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# *Caryospora duszynskii* (Apicomplexa: Eimeriidae) from the Speckled Kingsnake, *Lampropeltis holbrooki* (Reptilia: Ophidia), in Arkansas, with a Summary of Previous Reports

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The speckled kingsnake, *Lampropeltis holbrooki* Stejneger (*=L. getula holbrooki*) is a medium sized colubrid that ranges from southern Iowa south through Missouri, Arkansas, western Tennessee, eastern Oklahoma, eastern Texas, Mississippi, and Louisiana to the Gulf of Mexico (Conant and Collins 1998). In Arkansas, *L. holbrooki* can be found statewide where it inhabits forested woodlands and rocky hillsides in the Interior Highlands (Ozark and Ouachita mountains) to floodplains and swampy wetlands in the Gulf Coastal Plain (Trauth et al. 2004).

Much is known about the ecology of this snake (see Trauth et al. 2004); however, less is known about its coccidian parasites. Fully sporulated oocysts and free sporocysts of Sarcocystis montanaensis Dubey were reported in a naturally infected L. holbrooki from Benton County, Arkansas by Lindsay et al. (1992) where they determined this snake species was the definitive host in a previously unknown speckled kingsnake-prairie vole (Microtus ochrogaster) life cycle. However, after carefully examining the same isolate, Duszynski and Upton (2009) found minor differences in sporocyst size and in the primary sarcocyst wall and named it as a new species, Sarcocystis lampropeltii. In addition, Eimeria zamenis Phisalix has been reported from L. holbrooki in Illinois and Iowa (see Duszynski and Upton 2009). Herein, we document a new host record for another coccidian parasite of L. holbrooki as well as a summary of hosts of this coccidian.

Between March 2010-August 2011, 11 adult colubrid snakes, including 2 southern black racers, *Coluber constrictor priapus* from Polk County, 2 western ratsnakes, *Scotophis obsoletus* from Pike and Sevier counties, 1 prairie kingsnake, *Lampropeltis calligaster calligaster* from Hot Spring County, 2 *L. holbrooki* from Franklin and Pope counties, 1 Great Plains ratsnake, *Pantherophis* (*=Elaphe*) *emoryi* from

Pope County, Arkansas, 1 Great Plains rat snake, Pantherophis emoryi from McCurtain County, Oklahoma, and 2 Texas patchnose snakes, Salvadora grahamiae lineata from Johnson County, Texas were collected by hand and examined for coccidian parasites. Snakes were killed with an overdose of sodium pentobarbital (Nembutal®) and a mid-ventral incision was made to expose fecal contents. Feces was collected and placed in individual vials containing 2.5% (w/v) aqueous potassium dichromate ( $K_2Cr_2O_7$ ) and examined by light microscopy following flotation in Sheather's sugar solution (specific gravity = 1.30). Negative samples were discarded and a single positive sample with unsporulated oocysts was allowed 1 week of sporulation at room temperature (ca. 23°C) in a Petri dish containing a thin layer of 2.5% K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>. This sample was shipped to R.S. Seville and oocysts were concentrated with Sheather's sugar solution (sp. gr. 1.30) and examined using a compound microscope equipped with Nomarski interference-contrast (DIC) Thirty-six oocysts were photographed and optics. measured using Olympus Microsuite<sup>®</sup> software. Measurements are reported in micrometers (um) with means followed by the ranges in parentheses. Oocysts were ca. 71 days old when measured and photographed. Standardized abbreviations for characteristics of oocysts and sporocysts are per Wilber et al. (1998) as follows: oocyst length (L) and width (W), their ranges and ratios (L/W), micropyle (M), oocyst residuum (OR), polar granules (PG), sporocyst length (L) and width (W), their ranges and ratio (L/W), Stieda body (SB), substieda body (SSB), parastieda body (PSB), and sporocyst residuum (SR). Α photovoucher of a sporulated oocyst (Fig. 1) was accessioned into the United States National Parasite Collection, Beltsville, Maryland as USNPC 104376. A host voucher specimen was deposited in the Henderson State University Herpetology Collection (HSU),

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Arkadelphia, Arkansas as HSU 1517. Host taxonomy follows Collins and Taggart (2008, 2009), Pyron and Burbrink (2009) or the Reptile Database (Uetz 2011).

One of 11 (9%) of the snakes was infected with coccidia. A single L. holbrooki (female, 472 mm snout-vent length) collected on 23 April 2010 from 3.2 km S of Cass off St. Hwy 23. Franklin County (35.587387°N, 92.852596°W) was found to be passing oocysts of a coccidian fitting the description of Carvospora duszynskii Upton, Current and Barnard, 1). Oocysts were spheroidal 1984 (Fig. to subspheroidal,  $L \times W = 24.9 \times 23.3$  (22.0-27.5 × 21.2-25.6), L/W ratio 1.1 (1.0-1.1), PG present, oocyst wall bilayered, ~ 1.9 (1.7-2.2), rough outer 2/3 thickness with no OR or M; sporocysts were ovoidal,  $L \times W =$ 17.7 × 12.9 (15.4-19.2 × 11.5-13.9), L/W ratio 1.4 (1.3-1.5), SB and SSB prominent, PSB absent, SR composed of numerous spheroidal granules dispersed into small and large granules. No gross pathology was observed in this host.

