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

14. Pollen Grains, Landscapes, and Paleoenvironments

Sérgio Augusto de Miranda Chaves

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Pollen Grains, Landscapes, and Paleoenvironments

Sérgio Augusto de Miranda Chaves

Palynology is the branch of botany that studies pollen grains and spores. Palynology textbooks such as Wodehouse (1935), Faegri & Iversen (1950), and Erdtman (1952) represent the phase of diversification of palynological studies and the technical and theoretical foundations for the different lines of research developed since then.

Pollen grains and spores have been studied since the early 20th century with the aim of contributing to taxonomic studies and, more recently, to studies on the environment and paleoenvironments.

Erdtman (1952) laid the groundwork for current studies in palynology. His pioneering graphically represented the morphological details of pollen grains and spores and laid the foundations for basic methodological procedures for their extraction, still used today.

Palynology has increasingly proven to have great potential for contributing to research in applied botany. The various current studies in paleopalynology shed light on the “fossil” plants and plant associations and help reconstruct their history. According to Renault-Miskovsky & Petzold (1992), pollen grains are particularly resistant thanks to exine, or their outer “membrane”. They can thus be preserved indefinitely over the course of geological time. They also withstand the inherent chemical aggressions from methods used to extract pollen from sediments. According to the authors, the grain's structure, size, shape, or even position, the type and number of germinal openings, and the description of the exine's sculpture allow the identification of a plant's family, genus, or even species under light microscopy.

Pollen analysis specifically provides diverse data on the pollen rain from the surrounding vegetation, subject to seasonal variations. To determine the floral evolution of given vegetation, we should turn to pollen analysis of elements of diverse origins, such as turf bogs and lacustrine sediments. Such sediments reflect evolution in the regional vegetation over a longer period, sometimes thousands of years (Salgado-Labouriau, 1973; Faegri & Iversen, 1989).

Pollen analysis of coprolites is also a highly interesting tool, both in paleoenvironmental studies and those related to paleodiet and the medicinal use of given plants, among others. The limits of this type of analysis are related to the seasons of the year and the plants' flowering period in the past (Chaves & Renault-Miskovsky, 1996; Chaves, 1997).

Specialists from Europe and the Americas met at the Feces Facies Symposium in London in 2002. The symposium featured a rich series of contributions, ranging from environmental approaches to paleodiets, paleopharmacology of prehistoric populations, and coprological studies. The relevance of this meeting was consolidated in 2006 with the publication of a special issue of the *Journal of Palaeogeography, Palaeoclimatology, Palaeoecology: advances in the interpretation of pollen and spores in coprolites*. More recently, in September 2009, at the 5th International Symposium on Ethnobotany, scientists presented various research methodologies in coprology, paleobotany, and different studies on the plants used by prehistoric populations, especially in South America.

Importantly, the science of palynology has increasingly proven to contribute greatly to research on vegetation from the past and interdisciplinary studies with parasitology and paleoparasitology.

Thus, palynology and these other sciences represent veritable guiding threads in applied research on the reconstruction of paleoenvironment and important tools in current lines of research in applied ecology, especially related to the evolution of the human species and its environment, creating a new perspective that we refer to here as historical ecology. We now have a relevant body of published work on these themes by researchers involved in projects linking paleopalynology and parasitology. In the studies related to archaeology, pollen analysis aids researchers in the interpretation of data on various occupations by prehistoric populations. Pollen analysis of so-called anthropic sediments also allows evaluating human impact on the environment in activities related to the development of agriculture and various cultivars (Carrión et al., 2004; Sears, 1982; Faegri & Iversen, 1989; Lima Ribeiro & Barbieri, 2005).

The history of human occupation of the American continent is closely linked to another important history: that of the occupation of diverse and often inhospitable ecosystems in this new and final space of conquest, America. Without a doubt, part of the human migratory waves that peopled our continent followed the same path as their contemporary megafauna. Probably coming from Siberia, these human waves crossed the Bering Land Bridge and traversed the extensive tundra formations there, as well as in the Alaska region. They followed the corridor already open between the glaciers during the Wurm interglacial on the sandy-clay plains of North America; they passed the Amazonian equatorial vegetation until they reached the Northeast and Center of Brazil and moved on to the distant coastal areas of the Atlantic and Pacific in South America (this route is purely speculative, since new archaeological discoveries point to at least two more access routes to the continent!). A new model is needed to elucidate the peopling of America, especially since the recent publication of evidence on the Clovis industry and its real representativeness in the cultural history of the peopling of this continent (Waters & Stafford, 2007).

