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ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

FIRST REGISTER OF *CONTRACAECUM* SP. AND *EUSTRONGYLIDES* SP. (NEMATODA) IN *KINOSTERNON SCORPIOIDES* (LINNAEUS, 1766) (TESTUDINES: KINOSTERNIDAE) FROM NORTHEASTERN, BRAZIL

PRIMER REGISTRO DE *CONTRACAECUM* SP. Y *EUSTRONGYLIDES* SP. (NEMATODA) EN *KINOSTERNON SCORPIOIDES* (LINNAEUS, 1766) (TESTUDINES: KINOSTERNIDAE) DEL NORESTE DE BRASIL

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Titulillo: *Contracaecum* sp. and *Eustrongylides* sp. in *Kinosternon scorpioides*

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ABSTRACT

Kinosternon scorpioides (Linnaeus, 1766) popularly known in Brazil as "Jurará" is a species of chelon that serves as a food resource and source of income for some families in Northeastern Brazil. In that sense, the present study aimed to analyze the metazoan endoparasites infecting *K. scorpioides* from the Baixada Maranhense, Brazil. Fifteen adult specimens were captured using funnel traps in flooded fields around the Aurá River, close to the city of São Bento-Maranhão. Samples were transported to the Postgraduate Multi-User Research Laboratory- Laboratories in Postgraduate Research/Postgraduate in Animal Science located at the State University of Maranhão- UEMA for parasitological analyses. The results revealed the presence of larvae of *Contracaecum* sp. infecting the stomach, intestine, and liver, while *Eustrongylides* sp. was found encysted in the musculature, stomach, intestine, and inside an egg of a gravid female mud turtle. The findings of the present study constitute the first records of these nematodes parasitizing *K. scorpioides*. Since *K. scorpioides* is consumed as food for some families in the State of Maranhão, Brazil, and the presence of both parasites with hygienic-sanitary importance, complementary studies should be designed to allow us to understand the relationship between host parasites and whether they could have zoonotic relevance.

Keywords: Baixada Maranhense – endoparasites – helminths – Jurará – Nematoda - scorpion mud turtle

RESUMEN

Kinosternon scorpioides (Linnaeus, 1766), conocido popularmente en Brasil como “Jurará”, es una especie de quelonio que sirve como recurso alimenticio y fuente de ingresos para algunas familias del Nordeste de Brasil. En ese sentido, el presente estudio tuvo como objetivo analizar los endoparásitos metazoarios que infectan a *K. scorpioides* de la Baixada Maranhense, Brasil. Quince especímenes adultos fueron capturados utilizando trampas de embudo en campos inundados alrededor del río Aurá, cerca de la ciudad de São Bento-Maranhão. Las muestras fueron transportadas al Postgraduate Multi-User Research Laboratory- Laboratories in Postgraduate Research/Postgraduate in Animal Science ubicado en la Universidad Estatal de Maranhão - UEMA para análisis parasitológicos. Los resultados revelaron la presencia de larvas de *Contracaecum* sp. infectando el estómago, intestino e hígado, mientras que

Eustrongylides sp. Se encontró enquistado en la musculatura, estómago, intestino y en el interior de un huevo de una tortuga de lodo hembra grávida. Los hallazgos del presente estudio constituyen los primeros registros de estos nematodos parasitando a *K. scorpioides*. Dado que *K. scorpioides* es consumido como alimento por algunas familias en el Estado de Maranhão, Brasil, y la presencia de ambos parásitos con importancia higiénico-sanitaria, se deben diseñar estudios complementarios que nos permitan comprender la relación entre los parásitos hospedadores y si pudieran tener relevancia zoonótica.

Palabras clave: Baixada Maranhense – endoparásitos – helmintos – Jurará – Nematoda – tortuga de lodo escorpión

RESUMO

Kinosternon scorpioides (Linnaeus, 1766) popularmente conhecido no Brasil como "Jurará" é uma espécie de quelônio que serve como recurso alimentar e fonte de renda para algumas famílias no Nordeste do Brasil. Nesse sentido, o presente estudo teve como objetivo analisar os endoparasitas metazoários que infectam *K. scorpioides* da Baixada Maranhense, Brasil. Quinze espécimes adultos foram capturados usando armadilhas de funil em campos alagados ao redor do Rio Aurá, próximo à cidade de São Bento-Maranhão. As amostras foram transportadas para o Laboratório de Pesquisa Multiusuário de Pós-Graduação - Laboratórios de Pesquisa de Pós-Graduação/Pós-Graduação em Zootecnia localizado na Universidade Estadual do Maranhão - UEMA para análises parasitológicas. Os resultados revelaram a presença de larvas de Contracaecum sp. infectando o estômago, intestino e fígado, enquanto *Eustrongylides* sp. foi encontrado encistado na musculatura, estômago, intestino e dentro de um ovo de uma tartaruga fêmea grávida. Os achados do presente estudo constituem os primeiros registros desses nematoides parasitando *K. scorpioides*. Como *K. scorpioides* é consumido como alimento por algumas famílias no estado do Maranhão, Brasil, e a presença de ambos os parasitas com importância higiênico-sanitária, estudos complementares devem ser delineados para nos permitir entender a relação entre os parasitas hospedeiros e se eles podem ter relevância zoonótica.

