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First report of a morulated Ascaridoidea (Nematoda) egg in an avian coprolite from the Paleogene of the Paraíba Valley, State of São Paulo, Brazil

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Abstract

Ascaridoidea (Nematoda) is a widespread superfamily of nematodes that comprises gastrointestinal parasites from all major groups of vertebrates. Although this taxon probably emerged in the Carboniferous, its Brazilian fossil record includes mostly eggs, found in ancient remains, collected in paleontological and archeological sites from the Mesozoic and Cenozoic Eras. The Tremembé Formation (Oligocene of the Taubaté Basin) has become an important source for paleoparasitological studies in avian coprolites during the third decade of the 21st century, with reports of eggs only at only a single cell stage, of embryonic development. Here we present the first egg of Ascaridoidea preserved containing morula, from a bird coprolite recovered from the shales of the Tremembé Formation. Three coprolites, from the outcrop of Aligra Comércio de Argila S/A, Taubaté municipality (State of São Paulo), were rehydrated and subjected to spontaneous sedimentation. Based on morphological and morphometric features and diet and zoopaleontological context, the trace fossils were assigned to piscivorous birds. The egg found showed morphological characteristics typical of Ascaridoidea: namely spherical form, ornamented, and somewhat thick shell. Moreover, this superfamily includes several taxa that infect piscivorous birds and fish in heteroxenous life cycles and produce eggs with similar features as the egg found in the present study. The paleoparasitological information associated with the paleofaunistic diversity of birds and fish from the Tremembé Formation, reveal that the ancient Brazilian paleoenvironments provided subsidies for the rise and success of nematodes infecting these animals during the Paleogene.

Introduction

Ascaridoidea (*sensu* Hodda, 2022) is a widespread superfamily of nematodes comprising gastrointestinal parasites infecting mammals, including humans, birds, reptiles, amphibians, and fish (Hartwich, 2009). This taxon probably emerged in the Carboniferous parasitizing terrestrial tetrapods and, subsequently, moving to aquatic environments and infecting other hosts like bony and cartilaginous fishes (Li *et al*., 2018).

The Brazilian fossil record related to ascaridoids includes mostly eggs found in ancient remains collected in paleontological and archeological sites and, even though the egg morphology is rather uniform and unspecific, some species have been proposed based on these immature forms. For example *Ascarites rufferi*, was described from an egg found in a coprolite of Cynodontia, the taxonomic bridge between reptiles and mammals, which was recovered in the Santa Maria Formation, Paraná Basin, Brazil (Silva *et al*., 2014). *Bauruascaris cretacicus* and *B. adamantinensis* were proposed based on eggs found in coprolites of Crocodilomorpha from the Adamantina Formation (Bauru Group, Brazil) (Cardia *et al*., 2018; 2019). In addition, Poinar & Boucot (2006) described the species *A. priscus* and *A. gerus* from eggs in a single dinosaur coprolite. Ascaridoid parasitic forms have been more frequently reported in Cenozoic ancient remains dated from the Holocene (Gonçalves *et al*., 2003; Leles *et al*., 2008; Camacho *et al*., 2013; Jaeger *et al*., 2013a, 2013b; Sianto *et al*., 2014; Guedes *et al*., 2020; Iñiguez *et al*., 2022).

The Tremembé Formation (Oligocene of the Taubaté Basin), aged between 33.9 and 23.03 million years ago, is an intercalation between shale and clay layers with lacustrine origin, and it has become an important source for paleoparasitological studies in avian coprolites, providing

different well-preserved morphotypes of helminth eggs, protozoan cysts, and non-sporulated oocysts (Carmo *et al.*, 2023; Carmo *et al.*, 2024a). Currently, the helminth eggs from this geological unit have been reported only at a single cell stage of embryonic development (Carmo *et al.*, 2023). Here, we report the first egg of Ascaridoidea preserved containing morula, from a single bird coprolite recovered from the Paraíba Valley, as well as discuss some insights into the potential of its lower taxon of origin.