During the Holocene (10,000 BP), herbivorous mammals thrived in America due to the expansion of “pasture” land. This was the same period in which *Homo sapiens sapiens* expanded his territorial conquest, spreading across the American continent and developing countless cultural transformations related to the tropical ecosystems of South America.

Even in the Late Pleistocene, between 13,000 and 10,000 BP, such waves of human migrations to the American continent already achieved enormous adaptive success, especially in their subsistence economy based on hunting wild animals, fishing, and even gathering plants. According to Roberts (1994), in order to survive in a given territory, in a migratory movement that undoubtedly depended on natural resources like water, these hunter-fisher-gatherers needed an area of at least 75 km² for a group of 25 individuals. By the Late Pleistocene, a large and representative population of human beings (estimated at more than two million) moved in greatly relevant migratory flows across the five continents.

In addition to the adaptability of these human groups to the new ecological scenarios, other questions can be addressed, such as their way of life and subsistence, or their various relations with closely “related” groups, as well as the existence of possible intragroup hierarchical levels. But in-depth discussion of such questions is beyond our scope here. Just to present an overview of the importance of some sciences in the reconstitution of paleoenvironments is a difficult task in itself!

PALEOENVIRONMENTS

The reconstitution of Holocene and even Pleistocene paleoenvironments now relies on numerous sciences, indispensable for the enhancement of paleoenvironmental studies. These include dendrochronology, or the study of tree “rings” and their relations to climate changes, palynology, the study of pollen grains and spores, paleomalacology, the study of fossil mollusks, the study of phytoliths – silica particles deposited in the epidermis of plant leaves and stems, the study of diatomaceae, or microscopic algae (excellent indicators of environmental changes), and even the study of certain coleopters, insects that respond rapidly to climate changes.

Pollen and spores provide important information on past environments and possible floral arrays existing in a given geological period, helping answer questions on ancient climates, environments, and vegetation. Pollen grains, microstructures responsible for the fertilization of flowering plants, are scattered yearly in amounts that vary according to the type of plant. They can be carried to places distant from the area in which they were produced by air currents, insects, animals, even human beings. However, most pollen grains fall close to their original site, thus characterizing the local flora. Such pollen deposition can be accumulated on “traps” (slides smeared with petroleum jelly) placed near the ground to collect “pollen rain”.

We can extract pollen grains from recent land or lacustrine sediments or even from archaeological sediments using specific chemical procedures, and later analyze the relative frequency of their appearance in the samples, separating them into arboreal pollen (AP) and non-arboreal pollen (NAP). Therein begins the reconstruction of ancient environments (or paleoenvironments) by analyzing and interpreting pollen diagrams. Such diagrams are constructed using the identification of taxon markers for given plant types and/or plant associations representing various environments: very dry, very wet, flooded, forest, savannah, and others. Importantly, the sediments submitted to such analyses should be previously dated using methods such as C^{14} , for samples up to 40,000 years old.

PRESERVATION OF POLLEN

The preservation of pollen grains is a chapter in itself. Pollen has a virtually indestructible envelope, sexine, which consists of sporopollenin, a compound that resists even the strongest acids (like sulfuric and hydrofluoric). However, this outer layer does not resist sediments in which oxygen is present. Peat sediments, lakes, lagoons, and swamps provide excellent environments for its preservation (as long as these sediments have not undergone phases of drying, which would destroy the pollen grains due an aerobic medium).

Archaeological excavations can uncover pollen grains: inside the sediments; in funeral urns from various burials, especially in the stamens of flowers, often used as offerings (Chaves & Brancaglione, 2005); on the surface of objects handled by humans, such as wooden, pottery, or stone utensils; or on the surface and inside fossilized feces, known as coprolites.

Coprolites can contain pollens and spores on the inside or on the surface, when the pollen grains are deposited on the outer side, functioning as a veritable organic pollen trap (Chaves, 1996; Chaves & Reinhard, 2006). The pollen content and macroscopic and microscopic remains found in this rich organic material thus provides information on human and animal diet (Chame, 1988; Sobolik & Gerik 1992; Reinhard, Mrozowski & Orloski, 1986; Reinhard, Hamilton & Hevly, 1991; Chaves, 1994; Carrión et al., 2004; Chaves & Reinhard, 2006; Reinhard, Chaves & Iñiguez, 2008).

A more in-depth analysis of a coprolite requires identifying the animal that produced it, whether herbivore or carnivore, since the environmental interpretation resulting from this type of information is closely linked to the animal's eating habits.

