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First record of Acanthocephala parasites eggs in coprolites preliminary assigned to Crocodyliformes from the Adamantina Formation (Bauru Group, Upper Cretaceous), São Paulo, Brazil

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Abstract: This study presents the oldest record of Acanthocephala parasite eggs in coprolites preliminary assigned to Crocodyliformes, recovered in the region of Santo Anastácio Municipality, Southwestern São Paulo State. For this, a paleoparasitological investigation was carried out on 53 mineralized coprolites (complete or fragmented), with round shape or cylindrical shape of rounded or pointed ends, 0.2 - 3.9 cm in length x 0.1 - 2.4 cm in diameter, 3.7 grams in weight, and absence of food remains. Individual samples of the surface and internal portions of each coprolite were extracted by electric drill, dissociated with Cloridic Acid 10% solution, washed with Distilled Water, and filtered in granulometric screen Mesh / Tyler 325. After laboratory processing, the sediments retained on the granulometric screen was studied with Glycerin under optical microscopy, and the presence of four Acanthocephala eggs could be observed in sample of only one of these ichnofossils. All specimens were well preserved and showed 72.5 - 85 µm in length x 27.5 - 50 µm in width, elliptical shape, three concentric and thick shells, and embryos in their interior. This study inaugurates investigations and knowledge about Paleoparasitology in Crocodyliformes coprolites from the Bauru Group, Upper Cretaceous from the Paraná Basin.

Key words: Acanthocephala, Bauru Group, Crocodylomorpha, Helminth eggs, Upper Cretaceous.

INTRODUCTION

Paleoparasitology is developing as an important division of Paleobiology / Paleontology since beginning of XX Century (Hugot et al. 2014). Parasites fossils of animals have been found since Cambrian, around 500 million years ago (Araújo and Ferreira 2000).

Investigations about parasites in coprolites, including groups of extinct animals, can provide valuable information about the geological moment when these organisms were fossilized, as well as aspects of the interaction parasite-host, casually influencing evolutionary changes resulting in the modern morphotypes (Dentzien-Dias et al. 2013). Therefore, it is an investigation about origin, diversification and evolution of parasitism through geological time (Stock Da-Rosa 2008, De Baets et al. 2015).

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In this context, coprolites are rich source of information, because they provide, besides possible presence of parasites, biological aspects of their hosts, just like food habits, but also paleoenviromental and paleoclimatic data, allowing better understanding of this complex inter specific relation, involving parasite, host, ecosystem (Silva et al. 2014, Qvarnström et al. 2016, Dentzien-Dias et al. 2018). Additionally acquistion of this kind of fossilized material, associated to skeletons of extinct animals is common (Ferreira et al. 2011), though they received few attention (Oliveira and Santucci 2017), and they are not much explored yet for paleoparasitological investigations.

Paleoparasitological evidence in coprolites are rare. Dinosaurs, crocodilians, birds, sharks, cinodonts, mammals and other vertebrates are known to have intestinal parasites thru the analyses of their coprolites since Permian (Reinhard et al. 1986, Poinar and Boucot 2006, Fugassa et al. 2008, Dentzien-Dias et al. 2013, 2018, Wood et al. 2013, Hugot et al. 2014, Silva et al. 2014, Bajdek et al. 2016, Francischini et al. 2018). Even so, the few contributions, published until now in the Brazil and other countries, revealed new genera and species of parasites, and new records of hosts, demonstrationg important possibilities to be explored (Fugassa et al. 2008, Hugot et al. 2014, Silva et al. 2014), and abundant sources of knowledge.

This contribution is the oldest record of Acanthocepha helminths eggs, present in coprolites preliminary assigned to Crocodyliformes, chronologically related to Campanian / Maastrichtian, Upper Cretaceous, possibly between 80 and 70 millions years ago.

