

Hematozoa (Apicomplexa: Haemogregarinidae, Hepatozoidae) from Two Turtles (Testudines: Chelydridae, Emydidae) and Two Snakes (Ophidia: Colubridae, Viperidae), in Southeastern Oklahoma

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Hematozoans (*Haemogregarina*, *Haemoproteus*, *Hepatozoon* spp.) are intraerythrocytic parasites that infect various vertebrates (Telford 2009). In Oklahoma, hematozoans have been reported from birds (Janovy 1963; Lewis et al. 1975; Bay and Andrews 2009) and mammals (see Allen et al. 2011). Although commonly reported from reptilian hosts in the surrounding states of Arkansas (McAllister and King 1980; Daly et al. 1984; McAllister et al. 1995, 2014), Louisiana (De Giusti and Batten 1951; Herban and Yaeger 1969; Acholonu 1974; Lowichik and Yaeger 1987; Powell and Knesel 1993) and Texas (Hilman and Strandmann 1960; Wang and Hopkins 1965), it is surprising that with the ubiquity of these parasites that no published surveys, to my knowledge, have been conducted on reptiles from Oklahoma. However, one lingering problem has been the taxonomic identity of these hematozoans which requires knowledge of their complete life cycle including developmental stages in vector and definitive host leeches (Siddall and Desser 1991, 2001) and in hematophagous invertebrates (Smith and Desser 1997; Jacobson 2007). Nevertheless, I report new records for some hematozoans from three reptiles of the state, including photomicrographs and select measurements.

Between June 2013 and October 2015, the following 12 reptiles were collected from McCurtain County and, as part of a survey of their helminth parasites, were at the same time also examined for hematozoans: two each of

common snapping turtle (*Chelydra serpentina*), Mississippi mud turtle (*Kinosternon subrubrum hippocrepsis*), common musk turtle (*Sternotherus odoratus*), and eastern cooter (*Pseudemys concinna concinna*), and one each of southern copperhead (*Agkistrodon contortrix contortrix*), western cottonmouth (*Agkistrodon piscivorus leucostoma*), timber rattlesnake (*Crotalus horridus*), and western rat snake (*Pantherophis obsoletus obsoletus*). Reptiles were overdosed with an intraperitoneal injection of sodium pentobarbital (Nembutal®). The plastron was removed from turtles with a bone saw and a midventral incision was made on snakes to expose the viscera. Blood was obtained from their exposed heart by obtaining a sample using ammonium heparinized (75 mm long) capillary tubes and thin films were air-dried, fixed for 1 min in absolute methanol, stained for 20–30 min with Wright-Giemsa stain, and rinsed in phosphate buffer (pH = 7.0). Slides were scanned at 100× or 400× and when infected cells were found, photographs were taken and length measurements were made on intraerythrocytic parasites (20/form) using a calibrated ocular micrometer under a 1,000× oil immersion lens and are reported in micrometers as means ±1SD followed by the ranges. Host vouchers are deposited in the Arkansas State University Museum of Zoology (ASUMZ) Herpetological Collection, State University, Arkansas. Voucher slides of hematozoans are deposited in the Harold W. Manter Laboratory of Parasitology (HWML), Lincoln, Nebraska.

Four (33%) of the 12 individual reptiles, including *C. s. serpentina*, *P. c. concinna*, *C. horridus* and *P. obsoletus* were found to harbor intraerythrocytic hematozoans. The following were negative: *K. s. hippocrepis*, *S. odoratus*, *A. c. contortrix* and *A. p. leucostoma*. Data is presented below in an annotated format.

