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## **Handbook for the analysis of micro-particles in archaeological samples**

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### **Citation**

Henry, A. G. (2020). *Handbook for the analysis of micro-particles in archaeological samples*. Cham: Springer International Publishing.  
doi:10.1007/978-3-030-42622-4

Version: Publisher's Version

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Downloaded from: <https://hdl.handle.net/1887/3502447>

**Note:** To cite this publication please use the final published version (if applicable).

Interdisciplinary Contributions to Archaeology

Amanda G. Henry *Editor*

# Handbook for the Analysis of Micro-Particles in Archaeological Samples

 Springer

# **Interdisciplinary Contributions to Archaeology**

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Amanda G. Henry

Editor

# Handbook for the Analysis of Micro-Particles in Archaeological Samples



Springer

*Editor*

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ISSN 1568-2722

Interdisciplinary Contributions to Archaeology

ISBN 978-3-030-42621-7

ISBN 978-3-030-42622-4 (eBook)

<https://doi.org/10.1007/978-3-030-42622-4>

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# Preface

The microscope opens up a whole world that is otherwise hidden from the naked eye. Like any explorers of new worlds, those who study micro-particles will inevitably come across unknown objects. By 2015, my lab had acquired so many images of unknown but potentially informative micro-particles that we decided to bring together experts in the analysis of microscopic archaeological remains to see if we could help identify each other's mystery objects. That conference, the "Workshop on the Analysis of Micro-Particles in Archaeological Samples (WAMPAS)," held in December 2016 at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, was the inspiration for this book.

This edited volume would not have been possible without the hard work of the authors, for which they have my profound thanks. The authors represent both senior researchers who founded their area of research, and junior scientists who have been pushing the forefront of their field. Rather than discuss their own considerable contributions to the field, each agreed to focus instead on the micro-particle type they study, to provide a teaching guide and reference to other researchers. We hope you find it useful!

Leiden, The Netherlands

Amanda G. Henry

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**Amanda G. Henry** received her PhD in Hominid Paleobiology from the George Washington University, with a project looking at the consumption of plants by Neanderthals and early modern humans. She is broadly interested in plant foods and diet in the past, including the use of plant microremains such as starch grains and phytoliths as archaeological markers. She is currently an Associate Professor in the Faculty of Archaeology at Leiden University.



# Introduction: Micro-Particle Analysis in Archaeology



Amanda G. Henry

Nearly 20 years ago, Stephen Weiner drew the distinction between macroarchaeology—that is, the study of the large objects recovered during excavation, from temples down to stone tools—and microarchaeology—that is, the application of scientific techniques to examine the archaeological record beyond what is visible to the naked eye (Weiner 2010). Microarchaeology has enjoyed an increasing prominence in archaeological studies. Such techniques, ranging from the analysis of wear on stone tools using light microscopy, to the identification of lipids and residues using mass spectroscopy, to the recovery and identification of proteins and DNA, have enabled us to “see” features, habitats, and behaviors that would otherwise be invisible.

Of these techniques, the use of light microscopy to analyze micro-particles recovered from archaeological samples is of particular interest for multiple reasons. First, these micro-particles have the potential to provide fine-grained information about human behavior and/or about the environments humans used. Some types of micro-particles can provide species-level identification of the kinds of organisms that humans interacted with. For example, starch granules from wild grass seeds have been recovered from dental calculus of Neanderthals, providing direct evidence of the kinds of plants that these individuals ate (Henry et al. 2014). In another case, eggs from parasites that rely on fish as intermediate hosts were found in sediments from a Mesolithic Irish site, confirming the heavy consumption of fish during this period (Perri et al. 2018). Other micro-particles can provide fine-grained information about the environments in which humans lived. The changing diatom profiles in a Pleistocene site in East Africa record alternating wet-dry cycles that could

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A. G. Henry (ed.), *Handbook for the Analysis of Micro-Particles in Archaeological Samples*, Interdisciplinary Contributions to Archaeology,  
[https://doi.org/10.1007/978-3-030-42622-4\\_1](https://doi.org/10.1007/978-3-030-42622-4_1)

be linked to the hominin occupation of the area (Owen et al. 2008). Pollen records on Rapa Nui (Easter Island) document the increase in grass and non-native species, and the decrease in palm forests associated with the first arrival of humans to this island (Rull et al. 2013).

A second benefit of micro-particle analysis is that the micro-particles can remain intact and identifiable over archaeological time-spans, even in environments where other archaeologically important macroremains do not. Phytoliths, diatoms, and other siliceous micro-particles preserve well in acidic environments, in contrast to bones that often decay. Phytoliths have even been recovered from fossil dinosaur coprolites dating to the late Cretaceous (Prasad et al. 2005). Fibers, parasite eggs, and other micro-particles can be preserved in grave sediments, when few other grave goods are preserved (Juhola et al. 2019).

Finally, the analysis of micro-particles requires less investment in materiel and equipment than do other microarchaeological techniques, such as FTIR or proteomics. A transmitted light microscope capable of 400× magnification is the only major expense, putting the analysis of micro-particles well within the budget for most researchers.

However, even within this relatively well-established realm of micro-particle analysis using transmitted light microscopy, there remain problems in applying these techniques to archaeological samples. One of the main issues, and the one that this book is meant to address, is the problem of the “unknowns.” Such objects often far exceed the “known” micro-particles in any archaeological sample. Frequently, these unknown objects have distinctive, non-random shapes, indicating their likely biological origin. However, most remain unidentified, simply because a researcher familiar with starch granules may not know what a parasite egg or fungal spore looks like. This book is meant as a first reference for those who are analyzing micro-particles in archaeological samples, to help them understand the wide variety of micro-particles that they may encounter. It is not meant to provide detailed information about any one micro-particle type; other such focused books and resources exist and are included in the bibliography of each chapter. It is meant, however, to provide abundant figures and a sufficient description of a broad variety of micro-remain types so that the reader can begin to reduce their “unknowns.”

In the following chapters, the authors have provided information about “their” micro-particle type and how it’s formed, how it has been used archaeologically to provide information about human behavior and paleoenvironments, the methods used to isolate and identify the micro-particle, and the limitations or restrictions of each micro-particle type. This last point was of special importance given the sometimes fantastic claims made about such micro-particles in the archaeological record. Finally, there are abundant figures to help orient the novice, and multiple references provided for further reading.

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**Part I**  
**Micro-Particles Used Primarily for**  
**Reconstructing Environmental and**  
**Sedimentary Contexts**

# Coccoliths and Other Marine Microfossils in Microparticle Analysis



Jeremy R. Young

## 1 Introduction

Microfossils produced in freshwater and terrestrial environments such as diatoms and palynomorphs are widely used in Quaternary science, science-based archaeology and forensic science, and such uses are reviewed elsewhere in this volume. These microfossils are very widespread in human habitats and can provide excellent environmental data. In consequence, they have well-established roles and expertise on them is often present in departments of archaeology or quaternary studies.

Marine microfossils are less commonly used in this type of work and so may seem more esoteric. They are, however, abundant and diverse and while they may be uncommon in terrestrial sciences they play major roles in marine geological research. They can also provide important environmental data in coastal settings and for marine archaeology. Equally importantly, they are very common as rock constituents so can sometimes be used to help in paleoprovenancing of artifacts or building materials including pottery objects (Tasker et al. 2017, Wilkinson 2017, Williams et al. 2017).

They also can be introduced into non-marine environments, for instance from erosion of marine sediments. So, it would not be uncommon for someone studying microparticles to accidentally encounter marine microfossils. Hence, a basic familiarity would be useful.

One purpose of this paper is to provide a very broad overview of the major microfossil types and some of the ways in which they can be used. In addition, coccoliths are discussed in more depth. This is because they are both a particularly abundant and widespread group of marine microfossils and because they can be confused with some other important microparticle types, such as starch grains and dung spherulites.

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## 2 Overview of Marine Microfossils for Archaeologists

There is an immense diversity of marine microfossils and their study typically requires specialist knowledge. However, anyone involved in microparticle analysis would be well advised to be able to recognize the main groups (Fig. 1) and to be aware of the basic differences in their ecology, use, and mode of study as discussed below and summarized in Fig. 1.

### 2.1 *Foraminifera*

Foraminifera are single-celled animals which produce chambered shells. They are immensely abundant in the marine realm, forming a significant part of many ecosystems and contributing prolifically to the rock record. Indeed, they are probably the single most common type of fossils. They are also highly diverse with tens of thousands of species having been described. They occur throughout the fossil record from the Paleozoic to the present day and are very widely studied by geologists. The archetypal morphology is perhaps a coiled calcareous shell resembling a minute ammonite or gastropod but formed of a succession of chambers. They can, however, vary greatly in both morphology and shell composition. Notably, while calcite shells are most common, some groups form aragonitic shells and others agglutinate sand grains. Perhaps more importantly in terms of practical applications there are three major ecological subgroups within the foraminifera.

#### **Planktonic Foraminifera**

These are, as the name indicates, a group of plankton, living in the surface waters of the ocean. They typically have globular chambers and they are also known as globigerinids, after the common genus *Globigerina*. They are, with coccoliths, the prime components of calcareous deep-sea sediments (the *Globigerina* ooze of older textbooks). These deep-sea sediments provide a continuous record of earth history and so have played an immense role in paleoceanography (see, e.g., reviews of Schiebel and Hemleben 2005, Spezzaferri and Spiegler 2005). Planktonic foraminifera are central to the study of deep-sea sediments both as a means of dating the sediments and for recording environmental change via assemblage changes and from geochemical proxies such as oxygen isotopes. For example, the critical evidence for multiple glacial cycles during the Quaternary came from oxygen isotope records from planktonic foraminifera. Nonetheless, planktonic foraminifera are predominantly open ocean organisms and so are less likely to be useful in an archaeological context, other than as rock-forming components.



**Fig. 1** Microfossil groups. Illustrations of the main groups of living marine organisms which have extensive microfossil records. There are also some entirely extinct groups (e.g., conodonts) and many groups with less abundant fossil records but these are the main ones which it would be useful for anyone with an interest in microparticle analysis in archaeology to be familiar with. Ecologically they can be divided into three groups—phytoplankton, zooplankton, and benthos. Alternatively, they can be divided by hard-part composition into calcareous, siliceous, and organic categories, and for practical study this is perhaps more relevant

### Smaller Benthic Foraminifera

These are the most common foraminifera, they are similar in size to planktonic foraminifera (mostly 0.1–0.5 mm) but live on the seabed, i.e., are part of the benthos. The seabed environment is diverse in terms of, for example, substrate, water depth, water temperature, food flows, oxygen content, and other biota. This creates a wide range of ecological niches and benthic foraminifera exist in virtually all of them, with the result that thousands of species are present even in the modern fauna and tens of thousands in the fossil record. This diversity combined with their ubiquity means they are one of the most useful tools for reconstructing ancient environments. Moreover, they do extend into shallow marine settings and so can occur in coastal archaeological sites with marine intervals. For example, in the extended Ancient Human Occupation of Britain (AHOB) project smaller benthic foraminifera have been repeatedly used to characterize marine intervals within sites (e.g., Whittaker 1999; Whittaker and Parfitt 2017).

## **Larger Benthic Foraminifera**

Many foraminifera have developed the ability to host algal symbionts. This adaptation can be highly advantageous in low-nutrient high-light environments. Commonly, it also favors increased organism size and increasing shell complexity. This can result in shells several millimeters or even centimeters across with elaborate and beautiful internal structures. This evolution of large size and complex internal structures in symbiont-bearing foraminifera has been repeated independently dozens of times over the past few hundred millions of years giving rise to many separate groups of large benthic foraminifera. The best known of these are the nummulites which famously are the main constituents of the Giza Limestone, one of the principal building stones of the Egyptian pyramids. They are characteristic of shallow warm water environments such as carbonate banks and the fringes of reefs.

Larger benthic foraminifera typically have rather simple external morphologies but elaborate internal structures and so typically are studied in thin section. Detailed identification requires expert knowledge but can yield very useful biostratigraphic data.

## **2.2 *Ostracods***

Ostracods are minute crustaceans with two calcareous shells or valves. They are not especially familiar as organisms to non-specialists but are in fact very widely distributed. They are similar in size to smaller benthic foraminifera, occur in similar rock types, and can be prepared and studied using similar methods (typically rocks are disaggregated and wet sieved and the residue picked under a binocular microscope). Hence, they frequently are studied together with foraminifera. They also occur in a broadly similar range of environments but with a significant difference that they also occur in freshwater environments and are often abundant in nearshore settings. So in archaeological contexts ostracods are liable to be at least as common and useful as foraminifera. If bivalve-like shells less than 1 mm long are encountered, then it is likely they are ostracods and specialist study of them may be highly productive. For example, in the archaeological investigation of British hominid sites ostracods are routinely used in conjunction with smaller benthic foraminifera to characterize aquatic environments (Whittaker 1999; Whittaker and Parfitt 2017). Similar applications in Italian archaeology are reviewed by Mazzini et al. (2017).

## **2.3 *Diatoms and Radiolaria***

Diatoms are abundant, widespread, and diverse in freshwater settings and are widely used in archaeological work (e.g., Hill et al. 2017). They are, however, equally abundant and even more diverse in the marine realm. Benthic diatoms occur in shallow marine water and in the open ocean pelagic diatoms are the single most important



group of phytoplankton, especially in high productivity environments such as upwellings. The group is of relatively recent origin with the oldest unambiguous examples being Early Cretaceous in age (Sims et al. 2006) and only becoming ecologically prominent in the Cenozoic. Marine diatoms can provide equally rich data to non-marine diatoms when preserved, however, the diversity of diatoms means that diatomists will often specialize on either marine or non-marine. So different expertise may be needed if non-marine assemblages are to be analyzed. This expertise, as with other marine microfossils, is most likely to be found in the earth sciences. See also chapter “Diatom Microfossils in Archaeological Settings” by Stone and Yost in this volume.

Radiolaria are a second group of organisms with siliceous tests but they are unicellular zooplankton rather than phytoplankton (plant plankton). Lazarus (2005) provides a useful modern introduction to research on the group. They are typically around 0.1–0.2 mm across and have rather open radial structures in comparison to the finely perforate dish-like morphology of diatoms. They have a rich fossil record extending back into the Paleozoic, but they typically occur in the deep photic layer of open ocean environments and so are less likely to occur in archaeological contexts.

## 2.4 *Dinoflagellates*

Dinoflagellates are single-celled flagellates of the phylum Dinoflagellata. They are a common group of photosynthesising algae, especially in shelf-environments, although usually less common than diatoms. The vegetative cells do not normally leave a fossil record but within their life cycles they have a cyst-forming stage which is much more readily preserved. The cyst walls have similar composition to plant spores and pollen and like them are very durable. They are widely present in the fossil record and are an invaluable group for biostratigraphy, notably in petroleum geology. They also can provide useful paleoenvironmental data especially in shallow marine settings (see, e.g., review of Matthiessen et al. 2005). Abundances of dinoflagellate cysts in rock are, however, rather low and typically they require acid extraction techniques to prepare them for study. So, they are relatively unlikely to be encountered by accident as opposed to as part of a specialist palynological investigation.

## 3 Recognition of Coccoliths and Discrimination from Other Similar Sized Particles Which Produce Pseudo-Extinction Figures

### 3.1 *Introduction to Coccoliths*

Coccoliths are the smallest of the microfossils, being only 1–10  $\mu\text{m}$  across. Indeed, they are possibly the smallest organic remains that are routinely identified to species level. They are, however, enormously abundant. Individual coccoliths are calcareous

plates which form part of a composite exoskeleton, the coccosphere, of a single-celled alga, the coccolithophore.

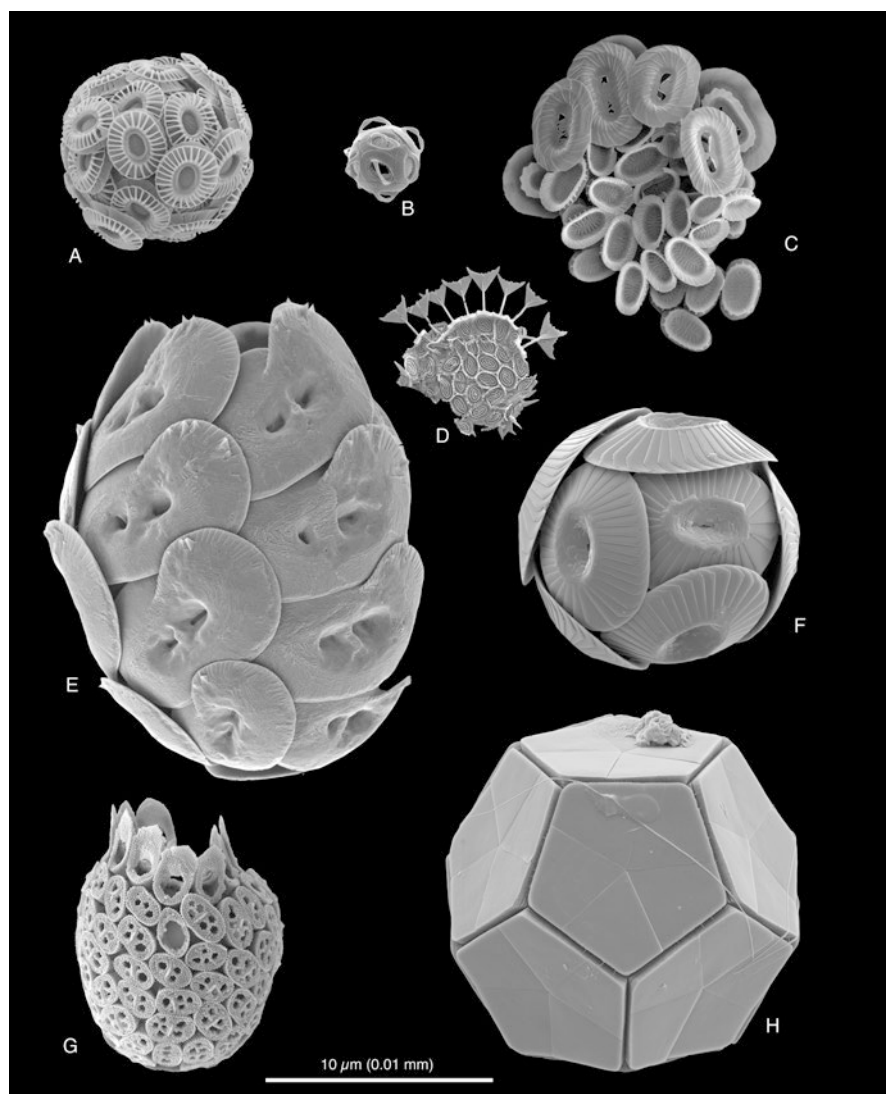
The coccolithophores themselves are a subgroup of the phylum Haptophyta, which are a group of predominantly marine flagellates. Morphologically they are fairly simple flagellates typically with spherical or ellipsoidal cells dominated by the nucleus, a pair of golden-brown chloroplasts, mitochondria, and golgi body. The flagellate stages are, however, distinctive with two similar smooth flagella and a third coiling, flagellum-like structure. This structure, the haptonema, is unique to them. Most haptophytes produce resistant organic scales, these are produced intracellularly in golgi-derived vesicles and then exocytosed to form a cell covering. In coccolithophores this process is modified with calcification occurring on the scales to produce much larger and more robust structures, the coccoliths (Young et al. 1999).

Coccolith formation, like scale formation, occurs inside the cell, within a golgi-derived vesicle. It is a remarkably finely controlled process with precise biotic determination of both crystal nucleation and growth. After coccolith formation has ended the coccolith vesicle, which can occupy much of the cell, migrates to the edge of cell and fuses with the cell membrane exocytosing the coccolith (Taylor et al. 2007). The coccolith then is emplaced within the coccosphere which is an extracellular structure (i.e., outside the cell membrane).

Coccolithophores are not an exceptionally diverse group with only about 250 known species (Young et al. 2003, 2005); however, they do show a remarkable range of cell morphologies. Coccolith morphologies are equally diverse, but the dominant coccolith types are circular or elliptical disks with more or less elaborate rims. They are formed of a radial array of complexly shaped interlocking calcite crystals.

The typical coccoliths just described are termed heterococcoliths and a complication is that many coccolithophores have a two-phase life cycle—producing heterococcoliths during the diploid phase of their life cycle and a different type of coccoliths, holococcoliths, during the alternate, haploid, phase (e.g., Young and Henriksen 2003; Billard and Inouye 2004). The holococcoliths are formed of numerous minute (ca. 0.1  $\mu\text{m}$ ) euhedral calcite rhombohedra (Fig. 2g). These holococcoliths are important biologically, but they have much lower preservation potential than heterococcoliths and so are much less likely to be encountered.

More significantly for microparticle analysis other coccolithophores produce calcareous structures which do not really correspond to either the heterococcolith or holococcolith model, for example, *Braarudosphaera* (Fig. 2h) and *Ceratolithus* in the modern plankton and *Discoaster* in the fossil record. These forms are collectively termed nannoliths and are studied along with coccoliths, although they probably have variable relationships to true coccoliths (see Young et al. 1999 for discussion). When it is necessary to explicitly include both coccoliths and nannoliths the terms calcareous nannofossil or calcareous nannoplankton can be used and these are very widely used in the geological literature. Indeed, geologists are liable to us the words coccolith and nannofossil almost interchangeably. Nannoliths can be abundant and useful. Typically they have rather simpler morphologies and are formed of fewer crystal units than heterococcoliths, and so can be more difficult to distinguish from non-biogenic particles.



**Fig. 2** Coccolithophore diversity. Seven extant species, collected from plankton samples, showing the wide range of shapes and sizes in both the complete coccospheres and their constituent coccoliths. (a) *Emiliana huxleyi*, (b) *Gephyrocapsa ericsonii*, (c) *Syracosphaera azureaplaneta*, (d) *Pappomonas borealis*, (e) *Helicosphaera carteri*, (f) *Coccolithus pelagicus*, (g) *Poricalyptra isselii*, (h) *Braarudosphaera bigelowii*. All images at the same scale

### 3.2 Geological Distribution

Coccoliths first occur in the Late Triassic (ca. 210 Ma) but at low abundances and diversities. They underwent a major radiation in the Early Jurassic and then became gradually more abundant and diverse through the Jurassic and Cretaceous (Bown et al. 2004). The Late Cretaceous was time of very high global sea levels which brought open marine sedimentary conditions onto the continental shelves and in particular resulted in chalk being a very widespread rock type. Late Cretaceous chalk is often predominantly (70–90%) composed of coccoliths and this was clearly a high point for the group in terms of both diversity and abundance.

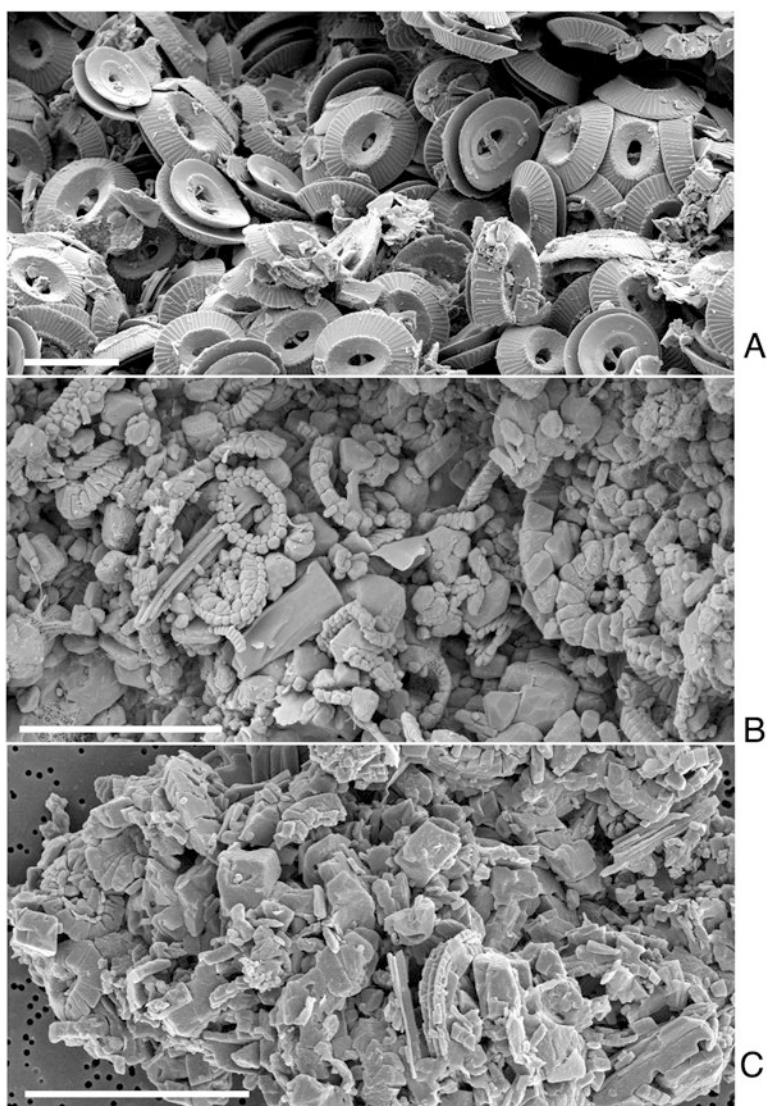
At the end of the Cretaceous (66 Ma), the mass extinction severely affected the group (e.g., MacLeod et al. 1997; Bown 2005). Only a very few species survived and none of the common Cretaceous species. Subsequently, there was a radiation of new forms during the Paleocene (66–56 Ma), but the new Cenozoic assemblages are distinctly different to the Mesozoic ones. During the Paleogene coccoliths probably recovered to similar abundance levels in the open ocean to those seen in the Cretaceous although lower sea levels mean that calcareous ooze/chalk sedimentation was largely confined to deep-sea sediments. Through the Neogene coccolithophores have become less abundant, possibly due to global cooling and/or competition with diatoms and now form a smaller proportion of deep-sea calcareous sediments (typical 10–30%) (Sucheras-Marx and Henderiks 2014).

### 3.3 Recognition of Coccoliths

*Hand specimen*—coccoliths are too small (<1/100 mm) to seen by the naked eye, with a hand lens or a binocular microscope. En masse they will appear as fine powder, with Late Cretaceous chalk being the archetypal example.

*Thin section*—typical rock thin sections are prepared to 35  $\mu\text{m}$  thickness, while coccoliths are only 5–10  $\mu\text{m}$  thick, so they are usually not easily seen but rather contribute to the very fine carbonate fraction—micrite. The presence of coccoliths can, however, be checked for by examining fine particles at the edge of the slide, in cross-polarized light at high magnification.

*Scanning electron microscopy*—coccoliths are near the limit of resolution even for electron microscopes but most instruments are capable of imaging them (Fig. 3). In pristine sediments identification is easy (Fig. 3a) but with even mild diagenesis dissolution and overgrowth can make the forms less regular and less obviously biogenic (Fig. 3b, c). However, the combination of a ring or disk-like shape, a radial structure formed of multiple crystals, and consistent rather complex morphologies are all strongly characteristic. If they are common it should also be relatively easy to confirm that they really are nannofossils by using standard references to identify the most abundant forms.



**Fig. 3** Coccolith appearance on SEM surfaces. Three rock samples showing coccoliths on broken surfaces. (a) Quaternary chalk from the Caribbean showing abundant pristine *Coccolithus pelagicus*. (b) A typical Cretaceous chalk surface (chalk fill of the Uffington White Horse, UK) showing numerous moderately well preserved ring-like coccoliths and rod-like nannoliths. (c) Poorly preserved coccoliths and nannoliths in a fragment of hard chalk, from base of a medieval artifact. Scalebars 10  $\mu\text{m}$

*Polarized light microscopy of strew mounts*—Coccoliths are formed of calcite which is the most birefringent common mineral ( $\delta = 0.172$ ) so they show bright interference colors in cross-polars. Indeed coccoliths and other nannofossils are typically only 1–5  $\mu\text{m}$  thick and so show bright first-order birefringence colors while most other minerals of this size, including clay, quartz, and biogenic silica, show very low birefringence. Moreover, since most heterococcoliths are radial arrays of calcite crystals they typically show pseudo-extinction crosses (Fig. 4a, b). These are readily seen in cross-polars and since different coccoliths have different crystal arrangements they also are useful for identifying species. So cross-polarized light is the standard routine technique used both for testing for the presence of coccoliths and for assemblage analysis.

Coccoliths are both abundant in rock samples and very readily transported so they can easily occur in a wide range of samples. Hence, in microparticle analysis it may be important to be able to confidently identify them. Also they might sometimes be confused with other microparticles which produce pseudo-extinction crosses, such as starch grains, dung spherulites, or diagenetic crystals. In general, however, such structures are formed by inorganic processes or by biologically mediated biomineralization under much less precise control than the biologically controlled biomineralization of coccoliths. This results in their structures being less regular in size and structure and less complex in morphology.

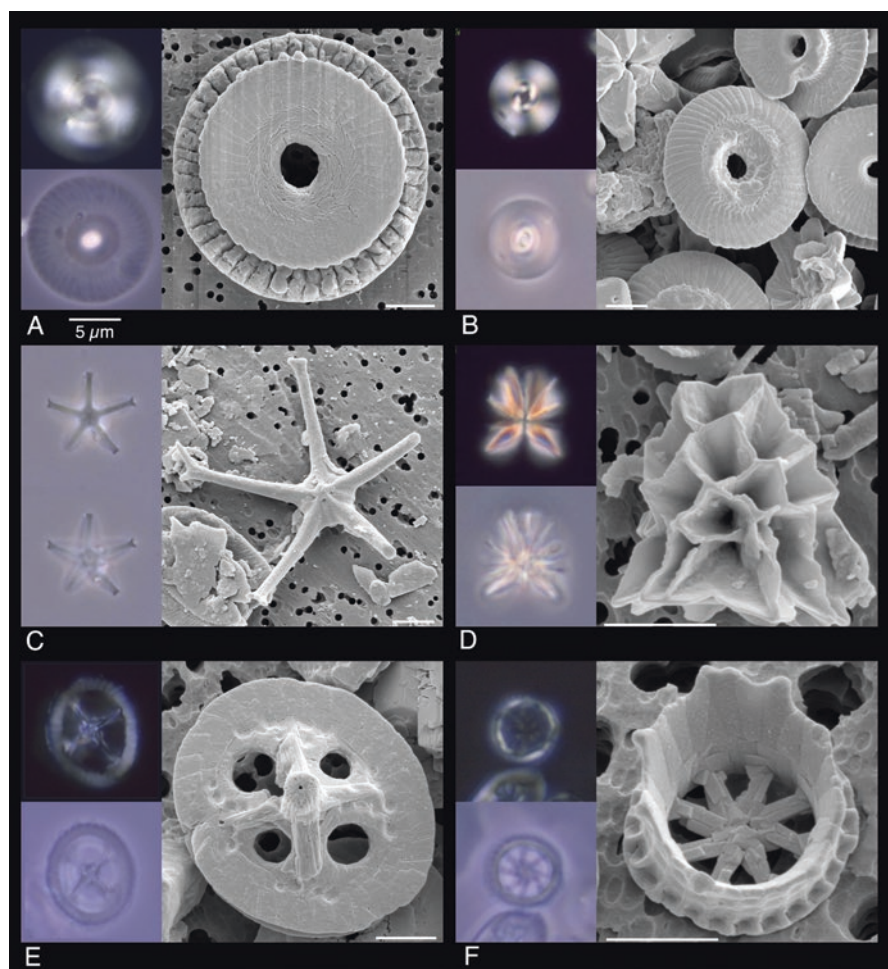
Coccoliths typically show ring like structures rather than simple radial structures, they are often regularly elliptical (Fig. 4b, e) rather than circular, usually any individual species in a sample will only show a two- to threefold range in dimensions, and they frequently show complex structures such as spines or central crosses (Fig. 4c, f). Careful focussing through specimens will often show that the morphology is disk-like rather than spherical and that the two sides of the disk are different. Identifying coccolith species in the light microscope requires some experience but, as in the SEM, if they really are coccoliths then it should be possible to find reasonable matches for common forms in a sample.

Nannoliths (e.g., Fig. 4c, d) can cause more problems since some nannoliths (e.g., *Ceratolithus*, *Minylithus*, *Quadrum*) have simple morphologies which are not obviously different from non-biogenic particles and others are compact radial forms which produce simple pseudo-extinction figures (e.g., *Sphenolithus*, *Fasciculithus*). However, it is unusual to have an assemblage in which nannoliths are present without coccoliths being at least as common. As a general rule, if all the specimens are only vaguely coccolith-like, with no unambiguous coccoliths present, then they are probably are not coccoliths.

### 3.4 Applications of Coccoliths in Archaeology and Related Fields

As with other marine microfossils there are two main types of occurrences and applications of coccoliths in archaeology; in situ coccoliths which can be used for environmental and age information and coccoliths contained in artifacts made of





**Fig. 4** Coccoliths in the light microscope. Images of six nannofossil species, each illustrated in cross-polarized light, phase contrast, and by scanning electron microscopy. The pairs of light microscope images are of the same specimen; the SEM image is of the same species but a different specimen. (a) *Calcidiscus macintyreii* (Pliocene heterococcolith); (b) *Cyclicargolithus floridanus* (Miocene heterococcolith); (c) *Discoaster pentaradiatus* (Pliocene nannolith); (d) *Sphenolithus moriformis* (Miocene nannolith); (e) *Grantarhabdus coronadventis* (Cretaceous heterococcolith); (f) *Rotelapillus crenulatus* (Cretaceous heterococcolith). All light micrographs at the same scale (scalebar below a); SEMs scalebar = 2  $\mu$ m

ceramics or rock which may be used for paleoprovenancing (e.g., Quinn 2017; Dunkley Jones et al. 2017). Since coccoliths are almost exclusively marine they are not common in archaeological settings and may be of low diversity. They can, however, sometimes be useful to determine if environments are marine and if they are present they may provide age data on the sediments. The use for age determination is due to the fact that the dominant subgroup, the reticulofenestrads (genera *Reticulofenestra*, *Gephyrocapsa*, *Pseudoemiliana*, and *Emiliana*) evolved remarkably rapidly during the Quaternary. So it is often possible to give ages to about 100 ka, or to determine for example which interglacial period a sample came from. This can be useful in ancient archaeological contexts.

For paleoprovenancing of artifacts, the great advantage of coccolith is that they are extremely small so minute scrapes of material can be studied. So, samples have been prepared from individual mosaic tesserae (e.g., Wilkinson et al. 2008), from the underside of stone objects, or even from the chalk ground of paintings (e.g., Perch-Nielsen and Plahter 1995). For example, the specimen shown in Fig. 2c was taken from the base of a medieval artifact, the Folkton Drum. Although the fragment is less than 0.05 mm across it contains enough identifiable coccoliths to prove it was from Late Cretaceous chalk, as opposed to the alternative identification of Permian Magnesian Limestone (Middleton et al. 2004).

The disadvantage of coccoliths for this type of work is that they usually can only give information on the geological age of the sample and coccolith-bearing rocks of a given age may be very widespread. Determining the geological age of a rock specimen is useful but will rarely uniquely determine its provenance. As another example, a study I was involved in with archaeologists from the British Museum concerned Egyptian stelae (engraved stone tablets) discovered on Malta (Young et al. 2009). It had been speculated that the tablets might have been made locally. Coccoliths from the samples proved to be Eocene in age, whereas the limestones on Malta are Middle Miocene in age. The Eocene age is typical of limestones used in Egypt and so supported the alternative hypothesis that the stelae were made in Egypt and transported to Malta (possibly as ship's ballast), as was supported by stylistic analysis of the stelae.

### 3.5 Sources of Further Information

There is a large specialist literature on fossil coccoliths. The most accessible reference work is the book *Calcareous nannofossil biostratigraphy* (Bown 1998) including particularly chapters on techniques (Bown and Young 1998), the Neogene (Young 1998), and Quaternary (Hine and Weaver 1998), an older but still valuable synthesis is provided by Perch-Nielsen (1985a, b) in the book *Plankton Stratigraphy* (Bolli et al. 1985). For discussion of broader topics there have been recent reviews on coccolith functional morphology (Monteiro et al. 2016), biogeographic distribution (Poulton et al. 2017), and ecology (Balch 2018). The single most accessible source is, however, provided by the website Nannotax ([www.mikrotax.org/Nannotax3](http://www.mikrotax.org/Nannotax3)).



This site has been developed over the past decade and includes a comprehensive taxonomic catalogue of original descriptions and illustrations linked to a database of modern taxonomic concepts. It includes ca. 20,000 images and covers all extant and fossil species. This is one of the most comprehensive databases available for any group of fossils and has become a standard reference source for workers in the subject. For non-experts, it allows easy look up of any taxon name to obtain reliable data on the modern taxon concept and geological range. Identification of taxa on the site requires some knowledge, but is facilitated by the ability to search by morphological characters and/or geological age, and by illustrated overviews which allow browsing through the taxonomy.

## 4 Conclusion

Marine microfossils are a very large and diverse group of organisms which can provide an immense amount of data. On the other hand, they are not usually present in terrestrial environments and so are only occasionally encountered in archaeological microparticle analysis. So, while it is useful for researchers undertaking such studies to be able to recognize marine microfossils there is relatively little requirement for any researcher to specialize in the study of marine microfossil in archaeology. Instead, it makes better sense to involve micropaleontologists with appropriate specialist expertise from geological backgrounds when there is need for this type of analysis.

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# Diatom Microfossils in Archaeological Settings



Jeffery R. Stone and Chad L. Yost

## 1 Introduction

The remains of diatom skeletons (as ecofacts) have been used as a component of archaeobiology for more than 70 years. Most of the earliest research arose from collaboration between diatomists and archaeologists (Battarbee 1988; Florin 1948). Since then, diatoms have increasingly been used to provide environmental and contextual information, both directly and indirectly, which has enhanced the relevance of their identification in archaeological settings (Battarbee 1988; Cameron 2013; Juggins and Cameron 2010; Mannion 1987; Miller and Florin 1989; Weiner 2010). As an environmental indicator for materials collected from archaeological sites, diatoms are probably under-utilized with respect to their potential; this is likely due to the need for expert analysis to provide reliable information given the immense species diversity of diatoms and the associated challenges in identification (Kociolek et al. 2015b). And, while the use of diatoms in archaeobiology has gained traction, the information they can provide is more commonly buried in technical reports (*see* The Digital Archaeological Record—<https://www.tdar.org/>) or as supporting information (Battarbee 1988; Cameron 2013) instead of being at the forefront of published findings.

Case studies exemplifying diatom archaeobiology have been integrated into several previous reviews (Battarbee 1988; Cameron 2013; Juggins and Cameron 2010; Mannion 1987). The intention of this paper is to provide a broad overview of the application of diatoms in archaeobiology and not just to focus on a few case studies.

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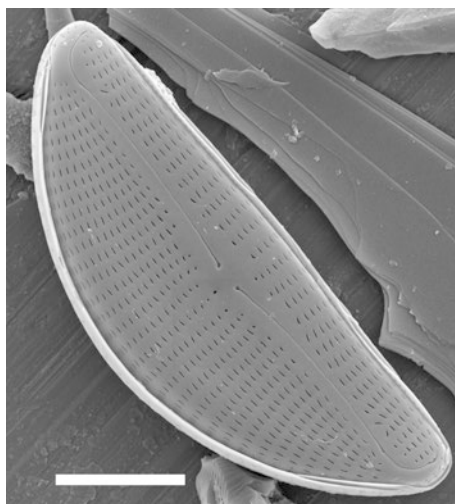
This includes providing non-specialists a guide to recognizing diatoms in their materials. To that end, this paper discusses what diatoms are, how they can be extracted for analysis, and how they can be distinguished from other siliceous microfossils. We also discuss why diatoms are powerful tools for providing environmental and contextual information and provide cautions regarding their use or interpretation. Owing to the complexity of diatom taxonomy and the diversity of skeletal forms, reviews such as this can not feasibly provide enough information to replace the need for a specialist. However, a better understanding of the strengths and weaknesses of using diatom skeletal remains can, hopefully, provide a better toolkit for exploring archaeological questions that diatoms may be uniquely capable of answering.

## 2 What Are Diatoms?

Diatoms are a microscopic, unicellular golden-brown algae that are common to all aquatic ecosystems (Round et al. 1990). Typically diatoms are silt-sized, with frustules ranging between 2 and 300  $\mu\text{m}$ ; a few very large diatom species can be up to 500  $\mu\text{m}$  in the longest dimension. However, diatoms can live singly or in a range of colony types that together can be large enough to be visible without the aid of a microscope (Spaulding et al. 2019). Most diatom species are identified by the characteristics of their siliceous cell wall, known as a frustule. Diatom frustules are composed of two valves. Each frustule has one valve that is slightly larger and older (epitheca) than the other (hypotheca). The valves fit together similar to a Petri dish or hatbox and are bound together by a series of linking siliceous bands, known as cingula (Battarbee et al. 2001). Diatom valves are perforated by a number of different types of apertures that can be used to communicate with the environment (Fig. 1). It is by these openings that diatoms are able to move, attach to substrates, and exchange waste products or nutrients (Kocielek et al. 2015a, b; Round et al. 1990).

In most aquatic ecosystems, diatoms are major contributors to the primary production (Armbrust 2009; Smol and Stoermer 2010). Although some diatoms can be heterotrophic (Ishii and Kamikawa 2017) or live in symbiotic relationships (Lee 2011), the majority of diatoms are autotrophic, requiring sunlight to grow (Battarbee et al. 2001; Round et al. 1990). Diatoms can reproduce very quickly, asexually, through binary fission. Each daughter cell takes one of the parent valves and creates one new valve internally prior to separating. Because the newly-formed valves are created internally, the daughter cells typically result in one cell remaining the same size (formed from the parent's epitheca) and one cell being slightly smaller (formed from the parent's hypotheca). The net effect is that the population clones itself quickly but gradually diminishes in size (Round et al. 1990). As the population size decreases, an environmental or size-related trigger occurs and the population switches to sexual reproduction. Sexual recombination results in the formation of auxospores, which regenerates the diatom cells to the largest size; this allows the population to return to asexual reproduction (Mann 2011). As a result of this

**Fig. 1** Scanning electron image of a diatom frustule (*Encyonema*); scale bar 5  $\mu\text{m}$



reproductive strategy, diatom species identification can occasionally be complicated when the full species size series does not occur in the materials being analyzed (Smol and Stoermer 2010).

Diatoms are thought to have evolved ~150 million years ago (Jurassic Period) in the marine realm and have subsequently developed an enormous species diversity (Armbrust 2009; Sims et al. 2006). Fossil diatoms are common components of freshwater lake deposits of Late Eocene (~35 million years ago) age and younger (Sims et al. 2006; Siver and Wolfe 2007; Wolfe et al. 2006; Wolfe and Edlund 2005). There is an immense number of diatom taxa, with more than 200 accepted genera and conservative estimates for extant species starting at around 20,000 (Guiry 2012) and ranging up to more than 2 million species (Spaulding et al. 2019). Diatoms form the base of the food web in many parts of modern oceans (Armbrust 2009) and in nearly all lake and river settings (Battarbee et al. 2001; Round et al. 1990).

Diatoms are highly adaptable and have evolved to live in nearly every known habitat where some light and at least periodic exposure to water is available (Smol and Stoermer 2010). Many diatoms have adapted to live in highly specialized extreme aquatic habitats, including those that are highly acidic (DeNicola 2000), highly basic (Kocielek and Herbst 1992; Verschuren et al. 1999), hot springs (Bonny and Jones 2003; Jones and Renaut 2006; Konhauser et al. 2004; Owen et al. 2008a, b), extremely low nutrient conditions (Cremer and Wagner 2003; Michelutti et al. 2003), within highly saline environments (Clavero et al. 2000; Kirkwood and Henley 2006), within sea ice (Ligowski et al. 1992), and at extremely high elevations (Jüttner et al. 2000; Nautiyal et al. 2004). Diatoms can even be found living in aerophylic environments such as tree bark (Kharkongor and Ramanujam 2014), tank bromeliad water pools (Killick et al. 2014), moist soils, moss, attached to cave walls (Lowe et al. 2013) and other surfaces where there is exposure to natural or artificial light (Johansen et al. 1981; Mcmillan and Rushforth 1985; St. Clair et al.

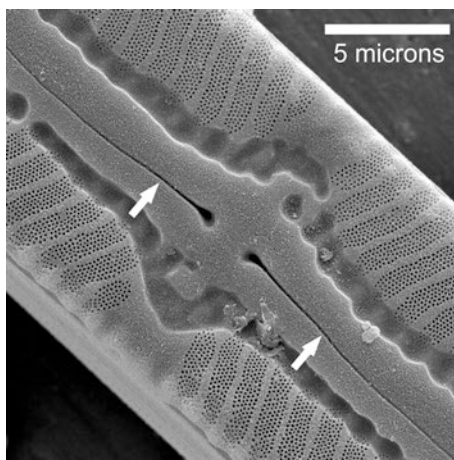


1981; St. Clair and Rushforth 1976). Owing to their high diversity and environmental specialization, diatoms are sensitive to a wide range of environmental parameters.

The morphology of diatom frustules is also often adapted to the environment in which the diatoms live. As such, many diatoms can be placed into broad ecological groups, based solely upon features of the frustule. For example, diatoms that live attached to substrates commonly have a raphe structure—a long slit-like opening in the valve—that is often used to attach the cell to a substrate or to sibling cells within a colony (Round et al. 1990). Benthic diatoms commonly use the raphe structure (Fig. 2) to move along the surface of substrates to which they are attached (Cohn and Weitzell 1996). Diatoms without raphe structures lack motility, and as such are either plankton or sessile. Additionally, diatoms are also commonly subdivided based upon the symmetry type of their valves. Diatoms with frustules that express symmetry around a point (centric) commonly lack raphe systems, whereas diatoms with frustules that express symmetry around a plane (pennate) are often subdivided into araphid and raphid morphological groups (Round et al. 1990). Similarly, many diatom frustules have distinct morphological structures called apical pore fields, from which the diatom may exude materials to allow it to attach to substrates. Since these morphological components of the frustule serve specific purposes, these and other features of the frustule can provide clues to the life habits of diatoms *even if the species is extinct*.

As mentioned above, diatoms may live in a very wide variety of environments. Diatom habits can broadly be divided into those that float in water (plankton) and those that live attached to a substrate (benthic). Since almost all diatoms require the presence of light to live, and deeper water habitats tend to have a very limited number of sunlit substrates, functionally this division separates most diatoms that are abundant in deeper water from those that are abundant in shallower water (Stone and Fritz 2004). However, the types of substrates to which diatoms attach can also vary widely, enhancing our ability to use them as environmental indicators. For

**Fig. 2** Scanning electron microscope image of a raphid pennate diatom (Pinnularia). Arrows indicate the slit-like external expression of the raphe system



example, some genera are known to exclusively attach to other biota (epizoic). In some cases, epizoic diatom taxa are highly selective of which biota they grow upon; for example, the marine genus *Tursiocola* grows exclusively on the skin of cetaceans (Frankovich et al. 2015) and *Synedra cyclopum* is an epizoic species commonly found living attached to crustacea in cool-water lakes (Patrick and Reimer 1966). Benthic diatoms often inhabit specialized microhabitats, such as living attached to plants or other macrophytes (epiphytic), attached to mud (epipellic), attached to rocks (epilithic), attached to sand (epipsammic), flowing water (rheophilic), within the near surface of soils (edaphic) or the many subaqueous environments discussed above (aerophilic). If this information is known for a diatom taxon, this greatly enhances our ability to interpret their presence in ancient aquatic or archaeological settings.

Diatom frustules are composed of rigid opaline silica; when the organism dies the skeleton is readily preserved as a fossil. This characteristic, combined with their wide distribution and sensitivity to environmental parameters (Fritz 1996), has resulted in the use of the diatoms to monitor or reconstruct a very large range of environmental and paleoenvironmental conditions. Examples of reconstructed parameters include water chemistry (Battarbee et al. 2001; Flower 2017; Fritz 1996), nutrients (Michel et al. 2006; Potapova et al. 2004; Potapova and Charles 2002), salinity (Fritz 1990), pH (Birks et al. 1990), light intensity (Vinebrooke and Leavitt 1996), water depth (Stone and Fritz 2004; Wigdahl et al. 2014), convective mixing (Saros et al. 2012; Stone et al. 2016), substrate type (Kingston et al. 1983), flowing water (Thoms et al. 1999), and to some degree water temperature, although mostly indirectly (Anderson 2000).

### 3 How Are Diatoms Useful for Archaeological Research?

Due to their small size, preservable fossil skeleton, and high concentration in both water and sediments such as lake muds and clays, diatoms are easily transported away from aquatic environments or ingested by migratory organisms that utilize water resources. Human settlements also tend to be established near easily accessible water resources. As such, humans tend to be major agents for the redistribution of living and fossil diatoms (Kociolek and Spaulding 2000). Diatoms can colonize practically any reservoir of water that meets their specific requirements for sunlight, nutrients, and aquatic or semi-aquatic environmental conditions (Battarbee et al. 2001). As a result, diatoms are capable of quickly becoming distributed widely. Invariably, this results in some fossil diatom materials ultimately preserved together with any archaeological remains (Battarbee 1988).

Fossil diatom materials have been collected and analyzed from a wide range of environments that might provide relevant archaeological information (Cameron 2013; Juggins and Cameron 2010; Mannion 1987; Winsborough 1995). Here we divide their use in archaeological settings into two broad groups: (1) examples where fossil diatom remains have been found in situ in sediments adjacent to human



settlements and thus used to provide environmental context; and (2) examples where diatom microfossil remains have been found directly within archaeological materials, typically introduced by human activities, and subsequently used to provide information about human culture, practices, or the surrounding environments from which diatom microremains have been imported (i.e., archaeobiology).

### ***3.1 Use of Diatom Remains from Sediment Records***

Traditional uses of fossil diatom assemblages as biological indicators from lacustrine, palustrine, or marine sediments have provided numerous examples of context regarding regional climate or local landscape changes that profoundly influenced prehistoric civilizations around the world (Jensen et al. 2004; Metcalfe and Davies 2007; Owen et al. 2008a, b; Tapia et al. 2003; Whitmore et al. 1996). Many of these examples highlight the use of diatoms to reconstruct changes in lake depth, using the ratio of planktonic to benthic diatom taxa found in sediments (Wolin and Stone 2010), linked to long-term changes in the regional hydroclimate. There are also many examples of diatoms collected from diatom-rich strata or diatomites within or adjacent to sites of archaeological interest, particularly in arid settings around spring deposits in the American Southwest (Blinn et al. 1994; Meltzer 1991; Winsborough 1995), lakes in Mexico (VanLandingham 2004, 2006) and lakes or springs in Africa (Ashley et al. 2009; Owen et al. 2008a, b, 2012; Robbins et al. 1996; Williams et al. 1979) and wetlands in the Balkans (Ognjanova-Rumenova 2008; Ognjanova-Rumenova and Zapryanova 1998); fossil diatom remains are often used in these settings as a primary tool for developing environmental context.

Fossil diatom remains in lacustrine sediments can also be used to provide information about how ancient human activities have modified the landscapes in which they lived. This can include intensive changes to aquatic environments, such as the impact of Late Holocene crannog development (O'Brien et al. 2005; Selby and Brown 2007) and the construction of dams, or more subtle changes, such as evidence of the influence of whaling practices in prehistoric communities (Douglas et al. 2004). Since diatoms are particularly sensitive to changes in nutrient flux to lake systems, diatoms have been used extensively, together with changes recorded in fossil pollen or phytoliths, to track land-use changes and agricultural practices (Bichet et al. 2013; Ekdahl et al. 2004, 2007; Mannion 1987; Ognjanova-Rumenova and Zapryanova 1998; Trombold and Israde-Alcantara 2005; Widgren et al. 2016), or cultural eutrophication (Fritz 1989; Gillson et al. 2018; Haworth 1985; Mannion 1987; Miller et al. 2004). Changes in fossil diatom assemblages have provided information on the timing of regional deforestation in a number of studies. These records often show changes in assemblages driven largely by diatom response to erosion or changes in regional runoff patterns. Diatoms have been shown to be particularly useful where anthropogenic deforestation has had a substantial influence on local water levels (Fritz 1989; Horrocks et al. 2007; Sniderman et al. 2009) or pH (Stabell 1993). Numerous examples of changes to the landscape produced by pre-

historic civilizations affecting local lake sedimentation or water quality, and reconstructed by fossil diatom analysis, are presented in detail in Mannion (1987).

Fossil diatom assemblages from lake sediments also can act as excellent archives of extreme flooding events. A number of researchers have taken advantage of this in archaeological settings to explore the effect of catastrophic hydrological events on Late Holocene prehistoric human settlements. Along the northwestern coast of the United States, abrupt shifts in the environment identified by using fossil diatom assemblages have been interpreted as indicators of earthquake-induced subsidence and tsunami events (Cole et al. 1996; Hemphill-Haley 1995; Sherrod 2001). These tsunami deposits are thought to have buried numerous occupation sites under a meter or more of sediment. Winsborough et al. (2012) provide a more direct example of catastrophic flooding events that affected Pachacamac settlements from sediment cores collected from a lagoon along the coast of Peru. In that study, fossil diatom remains were used together with other biological indicators, to provide evidence for four major floods alternating with severe droughts in the Andean highlands over the last 2000 years.

This approach has even been extended into the last several million years by analyzing ancient lake sediments and diatomite deposits to provide answers about global climatological phenomena that might have had a substantial impact on human evolution (Campisano et al. 2017; Cohen et al. 2007; Gasse 2000; Gasse and Street 1978; Stone et al. 2011). Jackson et al. (2015) provide an excellent example of how fossil diatom remains can provide answers to long-standing anthropological questions. This study exploited the occurrence of volcanic cryptotephra with Lake Malawi sediments to explore and ultimately *refute* the notion that the Toba Eruption (~75,000 years before present) had a significant bottleneck effect on early human populations. Their results showed no sustained large-scale changes in the fossil diatom assemblages, or other bioindicators within the sediments (Yost et al. 2018), prior to or after the eruption. This indicates that the lake and surrounding landscapes that early humans inhabited were not likely to have been catastrophically affected. More direct uses of changes in fossil diatom remains include providing information about the quality or type of water resources adjacent to early human settlements. For example, Ashley et al. (2009) showcase the use of fossil diatoms from spring deposits adjacent to Oldowan sites to provide information about whether hominid use of water resources changed during extensive wet or dry periods. Here diatoms were able to provide information regarding the water chemistry (fresh vs. brackish), which could in turn be used to reconstruct wet and dry periods. From this information, a pattern emerged regarding materials used and the frequency of artifacts in wetter and drier periods that helped determine how hominids were exploiting available resources.

Since diatoms are highly adapted to different environments along salinity gradients, marine and freshwater diatoms are usually easily distinguishable from each other. This has led to the use of diatom remains in coastal settings where human settlements occur to help determine whether local water resources were potable, brackish, or saline. These include some of the earliest studies of fossil diatom assemblages in archaeological settings (Mannion 1987) and extensive analysis of diatoms for archaeological environmental context in estuarine settings (Jansma

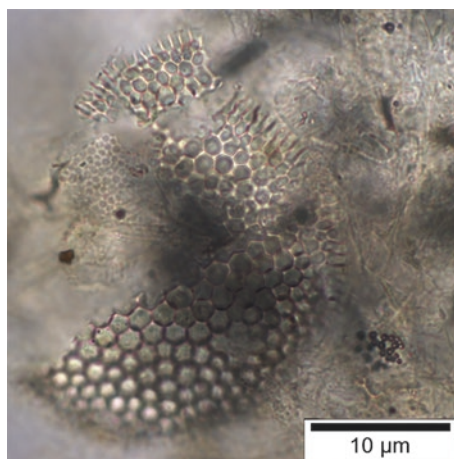
1982; Juggins 1992; Miller and Florin 1989; Milne et al. 1983; Nunez and Paabo 1990). This topic has been the focus of several prior reviews of the use of diatoms in archaeology; fossil diatom remains have provided information regarding the reconstruction of sea level, river quality, and tidal influence of the Thames during the early Roman and pre-Roman periods (Battarbee 1988; Juggins 1992; Juggins and Cameron 2010; Miller and Florin 1989). Here the presence of brackish-water species could be used to provide context for the timing of the tidal head of the Thames River into London. This transition could then be dated to provide information on the timing (Late Bronze Age) for this event and also provide some understanding of how rapid and continuous these transitions were. Using a similar understanding of the relationship between fresh and brackish-water taxa, diatoms have also been used to detect patterns of transgressive-regressive sequences affecting abandonment and reoccupation around the coastline of the Baltic Sea (Battarbee 1988; Juggins and Cameron 2010), prehistoric sites around the coast of the Netherlands (Battarbee 1988; Jansma 1982, 1990; Juggins and Cameron 2010; Mannion 1987), and ancient Roman harbors (Cameron 2013).

### 3.2 *Use of Diatoms in Archaeobiology*

As discussed above, diatoms are capable of colonizing practically any aquatic or semi-aquatic environment. This includes environments that are only occasionally wet or contain trace levels of nutrients essential for their growth (Cremer and Wagner 2003; Kharkongor and Ramanujam 2014; Killick et al. 2014; Lowe et al. 2013). As such diatoms can readily colonize human-built structures designed to retain or move water (Battarbee 1988; Cameron 2013; Juggins and Cameron 2010). Fossil diatom remains also have regularly been recovered from clay or rock materials fabricated from lake sediments and diatomite outcrops. One of the most common applications of diatoms in archaeobiology is as a provenance tool in the typology of archaeological materials (Battarbee 1988; Juggins and Cameron 2010). In these cases, something about the diatom assemblage provides information regarding the source of the clays or other earthen materials. Diatom microfossil remains are small enough to be incidentally digested with other foods and often can pass through digestive systems without damage to the frustule. Several studies have used fossil diatoms (together with phytoliths, starch grains, and many other microfossils discussed in this volume) collected directly from dental calculus (King et al. 2017), bones (Peabody and Cameron 2010), or fecal materials (Chicoine et al. 2015; Horrocks et al. 2003) to provide information about prehistoric diets or potentially food processing techniques (Fig. 3).

Human-built or human-dug structures used to import or retain water within settlements, such as privies, cisterns, canals, ditches (Jansma 1978), moats (Beneš et al. 2002; Keevill 2004; Nunez and Paabo 1990), aqueducts, wells (Neely et al. 1995), and other retention structures, are common sites where fossil diatoms have been found in archaeological settings (Battarbee 1988; Juggins and Cameron 2010). In these cases,

**Fig. 3** Fragmented frustule from a marine diatom (*Coscinodiscus*) recovered from human dental calculus, which could be used to determine the salinity of nearby water sources and suggesting a potential coastal diet



diatoms are usually assumed to have colonized structures and fossils are thought to represent in situ assemblages living in built environments (Juggins and Cameron 2010). Similar to lake sediment studies, analyses of fossil diatom assemblages in these materials have provided archaeologists with the environmental context of the water within the structures, such as the concentration of organic pollution or salinity (Keevill 2004). In some cases, context for the aquatic environment can provide important clues about the function of the structures, for example, the presence of marine diatom taxa has been used to help identify structures as fish traps, and marsh environments reconstructed from sediments a hand-dug structure was excavated from was used to help determine that it was actually a well (Godbold and Turner 1993; Neely et al. 1995).

As water resources are often ported into human settlements for cooking or other uses, fossil diatom remains can often be obtained from hearths (Risberg et al. 2002), occupation floors (Bar-Yosef et al. 1992), cooking areas, middens, or other sites where water collected from local resources are used (Battarbee 1988; Juggins and Cameron 2010; Mannion 1987) or food is processed (Denys 1992). As a result, it may also be possible to recover diatom microfossil remains from instruments used to process, store, or prepare foods, such as stone tools and cooking residue from steatite and ceramic containers (Fig. 4). Diatoms could also be useful in helping to identify plant resources such as roots and stems derived from aquatic habitats, particularly if they are epiphytic or edaphic taxa.

Because fossil diatoms are a common constituent of mud, diatoms have been collected from bricks (Flower 2017) and clay tablets (Tuji et al. 2014). Diatoms regularly have been analyzed from other fabricated ceramics, particularly pottery (Battarbee 1988; Gibson 1986; Håkansson and Hulthén 1988; Jansma 1982; Juggins and Cameron 2010). This technique suggests they could potentially be recovered from other commonly constructed items, such as pipes and figurines. There is a long tradition of using diatom remains in ceramics as a means to trace potential sources of clay (Battarbee 1988; Gibson 1986; Matiskainen and Alhonen 1984). Battarbee (1988) discusses several case studies where diatoms have been recovered from ceramics. Common uses include providing pottery typology, relating clay to local

**Fig. 4** Frustule of an aerophilic diatom (*Hantzschia*) collected from hearth materials in archaeological settings



sources, excluding local clay sources, and indicating preferences for clay types in pottery. Despite numerous examples of the use of diatoms recovered from ceramics, there are also quite a few potential pitfalls with this approach which can limit its effectiveness. These are discussed in detail below.

Diatom microfossils can also be recovered directly from human structures that use or are built within earthen materials, such as cave walls (Cennamo et al. 2012; Falasco et al. 2014), gravesites (Šálková et al. 2015), and dwelling structures with earthen floors or daubed with mud. The abandonment of archaeological features, such as pit houses, post holes, storage pits, or agricultural plots, also can lead to microhabitats favorable for the growth of diatoms. Diatom analyses of these materials can often provide information regarding the original sources of building materials or the nature of the aquatic environments from which they are derived.

For example, a recent study revisited a classic prehistoric wetland settlement in the UK known as Glastonbury Lake Village with a multi-proxy microfossil approach (Hill et al. 2019). This Iron Age site was a coastal settlement that had layers of occupation floors preserved. Prior research had assumed to be derived from nearby coastal mudflats; however, diatom analysis of these sediments showed that the origin was a purely freshwater source. These findings suggest that materials were transported from longer distances by settlers, despite the presence of local wetland mud sources.

A particularly interesting study is presented in Bathurst et al. (2010), which uses diatom microfossil remains to explore turf structures from an ancient Viking Age farmstead in Iceland. Although turf traditionally was used as one of the main building components in this region at the time, often there is little evidence of their existence remaining on the landscape today. In this study, fossil diatom remains imbedded in the turf were used not only to identify the presence of turf-based structures but also to discriminate different archaeological features based on the types of diatoms associated with them. Because a subset of diatoms can live in soils, attached to moist cave walls, and other subaerial habitats, it may be difficult to separate fossil diatoms occurring within materials being used from those of aerophilic species that may be in situ, having colonized the built environments.

Diatom frustules are siliceous and as such will not dissolve in most acids; they can pass through the digestive systems of organisms and can readily be recovered from dung (Golyeva 2012), human coprolites (Horrocks et al. 2003), or guano, to provide important information about landscape uses or cultural practices related to food sources. In cases where human coprolites are recovered, fossil diatoms recovered from these materials can also be used to help determine something about the diet of the people who consumed them. For example, the presence of marine diatoms in coprolites has been used to help determine whether people consumed marine fish (Horrocks et al. 2007). Diatom frustules are also capable of entering the lungs of organisms when water is aspirated and can pass through alveoli in the lungs and enter the bloodstream. There they eventually become deposited in the marrow of bones, allowing fossil diatom remains to potentially be recovered directly from the bones of organisms, including human remains (Peabody and Cameron 2010).

## 4 How Are Diatoms Recovered from Archaeological Samples

The diatom frustule is made of an opaline silica. This makes it easy to preserve and resistant to most acid-based chemical processing that might be used to extract diatom fossil materials from samples. Processing techniques required tend to be specific to the type of sample materials being analyzed. However, generally, diatom fossil extraction techniques are meant to reduce any materials that may obscure a complete view of the frustule in light microscopy or scanning electron microscopy. These techniques often follow a pattern of first digesting any organic materials and then removing carbonates or other easily dissolved materials. For most archaeobiological materials, additional treatments will be required to remove detrital grains, which can include filtering, timed settling, or heavy liquid separation techniques. For diatom frustules that are incorporated into artifacts or manufactured materials, additional chemical treatments may be required to extract diatom frustules. It should be noted that fossil diatom remains are readily observable in a microscope from simple wet mounts or smear slides, but typically require extraction from the sample for species-level identification.

### 4.1 *Extraction of Diatoms from Indurated or Ceramic Materials*

Where the diatom materials are highly indurated or not easily extracted through chemical processing techniques, sometimes diatom fossil materials can be recovered in sufficient concentrations by simply scraping or grinding the original materials. Afterward, processing techniques described above as appropriate for the material type can be used on the scraped or ground materials. This sort of approach has commonly been used when diatom frustules need to be extracted from diatomites (Ashley et al. 2009; Owen et al. 2004), where the concentration of diatom frustules makes it possible to avoid damaging most of the valves when separating them from the source rock.



Diatom frustules can be observed in microscopic thin sections, which is a common approach to analyzing ancient ceramics. However, species identification in thin sections is challenging. Valves can be obscured by clay particles and thin sectioning may result in diatom valves that are exposed in transected views (Håkansson and Hulthén 1986; Harris-Parks 2016). In most cases, diatom frustules can be extracted by dissolving fired clays without damage to the valves. When diatoms are being extracted from pottery or other clay-based artifacts, the original materials are typically broken into small pieces and placed in a weak concentration (10%) of phosphoric acid at a constant temperature of 50 °C for 7–10 days (Håkansson and Hulthén 1986). Because diatom frustules for some species are delicate, aggressive techniques may fragment or destroy fossil remains. Particular care should be taken to limit exposure to strong bases, which can result in the dissolution of opaline silica (Barker et al. 1994; Ryves et al. 2001) or extensive or aggressive agitation such as long exposure to intense vibrations in an ultrasonic water bath (Battarbee et al. 2001).

## 4.2 *Extraction of Diatoms from Sediments*

To recover fossil diatoms from lake sediments, peats, or other organic-rich materials, samples are often treated with a strong oxidizer (typically 30–35% hydrogen peroxide) or a strong acid, to digest the organic carbon components (Battarbee et al. 2001). In cases where there are substantial or resistant organic materials, nitric or sulfuric acid can be substituted for hydrogen peroxide or samples can be gently heated to accelerate the digestion (Battarbee et al. 2001; Serieyssol et al. 2010). After the digestion of organic materials is completed, the samples should be rinsed several times with reverse-osmosis purified water. After each rinse, samples can either be centrifuged or allowed to settle. The supernatant fluids may be decanted or removed with a sink aspirator between rinses. Removal of carbonates in the original materials can be accomplished by adding a weak acid (typically 10% hydrochloric acid) and afterward samples can be rinsed as above (Battarbee et al. 2001). Similar strong acid-digestion techniques can be applied for use on organic materials collected from bones or other organic tissues (Cameron 2013; Verma 2013).

In many cases, archaeological samples include a substantial fraction of sand- and silt-sized detrital components. Detrital grains cannot be easily removed through chemical processing treatments. Even after organic and carbonate materials are removed, detrital components can obscure the visibility of diatom frustules in microscopic slides or diatom concentrations within the samples are so low as to make detection difficult. Because sand-sized detrital components will settle more readily than silt-sized components, such as diatom frustules, timed settling techniques or in some cases filters can be used to separate diatom fossil materials from sand-sized detrital fractions (Battarbee et al. 2001). However, the frustules of most diatoms are silt-sized particles and these can often be difficult to sort from detrital silt particles. When this is an issue, fossil diatom samples can be concentrated by floating diatom fossil materials through heavy liquid extraction techniques. This technique capitalizes on the density differences between opaline siliceous materials

and detrital siliceous materials to float diatom frustules in heavy liquids that can be mixed to specific densities. Fossil diatoms and other opaline silica materials can be concentrated by extracting the materials from the boundary between two or more heavy liquids of variable densities (Tapia and Harwood 2002). This technique can also be utilized to extract diatom materials from samples that are rich in siliceous clays. Although not optimized for the recovery of large or delicate diatoms, phytolith extractions often contain significant numbers of diatoms and can inform on the potential success of a diatom-specific analysis. If low diatom concentrations are anticipated, especially for non-lacustrine or non-riverine contexts, dual phytolith-diatom extraction methods can be developed to better optimize diatom recovery.

### 4.3 Mounting Diatom Extractions for Analysis

Once the diatom particles are extracted and/or concentrated from the original materials, the extracted materials can be settled onto “number 1” type (or thinner) microscope coverslips and evaporated to dry (Battarbee et al. 2001). A specialized semi-permanent resin mountant with a high refractive index (e.g., Naphrax, Hyrax, Z-rax) cured by volatilizing admixed toluene on a hot plate is typically used to adhere the coverslips to microscope slides for appropriate viewing in light microscopes. To identify diatoms to species often requires high magnification (oil immersion, 600–1000× magnification) light microscopy or scanning electron microscopy imaging. For SEM imaging and analyses, diatom extractions are typically evaporated directly onto stubs or specialized attachable coverslips and sputter-coated with a conductive material to reduce charging (Battarbee et al. 2001).