*Caryospora duszynskii* was originally described from the eastern corn snake, *Pantherophis* (=*Elaphe*) *guttatus* from Georgia (Upton et al. 1984). Since then the species has been found in other North American colubrid snakes, including those in the genera *Lampropeltis*, *Masticophis*, *Pantherophis* and *Scotophis* (Table 1; Arkansas State University Museum of Zoology = ASUMZ). Upton et al. (1984) provided the first published photomicrograph and line drawing of an oocyst of *C. duszynskii*, which compare favorably to oocysts we describe herein (Figs. 1-2). We did observe some minor differences in measurements between the two isolates (Table 2), but all other morphological features were essentially the same. Perhaps the use of molecular tools, rather than relying on morphology alone, could help elucidate whether coccidians found are truly the same species or represent cryptic species in separate host species (Williams et al. 2010).

Modrý et al. (2005) recently demonstrated that mice (*Mus musculus*) are capable of indirectly transmitting infections of *C. duszynskii* to uninfected snakes (*P. guttatus* and *S. obsoletus*). Since speckled kingsnakes and other hosts of *C. duszynskii* primarily eat rodents (Green 1997), this finding may be an integral part of the natural history of these hosts. In addition, Modrý et al. (2005) demonstrated the direct transmission of *C. duszynskii* from *P. guttatus* to *P. obsoletus*. Interestingly, *L. holbrooki* in Arkansas has been shown to eat other reptiles (including hosts of *C. duszynskii*) and their eggs (Trauth and McAllister 1995). Additional studies are suggested to investigate this ecological phenomenon in other Arkansas snakes.

Table 1.	Seven	known	hosts	of	Caryospo	ora duszy	nskii.
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Host	State	Prevalence <sup>1</sup>	Reference
Pantherophis guttatus	Georgia	1/1 (100%)	Upton et al. (1984)
	Florida	2/3 (67%)	Modrý et al. (2005)
P. emoryi	Oklahoma <sup>2</sup> ; Texas <sup>3</sup>	2/2 (100%); 2/8 (25%)	McAllister (1989); McAllister et al. (1995) ; McAllister and Upton (pers. obs.)
Scotophis obsoletus	Missouri	1/1 (100%)	Upton et al. (1984)
	Texas	1/4 (25%)	McAllister (1989); McAllister et al. (1995)
Lampropeltis calligaster calligaster	Arkansas <sup>4</sup> ; Oklahoma	2/2 (100%); 1/1(100%)	McAllister et al. (1995) McAllister and Upton (pers. obs.)
L. holbrooki <sup>5</sup>	Arkansas	1/2 (50%)	This report
L. triangulum syspila <sup>5</sup>	Arkansas <sup>6</sup>	1/6 (17%)	McAllister and Upton (pers. obs.)
Masticophis flagellum flagellum	Arkansas	1/3 (33%)	Upton et al. (1994)

<sup>1</sup>Prevalence in collected samples = number infected/number examined (percent); prevalence values may not represent reality as larger sample sizes may yield more relevant prevalence.

<sup>2</sup>Collected on 29 September 1992 from Greer County, Oklahoma (ASUMZ 18601).

<sup>3</sup>Collected on 26 April 1991 from Jim Hogg County, Texas (host released).

<sup>4</sup>Collected on 29 June 1993 from Conway County, Arkansas (ASUMZ 19104).

<sup>5</sup>New host record.

<sup>6</sup>Collected on 30 June 1992 from Lee County, Arkansas (ASUMZ 18524); mixed infection with *Caryospora lampropeltis*.

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Table 2. Selected compara	ive measurements for (	3 isolates of	C. duszvnskii.
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Host	Oocysts <sup>1</sup>	Sporocysts <sup>2</sup>	Reference	
	$L \times w$ (range) $\mu m$	$L \times W$ (range) $\mu m$		
Pantherophis guttatus	25.7 × 24.3 (23.0-28.5 × 22.0-28.0)	18.3 × 14.8 (17.0-21.5 × 13.5-16.5)	Upton et al. (1984)	
Scotophis obsoletus	27.7 × 26.0 (25.6-29.6 × 24.8-28.0)	19.3 × 14.3 (18.4-20.8 × 13.6-15.0)	McAllister (1989)	
Lampropeltis holbrooki	24.9 × 23.3 (22.0-27.5 × 21.2-25.6)	17.7 × 12.9 (15.4-19.2 × 11.5-13.9)	This report	

<sup>1</sup>Oocyst L/W ratios = 1.1 (1.0-1.1) vs. 1.1 (1.0-1.1) vs. 1.1 (1.0-1.1). <sup>2</sup>Sporocyst L/W ratios = 1.2 (1.1-1.3) vs. 1.4 (1.3-1.4) vs. 1.4 (1.3-1.5).



Figure 1. Sporulated oocyst of *Caryospora duszynskii* from *Lampropeltis holbrooki* collected in Franklin County, Arkansas. PG = polar granule; SB = Stieda body.

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Figure 2. Line drawing of *Caryospora duszynskii* from *Pantherophis* spp. (Redrawn from Upton et al. 1984; see McAllister 1989, Fig. 16).

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