First Report of *Haemogregarina* sp. (Apicomplexa: Haemogregarinidae) from Razor-Backed Musk Turtle, *Sternotherus carinatus* (Testudines: Kinosternidae), from Oklahoma, with a Summary of Hematozoans from the Family Kinosternidae

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Abstract: Generally, little is known regarding the hematozoan parasites of turtles in Oklahoma. Here, we report a *Haemogregarina* sp. from one of two razor-backed musk turtles, *Sternotherus carinatus*, collected from McCurtain County. The host possessed a single infected erythrocyte containing a bean to ovoidal-shaped gamont. Although *S. carinatus* has been previously reported, albeit with very limited information with a *Haemogregarina* sp. from Texas, this is the first time a *S. carinatus* from Oklahoma has been reported with a hematozoan to include a photomicrograph and information about the infection. We also provide a summary of the hematozoans known from the family Kinosternidae, to date.

Introduction

The razorback musk turtle, *Sternotherus carinatus* (Gray) is a medium-sized kinosternid with a prominent, sharp mid-dorsal carapace keel, that ranges from the Pascagoula River System of southeastern Mississippi and Alabama west to the Brazos River Basin of eastern Texas and north to the southern Ouachita uplift, the West Gulf Coastal Plain, and Delta of Arkansas and southeastern Oklahoma (Iverson 1979; Powell et al. 2016). In Oklahoma, *S. carinatus* had been reported from eight counties of the southeastern part of the state (Sievert and Sievert 2021). It occurs in slow-moving rivers and streams, oxbows, and swamps, including habitat with abundant aquatic vegetation and soft substrate (Ernst and Barbour 1972). This turtle feeds almost exclusively on mollusks but will also opportunistically take insects, carrion, and aquatic vegetation (Trauth et al. 2004).

Haemogregarines are apicomplexan blood-inhabiting parasites with an obligatory heteroxenous life cycle. In the life cycle, asexual multiplication occurs in a vertebrate host whereas sexual reproduction occurs in a hematophagous invertebrate vector (Telford 2009). They are omnipresent in all types of vertebrates, from fishes to mammals. Species of *Haemogregarina* infects lower vertebrates as intermediate hosts and various leeches serve as definitive hosts. When data on their life cycle stages is unknown, taxonomic classification of haemogregarines can be challenging. Therefore, the basis in classification of taxa of haemogregarines is related to the sporogonic cycle (Telford 2009). In addition, when sufficient sample is available, the inclusion of a molecular approach is an

excellent tool that aids in the identification of these parasites by elucidating their evolutionary relationships.

A good deal of information is known about the ecology of *S. carinatus* (Iverson 1979; Iverson and Iverson 1980) but data on its parasites, particularly intraerythrocytic hematozoans is mostly lacking. Herban and Yaeger (1969) examined five *S. carinatus* from Louisiana but none were infected, and McAllister et al. (2016) did not find hematozoans in a single *S. carinatus* from Arkansas. Wang and Hopkins (1965) reported a *Haemogregarina* sp. from a *S. carinatus* from Texas but it lacked detailed information, specifically by not providing a photomicrograph or any mensural or morphological data of the infection. Here, we report a hematozoan from a razor-backed musk turtle from southeastern Oklahoma, including the first photomicrograph of the infection. In addition, we provide a summary of the hematozoans from North American kinosternid turtles.

Methods

On 13 September 2022, two adult male *S. carinatus* (133 and 143 mm carapace length) were collected alive with a dipnet from two locales in McCurtain County, one site at Lukfata Creek (34°03'08.3916"W,-94°48'11.9154"N) and another at Yanubbee Creek (34°02'45.6426"N, -94°43'19.761"W). They were overdosed with a concentrated solution of tricaine methanesulfonate (TMS-222) via an intraperitoneal injection. The plastron was removed with a bone saw, a mid-ventral incision was made to expose the viscera, and the internal organs were visualized. 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). Two slides were scanned at 100× or 400× and if any infected erythrocytes were observed after counting 5,000 cells, photographs (digital images) were taken using a Swift model M10 light microscope (Microscope Central, Feasterville, Pennsylvania) under a 1,000× oil immersion lens. A host voucher is deposited in the Eastern Oklahoma State Vertebrate Collection (EOSC), Idabel, Oklahoma. A photovoucher of the parasite is deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska.

