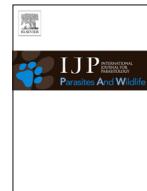




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Brief Report

Haemogregarine infections of three species of aquatic freshwater turtles from two sites in Costa Rica



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ABSTRACT

Twenty-five black river turtles (*Rhinoclemmys funerea*) and eight white-lipped mud turtles (*Kinosternon leucostomum*) from Selva Verde, Costa Rica were examined for haemoparasites. Leeches identified as *Placobdella multilineata* were detected on individuals from both species. All turtles sampled were positive for intraerythrocytic haemogregarines (Apicomplexa:Adeleorina) and the average parasitemia of black river turtles ($0.34\% \pm 0.07$) was significantly higher compared to white-lipped mud turtles ($0.05\% \pm 0.006$). No correlation was found between parasitemia and relative body mass of either species or between black river turtles from the two habitats. In addition, one scorpion mud turtle (*Kinosternon scorpioides*) examined from La Pacifica, Costa Rica, was positive for haemogregarines (0.01% parasitemia). Interestingly, parasites of the scorpion mud turtle were significantly smaller than those from the other two species and did not displace the erythrocyte nucleus, whereas parasites from the other two species consistently displaced host cell nuclei and often distorted size and shape of erythrocytes. This is the first report of haemogregarines in turtles from Central America and of haemogregarines in *K. leucostomum*, *K. scorpioides*, and any *Rhinoclemmys* species. Additional studies are needed to better characterise and understand the ecology of these parasites.

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1. Introduction

The haemogregarines (Apicomplexa:Adeleorina) are protozoan, intraerythrocytic parasites that infect a wide variety of vertebrates (Davies and Johnston, 2000; Telford, 2009) and are considered common haemoparasites of reptiles. There are currently four recognised genera of haemogregarines that infect reptiles and *Haemogregarina* is the most common genus reported in aquatic turtles. Since first described in European pond turtles (*Emys orbicularis*), numerous species of *Haemogregarina* have been documented; however, description of species based solely on vertebrate host stages is discouraged and should include stages in invertebrate hosts (Ball, 1967; Telford, 2009). In addition, molecular characterisation may provide additional data on host specificity and species diversity (Barta et al., 2012).

Among aquatic turtles, *Haemogregarina* spp. have been reported from numerous countries in North America, Europe, Asia, and in

Australia (Telford, 2009). For haemogregarines with known life cycles, leeches are the invertebrate hosts and vectors for parasites of aquatic turtles and ticks are hosts for parasites of terrestrial reptiles (Siddall and Desser, 1991, 2001; Cook et al., 2009). Although there are no previous reports of haemogregarines in aquatic turtles from Central America, *Placobdella* leeches have been reported on black river turtles from Costa Rica (Ernst and Ernst, 1977). In addition, haemogregarines have been reported in numerous species of aquatic turtles in North America and the Geoffroy's Toadhead turtle (*Phrynosoma geoffroyi*) in South America (De Campos Brites and Rantin, 2004; Telford, 2009).

Major conservation threats for chelonians in Costa Rica include habitat destruction, use of turtles for food, and harvesting of sea turtle eggs. Excluding sea turtles, there are eight native aquatic/terrestrial turtles in Costa Rica including three species of mud turtles (*Kinosternon* spp.), two species of wood/river turtles (*Rhinoclemmys* spp.), two species of sliders (*Trachemys* spp.), the South American snapping turtle (*Chelydra acutirostris*), and an introduced, established species (red-eared slider, *Trachemys scripta elegans*). Data on haemoparasites of aquatic turtles in Central America are scant; therefore, we conducted this study to determine if aquatic turtles of riverine and pond habitats in Selva Verde, Costa Rica,

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were infected with haemogregarines. Furthermore, because aquatic turtle health may be influenced by parasitism in Costa Rica where natural habitat is shrinking, we determined if habitat or body mass were associated with differences in prevalence or intensity of infection.

2. Methods

2.1. Study site

Selva Verde ($10^{\circ} 27' 4''\text{N}$, $84^{\circ} 4' 10''\text{W}$) is a Costa Rican eco-lodge and rainforest reserve that spans 500 acres of the Sarapiquí Canton in the northern Caribbean lowlands (Fig. 1). The reserve has obtained Costa Rica's Certification for Sustainable Tourism and is dedicated to maintaining biodiversity. However, the reserve itself is bordered by pineapple and banana agriculture to the east and cattle pasture to the west. Black river turtles and white-lipped mud turtles were trapped at two sites within Selva Verde: Site 1 was a natural creek that runs through the reserve and ends in the Sarapiquí River and Site 2 was a small man-made pond located across a highway and 0.45 km from the creek.

In addition, a single specimen of scorpion mud turtle (*Kinosternon scorpioides*) was collected from a small creek at La Pacifica ($10^{\circ} 28' 32''\text{N}$, $85^{\circ} 8' 41''\text{W}$), an ecological farm and hotel in the Guanacaste Province in western Costa Rica (Fig. 1). This site is classified as a deciduous, dry tropical forest surrounded by fragmented forest and commercial tilapia (*Oreochromis niloticus*) farms.

