Tetrathyridia of *Mesocestoides* sp. (Cestoda: Cyclophyllidea: Mesocestoididae) from Pickerel Frog, *Rana palustris* (Anura: Ranidae), with a Summary of Hosts from Oklahoma

Chris T. McAllister
Science and Mathematics Division, Eastern Oklahoma State College, Idabel, OK 74745

Stanley E. Trauth
Department of Biological Sciences, Arkansas State University (Emeritus), State University, AR 72467

Henry W. Robison
9717 Wild Mountain Drive, Sherwood, AR 72120

Abstract: We collected four pickerel frogs (*Rana palustris*) from McCurtain County, Oklahoma, and examined each for the cyclophyllidean cestode, *Mesocestoides* sp. A single (25%) *R. palustris* harbored tetrathyridia of this tapeworm. We document additional information on *Mesocestoides* sp. and report it for the first time from *R. palustris* in Oklahoma. In addition, we provide a summation of the amphibians and reptiles of Oklahoma reported to harbor this enigmatic tapeworm.

Introduction

The tetrathyridial or metacestode stage of the cyclophyllidean cestode, *Mesocestoides* Vaillant, 1863, occurs free in the body cavity and as encapsulations in tissues of a broad range of vertebrate second intermediate hosts such as amphibians, reptiles, and small rodents; the adult worm is found infecting definitive hosts including birds of prey, placental mammals (canids, felids, mustelids, and hyaenids), non-human primates, and rarely humans (Fuentes et al. 2003; Padgett et al. 2012; Montalbano Di Filippo et al. 2018). Although the classical literature on parasites from anuran amphibians of Oklahoma reported several hosts infected with cestodes (Trowbridge and Hefley 1934; Kuntz 1941; Kuntz and Self 1944), none reported any hosts with *Mesocestoides* sp. However, for more than a decade, our research consortium has reported *Mesocestoides* sp. tetrathyridia from several amphibians and reptiles of Oklahoma (see McAllister and Bursey 2004; McAllister et al. 2005, 2017, 2018a, 2018b, 2020). The purpose of this report is to document additional information on *Mesocestoides* sp. in a common anuran of the state. We also provide a summation of the amphibians and reptiles of Oklahoma reported to harbor this enigmatic tapeworm.

Methods

Between March 2018 and September 2021, four adult (1 male, 3 female, mean ± 1SD snout-vent length [SVL] = 65.8 ± 8.1, range 52–72) pickerel frogs, *Rana palustris* (LeConte, 1825), were collected by hand from Hochatown, McCurtain County, Oklahoma (34°10’17.0286”N, -94°45’5.7414”W), and examined for *Mesocestoides* sp. Specimens were euthanized with a concentrated tricaine methanesulfonate solution following accepted guidelines (SIH 2004) and a mid-ventral incision was made and the coelomic cavity

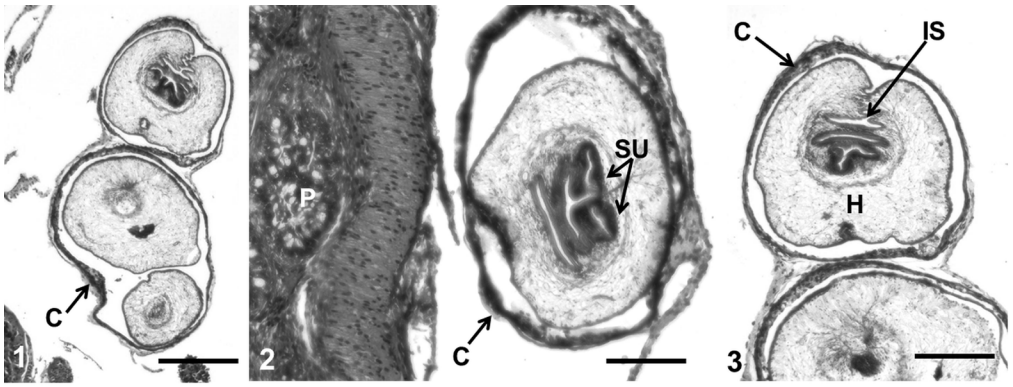
and various organs (placed in Petri dishes with saline) was examined with a stereomicroscope. When suspected encapsulated tapeworms were observed, they were excised with a portion of tissue and preserved in 10% (v/v) neutral buffered formalin. Tissues were prepared for light microscopy following Presnell and Schreibman (1997). The histological steps included the following: dehydrating tissue through a graded ethanol series, clearing in 100% xylene, infiltrating in paraffin wax overnight in a paraffin oven (56C), embedding in paraffin using plastic molds, sectioning on a rotary microtome into 10-µm strips (affixed onto glass slides with Haupt's adhesive prior to floating strips in 2% NBF on a slide warmer) and staining to reveal general cytology with Pollak trichrome stain for the enhancement of tissue. Coverslips were adhered to the microscopic slides using Permount. For photomicroscopy, specimens were examined and photographed with a Leica DM 2000 LED microscope (Leica Microsystems, Inc., Buffalo Grove, Illinois). A subset of sample was saved in 70% (v/v) DNA grade ethanol for future molecular analysis (VV Tkach, *pers. comm*.).