Materials and methods

Three coprolites were recovered from sedimentary rocks of the Tremembé Formation, Taubaté Basin, in 2023 at the outcrop of Aligra Comércio de Argila S/A, Taubaté municipality (State of São Paulo). Samples were rehydrated in 10 mL of 0.5% trisodium phosphate (Na_3PO_4) solution for 72 h (Callen, 1967; Fugassa *et al.*, 2006) and subjected to spontaneous sedimentation for 24 h (Hoffman *et al.*, 1934). A drop of sediment was placed on a glass slide, together with a drop of glycerine, covered with a coverslip (Ferreira *et al.*, 2014), and observed using a light microscope Nikon Eclipse Ei with image capture system PrimeCam Intervision 12. A total of 20 slides were mounted for each sample of coprolite. Parasitological identification was performed based on morphology and morphometry (Ferreira *et al.*, 2014); because there is no general literature for taxonomic identification of nematode eggs, the present identification was based on the expertise of the authors and comparisons with specific literature (see results and discussion for details). The avian origin of the coprolite was interpreted based on criteria adopted by Castro *et al.* (1988), Souto (2017), and Carmo *et al.* (2023). Two females of *Contracaecum* Railliet & Henry, 1912 (Ascaridoidea, Anisakidae) were collected from a single bird of the species *Nannopterum brasilianum*, and their uteri were dissected to obtain eggs that were used for comparison with the fossilized material. The genus *Contracaecum* was identified based on the following criteria: three well-developed labia, excretory pore at the base of subventral labium, presence of ventriculus with posterior appendix and intestinal caecum (Hartwich, 2009; Gibbons, 2010; Supplementary Figure S1). We also provided an micrography of an eimeriid oocyst (Fig. 1B), found by our research group in a previously analyzed sample, from the same geological formation, for comparisons.