MATERIALS AND METHODS

AREA OF INVESTIGATION AND SAMPLES

Among Crocodyliformes bones, 53 coprolites (29 complete and 24 fragmented) were recovered (Fig. 1), from Upper Cretaceous sedimentary rocks of the Bauru Group, Adamantina Formation (Fig. 2), Santo Anastácio Municipality, São Paulo State (Fig. 3), collected between 1988 and 1997 (Bertini 1993). They were preserved in glass flasks and housed in "Museu de Paleontologia e Estratigrafia Paulo Milton Barbosa Landim", São Paulo State University - UNESP, Rio Claro *Campus*.

These ichnofossils, preserved by phosphatization, showed in general 1.3 cm in average length (range: 0.2 - 3.9), 0.8 cm in average diameter (range: 0.1 - 2.4), 3.7 grams in average weight (range: 1.0 - 20.0), round shape or cylindrical shape with rounded or pointed ends. This morphology and the absence of food remains, such as bone fragments, are typical characteristics of Crocodylomorpha coprolites, due to the efficacy of the digestive system of these archosaurs (Souto 2010, Lucas et al. 2012, Milàn 2012, Dentzien-Dias et al. 2018). In addition, some coprolites exuded during their laboratory processing a strong odor of uric acid, similar to that observed in the feces of modern crocodilians.

The Adamantina Formation is one of the most important geological units from the Upper Cretaceous Bauru Group in the Western São Paulo State, which comprises predominantly fluvial deposits (Fernandes and Coimbra 2000, Oliveira and Santucci 2017), with a rich fossil assemblage represented by an abundance and diversity of crocodylomorphs (more than 10 different groups) and other vertebrates (fishes, lizards, testudines, dinosaurs and mammals), invertebrates (gastropods, bivalves and ostracods), plant fragments and palynomorphs. Additionally, vertebrate ichnofossils (coprolites and fossil eggs) are often found in this same locality in association with crocodylomorphs bones (Bertini et al. 1993, Azevedo et al. 2000).

The fossil locality, where these coprolites were recovered, was firstly prospected by L. I. Price during 1940s (Campos and Castro 1978), and it is situated on the outskirts of Santo Anastácio, Southwestern

Figure 1 - Some coprolites preliminary assigned to Crocodyliformes recovered from the Bauru Group, Adamantina Formation, Santo Anastácio Municipality, São Paulo State.

São Paulo State. Between 1988 and 1997, it was an abandoned quarry, however, currently it is part of the urban area of this municipality, being equivalent to a quarter of it (Fig. 4). Lithologically it is composed by medium to coarse conglomeratic sandstones, with centimetric and decimetric mudstones peebles of fluvio-lacustrine origin (Fig. 2).

LABORATORIAL ACTIVITIES

The morphometric measures of each coprolite were obtained through caliper rule, and its weight was gotten individually, with a digital balance.

Superficial and internal fragments of each coprolite were extracted with an electric drill, according to Silva et al. (2014), until acquisition of some macerated material. As paleoparasitological processing required the use of individual samples with approximately 1 gram of each coprolite, several of these were totally destroyed. This product obtained was packaged in polipropilene tubes, Model Falcon, capacity of 15 mL, rightly identified.

It was added a Cloridic Acid solution, concentration 10 %, according to Ferreira et al. (2011). After this dissociation process, the reaction