## 5 How Are Diatoms Distinguished from Other Microfossils?

Diatom frustules are always siliceous, silt-sized particles, and tend to be highly symmetrical either around one or more points (centric, *see*, e.g., Plate 1) or one or more planes (pennate, *see*, e.g., Plates 2, 3, 4, 5, 6, and 7), and as such can be quickly distinguished from most other biological remnants in any setting (Kocielek et al. 2015a, b). However, other organisms with siliceous elements or skeletons produce microfossil remains that have the potential to be confused with diatom frustules. As discussed above, diatom frustules are composed primarily of *two valves*, which when found together as whole frustules clearly will distinguish diatoms from all other siliceous microfossil remains. However, often in materials where diatoms are preserved as fossils, the linked cingula that hold the frustule together will disarticulate, resulting in a separation of the two valves (Battarbee et al. 2001); also, depending on how the materials have been processed to extract or concentrate the diatom fossils and the quality of the fossil preservation in the original materials, diatom remains recovered from archaeological settings frequently can be highly fragmented (Battarbee 1988; Håkansson and Hulthén 1988; Mannion 1987). Together, these factors can compli-



**Fig. 5** Example of a diatom fragment (Naviculoid) collected from archaeological settings. The presence of a raphe indicates this as a benthic (shallow water) taxa



cate the identification of fossil diatoms substantially (Fig. 5). The diversity of forms in living and fossil diatom species is also immense, which makes identification to species level challenging enough that commonly an expert is required, but the characteristics and features that can be used to identify that a particle is a diatom frustule, rather than another siliceous microfossil, are fairly limited.

Diatom frustules are never completely isodiametric. As a result, diatoms typically can be viewed in orientations where either the valve face is exposed (referred to as “valve view”—see Plate 1, Fig. m) or where the cingula are exposed (referred to as “girdle view”—see Plate 1, Fig. l) and the valve face is seen in profile (Round et al. 1990). For many diatoms, species identification is primarily accomplished in a light microscope by features visible when the frustule is *in valve view*, although characteristics of diatoms in girdle view may also be very important for the identification of some genera (Battarbee et al. 2001; Round et al. 1990; Spaulding et al. 2019), for example, *Aulacoseira* (see Plate 1, Figs. t, u).

Another common feature of pennate diatom frustules that is helpful to distinguish them is the presence of a raphe structure (Kociolek et al. 2015a). The raphe system is usually nested within a thickened section of the valve (sternum), which, because of the additional silica, tends to be one of the last components of the diatom valve to dissolve. As a result, even poorly preserved fossil diatom valves can sometimes be readily identified and used to provide some information regarding aquatic environments (Barker 1992; Battarbee et al. 2005; Ryves et al. 2001, 2002).

### ***5.1 Other Common Microfossils That Have the Potential to Be Confused with Diatoms***

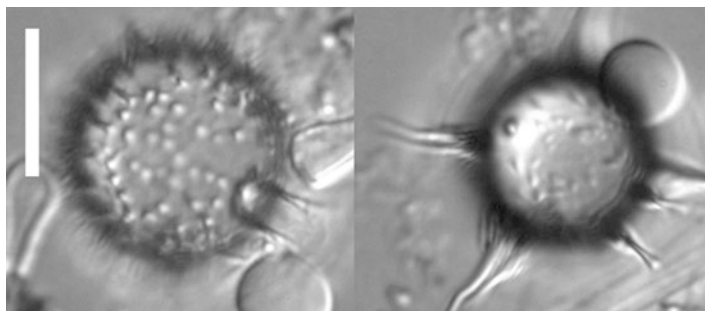
In terrestrial settings, there are three other major groups of siliceous microfossil remains that, because of their composition and size, could potentially be mistaken for a diatom by a non-expert. Diatoms are commonly found together in aquatic and

semi-aquatic settings with all three groups: chrysophyte cysts or scales, sponge spicules, and phytoliths. In deep marine environments, there are other siliceous microfossils, such as silicoflagellates and radiolaria, which potentially might be confused with diatoms but are less likely to be found in abundance in most archaeological settings. Most other microfossil remains that have strongly symmetrical forms or surface ornamentation that might be confused with diatoms, such as pollen, pediatrum, cosmarium, desmids, or other green algae, can occasionally persist through organic digestion processes that are used to extract diatom microfossils but generally can be excluded by non-experts based upon composition.

### Chrysophyte Scales and Cysts

Chrysophytes are a major group of single-celled golden-brown algae that are common in freshwater systems. Like diatoms, they are photosynthetic, although almost all chrysophytes are facultatively heterotrophic when inadequate sunlight is available (Siver 2015). A subset of chrysophytes (synurophytes) also can cover the surface of their cells with fine siliceous scales that often have fine ornamentation that might be confused with a fragment of a diatom frustule, but the scales tend to be much smaller than most diatom species (typically less than 5  $\mu\text{m}$ ) and while they can be perforated, unlike diatom frustules they tend to lack symmetry and do not have other features and structures common to diatom valves (Zeeb and Smol 2001). Like diatoms, many chrysophytes have environmental preferences and tolerances that allow them to be used to provide information about past aquatic settings (Charles et al. 1991; García-Rodríguez 2006; Pla and Catalan 2005; Rühland et al. 1999; Zeeb et al. 1994).

Many synurophyte algae can enter a resting stage triggered by sudden changes in the environment or by population density, creating a spherical stomatocyst (Siver 2015). Chrysophyte cysts rarely share obvious characteristics with the encysting organism, and as a result, a very small fraction of the cysts have been clearly linked with the encysting species (Fig. 6). Chrysophyte stomatocysts are siliceous in composition and typically range in size from 2 to 30  $\mu\text{m}$ , and as such could potentially

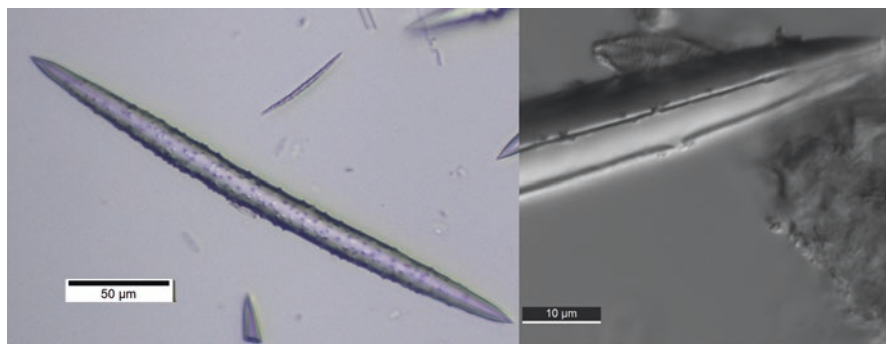


**Fig. 6** Examples of highly ornamented chrysophyte stomatocysts. Scale bar is 10  $\mu\text{m}$

be mistaken for a diatom valve. Stomatocysts are generally hollow and globe-shaped, but some synurophytes can form oval or flattened cysts (Duff et al. 1995; Zeeb and Smol 2001). The interior of the cyst is always smooth, but the external surface of some stomatocysts can be highly ornamented with ridges, spines, and other features. However, stomatocysts can always be distinguished from diatoms and other siliceous microfossils by the presence of a distinct aperture (pore) that defines the anterior hemisphere of the cyst (Siver 2015). Pores may also be surrounded by a thickened rim (collar) of varying complexity. For a more complete description of synurophyte algae and the structural components of stomatocysts, see Duff et al. (1995) and Siver (2015).

### Sponge Spicules

Sponges are large multicellular filter-feeding organisms that can be found in fresh and saltwater ecosystems. There are several thousand known species of sponges; most of these are found in marine settings (Hooper and van Soest 2002). While there is no risk of mistaking a sponge for a diatom, the body of siliceous sponges commonly includes numerous spicular elements that can act as supporting structures and help deter predators (Uriz et al. 2003). Sponge spicules can range in size from those that are visible to the naked eye down to microscopic components known as microscleres (Fig. 7). Microscleres are commonly of hydrated silica composition and roughly within the size range of diatoms, although most tend to be larger than freshwater diatom taxa. Microscleres are often highly symmetrical and occur in a very large range of geometric shapes (Uriz et al. 2003). Sponge spicules can also be highly ornamented with spines, knobs, radiating elements, and other granular components that could appear, superficially, to resemble diatoms in a light microscope. Most microscleres can be distinguished from diatom microfossil remains by their needle-like shape and an obvious hollow axial canal, which is visible in light



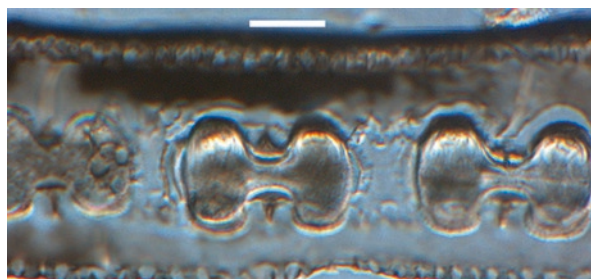
**Fig. 7** Examples of light microscope images of sponge spicules; right image showcasing the axial canal

microscopy. Like other microfossil remains, sponge spicules can also provide some information regarding past aquatic environmental conditions (Guerreiro et al. 2018; Kahlert and Neumann 1997).

## Phytoliths

Phytoliths are microscopic mineralized particles that form within plants. Like sponge spicules, most phytoliths provide support for the plant or protection from predators. Most phytoliths are composed of rigid opaline silica and thus have the capacity to be preserved long after the plant that formed them has expired. Phytoliths can form within plant cells or between multiple cells, which can result in some phytoliths having highly symmetrical shapes (Fig. 8). Many phytoliths are robust and capable of surviving through burning of the original plant materials and as such can often be readily transported from terrestrial landscapes into aquatic or semi-aquatic landscapes where diatom microfossil remains are common (Jackson et al. 2015; Yost et al. 2018). Most phytoliths lack symmetry or surface ornamentation that could be mistaken for a diatom. However, fragments of leaf phytoliths from riverweeds (*Podostemaceae*), sedge achenes (*Cyperaceae*), and hackberry drupes (*Celtis* sp.), for example, could potentially be confused for fragments of poorly preserved diatoms by a non-specialist (see Piperno (2006) for images of these phytoliths). Phytoliths have been used extensively for reconstructing information about past human settlements and are progressively being used more frequently to infer past landscape environments (Ball et al. 2016; Yost et al. 2013, 2018); for a more detailed description of phytoliths and their uses in archaeological settings, see chapter “Phytolith Analysis in Paleoecology and Archaeology” by Cabanes in this volume.

**Fig. 8** Example of light microscope image of phytoliths observed in life position in charcoal fragments from Lake Malawi. Scale bar is 10  $\mu$ m



## 6 What Are the Potential Biases in Fossil Diatom Remains?

The size ranges, the vast diversity of forms, and subtle or cryptic differences between species mean that even for experts, diatom taxonomy is often quite challenging. Analysis of the fossil remains of diatoms almost always requires the use of a high-resolution light microscope, or in some cases, even scanning electron microscopy to correctly distinguish between diatom species. As a result, in most cases, diatom identification usually requires analysis by a specialist. Endemic species are fairly common (Kociolek and Spaulding 2000) and regional taxonomic guidebooks of diatom flora are relatively rare in remote places, which can increase the difficulty in correctly identifying species (Battarbee 1988; Battarbee et al. 2001).

In highly saline or alkaline lake settings, where pH values of 9 or greater are common, diatom frustules can dissolve, potentially biasing the sediment record or in some cases removing all traces of fossil diatoms (Barker 1992; Battarbee et al. 2005; Ryves et al. 2001). Large diatoms may also become fractured by mechanical means, particularly when materials containing fossil diatoms are transported, eroded, or crushed. When analyzing diatom assemblages for environmental information, most diatomists use a number of techniques to ensure that the data they are generating are not biased by small sample sizes. Typically 200–500 diatoms are identified when analyzing sediment assemblages, to provide a robust interpretation (Battarbee et al. 2001). In coastal settings, planktonic taxa found in sedimentary assemblages may represent specimens washed in by tides. More commonly in these cases, benthic taxa are given more weight in analyses, since they likely represent autochthonous assemblages. Additionally, exotic markers of known, constant quantities (such as microspheres or exotic pollen) are often added to each sample after processing to provide a means of determining changes in diatom concentration between samples and may reflect the preservation biases discussed above.

As we have attempted to showcase through numerous examples described above, diatoms are useful as environmental indicators because their skeletal remains are siliceous, and as such, they are highly resistant to most degradation. However, this also means that they may persist in environments long after the organism dies and thus may not be representative of the environments in which they are ultimately found. Because microfossil remains of diatoms are often found in low concentration in archaeobiological analyses, there always lies the possibility the materials recovered from ancient deposits are reworked from even older deposits and are inherited in environments where human settlements occur (Battarbee 1988).

As mentioned above, diatoms are readily transported as living cells but can also be remobilized as fossil components by a variety of means within an environment. As such, diatom fossils have a strong potential to be introduced as contaminants. For example, it is possible that diatoms from desiccating lake basins and river valleys can be transported by wind to nearby archaeological sites. Under these conditions, sudden increases in fossil diatom remains may indicate drier rather than wetter conditions. Humans can also act as agents for mobilizing diatom fossil materials from local lake mud sources, either intentionally or inadvertently. For example, fossil diatoms in sediments can be introduced into human settlement environments as contaminants to structures that are commonly trampled by humans, such as on

dwelling floors (Bathurst et al. 2010). Thus, the presence of diatoms in terrestrial contexts must always be interpreted cautiously. Additionally, trade was an important part of most ancient societies. There is great potential for materials containing diatoms to be transported substantial distances before ultimately being incorporated into archaeological settings (Juggins and Cameron 2010).

Although the analysis of diatom fossils recovered from ceramics and other artifacts is a popular use for multiple purposes, it is rarely as straightforward as it may first seem. Fossil diatom remains may be difficult to extract or identify because of their low concentrations within the materials used (Håkansson and Hulthén 1988). Clays used in pottery also may be derived from multiple sources; in some cases, this is done intentionally in the fabrication process, mixing local and regional sources (Kligmann and Calderari 2012). Fossil diatom remains within ceramics also may be destroyed or substantially altered by heating during the firing of ceramics, particularly when fired at higher temperatures (Battarbee 1988; Kligmann and Calderari 2012). Determining the provenance of ceramics through diatom microfossil remains also requires a significant understanding of local and regional clay sources and the diatoms found therein (Battarbee 1988; Juggins and Cameron 2010; Kligmann and Calderari 2012; Quinn 2008); considering all of these issues, Kligmann and Calderari (2012) suggest that using fossil diatoms from ceramics may actually only have very limited usefulness, except in cases where it is used to refute local ceramic provenances.

Finally, where diatoms are found directly within human remains, there is always the potential to over-interpret their meaning. For example, recent studies have shown that dental calculus may not be entirely representative of foods consumed, since there are many other ways in which microfossils may become trapped in human mouths or incidentally digested (Radini et al. 2017). For example, fossil diatom remains recovered from dental calculus may be derived from dust or grit that is inadvertently introduced during the preparation or storage of foodstuffs. Understanding the potential biases of small sample populations is critical because diatoms often occur sparsely in archaeological settings.

## 7 Conclusions

Despite the use of diatoms in archaeobiology for over 70 years, diatoms are still an underutilized ecofact in archaeological investigations. In the past, diatoms have typically been used to provide a paleoenvironmental context for archaeological sites, but they have much more to offer. The developing subfield of ceramic petrography and a steady increase in the analysis of dental calculus, coprolites, and cooking residue provide a few examples of emerging areas of diatom microfossil recovery. However, given that diatoms can be found in most archaeological contexts, their full potential in archaeology has yet to be realized.

**Acknowledgments** This manuscript has been improved by helpful feedback from Karlyn S. Westover and Erika L. Smith. Some additional useful articles and techniques for diatoms in archaeology were suggested by Barbara M. Winsborough.



## Description of the Plates

The following selection of plates is meant to showcase a wide range of diatom images to give readers enough examples to be able to distinguish diatoms from other microfossils. For a more complete collection of diatom images that could be used for genus or species level identification, we recommend visiting online guides representative of regional flora, such as Diatoms of the US (Spaulding et al. 2019) or Freshwater Diatom Flora of Britain and Ireland (Jüttner et al. 2018).

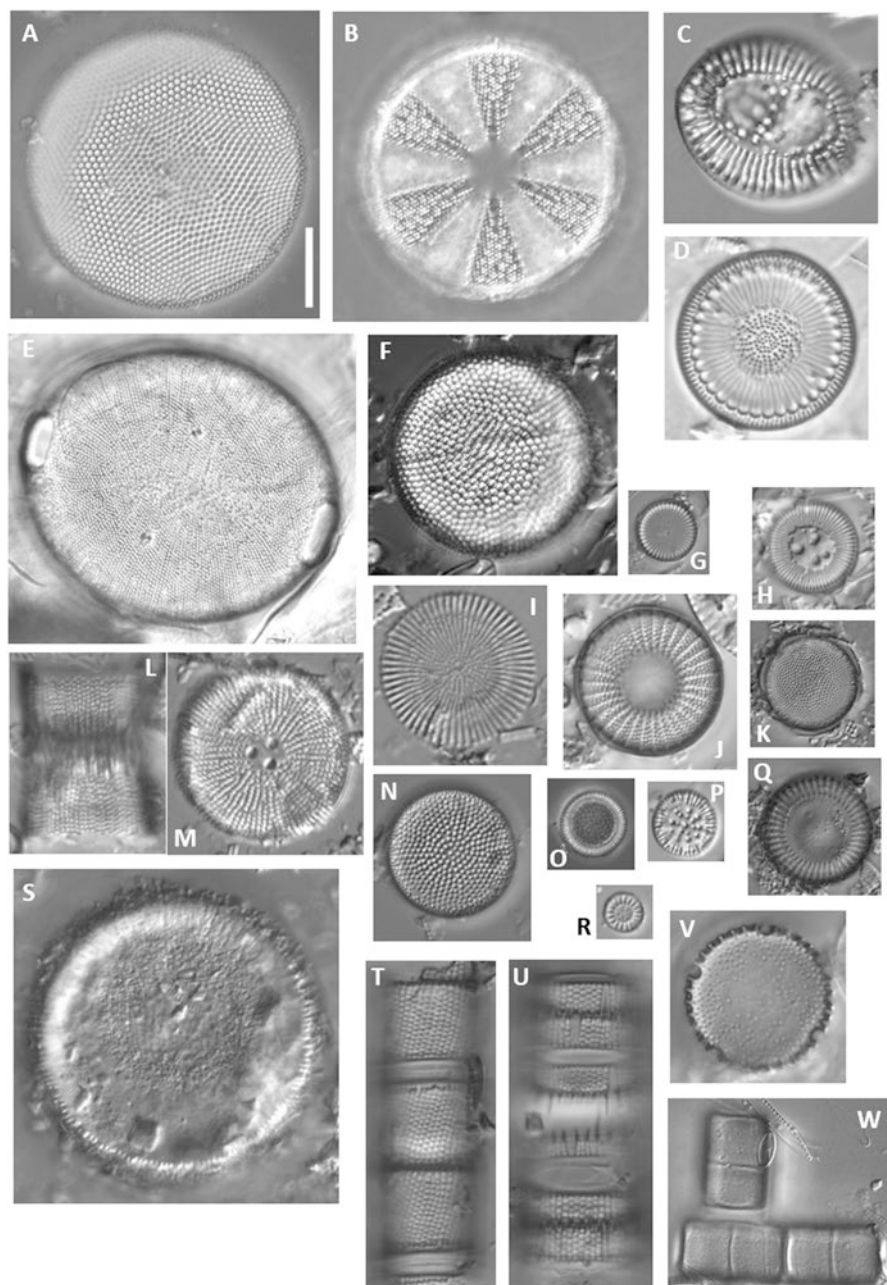
The following images have been arranged into broad groups, starting with “centric” diatoms. Centric diatoms have symmetry around one or more points (*see*, e.g., Plate 1). Most centric diatoms live either as solitary planktonic organisms, representing open water (Plate 1, Figs. a–d, f–k, n–r) or filamentous colonies representing environments ranging from open water (Plate 1, Figs. t–v), to flowing water (Plate 1, Figs. e, w), to aerophylic habitats (Plate 1, Fig. l, m, s).

The remaining images represent “pennate” diatoms, which are characterized by symmetry around one or more planes (*see* Plates 2–7). Pennate taxa can be further grouped by the absence/presence of their raphe structure on one or both valves, the type of raphe present, and the type of valve symmetry typically expressed. Most araphid pennate diatoms (Plate 2) are colonial (*see* Plate 2, Figs. i, s) and may represent either shallow or deep water environments. In contrast, most diatoms with one or more valve with a raphe structure present (Plates 3–7) typically represent aquatic environments where benthic substrates are sunlit.

All images in the plates were collected using a light microscope outfitted with differential interference contrast, collected at 1000× magnification. All images for the plates are identically scaled; all scale bars shown in Plates 1–7 are 10 μm in length.

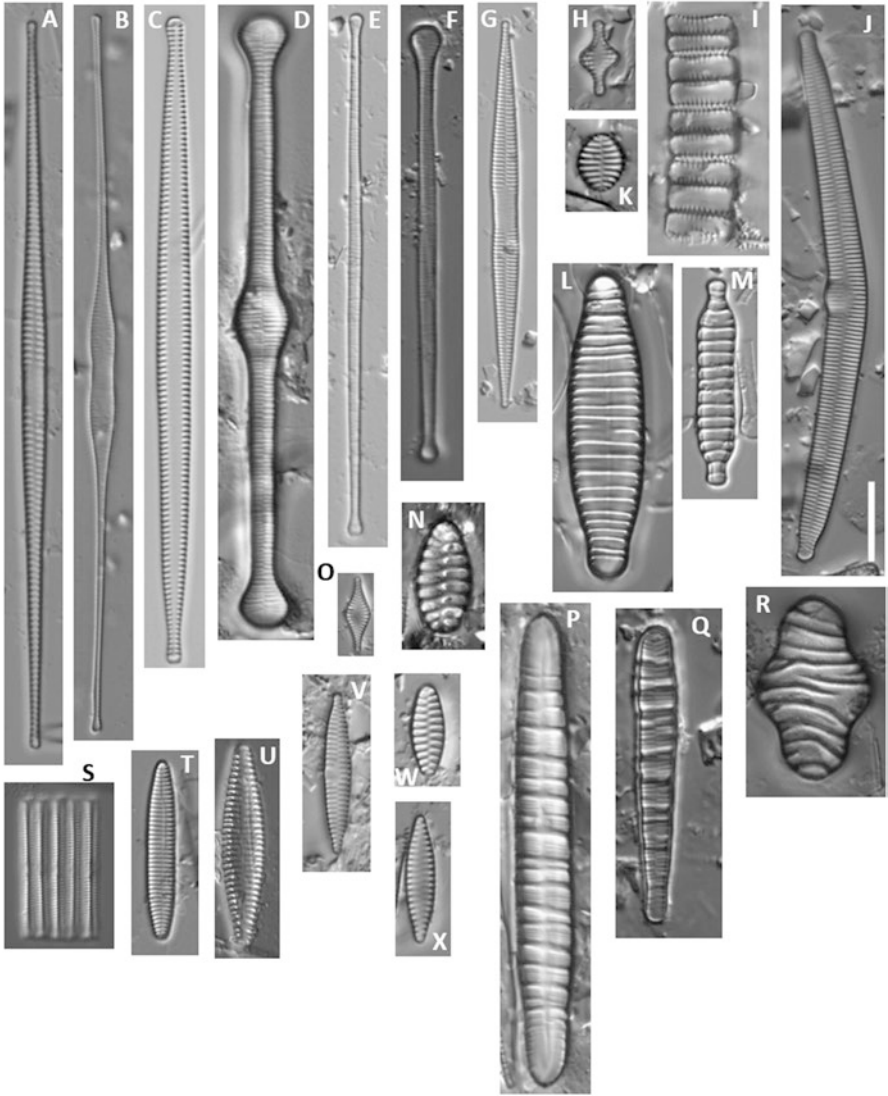
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**Plate 1** (continued) view, collected from a stream in Iowa, US. (f) *Coscinodiscus* sp.—Valve view, collected from Saginaw Bay, Michigan, US. (g) *Cyclostephanos invisitatus*—Valve view, collected from the Des Moines River, Iowa, US. (h) *Lindavia ocellata*—Valve view, collected from June Lake, California, US. (i) *Cyclostephanos malawiensis*—Valve view, collected from Lake Malawi, Malawi, Africa. (j) *Stephanodiscus* sp. —Valve view, collected from Foy Lake, Montana, US. (k) *Thalassiosira* sp.—Valve view, collected from Paleolake Mababe, Botswana, Africa. (l) *Orthoseira roseana*—Girdle view, collected from Emerald Lake, Wyoming, US. (m) *Orthoseira roseana*—Valve view, collected from Hidden Lake, Montana, US. (n) *Actinocyclus normanii*—Valve view, collected from the Ohio River, US. (o) *Lindavia* sp.—Valve view, collected from Lake Malawi, Malawi, Africa. (p) *Lindavia radiosa*—Valve view, collected from Hidden Lake, Montana, US. (q) *Cyclotella meneghiniana*—Valve view, collected from Paleolake Mababe, Botswana, Africa. (r) *Discostella stelligera*—Valve view, collected from Beauty Lake, Wyoming, US. (s) *Ellerbeckia arenaria*—Valve view, collected from Beauty Lake, Wyoming, US. (t) *Aulacoseira* sp.—Girdle view, collected from Miocene diatomite deposits, Idaho, US. (u) *Aulacoseira muzzanensis*—Girdle view, collected from the Mississippi River, US. (v) *Aulacoseira muzzanensis*—Valve view, collected from the Mississippi River, US. (w) *Melosira varians*—Girdle view, collected from Union Slough, Iowa, US



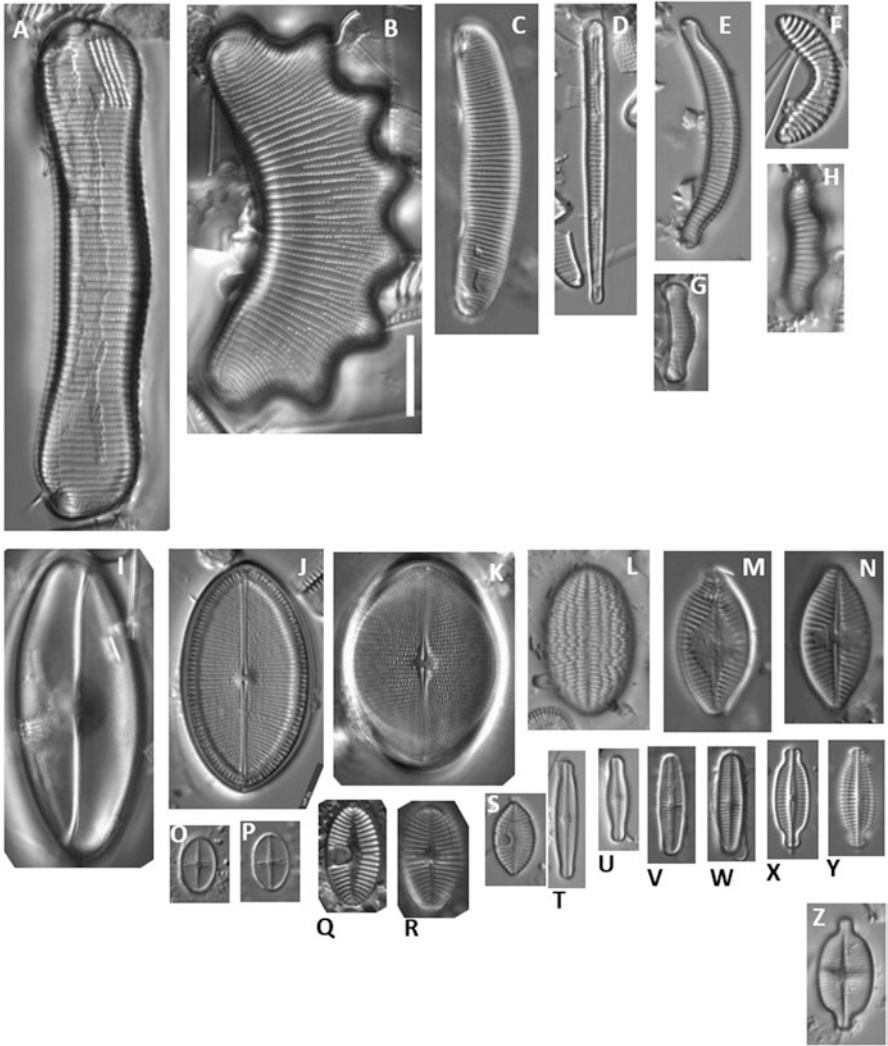
**Plate 1** Examples of centric diatoms. (a) *Coscinodiscus* sp.—Valve view, collected from the Patos Lagoon, Brazil. (b) *Actinopterychus splendens*—Valve view, collected from the Patos Lagoon, Brazil. (c) *Lindavia* sp.—Valve view, collected from Miocene diatomite deposits, Idaho, US. (d) *Lindavia intermedia*—Valve view, collected from June Lake, California, US. (e) *Pleurosira laevis*—Valve





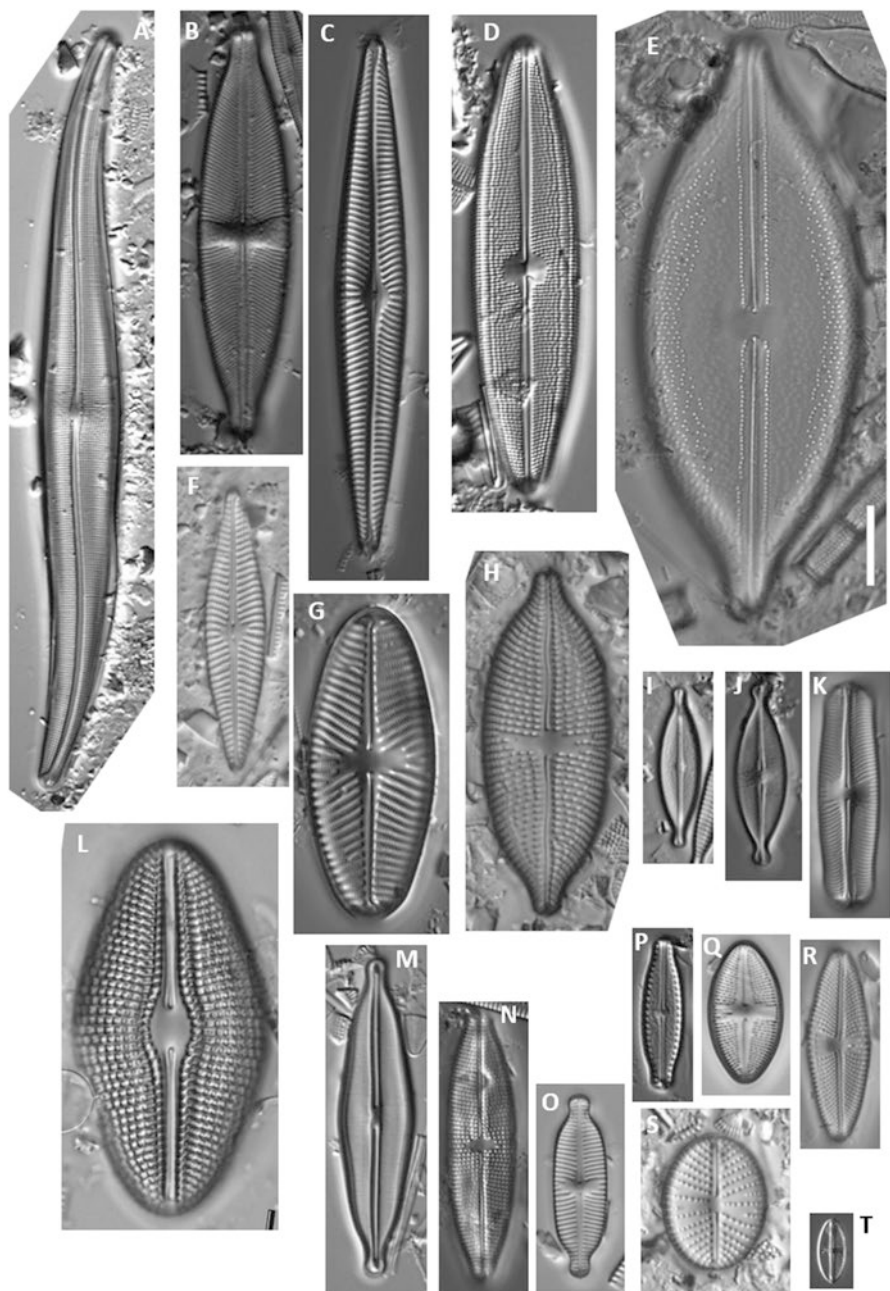
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**Plate 2** Examples of Araphid Pennate diatoms. (a) *Ulnaria* sp.—Valve view, collected from Iowa, US. (b) *Fragilairia crotonensis*—Valve view, collected from Gavins Point Dam, South Dakota, US. (c) *Tabularia fasciculata*—Valve view, collected from Lazy Lagoon, Iowa, US. (d) *Tabellaria fenestrata*—Valve view, collected from Cumbres Bog, New Mexico, US. (e) *Distirionella incognita*—Valve view, collected from the Flathead River, Montana, US. (f) *Asterionella formosa*—Valve view, collected from Saginaw Bay, Michigan, US. (g) *Ulnaria* sp.—Valve view, collected from the Des Moines River, Iowa, US. (h) *Pseudostaurosira pseudoconstruens*—Valve view, collected from Mt. Everest, Tibet. (i) *Staurosira construens*—Colony, girdle view, collected from June Lake, California, US. (j) *Hannaea arcus*—Valve view, collected from Mt. Everest, Tibet. (k) *Staurosira construens* var. *venter*—Valve view, collected from June Lake, California, US. (l) *Diatoma vulgaris*—Valve view, collected from Antelope County, Nebraska, US. (m) *Meridion anceps*—Valve view, collected from Emerald Lake, Wyoming, US. (n) *Staurosirella martyi*—Valve view, collected from Saginaw Bay, Michigan, US. (o) *Pseudostaurosira parasitica*—Valve view, collected from Cumbres Bog, New Mexico, US. (p) *Odontidium* sp.—Valve view, collected from Mount Everest, Tibet. (q) *Meridion circulare*—Valve view, collected from Saginaw Bay, Michigan, US. (r) *Tetracyclus glans*—Valve view, collected from Emerald Lake, Wyoming, US. (s) *Fragilaria* sp.—Colony, girdle view, collected from Lazy Lagoon, Iowa, US. (t) *Stauroforma exiguaformis*—Valve view, collected from Beauty Lake, Wyoming, US. (u) *Staurosirella africana*—Valve view, collected from Paleolake Mababe, Botswana, Africa. (v) *Fragilaria vaucheriae*—Valve view, collected from June Lake, California, US. (w) *Staurosirella pinnata*—Valve view, collected from Mount Everest, Tibet. (x) *Pseudostaurosira* cf. *brevistriata*—Valve view, collected from Mount Everest, Tibet



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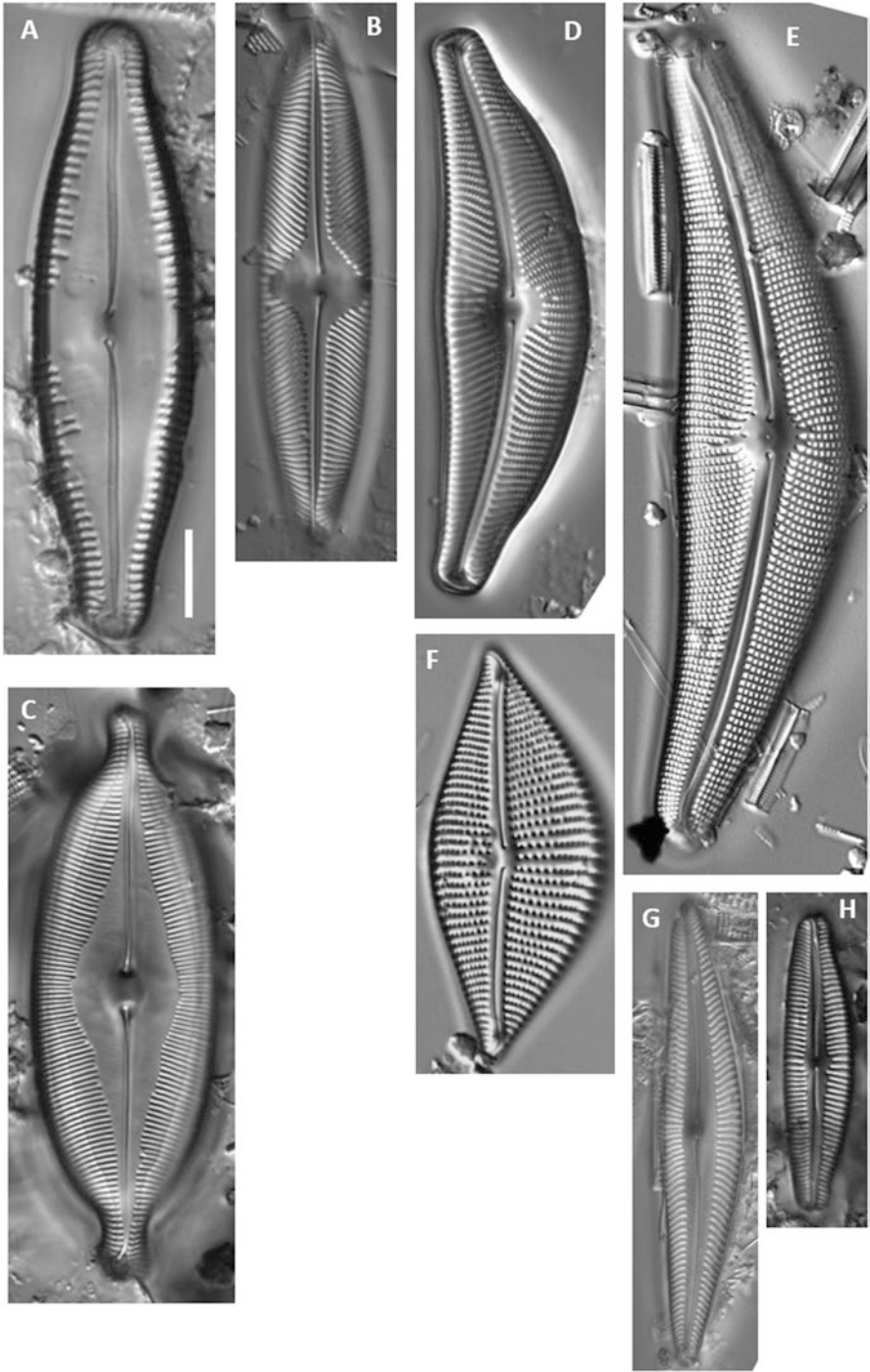
**Plate 3** Examples of Eunotioid diatoms. (a) *Eunotia* sp.—Valve view, collected from Cumbres Bog, New Mexico, US. (b) *Eunotia diadema*—Valve view, collected from Victoria Island, Canada. (c) *Eunotia faba*—Valve view, collected from Victoria Island, Canada. (d) *Peronia* sp.—Valve view, collected from Victoria Island, Canada. (e) *Eunotia* sp.—Valve view, collected from Victoria Island, Canada. (f) *Semiorbus hemicyclus*—Valve view, collected from Victoria Island, Canada. (g) *Eunotia* sp.—Valve view, collected from Victoria Island, Canada. (h) *Eunotia* cf. *perpusilla*—Valve view, collected from Victoria Island, Canada. Examples of Monoraphid diatoms. (i) *Eucoconeis* sp.—Valve view, collected from Victoria Island, Canada. (j) *Cocconeis placentula*—Valve view, raphe valve, collected from Saginaw Bay, Michigan, US. (k) *Cocconeis pediculus*—Valve view, raphe valve, collected from Saginaw Bay, Michigan, US. (l) *Cocconeis placentula*—Valve view, rapheless valve, collected from June Lake, California, US. (m) *Planothidium holstii*—Valve view, rapheless valve, collected from Beauty Lake, Wyoming, US. (n) *Planothidium holstii*—Valve view, raphe valve, collected from Beauty Lake, Wyoming, US. (o) *Psammothidium* sp.—Valve view, rapheless valve, collected from Beartooth Lake, Wyoming, US. (p) *Psammothidium* sp.—Valve view, raphe valve, collected from Beartooth Lake, Wyoming, US. (q) *Planothidium joursacense*—Valve view, rapheless valve, collected from Kintla Lake, WY, US. (r) *Planothidium joursacense*—Valve view, raphe valve, collected from Kintla Lake, Wyoming, US. (s) *Planothidium* sp.—Valve view, rapheless valve, collected from Beartooth Lake, Wyoming, US. (t) *Achnanthidium* sp.—Valve view, raphe valve, collected from Emerald Lake, Wyoming, US. (u) *Achnanthidium* sp.—Valve view, raphe valve, collected from Mouth Everest, Tibet. (v) *Rossithidium pussilum*—Valve view, raphe valve, collected from Beartooth Lake, WY, US. (w) *Rossithidium pussilum*—Valve view, rapheless valve, collected from Beartooth Lake, WY, US. (x) *Karayevia nitidiformis*—Valve view, raphe valve, collected from Beauty Lake, WY, US. (y) *Karayevia nitidiformis*—Valve view, rapheless valve, collected from Beauty Lake, WY, US. (z) *Achnanthidium exiguum*—Valve view, raphe valve, collected from June Lake, California, US





**Plate 4** Examples of Naviculoid diatoms. (a) *Gyrosigma* sp.—Valve view, collected from Kinta Lake, Wyoming, US. (b) *Stauroneis* sp.—Valve view, collected from Victoria Island, Canada. (c) *Navicula radiosa*—Valve view, collected from Center Lake, Iowa, US. (d) *Neidium* sp.—Valve view, collected from Victoria Island, Canada. (e) *Anomoeoneis* sp.—Valve view, collected from the St. Mary of the Woods Campus, Indiana, US. (f) *Navicula* sp.—Valve view, collected from June Lake, California, US. (g) *Navicula reinhardtii*—Valve view, collected from Saginaw Bay, Michigan, US. (h) *Aneumastus rostratus*—Valve view, collected from June Lake, California, US. (i) *Brachysira vitrea*—Valve view, collected from Foy Lake, Montana, US. (j) *Nupela impexiformis*—Valve view, collected from Emerald Lake, Wyoming, US. (k) *Sellaphora pupula*—Valve view, collected from Emerson Bay, Iowa, US. (l) *Diploneis* sp.—Valve view, collected from Hidden Lake, Montana, US. (m) *Frustulia* sp.—Valve view, collected from Victoria Island, Canada. (n) *Mastogloia lacustris*—Valve view, collected from Foy Lake, Montana, US. (o) *Placoneis* sp.—Valve view, collected from Mouth Everest, Tibet. (p) *Pulchella schwabei*—Valve view, collected from Beauty Lake, Wyoming, US. (q) *Luticola* sp.—Valve view, collected from Mouth Everest, Tibet. (r) *Luticola* sp.—Valve view, collected from the Ohio River, US. (s) *Cavinula scutelloides*—Valve view, collected from June Lake, California, US. (t) *Sellaphora* sp.—Valve view, collected from Lake Malawi, Malawi, Africa

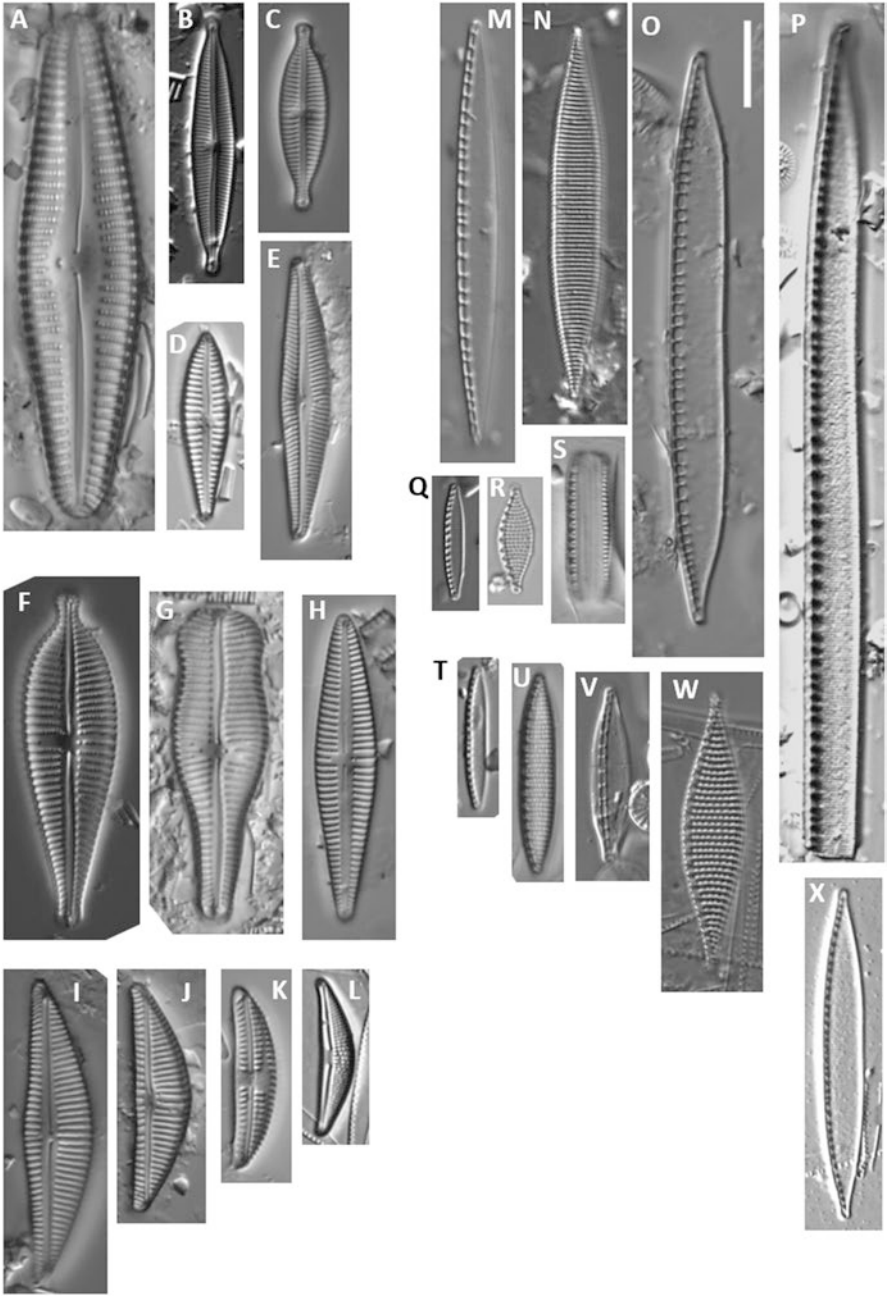




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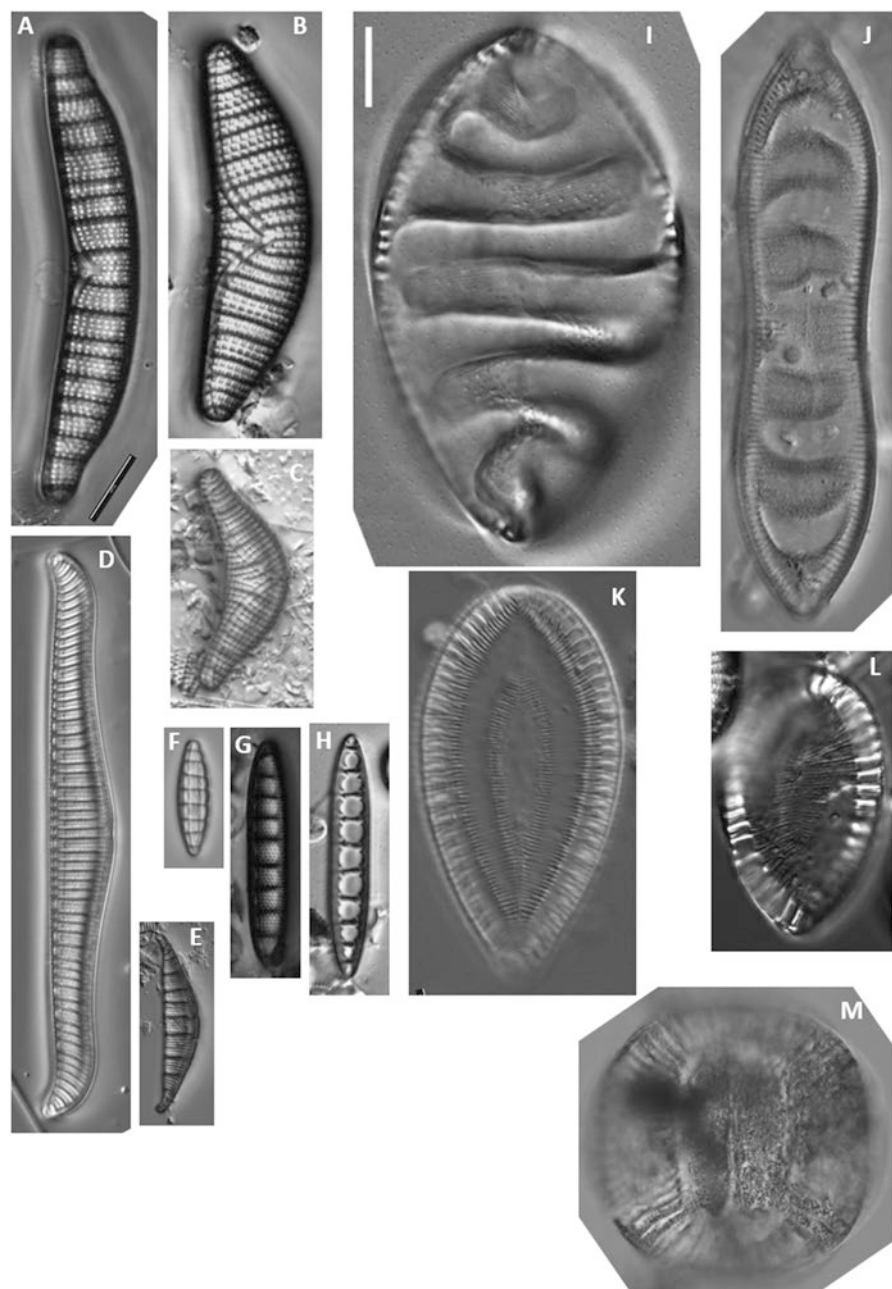
**Plate 5** Examples of Pinnularioid diatoms. (a) *Pinnularia* sp.—Valve view, collected from Lake Malawi, Malawi, Africa. (b) *Pinnularia* sp.—Valve view, collected from Foy Lake, Montana, US. (c) *Caloneis amphisbaena*—Valve view, collected from Iowa, US. Examples of Cymbelloid Diatoms. (d) *Cymbella* sp.—Valve view, collected from Saginaw Bay, Michigan, US. (e) *Cymbella* **Plate 5** (continued) sp.—Valve view, collected from Gar Lake, Iowa, US. (f) *Encyonema triangulum*—Valve view, collected from High Lake, Iowa, US. (g) *Cymbella* cf. *prisca*—Valve view, collected from Paleolake Mababe, Botswana, Africa. (h) *Cymboplectura subaequalis*—Valve view, collected from Foy Lake, Montana, US





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**Plate 6** Examples of Cymbelloid, Gomphonemoid, and Amphoroid diatoms. **(a)** *Oricymba* sp.—Valve view, collected from Mount Everest, Tibet. **(b)** *Encyonopsis* sp.—Valve view, collected from Foy Lake, Montana, US. **(c)** *Gomphonema* sp.—Valve view, collected from Mount Everest, Tibet. **(d)** *Gomphonema* sp.—Valve view, collected from the Pantanal, Brazil. **(e)** *Navicymbula pusilla*—Valve view, collected from Foy Lake, Montana, US. **(f)** *Gomphonema* cf. *sphaerophorum*—Valve view, collected from Marion, Kentucky, US. **(g)** *Gomphonema truncatum*—Valve view, collected from June Lake, California, US. **(h)** *Gomphonema* sp.—Valve view, collected from Mount Everest, Tibet. **(i)** *Encyonema silesiacum*—Valve view, collected from Foy Lake, Montana, US. **(j)** *Encyonema* sp.—Valve view, collected from Mount Everest, Tibet. **(k)** *Amphora* cf. *pediculus*—Valve view, collected from June Lake, California, US. **(l)** *Halamphora veneta*—Valve view, collected from Union Slough, Iowa, US. Examples of Nitzschioid diatoms. **(m)** *Nitzschia recta*—Valve view, collected from Maybury Creek, Michigan, US. **(n)** *Nitzschia* sp.—Valve view, collected from Saginaw Bay, Michigan, US. **(o)** *Nitzschia* sp.—Valve view, collected from Lake Ohrid, Macedonia. **(p)** *Nitzschia* sp.—Valve view, fragment, collected from Hidden Lake, Montana, US. **(q)** *Nitzschia* sp.—Valve view, collected from Kettle Lake, South Dakota, US. **(r)** *Nitzschia* sp.—Valve view, collected from Kabua Gorge diatomites, Kenya, Africa. **(s)** *Nitzschia* sp.—Girdle view, collected from Union Slough, Iowa, US. **(t)** *Nitzschia* sp.—Girdle view, collected from Union Slough, Iowa, US. **(u)** *Nitzschia* sp.—Girdle view, collected from Kettle Lake, South Dakota, US. **(v)** *Nitzschia* sp.—Girdle view, collected from Herd Lake, Idaho, US. **(w)** *Nitzschia* sp.—Girdle view, collected from Lake Tanganyika, Tanzania, Africa. **(x)** *Nitzschia* sp.—Girdle view, collected from Union Slough, Iowa, US





**Plate 7** Examples of Epithemioid diatoms. (a) *Epithemia* sp.—Valve view, collected from Lake Okoboji, Iowa, US. (b) *Epithemia turgida*—Valve view, collected from Iowa, US. (c) *Epithemia smithii*—Valve view, collected from June Lake, California, US. (d) *Rhopalodia gibba*—Valve view, collected from Union Slough, Iowa, US. (e) *Rhopalodia gibberula*—Valve view, collected from Paleolake Mababe, Botswana, Africa. (f) *Denticula* cf. *tenuis*—Valve view, collected from Mount Everest, Tibet. (g) *Denticula* sp.—Valve view, collected from Iowa, US. (h) *Denticula* sp.—Valve view, costae valve, collected from Iowa, US. Examples of Surirelloid diatoms. (i) *Cymatopleura* sp.—Valve view, collected from Upper Gar Lake, Iowa, US. (j) *Cymatopleura solea*—Valve view, collected from Paleolake Mababe, Botswana, Africa. (k) *Surirella* sp.—Valve view, collected from Dugout Creek, Iowa, US. (l) *Surirella iowaensis*—Valve view, collected from High Lake, Iowa, US. (m) *Campylodiscus clypeus*—Valve view, collected from Paleolake Mababe, Botswana, Africa

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# Non-Pollen Palynomorphs



Lyudmila S. Shumilovskikh and Bas van Geel

## 1 What Are Non-Pollen Palynomorphs?

Non-pollen palynomorphs (NPP) are microscopic objects of about 10–250  $\mu\text{m}$  in size, which can be found along side pollen during routine pollen counting. These “extra” microfossils in pollen slides (van Geel 2001) have an outer organic wall with a characteristic morphology, which is resistant to decomposition processes and standard palynological laboratory treatments. NPP consist of a large inhomogeneous group of remains of organisms, their parts or life stages, which lived in past environments and therefore represent a “memory of former, often strictly local environmental conditions” (van Geel 2001).

Microfossils in pollen slides have received attention since the very beginning of palynology as science. Karl Rudolph published in 1917 his work “Untersuchungen über den Aufbau Böhmischer Moore. I. Aufbau und Entwicklungsgeschichte Südböhmischer Hochmoore” (Studies on the composition of Bohemian peatlands. I. Composition and development history of South-Bohemian peat bogs), where besides pollen and botanical macroremains he described and drew microfossils of animal and fungal origin and used them in paleoenvironmental reconstructions (Rudolph 1917). For example, he correctly identified testate amoebae (rhizopods), drew “Hochmoortönchen” that he identified as coming from the cocoons of *Nephelis* (the correct identification as a shell of the testate amoeba *Amphitrema flavum* was found later), and also presented other animal remains (spermatophores of copepods, oocytes of Rhabdocoela) without a taxonomic identification. In 1929, Herbert Hesmer published an article “Mikrofossilien in Torfen” (“Microfossils in peat deposits”), where he

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provided information on ecology and in some cases also microphotographs of different microfossils such as pollen infected with spores of the chytrid *Olpidium pendulum*, conidia of *Helicosporium*, teleutospores, different species of testate amoebae, spermatophores of copepods, the egg of *Macrobiotus echinogenitus*, and the shell of the heliozoan *Clathrulina elegans*. Later on, David G. Frey (1964) published in his work “Remains of animals in Quaternary lake and bog sediments and their interpretation” a comprehensive overview of the microfossils of animal origin from Holocene lacustrine sediments and peat deposits.

Systematic studies on NPP only began recently, particularly by the work of the second author and his colleagues of the Hugo de Vries Laboratory at the University of Amsterdam (e.g., Bakker and van Smeerdijk 1982; Pals et al. 1980; Kuhry 1985; van der Wiel 1982; van Geel 1978; van Geel et al. 1981, 1983, 1986, 1989). During routine pollen counting, they documented all unknown “extra” microfossils under a type number (Type 1, Type 2, Type 3 etc.). Morphological descriptions of NPP were provided with microphotographs and where possible assignment to extant taxa, in combination with discussions on their stratigraphic position and indicator values. The identification was carried out with help of biological literature and consulting of colleagues in mycology, phycology, zoology, and plant anatomy. Even though the majority of the types have not yet been identified, they can be used as paleoecological indicators. This systematic approach of NPP documentation led to a strong development of the field in the last 40 years, and the total amount of NPP types exceeds 1300 (Miola 2012).

While NPP represent a large group of resistant remains of any kind of organisms, taxonomically they belong to a wide variety of groups such as cyanobacteria, green algae, cysts of dinoflagellates (dinocysts), vascular plants, testate amoebae, rotifers, tardigrades, Cladocera (water fleas), Rhabdocoela (flat worms), and fungi from Ascomycetes, Basidiomycetes, Hyphomycetes, and Zygomycetes. Some of these groups, like dinocysts, Cladocera, or testate amoebae are subject of a special analysis, but they can also be considered as NPP in routine palynological studies. Here we concentrate on the NPP that are important for environmental reconstructions at, or close to archaeological sites in connection with human impact. Therefore, only a limited number of NPP groups is presented and discussed here with illustrations and environmental information. The presented examples are chosen to reflect the broad range of NPP analyses and their paleoecological use.

## 2 What Do Non-Pollen Palynomorphs Tell Us About Environments?

NPP can be found in any type of sediment, from aquatic to terrestrial environments (Table 1). The range of the types tracing special human activities can differ, depending on the type of archive.



**Table 1** Appearance of NPP in different archives

NPP group	Archive type			
	Marine	Lacustrine	Peat	Soil
<i>Algae</i>				
Green algae	(+)	++	+	(+)
Cyanobacteria	(+)	++	(+)	(+)
Cysts of dinoflagellates	++	(+)	–	–
Prasinophyta	++	–	–	–
<i>Vascular plants</i>				
Stomata	(+)	+	++	(+)
Epidermis	–	(+)	++	–
<i>Zoological remains</i>				
Rotifera (eggs, shells)	–	+	++	–
Testate amoebae	(+)	+	++	(+)
Chironomids (head capsules)	–	++	(+)	–
Tardigrades (eggs, fragments)	(+)	(+)	++	(+)
Copepods (fragments, spermatophores)	–	++	+	–
Cladocera (fragments)	–	++	+	–
Helminths (eggs)	+	+	+	+
Rhabdocoela (oocytes)	–	++	+	–
Insects (fragments)	+	+	+	+
<i>Fungi</i>				
Coprophilous fungi	(+)	+	++	+
Ligninolytic fungi	(+)	+	+	+
Carbonicolous fungi	(+)	+	+	+
Rust fungi	(+)	+	++	(+)
Smut fungi	(+)	+	++	(+)
Arbuscular mycorrhizal fungi	(+)	+	++	+++
<i>Fibers</i>	–	–	–	+
<i>Unidentified NPP</i>	+	+	+	+

## 2.1 Soils

Soils are the most frequent archives in archaeology. However, due to rapid decomposition of pollen in soils and taphonomic processes, palynological analysis is rather seldom applied for archaeological layers in sites with dry preservation (Dimbleby 1985). This is possibly a reason that there are just a few studies of NPP from dry soil contexts (van Geel et al. 2003; Kvavadze and Gagoshidze 2008; Kvavadze and Kakhiani 2010; Kvavadze et al. 2010, 2015; Gavrilov et al. 2016; Wieckowska-Lüth and Heske 2019). However, due to restricted dispersal possibilities of spores, NPP and especially fungal spore analysis often provide a very local signal, which can be directly connected to human activities. Van Geel et al. (2003) highlighted richness and good preservation of spores of coprophilous (feces inhabiting) fungi in archaeological sites, which clearly indicate the presence of animals

near the sample site, providing important information to animal husbandry, especially when the animal bones are not preserved. Combined phytolith, pollen, and NPP studies of the medieval settlement Zhankent in Kazakhstan (Gavrilov et al. 2016) demonstrated the use of dung for mud brick construction, while places of organic and dung deposition could be identified. Several studies were undertaken on material from burials where microremains of textile fibers were documented and identified (Kvavadze and Gagoshidze 2008; Kvavadze and Kakhiani 2010; Kvavadze et al. 2010, 2015). Studies from the Early Bronze Age Paravani Kurgan (southern Georgia) reveal a cover of the burial chamber and ceremonial wagon with flax textile, while pollen spectra and numerous mite remains indicate early summer as the season of burial (Kvavadze and Kakhiani 2010). In the Bedeni Plateau (southern Georgia), pollen and NPP studies indicated that honey and medical plants were involved in the funerary process (Kvavadze et al. 2015). In the late Bronze Age burials from Saphar-Kharaba (southern Georgia), clothing were made of flax and cotton, indicating trade relations between Caucasus and India (Kvavadze et al. 2010). The character of microscopic fiber remains from the weaving workshop Dedoplist Gora palace (AD first century) showed production of cotton and flax fabrics mixed with silk, revealing the involvement of the Caucasus in the “Silk Road” trade (Kvavadze and Gagoshidze 2008). The study of Wieckowska-Lüth and Heske (2019) is another example of investigations on NPP from archaeological sites with dry preservation. The study deals with on-site living conditions during the Late Bronze Age/Early Iron Age at the Hünenburg hillfort–settlement complex. In this study, the analysis of the horizons of pit findings shows microscopic (NPP and pollen) evidence for stockpiling, plant processing, waste management, and hygienic conditions within the settlement area.

## 2.2 Caves

NPP studies have been successfully applied in archaeological and paleontological cave deposits. Thus, Kvavadze et al. (2009) found wild flax fibers in the Upper Paleolithic layers of Dzudzuana Cave (Georgia), indicating production of cords for hafting stone tools, weaving baskets, or sewing garments. In Pleistocene (1.5 million to 200 ka years ago) cave sequences from the Trinchera area (Sierra de Atapuerca, Burgos), algal palynomorphs helped to identify dry and wet local conditions of deposition, *Glomus* spores indicated increased erosion phases, spores of coprophilous fungi suggested the presence of herbivores, and saprophytic fungi point to accumulation of organic matter possibly due to the presence of hominins or animals in the cave (Expósito et al. 2017).

## 2.3 Peat Bogs

Peat bogs represent rich archives of NPP, characterized by high diversity, high abundance, and good preservation. In raised bogs located at relatively large distances to settlement sites, human indicators, for example spores of coprophilous fungi, are rare, even during the intense regional human occupation (van Geel et al. 2003). However, small peat bogs (<2 ha) can be used for reconstruction of pastoral history. For example, combining a modern analogue NPP data set with fossil NPP spectra, Cugny et al. (2010) reconstructed grazing pressure phases, alternating with abandonment of the territory over the last 700 years in the Sourzay basin, western Pyrenees (France). Using NPP, Blackford et al. (2006) investigated the impacts of hunter-gatherers on vegetation during the Mesolithic period. Also in the Swiss Alps, agro-pastoral activities in the past were reconstructed using NPP from a peat deposit (Dietre et al. 2017).

## 2.4 Archaeological Water-Logged Sites

Archaeological water-logged sites represent the best opportunity to trace the economy of the past societies or change in settlement environments (van Geel et al. 1983, 1986; Bos et al. 2005; Revelles et al. 2016). Thus, combined macrofossils, diatoms, and NPP studies from the deposits near Enkhuizen (The Netherlands) confirmed the hypothesis about abandonment of Bronze Age settlements due to a rise of the water table (van Geel et al. 1983). Paleoecological investigations of a Late Bronze Age watering-place at Bovenkarspel (the Netherlands) reveal the presence of spores of coprophilous fungi and eggs of *Trichuris*, indicating the presence of feces in the fill (Buurman et al. 1995). Furthermore, construction of medieval dikes in coastal areas of the Netherlands was clarified by NPP studies, showing inundation of the area by saline and brackish water, indicated by foraminifer linings, during storms already in the thirteenth century (van Geel 1983, 1986). Studies from the early medieval urban habitats of Gdańsk (N. Poland) reveal poor sanitary conditions, confirmed by the presence of intestinal parasite eggs (*Trichuris* and *Ascaris*) and a high frequency of spores of coprophilous fungi (*Sordaria*, *Sporormiella*, *Chaetomium*) in the sediments (Święta-Musznicka et al. 2013). Revelles et al. (2016) applied NPP analysis to the waterlogged site La Draga (Spain), reconstructing a change from lacustrine to a wetland environment, where settlement formation started. Increased fungal decomposition could be linked to the collapse of pile dwellings, increased erosion possibly induced by deforestation, and final abandonment of the settlement and spread of eutrophic peatlands. Bos et al. (2005) reconstructed trampling and/or eutrophication resulting from the presence of large herbivores and people at a Mesolithic site in the Netherlands.

## 2.5 Lakes

Lakes represent mostly off-site archives and reflect environmental changes in a rather large area around the lake as well as in situ. Gauthier et al. (2010) reconstructed medieval farming activities in southwestern Greenland indicated by phases of pastoral activities and increased erosion alternating with abandonment of the area. McAndrews and Turton (2010) described and identified spores of crop-parasitizing smuts and rusts, which recorded two phases of crop cultivation in Crawford Lake (Ontario, Canada) in the second millennium AD. NPP studies of human impact of Early Neolithic communities in NE Iberia (Revelles and van Geel 2016) demonstrated that deforestation caused wood decay, increased erosion, and changing turbidity of lake water. Furthermore, soil disturbance and increased pasture were linked with local habitation during the Late Neolithic—Chalcolithic. NPP studies on the sediments from Lake Durankulak (Bulgaria) revealed Early Bronze Age activities of nomadic tribes including stock-breeding and grazing as well as Late Bronze Age and Early Iron Age occupation phases (Marinova and Atanassova 2006). Human impact on freshwater resources was traced by studies of algal and cyanobacterial palynomorphs in Poland (van Geel et al. 1994), in Switzerland (Hillbrand et al. 2014), as well as in the Great Lakes region (McCarthy et al. 2018) and in Cook's Bay (Danesh et al. 2013) in Canada, reflecting cultural eutrophication and soil erosion in the catchment.

## 2.6 Marine Sediments

Marine sediments are rather difficult to connect to human activities due to a very big source area for terrestrial NPP (e.g., Mudie et al. 2010). However, the first occurrence of new species of dinoflagellates (dinocysts) in the sea can indicate on one hand a merchant transport and therefore possible trade with distant areas and on the other hand a very early human-induced invasion of some species in the area (e.g., Marret et al. 2009; Shumilovskikh et al. 2016b). Human-induced changes in ecosystems can be traced in ancient harbors, which, as economic centers and points for naval navigation, represent important archives of geological, biological, and archaeological information (e.g., Morhange et al. 2014). For example, NPP studies from the ancient harbor of Elaia (western Turkey) provided new information on human-environment interactions in the Eastern Mediterranean since the last 7500 years (Shumilovskikh et al. 2016b). A marine lagoon indicated by dinocysts and foraminifer linings was transformed to a harbor in Hellenistic times. The flourishing period of the harbor shows increased soil erosion and pastoral farming, indicated by *Glomus* spores and spores of coprophilous fungi, respectively. Helminth eggs point to the presence of sewerage in the city, leading to a continuous eutrophication of the harbor through time, indicated by changes in dinocyst assemblages. Furthermore, maritime contacts with the Black Sea and Marmara Sea are suggested from the temporal presence of the Black Sea endemic dinocyst species *Peridinium ponticum*.

