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A NEW SPECIES OF *CARYOSPORA* (APICOMPLEXA: EIMERIIDAE) FROM THE FLATHEAD SNAKE, *TANTILLA GRACILIS* (OPHIDIA: COLUBRIDAE), IN SOUTHEASTERN OKLAHOMA

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Abstract

A single flathead snake, *Tantilla gracilis*, collected in early October 2010 from Choctaw County, Oklahoma, was found to harbor an undescribed species of *Caryospora*. Oocysts of *Caryospora choctawensis* n. sp. were spherical to subspherical, 15.8×15.0 ($14\text{--}18 \times 14\text{--}16$) μm with a thick bilayered wall and a shape index (length/width) of 1.1. A micropyle and an oocyst residuum are absent but prominent Stieda and bubble-like sub-Stieda bodies were present as well as a bilobed polar granule near the oocyst wall. Sporocysts were ovoidal, 10.8×9.0 ($10\text{--}12 \times 8\text{--}9$) μm with a shape index of 1.3. The sporocyst residuum was spherical and composed of a cluster of granules often membrane-bound. This is the second time a caryosporan species has been reported from *T. gracilis*, but the first coccidian ever described from a reptilian host in Oklahoma. Additional *T. gracilis* from Arkansas (n = 6), Oklahoma (n = 1), and Texas (n = 7) were examined and a single specimen from Newton County, Arkansas, harbored *Caryospora gracilis* Upton, McAllister, Trauth, and Bibb, 1992, previously reported from *T. gracilis* collected in Arkansas and Texas.

Upton et al. (1992) provided descriptions of 2 new species of coccidia (*Caryospora gracilis* and *Isospora wilsoni*) from flathead snakes, *Tantilla gracilis* Baird and Girard, 1853, collected in Arkansas and Texas. Since that time, to our knowledge, no other coccidia have been reported from this snake (see Duszynski and Upton, 2009). Herein, we provide a description of a new species of *Caryospora* from *T. gracilis* from southeastern Oklahoma.

MATERIALS AND METHODS

Between April 2001 and October 2011, 15 adult *T. gracilis* (> 100 mm snout–vent length [SVL]) were collected by hand from various sites in Arkansas (Carroll, Garland, Izard, Logan, Newton, and Union counties, n = 6), Oklahoma (Choctaw County, n = 2), and Texas (Bandera, Edwards, and Kimble counties, n = 7), and examined for coccidia. Specimens were placed in individual plastic bags on ice and returned to the laboratory within 24 hr for processing. Snakes were killed with an overdose of sodium pentobarbital (Nembutal, Eli Lilly, North Chicago, Illinois) solution and a mid-ventral incision was made to expose rectal contents. Fresh feces were collected from the rectum and placed in individual vials containing 2.5% (w/v) aqueous potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) and examined for coccidia by light microscopy after flotation in Sheather's sugar solution (sp. gr. 1.20). Two positive samples were allowed to complete sporulation at room temperature (23 C) in Petri dishes

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containing a shallow layer of 2.5% $K_2Cr_2O_7$ for 5 days and both were sent to 1 of us (RSS) for further examination. Sporulated oocysts were again isolated by flotation (as above) and measurements were taken on 10 oocysts (4 unsporulated oocysts were also measured) using a calibrated ocular micrometer and reported in micrometers (μm) with means followed by the ranges in parentheses; photographs were taken using Nomarski interference-contrast optics. Oocysts were either ~300-days-old (n. sp.) or ~3,650-days-old when measured and photographed. Descriptions of oocysts and sporocysts follow the guidelines of Wilber et al. (1998): oocyst (O) length (L) and width (W), their ranges and ratios (L/W), oocyst wall (OW), micropyle (M), oocyst residuum (OR), polar granules (PG), sporocyst (SP) length (L) and width (W), their ranges and ratio (L/W), Stieda body (SB), sub-Stieda body (SSB), para-Stieda body (PSB), sporocyst residuum (SR), sporozoites (SZ), anterior refractile body (ARB), posterior refractile body (PRB) and nucleus (N). Photovouchers of sporulated oocysts were accessioned into the United States National Parasite Collection (USNPC), Beltsville, Maryland. Voucher specimens of snakes were deposited in the Arkansas State University Museum of Zoology (ASUMZ), State University, Arkansas, as ASUMZ 25982, 25985, and 32625 and the Angelo State University Natural History Collection (ASNHC), San Angelo, Texas, as ASNHC 14133.

