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A New Host for *Caryospora lampropeltis* (Apicomplexa: Eimeriidae) from the Eastern Hognose Snake, *Heterodon platirhinos* (Ophidia: Colubroidea: Dipsadinae), from Arkansas, U.S.A., with a Summary of Hosts of This Coccidian

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Abstract

Between May 2012 and July 2013, four eastern hognose snakes (*Heterodon platirhinos*) were collected from Arkansas ($n = 2$) and Oklahoma ($n = 2$), U.S.A., and examined for coccidians. A single *H. platirhinos* from Arkansas was found to be passing oocysts of *Caryospora lampropeltis* Anderson, Duszynski, and Marquardt. Oocysts of *C. lampropeltis* were spheroidal to slightly subspheroidal with a rough, colourless, bi-layered wall, measure $23.5 \times 22.8 \mu\text{m}$, and have a length/width (L/W) ratio of 1.0; both micropyle and oocyst residuum were absent, but a prominent polar granule was present. Sporocysts are ovoidal, $16.8 \times 12.8 \mu\text{m}$, L/W 1.3; a prominent Stieda and subStieda body was present; a sporocyst residuum was present and composed of numerous spheroidal granules dispersed into small and large granules. Sporozoites lie lengthwise and parallel in a semi-spiral in sporocyst; a spheroidal anterior refractile and posterior refractile body is present; a single nucleus is located between the 2 refractile bodies. This represents the first report of a caryosporan reported from *H. platirhinos* as well as the only known coccidian from this host. A summary of hosts of *C. lampropeltis* is provided.

Keywords

Arkansas; *Heterodon platirhinos*; eastern hognose snake; Apicomplexa; Ophidia; *Caryospora lampropeltis*; coccidian

The eastern hognose snake, *Heterodon platirhinos* Latreille is a medium-sized rear-fanged reptile that is found in a variety of terrestrial habitats in the United States from extreme New Hampshire south to Florida and west to Minnesota, South Dakota, Kansas and Texas (Conant and Collins, 1998). This snake feeds primarily on toads and frogs (and rarely

salamanders) but will also take insects (Trauth, 1982; Ernst & Barbour, 1989; Trauth & McAllister, 1995). Hognose snakes are known for feigning death or “playing dead” and as a result of their bluffing behaviors have earned colloquial names such as “hissing adder”, “blow viper”, “spreading adder”, “hissing sand snake”, and “puff adder.”

Although much is known about the ecology (Blem, 1981) and helminth parasites of *H. platirhinos* (see Ernst and Ernst, 2006), nothing, to our knowledge, is known about coccidia of this snake (see Duszynski and Upton, 2009). Upton and McAllister (1990) examined a single *H. platirhinos* from Texas and did not find coccidia. Daszak et al. (2011) recently reported a new eimerian from the related western hognose snake, *Heterodon nasicus* Baird and Girard from Texas. Here, we provide additional information on *Caryospora lampropeltis* Anderson, Duszynski, and Marquardt, reported for the first time from *H. platirhinos*, and a summary of previous hosts of this coccidian.

Between May 2012 and July 2013, 4 *H. platirhinos* were collected by hand from Izard and Ouachita counties, Arkansas ($n = 2$) and Latimer and McCurtain counties, Oklahoma ($n = 2$) and examined for coccidia. Specimens were placed in individual collection bags on ice and following the guidelines for the humane treatment of research animals (HACC, 2004) they were killed with an overdose of concentrated tricaine methanesulfonate (TMS-222) and a mid-ventral incision was made to expose intestinal contents. Fecal samples from the rectum were placed in individual vials containing 2.5% (w/v) aqueous potassium dichromate ($K_2Cr_2O_7$) and examined for coccidia by light microscopy after flotation in Sheather's sugar solution (specific gravity = 1.30). Negative samples were discarded and a single positive sample with unsporulated to partially sporulated oocysts was allowed 1 wk to sporulate at room temperature (ca. 23°C) in a Petri dish containing a thin layer of 2.5% (w/v) $K_2Cr_2O_7$. This sample, after flotation, was examined again using a compound microscope with Nomarski interference-contrast (DIC) optics. Measurements and photographs were taken on 13 sporulated oocysts using Olympus cellSens® software. Measurements are reported in micrometers (μm) with means followed by the ranges in parentheses. Oöcysts were ca. 240 days old when measured and photographed. Descriptions of oocysts and sporocysts follow guidelines of Wilber et al. (1998). Host vouchers were accessioned into the Arkansas State University Museum of Zoology (ASUMZ), Herpetological Collection, State University, Arkansas, USA. Photovouchers of sporulated oocysts were accessioned into the United States National Parasite Collection (USNPC), Beltsville, Maryland, USA. Snake taxonomy follows Burbrink (2001), Pyron et al. (2011) and the TIGR reptile database (Uetz and Hošek, 2013).

One of 4 (25%) *H. platirhinos* was found to be passing oocysts of *C. lampropeltis*; the description follows.