In intensively used coastal areas, agricultural practices can be easily traced. For example, Zong et al. (2007) studied NPP from coastal marshes close to the site Kuahuqiao (lower Yangtze region, East China) regarding the origin of wet rice cultivation systems. This detailed paleoecological study shows that the earliest rice cultivation system was located in slightly brackish coastal reed-swamps and rice cultivation involved clearance and management of the coastal marsh vegetation by fire. A marine inundation ended the settlement and rice cultivation in this environmentally unstable coastal ecosystem.

As we show with examples above, the environmental reconstructions based on NPP are very diverse and provide different insights on human life, activities, and environment, helping to obtain a deeper understanding of human-environment interactions in the past. Especially efficient is the usage of NPP in a multi-proxy approach, allowing us to test indicative values of different NPP types. We encourage palynologists working with archaeological material to pay special attention to these “extra microfossils.”

### **3 How Can Non-Pollen Palynomorphs Be Recovered from Archaeological Samples?**

NPP analysis began and is still used as a supplementary method to traditional pollen analysis (van Geel 2001). Therefore, recovery of NPP from archaeological samples occurs with the help of a standard methodology of pollen sample preparations. However, the “standard” methodology is a combination of different laboratory treatments (Moore et al. 1991) and varies from one laboratory to another. The laboratory preparation of samples depends on the sediment composition and can include treatments with hydrochloric acid to dissolve carbonates, potassium hydroxide or sodium hydroxide to disaggregate the sediment, fluoride acid to dissolve silicates or different heavy liquids to remove the minerogenic part, acetolysis to degrade cellulose, sieving on 200–250 µm meshes to remove large pieces, sieving on meshes with size of 1–10 µm and ultrasonication to remove small particles. Such a great variety of methods implies different preservation of pollen and also NPP.

The first NPP studies were performed on material treated with harsh laboratory methods (van Geel 2001; Chambers et al. 2011). Nowadays more and more studies compare the influence of lab preparation methods on the palynomorph assemblage composition and highlight the sensitivity of remains to chemical procedures, especially to acetolysis (Enevold et al. 2019). Special attention to the laboratory preparation was given to specific NPP groups such as fungal spores generally, spores of coprophilous fungi, algae, and testate amoebae.

McAndrews and Turtton (2010) compared the influence of gentle (KOH, HCl, sieving at 10 and 200 µm) and harsh (KOH, HCl, sieving on 10 and 200 µm, hot HF, hot acetolysis mixture) laboratory processing on fungal spores. They demonstrated that fungal spore abundance was six times lower when using the harsh method, indicating poor resistance of fungal spores to acetolysis in comparison to pollen grains.

Van Asperen et al. (2016) compared four preparation methods of spores of dung fungi: boiling with sodium hydroxide, sieving at 125 and 6  $\mu\text{m}$ , treatment with hydrochloric acid, and acetolysis. The study demonstrated that fungal spores are not preserved uniformly: hyaline and unpigmented spores degrade easily, while pigmented thick-walled spores experience different impact of the different methods. The most diverse assemblage was achieved by sieving only. For paleoecological material, the authors suggest to use the less corrosive method starting with sieving and swirling, then boiling in KOH and treatment with HCl, then heavy liquids, then boiling in NaOH and no acetolysis.

Methodological studies on algal remains also emphasize the destructive nature of acetolysis. Marret (1993) shows destruction of the cysts of protoperidinioid dinoflagellates. Mertens et al. (2009) suggest to avoid the use of oxidation, KOH, warm acids, acetolysis, large ( $>15\ \mu\text{m}$ ) mesh sizes, and long ( $>1\ \text{min}$ ) ultrasonication for determination of reproducible absolute abundances. Acetolysis also decreases the abundance of desmids and biases the assemblage toward more robust forms (Riddick et al. 2016). Loricae of euglenoid algae *Trachelomonas*, an important indicator of organic pollution, get dissolved by acetolysis (Shumilovskikh et al. 2019). In contrast, green algae such as *Pediastrum*, *Botryococcus*, or spores of Zygnemataceae, all of which have a cell wall which is reinforced with sporopollenin-like material, are resistant to acetolysis (Jankovská and Komárek 2000; van Geel 2001; Riddick et al. 2016).

Several tests were performed also on testate amoebae. Swindles and Roe (2007) tested dissolution of testate amoebae under acidic conditions and showed a large variability in dissolution sustainability of taxa. However, comparison of water-treated samples with palynological ones demonstrated that many taxa are destroyed in pollen preparations, but remaining taxa show similar abundance to the water-treated samples, suggesting that preserved assemblages, while reduced, are not biased and therefore can be used for paleoenvironmental reconstructions (Payne et al. 2012).

Comparison of the extraction techniques on different NPP groups demonstrates different resistance of taxa to laboratory preparation and achievement of higher diversity by using non-destructive methods. In paleoecological studies, the choice of the applied techniques depends on the sediment composition, influencing the NPP assemblages and abundance. When interpreting NPP records, one should be aware of this problem.

NPP data are usually represented in the form of diagrams, either together with pollen data or separately. NPP data can be calculated as percentage of the pollen sum or concentrations, or – in case of a good age-depth model – accumulation rates (influx). NPP taxa are often grouped according their ecological affinities.

## 4 How Can Non-Pollen Palynomorphs Be Identified?

A specific and formal NPP identification seems to be rather difficult because the microfossils represent just a part of an organism or a specific life stage, while the biological taxonomy is based mostly on the complete organism. Therefore, specialized

literature of algologists, mycologists, and zoologists can only be used to a limited extent. Furthermore, an atlas of Quaternary NPP is still not available.

The best printed overview of the NPP with descriptions and photographs is a compilation from previous publications by Bas van Geel and colleagues and initial reports of the Hugo de Vries-Laboratory “A study of non-pollen objects in pollen slides. The types as described by Dr. Bas van Geel and colleagues” (van Hove and Hendrikse 1998). Miola (2012) reviewed the “classical” NPP literature and provided a list of more than 1300 NPP described until 2011. This work provides the first standardization of the NPP naming between different laboratories and is widely used in NPP research. In order to systematize the NPP, an NPP webpage is established <http://nonpollenpalynomorphs.tsu.ru/>, including a database with search function by morphological criteria and an NPP gallery as a visualization tool. The basis for the search function is the recording system suggested by Coles (1990).

Nevertheless, there are efforts in identification of different groups. Below we provide an overview of the most common NPP applicable for archaeological studies and references for identification.

## 4.1 Cyanobacteria and Algae

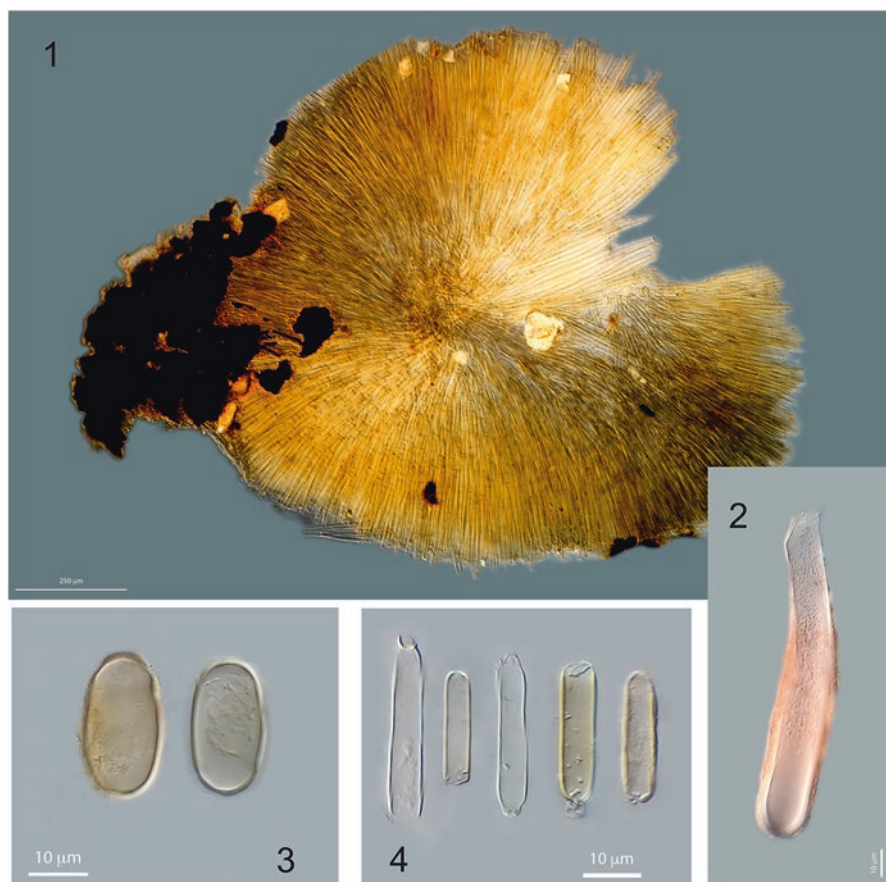
### Cyanobacteria

Cyanobacteria (formerly blue green algae) are photosynthetic prokaryotes able to produce oxygen and to fix nitrogen. They can be found in terrestrial and aquatic habitats and are known for causing blooms, which can be toxic. In fossil material, cyanobacteria, especially akinetes of *Aphanizomenon* (HdV-600 by Ralska-Jasiewiczowa and van Geel 1992 and van Geel et al. 1994) and *Anabaena* (HdV-601 by van Geel et al. 1994), showed phosphate-eutrophication of a Medieval lake as a consequence of intensification of farming and fertilization of the area around the lake (van Geel et al. 1994, 1996). There is no identification key for fossil Cyanobacteria (Fig. 1), and we refer to the descriptions in Ralska-Jasiewiczowa and van Geel (1992), van der Wiel (1982), and van Geel et al. (1983, 1994, 1996).

### Colonial Green Algae

Colonial green algae, *Pediastrum* and *Botryococcus*, are very well-known objects in palynology (Fig. 2). Besides them *Coelastrum* (UG-1233, Gelorini et al. 2011), *Tetraedron* (HdV-371, Bakker and van Smeerdijk 1982), and *Scenedesmus* (HdV-770, Bakker and van Smeerdijk 1982; UG-1239, Gelorini et al. 2011) occur in pollen slides. Different species of *Pediastrum* demonstrate different responses to environmental parameters such as turbidity, water chemistry, nutrient status, pH, and geography. Therefore, *Pediastrum* is a good indicator for recording changes in the trophic status of a lake (e.g., Nielsen and Sørensen 1992; Jankovská and Komárek 2000). Identification and paleoecological interpretation of the fossil green algae can be done



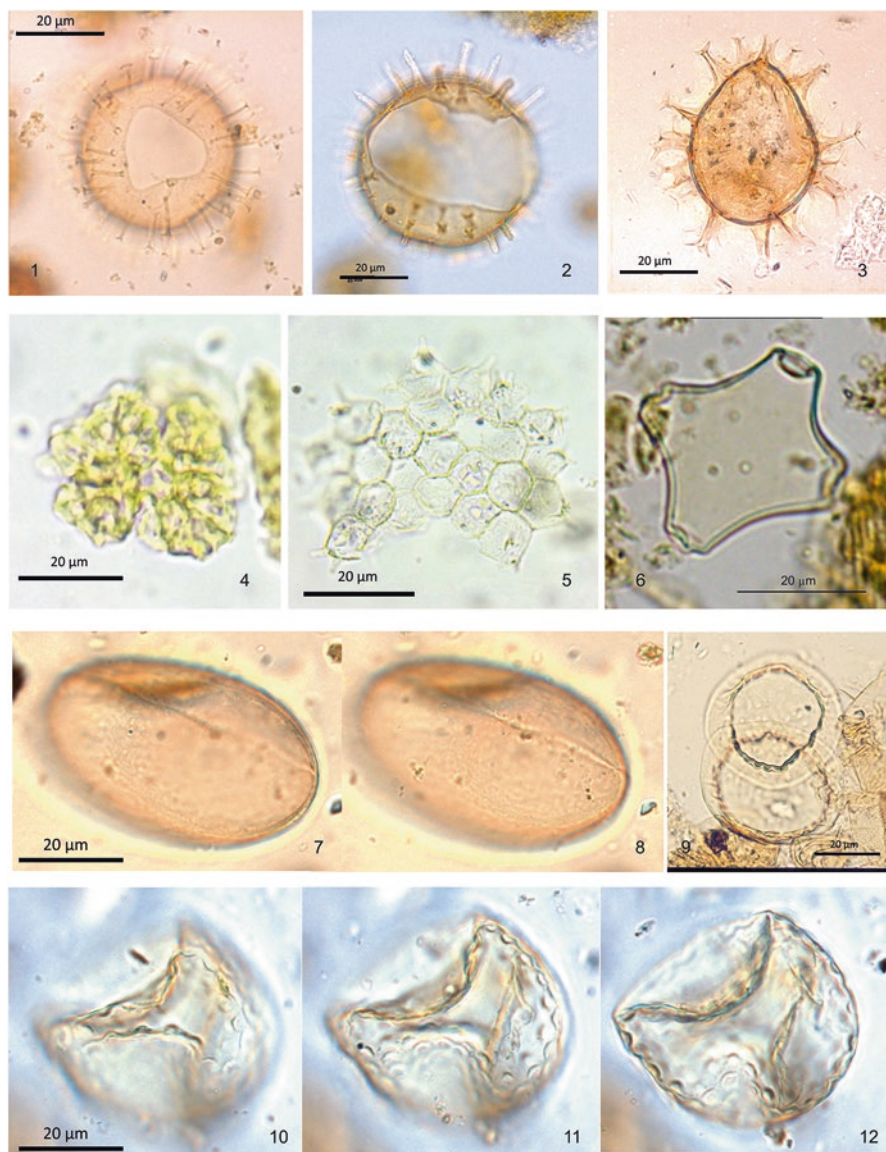


**Fig. 1** Remains of Cyanobacteria: (1) *Gloeotrichia*-type, colony; (2) *Gloeotrichia*-type, sheath; (3) *Anabaena*, akinetes; (4) *Aphanizomenon*, akinetes

using overview works of Komárek and Marvan (1992), Jankovská and Komárek (2000), and Komárek and Jankovská (2001).

### Conjugatophyceae: Zygnematales

Conjugatophyceae are charophycean green algae reproducing by conjugation to produce resting spores with a sporopollenin-like wall. They contain two orders: Zygnematales and Desmidiiales. Zygnemataceae are unbranched filamentous green algae, inhabiting shallow, stagnant, oxygen-rich freshwater lakes, ponds, small pools, or wet soils. A few species inhabit salt water, but there are no marine representatives. During their sexual reproduction, Zygnemataceae produce thick-walled zygosporangia, which can fossilize and be found in the sediment archives. Apart from



**Fig. 2** Algal remains: (1) *Operculodinium centrocarpum*, dinocyst (Shumilovskikh et al. 2013); (2) *Lingulodinium machaerophorum*, dinocyst (Shumilovskikh et al. 2013); (3) *Spiniferites* sp., dinocyst (Shumilovskikh et al. 2013); (4) *Botryococcus*, colonial green alga; (5) *Pediastrum*, colonial green alga; (6) *Mougeotia*, zygospore; (7, 8) *Spirogyra*, zygospore or aplanospore; (9) *Debarya*, zygospore; (10–12) *Zygnema*-type, zygospore or aplanospore

zygospores, asexual resting spores (aplanospores) can also be formed. The spores allow the algae to overcome unfavorable conditions such as drying of the sediment surface during summer or freezing during winter. In most cases, the morphological characteristics of zygospores and aplanospores are necessary for identification to the species level. For information on extant Zygnemataceae, reference is made to Transeau (1951), Randhawa (1959), Kadlubowska (1984), and Hoshaw and McCourt (1988). Fossil spores recorded in pollen slides are: *Mougeotia*, *Zygnema*-type, *Spirogyra*, *Debarya* (Fig. 2). They have been described and their use as paleoenvironmental indicators was started by van Geel (1976). However, until now there is no formal identification key for spores of Zygnemataceae, and the descriptions and photographs of these fossil spores occur only in a handful of papers such as Ellis-Adam and van Geel (1978), van Geel and van der Hammen (1978), van Geel and Grenfell (1996), van Geel (1976, 1979, 2001), and van Geel et al. (1989, 1994).

### Conjugatophyceae: Desmidiaceae

Other representatives of Conjugatophyceae are the unicellular green algae Desmidiaceae. They inhabit mostly freshwater and may be found between *Sphagnum* mosses in peat bogs. Their cells are divided into two symmetrical parts separated by an isthmus. However, in the fossil stage they are represented by a half of the cell. Several genera of Desmidiaceae were recorded in NPP studies: *Penium* (HdV-66, van Geel 1978), *Cosmarium* (HdV-304, several types of HdV-332, *Cosmarium* spec. by Kramer et al. 2010), *Closterium* (HdV-60), *Staurostrum* (HdV-983, Carrion and van Geel 1999). Together with other algal palynomorphs, desmids can be used as proxies of human impact on lacustrine ecosystems (McCarthy et al. 2018). Identification of the fossil remains to genus or species level is possible by using algological literature (Coesel and Meesters 2007, 2013; Štátný 2010, 2013).

### Cysts of Dinoflagellates

Cysts of dinoflagellates (dinocysts) are found mostly in marine sediments but also in freshwater. They represent resting stages in the life cycle of dinoflagellates that are microscopic unicellular algae belonging to the phylum Dinoflagellata. Two systems exist for the identification of the dinoflagellates: (1) for biologists based on motile cells and (2) for paleontologists based on resting stages (Fig. 2). Since only 10–20% of dinoflagellate species produce cysts composed of highly resistant dinosporin, dinocyst assemblages provide only limited information about the former ecological community. Nevertheless, numerous studies on the distribution of dinocysts highlight their usefulness for reconstruction of salinity, temperature, ice-cover, and productivity or eutrophication (de Vernal and Marret 2007; Marret and Zonneveld 2003; Zonneveld et al. 2013). Dinocyst studies belong to a special branch of palynology, and several common identification keys for Quaternary dinocysts

exist as papers or online resources (Rochon et al. 1999; Mudie et al. 2017; <https://www.marum.de/Karin-Zonneveld/dinocystkey.html>).

## 4.2 Fungi

Fungal remains often occur in the pollen samples in the form of thick-walled normally dark-colored spores or dark-colored hyphae. While hyphae cannot be identified morphologically, the varieties of the fungal spores have distinct morphological characteristics. Fossil fungi are known since the Proterozoic (Taylor et al. 2015), and they are actively studied in geology (e.g., Elsik 1976; Graham 1962; Taylor and Osborn 1996). A special system has been developed for the description of fossil fungal remains (Elsik 1983) summarized by Kalgutkar and Jansonius (2000), presented in the online database of fossil fungi: [https://advance.sciences.sfu.ca/fungi/fossils/Kalgutkar\\_and\\_Jansonius/](https://advance.sciences.sfu.ca/fungi/fossils/Kalgutkar_and_Jansonius/).

While working with deep-time geology, it is difficult to establish the connection of fossil finds to the modern mycoflora, but this is possible to do with Quaternary material. Taxonomically the majority of fungal spores recorded in paleoecological studies belong to Ascomycetes, Basidiomycetes, and Hyphomycetes (van Geel 2001), and in rare cases to Zygomycetes (e.g., zygospores of *Mucor* sp. described by Shumilovskikh et al. 2015a). However, for interpretation purposes, they are usually grouped by their nutritional strategies rather than by taxonomy: (1) saprotrophs, general or specialized on dung (coprophilous fungi), wood (lignicolous fungi), charred material (carbonicolous fungi), or soil; (2) parasites such as rusts and smuts; and (3) mycorrhizal fungi (Webster and Weber 2007).

### Coprophilous Fungi

Coprophilous fungi represent a large group of saprophytic fungi able to grow on dung or soil contaminated with dung. The coprophilous fungi inhabit or are associated with dung of wild and domesticated mammals, both herbivores and carnivores, of birds and rarely of other vertebrates and invertebrates. Spores of many coprophilous taxa germinate after passage through the digestive tract (Webster 1970), while germination, hypha growth, and sporulation occur in the fresh dung deposited in warm and moist conditions. Identification of coprophilous fungi is based on the morphology of fruiting bodies and spores. There are several general and specialized identification keys (Ahmed and Cain 1972; Bell 1983, 2005; Doveri 2007; Krug and Cain 1974; Luck-Allen and Cain 1975; Lundqvist 1972; Mirza and Cain 1969).

Taxonomically, coprophilous fungi belong to Ascomycetes, Basidiomycetes, Zygomycetes, and Myxomycetes (Krug et al. 2004), but almost exclusively Ascomycetes produce dark-colored thick-walled spores resistant to decomposition and laboratory treatments. Davis (1987), for the first time, used spores of *Sporormiella* for the reconstruction of herbivore density and megaherbivore

extinction (see also Davis and Shafer 2006). Later paleoecological studies provided descriptions of a spectrum of spores of coprophilous fungi (Table 2; Fig. 3) and highlighted their potential for the reconstruction of the local presence of herbivores as well as for pastoral activities (e.g., Blackford et al. 2006). It is worth to mention that sometimes *Sporormiella* is rare, or even absent, while other spore types of coprophilous taxa are of regular occurrence. This means, there are no good reasons to just and only make records of *Sporormiella*. Among more than 150 genera of coprophilous ascomycetes (Krug et al. 2004), 16 spore types are known from the late Pleistocene and Holocene sediments. Some of these genera are represented by several NPP types (Table 2). Until now there is only one identification key created for paleoecological use covering several types of *Podospora* spores (Schlütz and Shumilovskikh 2017). For identification of other genera, it is best to use the mycological literature mentioned above, original descriptions of NPP types (Table 2), and studies on the influence of laboratory preparation methods on spores of coprophilous fungi (van Asperen et al. 2016).

It is important to mention that coprophilous fungi are first of all saprophytic fungi and their genera include species with different degrees of coprophily as many of the species inhabit soils (e.g., Watanabe 2010; Guarro et al. 2012; Perrotti and van Asperen 2019). Krug et al. (2004) suggested the division of genera into three groups: (1) species predominantly coprophilous or occupying dung-impregnated litter, but for which the genus may include one or more non-coprophilous species; (2) species primarily found on other substrates, but for which the genus may contain one or more coprophilous species or records; (3) genera typically with many coprophilous and non-coprophilous species (Table 2). For interpretation of the coprophilous fungal record, one should be aware of this problem. In order to identify some of the coprophilous fungal spores to species level, more review and laboratory work on spores is required. As the studies on *Podospora* spores show (Schlütz and Shumilovskikh 2017), spores of different genera can be grouped together or in some cases even species can be identified. Similar work would be worth to do on other genera with variable spore morphology such as *Apiosordaria*, *Arnium*, *Bombardioidea*, *Cercophora*, *Delitschia*, *Gelasinospora*, *Saccobolus*, *Sporormiella*, and *Trichodelitschia*. Unless such reviews have been done, the source of the coprophilous fungal spores should be interpreted with caution and with reference to recent mycological literature (e.g., Doveri 2007; Guarro et al. 2012; Watanabe 2010).

## Carbonicolous Fungi

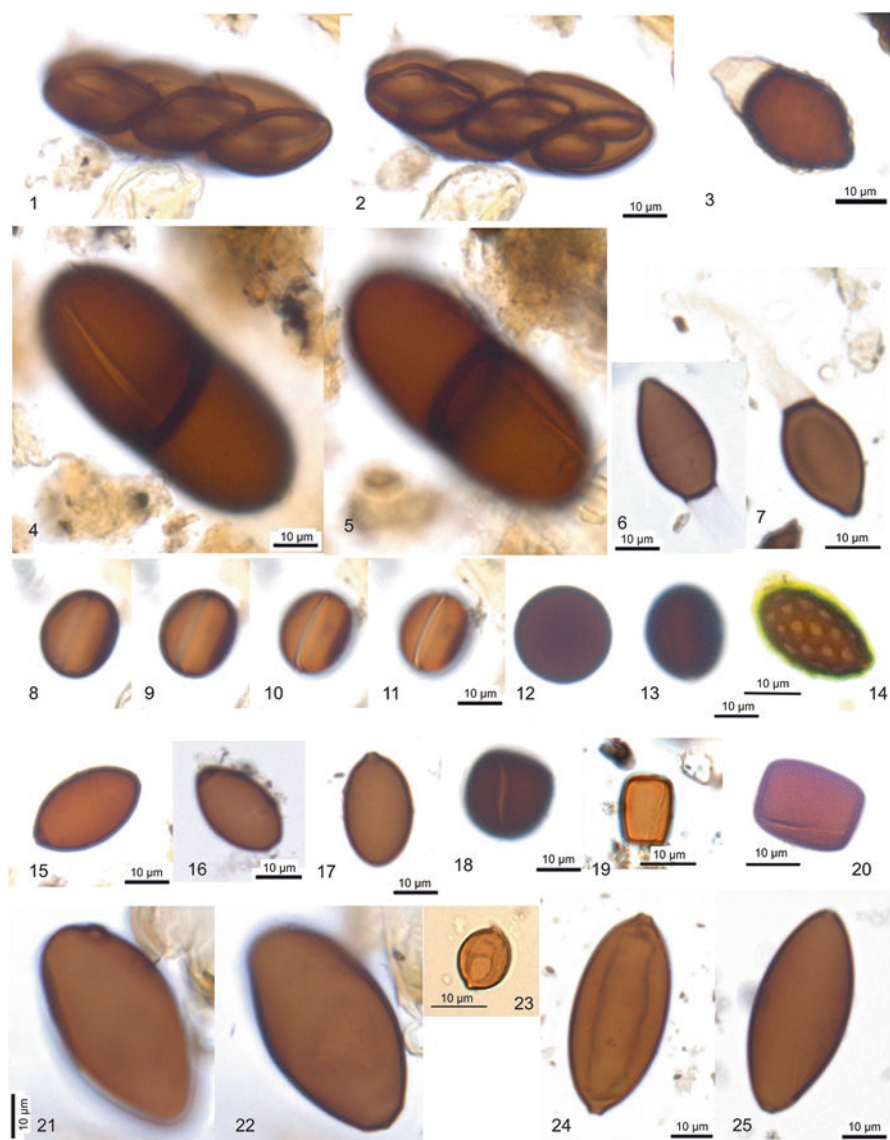
Carbonicolous fungi can be found only or preferably on burned substrates. There are several links: heat from fire stimulates spore germination or triggers the fungus to produce fruiting bodies; fire kills microorganisms that are competitive or antagonistic to carbonicolous fungi; fire changes soil chemistry by increasing alkalinity through ash (e.g., McMullan-Fisher et al. 2011; Widden and Parkinson 1975). In paleoecological studies, ascospores of fungi *Gelasinospora* (Fig. 3) and *Neurospora* were identified as taxa of interest through the work of van Geel (1978) and Bakker



**Table 2** Overview of the genera of coprophilous fungi documented in paleoecological studies with their degree of coprophily (based on Krug et al. 2004) and associated NPP types (<http://nonpollenpalynomorphs.tsu.ru/>)

Fungal genus	Degree of coprophily	Associated NPP types
<i>Apiosordaria</i>	Primarily on other substrates	HdV-169 (van Geel et al. 1983), UG-1171 (Gelorini et al. 2011)
<i>Arnium</i>	Predominantly coprophilous	HdV-261 and HdV-262 (van Geel et al. 2003)
<i>Ascodesmis</i>	Primarily on other substrates	UG-1285 (Gelorini et al. 2011)
<i>Bombardioidea</i>	Predominantly coprophilous	HdV-575 (Bos et al. 2005)
<i>Cercophora</i>	Coprophilous and non-coprophilous	HdV-112 (van Geel et al. 1981), HdV-1013 (van Geel et al. 2011), UG-1183 (Gelorini et al. 2011)
<i>Chaetomium</i>	Coprophilous and non-coprophilous	HdV-7A (van Geel 1978), HdV-1009 (van Geel et al. 2011)
<i>Coniochaeta</i>	Coprophilous and non-coprophilous	HdV-172 (van Geel et al. 1983), TM-16 and TM-211 (Cugny et al. 2010), UG-1208 (Gelorini et al. 2011)
<i>Delitschia</i>	Predominantly coprophilous	BRN-4 (Feeser and O'Connell 2009), TM-6 and TM-23A-B (Cugny et al. 2010), UG-1066 (Gelorini et al. 2011)
<i>Gelasinospora</i>	Coprophilous and non-coprophilous	HdV-1 and HdV-2 (van Geel 1978), HdV-528 (van Geel et al. 1986), HdV-1093 and HdV-1351 (van Geel et al. 2011), UG-1093 and UG-1139 (Gelorini et al. 2011)
<i>Neurospora</i>	Primarily on other substrates	HdV-55C (van Geel 1978)
<i>Podospora</i>	Predominantly coprophilous	BRN-6 and BRN 9 (Feeser and O'Connell 2009), HdV-368 (van Geel et al. 1981), HdV-466 (Kuhry 1985), TM-110 (Cugny et al. 2010), <i>Podospora curvispora</i> (Shumilovskikh et al. 2016a, b), several <i>Podospora</i> types (Schlütz and Shumilovskikh 2017)
<i>Saccobolus</i>	Predominantly coprophilous	<i>Saccobolus minimus</i> -type (Shumilovskikh et al. 2016a, b)
<i>Sordaria</i>	Predominantly coprophilous	HdV-55A (van Geel 1978), HdV-1012 (van Geel et al. 2011), UG-1178 and UG-1180 (Gelorini et al. 2011)
<i>Sporormiella</i>	Predominantly coprophilous	HdV-113 (van Geel et al. 2003)
<i>Trichodelitschia</i>	Predominantly coprophilous	HdV-546 (van Hofwegen 1983)

and van Smeerdijk (1982). The occurrence of these spores correlated with the presence of charred material, highlighting their potential as indicator of local fires, which was tested by later works (e.g., Blackford et al. 2006; Innes et al. 2004). Nevertheless, both genera contain coprophilous species as well (Table 2).



**Fig. 3** Ascospores of coprophilous and carbonicolous fungi: (1, 2) *Saccobolus*; (3) *Apiosordaria*; (4, 5) *Delitschia*-type, (6, 7) *Cercophora*-type; (8–13) *Coniochaeta*; (14) *Gelasinospora retispora*; (15, 16) *Sordaria*-type (HdV-55A) (*Podospora inflatula*-type sensu Schlütz and Shumilovskikh 2017); (17) *Sordaria*-type (HdV-55A); (18–20) *Sporormiella*-type; (21, 22) *Podospora*-type (HdV-368) (*Podospora decipiens*-type sensu Schlütz and Shumilovskikh 2017); (23) *Chaetomium*-type; (24, 25) *Arnium*-type



## Rusts

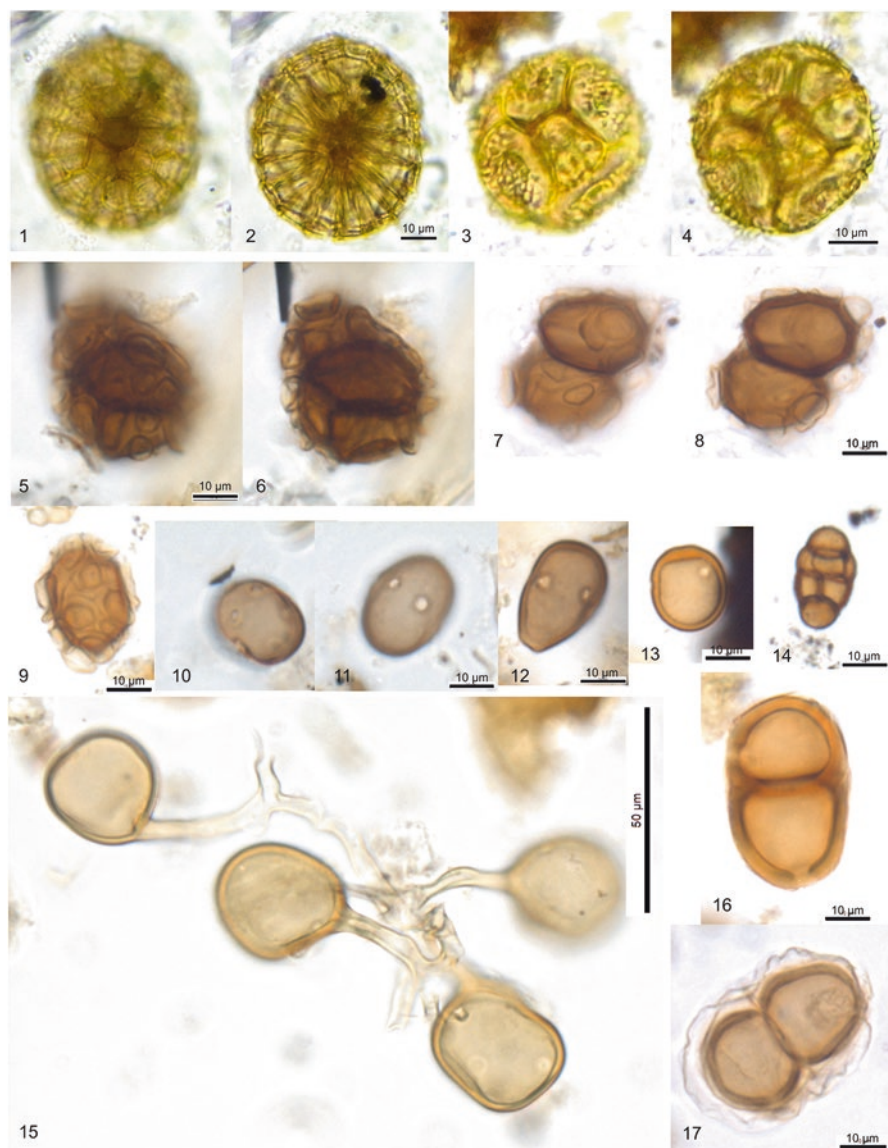
Rusts (Pucciniales, formerly Uredinales) are a specialized parasitic group of Basidiomycetes which are found on various plant species. They have a complex life cycle with several stages on different hosts: uredospores (urediniospores), teleutospores (teliospores), basidiospores, spermatia, aeciospores. Uredospores (summer spores) and teleutospores (winter spores) have a thick wall that is resistant to biological degradation and palynological laboratory treatments (Fig. 4). Teleutospores of rusts (*Puccinia*) are known NPP types: HdV-357 (van Geel et al. 1981), HdV-529 (van Geel et al. 1986). Furthermore, summer and winter spores parasitizing cereals, such as *Puccinia sorghi*, *P. graminis*, and *Uromyces appendiculatus*, were used for the reconstruction of Canadian agriculture in the second millennium AD (McAndrews and Turton 2010). Further morphological studies are required to develop an identification key of the spores. For mycological literature, we refer to Grove (1913), Ellis and Ellis (1997), and Kuprevich and Ulyanishchev (1975).

## Smuts

Smuts (Ustilaginales) represent another big group of plant parasitic Basidiomycetes that grow mainly on angiosperms, especially on monocots, with some exceptions for saprophyagy (Vánky 2013). Due to their specialization on different plant species, spores are important indicators for the presence of host plants. Thus, McAndrews and Turton (2010) identified *Ustilago maydis* and *Tilletia* cf. *caries* and used them as an indication for crop plants. The presence of *Glomosporium leptideum* in harbor sediments indicated the presence of *Chenopodium* in the surrounding area (Shumilovskikh et al. 2016b). Other spore balls of smut fungi were described from paleoecological contexts (Fig. 4): *Thecaphora* (HdV-364) by van Geel et al. (1981), *Urocystis* (UG-1079) by Gelorini et al. (2011), and *Thecaphora* sp. by Shumilovskikh et al. (2016b). However, a comprehensive overview and identification key of these spores in paleoecological contexts is still missing and we refer to mycological literature for further details (Schellenberg 1911; Vánky 1985, 2013; Vánky and Shivas 2008).

## Other plant parasitic fungi

There are also plant parasitic fungi in other taxonomic groups. For example, *Pithomyces* is a genus of the dematiaceous Hyphomycetes (Ellis 1971). The conidiospores of *Pithomyces chartarum* were described from lagoon sediments of Hellenistic times (Fig. 4; Shumilovskikh et al. 2016b). *P. chartarum* is a largely saprophytic fungus (Ellis 1971) and a pathogen of cereals (Chong and Sheridan 1982; Tóth et al. 2007). Isolates of *P. chartarum* produce the mycotoxin sporidesmin causing facial eczema (Collin et al. 1998), known in New Zealand and Europe. It appears when *P. chartarum* sporulates profusely after rain in late summer and autumn (van Wuijckhuise et al. 2006; Di Menna et al. 2010).



**Fig. 4** Spores of plant parasitic fungi: (1, 2) *Glomosporium leptideum*, teliospore ball of smut fungi (Shumilovskikh et al. 2016b); (3, 4) *Thecaphora*, teliospore ball of smut fungi (Shumilovskikh et al. 2016b); (5–9) *Urocystis*, teliospore ball of smut fungi; (10–13) uredospores of rust fungi; (14) *Pithomyces chartarum*, conidiospore; (15) *Glomus*-type, chlamydospores; (16, 17) *Puccinia*-type, teliospores of rust fungi

## Mycorrhizal Fungi

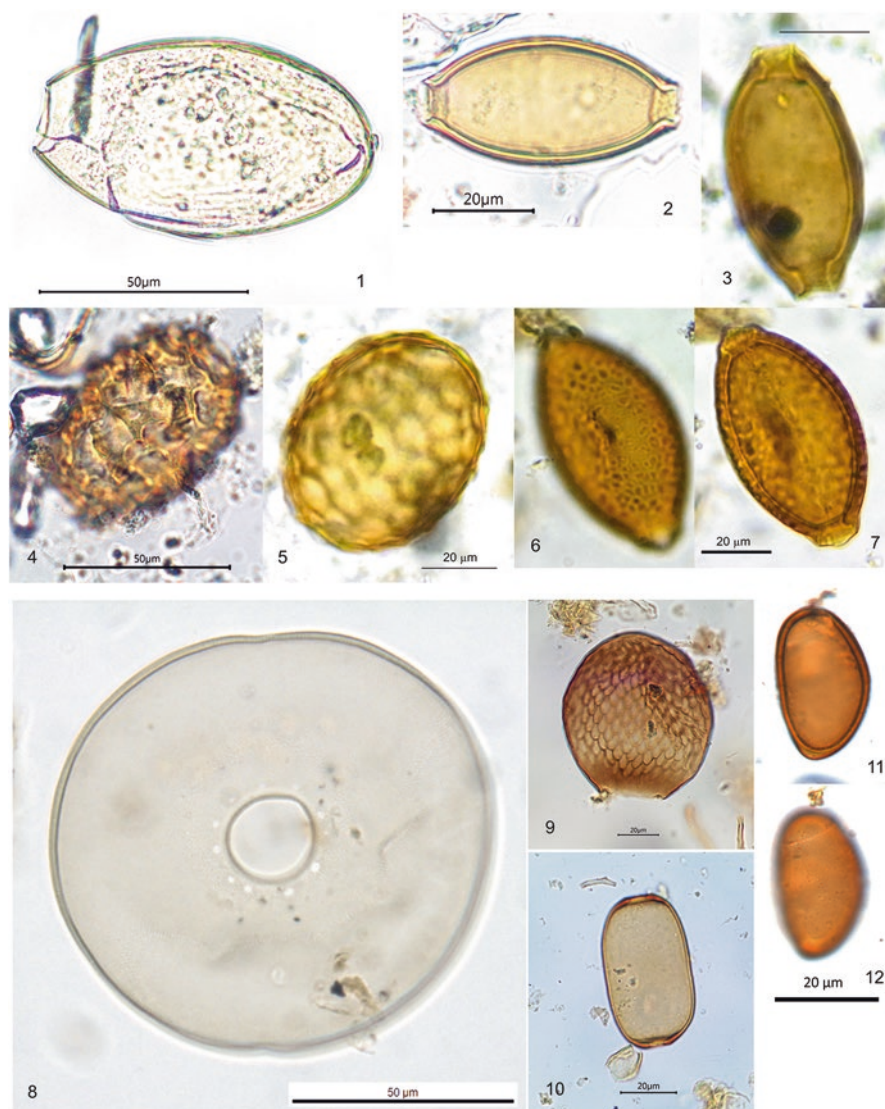
The most important representatives of mycorrhizal fungi in paleoecological research are chlamydospores of arbuscular mycorrhizal fungi (AMF) of Glomeromycota. In paleoecological studies, spores of different groups of Glomeromycota are mostly merged under *Glomus*-type or just named *Glomus* (Fig. 4). Several NPP types are attributed to this group: HdV-207 (van Geel et al. 1989), HdV-1103 (van Geel et al. 2011), UG-1103, and UG-1291 (Gelorini et al. 2011). Spores of several *Glomeromycota* species from peat material are shown by Kołaczek et al. (2013). AMF produce spores below the ground and therefore their occurrence in lacustrine and marine sediments is used as indicator of the soil erosion in the catchment (e.g., Bos et al. 2005; Gauthier et al. 2010; Miehe et al. 2009; Shumilovskikh et al. 2016b; van Geel et al. 1989), while in peat and soil sediments it can occur through roots growth into the sediment (Kołaczek et al. 2013).

## 4.3 Zoological Remains

In comparison to an intensive use of cyanobacteria, algae, and fungi in palynological research applied to solve archaeological questions, microscopic animal remains are less represented. The most important of them are helminth eggs and testate amoebae.

### Helminth Eggs

Helminth eggs (Fig. 5) are often found in sediments, indicating the presence of parasitic worms, their host, and the disease in the area. In palynological studies, especially very resistant eggs such as of *Trichuris* (HdV-531 by van Geel et al. 1986; Brinkkemper and van Haaster 2012; Fernandes et al. 2005; Florenzano et al. 2012; Le Bailly et al. 2007; Maicher et al. 2017), *Ascaris* (Brinkkemper and van Haaster 2012), *Capillaria* (Shumilovskikh et al. 2016b; Bosi et al. 2011; Le Bailly et al. 2007), *Dicrocoelium* (Shumilovskikh et al. 2016a; Le Bailly and Bouchet 2010; Bosi et al. 2011; Florenzano et al. 2012), and *Diphylobothrium* (Le Bailly et al. 2007; Florenzano et al. 2012) occur. While harsh palynological laboratory treatments might change the morphology of the eggs, identification of resistant helminth eggs is still possible (e.g., Lardín and Pacheco 2015). Studies on helminths eggs from sediments and latrines have developed to a special field of paleoparasitology, where gentle methods of sample preparation are used (see chapter “Parasite Microremains: Preservation, Recovery, Processing, and Identification” by Camacho, Perry and Reinhard, this volume).



**Fig. 5** Zoological remains: (1) *Hesciola hepatica*, egg; (2, 3) *Trichuris*, egg (Shumilovskikh et al. 2016b); (4) *Ascaris*, unfertilized egg; (5) *Ascaris*, fertilized egg; (6, 7) *Capillaria*, egg (Shumilovskikh et al. 2016b); (8) *Arcella* sp., testate amoeba; (9) *Assulina* sp., testate amoeba; (10) *Amphitrema flavum*, testate amoeba; (11, 12) *Dicrocoelium* sp., egg

### Testate Amoebae

Testate amoebae are a group of eukaryotic microorganisms with a decay-resistant shell, which can get fossilized (Fig. 5). While studies on testate amoebae are a subject of specialized analysis (Charman et al. 2000; Mitchell et al. 2008), the shells

can be identified from pollen slides and used for reconstructions of hydrological changes, eutrophication, or pollution (Groszpietsch 1952; Mazei and Tsyganov 2006; Payne et al. 2012; Swindles and Roe 2007). Several NPP types were assigned to testate amoeba such as *Amphitrema flavum* (HdV-31A), *A. wrightianum* (HdV-31B) by van Geel (1978), HdV-186 (van Geel et al. 1983), t-22 (Kuhry 1988), *Assulina muscorum* (HdV-32A), and *A. seminulum* (HdV-32B) (van Geel 1978).

## 5 Potential Problems and Biasing Factors

When interpreting NPP records, one should be aware of several potential problems and biasing factors such as a choice of the laboratory treatment, identification bias, and taphonomic problems.

As discussed in the methodological section, sample preparation can significantly influence the diversity of NPP assemblages and composition. In general, less harsh methods are suggested to use in order to get more diverse spectra (Marret 1993; McAndrews and Turton 2010; Payne et al. 2012; Riddick et al. 2016; van Asperen et al. 2016; Enevold et al. 2019). However, using different laboratory approaches makes comparison of the NPP assemblages between sites challenging.

NPP identification is the next problem. As we have shown above, the absence of an NPP atlas for Quaternary sediments makes identification a time-consuming process, where one should be aware of several hundreds of papers with NPP descriptions and general biological literature about very different groups of organisms. One step forward represents the NPP database, which is in development. Furthermore, more studies on biological affinities of NPP should be conducted in order to understand the ecological value of these objects. Recently, several review studies were carried out on fungal spores (e.g., Hawksworth et al. 2016; Schlütz and Shumilovskikh 2013, 2017; Shumilovskikh et al. 2015b; van Geel et al. 2006), highlighting the potential of these types for paleoenvironmental reconstructions but also emphasizing restrictions in interpretation. In addition, further research is required for understanding of the association between modern-day NPP assemblages and current environmental conditions and/or taphonomic processes (e.g., Prager et al. 2006; Blackford and Innes 2006; Medeanic 2006; Cugny et al. 2010; Ghosh et al. 2017).

Understanding of the NPP origin is crucial for environmental reconstructions. The best example is provided by a study on spores of the arbuscular mycorrhizal Glomeromycota (*Glomus*) (Kołaczek et al. 2013), which, when found in lacustrine sediments, are very good indicators of erosion in the catchment. But the results of that study also show that application of *Glomus* to peat sediments is highly questionable, because the spores may originate from mycorrhiza on the roots of a plant growing at the sampling site. Furthermore, NPP often provide a very local signal, making an intercorrelation between sites difficult (Expósito et al. 2017). Revelles et al. (2016) show that NPP distribution is mainly influenced by taphonomic or anthropic processes rather than by natural ecological or climate dynamics. The dispersal of the fungal spores that we find is less efficient in comparison with the pollen



of wind-pollinated trees, possibly due to the position of fruiting bodies mostly close to the ground (van Geel et al. 2003).

One of the possible solutions to trace taphonomic problems is the application of a multiproxy analysis. Thus, comparison of frequencies of coprophilous Coleoptera and percentages of coprophilous fungi from the sediment core Lac des Lauzons (French Alps) show a complete discrepancy: dung beetles are abundant in the lower lacustrine part of the sediment and disappear to the top, where coprophilous fungal spores increase (Ponel et al. 2011). Even by the same grazing pressure, this change was explained by the authors as a change from open water environment, where beetles could fall down and sink, to peat, where locally produced spores could get embedded.

## 6 Conclusion

NPP-analyses open up new opportunities for more detailed environmental reconstructions in the frame of archaeological contexts. With regard to archaeological stratigraphies, the analysis of NPP can provide information about temporal and/or spatial changes in specific conditions, thus helping to reconstruct how former structures and settlement areas have been used in the past. NPP are often better preserved than pollen at sites with dry deposition, making them valuable indicators for on-site characterizations at archaeological locations. As a whole, combination of NPP with pollen and with geochemical and sedimentary records can provide a more inclusive picture of environmental conditions and human behavior.

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**Part II**  
**Micro-Particles Used Primarily for**  
**Analysis of Human Behavior: Diet, Health,**  
**Plant Use, Animal Husbandry, and More**

# Starch Granules as Markers of Diet and Behavior



Amanda G. Henry

Starch granules, the energy-storage molecules produced by plants, are increasingly used in archaeological studies as markers of human diet and food processing. While starch granules are organic in nature, their semicrystalline structure likely contributes to their preservation in some protected archaeological contexts, such as within dental calculus or pottery crusts. However, many naturally-occurring and human-induced processes can damage or destroy starches, so a detailed understanding of how starches are formed and ultimately preserved is necessary to accurately translate the starch record into meaningful statements about human behavior.

## 1 Starch Granule Formation

Many different kinds of plants produce starch granules, including lower plants (mosses, ferns, etc.) and higher plants. Most starch is produced directly in the chloroplasts as a means of immediate energy storage, to be used during the night or at other times when energy is needed (BeMiller and Whistler 2009). These short-term starches are called transient starches and are of limited use in archaeological analysis because of their very small size and lack of diagnostic features (Haslam 2004). Higher plants have specialized organelles, called amyloplasts, in which they produce larger “storage” starches for long-term energy storage (Sivak and Preiss 1998). These starches are often produced in those parts of plants that require energy other than that created by photosynthesis after a long period of inactivity, such as underground storage organs (e.g., tubers, corms, and bulbs), seeds, and fruits.

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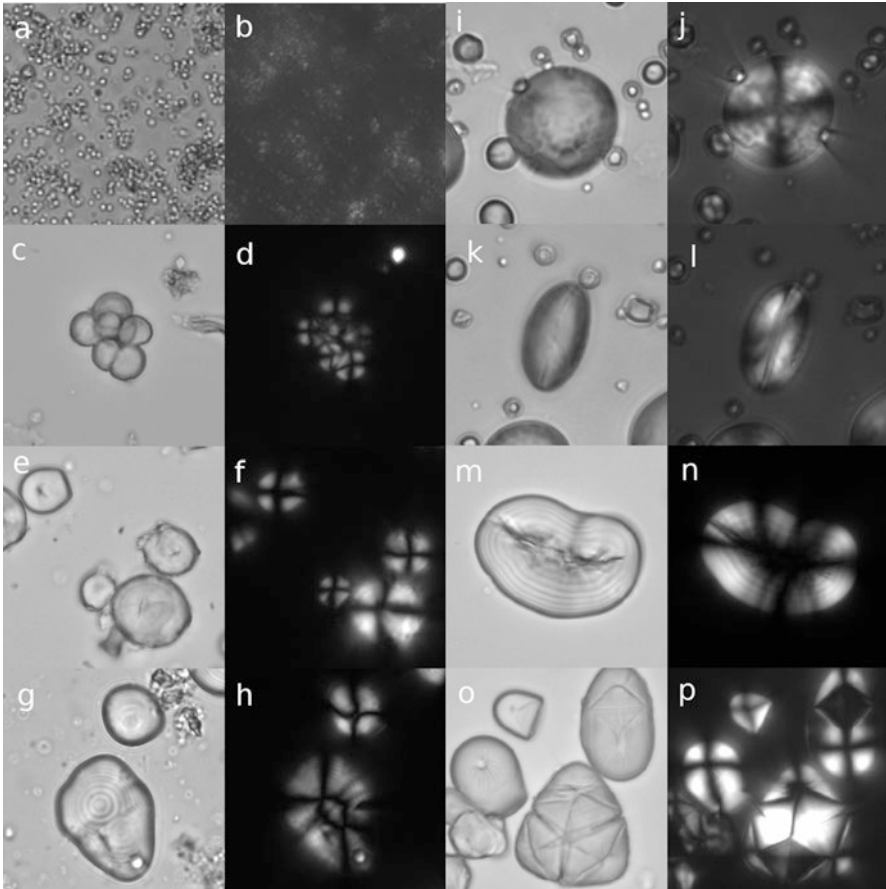
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A. G. Henry (ed.), *Handbook for the Analysis of Micro-Particles in Archaeological Samples*, Interdisciplinary Contributions to Archaeology,  
[https://doi.org/10.1007/978-3-030-42622-4\\_5](https://doi.org/10.1007/978-3-030-42622-4_5)

While the formation of starches is broadly similar among all plants, details of the process differ among taxa. Many of these details are under genetic control and give rise to the variation that allows us to identify the taxonomic origin of many starches. In simple terms, plants produce carbon sugars during photosynthesis, which are transferred to the amyloplasts and converted into two kinds of polysaccharides—amylose and amylopectin. Amylose is a straight-chained molecule and accounts for roughly 20–35% of the mass of the starch, though this amount can range from less than 15% to greater than 40% depending on the plant species or breed (BeMiller and Whistler 2009). Amylopectin is a heavily branched molecule and makes up the backbone of the starch. These polysaccharides are built together into a semi-crystalline structure known as a starch granule or grain.

The pattern of formation provides the morphological features which are used to identify starches (Fig. 1). The formation begins at a nucleus, called the hilum (pl. hila), and the sugar chains extend radially from this point. As starches grow, the carbohydrate chains can be deposited evenly in all directions, leading to a starch with a centric hilum, or they can be deposited preferentially on one side, leading to starches with an eccentric hilum. Throughout the starch there are alternating bands of higher and lower crystallinity. In some taxa, some of these layers of varying crystallinity are visible as darker and lighter bands, which are called lamellae. The level of hydration of the starch and the refractive index of the mounting medium can change the visibility of the lamellae. The alternating layers also result in one of the diagnostic features of the starch granules, that is, the appearance of an extinction or polarization cross when viewed under cross-polarized light. Finally, the manner of growth in the cell can determine both the overall shape of the starches as well as features of their surface. If there is one starch per amyloplast, the starches are called simple starches, but if several starches grow in the same amyloplast, they are called compound starches. Often there are several amyloplasts within a single plant cell, and the starches can press against each other as they grow. When the starches are all generally the same size this leads to polyhedral starches with flat pressure facets, such as in maize. If the starches are bimodal, as is the case with the Triticeae, this results in the larger starches having a golf ball-like pockmarked appearance, with surface dimples where the smaller starches pressed against them.

While much of the variation among taxa is under genetic control, it is important to note that starches, like phytoliths, are subject to problems of multiplicity and redundancy. Starch morphologies can considerably overlap among both closely and distantly related taxa [for example, the similarities among the Triticeae (Piperno et al. 2004), and between the Triticeae and *Capsicum* (Perry et al. 2007)]. There can be several starch morphologies present in each plant, both within a single organ [e.g., the bimodal starch types seen in the seeds of Triticeae (Stoddard 1999)], and between organs of the same plant (seeds and roots, or seeds and pollen). The shape of starches can change as the plant ages, as well (Evers 1971; MacGregor and Ballance 1980). Care must be taken when developing a reference collection to sample broadly across taxa, as well as deeply within a species, in order to capture the full range of potential morphologies (see for example Lentfer 2009).



**Fig. 1** Starches from modern plants demonstrating their variation. All boxes 50  $\mu$ m each side. (**a**, **b**) *Atriplex halimus* seed starches (many of the starches in the Chenopodiaceae family are very small compound granules that fracture into tiny component granules); (**c**, **d**) *Malva sylvestris* root starch; (**e**, **f**) *Nuphar lutea* rhizome starch, with distinctive small linear to stellate fissures at the hilum; (**g**, **h**) *Trapa natans* seed starch, with one simple starch with eccentric hilum and one compound starch, both with very visible lamellae near the hilum; (**i**, **j**) *Triticum aestivum* seed starch showing the bimodal size distribution and surface dimples on the large granule. (**k**, **l**) *Triticum aestivum* seed starch viewed from the side, showing the equatorial groove; (**m**, **n**) *Lens culinaris* seed starch with the characteristic longitudinal cleft fissure, very visible lamellae, and disrupted cross with multiple arms; (**o**, **p**) *Babiana cedarbergensis* corm starch, showing the variation in forms present in this species. Upper right is an ovoid compound with two unequal components; lower right a triangular compound with three equal components. Upper and middle left, isolated component granules. The hila are usually refractive dots and are often marked with lines or fissures that extend from the hilum to the corners of the granules. This feature is common to several members of the Iridaceae

## 2 Starch as an Archaeological Marker

While starch has a long history of study and use as a component of food, its application as an archaeological marker developed only in recent times. The consistent morphological differences of starch granules produced by certain taxa were studied in the late nineteenth century by a variety of continental scholars (e.g., Nägeli 1858). In 1913, an American medical doctor by the name of Edward Tyson Reichert published a large compendium of figures and descriptions of starches (Reichert 1913), most of which had been produced by his two “assistants,” Dr. Elizabeth E. Clark and Dr. Martha Bunting. Reichert was interested in how biochemical markers, including starches, proteins, and the like, might be used to uniquely identify species. In the two-volume, nearly 900-page treatise (which Reichert nevertheless referred to as a “report of a preliminary investigation”), Reichert, Clark, and Bunting provided detailed morphological and biochemical descriptions of starches from a large number of taxa. Their systematic approach confirmed that many starch morphologies were unique to specific taxa, and their research laid the foundation for the use of starch as a marker in archaeology.

In the late 1920s, Johannes Grüss found starch granules, bacteria, and yeast cells in the residues on Egyptian amphora that had been used to store beer (Grüss 1929). This was one of the first studies to use starch analysis to identify not only food residues, but also the food processing steps involved: Grüss identified marks on the starches that indicated they had been sprouted, further clarifying the beer-making procedure. Despite this early progress, it wasn’t until much later, after Donald Ugent reported starch granules in desiccated tubers from Peru (Ugent et al. 1982), that the potential for starch analysis to assist in the identification of ancient plant materials was more broadly adopted. The feasibility of the analysis of individual starches isolated from residues on stone tools was first explored by an honor’s thesis at the University of Queensland in 1988 (Higgins 1988), and publications in high-impact journals quickly followed (Fullagar et al. 1996; Loy et al. 1992; Piperno and Holst 1998). Since that time, starch granules have been recovered from a variety of archaeological materials and used to document many aspects of human interaction with plants (Barton and Matthews 2006).

Because of its abundance in many of the foods targeted by humans, starch has the potential to be found in almost any context in which people processed, prepared, or consumed food items. Starch can therefore be recovered from plant processing tools such as grindstones and cooking utensils such as pottery. Starch from barley has been found on early (c. 3000 BC) funnels and beakers from the Mijiaya site in North China (J. Wang et al. 2016b) as well as on vessels from several sites in middle Egypt that had been used to make or store beer from c. 2000–1200 BC (Samuel 1996a). Starch granules, in combination with phytoliths, micro-charcoal, and fungal remains, were found in charred cooking pot residues on pottery from the pampas in Argentina (Musaubach and Berón 2017), showing the consumption of both wild and domesticated plants. Furthermore, this study highlighted the importance of considering multiple kinds of microparticles and other residues (in this case, fatty acids

analyzed by gas chromatography)—the more detailed analysis allowed the authors to confirm the use of maize (*Zea mays*) and to explore how it had been processed.

Starch is also preserved on tools. Analysis of flakes from Neanderthal sites in France confirmed their use of starchy plants (B. L. Hardy 2004; B. L. Hardy and Moncel 2011). Several grindstones from Gravettian contexts within sites in Italy, Russia, and the Czech Republic also preserved starch from a variety of plants, including *Typha* spp. (Aranguren et al. 2007; Revedin et al. 2010). This evidence of flour-making suggested that advanced processing techniques of starchy plant material were common well before agriculture. Starches found on grindstones from the Last Glacial Maximum in China also indicated the use of a large number of taxa, suggesting an increased reliance on plant foods during this period of climatic challenges and general resource scarcity (Liu et al. 2013). Starches recovered from stone tools in highland Ecuador demonstrated the early dispersal and use of maize (Pagán-Jiménez et al. 2015). Stone flakes and grindstones are not the only tools where starch may be found—a recent study has also found starches from maize and other local foods on shell scrapers from the Bahamas (Ciofalo et al. 2018).

Starches have been recovered from sediments and used to reconstruct site-use patterns (Balme and Beck 2002) though there are many potential sources of bias with this approach (Haslam 2004; Hutschenreuther et al. 2017). The potential pathways by which starches could be preserved in sediments (such as adhesion to clay particles as proteins do, for example) are not yet understood, and further research on these are needed.

Dental calculus is increasingly used as a target for starch recovery, for several reasons. Calculus forms during the lifetime of the individual, and as it forms it traps food remains within a matrix that is composed of bacterial proteins and mineral crystals (predominantly calcium phosphate in several different mineral forms) (Jin and Yip 2002; Lieverse 1999). The calculus becomes increasingly mineralized during the individual's lifetime, and after the death of that individual it remains a relatively stable environment. This seems to provide a protected environment that contributes to the long-term preservation of starches. Furthermore, because calculus forms in the mouth, it is a record of the kinds of plants that have been directly introduced into the oral cavity. This will predominantly represent food items, but other activities can also be recorded, such as oral processing of plant-based building materials (e.g., basket making) or non-dietary plant use (medicinal/recreational) (Radini et al. 2017). Other microparticles such as charcoal, phytoliths, fungal spores, pollen, and mineral particles may also be recovered from calculus, and may likewise represent both consumption and non-dietary behaviors (K. Hardy et al. 2016; Mackie et al. 2017; Radini et al. 2019). Starch granules have been recovered from calculus from Neanderthals (Fiorenza et al. 2015; K. Hardy et al. 2016; Henry et al. 2011, 2014; Power et al. 2018) and from early modern humans in Europe (Power et al. 2015a, 2016). Starch from calculus has also provided a wealth of information about trade networks, and the spread of people and agriculture across the Americas, including Peru (Piperno and Dillehay 2008), Brazil (Boyadgian et al. 2016), and Mexico (King et al. 2017). Calculus is rarely examined for starches alone; instead researchers will combine methods, for example, looking at starches

and other microremains together (Cummings et al. 2018; e.g., Tromp and Dudgeon 2015), or combining starch analysis with other methods of dietary reconstruction, such as stable isotope analysis on collagen (T. T. Wang et al. 2016a), MS-based analysis of residues in the calculus (Gismondi et al. 2018), or analysis of calculus proteins and DNA (Warinner et al. 2014). In general, studies that combine analysis and identification of starches with other methods provide the most complete information about diet and behavior in the past.

One of the aspects of starch granules that makes them a useful tool for archaeologists interested in food behavior is that the physical appearance of starches changes as a result of processing. These changes, including things like swelling, cracking, and partial gelatinization (the irreversible breakdown of bonds among linked amylose and amylopectin chains, leading to the loss of crystallinity) appear as the result of different kinds of processing. For example, Babot (2003) demonstrated that air drying, roasting, charring, freezing, and milling all produced distinctive damage to plants native to Central and South America including potatoes, maize, and beans. Similar results were shown for North American food plants (Messner and Schindler 2010) and a variety of Old World domesticated species (Henry et al. 2009; J. Wang et al. 2017). Though modern samples may predictably be altered by processing, interpreting damage on archaeological materials remains challenging. In contexts where the likely processing steps are well known, damage to starch has been a useful indicator of the exact steps used. For example, processing marks on the starches preserved in Egyptian pottery crusts showed that the brewers used malted grain and heated the wort at different temperatures depending on the brew (Samuel 1996b). Partially gelatinized starches recovered from dental calculus of Neanderthals suggest they consumed grains, including wild relatives of wheat and barley, that had been heated in the presence of water (Henry et al. 2011). However, many changes to starch morphology can occur for reasons other than food processing, including taphonomic effects and laboratory processing conditions (discussed below). A full understanding of these other processes is necessary before starch damage is used to interpret things like multistep processing.

### 3 Methods to Recover Starch

#### 3.1 *From Modern Reference Materials*

The first step in any analysis must be to create a reference collection of starches from modern plants. For any region, it is best to sample not only the starchy parts of the plants of interest (e.g., the seeds from wheat or barley) but also any other potentially starch-containing plant organs (e.g., wheat pollen), as well as starchy parts of other native species (e.g., the seeds of “weed” grasses such as goat grass). Starch granule size and morphology can vary slightly among plants of different ages and from different growing conditions, so it is important to include samples from multiple individual plants.



The sampling of starches from modern plants is relatively simple. The starchy part of the plant is cut to expose the interior. A scalpel is used to scrape a very small amount off the plant directly onto the slide. Large chunks are flattened with the side of the scalpel or removed. A 10–12% solution of glycerin in distilled water is added (higher glycerin concentrations may obscure important features of the starches) and a cover glass is put over the sample. It may be necessary to gently press down on the cover glass to further disperse the starches. The samples are then examined at 200–400 $\times$  magnification under a transmitted light microscope which has two polarization filters that are capable of cross-polarizing. A microscope with an x-y stage is preferred but it is possible to use a circular stage.

The starches should be examined, photographed at multiple different focal planes, and described. There is no peer-reviewed published terminology, but many in the field contributed to the International Code for Starch Nomenclature in 2011, which is available online (ICSN 2011). In general a description should record if the starch forms singularly or in compounds, the overall shape in both two and three dimensions, the position and visibility of the hilum, the appearance of the lamellae, the presence and appearance of fissures, the texture of the surface, the appearance of the polarization cross, and whether the collection of starches from a single plant part has one starch type (isomorphic) or several distinct types (heteromorphic). Most plants will show some variation with their starches, so it is important to document the degree and frequency of this variation (e.g., using terms like most, some, rare, etc.). Ideally, these would be supported by actual frequency counts, but this is not possible in many cases, and not always useful in distinguishing starch types.

### 3.2 *From Dental Calculus*

The methods to recover starch granules from dental calculus have varied. Most protocols begin with removing the calculus from the tooth using a dental pick or scalpel, and chemically or physically breaking apart the calculus if necessary, and then examining using light microscopy or scanning electron microscopy (SEM). In cases where the calculus deposit is small, and the tooth is separated from the bone, then some practitioners recommend a kind of “wash,” using dilute HCl or water and sonicating the entire tooth (Boyadjian et al. 2007). While this method often results in the recovery of a large number of starches, it may be problematic in that it is more difficult to control or test for contamination, and when HCl is used, this process can damage the enamel surface of the tooth. Other kinds of pre-sampling contamination removal include brushing with a soft, single-use toothbrush.

If the calculus deposit is large enough, it should be removed in a large, single chunk. A dental scaler or scalpel is used to pull the calculus off the tooth, usually onto a prepared sampling surface, such as a piece of autoclaved laboratory aluminum foil, a folded piece of weighing paper, or directly into a microcentrifuge tube. After collection, some researchers further decontaminate the calculus

using, for example, multiple washes in distilled water (Tavarone et al. 2018) or weak sodium hydroxide (Soto et al. 2019). However, such methods are still problematic in that it is unclear if contaminating starches are fully removed in the former case, and whether the method would work on real dental calculus in the latter case. Further testing to refine decontamination methods is needed. After cleaning, the majority of the calculus chunk can be chemically or physically broken down. Physical disaggregation by grinding in a mortar (preferably made from agate) can reduce the particle size but may damage starches and may leave starch and other microparticles trapped in the calculus matrix. Mechanical disaggregation using sonication has proven useful for releasing trapped particles from the calculus (Radini et al. 2019). Chemical processing can be more effective at releasing the starches but may also damage them if too strong solutions are used for too long. Some researchers have used dilute (~2%) HCl to break up the calculus, but a recent paper has demonstrated that EDTA provides better results and does not damage the starch (Tromp et al. 2017). The broken-down calculus is then mounted and examined under transmitted light microscopy, usually at 400× magnification. Many mount the calculus in a glycerin solution (10–12%), but some mount directly in EDTA or HCl in order to observe the dissolution of the calculus to help determine that the starches are directly released from the calculus.

### 3.3 *From Pottery*

Pottery vessels were often used as food storage and processing implements, and therefore can contain a large amount of starch. Sometimes starches are preserved in the charred food crusts remaining inside cooking vessels. This crust is removed with a scalpel or other blade. Some researchers then dissolve the crusts in 10% H<sub>2</sub>O<sub>2</sub> to free the starches (e.g., Saul et al. 2012); others use density separation by heavy liquid flotation to separate the starches from other material (e.g., J. Wang et al. 2016b), some do both (e.g., García-Granero et al. 2017). Heavy liquid separation relies on the general principle that starches have a specific gravity of around 1.2 g/cm<sup>3</sup>, and will therefore float when in a solution that has a specific gravity of 1.3 g/cm<sup>3</sup>. Sodium polytungstate (SPT) is the most often used heavy liquid, but it should be noted that SPT can damage starches if they are left in solution for too long. The density separation process therefore requires a centrifuge to pull down the denser material through the SPT, leaving the starches floating on top. The resulting SPT/starch mixture is transferred to a new tube and diluted with enough water to lower the specific gravity below 1.2, and centrifuged again to concentrate the starches.

Starches may also be preserved on the surfaces of the pottery in the absence of food crusts. In such cases, it may be possible to wash a small area with distilled water and a sterile brush, and to collect and analyze the resulting liquid suspension (Duke et al. 2018). Finally, smaller sherds can be sonicated in beakers with a small

amount of water to extract starches. The starches are then separated from the remaining suspension in the beaker using heavy liquid flotation. It is especially important to collect contamination control samples (see below) when using either of the latter water wash methods, as the sampled surfaces often have no protection from the surrounding environment.

