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A new eimerian (Apicomplexa: Eimeriidae), from ornate box turtle, *Terrapene ornata* (Testudines: Emydidae) from northwest Arkansas, USA

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Abstract

A new species of *Eimeria* (Apicomplexa: Eimeriidae) collected from an ornate box turtle, *Terrapene ornata* (Agassiz) from Arkansas, USA, is described. Oocysts of *Eimeria doddi* n. sp. are ovoidal to ellipsoidal with a smooth, light to darker brown, bi-layered wall, measure 21.1×14.0 μm , and have a length/width (L/W) ratio of 1.5; both micropyle and oöcyst residuum are absent, but a polar granule is present. Sporocysts are ellipsoidal, 9.9×6.1 μm , L/W 1.6; the Stieda body is present, but the sub-Stieda and para-Stieda bodies are absent and the sporocyst residuum is composed of small granules in a cluster. Sporozoites have a spheroidal anterior refractile body, a subspheroidal posterior refractile body, and one centrally-located nucleus. This is the third description of an eimerian from the turtle genus *Terrapene* and the second from *T. ornata*. In addition, we report *Eimeria ornata* McAllister & Upton, 1989 from *T. ornata* from Texas.

Introduction

There are six species of box turtles belonging to the genus *Terrapene*, *T. bauri* (Taylor), *T. carolina* (Linnaeus), *T. coahuila* Schmidt & Owens, *T. nelsoni* Stejneger, *T. ornata* (Agassiz)

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethical standards

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed.

and *T. mexicana* (Gray) (Powell et al., 2016; Uetz & Hošek, 2016). One of these, the ornate box turtle, *T. ornata* ranges from Indiana, Wisconsin and Wyoming southward to the Gulf Coast of Louisiana and Texas and westward into southeastern Arizona to the state of Sonora, México (Powell et al., 2016). In Arkansas, *T. ornata* is known from isolated records in nine counties of the northwestern and central parts of the state (Trauth et al., 2004). Populations in Arkansas are listed as S2 (impaired) by NatureServe (NatureServe, 2015).

Although much is known about the natural history and ecology of North American box turtles (Dodd, 2001), only three coccidian parasites have been reported as follows: *Eimeria carri* Ernst & Forrester, 1973 from *T. carolina carolina* from Alabama and Florida, USA, and *T. mexicana triunguis* (= *T. c. triunguis*) from Arkansas, USA; the ubiquitous *Eimeria mitraria* (Laveran & Mesnil, 1902) from *T. m. triunguis* from Arkansas; and *Eimeria ornata* McAllister & Upton, 1989 from *T. m. triunguis* from Arkansas and Oklahoma and *T. ornata* from Texas, USA (Ernst & Forrester, 1973; McAllister & Upton, 1989; McAllister et al., 1994, 2015; Duszynski & Morrow, 2014). To date, nothing has been published on coccidia from *T. ornata* from Arkansas. The present study provides a description of an eimerian infecting *T. ornata* in Arkansas, USA.

Materials and methods

During May 2014 and again in May 2016, three adult *T. ornata* were collected by hand from Johnson County, Texas (n = 1) and Benton County, Arkansas (n = 2). Fresh fecal samples were placed in individual vials containing 2.5% (w/v) aqueous potassium dichromate (K₂Cr₂O₇). Samples were examined for coccidia using the Olympus BX53 light microscope after flotation in Sheather's sugar solution (specific gravity = 1.30). Measurements were taken on 70 sporulated oocysts from a single turtle using Olympus® cellSens 1.14 digital software and reported in micrometers (µm) with the ranges followed by the means in parentheses; photographs were taken using Nomarski interference-contrast optics. Oocysts of the new species were c.35 days old when measured and photographed. A host photovoucher was accessioned into the Arkansas State University Museum of Zoology (ASUMZ) Herpetological Collection, State University, Arkansas, USA. Photosyntypes of sporulated oocysts were accessioned into the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, Nebraska, USA. Turtle taxonomy follows the reptile database (Uetz & Hošek, 2016).

Results

Two of the three (67%) *T. ornata* were found to be passing coccidian oocysts. One of one (100%) from Texas harboured *Eimeria ornata* and one of two (50%) from Arkansas was infected with an eimerian that we describe here as new.

Eimeria doddi n. sp

Type-host: Ornate box turtle, *Terrapene ornata* (Agassiz, 1857) (Reptilia, Testudines, Emydidae) (adult female, 83 mm carapace length, symbiotype ASUMZ 33573 collected 11.x.2016).

Type-locality: 5 km NE Maysville, Benton County, Arkansas, USA (36.436304°N, 94.558736°W).

Type-material: Photosyntypes of sporulated oocysts are deposited as HWML 102078.

Prevalence: In 1 of 3 (33%) overall; in 1 of 2 (50%) Benton County, Arkansas, USA.