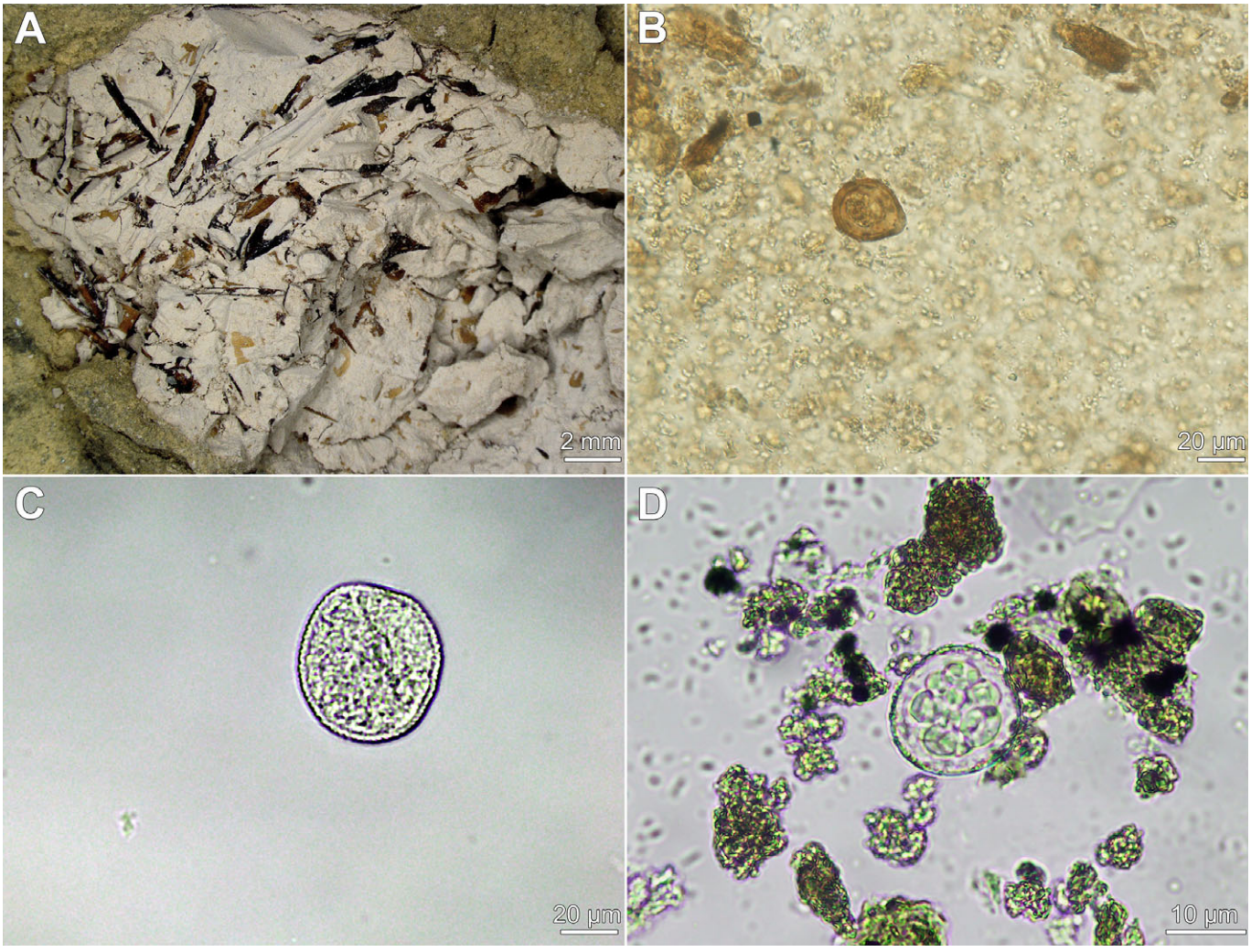

Figure 1. Avian coprolite with fish bone fragments (A), eimeriid oocyst (B), *Contracaecum* egg (C), and fossilized egg of Ascaridoidea (C) from the Tremembé Formation, Taubaté Basin, State of São Paulo, Brazil.

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Results and discussion

Coprolites were ovoid, grayish-yellow in colour, 10.80- to 28-mm long and 6.93- to 16.30-mm wide, containing fragmented remains of fishes (Fig. 1A). Based on morphological and morphometrical features, diet remains, and zoopaleontological context, the samples were assigned to piscivorous birds (Avialae, Neornithes). Some bird species may have benefited from periodic fish mortality because of seasonality (dry and wet seasons), which possibly supported the great success of this feeding habit in the Paraíba Valley during the Oligocene (Olson & Alvarenga, 2002).

A single slide from a single coprolite was positive for the parasite form, which was represented as a spherical egg, measuring 34.21 × 32.44 µm, containing morula, translucent, with somewhat thick and ornamented shell (Fig. 1D). The identification of immature parasitic forms (*i.e.*, eggs and larvae), preserved without adult worms or genetic material, is rather difficult and generalist because these forms hold ancestral ontogenetic traces that are common within a higher taxa (*i.e.*, family, order), show homogeneous morphology and lack specific traits (Carmo *et al.*, 2024b). Nevertheless, the egg exhibited morphological features typical of the Ascaridoidea nematodes (i.e., the shell with ornamentations [resembling a mammillated layer] and the pronounced spherical form). These characteristics are especially common in eggs from representatives of Ascarididae, Anisakidae, and Raphidascarididae, all belonging to Ascaridoidea (Koie & Fagerholm, 1995; Anderson, 2000; Carrera-Játiva *et al.*, 2014; Carvalho, 2020).

The family Anisakidae was of particular interest in the present context because it includes some parasites that use piscivorous birds as definitive hosts and fish as intermediate or paratenic hosts, as well as produce eggs with similar morphometry and morphology as that found in the present study (Koie & Fagerholm, 1995; Anderson, 2000; Carrera-Játiva *et al.*, 2014; Carvalho, 2020). The present egg was quite similar to those reported by Carrera-Játiva *et al.* (2014), and mainly to the morulated stages reported by Huizinga (1967), all assigned to the genus *Contracaecum*.

The *Contracaecum* eggs (54.36 – 61.27 × 43.82 – 50.04 µm; Fig. 1C) recovered from the dissected females were found in an initial stage of development, before morulation, similar to those reported by Huizinga (1967). Although the stages of development from these eggs were different, they showed morphological similarities such as an oval to spherical shape and an ornamented and relatively thinned shell that is typical from nematodes that have aquatic life cycles. Moreover, differences observed in the egg size between the fresh and the fossilized materials may be accounted for by intrinsic features of females from each species. According to Herreras *et al.* (2007), spatial constrictions imposed by uterine size may influence the number and size of eggs produced by females of anisakid nematodes.

Although the parasitic form reported here may have a size similar to that of some oocysts from Eimeriidae, previously found in the Tremembé Formation (Carmo *et al.*, 2024a; Fig. 1B), it lacks common traits such as micropyle and micropyle cap. Moreover, we believe that the internal embryonic structure represents morula rather than developing sporocysts. In fact, more than 10 oocyst morphotypes were described in the Tremembé Formation, and none had preserved sporocysts or sporozoites (Carmo *et al.*, 2024a). In this sense, sporocysts or sporozoites most likely degrade before feces fossilization in this paleoenvironment, or the abiotic conditions essential of sporulation (*e.g.*, heat, humidity, oxygenation) were absent (Fayer, 1980; Berto *et al.*, 2014).