Paleocoprology, or the study of coprolites, also yields precious data on the ancient demography of a given site, since it is possible to extract specifically male and female steroids from coprolites, in addition to mitochondrial DNA (mtDNA), used for taxonomic studies and studies on kinship and evolution (Sobolik et al., 1996). Paleoparasitology, a science that works closely with coprology, also produces information on parasitic diseases and possible migratory routes travelled by ancient human and animal populations (Araújo et al., 1988; Araújo, Rangel & Ferreira, 1993; Ferreira et al., 1987).

By studying the pollen in coprolites, palynologists obtain information that they analyze together with other tests to allow paleoenvironmental reconstruction, even constructing a possible scenario in which the prehistoric humans lived, relating them to given time periods. The study of pollen grains and other microresidues in coprolites allows inferences on diet and the medicinal uses of specific plants (Sobolik & Gerik, 1992; Reinhard, Mrozowski & Orloski, 1986; Reinhard, Hamilton & Hevly, 1991; Chaves, 1994, 2000a; Carrión et al., 2004; Chaves & Reinhard, 2006). When making paleoecological interpretations based on coprolites, the palynologist should be alert to two important methodological issues. The first is when the pollen is found inside the coprolite, meaning that it was ingested with the solid and/or liquid food of the human or animal that produced the coprolite.

The second is when the pollen rain was deposited on the coprolite's outer surface. In this case the feces that turned into a coprolite acted as a "trap", collecting the pollen from plant species in the immediate environment (mainly trees), as well as from plants found at long distances. The latter pollens were brought by air currents. If produced by a carnivorous animal, the coprolite can contain pollens from the stomach of prey consumed by it, herbivores for example. Birds and insects ingested by carnivores can represent vegetation located many kilometers away from the collection site. Pollen recovered from a coprolite's surface can indicate the taxa of plants that grew at (or close to) the point where the coprolite was found (Carrión et al., 2004; Putman, 1984).

Great caution is thus needed when making environmental interpretations based on coprolites. A mistaken diagnosis, especially failing to consider these two types of impregnation of coprolites by pollens, can lead ecological (or paleoecological) interpretations to erroneous and hasty conclusions.

Phytoliths are also excellent microresidues used for paleoenvironmental reconstruction. The application of phytolith analyses and the examination of these silicon particles increased considerably beginning in the 1970s. According to Jones & Handreck (1967), the silica constituting phytoliths is deposited in the epidermis and other parts of the plants.

In the different taxa, phytoliths display distinct shapes, which allow diagnosing them and consequently identifying the plant. According to Piperno (1991) and Pearsall (1989), various families of monocotyledons and dicotyledons produce abundant amounts of phytoliths. Piperno (1991) identified some current phytoliths in the following families of monocotyledons: Bromeliaceae, Cannaceae, Cyperaceae, Poaceae, Heliconiaceae, Marantaceae, Musaceae, Orchidaceae, Arecaceae, and Zingiberaceae. This same study also indicated the production of phytoliths in the following

families of dicotyledons: Acanthaceae, Annonaceae, Aristolochiaceae, Burseraceae, Cannabaceae, Chloranthaceae, Chrysobalanaceae, Asteraceae, Cucurbitaceae, Dilleniaceae, Euphorbiaceae, Loranthaceae, Magnoliaceae, Moraceae, Piperaceae, Rosaceae, Sterculiaceae, Ulmaceae, Urticaceae, and Verbenaceae.

Piperno (1991), Alexandre, Colin & Meunier (1994), and Alexandre (1996) describe phytolith analyses aimed at palaeoenvironmental reconstruction. These articles present research conducted in the tropics and contribute substantially to the reconstruction of tropical environments, since certain taxa are even ‘invisible’ in a palynological diagnosis. Under scanning electron microscopy (SEM), the surface of phytoliths from a soil stratigraphic profile shows that marks of changes, and consequently their age, increase regularly with depth. Their distribution can be interpreted with paleogeographic studies (Alexandre, 1996). Piperno (1991) reported evidence that phytolith analysis can distinguish corn from teosinte. Mulholland (1989) also separated types of corn and wheat in North America. Piperno (1991), Pearsall (1989), and Pearsall & Piperno (1990) published a method for maize identification in the New World.

Phytolith analysis can also identify other cultivars: arrowroot (*Canna edulis*) (Cannaceae), (*Maranta arundinacea* L.) (Marantaceae), and tigernut (*Cyperus esculentus* L.) (Cyperaceae) (Pearsall, 1989). According to Piperno (1991), phytolith analysis identifies a significant number of wild species used by prehistoric New World human populations, e.g., some palm trees, bamboos, and wild plantains or *Heliconia* (Musaceae). Unfortunately, many New World root cultivars such as manioc and potato cannot be diagnosed by phytolith analysis.