Sporulation time: Unknown; oocysts were already sporulated when received by the senior author.

Site of infection: Unknown; oocysts recovered from faeces.

Etymology: The specific epithet is given in honor of C. Kenneth Dodd, Jr., (University of Florida-Gainesville, Florida, USA), American herpetologist and conservationist for his many works on the natural history and ecology of North American box turtles.

Description (Figs. 1, 2A–D)

Sporulated oocyst: Oocyst ($n = 70$) light to darker brown, smooth, ovoidal to ellipsoidal; $16\text{--}24 \times 11\text{--}16$ (21.1×14.0), length/width (L/W) ratio 1.3–1.8 (1.5). Wall bilayered, c.1.1 thick, inner layer c.0.5, outer layer c.0.6, upper pole wrinkles easily in Sheather's sugar solution. Micropyle and oocyst residuum absent, polar granule present.

Sporocyst: Sporocyst ($n = 146$) four, colourless, smooth, ellipsoidal $8\text{--}12 \times 4\text{--}7$ (9.8×6.1); L/W ratio 1.3–2.3 (1.6); wall single-layered, c.0.4 thick. Indistinct Stieda body present, sub-Stieda and para-Stieda bodies absent; sporocyst residuum formed by small granules of different sizes in a cluster.

Sporozoites: Sporozoites (not measured) two, sausage-shaped; with a spheroidal anterior refractile body and subspheroidal posterior refractile body and one centrally located nucleus.

Remarks—We limit the comparison of our new form only to those eimerian species from box, pond and water turtles of the family Emydidae (see Duszynski & Morrow, 2014). Neither one of the two eimerians (*E. carri* or *E. ornata*) previously described from *Terrapene* spp. box turtles, are at all similar to the new species as they possess L/W ratios of 1.1 (1.0–1.2) and 1.1 (1.0–1.3) vs. 1.5 (1.3–1.8), respectively. Therefore, only a single eimerian, *Eimeria scriptae* Sampson & Ernst, 1969, from the red-eared slider, *Trachemys scripta elegans* (Wied-Neuwied) from an unknown locality in the Mississippi River Valley is remotely similar to the new species; however, it possesses a micropyle as well as having larger oocysts ($24.2 \times 13.7 \mu\text{m}$) and no polar granule, all three morphological characters differ from those of the new species.

Based on the differences in morphological and morphometric characteristics noted in the present study, *E. doddi* is considered a species new to science. Moreover, it is the second eimerian reported from the ornate box turtle as well as the fourth species reported from Arkansas box turtles.

***Eimeria ornata* McAllister & Upton, 1989**

Host: Ornate box turtle, *Terrapene ornata* (Agassiz, 1857) (Reptilia, Testudines, Emydidae) (adult female, 123 mm CL, photovoucher ASUMZ 33578 collected 10.x. 2014).

Locality: Off county road 1120, Charles Russell Ranch, Johnson County, Texas, USA (32° 18' 46.2054"N, 97° 36' 5.8392"W).

Material: Photovouchers of sporulated oöcysts are deposited as HWML 102079.

Prevalence: One of 3 (33%) overall. One of 1 (100%) Johnson County, Texas, USA.

Sporulation time: Endogenous. Oöcysts passed fully sporulated.

Site of infection: Unknown. Oöcysts recovered from feces.

Description (Figs. 2E–H)

Sporulated oöcyst: Oöcyst ($n = 10$) colourless, smooth, subspheroidal to ellipsoidal; 12–16 $\times$ 12–13 (14.2 $\times$ 12.5), length/width (L/W) ratio 1.0–1.2 (1.1). Wall unilayered, $c.1.0$ thick. Micropyle absent but subpheroidal oöcyst residuum (3.4 $\times$ 3.2) and single polar granule present.

Sporocyst: Sporocyst ($n = 10$) four, colourless, smooth, ovoidal, 6–9 $\times$ 4–6 (8.1 $\times$ 4.7); L/W ratio 1.5–2.2 (1.8); thin wall unilayered. Indistinct Stieda body present, nipple-like, sub-Stieda and para-Stieda bodies absent; sporocyst residuum formed by scattered granules of different sizes in a cluster or loose.

Sporozoites: Sporozoites (not measured) two, sausage-shaped; with a spheroidal anterior refractile body and spheroidal posterior refractile body and one centrally located nucleus.

Remarks—It has been over 25 yr since *E. ornata* has been described (McAllister & Upton, 1989) and over 20 yr since it has been reported from additional *T. ornata* from Texas (McAllister et al., 1994). The type locality of *E. ornata* in Texas is Ellis County (McAllister & Upton, 1989) and this site is relatively close (55.5 km [34.5 mi] SSW) to the new Texas locality (Johnson County) reported herein. Interestingly, the measurements of this isolate of *E. ornata* differ in oöcyst and sporocyst size when compared to the original description (Table 1). In addition, oöcysts of *E. ornata* from various three-toed box turtles (*T. m. triunguis*) from Arkansas and Oklahoma show differences in size when compared to oöcysts of our isolate (Table 1). However, all other morphologies are similar and we believe this new isolate represents a smaller morphotype of *E. ornata*.

