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Haemogregarina sp. (Apicomplexa: Eucoccidiorida: Adeleorina) from Eastern Spiny Softshell, *Apalone spinifera spinifera* (Testudines: Trionychidae), from Arkansas

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Cover Page Footnote

The Arkansas Game and Fish Commission provided a Scientific Collecting Permit to CTM. We thank Drs. Scott L. Gardner and Gabor Racz (HWWL), and Renn Tumblison (HSU) for expert curatorial assistance. We also thank Uland Thomas (Chicago, IL) and Dr. David Neely (Tennessee Aquarium, Chattanooga, TN) for assistance in collecting.

***Haemogregarina* sp. (Apicomplexa: Eucoccidiorida: Adeleorina) from Eastern Spiny Softshell, *Apalone spinifera spinifera* (Testudines: Trionychidae), from Arkansas**

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Running Title: Haemogregarine from *Apalone spinifera*

Haemogregarines are intraerythrocytic parasites that infect various vertebrates but are most commonly reported from aquatic turtles with leeches serving as the only known invertebrate hosts and vectors (Telford 2009). Numerous turtles from all the surrounding states of Arkansas (except Mississippi) have been reported to serve as hosts, including some from Louisiana (Degiusti and Batten 1951; Herban and Yaeger 1969; Acholonu 1974), Missouri (Smith *et al.* 1983) Oklahoma (McAllister 2015), Tennessee (Edney 1949) and Texas (Wang and Hopkins 1965). Hematozoan parasites have been identified in Arkansas turtles (McAllister and King 1980; McAllister *et al.* 1995, 2014, 2016), but nothing is known about those of spiny softshell turtles in the state. Here we report a new host record and the first photomicrographs of a haemogregarine from a common softshell turtle in Arkansas.

A single juvenile eastern spiny softshell, *Apalone spinifera spinifera* (carapace length = 145 mm) was collected by hand on 21 April 2017 from Crow Creek at Madison, St. Francis County (35°00'45.12"N, 90°44'16.71"W). It was killed by an intraperitoneal injection of sodium pentobarbital (Nembutal®) following accepted guidelines (SIH 2004). A bone saw was used to remove the plastron to expose the heart. Blood was obtained by making a small incision in the heart and taking a sample using an ammonium heparinized (75 mm long) capillary tube. Thin films were smeared onto glass slides, 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. Length and width (L × W) measurements on gamonts of an intraerythrocytic parasite (*n* = 20) using a calibrated ocular micrometer under a 1,000× oil immersion lens are reported in micrometers (μm) as means ±SD followed by the ranges in parentheses. A photographic voucher of the host was deposited in the Henderson State University Vertebrate Collection,

Arkadelphia, Arkansas. A voucher slide was deposited in the Harold W. Manter Laboratory (HWML) of Parasitology, University of Nebraska, Lincoln, Nebraska.

The red-blood cells of the *A. s. spinifera* was found to possess intraerythrocytic hematozoans (HWML 216010) thought to represent a *Haemogregarina* sp. Gamonts were kidney-bean shaped with a length of 12.5 ± 0.5 (11.5–13.0) μm and width of 8.0 ± 0.2 (7.8–8.2 × 5.8–6.0) μm (Figs. 1A–B). The nucleus of the parasite was elongate-ellipsoidal and measured 7.3 ± 0.2 × 5.4 ± 0.2 (7.0–7.5 × 5.3–5.6) μm (Fig. 1B). Intensity of infection revealed 1–2 gamont(s) infected erythrocyte(s)/20 microscopic fields.

Figures 1A–B. Gamonts of *Haemogregarina* sp. from *Apalone spinifera*. (A) Kidney-bean shaped gamont (*). (B) Another kidney-bean shaped gamont (*) showing dark-staining ellipsoidal-elongate nucleus. Scale bars = 10 μm.

Compared to other aquatic turtles, little has been published on haemogregarines of spiny softshell turtles (Ernst and Ernst 1979). Apparently the first to report a haemogregarine was Edney (1949) who reported *H. stepanowi* Danilewsky in 3 of 4 (75%) *A. s. spinifera* from middle Tennessee. The life cycle of this parasite involves transmission by leeches as described by Reichenow (1910). Later, Wang and Hopkins (1965) reported a *Haemogregarina* sp. in a single Texas spiny

softshell, *A. s. emoryi* from eastcentral Texas, and Herban and Yaeger (1969) found *H. stepanowi* in 3 of 5 (60%) western spiny softshells, *A. s. hartwegi* from Louisiana.

To date, 8 species of turtles have been reported from Arkansas to harbor hematozoans (McAllister and King 1980; McAllister *et al.* 1995, 2014, 2016) including: common snapping turtle (*Chelydra serpentina*), alligator snapping turtle (*Macrochelys temminckii*), southern painted turtle (*Chrysemys dorsalis*), eastern river cooter (*Pseudemys concinna*), red-eared slider (*Trachemys scripta elegans*), common map turtle (*Graptemys geographica*), Mississippi mud turtle (*Kinosternon subrubrum hippocrepis*), and stinkpot (*Sternotherus odoratus*). There are 17 species and subspecies of aquatic turtles within 4 families in Arkansas (Trauth *et al.* 2004) and 9 species are yet to be reported as hosts of hematozoans. Obviously, more work needs to be done in surveying additional turtles, including smooth softshell (*A. mutica*) in the state (and elsewhere), for these apicomplexan parasites.

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