Parasite eggs, larvae, and DNA can also be recovered from preserved coprolites. However, in other sites, when the coprolites are “contaminated” by rain, a sporadic type of decomposition occurs in aerobic circumstances. Coprolites in such open environments display low preservation or “pollen richness”, and mites, fungal spores, and hyphae of various forms. Active growth of hyphae occurs in such deposits, and archaeologists have acquired pulmonary infections at least once, apparently from excavating coprolites (Bouchet et al., 2003).

Well-preserved coprolites do not differ morphologically from recently deposited feces. They can be fragmented or even dissolved in the archaeological layers or in the soil, as in the case of latrines. After defecation, the feces are exposed to many climatic factors. As mentioned, rain and moisture can alter the fecal morphology, potentially leading to erroneous paleoenvironmental interpretations.

PALEOPHARMACOLOGY, PALYNOLOGY STUDIES, AND THE BRAZILIAN PHARMACOPEIA

Paleopharmacology studies residues with medicinal uses found in places such as archaeological sites (Reinhard, Hamilton & Hevly, 1991; Chaves & Reinhard, 2003, 2006).

The groundbreaking theoretical work for this field of study was by Ortiz de Montellano (1975), who conducted a detailed survey of medicinal plants used by the Aztecs and identified in a historical codex. Coprolite analysis has obvious implications for this field of research and can extend our knowledge on the ancient use of medicinal plants. As reviewed by Riley (1993), numerous researchers have drawn on seeds to identify the prehistoric use of anthelmintics.

Parasitism is a complex relationship. Ancient peoples and civilizations developed medicinal “treatments” aimed at eliminating various parasitic infections based on knowledge of a given pharmacopeia. We believe it is possible recover the type of use and/or evidence of use of a given purportedly medicinal plant in archaeological sites, as well as to establish relationships between its use and specific circumstances. This type of study should adopt a multidisciplinary approach, involving paleoethnobotanists, paleoparasitologists, paleopathologists, and pharmacologists, among others.

The archaeology of the *sertão* or semiarid backlands of Brazil provides a rich source of information on the first American cultures. The *sertão* includes the *caatinga* or scrub forest region encompassing parts of the current States of Piauí, Ceará, Rio Grande do Norte, Alagoas, Paraíba, Pernambuco, Sergipe, Bahia, and Minas Gerais. In the *sertão*, the cultural tradition of Northeast Brazil was documented by burials, rock art, and the presence of associated artifacts in various well-dated locations (Guidon et al., 1994; Martin, 1996; Lessa & Guidon, 2002).

Human coprolites, dated 8,500 to 7,000 BP, were collected from the rock shelter at the Boqueirão da Pedra Furada site in Piauí State, Brazil. These dates correspond to the Serra Talhada I and II cultural traditions, the Northeast tradition, or a Paleoindian culture from Northeast Brazil. Parasitological analysis of these coprolites identified parasite eggs. Analysis of the skeletons and hair of individuals from this tradition shows a variety of diseases such as osteodistrophy, dental deformations, and head lice.

Palynological analysis of the coprolites showed 12 potentially medicinal genera. The data obtained from the pollen analyses were critically assessed to identify the therapeutic potential of the plants represented by the pollens.

The most representative cases involved the presence of three genera, *Anacardium*, *Borreria*, and *Terminalia*, with these plants used for medicinal purposes such as treatment of symptoms from intestinal parasites (Chaves & Reinhard, 2006).

Analysis of the coprolites, skeletons, and hair samples provided evidence of ancient diseases that required knowledge of the use of traditional medicinal plants to treat this population related to the Northeast tradition. Araújo et al. (2000) found louse eggs on single hair shafts dated 10,000 BP. The repellents or so-called “remedies” would have reduced the discomfort caused by these parasites.

According to Reinhard et al. (2001), the oldest hookworm infections were diagnosed in coprolites from the Northeast tradition. According to the authors, some other helminths also parasitized individuals from this culture (Gonçalves, Araújo & Ferreira, 2003). Anthelmintics and treatments for symptoms of this infection would have been necessary. Lessa & Guidon (2002) found evidence of both a degenerative disease and an oral disease that required the use of analgesics.

Reinhard, Hamilton & Hevly (1991) proposed the use of pollen concentration to identify medicinal plants in coprolites and burials. According to the authors, the discovery of *Salix* (willow), *Larrea* (creosote), and *Ephedra* (Mormon tea) in high percentages and concentrations indicated their use for specific treatments. A principle established by these studies is the value of pollen concentration to identify intentional consumption of anemophilous plants (airborne pollination) and entomophilous plants (pollination by insects). Knowledge of pollen concentration is needed to determine whether the amenophilous types were aggregated by chance in the coprolites or consumed intentionally with pollen-rich medicinal plants.

