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C.T. McAllister
Northeast Texas Community College, Mt. Pleasant, Texas

H.W. Robison

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New Host Records of Apicomplexan Blood Parasites (Haemogregarinidae and Hepatozoidae) Infecting Two Reptiles (Testudines; Ophidia) from Arkansas

C.T. McAllister[1*], and H.W. Robison[2]

[1]*Division of Natural Sciences, Northeast Texas Community College, 2886 FM 1735, Chapel Hill Road, Mt. Pleasant, Texas 75455*
[2]*602 Big Creek Drive, Sherwood, AR 72120*

[*]Correspondence: cmcallister@se.edu

Running Title: Hemoparasites from Midland Smooth Softshell and Western Milksnake

Abstract

Relatively few records of apicomplexan blood parasites from reptiles in Arkansas have been published although the effects of these parasites on reptilian health may be of concern. Using photomicrographs we describe the morphotypes of parasite gamonts found in blood samples from the Midland smooth softshell turtle, *Apalone mutica mutica*, and a western Milksnake *Lampropeltis gentilis* from Arkansas. The turtle possessed four distinct morphological gamont forms of a *Haemogregarina* sp. The snake possessed two morphological forms of gamonts of a *Hepatozoon* sp. Both infections are new host records and the western milksnake has not been described as a host elsewhere for a *Hepatozoon* sp. prior to this study. These findings show the need for more surveys to help describe the diversity of this group of hemoparasites in the state. In addition, we provide a summary of hemoparasites from American members of the softshell family Trionychidae and from the reptiles of the state as well, to date.

Introduction

Haemogregarines are apicomplexan blood-inhabiting parasites with an obligatory heteroxenous life cycle. The life cycle includes two distinct stages, an asexual multiplication stage occurs in a vertebrate host whereas a sexual reproduction stage occurs in a hematophagous invertebrate vector (Telford 2009). These blood-inhabiting parasites are omnipresent in all types of vertebrates, from fishes to mammals.

A good deal of information is available on the natural history and ecology of *A. mutica* (Webb 1973); however, there is only a single report of intraerythrocytic hematozoans from this host. Wang and Hopkins (1965) examined four *A. mutica* from Brazos County, Texas, and all were infected; however, no detailed information was provided and photomicrographs are lacking.

Likewise, much is known about the biology *L. gentilis* (Williams 1994). This species comprised some of the formerly recognized subspecies of *L. triangulum* (Ruane *et al.* 2014). Nothing, to our knowledge, is known about hematozoans of *L. gentilis*.

The goal of this study was to provide morphological descriptions to determine putative identifications of hemoparasites from a smooth softshell and western milksnake, including the first photomicrographs of the infections. In addition, we provide a summary of the hematozoans from North American trionychid turtles and reptiles of the state.

Methods

Reptile collection and blood sample preparation

On 15 March 2021, an adult male *L. gentilis* (snout-vent length = 450 mm) was collected by hand from the slate mine at the Ouachita Mountains Biological Station (OMBS), Polk County (34°27'43.4484"N, -93°59'54.3264"W) and on 23 February 2022, an adult female *A. m. mutica* (carapace length = 285 mm) was collected from a hoop net set in the Black River at Black Rock, Lawrence County (36°06'53”W, -91°04'25.73”N). These individuals were transported to the laboratory where blood samples were obtained for parasite detection. Both reptiles were overdosed with a concentrated solution of tricaine methanesulfonate (TMS-222) via an intraperitoneal injection per SIH (2004) guidelines. Blood from the heart by obtaining from both using ammonium heparinized capillary tubes (75 mm long) and thin films were made. These samples were air-dried, fixed

for one minute in absolute methanol, stained for 20–30 minutes with Wright-Giemsa stain, and rinsed in phosphate buffer (pH = 7.0).

Host voucher specimens are deposited in the Eastern Oklahoma State Vertebrate Collection (EOSC), Idabel, Oklahoma. We followed the common and scientific names of North American turtles and snakes of Crother (2017).