**Apicomplexa: Adeleorina:
Haemogregarinidae**

Haemogregarina sp. Danilewsky, 1885 – About 5% of the red blood cells (rbc's) of a *C. serpentina* (adult male, 350 mm carapace length [CL], collected in April 2015 from the vicinity of Holly Creek, 33.96814°N, 94.804255°W) contained an intraerythrocytic hematozoan thought to belong to the genus *Haemogregarina* (HWML 101841). Three morphological types (Figs. 1A–B) were observed and measurements are given for small, medium and large types (Table 1). Ovoidal to bean-shaped gamonts were most often observed (Fig. 1A). These were very similar to morphological types described, but

not figured, from Arkansas red-eared sliders (*Trachemys scripta elegans*) by McAllister and King (1980). McAllister et al. (1995, Fig. 3) also reported large immature gamonts from an alligator snapping turtle (*Macrochelys temminckii*) from Arkansas similar of those from *C. serpentina*. The common snapping turtle has been commonly studied and previously reported as a host of various hematozoans from Illinois, Iowa, Kentucky, Louisiana, Massachusetts, Ohio, Tennessee, Texas, and Ontario, Canada (Hahn 1909; Roudabush and Coatney 1937; Edney 1949; Wang and Hopkins 1965; Marquardt 1966; Herban and Yeager 1969; Desser 1973; Acholonu 1974; Paterson and Desser 1976; Strohle and Christensen 1984; Siddall and Desser 1991, 1992; Brown et al. 1994).

Less than 1% of the rbc's of an adult male (270 mm CL) *P. c. concinna* collected in September 2015 from 8.0 km N of Broken Bow off US 259 (34.091873°N, 94.739463°W) harbored a hematozoan (HWML 101842) also

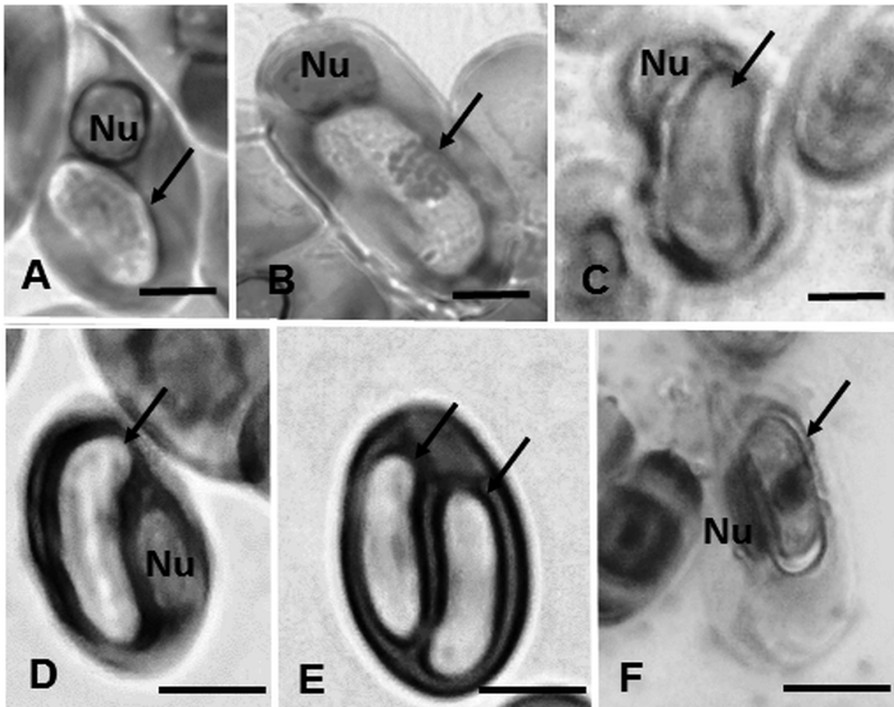


Figure 1. Photomicrographs of hematozoans (arrows) from four Oklahoma reptiles. A–B. Gamonts from *Chelydra serpentina*. C. Gamont from *Pseudemys concinna concinna*. D–E. Single (D) and pair (E) of macrogamonts from *Pantherophis obsoletus obsoletus*. F. Gamont from *Crotalus horridus*. Abbreviation: Nu (nucleus of host rbc). Scale bars = 10 μ m.