Palavras-chave: Baixada Maranhense – endoparasitas – helmintos – Jurará – Nematoda – tartaruga-escorpião

INTRODUCTION

Maranhão is a State that encompasses three of the main Brazilian morphoclimatic domains: Amazon, Caatinga, and Cerrado. The State represents an ecotone zone between these biomes, with 14 distinct vegetation types (IBGE, 2004), reflecting the transition between humid forests and semi-arid habitats (Da Silva, 2011).

The Baixada Maranhense region, placed in Maranhão, is located on the easternmost limit of the Amazon biome, forming part of the Brazilian Legal Amazon. This region is characterized by a landscape made up of lakes, fields, floodplains, igapós, landfills, river-marine systems, and dry land. The floodable natural fields of Baixada Maranhense are extremely complex environments from an ecological point of view, with very diverse structures and functioning (Costa-Neto *et al.*, 2002; Pinheiro, 2013).

Due to the recognized importance of the region's floodable natural fields, the Government of the State of Maranhão established the Baixada Maranhense Environmental Protection Area (APA) with Decree No. 11,900 of June 11, 1991 (State of Maranhão, 1991). In 2000 it was designated by the National Wetlands Committee (CNZU) as a Ramsar Site: regions that provide fundamental ecological services - meeting water and food needs - for species of fauna and flora and human populations, rural and urban. In addition to regulating the water regime of vast regions, they function as a source of biodiversity at all levels. It has the largest set of lake basins in the

Northeast, which contribute to high fishing productivity, the main basis of food and income support for its population (Costa-Neto *et al*., 2002).

Chelonians are animals that perform important ecosystem functions and are intrinsically linked in many traditional cultures, from the consumption and seed dispersal of various tree species to the important social role of communities that use river systems as a means of subsistence (Alves *et al*., 2008; Rhodin et al., 2018). *Kinosternon scorpioides* (Linnaeus, 1766) popularly known as the scorpion mud turtle and commonly named in Brazil as “Jurará” is a species of chelon widely distributed in the Neotropical region, occurring from Panama, along the Caribbean mountain range to Brazil, covering the entire Amazon forest, eastern mountain ranges of Colombia, Ecuador, and Peru.

Historically, many species of chelonians in different parts of the world have great nutritional, economic, and cultural importance, with their eggs, meat, viscera, fat, and shell being used intensively by humans (Van Dijk *et al*., 2014). In Baixada Maranhense, species such as *Phrynops geoffroanus* (Schweigger, 1812) and mainly *Kinosternon scorpioides* serve as a food resource and source of income for some families, being captured illegally, in adult and sub-adult form alive and hanging, and sold mainly from May to August, when females appear ovate. Wild populations are used for consumption as a regional delicacy, both for subsistence and recreational consumption (Machado Júnior *et al*., 2006; Medeiros, 2016).

According to Giari *et al.* (2022) in aquatic environments there exist metazoan parasites with zoonotic potential that can cause strong negative impacts on the safety and quality of the meat. These parasites can be of public health interest and can compromise the health of the consumers. Humans can be infected accidentally with zoonotic parasites by the ingestion of raw or poorly cooked meat (Giari *et al*., 2022).

Due to the consumption of *K. scorpioides* meat by the Brazilian population and the presence of parasites with zoonotic potential in aquatic organisms, the present study aimed to analyze the metazoan endoparasites infecting *K. scorpioides* from the Baixada Maranhense, Brazil.

MATERIAL AND METHODS

The study was carried out around the Aurá River, located in the Baixada Maranhense Environmental Protection Area (APA), in the Northeast of Brazil, on the eastern edge of the Amazon. This area represents a wetland of international importance (Ramsar Site), bringing together a set of river basins and flooded fields, and seasonal periods with great contrasts in temperature, humidity, and rainfall (Costa-Neto *et al*., 2002).

The collections were carried out in flooded fields around the Aurá River, city of São Bento-Maranhão (2°42'08,1"S, 44°51'40,3"W and 2°40'60,0"S, 44°49'00,0"W) (Fig.1). The animals were captured using funnel traps (Secco *et al*., 2013; Balestra *et al*., 2016).