### ***3.4 From Stone Tools***

Stone tools have also been targeted as potential sources of ancient starches. Some analysts have directly examined the surface of the tools under a dissecting microscope with cross polarized light (e.g., B. L. Hardy 2004). This method allows a better understanding of the distribution of residues across the surface of the tool but often does not have high enough magnification to identify the botanical origin of the starches. Instead, the residues must be removed from the tool and examined using transmitted light microscopy. Flaked stone tools are often sonicated, as with pottery sherds, and the resulting liquid centrifuged or evaporated to concentrate the residues. A spot sampling method may also be employed, in which a small amount of water is placed in a spot of known area (e.g., a circle with a 1 cm diameter). The water is agitated with a pipette and then collected. The washing of that spot is repeated several times and all the water collected together. This method may not extract all of the starches but can provide some information about the variable distribution of starches across the tool, when multiple spot-samples from the same tool are compared. For grindstone tools, a multi-step processing method can help to provide a means of contamination control. The grindstone can first be dry brushed, then wet brushed, then “picked” (a probe is inserted into visible pits in the tool surface to pull out embedded material), and finally sonicated (as described in Piperno 2006). If the tool is too large to fit into a sonicator, then an electric ultrasonic toothbrush can also be used (Liu et al. 2018). The material removed at each sampling step is individually examined, with the dry and wet brushing samples representing more likely contaminated samples and the picking and sonication samples more likely to contain a majority of endemic starches.

### ***3.5 From Bone***

Bone can also be sampled for starches using the spot sampling method as described for stone tools. This is a useful method for testing for contamination. Bones are less likely to have been part of plant food processing procedures, but are often handled in the same laboratory settings as tools and teeth, and therefore can provide a record of post-excavation contamination.

### 3.6 *From Sediments*

Sediments have been examined for starches, and some have used this method to explore differences in site activity areas (Balme and Beck 2002). Usually the sediment samples are minimally processed, and heavy liquid flotation is used to separate the starch from the heavier sediment particles. However, there are concerns about how small changes in sediment properties might affect the long-term survival of these starches (Haslam 2004; Hutschenreuther et al. 2017), so more research is needed before starches in unprotected environments in sediments are regularly used.

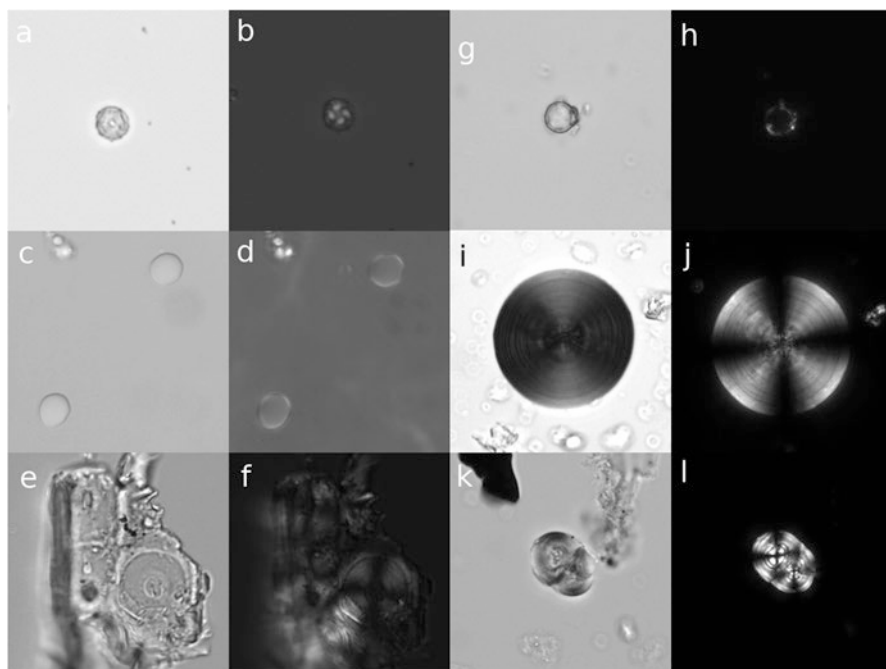
## 4 Identifying Starch Granules

The most common methods for identifying starches rely on the comparison of the starch morphology to a reference collection. This is usually done by “expert analysis,” though recently some have attempted computer image processing methods that allow post-hoc assignment of the starches to certain types or species (Coster and Field 2015; Liu et al. 2014; Wilson et al. 2010). While there is great potential with these kinds of computerized methods, they are limited in that most computer-based image processing programs can only “see” the starch in two dimensions, while the three-dimensional information is lost. This means that automated algorithms have less success identifying taxa in which the starches have very different outlines in plane view and in side view.

The features that can reliably differentiate starches are formation type, shape, appearance and position of the hilum, appearance of the extinction cross, appearance of the lamellae, and surface features. If possible to see clearly, the formation type of the starch should be described as either simple or compound. If compound, the shape of both the overall compound starch and the individual component granules should be described, as well as the frequency with which the compound breaks into components (i.e., how often the component granules are seen alone). Both the two-dimensional and three-dimensional shape of the starch should be described. Two-dimensional terms should be used especially when the shape varies among the axes of the granule. For example, a starch may be circular in plane view, but compressed or flattened in side view, and lenticular in 3d. Usually if a 2d shape is encompassed within a 3d shape, it is not necessary to use both—a spherical starch will always be circular in 2d, and a polyhedral starch will be polygonal in 2d. The hilum is usually described as centric or eccentric and visible or invisible. A visible hilum can be a single refractive point, or marked by one or more small fissures, or an open vacuole. The extinction cross always centers on the hilum, and can therefore also be centric or eccentric. The general orientations of the arms of the cross are determined by the orientation of the two polarization filters on the microscope, but the arms can be thick or thin, straight or bent, invisible at the center, or widening toward the margins of the starch. The lamellae can be visible or invisible. If visible, they can vary in thickness, density, and completeness around the starch. Fissures

can appear at the hilum, as single lines, T-shaped, Y-shaped, X-shaped, or stellate cracks. Some fissures appear at the margins of granules rather than at the hilum. When describing fissures, care should be taken to differentiate those that are naturally forming in the starch and therefore potentially diagnostic from those that are caused by damage. The surface of the starch may be marked by pressure facets of various kinds, or may be smooth or textured. Finally, some taxa have distinctive features which do not fall into the previous categories. Some Triticeae have an equatorial groove, which is a thin crease that runs around the circumference of the mostly lenticular starches (Fig. 1k). In many Fabaceae, there is a very large, ragged fissure that divides the granule along the long axis—this is known as the longitudinal cleft fissure or LCF (Fig. 1m, n). Some of the Iridaceae have two distinct lines or creases that run from the hilum to the distal marginal corners of the hemispherical or sub-triangular component granules (Fig. 1o, p). All of the terms used above, as well as several other useful terms are defined by the ICSN (2011). Size is sometimes used to differentiate starches, but care must be taken here, because the size ranges of many species overlap considerably, and the size of starches can change as the plant ages. As mentioned above, creating a reference collection of sufficient depth and breadth is necessary for confident identification. It is always recommended for the researcher to indicate their degree of confidence in their identification. It is more accurate to state that an archaeological starch has morphology that is consistent with that of a modern reference starch, than to say that an archaeological starch is from a particular plant.

There are several objects that are of a similar size to, and that share some features with starches. These objects can occur in a variety of archaeological contexts and can confuse starch researchers. First, coccoliths can often occur in archaeological samples (see chapter “Coccoliths and Other Marine Microfossils in Microparticle Analysis” by Young, this volume), and their flat appearance is not always immediately visible (starches are almost always thicker and less plate-like than coccoliths, but it can be difficult to turn coccoliths to see their complete shape). Coccoliths also often have a central point that looks like a hilum, and a polarization cross. However, unlike in starches, this polarization cross will always appear twisted (Fig. 2a, b). Small bubbles of oil or air occasionally have a birefringence pattern at the very margin of the bubble, but these bubbles lack internal features that identify starches and the cross never appears at the center (Fig. 2c, d). Fibers or cell walls from soft woods often have bordered pits, which can sometimes appear as a row of donut-like shapes. When viewed under cross-polarized light, they have an extinction cross. However, starches do not form attached to a fiber, and the central hole of the bordered pit is usually much larger than the hilum of a starch. Furthermore, the fiber itself is flat, and the margin of the bordered pit is usually diffuse (Fig. 2e, f). Note that while bordered pits come from wood, they are very common in modern paper products and therefore unreliable markers of past use of wood. Undecorated chrysophycean stomatocysts (see chapter “Diatom Microfossils in Archaeological Settings” by Stone and Yost in this volume) have a solid outer shell that sometimes cross polarizes (Fig. 2g, h). They are distinguished from starches in that they never have a cross in the center (in fact, they have a hollow center) and they often have a



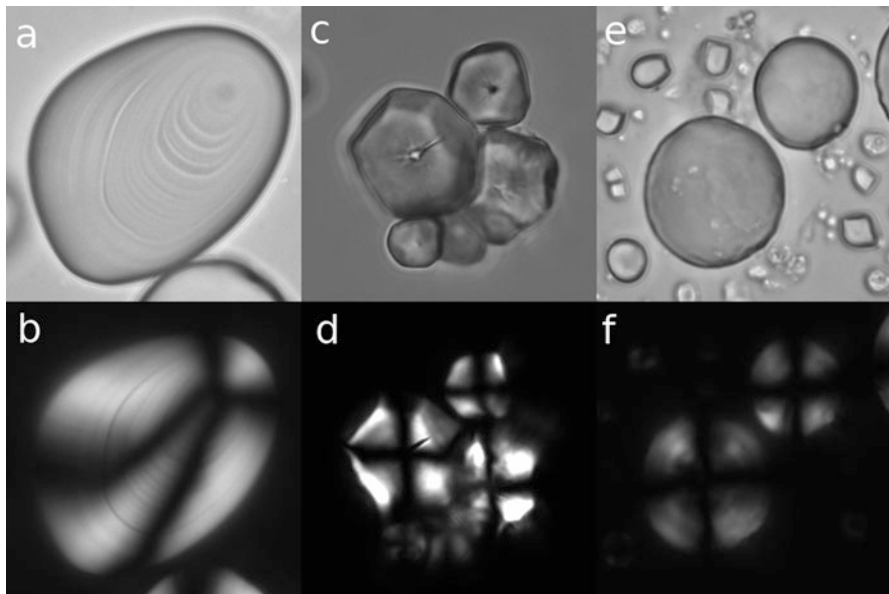
**Fig. 2** Microremains that may be confused with starch grains. All boxes 50  $\mu\text{m}$  each side. (a, b) Coccoliths from a limestone cave context. Note the twisted cross; (c, d) oil bubbles from *Glycine max* (soy beans). Note the flat appearance and poor polarization; (e, f) fibers or cell walls from soft wood. (g, h) Undecorated chrysophycean stomatocysts; (i–l) spherulites of other non-starch polymers. Note the refractive quality when viewed under single-polarized light

single pore in the outer wall. Finally, spherulites of other non-starch polymers can be observed on slides (Fig. 2i–l). These objects often have quite distinctive concentric rings when viewed under cross-polarized light, and show some degree of refractive quality when viewed under single-polarized light (note the darker areas in the “brightfield” images of these objects). They often appear in pairs or groups (as in Fig. 2k, l), and often disappear from a slide after some time. While it is currently unclear what these objects are, they are not starch.

## 5 Potential Problems in Starch Research

### 5.1 Contamination: Modern and Ancient

Contamination of ancient material by modern starch is the largest challenge facing starch research today. Starch granules are ubiquitous in modern environments. They are present in our foods, used in a large number of industrial processes, and added to a variety of products including paper, soaps, and adhesives. There are no reliable



**Fig. 3** Common contaminant starches. All boxes 50  $\mu\text{m}$  each side. (a, b) *Solanum tuberosum* (potato); (c, d) *Zea mays* (maize / corn); (e, f) *Triticum aestivum* (wheat)

visual markers that can differentiate modern and ancient starches, so once modern starch has been introduced to a sample, it is impossible to tell which starches are endemic, and which are contamination. Some of the species most interesting for archaeologists are also the most commonly found as contaminants, including wheat, potato, and maize (Fig. 3). Starch researchers must therefore use protocols that minimize the risk of contamination, test sampling and work spaces for contamination, take abundant control samples, and, importantly, publish the results of these sampling and control tests in full detail to ensure that others can fully trust their results.

Field, laboratory, and museum settings often have contaminating starches (Crowther et al. 2014), and the collection of samples should be done in a manner to minimize contamination. While it may not be possible in all situations, a strict sampling protocol that makes use of full-body covering and a positive-pressure tent may help ensure that environmental and personal starches cannot contaminate artifacts during excavation (Mercader et al. 2017). Autoclaved tools should be used whenever possible, and cleaned with a 5% sodium hydroxide solution and rinsed where not (Gismondi et al. 2018). Laboratory supplies such as soap, paper towels, gloves, foil, and plastic ware should be tested prior to use. Liquids can be mounted directly onto slides and examined. Solids can be spot-washed (as described for stone tools, above) or, if small enough, washed directly. The material is placed in a 50 ml tube with approximately 30 ml of distilled water. The tube is vigorously shaken, and the material removed using autoclaved forceps. The tube is centrifuged and the



supernatant removed, and the remaining pellet mounted and examined. The areas for the preparation of modern samples and reference material should be physically separated from those where ancient material is handled, preferably in separate rooms. The preparation and analysis areas should be cleaned regularly (once a week if possible) with water and a 5% sodium hydroxide solution (Crowther et al. 2014). Additionally, once a month the containers of mounting media (water and glycerin) should be tested, emptied, and replaced.

While the cleaning is important, it may not be sufficient to remove all of the potential contaminants. A “wipe test” performed before and after the weekly cleaning can help assess the background contamination level. A wipe test involves spraying a small amount of distilled water onto an area of known size (for example, by using a cleaned plastic frame that outlines a  $20 \times 20$  cm square) on the main sample processing areas. A kimwipe held by autoclaved forceps is then used to carefully and deeply wipe the surface. The kimwipe is then transferred to a 50 ml tube containing roughly 20 ml of distilled water and allowed to rest for 1 min. After this, the forceps are used to first swish the kimwipe around in the water and then squeeze the wipe against the walls of the tube to remove as much water as possible. The tube is centrifuged 3k rpm for 7 min, and most of the supernatant removed with a pipette, leaving roughly 50–100  $\mu$ l in the tube. The remaining volume is estimated by sucking it up into a pipette set to 150  $\mu$ l, and reducing the volume setting of the pipette until the liquid is just at the very end of the tip and no air remains. Finally, the entire volume is mixed with a pipette, and 10  $\mu$ l of the liquid is mounted and examined. Knowing the volume remaining allows the calculation of the total number of contaminants found in the  $20 \times 20$  cm square. Publications of archaeological material should also include the results of the wipe tests that occurred in the same periods in which the archaeological material was examined.

For each investigation of archaeological materials, it is important to collect control samples from the site, from the museum (when applicable), and from the laboratory. Site control samples include things like unmodified animal bones, surrounding sediments, and human cortical bone surfaces (when collecting calculus, for example, I like to do a water wash of the surface of the ascending ramus of the mandible). These materials can record both ancient and modern contamination sources, since they will be exposed to similar burial settings, and often handled by excavators, archaeologists, and curators in a manner similar to the target samples.

Many researchers in the field are working toward better methods for establishing authenticity criteria for ancient starches, including exploring the potential for molecularly identifying starches, and understanding the taphonomic pathways by which starches may be preserved (Mercader et al. 2018). This remains an area of active research.

## 5.2 *Other Issues*

While starch granules can be strong markers of the presence of certain plants within the diets of individuals or populations, there are limits to kinds of questions that starch granules can be used to answer. Starch granules are not present in all plants, and the relative abundance of starches in an archaeological sample does not directly reflect the frequency of consumption of that plant, even when controlling for variable rates of starch production within the plant (Leonard et al. 2015; Power et al. 2015b). Therefore, starches should not be used to discuss the exact number of plants in the diet, nor can they be used to discuss the relative importance of particular species. Questions of diet breadth (i.e., the number of different plants consumed) can be explored, but only when considering a large enough number of samples from a single population (Leonard et al. 2015). Finally, because starch granules can be easily removed from the archaeological record, the absence of a particular type of starch does not necessarily indicate that the plant was not consumed. Researchers must take care that their research question is one that can be answered by these microremains.

There are currently several published and online databases of starch granules, but all such databases are organized by taxon rather than by shape, in part because of the difficulty of agreeing on terms for shapes, and in part because starches from single plants often have variable morphologies. This is an area where the starch community could improve, potentially by using data-sharing platforms.

Furthermore, starch research in archaeology is still developing and there remain methodological concerns to resolve. For example, the use of heavy liquid flotation can be extremely valuable, especially for “dirty” samples that contain many non-starch particles, such as pottery crusts and sediment samples. However, recent work from the author’s laboratory has demonstrated that heavy liquid flotation can heavily bias the recovered starch assemblages, especially against partially gelatinized and damaged starch granules (Henry et al. 2016). The starch sample processing method therefore needs to be tailored to the research question and the sample type. If food processing is of interest to the researcher, then heavy liquid flotation should be avoided. Also, methods to test for and minimize the impact of contamination are not equally applied, and several authors have raised concerns with the authenticity of ancient starches. Further testing for contamination and publication of these contamination tests are needed for ancient starch research to become more solidly established.

## 6 Conclusion

While the potential problems described in this last section deserve serious consideration, if they are properly addressed, starch research can be a powerful tool for exploring questions about human food preferences and processing.

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# Ash and Dung Calcitic Micro-remains



Shira Gur-Arieh and Ruth Shahack-Gross

## 1 Introduction

Ash pseudomorphs and dung spherulites are calcitic micro-remains found in plant ashes and animal dung, respectively. In practice, plant ashes, especially from the burning of wood, appear in the archaeological record as early as 1 million years ago (Berna et al. 2012). Herbivore dung is found in large quantities in archaeological sites since the domestication of herd animals, sometime around 11,000 years ago (Vigne 2011), while burned dung remains may also be found associated with the use of dung as a fuel material. In the following chapter, we will introduce each of these micro-remains (in terms of their formation and composition) and their significance to the interpretation of the archaeological record, followed by a description of their extraction and identification under the microscope. Finally, other micro-remains with similar appearance that may be confused with ash pseudomorphs and dung spherulites as well as other possible biasing taphonomic factors will be discussed.

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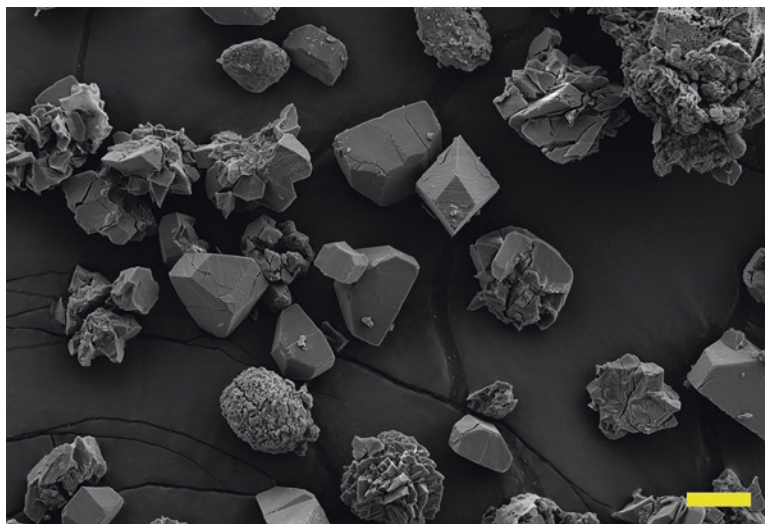
A. G. Henry (ed.), *Handbook for the Analysis of Micro-Particles in Archaeological Samples*, Interdisciplinary Contributions to Archaeology, [https://doi.org/10.1007/978-3-030-42622-4\\_6](https://doi.org/10.1007/978-3-030-42622-4_6)

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## 1.1 Formation and Composition of Ash Pseudomorphs

In general, plant ash is composed of inorganic minerals and salts that are left after most of the organic material has oxidized, typically at temperatures in excess of 400–500 °C and with sufficient oxygen. The typical color of ash is gray-white and it may also include charcoal—resulting in darker colors—where incomplete combustion of organics occurs. Ash pseudomorphs are common in the ash of dicotyledonous plants (hereafter, dicots), while siliceous phytoliths are rare. The opposite is true for monocotyledonous plants (hereafter, monocots), where phytoliths are very common and ash pseudomorphs are rare or entirely absent. Due to the presence of alkaline materials, ashes (especially those produced by the combustion of dicots) typically have a pH that ranges between 9.5 and 13 (Etiégni and Campbell 1991).

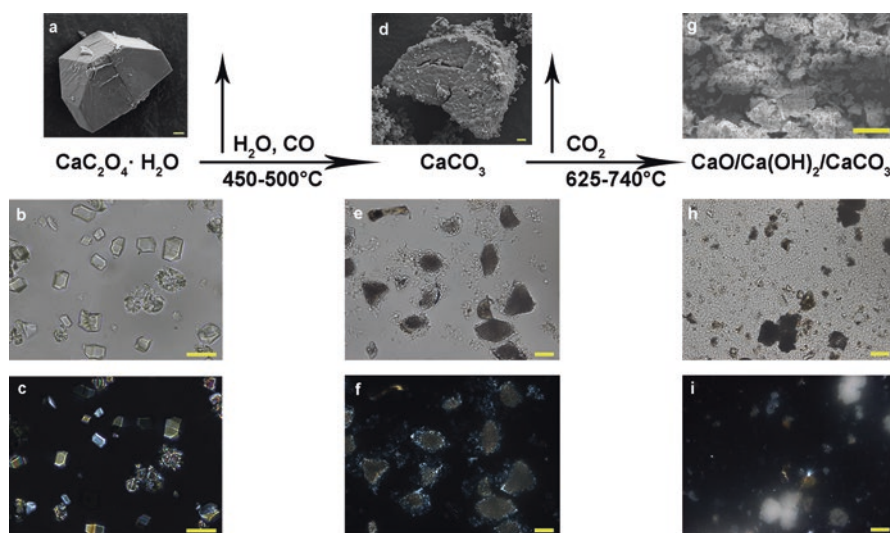
Ash pseudomorphs come from, and take the shape of, pre-existing calcium oxalate (hereafter, CaOx) crystals (Fig. 1) that are abundant bio-minerals in higher plants (Franceschi and Horner 1980). The most abundant forms of this mineral are the monohydrate form called whewellite ( $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$ ) and the dihydrate form called weddellite ( $\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ ) (Weiner 2010). CaOx crystals are found in all the main groups of photosynthesizing organisms, from angiosperms and gymnosperms to lower vascular plants and algae but are not found in all plants. They are formed from internally synthesized oxalic acid ( $\text{C}_2\text{H}_2\text{O}_4$ ) and calcium (Ca) derived from the environment. They are deposited intercellularly or extracellularly in a variety of shapes and sizes (10–50  $\mu\text{m}$ ), in most plant organs and tissues (Franceschi and Nakata 2005). There are five main morphologies of CaOx crystals in plants (Franceschi and Horner 1980, and see below), and these morphologies and their



**Fig. 1** SEM image of calcium oxalate rhombs and druses extracted from oak leaves (*Quercus* sp.). Scale = 10  $\mu\text{m}$

distribution within plants are highly variable. Some plants may produce only one crystal shape, while others produce different crystal shapes in different organs or even in the same organ. Although there is a big range of crystal shapes between species, their morphology and distribution within each species is consistent as it is highly genetically regulated (Franceschi and Nakata 2005). While crystal size may be determined by the type of cell in which it forms, calcium availability and other environmental conditions may also affect its size (Franceschi and Nakata 2005). Many hypotheses regarding CaOx crystal function in plants have been suggested over the years, of which the more accepted ones are to regulate calcium levels, to encapsulate toxic metals (aluminum, heavy metals), and as a protective mechanism against herbivory (Franceschi and Horner 1980; Franceschi and Nakata 2005).

In general, CaOx crystals rarely preserve in the archaeological record as they are rapidly recycled (Weiner 2010; Dauer and Perakis 2014). Therefore, we usually encounter their pseudomorphs which form during combustion. Thermogravimetric analysis has shown that CaOx crystals release structural water at temperatures up to 200 °C and then release carbon monoxide (CO) at about 450–500 °C, a process which alters their composition into calcite ( $\text{CaCO}_3$ ) while their original shape remains intact (Shahack-Gross and Ayalon 2013) (Fig. 2a–f). Keeping the shape while having a new chemical composition is why they are called pseudomorphs (the generic term should be “calcite pseudomorphs after calcium oxalate crystals” yet in archaeology we simply refer to them as “ash pseudomorphs”).



**Fig. 2** (a) SEM image of CaOx-twinned rhombs from oak leaves (*Quercus* sp.), Scale = 10  $\mu\text{m}$ . (b) Oak leaf CaOx crystals in PPL. Scale = 20  $\mu\text{m}$ . (c) Same as b, in XPL. (d) SEM image of ash pseudomorph of twinned rhombs burnt at 550 °C for 4 h, Scale = 10  $\mu\text{m}$ . (e) Oak leaf ash pseudomorphs burnt at 550 °C for 4 h in PPL, Scale = 20  $\mu\text{m}$ . (f) Same as e in XPL. (g) SEM image of oak ash burnt at 900 °C for 1 h, Scale = 50  $\mu\text{m}$ . (h) Oak leaves ash pseudomorphs burnt at 900 °C for 1 h in PPL, Scale = 20  $\mu\text{m}$ . (i) Same as h in XPL

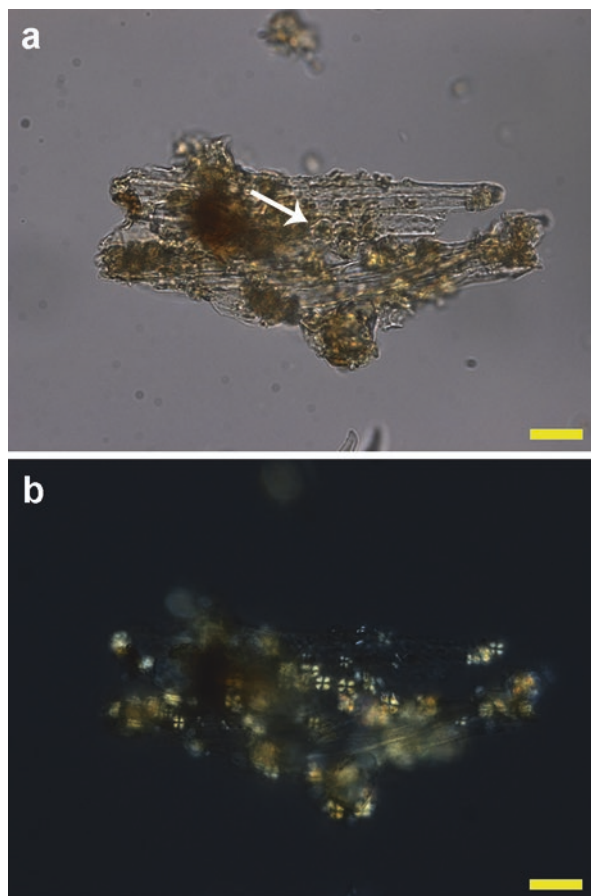
If ash forms at temperatures in excess of 500 °C, further chemical change will occur. When the combustion temperatures reach the range of 625–740 °C, CO<sub>2</sub> is released and quicklime (CaO) is formed. This material readily absorbs water and atmospheric CO<sub>2</sub> after cooling down and eventually reforms as calcite (Shahack-Gross and Ayalon 2013). At this stage, some of the pseudomorphs may lose their distinctive shape (Fig. 2g–i).

By identifying the presence of ash pseudomorphs, archaeologists can infer the burning of plant materials in antiquity. For CaOx and ash pseudomorph identification under the petrographic microscope, see section “Calcium Oxalate and Ash Pseudomorphs” below. In addition to identification under the petrographic microscope, other methods of ash characterization are in use, including (1) elemental analysis based on the dominance of calcium (Canti 2003), (2) FTIR analysis based on the grinding curve method (Regev et al. 2010), and (3) isotopic analysis based on distinctive stable carbon and oxygen signatures (Shahack-Gross and Ayalon 2013). Micromorphologically, the presence of ash pseudomorphs in association with microcharcoal and burned lumps of soil is taken as strong evidence for combustion feature identification (Mentzer 2014; Mallol et al. 2017).

## 1.2 Formation and Composition of Dung Spherulites

Dung of herbivores is composed mainly of organic material with inorganic inclusions. Depending on the animal and its diet, the inorganic components include various archaeologically important micro-remains such as siliceous phytoliths (see chapter “Phytolith Analysis in Paleoecology and Archaeology” by Cabanes, this volume) and CaOx crystals (this chapter, above) originating from the animal vegetal fodder, small amounts of geogenic minerals (e.g., quartz, calcite, clay) that are ingested with food and water, diatoms (also originating from ingested water, see chapter “Diatom Microfossils in Archaeological Settings” by Stone and Yost, this volume), and dung spherulites (Shahack-Gross 2011). Dung spherulites are radially forming microscopic (5–20 µm) spheres found in animal dung, especially ruminants (Fig. 3). They were first identified in Neolithic layers at the rock shelter *La Baume de Ronze*, in France by Brochier (1983) who later demonstrated that they are formed in the small intestine of sheep (Brochier et al. 1992). Later, Canti (1999) demonstrated that they are produced in large numbers by ruminants (sheep/goat, cow, deer), in much lower numbers by omnivores and carnivores (pig, human, badger, dog, cat), and were completely absent from the dung of caecal digesters (rabbit, hare, horse). Shahack-Gross et al. (2003) suggested that their composition upon secretion is monohydrocalcite (CaCO<sub>3</sub>·H<sub>2</sub>O), which is a relatively unstable form of carbonate, and Canti (1997) showed that they are composed mostly of calcite (CaCO<sub>3</sub>) which is covered by an outer coating of organic matter (for SEM images of dung spherulites, see Canti 1997; Canti and Nicosia 2018). For identification under the petrographic microscope, see section “Dung Spherulites.”

**Fig. 3** Phytoliths and dung spherulites from fresh cow dung from Uzbekistan, (a) PPL, (b) XPL. Scale = 20  $\mu\text{m}$ . Arrow points to the location of more visible spherulites in PPL



Upon burning in low temperatures (up to 650 °C in an oxidizing environment), the organic coating decomposes. Between 500 and 700 °C under reducing conditions, some spherulites may darken and expand (Canti and Nicosia 2018), but as they are composed of a calcareous mineral, they will decompose to CaO and lose their characteristic morphology and optical properties when exposed to temperatures above 650–700 °C (Shahack-Gross 2011).

Numerous studies have contributed to the accumulating data on spherulite concentrations in the dung of different animal species from various environments (Table 1). The quantitative published data currently available indicate that ruminants are the main spherulite producers and that there is considerable variability in spherulite production within species (e.g., in sheep dung; Table 1). Canti (1999) attempted to relate spherulite production to the animal type of digestive system (i.e., ruminant vs. omnivores, carnivores, and caecal digesters), seasonality (i.e., increased spherulite production in spring and early summer), and calcium availability in soils where animals graze, which also relates to soil pH. He demonstrated a tendency for

**Table 1** Micro-remains concentrations in modern dung reference samples from various studies

Sample description	Location	Phytolith concentration (10 <sup>6</sup> /g)	Dung spherulite concentration (10 <sup>6</sup> /g)	Ash pseudomorph concentration (10 <sup>6</sup> /g)	Source
Cow	Sivasoy, Uzbekistan	78.40	9.90	6.2	Gur-Arieh et al. (2013: 4341, Table 3) <sup>ab</sup>
Cow	Tolly, Uzbekistan	39.10	94.50	6.4	
Sheep/goat	Sivasoy, Uzbekistan	28.90	256.80	19.7	
Sheep/goat	Tolly, Uzbekistan	46.80	420.80	4.8	
Cow	Western Ghats, South India	69	1.63	0.75	Gur-Arieh et al. (submitted) <sup>ab</sup>
Sheep	Yatir forest, Israel	24.91	1.68	0	Dunseth (2013, Table 6.2) <sup>ab</sup>
Sheep/goat (free-ranging + supplements)	Uzbekistan	58.03	424.89	1.659	
Cow	Tell es-Safi/Gath, Israel	32.55	33.74	0.409	
Sheep/goat (December 2015)	Wadi Zeitan, Israel	42.70	718.83	1.239	Dunseth et al. (forthcoming) <sup>ab</sup>
		98.19			
Black dwarf goats (free-ranging, 1984)		71.54	615.78	1.672396172	Shahack-Gross and Finkelstein (2008: 976, Table 2) <sup>cd</sup>
Camel (free-ranging, 1984)	Nahal Avdat (Wadi er-Ramliyyeh), Israel	0.20	37.00	–	
Donkeys (free-ranging, 1984)	Nahal Avdat (Wadi er-Ramliyyeh), Israel	4.90	100.00	–	
Goats (spring 2005)	Nahal Avdat (Wadi er-Ramliyyeh), Israel	1.80	90.00	–	
Camels (spring 2005)	Nahal Agrab, Israel	9.60	200.00	–	
Camels (winter 2005)	Nahal Agrab, Israel	5.20	4.00	–	
Sheep (free-ranging, spring 2005)	Nahal Haroa, Israel	0.90	13.00	–	
Sheep (free-ranging + supplements, summer 2005)	Yatir, Israel	6.30	5.00	–	
	Yatir, Israel	4.80	0.30	–	
Black dwarf goats (in captivity, 2006)	Tel Aviv University Zoological Gardens, Israel	4.80	0.00	–	
Ibex (free-ranging, autumn 2006)	Avdat, Israel	3.60	62.50	–	

Cattle dung (livestock penning)	Near Althiburos/Oued el Médéina and the village of Dahmani, Tunisia	119.00	235.00	–	Portillo et al. (2012) <sup>c,d</sup>
Sheep/goat dung (fallow barley field)	Near Althiburos/Oued el Médéina and the village of Dahmani, Tunisia	129.00	541.00	–	
Sheep/goat dung (oued)	Near Althiburos/Oued el Médéina and the village of Dahmani, Tunisia	136.00	145.00	–	
Cattle dung (oued)	Near Althiburos/Oued el Médéina and the village of Dahmani, Tunisia	97.00	446.00	–	
Goat dung (road margin)	Near Althiburos/Oued el Médéina and the village of Dahmani, Tunisia	118.00	192.00	–	
Dog dung	Near Althiburos/Oued el Médéina and the village of Dahmani, Tunisia	14.00	7.00	–	
Pig dung (livestock penning)	NE Spain	47.00	6.00	–	
Sheep/goat fresh dung pellet from fallow wheat field	Seker al-Aheimar, Syria	141.00	249.00	–	Portillo et al. (2014) <sup>c,d</sup>
Cattle fresh dung pellet from fallow wheat field	Seker al-Aheimar, Syria	133.00	145.00	–	
Sheep/goat fresh dung pellet from livestock penning	Seker al-Aheimar, Syria	145.00	400.00	–	
Hen fresh dung pellet from corral	Seker al-Aheimar, Syria	12.00	48.00	–	
Dog fresh dung pellet	Seker al-Aheimar, Syria	8.00	15.00	–	
Mice fresh dung pellet from open-air storage area	Seker al-Aheimar, Syria	1.40	0.00	–	
Sheep/goat fresh dung from livestock penning	Althiburos, Tunisia	135.90	529.00	0.5	Portillo et al. (2017) <sup>c,d</sup>
Cattle ( <i>n</i> = 33)	England	–	101	–	Canti (1999) <sup>d</sup>
Goat ( <i>n</i> = 79)	England	–	105	–	
Deer ( <i>n</i> = 18)	England	–	73	–	
Hare/rabbit ( <i>n</i> = 9)	England	–	0	–	

(continued)



**Table 1** (Continued)

Sample description	Location	Phytolith concentration (106/g)	Dung spherulite concentration (106/g)	Ash pseudomorph concentration (106/g)	Source
Horse ( <i>n</i> = 9)	England	–	0	–	
Pig ( <i>n</i> = 7)	England	–	12.5	–	
Badger ( <i>n</i> = 10)	England	–	0.63	–	
Bird ( <i>n</i> = 8)	England	–	7.3	–	
Ape + human ( <i>n</i> = 9)	England	–	12.5	–	
Fox ( <i>n</i> = 6)	England	–	4	–	
Dog ( <i>n</i> = 11)	England	–	2	–	
Cat ( <i>n</i> = 11)	England	–	0	–	
Goat/sheep 1	Bestansur, Iraqi Kurdistan	47	1194	–	Eliot et al. (2014) <sup>e</sup>
Goat/sheep 2	Bestansur, Iraqi Kurdistan	59	2210	–	
Goat/sheep 3	Bestansur, Iraqi Kurdistan	69	962	–	
Goat/sheep 4	Bestansur, Iraqi Kurdistan	69	1456	–	
Goat/sheep 5	Bestansur, Iraqi Kurdistan	63	1554	–	
Goat/sheep household 1	Bestansur, Iraqi Kurdistan	48	4	–	
Cow dung house 2	Bestansur, Iraqi Kurdistan	96	0	–	
Cow dung house 2	Bestansur, Iraqi Kurdistan	108	0	–	
Cow dung house 1	Bestansur, Iraqi Kurdistan	99	2	–	
Cow dung house 1	Bestansur, Iraqi Kurdistan	51	0	–	
Cow dung house 1	Bestansur, Iraqi Kurdistan	95	1	–	

There is a difference in extraction and quantification methods between the different studies which makes an absolute comparison impossible

<sup>a</sup>Quantification of phytoliths concentrations following Katz et al. (2010)

<sup>b</sup>Quantification of dung spherulites and ash pseudomorphs concentrations following Gur-Arieh et al. (2013)

<sup>c</sup>Quantification of phytoliths concentrations following Albert et al. (1999)

<sup>d</sup>Quantification of dung spherulites concentrations following Canti (1999)

<sup>e</sup>Total numbers of spherulites and phytoliths counted in 20 fields of view at 400× magnification

positive correlation between spherulite production and grazing on alkaline soils, but exceptions for this pattern were noted (i.e., spherulites were absent from certain dung samples collected from areas with alkaline soils, and on the other hand, they were present in samples collected from areas where animals grazed on acidic soils). Along this line, low concentrations of dung spherulites in cow dung from India were explained as relating to animal diet and the local acidic sediments (Lancelotti and Madella 2012; Gur-Arieh et al. 2018). It has also been suggested that dung spherulites are produced in relation to intestinal microbiome activity (Shahack-Gross 2011; Canti and Brochier 2017). A systematic study on the controlling factors in spherulites production is still needed.

By identifying the presence of dung spherulites, archaeologists can infer the presence of dung remains at archaeological sites. In addition to identification under the petrographic microscope, a recent observation indicates that sediments enriched in dung spherulites may be identified using the calcite grinding curve method (Dunseth and Shahack-Gross 2018). In addition, for the general identification of dung, the enrichment in phosphorus and in  $^{15}\text{N}$  has been reported and is in practical use (review in Shahack-Gross 2011).

## 2 Archaeological Significance

### 2.1 Ash Pseudomorphs

As mentioned above, CaOx crystals are rarely preserved in the archaeological record. Moreover, their limited morphological variability does not allow precise taxonomic identification (Prychid and Rudall 1999). However, when combined with other markers, or when used to explore specific questions, they can be useful markers of some plant taxa. They sometimes preserve within charred woody tissues (Mallol et al. 2017). Danielson and Reinhard (1998) and Reinhard and Danielson (2005) analyzed coprolites of prehistoric hunter-gatherer and horticultural groups who lived in the southwestern deserts of North America and proposed that CaOx crystals in the coprolites originated from staple plant foods of these communities, such as prickly pear and agave. Zhang et al. (2014) used CaOx druse shapes and sizes together with other markers to differentiate modern tea plants (*Camellia sinensis* L.) from other plants in the tea family (Theaceae) and common non-Theaceae plants. Building on their study, Lu et al. (2016) used CaOx crystals together with chemical markers for the identification of decayed tea leaves found in two burial sites in Xi'an, Sha'anxi Province, China and Ngari district, western Tibet, dated to 2100 BP and 1800 BP, respectively.

An important question in any study of CaOx from archaeological contexts is whether the find is a rare well-preserved occurrence or a post-depositional intrusion (see chapter “Starch Granules as Markers of Diet and Behavior” by Henry and “Pollen and Archaeology” by Bakels, this volume, with similar concerns). While

CaOx crystals have been reported from ancient sediments, such as those associated with Acheulian stone tools at Olduvai Gorge (Dominguez-Rodrigo et al. 2001), there remain concerns that such open contexts may be affected by contamination. Preservation of intact archaeological CaOx crystals is more likely but still not assured in complete artifacts (e.g., coprolites) or sealed environments (such as dental calculus). Researchers must take care to assess potential sources of contamination by taking control samples. Direct radiocarbon dating of such CaOx finds may allow distinguishing between original and post-depositional micro-remains.

In practice, it is more common to encounter in the archaeological record the pseudomorphs of calcite after CaOx in the form of ashed plant matter. Wood ash (and in later periods also dung ash) is an important source of information regarding human use of fire, which has long been identified as a major milestone in human evolution. Roebroeks and Villa (2011) discuss evidence for use of fire along prehistory and address the debate over the earliest use of fire vs. control of fire. The presence of ash in Lower Paleolithic sites is scarce. Berna et al. (2012) identified ash pseudomorphs and burnt bones in micromorphological thin sections from an early Acheulean occupation dated to approximately 1.0 million years ago at Wonderwerk Cave in South Africa. Karkanas et al. (2007) and Shahack-Gross et al. (2014) demonstrated the habitual use of fire in the late Lower Paleolithic Qesem Cave in Israel (420–200 kya). By the Middle Paleolithic, use of fire is widespread and numerous hearths have been identified. These provide information on spatial organization and social practices (e.g., Aldeias et al. 2012; Courty et al. 2012; Goldberg et al. 2009; Karkanas and Goldberg 2010; Macphail and Goldberg 2000; Mallol et al. 2010; Meignen et al. 2007). For more sites, see Mentzer 2014, Table 3).

In later periods, such as the Upper Paleolithic, Mesolithic, and Neolithic, there is less focus on the identification of ash, as it is accepted that the habitual use of fire was well established (Roebroeks and Villa 2011); rather, discussions about human behavior in relation to hearths are common (Milek 2012; Out 2012).

Apart from the utility of ash to address issues concerning fundamental questions related to early use of fire and hominin site use and spatial organization, ash is an important artifact that is associated with all types of pyro-technology—cooking, plasters and mortars, pottery, metal, and glass production—all requiring fuel. Therefore, the identification of fuel sources in hearths, ovens, kilns, and furnaces can provide an important insight into the choice of fuel by humans and by extension to human–environment interactions. For example, Gur-Arieh et al. (2014) and Eliyahu-Behar et al. (2017) identified the use of both wood and dung fuels in Iron Age and Early Bronze Age cooking installations in Israel, respectively. Kahlenberg and Shahack-Gross (2016) identified wood to be a major fuel used in the hearths of the lower city of Late Helladic IIIC Tiryns.

The ability to detect changes in the choice of wood vs. dung in the archaeological record is possible when the ratio of ash pseudomorphs and dung spherulites is quantified (Gur-Arieh et al. 2013). Dung spherulites as an indicator for the use of dung

as fuel are found in many sites from the Early Neolithic Period onwards (e.g., Stiner et al. 2014; Gur-Arieh et al. 2014; Portillo et al. 2014). Other sources of fuel, such as peat or coal, are known from Europe (Branigan et al. 2015; Deforce et al. 2007; Théry et al. 1996). Experimental work on modern peat ashes showed variable results regarding the presence of ash pseudomorphs. While Braadbaart et al. (2012) identified their presence in peat from the Netherlands, they were not identified in peat from Iceland (Simpson et al. 2003).

The choice of fuel type may not only indicate environmental availability but may also reflect technological need (i.e., high temperature, steady combustion) or cultural preferences (e.g., the preference of wood over dung by Maasai herders despite the ubiquity of the latter; Shahack-Gross et al. 2004) and taboos (e.g., the avoidance of Fang villagers in Equatorial Guinea from using specific tree species; Picornell Gelabert et al., 2011). An example of the complex interaction between fuel availability, technological needs, and cultural preference is Sillar's (2000) ethnographic study of household pottery production in the Peruvian and Bolivian Andean highlands. He showed how in this wood depleted environment, the local population relies on the use of cow, sheep, and llama dung for a variety of domestic (cooking), agricultural (manuring) as well as industrial (pottery firing) purposes. Sillar (2000) showed that using dung for pottery firing took precedence over fueling for other tasks. Explanations given to this strong preference of dung as fuel for pottery firing included the steady heat produced by dung, its porosity that allowed airflow to maintain oxidizing conditions during pottery firing, and the fact that the dung ashes created an isolating layer on top of the fire, preventing heat loss.

Except for ash being a product of burning activities, this material is also used secondarily. One known use of ash is for floor maintenance (Milek 2012). Ash (and lime) has been used to sanitize latrines and cesspits (Baeten et al. 2012). Ash is used as a fertilizer in agricultural fields (Portillo et al. 2017). Ash was also used as an alkali flux in glass production lowering the quartz melting point (e.g., Tanimoto and Rehren 2008; Babalola et al. 2018).

Lastly, ash and its mineralogical transformations in cave sites serve as indicators for site formation processes and diagenesis. Weiner (2010) explains the chain of formation of phosphate minerals in association with wood ash in prehistoric caves such as Kebara and Hayonim, Israel. In these examples, under acidic conditions related to degradation of bat guano, ash reacts with phosphate and transforms mineralogically. This process results in the loss of the characteristic ash pseudomorphs, or the complete dissolution of ash. Shahack-Gross et al. (2008a) showed that ash pseudomorphs remain well-preserved under dry sheltered conditions at Amud Cave (Israel) while localities affected by alkaline hydraulic systems undergo dissolution and re-precipitation of calcite resulting in partial to complete loss of ash pseudomorphs (also at Qesem Cave, Karkanas et al. 2007). Overall, the presence/absence of ash and state of preservation of ash pseudomorphs are largely dictated by the burial conditions.

## 2.2 *Dung Spherulites*

Dung spherulites are the strongest stand-alone marker for the presence of dung in the archaeological record. Other markers, such as elevated phosphate concentrations or elevated  $^{15}\text{N}$  values, cannot stand alone and must be accompanied by a suite of other indicators to supply unequivocal identification of dung. Coprophilous fungal spores are also a useful dung marker, though they are airborne and some genera do not grow exclusively on dung (Morandi 2018; van Geel et al. 2003; also see chapter “Non-pollen Palynomorphs” by Shumilovskikh and van Geel, this volume). Once dung has been identified at a site, the context of the find may reveal primarily socioeconomic and other types of information. First and foremost, it is a marker for animal penning (see criteria in Shahack-Gross 2017). The identification of pens within archaeological sites informs on site structure and organization of space. The presence of pens on site indicates a pastoral or agro-pastoral subsistence base, while the absence of pens indicates economy based on hunting and gathering or an urban consumer society. Determining the presence of pens in sites dating to the beginning of the Neolithic or just before will provide interesting information on the domestication process of livestock and Neolithization (Shahack-Gross 2011 and references therein). The use of dung as fuel is one of the markers for the utilization of secondary products. The debate between Miller (1996, 1997) and Hillman et al. (1997) regarding whether seeds identified in Neolithic deposits originate from dung used as fuel or signify remains of human diet has not yet been resolved. The presence or absence of dung spherulites in such debated contexts may crucially supply a solution.

Dung spherulites in livestock enclosures, especially ruminant enclosures, have been identified in sites in South-West Asia (e.g., Albert and Henry 2004; Matthews 2005; Shahack-Gross et al. 2005; Shahack-Gross and Finkelstein 2008; Dunseth et al. 2016, 2018), Africa (di Lernia 2001; Boles and Lane 2016), Europe (e.g., Brochier et al. 1992; Égüez et al. 2016; Koromila et al. 2018; Macphail et al. 1997; Polo-Díaz et al. 2016), and South America (Korstanje and Cuenya 2010). In addition to its importance as fuel source and manure, dung was, and still today is, used for construction. Dung spherulites have been identified in various remains of mud bricks (Love 2012), plaster floors (Karkanas 2006; Portillo et al. 2014), and mud constructed feature walls (Macphail et al. 2007; Viklund et al. 2013; Stiner et al. 2014), indicating its use as a construction material from the Early Neolithic.

Dung spherulites, forming within the intestines, have a carbon isotopic composition that seems to reflect that of the ingested type of vegetation (i.e., C3 or C4; Shahack-Gross et al. 2008b). Therefore, they may serve as indirect proxies for environmental changes or changes in grazing habits of livestock. Direct measurement of their chemical and isotopic compositions, if further developed, may be useful for radiocarbon dating and environmental reconstructions.

Lastly, dung spherulites are extremely sensitive to humidity and dissolve quite rapidly by rain. Their presence must therefore indicate a rather good state of preservation, from the mineralogical point of view, at a site.

### 3 Practical Analysis

This section will focus on the practical parts of the analysis of ash pseudomorphs and dung spherulites through optical microscopy. We first present the method used to recover these calcitic micro-remains from ashes and sediments/soils and then describe how they are identified using a polarized light microscope.

#### 3.1 Recovery

To determine the presence/absence of ash pseudomorphs and dung spherulites, the simplest procedure is preparing a grain mount by mixing about 1 mg of sediment with a mounting medium such as Entellan new (Merck) which has a refractive index of 1.490–1.500. This procedure is easy to conduct on-site (requires a petrographic microscope on-site) and is useful for providing a first approximation of the type of sediment excavated.

Determination of the absolute concentration of micro-remains provides more insight into the archaeological sediments and site. Until recently, most methods quantified only spherulites but not ash pseudomorphs and did not provide a clear means to compare quantities within or between sites. Instead, they provided estimations of the percentage of area taken by spherulites (Canti 1997) or color measurement on thin sections using image analysis software (Simpson et al. 2003). A recently developed method (Gur-Arieh et al. 2013) provides a means to both (1) conduct inter- and intra-site comparisons and (2) determine the relative quantities of wood ashes and dung ashes where they were used together. Furthermore, using this method provides absolute counts of both spherulites and ash pseudomorphs in one procedure, using a low-viscosity liquid (sodium polytungstate) that allows the researcher to evenly spread the sample under the coverslip, promoting the precision of the method. To carry out this procedure, one should have available an analytical scale, ultrasonic bath, and automatic pipettes. It is therefore often carried out in the laboratory, rather than on-site. This method has been so far used in various archaeological contexts worldwide (Friesem et al. 2014; Alonso-Eguíluz et al. 2017; Eliyahu-Behar et al. 2017; Gur-Arieh et al. 2014; Polo-Díaz et al. 2016; Dunseth et al. 2018).

The method comprises the following steps:

1. Lightly homogenize the sediments according to need using a pestle and mortar. Do not grind forcefully as this may result in the breakage of micro-remains.
2. Weigh between 10 mg (of ash) and 50 mg (of archaeological sediment).
3. Sieve the sediments through a 150- $\mu$ m mesh to remove large particles. If there are larger sediment aggregates, it is possible to lightly disaggregate them using the edge of a spatula.
4. Place the sieved sediments in a 0.5-ml Eppendorf tube and add 500  $\mu$ l sodium polytungstate (SPT) solution with a 2.4 g/l density.

5. Vortex the sample and sonicate for 10 min to further disaggregation.
6. After sonication, vortex each sample for a few seconds to ensure that the sediment is homogenously suspended in the SPT solution and immediately draw out from the center of the tube 50  $\mu$ l using a pipette.
7. Place the drawn-out solution on a microscope slide and cover with a 24  $\times$  24 mm coverslip.

The sample is ready for counting. Use a polarizing light microscope at  $\times 400$  magnification. Count the micro-remains in 10–40 randomly distributed fields of view (depending on the density of micro-remains in the sample). Use plane-polarized light (PPL) to identify and count ash pseudomorphs and cross-polarized light (XPL) to identify and count dung spherulites in the same field of view. Notice that Gur-Arieh et al. (2013) counted ten fields and provided an analytical error of ca. 30%. This large error means that significant differences among samples should be sought in order of magnitude (i.e., a sample containing 30,000 dung spherulites is significantly different from a sample containing 300,000 dung spherulites but cannot be statistically differentiated from a sample containing, e.g., 80,000 dung spherulites). The more fields of view counted the lower analytical error expected. The re-calculated analytical error should be sought by preparing and counting the same sample at least three times, see Gur-Arieh et al. (2013) for more details.

Although it was shown by Gur-Arieh et al. (2013) that within an hour there is no visible change to the calcitic micro-remains in SPT, over time they are likely to dissolve due to the acidity of the SPT solution. In addition, SPT slowly crystallizes as the sample dries out. Therefore, it is recommended to complete the quantification within 1 h of sample preparation.

To calculate the concentration of each micro-remain, the following formula is used:

$$\frac{\Sigma \text{ of microremains counted}}{\text{Area counted (no. of field counted} \times \text{Area of 1 field of view)}} \times \text{Area of the slide} = \text{no. of microremains on the slide (50 } \mu\text{l)} = A \quad (1)$$

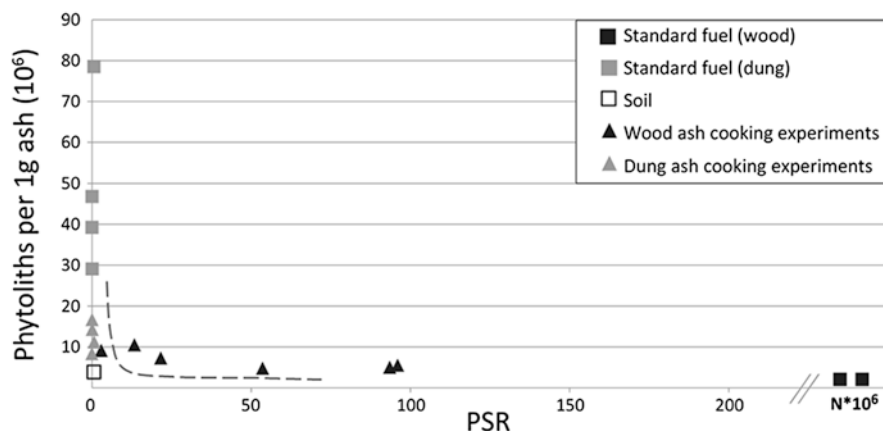
$$\frac{10A = \text{no. of microremains in all the solution (500 } \mu\text{l)}}{\text{Initial sample weight (mg)}} \times 1000 \quad (2)$$

$= \text{no. of microremains in 1 gr sediment}$

When in part (1), the area of the slide ( $\text{mm}^2$ ) equals 24 mm  $\times$  24 mm = 576.

When interested in looking into the concentrations of calcitic micro-remains and phytoliths (see this volume chapter “Phytolith Analysis in Paleoecology and Archaeology” by Cabanes), Gur-Arieh et al. (2013) proposed calculating a value nicknamed pseudomorphs to spherulites ratio (PSR). This ratio can help identify the fuel source. The ratio is below 1 in dung ash due to the abundance of dung spherulites and in the range of hundreds of millions (when arbitrarily considering the presence of 1 dung spherulite) in wood ash due to the abundance of ash pseudomorphs





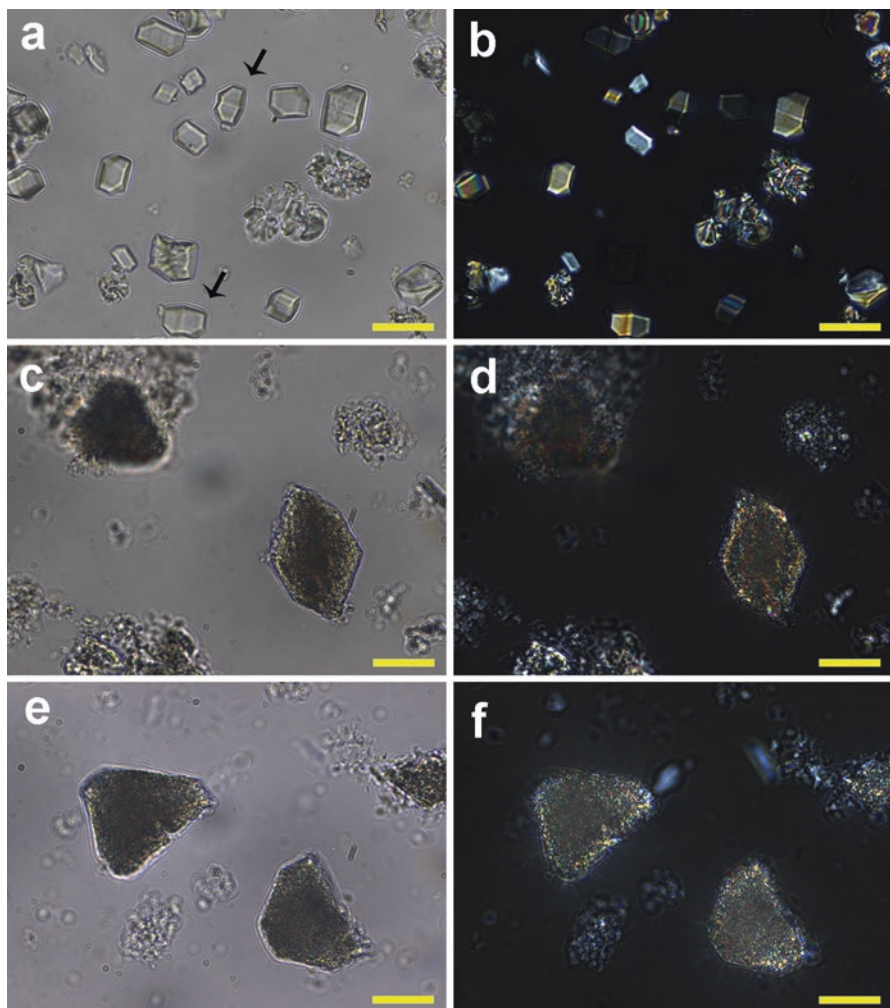
**Fig. 4** Chart showing the relationship between PSR and phytolith concentrations in dung and wood ash standards produces experimentally in a muffle furnace, and ethnographic samples collected from hearths and ovens fuelled with dung or wood in Uzbekistan (based on data from Gur-Arieh et al. 2013). The dashed line represents a mixing curve of the fuel standards

and absence of dung spherulites. As dung produced by livestock grazing on grasses includes high concentrations of phytoliths, and wood ash is composed of low concentrations of phytoliths, a distinct pattern is obtained by plotting PSR vs. phytolith concentration values (Fig. 4). Furthermore, this scheme allows one to identify cases where both wood and dung have been used as fuel, with a PSR value above 5 for wood dominated ash and below 1 for dung dominated ash. Gur-Arieh et al. (2014) have demonstrated that the range between 1 and 5 PSR values represents either partially dissolved dung dominated ash or a mixture which is not dominated by either wood or dung ashes. Gur-Arieh et al. (2014) and Eliyahu-Behar et al. (2017) used this method to demonstrate the alternating use of both wood and dung in Bronze and Iron Age cooking installations (hearths and ovens) in Israel, while Dunseth et al. (2016, 2018) used it to identify mixed fuel use at the Intermediate Bronze Age site of Mashabe Sade and the Early and Intermediate Bronze Age phases of the site of Nahal Boker 66 in Israel.

### 3.2 Visual Identification

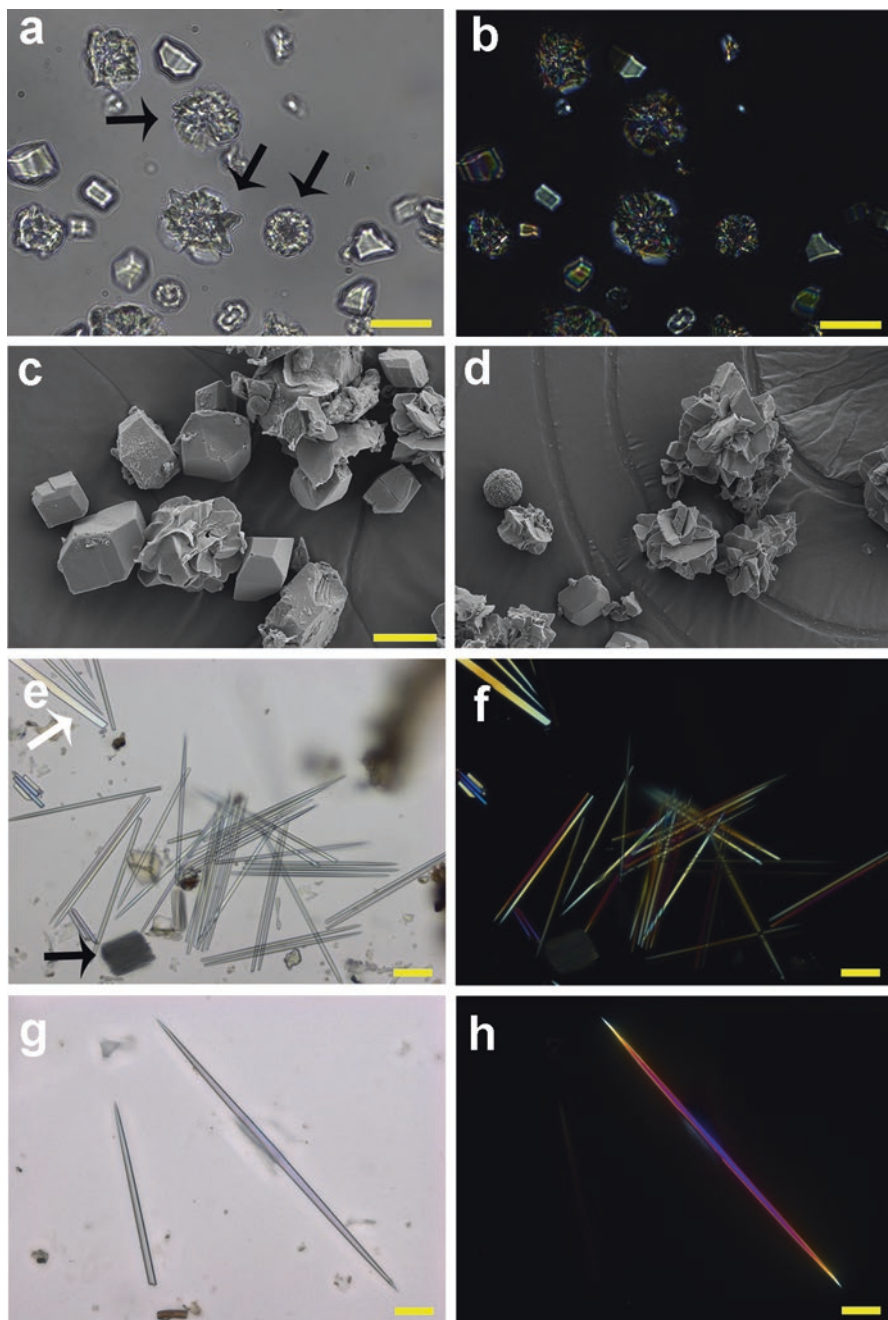
#### Calcium Oxalate and Ash Pseudomorphs

CaOx crystals range in size from 10 to 50  $\mu\text{m}$  and are euhedral with smooth faces (Canti 2003; Shahack-Gross and Ayalon 2013). They are transparent under plane-polarized light (PPL), while under crossed-polarized light (XPL) they show interference colors similar to those of calcite, i.e., high order pastel pink, green, and creamy white (Durand et al. 2010). Calcium oxalates appear in five main



**Fig. 5** (a) Oak leaf calcium oxalate rhombs and twinned rhombs (arrows) under PPL, Scale = 20  $\mu\text{m}$ . (b) Same as **a** under XPL. (c) Oak leaf ash pseudomorph rhomb under PPL, Scale = 20  $\mu\text{m}$ . (d) Same as **c** under XPL. (e) Oak leaf ash pseudomorph twinned rhomb under PPL, Scale = 20  $\mu\text{m}$  (f) Same as **e** under XPL

morphologies; single or twinned rhombohedral or prismatic crystals (Fig. 5), druses which are radial aggregates of prismatic crystals (Fig. 6a–d), raphides which are bundles of needle-shaped crystals (Fig. 6e–h), styloids which are elongated crystals with pointed ends (Fig. 6e–f) and crystal sand which is composed of small angular crystals (Franceschi and Horner 1980, Fig. 12; Durand et al. 2010). The wood of most trees and shrubs, and the leaves of most deciduous trees contain mainly druses and prismatic crystals (Durand et al. 2010), while monocot plants contain mostly raphides as well as styloids and druses (Prychid and Rudall 1999). Although CaOx



**Fig. 6** (a) Oak leaf calcium oxalate druses (arrows) under PPL, Scale = 20  $\mu$ m. (b) Same as a under XPL. (c) SEM image of calcium oxalate druse extracted from oak leaves, Scale = 10  $\mu$ m. (d) SEM image of calcium oxalate druse extracted from oak leaves, Scale = 20  $\mu$ m. (e) Calcium oxalate raphides and styloid (white arrow) extracted from a *Polygonatum multiflorum* rhizome, under PPL. At the bottom, a bundle of raphides is visible (black arrow), Scale = 20  $\mu$ m. (f) Same as e under XPL. (Photographs courtesy of A.G. Henry). (g) Calcium oxalate raphides extracted from a *Polygonatum multiflorum* rhizome, under PPL, Scale = 20  $\mu$ m. (h) Same as g under XPL. (Photographs courtesy of A.G. Henry)

crystals appear in many herbaceous monocots, they are normally absent from the Poaceae family except for taxa in the genus *Panicum* (for a full list of CaOx crystal occurrence in monocot plant families see Prychid and Rudall 1999).

When burnt at low temperatures (ca. 400–600 °C), the general rhombohedral (or other) shape of the single CaOx crystal is retained; however, it is now composed of many nanometer-sized fused crystallites of calcite (Canti 2003; Shahack-Gross and Ayalon 2013). This change in the mineral composition and size of the crystals affects the optical properties of the ash pseudomorph. While the unburned CaOx crystal is transparent, its pseudomorph is dark gray under PPL, which is typical of micritic calcite. Under XPL, they are dark gray-brown, spotted with occasional second-order yellow-red-blue interference colors. Due to the varied orientation of the calcite crystallites, a full ash pseudomorph never goes completely extinct but rather “twinkles” as various crystallites extinct in different rotation angles.

After exposure to high temperatures (>700 °C), most ash pseudomorphs disintegrate into small particles, and the few surviving pseudomorphs that still retain their original form lose the smooth sharp appearance of their edges. In PPL, they are transparent to light brown (Fig. 2h), and under XPL, they are gray with white specks and have a fuzzy appearance (Fig. 2i and Shahack-Gross and Ayalon 2013).

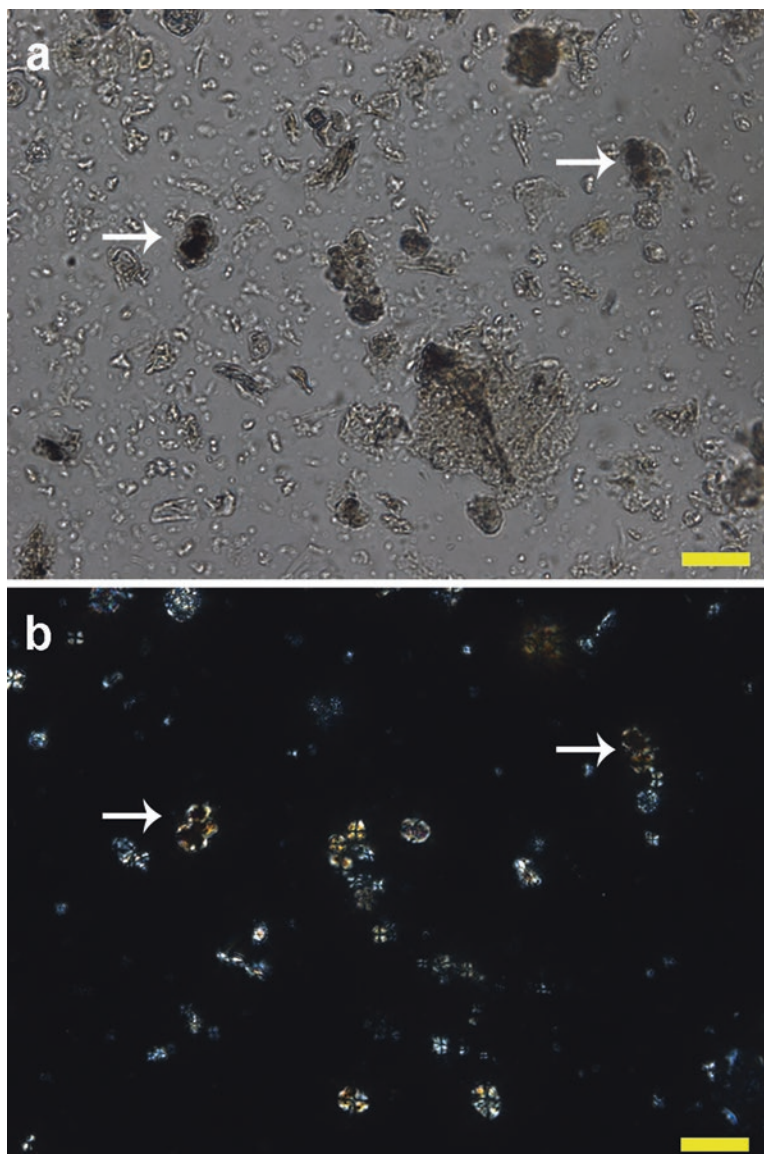
Ash pseudomorphs may be confused, under the petrographic microscope, with geogenic micritic calcite such as chalk or pedogenic nodules. For that reason, one should be aware of the lithology in the vicinity of the studied archaeological site and sample regional control rocks and sediments/soils for comparison. Complementary methods such as FTIR spectroscopy (Regev et al. 2010; Poduska et al. 2011) and stable isotope analysis (Shahack-Gross et al. 2008a; Shahack-Gross and Ayalon 2013) can be used to obtain more secure identification of calcite origins at archaeological contexts.