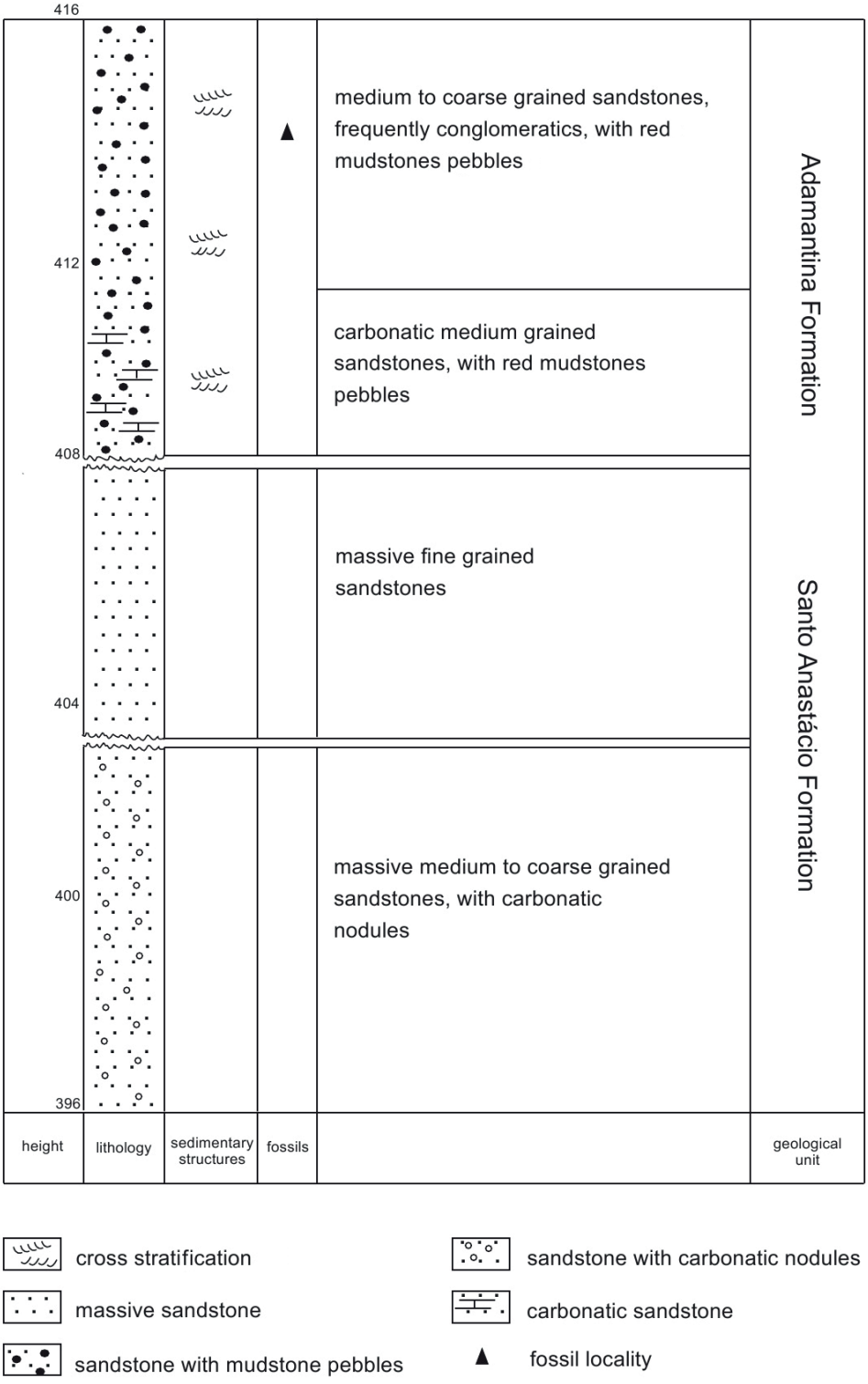

Figure 2 - Columnar section from the abandoned quarry on the outskirts of the Santo Anastácio Municipality, Southwestern São Paulo State.