Fossilization is a singular phenomenon that takes place under specific conditions, which partially explains the lack of parasitological studies on material found in paleontological sites (Dentzien-Dias *et al.*, 2013; Carmo *et al.*, 2024b). Although we found only one egg in the present coprolites, the record of a morula reveals good potential for preservation of parasitic forms in the Tremembé Formation. In addition, the identification of the Ascaridoidea egg provides insights on the biogeographic distribution of this taxon, in the Brazilian territory, during the Oligocene.

The paleoparasitological information associated with the paleofaunistic diversity of birds and fishes from the Tremembé Formation (Carmo *et al.*, 2024c) indicate that the ancient Brazilian paleoenvironments provided conditions for the rising and success of nematodes infecting such hosts during the Paleogene.

Previous paleoparasitological studies from the Tremembé Formation were performed in coprolites exclusively from the site Fazenda Santa Fé (Carmo *et al.*, 2023). Therefore, this is the first parasitological analysis in trace fossils from the outcrop of Aligra Comércio de Argila S/A, providing new perspectives related to the paleoparasitological studies in this lithostratigraphic unit, and expanding the knowledge on helminth infections in birds that inhabited the Paraiba Valley millions of years ago.

Supplementary material. The supplementary material for this article can be found at http://doi.org/10.1017/S0022149X24000610.

Conflict of interest. The authors declare no conflicts of interest.

Author contribution. G.M.d.C.: conceptualization, methodology, investigation, writing-original draft, writing-review & editing, funding acquisition. A.H. d.S.G.L.: methodology, investigation, writing-original draft. J.F.P.: writing-original draft. S.d.S.L.: validation, resources, supervision, funding acquisition. H.I.d.A.-J.: validation, resources, supervision. F.B.P.: validation, resources, supervision, funding acquisition.

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References

Anderson, R.C. (2000) Nematode parasites of vertebrates: their development and transmission. CABI Publishing, New York.

Berto, B.P., Mcintosh, D., and Lopes, C.W.G. (2014) Studies on coccidian oocysts (Apicomplexa: Eucoccidiorida). *Revista Brasileira de Parasitologia Veterinária* **23**(**1**): 1–15. https://doi.org/10.1590/s1984-29612014001

Callen, E.O. (1967). Analysis of Tehuacan coprolites. In: Macneish, R.S., Byers, D.S. The Prehistory of the Tehuacan Valley: Volume One Environment and Subsistence, University of Texas Press, Great Britain, pp 261–289.

Camacho, M., Pessanha, T., Leles, D., Dutra, J.M.F., Silva, R., Souza, S.M., and Araujo, A. (2013) Lutz's spontaneous sedimentation technique and the paleoparasitological analysis of sambaqui (shell mound) sediments. *Mem Inst Oswaldo Cruz* **108**: 155–159. https://doi.org/10.1590/0074-0276108022013005

Carrera-Játiva, P.D., Rodríguez-Hidalgo, R., Sevilla, C., and Jiménez-Uzcátegui, G. (2014) Gastrointestinal parasites in the Galápagos Penguin Spheniscus mendiculus and the flightless cormorant Phalacrocorax Harrisi in the Galápagos Islands. *Marine Ornithology* **42**(**1**): 77–80.

Cardia, D.F.F., Bertini, R.J., Camossi, L.G., and Letizio, L.A. (2018) The first record of Ascaridoidea eggs discovered in Crocodyliformes hosts from the Upper Cretaceous of Brazil. *Revista Brasileira de Paleontologia* **21**(**3**): 238–244. https://doi.org/10.4072/rbp.2018.3.04

Cardia, D.F.F., Bertini, R.J., Camossi, L.G., and Letizio, L.A. (2019) Two new species of ascaridoid nematodes in Brazilian Crocodylomorpha from the Upper Cretaceous. *Parasitology International* **72**: 1–5. https://doi.org/10.1016/j.parint.2019.101947

Carmo, G.M., Garcia, R.A., Vieira, F.M., Lima, S.S., Araújo-Júnior, H.I., and Pinheiro, R.M. (2023) Paleoparasitological study of avian trace fossils from the Tremembé Formation (Oligocene of the Taubaté Basin), São Paulo, Brazil. *Journal of South American Earth Sciences* **125**: 1–8. https://doi.org/10.1016/j.jsames.2023.104319