Captured animals presented 13,73 cm. mean carapace length, 9,17 cm. mean carapace width, 12,60 cm. mean plaston length, 7,97 cm. mean plaston width, 4,87 cm. mean height and 342,40 g. mean weight. Samples were transported at the Postgraduate Multi-User Research Laboratory- Postgraduate Multi-User Research Laboratory-Laboratories in Postgraduate Research/Postgraduate in Animal Science located at the State University of Maranhão- UEMA Campus Paulo VI. The samples were collected in the region of the Municipality of São Bento-MA, belonging to the APA of Baixada Maranhense, Sítio Ramsar.

The musculature was analyzed and carefully observed. Internal organs were analyzed using a stereoscope Leica EZ4. Organs were placed in Petri dishes with distilled water, and with the aid of tweezers and needles, the tissue was examined by making fine cuts that allowed to observe inside them. In the presence of any parasites, these were removed with tweezers and preserved in ethanol 70% for posterior taxonomic identification.

For taxonomic identification, cysts with nematodes were placed in Petri dishes, and then, with the aid of needles, the cysts were broken, liberating the larvae, and then preserved in 70% ethanol for posterior analyses. For the identification of nematodes, they were placed into glass slides with Lactic Acid, which is used for the clarification of parasite tissue and consequent visualization of external structures and internal organs (Morey, 2019). All parasites mounted in slides were observed under an optical microscope Leica DM750. Based on the morphological characteristics of the parasites, the identification was made using the information of Moraveck (1998) and Morey *et al.* (2022). Parasitological indices were calculated according to Bush *et al.* (1997).

Ethic aspects: The collection of samples was carried out under the permissions granted by the Biodiversity Authorization and Information System (SISBIO), number 85805-1.

RESULTS

The nematodes *Contracaecum* sp. and *Eustrongylides* sp. were found parasitizing *K. scorpioides*. *Contracaecum* sp. was found in the stomach, intestine, and liver (Fig. 2, while *Eustrongylides* sp. was found encysted in the musculature (Fig. 3) stomach, intestine and inside the egg of a gravid female *K. scorpioides* (Fig. 4). Both parasites were found encysted, being necessary to break the cyst to liberate the larvae. Parasitological indices are presented in Table 1.

Contracaecum sp. medium-sized nematodes, opaque-white when alive. Transversely striated cuticles present and more distinct at the extremities of the body, with the anterior region interrupted by a short, lateral line. The cephalic extremity is rounded with a small, ventral cuticular tooth. The oesophageal muscle is narrow, the ventricle is small and rounded, and the ventricular appendix is a short, long intestinal caecum, extending anteriorly to the nerve ring. Tail conical without mucron (Fig. 5A-C).

Eustrongylides sp. presented a cephalic extremity with a small oral cavity surrounded by 12 cephalic papillae of similar size arranged in two concentric rings, and genital primordia in the posterior part of the body (Fig. 5D-F).

According to the results of the present study, it can be assumed that in the life cycle of *Eustrongylides* sp. and *Contracaecum* sp., different organisms are involved: invertebrates play the role of intermediate hosts, while fish and *K. scorpioides* act as paratenic hosts. Finally, given that *K. scorpioides* are preyed upon by Amazonian alligators, it can be inferred that they act as final hosts for these nematodes in territories of the Baixada Maranhense, Maranhão, Brazil (Fig. 6).

DISCUSSION

Studies focusing on South American turtles are still recent and scarce (Bursey & Brooks, 2011; Mascarenhas *et al*., 2013). Few studies have examined the nematodes parasites of *K. scorpioides* in Brazil: *Serpinema magathi* from specimens collected in Pará (Alho, 1965); Freitas and Dobbin Jr. (1971) from Pernambuco; *S. monospiculatus* from specimens collected in a semiarid region in the Northeast (Pereira *et al*., 2018): *S. pelliculatus* was found from the gastrointestinal tract of specimens collected in Baía do Capim (municipality of Abaetetuba) and Ilha do Marajó (municipality of Soure), Pará, Brazil (Silva *et al*., 2023). These parasites are commonly found in freshwater turtles worldwide (Hidalgo-Vila *et al*., 2009; Bursey & Brooks, 2011; Moraga *et al*., 2012; Mascarenhas *et al*., 2013; Mascarenhas & Muller, 2015).

For northeastern Brazil, the nematodes *Serpinema magathi and Spiroxys figueiredoi* have been found in the digestive tract of *K. scorpioides* (Viana *et al*., 2016). In the present study, none of these nematodes were found on specimens captured in the municipality of São Bento, Maranhão, Brazil. In the present study, the findings of *Contracaecum* sp. and *Eustrongylides* sp. are the first records of *K. scorpioides*.