Results

One of two *S. carinatus* (133 mm CL) was found to be infected with an intraerythrocytic hematozoan. Information on the infection is presented below.

Apicomplexa Levine, 1970

Adeleorina Léger, 1911

Haemogregarinidae Léger, 1911

***Haemogregarina* sp. Danilewsky, 1885** – A single red blood cell (rbc) containing a gamont of a *Haemogregarina* sp. (Fig. 1, HWML 216885) was found infecting the host from Lukfata Creek. The gamont was ovoidal to bean-shaped but the infected erythrocyte was abnormally shaped and not the typical nucleated biconcave disc (Fig. 1); therefore, it was not possible to get an accurate length × width measurement. However, the morphology and size (Fig. 1) of this infected rbc had obviously undergone considerable aberrant changes. Hypertrophy of the cell clearly resulted in added intraerythrocytic volume from the gamont or may represent an erythrocyte adaptation to the presence of the parasite (Al-Quraishy et al. 2021).

Wang and Hopkins (1965) noted that in 24 of 33 (73%) infected turtles they examined from Brazos County, Texas (species not specified), less than 1% of the host erythrocytes possessed haemogregarines, including one of 142,000 erythrocytes (0.0007%) in one turtle, and one of 205,000 erythrocytes (0.0005%) in another. Here, we found an almost nearly undetectable intensity in *S. carinatus*. Interestingly, despite high prevalence of many turtles reported with haemogregarines, less than 1% of erythrocytes are infected on average (Davis and Sterrett 2011; Rossow et al. 2013; Nordmeyer et al. 2020) and

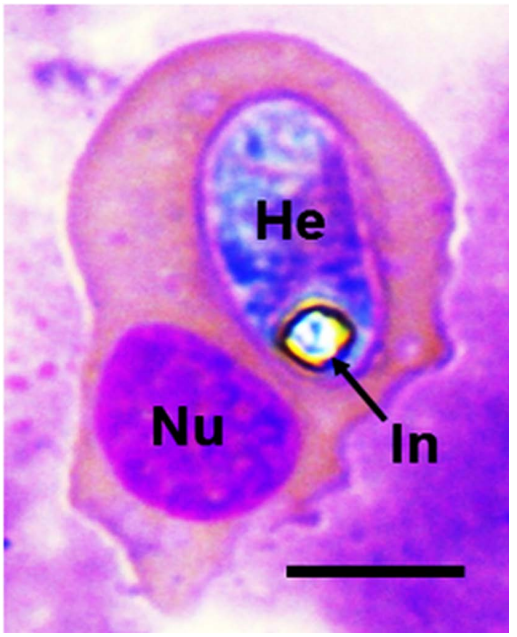

Figure 1. Photomicrograph of *Haemogregarina sp.* from *Sternotherus carinatus* from Oklahoma. Abbreviation: He (haemogregarine); In (inclusion); Nu (nucleus of host rbc). Scale bar = 5 µm.

sometimes only a single gamont is observed (Marquardt 1966; this study).

The mud turtle family Kinosternidae Agassiz includes 36 species/subspecies and four genera (van Dijk et al. 2011). In the Americas, the semiaquatic mud turtle genus *Kinosternon* Spix containing 31 species/subspecies ranges from New England to northern Argentina and the aquatic musk turtle genus *Sternotherus* Bell *in* Gray with five species/subspecies ranges from southern Ontario south to the Gulf states (van Dijk et al. 2011; Powell et al. 2016). Hematozoans have been previously reported from only nine of 36 (25%) species/subspecies of kinosternids (Table 1) with the eastern musk turtle or stinkpot, *Sternotherus odoratus* (Latreille, *in* Sonnini and Latreille) being reported most often as host, including specimens collected from Arkansas, Georgia, Illinois, Kentucky, Massachusetts, North Carolina, and Tennessee. This is not too unexpected as *S. odoratus* is the most widely ranging and perhaps most often collected kinosternid and is very common being found in 32 of 50 (64%) US states as well as Ontario and Québec, Canada (van Dijk et al. 2011).

