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Running Title: Coccidia of Turtles

Abstract

We collected 50 three-toed box turtles (*Terrapene carolina triunguis*) from 9 counties of Arkansas and 4 counties of Oklahoma, and examined their feces for coccidial parasites. Nine of 24 (38%) turtles from Arkansas and 8 of 26 (31%) from Oklahoma were found to be passing oocysts of *Eimeria ornata*. This represents two new geographic distributional records for this coccidian. Measurements of individual isolates of *E. ornata* as well as morphological characteristics are provided with comparison to its original description and to another *Terrapene* coccidian, *Eimeria carri*. In addition, we noted an adelid pseudoparasite being passed by a single *T. c. triunguis* from Oklahoma that likely represents a parasite of arthropods.

Introduction

Much has been written on the natural history and ecology of North American box turtles, *Terrapene* spp. (Dodd 2001). There is also a great deal of information available on their endoparasites (Ernst and Ernst 1977, and others). However, little is known about coccidian parasites of box turtles. McAllister and Upton (1989a) were the first to summarize the coccidians (Apicomplexa) of turtles and, more recently, Duszynski and Morrow (2014) provided a summation on the coccidia of turtles of the world. In the genus *Terrapene* Merrem only 3 species of coccidia have been described and/or reported as follows: *Eimeria carri* Ernst and Forrester originally reported from the eastern box turtle, *Terrapene carolina carolina* from Alabama and Florida (Ernst and Forrester 1973) and later found in three-toed box turtles, *Terrapene carolina triunguis* from Arkansas (McAllister et al. 1994); the second coccidian is *Eimeria ornata* McAllister and Upton reported from *Terrapene ornata ornata* from Texas (McAllister and Upton 1989b); and the third is the ubiquitous *Eimeria mitraria* (Laveran and Mesnil) Široký, Kamler and Modrý, reported from *T. c. triunguis* from Arkansas (McAllister et al. 1994). To date, as far as we know, neither of the first two species has been reported from additional turtles or additional US states. Here, we report, for the first time, *E. ornata* from *T. c. triunguis* from Arkansas and Oklahoma, and provide comparative measurements of individual isolates as well as data on their morphological characteristics.

Materials and Methods

Between March 2012 and May 2015, we collected 50 juvenile and adult *T. c. triunguis* by hand or as salvaged road-killed (DOR) specimens in nine (Benton [*n*=1], Boone [*n*=1], Fulton [*n*=1], Little River [*n*=2], Marion [*n*=2], Montgomery [*n*=1], Pike [*n*=4], Pope [*n*=1], Union [*n*=11]) counties of Arkansas and four (Latimer [*n*=4], Le Flore [*n*=3), McCurtain [*n*=18], Pushmataha [*n*=1]) counties of Oklahoma. In the laboratory, live turtles were held in 38L glass terrariums and, immediately after defecation, each fecal sample was placed in a vial containing 2.5% (w/v) aqueous potassium dichromate solution ($K_2Cr_2O_7$); these turtles were released back into the wild. Feces were obtained from DOR turtles by taking samples directly from the rectum. Following an initial examination, all positive samples were transferred to Petri dishes containing a thin layer of $K_2Cr_2O_7$ and allowed to sporulate completely for up to 1 wk. Following sporulation, oocysts were concentrated by flotation in a modified Sheather's sugar solution (sp. gr. 1.30) and examined using light microscopy, photographed with Nomarski interference-contrast optics, and measured with a calibrated ocular micrometer or Olympus© cellSens 1.7 digital software.

Measurements are reported in micrometers (µm) with means followed by the ranges in parentheses. Descriptions of oocysts and sporocysts follow guidelines of Wilber et al. (1998) as follows: 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). A host photovoucher was accessioned into the Arkansas State University Museum of Zoology, Herpetology Collection (ASUMZ), State University, AR as ASUMZ 32041. Photovouchers of sporulated oocysts were accessioned into the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, NE as HWML 101825-101827.

Results

Nine of 24 (38%) three-toed box turtles from Arkansas and 8 of 26 (31%) from Oklahoma were found to be passing oocysts of *E. ornata* (Fig. 1); overall prevalence was 17 of 50 (34%).

A description of the oocysts (n = 89) we observed are as follows: sporulated oocyst with four sporocysts; shape spheroidal to subspheroidal; smooth uni-layered wall, colourless, ~ 0.5 thick; L × W: 18.2 × 16.0 (17–19 × 15–17); L/W: 1.1 (1.1–1.2); M absent, OR present as compact granulated spheroidal mass frequently surrounded by membranous sac, PG (1-2) present. SP ellipsoidal to elongate, smooth uni–layered wall; L × W: 11.3 × 5.2 (11–12 × 5.1–5.4); L/W: 2.2 (2.0–2.3); SB present as a distinct point on end of SP, SSB, PSB absent; SR: composed of small granules dispersed throughout; SZ: (not measured) sausage-shaped, lying lengthwise in SP with large and distinct subspheroidal ARB and PRB; N slightly off center of SZ.