Since *K. scorpioides* is an omnivorous species that feeds on algae, plants, seeds, and a variety of insects, mollusks, fish, and small amphibians (Berry & Ivwerson, 2001; Berry & Iverson, 2011), its feeding habits facilitate the infection of this freshwater turtle by different parasitic nematode larvae present in aquatic environments, such as *Contracaecum* spp. and *Eustrongylides* spp.

Contracaecum larvae show low specificity, are often found in abnormal hosts, and do not develop into adults in turtles (Shubber *et al*., 2020). In this sense, it can be assumed that *K. scorpioides* acquired the nematodes by feeding on fishes in their ecosystems, a fact that was supported by a personal observation made in the field during the capture of the animals in this study.

In the life cycle of *Eustrongylides* spp. freshwater oligochaetes act as intermediate hosts; fish as paratenic hosts and birds as fin7al hosts (Spalding, 2008). *Eustrongylides* spp. can infect poikilothermous hosts, as was demonstrated by experimental infections with piscine, amphibians, and reptilian hosts (Cooper *et al*., 1978). The presence of nematode larvae in *K. scorpioides* indicates that this turtle acts as the paratenic host of *Contracaecum* sp. and *Eustrongylides* sp. Natural predators of this mud turtle are documented as jaguars (Savage, 2002), owls (Pereira *et al*., 2007), vultures, green iguanas (Acuña-Mesén, 1998), and alligators (Savage, 2002). The presence of *Eustrongylides* sp. inside the egg of a gravid female *K. scorpioides* can be assumed as a strategy of the parasite to reach easily the final host, since eggs deposited by females are more vulnerable to predators, being predated by birds, terrestrial mammals, and reptiles, facilitating the contact of the nematode with the final host.

In that way, due to the feeding habitat of this chelonian, it is assumed that invertebrates are the intermediate hosts, fish, and *K. scorpioides* paratenic hosts, and alligators are the final hosts.

Kinosternum scorpioides from the Baixada Maranhense are parasitized by the nematodes *Contracaecum* sp. and *Eustrongylides* sp. Since *K. scorpioides* is consumed as food for some families in the State of Maranhão, Brazil, and the presence of both parasites with hygienic-sanitary importance, complementary studies should be designed to allow us to understand the relationship between host parasites and whether they could have zoonotic relevance.

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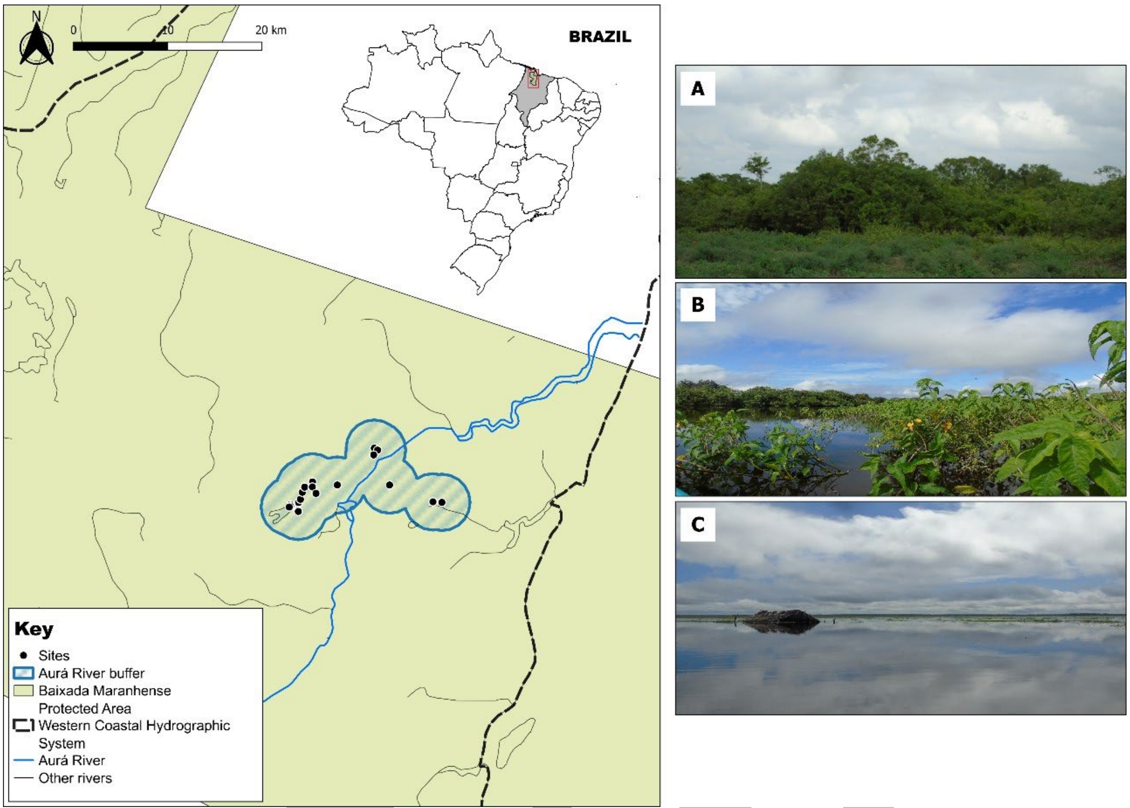