Discussion

Duszynski & Morrow (2014) summarized the coccidians from turtles of the world. Here, we provide an update with what we believe to be three species known from North American box turtles from Arkansas, Oklahoma and Texas (Table 1).

Together, the states of Arkansas, Oklahoma and Texas support 59 species/subspecies of turtles (Trauth et al., 2004; Sievert & Sievert, 2011; Dixon, 2013) and, although several have been reported to harbor coccidia (see Duszynski & Morrow, 2014), many remain to be surveyed. Indeed, it is very likely that in the course of future surveys we will increase our knowledge of turtle coccidians by reporting new host and geographic distributional records, as well as including the possibility of discovering additional species new to science.

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Fig. 1.
Schematic line drawing of *Eimeria doddi* n. sp. Bar: 5 μ m.

Fig. 2. Nomarski-interference contrast microscopy of sporulated oocysts (A–D) of *Eimeria doddi* n. sp. and *Eimeria ornata* (E–H). (OR) oocyst residuum; (OW) oocyst wall; (PG) polar granule; (SR) sporocyst residuum; (SB) Stieda body. Bars: 10 μm.

Comparison of the sporulated oocysts of three eimerians (except *E. mitraria*) from North American box turtles, *Terrapene* spp.

Table 1

<i>Eimeria</i> spp.	Host (current name) Locality	Oocyst shape, size, features ^{*,†}	Sporocyst shape, size, features ^{*,†}	References
<i>E. curri</i>	<i>Terrapene carolina</i> (Linnaeus) Alabama, Florida, USA	Subspheroidal to ellipsoidal 15.9 × 14.5; L/W 1.1 12–20 × 12–16 M, PG: both – OR: +	Ellipsoidal 11.1 × 5.2; L/W 1.4 10–13 × 4–6 SB, SR: both + SSB, PSB: both –	Ernst & Forrester, 1973
<i>E. doddi</i>	<i>Terrapene ornata</i> (Agassiz) Arkansas, USA	Ovoidal to ellipsoidal 20.6 × 13.8; L/W 1.5 16–23 × 11–16 M, OR: both – PG: +	Ovoidal 9.8 × 6.1; L/W 1.6 (8–12 × 5–7) SB, SR: both + SSB, PSB: both –	This study
<i>E. ornata</i>	<i>T. ornata</i> Texas, USA	Subspheroidal to ellipsoidal 17.9 × 15.7; L/W 1.1 16–21 × 14–18 M: – OR, PG: both +	Elongate 11.1 × 5.4; L/W 2.1 9–13 × 5–6 SB, SR: both + SSB, PSB: both –	McAllister & Upton, 1989
<i>E. ornata</i>	<i>T. ornata</i> Texas, USA	Subspheroidal to ellipsoidal 14.2 × 12.5; L/W 1.1 12–16 × 12–13 M: – OR, PG: both +	Ellipsoidal to elongate 8.1 × 4.7; L/W 1.8 6–9 × 4–6 SB, SR: both + SSB, PSB: both –	This study
<i>E. ornata</i>	<i>Terrapene mexicana</i> (Gray) Arkansas, USA [‡]	Spheroidal to subspheroidal 18.7 × 16.1; L/W 1.2 M: – OR, PG: both +	Elongate 11.1 × 5.3; L/W 2.1 SB, SR: both + SSB, PSB: both –	McAllister et al., 2015
<i>E. ornata</i>	<i>T. mexicana</i> Oklahoma, USA	Spheroidal to subspheroidal 18.2 × 16.2; L/W 1.1 M: – OR, PG: both +	Elongate 11.5 × 5.1; L/W 2.3 SB, SR: both + SSB, PSB: both –	McAllister et al., 2015

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* Measurements in μm .

[†] Descriptions of oöcysts and sporocysts follow guidelines of Wilber et al. (1998) as follows: oöcyst length (L) and width (W), their ranges and ratios (L/W), micropyle (M), oöcyst residuum (OR), polar granule(s) (PG), sporocyst (SP) length (L) and width (W), their ratio (L/W), Stieda body (SB), sub-Stieda body (SSB), para-Stieda body (PSB), and sporocyst residuum (SR).

[‡] Measurements represent averages from four isolates of *E. ornata* from *T. mexicana* (= *T. c. triungius*) from Arkansas (Fulton, Pike and Union cos).

[§] Measurements represent averages from two isolates of *E. ornata* from *T. mexicana* (= *T. c. triungius*) from Oklahoma (McCurtain County).