Although it seems that CaOx shapes can be useful for the identification of certain genera such as *Pinus*, *Tilia*, and *Camellia* (Brochier 1991, 1995; Zhang et al. 2014; Canti and Brochier 2017), one should consider the possibility in which the remains of several plant species were mixed together, such as in combustion features. More systematic work on CaOx crystal shapes is still required to evaluate their full potential for archaeological research.

## Dung Spherulites

Dung spherulites range in size between 5 and 20 µm. Under PPL, they are hard to detect. Under XPL, they usually have low-order interference colors with small spherulites exhibiting gray to white interference colors and larger spherulites exhibiting interference colors of up to second-order green. One of their most prominent characteristics is their fixed cross of extinction. Upon stage rotation, the cross arms oriented on the north-south east-west stay in position (Canti 1997, 1998; Canti and Brochier 2017). This feature is due to their radial structure, caused by acicular crystallites surrounding a nucleus (Canti 1997).





**Fig. 7** Ash produced from modern goat dung burnt at 500 °C for 4 h in a muffle furnace. (a) PPL. Arrows point to darkened spherulites. Notice the distorted shape of the spherulite on the left, (b) XPL, Scale = 20  $\mu\text{m}$

Canti and Nicosia (2018) noticed that when burned at temperatures between 500 and 700 °C, especially under anoxic conditions, some spherulites may darken (partially or almost completely) and thus become visible also under PPL as dark brown to black round bodies (Fig. 7a). They also noticed that some of the spherulites may

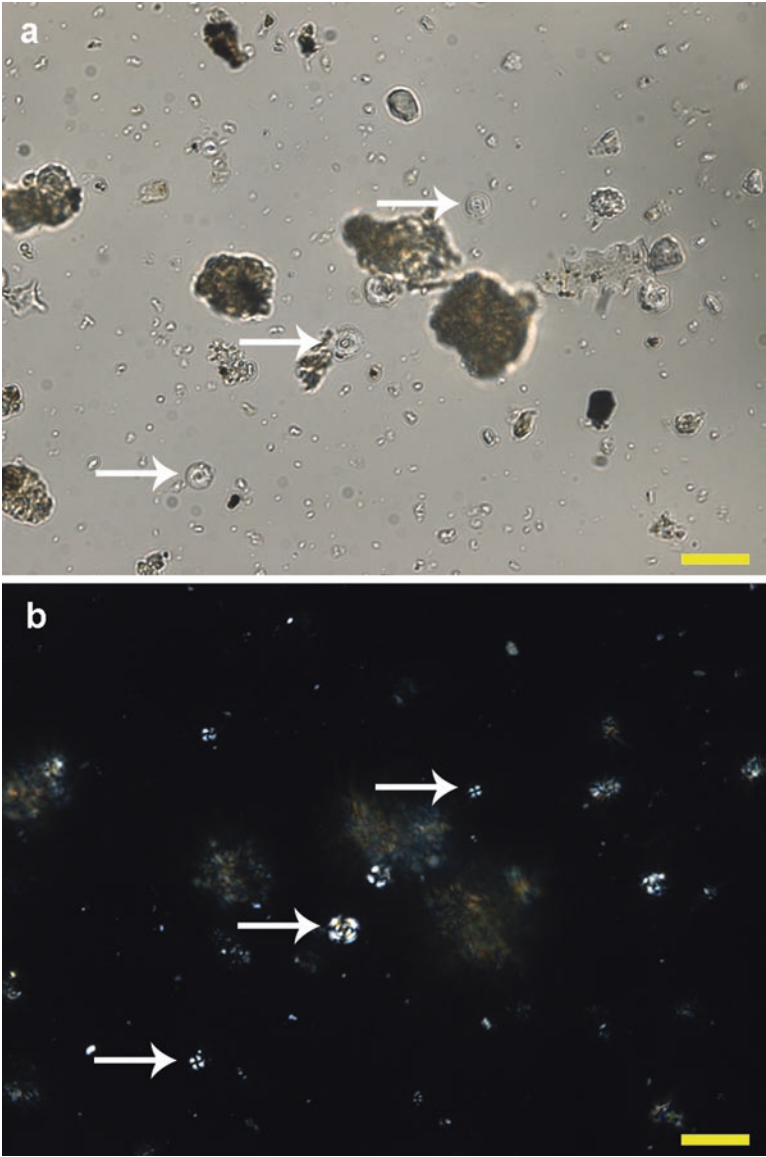
expand and reach up to 25  $\mu\text{m}$  in diameter and some may lose their round shape and distort to a lobe-shaped body. They suggested that the darkening might be partially due to the charring of organic matter that was incorporated in the spherulites matrix, or due to the creation of a compound relief resulting from crystal separation (somewhat similar to the darkening of  $\text{CaOx}$  crystals upon combustion). Under XPL, the darkened spherulites remain opaque but the interference colors of the spherulite can be seen beyond the darkened area (Fig. 7b). The spherulite cross of extinction is no longer visible in darkened spherulites.

While dung spherulites are very indicative once one learns to recognize them, they can be confused with other microscopic spherulites, especially coccoliths from chalk where archaeological sites are located on a chalk lithology and certain starch granules. Additionally, they may be mistaken for spherulites of gypsum which form by hydration of anhydrite, such as where the gypsic ash of tamarisk wood occurs (Shahack-Gross and Finkelstein 2008).

### Look-Alikes

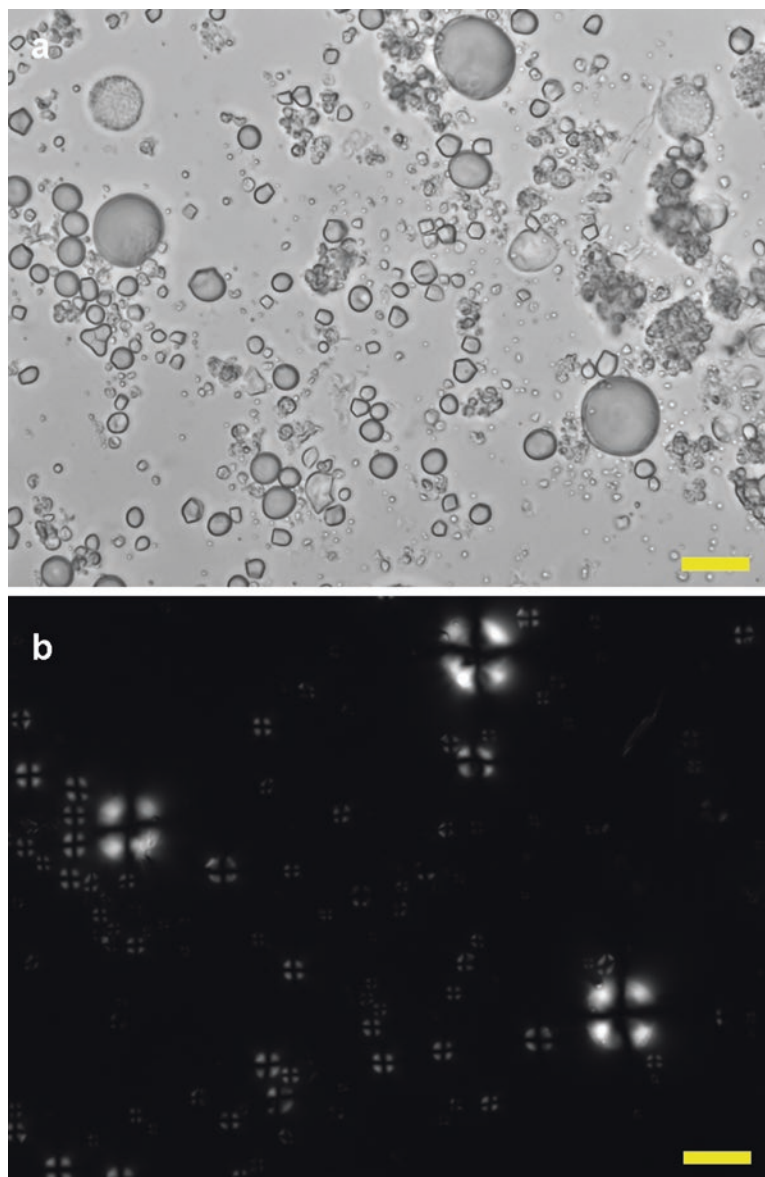
Coccoliths are the individual plates of unicellular planktonic algae (Coccolithophores), common in saline seas and are a major component of chalk and marl (for more detailed information, see chapter “Coccoliths and Other Marine Microfossils in Microparticle Analysis” by Young in this volume). They are composed of calcite, come in a wide diversity of forms (Cros and Fortuño 2002), and they generally range in size between 1 and 15  $\mu\text{m}$  (Tyrrell and Young 2009). Some coccoliths are composed of radial acicular calcite and therefore exhibit under XPL a cross of extinction that can be confused with dung spherulites (Fig. 8). Yet, the arms of the cross of coccoliths are inclined at both ends, unlike the straight arms of the cross of extinction of dung spherulites. In addition, upon stage rotation, the cross arms of coccoliths distort, as opposed to the cross of dung spherulites which stay unchanged on rotation. In PPL, they are usually transparent, ring-shaped, and sometimes exhibit a light greenish color. Their interference colors in XPL are first-order white (Fig. 8b). Therefore, to differentiate between dung spherulites and coccoliths, one should check whether the arms of the cross are straight or inclined, if they distort or stay fixed when the stage is rotated, and whether the micro-remain is visible and has a ring shape. Familiarity with the site sediments and rock formation will also help to determine the possible occurrence of coccoliths in the area, and therefore, off-site control samples are essential.

Starch granules are the energy storage units of plants. They are an important dietary component for humans and herbivores and can thus sometimes be found in the same context as dung spherulites, either in kitchens or in animal enclosures. While starch granules have a variety of morphologies depending on the plant origin (for a full description, see chapter “Starch granules as markers of diet and behavior” by Henry, this volume), some starch granules can be confused with dung spherulites as they also have a dark cross of extinction with straight arms (Maltese cross) (Fig. 9). However, opposed to dung spherulites, the cross arms of starch granules



**Fig. 8** Micrographs of coccoliths from the Eocene chalk bedrock where Tell es-Safi/Gath, Israel is located. **(a)** PPL. Arrows point to coccoliths. **(b)** XPL, Scale = 20  $\mu$ m





**Fig. 9** Micrograph of *Triticum* sp. (wheat) starch, (a) PPL. (b) XPL, Scale = 20  $\mu\text{m}$ . (Photographs courtesy of A.G. Henry)

distort upon stage rotation. In addition, starch granules have first-order white interference colors while dung spherulites also exhibit yellow, orange, and second-order green interference colors and are rarely all white.

## 4 Preservation Potential and Biasing Factors

The preservation potential of CaOx is generally low due to the organic nature of the oxalate molecule and thus palatability to the soil microbiome. Ash pseudomorphs and dung spherulites on the other hand are composed of calcite, an inorganic mineral, and therefore have a good potential for preservation in alkaline archaeological sediments. Still, their preservation depends on various taphonomic factors, including the type of site (open vs. sheltered), water availability (moist vs. dry deposits and environments), rapidity of burial and intrinsic differential preservation related to the ratio between micro-remain surface area and bulk volume.

Both ash pseudomorphs and dung spherulites are reported to be found in abundance in sheltered sites, i.e., caves and rockshelters (e.g., Brochier et al. 1992; Macphail et al. 1997; Weiner 2010). As mentioned above, calcitic ash either transforms into phosphate compounds or dissolves completely in caves inhabited by bats where acidic conditions develop in the cave sediments (Shahack-Gross et al. 2004). In karstic caves dominated by the bicarbonate system, calcitic remains and micro-remains are expected to preserve. Preservation will be reduced under moist conditions but quite good under dry conditions (e.g., Shahack-Gross et al. 2008a). Calcitic ash deposited in open-air sites (e.g., hearths, refuse pits/heaps) is less likely to preserve over time as it will be exposed to wind deflation, bioturbation, and water erosion that may also dissolve the calcitic components. Mallol et al. (2007) demonstrated the striking difference in preservation of two 1-year-old hearths, one located in the open area of an abandoned Hadza camp and the other in the entrance of a hut. Almost nothing, save for the stones marking the hearth contour and few microscopic charcoal fragments remained in the outdoor hearth, while ash pseudomorphs were still present in the indoor hearth. However, in cases where rapid burial of combustion features takes place, the chances for ash preservation are higher. For example, Friesem et al. (2014) suggested that well-preserved ash in the Middle Paleolithic open-air site of Nesher Ramla (Israel) is related to rapid colluvial infilling of this site which is located within a sinkhole. Other open-air sites where rapid burial (or accumulation) of sediments takes place are stratified mounds (*tells*). Gur-Arieh et al. (2014) suggested that the state of preservation of ashes found in ovens relates to the rapidity of *tell* formation, with Tel Megiddo having a faster accumulation and better preservation of ash relative to Tell es-Safi/Gath.

From the Neolithic and with animal domestication, herding, and penning, cave sites and rock shelters were sometimes used for stabling, resulting in thick deposits of animal dung (e.g., Boschian and Montagnari-Kokelj 2000; Macphail et al. 1997).

Presumably, the degradation of organic dung matter will result in the formation and release of organic acids into the sediments (yet, this was not studied systematically, to the best of our knowledge). The presence of acids may lead to the dissolution of calcitic dung spherulites as well as calcitic ash pseudomorphs if these were present in such cave stabling sites. Milek and Roberts (2013) raised this possibility in their study of a Viking age house in Iceland.

Where dung is burnt, either as part of stabling floor burning (which is a common practice to prevent the spread of vermin) or as fuel material, its calcitic micro-remains are more likely to preserve than in organic-rich dung deposits. Dung ash and its microscopic remains are then prone to the same post-depositional and diagenetic processes as outlined above for ash pseudomorphs, again depending on the type of site, moisture level, pH, and rapidity of burial. Notably, dung spherulites are most abundant in archaeological sites in arid environments (e.g., Dunseth et al. 2016, 2018).

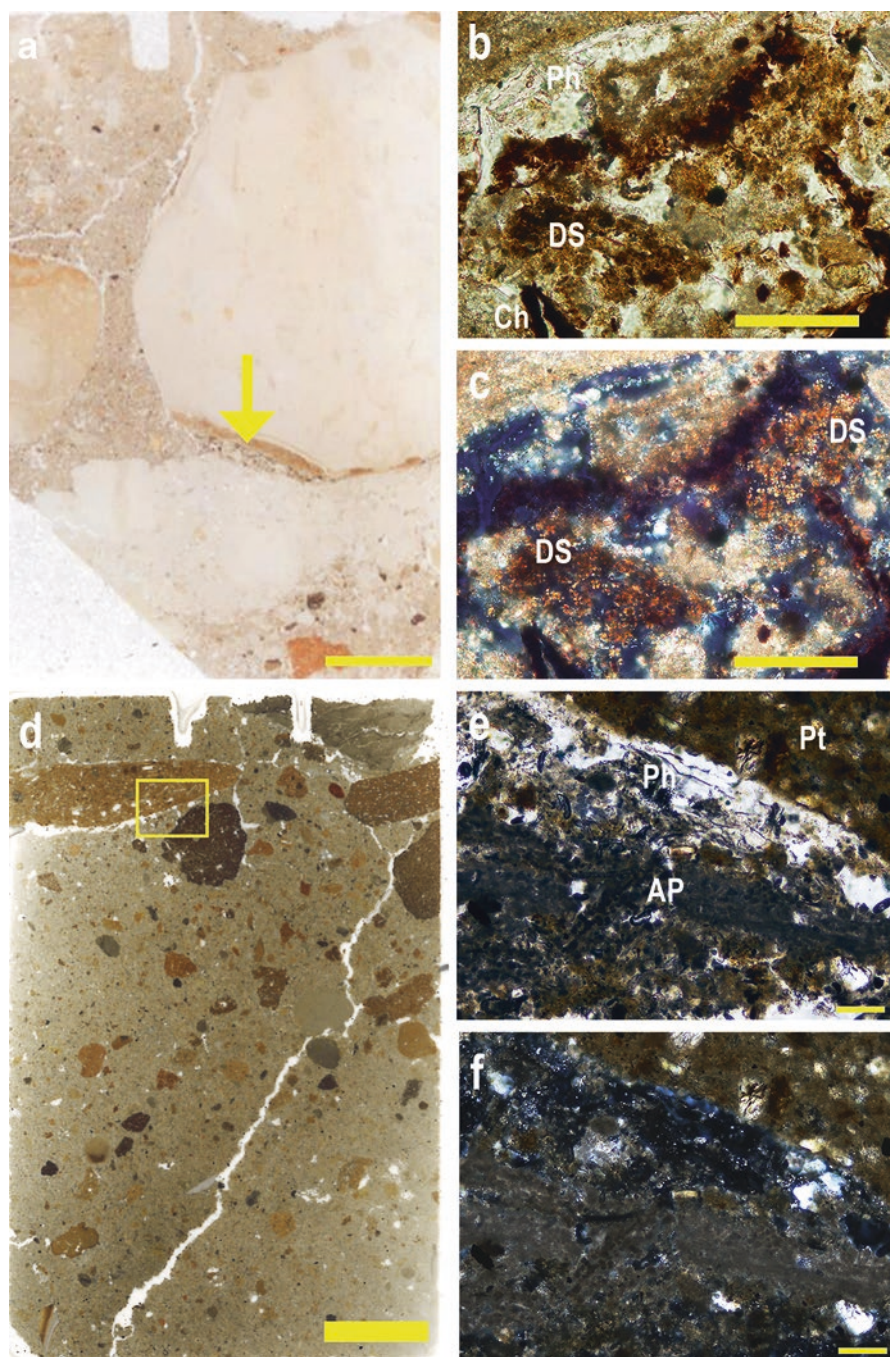
Gur-Arieh et al. (2014) conducted a controlled dissolution experiment and showed empirically that dung spherulites dissolve faster than ash pseudomorphs in acidic conditions. They explained this observation in relation to the larger surface/bulk ratio of dung spherulites, i.e., these are more prone to dissolution than ash pseudomorphs. As a result of this differential rate of dissolution, the PSR values of ashes composed originally from a mixture of dung (spherulites) and plant pseudomorphs may increase. This change in PSR poses certain interpretational difficulties regarding the dominance of one type of fuel over the other (see more in Gur-Arieh et al. 2014).

The preservation potential of calcitic microremains can vary on a micro-environmental level. For example, Gur-Arieh et al. (2014) identified well-preserved dung spherulites and ash pseudomorphs in sheltered localities within poorly preserved combustion features, such as underneath stones and potsherds (Fig. 10). Similarly, micro-remains may preserve in micro-environments such as CaOx within charred woody tissues (Mallol et al. 2017) or within dental calculus (Power et al. 2014).

Lastly, changes in micro-remain concentrations may also occur due to post-depositional mixing. For example, dung spherulite concentrations may decrease in open-air sites located in arid environments where aeolian dust accumulates and later incorporates through bioturbation into the archaeological dung deposit (e.g., Shahack-Gross et al. 2014).

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**Fig.10** (Continued) pebble above a chalk foundation. Arrow points to the location where images **b** and **c** were obtained. Scale = 1 cm. **(b)** Microphotograph of sediment below hearth pebble showing phytolith (Ph), dung spherulites (DS), and micro-charcoal fragments (Ch) (PPL). The edge of the pebble is visible at the top left corner. Scale = 100  $\mu$ m. **(c)** Same image as in **(b)** but in XPL showing aggregates composed of dung spherulites preserved under the pebble (DS). **(d)** Scan of a thin section showing the fill inside an Iron Age oven 121110 from Tell es-Safi/Gath. Yellow rectangular shows the location where images **e** and **f** were obtained. Scale = 1 cm. **(e)** Microphotograph of sediment below a potsherd inside oven fill showing phytolith (Ph) and ash pseudomorphs (AP). The potsherd is visible at the top-right corner (Pt). Scale = 100  $\mu$ m. **(f)** Same image as **(e)** but in XPL



**Fig. 10** Micromorphological observations from Iron Age cooking installations; (a) Scan of a thin section produced from an Iron Age pebble hearth 132029 from Tell es-Safi/Gath, showing a limestone



In conclusion, calcitic micro-remains of ash pseudomorphs and dung spherulites are valuable sources of archaeological information pertaining primarily to human use of fire, pyrotechnology of various types, livestock rearing and penning as well as site structure and human–environment interactions. Current extraction methods allow quantification and therefore comparison across and within sites. Ambiguities in identification based on optical properties, shapes, and sizes of these micro-remains are rather few. Taphonomic processes and preservation state should be considered before reaching archaeological interpretations.

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# Identification of Natural Fibers



Walter F. Rowe

## 1 Introduction

Fibers occur widely in nature. Vegetable fibers may facilitate the dispersal of seeds; they may reinforce plant stalks and leaves. Many animals also produce fibers: the possession of hair or fur is a defining characteristic of mammals. Many arthropod species produce fibers for webs or cocoons. These natural fibers may be dispersed into the environment as a part of their biological functions (e.g., seed hairs and spider webs) or released into the environment through the decay of plant or animal remains. When environmental conditions are favorable, natural fibers may be preserved with little alteration and retain both the external and internal features needed for their identification. Natural fibers can provide clues to past environments, in much the same way as plant phytoliths and pollen. Humans have been exploiting plants and animals for tens of thousands of years. Fibers recovered at archaeological sites may shed light on human diet (e.g., fiber remains at hearths, in cooking vessels, in dental calculus, and in coprolites), on human technology (medicine, textile production, cordage production, fiber reinforcements), and on cultural practices (fashion, costuming, burial practices). If the plant and animal sources of the fibers recovered at a site are not indigenous species, the fibers may be evidence of the existence of ancient trade routes such as the Silk Road.

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A. G. Henry (ed.), *Handbook for the Analysis of Micro-Particles in Archaeological Samples*, Interdisciplinary Contributions to Archaeology, [https://doi.org/10.1007/978-3-030-42622-4\\_7](https://doi.org/10.1007/978-3-030-42622-4_7)

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## 2 Origins of Fibers

This discussion will focus on natural fibers occurring as components of textiles (woven, knitted, knotted, and felted) and cordage. Fibers put to these uses have been the most extensively studied of all natural fibers. Barber (1991) has summarized the history of the domestication of the major natural fibers: flax, hemp, ramie, wool, silk, and cotton. The modern textile industry has developed analytical schemes for the identification of all types of fibers, both natural and man-made, used in modern textiles (Luniak 1953; American Association of Textile Chemists and Colorists 2018). Archaeologists and paleoethnobotanists have also carried out research on the identification of natural fibers recovered during the course of archaeological excavations (Raheel 1994; Pearsall 2000). Because textile fibers and animal hairs also constitute an important type of transfer trace evidence, forensic examiners have also developed schemes for the identification these types of fibers (Palenik 2018; Eyring and Gaudette 2005; Fergusson and Hemming 2018).

Textile fibers may be classified as either natural or man-made (Luniak 1953; Eyring and Gaudette 2005; American Association of Textile Chemists and Colorists 2018; Fergusson and Hemming 2018). Naturally occurring fibers may be subdivided into cellulosic (vegetable), proteinaceous (animal), and mineral fibers. Vegetable fibers are composed of cellulose (a polymer of anhydroglucose units linked by 1,4- $\beta$ -glucosidic bonds), hemicelluloses (complex polysaccharides), lignins (complex polymers of phenylpropane units), pectins (high-molecular-weight carbohydrates), waxes, fats, and various water-soluble compounds (Fergusson and Hemming 2018). Lignins are constituents of cell walls and are present in high concentrations in woody tissue (Gargulak et al. 2015). Staining of lignin using phloroglucinol reagent is a useful test for the identification of vegetable fibers (American Association of Textile Chemists and Colorists 2018; Palenik 2018).

Vegetable fibers can be categorized according to the part of the plant the fibers come from: seed fibers, bast fibers, and leaf fibers (Young 2003; Eyring and Gaudette 2005; Fergusson and Hemming 2018).

Seed fibers come from the seeds of plants. The principal seed fibers currently being produced are cotton, kapok, and coir (which comes from the husks of coconuts) (Fergusson and Hemming 2018). Cotton is of course widely used in the production of textiles; cotton is also used to make sewing thread and twine. Cotton was domesticated for fiber production in India as early as the Third Millennium BCE, based both on fiber impressions at Mohenjo Daro and Harappa and on the identification of actual fibers recovered at Mohenjo Daro (Barber 1991). Kapok fibers are generally too short to spin into yarns and have mainly been used as filling (Fergusson and Hemming 2018). The best quality coir fibers are used in ropes, twines, fish netting, and matting. Coarser coir fibers are used in brushes, brooms, and door mats. Cattail, thistle, and milkweed also produce seed fibers, but these have rarely been used in the production of textiles or cordage (Palenik 2018).

Bast fibers come from the stems of dicotyledonous plants. Bast fibers occur in bundles in the plant stalks; they lie under the outer bark and comprise a sort of inner

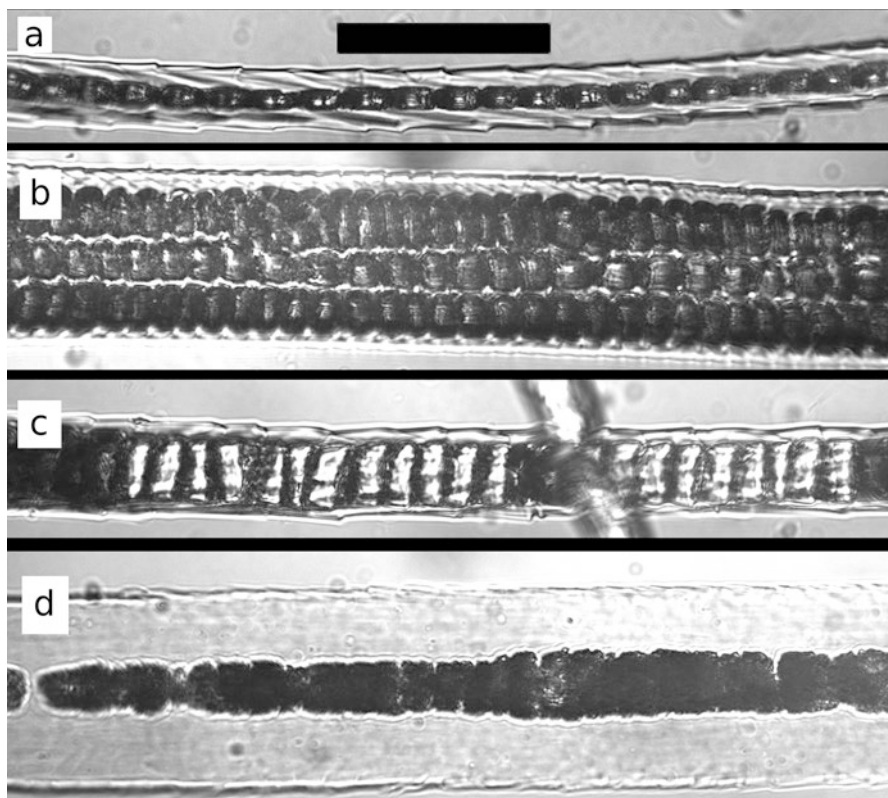
bark surrounding the woody central region of the plant stem. Bast fiber bundles are released by bacterial or chemical degradation of the pectins that hold them together. This process is called retting. Flax, ramie, hemp, kenaf, and jute are the main commercial bast fibers. Flax, hemp, and ramie were all widely exploited as sources of fiber by Neolithic and Bronze Age cultures (Barber 1991; Fergusson and Hemming 2018).

Leaf (“hard”) fibers come from the leaves of various tropical or subtropical plants. The main commercial leaf fibers are sisal, henequin, abaca (Manila hemp), and New Zealand hemp. The leaf fibers are freed by scraping away the leaf tissue. Esparto fibers come from the leaves of two different species of grass and are released by a process similar to the retting of flax and hemp (Barber 1991; Fergusson and Hemming 2018).

The three types of vegetable fiber that are commonly designated hemp (hemp, Manila hemp, and New Zealand hemp) come from different species of plant. This fact emphasizes the importance of creating fiber reference collections using only botanically authenticated samples, not fibers obtained from commercial sources.

Proteinaceous (animal) fibers include hairs and silk. Hair is one of the defining characteristics of mammals. These proteinaceous filaments grow out of specialized structures called follicles located in the dermal (innermost) layer of the skin. They are composed of the protein keratin. Fur-bearing animals typically have three types of hair: tactile hairs, very coarse hairs that grow out of the animal’s muzzle; guard hairs, the coarse hairs that form the animal’s outer coat; and down hair, finer hairs that lie close to the skin. Humans have fine, down-like vellus hair and also coarser terminal hair (head, pubic, chest, and axillary hair). The structure of hairs may be compared to that of a lead pencil. The lead in the pencil corresponds to the medulla, a column of specialized cells in the center of the hair shaft. These cells frequently shrink and become infiltrated with air. When observed using a transmitted light microscope, the air-infiltrated cavities appear dark; when observed with reflected light, the air-filled medullas will appear white. The morphologies of the dark medullas observed microscopically provide information about the species of the animal that provided the hair. Some of the common types of medullas that occur in animal hair include uniserial ladders (Fig. 1a), multiserial ladders (Fig. 1b), vacuolated medullas (Fig. 1c), amorphous medullas (Fig. 1d), and lattice medullas (Fig. 2). The cortex of the hair surrounds the medulla. This layer corresponds to the wood in the lead pencil. The cortex is composed of spindle-shaped cells held together by specialized matrix proteins. The cells are filled with a semicrystalline form of keratin. The cortical layer may contain pigment granules comprised of melanin (a dark brown pigment) or pheomelanin (a reddish pigment). This layer may also contain small, air-filled cavities called cortical fusi and ovoid bodies, which are believed to be undispersed melanosomes lying between cortical cells. The outermost layer of the hair is the cuticle. This layer is composed of a less crystalline form of keratin than the cortex and consists of a series of overlapping scales. The pattern of scales varies among different species of animal and is used to identify the species of animal the hair came from. There are three general types of scale patterns: coronal (resembling a stack of paper cups, Fig. 3a), spinous or petal-like



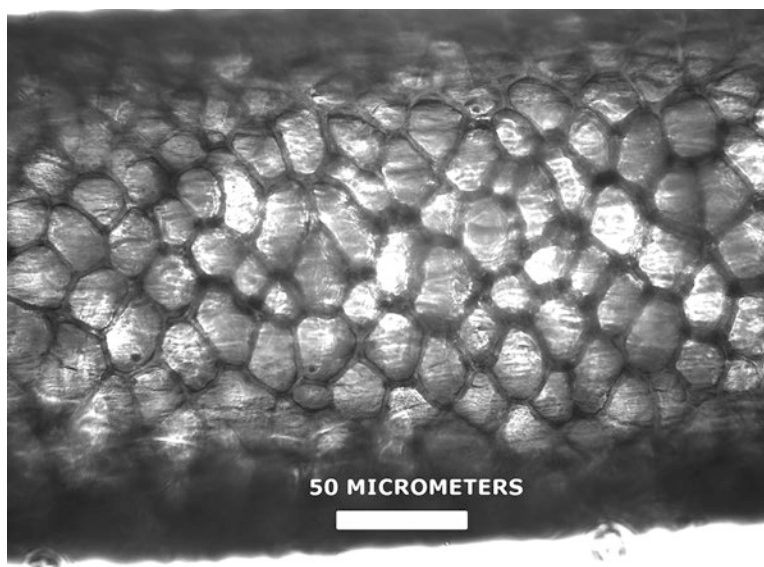


**Fig. 1** Examples of various medullas, all photomicrographs taken by the author. (a) Uniserial ladder medulla. Muskrat hair. (b) Multiserial ladder medulla. Rabbit hair. (c) Vacuolated medulla. Fox hair. (d) Amorphous medulla. Cow hair. The black scale bar at the top is 50  $\mu\text{m}$  and applies to the entire image

(Fig. 3b), and imbricate (resembling shingles on a roof, Fig. 3c) (Brunner and Cornan 1974; Hicks 1977; Appleyard 1978; Robertson 1998; Bisbing 2002).

Domesticated sheep are the main source of animal hairs used in the construction of textiles. These hairs have different names according to their diameters. Hairs in the range of 100–250  $\mu\text{m}$  diameter are referred to as *kemps*. Hairs with diameters in the range 50–100  $\mu\text{m}$  are simply called hair. Finer hairs are designated *wool*. Wool fibers with diameters in the range 30–60  $\mu\text{m}$  are considered medium wool, while wool fibers with diameters under 30  $\mu\text{m}$  are classified as fine wool. Kemps are almost impossible to spin into yarn by themselves: they are too brittle and stiff. Hairs can be spun if mixed with finer fibers. Dark medullas are present in kemps and occasionally in hairs, but not in wool. Wool fibers may be obtained by shearing the sheep or by plucking fibers from the animal's coat. Wool fibers can be prepared for spinning in one of two ways: combing or carding. Combing causes the fibers to lie



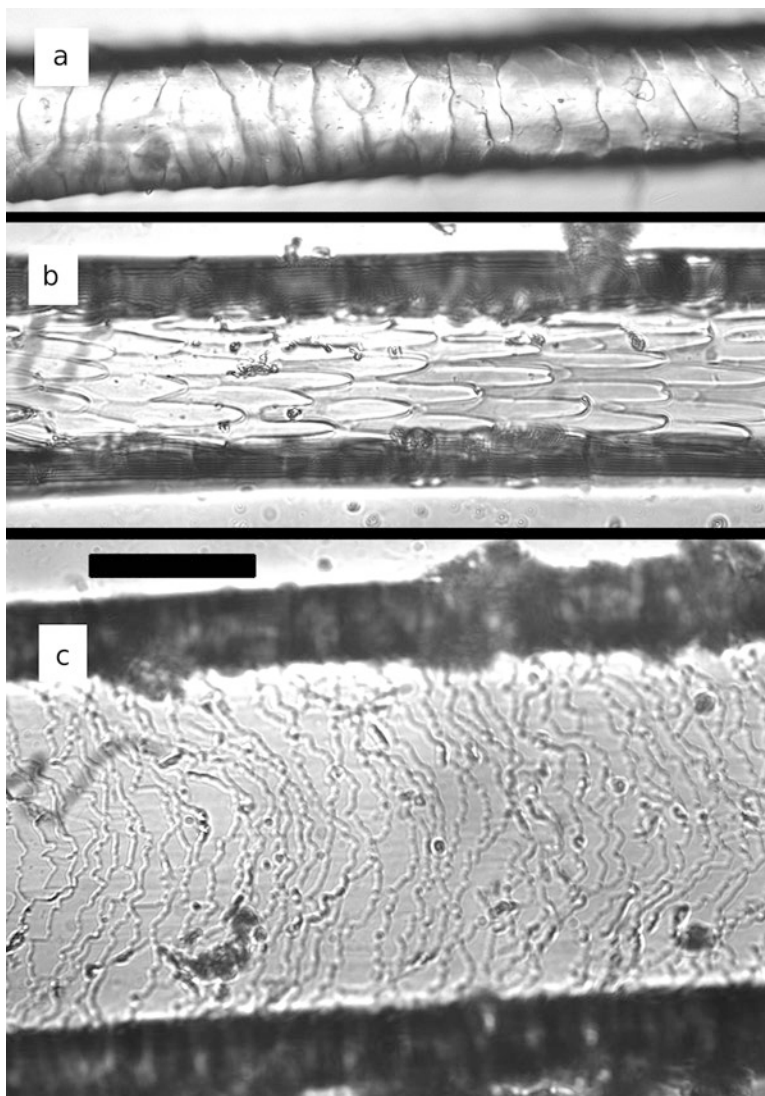


**Fig. 2** Lattice medulla. Deer hair. (Photomicrograph by the author)

more or less parallel (like bast fibers); the resulting yarn (*worsted*) is hard and strong. Carding produces fluffy, elastic *woolen* yarn (Barber 1991).

Silk is composed of the protein fibroin. It is produced by a variety of insects belonging to the order *Lepidoptera*. Commercial production of silk has focused on silk harvested from the cocoons of silk moths such as *Bombyx mori*. Raw silk has an elliptical cross section with two strands of fibroin embedded in a gummy protein called sericin. The fibroin filaments run side-by-side and have cross sections ranging from triangles with rounded corners (*Bombyx* silk) to wedges (Tussah silk) to roughly triangular with the apex of the triangle elongated and bent (*Anaphe* silk). Degumming of silk removes sericin and frees the fibroin filaments. The earliest direct evidence for the use of silk in fabrics comes from fiber pseudomorphs (see below) on the surfaces of Shang Dynasty bronzes (Barber 1991; Fergusson and Hemming 2018).

Mineral fibers belong to one of the six asbestiform minerals (McCrone 1980). Minerals in two silicate mineral groups crystalize as long, thin fibers: serpentines and amphiboles. Chrysotile (white asbestos) is the sole asbestiform mineral belonging to the serpentine group; its fibers are soft and curly. The amphibole group of asbestiform minerals includes amosite (brown asbestos), crocidolite (blue asbestos), tremolite (green asbestos), anthophyllite, and actinolite. Although widespread use of asbestos fibers has been a feature of modern technology, asbestos fibers were used in antiquity as wicks for oil lamps and in woven textiles (Evans 1906; Browne 2003). Asbestos fibers may be a form of modern contamination at a site; however, asbestiform minerals may be encountered during excavations (Richardson 1987).



**Fig. 3** Examples of various scale patterns, all are photomicrographs taken by the author. (a) Coronal scale pattern. Dry-mounted wool fiber. (b) Spinous or petal scale pattern. Cast of mink hair. (c) Imbricate scale pattern. Cast of mink hair

Man-made fibers may be organic or inorganic. The organic fiber types include regenerated fibers and wholly synthetic fibers. Regenerated fibers are produced from naturally occurring polymers. For example, rayon, acetate, and triacetate fibers (the most common regenerated fibers) are produced from cellulose. In the case of rayon, the structure of cellulose is partially broken down chemically and then reconstituted as a polymer. Wood pulp is the major source of the cellulose used in the

production of rayon. Cellulose acetate and cellulose triacetate are often considered types of rayon. However, their production does not involve the disruption of the backbone of the cellulose polymer. Instead, the hydroxyl groups on the  $\beta$ -D-glucose moieties comprising cellulose are acetylated to differing degrees: to produce cellulose triacetate, all three hydroxyl groups are acetylated, while acetylation of two out of every three hydroxyls yields cellulose acetate. Azlon is the name given to regenerated fibers produced from natural protein. The protein used in the production of these fibers can come from milk, soy beans, peanuts, or corn (maize). Regenerated protein fibers were produced commercially for a time in the mid-twentieth century; however, the mechanical strength of the fibers when wet proved to be unacceptably low. Regenerated protein fibers could not compete with wholly synthetic fibers produced from petrochemicals and their commercial production ceased in the 1950s. However, the growing movement for the development of products that use renewable resources may lead to a revival of azlon production. Synthetic fibers (acrylics, aramids, modacrylics, nylons, olefins, and polyesters) are generally derived from petrochemicals. They can occur as staple fibers or as continuous filaments. Glass fibers and mineral wool are examples of man-made inorganic fibers (Fergusson and Hemming 2018; Eyring and Gaudette 2005).

Man-made or manufactured fibers are of limited interest to archaeologists and paleoethnobotanists, as these fibers would represent modern contamination of the sites they may be investigating. Historical archaeologists, on the other hand, may encounter man-made fibers at early twentieth century sites. The presence of such fibers can aid in the dating of the horizons in which they are found because the dates of introduction of various man-made fibers are well established. The author was asked to examine textile fragments recovered from the lowest horizon of a deposit believed to date from the 1920s. The fragments included nylon hosiery and a printed cellulose triacetate woven textile. Nylon and triacetate were both produced only after World War II.

### 3 Uses of Fibers

Archaeobotanists and paleoethnobotanists examine hairs and fibers from a number of different perspectives. Animal and vegetable fibers recovered from a site may provide clues to the indigenous flora and fauna at the time of the site's formation. Because natural fibers are less likely to survive than phytoliths and pollen, those microtraces are more likely to be useful as guides to past environments. Knowledge of past environments in turn may provide clues about past climate. Identification of fibers in textiles or cordage may provide information about a culture's exploitation of local resources (Raheel 1994). Early civilizations tended to exploit one particular type of fiber: Egypt, flax; India and Peru, cotton; China, silk; and Mesopotamia, wool. If the fibers did not come from indigenous flora and fauna, their presence may be indicative of the existence of ancient trade routes like the Great Silk Road. Fibers in dental calculus and human and animal coprolites may provide information about

ancient diets and material processing (Blatt et al. 2011; Buckley et al. 2014; Radini et al. 2016; Hardy et al. 2017).

Natural fibers have been put to a wide range of uses by humans. These fibers have been used to provide clothing, shelter, and transportation. Loose fibers may be used to make felts as well as filling inside garments. Loose fibers can also be used as packing to seal gaps in boat hulls or dwellings. Once humans discovered the technology of spinning fibers into strings and yarns, a multitude of materials could be constructed (Hardy 2008). Yarns can be used to produce a wide variety of textiles, including woven textiles, knitted textiles, and knotted textiles (e.g., laces). Textiles may range from mundane, utilitarian items to high prestige objects. Fibers may also be used to construct ropes and other cordage. Yarns as well as individual fibers can be used to construct bags and baskets. Natural fibers have been used to make brushes and brooms. Natural fibers have been used as reinforcements for wall paintings (Ma et al. 2018). Until the introduction of wood pulp, paper was made of the same vegetable fibers as textiles. In fact, before the introduction of wood pulp, rags were a major source of fibers for paper (Hunter 1947). Those who work with ancient fibers should be aware of modern items that contain natural fibers and are potential sources of contamination. Forensic fiber examiners have long been aware that those collecting fibers and those analyzing them are potential sources of contamination (Eyring and Gaudette 2005).

## 4 Collection of Fibers

Fibers may occur at archaeological sites as components of intact manufactured objects, as components of fragments of manufactured objects, or as individual fibers (Good 2001). Fibers may have suffered serious environmental alteration (e.g., severe oxidation, microbial attack, carbonization, or mineralization). Impressions of fibers may also be found on ceramics. The recovery of intact or fragmentary items will depend on the condition of the constituent fibers. If the fibers retain most of their tensile strength, the items can be lifted from their findspots and appropriately packaged for later analysis and conservation. Degraded items may have to be removed in blocks of soil which will be later examined in a laboratory setting. Waterlogged items can be frozen for transport to a conservation laboratory. Recovered items may require treatment with appropriate biocides to prevent degradation by fungi or insects. Conservation treatments may interfere with microscopical or other instrumental analyses.

Forensic scientists and crime scene investigators have developed a number of methods for the collection of individual fibers (Eyring and Gaudette 2005). The simplest approach is to search surfaces with a bright light, a magnifying glass, and a pair of tweezers. This approach is obviously time-consuming. Loose fibers may be lifted from surfaces using special adhesive lifters, low-adhesive fingerprint lifting tape, lint rollers, packaging tape, or post-it-notes. The lifts can be placed on clear plastic sheets such as document protectors for protection from contamination. The

clear plastic sheets facilitate rapid searching of the recovered fibers for fibers likely to match the fibers comprising items of clothing or other textiles submitted as evidence. Vacuum cleaners equipped with filters can also be used to collect fibers and other particulate trace evidence. In the forensic science laboratory, fibers can be recovered by scraping: items of clothing or other textile evidence are suspended over a large table covered with sheets of brown paper. Particles are scraped off the fabric surface using large metal spatulas. Scraping requires a dust-free, draft-free room. The author's personal experience with scraping has been that fibers tend to cling to the surfaces of metal spatulas or remain suspended in air. Scraping tends to be better suited to other forms of trace evidence, such as soil or glass particles. In general, the methods of fiber recovery employed by forensic scientists and crime scene investigators are applicable to loose fibers on surfaces which themselves do not shed copious amounts of particles. These methods do not lend themselves to fiber recovery at excavations, where a large number of other particles (e.g., soil particles) would also be collected.

Individual fibers and textile fragments can be manually recovered during excavation when they are encountered. This is sometimes referred to as *in situ* recovery. To insure the most complete recovery of artifacts possible, soil from the excavation is passed through a series of screens (van de Guchte and Edging 1994). Only large textile fragments are likely to be recovered by this process. Individual fibers may pass through the openings in the screens. Further artifact recovery can be obtained by applying flotation methods using water as the floatation medium. Botanical remains such as seeds, leaves, and stems float and can be decanted or skimmed from the surface of the water. With the exception of polyethylene, all currently used fibers (including all natural fibers) are denser than water and therefore do not float unless air is trapped inside the fibers (as in the lumen of cotton fibers) (Luniak 1953). Aqueous solutions of zinc chloride have been suggested as floatation media for the recovery of denser artifacts; however, the added expense of zinc chloride and the disposal problems associated with its use would preclude the use of such solutions except in small-scale recovery projects (van de Guchte and Edging 1994). The lack of archaeological recovery techniques specifically for natural fibers indicates an area for further research.

## 5 Preservation of Fibers

Plant and animal fibers, like most organic material, are subject to rapid decomposition through the action of microorganisms. Common archaeological contexts do not provide environments in which these fibers are likely to survive. Special conditions may slow down or completely inhibit the activity of microorganisms, leading to fiber survival: arid climates and salt deposits which desiccate materials; bogs which provide anaerobic waterlogged environments rich in tannins; and cold environments in which water is frozen. Different environments may favor survival of one type of fiber over another: animal fibers tend to survive in environments where vegetable

fibers do not and vice versa. As a rule of thumb, cellulose fibers tend to be better preserved in alkaline conditions, while animal fibers tend to survive better in slightly acidic conditions.

Animal fibers tend to survive better in soil than vegetable fibers. There are fewer microorganisms that consume proteins than consume vegetable fibers. However, hairs are broken down by keratinolytic fungi. The spores of these fungi are widespread in soil and readily dispersed through the air. Once the spores attach to the surfaces of hairs, they generate fine filaments or hyphae. These hyphae initially extend along the surface of the hair, raising the edges of cuticular scales and detaching them from the hair surface. Eventually, the hyphae burrow into the hairs until they reach the medulla where they spread laterally, eventually consuming the medulla. The burrowing of the hyphae involves a combination of enzymatic dissolution of keratin and mechanical separation of the cells comprising the hair. Insects also attack hairs and other animal fibers. Moths readily attack woolen garments and dermestid beetles consume hair and other sources of keratin (Rowe 1997).

The forms of fibers (if not their original chemical makeup) may be preserved through carbonization or through mineralization from contact with metal artifacts. There are two forms of mineralized fibers: positive casts and negative casts. A positive cast is created when minerals form within the fibers; negative casts result from mineral deposition on the surfaces of fibers. Laboratory experiments with fiber mineralization (Gillard et al. 2013) have shown that sufficient traces of the organic matrix of cellulosic and proteinaceous fibers remain to permit the differentiation of these fibers by FTIR microscopy. These authors suggested that true pseudomorphs in which total replacement of the organic matrices of fibers by inorganic minerals has taken place are rare.

Much ancient botanical material is recovered from hearths and firepits in a charred state. Fragments of charred textiles may survive from cremations and from the burning of structures (e.g., dwellings and storehouses). However, the effects of elevated temperatures on natural fibers have only been studied to a limited degree. Helbaek (1963) reported on his examination of fibers from the so-called Burnt Burial excavated from Level VI at Catal Huyuk, an early Neolithic site in Anatolia (also written Çatalhöyük). These fibers appeared to have been carbonized by elevated temperature (hence the name Burnt Burial). Helbaek tentatively identified the fibers as being of animal origin based on what appeared to be surface scales. Ryder (1965) disputed both the identification of the fibers as being of animal origin and their “carbonization” being the result of burning. First of all, the fiber diameters were more consistent with those of flax fibers. Treatment of “carbonized” fibers with dilute alkali removed a black coating from the fibers to reveal the transverse dislocations characteristic of bast fibers. Dilute alkali would have dissolved wool fibers. The black coating was the result of normal decay processes. Srinivasan and Jakes (1997) reported on the effect of charring on Indian hemp (*Apocynum cannabinum* L.) fibers. Fiber bundles were exposed to elevated temperatures (250 and 350 °C) in air and in vacuo. Charring was found to decrease the fiber diameters; charring also precluded the examination of internal and external morphological features of the fibers using either transmitted or reflected light microscopy. The authors concluded that changes



in fiber morphology brought about by carbonization would make fiber identification even with scanning electron microscopy difficult.

Henson and Rowe (2002) heated male and female human head hairs in air to temperatures ranging from 240 to 300 °C. These authors found that heating increased the diameter of the hairs and bubble-like cavities. These changes could result in an inexperienced examiner misidentifying human hairs as animal hairs. Clearly more research needs to be conducted on the effect of elevated temperatures on both vegetable and animal fibers.

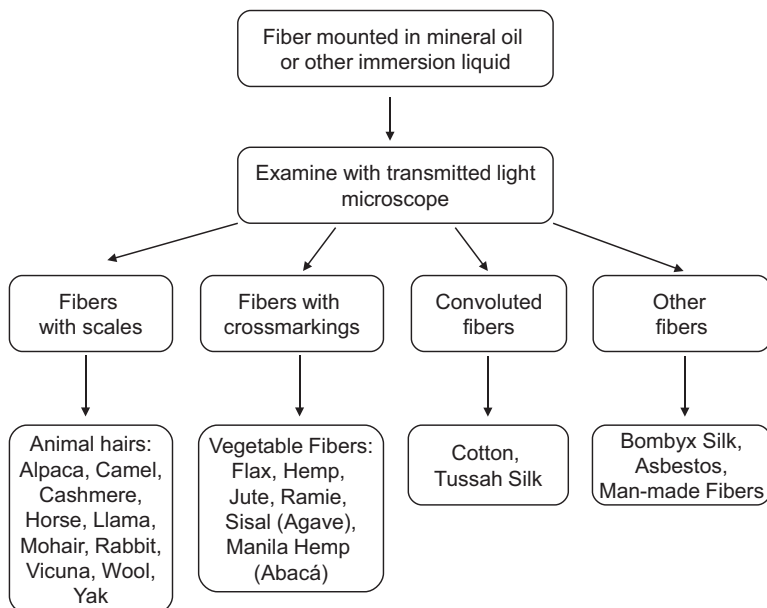
## 6 Identification of Fibers

At the outset, an archaeologist or ethnobotanist attempting to identify natural fibers from an archaeological context should be aware that very little external expertise may be available to aid in this endeavor. Forensic science laboratories have largely lost the ability to identify natural fibers (concentrating instead on man-made fibers). This is due to changes in the commercial use of fibers and to the de-emphasis on trace evidence in general caused by the advent of DNA profiling. Textile chemists and colorists tend to focus on fibers currently of commercial importance. A laboratory that undertakes the identification of fibers should have detailed, validated protocols for the recovery of fibers, their labeling and packaging, and their protection against contamination and further deterioration, as well as protocols for the identification of natural fiber. It should also have traceable reference collections of natural and man-made fibers. Collections of commercially mounted microscope slides, published fiber descriptions, and collections of fiber photomicrographs, while useful, do not permit the validation of laboratory procedures for fiber recovery and analysis. Microtrace LLC (Elgin, IL) sells collections of animal hair and fur and natural fibers. The McCrone Group has made the McCrone Atlas of Microscopic Particles, a searchable microscopy database, available online ([mccroneatlas.com](http://mccroneatlas.com)). Fiber atlases and fiber collections tend to focus on fibers of present commercial importance, not fibers that may have been exploited in the past by indigenous populations. Certain laboratory equipment is also required. A polarizing microscope is highly desirable for general fiber identification (American Association of Textile Chemists and Colorists 2018; Palenik 2018). The microscope should be capable of magnifications from 100 to 500× (American Association of Textile Chemists and Colorists 2018). A serviceable polarizing microscope can be constructed from a standard compound microscope by placing a small square of polarizing filter over the microscope's illuminator and a second on top of the sample slide. A stereomicroscope (preferably one mounted on a boom) is useful for examination of textile weaves and yarn twists, as well as preliminary sampling of fibers (Palenik 2018). A microspectrophotometer is useful for comparing colors of fibers but does not analyze dyes in situ. Micro-FTIR and micro-Raman instruments are essential for the nondestructive analysis of man-made fibers, but these instruments have more limited utility in the analysis of natural fibers (specifically in the differentiation of types

of bast fibers). Some researchers have relied on scanning electron microscopy rather than light microscopy for fiber identification; however, most research budgets will not accommodate such expensive equipment.

Identification of fibers begins with a preliminary survey of the material submitted for analysis (American Association of Textile Chemists and Colorists 2018). The examiner first notes the form (loose fibers, felts, yarns, woven fabric, knitted fabric, knotted fabric, and so forth), color, fiber length and fineness, uniformity, and probable end-use. Warp and filling (weft) yarns of woven fabrics must be sampled separately, because these may be composed of different fibers. In general, samples should be taken reflecting the different colors, lusters, sizes, and textures present in the material. While in many instances fibers can be identified without pretreatment, the American Association of Textile Chemists and Colorists method calls for the removal of adherent materials such as starch, waxes, oils, and other coatings by heating the fiber in warm to hot distilled water. If treatment with hot water fails to remove interfering material, extraction with organic solvents or treatment with 0.5% hydrochloric acid or 0.5% sodium hydroxide may be successful. However, proteinaceous fibers such as human hair, wool, silk, and azlon are likely to be damaged by sodium hydroxide. Individual cells can be separated from vegetable fiber bundles by treating the bundles with 0.5% sodium hydroxide. Usually dyeing does not interfere with microscopical examination; however, dyes can be stripped from fibers by heating them at 50 °C for half an hour in hydrosulfite-caustic solution (recipe at the end of the chapter). This dye-stripping procedure is appropriate to remove dyes that interfere with the microscopical examination of the fibers; dye analysis involves different extraction procedures that do not disrupt the dye structures. The American Association of Textile Chemists and Colorists method for the identification of fibers calls for mounting fibers in mineral oil for microscopical examination. This initial survey of the fiber sample seeks to place the fiber in one of four categories: fibers with scales, fibers with cross markings, twisted fibers, and other fibers (Fig. 4). Fibers with scales include human hairs, as well as wool, cashmere, mohair, camel hair, horse hair, and porcine hair (Fig. 5a, b). Fibers with cross markings are bast fibers such as flax, hemp, jute, and ramie (Fig. 5c, d). Twisted fibers are cotton and silk (Fig. 5e, f). The “other fiber” category includes all the man-made fibers. A cautionary note is in order. Fibers recovered in archaeological contexts may have undergone changes that alter important diagnostic features. For example, human hairs as well as other animal hairs may have lost their scales (Carr et al. 2008). These changes may lead to misidentification of fibers.

In the American Association of Textile Chemists and Colorists method of analysis, bundles of fibers in each of the four groups are examined in cross section. A steel plate with a small hole drilled in it can be used to prepare temporary cross sections. A thin wire is used to pull a bundle of fibers through the hole in the plate. The bundle should completely fill the hole. A razor blade is used to cut off the excess fibers on both sides of the plate. The plate is then placed on the stage of the microscope for examination in air and under an immersion liquid. A Hardy fiber microtome can also be used to prepare permanent cross sections. These methods of

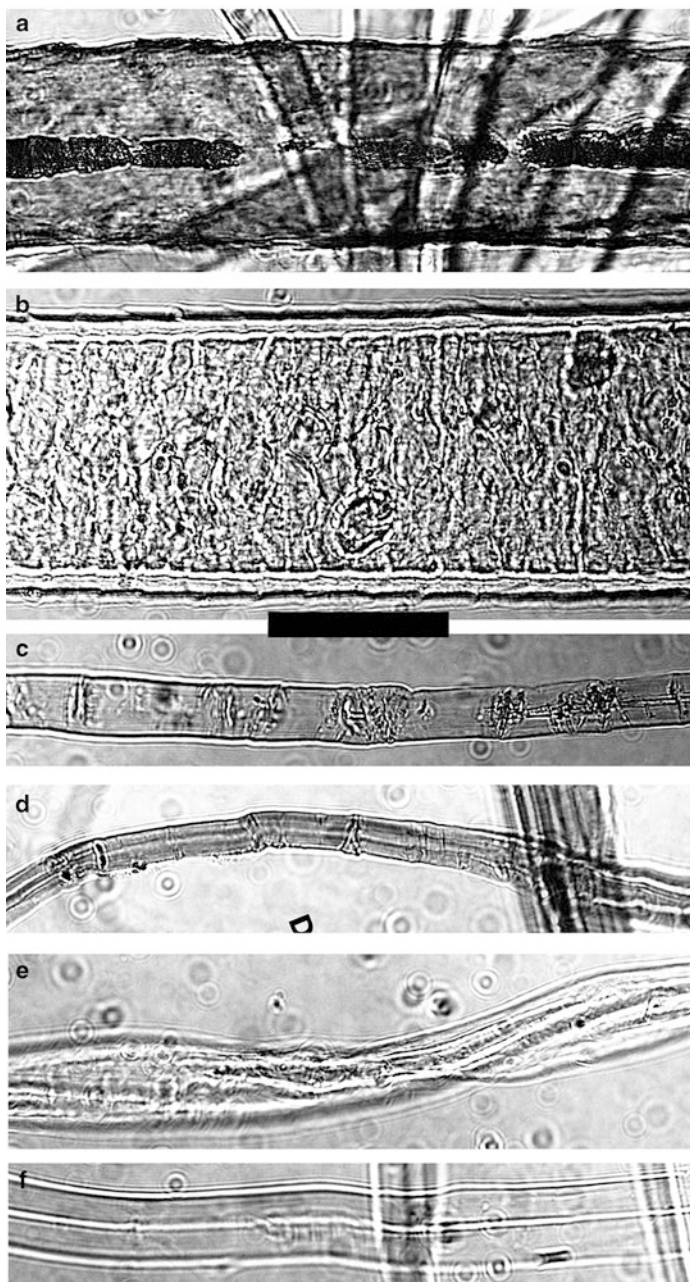


**Fig. 4** American Association of Textile Chemists and Colorists analytical scheme for fiber identification (AATCC Technical Manual 2018). (Diagram by the author)

preparing fiber cross sections require practice to attain acceptable cross sections; they are also destructive.

The American Association of Textile Chemists and Colorists method for fiber identification calls for fibers with scales to be examined longitudinally noting the characteristics of the medulla, cortex, and cuticle. The occurrence, type, and width of the medulla should be noted. Is the medulla usually present in the fibers, seldom present or never present? For the cuticle, critical features noted are how pronounced the scale edges are, whether the scales are coronal or imbricate and whether the scale edges are smooth or serrated. The fiber cross sections are examined to determine the fiber contour, the medulla contour, and the distribution of pigment. The fiber analyst concludes the fiber identification by comparing the features of the unknown fiber to photomicrographs provided in the *AATCC Technical Manual*.

In order to better visualize the cuticular scale patterns, the analyst may wish to modify the American Association of Textile Chemists and Colorists method somewhat at this point. If the fiber is not heavily dyed or pigmented, it can be dry-mounted (placed on a microscope slide and just covered with a coverslip): the scale pattern will be readily apparent. Alternatively, a cast of the scale pattern can be made by applying a thin layer of clear fingernail polish to a microscope slide and laying the fiber down on the surface of the drying fingernail polish. Once the fingernail polish is dry, the fiber can be removed. The cast can best be viewed under a coverslip (but without any mountant or immersion liquid).

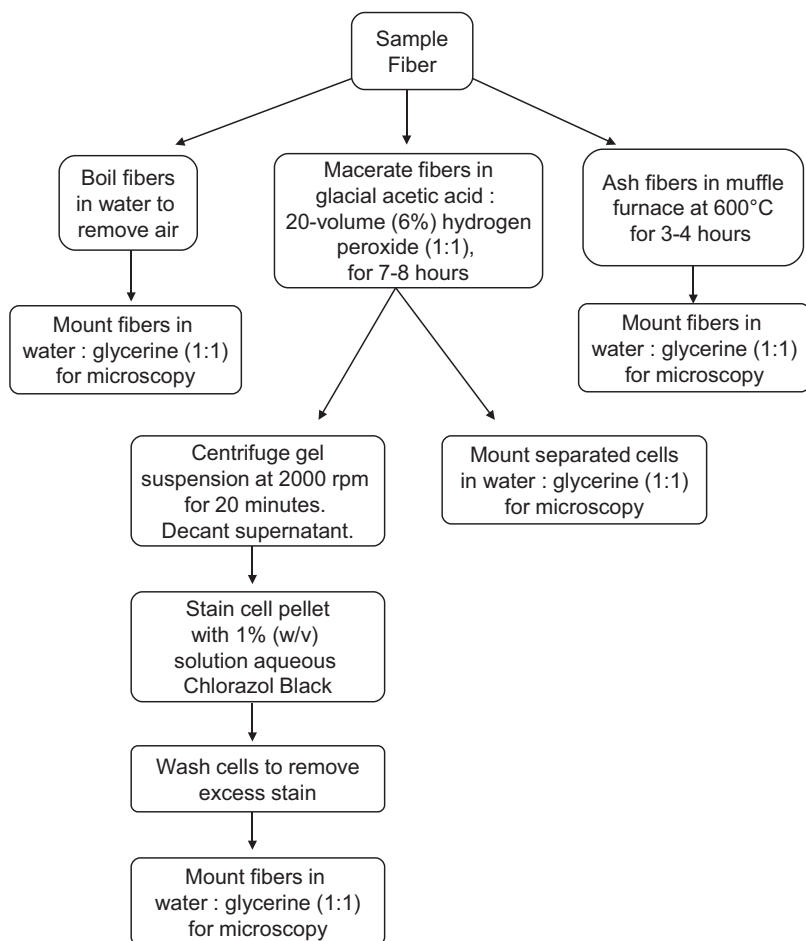


**Fig. 5** Examples of natural fibers, all photomicrographs by the author. Scaled fibers include (a) Wool and (b) Goat fibers. Fibers with cross markings include (c) Hemp and (d) Jute fibers. Note the dislocations along the fiber. Twisted fibers include (e) Cotton fibers and (f) Raw (Tussah) silk. The scale bar in (e) is 50  $\mu\text{m}$  and applies to the entire image)

The fibers in the remaining three categories delineated in the American Association of Textile Chemists and Colorists method are also examined longitudinally and in cross section. Longitudinal mounts and cross sections are prepared as described above. Fibers with cross markings are examined for the ratio of the width of the internal lumen to the width of the fiber, for nature of the cell ends (sharp, forked, or blunt) and for the cross-sectional shapes of the fiber (sharp polygon, rounded polygon, or elongated polygon). Catling and Grayson (1998) have demonstrated that the ends of the cells of vegetable fibers are not diagnostic features useful for identification. American Association of Textile Chemists and Colorists (2018) also recommend the use of staining tests with zinc-chloro-iodide and acid phloroglucinol to aid in the differentiation of the vegetable fibers in the category of fibers with cross markings. Zinc-chloro-iodide stains hemp, ramie, and cotton violet; flax stains brownish violet; and jute stains brown. Phloroglucinol reagent stains woody fibers magenta. The recipes for zinc-chloro-iodide and phloroglucinol reagents are at the end of the chapter.

Catling and Grayson (1998) have published a procedure for the identification of vegetable fibers (Fig. 6). The *technical* fiber (the fiber as it is used in textiles or cordage) is examined microscopically in a temporary mount. Technical bast and leaf fibers are bundles of cells; seed fibers are single cells and do not require an elaborate analysis scheme. A portion is macerated to release the cells comprising it (the fiber *ultimates*) for microscopical examination. Inorganic inclusions are obtained for analysis by ashing the technical fiber. The entire analytical procedure requires up to 8 h to complete. Palenik (2018) has published a quicker procedure for bast and leaf fibers (Fig. 7).

It should be noted that the correct identification of bast fibers requires considerable skill. Barber (1991) has pointed out that many finds of textiles confidently identified as linen have been later determined to be comprised of nettle fibers. These include the textiles of the great Oseberg ship find collections. Haugan and Holst (2014) carried out a comprehensive microscopical study of flax, hemp, and nettle fibers. They looked at all the features that are supposed to allow differentiation of these fibers: cross section, diameter, lumen diameter, dislocations, and cross markings. Samples of each fiber type contained individual fibers having microscopical features supposedly diagnostic of one of the other fiber types. The Herzog test or the red plate test can be used to differentiate bast fibers. This test employs a polarizing microscope and a first-order 530-nm retardation plate. First-order 530-nm retardation plates are common accessories that come with polarizing microscopes. These plates can also be purchased from most suppliers of microscope accessories. The Herzog test is based on the orientation of the cellulose microfibrils in the thickest parts of the fibers' three secondary cell walls (S1, S2, and S3) (Haugan and Holst 2013). The microfibrils in the cell walls may be Z-twist, S-twist, or parallel to the fiber axis. In flax, hemp, and ramie, S1 is Z-twist and S2 is S-twist; S3 is Z-twist in flax, while in ramie and hemp, the microfibrils in S3 are almost parallel to the fiber axis. Because the relative thicknesses of S1, S2, and S3 are different, hemp is overall Z-twist and flax and ramie are overall S-twist. To perform the Herzog test, a bast fiber is placed between crossed polarizing filters in a position of extinction (parallel

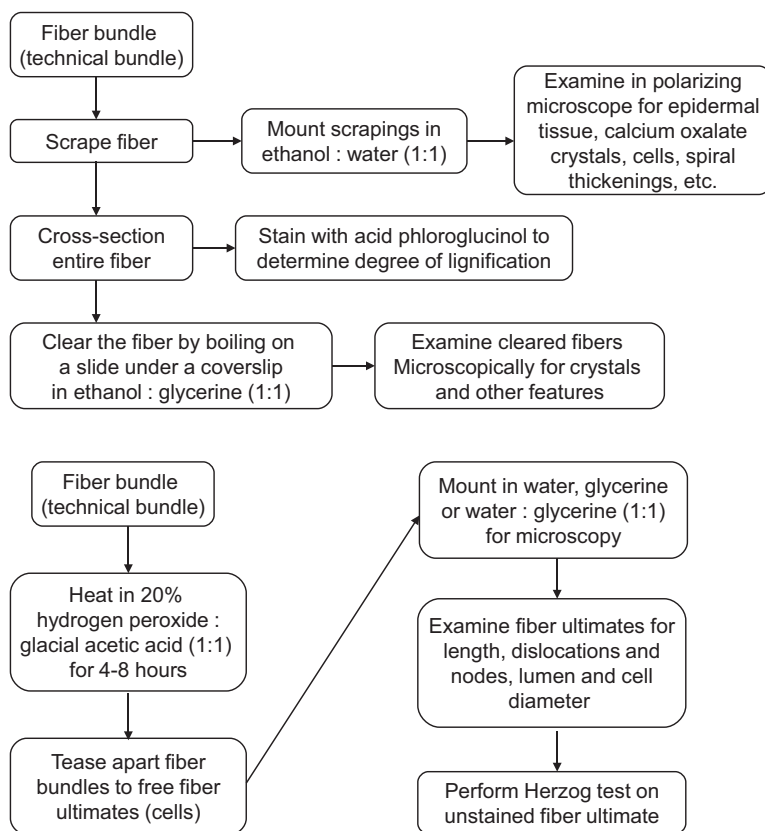


**Fig. 6** Catling and Grayson's analytical scheme for the examination of vegetable fibers (Catling and Grayson 1998). (Diagram by the author)

to the polarizer or analyzer directions). When a first-order retardation plate is inserted at a 45° angle, the fiber turns blue or yellow (depending on the fiber-orientation relative to the accessory plate). Palenik (2018) strongly recommends that fiber microscopists become familiar with the Herzog test by examining known samples of bast fibers before attempting to apply the test to unknown fibers. As will be discussed later, infrared and Raman spectrometries can also be used to differentiate bast fibers.