Figure 1. Study area. Identification of collection points distributed along the Aurá River (blue) A-Forest areas; B- Flooded fields; C- Water areas (River). Source: IBGE (2018); ZEE (2019); MapBiomas (2021).

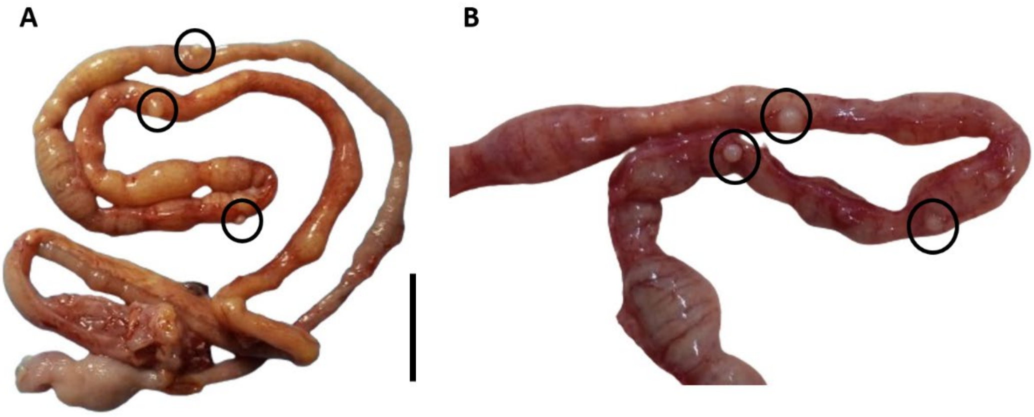

Figure 2. A. Encysted *Contracaecum* sp. in internal organs of *Kinosternon scorpioides*. Black circles present the cyst of the parasites. Scale bar: 2 cm.

