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A new coccidian (Apicomplexa: Eimeriidae), from midland brown snake, *Storeria dekayi wrightorum* (Ophidia: Colubridae) from Arkansas, USA

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Abstract

A new species of coccidian (Protista: Apicomplexa: Eimeriidae) collected from a midland brown snake, *Storeria dekayi wrightorum* from Arkansas, USA, is described. Oocysts of *Isospora holbrooki* n. sp. are subspherical to ovoidal with a smooth, colourless, bi-layered wall, measure $27.1 \times 24.0 \mu\text{m}$, and have a length/width (L/W) ratio of 1.1; both micropyle and oöcyst residuum are absent, but a polar granule is present. Sporocysts are ovoidal, $14.8 \times 10.0 \mu\text{m}$, L/W 1.5; the Stieda body is nipple-like, the sub-Stieda body is ellipsoidal and the sporocyst residuum is composed of coarse granules in a cluster. Sporozoites have a spheroidal anterior refractile body, a subspheroidal posterior refractile body, and one centrally-located nucleus. This is the first description of an isosporan from the snake genus *Storeria* as well as the largest oocysts and sporocysts of any previous snake isosporan to date.

Introduction

The midland brown snake, *Storeria dekayi wrightorum* is a small colubrid snake that ranges from eastern Wisconsin and the upper peninsula of Michigan to the Carolinas and south to the Gulf Coast (Conant & Collins, 1998). In Arkansas, midland brown snakes occur throughout most of the state where they are found beneath rocks and logs on moist forest litter, particularly in ravines, on hillsides, in river flood plains and even in disturbed areas littered with trash and in flowerbeds inhabited by earthworms (Trauth et al., 2004; Sievert & Sievert, 2011). This fossorial snake feeds primarily on earthworms with slugs and soft-bodied arthropods also being taken (Ernst & Barbour, 1989; Johnson, 2000). Although much is known about the natural history of *S. d. wrightorum* (Christman, 1982; Ernst & Barbour, 1989), a few papers have been published on its helminth parasites (see Ernst and Ernst, 2006), but very little is known about the coccidia (Duszynski & Upton, 2009) of this snake. Wacha & Christiansen (1974, 1975 [abstract]) examined 11 and 16 Texas brown snakes (*Storeria dekayi texana*) from Iowa and none were infected with coccidia, and a single *S. d. texana* from Texas was examined by Upton & McAllister (1990) but was not found to be

passing oöcysts. However, Graczyk et al. (1996) reported a *Cryptosporidium* sp. from the stomach of *S. dekayi*. The present study provides a description of an isosporan coccidian infecting a *S. d. wrightorum* in Arkansas, USA.

Materials and methods

Between November 2011 and November 2014, four adult *S. d. wrightorum* (range 145–148 mm snout-vent length [SVL]) were collected by hand from Searcy ($n = 1$) and Union ($n = 2$) counties, Arkansas, and Atoka County ($n = 1$) Oklahoma, and examined for coccidia. Fecal samples from the rectum were placed in individual vials containing 2.5% (w/v) aqueous potassium dichromate ($K_2Cr_2O_7$). Samples were examined for coccidia by light microscopy after flotation in Sheather's sugar solution (specific gravity = 1.30). Measurements were taken on 10 sporulated oöcysts from a single snake using a calibrated ocular micrometer or Olympus® cellSens 1.7 digital software and reported in micrometers (μm) with the ranges followed by the means in parentheses; photographs were taken using Nomarski interference-contrast optics. Oöcysts were $c.225$ days old when measured and photographed. A host voucher was accessioned into the Arkansas State University Museum of Zoology (ASUMZ) Herpetological Collection, State University, Arkansas, USA. Photosyntypes of sporulated oöcysts were accessioned into the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, Nebraska, USA.

Results

One of the four (25%) *S. d. wrightorum* were found to be passing coccidian oöcysts that we describe here as new.

Isospora holbrooki n. sp

Type-host—Midland brown snake, *Storeria dekayi wrightorum* Trapido, 1944 (Reptilia, Ophidia, Colubridae) (adult male, 146 mm SVL, symbiotype ASUMZ 33362 collected 4.xi. 2014).