We also found one of 26 (4%) turtles from Oklahoma to be passing oocysts of an unknown genus of coccidian with oocysts containing many (>12) sporocysts (Fig. 2). We consider this an adelid pseudoparasite that was likely ingested by this turtle with its arthropod host.

Discussion

Herein, we have provided the largest survey, to date, on coccidia of *T. c. triunguis*. In comparing our samples of *E. ornata* to those originally described by McAllister and Upton (1989b) from a different host (*T*

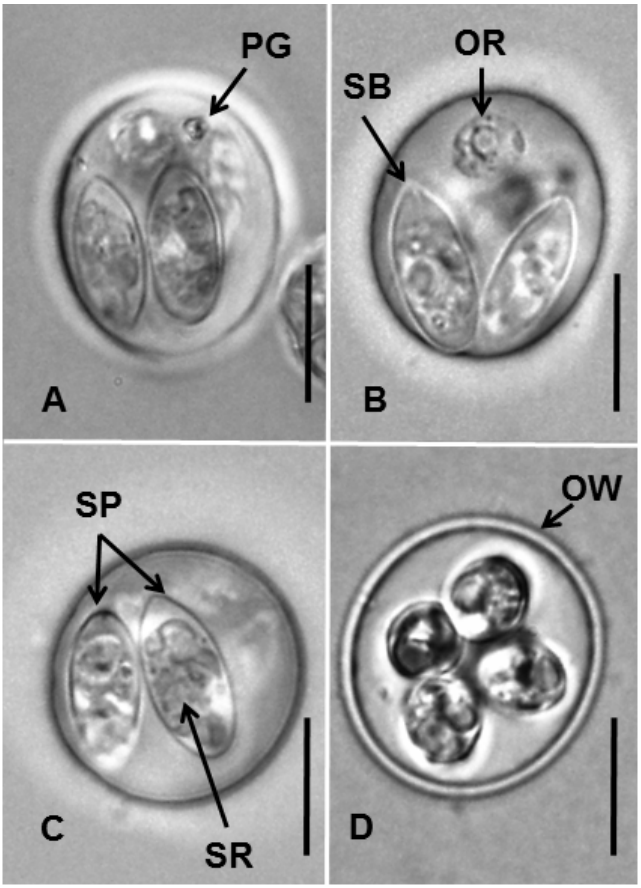

Figure 1. Sporulated oocysts of *Eimeria ornata*. A. Oocyst from isolate Tct1 showing polar granule (PG); B. Oocyst from isolate Tct6 showing oocyst residuum (OR) and point on Stieda body (SB); C. Oocyst from Tct6 showing typical elongate shape of sporocysts (SP) and sporocyst residuum (SR); 4. Oocyst from isolate Tct33 showing single layered oocyst wall (OW). Scale bars = 10 µm.

o. ornata) and locality (Texas) we observed the following: oocyst and SP shape and size (17.9 × 15.7 µm, 11.1 × 5.4 in original description) were similar as well as L/W ratios, and SR; differences were observed in the appearance of the OR as we did not see a vacuole in the middle but closer to the edge, and the oocyst wall was listed originally as being thicker at 1.0 µm. However, these differences are minor and could be the result of using different microscopic optics.

We did observe some differences in sizes of oocysts and SP between the 6 isolates that were measured (Table 1). However, these differences are not considered significant and we are confident that all isolates represent *E. ornata*.

Duszynski and Morrow (2014) argue that *E. carri* and *E. ornata* may represent the same species. We disagree because there are enough differences in oocysts between the two, particularly in the fact that *E. carri* has never been reported to have polar granules,

Figure 2. Unknown adelid coccidian containing many sporocysts from *Terrapene carolina triunguis* from Oklahoma. Scale bar = 10 µm.

but *E. ornata* often does, and oocysts are smaller in *E. carri* by at least 2.0 µm and average 15.9 × 14.5 µm. In addition, there are also some minor structural differences, marked differences in sporulation, and no photomicrograph of *E. carri* exists. Ideally, molecular confirmation would be needed to resolve this question.

In conclusion, we have provided additional information on the turtle coccidian, *E. ornata*. We document 2 new geographic distribution records for this coccidian. Although there is information on coccidia of other turtles in Arkansas, including *Chelydra serpentina* (McAllister et al. 1990), *Macrochelys temminckii* (Upton et al. 1992) and various emydid, kinosternid, and trionychid turtles (McAllister et al. 1994), additional surveys are recommended on other turtles of the state and, especially, those from Oklahoma.