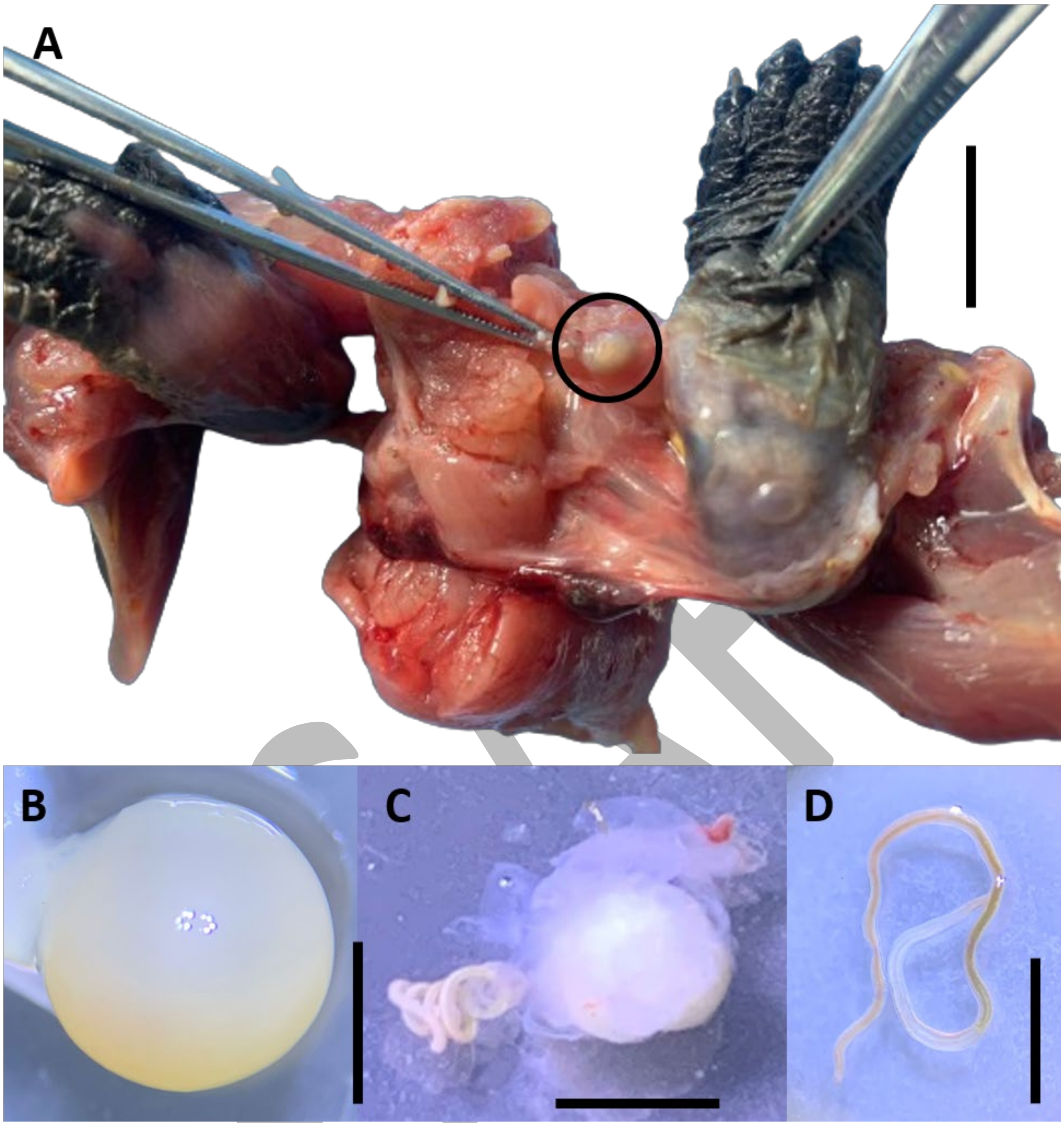

Figure 3. A. *Eustrongylides* sp. in the musculature of *Kinosternon scorpioides*. B. Cyst. C. *Eustrongylides* sp. being released from a cyst. D. Free *Eustrongylides* sp. Scale bar: A, B = 2 cm. C-E = 0.5 cm

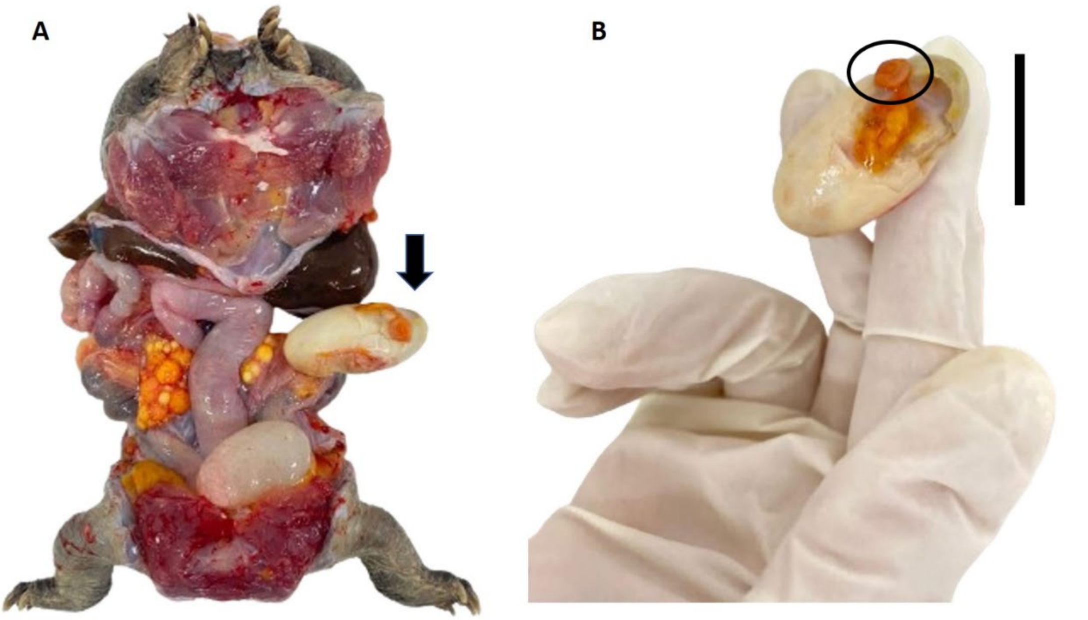

Figure 4. A. *Eustrongylides* sp. in an egg of *Kinosternon scorpioides*. The black arrow indicates the egg with the parasite. B. Broken egg showing the nematode inside. Scale bar: 2 cm.