Much is known about current American and Brazilian indigenous pharmacopeia (Gonzalez, 1965; Martius, 1939; Pardal, 1937; Vogel, 1973), but paleopharmacology is still taking its first steps towards elucidating the prehistoric use of medicinal plants (Chaves & Reinhard, 2006).

The *Código Farmacêutico Lusitano*, or Lusitanian Pharmaceutical Code, dated 1835, written by Dr. Agostinho Albano da Silveira Pinto, was approved as Brazil's official pharmacopeia. However, the first official pharmacopeia written by physicians and pharmacists was the *Farmacopeia Portuguesa*, or Portuguese Pharmacopeia, dated 1876.

PALEOENVIRONMENTAL RESEARCH IN BRAZIL

Paleoecological studies aimed at the paleoenvironmental reconstitution of the Brazilian Quaternary through palynomorphs have been conducted in various regions of the country. Thus, the existence of different scenarios and the development of a variety of landscapes led to various theories on the installation of inter-tropical vegetation.

Beginning in the Holocene, the development of tropical vegetation, plant species migrations, and at the same time the expansion of dry vegetation from Brazil were determined by edaphic and climatic factors and anthropic influences (Ab'Saber, 1980).

The following is a summary of some representative studies on paleoclimates in the Brazilian territory.

The South region

Palynological studies in sediments from the coastal plain in Capão do Leão, Rio Grande do Sul State, Brazil, showed changes in the vegetation and increase in temperature and humidity at 6,700 BP. Around 5,000 BP, there was a peak rise in sea level, followed by the development of tropical flooded forest similar to the present (Neves & Lorscheitter, 1997).

Studies by Behling (1998) in Santa Catarina State in southern Brazil showed low slopes on the Rio do Rastro and Boa Vista mountain ranges with a phase of subtropical climate and evidences of frosts between 14,000 and 10,000 BP.

In these same areas, and in low-lying regions of the hill known as Morro da Igreja, the climate was tropical from 10,000 to 3,000 BP, and in the higher regions the climate was dry and hot. In Poço Grande, during this same period, the climate was also tropical, and there was a climate variation that began with a dry period at 4,840 to 4,590 BP, followed by an increase in humidity around 4,590 to 4,260 BP, and reaching a phase with high humidity between 4,260 and 3,525 BP. From 3,000 BP until today, a tropical climate was established in the lowlands. From 2,900 to 1,000 BP, on the upper part of the mountains and Morro da Igreja, there was a cooler and more humid climate than in the previous period. From 1,000 BP to the present it has remained cool, but with an increase in humidity.

Analyses of sediments collected along the Paraná River showed a dry phase from 40,000 to 8,000 BP, followed by a more humid phase from 8,000 to 3,500 BP. From around 3,500 to 1,500 BP, the dry conditions returned, followed by a new increase in humidity after 1,500 BP, reaching current conditions (Stevaux, 1997).

In studies in the Campos Gerais Mountains, Behling (1997a, 1998) observed a phase between 12,480 and 9,660 BP with cold and dry climate and harsh frosts in the winter in the higher regions. In the valleys, from 9,660 to 2,850 BP, the climate was tropical and relatively humid, with long dry seasons, which probably favored the expansion of *Araucaria* in an area of field vegetation. There was a humid climate with a dry season from 2,850 to 1,530 BP, followed by an increase in humidity.

Studies by Behling, Coehn & Lara (2001) in São Francisco de Paula, in the South of Brazil, recorded a period from around 7,500 to 4,000 BP with a markedly drier climate than at present. Around 4,000 BP, milder climatic conditions were observed, with the appearance of field vegetation and areas with the installation of *Araucaria* forest. The forest's expansion is documented since 1,060 BP and the expansion of *Araucaria angustifolia* (Bert.) Kentz trees since 850 BP. The region currently presents fields of pastures and altered *Araucaria* forest.

Behling (2002) published an interesting summary of the open landscapes in the Late Quaternary in the South and Southeast of Brazil. In regions currently covered by mixed rainforest, with individual *Araucaria* trees, an expansion occurred from so-called refuges, occupying field areas. This process has been relatively recent, beginning around 4,000 BP.

The Southeast region

Studies in the São Paulo Basin by Takiya & Ybert (1991) showed a dry phase from 5,500 to 2,500 BP.