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Literature Cited

Dodd CK Jr. 2001. North American box turtles: A natural history. Norman (OK): University of Oklahoma Press. 231 p.

Duszynski DW and **JJ Morrow**. 2014. The biology and identification of the coccidia (Apicomplexa) of turtles of the world. Boston (MA): Academic Press. 210 p.

Ernst EM and **CH Ernst**. 1977. Synopsis of the helminths parasitic in native turtles of the United States. Bulletin of the Maryland Herpetological Society 13:1-75.

Ernst JV and **DJ Forrester**. 1973. *Eimeria carri* sp. n. (Protozoa: Eimeriidae) from the box turtle, *Terrapene carolina*. Journal of Parasitology 59:635-636.

McAllister CT and **SJ Upton**. 1989a. The coccidia (Apicomplexa: Eimeriidae) of Testudines, with description of three new species. Canadian Journal of Zoology 67:2459-2467.

McAllister CT and **SJ Upton**. 1989b. *Eimeria ornata* n. sp. (Apicomplexa: Eimeriidae) from the ornate box turtle, *Terrapene ornata ornata* (Reptilia: Testudines), in Texas. Journal of Protozoology 36:131-133.

McAllister CT and **SJ Upton**. 1990. Coccidian parasites (Apicomplexa: Eimeriidae) of Chelydra serpentina (Testudines: Chelydridae) from Arkansas and Texas, U.S.A., with descriptions of *Isospora chelydrae* sp. nov. and *Eimeria serpentina* sp. nov. Canadian Journal of Zoology 68:865-868.

McAllister CT, **SJ Upton** and **SE Trauth**. 1994. New host and geographic records for coccidia (Apicomplexa: Eimeriidae) from North American turtles. Journal of Parasitology 80:1045-1049.

Upton SJ, **CT McAllister** and **SE Trauth**. 1992. Description of a new species of *Eimeria* (Apicomplexa: Eimeriidae) from the alligator snapping turtle, *Macroclemys temminckii* (Testudines: Chelydridae). Journal of the Helminthological Society of Washington 59:167-169.

Wilber PG, **DW Duszynski**, **SJ Upton**, **RS Seville** and **JO Corliss**. 1998. A revision of the taxonomy and nomenclature of the *Eimeria* spp. (Apicomplexa: Eimeriidae) from rodents in the Tribe Marmotini (Sciuridae). Systematic Parasitology 39:113-135.

Table 1. Comparative data on isolates of *Eimeria ornata* from *Terrapene carolina triunguis* (Tct). Eighty-nine oocysts were photographed, the mean for all oocysts measured (n = 86) L × W (µm) was 18.2 × 16.0 and the mean for all sporocysts measured (n = 113) L × W (µm) was 11.3 × 5.2.

Isolate[1-6]	Oocysts	L/W	Sporocysts	L/W
	Mean L × W (µm)		Mean L × W (µm)	
Tct1-Pike Co., AR (n = 5)[1]	19.3 × 16.1 (n = 4)	1.2	11.4 × 5.3 (n = 10)	2.2
Tct2-Pike Co., AR (n = 30)[2]	18.5 × 15.8 (n = 29)	1.2	11.0 × 5.4 (n = 42)	2.1
Tct6-McCurtain Co., OK (n = 24)[3]	17.4 × 15.6 (n = 24)	1.1	11.4 × 5.1 (n = 30)	2.2
Tct21-Union Co., AR (n = 5)[4]	16.9 × 15.4 (n = 4)	1.1	11.5 × 5.1 (n = 4)	2.2
Tct30-Fulton Co., AR (n = 8)[5]	18.2 × 16.5 (n = 8)	1.1	10.9 × 5.1 (n = 7)	2.2
Tct33-McCurtain Co. OK (n = 17)[6]	18.9 × 16.7 (n = 17)	1.1	11.5 × 5.1 (n = 20)	2.3

[1]Little Missouri Bridge W of Daisy off US 70 (34° 14' 22.6428”N. 93° 50' 3.4944”W), collected 26 April 2012.

[2]vicinity of Kirby off US 70 (34° 15' 5.562"N, 93° 39' 29.559"W), collected 26 April 2012.

[3]Lukfata off Memorial Street (34° 00' 15.8394N", 94° 45' 28.8108"W), collected 30 May 2013.

[4]Grady Bell Road, El Dorado (33° 12' 59.6514"N, 92° 35' 9.2142"W), collected 4 April 2012.

[5]Big Creek, SE of Mammoth Spring off US 63 (36° 26' 19.5282"N, 91° 29' 46.7082"W), collected 29 July 2013.

[6]Idabel off US 259 (33° 55' 19.473"N, 94° 46' 14.7714"W), collected 16 June 2013.