
First Report of a *Hepatozoon* sp. (Apicomplexa: Adeleina: Hepatozoidae) from Midland Water Snake, *Nerodia sipedon pleuralis* (Ophidia: Colubridae), from Missouri

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Intraerythrocytic hematozoans of the genus *Hepatozoon* (Miller, 1908) occur in the gamont stage in birds, mammals, and all groups of reptiles (Telford 2009). For example, one group of reptiles, the North American watersnakes of the genus *Nerodia*, are important hosts of a variety of these parasites (Gibbons and Dorcas 2004). The northern watersnake, *Nerodia sipedon sipedon* (Linnaeus, 1758) has been previously reported to harbor *He. sipedon* Smith, Desser, and Martin, 1994 from Ontario, Canada (Smith et al. 1994; Smith and Desser 1998). However, another widely ranging subspecies, the midland watersnake, *N. s. pleuralis* (Cope, 1892), has not previously been reported as a host nor has any snake, to our knowledge, been reported from Missouri with these hemoparasites. Here, we document, with photomicrographs and select measurements, the first report of a *Hepatozoon* sp. in *N. s. pleuralis*, as well as documentation, to our knowledge, from any ophidian host in Missouri. In addition, a summary of intraerythrocytic hematozoans of North American watersnakes and swampsnakes is provided.

Between April 2012 and June 2016, 10 *N. s. pleuralis* were collected by hand or with snake tong from Franklin ($n = 1$), Fulton ($n = 1$), Garland ($n = 1$), Independence ($n = 5$), Marion ($n = 1$), and Montgomery ($n = 1$)

counties, Arkansas; they were overdosed with an intraperitoneal injection of sodium pentobarbital (Nembutal®). In addition, a single specimen of *N. s. pleuralis* found recently dead (no petrification) was collected on 11 June 2017 from Franklin County, Missouri. A midventral incision was made on each snake to expose the heart and blood was obtained by puncturing the ventricle with a .22 gauge needle, with samples being drawn into an ammonium heparinized (75 mm long) capillary tube. Thin films were air-dried, fixed for one minute in absolute methanol, stained for 20–30 minutes with Wright-Giemsa stain, and rinsed in neutral-buffered phosphate buffer (pH = 7.0). Slides were scanned at 100× or 400× and when infected cells were found, photographs were taken and length and width (L × W) measurements of gamonts were made of intraerythrocytic parasites ($n = 20$) using a calibrated ocular micrometer under oil immersion lens at 1,000×. A host voucher was deposited in the Henderson State University (HSU) collection, Arkadelphia, Arkansas, as HSU 1950. A voucher slide of a hematozoan was deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska.

One of 11 (9%) of the *N. s. pleuralis* (adult, 425 mm snout-vent length [SVL]), collected from Little Indian Creek, Franklin County,

Missouri (38° 14' 07.008"N, 90° 56' 23.1792"W) was found to harbor an intraerythrocytic hematozoan (HWML 139358); all of the 10 snakes from Arkansas were negative. Less than 0.5% of the erythrocytes contained elongate-shaped (unrecurved) thin gamonts (Figs. 1A–C) thought to belong to the genus *Hepatozoon*. Measurements are as follows: $L \pm 1SD$ (range) $\times W \pm 1SD$ (range) = 19.5 ± 1.0 (19–21) $\times 4.0 \pm 0.6$ (3.4–5.0) μm . These measurements and morphologies are similar to those provided by Smith et al. (1994) for *He. sipedon*; these authors also reported a parasitemia of 0.2% in naturally-infected host snakes that is similar to our observed prevalence in erythrocytes.

Smith (1996) considered all hemogregarines of snakes to be members of the genus *Hepatozoon*. However, specific identification of these hematozoans is difficult as the complete life cycle including developmental stages in hematophagous invertebrates must be known (Jacobson 2007; Smith and Desser 1997). *Hepatozoon sipedon* uses culicine vectors, *Culex pipiens* and *C. territans* (Smith et al. 1994); unfortunately, these vectors were not examined in this study.