Carmo, G.M., Berto, B.P., Pereira, F.B., Lima, S.S., Araújo-Júnior, H.I., and Pinheiro, R.M. (2024a) Protozoan parasites of birds from the Tremembé Formation (Oligocene of the Taubaté Basin), *São Paulo, Brazil*. International Journal of Paleopathology **45**: 46–54. https://doi.org/10.1016/j.ijpp.2024.04.003

Carmo, G.M., Lima, S.S., Araújo-Júnior, H.I., and Pereira, F.B. (2024b) Paleoparasitological contributions to the study on ancient infections of hominids and other vertebrates in Brazil: a review. *Paleontologia em Destaque - Boletim da Sociedade Brasileira de Paleontologia* **38(79)**:45–61. https://doi.org/10.4072/paleodest.2023.38.79.04

Carmo, G.M., Lima, S.S., Araújo-Júnior, H.I., Pinheiro, R.M., Melo, D.J., and Couto-Ribeiro, G. (2024c) Paleo-faunistic checklist of the Tremembé Formation (Oligocene of the Taubaté Basin, Paraíba Valley, Brazil). *Terrae Didatica* **20**: 1–11. https://doi.org/10.20396/td.v20i00.8674375

Carvalho, E.L., Santana, R.L.S., Gonçalves, E.C., Pinheiro, R.H.S., and Giese, E.G. (2020) First report of *Anisakis* sp. (Nematoda: Anisakidae) parasitizing Muscovy duck in Marajó Island, state of Pará, Brazil. *Braz J Vet Parasitol* **29** (**2**): 1–9. https://doi.org/10.1590/S1984-29612020015

Castro, A.C.J., Fernandes, A.C.S., and Carvalho, I.S. (1988) Coprólitos de aves da Bacia de Taubaté, SP. In: Congresso Brasileiro de Geologia, 35, 1988, Belém. Anais […] Belém: Sociedade Brasileira de Geologia. v. 6. p. 2358–2370. Available at: https://www.researchgate.net/publication/236345336_Coprolitos_de_aves_da_Bacia_de_Taubate_SP. Accessed 14 March 24.

Dentzien-Dias, P.C., Poinar, Jr G., Figueiredo, A.E.Q., Pacheco, A.C.L., Horn, B.L.D., and Schultz, C.L. (2013) Tapeworm eggs in a 270 million-year-old shark coprolite. *PLOS ONE* **8**(**1**): 1–4. https://doi.org/10.1371/journal.pone.0055007

Fayer, R. (1980) Epidemiology of protozoan infections: the coccidia. *Veterinary Parasitology* **6**(**1-3**): 75–103. https://doi.org/10.1016/0304-4017(80)90039-4

Ferreira, L.F., Reinhard, K.J., and Araújo, A. (2014) *Foundations of Paleoparasitology*. Fiocruz, Rio de Janeiro.

Fugassa, M.H., Araújo, A., and Guichón, R.A. (2006) Quantitative paleoparasitology applied to archaeological sediments. *Mem Inst Oswaldo Cruz* **101** (**Suppl.II**): 29–33. https://doi.org/10.1590/s0074-02762006001000006

Gibbons, L.M. (2010) Keys to the Nematode parasites of vertebrates: Supplementary Volume. Wallingford: CABI Publishing.

Gonçalves, M.L.C., Araújo, A., and Ferreira, L.F. (2003) Human intestinal parasites in the past: new findings and a review. *Mem Inst Oswaldo Cruz* **98**: 103–118. https://doi.org/10.1590/S0074-02762003000900016

Guedes, L., Borba, V.H., Camacho, M., Neto, J., Dias, O., and Iñiguez, A.M. (2020) African helminth infection out of Africa: paleoparasitological and paleogenetic investigations in Pretos Novos cemetery, Rio de Janeiro, Brazil (1769–1830). *Acta Tropica* **205**: 1–6. https://doi.org/10.1016/j.actatropica.2020.105399

Hartwich, G. (2009) Ascaridida: Ascaridoidea. In: Anderson, R.C., Chabaud, A. G., Willmott, S. (eds). Keys to the Nematode parasites of vertebrates: Archival Volume, CABI Publishing, Wallingford, pp 309–323.