McCrone (1974, 1980) has developed an analytical protocol for the identification of asbestiform minerals using a polarizing microscope equipped with a dispersion staining objective. Dispersion staining objectives are relatively inexpensive and serviceable ones may be made by painting opaque dots in the back lenses of regular lenses. In McCrone's procedure, suspected asbestos fibers are selected from the





**Fig. 7** Palenik's analytical scheme for the examination of bast and leaf fibers (Palenik 2018). (Diagram by the author)

sample and mounted in a series of high dispersion immersion liquid. Dispersion staining colors are observed as the polarizing filter below the stage is slowly rotated. The colors observed with each immersion liquid serve to identify the asbestiform mineral comprising the fiber. The procedure is designed so that chrysotile, the most common form of asbestos used commercially, is identified first. Care must be exercised in handling suspected asbestos fibers.

## 7 Permanent Mounting of Fibers

Permanent mounting of fibers reduces the likelihood of fibers being lost. Permanently mounting fibers also means that they are available for later examination with minimal preparation. The ideal mounting medium has a number of desirable characteristics (Cook and Norton 1982):

- No alteration of fibers or dyes
- Ease of use (not too viscous or too runny; no heating required)
- Rapid setting
- No “cold flowing”
- No formation of bubbles or crystals to interfere with microscopic examinations
- Refractive index suitable for the examination of internal features of fibers
- Easy recovery of fibers from the hardened mountant
- Solubility in nontoxic solvent
- No color
- No fluorescence
- No yellowing or shrinkage with age
- Low cost

From the standpoint of the microscopic examination of vegetable fibers and hairs, the refractive index of the mounting medium is the most significant. If tape is used to recover fibers, it is desirable that the solvent in which the mountant is dissolved also be able to dissolve the tape adhesive. If DNA profiling of hairs is a possibility, the mountant should also not interfere with that process.

No mounting medium meets all of these criteria. For example, Canada balsam has been used for many years as a general mounting medium for tissue sections, as well as hairs and fibers. It is very cheap; however, Canada balsam yellows with age and becomes brittle. Permount (a polymer of  $\alpha$ -pinene,  $\beta$ -pinene, dipentene, and  $\beta$ -phellandrene dissolved in toluene) does not yellow with age but it does have a slight yellow color and is faintly fluorescent. Many mountants have been evaluated by forensic hair and fiber examiners (Roe et al. 1991; Grieve and Deck 1995; Wiggins and Drummond 2007). Wiggins and Drummond (2007) have concluded Entellan New and Eukitt mountants are suitable for use with both hairs and fibers.

## 8 Documentation

The advent of digital photomicrography has made the documentation of the microscopical appearance of fibers more convenient than in the past. Ten megapixel and 14 megapixel digital eyepiece cameras can now be purchased for less than \$400. These are capable of publication quality images.

## 9 The Future of Fiber Examinations

While microscopical examinations of natural fibers will remain essential for the paleoethnobotanist and archaeologist, other technologies can complement the basic polarized light microscope. Scanning electron microscopy (SEM) permits detailed analysis of the surfaces of carbonized fibers and fiber pseudomorphs. Cuticular

scale patterns can identify the source of an animal hair down to the genus level and in some cases down to the species level. The nodes or dislocations and cross-markings characteristic of bast fibers can be easily seen on their surfaces. However, further differentiation of bast fibers may not be possible based on SEM electronmicrographs, because such differentiation requires observation of internal characteristics such as the widths of the lumen.

Infrared microscopy has become a common analytical tool for the identification of fibers. In infrared microscopy, an infrared microscope is used to observe fibers in either transmission mode or reflection mode. Infrared light is passed from the microscope to the bench of a Fourier transform infrared spectrometer which produces an infrared spectrum of the fiber. In infrared spectrometry, internal vibrations of molecules are excited and the frequencies at which light is absorbed provide information about the chemical bonds comprising the molecules of the sample. In the case of polymers, such as cellulose and keratin the major contributors to their infrared spectra are the vibrations of the basic monomeric units. At the present time, infrared microscopy is confined to the laboratory: the equipment is bulky and the low infrared light levels involved require liquid nitrogen cooled detectors. Attenuated total reflectance Fourier transform infrared (ATR FTIR) spectrometry has emerged as a routine analytical tool in many fields (forensic drug analysis, materials analysis, museum conservation). In ATR FTIR, a beam of infrared radiation is directed through a crystal having a high index of refraction and undergoes complete internal reflection at one of the crystal's faces. If a sample is pressed against that face, its molecules will absorb infrared radiation at frequencies corresponding to the molecules' internal vibrations. With ATR FTIR sample preparation is minimal: solids such as powders, bulk plastics, and fibers simply need to be pressed against the ATR crystal's face. Several portable ATR FTIR units have been developed for field use. Weiner (2010) has described the routine use of a portable ATR FTIR instrument at archaeological sites; this author has also created a downloadable library of infrared spectra of materials likely to be encountered at archaeology sites. As indicated earlier, differentiation of bast fibers such as flax and ramie has proven to be a problem for archaeologists and paleoethnobotanists. Garside and Wyeth (2003) examined the ATR FTIR spectra of cotton, flax, hemp, jute, ramie, and sisal. The ratios of the peaks at 2900, 1595, and 1105  $\text{cm}^{-1}$  were found to differentiate the six types of fiber. The authors' interpretation of these three peaks was that the C–H stretch at 2900  $\text{cm}^{-1}$  represents the total organic content of the fiber (C–H bonds being present in cellulose, lignin, and pectins), while the 1595  $\text{cm}^{-1}$  peak represents the lignin content and the 1105  $\text{cm}^{-1}$  peak represents the cellulose content.

Raman scattering is another vibrational spectroscopy that can also be used to identify natural fibers. It relies on inelastic scattering of photons from the molecules being analyzed. Because hydrogens and the bonds involving them have low electron densities, the vibrations of C–H bonds are not readily excited by Raman scattering. The parts of molecules containing carbon–carbon, carbon–oxygen, and carbon–nitrogen bonds are more likely to be excited by Raman scattering. Raman spectroscopy thus provides more information about the skeleton of the molecule being analyzed than FTIR. In this sense, FTIR and Raman spectroscopy

are complementary techniques. In this light, it is interesting to compare the work of Garside and Wyeth (2003) with that of Edwards et al. (1997). The latter researchers analyzed the Raman spectra of flax, ramie, jute, cotton, kapok, coir, and sisal fibers. The fibers could be differentiated from one another using the ratios of the Raman peaks at Raman shifts of 1121, 1096, and  $2900\text{ cm}^{-1}$ . While Raman spectroscopy has some value for the differentiation of vegetable fibers, its greatest value in the examination of ancient fibers stems from its ability to identify dyes and other colorants. Dyes and pigments are strong Raman scatterers. The Raman spectra of dyed fibers will typically show both the Raman spectrum of the polymer comprising the fiber and the Raman spectra of the dyes in the fiber. The intensity of Raman spectra can be enhanced by many orders of magnitude by treating samples with metal colloids. This effect is called surface enhanced Raman scattering (SERS); it has been widely applied in the field of art conservation for the identification of artists' pigment (Edwards and Vandenabeele 2016).

Raman instrumentation has undergone significant improvement in recent years. The footprints of the instruments have shrunk considerably. There are now even handheld Raman spectrometers that can be taken to the field. Although these instruments are intended primarily for geological prospectors and for first responders, they are could be adapted for archaeological field work. A number of instrument manufacturers have produced Raman microscope systems well suited for the analysis of fibers and other microtraces. These Raman microscope systems are compact enough for field work. There are, however, problems with the routine use of Raman spectroscopy. Raman scattering uses lasers to excite the scattering phenomenon. The lasers are sufficiently powerful that they can char the samples being analyzed. The laser wavelengths used often excite fluorescence in the samples. Indeed, background fluorescence is a ubiquitous problem with Raman spectroscopy.

Good (2001) suggested that recent advances in DNA technology such as the polymerase chain reaction (PCR) would provide new avenues for the analysis of natural fibers. In the 1980s, several research groups demonstrated that sufficient DNA could be extracted from hair shafts to produce DNA profiles (Higuchi et al. 1984, 1988; Kalbe et al. 1988). Hamlyn et al. (1992) showed that species-specific DNA PCR probes could be created to facilitate the identification of specialty animal fibers. A sheep-specific probe was the first to be produced. The same research group also developed a goat-specific probe (Nelson et al. 1992) and probes for South American Camelids (Hamlyn et al. 2001). Subramanian et al. (2005) have developed a PCR technique based on mitochondrial single nucleotide polymorphisms (SNPs) to differentiate wool and cashmere fibers. Similar research has not been reported on vegetable fibers. This may be due to the fact that animal hairs contain substantially more DNA than vegetable fibers. At this time, the applicability of PCR-based DNA methods to ancient animal fibers has yet to be demonstrated.

## 10 Conclusion

Polarized light microscopy remains the simplest yet most powerful tool for the identification of natural fibers. The Herzog test allows experienced fiber analysts to differentiate the commonly encountered bast fibers. Archaeologists and paleoethnobotanists will be able to use portable FTIR and Raman spectrometers at dig sites so that fibers in artifacts can be analyzed as soon as recovered. Advances in DNA technology will permit the identification of virtually all animal fibers. Advances in DNA technology may even make possible the identification of vegetable fibers.

## 11 Reagent Recipes (American Association of Textile Chemists and Colorists 2018)

Hydrosulfite-caustic solution: Dissolve 2 g of sodium hydrosulfite and 2 g of sodium hydroxide in 100 mL distilled water.

Zinc-chloro-iodide reagent: Dissolve 20 g of zinc chloride in 10 mL distilled water. Dissolve 2.1 g of potassium iodide and 0.1 g iodine in 5 mL distilled water. Add the potassium iodide/iodine solution to the zinc chloride solution.

Phloroglucinol reagent: Dissolve 2 g of phloroglucinol in 100 mL of distilled water. Mix with an equal volume of concentrated hydrochloric acid.

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# Parasite Microremains: Preservation, Recovery, Processing, and Identification



Morgana Camacho, Angela Perri, and Karl Reinhard

## 1 Paleoparasite Microremains and Their Formation: An Introduction

Archaeological parasitology focuses on the recovery and interpretation of all types of parasite remains. Parasitology is a multiphyletic science that includes any organism that is found in or on another host organism and derives nutrients from that host. Parasites include a wide range of organisms ranging from single-celled protozoa to several types of worms and arthropods such as fleas. In archaeological sediments, eggs from intestinal worms are most commonly found because protozoa cysts usually do not preserve in archaeological materials. In these contexts, ancient protozoa infection is detected by applying enzyme-linked immunosorbent assay (ELISA) and molecular biology techniques. Arthropods such as fleas and lice require excellent preservation conditions and are usually found only on mummified human remains (Reinhard and Buikstra 2003). For these reasons, this chapter focuses on helminth eggs. Helminths include nematodes (roundworms), trematodes (flukes), cestodes (tapeworms), and acanthocephalans (thorny-headed worms).

The life cycles of parasites often involve high mortality. Therefore, most common parasites release thousands of eggs into the environment daily. The structures of the eggs that are released into the environment are durable. Particularly for fecal-borne nematode eggs that require days or weeks in the open environment for

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embryonation, durable eggs ensure larval survival for months to years and increase the chance of infecting a suitable host. High fecundity and durable egg structure are shared by many helminths. Nematode eggs have three common layers: an outer vitelline layer, a main chitinous layer, and an inner lipid layer (Wharton 1980; Wharton and Jenkins 1978). Trematode eggs are composed of a keratin protein. Cestode eggs are composed of protein, although diphylobothriids' eggs (operculated eggs of flukes and tapeworm) are made of sclerotin protein (Smyth and McManus 2007: 172). As the eggs develop within the parasites, the eggshells harden to durable structures when they are released in the environment. This is noteworthy for parasitologists involved in mummy analyses. Increased egg pliability and decreased size have been noted for nematode eggs (Morrow et al. 2014) and cestode eggs (Reinhard and Urban 2003) in mummies due to the fact that immature eggs become released in the mummies' intestinal tracts as the intestinal walls break down.

Acanthocephalans are different from the other classes of helminths. The embryo, or acanthor, is enclosed by a series of four or five envelopes (Nikishin 2001, 2004). One of the outer envelopes is composed of keratin-type protein which provides some durability to the eggs. Acanthocephalan eggs are readily identifiable as such due to the multiple envelopes and the acanthor larva, which is armed with resilient hooks. Archaeologically, acanthocephalan eggs are not as commonly encountered as other helminths, probably due to decreased fecundity. However, where evidence of pig domestication is found, or in regions where humans eat insects, these thorny-headed worms are more evident (Fugassa et al. 2011).

## 2 Paleoparasitology in Archaeological Contexts

The first archaeoparasitological analysis was undertaken by Ruffer (1910) on Egyptian mummies, but it was not until the 1960s and 1980s that standardization of the field took place. Before this period, parasitology in ancient contexts was moving from solely being used as an indicator of pathology and/or disease, particularly in mummy studies, to the use of parasites as important indicators of past lifeways, such as general living conditions, diets, and cooking methods. The history of archaeoparasitological analysis and its growth into the current field have been reviewed by several researchers (Reinhard 1990, 1992, 2017; Araújo et al. 2008; Dittmar 2009; Dittmar et al. 2012).

Stemming from its initial use in mummy research, one of the most obvious applications of archaeoparasitology has been as an indicator of human (and animal) health and disease through time (Reinhard 1988). Barrett et al. (1998) suggested that infectious disease has passed through distinct paleoepidemiologic transitions, consisting of three phases: (1) a "Paleolithic Age Baseline" in which humans during the Paleolithic period existed in small numbers and therefore could not support many infectious agents; (2) the transition into the Neolithic period when humans began to form large settlements, to domesticate livestock animals, to practice agriculture, and to accumulate large amounts of human and animal waste; and (3) the

Industrial Revolution, which saw the control of infectious disease and a reduction of parasite findings in archaeological contexts (Reinhard and Pucu, 2014; Reinhard et al. 2013). While they act as potentially important indicators of human health and disease (e.g., Perri et al. 2018), inferences from archaeoparasitological findings can be difficult to evaluate given the other pathoecological factors involved, including the nutritional status of the population, population size, immune status, and the other infectious organisms present in the host population.

Inferences related to living conditions which assist in parasites completing their life cycles may be more informative than the disease implications of the parasites. For example, the finding of parasites that rely on fecal–oral transmission, such as *Trichuris trichiura*, may indicate fecal contamination of the local environment, including water sources. Similarly, the presence of pinworms (*Enterobius vermicularis*) suggests personal hygiene challenges and cramped living conditions. Findings of fecal-borne parasites as a result of poor sanitation practices have been documented throughout time and in many locations (Reinhard et al. 1986; Faulkner 1991; Warnock and Reinhard 1992; Reinhard 2017; Matsui et al. 2003; Mitchell 2015), with a recent strong focus in the Roman and Medieval periods (Mitchell and Tepper 2008; Mitchell et al. 2008, 2011, 2013; Yeh et al. 2014; Mitchell 2017). Since 2016, researchers in central Russia and Siberia defined new patterns in infection. Host switching of beef tapeworm infection from reindeer to a human raised the possibility that archaeological parasitology can contribute to the new Stockholm Paradigm of ecological fitting, host switching, and emergent disease (Slepchenko et al., 2016; Reinhard and Slepchenko, 2018). From the 18th to the 20th centuries, Russian and native populations exhibited contrasting infection patterns (Slepchenko et al., 2019a). Only more recent people were infected with *A. lumbricoides* and *E. vermicularis*. In the earlier native sites, *Diphyllobothrium* sp. dominated the parasite spectrum with rarer occurrence of *Taenia* sp. For the 18th to 13th centuries, researchers consistently found three species of fish borne parasite showing that raw or undercooked fish was commonly eaten throughout this 400 year period (Slepchenko et al., 2019b). Studies of the changes in parasitism from premodern to contemporaneous times were also completed in South Korea (Seo et al., 2014a, 2014b). The South Korean methods from field excavation to autopsy were completely reviewed (Seo et al., 2014a). Case studies revealed infection with *Clonorchis sinensis*, *A. lumbricoides*, *T. trichiura*, *Metagonimus yokogawai*, *Strongyloides stercoralis*, *Trichostrongylus* spp., *Enterobius vermicularis* and *Paragonimus westermani* (Seo et al. 2007, 2008; Shin et al., 2009a, 2011a). The next papers explored the relevance of archaeological finds to modern distributions of parasites. One parasite, *G. seoi*, is of particular interest because the Joseon distribution of the species was broader than that of today (Shin et al., 2012). This total data set (Seo et al., 2014a, 2014b) was sufficiently robust to compare to recent South Korean parasite epidemiology. Fluke infections were much more common archaeologically than in 1961. In contrast, the prevalence of the fecal born parasites *A. lumbricoides* and *T. trichiura* did not show a marked change between Joseon people and their 1961 descendants. A wider perspective of ancient Korean parasitism has been developed through the analysis of sediments from sites belonging to the Silla and Baekje Kingdoms, which

overlapped in time (from roughly 18BC to 660AD) but occupied different parts of the Korean peninsula (Kim et al., 2016; Seo et al., 2016; Shin et al., 2009a, 2014b). Geohelminths were evident only in large cities but were absent at the smaller cities. This study was the first to suggest that in ancient Korea, egg contamination and infection risk was related to population density.

Other important archaeological applications for parasite findings include those related to diet, migration, and environment. Because many zoonotic parasites show high specificity for particular intermediate hosts, the identification of these parasites in archaeological contexts can provide direct evidence of the consumption of their host animals. For example, the identification of eggs from species of the *Diphyllbothrium* genus, which use fish as an intermediate host, in human coprolites indicates the consumption of infected fish (Ferreira et al. 1984; Bathurst 2005; Le Bailly et al. 2005; Le Bailly and Bouchet 2013; Perri et al. 2018). It also indicates these fish were eaten raw, undercooked, inadequately salted, incompletely smoked, or otherwise cured in a way that did not kill the tapeworm larvae.

After domestication, the consumption of livestock animals became a regular occurrence, and human infection with the tapeworms of these animals, especially those of cow and pig, due to improperly cooked meat, is found in archaeological contexts (Nemeth 1998; Matsui et al. 2003; Sianto et al. 2009; Le Bailly et al. 2010; Reinhard et al., 2013; Nezamabadi et al. 2013). Parasites may also help identify human consumption of species not otherwise identified in the faunal record, as seen in the identification of lizard parasites, indicating a long history of reptile consumption, in northeastern Brazil (Sianto et al. 2012). Equally, they may indicate the accidental consumption of parasite eggs present on unwashed water plants (e.g., watercress; Herrmann 1986), the consumption of grain beetles associated with agricultural products (Reinhard et al. 1987), and the practice of insectivory in ancient populations (Fry 1977).

Parasite eggs can also be used as an important indicator of human migration, movement, and trade (Slepchenko et al., 2019a, b). Parasites with restricted geographic ranges may be carried and deposited by their human hosts to locations in which that parasite is not infective, due to being unable to complete their life cycles, and thus being ineffective in parasitizing hosts. For example, Reinhard et al. (1987) report finding *T. trichiura* eggs from latrine soils at a prehistoric site in Flagstaff, Arizona. This species requires 21 days in warm, moist, shaded soils to complete its life cycle and become infective; yet this location would have had very dry soils inhospitable to the parasite. Reinhard and colleagues thus suggested that the presence of *T. trichiura* at the site represented a case of seasonal transhumance, with the human inhabitants moving periodically, bringing this particular parasite to the site from another wetter location (Reinhard et al. 1987). Similarly, Araújo and colleagues (1983) reported finding the marine fish tapeworm *Diphyllbothrium pacificum* (syn. *Adenocephalus pacificus*) in human coprolites from a site in Chile 40 km from the coast and 950 m asl, suggesting human movement between the mountains and coast or trade of marine fish between the regions.

Because of the particular ecological requirements in each parasite species, the identification of parasite microremains from archaeological sites, especially in the

case of the presence of multiple species, can help researchers reconstruct the location environment at the time of infection. They can also help track ecological and environmental change at site over time. For example, some parasites require time in the soil to mature and move to the next stage of their life cycles, and many of these require moist conditions for this process. Several researchers have noted the presence of parasites requiring moist soil at archaeological sites, which are currently dry, suggesting that in the past, these sites may have seen moister conditions, perhaps naturally or due to human intervention with irrigation (Moore et al. 1974).

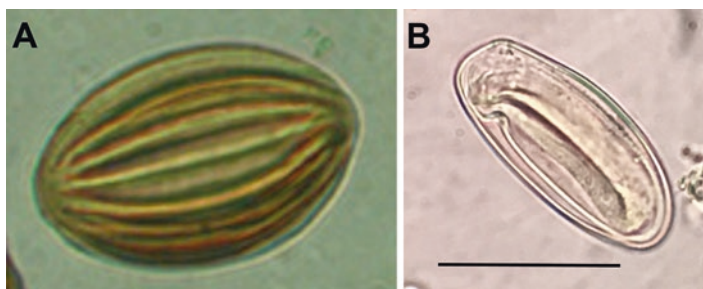
### 3 Identification and Artifacts

It is important for parasitologists working in archaeological sites to have some familiarity with palynology as pollen can easily be confused with parasite eggs (Camacho and Reinhard, 2019). Palynology is the study of pollen and spores, and palynologists have included parasite eggs as interpretable microfossils for decades, as reviewed by Brinkkemper and Van Haaster (2012). Beginning in the 1980s, palynologists began categorizing parasite eggs as non-pollen palynomorphs (Camacho and Reinhard, 2019). Palynomorphs are organic-walled microfossils resistant to palynological processing methods. Van Geel et al. (1983) and Pals and Van Dierendonck (1988) were the first to assign a non-pollen palynomorph type numbers to a parasite eggs (see also chapter “Non-Pollen Palynomorphs” by Shumilovskikh and van Geel, this volume).

The origins of archaeological parasitology have a source in palynological studies, especially when applied to sediments from archaeological sites (Hevly et al. 1979; Kuijper and Turner, 1992). The use of palynological processing to recover eggs goes on to recent times, and the interrelation of the two fields was recently formalized (Florenzano et al. 2012).

All of these studies relied on the expertise of palynologists to differentiate pollen grains from parasite eggs. This is necessary because pollen is ubiquitous in archaeological deposits, often in millions of pollen grains per gram of sediment (Reinhard et al. 2006). The value of this interdisciplinary approach is that researchers have experience in distinguishing the morphology of parasite eggs from pollen grains. There are tens of thousands of distinct pollen types (Traverse 2007). Some of these types can resemble parasite eggs, such as *Enterobius* eggs (Fig. 1). Familiarization with all parasite egg forms and potential confounding artifacts is critical for analysis in paleo- and archaeoparasitology. There are a number of references available to help identify parasite microremains and recommended for researchers in the area to familiarize in order to not confound parasites eggs with microresidues with similar shape and size (Fig. 2) (Spencer and Monroe 1961; Melvin and Brooke 1989; Peters and Gilles 1995; Ash and Orihel 1997).





**Fig. 1** Pollen grains of the *Ephedra* genus are sometimes wrongly diagnosed as *Enterobius vermicularis* eggs: (a) an *Ephedra* sp. pollen grain; (b) an *E. vermicularis* egg

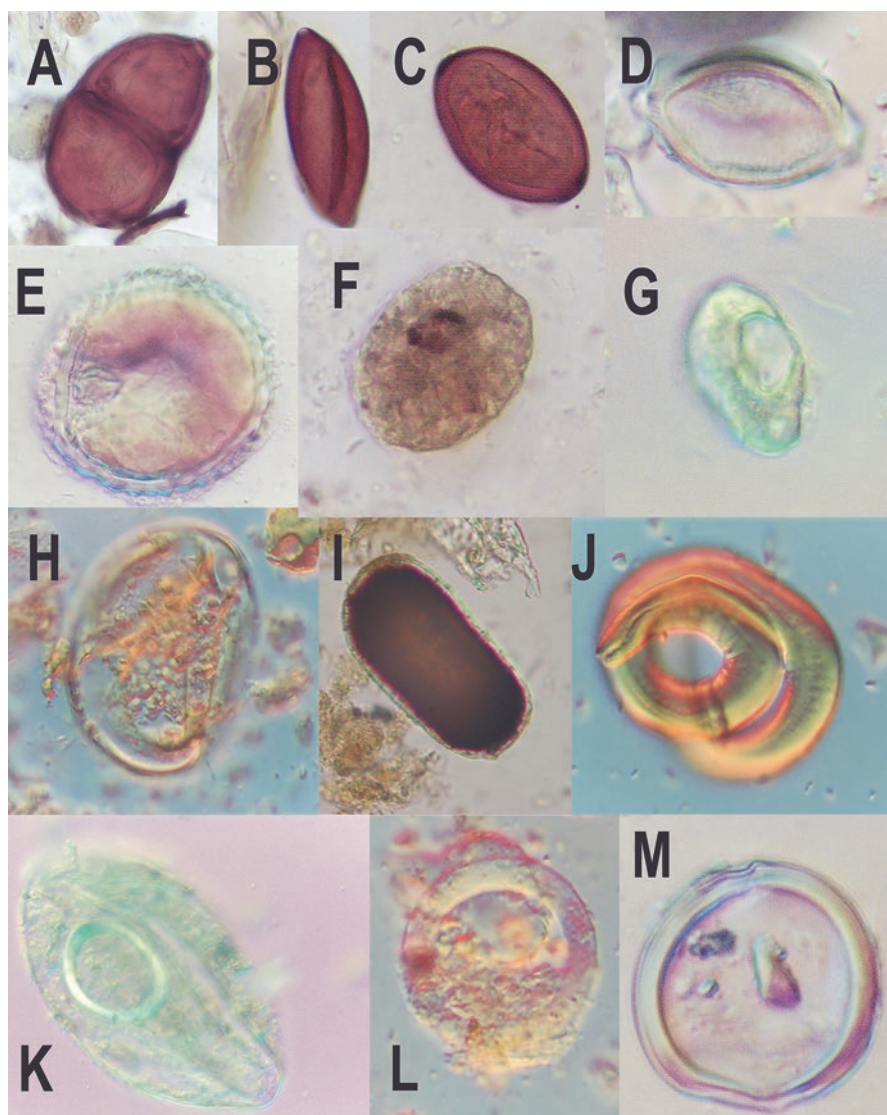
#### 4 Paleoparasite Recovery, Lab Methods, and Lab Supplies

Sample collection and transport are preliminary considerations in the field. The use of personal protective equipment is necessary to avoid contamination during sample collection. Disposable lab jackets, head protection, facemask, foot protection, and powder-free gloves are essential in this process. Powder-free gloves are important, since gloves that present powder may contaminate samples with starch that can interfere in the dietary data interpretation. Materials used to collect the samples need also to be disposable. Multiple sizes of plastic spoons and wood sticks are used. Metal instruments can be used only once without washing them; however, they can be re-used after adequate cleaning with hypochlorite (Sianto et al. 2011).

The amount of material to be collected should range between 100 and 300 mL, depending on how mixed fine sediments are with large inclusions. Whirl-Pak® plastic bags and sterile plastic tubes with screw caps are generally used to store samples for transportation. In case samples are stored in a plastic bag, it is recommended to put the bag containing the sample inside another bag and to use two labels, one for each bag. This is because bags with just a single label on the bag exterior can be separated from the label in transit. The double bag with double label approach ensures that no sample is found without identification. Ideally, a tag, adhesive label, or written label on the exterior of an outer bag should be matched by an interior label inserted between the outer plastic bag and an interior bag that contains the actual sample.

Transportation must be made considering the conditions in which the samples were in the moment that they are collected from the archaeological site. If samples were humid or dry, it is necessary to transport them to the lab in the same conditions. For samples that will be analyzed by molecular methods, it is recommended to use aluminum foil to completely cover the bags or tubes containing the samples, in order to avoid exposure to light, which can damage ancient DNA (aDNA) (Sianto et al. 2011).

Preliminary processing methods for shaft feature analysis were developed by Reinhard and colleagues (1986). Warnock and Reinhard (1992) formalized a method



**Fig. 2** Microremains that are not ancient parasites. (a–c) Coprophagous spores that were confused with trichurid eggs; (d) equatorial view of a pollen grain that was confused with a *trichurid* egg; (e) hypogeous fungal spores similar to an ascarid egg; (f) ill-defined structure suggested to be a protozoan cyst; (g) a plant cell identified as an *Iodamoeba* cyst; (h) a pollen grain associated with debris that looked like a nematode egg; (i) an air-filled lignified plant cell that was confused with a hookworm egg; (j) a free-living nematode juvenile; and (k–m) birefringent xylem rings which often are confused with parasitic structures. (k) A ring associated with the maize pollen grain. In bright field, a worker suspected that the ring was integral to the pollen and believed that this was a parasite structure. Polarized light shows that these are indeed two separate structures that are fortuitously associated. (l, m) Rings that look like taeniid eggs. (Images by Agustín Jimenez)

for simultaneous recovery of seeds, parasite eggs, and pollen grains from the same samples. Later study by another researcher (Bain 2001) confirmed the utility of the methods described for latrine sediments. These methods are further described in this chapter.

Reinhard (2017) stressed that interdisciplinarity is essential in scientific coprolite analysis. An ideal lab should be equipped to recover all types of dietary, environmental, and parasitic microfossils. No evidence should ever be thrown away. In addition, it should be equipped for molecular sampling. Camacho and coauthors (2018a) specified and described several acceptable methods for interdisciplinary processing. However, they specified two methods that are not interdisciplinary, these being the 1919 Lutz spontaneous sedimentation method and the more recent Reims method. The methods that are appropriate are the Callen, Searcey, and sediment methods (Camacho et al. 2018a).

The pathoecology laboratory at the University of Nebraska–Lincoln was designed for micro- and macrofossils analysis using all methods. Its design was based on that of the Texas A&M Palynology Laboratory. Both have distilled water outlets, acid-resistant plumbing flume scrubbers to remove airborne chemicals, multiple safety eyewashes, showers, and large countertop work spaces. To process soil samples, they are equipped with large, deep sinks, vortex stirrers, hot plates, metric balances, sonicator, fireproof chemical storage, research and teaching microscopes, and a large open work area equipped with desktop computers and printers. Dissecting microscopes are available for macrofossil identification. Both labs have “dirty” and clean rooms for preprocessing and microfossil processing. Extensive archive space is dedicated to storing original bags of samples and vials of extracts.

The ideal lab should be prepared to be a standalone analysis facility and a hub for receiving and sending samples to other labs. This is especially important for molecular analysis. The facility should be equipped in a way such that samples can be opened, subsampled, and then divided into sterile containers in a particulate contamination-free environment. In the modern world of coprolite analysis, samples may arrive from molecular lab having been verified as human by aDNA analysis. Conversely, samples may be recovered from the field, delivered to the coprolite lab, and then subsampled for molecular analysis. In the latter case, samples for molecular analysis should be immediately separated and stored. Separate sterile gloves, scalpel blades, and scales dishes should be available for each sample. There should be ample work surfaces that can be easily cleared and sterilized for subsampling. To prevent particulate contamination, the lab should be a positive pressure facility with air filtered of particles less than a micrometer. In ideal settings, a sterile glove area could be available, either within a fume hood or fabricated on an analysis counter surface. Climate control should be able to reduce temperature and humidity to levels not conducive for microbial growth. The lab fume hood should be large enough to hold 16 600 mL beakers and reagents. A six-foot (2 m) long fume hood is recommended as ideal.

For simple rehydration and screening of coprolites (Van Cleave and Ross 1947; Callen and Cameron 1960; Santoro et al. 2003; Sianto et al. 2005), mummy intestinal contents (Kumm et al. 2010; Piombino-Mascalì et al. 2013; Shafer et al. 1989),

or mummy intestine sections (Morrow et al. 2014; Reinhard 2017; Searcey et al. 2013), the basic needs are similar. For these analyses, equipment should include vortex stirrers; metric balances; sonicator; stir plates; *Lycopodium* spore tablets; screens of 250–300  $\mu\text{m}$  mesh; beakers of 600, 300, and 50 mL sizes; filter drying paper; disposable gloves; glass stir rods; micro-spatulas with round and tapered ends; and eye protection. Centrifuges will be needed for both glass 12 mL and polypropylene 50 mL tubes. In some cases, it is useful to have a microcentrifuge with 1.5 and 2.0 mL tubes. Wash bottles for separating microremains from macroremains are needed. Reagents should include alcohol, trisodium phosphate, and dilute hydrochloric acid. In some cases, potassium hydroxide is needed to disaggregate coprolites from mummies.

For chemical processing of sediments, all of the above will be needed. These methods have been detailed extensively (Fisher et al. 2007; Florenzano et al. 2012; Hevly et al. 1979; Kuijper and Turner 1992; Warnock and Reinhard 1992). However, the more intensive use of acids requires more reagents and safety material. Calcium gluconate cream for hydrofluoric acid exposure, acid aprons, suitable sizes of gloves to ensure effective double gloving, and labeled reagent disposal bottles are necessary. Vented chemical storage cabinets must draw fumes into the fume hood area and/or to the outside. Additional necessary reagents are acetic anhydride, glacial acetic acid, hydrofluoric acid, and sulfuric acid. For hydrofluoric acid processing, stable polypropylene 500 mL beakers are needed.

The lab microscopes should be able to use bright field. However, diagnosis is more certain if it has additional capabilities. Basic polarized light will help to distinguish dubious objects of lignin and starch. Differential interference contrast (DIC) or Nomarski microscopy can be used to image details of the eggs and larvae. For standard counting and diagnosis of parasites in the University of Nebraska Pathoecology Laboratory, the Zeiss JenaVal microscope equipped with various types of phase contrast imaging including Nomarski phase, dark, and light field phase contrast, is used. This older instrument has been upgraded for digital image capture with an Olympus OM-D E Mark II, a C-mount adapter, and a C-ring. Olympus Capture Image software is available for Mac and PC from the Olympus website. In this way, older instruments with high-quality optics that were produced before the digital era can be made into high-quality digital capture tools.

The second instrument is a new Nikon Eclipse compound microscope equipped with a Lumenera Infinity-1 camera working in conjunction with Infinity Image Capture software. The pathoecology lab partners with the The University of Nebraska–Lincoln's Manter Laboratory of Parasitology. This lab is equipped with a Zeiss Axioplan 2 Imaging system on an automated microscope system that facilitates scanning and counting.

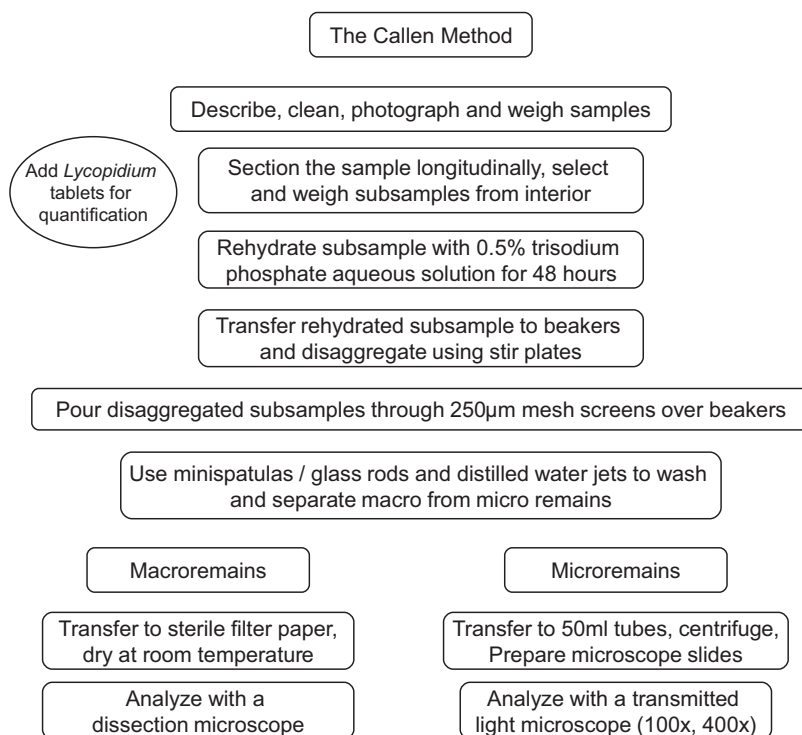
## 5 Laboratory Processing

A recent paper (Camacho et al. 2018a) reviews processing methods for coprolites and mummies. That review recognizes two periods of research. In the first period of the latter half of the twentieth century, researchers were focused on presence or absence of infection in studies of individuals. As large numbers of coprolites and mummies were analyzed, by 1977, prevalence studies emerged and comparisons between cultures, subsistence patterns, and geographical areas were established. Assessment of intensity of infection was rarely done and was limited to a single study by Jones (1983). In the first decade of the twenty-first century, researchers showed more interest in determining the pathology caused by infection. Recognizing that relatively few individual infections provoke disease, a new interest emerged in assessing the relative infection intensity represented in mummies and coprolites.

Several papers demonstrated that clinical methods were not consistently satisfactory for mummies and coprolites (Camacho et al. 2018a). For presence/absence and prevalence studies, rehydration rapidly became standard and was the first step for other methods. The most common method was rehydration and screening such that macroscopic and microscopic remains were separated. These remains were then used for parallel diet and parasite analyses. This was the Callen method which was adaptable to infection intensity analyses (Fig. 3) (Camacho et al. 2018a). Often, empty mummy intestinal sections are recovered. The Searcey method was developed for such remains (Camacho et al. 2018a). The intestine section is rehydrated. Microfossils from the exterior are recovered and used as a control. Then the section is opened, and microfossils are recovered from the interior including parasite eggs, pollen, starch, and other microfossils (Fig. 4).

The comparative evaluation of parasitological techniques as applied to ancient samples has been a long-standing area of study (Dufour and Le Bailly 2013; Reinhard et al. 1986). Coprolites, for example, are not preserved equally, even from sites of the same culture and in the same region, and even from the same structure as discussed for Aztec rooms 219 and 225 (Camacho et al. 2018b). This fact is demonstrated by Ancestral Puebloan coprolites from the Colorado Plateau. Coprolites from the same latrine, but different strata, were either mineralized with calcium carbonate or alternatively preserved by desiccation (Camacho 2017). Depending on the preservation of the sample, different processes are used before the microscopic analyses, including rehydration and dilute hydrochloric acid processing techniques (Camacho 2017). For desiccated coprolites, the Callen standard rehydration technique with added *Lycopodium* spores was used. For mineralized samples, the HCl Callen method was used. Both methods are described by Camacho et al. (2018a).

Processing methods for sediment samples have been derived from palynology and modifications of standard parasitology procedures. Much of this work was extensively reviewed in the early years of the field, and detailed descriptions of several methods were presented (Reinhard et al. 1986).



**Fig. 3** Flowchart illustrating the Callen method

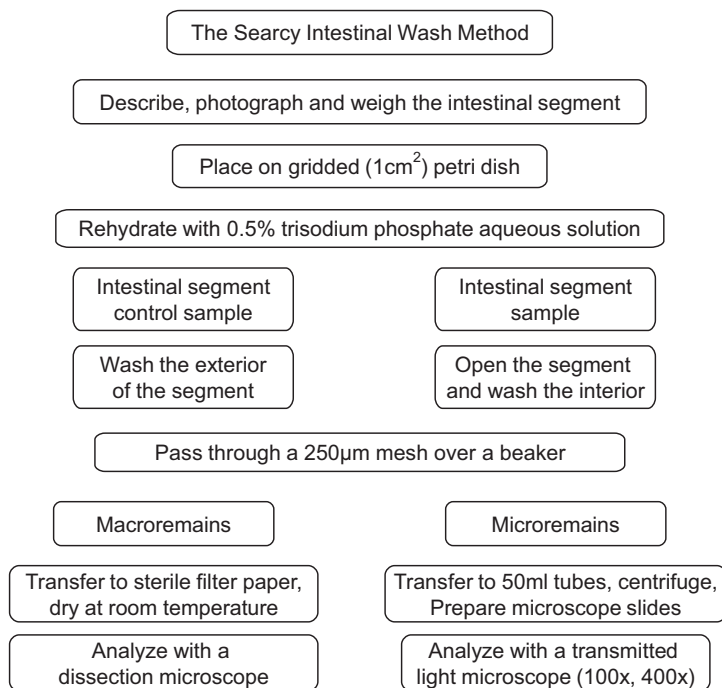
## 6 Recommended Methods for Paleoparasite Recovery from Sediments

Two methods for the recovery of paleoparasite eggs from sediment samples are recommended. For this purpose, sediment refers to loose or consolidated soils from archaeological features. Two comparable methods were developed around the same time periods: the modified Stoll technique (Jones 1982, 1985) and the modified palynology method—the swirl technique (Warnock and Reinhard 1992; Florenzano et al. 2012).

### 6.1 Jones Modified Stoll Method

Jones's original method (Fig. 5) is as follows: 3 g of sediment is disaggregated in 42 mL of water. Then the fluid is filtered through a 250 µm mesh. Microscope slides are prepared by measuring 0.15 mL aliquots of the filtrate onto standard 3" by 1" slides. Two or three drops of warmed glycerine jelly are added to the slide and covered with a 50 × 22 mm coverslip. The slides are scanned and all parasite eggs are





**Fig. 4** Flowchart illustrating the Searcy method

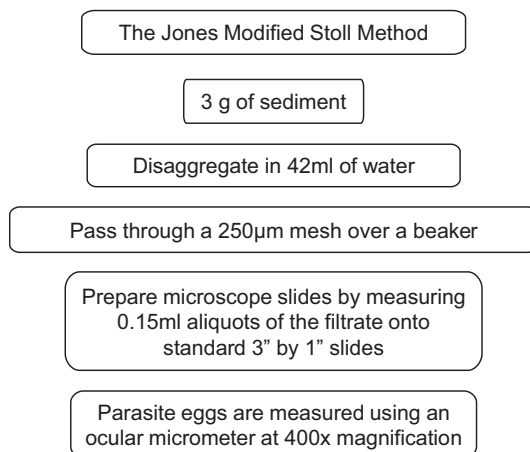
measured using an ocular micrometer at 400× magnification. This method has the advantage that all potential microremains of the correct size range (pollen, starch, phytoliths, spherulites, etc.) will be preserved and potentially visible in the sample. Heavier processing methods such as the palynological method below may lead to the destruction of the more fragile microremains (e.g., starch grains).

## 6.2 *Palynological Method Derived from Warnock and Reinhard (1992)*

Palynological processing involves digestion of inorganic components while preparing for quantification by adding known quantities of exotic spores or pollen. It offers the advantage over the Stoll method of liberating microfossils while eliminating over 95% of the organic and inorganic components of sediment samples.

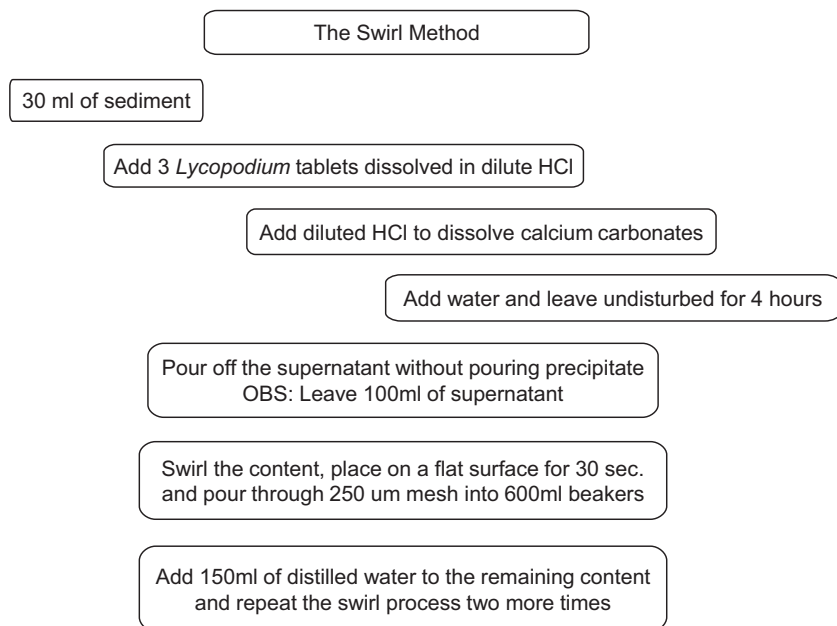
Quantification of microfossils is facilitated by calculating concentrations of objects by using added *Lycopodium* spores. By introducing a known number of *Lycopodium* spores into weighed samples, the concentrations of microfossils can be calculated per gram or milliliter of sediment (Reinhard et al. 2006). If the samples come from an area where *Lycopodium* grows endemically, then *Eucalyptus* pollen tablets are used. *Lycopodium* tablets can be purchased at the Department of Geology,

**Fig. 5** Flowchart demonstrating the Jones modified Stoll method



Lund University. Alternatively, black ceramic microspheres can be used for quantifications in microfossil analyses (Kitaba and Nakagawa 2017).

First, 60 mL of fine sediment of each sample is screened through a 1.0 mm mesh. From the screened sediment, 30 mL of each sample is measured out for analysis. These are weighed so that parasite egg concentrations can be calculated in terms of eggs per milliliter and eggs per gram. In order to perform quantification of microfossils in the samples, *Lycopodium* tablets are introduced according to the volume of sample measured. Since 30 mL of sediment is weighed, three *Lycopodium* tablets are used (one for every 10 mL of sediment). Before introducing the tablets, it is necessary to dissolve them in dilute hydrochloric acid (HCl) in 50 mL beakers. After weighing, the 30 mL sediments from each archaeological sample is added to individual 600 mL beakers and labeled with that sample's assigned laboratory number. Preliminary observations are made regarding sediment color, texture, and soil make up. The dissolved *Lycopodium* spores are added to the sediment by thoroughly rinsing the 50 mL beakers three times into the 600 mL beakers containing the sediments. Then ten drops of 30–40% HCl are added to test whether or not the samples need to be treated with this acid. If a reaction is noted, the reactive samples are treated with dilute HCl until calcium carbonates are completely dissolved and the microfossils are liberated. This is signaled by a cessation of bubbling or fizzing reaction. Twenty milliliters of HCl is added at a time. When the addition of more acid does not elicit a continuing reaction, then this stage is concluded. It is important to be aware that fragments of shell that are not removed from the sediment will continue to react, even when the general reaction stops. Distilled water is added when the reaction between the acid and the carbonates stops. At this stage, the sediment is prepared to decant. The beakers are left undisturbed for at least 4 h. After this time, the majority of the supernatant is poured off of the precipitate. It is important to do this carefully, not pouring off the precipitate. This can be done by leaving 100 mL of supernatant in the beaker and to stop pouring if the precipitate begins to move toward the beaker spout.



**Fig. 6** Flowchart demonstrating the Swirl method

At this point, one applies the swirl technique, which removes the heavy, nonorganic particles from the samples and separates macrofossils, such as seeds, on the screens (Fig. 6). The precipitate and 100 mL of supernatant are transferred to 300 mL beakers. For each sample, a clean, labeled 600 mL beaker must be set out and covered with a 250  $\mu$ m mesh. The contents of the 300 mL beakers are swirled until all particles are in suspension. The beakers are placed on a flat surface for 30 s. After 30 s, the fluid from the beakers is poured through 250  $\mu$ m mesh screens into 600 mL beakers. After this process, 150 mL of water is added to the beakers containing the precipitate, and the swirl technique is repeated two more times. By third swirl, the supernatant should be translucent.

The sediments are washed three times in distilled water. Then the screened fluids are concentrated by centrifugation in 50 mL centrifuge tubes. Then the sediments are transferred into labeled 500 mL polypropylene beakers. After that, 50–100 mL of 48% hydrofluoric acid is added to each beaker, and the sediments are thoroughly mixed in the acid. The samples are left in the hydrofluoric acid for 24 h and are stirred occasionally during this period. The samples must be examined periodically to prevent drying. If the acid shows evaporation, then more acid must be added. Then, the sediments are concentrated by centrifugation in 50 mL polypropylene centrifuge tubes. The sediments in the tubes are then washed many times in distilled water until the supernatant is clear. This can take as many as 30 sequential centrifugations. Each one involves centrifuging the tubes at high rpm for 1 min. The supernatant is poured off. The sediments are suspended with a vortex stirrer. Distilled

water is added to the 45 mL gradation on the graduated tube. Then the centrifugation is performed, and the translucence of the supernatant is assessed. If cloudiness is observed, then the supernatant is decanted, and the process of adding water, vortex stirring, centrifugation, and visual examination is repeated. Once the supernatant is clear, the precipitate is transferred to achievable one dram vials.

The macrofossils on the screens are examined especially for the presence of seeds. Sometimes, ectoparasites, especially louse eggs on hair shafts, are recovered with macrofossils. The screened macroscopic remains are dried and transferred to Petri plates marked with 1 cm grids. The macrofossils are distributed over the grids and counted.

For archaeoparasitological analysis, drops of the sediments are transferred to glass microscope slides with Pasteur pipettes or applicator sticks. The sediment drops are mixed with glycerin and covered with glass cover slips. A minimum of six preparations are examined, and a minimum of 25 *Lycopodium* spores are counted for each sediment sample. Parasite eggs and added *Lycopodium* spores are counted. The concentrations of eggs are determined by the ratio of eggs to the known number *Lycopodium* spores added to the samples. Identification of the genera of the parasite eggs is done by morphological analysis. Concentrations of parasites, pollen grains, and starch grains are calculated with the following formula: **Microfossil concentration** =  $((f/m) \times e)/v$ , where  $f$  = microfossils counted,  $m$  = marker *Lycopodium* spores counted,  $e$  = *Lycopodium* spores added, and  $v$  or  $w$  = volume or weight of sediment.

Labs must have adequate storage space to archive the vials of sediment after analysis. Slides can also be kept, but microscope slides in glycerin have a storage life of 20–30 years. In contrast, samples stored on one dram vials in glycerin have an indefinite storage life, if the vials are sealed with parafilm and screw caps. More recently, Poly-Seal screw caps have been introduced, and these seem to offer an alternative to parafilm.

Chemical digestion of sediment components uses reagents selected to target the calcium carbonate, silica, and cellulose. These three components are present in most sediment samples and are removed sequentially. Hydrochloric acid is the first reagent used, and it targets calcium carbonate. Calcium carbonate forms a matrix that traps microfossils. For latrines in the Americas, lime or seashells were often privy additives, forming calcium carbonate over time even in environments where it does not form naturally. By breaking down the calcium carbonate, the microfossils are released. Silica is a second common component of sediment often present as loose sand or more rarely clay. Hydrofluoric acid has long been used to break down hard matrices to liberate organic remains. It reacts with the sand, silicon dioxide, to produce the gas silicon tetra fluoride and liquid water. After the silica is dissolved, the remaining sediments are organics. At this point, the samples can be scanned and parasite eggs counted along with *Lycopodium* spores.

## 7 Preparing Microscope Slides

Slides should be prepared with a curation life of 20–30 years. Permanent mounts can be made with glycerin, glycerine jelly, or Canada balsam. In the pathoecology lab, it is preferred to mount in glycerin and to seal the slides with commercial, clear nail lacquer. Slides so prepared have a storage life of up to 30 years. The glycerin preference related to the fact that all of the thousands of parasitology extracts are curated in one dram vials in glycerin. Therefore, if a slide is broken or dried out after some decades, new slides are easily and rapidly prepared from the curated vials. The slides should be available to visiting scholars and also be available for mailing to distant consultants for joint research. Recently, it has become fashionable to share TIFF or JPEG images for collaboration. This is appropriate if the images are of good quality, and the specimen has not ambiguous features. However, out of focus images or those of plant cells or sand should really be shown to a collaborator as microscope slides. Water mounts are not a permanent storage solution.

## 8 Distribution of Parasite Eggs

Parasite eggs are not randomly distributed across the landscape. Eggs, larvae, and cysts are initially concentrated in areas of deposition. Over time, they may percolate in any direction depending mostly on fluid movement in sediments. Therefore, field archaeologists should be attuned to the places parasite eggs are most likely to be deposited. This should include latrine features of all sorts (barrels, shaft features, abandoned habitations), burials, drains, streets, and trash. In addition to the samples being analyzed for paleoparasites, control samples should be taken laterally and vertically with respect to the evidentiary sample. For burials, evidentiary samples are in the sacral area, and control samples should be taken of the burial fill, usually from the head and foot region and from fill just outside the burial. Methods for burial microfossil sampling have been presented by Reinhard et al. (1992) and Berg (2002). Urban landscape sampling strategies have been presented by several researchers (Fisher et al. 2007; Jones 1982, 1985; Reinhard and Pucu 2014; Trigg et al. 2017).

Latrines, or “night soil,” deposits can provide important information about parasitic loads and household health. Sediments from latrines must be sampled stratigraphically to identify levels that are parasite-rich (Reinhard 2017). Sometimes, diet-related micro- and macroremains, such as pollen from *Brassica* and *Fagopyrum* and seeds from *Rubus* species, can signal fecal deposits, even when parasite eggs are rare. Importantly, pollen and seed evidence of fecal deposits can potentially be used to define households with low levels of parasite infections (Bain 2001). If pollen and seeds indicate night soil presence, but if few or no parasite eggs are found, this suggests a low level of infection. In contrast, night soil deposits with high numbers of parasite microfossils signal an infected household. By assessing the numbers of eggs present in fecal deposits from latrines of different households, one can compare

relative levels of infection. Similarly, by sampling a number of shaft features dated to long periods, one can identify emergence and control of parasites. Control and reduction of parasite counts can be linked to individual household behaviors or community action that limited infection (Fisher et al. 2007; Trigg et al. 2017).

## 9 Percolation Movement of Paleoparasite Remains and Sediment Constitution

Reinhard (2017) emphasizes that parasites in archaeological contexts occur in abundance in limited contexts. The author also references studies that show percolation of microfossils, including parasite eggs, through sediments. Therefore, parasite eggs should be numerous from intact contexts. Discoveries of one parasite-like structure during extensive examination of sediments are likely to be contaminants or misdiagnoses.

Percolation is a phenomenon that interferes directly with the findings of parasite eggs and other microremains in ancient sediment samples. There are two issues that need to be considered when one analyzes sediment samples. The first is the protection that it is usually given to the microremains by the coprolites. When dried feces are formed in the environment, the parasite and diet microremains are protected inside the coprolite, thus contributing to their preservation and concentration. The second issue is the dispersion of microremains through the archaeological strata, which leads to problems like the loss of microremains that are carried by water percolation or animal movement to the deep strata. This can contribute to the degradation of the microremains. Even if the microremain resists degradation and can be found, it becomes hard to contextualize the finding and to establish its antiquity, sometimes causing a misinterpretation in the finding's chronology. Factors like soil components and consistency, rainfall, and water movement of nearby sources need to be evaluated individually in order to establish the interference of percolation at each site context. Though many authors consider water percolation as an important taphonomic factor that interferes in microremains findings in archaeological sites, experimental evaluations have not been extensively done (Dent et al. 2004; Araújo et al. 2011; Rácz et al. 2015; Camacho et al. 2016; Morrow et al. 2016).

One type of site used as a model to study the influence of water percolation in ancient parasite findings is the Brazilian shellmounds or *sambaquis*, as they are known in Brazil. The people that built them, *sambaquieiros*, represent several different cultures (Roksandic et al. 2014). *Sambaquis* are archaeological sites intentionally built throughout most of the Brazilian coast by hunter-fisher-gatherers that inhabited this region from 10,000 to 2000 years Before Present (B.P.) (Gaspar et al. 2008). These sites were built near water sources like rivers, lakes, and bays, in which a valuable subsistence ecosystem could be found. *Sambaquis* are especially characterized by the constant presence of shells, one of the materials used in their construction. Some sites could reach 30 m high and 500 m long, and archaeologists believe that they were landmarks for shellmound people



(Gaspar et al. 2008). Burials and evidence of habitation constructions can be found at these sites, which indicate that shellmound people probably used these sites both as a ritual and dwelling place.

Fish bones, clay, sand, charcoal, and ashes were used as materials to build these sites in addition to shells. Due to the extremely moist and hot environment, coprolites do not preserve in this environment. Dietary data are obtained from dental calculus and stable isotopes analyses (Bastos 2014; Boyadjian et al. 2016; Wesolowski et al. 2010). Parasitological data have been obtained from the analyses of sediments collected from the pelvic girdle of the burials and compared with the results from control sediments. Despite the large number of burials and evidence of daily activities, findings of parasite eggs and DNA are rare. One hypothesis suggested to justify this rarity is the possible influence of water percolation through the archaeological layers of shellmounds, since the proximity of these sites with water sources could subject them to variations in water level, besides constant rainfall, which is normal at the Brazilian coast in all seasons. However, the experiment performed indicated an intense biodegradation instead of water percolation as the main factor that probably contributes to the rarity of parasite remains findings in *sambaquis*.

Previously, processing of 121 shellmound samples was performed with a parasitological technique specific to the analyses of sediment (Caldwell and Caldwell 1928). Despite the specificity of the technique, no parasite remains were recovered. For this reason, together with the percolation experiment, three parasitological techniques were evaluated in order to test which one(s) could be considered the best option to analyze sediment samples. The techniques were selected based on their function: one to concentrate (spontaneous sedimentation), described by Lutz (1919); and two based on the flotation process (modified centrifugal flotation with sucrose solution—specific gravity (sg) = 1300, and centrifugal flotation with zinc sulfate solution—sg = 1180) with different specific gravities that allow to recover objects based on their density (Sheather 1923; Faust et al. 1939; Hubber et al. 2003).

Results showed that sediment constitution seems to interfere directly in the choice of the parasitological technique. The spontaneous sedimentation technique was more efficient in sandy soil, while the modified centrifugal flotation with sucrose solution (sg = 1300) was more efficient in clay soil. This result demonstrates the importance of the evaluation of soil components to determine the best option for parasite recovery (Camacho 2013).

## 10 Taphonomic Factors Affecting Paleoparasite Recovery

Taphonomy refers to factors that influence in the transition of organic matter from biosphere to the lithosphere (Lyman 2010). There are several variables that are considered both in the macro- and the microenvironments, and conditions need to be individually evaluated at each archaeological site (Morrow et al. 2016). A few studies concerning the influence of taphonomic factors in the

degradation of parasite eggs in ancient material have been published (Reinhard et al. 1986; Leles et al. 2010; Camacho et al. 2013; Rácz et al. 2015; Morrow et al. 2014, 2016). In these studies, variables like the coprolite rehydration process, the interference of coprophilous fungi, and soil pH were suggested to explain rarity of findings in some archaeological sites. Still, taphonomy remains as a subject that requires investigation regarding the influence of each factor in parasite egg degradation. Experimental studies are mostly complex, and the factors must be evaluated individually.

Nonetheless, researchers of ancient parasites are increasingly interested in taphonomy since all findings and absences demonstrated through the analyses of archaeological materials are the result of the influence of taphonomic processes that may have contributed to the preservation or degradation the materials. Morrow et al. (2016) published a pioneering study in which five taphonomic categories are suggested as an organization structure to work with each site's unique taphonomic conditions. With the five categories developed by the researchers, one can evaluate on a relative basis the abiotic, contextual, anthropogenic, organismal, and ecological factors based on observations of the studied sites and consider which ones contributed to the degradation of the parasite remains. The abiotic category involves all the nonliving environmental conditions; the contextual involves the particularities related to the material from which the parasites were recovered (coprolites, sediments, mummies, jars, etc.); the anthropogenic refers to the influences of human interaction with the ancient material from the moment of its deposition to the collection, transportation, and analysis; the organismal is related to each parasite (life cycle, egg resistance), and the ecological refers to the influence of organisms in the parasite remains (fungi, nematode larvae, mites).

In a general sense, biotic conditions are the most important for helminth egg taphonomy. However, each site offers a different environmental perspective that makes the taphonomic discussion specific. Coprophilous fungi are suggested as the main ecological factor responsible for parasite egg degradation in archaeological sites (Reinhard et al. 1986; Leles et al. 2010; Camacho et al. 2013; Rácz et al. 2015). For a long time, coprophilous fungi were believed to be the main factor that interfered in parasite egg degradation in shellmounds (Leles 2010; Teixeira-Santos 2010; Camacho et al. 2013). However, no coprophilous fungi spores were found in the *sambaqui* sites characterized by extreme parasite egg degradation (Camacho et al. 2016). Instead, bacteria spores of *Bacillus* and correlates were observed in one of these shellmounds. There are some soil bacteria of this group that are used for insect control, since it produces chitinases that break down the chitin present in insects' exoskeleton (Bravo et al. 2007; Schnepf et al. 1998; Maagd et al. 2001; Minard et al. 2013). Parasite eggs are also made of chitin, thus indicating the potential of this bacterium to degrade the eggshells. Experimental evaluation of this process is in progress. Soil nematodes also seem to be an ecological factor that interferes in parasite egg preservation (Camacho et al. 2013). The relationship between nematodes and parasite egg degradation is still not proven; yet previous study relates them with the decomposition of soil protozoans, thus indicating their potential as taphonomic factors (Georgieva et al. 2005).

Mites also appear to be important in taphonomic processes affecting coprolites and parasite egg preservation (Camacho et al. 2018b). Mites are related in the literature to free-living nematode predation through several lab experiments (Epsky et al. 1988; Martikainen and Huhta 1990; Muraoka and Ishibashi 1976; Read et al. 2006; Santos et al. 1981). Despite this proven correlation, the role of mites in the degradation of parasite eggs is still not proven experimentally. Mites are frequently seen in Ancestral Pueblo coprolite samples, and for this reason, the correlation between the presence of this taphonomic agent and the degradation of pinworm eggs was evaluated in two Ancestral Pueblo sites at Salmon Ruins and Aztec Ruins (Camacho et al. 2018b). All latrines at the sites presented mites in different quantities. A statistical regression was made in order to verify the correlation between the presence of mites and pinworm egg degradation in these sites. Results showed that mites were correlated with pinworm egg degradation in locations where the preservation was the worst.

The general preservation found in Ancestral Pueblo sites is excellent (Reinhard and Bryant 2008) due to these sites being located in dry environments, described as the best kind of condition to preserve parasite remains. Pinworm eggs, considered fragile and rare in ancient parasites studies, are easily recovered from these sites (Reinhard et al. 2016; Camacho et al. 2018a). However, egg preservation in open pueblo sites is not as good as at sites inside caves (Camacho and Reinhard 2019). Explanations for the different levels of preservation were previously discussed, but no correlation was proven (Reinhard et al. 1986). These include good preservation in desiccated coprolites (when desiccation is rapid), cool and dry environments, and most latrine soils. Bad preservation is observed in environments with decomposition due to fungal organisms and freeze/thaw cycles (Reinhard et al. 1986). Taphonomic factors still need to be individually evaluated with experiments. In some cases, in the same site, different conditions can be found. This requires caution when one aims to interpret the taphonomic scenery and consequently the infection patterns observed in archaeological sites.

## 11 Conclusion

Parasitology became an archaeological science decades ago. It is relevant to the interpretation of a variety of extinct human activities and infection patterns with health consequences. Over the last 30 years, numerous reviews assessed emerging applications, theories, and models of parasitology to archaeology. Until quite recently, one could count on accuracy in diagnosis as a foundation for archaeological parasitology. As an archaeological science, multidisciplinary researchers from the 1960s onward were more versed in distinguishing plant cells, fungal bodies, sand grains, and general detritus from true parasite eggs. In the last decade, diagnostic precision eroded. The goal of this chapter is to orient those interested in the field with the variety of structures that can be confused with eggs.

Although taphonomy was recognized as a significant force affecting egg recovery and morphology for many years, only once in the twentieth century did parasitologists combine their expertise to address this subject. As reviewed in this chapter, aspects of taphonomy have become the focus of intense scrutiny, experimentation, and modeling. These studies, summarized here for the first time, will guide the community toward establishing principles of preservation applicable in all types of archaeological remains in which eggs can be recovered.

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**Part III**  
**Micro-Particles Used Widely for Both**  
**Environmental and Behavioral**  
**Reconstruction**



Corrie Bakels

## 1 Introduction

Pollen is derived from plants. It is the microscopic particle that transports the male seed cells formed in the anther to the pistil with the female egg cell. In most plants, the particle has to travel from one flower to another, in many cases even from one plant to another. Its name is borrowed from Latin and means flour. The word has no plural. When needed, its alternative, pollen grain, provides the plural: pollen grains. The study of pollen is known as palynology. Most pollen is transported through the air; only pollen from some aquatic plants is transported by water. Air transport is provided by wind, by insects, and in some cases by bats or other animals. To protect the seed cells from the outer world, they are encased by a wall which is very resistant to chemical and physical attack. Its main constituent is a substance called sporopollenin. Only bacteria and fungi in a damp environment with access to air (oxygen) are able to break down the sporopollenin. It is this outer wall that makes pollen of interest to archaeologists, because it does not decay readily and makes fossilization possible. The wall is sturdier in pollen that has to travel through the air than in pollen that is transported by water.

The size of the grains differs per plant species. Most grains range in size between 20 and 50  $\mu\text{m}$ , but other sizes are found as well. Small grains, 5  $\mu\text{m}$ , are for instance produced by forget-me-nots (*Myosotis*), while very large ones, 350  $\mu\text{m}$ , are produced by cucumbers (Cucurbitaceae). Generally, pollen are produced and released in large quantities. The greatest numbers are provided by those plants that have to rely on wind and air currents for their pollen dispersal. Pollen, especially airborne pollen, can travel far. An Atlantic weather ship 450 km off the Norwegian coast and 1700 km from the nearest forests with oak reported 5.5 grains of pine, 4.7 of birch,

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0.8 grains of oak, and 5.5 grains of grasses per square centimeter per season (Faegri and Iversen 1989). Grains are even found on the North and South Pole (Kappen and Straka 1988; Ukraintseva and Sokolov 2003).

If the outer wall had been the same shape for every plant, pollen would not have been of interest despite their ability to fossilize. But fortunately there are differences in their form and outer sculpture that make it possible to identify the plants that released the grains (see Plates 1 and 2 at the end of the chapter). However, in some plant families, the diversity is less well developed than in others. Pollen of grasses (Poaceae), for instance, looks almost always the same, and even in families where more differences are observed, identification at the species level is rare. In this aspect, pollen analysis offers less exactness than the analysis of macrobotanical remains such as fruits and seeds. On the other hand, pollen travels much further from its parent plant and bears witness of its occurrence even at a distance.

Lagerheim (1902) was one of the first scientists to study pollen retrieved from sediments, especially peat. He recognized the importance of pollen for the study of past vegetations. Von Post (1916) combined the results from pollen counting with the stratigraphy of the sediment from which the pollen grains were obtained. He invented the pollen diagram and is regarded as the founding father of pollen analysis. The year 1916 is considered as the year of birth of palynology.

## 2 Uses of Pollen Analysis in Archaeology

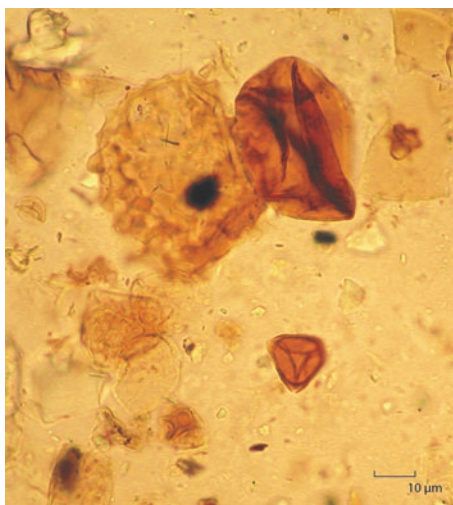
In the following, the information based on pollen analysis and of interest to archaeologists will be discussed on three levels: the single species, the combination of several species within one single time slice (a pollen spectrum), and the combination of a series of slices through time (Sect. 2.3).

### 2.1 The Single Species

As written above, identification up to the level of plant species is only possible in a minority of cases. Identification at the level of genus is more common, but in regions where a genus counts only one species, the genus can be treated as species. Such is the case of ivy (*Hedera*), holly (*Ilex*), and mistletoe (*Viscum*) in northwest Europe. The plants are important climate indicators, a characteristic that has been recognized by the Danish palynologist J. Iversen.