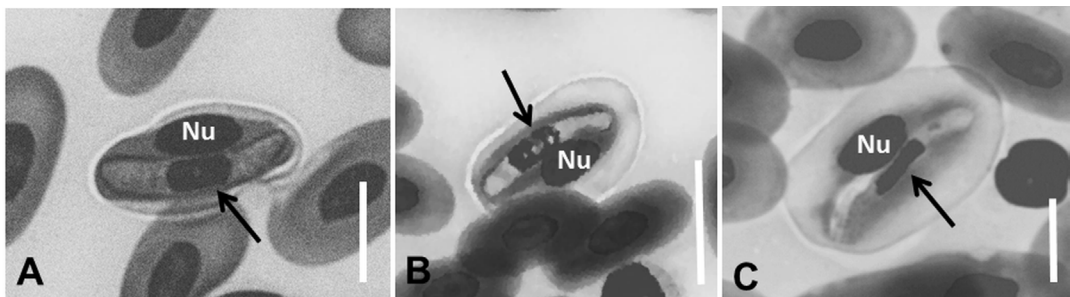
Several species of North American watersnakes, including at least eight species/subspecies of *Nerodia* and the North Florida swampsnake, *Liodytes (=Seminatrix) pygaea pygaea* (Cope, 1871) host these parasites, most identified as “haemogregarines” (Table 1). To date, there have been no hematozoans reported from three species of watersnakes, *N. clarkii* (Baird and Girard, 1853), *N. cyclopion* (Duméril,

Bibron, and Duméril, 1854), and *N. harteri* (Trapido, 1941), two species of crayfish snakes, *Regina grahamii* Baird and Girard, 1853, and *R. septemvittata* (Say, 1825) or two species of swampsnakes, *L. alleni* (Garman, 1874), and *L. rigida* (Say, 1825). Without life cycle data, the inclusion of molecular characterization (DNA sequences) would be particularly helpful in the identification of some hematozoans (see Allen et al. 2011; Cook et al. 2014; Maia et al. 2014) which have limited morphological traits.

As far as we can tell, the only previous report of hemoparasites from any Missouri reptile is that of Smith et al. (1983) who reported a *Haemogregarina* sp. from a red-eared slider, *Trachemys scripta elegans* (Wied, 1838) from Bollinger County. We therefore document a *Hepatozoon* sp. from *N. s. pleuralis* and, in Missouri, for the first time.

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Figures 1A–C. Photomicrographs of a *Hepatozoon* sp. from *Nerodia sipedon pleuralis* from Missouri. A–B. Typical banana-shaped gamonts from two separate erythrocytes. C. Slender more attenuated gamont morph. Abbreviation: Nu (nucleus of host rbc). Scale bars = 10 μm .

Table 1. Reports of hematozoans from North American watersnakes and swampsnakes.

Host	Parasite	Prevalence*	Locality	Reference
<i>Liodytes pygaea pygaea</i>	<i>He. floridana</i>	1/4 (25%)	FL	Telford et al. (2001)
	<i>He. seminatrici</i>	1/4 (25%)	FL	Telford et al. (2001)
<i>Nerodia erythrogaster</i>	<i>He. serpentium</i>	1/11 (9%)	TX	Hilman and Strandtmann (1960)
	“Haemogregarine”	10/20 (50%)	LA	Lowichik and Yaeger (1987)
<i>N. fasciata confluens</i>	“Haemogregarine”	25/26 (96%)	LA	Lowichik and Yaeger (1987)
<i>N. f. pictiventris</i>	<i>Hepatozoon</i> sp.	1/1 (100%)	FL	Wozniak et al. (1998)
	<i>Haemogregarina floridana</i>	1/3 (33%)	FL	Telford et al. (2001)
	<i>He. fasciatae</i>	3/5 (60%)	FL	Telford et al. (2001)
	<i>He. pictiventris</i>	8/10 (80%)	FL	Telford et al. (2001)
<i>N. floridana</i>	<i>He. floridana</i>	3/4 (75%)	FL	Telford et al. (2001)
<i>N. rhombifer</i>	“Haemogregarine”	1/1 (100%)	AR	Daly et al. (1984)
		1/1 (100%)	LA	Lowichik and Yaeger (1987)
<i>N. sipedon pleuralis</i>	<i>Hepatozoon</i> sp.	1/1 (100%)	MO	This report
<i>N. s. sipedon</i>	<i>Haemogregarina</i> sp.	1/2 (50%)	CA†	Fantham and Porter (1954)
	“Haemogregarine”	8/29 (28%)	OH	Hull and Camin (1960)
	<i>He. sipedon</i>	18/26 (69%)	CA§	Smith et al. (1994)
<i>N. taxispilota</i>	“Haemogregarine”	1/1 (100%)	UN	Roudabush and Coatney (1937)
<i>Nerodia</i> sp.#	“Haemogregarine”	3/5 (60%)	IL	Marquardt (1966)

*Number infected/number examined (%).

†Quebec, Canada.

§Ontario, Canada.

||Unknown; locality not given.

#Species not identified; *N. erythrogaster*, *N. rhombifer*, *N. s. pleuralis*, and *N. s. sipedon* all occur in Illinois (see Powell et al. 2016).

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