Herreras, M.V., Montero, F.E., Marcogliese, D.J., Raga, J.A., and Balbuena, J. A. (2007) Phenotypic tradeoffs between egg number and egg size in three parasitic anisakid nematodes. *Oikos* **116**(**10**):1737–1747. https://doi.org/10.1111/j.0030-1299.2007.16016.x

Hodda, M. (2022) Phylum Nematoda: a classification, catalogue and index of valid genera, with a census of valid species. *Zootaxa* **5114**(**1**): 001–289. https://doi.org/10.11646/zootaxa.5114.1.1

Hoffman, W.A., Pons, J.A., and Janer, J.L. (1934) The sedimentation concentration method in schistosomiasis mansoni. *Puet Rico J Publ Health Trop Med* **9**: 283–289.

Huizinga, H.W. (1967) The Life Cycle of Contracaecum multipapillatum (von Drasche, 1882) Lucker, 1941 (Nematoda: Heterochelidae). *The Journal of Parasitology* **53**(**2**):368–375. https://doi.org/10.2307/3276593

Iñiguez, A.M., Brito, L., Guedes, L., and Chaves, S.A.M. (2022) Helminth infection and human mobility in sambaquis: paleoparasitological, paleogenetic, and microremains investigations in Jabuticabeira II, Brazil (2890±55 to 1805±65 BP). *The Holocene* **32**: 200–207. https://doi.org/10.1177/09596836211060490

Jaeger, L.H., Taglioretti, V., Dias, O., and Iñiguez, A.M. (2013a) Paleoparasitological analysis of human remains from a European cemetery of the 17th–19th century in Rio de Janeiro, Brazil. *International Journal of Paleopathology* **3**: 214–217. https://doi.org/10.1016/j.ijpp.2013.04.001

Jaeger, L.H., Taglioretti, V., Fugassa, M.H., Dias, O., Neto, J., and Iñiguez, A. M. (2013b) Paleoparasitological results from XVIII century human remains from Rio de Janeiro, Brazil. *Acta Tropica* **125**: 282–286. https://doi.org/10.1016/j.actatropica.2012.11.007

Koie, M., and Fagerholm, H.-P. (1995) The life cycle of *Contracaecum osculatum* (Rudolphi, 1802) sensu stricto (Nematoda, Ascaridoidea, Anisakidae) in view of experimental infections. *Parasitol Res* **81**(**6**): 481–489. https://doi.org/10.1007/bf00931790

Leles, D., Araújo, A., Ferreira, L.F., Vicente, A.C.P., and Iñiguez, A.M. (2008) Molecular paleoparasitological diagnosis of *Ascaris* sp. from coprolites: new scenery of ascariasis in pre-Columbian South America times. *Mem Inst Oswaldo Cruz* **103**: 106–108. https://doi.org/10.1590/s0074-02762008005000004

Li, L., lü, L., Nadler, S.A., Gibson, D.I., Zhang, L.P., Chen, H.X., Zhao, W.T., and Guo, Y.N. (2018) Molecular phylogeny and dating reveal a terrestrial origin in the early Carboniferous for Ascaridoid Nematodes. *Syst Biol* **67**(**5**): 888–900. https://doi.org/10.1093/sysbio/syy018

Olson, S.L., and Alvarenga, H.M.F. (2002) A new genus of small teratorn from the Middle Tertiary of the Taubaté Basin, Brazil (Aves: Teratornithidae). *Proc Biol Soc* **115**(**4**): 701–705.

Poinar, Jr G., and Boucot, A.J. (2006) Evidence of intestinal parasites of dinosaurs. *Parasitology* **133**(**2**): 245–249. https://doi.org/10.1017/S0031182006000138

Sianto, L., Souza, M.V., Chame, M., Luz, M.F., Guidon, N., Pessis, A., and Araújo, A. (2014) Helminths in feline coprolites up to 9000 years in the Brazilian Northeast. *Parasitology International* **63**: 851–857. https://doi.org/10.1016/j.parint.2014.08.002

Silva, P.A., Borba, V.H., Dutra, J.M.F., Leles, D., Da-Rosa, A.A.S., Ferreira, L. F., and Araujo, A. (2014) A new ascarid species in cynodont coprolite dated of 240 million years. *Anais da Academia Brasileira de Ciências* **86**(**1**): 265–269. https://doi.org/10.1590/0001-3765201320130036

Souto, P.R.F. (2017) *Icnologia de Paleovertebrados*. Rio de Janeiro: Letra Capital.