Iversen combined the occurrence of flowering *Hedera*, *Ilex*, and *Viscum* with the data provided by nearby weather stations in Europe. He found that their distribution was governed by temperature, in the case of *Hedera* and *Ilex* the mean temperature of the coldest month, and in the case of *Viscum* the mean temperature of the warmest month. *Hedera* does not flower (and release pollen) below  $-1.7^{\circ}\text{C}$ , *Ilex* not below  $-0.5^{\circ}\text{C}$ , and *Viscum* not below  $+15.5^{\circ}\text{C}$  (Iversen 1944; Zagwijn 1994). As their

**Fig. 1** The triangular pollen grain of clove (*Syzygium aromaticum*) in the fill of a cesspit. The object with warts diagonally above the grain is an egg of *Ascaris* (see chapter “Non-pollen Palynomorphs” by Shumilovskikh and van Geel, this volume) (Photo Biax Consult).  
Bar = 10  $\mu$ m



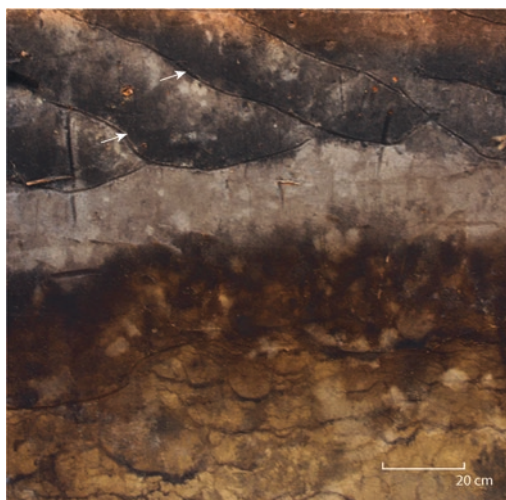
pollen is known not to travel great distances, detection of their pollen in an off-site deposit contemporaneous with an archaeological site provides information about the temperature of the period. Off-site is necessary, because on-site situations may concern imports, though in the case of these three plants this is not very likely.

Another example of the merit of species identification is the fact that some plants, valuable to people, can only be detected by their pollen, because other parts are not preserved. This applies, for instance, to some spices and condiments. An example is clove (*Syzygium aromaticum*), the flower bud of a tree that has its natural habitat in the Moluccas, an Indonesian archipelago. The dried bud is used worldwide. Cloves have never been found as macroremains in archaeological contexts, even not water-logged ones. But the buds contain pollen and this is preserved in, for instance, medieval and later cesspits with a water-logged fill (Fig. 1).

## 2.2 Pollen in a Spectrum

All pollen grains retrieved from a single sample taken together constitute a so-called pollen spectrum. They represent the plants releasing pollen within a certain time slice. The length of this period depends on the context. A sample taken from a deposit formed within a short time differs from a sample from a deposit that formed slowly. For example, a sample from a thin layer of sediment at the bottom of a lake that took centuries to form will contain pollen from those centuries while a layer of plant matter dumped into a ditch may represent 1 year. A spectrum is composed of several pollen types. Some of these represent a species; others, a genus; and still others, a family. In archaeology, pollen spectra are used in several ways. The following studies may serve as an example.

**Fig. 2** Section of a sod-built barrow. In this case, the original surface was removed till the yellow subsoil was reached. The sods lie upside down in a slanted position. Their surface is indicated by an arrow. Bar = 20 cm. (Photo Q. Bourgeois)



As stated above, pollen does preserve in many environments, but not where sufficient moisture in combination with oxygen allows bacteria and fungi to destroy sporopollenin. Pollen deposited on the surface in forest or grassland, for instance, decays. This process takes one to several decades (Heim 1970). In cases where the surface is suddenly covered by a sediment blocking the access of air, the process is stopped. This implies that the uppermost part of an ancient buried soil contains pollen released by the vegetation present at the time of burial. This fact is used to reconstruct the vegetation present during the construction of barrows. The pollen grains found belonged to the plants smothered by the barrow and to the surrounding vegetation of which pollen had become trapped in these plants. Pollen from nearby and even remote localities ultimately falls down on earth and mixes with the locally produced pollen. Most of this pollen coming from elsewhere is pollen washed out from the air during rainfall. Local and non-local pollen together make up the spectrum detected under the barrow, though pollen from the actually buried plants predominates.

In this way, it could be established that many Bronze and Iron Age barrows constructed on Pleistocene sands in the Netherlands had been built on existing open patches in an otherwise forested landscape and that these patches were covered with heath (*Calluna*). These areas were not cleared by felling trees for the purpose of creating the barrow as was thought previously. As such heath covered areas are not natural, human interference with the landscape must have preceded the erection of the barrows, presumably by herding livestock for a considerable period of time (Doorenbosch 2013). The building material of the barrows consisted of sods, and because their surface was also smothered in the process of building, pollen retrieved from sods provided information on the place where they were cut (Fig. 2). In the case of the barrows, it became clear that they had been cut close by (Casparie and Groenman-Van Waateringe 1980; Doorenbosch 2013). This kind of investigation is not restricted to barrows. Any structure built on an original surface and/or

constructed with the help of sods, such as banks, dykes etc., may reveal information on former vegetation.

Other examples of the use of spectra are studies of the contents of vessels. Pollen analysis is reported to be successful when the vessels were found in particular environmental conditions: absolutely dry, waterlogged, near or below freezing point, or with a high toxicity (Rösch 2005). The last mentioned factor is present in the case of the contents of copper or bronze vessels. An interesting study concerns the bronze vessels found in the Iron Age princely burial of Glauberg, Germany. Rösch investigated the contents of two pitchers and detected a series of herb pollen which could without any doubt be attributed to the presence of honey (Rösch 1999). Honey contains pollen from the flowers visited by the bees, a fact that at present is used to assess the origin of honey sold in shops in order to detect fraud. In the Iron Age, drinks made from honey (mead) or flavored with honey were quite common, but what makes the Glauberg spectra especially interesting is that honey from different kinds of environment was involved. The flowering plants could not have been part of a single kind of vegetation type. Reconstruction of the several different vegetations present in the far surroundings of Glauberg revealed that the places where the plants grew were too far apart to have been visited by one bee population. Honey bees have a certain range in which they visit flowers and this is rather restricted. This implies that several bee keepers must have contributed honey to make the drinks. The alternative, of one bee keeper traveling around with the bees as is quite common today, is considered less probable. The pollen grains suggest that the bee keepers lived at distances of 20–90 km from Glauberg. If the honey was part of the tribute given to the prince buried at Glauberg, the distances may give an indication of the extent of his domain (Rösch 1999).

A third example of what single spectra may bring is the analysis of coprolites, i.e., fossilized excrements. The droppings of carnivores and omnivores in particular are able to turn into stony objects, due to the abundance of calcium phosphate originating from consumed bone, which assists in the formation of coprolites. Coprolites contain pollen, and the origin of this pollen may be ingestion with food (stomach contents of prey or plant matter), with water, by licking fur, and by breathing. Much pollen is caught in nasal mucus and swallowed with it. Vermeeren and Kuijper (1993) studied coprolites from Roman dogs found in The Hague, Netherlands, and concluded that their pollen content reflected both what they had eaten and also the environment in which the dogs had nosed around. Not represented was the season in which they left their droppings. They based their conclusion on the study of recent fox droppings collected during 2 years in a dune area not far from The Hague, which had an environment similar to that during the Roman period as ascertained by other means. Recent dog droppings were unsuitable for the study, as modern dog food is quite different.

Other animals well-known for their coprolites are hyenas. Pleistocene hyena coprolites from Montenegro and Serbia provided data on the vegetation in a region where other pollen sources are lacking (Argant and Dimitrijević 2007). Comparison of the pollen content of hyena coprolites from Equus Cave, Taung, South Africa with the pollen content of the cave's sediments showed that the coprolites

represented a far wider area than pollen retrieved from the cave. This is explained by the wide range of hyenas, 15 km, while the sediments in the cave seem to have trapped very local pollen (Scott 1987). Coprolites from different stratigraphic levels, and therefore from different periods, provided even a vegetation history of the Taung area.

A fourth example is the use of pollen analysis to detect the place where an object may have been before it was found. The technique was used, for instance, to follow the travels of the “Ice Man,” the frozen remains of a man found in Ötztal, Italy. Groenman-Van Waateringe (1991) studied pollen trapped in his clothing. But a still more famous example is the study of the shroud of Turin, a Christian relic that is said to be the burial cloth of Christ. Several analyses have been carried out to prove that this cloth has been in Jerusalem, a discussion that has not yet come to an end (Boi 2017 and the literature mentioned there).

### 2.3 *The Pollen Diagram*

When spectra from a single location but of different age are stacked in a chronological order, the result is called a pollen diagram. The pollen diagram is a graph, and the combination of spectra in one single graph asks for an adjustment of the data offered. This is necessary because raw numbers cannot be compared. A slow forming sediment catches pollen from a longer period than a fast forming one. As pollen is commonly extracted from a definite sample volume (or sometimes weight), the number of pollen grains present in a sample from a sediment that had been slow in forming is higher than that found in a deposit that formed over a short period of time. The resulting discrepancy can be corrected with a multitude of  $^{14}\text{C}$  dates, but this is expensive and not always possible. The solution is to revert the numbers into percentages, but then another problem turns up, i.e., overrepresentation of pollen shed by plants growing near the place of sampling. The majority of pollen lands close to the parent plant, even in the case of wind-pollinated species. Therefore, the pollen grains deriving from such plants are not used in the summing up of numbers to calculate percentages. Those that are used constitute the so-called pollen sum. As most sampling for pollen diagrams takes place in marshy areas, peats, or lakes, pollen from water-, marsh- or waterside-plants are left out of the pollen sum. In those cases, the resulting sum is a dryland (also called upland) pollen sum composed of trees, shrubs, and herbs that grow on dry locations. Per spectrum, their numbers are added up to provide the sum that serves as 100%. The numbers of wetland plant pollen are also converted into percentages based on this sum and these percentages can exceed 100%, a fact that is confusing for people not familiar with pollen diagrams. For example, in a sample with 40 grains of oak, 50 grains of hazel, 10 grains of cereals, and 200 grains of alder, retrieved from alder carr peat, the respective percentages are 40%, 50%, 10%, and 200%, because the alder must have been a very local pollen shedder. Numbers of local types are converted to percentages based on the upland types (which functions as an independent standard) to enable

the study of changes in the wetland vegetation. If it is impossible to know the local plants beforehand, for instance when dealing with sections in dry sediment, excluding plants is impossible. In such cases, all counted material is taken together in a so-called total pollen sum. It is always wise to be informed of the pollen sum used when comparing percentage diagrams from different publications, as values differ when the composition of the sum is not identical.

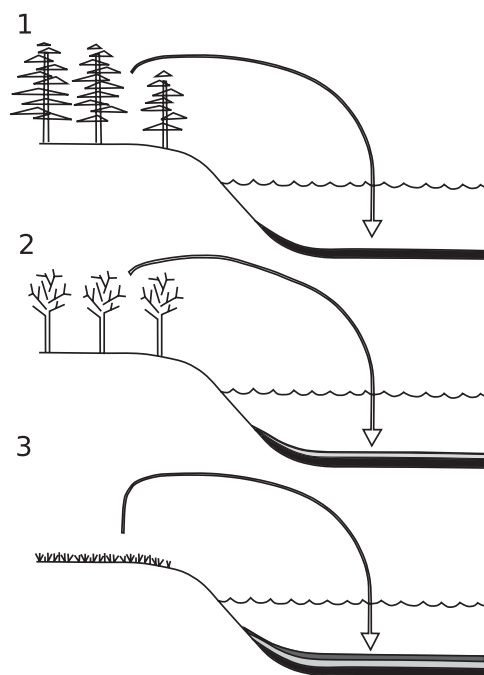
As mentioned above, numbers are commonly reverted to percentages, but sometimes knowledge of the real pollen influx, i.e., the number of pollen grains falling on a unit area of sediment in a given time, is required. This is, for instance, the case when the number of plant species or families included in the pollen sum is low and the percentages of one type of pollen grain show a sudden rise. A rise might imply an increase in importance of the plant releasing this pollen, but it is also possible that other plants are disappearing without any increase of the plant showing a rise. In such cases, the rise is caused by the fact that percentages represent relative values. To solve this problem, pollen diagrams in which real numbers are presented per unit of volume or weight are combined with a number of dates providing a time scale. Such diagrams offer the best possible information on the influx and are called influx diagrams. The idea behind this is that if the importance of the plant in question stays the same, its pollen influx value also stays the same (but see biases mentioned at the end of this chapter).

The main purpose of making pollen diagrams is to reconstruct the vegetation through time. When a basin fills up with sediment, the deposits at the bottom reflect an older period than the subsequent layers. The topmost one is the youngest. Pollen trapped in the sediments represent a history and if the sediments were continuously waterlogged, this pollen is preserved (Fig. 3). The pollen-based reconstruction of the vegetation history contributes to the knowledge of the past in several ways. In the following, examples are given and these are also used to show the different ways in which percentage diagrams are illustrated or visualized in the literature.

One common aim is the reconstruction of climate and climate history. As mentioned before, by their presence or absence some plants can provide data on climatic aspects, but it is more common to look at changes in entire vegetations. The climatic fluctuations at the end of the Pleistocene were detected in this way. Figure 4 presents a diagram for north-central Europe (after Van der Hammen 1949). The percentages of trees (arboreal pollen commonly given as AP), heather (*Ericales*) and all herbs except heather (other herbs), are stacked together to reach 100%, but as the development of individual tree species may be of importance, their curves are drawn within the AP part where they all figure as separate curves. This is a quite common way of presenting a pollen diagram. The values of each spectrum are connected by straight lines. The individual curves of the trees can cross each other, and to make it possible to follow each of them, the position of the values entered is not drawn as a simple black dot but indicated by a symbol. In Europe, some symbols are standardized (Fig. 5).

The diagram is horizontally split into zones. This is customary in pollen diagrams and serves to make the description and interpretation easier. A zone is a part of the diagram where the pollen assemblage as a whole differs from the pollen

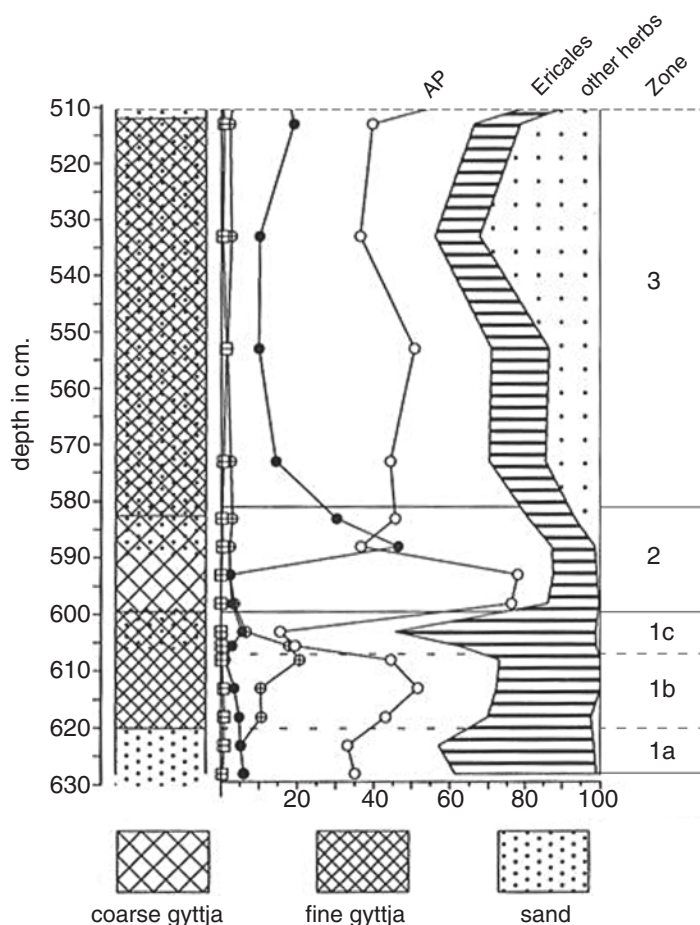




**Fig. 3** The build-up of a history of vegetation record. Pollen released by vegetation 1 is preserved under water-logged circumstances on the bottom of a basin; its successor vegetation 2 left its pollen in a sediment on top of the bottom-layer; pollen from vegetation 3 is preserved in the next layer, etc.

assemblage below and above. Zone boundaries can be drawn visually or with the help of a statistical program. In principle, a zone has meaning only for the diagram it was drawn for. But if the same kind of zone appears time and again in pollen diagrams, zones acquire a more general meaning.

In zone 1a trees are relatively few, but in zone 1b a rise in willow and birch pollen percentages is observed (Fig. 4). This is explained by a climate change which allows the growth of shrubs and trees. In zone 1c tree and shrub percentages fall again which is explained by a return to a colder climate. In zone 2 the climate changes again toward a situation sufficiently warm for trees, in this instance birch and later pine, as the period lasts long enough for pine seed to reach the region and for pine to outcompete birch. In zone 3 the climate deteriorates again. The zones drawn in



**Fig. 4** Pollen diagram reflecting climate-steered vegetation change at the end of the Pleistocene. Location Hijkermeer (Drawing after Van der Hammen 1949). For symbols, see Fig. 5

this diagram are recognized in other regions as well, and they have since acquired names. Zone 1a is called the Earliest Dryas, the first period with a warmer climate is known as the Bølling interstadial, zone 1c is the Earlier Dryas, and the second warm period is called the Allerød interstadial. Zone 3 is the Late Dryas. The name Dryas is derived from an Arctic plant of which leaves were found in the deposits; the others are derived from Danish locations where they were first detected in a pollen diagram. It goes without saying that such climatic fluctuations did not affect only plants but animals and humans as well.

**Fig. 5** The most common pollen symbols used in north and west Europe

X	<i>Abies</i>	spruce
□	<i>Alnus</i>	alder
○	<i>Betula</i>	birch
▲▽	<i>Carpinus</i>	hornbeam
◆	<i>Corylus</i>	hazel
▲	<i>Fagus</i>	beach
△	<i>Picea</i>	fir
●	<i>Pinus</i>	pine
⊕	<i>Salix</i>	willow
■	<i>Quercetum mixtum</i>	deciduous forest
—	<i>Quercus</i>	oak
----	<i>Tilia</i>	lime
.....	<i>Ulmus</i>	elm

Vegetation history is also of importance to assess the natural resources available to humans and to describe the influence of humans on their environment. The vegetation during a single narrow time slice can be reconstructed through single spectra, but long-term developments such as the impact of human behavior on the landscape can only be studied through pollen diagrams. One of the main activities of humans is obtaining wood, felling trees, and creating more open space. These are precisely activities that can be detected in pollen diagrams. Where people live, trees disappear.

A famous example is the vegetation history of Easter Island (Rapa Nui). At present this remote island is covered by grassland and some small stands of imported tree species. But this was not always so. Pollen diagrams reveal that in the past palm-dominated forests were present (Rull et al. 2013). The diagram depicted in Fig. 6 shows the vegetation history before and after the arrival of the first settlers, mainly Polynesians (European contact dates from AD 1720). The diagram is an example of another common style in presenting diagrams: the resolved diagram composed of individual curves called saw blades (connection of the separate values makes the individual graphs look like saws). The arrival of the first humans is marked by pollen of *Verbena littoralis*, a plant known to be linked to Polynesian activities and not naturally growing on the island. This kind of pollen is also known as anthropogenic indicator. Charcoal particles, a proxy for the use of fire, increased in number. Tree pollen percentages (Palmae) declined gradually and those of grasses (Poaceae) increased. In the end the effect is a landscape bare of trees and this is attributed to deforestation by humans.

Such declines can also be sudden events as will be shown in the following example. It serves also to present a third common way to draw a pollen diagram, which is the stacking of bars. This kind of diagram is mainly used by scientists who are of

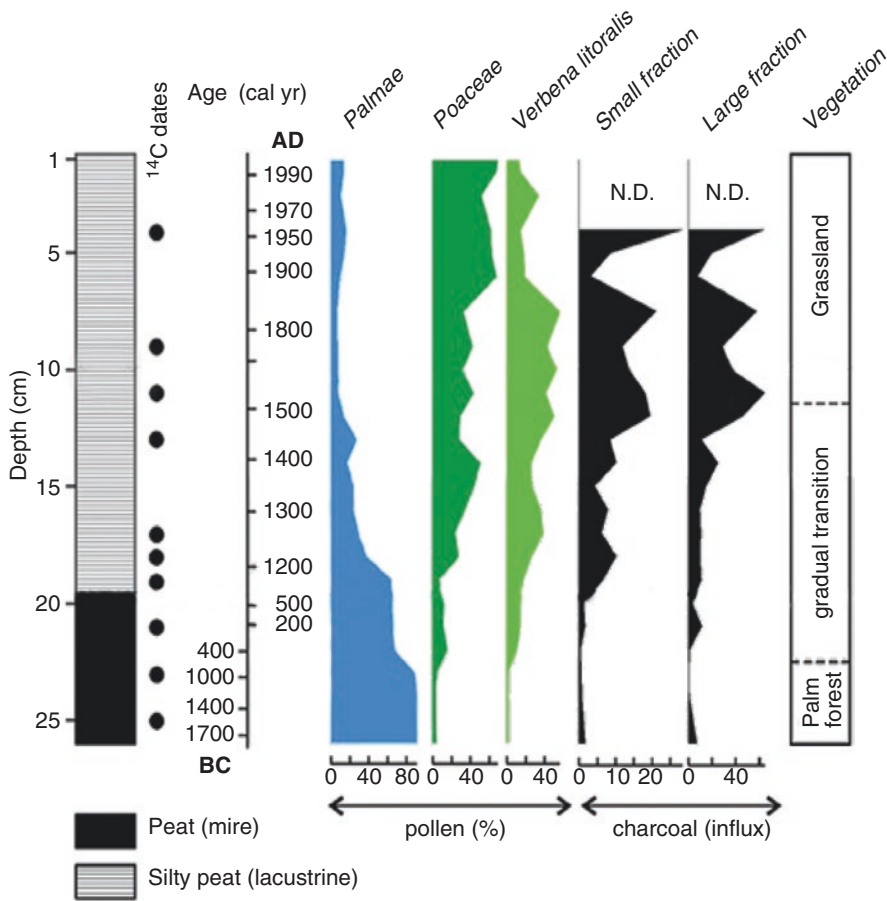


Fig. 6 Pollen diagram from Lake Raraku, Easter Island. (After Rull et al. 2013)

the opinion that separate findings (spectra) should not be linked by lines. Presented in Fig. 7 is a diagram that shows a sudden fall in tree pollen (AP) percentages accompanied by plants indicating cereal fields. Shown are the percentages of cereals excluding rye (*Cerealia minus Secale*), rye (*Secale cereale*), cornflower (*Centaurea cyanus*), goosefoot family (*Chenopodiaceae*), and wild grasses (*Poaceae*). The abrupt change in the vegetation is not due to a hiatus in the deposits, as confirmed by the  $^{14}\text{C}$  dates. It can be linked to the founding of a medieval rural estate, of which the huge barns were excavated in the close vicinity (Bakels 2017).

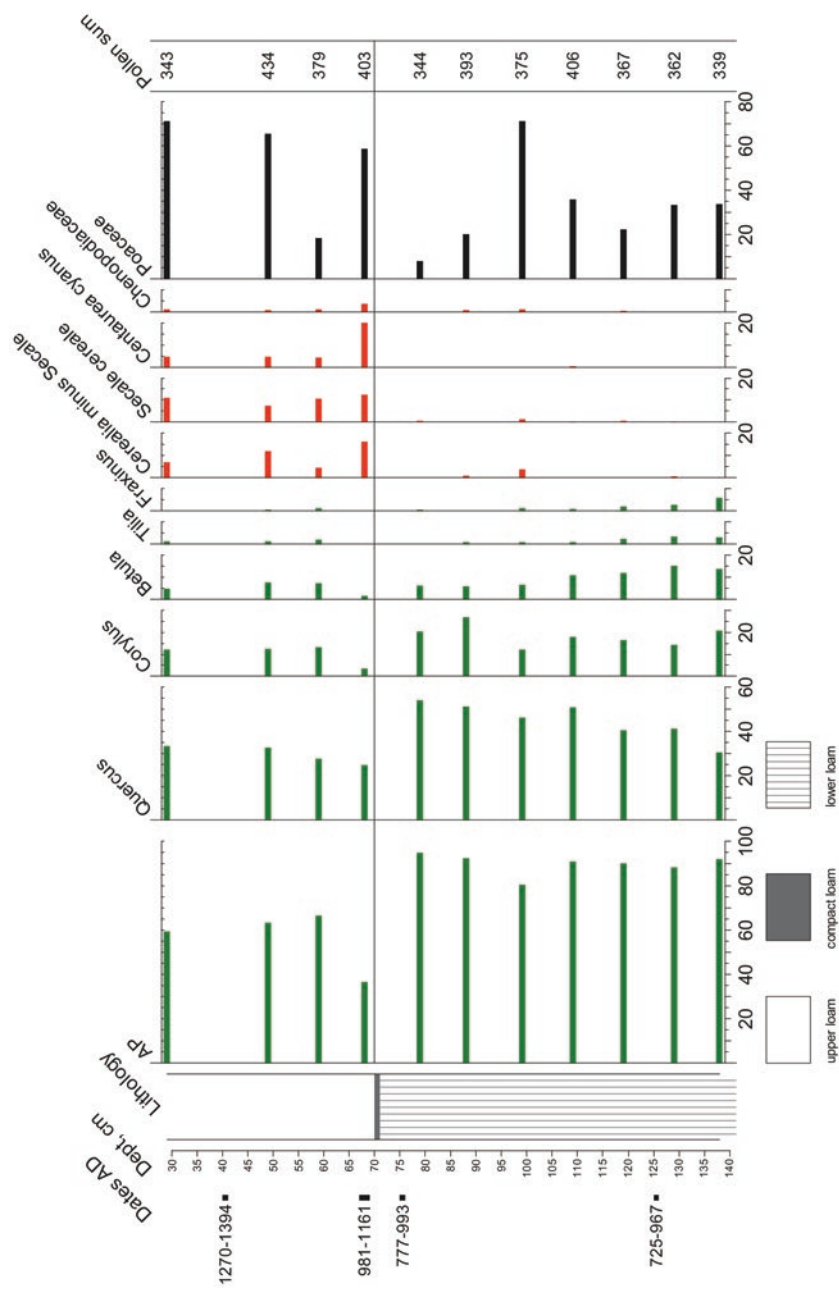


Fig. 7 Pollen diagram from Swalmen. (After Bakels 2017)

### 3 Sampling for Pollen and the Subsequent Treatment of the Samples

#### 3.1 *Types of Samples*

The most common source of ancient pollen is a wet sediment that has never dried out during its existence. Suitable off-site locations are silted-up former river beds, lakes, peats, etc. Every location has a distinct catchment area for pollen. For example, pollen obtained from a sediment in the center of a large lake represents an area with a radius of several kilometers around the place of sampling. Pollen obtained from a small pond may represent an area with a radius of c. 1 km (Sugita et al. 1999 and the references therein). When the aim of the research is to study the vegetation in the near surroundings of a settlement, a sampling place not further away than 1 km is advisable. When the impact of a large population on the wider environment is the goal, sampling a large lake or bog will provide the required information. Nevertheless, a series of diagrams obtained from small basins dispersed over the landscape will provide a better resolution. The resulting mosaic of small-scale reconstructions offers more detail than the generalized diagram from a large basin. An example is offered by the study of the Flögeln area, Germany (Behre and Kucán 1986). However, this approach is far more expensive. Moreover, some types of landscape offer better opportunities for pollen analysis than others. This is mainly due to the hydrology of the land. In well-drained regions, the right places are very difficult to find and often almost or totally absent, due to the lack of stagnant water which provides the right environment for the preservation of fine sediments and plant matter. The ideal sampling strategy is almost never achieved.

Less common sources of off-site pollen are buried soils and colluvial deposits. A special case of the study of soils is mentioned above in the section on pollen in a spectrum and regards buried surfaces, but in certain types of soils, especially acid soils, pollen is preserved without the sealing-off from the air by structures on top. Such soils show pollen stratification, though the mechanism behind this is still not entirely understood. Nevertheless they can serve to make pollen diagrams and yield information on past environments (Moore et al. 1991). For instance, paleosol studies of loess deposits offer insight in the past vegetation of desert and desert-steppe landscapes including the cold versions of these kinds of environment. They are often used when studying the Paleolithic (see for instance Rousseau et al. 2001; Tang et al. 2017), but need not be restricted to this period (Paez and Prieto 1993).

A special version is the anthropogenic soil type known as “plaggen soil,” an arable soil originating from manuring arable fields with sods (plaggen in Dutch and German). These sods were cut in the environment, brought to the stable where they served as litter, and, once drenched with fecal matter, spread out over the fields. The resulting soils are rich in well-preserved pollen (Groenman-Van Waateringe 1988; Bakels 1988).



Colluvia present another example of a dry kind of deposit from which pollen diagrams can be produced. An example is offered by a study of a part of NW Spain where suitable wet deposits are lacking, but colluvia provide the records for assessing prehistoric land use (Kaal et al. 2013).

On-site water-logged sources of pollen are moats, wells, and cesspits. Moats and wells are sometimes used to reconstruct the environment of a site, but it must be kept in mind that their fill is not only derived from areas around the site. Their fill contains also matter thrown in by the inhabitants or fallen in from the banks or yards next to the place of sampling. Such material is rich in pollen and does not necessarily reflect the environment. The main component of cesspits is household waste and food remains. Their content serves to reconstruct human behavior rather than the natural environment.

On-site dry environments commonly produce pollen data that are single spectra, but true diagrams can be obtained from, for instance, cave deposits. Although the interpretation of the pollen records from caves is subject to some debate (Carrión et al. 1999; Fiacconi and Hunt 2015), they result in views on the local environment.

### 3.2 *Sampling for Pollen*

The nature of sampling is determined in part by the nature of the archaeological material, and by the questions the researcher seeks to answer. Individual samples from sediments are taken with the help of a spoon or putty knife. Another possibility is to push a tube into the material. Attention should be paid to contamination by older, younger, or even surface material. Surfaces that were exposed for some time to the air should be avoided, because of the pitfalls connected with degradation (see the section on biases below). Sample size depends on the kind of matrix, but volumes the size of a small fist are almost always sufficient, even when taking into account accidental losses during the processing of the samples. The absolute minimum size is 1 cm<sup>3</sup>. If the sample must also provide macroremains to allow <sup>14</sup>C dating, larger samples are advisable. All this concerns “everyday” situations. In special cases, like dealing with coprolites, the contents of vessels or the surface of textiles, the sample size is dependent on the availability of the material and the nature of the source. The method to be applied has to be devised according to the situation. Scalpels or adhesive tape may be of use.

Samples can be stored in plastic bags or glass containers. It is best to store them in cold storage or another cool place, but if the samples are processed within a reasonable time, the absence of cool storage is not dramatic. Deep freezing is not needed, except for a very long period of storage.

If the aim is to make a pollen diagram of a section, the section can be sampled in the same way. Sections can also be sampled by hammering in monolith tins (Fig. 8). Common tins are made of zinc and are 50 cm long, 15 cm wide, and 10 cm deep.



**Fig. 8** Monolith tins in a section. (Photo Archol B.V.)

Two holes in the back may serve to let air out during hammering. Rubber hammers are preferred as they will not damage the tin. The tins are marked on the outside to indicate top and bottom, and wrapped in airtight plastic foil. Sections longer than 50 cm are sampled with tins in parallel lines with an overlap. The overlap is necessary because the sediment caught in the end of tins is always disturbed. More often than not, precisely these ends represent an interesting horizon, not remarked during sampling. The overlap ensures that this horizon can be sub-sampled in the second box. After the hammering, the tins are cut loose with a long, sharp knife. If a

sediment, such as stiff clay, resists hammering in tins, the outline of the tin can be marked on the section's surface and the surrounding sediment cut away to leave a prominence exactly the size of the tin. The tin is then gently put over the prominence.

Sections are the preferred sampling sites, because they offer a good view on the deposit with its possible horizons and local differences, but sampling sections cannot always be achieved. In such cases, coring is the only way to obtain samples. Many types of corers are on the market. There are side-filling corers and end-filling corers. Some can be operated manually; others require more machinery. Each aim and each location requires its own kind of corer. Cores are usually stored in PVC pipes. If their contents are to be kept for a longer period, they should be stored in a cool place or a fridge. More information on sampling may be found in textbooks on palynology such as those by Faegri and Iversen (1989) and Moore et al. (1991).

### 3.3 Laboratory Processing

Processing is done in a specialized laboratory. Individual samples or sub-samples taken from cores are treated with a series of chemicals with the ultimate goal to remove all kinds of matter except pollen. As mentioned before, pollen is very resistant to chemicals. A common sample size is 1 cm<sup>3</sup>. If it is desirable to establish the number of pollen grains per 1 cm<sup>3</sup> of sediment or peat, a tablet with a known number of pollen of an exotic species not likely to be found in the deposit is added before treatment (method Stockmarr 1971). A common exotic is not a pollen type but the spore of *Lycopodium* (a club moss). Recently some researchers have advocated for the use of black spherical ceramic balls (Kitaba and Nakagawa 2017), as these are easily recognizable and exotic in all samples, unlike *Lycopodium*. Both *Lycopodium* and ceramic balls are commercially available.

The exact treatment depends on the kind of sediment or peat. Recipes can be found in all handbooks on pollen analysis, for instance in Faegri and Iversen (1989). Many laboratories use their own varieties. The main steps are deflocculation, removal of large particles, removal of humic acids (unsaturated organic soil colloids), calcium carbonate, mineral particles, and plant material (cellulose) other than pollen. Deflocculation and removal of humic acid is often achieved in the same step: treatment with 10% KOH. Coarse particles are removed by very fine sieving. A treatment with 10% HCl dissolves CaCO<sub>3</sub>. Mineral particles are heavier than organic material and can be removed by specific gravity separation in a liquid with a specific gravity of 2.0. The alternative is to dissolve them in cold or hot HF. Cellulose is removed by boiling in a mixture of nine parts anhydric acetic acid and one part concentrated H<sub>2</sub>SO<sub>4</sub>, a treatment called acetolysis. Between all steps, thorough washing with water or other liquids is necessary. The procedure implies the use of fume hoods and a centrifuge. Coprolites may be treated in the same way, but good results are also obtained by scraping off the surface to avoid contamination with recent matter, dissolving (part of) them in phosphoric acid (85% for 3–4 h) and to continue with the specific gravity and acetolysis procedure.

The result is preserved in glycerol or silicone oil. In ideal cases the result is pollen together with material that is as resistant as pollen such as non-pollen palynomorphs (NPP) and charcoal (see chapter “Non-pollen Palynomorphs” by Shumilovskikh and van Geel and chapter “Microscopic Charcoal Signal in Archaeological Contexts” by Marquier and Otto, this volume). Pollen can be stained to make them more visible. A red stain (fuchsin or saffranin) is the usual one. For analysis, a drop of the material is mounted on a glass slide and covered by a cover-slip. Mounts can be sealed by paraffin, nail polish, or other sealing materials.

The subsequent identification and counting of pollen requires a high-quality microscope and magnifications of at least  $\times 400$ . For some identifications a magnification of  $\times 1000$  is needed, or phase contrast microscopy. The use of a reference collection of recent pollen obtained from the flora of the region under study is highly recommendable. This pollen should resemble the pollen encountered in the sediments, which implies the removal of all matter except sporopollenin. This is achieved by acetolysis of the recent pollen grains. In addition to the reference collection, books with identification keys and with photographs are available, though for parts of the world where pollen analysis has only few practitioners, these kinds of support fall sadly short. A world list of Quaternary pollen and spore atlases is published by Hooghiemstra and Van Geel (1998).

For the drawing of a pollen diagram, several versions of software are on the market. A universally used program is Tilia for the calculations and TiliaGraph for the actual drawing (Grimm 2015), but other programs exist as well.

## 4 Biasing Factors

The main bias in pollen analysis is that pollen does not exactly represent the former vegetation. Some pollen does not preserve because of a lack of (sufficient) sporopollenin in their outer wall. This is for instance a problem of rushes (*Juncus*). Differences in preservation lead also to other biases in counts.

Some pollen types disappear faster from the records than others. One of the last types to remain are Asteraceae section Cichoridae, which can dominate in samples with bad preservation. Degradation of pollen types does not follow a fixed pattern, because pollen that disappears readily in one type of soil may still be found in another kind of soil, while with other pollen types this is the reverse. Experiments with burying recent pollen in different soil types have been carried out to gain an insight in this kind of bias. For instance, spores of the fern *Polypodium* are very resistant in river clay, but in *Sphagnum* and sedge peats they are susceptible to decay. Pollen from sweet gale (*Myrica*) disappears readily from river clay, but survive in peat (Havinga 1984). Phuphumirat et al. (2015) found in their study of mangroves that each depositional setting provided a unique set of variables with regard to the preservation of the pollen shed by specific mangrove tree species.

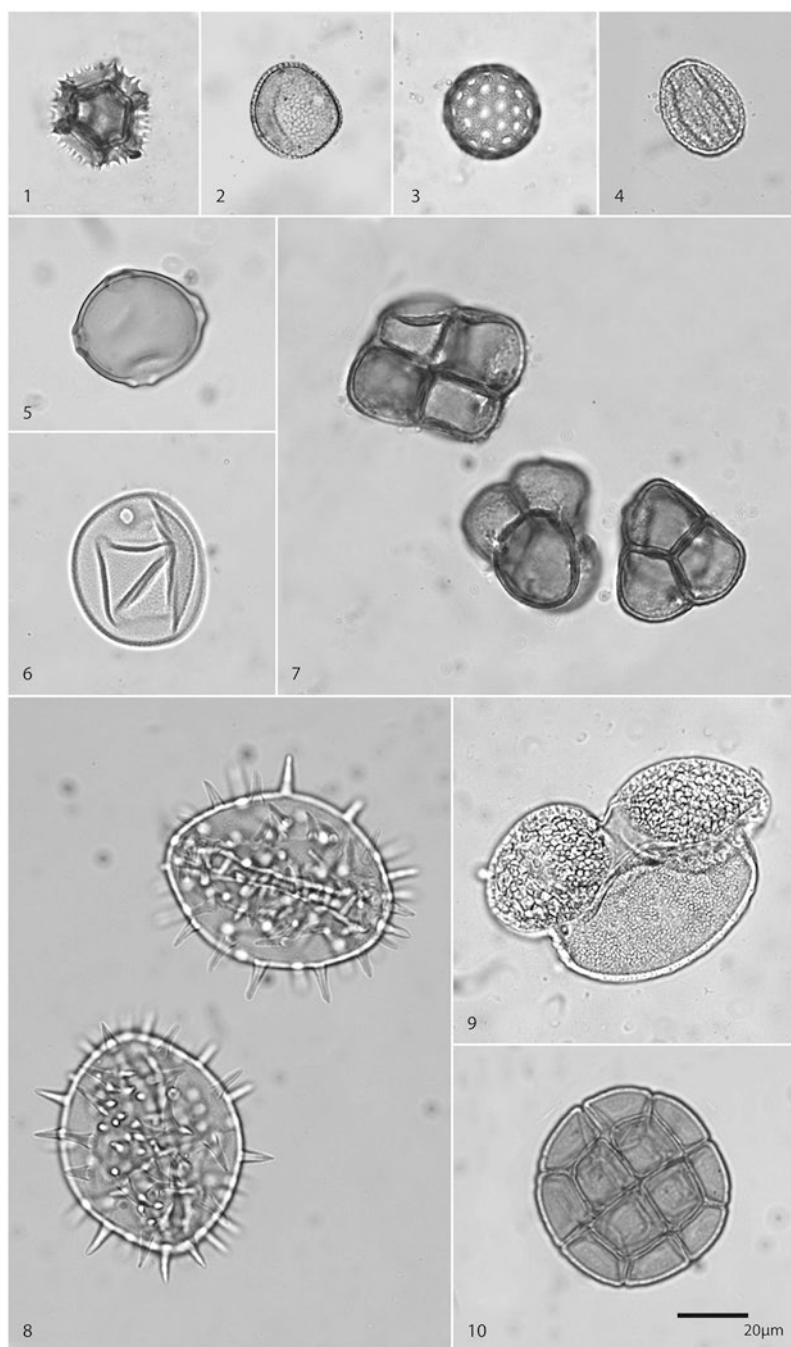
Another bias is offered by the difference in pollen production per plant. Species that rely on airborne pollen produce more pollen than species that are pollinated by

animals. Light plays a role as well. A wind-pollinated tree like oak produces far more catkins and pollen when standing free than when being part of a dense forest. The study of recent vegetations in combination with the study of the pollen they release provides insight in this kind of problem (see for instance Heim 1970; Broström et al. 2008; Doorenbosch 2013). Attempts to establish general applicable correction factors have failed so far, but software has been developed and is continuously adapted to cope with this problem and make the modeling of past vegetations possible (see for instance Middleton and Bunting 2004).

A last problem to be mentioned is the fact that some animals actually bury pollen. This is mainly a problem connected with the palynology of non-wetland situations. Some bees, for instance, dig holes to lay their eggs in and provide these burrows with pollen to feed the larvae. Such cases may distort the result of analyses (Bottema 1975).

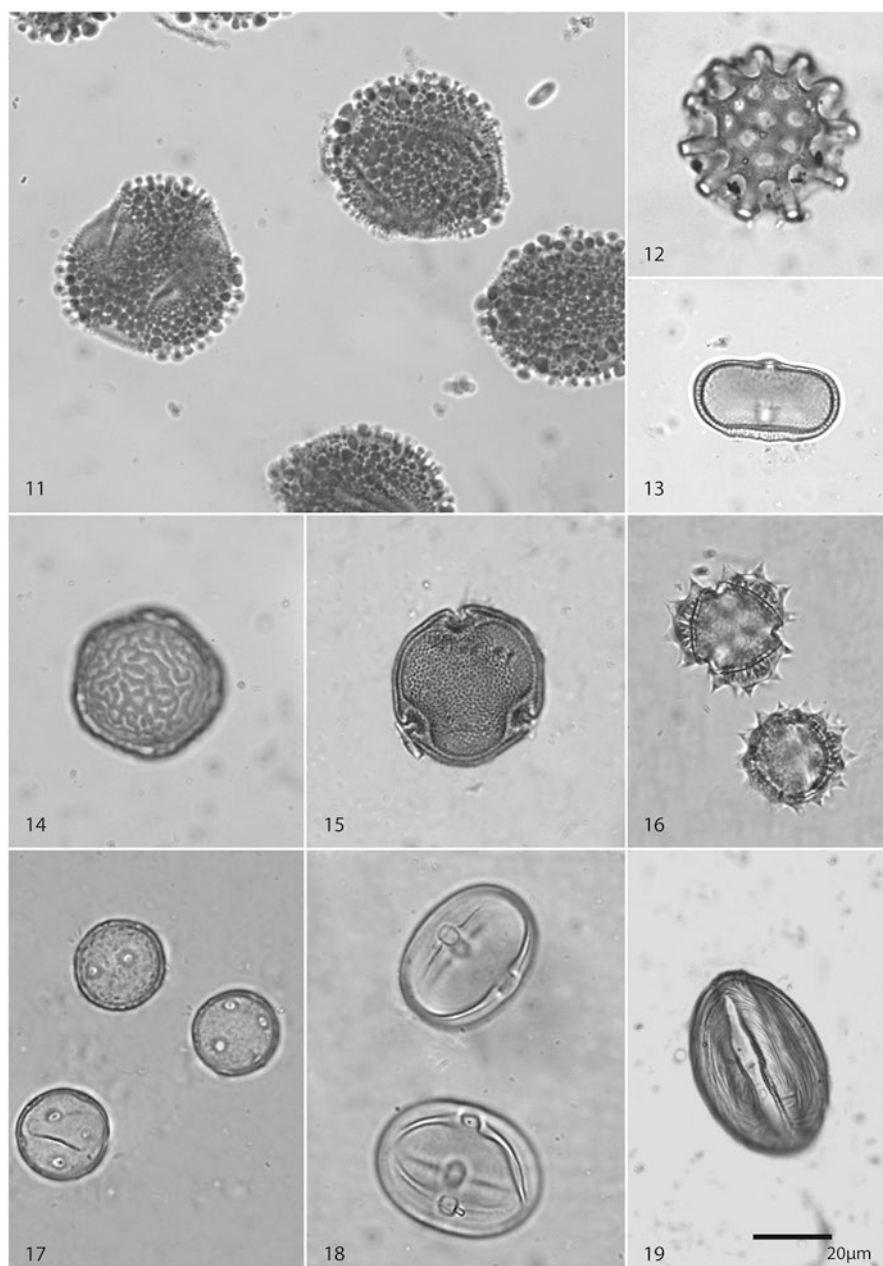
## 5 Conclusion

Pollen analysis can offer important contributions to archaeological research. The study of pollen provides far more than the vegetation reconstruction of environments, a goal pollen analysis is commonly associated with. Found in a context with other remains of past human activity, pollen grains can shed light on human behavior, for instance food habits or the movement of goods. They are valuable traces of the human past, related to nature, culture, or a combination of both.



**Plate 1** Images of pollen types. Examples of pollen grains, all shown in the same size. Photos by C. Bakels. 1 *Hypochaeris radicata* L.; 2 *Sparganium erectum* L.; 3 *Chenopodium bonus-henricus* L.; 4 *Quercus robur* L.; 5 *Carpinus betulus* L.; 6 *Molinia caerulea* (L.) Moench; 7 *Calluna vulgaris* (L.) Hull; 8 *Nuphar lutea* (L.) Sm.; 9 *Pinus cembra* L.; 10 *Acacia podalyriifolia* A. Cunn.





**Plate 2** Images of pollen types. Examples of pollen grains, all shown in the same size. Photos by C. Bakels. 11 *Ilex aquifolium* L.; 12 *Polygala vulgaris* L.; 13 *Foeniculum vulgare* Mill.; 14 *Ulmus minor* Miller; 15 *Tilia vulgaris* Hayne; 16 *Leucanthemum vulgare* Lamk.; 17 *Plantago lanceolata* L.; 18 *Vicia cracca* L.; 19 *Menyanthes trifoliata* L.



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# Microscopic Charcoal Signal in Archaeological Contexts



Laurent Marquer and Thierry Otto

## 1 Introduction

Charcoal in archaeological contexts is the product of the exploitation and use of wood fuel resources (Théry-Parisot 2001; Asouti and Austin 2005; Scott and Damblon 2010; Henry and Théry-Parisot 2014). The panel of firewood management practices is specific to each society, population, and group, as well as to regional and local environmental and climate contexts.

Charcoal consists of inorganic carbon compounds resulting from an incomplete combustion of plant remains (Goldberg 1985), mainly wood but it can also refer to other plant materials. Charcoal is generally inert and has been recovered from sediments dating back to a million years or more (Scott and Glasspool 2007). However, the fragile or brittle consistency of charcoal resulting from its highly porous structure is a major cause of fragmentation.

Charcoal fragmentation mainly takes place during combustion and post-depositional processes (Théry-Parisot et al. 2010). Fragmentation is not assumed to significantly affect the paleoecological and firewood management information that can be obtained from charcoal assemblages (e.g., Chabal 1990; Chravzez et al. 2014; Arranz-Otaegui 2017). The initial fuel assemblages that have been used to feed a fireplace can therefore realistically be assessed in an archaeological context. These assemblages reflect opportunistic or voluntary selections of particular tree and shrub species, specific parts of wood (e.g., branches, trunk, bark, and

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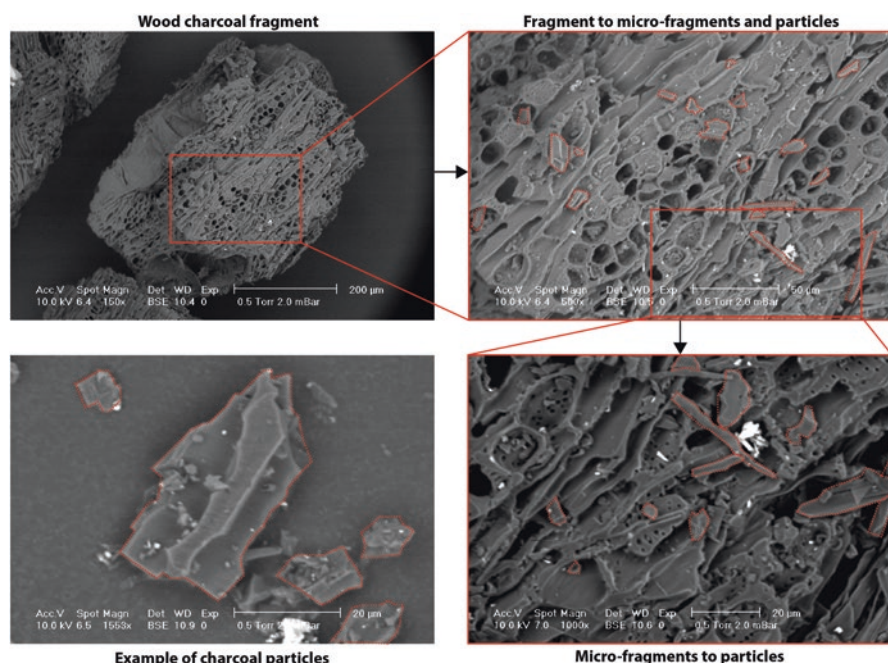
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brushwood), and wood characteristics (e.g., green, decayed, and deadwood). However, intense and frequent impacts of post-depositional processes on charcoal might result in a scarcity or apparent absence of residues. This is particularly true in many European Paleolithic sites, although the use of other fuel needs to be further considered (Villa et al. 2002; Th  ry-Parisot 2001, 2002; Costamagno et al. 2005; Th  ry-Parisot et al. 2005; Beresford-Jones et al. 2010; Clark and Ligouis 2010; Marquer et al. 2010a, 2012, 2015).

A method for extraction and quantification of charcoal pieces, fragments, and particles has been developed over the last decade and applied to combustion features from Paleolithic sites (Marquer 2009, 2010, 2013; Marquer et al. 2010a, b, 2011, 2012, 2015; White et al. 2017). This approach provides quantitative estimates of the charcoal signal (pieces, fragments, and particles) present in three sediment fractions, i.e., the macroscopic ( $>0.5$  mm), mesoscopic ( $0.5\text{--}0.16$  mm), and microscopic ( $<0.16$  mm) fractions. This approach has shown that charcoal can be at the highest concentration in the smallest sediment fractions when it is apparently absent in archaeological contexts. This means that all sedimentary fractions need to be considered when hearth residues are explored in order to understand fuel management strategies.

It is well known that charcoal is black and opaque. Large pieces ( $>0.25$  mm) are easily recognized under the microscope because they retain visible wood anatomy. Charcoal fragments, micro-fragments, and particles ( $<0.25$  mm) retain only parts of a few wood cells and structures (Fig. 1), and their identification as charcoal is sometimes difficult under a stereo-microscope or transmitted light illumination. The further we go into the smallest fractions, the more difficult it is to differentiate charcoal particles from other potential confusants, such as burned bone and dark minerals. A manual for the observation (pictures and descriptions) of charcoal fragments, micro-fragments, and particles has never been developed so far.

The present chapter aims at providing a manual for the observation of charcoal, from large pieces to the smallest particles based on modern samples. These samples correspond to a panel of wood and grass charcoal that have been manually crushed and sieved in order to obtain six size classes:  $>1$  mm (charcoal pieces),  $1\text{--}0.5$  mm (limit of taxa identification based on charcoal pieces),  $0.5\text{--}0.25$  mm (limit between charcoal pieces and fragments),  $0.25\text{--}0.1$  mm (limit between charcoal fragments and micro-fragments),  $0.1\text{--}0.05$  mm (charcoal particles), and  $<0.05$  mm (limit of charcoal observation). The same approach has been applied to modern samples of burnt bones to discuss the major criteria to differentiate charcoal from burnt bones; burnt bones are considered as the main confusants. Each class is observed under the microscope, and the major criteria for identification are discussed and illustrated with photographs. We further provide (1) a definition of what the charcoal signal means in an archaeological context; (2) an overview of taphonomy that causes charcoal fragmentation; (3) a review of charcoal sampling, extraction, observation, and quantification protocols; and (4) a discussion about why the charcoal signal is useful for archaeologists.



**Fig. 1** Scanning electron microscope (SEM) observations of wood charcoal fragmentation, from a charcoal fragment that preserves all wood anatomical features to micro-fragments and particles that are parts of anatomical features, and then the smallest particles. An example of SEM view of charcoal particles is also shown. Largest charcoal particles are underlined using red dashed lines. These observations raise the question about how far can we go into the observation of charcoal micro-fragments and particles to get reliable information for archaeologists. Note that few fragmentations are observed here when charcoal in archaeological contexts can be entirely crushed into thousands of particles. SEM observations have been done by Laurent Marquer and Brigitte Deniaux

## 2 Charcoal Signal

The charcoal signal corresponds to all pieces, fragments, micro-fragments, and particles of charcoal that are observed from a single sample (Marquer 2010). This signal has different meanings depending on the depositional context. In geological contexts, this signal corresponds to fire events (Scott 2009, 2010), i.e., natural or anthropogenic vegetation fires. In those contexts, charcoal provides reliable information for the estimation of vegetation changes and the frequency and intensity of fire events (Vernet 1973; Patterson et al. 1987; Vernet and Thiébault 1987; Clark 1988, 1990; MacDonald et al. 1991; Thinnon 1992; Millspaugh and Whitlock 1995; Carcaillet 2007; Power et al. 2008; Marlon et al. 2013; Daniau et al. 2019). In archaeological contexts, the charcoal signal informs archaeologists about the collection of plants, peat, and/or herbivore dung for specific activities related to fireplaces

(e.g., Miller 1984; Asouti and Austin 2005; Th  ry-Parisot 2001, 2002; Scott and Damblon 2010; Th  ry-Parisot et al. 2010; Braadbaart et al. 2017; Lancelotti 2018).

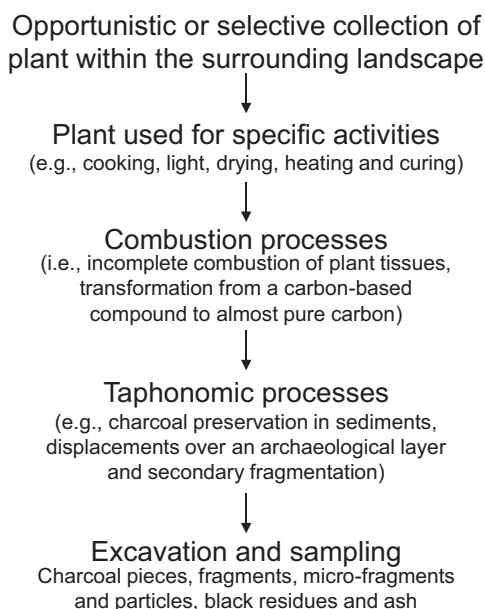
Until recently, archaeologists and archaeobotanists did not have specific interest in charcoal micro-fragments and particles as most charcoal information was obtained from pieces and the largest excavated fragments, so further studies were not required. However, the smallest charcoal residues offer unique information about plant fuel gathering when the charcoal signal is reduced to the smallest sediment fractions (Marquer 2010; Marquer et al. 2010a, 2012, 2015; see Sect. 6).

### 3 Taphonomy: From Plants to Charcoal Fragments and Particles

#### 3.1 From Plants to Charcoal

Charcoal assemblages correspond to the collection of plant material (e.g., wood and grass) and derivatives (e.g., leaves, roots, seeds, peat, and dung) that have been used intentionally or accidentally in fireplaces (Fig. 2). The choice of plant fuel is influenced by cultural and environmental factors (Th  ry-Parisot et al. 2010). Plant fuel gathering can be intentional in order to obtain particular combustion properties, or it can be opportunistic and therefore reflect only the availability of local resources (e.g., Otto 1993; Dotte-Sarout et al. 2015; Picornell-Gelabert et al. 2011, 2017; Picornell-Gelabert 2017). The duration of human occupations further affects the

**Fig. 2** Charcoal assemblages result from ante- and post-depositional processes





collection of plant fuel and therefore charcoal assemblages (Théry-Parisot and Texier 2006). The initial characteristics of the plant fuel (e.g., density, caliber, water content, chemical compounds, and physiology) impact the physical and chemical processes of combustion (Théry-Parisot 2001).

Combustion or charcoalfication (i.e., the transformation of a carbon-based compound to almost pure carbon) is the process by which plants are transformed into charcoal (e.g., Beall 1972; Goldberg 1985; Pyne et al. 1996; Braadbaart and Poole 2008). This process starts when plants are exposed to temperatures between 280 and 500 °C, and the supply of oxygen is limited. A complete combustion driven by high supply of oxygen takes place above 500 °C resulting in the conversion of plants into ash. Several factors determine the morphological, physical, and chemical properties of the final combustion residues, including the characteristics of the initial plant assemblages, the intensity and velocity of the fire, and the length of time the plant is in the fire (Braadbaart et al. 2007, 2009; Braadbaart and Poole 2008).

Physical transformations from plants to charcoal occur during both endothermic (e.g., dehydration) and exothermic phases (e.g., pyrolysis and ignition stage) of combustion, which cause changes such as discoloration, shrinkage, reduction of wall cells, cell cracks, and anatomical distortions (Beall et al. 1974; Schweingruber 1978; Thinnon 1992; Zicherman and Williamson 1981; Vaughan and Nichols 1995; Allué et al. 2009). Chemical transformations occur during the degradation of cellulose and lignin via the release of volatile compounds. Both physical and chemical processes generate physical alterations of plant anatomy that favor fragmentation, such as fractures and thinning of wall cells.

Once the combustion ends, charcoal pieces conserve plant anatomy, and they are considered as chemically inert and therefore resistant to decay (Scott and Jones 1991; Nichols et al. 2000; Scott and Glasspool 2007). However, charcoal is not a pure form of carbon, and it might be affected by diagenetic alterations (Smernik et al. 2000; Antal and Gronli 2003; Cohen-Ofri et al. 2006; Ascough et al. 2008). The final product of combustion is a mix of charcoal pieces, fragments, micro-fragments, and particles, but also of ash and soot.

### ***3.2 From Charcoal Fragments to Particles***

After combustion, charcoal pieces and fragments are highly sensitive to mechanical stresses caused by cell cracks, thinning of wall cells, and anatomic distortions, all of which are key factors influencing the extent of charcoal secondary fragmentation. Species-specific wood characteristics (i.e., density and porosity) should also be considered. Fragmentation does not seem to significantly influence our ability to identify assemblages of plant species that produced the charcoal (Chabal 1990, 1992, 1997).

Mechanical stresses on charcoal are caused by post-depositional processes, including both natural and human-induced actions. The natural processes include the action of water and wind, characteristics of the sediments (surface exposure,



moisture content, and compaction), activities by earthworms and insects, mineral inclusions (e.g., silica, carbonates, or oxides), and growth of plant roots and fungus (Courty and Fédoroff 1983; Clark 1988; Greenlee 1992; Thinon 1992; Carcaillet and Talon 1996; Gabet et al. 2003; Lynch et al. 2004; Scott 2010; Théry-Parisot et al. 2010). In the last glacial period, charcoal assemblages were also affected by the freeze-thaw cycle, which is a natural action that causes successive mechanical stresses (Théry-Parisot 2001; Chrzavzez et al. 2014). The consequences of these actions are fragmentation of charcoal and the horizontal and/or vertical dispersion of all residues. The human-induced factors are those related to daily activities close to hearths, in particular cleaning and trampling (Goldberg and Berna 2010; Miller et al. 2010). Cleaning leads to removal of larger charcoal pieces and the dispersion of particles over a larger area, while trampling results in the breakage of the charcoal. Degradation of charcoal via diagenetic processes is rare and mostly related to alkaline soil conditions (Cohen-Ofri et al. 2006; Braadbaart et al. 2009).

Note that further fragmentations can occur during the sampling and extraction protocols (e.g., Brady 1989; Greenlee 1992; Marquer et al. 2010a; Arranz-Otaegui 2017).

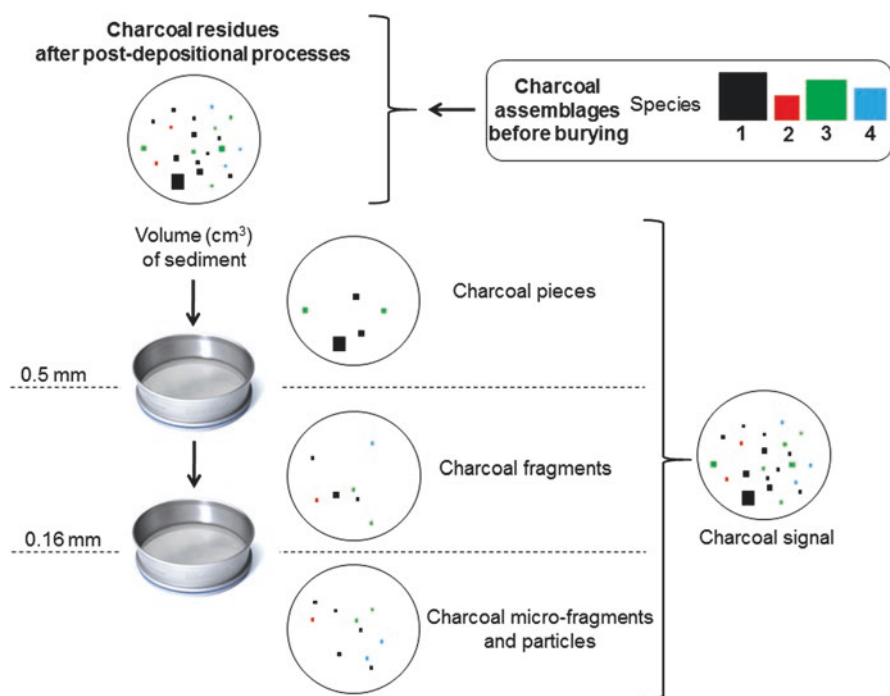
## 4 Methodological Approach

### 4.1 Sampling

The common sampling strategy in archaeological contexts is to collect each charcoal piece during excavations in the same manner as other materials such as lithics and bones, and/or to retrieve them after flotation and sieving of residual archaeological sediments. The smallest fragments and particles are usually not recovered through these approaches. Depending on the research objectives, looking at charcoal pieces and the largest fragments is appropriate for acquiring a fair image of charcoal assemblages resulting from initial plant gathering. These traditional charcoal analyses focus on large spatial areas and tend to avoid combustion features in order to get a reasonable picture of local vegetation (Chabal 1992). However, it is necessary to focus on combustion features if the research project aims at studying fuel management (e.g., Vidal-Matutano et al. 2015, 2017; Vidal-Matutano 2017). In cases where a more in-depth view of wood gathering is desired, or if charcoal appears to be absent from combustion features and archaeological layers, other sampling strategies are needed.

One such sampling strategy to recover a more complete charcoal signal (Fig. 3; Marquer 2010; Marquer et al. 2010a, 2012, 2015) consists of collecting raw sediments from well-understood geoarchaeological contexts, such as hearths, archaeological features, and/or layers.

Analyzing the charcoal signal requires that the researcher carries out horizontal and vertical sampling within each combustion feature to avoid potential biases



**Fig. 3** Sieving processes to extract charcoal pieces, fragments, micro-fragments, and particles in order to reconstitute the charcoal signal (sum of all fractions). The charcoal signal is expected to reflect charcoal assemblages after post-depositional processes

caused by non-homogeneous dispersion of fragments, micro-fragments, and particles within the feature. Continuous samples can be horizontally collected over a large area of an archaeological layer to explore the spatial distribution of charcoal residues. Samples can also be retrieved vertically within an archaeological layer to discuss taphonomy or temporal distribution of residues.

## 4.2 Extraction and Observation

Charcoal residues are extracted by using a succession of sieves (Fig. 3). It is important to measure the initial volume of sediment that is used for sieving. Note that the possibilities to extract macroscopic charcoal from sediments for botanical identification are higher with large volumes of sediment. A volume of 100–500 cm<sup>3</sup> is a good compromise between the time needed to process the samples and the reliability of the results. There are two options for sieving—water-assisted screening or dry screening. Dry screening is expected to produce a lower amount of mechanical stress on the charcoal than water screening, because water adds tension due to the

hydration/dehydration of charcoal. However, water screening is essential for sediment matrices with high amounts of clay, organics, and/or ash. Sieving phases need to be carefully realized to reduce re-fragmentation of charcoal through further mechanical stresses, e.g., compression.

The first sieving is carried out at 0.5 mm (Fig. 3) in order to extract pieces and larger fragments of charcoal. These pieces and fragments are manually retrieved using forceps under a stereo-microscope, and they are observed under reflected light illumination for botanical identification. The second sieving is realized at 0.16 mm, as this diameter represents the limit between fragments and micro-fragments/particles. The fraction 0.5–0.16 mm is observed under a reflected light microscope by using microscope slides adapted with adhesive to glue fragments and micro-fragments; the volume of sediment that is deposited on each slide should be measured. For the fraction smaller than 0.16 mm, a volume of the remaining sediments is treated using a traditional palynological approach to eliminate mineral and organic fractions within the sediment (Asselin and Payette 2005). Chemical processes using hydrochloric acid (HCl), hydrofluoric acid (HF), and potassium hydroxide (KOH) eliminate, respectively, carbonates, siliceous compounds, and organic matter. Zinc chloride ( $\text{ZnCl}_2$ , density 2.1) is used to separate charcoal particles from compounds with higher density than charcoal particles. Cold HCl treatment is preferable to hot treatment in order to minimize charcoal alteration during the chemical processes. Note that treatment to bleach the organic matter can be applied to make a clear distinction between burnt and unburnt elements (Clark 1984). The pros and cons of using a palynological approach for charcoal particle analysis in archaeological contexts are discussed in Marquer (2009) and Marquer et al. (2011). Transmitted light illumination is used for the observation of micro-fragments and particles. It is important to record all sediment volumes at each step of the sieving and observation processes in order to sum up all information and thereby assess the charcoal signal (Fig. 3).

Another approach (unpublished but applied in White et al. 2017) has been developed for the observation of micro-fragments and particles to avoid the use of chemicals and therefore view charcoal particles under reflected light microscope.

### 4.3 Quantification

Traditional charcoal analyses use counts, frequencies, or weights of charcoal to estimate abundance. Our approach aims at quantifying abundance using charcoal concentration in each of the three sediment fractions and then sums them up to assess the charcoal signal (Fig. 3). This approach can be applied to other combustion residues such as burnt bones for comparison (Marquer et al. 2010a, 2012, 2015; White et al. 2017).

Quantifying abundance using charcoal concentration is the most appropriate method because measures of charcoal residues from different size classes cannot be summed up when they are expressed as counts, frequencies, or weights. It is a

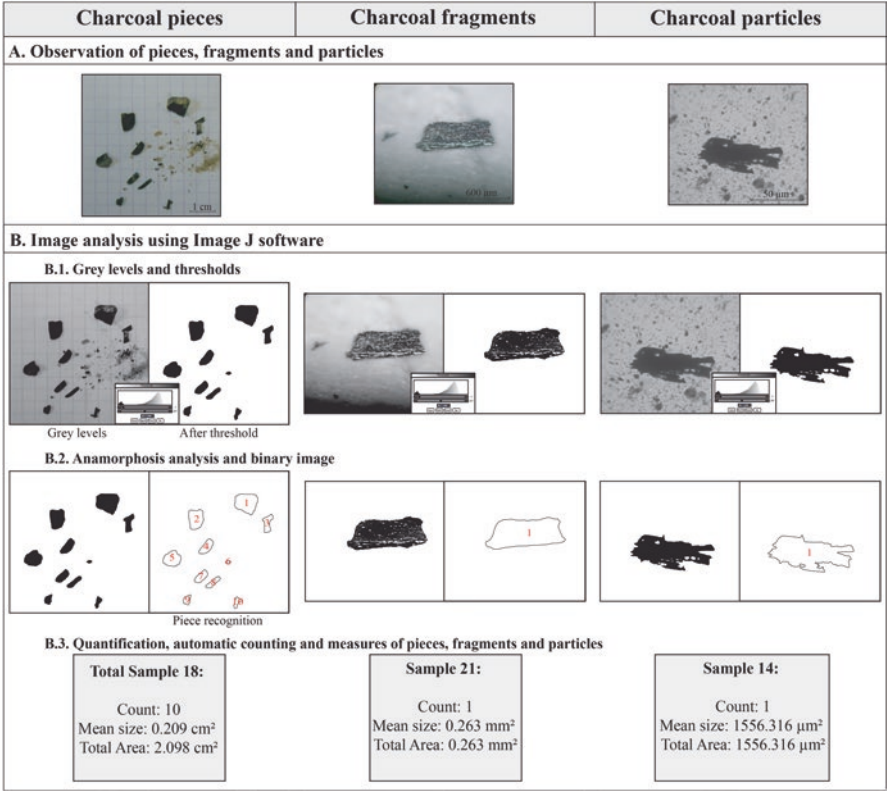
circular reasoning to sum up counts and frequencies from different size classes because fragments and particles are derived from the largest pieces. Furthermore, counts would not be relevant for comparison of the relative abundances of charcoal versus other combustion residues such as burnt bones due to differential fragmentations between the combustion residues. The use of weight is realistic when pieces or fragments are considered, but it is not when micro-fragments and particles are analyzed. Considering all of the above issues, we calculate the concentration of combustion residues by estimating the areas of pieces, fragments, and particles and then adding them up to assess the charcoal signal (Marquer 2009, 2010; Marquer et al. 2010a, 2012, 2015).