A voucher host specimen was deposited in the Eastern Oklahoma State College Vertebrate Collection, Idabel, Oklahoma. Voucher specimens of *Mesocestoides* sp. were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska. We generally adopt the amphibian and reptile taxonomy of Crother et al. (2017), except we follow Yuan et al. (2016) for North American *Rana* spp., rather than *Lithobates*.

Results and Discussion

A single gravid female *R. palustris* (73 mm SVL) harbored tetrathyridia (HWML 216710) in its mesenteries (Figs. 1, 3) and near the pancreas (Fig. 2). Tetrathyridia possessed the characteristic features of a single invaginated scolex (Fig. 2), a generally deep invagination canal (Fig. 3), a prominent single excretory pore at the end opposite the scolex, and a solid hindbody (Fig. 3). None of the tetrathyridia possessed any anomalies such as those reported in some aberrant acephalic tetrathyridia from other hosts, including a divided scolex, somatic bud, or any tegumental or excretory irregularity (see review by Conn et al. 2011).

The complete life cycle of *Mesocestoides* remains an enigma. Several authors have suggested that terrestrial (perhaps coprophagic) arthropods, including coleopterans (dung beetles), hymenopterans (ants), blattodeans (roaches), and mites could serve as potential



Figures 1–3. *Mesocestoides* sp. tetrathyridia from *Rana palustris*. (1) Three tetrathyridia from mesenteries; two in single capsule (arrow). (2) Single tetrathyridium from near pancreas; note capsule and suckers (arrows). (3) Two tetrathyridia from mesenteries; note capsule and invaginated scolex (arrows). Abbreviations: capsule (C); solid hindbody (H); IS (invaginated scolex); pancreas (P); suckers (SU). Scale bars (1) 1 mm; (2-3) 250 µm.

Table 1. Amphibians and reptiles of Oklahoma* reported as hosts of *Mesocestoides* sp.

Host (Class, Order, Family, Species)	Prevalence†	Reference(s)
Amphibia		
Caudata		
Plethodontidae		
Plethodon sequoyah	3/14 (21%)	McAllister and Bursey (2004)
Anura		
Bufonidae		
Anaxyrus fowleri	1/1 (100%)	McAllister et al. (2020)
Scaphiopodidae		
Scaphiopus hurterii	3/14 (21%)	McAllister et al. (2005)
Spea bombifrons	2/3 (67%)	McAllister et al. (2005)
Ranidae		
Rana catesbeiana	1/18 (6%)	McAllister et al. (2017)
Rana palustris	1/5 (20%)	This report
Rana sphenocephalus utricularius	21/74 (28%)	Vhora and Bolek (2015)
	1/1 (100%)	McAllister et al. (2020)
Reptilia		
Squamata		
Scincidae		
Scincella lateralis‡	1/1 (100%)	McAllister et al. (2018b)‖
Colubridae		
Heterodon platirhinos	1/1 (100%)	McAllister et al. (2020)
Thamnophis sirtalis sirtalis	2/2 (100%)	McAllister et al. (2018a)

*All hosts were collected in McCurtain County except spadefoot toads came from the University of Oklahoma Biological Station (Marshall County) and *R. s. utricularius* were collected from Stillwater, Payne County (Vhora and Bolek 2015).

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

‡Harbored pre-tetrathyridia with evaginated scolex and neck region.

‖McAllister et al. (2014b) also examined 20 *S. lateralis* from McCurtain County and none were infected with *Mesocestoides*.

first intermediate hosts (see Sapp and Bradbury 2020). However, it has also been proposed that *Mesocestoides* could perhaps develop through a simple two-host (diheteroxenous) life cycle rather than an obligate three-host cycle (triheteroxenous) by utilizing only vertebrates as the intermediate host (McAllister et al. 2018b).

McAllister et al. (1995) provided a survey of the parasites of *R. palustris* from the southern part of its range. In that study, two specimens were collected in Le Flore County, Oklahoma, but they only harbored nematodes, *Abbreviata* sp. However, a single specimen of 24 (4%) collected in the same survey from Arkansas had encapsulated *Mesocestoides* sp. tetrathyridia in its mesenteries. Numerous other surveys of *R. palustris* collected in other parts of it range, including Illinois, Indiana, Maine, Massachusetts, Missouri, North Carolina, Texas, and Wisconsin, and Ontario, Canada, did not report the presence of this tapeworm in any hosts (see McAllister et al. 1995, their Table 2). Therefore, *Mesocestoides* appears to be a rarely encountered helminth of pickerel frogs and generally of other North American anurans as well (McAllister and Conn 1990; McAllister et al. 2014b, 2017).

In Oklahoma, to date, 10 species of amphibians and reptiles, including a single salamander, six species of anurans, a skink, and two colubrid snakes have been reported to harbor *Mesocestoides* sp. (Table 1). As Oklahoma supports 157 species/subspecies of amphibians and reptiles (Sievert and Sievert 2011), only 6% have been reported in the state infected with *Mesocestoides*, so there is plenty of opportunity to discover this unusual parasite by surveying additional herpetofauna.

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