestoides* sp. from *R. catesbeiana*.

Between May 2013 and September 2016, 18 juvenile and adult American bullfrogs (mean \pm 1SD snout-vent length [SVL] = 65.3 ± 38.1 , range 35–102 mm) were collected by hand or dipnet from Little River ($n = 7$) and Polk ($n = 7$) counties, Arkansas, and McCurtain ($n = 4$) County, Oklahoma. Specimens were placed in individual collection bags on ice and following the guidelines for the human treatment of research animals (HACC 2004) were overdosed by immersion in a concentrated chloretone (chlorobutanol) solution. A mid-ventral incision was made from throat to cloaca and the viscera and body cavity examined for *Mesocestoides* sp. When suspected encapsulated cestodes were observed, they were excised with a bit of tissue and preserved in 10% neutral buffered formalin. Other specimens were teased from mesenteries and preserved in 70–90% (v/v) DNA grade ethanol. For light microscopy, we used standard histological techniques to prepare the tissue preserved in 10% neutral-buffered formalin for light microscopy following Presnell and Schreibman (1997). For photomicroscopy, we used a Nikon Eclipse 600 epifluorescent light microscope with a Nikon DXM 1200C digital

camera (Nikon Instruments Inc., Melville, NY). A host voucher specimen was deposited in the Arkansas State University Herpetological Museum (ASUMZ), State University, Arkansas. Voucher specimens of *Mesocestoides* sp. were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska. We follow Yuan et al. (2016) in the taxonomic use of the genus *Rana* (rather than *Lithobates*) for North American ranid frogs.

Results and Discussion

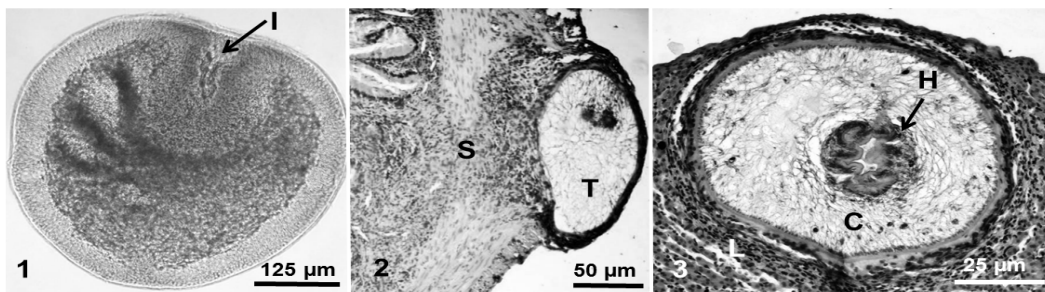
One of 18 (6%) *R. catesbeiana* was found to be infected with encapsulated and free tetrathyridia of *Mesocestoides* sp. as follows.

Cestoda: Cyclophyllidea: Mesocestoididae
Mesocestoides sp. Vaillant, 1863 (Figs. 1–3)

Host and locality: American bullfrog, *Rana catesbeiana* (ASUMZ 33580, 102 mm SVL, adult male) collected on 29 June 2016 from Yanubbee Creek off county road N4680 at Broken Bow, McCurtain County, Oklahoma (34° 02' 45.6426" N, 94° 43' 19.761" W).

Prevalence and intensity: 1/18 (6%) overall; 1/4 (25%) McCurtain County, Oklahoma; tetrathyridia too numerous to count.

Site of infection: Free tetrathyridia in body cavity (Fig. 1); encapsulations in intestinal tract (Fig. 2), liver (Fig. 3), and spleen.



Figures 1–3. Tetrathyridia of *Mesocestoides* sp. from *Rana catesbeiana*. 1. Stained whole mount of free tetrathyridium from body cavity showing invaginated scolex and deep invagination canal (I). 2. Low power microscopic view showing stained histological section of encapsulated tetrathyridium (T) on serosal surface (S) of intestinal tract. 3. Higher power microscopic view of stained histological section of tetrathyridium in host-derived fibrotic capsule in liver. Note the solid hindbody characteristic of tetrathyridia of *Mesocestoides*. Abbreviations = calcareous corpuscles (C); scolex holdfast (H).

Morphological and histological features: Tetrathyridia possessed characteristic individual features of a single invaginated scolex, a generally deep invagination canal (Fig. 1), calcareous corpuscles, and a solid hindbody (Figs. 1–3). No tetrathyridium possessed a divided scolex, somatic bud, or any tegumental or excretory anomalies such as those reported rarely from tetrathyridia in some aberrant acephalic tetrathyridia from other host species (Conn et al. 2010; see also recent review by Conn et al. 2011).

Additional Oklahoma records: CAUDATA: Sequoyah slimy salamander, *Plethodon sequoyah* Highton, 1989 (McAllister and Bursey 2004); ANURA: Hurter's spadefoot, *Scaphiopus hurterii* Strecker, 1910, and plains spadefoot, *Spea bombifrons* (Cope, 1863) (McAllister et al. 2005).

Additional reports, anurans: see summary by McAllister et al. (2014).

Other reported herpetofauna: See recent summary by Bursey et al. (2012) which includes a variety of hosts, including amphibians and reptiles from the Asian, Australo-Papuan, Ethiopian, Nearctic, Neotropical, and Palearctic regions.

Geographic range: The genus is cosmopolitan (Schmidt 1986).

Type species, type host and type locality: *Mesocestoides ambiguous* (Mammalia: Carnivora), small-spotted genet, *Vivera genetta*, Africa (Vaillant 1863).

Specimens deposited: HWML 102076–102077 (slides).

The life cycle of *Mesocestoides* spp. has been and remains an enigma. Current thought of most researchers is that it requires at least three hosts (i.e., a vertebrate definitive host, a vertebrate second intermediate host, and a supposed arthropod first intermediate host) (Rausch 1994), though this has not been verified and the putative first-intermediate host is not known.

Indeed, it is not unusual to find encapsulated and free tetrathyridia in the body cavity and various organs of amphibians, reptiles and rodents (Padgett and Boyce 2004) as well as some birds (Skirnisson et al. 2016), all of which have been assumed to be second-intermediate hosts. In our sample, there was no morphological, histological or other evidence of asexual proliferation. Thus, these findings are consistent with the absence of asexual reproductive capacity reported in our earlier studies (for example, see McAllister et al. 1989, 1992, 2005, 2013a, b, 2014, 2015; Conn and McAllister 1990; McAllister and Conn 1990), and corroborate the rarity of asexuality for tetrathyridia in general (Conn 1990; Conn et al. 2011).

We cannot offer any explanation for the absence of *Mesocestoides* in *R. catesbeiana* from all previously published surveys. This frog has been reported to prey on a great diversity of invertebrate and vertebrate fauna (see Table SP-3 of Casper and Hendricks 2005). The study site noted herein (Yanubbee Creek) is not unlike other watersheds in Arkansas or Oklahoma where we have collected *R. catesbeiana* in the past. Perhaps some unknown intermediate host occurs at that site and additional research will be required to investigate this unusual finding in a common, widely-ranging, anuran.

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