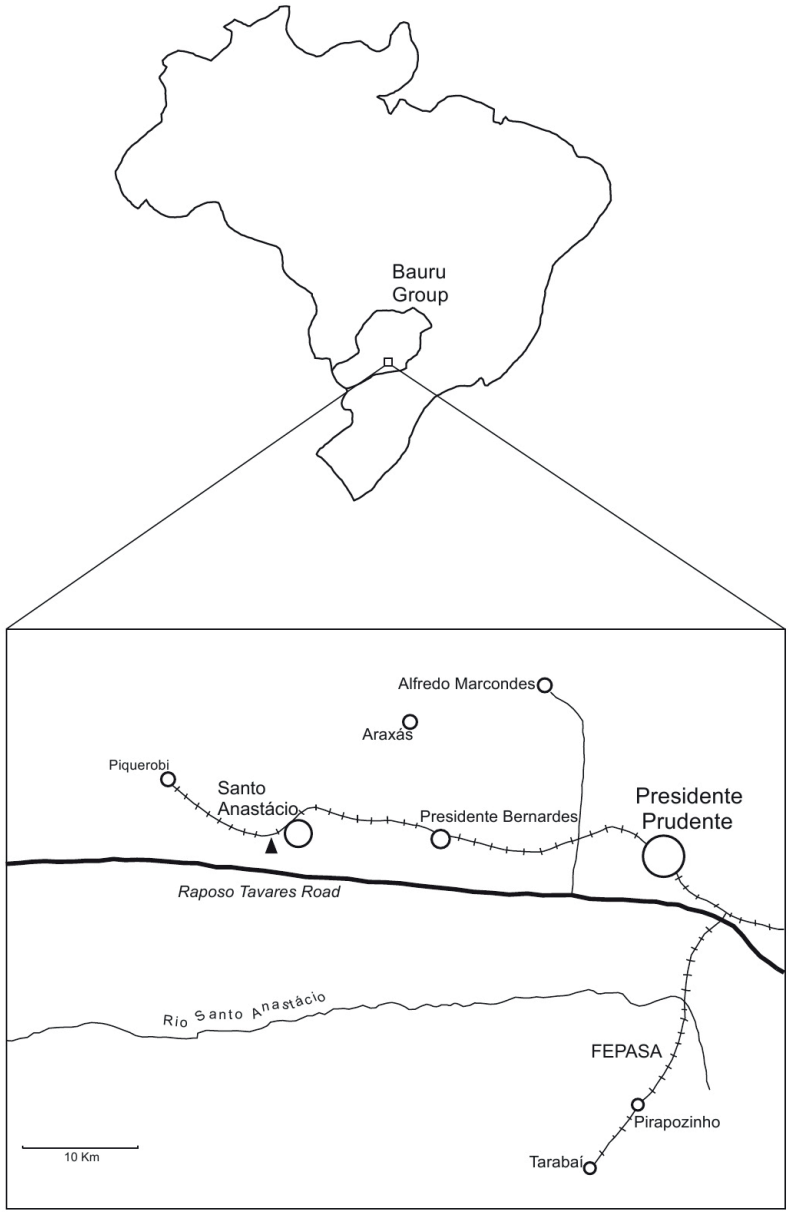

Figure 3 - Geographical situation of the fossil locality where the coprolites were recovered; Fossil locality (▲).

was interrupted adding Distilled Water in double volume.

The solution obtained crosses through successive washing with Distiled Water, followed by screening in granulometric screen Mesh / Tyler 325, according to Bouchet et al. (1999). The sediment of each sample, retained on the granulometric screen, was again washed in Distilled Water. To each drop of material was added three drops of Glycerin, between lamina and thin microscopic slide. Finally it was utilized an optical microscope, in the increase from 100 to 400 times.

RESULTS

From a total of 53 coprolites analyzed, only a single sample (Fig. 5) was positive for eggs of

Figure 4 - The abandoned quarry Southwestern Santo Anastácio City, São Paulo State; an image from 1990.

Figure 5 - Positive coprolite for Acanthocephala eggs in paleoparasitological examination from Crocodylomorpha host recovered in Santo Anastácio Municipally, São Paulo State, Brazil.

Acanthocephala helminths (Fig. 6). This coprolite was fragmented, probably a rounded end, showed 1.6 cm in length, 1.0 cm in diameter and 4 grams in weight. In addition, other inclusions were observed under optical microscopy in this ichnofossil, such as eggs of Ascaridoidea nematodes and fungal microconidias.

All four eggs of Acanthocephala (Figs. 6-7) were well preserved and showed elliptical shape, containing in their interior an embryo, named "Acanthor", with 42.5 - 45.0 µm long by 12.5 - 17.5 µm wide, elliptical shape and inconspicuous hooks in the anterior portion of their body, wrapped by three distinguished, concentric and thick shells. The individual measures of each egg were obtained with micrometric ocular, achiveing following values. Egg 1: 85 µm in length x 47.5 µm in width (Fig. 6a). Egg 2: 75 µm in length x 27.5 µm in width (Fig. 6b). Egg 3: 82.5 µm in length x 50 µm in width (Fig. 6c). Egg 4: 72.5 µm in length x 35 µm in width (Fig. 6d).