Parasite identification

Parasites were detected by scanning blood smears from two slides per specimen at 100× or 400× to detect infected erythrocytes in a 5,000 cell count. From these slides, photographs of infected cells were taken using a Swift model M10 light microscope (Microscope Central, Feasterville, Pennsylvania) under a 1,000× oil immersion lens. Measurements were made of 10 gamonts/form when available using a calibrated ocular micrometer under a 1,000× oil immersion lens. Taxonomic identification of the apicomplexan parasites were made mostly on the gametocyte morphology and morphometric calculations using (Smith 1966), Telford (2009), and McAllister *et al.* (2016) to get to the lowest taxonomic level. Gamont shape follows descriptions by Hull and Camin (1960).

Photovouchers of the parasites are deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska.

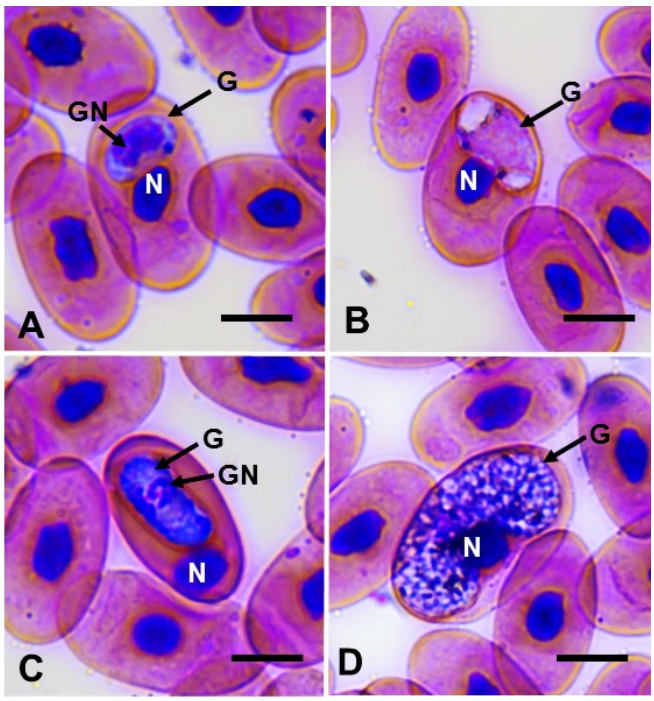
Results

Erythrocytes from the turtle contained four gamont morphotypes (Table 1, Figs. 1A-D; HWML 216942). These morphotypes represented gamonts of a species of *Haemogregarina*. Erythrocytes from the snake contained two gamont morphotypes (Figs. 2A-B; HWML 216943), although not enough gamonts were observed so measurements were not possible. These morphotypes represented gamonts of a species of *Hepatozoon*. No other developmental stages were observed in thin blood smears of either hemoparasite. Detailed information on the infections is presented below.

APICOMPLEXA LEVINE, 1970
ADELEORINA LÉGER, 1911
HAEMOGREGARINIDAE LÉGER, 1911

***Haemogregarina* sp. Danilewsky, 1885** – The four gamont morphotypes observed (Table 1), included a kidney bean-shaped recurved form (Fig. 1A), an ovoidal-shaped form (Fig. 1B), an elongate-shaped form (Fig. 1C), and a large (granular) comma-shaped form (Fig. 1D). This latter form greatly enlarged the host erythrocyte (Fig. 1D). Gamonts were enclosed in a thin parasitophorous vacuole.

Remarks: Wang and Hopkins (1965) reported a *Haemogregarina* sp. from four *A. m. mutica* from Brazos County, Texas. Unfortunately, no mensural or morphometric data was provided specific to those hosts as well as lack of a photomicrograph of the infection. We are unaware of any additional previous reports of *Haemogregarina* from *A. m. mutica*. On the other hand, haemogregarines have been previously reported from various spiny softshells (*A. spinifera*) from Arkansas, Louisiana, Tennessee, and Texas (Table 1).



Figures 1A-D. *Haemogregarina* sp. from *Apalone mutica mutica*. (A) Kidney-bean shaped recurved gamont. (B) Ovoidal-shaped gamont. (C) Elongate-shaped gamont; note displacement of host erythrocyte nucleus. (D) Large comma-shaped granulous gamont. Scale bars = 10µm. Abbreviations: G (gamont); GN (gamont nucleus); N (host erythrocyte nucleus).