2.2. Animal capture and sampling

Turtles were captured in $81 \times 51 \times 30$ cm nylon mesh crab traps (Promar, Gardena, CA) baited with banana, papaya, and tuna.

Two traps were used simultaneously at each site. The traps were initially monitored every 50 min at both sites, but the frequency of monitoring was increased to every 15–30 min in the creek due to the higher frequency of captures of black river turtles at this site.

Upon capture, turtles were placed in individual holding containers and the following physical parameters were collected: body weight, curved carapace and plastron length and width. Each individual was identified to species (Savage, 2005), marked with identification numbers on the carapace with nail polish, and photographed. A sample of blood was collected from either the ventral tail vein or the subcarapacial sinus, depending on species, and placed in tubes lined with lithium heparin (Becton, Dickinson and Company, Franklin Lakes, NJ) (Hernandez-Divers et al., 2002). The skin of the head, limbs and tail of each turtle was examined for leeches. Collected leeches were identified using morphologic characters (Sawyer, 1986). All black river turtles were fully examined for leeches, but only two white-lipped mud turtles could be examined because complete physical exams required general anesthesia (5 mg/kg propofol IV; PropoFlo™, Abbott, Abbott Park, IL) to open the double-hinged shell, which proved impractical for the rest of the animals. After processing, all black river turtles and non-anesthetized white-lipped mud turtles were immediately released near the site of capture. The time until release for anesthetized white-lipped mud turtles was increased to three hours to ensure full recovery before return to their normal environment.

Two thin blood smears were immediately made for each turtle, air dried, fixed in 100% methanol, and stained with a modified Giemsa stain (DipQuick, Jorgensen Laboratories, Inc., Loveland, CO). Parasitemia was determined by counting the number of parasites observed during examination of at least 5,000 erythrocytes (Davis and Sterrett, 2011). If no haemogregarines were observed during the first screening, a second count was conducted with the other slide (total of 10,000 erythrocytes examined).

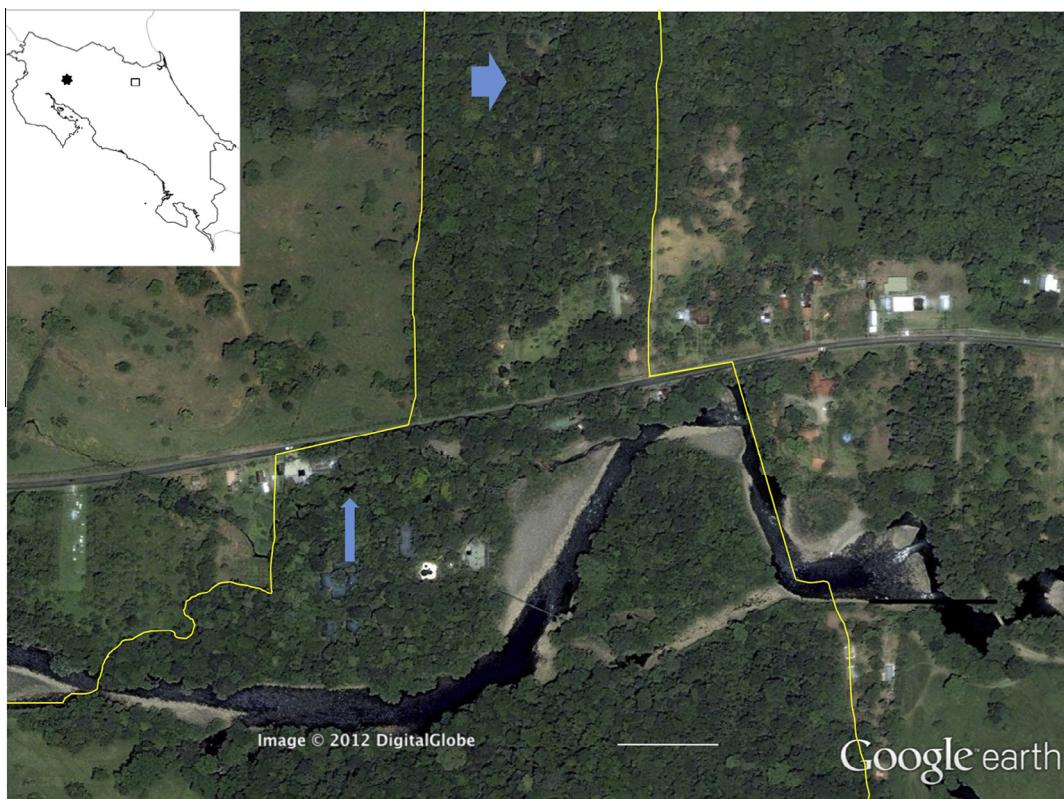


Fig. 1. Satellite image of Selva Verde and surrounding area showing the property of Selva Verde (outlined in yellow) with the creek site marked with narrow arrow and the pond marked with the thick arrow. White bar is 100 m. Inset: map of Costa Rica showing location of Selva Verde (black box) and La Pacifica (black star).

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