In the Middle Paraíba do Sul River Valley in São Paulo State, Garcia (1994) suggested a cold and dry climate in the Late Pleistocene, with irregular and intense rains, and a more humid climate on the mountaintops. There was a hot and humid climate around 9,500 BP, with heavier precipitation than at present. From 9,500 to 8,000 BP, the climate changed to hot and dry, and in the following phase, from 8,000 to 6,400 BP, it changed to cooler and more humid.

Barros et al. (1993), in Bananal, São Paulo State, in the Middle Paraíba do Sul Valley, found similar situations to the present (namely humid) in the Early Holocene. In a phase prior to the Holocene, the drier climate was characterized by elements of open vegetation.

In the same region, Barros (1996) suggests that the transition from the Pleistocene to the Holocene was marked by instability in the climate and vegetation, with evidence of an open swampy or marshy environment with soaked soils. The same author identifies an attempt at the installation of plant cover with the presence of various pioneering species, but without evidence of a dense forest.

Analyses by Behling (1997b, 1998) in the Morro de Itapeva region in São Paulo State covering the period between 35,000 and 17,000 BP detected the existence of a cooler and drier climate than at present. The climate remained cool, but drier, between 17,000 and 10,000 BP, while in the following phase, from 10,000 to 3,000 BP, it was hotter and more humid on the eastern slope, and hot and dry on the higher ground. An increase in humidity was observed beginning at 3,000 BP, with large numbers of *Araucaria* and *Podocarpus*.

Luz (1997), analyzing a core from Lagoa de Cima, Rio de Janeiro State, suggested a hot and relatively humid phase prior to 6,000 BP. In the following phase, in the millennium starting at 6,000 BP, the climate remained hot, but seasonally dry. Around 4,000 BP there was an increase in humidity, followed by the beginning of a drier period.

In the Middle Rio Doce Valley, Minas Gerais State, from 10,000 to 8,900 BP, Ybert, Albuquerque & Turcq (1997) suggested a dry climate with *cerrado* (savannah) vegetation, followed by a period with higher humidity around 8,500 BP, as evidenced by elements of semi-deciduous and gallery vegetation, in addition to the development of a lake.

Chaves (1997), analyzed deposits from the slopes of the Middle Paraíba do Sul Valley, also in Bananal, on the border between São Paulo and Rio de Janeiro States, and identified pollen grains that mark environmental changes associated with anthropic processes in the late 19th century, at the peak of the replacement of native forests with coffee plantations.

Analyses of sediments from Lagoa Salgada, Rio de Janeiro State, showed a dry period around 2,500 BP between humid periods (Toledo, 1998).

Based on studies in Lago do Pires, Minas Gerais State, Behling (1998) suggested five climatic phases: 1) 9,720 to 8,810 BP, dry climate with low precipitation; 2) higher humidity around 8,810 to 7,500 BP; 3) the same conditions as the first phase from 7,500 to 5,530 BP; 4) less dry from 2,780 to 140 BP; and 5) the last phase, from 140 BP to the present, characterized by deforestation and the use of fire attributed to human presence in the region.

Coelho (1999), analyzed sediments from Sepetiba Bay, Rio de Janeiro State, and observed four climate changes: 1) 6,300 to 4,650 BP, a humid phase indicated by elements of dense rainforest; 2) 4,650 to 1,350 BP, a drier period shown by the expansion of open vegetation; 3) 1,350 to 45 BP, a return to characteristics of higher humidity, identified by an increase in forest elements; and 4) from 45 BP to the present, characteristics of a drier environment were attributed to the greenhouse effect (artificial decrease in humidity) and to changes in the vegetation due to deforestation by man.

Barth et al. (2004) and Barth, São-Thiago & Barros (2006) analyzed sediments from Guanabara Bay, Rio de Janeiro, dated 4,210 and 1.760 ± 50 BP, respectively, the latter study attesting to the existence of mangroves during this period.

The Central-West region (Central Plateau)

In the Middle Holocene, the intense action of fire contributed greatly to the appearance of the *cerrado* (savannah) landscape in Brazil. The installation of this type of vegetation in the Central Plateau of Brazil was due mainly to the arid summer season and fire in that region (Vernet et al., 1994).

Ledru (1998) found evidence of climatic oscillations in the Central Plateau, including a milder and more humid climate around 9,000 BP.

In the region around Brasília, pollen analyses by Ribeiro (1994) showed an initial humid and cool phase from 25,790 to 23,380 BP. From 23,380 to 21,450 BP there was an increase in humidity and a decrease in temperature. Characteristics of a dry climate appeared from 21,450 to 7,220 BP, followed by a hot, humid phase, quite similar to the present.