HEPATOZOIDAE WENYON, 1926

***Hepatozoon* sp. Miller, 1980** – Ellipsoidal to elongate gamonts was observed as well as a slender host erythrocyte nucleus that was pushed to the edge of the cell.

Remarks: This is the first report of a hematozoan parasite from *L. gentilis*. Smith (1996) reported a *Hepatozoon* sp. from a single eastern milksnake, *Lampropeltis triangulum* (Lacépède) from Ontario, Canada. Langmann (1899) examined three *L. triangulum* from New York but none were infected. In

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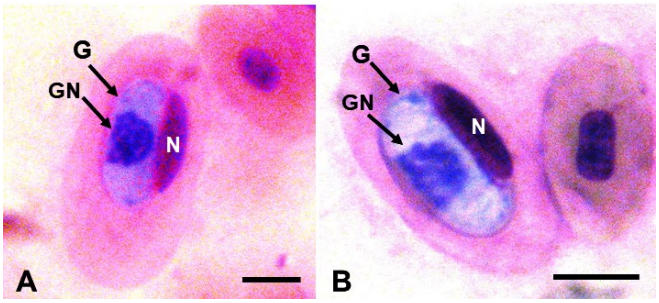

Figure 2. *Hepatozoon* sp. from *Lampropeltis gentilis*. (A) Ovoidal to kidney bean-shaped gamont. (B) Ellipsoidal to elongate gamont. Note N is abnormally shaped, slender, and elongated. Abbreviations: gamont (G); gamont nucleus (GN); host erythrocyte nucleus (N). Scale bars = 5µm.

addition, McKinstry (1973) examined nine *L. triangulum* from Pennsylvania but did not find hematozoans. Interestingly, hematozoans have been previously reported from related speckled kingsnakes, *Lampropeltis getulus* Stejneger (Hull and Camin 1960; Telford 2010).

Discussion

Species of *Haemogregarina* infect lower vertebrates as intermediate hosts and various leeches serve as definitive hosts. Taxonomic classification of haemogregarines can be challenging when data on their life cycle stages is unknown. Therefore, the basis in classification of taxa of haemogregarines is related to their sporogonic cycle (Telford 2009). In addition, the inclusion of a molecular approach is an excellent tool when sufficient sample is available, which can aid in the identification of these hematozoans by interpreting their evolutionary relationships.

Apicomplexans of the genus *Hepatozoon* include about 121 species described from more than 200 snake species (Smith *et al.* 1999; Han *et al.* 2015). Members are characterized by a life cycle with localization of the gamont stage in erythrocytes, sporogonic development with formation of large polysporocystic oocysts in the hemocoel of haematophagous mosquitoes, development of cysts in the liver or lung of lizards or frogs that has ingested an infected mosquito, and merogonic development in various visceral organs of a snake that has eaten the intermediate vertebrate host (Smith 1996). Except for a few taxa in which life-cycles have been clarified, the majority of species are known only from the description of the most familiar stage, the intraerythrocytic gamont in the host snake.

The softshell turtle family Trionychidae Fitzinger includes three species and seven subspecies in North America (van Dijk *et al*. 2011). Hematozoans have been previously reported from two of three (67%) of these species (Table 2). However, the Florida softshell, *Apalone ferox* (Schneider) has yet to be reported as a host of hematozoans.

There are 19 species and subspecies of turtles that inhabit Arkansas (Trauth *et al*. 2004). To date, haemogregarines have been reported from 10 (53%) species/subspecies (Table 3). Since turtles are hosts of numerous described and potentially novel hematozoans (see Ernst and Ernst 1979, Table 2), additional surveys on larger samples of turtles from the state need to be carried out as nine species/subspecies remain unexamined for hematozoans.

Concerning the snakes of Arkansas, in which 45 species/subspecies occur, although other species have been surveyed, only five species (11%) have been reported with hematozoans (Table 2); therefore, numerous species remain to be examined for hematozoans. Conducting surveys on these snakes, yet to be examined or found to be infected, would likely yield new host and geographic distributional records for hematozoa, including the possibility of discovering new species if life cycle studies as well as nested PCR and DNA sequencing are included.

Table 1. Measurements of four haemogregarine morphotypes found in erythrocytes of *Apalone mutica mutica*.

