First Report of *Eimeria hydrophis* (Apicomplexa: Eimeriidae) from Plain-Bellied Watersnake, *Nerodia erythrogaster* (Serpentes: Colubridae: Natricinae), from Oklahoma

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Abstract: There are several reports of coccidian parasites (Apicomplexa) from plain-bellied watersnakes, *Nerodia erythrogaster* collected from various locales in Arkansas and Texas. However, there are no reports of coccidians from this host or any other watersnake in Oklahoma. Here, we document, for the first time, *Eimeria hydrophis* from a *N. erythrogaster* collected in Oklahoma, as well as provide the first photomicrographs of the coccidian.

Introduction

The plain-bellied watersnake, *Nerodia erythrogaster* (Forster, 1771) is a large, heavily-bodied reptile that ranges from the Delmarva Peninsula south to northern Florida and westward to Oklahoma, New Mexico, Texas, and adjacent México; there are isolated populations in the upper Midwest (Powell et al. 2016). In Oklahoma, this snake is found statewide (Sievert and Sievert 2011). It inhabits a wide variety of wetlands, but mostly larger, more permanent watersheds, river bottoms, floodplains, marshes, sloughs, swamps, and the edges of man-made ponds and lakes (Gibbons and Dorcas 2004). The taxon was formerly recognized as having four subspecies; however, using mitochondrial data, Makowsky et al. (2010) found little support for subspecific recognition and concluded this taxon represents a single, widespread species. We therefore follow Crother et al. (2017) in not recognizing any subspecies.

Several coccidian parasites have been previously reported from *N. erythrogaster* (Table 1), including *Eimeria attenuata* Wacha and Christiansen, 1974, *E. conanti* McAllister and Upton, 1989, *E. cyclopion* McAllister, Upton, and Trauth, 1990, an *E. helmisophis*-like coccidian, *E. hydrophis* Wacha and Christiansen, 1974, *E. natricis* Wacha and Christiansen, 1974, *E. sipedon* Wacha and Christiansen, 1975, and *E. tenuis* Upton and McAllister, 1988 (Duszynski and Upton 2009; McAllister et al. 2017). Here, we document a new geographic record for *E. hydrophis* from a *N. erythrogaster* collected from Oklahoma, and provide the first photomicrographs of the coccidian.

Methods

Between April 2012 and August 2020, four *N. erythrogaster* (660–745 mm snout-vent length [SVL]), two broad-banded watersnakes, *Nerodia fasciata confluens* (Blanchard, 1923) (178–223 mm SVL), and five northern diamond-backed watersnakes, *Nerodia rhombifer rhombifer* (Hallowell, 1852) (655–785 mm SVL) were collected from several sites in McCurtain County, and examined

for coccidians. They were killed with an intraperitoneal injection of sodium pentobarbital (Nembutal®). A mid-ventral incision was made and feces from the rectum was collected and placed in an individual vials containing 2.5% (w/v) potassium dichromate ($K_2Cr_2O_7$). Because the oocyst walls of some watersnake coccidians are known to wrinkle in sugar solutions used for flotation (Wacha and Christiansen 1974), an initial flotation was done in Sheather's sugar solution (specific gravity = 1.30), the coverslip was removed, and then rinsed with tap water. The sample was centrifuged (430×g for 10 min.) and wet mounts were examined for coccidia using an Olympus BX43 microscope with Nomarski DIC. Measurements were taken on 10 sporulated oocysts using a calibrated ocular micrometer and Lumenera Infinity Analyze software (Teledyne Lumenera, Ottawa, Ontario, Canada) and reported in micrometers (µm) with the means followed by the ranges in parentheses; photographs were taken using brightfield optics. Oocysts were 60 days old when measured and photographed. Descriptions of oocysts and sporocysts follow the standard guidelines of Wilber et al. (1998) including: oocyst length (L) and width (W), their ranges and ratios (L/W), micropyle (M), oocyst residuum (OR), polar granule(s) (PG), sporocyst length (L) and width (W), their ranges and ratio (L/W), sporocyst (SP), Stieda body (SB), substieda body (SSB), parastieda body (PSB), sporocyst residuum (SR), sporozoites (SZ) anterior (ARB) and posterior (PRB) refractile bodies, and nucleus (N).

Standard common and scientific names follow Crother et al. (2017). A host voucher was deposited in the EOSC collection, Idabel, Oklahoma. Photovouchers of coccidia were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska.

Results

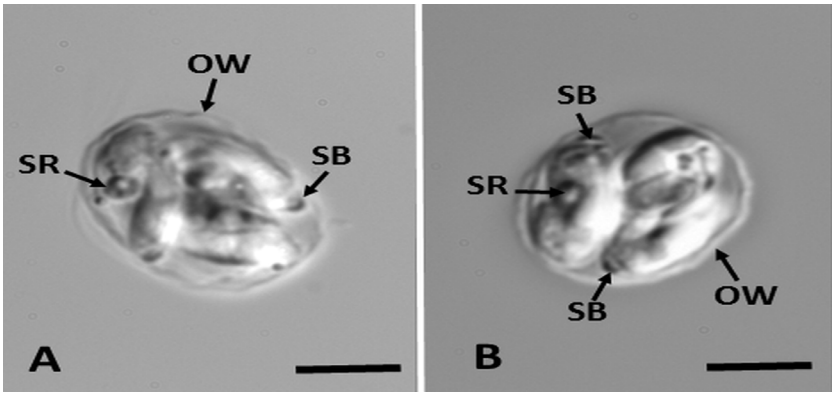
A single *N. erythrogaster* was found to be passing coccidia; none of the other 10 watersnakes were infected. Detailed information on the sample follows.