DISCUSSION

Paleoparasitology can reveal important informations about relations parasite / host in the geological past, allowing to extrapolate data concerning paleoclimatic, paleoenvironment and evolutionary investigations, based on kind of parasitic infection (Ferreira et al. 2008, 2011).

The characteristic morphology of the eggs, presenting multiple layers (shells), elliptical shape, containing "Acanthor" in their interior, safely allowed to identify them as belonging to Acanthocephala, a group of parasites helminths, phylogentically related to Rotifera. Adult acanthocephalans can measure from milimeters to 65 cm long showing, as main common characteristic, a reversible proboscis, equiped with spines. They fix to the intestinal mucous membrane, where they feed directly absorbing nutrient from their definitive hosts, as fishes, amphibians, reptiles, birds and mammals (García-Varela and Pérez-Ponce de León 2015, Mehlhorn 2016).

The parasites belonging to this taxonomic groups show complex life cicles, comprehending at last two hosts species, the first always represented by an invertebrate, the second by a vertebrate. The crocodylomorphs also take part in the ensemble of the vertebrate hosts of these parasites (Kennedy 2006, Santos et al. 2013).

It is possible this group of parasites emerged during the chronological interval Cambrian - Ordovician (Littlewood and Waeschenbach 2015, Poinar 2015), with arising of the arthopods hosts.

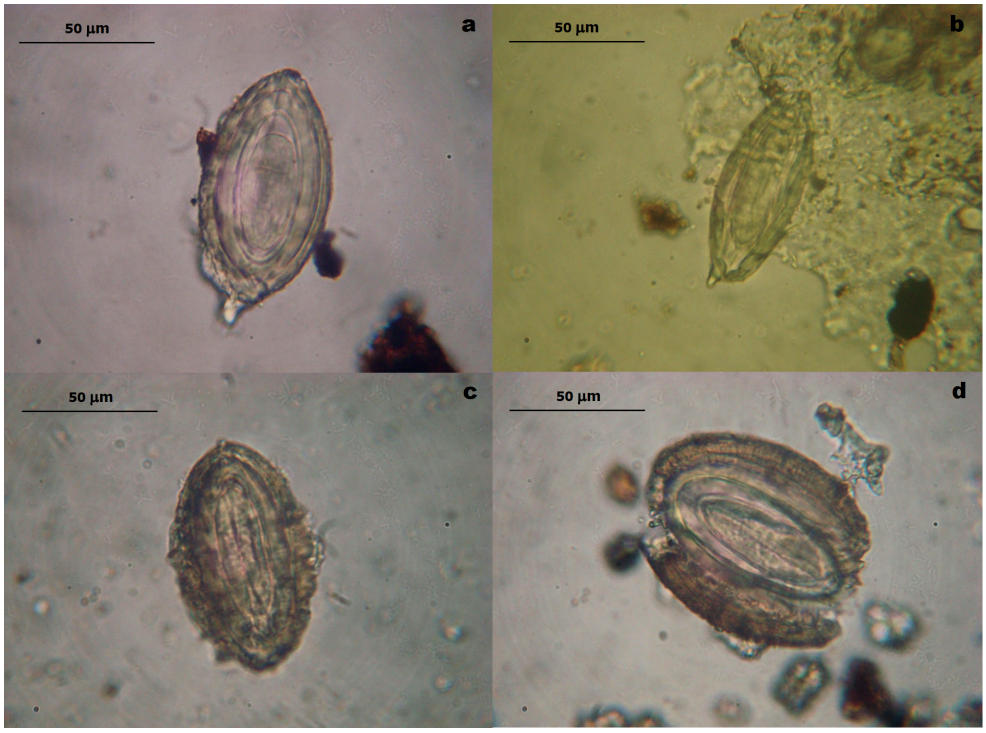

Figure 6 - Photomicrographs of Acanthocephala eggs recovered in coprolite preliminary assigned to Crocodyliformes from the Adamantina Formation, Bauru Group, Upper Cretaceous, Santo Anastácio Municipality, São Paulo State, Brazil (400 X magnification). **a.** Egg 1; **b.** Egg 2; **c.** Egg 3; **d.** Egg 4.