Pollen analyses by Ferraz-Vicentini & Salgado-Labouriau (1996) and Salgado-Labouriau (1997) in Cromínia, Goiás State, showed vegetation similar to the present and a hot and semi-humid climate in the period prior to 32,400 BP. From 32,400 to 20,000 BP, the decrease in tree and bush pollen types was attributed to a phase that was probably more humid and cooler than the present. From 18,500 to 11,300 BP, the decrease in the concentration of palynomorphs suggests a drier and perhaps cooler phase in the Late Pleistocene. This climatic condition also lasted from 10,500 to 7,700 BP, with a slight increase in temperature.

From 6,680 to 3,500 BP, recovery of the vegetation suggests an increase in humidity. Pollen analyses in animal coprolites from the archaeological site in Santa Elina, Mato Grosso, dated 4,000 to 2,000 BP, showed transitional vegetation (from *cerrado* to *cerradão*) during this period (Chaves, 2000b).

The North and Northeast regions

In the Amazon region, pollen analyses published by Martin et al. (1993) indicate four periods of dry climate in the last 60,000 years. According to the authors, the numerous climatic oscillations were caused by El Niño-type phenomena.

Also in the Amazon, studies by Sifeddine et al. (1994) in palynology and sedimentology showed that the fluctuations observed in the vegetation during the climatic events of the Early Holocene persisted during the recent Holocene, but with less intensity.

Authors like Colinvaux (1996), Oliveira (1996), and Behling (1998), using palynological studies, suggested a cooler climate in the Amazon forest from 40,000 to 10,000 BP compared to the present. Colinvaux, Oliveira & Bush (2000) review the past existence of refuges in the region.

According to Behling (1996), the large amount of carbonized particles found in sediments prior to 9,530 BP was due to human presence in the Amazon.

Behling & Costa (2001) analyzed the pollen content of sediments from Lago Crispim (in northeastern Pará State) dated to 7,640 BP and observed variations in the vegetation close to the study area, controlled by oscillation in the sea level. Along the core that was analyzed, the sedimentological and palynological records and geochemical elements assisted the interpretation of the floral composition controlled by the marine regression and transgression,

as evidenced by the advance and retreat of mangroves and the moment of installation of palm tree vegetation. The presence of carbonized particles on the coastal region during the Late Holocene was interpreted as manmade.

Studies by Behling, Coehn & Lara (2001) used pollen analysis of three sediment cores to evaluate changes and the dynamics of the mangrove ecosystem on the Bragança Peninsula, a coastal region in northeastern Pará. Installation of the mangrove vegetation began at different times in three sites: 5,120 BP in Campo Salgado, 2,170 BP in Bosque de Avicennia, and 1,440 BP in Furo do Chato, characterizing the Middle Holocene on the Bragança Peninsula as having its higher areas covered by mangroves. The change from mangrove to tidal vegetation in these higher areas around 420 BP was interpreted as natural encroachment of the sea level rather than the result of human action.

Sifeddine et al. (2003) analyzed a core from Lagoa do Caço, Maranhão, and observed higher frequencies of *Podocarpus* in the Late Pleistocene than at present. The increase in *Podocarpus*, followed by successive increases in various pioneering species such as *Didymopanax*, *Melastomataceae/Combretaceae*, and *Cecropia*, suggests a progressive increase in humid and cool climatic conditions.

For the Northeast region, the paleoclimatic data are still scarce. Guerin et al. (1993), Chaves (1994), and Chaves & Renault-Miskovsky (1996) have documented the existence of a humid regional phase in the Late Pleistocene and between 9,000 and 7,000 BP.

Chaves & Renault-Miskovsky (1996) used pollen analysis of animal coprolites to provide data that allowed tracing a paleoclimatic and paleoenvironmental picture for the region of São Raimundo Nonato, Piauí. The authors suggest attenuation in the last Holocene arid crisis between 8,700 and 7,000 BP. At that time, the landscape in the São Raimundo Nonato region was very different from today, with *cerrado-cerradão*-type vegetation. These records also reveal a climate that was slightly cooler and less dry than today.

One of the few specific studies on the paleoenvironment in what is now the State of Ceará was on the coastal area 90 km from the capital city of Fortaleza. Pollen was analyzed from marine sediments, showing glacial episodes and climate changes.

Pollen analyses by Oliveira Barreto & Suguio (1999) in turf bogs dated $10,990 \pm 80$ BP provided a history of changes in the vegetation and climate in the Icatu River Valley in the central São Francisco River region.