Type-locality—El Dorado, Arkansas, USA (33° 12' 32.439"N, 92° 35' 24.6696"W).

Type-material—Photosyntypes of sporulated oocysts are deposited as HWML 101833.

Prevalence—1 of 4 (25%).

Sporulation time—Unknown. Oöcysts were already sporulated when received by the senior author.

Site of infection—Unknown. Oöcysts recovered from feces.

Etymology—The specific epithet is given in honor of Dr. John Edwards Holbrook (1796–1871), American zoologist, naturalist, physician, and considered the father of American herpetology. In addition to describing the host species (*S. dekayi*), he provided the

first comprehensive illustrated account of North American amphibians and reptiles in the two editions of his *North American Herpetology*.

Description (Figs. 1-2)

Sporulated o cyst

O cyst shape ($n = 10$) colourless, smooth, subspheroidal to ovoidal; $25\text{--}30 \times 20\text{--}29$ (27.1×24.0), length/width (L/W) ratio 1.0–1.3 (1.1). Wall bilayered, *c.* 1.1 thick, inner layer *c.* 0.4, outer layer *c.* 0.7. Micropyle and oöcyst residuum absent, polar granule present.

Sporocyst

Sporocyst ($n = 10$) two, colourless, smooth, ovoidal, $13\text{--}18 \times 9\text{--}11$ (15.4×10.0); L/W ratio 1.4–1.7 (1.5); wall single-layered, *c.* 0.4 thick. Prominent Stieda body present, nipple-like, sub-Stieda body present, ellipsoidal, para-Stieda body absent; sporocyst residuum formed by coarse granules of different sizes in a cluster.

Sporozoites

Sporozoites (not measured) 4, 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 isosporan species previously described from snakes of the family Colubridae. In an abstract, Wacha & Christiansen (1975) reported an unnamed isosporan with ovoidal to ellipsoidal o cysts measuring 10.9 ($10\text{--}11$) $\times$ 8.5 ($8\text{--}9$) μm from a group of colubrid snakes, including glossy snake (*Arizona elegans*), North American racer (*Coluber constrictor*), and gopher snake (*Pituophis catenifer*); however, these o cysts are more than 2.5-fold smaller than the new species. We suspect this isosporan is more likely a species from lizards or birds that were eaten by, and just passing through, the digestive system of these predatory snakes. In addition, the new species differs significantly in dimensions to all other isosporans described from colubrid snakes as follows: *Isospora wilsoni* Upton, McAllister, Trauth & Bibb, 1992 from the flathead snake, *Tantilla gracilis* from Arkansas, USA possesses subspheroidal o cysts measuring 17.2×17.0 ($15\text{--}19 \times 15\text{--}19$) μm , significantly smaller than *I. holbrooki* (Upton et al., 1992); *Isospora cenchoae* Asmundsson, Upton & Freed, 2001 from the Amazon basin tree snake, *Imantodes lentiferus* from Ecuador also has smaller o cysts, 21.6×15.0 ($20\text{--}23 \times 14\text{--}16$) (Asmundsson et al. 2001). The only similarly-sized species is *Isospora kiamichiensis* McAllister, Seville & Connior, 2015, from the eastern coachwhip, *Coluber flagellum* from Oklahoma, USA (McAllister et al., 2015a); however, it does not possess a polar granule (the new species does) and oöcysts are smaller 25.0×22.2 ($20\text{--}28 \times 19\text{--}24$) μm . Therefore, none of the previous isosporans from colubrid snakes has been described with a combination of morphological characters descriptive of the new species.

Based on the differences in morphological and morphometric characteristics noted in the present study, *I. holbrooki* is considered a species new to science. Moreover, it is the first isosporan reported in the Midland brown snake, *S. d. wrightorum*.