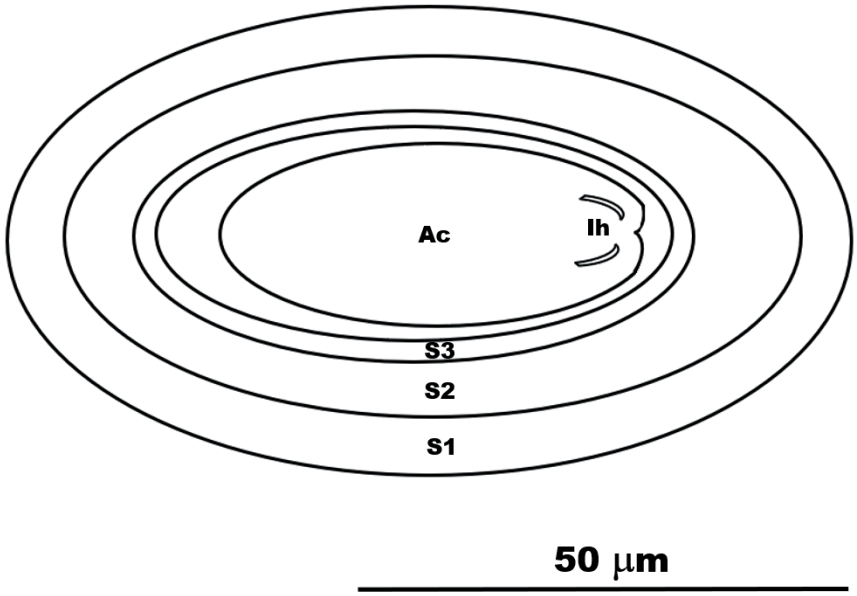

Figure 7 - Representative drawing showing the morphology of Acanthocephala egg in coprolite preliminary assigned to Crocodyliformes dated around 80-70 million years old. Abbreviations: **S1** = first shell; **S2** = second shell; **S3** = third shell; **Ac** = acanthor; **Ih** = inconspicuous hooks in the anterior portion of acanthor.

However some investigations in the Brazil and other countries identified Acanthocephala eggs only in coprolites of almost 10,000 years old belonging to humans (Horne 2002, Gonçalves et al. 2003, Fugassa et al. 2011), xenarthrans and carnivorous mammals (Ferreira et al. 1989, Noronha et al. 1994, Sianto et al. 2014, Mowlavi et al. 2015, Beltrame et al. 2016), and birds of prey (Beltrame et al. 2015).

Predatory habits can explain the way of acquistion of these parasites, suggesting Crocodyliformes hosts, obtaining parasitism feeding on crustaceans and / or fishes.

This group of parasites is found in hot tropical climates, demonstrating the climatic characteristic of the Bauru Group during Upper Cretaceous, on Southeastern Brazil (Bertini 1993).

The acanthocephalans found in modern reptiles show morphological and morphometric relations (elliptical shape, appearance of shells and size) with those encountered in this investigation (Jacobson 2007, Taylor et al. 2015), demonstrating possibly present reptilian amniotes acquired parasitism through evolutive heritage from their primitive ancestors.

Paleoparasitological findings obtained through this investigation, utilizing Crocodyliformes coprolites coming from Bauru Group, Adamantina Formation of the Southwestern São Paulo State, mention the most ancient record of Acanthocephala described in the literature, recovered in coprolites dating around 80 to 70 million years, probably Campanian age of the Upper Cretaceous.

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AUTHOR CONTRIBUTIONS

D.F.F. Cardia worked on paper design, data interpretation and writing. R.J. Bertini worked on the final essay and critical review. L.G. Camossi and L.A. Letizio worked on methodology and writing.

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