Part II - Parasite Remains Preserved in Various Materials and Techniques in Microscopy and Molecular Diagnosis

9. The Study of Microinclusions in Coprolites

Paulo Roberto de Figueiredo Souto

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A grayscale electron micrograph of a parasite, likely a merozoite, showing its internal structure and organelles. The parasite is elongated and has a distinct nucleus and other internal components. The background is a granular, textured surface.

part II

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9

The Study of Microinclusions in Coprolites

Paulo Roberto de Figueiredo Souto

Coprolites nearly always contain undigested residues from feeding activity. These may include the hard parts of prey (bones, teeth, scales, otoliths, keratins, and chitins) and plant remains (wood fragments, cuticles, phytoliths, and seeds). These inclusions are in fact the best proof of the identity of these structures.

The occurrence and investigation of organic microstructures in coprolites more than 3 million years old are more complex, when compared to the analysis of excreta in mummified state. The advanced state of lithification that the structure undergoes during fossilization requires a more painstaking process to recover these remains. The analysis of included organic particles in this case depends on the degree of dissociation allowed by the coprolite's matrix and involves more complex methodologies.

When the ingested organic microparticles withstand environmental conditions, the cell structures and tissues can be preserved inside the coprolite and thus be separated from the matrix and examined individually.

When permineralization has occurred with calcite, dissolution with acid treatment can damage or even destroy identifiable structures. However, when the particles are resistant, for example in carapaces, spores, and plant cuticles, the possibility of identification increases. Thus, most studies of coprolite microinclusions begin with thin sections; even though the material loses its three-dimensional structure, one can identify a significant variety of organic material.

Renault & Bertrand (1895) were the first to report the presence of microorganisms in coprolites produced by vertebrates during the Permian in France, identifying an estimated 12 to 140 thousand bacteria per mm³. Later, in the 20th century, two studies were published on fossil microbial activity in coprolites from the Triassic in Arizona (Lipman & McLees, 1940) and in coprolites from the Eocene in Wyoming (Bradley, 1946), USA, in which the coprolite matrix was laminated and dissolved, allowing the identification of silicified enteric bacteria in the form of cocci, rods, and ovoids, as well as fungal spores and freshwater unicellular algae.

Also in the 20th century, Stokes (1964) and Chin & Gill (1996) used the thin section technique to reveal the inside of herbivorous dinosaur coprolites with the presence of plant fragments and large amounts of silicified amorphous material, attributed to possible bacterial structures.

In recent years, studies using chemical dissolution of the coprolite matrix (Hollocher et al., 2001; Baszio & Richter, 2001) revealed biomineralized inclusions associated with feeding and decomposing activity produced by bacteria and invertebrates (Wilby & Martill, 1992; Richter & Baszio, 2001; Prasad et al., 2005). More recently, molecular studies have proven to be a promising field of research in the area of parasitology by providing evidence on the presence of intestinal parasites in coprolites (Poinar Jr., 2007; Poinar Jr. & Boucot, 2006). Such evidence indicates that insects were the vectors of pathogens such as the etiological agents of malaria and leishmaniasis, and that associated with other intestinal parasites like protozoa and helminths, such pathogens acted as one possible cause of the extinction of many groups, including dinosaurs.

METHODOLOGY

Preservation of material in the form of a coprolite is such a rare event that it requires the adoption of analytical procedures that allow maximum yield from the material (Amstutz, 1958; Häntzschel, El-Baz & Amstutz, 1968).

One of the most widely used procedures in studying coprolite microinclusions is thin sections. Longitudinal sections (i.e., along the longest plane) are recommended for the best yield. The specimen is cut in half with an electric saw, and each half is cut again in one or two sections. The sections are attached to glass slides with epoxy glue and polished down to a thickness of approximately 1.5 to 3.5 mm, according to the degree of translucidity allowed by the section (Hirsch, 1979).