Discussion

Duszynski & Upton (2009) summarized the isosporans from snakes of the world. Here, we provide an update with what we believe to be seven valid isosporans (Table 1) and agree with these authors that some of those former isosporans from snakes should be considered *species inquirenda*. They include *Isospora guzarica* Davronov, 1985, from the spotted desert racer, *Platyceps* (= *Coluber*) *karelini* from Republic of Uzbekistan (Davronov, 1985), which is thought to be a *Sarcocystis* sp. (Upton et al., 1992; Duszynski & Upton, 2009), *Isospora neivai* Pinto and Maciel 1929 from the jaracaca, *Bothrops jaracaca* from Brazil (Pinto & Maciel, 1929) and *Isospora guersae* Yakimoff & Matschoulsky, 1937 from the Levantine viper, *Macrovipera lebetina* from Uzbekistan (Yakimoff & Matschoulsky, 1937; Duszynski & Upton, 2009).

The state of Arkansas supports 45 species/subspecies of snakes (Trauth et al., 2004) and, although several have been reported to harbor coccidia (see Duszynski & Upton, 2009; McAllister et al., 2011, 2015a, b), many remain to be surveyed. Indeed, it is very likely that additional species will be discovered in the course of future surveys, including those new to science.

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Fig. 1.
Schematic line drawing of *Isospora holbrooki* n. sp. Bar: 5 μ m.

Fig. 2. Nomarski-interference contrast microscopy of sporulated oocysts (A–C) of *Isospora holbrooki* n. sp. Arrowhead indicates bilayered oocyst wall; (arb) anterior refractile body; (n) nucleus; (pg) polar granule; (prb) posterior refractile body; (s) Stieda body; (ss) sub-Stieda body. Bars: 10 μ m.

Table 1

Comparison of the sporulated oöcysts of valid isosporans from snakes of the world

<i>Isopora</i> spp.	Type host (current name) Locality	Oöcyst shape, size, features* [†]	Sporocyst shape, size, features* [†]	References
<i>I. cenchoae</i>	<i>Imantodes lentiferus</i> Ecuador	Ovoidal to ellipsoidal 21.6 × 15.0; L/W 1.4 20–23 × 14–16 M, OR: both – PG: +	Ovoidal 11.5 × 8.3; L/W 1.4 10–13 × 8–9 SB, SSB, SR: all +	Asmundsson et al., 2001
<i>I. colubris</i>	<i>Hierophis viridiflavus</i> Italy	Spheroidal to subspheroidal 17.1 (15–20); L/W 1.0 M, OR, PG: all –	Lemon-shaped 11.3 × 7.9; L/W 1.4 (11–12 × 7–8) SB, SSB, SR: all +	Matuschka, 1986
<i>I. dipsasi</i>	<i>Dipsas vermiculata</i> Ecuador	Spheroidal 15.0 (13–18); L/W 1.0 M, OR: both – PG: +	Lemon-shaped 11.2 × 7.8; L/W 1.4 10–12 × 7–9 SB, SSB, SR: all +	Asmundsson et al., 2001
<i>I. holbrooki</i>	<i>Storeria dekayi wrightorum</i> Arkansas, USA	Subspheroidal to ovoidal 27.1 × 24.0; L/W 1.1 25–30 × 20–29 M, OR: both – PG: +	Ovoidal 15.4 × 10.0; L/W 1.5 13–18 × 9–11 SB, SSB, SR: all +	This study
<i>I. kiamichiensis</i>	<i>Coluber flagellum flagellum</i> Oklahoma, USA	Spheroidal to subspheroidal 25.0 × 22.2; L/W 1.1 20–28 × 19–24 M, OR, PG: all –	Ovoidal 13.9 × 9.4; L/W 1.5 11–16 × 8–10 SB, SSB, SR: all +	McAllister et al., 2015a
<i>I. natricis</i>	<i>Natrix natrix</i> Uzbekistan	Spheroidal 14.4 (12–16); L/W 1.0 M, OR, PG: all –	Ovoidal 10.8 × 5.4 SB, SSB, SR: all –	Yakimoff & Gousseff, 1935
<i>I. wilsoni</i>	<i>Tantilla gracilis</i> Arkansas, USA	Spheroidal to subspheroidal 17.2 × 17.0; L/W 1.0 15–19 × 15–19 M, OR, PG: all –	Ovoidal 10.9 × 8.4; L/W 1.3 6.5–9 × 4–7 SB, SSB, SR: all +	Upton et al., 1992

* 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), and sporocyst residuum (SR).