Apicomplexa: Eimeriidae

Eimeria hydrophis Wacha and Christiansen, 1974 (Fig. 1)

Description: Oocyst shape: subspheroidal to ellipsoidal; number of walls: 2; characteristics: smooth, walls wrinkle readily even in diluted Sheather's sugar solution; (L × W) (*n* = 10): 13.4 × 11.5 (11–16 × 10–13), L/W ratio: 1.2 (1.1–1.4); M, OR, PG: all absent. Sporocyst shape: ellipsoidal; (L × W) (*n* = 10): 9.6 × 4.8 (7–12 × 4–5), L/W 2.0 (1.6–2.4); flattened SB present; SSB, PSB: both absent; SR: present; composed of small, compact sphere of granules; SZ: Sausage-shaped (not measured); ellipsoidal ARB and PRB present, N in middle of SZ.

Type host: Northern watersnake, *Nerodia sipedon sipedon* (L., 1758).



Figures 1A-B. Sporulated oocysts of *Eimeria hydrophis*. Abbreviations: OW (oocyst wall); SB (Stieda body); SR (sporocyst residuum). Note wrinkled OW. Scale bars = 5 µm.

Table 1. Coccidians reported from plain bellied watersnake, *Nerodia erythrogaster*.*

Coccidian	Prevalence†	State	Reference(s)
Eimeria attenuata	2/20 (10%)	Arkansas	McAllister et al. (1995)
	2/23 (9%)	Texas	McAllister and Upton (1989)
E. conanti	2/20 (10%)	Arkansas	McAllister et al. (1995)
	2/23 (9%)	Texas	McAllister and Upton (1989)
E. cyclopion	1/1 (100%)	Arkansas	McAllister et al. (1990)
	2/9 (22%)	Arkansas	McAllister et al. (2017)
E. helmisophis-like‡	3/20 (15%)	Texas	McAllister and Upton (1989)
	5/20 (25%)	Arkansas	McAllister et al. (1995)
	1/2 (50%)	Texas	McAllister et al. (1995)
E. hydrophis	2/20 (10%)	Arkansas	McAllister et al. (1995)
	1/4 (25%)	**Oklahoma**	**This report**
	2/23 (9%)	Texas	McAllister and Upton (1989)
E. natricis	2/20 (10%)	Arkansas	McAllister et al. (1995)
E. sipedon	11/20 (55%)	Arkansas	McAllister et al. (1995)
	3/23 (13%)	Texas	McAllister and Upton (1989)
E. tenuis	2/22 (9%)	Arkansas	McAllister et al. (1995)

*Includes subspecies previously known for *N. erythrogaster*, including yellow-belly watersnake (*N. e. flavigaster*) and blotched watersnake (*N. e. transversa*).

†Number infected/number examined (%).

‡It is doubtful this coccidian is the same species found in worm snakes (*Carphophis* spp.) (see McAllister et al. 1995).

Type specimen: None deposited by Wacha and Christiansen (1974).

Type locality: USA: Iowa: Louisa County, 5 mi S Muscatine at Windy Hills Shooting Refuge.

Prevalence: 5/14 (36%).

Site of infection: Intestine.

Other hosts and localities: *N. erythrogaster*, Johnson County, Texas (McAllister and Upton 1989); midland watersnake, *Nerodia sipedon pleuralis* (Cope, 1892), Arkansas (McAllister et al. 1995); Brazo's River watersnake, *Nerodia harteri* (Trapido, 1941), Somervell County, Texas (McAllister and Upton 1989); *N. s. sipedon*, Bremer, Louisa, and Van Buren counties, Iowa, *N. r. rhombifer* (Hallowell, 1852), Muscatine County, Iowa (Wacha and Christiansen 1974, 1975).

Present host: *N. erythrogaster*.

New locality: USA: Oklahoma: McCurtain County, Hochatown (34° 09' 55.152'' N, 94° 45' 35.8776'' W).

Specimen deposited: HWML photovoucher 216631.

Prevalence: 1/11 (9%) overall; 1/4 (25%) *N. erythrogaster*.

Site of infection: Oocysts found in feces; bile contents did not contain oocysts. Wacha and Christiansen (1974) also recovered oocysts of *E. hydrophis* only in feces, and not bile, which indicated the site of endogenous development was the intestinal tract.

Remarks: Oocysts from the present sample were not significantly different than those described originally by Wacha and Christiansen (1974) from *N. s. sipedon* from Iowa. Our oocysts were, on average, slightly smaller in length (13.4 × 11.5 *vs.* 15.4 × 10.9 µm) but sporocysts were nearly identical in average sizes (9.6 × 4.8 *vs.* 10.3 × 4.9 µm) as well as all other morphological and mensural characteristics fitting *E. hydrophis*.

Discussion

There are several comprehensive surveys of various North American watersnakes for coccidian parasites from the surrounding states of Arkansas and Texas as well as Iowa (see summary in Duszynski and Upton 2009). Prevalence of infection in eight coccidians of *N. erythrogaster* is usually low depending on the eimerian species found and is 43 of 270 (16%) overall (Table 1).

In conclusion, a modest sample of three species have been examined to date for coccidians in Oklahoma, and information on a single infected host has been provided herein. As all four of the *Nerodia* spp. that occur in the state have been reported to harbor several coccidians from other states (Duszynski and Upton 2009), future surveys will surely provide new geographic distribution records as well as the possibility of discovering new species as additional Oklahoma watersnakes are examined.

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