González et al. (2019) examined three scorpion mud turtles, *Kinosternon scorpioides* (L.) from Colombia for hematozoans, but none were infected. We are also unaware of any reports of hematozoans from the remaining two genera (and three species) of kinosternids, namely *Claudius* (Cope) of Belize, Guatemala, and México, and *Staurotypus* Wagler from El Salvador, Guatemala, and México (van Dijk et al. 2011). For example, Nordmeyer et al. (2020) examined six captive northern giant musk turtles, *Staurotypus triporcatus* Wiegmann from Chiapas, México, but no hematozoans were observed.

There are 18 species and subspecies of turtles that inhabit Oklahoma (Sievert and Sievert 2021). Since turtles are hosts of numerous described and potentially novel hematozoans (Ernst and Ernst 1979), additional surveys on larger samples of turtles from the state need to be carried out as several species should be examined for hematozoans. Moreover, the inclusion of molecular characterization (DNA sequences) would be particularly helpful to identify some hematozoans which have limited morphological traits. As such, new host and geographic distributional records could be found, including the possibility of discovering new species.

Finally, Wang and Hopkins (1965) reported that one of two *S. carinatus* harbored *Haemogregarina* sp. Unfortunately, no detailed information was provided on this infection as well as lacking a photomicrograph. Therefore we document novel information herein and the first photomicrograph of a *Haemogregarina* from *S. carinatus*, as well as the first time this host has been reported with an infection from Oklahoma.

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The Oklahoma Department of Wildlife Conservation issued Scientific Collecting

Table 1. Hematozoans (*Haemogregarina* sp.) reported from turtles of the family Kinosternidae.

Host	Prevalence*	Locality	Reference
	***Kinosternon* spp.**		
K. leucostomum	8/8 (100%)	Costa Rica	Rossow et al. (2013)
K. scorpioides albogulare	1/1 (100%)	Costa Rica	Rossow et al. (2013)
K. s. cruentatum	1/1 (100%)	Central America†	Plimmer (1913)
		North America†	Plimmer (1916)
K. sonoriense	2/3 (67%)	Alabama‡	Nordmeyer et al. (2020)
K. subrubrum hippocrepis	1/2 (50%)	Texas	Wang and Hopkins (1965)
	3/5 (60%)	Louisiana	Herban and Yaeger (1969)
	7/7 (100%)	Louisiana	Acholonu (1974)§
	1/4 (25%)	Arkansas	McAllister et al. (2016)
K. s. subrubrum	2/2 (100%)	North Carolina	Hahn (1909)
	***Sternotherus* spp.**		
S. carinatus	1/2 (50%)	Texas	Wang and Hopkins (1965)
	1/2 (50%)	Oklahoma	This study
S. minor	1/2 (50%)	Tennessee	Edney (1949)‖
S. odoratus	3/5 (60%)	Massachusetts, North Carolina	Hahn (1909)
	1/1 (100%)	Tennessee	Edney (1949)‖
	1/1 (100%)	Illinois	Marquardt (1966)
	26/27 (96%)	Kentucky	Strohlein and Christensen (1984)
	8/9 (89%)	Georgia	Davis and Sterrett (2011)
	3/7 (43%)	Arkansas	McAllister et al. (2016)
	1/3 (33%)	Texas	Nordmeyer et al. (2020)

*Prevalence = infected/examined (%).
†Exact locality not specified.
‡Captive specimen from Guthrie Turtle Farm, Birmingham, Alabama; host range includes Chihuahua and Sonora, México, and Arizona and New Mexico (Powell et al. 2016).
§Identified as *Haemogregarina pseudemydis* Acholonu, 1974, based solely on gamont stage (see Acholonu 1974).
‖Identified as *Haemogregarina stepanowi* Danilewsky, 1885, based solely on gamont stage (see Edney 1949).

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