If the specimen is larger than a conventional slide, larger glass slides should be made in advance, adapted to the specimen's size. The sections are later exposed to transmitted and polarized light under a binocular light microscope coupled to a digital camera, photographing with lenses of different magnifications to investigate for the presence of organic inclusions.

Petrified microorganic inclusions in the feces are separated by chemical treatment, i.e., application of 10% HCL (hydrochloric acid) to dissolve the carbonate crystals. The particles are later washed and centrifuged. The residues are subsequently resuspended twice in 40% HF (hydrofluoric acid) to remove silicates, allowing them to settle for 12 hours. The precipitate is washed at least twice with bidistilled water, centrifuged, and examined under light microscopy (Hansen & Gudmundsson, 1979).

MICROINCLUSIONS IN COPROLITES FROM ARCOSAURS

The coprolites from Pinheiros in the municipality of Candelária, Rio Grande do Sul State, Brazil, date to 230 million years at the beginning of the Mesozoic (Middle Triassic). These coprolites are single or clustered masses in excellent state of preservation, with predominantly clustered ovoid shapes associated with a smaller number of single cylindrical shapes.

The paleofauna found in this sediment consists mainly of dicynodonts (mammal-like reptiles). When these coprolites were first discovered, they were mistakenly thought to be eggs or concretions (Von Huene, 1990). However, later studies revealed their true biological nature (Souto, 2000). In addition to the peculiar structural appearance of large clusters, these coprolites provide important information for understanding paleoecological relations during Gondwana. Specimens analyzed with the thin section technique displayed palynomorphs and plant remains belonging to the Cycadaceae (Plate 1: figures 1a, 1b, 1c, 1d).

MICROINCLUSIONS IN FISH COPROLITES

Fish coprolites are frequently found in many sedimentary units in Brazilian territory. The most important and representative record of fish coprolites belongs to the Araripe Basin in Ceará State. Located in Northeast Brazil, this basin contains the majority of the outcrops in Ceará. Its origin is related to the opening of the South Atlantic Ocean, characterizing an epicontinental sea at that time. These sediments contain the preservation of both numerous amounts of fish and fossil records of pterosaurs, chelonians, and dinosaurs.

The main sedimentary unit with abundant coprolites belongs to the Santana Formation (Lower Cretaceous). They are found mainly in the Ipubi members, included between black shale layers and the Romualdo Formation, preserved inside limestone nodules. Cylindrical and ovoid structures are present in both units.

The coprolites in this region are mainly associated with fossil fish. The specimens' good state of preservation indicates autochthonous origin. As for the fossilization process, they display permineralization of the internal structures by calcite minerals. The chemical composition, determined by X-ray fluorescence, consists of calcium phosphate. Thin section microscopic analysis revealed the presence of radiolarians, ostracods, and worms (Plate 1: figures 1e, 1f, 1g, 1h; Plate 2: figures 2a, 2b, 2c).

MICROINCLUSIONS IN REPTILE COPROLITES

Reptile coprolites have been found in the Paraná and São Luís Basins. The Paraná Basin includes Northeast Argentina, Central-South Brazil (from Mato Grosso to Rio Grande do Sul State), Eastern Paraguay, and Northern Uruguay. The São Luís Basin is located in northern Maranhão State. In both cases, the coprolites are associated with sediments deposited during the Upper Cretaceous.

In the São Luís Basin, coprolite specimens were collected in the outcrop known as Laje do Coringa, corresponding to the Itapecuru Formation of the same basin. The site is located in the eastern part of Cajual Island, in the Bay of São Marcos in the State of Maranhão. The lithology of Laje consists mainly of rudaceous deposits varying from lithic conglomerates (pebbles, cobbles, and fragments) to extensive bone beds. The coprolites occur included or loose in the sediment of the Laje and are associated with abundant bone fragments from dinosaurs, crocodylomorphs, and fish. Some fifty specimens have been described, identifying coprolites with ovoid, cylindrical, conical, and spiral shapes. Thin section examination revealed (in addition to permineralization with ferrous oxide crystals) the presence of scales, worms, and plant remains included in the coprolite matrix (Plate 2, figures 2d, 2e, 2f, 2g).