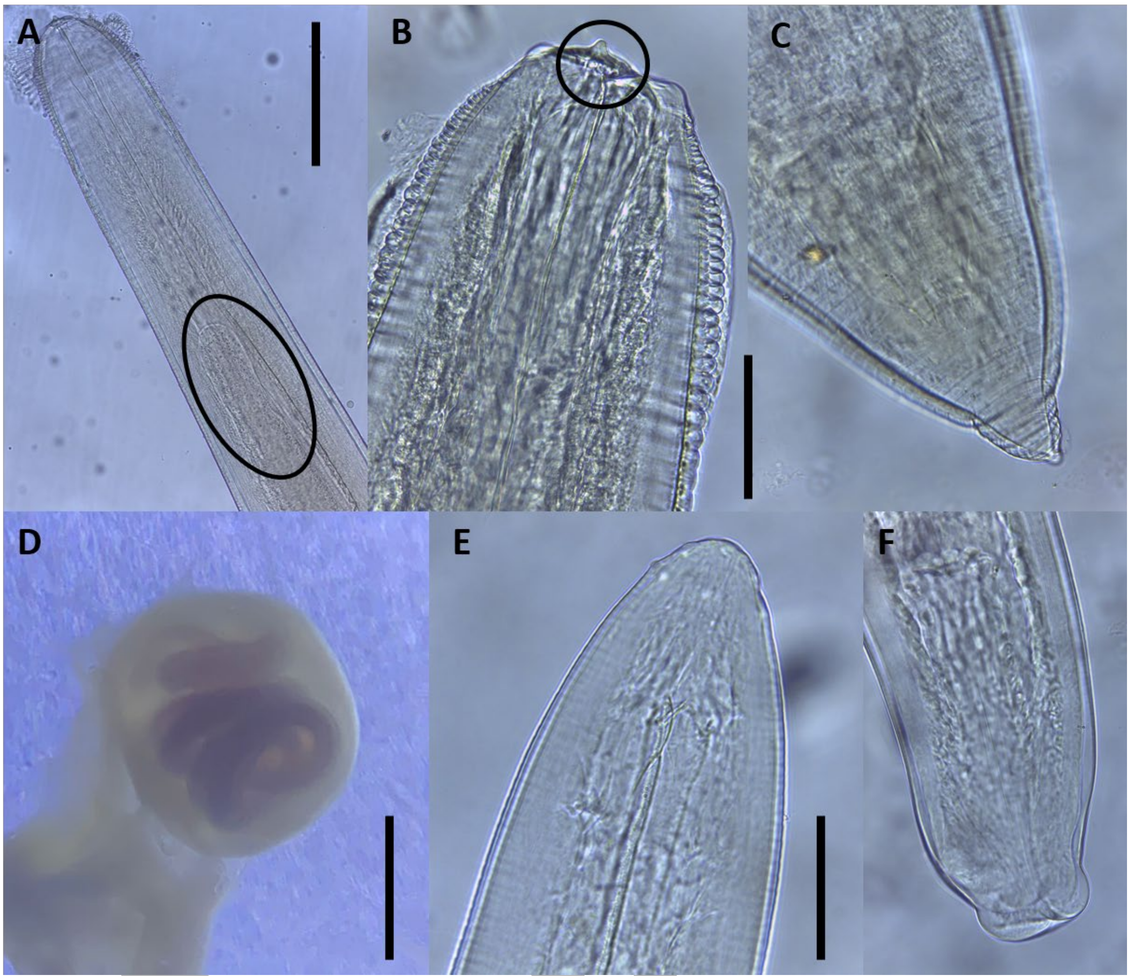

Figure 5. A. Microphotography of the anterior part of *Contracaecum* sp. Dark circle showing intestinal cecum. B. Microphotography of larval tooth (dark circle). C. Microphotography of the tail of *Contracaecum* sp. D. Encysted *Eustrongylides* sp. E. Microphotography of the anterior part of *Eustrongylides* sp. F. Microphotography of the tail of *Eustrongylides* sp. Scale bar: A = 50 μm. B, C = 20 μm. D = 500 μm. E, F = 20 μm.