The palynological profile of Saquinho (in the São Francisco Valley) can be divided into five zones. The first, called zone SA1, corresponding to the period from 10,990 to 10,540 BP, showed pollen from taxa currently found in the Amazon and Atlantic forests, as well as mountain taxa, suggesting humid climatic conditions and lower temperatures. From 10,540 to 6,790 BP (zone SA2), the predominance of *Mauritia* and *Ilex* pollen type suggests a progressive warming and high humidity. From 8,910 to 6,790 BP, there was a progressive decline in forest taxa and a gradual increase in *caatinga* (scrub forest) and *cerrado* taxa in the landscape. From 6,790 to 6,230 BP (zone SA3), no palynomorphs were found, which was attributed to seriarid conditions. The period from 6,320 to 4,535 BP (zone SA4) was marked by the return of gallery forest, *caatinga*, and *cerrado* taxa, indicating a more humid climate. Finally, zone SA5, from 4,535 BP to the present, shows stabilization of the current climate and vegetation.

To conclude this chapter, we can say that palynology and the other disciplines discussed here are veritable guiding threads in applied research on paleoenvironmental reconstruction, serving as important tools in current studies in applied ecology, especially related to the evolution of humans and their environment, thus establishing a new approach that we refer to here as historical ecology.

METHODOLOGY FOR POLLEN ANALYSIS IN ARCHAEOLOGICAL MATERIAL

Samples of sediments selected for pollen analysis receive the following chemical treatment:

1. Place 2.0 cm³ of sediment in a 100 ml beaker and add 50 ml distilled water to dissolve the material.
2. Sift the material in a 200 μ m screen to remove plant remains, sand, and other materials. Then add two tablets of exotic spore (*Lycopodium* – each with 12,100 spores, for subsequent calculation of the absolute frequency of palynomorphs – Stockmarr, 1971).
3. Transfer the solubilized material to plastic centrifuge tubes to discard the water.
4. Add hydrofluoric acid (HF) 40% to eliminate silicates and let stand for 24 hours (repeat this step when necessary).
5. Add hydrochloric acid (HCl) 50% to eliminate fluorosilicates formed in the previous reaction; boil for 15 minutes.
6. Add potassium hydroxide (KOH) 10% to eliminate unwanted plant elements.
7. Add acetic acid (CH₃COOH) for dehydration of the material (repeat this step).
8. Add 5 ml of mixture for acetolysis: 4.5 ml HOOC-COOH P.A. (acetic anhydride) and 0.5 ml H₂SO₄ P.A. (sulfuric acid), for dissolving part of the organic matter, especially the cellulose, and acetylation of the exine (Erdtman, 1952); boil for 4 minutes.
9. Wash the material in the tube with 10 ml distilled water (repeat this step).
10. Add 5 ml of zinc chloride (ZnCl₂) to separate palynomorphs from the remaining material, transfer supernatant with palynomorphs to another centrifuge tube (this is the only step in which the supernatant is saved).
11. Add hydrochloric acid (HCl) 10% to allow sedimentation of palynomorphs, recovering them.
12. Wash the material in the tube with 10 ml distilled water (repeat this step).
13. Add glycerinated water (1:1), let stand for 30 minutes before mounting slides.
14. Mount permanent slides with glycerin gel (Kisser, 1935 apud Erdtman, 1952).

Note: at the end of each step, centrifuge for 5 minutes (1500 rpm) and discard supernatant, except in the zinc chloride phase.

The following are some examples of results from slides prepared with this technique. Figure 1 shows sweet potato pollen from a site in Mato Grosso, Brazil, dated 2,000 BP

Figure 1 – Pollen from sweet potato *Ipomoea batatas* (x 1.000), from archaeological sediments dated to 2,000 BP from site in Santa Elina, Mato Grosso, Brazil

Photograph: Sérgio de Miranda Chaves.

Figure 2, however, shows a phytolith under scanning electron microscopy, and Figure 3 is pollen from the archaeological region in southeastern Piauí, both dated c. 8,000 BP.

Figures 2 and 3 – Phytolith of *Poaceae* and pollen of *Justicia* sp. (Acanthaceae) (x 1.000) found in sediments from Quari Lagoon, Piauí, dated $8,770 \pm 55$ BP

Photograph: Sérgio de Miranda Chaves.

Figure 4 shows another phytolith from the same region of Piauí, still unidentified.

Figure 4 – Unidentified phytolith (x 400) found in sediments from Quari Lagoon, Piauí, dated $8,770 \pm 55$ BP

Photograph: Sérgio de Miranda Chaves.

Figures 5 and 6 show recent pollen grains with characteristic morphology, easy to diagnose.

Figure 5 – *Bauhinia* sp. (recent pollen), polarized light (x 400)

Photograph: Sérgio de Miranda Chaves.

Figure 6 – *Zea mays* (recent pollen) (x 400)

Photograph: Sérgio de Miranda Chaves.

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