Table 1. Length measurements of hematozoans found in *Chelydra serpentina* and *Pantherophis obsoletus obsoletus* from Oklahoma.

Host/morphological type	Mean length (μm)	Size range (μm)
<i>C. serpentina</i>		
Small	12.2 \pm 1.2	10–15
Medium	20.0 \pm 1.2	16–21
Long	31.6 \pm 2.2	28–37
<i>P. o. obsoletus</i>		
Long slender	18.2 \pm 1.4	17–20

thought to belong to *Haemogregarina* (Fig. 1C). River cooters have previously been reported to be infected with hematozoans from Louisiana (Herban and Yeager 1969; Acholonu 1974), Illinois (Marquardt 1966), Tennessee (Edney 1949) and Texas (Hopkins 1965). Haemogregarines are most commonly reported from aquatic turtles with leeches serving as the only known invertebrate hosts and vectors (Telford 2009). We report these hematozoans from two turtles in Oklahoma for the first time.

Hepatozoidae

Hepatozoon sp. Miller, 1908 – About 10% of the rbc's of a *P. o. obsoletus* (adult male, 793 mm snout-vent length [SVL], collected in October 2013 from Beavers Bend State Park, 34.126576°N, 94.674858°W) contained an intraerythrocytic hematozoan (HWML 101843) thought to belong to the genus *Hepatozoon* (Figs. 1D-E). Measurements of a single form of the parasite is provided in Table 1. Hematozoans were reported previously from various subspecies of *P. obsoletus* from Arkansas, Florida, Illinois and Louisiana (Marquardt 1966; Daly et al. 1984; Lowichik and Yeager 1987; Telford et al. 2001, 2004, 2005) and from captive rat snakes from zoos in Illinois and Ohio (Hull and Camin 1960).

In addition, a single *C. horridus* (adult male, 1,030 mm SVL, collected on 3 June 2013 from the Eastern Oklahoma State College Campus,

Idabel, 33.920662°N, 94.777173°W) was found to be infected (~ 1% of rbc's) with another hematozoan (HWML 101844), also thought to belong to the genus *Hepatozoon* (Fig. 1F). Hematozoans were reported previously from 4% of the erythrocytes of *C. horridus* from the Catskill Mountains (specific locality unknown) (Fantham and Porter 1954) and *Hepatozoon horridus* and *H. sauritus* was documented in one of eight (13%) timber rattlesnake from Florida (Telford et al. 2008). Gamonts of *H. horridus* and *H. sauritus* were reported by these authors to measure 15.7 \times 5.1 (13–17 \times 4–6) and 16.6 \times 4.1 (15–19 \times 3.5–6) μm respectively, and, although there were not enough infected erythrocytes in the present sample for comparative measurements, length of two gamonts were well within their ranges.

Smith (1996) considered all hemogregarines of snakes to be members of the genus *Hepatozoon* even in the absence of life-cycle data to the contrary. However, with little evidence that snakes are infected by species of *Haemogregarina*, Telford et al. (2001) described, with some reservation, *Haemogregarina floridana* from Florida green water snake (*Nerodia floridana*), Florida water snake (*Nerodia fasciata pictiventris*), and North Florida swamp snake (*Liodytes pygaea*) from Florida and South Carolina. I therefore document the two hematozoans from Oklahoma

snakes herein to represent *Hepatozoon* spp.

Turtles and snakes are hosts of numerous described and potentially undescribed hematozoans (Ernst and Ernst 1979; Telford 2009). Since Oklahoma supports 18 species and subspecies of turtles and 46 species and subspecies of snakes (Sievert and Sievert 2011), additional surveys on larger samples of turtles and snakes from the state need to be done as several species should be examined for hematozoans. Moreover, the inclusion of molecular characterization (DNA sequences) would be particularly helpful to identify some hematozoans (see Allen et al. 2011; Cook et al. 2014; Maia et al. 2014) which have limited morphological traits. As such, new host and distributional records could be found, including the possibility of discovering new species.

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