RESULTS

Two of 15 (13.3%) *T. gracilis* were found to be passing coccidian oocysts in their feces; 1 (ASUMZ 25982) collected on 20 April 2001 from 6.4 km N of Pruitt off St. Hwy. 7 in Newton County, Arkansas (36.125764°N, 93.124237°W) possessed oocysts (photosyntypes, USNPC 104841) of *Caryospora gracilis* Upton, McAllister, Trauth, and Bibb, 1992 (Fig. 1) previously reported from *T. gracilis* in Arkansas and Texas. Spherical to subspherical oocysts (n = 30) with a moderately pitted wall (Fig. 1) measured 23.4×22.4 (20–25 $\times$ 19–24) μm and ovoidal sporocysts were 16.1×12.2 (15–18 $\times$ 11–14) μm . These measurements, even after 10 yr (~3,650 days) in $K_2Cr_2O_7$ solution and uncontrolled temperatures, accord well with the original description of *C. gracilis* provided by Upton et al. (1992). One of the *T. gracilis* from Choctaw County, Oklahoma, was passing oocysts of an undescribed species of *Caryospora*, described herein as new.

DESCRIPTION

Caryospora choctawensis n. sp

Diagnosis—Oocyst (n = 10) shape spherical to subspherical L $\times$ W 15.8×15.0 (14–18 $\times$ 14–16); L/W 1.1 (1.0–1.2); OW thick and smooth, bilayered. M and OR absent but prominent SB and bubble-like SSB present, bilobed PG near OW. SP ovoidal, L $\times$ W 10.8×9.0 (10–12 $\times$ 8–9); L/W 1.2 (1.0–1.5). SR present, with many granules in compact mass. SZ elongate, arranged head-to-tail within SP. Each SZ with centrally located, spherical to ellipsoidal ARB and spherical, rarely subspherical, PRB. N located between RB. Unsporulated oocysts (n = 4) slightly larger, 16.4×16.0 (16–17 $\times$ 15–17); L/W 1.0 (1.0–1.1).

Taxonomic summary

Type host: *Tantilla gracilis* Baird and Girard, 1853, flathead snake, adult male (SVL = 122 mm), collected on 9 October 2010 by C. T. McAllister. Symbiotype deposited as ASUMZ 31625.

Other hosts: None.

Secondary host: Unknown.

Type specimens: Phototype deposited as USNPC 104842.

Type locality: USA: Oklahoma, Choctaw County, Fort Towson Historic site Post Cemetery off US 70 on county road E2060 (34.025801°N, 95.258803°W).

Prevalence: Found in 1 of 15 (6.7%) of the snakes examined; 1/2 (50%) Choctaw County, Oklahoma; 0/6 (0%) Arkansas; 0/7 (0%) Texas.

Sporulation: Exogenous. All oocysts were passed unsporulated or partially sporulated and some became fully sporulated within 5 days at ca. 23 °C; others never became fully sporulated.

Prepatent and patent periods: Unknown.

Site of infection, definitive host: Unknown. Oocysts recovered from rectal contents and feces.

Site of infection, secondary host: Unknown.

Endogenous stages, definitive host: Unknown.

Endogenous stages, secondary host: Unknown.

Cross-transmission: None.

Pathology, definitive host: Unknown.

Pathology, secondary host: Unknown.