Morphotype	Length of gamont (µm) Mean ± SD (range)	Width of gamont (µm) Mean ± SD (range)
Kidney-bean recurved shaped form	11.6 ± 1.2 (10.4–13.6)	4.6 ± 0.5 (4.0–5.6)
Ovoidal-shaped form	24.0 ± 1.4 (22.0–25.6)	5.0 ± 0.5 (4.2–5.8)
Elongate-shaped form	24.6 ± 1.6 (22.4–26.0)	4.6 ± 0.6 (4.4–5.2)
Comma-shaped granulous form	48.8 ± 2.0 (42.0–50.4)	7.8 ± 1.0 (6.4–8.2)

Table 2. Hematozoans (*Haemogregarina* sp.) reported from turtles of the family Trionychidae in North America.

Host	Prevalence*	Locality	Reference
Apalone mutica mutica	4/4 (100%)	Texas	Wang and Hopkins (1965)
	1/1 (100%)	Arkansas	This study
A. spinifera emoryi	1/1 (100%)	Texas	Wang and Hopkins (1965)
A. spinifera†	4/4 (100%)	Louisiana	Acholonu (1974)‡
A. s. spinifera§	3/5 (60%)	Louisiana	Herban and Yaeger (1969)
	3/4 (75%)	Tennessee	Edney (1949)‖
	1/1 (100%)	Arkansas	McAllister and Robison (2019)

*Prevalence = infected/examined (%).

†Host subspecies not specified but collection locality (Baton Rouge, East Baton Rouge Parish) is within the range of *A. s. pallida* (Powell *et al*. [2016, their fig. 85]).

‡Hematozoan identified as *Haemogregarina pseudemydis* Acholonu, 1974, based on merogony and gametogony stages (see Acholonu 1974).

§Host originally reported as *A. s. hartwegi*; McGaugh *et al*. (2008) synonymized *A. s. hartwegi* with *A. s. spinifera*.

‖Hematozoan identified as *Haemogregarina stepanowi* Danilewsky, 1885, based solely on gamont stage (see Edney 1949).

Table 3. Hematozoans reported from reptiles of Arkansas.

Host (Order, Family)	Prevalence*	County	Reference
	***Haemogregarina* sp.**		
Testudines: Chelydridae			
Chelydra serpentina	1/1 (100%)	Lincoln	McAllister *et al*. (2016)
Macrochelys temminckii	1/2 (50%)	Ouachita	McAllister *et al*. (1995)
Emydidae			
Chrysemys dorsalis	1/1 (100%)	Pulaski	McAllister *et al*. (2016)
Graptemys geographica	1/1 (100%)	Marion	McAllister *et al*. (2014)
Pseudemys concinna concinna	1/2 (50%)	Perry	McAllister *et al*. (2016)
Trachemys scripta elegans	25/25 (100%)	Lonoke	McAllister and King (1980)
Kinosternidae			
Kinosternon subrubrum hippocrepis	1/4 (25%)	Ouachita	McAllister *et al*. (2016)
Sternotherus odoratus	3/7 (43%)	Lincoln	McAllister *et al*. (2016)
Trionychidae			
Apalone mutica mutica	1/1 (100%)	Lawrence	This study
A. spinifera spinifera	1/1 (100%)	St. Francis	McAllister and Robison (2019)
	***Hepatozoon* sp.**		
Ophidia: Colubridae			
Lampropeltis gentilis§	1/1 (100%)	Polk	This study
Nerodia rhombifer	1/1 (100%)	Central AR†	Daly *et al*. (1984)
Panterophis obsoletus	1/1 (100%)	Central AR†	Daly *et al*. (1984)
Thamnophis proximus proximus	1/1 (100%)	Central AR†	Daly *et al*. (1984)
Viperidae			
Agkistrodon piscivorus	1/2 (50%)	Lincoln	McAllister *et al*. (2016)
	1/3 (33%)	Independence	McAllister *et al*. (2016)

*Prevalence = infected/examined (%).

†County not specified.

§This species was comprised of the formerly recognized subspecies *L. t. amaura* (part), *L. t. annulata* (part), *L. t. celaenops, L. t. multistriata, L. t. syspila* (part), and *L. t. taylori,* and (see Ruane *et al*. 2014).

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