The coprolites found in the Paraná Basin are associated with reptiles, mainly the group of crocodylomorphs. The material comes from sediments that correspond lithologically to the Adamantina Formation, deposited during the Upper Cretaceous, and have been collected in different outcrops in the western region of the State of São Paulo. Numerous macroscopic food traces have been observed. However, thus far thin section examination has only revealed the presence of micro-bivalves.

MICROINCLUSIONS IN MAMMALIAN COPROLITES

During the Cenozoic, the majority of coprolite findings are related to the expansion of mammals on dry land environments. The most significant records include the São José de Itaboraí Basin, located in São José in the town of Itaboraí, Rio de Janeiro State.

This basin consists of banded limestones vertically cut by fissures, filled with dark clays and marls. The basin is known for the fossil assemblage consisting of a rich fauna of mammals and pulmonate gastropods, besides plant remains (Bergqvist, Moreira & Pinto, 2006), and more recently by the presence of fossilized feces (Souto, 2007).

Coprolites were collected in the southern part of the basin, in the layers of grayish banded limestones in the dissolution channels, where vertebrate bones are also present. In stratigraphic terms, these layers belong to lower levels of the sequence and correspond to the episode of sedimentation that occurred during the Paleocene, in the Early Cenozoic.

In terms of their origin, the fact that the majority of the coprolites display breaks or are fragmented allows them to be classified as para-allochthonous. Preserved on the outer part of some specimens are marks from the excretory musculature, compaction cavities, and pin-shaped polarities that show the material's biological identity. Since the internal structure is too brittle, observations were made with chemical treatment, revealing the presence of spores and grass phytoliths. The latter is an unprecedented element for this sequence of the fossil record (Plate 2, figures 2h, 2i).

FINAL REMARKS

The relevance of coprolites for research has only recently emerged in the scientific scenario, when compared to the longer existence of other areas in paleontology. The implementation of new techniques (Poinar et al., 1998) such as molecular coproscopy and the specialization of studies on coprolites elsewhere in the world have fostered new and highly relevant research paths for understanding the biological interactions of the past. In Brazil, although coprolite findings have increased significantly (Souto, 2008), only in recent years has it been possible to establish adequate and permanent work procedures for highly petrified specimens. The findings cited above bear witness to future directions in the field of paleoparasitology. The presence of parasite microinclusions thus appears as a promising research trend for the coming years.

Plate 1 – coprolites of mammal-like reptile and fish

1a: Coprolite MCT 1517r, longitudinal section.

1b: Fern sporangium under transmitted light, magnified 200x.

1c: Coprolite MCT 1528r, longitudinal section.

1d: Conifer seed under transmitted light, 100x magnification.

1e: Coprolite UFRJ-DG 213-IcV, associated with limestone nodule.

1f: Ostracod shells under transmitted light, magnified 250x.

1g: Coprolite UFRJ-DG 290-IcV, associated with limestone nodule.

1h: Radiolarians under transmitted light, 100x magnification.

Plate 2 – Coprolites and food remains

2a: Coprolite UFRJ-DG 291-IcV, included in limestone nodule.

2b: Scolecodont under transmitted light, 100x magnification.

2c: Body of worm under transmitted light, 250x.

2d: Coprolite UFRJ-DG 265-IcV.

2e: Xylem of *Paradoxopteris* sp. fern under transmitted light, 200x.

2f: Coprolite UFRJ-DG 260-IcV.

2g: Worm-like element in transmitted light, 100x.

2h: Herbivore coprolite MCT 3066m.

2i: Fungal spore and grass phytolith under transmitted light, 250x.

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