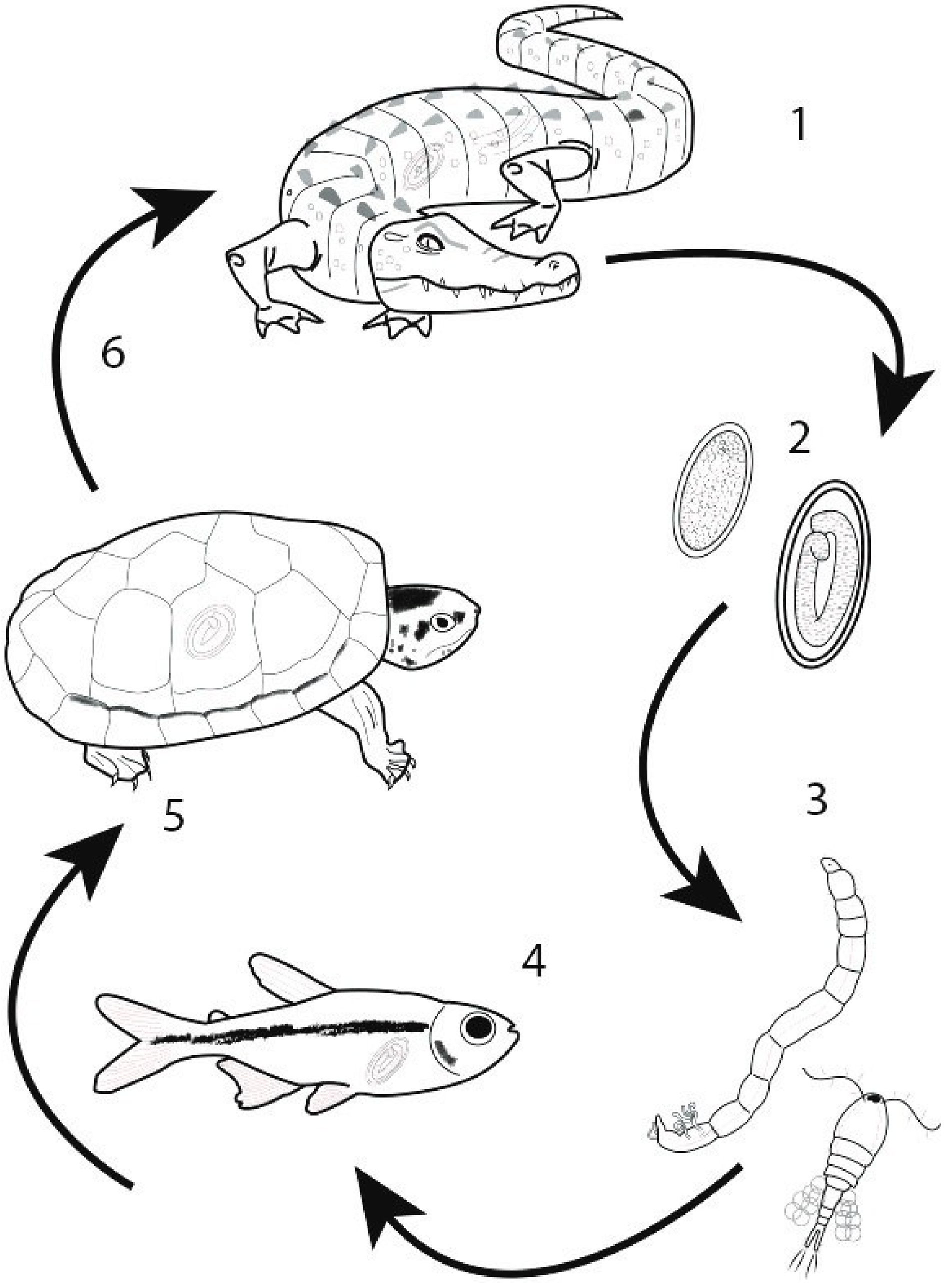

Figure 6. The life cycle of Nematoda larvae of *Eustrongylides* sp. and *Contracaecum* sp. 1. Nematoda eggs are liberated from the alligator. 2. Eggs with infective L1 larvae. 3. Eggs are consumed by an annelid (for *Eustrongylides* sp.) or for a copepod (for *Contracaecum* sp.). Nematoda larvae develop inside the invertebrates from L1 into L2 and L3. Invertebrates act as intermediate hosts. 4. Fish consume the invertebrates with

Nematoda larvae. Fish are paratenic hosts. 5. Fish are consumed by *Kinosternum scorpioides.* The mud turtle acts as a paratenic host. 6. *Kinosternon scorpioides* are consumed by an alligator. The alligator acts as the final host of Nematoda larvae of *Eustrongylides* sp. and *Contracaecum* sp.

Table 1. Parasitological indices of endoparasites reported in Kinosternon scorpioides. AS = Analyzed specimen. PS = Parasitized specimen. P% = prevalence. TNP = total number of parasites. mI = mean intensity of infection. mA = mean abundance of infection.

Parasite	**AS**	**PS**	**P%**	**TNP**	**mI**	**mA**
Contracaecum sp.	15	11	73.33	19	1.72	0.73
Eustrongylides sp.	15	12	80	32	2.66	2.13