Apicomplexa: Eimeriidae

Caryospora lampropeltis

Anderson, Duszynski, and Marquardt, 1968 (Figs. 1–2)

Description of oocysts, sporocysts, and sporozoites

Oocyst ($n = 13$) colorless, spheroidal to slightly subspheroidal, 23.5×22.8 ($21\text{--}26 \times 20\text{--}26$); length/width (L/W) ratio 1.0 (1.0–1.1). Wall bi-layered (ca. 1.5); finely-pitted outer layer, ca. 0.8–1.1, inner layer is ca. 0.4–0.7. M, OR absent; PG present. SP ($n = 13$) 1, colorless, smooth, ovoidal, 16.8×12.8 ($16\text{--}18 \times 11\text{--}14$); L/W ratio 1.3 (1.2–1.5); wall single-layered. Prominent SB and SSB body present, PSB body absent; SR composed of numerous spheroidal granules dispersed into small and large granules between SZ. SZ (not measured) 8, lying lengthwise and parallel in a semi-spiral in SP; single spheroidal ARB and spheroidal PRB, with single N located between them.

Host and locality

Eastern hognose snake, *Heterodon platirhinos* Latreille in Sonnini & Latreille, 1801 (Ophidia: Dipsadidae), ASUMZ 32373, collected on 25 October 2012 from 5.5 km SW of Chidester at junction of St. Hwys. 57 and 76, Ouachita County, Arkansas, U.S.A. ($33^{\circ}39'51.1''\text{N}$, $93^{\circ}03'29.52''\text{W}$).

Prevalence

1/4 (25%).

Additional Arkansas records

Red milk snake, *Lampropeltis triangulum sypila* (Cope, 1889), Lee County (McAllister et al., 1995).

Type host and locality

Prairie kingsnake, *Lampropeltis calligaster calligaster* (Harlan, 1827), Jackson County, Illinois (Anderson et al., 1968).

Other reported hosts

See Table 1.

Geographic range

See Table 1.

Specimens deposited

USNPC 107228 (photo voucher).

Sporulation

Oocysts were passed unsporulated or partially sporulated in feces and were fully sporulated after 1 wk in 2.5% (w/v) $\text{K}_2\text{Cr}_2\text{O}_7$ at room temperature (ca. 23°C).

Site of infection, definitive host

Unknown. Oocysts were recovered from feces.

Remarks

We did observe some morphological difference in the nature of the SR granules between our isolate and those reported in *C. lampropeltis* by Anderson et al. (1968); however, granules can potentially change over time and there is no record of the time period between sample collection and photography/morphometry of coccidia in the original description. All measurements of our isolate of *C. lampropeltis* compare well with those of Anderson et al. (1968), both morphologically and morphometrically. In addition, *C. lampropeltis* and our isolate occur in snake hosts in the same geographic region (i.e., Arkansas, U.S.A.). The only other similar species are *Caryospora guatemalensis* Seville, Asmundsson, and Campbell, 2005 from a scarlet kingsnake, *Lampropeltis triangulum* from Guatemala (Seville et al., 2005) and *Caryospora duszynskii* Upton, Current and Barnard, 1984 from various colubrid snakes (*Coluber* [= *Masticophis*] *flagellum*, *Lampropeltis* spp., *Pantherophis* spp.) in Arkansas, Florida, Georgia, Missouri, Oklahoma and Texas (Upton et al., 1984; Mod y et al., 2005; McAllister et al., 2011). Compared to *C. lampropeltis*, the former species differs in having a heavily-pitted oocyst wall and the latter species possesses larger oocysts. Based on the above similarities, we conclude that our isolate is indeed *C. lampropeltis*. Perhaps the use of molecular tools, rather than relying on morphology alone, could help elucidate whether coccidians found among various snakes are truly the same species or represent cryptic species in separate hosts (Williams et al., 2010).

Interestingly, Wacha and Christiansen (1974), in a survey of 117 colubrid snakes from Iowa that included some potential hosts of *C. lampropeltis*, did not find any to be passing this coccidian. In addition, we have recently examined 35 additional snakes, including those previously reported as hosts of *C. lampropeltis* (see Table 1) from Arkansas, Oklahoma and Texas, and have not yet found any to be passing *C. lampropeltis*. This coccidian may simply exhibit a low prevalence of infection and, therefore, is challenging to find select snakes passing this species. Additional study is suggested using larger sample sizes to help answer this and other information about the caryosporans of snakes.

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Figures 1–2.

DIC photomicrographs of oocysts of *Caryospora lampropeltis*. **1.** Oocyst showing polar granule (PG), Stieda body (SB), and sub-Stieda body (SSB). **2.** Oocyst showing finely-pitted outer oocyst wall (OW), sporocyst (SP), and sporocyst residuum (SR). Scale bars = 10 μ m.

Table 1

Previous hosts, localities, and select measurements for oocysts and sporocysts of *Caryospora lampropeltis*.

Host	Locality	Oocysts (L × W) μm	Sporocysts (L × W) μm	Reference
<i>Heterodon platirhinos</i>	Arkansas	23.5 × 22.8 (21–26 × 20–26)	16.8 × 12.8 (16–18 × 11–14)	This study
<i>Lampropeltis c. calligaster</i> [*]	Illinois	23.3 (20–25)	17.1 × 12.3 (15–19 × 11–13)	Anderson et al. (1968)
<i>Lampropeltis holbrooki</i> [†]	Texas	–	–	McAllister et al. (1995)
<i>Lampropeltis triangulum sypila</i>	Arkansas	–	–	McAllister et al. (1995)

^{*} Type host.

[†] This host was originally reported as *Lampropeltis getula splendida*.