Etymology: The specific epithet reflects the county of collection of the host (*-ensis*) and the Choctaw Nation of Oklahoma. It is given in honor of a tribe of farmers who lived in what is now the southeastern United States until 1830 when most tribal members were forcibly moved to southeastern Oklahoma in “the Trail of Tears.”

Remarks

It has been 2 decades since Upton and associates described *Caryospora gracilis* from *T. gracilis* collected in Hood County, Texas, and Crawford County, Arkansas (Upton et al., 1992). Measurements of oocysts of our isolate of *C. gracilis* reported herein and those reported in the original description of *C. gracilis* (Upton et al., 1992) are considerably larger than those of the new species (23.4–23.7 × 22.4–22.5 vs. 15.8 × 15.0 μm) and *C. gracilis* also possesses an OR, a structure found in no other caryosporan species from snakes (see Duszynski and Upton, 2009), as well as a very large SR. Interestingly, 2 other caryosporans, *C. relictæ* Telford, 1997, and *C. tantillæ* Telford, 1997, are known from Florida or Peninsula crowned snakes, *Tantilla relictæ* Telford, 1966, collected from Marion County, Florida (Telford, 1997). However, they differ from *C. choctawensis* as follows: both the oocysts and sporocysts of *C. relictæ* (OL × OW: 18.5 × 17.6 μm; SL × SW: 14.8 × 11.4 μm) and *C. tantillæ* (OL × OW: 19.6 × 18.9 μm; SL × SW: 15.1 × 11.6 μm) are considerably larger. In addition, the recently described *Caryospora durelli* Daszak, Ball, Streicker, Jones, and Snow, 2011 from Round Island boas, *Casarea dussumieri* (Schlegel) from Mauritius also possess considerably larger oocysts and sporocysts (OL × OW: 19.2 × 18.2 μm; SL × SW: 14.7 × 11.0 μm) and differ from *T. gracilis* in host systematics and geographical distribution as well. To our knowledge, *C. choctawensis* possesses the smallest oocysts of any caryosporan described to date from North American snakes (Duszynski and Upton, 2009).

DISCUSSION

We provide additional measurements and a photomicrograph of a second isolate of *C. gracilis*. Although several coccidians, including *Caryospora* spp. have been described or reported from snakes collected from states bordering Oklahoma, i.e., Arkansas and Texas (Upton et al. 1992, 1994; McAllister and Upton, 1995; Duszynski and Upton, 2009), we know of no coccidia whose new species description has been based on any snake collected in Oklahoma. Therefore, we provide the first description of a coccidian species from a common snake found in the state. At present, at least 60 species and subspecies of snakes are found in Oklahoma (Sievert and Sievert, 2011) and many remain to be examined for coccidia. To our knowledge, the only snakes thus far reported to harbor coccidia from Oklahoma include the prairie kingsnake, *Lampropeltis calligaster calligaster* and great plains ratsnake, *Pantherophis emoryi* (both species having *Caryospora duszynskii* Upton, Current and Barnard, 1984) and rough earth snake, *Virginia striatula* (with *Eimeria striatula* Upton and McAllister, 1990) (McAllister and Upton, 1995, their Table I; McAllister et al., 2011). Further surveys on coccidians from snakes of Oklahoma are certainly warranted and additional species will undoubtedly be reported and described in the future.

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Figures 1–3.

Coccidians from *Tantilla gracilis*. (1) Nomarski interference-contrast photomicrograph of an oocyst of *Caryospora gracilis* Upton, McAllister, Trauth, and Bibb, 1992. Abbreviation: OW (oocyst wall). (2–3) Nomarski interference-contrast photomicrographs of oocysts of *Caryospora choctawensis* n. sp. Abbreviations: OW (oocyst wall); PG (polar granule); SB (Stieda body); SSB (sub-Stieda body).

Figure 4.
Composite line drawing of an oocyst of *C. choctawensis* n. sp.