Estimation of particle areas has been commonly performed in charcoal particle analysis by using image analysis software (MacDonald et al. 1991; Szeicz and MacDonald 1991; Horn et al. 1992; Earle et al. 1996; Clark and Hussey 1996). Results are expressed as the total area of all particles in  $\text{cm}^2$ ,  $\text{mm}^2$ , or  $\mu\text{m}^2$ . Quantification of charcoal particles expressed as size and area measurements is a more reliable estimate of charcoal content than particle number, in particular for multiple-site studies (Halsall et al. 2018). Marquer (2009, 2010) and Marquer et al. (2015) have developed this approach via an automatic estimation of the total areas of pieces, fragments, and particles (Fig. 4). Outputs are expressed as concentrations of charcoal ( $\text{cm}^2$  of charcoal for  $1 \text{ cm}^3$  of sediment) in each fraction that are summed up to obtain the charcoal signal (Fig. 3). All the sediment volumes recorded at each step of the sieving and observation processes are used for this calculation.

## 5 Key for Charcoal Observation from Experimental Materials

### 5.1 Modern Reference Dataset

Modern plant samples have been collected from the regional vegetation near Toulouse (France). These samples correspond to a panel of plant species that considers different plant anatomical features (e.g., hardwood vs. softwood and dicots vs. monocots) and proprieties (e.g., green vs. decayed woods and trees vs. shrubs). The collected plants are four gymnosperms (softwood): pine (*Pinus sylvestris* L.), fir (*Abies alba* Mill.), juniper (*Juniperus communis* L.), and yew (*Taxus baccata* L.); six dicots including four broadleaved trees (hardwood): oak (*Quercus robur* L.), willow (*Salix alba* L.), fig (*Ficus carica* L.), and Rosaceae (*Prunus avium* L.); two shrubs: box (*Buxus sempervirens* L.) and *Clematis vitalba* L.; and one monocot: giant cane (*Arundo donax* L.). Most of the wood taxa can be found during the last Glacial and Paleolithic periods. Decayed wood from oak and fig trees has been collected to explore the potential influences of green versus altered woods on the observation of charcoal residues. Fresh long bones (diaphysis and epiphysis) from a cow (*Bos*) have been selected as potential confusants for charcoal.



**Fig. 4 (A, B)** Automatic counting and estimations of the total areas of charcoal pieces, fragments, and particles using image analysis software (e.g., ImageJ 1.51u). (Adapted from Marquer 2010 and Marquer et al. 2015)

Plant and bone samples were isolated into individual aluminum containers. Each sample was covered by pure sand (e.g., Fontainebleau sand) to limit the supply of oxygen and avoid complete combustion of samples. Samples were then exposed to heat at 550 °C using an oven. We followed the recommendation of Orvis et al. (2005), although we did not saturate the sand with water. This laboratory production of combustion residues tends to mimic burning as it occurs in fireplaces. We consider this protocol relevant in order to explore the diversity of charcoal “morphotypes” in successive size classes. Testing various protocols based on different combustion characteristics would certainly result in the same “morphotypes” as the ones we present here. The residues of these combustion processes were manually crushed (heterogeneous crushing) using ceramic mortars to simulate natural fragmentation. We regard heterogeneous crushing as a more realistic simulation of natural fragmentation in sediments than homogeneous crushing using a press machine. The use of a press machine applies automatic and constant pressures on samples which would result in complete fragmentation of pieces into particles. Fragmented

samples were sieved at different mesh sizes to obtain various size intervals in order to observe pieces, fragments, micro-fragments, and particles of charcoal, i.e., >1 mm (charcoal pieces), 1–0.5 mm (limit of taxa identification based on charcoal pieces), 0.5–0.25 mm (limit between charcoal pieces and fragments), 0.25–0.1 mm (limit between charcoal fragments and micro-fragments), 0.1–0.05 mm (charcoal particles), and <0.05 mm (limit of charcoal observation).

Charcoal residues from the six size classes were observed using Leica DMR (transmitted light illumination) and DMRB (reflected light illumination) microscopes at 200× and 400× magnifications. In charcoal analysis, pieces and fragments are identified using reflected light in order to observe key characteristics of wood anatomy. In microscopic charcoal analysis, particles are commonly seen using the transmitted light to distinguish charcoal particles from other confusants. However, if charcoal particles can be recognized using a transmission microscope, the observation of wood anatomy criteria is very limited. Reflected light illumination provides the best views of wood anatomy characteristics and a differentiation between charcoal particles and unburnt organic matters. Therefore, reflected light microscopy would be a more appropriate option to have a look at charcoal fragments and particles than transmitted light microscopy for recognition of burnt elements and wood anatomical criteria. Charcoal particles are hereafter observed under both reflected and transmitted light illumination.

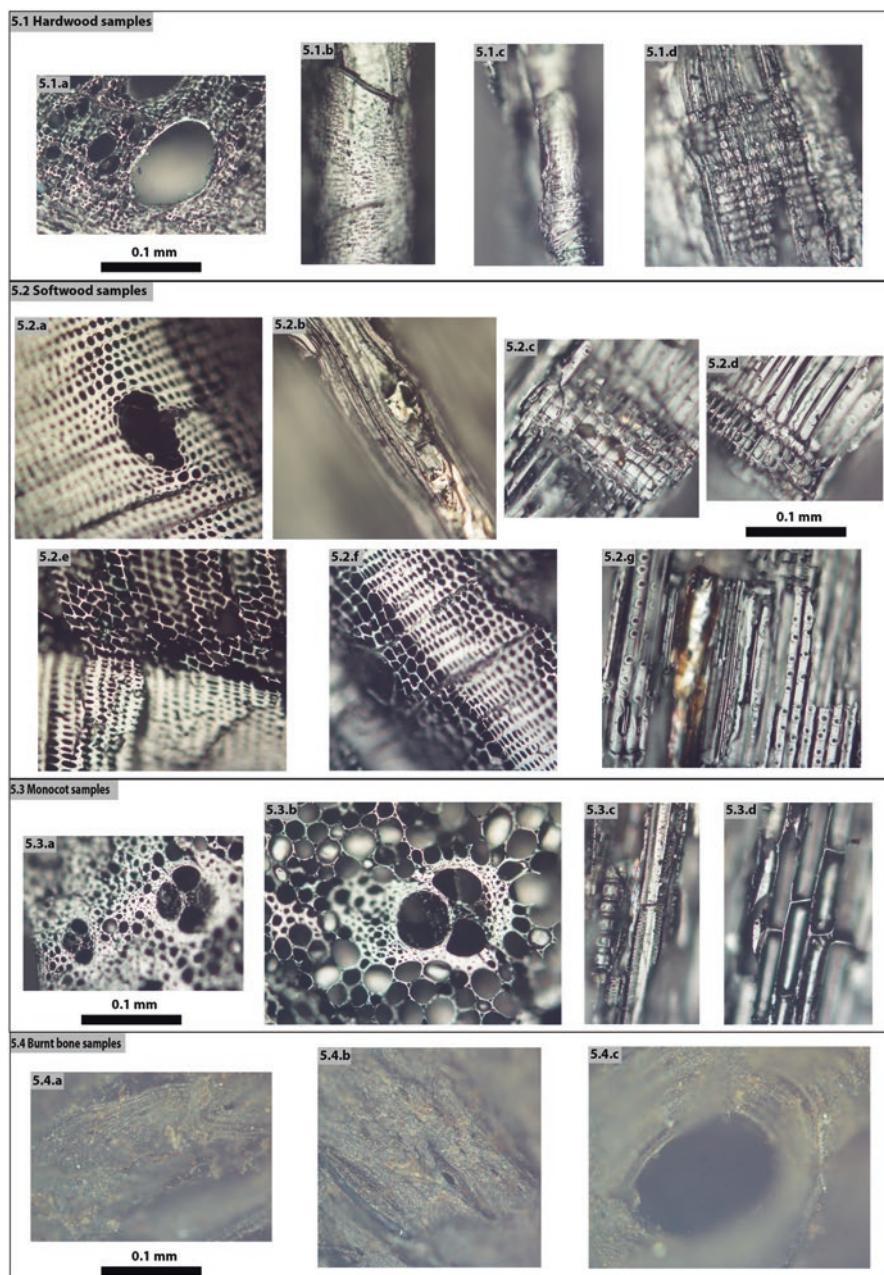
In previous studies, we have explored the morphological characteristics of charcoal and burnt bones by using a scanning electron microscope (SEM). SEM analysis provides the best observations and unequivocal identifications (Cartwright 2015); however, SEM cannot be used as routine analyses for observation and quantification. Therefore, we focus, in the present chapter, on reflected and transmitted light illuminations that are the most common illuminations used for archaeological and environmental studies.

For the microscopic observation, a sample of each size interval was individually spread out over a microscope slide (glass slide) adapted with a gum arabic coating to glue combustion residues. A camera, Nikon DS-Fi2, has been used to create images for an illustrated key for charcoal observation.

## 5.2 Charcoal Pieces

Recognition of charcoal pieces from other elements (burnt or unburnt elements) is easy for size classes higher than 0.25 mm because of the observation of plant anatomical features (Fig. 5). These features can be viewed along specific anatomical sections of the plant (i.e., cross, radial, and tangential sections) from which criteria for botanical identification can be seen, e.g., vessels, rays, pits, fibers, and parenchyma. Key publications and atlases about European wood anatomy provide the species-specific characteristics for the description and identification of wood remains (e.g., Schweingruber 1990; Thinin 1994a, b, c; Vernet et al. 2001; IAWA 1989, 2004). We focus here on the major characteristics that are needed for the





**Fig. 5** Observation of charcoal pieces (>0.25 mm). This figure shows examples of the three anatomical sections of the plant, i.e., cross, radial, and tangential sections, as well as critical features for botanical identification. All pictures have been taken under reflected light microscope at 200× magnification. 5.1 Hardwood (*Quercus robur* L.): (a) vessels and rays in cross section; (b) vessel



differentiation between hardwood (dicotyledonous wood), softwood (coniferous wood), and monocots (Fig. 5). These characteristics are important for the understanding of fragmentation patterns (e.g., breakage along specific anatomical sections and/or from particular anatomical features) and morphotypes resulting from fragmentation.

A major characteristic to differentiate hardwood from softwood is the organization of anatomical features (Fig. 5). Hardwood has more complex features than softwood, in particular hardwood has numerous pores, while softwood has very few of them and is mainly characterized by tracheids (i.e., longitudinal cells for sap conduction). Softwood can also have fusiform rays that correspond to resin canals. Parenchyma characteristics, earlywood to latewood transitions, and cross-field pitting, among other criteria, can further be used to distinguish hardwood from softwood.

Monocots essentially differ from dicots (i.e., hardwood and softwood) via the absence of wood features (Fig. 5), such as the formation of different rings resulting from a secondary growth. Monocots have scattered vascular bundles (plant tissues used for the transport of soluble organic compounds, water, and nutrients; xylem and phloem cells) throughout the stem while dicots have the vascular bundles arranged in a ring. These characteristics can be viewed in cross section only.

All wood anatomical features are observable for sizes of charcoal ranging from >1 to 0.25 mm, i.e., our size intervals >1, 1–0.5, and 0.5–0.25 mm.

### 5.3 Charcoal Fragments and Micro-Fragments

Charcoal fragments and micro-fragments correspond to the size interval 0.25–0.1 mm. A fragment is a portion of a charcoal piece along one of the three anatomical sections (i.e., cross, radial, or tangential section) when a micro-fragment is a portion of a fragment (Fig. 6). The three anatomical sections together cannot therefore be distinguished from a single fragment or micro-fragment; charcoal residues are too small to observe such features joined together. Furthermore, the tree rings are not preserved and vessels and rays are isolated from one another. This hampers the botanical identification of the species because important information such as the number of rays per millimeter in the tangential section or earlywood to

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**Fig. 5** (continued) in radial section with pits between vessels and perforation plate between two vessel cells; (e) vessel in radial section with cross-field pitting and pits between vessels; (d) rays. 5.2 Softwood (*Pinus sylvestris* L.): (a) resin canal in cross section; (b) resin canal in tangential section; (c) pits and cross-field pitting in radial section; (d) bordered pits of tracheids and cross-field pitting in radial section; (e) earlywood to latewood transition in cross section; (f) earlywood to latewood transition in cross section with one resin canal to the left; and (g) resin canal in radial section with bordered pits of tracheids. 5.3 Monocots (*Arundo donax* L.): (a) two vascular bundles in cross section; (b) one vascular bundle in cross section; (c) vessel cells with pits in longitudinal section; and (d) parenchyma cells in longitudinal section. 5.4 Burnt bones (diaphysis and epiphysis) as potential confusants: (a) epiphysis surface; (b) diaphysis surface; and (c) concentric lamellae with central canal



**Fig. 6** Observation of charcoal fragments and micro-fragments (size interval 0.25–0.1 mm). This figure shows examples of anatomical features that are still discernible. The observation of these

latewood transitions in the cross section cannot be assessed. Botanical identification is hazardous for residues below a size of 0.25 mm. Nevertheless, hardwood, softwood, and monocots can still be distinguished, although there are few criteria.

Criteria that are discernible from fragments and micro-fragments are vessels, tracheids, rays, fibers, parenchyma, cross-field pitting, and the smallest features such as pits and spiral cell wall thickenings in vessels (Fig. 6). Softwood can be easily recognized based on cell organization, i.e., complex aggregates of pores (hardwood) can be distinguished from joint tracheids (softwood), and the observation of parts of fusiform rays (resin canals) and cross-field pitting or specific parenchyma criteria further help to discriminate softwood from hardwood and monocots.

The distinction between hardwood and monocots can be sometimes difficult if no key anatomical criteria are observed. This is mainly caused by the fact that cross sections are badly preserved at this microscopic scale, which prevents the observation of scattered vascular bundles and the absence of rings resulting from a secondary growth that characterize monocots. Indeed, no cross sections were observed in monocot samples, and hardwood and softwood samples show that fragmentation seems to favor the observation of charcoal residues into radial and tangential sections rather than cross section. Generally, then, fragments and micro-fragments that show anatomical characteristics of cross section are fewer than those preserving radial and tangential sections. No distinct fragmentation patterns have been observed between hardwood, softwood and monocots, trees and shrubs, or decayed and greenwoods; however, a quantitative analysis might help to explore this issue.

The observation of anatomical features in the size interval 0.25–0.1 mm provides a reliable recognition of charcoal fragments and micro-fragments from other confusants such as burnt bones (Fig. 6).

## 5.4 Charcoal Particles

Charcoal particles correspond to all residues <0.1 mm. Samples from the size interval 0.1–0.05 mm show that sometimes radial and tangential sections and anatomical features can be recognized (Fig. 7). A distinction between angiosperms (i.e.,

←  
**Fig. 6** (continued) features provides a distinction between hardwood, softwood, and monocots, as well as from other confusants such as burnt bones. All pictures have been taken under reflected light microscope at 200×. 6.1 Hardwood (*Quercus robur* L.): (a) vessel in radial section with pits between vessels and perforation plate between two vessel cells; (b) tracheids in radial section with pits; (c) rays and fibers in tangential section; (d) part of a vessel with pits and cross-field pitting in radial section (decayed wood); and (e) part of a vessel with pits in radial section (decayed wood). 6.2 Softwood (*Pinus sylvestris* L. and *Taxus baccata* L.): (a, b) tracheids and rays in tangential section (*Pinus sylvestris* L.); (c) cross-field pitting in radial section (*Pinus sylvestris* L.); (d) bordered pits of tracheids in radial section (*Pinus sylvestris* L.); (e, f) bordered pits of tracheids and spiral cell wall thickenings in vessels in radial section (*Taxus baccata* L.); and (g) tracheids of pits, cross-field pitting, and spiral cell wall thickenings in vessels in radial section (*Taxus baccata* L.). 6.3 Monocots (*Arundo donax* L.): (a) parenchyma cells in cross section; (b) parenchyma; and (c, d) vessel cells with pits in longitudinal section. 6.4 Burnt bones (diaphysis and epiphysis) as potential confusants: (a, b) diaphysis surface; (c) epiphysis surface

hardwood + monocots) and softwood can be possible when vessels, tracheids, cross-field pitting, or bordered pits are observed. Fiber and parenchyma particles are rare. Bordered pits are usually visible along radial and tangential sections, and given that these sections appear to be more preserved than cross sections, the bordered pits are easily discernible in our samples. In the size interval  $<0.05$  mm, only the largest pits of vessels, tracheids, and spiral cell wall thickenings in vessels are observed (Fig. 7). This reduces the possibilities to distinguish softwood from hardwood and monocots.

Hardwood has more complex anatomical features (such as a greater number of pores) than softwood, which is essentially characterized by tracheids. Charcoal particles from softwood mostly have acicular shapes (Fig. 7), that is, elongated and cylindroid, due mostly to the shape of the tracheids, which are elongated cells with tapering ends. Acicular shapes are abundant in charcoal residues smaller than 0.05 mm. The proportion of particles with acicular shapes from hardwood and monocot samples is few in comparison to softwood samples.

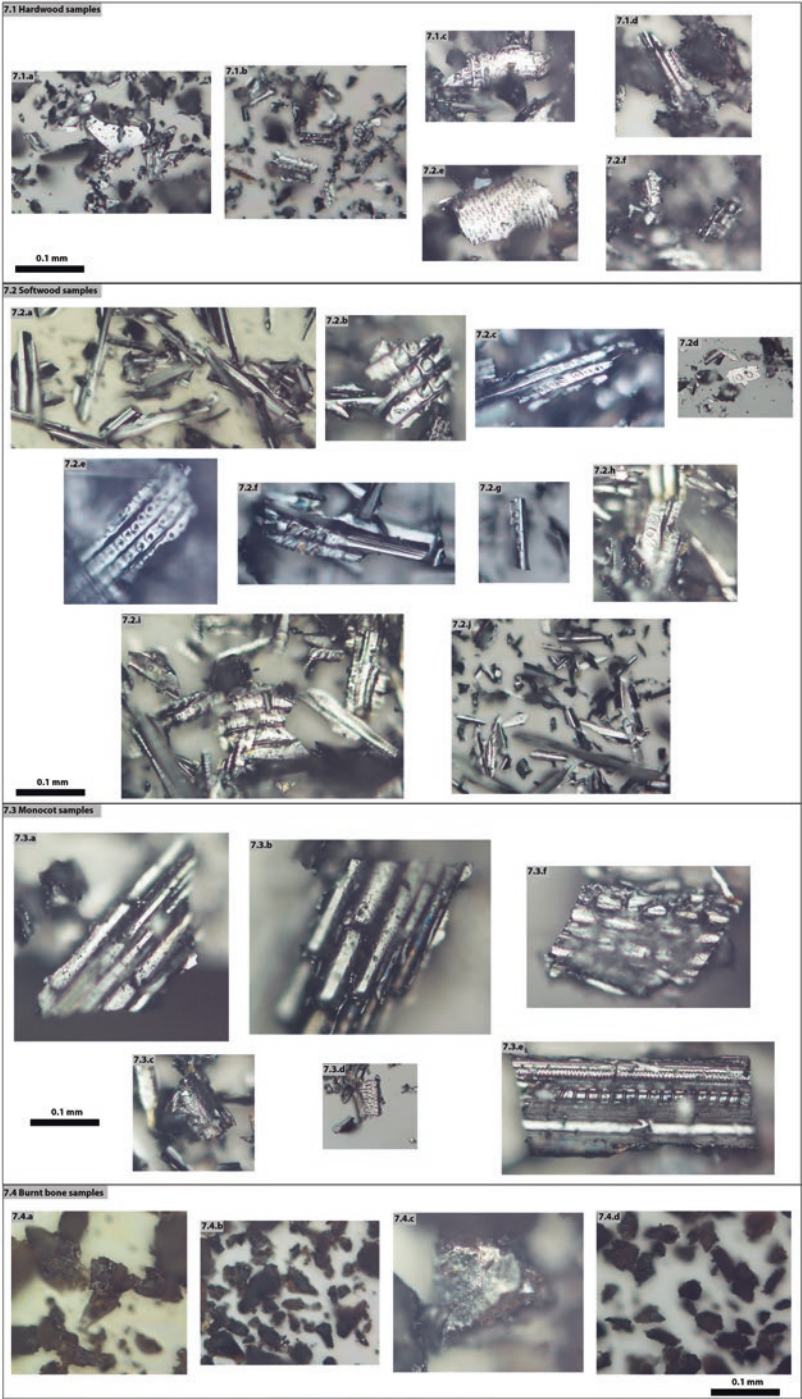
Our observations show that fragmentation might act preferentially on the less resistant parts of cell walls such as at the boundary between two cells of tracheids and vessels, particularly at the perforated end walls of vessels and the tapering ends of tracheids. Along radial sections, the less resistant parts appear to parenchyma and at the limits between vertical and horizontal cells of rays. A preferential fragmentation along those less resistant parts might explain why charcoal particles are essentially viewed along radial and tangential sections rather than cross sections. This means that fragmentation of charcoal pieces and fragments might mainly break at right angles along the vessels, tracheids, and rays; this would confirm the observations realized by Umbanhowar and McGrath (1998). Chravzev et al. (2014) have further shown that the intensity of fragmentation is related to charcoal porosity, i.e., porous areas with large vessels or radial pores and large rays are prone to fragmentation.

In addition to the differences among plant types described above, we also observed differences between charcoal particles from decayed (oak and fig trees) and greenwoods. The wood anatomical features can still be seen; however, cell

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**Fig. 7** (continued) reflected light microscope at 200 $\times$ . 7.1 Hardwood (*Quercus robur* L.): (a, b) large views of various particles and morphotypes; (c) part of the vessel with pits and cross-field pitting; (d) tracheids in radial section with pits; and (e, f) part of the vessel with pits. 7.2 Softwood (*Pinus sylvestris* L. and *Taxus baccata* L.): (a, b) large views of various particles (parts of tracheids and fibers) and morphotypes (elongated and acicular shapes), *Pinus sylvestris* L.; (b) cross-field pitting with pits in radial section (*Pinus sylvestris* L.); (c, e, g) bordered pits of tracheids in radial section (*Pinus sylvestris* L.); (d) bordered pits of tracheids in radial section (*Taxus baccata* L.); (f) cross-field pitting with pits and bordered pits in radial section (*Pinus sylvestris* L.); (h) bordered pits of tracheids and spiral cell wall thickenings in vessels in radial section (*Taxus baccata* L.); (i) cross-field pitting with pits and spiral cell wall thickenings in vessels in radial section (*Taxus baccata* L.); and (j) view of various particles without specific anatomical features observed. 7.3 Monocots (*Arundo donax* L.): (a, b) parenchyma cells in longitudinal section; (c, d) parts of vessel cells with pits in longitudinal section; (e) vessel cells with pits in longitudinal section; and (f) parenchyma. 7.4 Burnt bones (diaphysis and epiphysis from cow) as potential confusants: (a, b) diaphysis particles; (c, d) epiphysis particles





**Fig. 7** Observation of charcoal particles (<0.1 mm). This figure shows examples of particle morphologies and anatomical features that are still discernible. All pictures have been taken under

walls are badly preserved and pits are difficult to distinguish in the case of decayed wood samples.

Charcoal particles result from the fragmentation of pieces, fragments, and micro-fragments. Distinction between softwood and angiosperms is possible if key anatomical features are observed. Reflected light illumination provides observation of plant anatomical characteristics and reflectance of surfaces that help to confirm the vegetal and burnt origin of particles. Observations are difficult using transmitted light illumination, because the identification of material as burnt particles is sometimes unclear, and plant anatomical features are not visible except when cells or pits are preserved. In that case, the finest charcoal residues are classified as morphotypes.

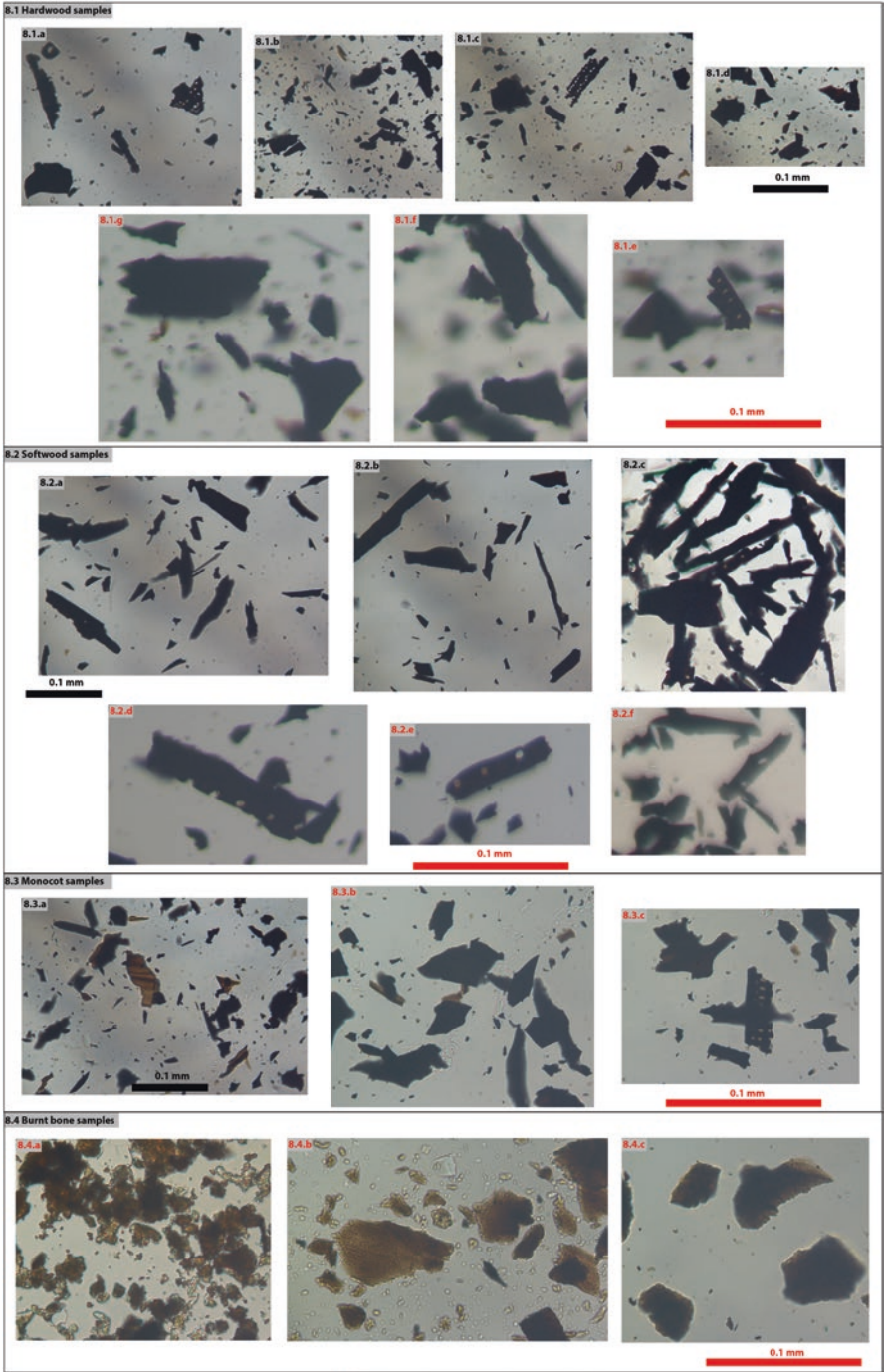
Charcoal particles under transmitted light illumination are visually recognizable as opaque, angular, and usually planar and black particles; sometimes parts of plant features are still preserved (Fig. 8). Descriptions of all morphotypes that can be found in “natural” contexts have been previously published (e.g., Enache and Cumming 2006, 2007; Jensen et al. 2007). The main morphotypes in archaeological contexts are (1) plant cells (i.e., plant features), (2) angular shapes, (3) elongated shapes, (4) no specific shapes, and (5) not totally black and opaque particles (Figs. 8 and 9; Marquer 2009, 2010). Angular shapes and no specific shapes are the most abundant based on wood and herbaceous samples and fossil samples (Fig. 9). Particles with elongated shapes and remains of plant features are more abundant in herbaceous samples. Note that these studies (Fig. 9; Marquer 2009, 2010) only considered morphotypes of hardwood as charcoal samples, and the results would have been different if softwood has been used. In particular, the proportion of elongated shapes would have been higher (see above).

Few morphotypes are observed in archaeological contexts in comparison to natural contexts because other parts of plants such as parts of roots and leaves can be abundant in lake, soil, or peat samples (Courtney-Mustaphi and Pisaric 2014). Plants in archaeological contexts result from plant selection, and more particularly wood as fuel. Roots and leaves are probably accidentally added to hearths and their initial abundances can be assumed to be much lower than those of wood, which is expected to have been intentionally used as fuel. Pine cones might have further been

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**Fig. 8** (continued) (black scales and labels) and 400× (red scales and labels) magnifications. 8.1 Hardwood (all species): (a–d) views of particles with black color, opaque, and angular shape characteristics (most of them), no specific shapes, elongated shapes, and few of them have plant features still visible; (e) particle with black, opaque, and angular shape characteristics, plant features are observed; (f, g) particles with black, opaque, and angular shape characteristics, some of them are elongated. 8.2 Softwood (all species): (a–c) views of particles with black color, opaque, and angular shape characteristics, most of them are elongated; (d–f) particle with black color, opaque, and angular/elongated shape characteristics and remains of plant features. 8.3 Monocots (*Arundo donax* L.): (a, b) views of particles with black color, opaque, and angular shape characteristics (most of them), no specific shapes, elongated shapes, and few of them are not totally black and opaque; (c) particle with black color, opaque, and angular/elongated shape characteristics and remains of plant features. 8.4 Burnt bones (diaphysis and epiphysis from cow) as potential confusants: (a–c) black or brown particles with transparent parts on their edges: burnt bone particles generally have splinter shapes but they do not have clear breakages like charcoal particles





**Fig. 8** Observation of charcoal particles (<0.1 mm) using transmitted light illumination. This figure shows examples of different morphotypes. All pictures have been taken at 200×

used as fuel in some specific cases (Carrión et al. 2008), and therefore, residues from pine cones might also be found in the microscopic fractions of sediment; this has not been investigated so far.

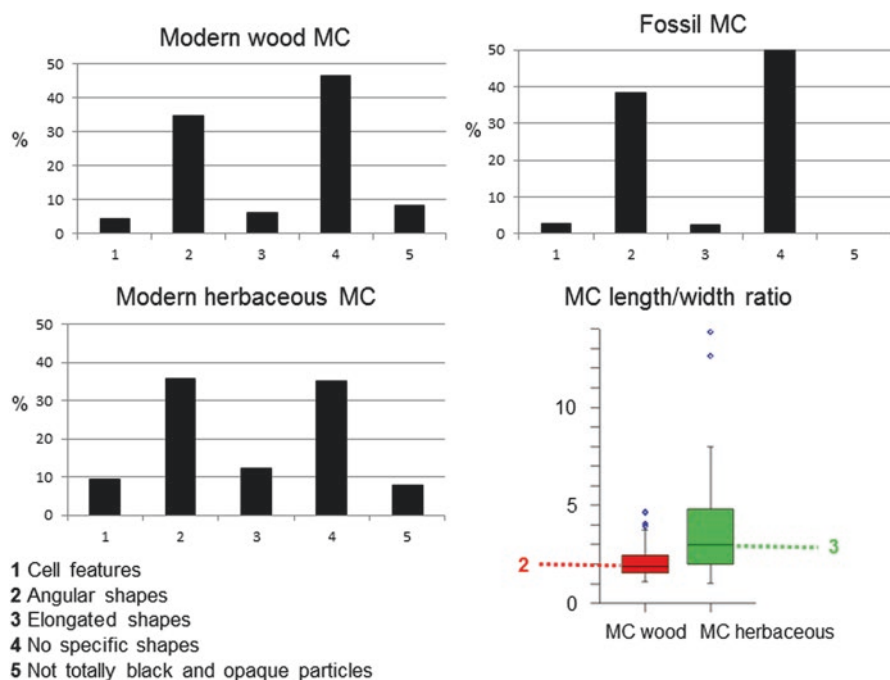
A method has been developed based on particle morphotypes (length-to-width ratio) to attempt a differentiation among wood, leaves, and herbaceous material (Umbanhowar and McGrath 1998; Crawford and Belcher 2014). The results show that the highest ratios in a range of 3.5–4.5 can be related to herbaceous material, while ratios of 2–2.5 correspond to wood. We have tested this method using our approach, and the outcomes were similar to the previously published results, with a value of 2.15 for wood samples and 3 for herbaceous material (Fig. 9; Cui 2008). The use of the length-to-width ratio needs however further experiments to fully understand its meaning and potential.

### 5.5 *Burnt Bones as Potential Confusants of Charcoal Residues*

Recognition of burnt bones is easy for the size classes higher than 0.25 mm because the observation of charcoal shows the common anatomical features of plants (Fig. 5), e.g., vessels, rays, pits, fibers and parenchyma. The distinction of fragments and micro-fragments (size interval 0.25–0.1 mm) of burnt bones from those of charcoal is also straightforward due to the recognition of plant anatomical features (e.g., vessels, tracheids, rays, fibers, parenchyma, and cross-field pitting; Fig. 6), although the largest anatomical structures are not joined together in residues of these sizes.

Anatomical features, such as trabecular structures, volumes of epiphyseal parts, and concentric lamellae with central canals, are also visible (until sizes of 0.1 mm) from burnt bone residues. Burnt bones in all sizes ranging from pieces to micro-fragments present several color degrees ranging from beige to white and black, and they usually have compact or spongy morphology. Weathering, fossilization, and other sources than combustion can also result in the discoloration of bones (Stiner et al. 1995; Cain 1995).

The differentiation of burnt bone and charcoal particles (<0.1 mm) is thought to be difficult; however, the use of reflected light microscopy and specific morphological criteria disentangles many of the potential misidentifications. There is no problem to differentiate burnt bones from charcoal when charcoal still has remains of plant anatomical features (Fig. 7). Nevertheless, most of the time these features cannot be observed, and identification relies on key morphotypes and the use of reflected light microscopy to view remains of plant characteristics on the surfaces of particles. Only the morphotype information can be used under transmitted light illumination, and this increases misidentifications. However, charcoal particles are black and opaque while burnt bone particles are partially black or brown with transparent parts on their edges (Fig. 9). Charcoal particles are further characterized by net breakages mainly resulting in particles with angular shapes. Burnt bone particles often have splinter shapes but they do not have clear breakages (Fig. 9).



**Fig. 9** Relative abundances of the different morphotypes of charcoal particles (MC) expressed as percentages. Abundances have been estimated based on wood (hardwood) and herbaceous modern samples and fossil samples (figures are adapted from Marquer 2009, 2010). The length-to-width ratio for wood and herbaceous charcoal particles is also shown (figure adapted from Cui 2008)

In archaeological contexts, dark minerals and unburnt plant fragments may sometimes be confused with charcoal particles. However, dark minerals are usually transparent and shiny with crystalline form, for example, the octahedral or cubic shape of pyrite. Plant micro-remains have a range of colors from brown to dark, but they are never opaque and totally black with net breakages. Burnt bones are the confusants that can lead to the most misidentifications; however following our identification criteria, the distinction from charcoal is not problematic.

## 6 Archaeological Applications of the Microscopic Charcoal Signal

### 6.1 A Taphonomic Indicator

Charcoal fragmentation and the related production of fragments, micro-fragments, and particles are caused by mechanical stresses on charcoal structures (see the section on Taphonomy). The intensity of post-depositional processes might be linked

to the strength of these mechanical stresses. It may be possible, therefore, to use the concentration of charcoal residues in the finest fractions as a proxy for the overall degree of post-depositional alteration of the sediments. The greater the concentration of fine charcoal residues, the greater the amount of alteration. Of course, there are many factors at play, and further exploratory research needs to be done.

Signals of charcoal and other combustion residues can be analyzed for a succession of samples spread out vertically and/or horizontally within fireplace features and archaeological layers (Marquer et al. 2010b; Marquer 2013; White et al. 2017). Based on such sampling strategies and our quantitative approach, it becomes possible to test hypotheses related to the degree of conservation of hearths and charcoal and other combustion residues, and to the spatial distribution of residues over human settlements in particular to explore the potential locations of fireplaces when those are apparently absent. The charcoal signal can therefore be considered as a taphonomic indicator for comparison with geoarchaeological studies.

The recovery of charcoal residues is of great interest for dating. This offers materials for  $^{14}\text{C}$  dating by increasing opportunities to retrieve charcoal fragments of sufficient sizes for dating (Marquer et al. 2012). Furthermore, a potential development might be to use a micro-manipulation technique for the isolation of charcoal particles from sediments without using any chemicals, such as the one that can be used for diatoms, pollen, and phytoliths (Snelling et al. 2013). While time-consuming, this method might offer  $^{14}\text{C}$  dating in many archaeological contexts where the largest pieces of charcoal are not preserved.

## 6.2 *An Environmental Indicator*

Identification of plant species from charcoal assemblages ( $>1$  mm) provides environmental information about the presence of plants within a small region surrounding the site, but only those that were gathered by humans. Plant species can reflect past climate when the collected wood comes from species that are sensitive to climate conditions. Furthermore, it has been assumed that charcoal assemblages might be a reliable image of vegetation abundance (Chabal 1991). This is based on the hypothesis that societies might have gathered most of the plants in the local/regional environments. This assumption is likely to be valid in specific archaeological contexts such as the Paleolithic periods when plant biomass was limited due to glacial climate conditions (e.g., Allué et al. 2018; Vidal-Matutano et al. 2018; Carrión et al. 2019).

Botanical identification of fragments and micro-fragments is however difficult below sizes of 0.25 mm, because key anatomical criteria are limited on a single fragment or micro-fragment. Hardwood, softwood, and monocots can sometimes be distinguished until sizes of 0.1 mm (see Sect. 5). This differentiation provides information about the presence of broadleaved and conifer trees in the vegetation. The relative proportions of broadleaved and conifer plants can indicate changes in vegetation composition or in human activities due to social factors. Below a size of 0.1 mm, environmental information cannot be directly estimated. However, the

length-to-width ratios of particles can be used as an indicator of vegetation type (see Sect. 5.4).

Microscopic charcoal analysis further provides a quantitative approach to estimate the relative proportions of different types of fuel that have been used by humans (Marquer 2010; Marquer et al. 2010a, 2012, 2015). This offers the opportunity to explore indirect evidence of environmental changes by using a ratio between charcoal and other fuel residues. This ratio might reflect human adaptations via modifications in fuel management due to environmental changes. For example, the use of alternative fuels (e.g., bones, peat, or dung) might have been used as complements of wood in fireplaces when wood cover was greatly reduced in the regional vegetation caused by unfavorable climate conditions (e.g., the last glacial maximum). However, these environmental interpretations based on particles from charcoal and combustion residues need further experiments and applications to assess their degrees of accuracy; the relative proportions of different types of fuel might also result from human behaviors, see hereafter.

### 6.3 *An Indicator of Human Behavior*

By studying the charcoal signal, we can better understand which wood and plant species have been used in hearths (see the previous sub-section); this can provide insights into firewood management practices. Charcoal pieces and fragments provide additional information about the characteristics of collected plants such as nature of wood pieces (e.g., branches, trunk, bark, and brushwood), sizes of wood pieces (e.g., caliber of the largest pieces), and wood physiology (e.g., greenwood, decayed wood, or driftwood). Such information cannot be assessed based on micro-fragments and particles.

The smallest charcoal residues can indicate the presence of relative mixture of grass and wood, and also offer the opportunity to provide comparable quantitative information about the abundance of different combustion residues, by using the same measurement unit expressed as  $\text{cm}^2$  of charcoal or burnt bones (or other types of combustion residues) for  $1 \text{ cm}^3$  of sediment. Concentration ratios between different combustion residues as well as compositional spectra of those might be considered; however, further experiments into this are needed. Ash analyses might further complement the fuel compositional spectra (Braadbaart et al. 2017).

Combustion processes such as hearth temperature can also be studied based on charcoal pieces, fragments, micro-fragments, and particles. Combustion temperature is thought to be related to the reflectance of charcoal residues (e.g., Scott et al. 2000; Scott and Glasspool 2005; Braadbaart and Poole 2008; McParland et al. 2009). Likewise, the color of burnt bones can be related to the intensity of heat that the bone reached (Stiner et al. 1995; Cain 1995). This might inform on fire intensity and/or velocity and time exposure. New knowledge on both variety of fuels that have been used in hearths and data about combustion processes offers the opportunity to explore the functionality of fireplaces such as cooking, light, drying, heating,

curing, or cleaning, and their diversifications over the habitats. These aspects can provide an important window to explore the degree of organization within a human group. Furthermore, exploration of changes to these functions through time due to societal or environmental changes can help archaeologists assess resilience and technological adaptation within human groups.

## 7 Conclusion

Charcoal assemblages can inform archaeologists about the use of wood fuel resources and other activities related to fireplaces. However, the quality of our interpretation of past fuel management depends on reliable information about charcoal assemblages.

Charcoal assemblages are closely related to taphonomy. Charcoal is very fragile in comparison to other combustion residues such as burnt bones. This is essentially due to the high porous structures (e.g., vessels and tracheids) of charcoal, compared to the compact morphology of bones. In archaeological contexts, charcoal pieces can be broken into fragments, micro-fragments, and particles. However, charcoal has extremely inert stable properties that favor its preservation, and therefore, remains of the presence of charcoal should be found if they were once abundant in human settlements. In that case, the absence or scarcity of charcoal in some archaeological sites might be just an illusion. This means that fragmentation of combustion residues results in incomplete information for archaeologists when only the largest pieces and fragments are considered. Note that data based on the largest pieces provide a fair and true sampling of the complete charcoal profile when they are abundant in archaeological contexts. However, fragmentation is a critical concern for the interpretation of combustion residues when charcoal is rare or apparently absent from hearths or archaeological features.

The microscopic charcoal analysis can therefore be considered as a strategy to minimize the impact of sample incompleteness and bias related to combustion residues in archaeological contexts. By taking into account the consequences of taphonomy, microscopic charcoal analysis provides a reliable assessment of firewood and fuel management practices and the related resilience of societies through time. Microscopic charcoal analysis can further offer additional information about the intensity of taphonomical processes, and opportunities for radiocarbon dating.

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# Phytolith Analysis in Paleoecology and Archaeology



Dan Cabanes

## 1 Introduction

The word phytolith comes from the Greek phyto (φυτό) and lithos (λίθος), meaning literally stones formed in plants. Phytoliths can be formed by calcium oxalate or opal, though in the scientific literature the term phytolith is almost exclusively applied to opaline phytoliths. Phytoliths can accumulate naturally in environments such as paleosoils, lakes, and other natural sedimentary deposits and they may constitute a very abundant element in archaeological sediments, to the point that some archaeological layers are formed almost exclusively by phytoliths (Albert et al. 2008).

Phytoliths can be used as a proxy for past vegetation and they provide information about paleoecology and human behavior. Phytoliths have been known since the nineteenth century (see Ehrenberg 1843, 1854 in Hart 2016; Piperno 1988), yet until relatively recently they have not enjoyed the popularity of other micro-botanical remains. Since 1997, the amount of phytolith-related publications has grown exponentially and continues growing nowadays (Hart 2016). Currently, the number of papers published annually on phytoliths is well above 100, and the number of phytolith specialists is increasing constantly. The main applications for conventional phytolith analyses are archaeology and paleoecology. However, phytoliths are of interest also in botany, chemistry, structural biology, geology, paleontology, soil sciences, and agricultural sciences, among others. Phytoliths have a critical function in plant biology, they have a central role in the global cycle of silicon and in carbon sequestration, and they can be applied to forensic analyses (Alexandre et al. 1997a; Derry et al. 2005; Epstein 1994; Farmer et al. 2005; Gérard et al. 2008; Ma and

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Yamaji 2006; Marumo et al. 1986; Parr and Sullivan 2005; Street-Perrott and Barker 2008).

The present chapter aims to be a short introduction to phytolith research in paleoecology and archaeology. The information appearing here is based on the current literature, but I would like to contribute as well with the observations I have gathered after several years of experience in the field and in the laboratory.

## 2 Phytolith Formation and Cycle

Phytoliths are the result of a biomineralization process by which certain plants accumulate silica at the cellular level (Perry et al. 2007). Silicon is one of the most abundant elements in the Earth's crust and plants absorb it in the form of monosilicic acid ( $\text{Si}(\text{OH})_4$ ) through their roots, together with water and other nutrients. Silicon is then deposited in the form of opal or hydrated silica ( $\text{SiO}_2 \cdot n\text{H}_2\text{O}$ ) into the aerial parts of the plants, particularly, but not exclusively, in those cells related to transpiration (Piperno 2006). In addition to opal, phytolith composition can include small amounts of impurities including Al, Fe, Mn, Mg, P, Cu, N, and organic carbon (Bartoli and Wilding 1980; Jones and Beavers 1963). The presence of small amounts of occluded carbon has been the basis for  $^{14}\text{C}$  dating of phytoliths (Wilding 1967).

Opal can accumulate in the plant's intercellular space, in the cell walls, or it can infill the cells adopting the morphology of these cells (Piperno 1988, 2006). In other words, opaline phytoliths can be considered a sort of mold or cast showing part of original cell morphology. Thus, the different phytolith morphotypes can be used to identify the original biological tissue where they form or the plant taxa producing them (Mulholland and Rapp Jr. 1992).

Although phytolith production is directly regulated by plant genetics (Piperno et al. 2002; Zheng et al. 2003), environmental factors including soil type, evapotranspiration, and amount of water available can affect the accumulation of phytoliths and the degree of silicification in plants (Quigley and Anderson 2014; Rosen and Weiner 1994). Phytolith formation in plants has an important role in the cycle of silica. Plants can obtain the silica by weathering silicon minerals in soils, but often they can also "recycle" previously deposited phytoliths (Conley 2002; Farmer 2005; Farmer et al. 2005; Meunier et al. 2001). In fact, in forest environments, the contribution of previously deposited phytoliths to the cycle of silica is much higher than that of the mineral fraction (Alexandre et al. 1997a; Bartoli and Wilding 1980).

The function of phytoliths in plants has been a debated issue (Strömberg et al. 2016). The main hypotheses explaining silica accumulation in plants describe phytoliths either as a growth promoter or a defensive mechanism. Plants can use silica instead of carbon-based compounds as structural support (Raven 2008). Silica is more cost effective than carbon and could have allowed plants to compete for light and space, but also might have re-enforced the cell walls preventing their collapse during droughts (Strömberg et al. 2016). Opaline phytoliths could be also an adaptive response of plants to herbivores and parasites, making the organic tissue of the

plants less digestible and accessible (Lucas et al. 2000; Massey et al. 2006). Other hypotheses suggest that phytoliths could be the result of the excretion of a substance, such as silica, that in excess could be toxic to plants (Piperno and Pearsall 1993). Strömberg et al. (2016) produced a thoughtful review of the evidence and concluded that at least the accumulation of phytolith in grasses was not the result of a defense mechanism against large mammalian herbivores. Recent research showed that silica in plants could help to stabilize amorphous calcium carbonate when producing cystoliths, which act as light scatters enhancing photosynthesis (Gal et al. 2012; Pierantoni et al. 2018). In addition, silica deposits reduce the amount of UV radiation protecting the plant tissues (Pierantoni et al. 2017).

Phytolith deposition in soils and sediments is ruled by the classical taphonomical process including necrolysis, which is the disarticulation and decomposition of the organic plant remains; biostratinomic processes, which encapsulate all the process previous to the burial of the plant; and fossil diagenetic processes, which comprise all the post-depositional processes that might affect the remains once buried (Madella and Lancelotti 2012). Phytoliths are deposited in sediments when the plant dies and decompose, releasing the phytoliths from the organic material surrounding them. Because of their inorganic nature, phytoliths can survive for millions of years in favorable burial environments. In addition, to some extent, they are resistant to fire and to the digestive tract of most mammals. Thus, they can also enter into the sedimentary record even when the plants are burnt or digested (Shahack-Gross 2011). Phytolith can also accumulate in the dental calculus of plant consuming animals, including humans. In a study on chimpanzee calculus, Power et al. (2015) discovered that phytolith abundance in calculus actually reflects the proportions of plants in the diet and, therefore, they can be used to study diet in fossil populations (Henry et al. 2011, 2012; Power et al. 2018).

In extreme conditions, such as desert storms or catastrophic wildfires, phytoliths can suffer long-distance transport (Piperno 1988), but generally their deposition is considered to be *in situ* and their horizontal and vertical movement is not significant (Grave and Kealhofer 1999; Piperno 2006). However, the movement of phytoliths down through a soil profile depends on soil granulometric characteristics and the size of the individual phytoliths, with smaller phytoliths being easily transported in soils with bigger particle sizes (Fishkis et al. 2010, 2009). Bioturbation, seismic activity, or any other post-depositional process affecting the sediments will also affect the phytoliths in them (Cabanes et al. 2012). From my own experience, it is difficult to recover phytoliths from sediments with a particle size bigger than fine sand, whereas they will have little translocation if the particle size is silt or clay. The inorganic nature of phytoliths makes them resistant to the weathering process that most organic vegetal remains cannot survive. Phytoliths can survive fire and acidic pH environments, yet they will be affected by temperatures above 700 °C and by alkaline pH (Cabanes and Shahack-Gross 2015; Cabanes et al. 2011; Fraysse et al. 2006a, b, 2009; Jones and Beavers 1963; Loucaides et al. 2010, 2008; Piperno 1988, 2006).

### 3 Sampling Strategies, Extraction Methods, Identification, and Quantification

#### 3.1 *Sampling Strategies*

Phytolith sampling will depend on the questions to be answered and on the methods used for extracting phytoliths. In archaeology and paleoecology, sediment samples can be collected from exposed excavation surfaces, profiles, cores, and archaeological artifacts. Additionally, phytoliths can be recovered from dental calculus (Henry et al. 2011, 2012; Henry and Piperno 2008; Power et al. 2018, 2015). Phytoliths could be extracted as well from modern plants and soil samples to build modern reference collections (Esteban et al. 2017c; Iriarte and Paz 2009; Mercader et al. 2009, 2011; Novello et al. 2018; Tsartsidou et al. 2007). In paleoecology, where the major aim is to detect diachronic changes, samples will be normally collected at regular intervals in exposed profiles and cores (Piperno 2006). Another approach can be to sample along several trenches to produce a spatial distribution of the phytolith assemblages and, in this way, reconstruct the distribution of plants in the past landscape (Bamford et al. 2006). In archaeology, the sampling can either focus on exposed profiles to study the diachronic use of plants or on different features and exposed excavation surfaces to describe the spatial pattern of human activities (Henry et al. 2004; Portillo et al. 2014; Regev et al. 2015; Rodríguez-Cintas and Cabanes 2017; Tsartsidou et al. 2008, 2009). In both cases, it is important to have a well-defined sampling strategy that includes multiple samples from the same layer or feature. These repeated samples can be used to determine the reproducibility of the extraction method applied and to evaluate the statistical significance of the differences observed in the phytolith assemblages.

The most important samples to collect during phytolith sampling are the control samples. Control samples in archaeological sites can be external or internal. Control samples will give information about the background noise in phytolith assemblages, allowing a proper interpretation of the anthropogenic assemblages. These are the phytoliths that have been accumulated by natural agents in the case of the internal control samples or modern phytoliths that could have been translocated in the case of external control samples. External samples are those from outside the site itself and can include the topsoil covering the site or different soil exposures around the site area. Internal control samples can be collected preferably from sterile layers within the site, with no evident signs of human activities. Less ideal locations are empty spaces between archaeological features and construction materials. Construction materials can be used, especially in archaeological sites with intense human occupations, such as tell sites in the Levant, where sterile layers barely exist. In these sites, mudbricks, adobe, or terra pisé walls were often made using local sources of clay that could include phytoliths (Albert et al. 2008; Cabanes et al. 2012; Regev et al. 2015). Frequently, these construction materials are degraded or eroded (Friesem et al. 2011, 2014a) releasing their phytolith content, and potentially mixing up with the phytolith assemblages produced by plants brought to the site by

humans. Therefore, it is important to differentiate among phytolith assemblages included in clays used for construction materials and phytolith assemblages produced by human activities. Note that certain construction materials such as thatched roofs will produce a very specific phytolith assemblage that should not be confounded with other activities involving plants (Friesem et al. 2014b; Regev et al. 2015). Control samples are particularly important in Paleolithic sites where plant-related human activities can be difficult to identify due to their low impact on the sedimentary record (Rodríguez-Cintas and Cabanes 2017).

Depending on the extraction method, different amounts of sediment ranging from 100 to 50 g to 30–50 mg will be needed. It is a good practice to collect more sample than needed for backup in case of an incident during the sample transport or extraction, also as for being able to replicate the sample if necessary. Note that depending on the research question it might be necessary to compromise between resolution and backup. Whereas collecting more sample than needed will secure a backup, the resolution of the study will be compromised by pooling and homogenizing sediment that can contain different phytolith assemblages. Prior to collecting the samples, it is important to clean the excavation surface or profile to avoid cross-contamination. In archaeology, it is essential to photograph the area sampled before and after the sampling. It is also important to locate the samples collected in the three-dimensional grid and include them into the excavation general database so they can be correlated with other archaeological remains. Although this may seem a standard practice, the integration of macro- and micro-remains in the archaeological interpretation is still nowadays a rare habit. Sampling from archaeological artifacts such as grinding stones, pottery, or mills might need to be performed in the laboratory under controlled conditions (Piperno 2006). It is essential in these cases to collect samples from the surroundings of the objects to differentiate the phytolith assemblage from the artifacts from that of the sediments. Since phytoliths have an inorganic nature, phytolith samples do not need special storage conditions and can be stored at room temperature in sealed plastic bags or tubes. If phytolith samples are collected for  $^{14}\text{C}$  dating, the storage conditions have to adapt to the standard procedures in  $^{14}\text{C}$  dating. Once the samples are collected, a good practice is to allow them to air dry overnight in a closed room during the fieldwork and then to dry them in a drying oven as soon as the sediments arrive at the laboratory. This will avoid the development of fungi and bacteria, in addition to ready the samples for analysis.

### **3.2 Extraction Methods**

#### **Extraction Methods from Modern Plants**

Since phytolith analysis is based on the identification of the original cell morphology, reference collections from modern plants are an essential part of phytolith research. When building the reference collection, one should consider sampling the whole plant, as well as the different tissues forming the plant, to identify whether

the plant was used as a whole or if some tissues were selected. It can be also a good practice to sample the same taxon in different ecological settings to cover the potential phytolith variability produced by different growing conditions.

Phytolith extraction from modern plants consists in accelerating the decay of the organic material surrounding the phytoliths. All the extraction methods from modern plants include three preliminary steps:

1. Washing the plant material with soap and using ultrasounds to eliminate possible contaminants attached to the surface of the plants. Note that among the contaminants, we can find also phytoliths from other plants (Tsartsidou et al. 2007).
2. Drying the plant material to eliminate the differences in water content previous to weighing the initial sample. This can be achieved efficiently by drying the plant material at 110 °C over 72 h.
3. Weighing the initial plant material and the final residue extracted to calculate phytolith concentrations in the plant studied.

The most common methods used to extract phytoliths from modern plants are the dry ashing method and the wet ashing method (Parr et al. 2001b). The dry ashing method consists of incinerating the plant material after it has been washed and dried. The time and temperature in the dry ashing method could vary, but normally do not go over 4 h and 550 °C. Note that time and temperature for dry ashing have to be adapted to the initial volume of plant material, otherwise, an excess of time/temperature can produce melted phytoliths. Also, it is necessary to provide enough oxygen when burning the samples to eliminate the maximum amount of organic material. One of the most common mistakes when extracting phytoliths using the dry ashing method is to introduce huge quantities of plant material in muffle ovens that have a relatively small room. An efficient approach is to divide the original sample into smaller quantities and use open crucibles. Note that some cross-contamination can occur if the temperature increase is too fast and flames appear. Therefore, I would suggest using a low rate of temperature increase and only one type of sample per incineration. Since plant ashes can contain different amounts of calcium carbonate, a simple addition of a few milliliters of 1 N HCl will suffice to eliminate the calcium carbonate. If charcoal is still present it can be eliminated with a second ashing after the HCl process, or by applying hydrogen peroxide in a hot bath. Wet ashing consists normally of applying nitric acid saturated with potassium chlorate ( $\text{KClO}_3$ ) to the plant material in a hot bath. Nitric acid can be eliminated by centrifugation and washed out with distilled water or with pure ethanol, but it needs to be watched and stirred continuously under the fume hood (Parr et al. 2001b). Both methods provide similar results in terms of the morphology of individual phytoliths, but using the wet-ashing extraction method will produce a lower amount of anatomically connected phytoliths (Jenkins 2009; Parr et al. 2001b). A variant of the wet ashing method includes acetolysis, which uses a combination of acetic anhydride and sulfuric acid to remove organic material (Costa et al. 2016). Microwave digestion can accelerate the processes and reduce costs when using the wet-ashing method (Parr et al. 2001a). When comparing dry and wet ashing methods in terms of productivity, time consumption, and laboratory equipment the dry ashing method seems to be a more efficient approach to prepare a phytolith reference collection.



## Phytolith Extraction from Soils and Archaeological Sediments

The process of extracting phytoliths from sediments and soils aims to isolate the maximum quantity of phytoliths possible from the organic and mineral components of the sample. When extracting phytoliths from sediments and soils, one should be aware that there is no single extraction method that will work in every archaeological site, and most probably the extraction method will have to be adapted to the characteristics of the sediment studied. Currently, there are several available methods to extract phytoliths from soils and sediments (Albert et al. 1999; Calegari et al. 2013; Katz et al. 2010; Lentfer and Boyd 1998; Lombardo et al. 2016; Madella et al. 1998; Parr 2002; Powers and Gilbertson 1987; Rovner 1972; Zhao and Pearsall 1998). Some of them are focused on only phytolith extraction, whereas others try to combine phytolith extraction with the recovery of other micro-remains, such as pollen, starches, or spores (Coil et al. 2003; Lentfer and Boyd 2000). These extraction methods vary greatly in the initial amount of sample needed, from tens of grams to tens of milligrams, and the duration of the process, from several days to few minutes. However, all of them have several steps in common, though they are not necessarily applied in the order presented here:

1. Weigh the dry sample. It is important to know the initial amount of sediment used to calculate the concentrations of phytoliths in the sample. To avoid errors due to differences in the water content of the sediments, the samples should be dried prior to weighing.
2. Disaggregate the initial sample either by physical or chemical means. Sediment samples can be disaggregated by gently using a pestle and a mortar, although an excess of grinding could damage the phytoliths. Alternatively, disaggregation can be done by deflocculation using sodium hexametaphosphate (Piperno 2006), EDTA ( $C_{10}H_{16}N_2O_8$ ), or sodium bicarbonate ( $NaHCO_3$ ).
3. Remove carbonates using acid. Normally carbonates are removed using hydrochloric acid (HCl) at different concentrations. A combination of HCl and nitric acid ( $HNO_3$ ) can be used to provide better results when organic matter and phosphates are present. The concentration and amount of HCl will change depending on the method used and the initial amount of sediment to be processed. Sometimes HCl is applied in a hot bath to accelerate the process, but if the concentration of acid is adapted to the composition of the sample, this step is unnecessary. The excess of acid can be removed by adding distilled water to the solution, centrifuging, discarding the supernatant, and repeating this process several times. Centrifuging times and speed will vary depending on the type of centrifuge used, the length of the tubes used, and the amount of sample processed.
4. Eliminate the excess of organic matter. Some samples might have an excess of organic matter that will make phytolith identification more difficult. In some cases, the original organic matter might still encapsulate the phytoliths, making it impossible to identify its morphology. Organic matter can be removed by chemical attack or by burning. Commonly, either  $HNO_3$  or hydrogen peroxide ( $H_2O_2$ ) is used to remove organic matter from the sediments (Albert et al. 1999;

Piperno 2006), but these processes are time intensive and might be dangerous. Alternatively, samples can be incinerated in a muffle furnace at 500 °C to eliminate the excess of organic matter (Rosen 2001). When using the fast extraction method (Katz et al. 2010), I have been able to eliminate the excess of organic matter by heating few milligrams of the sample at 500 °C for 1 min and then letting the furnace cool down. The major advantage is that the organic matter can be removed in a couple of hours in a cheap and safe process that does not require constant attention. Obviously, the major disadvantage is that any information regarding the thermal alteration of the phytoliths will be lost.

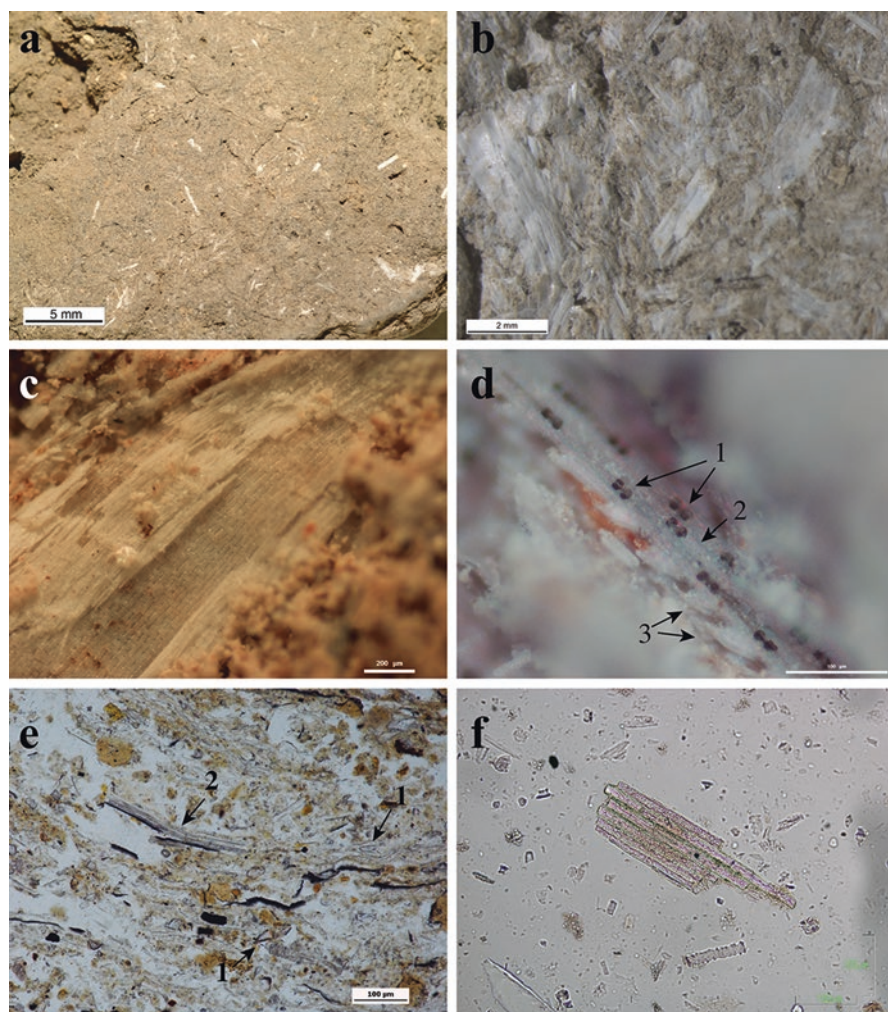
5. Isolate the phytoliths from the rest of the sedimentary components using a heavy liquid. Phytoliths have a specific gravity ranging from 1.5 to 2.3 (Piperno 2006) and can be separated from lighter and heavier fractions of sediment using a heavy liquid. There are several commercial heavy liquids available, but in the recent years, sodium polytungstate (SPT) has acquired a prominent role. This heavy liquid is easy to prepare, requiring only distilled water and a stirrer, it is safer to use than other heavy liquids and it helps to further deflocculate the clays (Katz et al. 2010). The basis of separation by heavy liquids is gravity decantation, which can be accelerated by centrifuging the sample. Also, additional sonication can help to further isolate phytoliths from other elements (Katz et al. 2010; Lombardo et al. 2016).
6. Mount and store the sample. The last step of phytolith extraction is to mount them into a slide for microscope observation or to store them for further analyses. Mounting media varies as much as the phytolith extraction methods, and it can be permanent or temporary. Some methods even use SPT as a temporary mounting media, eliminating the requirement for additional products (Katz et al. 2010). In general, all mounting media used for phytoliths should have two basic properties: (1) the refractive index should be sufficiently different from that of phytoliths so they can be observed clearly. Note that phytoliths' refractive index depends on their hydration state and it can range from 1.427 to 1.487 (Elbaum et al. 2003); (2) the mounting media should permit the rotation of the phytoliths so that their three-dimensional attributes can be seen.

A general rule of thumb when choosing a phytolith extraction method is that those with fewer steps will likely produce fewer errors. Each time a solution containing phytoliths is transferred, part of the sediment might be lost increasing the potential final error. Also working with solutions rather than with dry sediment helps to minimize the potential loss of phytoliths in the laboratory. Nevertheless, each type of sediment is different and additional steps might be needed to efficiently extract phytoliths. If several steps are used, weighing the sample at different stages of the process can give information about the composition of the samples in percentage of carbonates, organic material, and acid insoluble fraction (AIF) (Cabanes et al. 2007, 2009).

### 3.3 *Phytolith Identification*

Normally phytoliths are not visible to the naked eye, but when they are present in large quantities they can be observed in the so-called phytolith rich layers. These layers can have a thickness that ranges from millimeters to centimeters, a color that ranges from gray to white, and a loose texture (Albert et al. 2008; Regev et al. 2015; Shahack-Gross et al. 2005). Sometimes, phytoliths can be identified in the form of millimetric white fibers in the occupation surfaces (Fig. 1a–d). Phytoliths can be detected in thin sections, although their identification is more complex than in regular preparations (Vrydaghs et al. 2017) (Fig. 1e). Once embedded in the thin section phytoliths cannot be rotated and depending on their orientation with respect to the cutting plane their identification might not be feasible. Under the microscope, the color of the phytoliths can range from translucent to violet in most mounting media. Dark-colored phytoliths can appear if the plant has been burnt (Parr 2006) (Fig. 1d), but phytoliths showing a normal violet-like coloration can be burnt as well. The change in coloration can be only expected if there is enough organic matter trapped in the opaline matrix. Interestingly, phytoliths extracted with the wet ashing method have a refractive index that ranges from 1.427 to 1.440, whereas phytoliths extracted using the dry ashing method have a refractive index that ranges from 1.438 to 1.487 (Elbaum et al. 2003). The change in the refractive index of phytoliths is produced by the loss of water with the increasing temperature and can be used to identify burnt phytoliths in the archaeological record regardless of their coloration (Elbaum et al. 2003). Phytolith identification can be performed using a biological microscope at 200 $\times$  and higher magnifications, although a petrographic microscope with polarizing light and rotating stage can be more convenient when learning to identify phytoliths. The opal forming the phytolith is an amorphous mineral. Thus, phytoliths are isotropic and do not transmit light in cross-polarized light and, consequently, they should be invisible when using crossed nicols.

Phytolith analysis is based on the identification and quantification of the different morphotypes. Individual phytoliths should be named following the International Code for Phytolith Nomenclature (ICPN) (Madella et al. 2005). The ICPN uses a hierarchical system of up to three words to name a phytolith morphotype. The naming system provides information at taxonomic, anatomical, and/or morphological level. The morphotype name will begin with the taxonomical information if the phytolith can be unequivocally attributed to a taxon. If a morphotype is produced by different taxa, but its production is restricted to a plant organ or tissue, then the name should begin with the anatomical information. Finally, if neither taxonomical nor anatomical information is available the name will begin with a morphological descriptor. In addition, phytoliths can have different types of decoration that helps to distinguish them and this information can be added to the name. Regardless of the information level provided by the naming system, a three-dimensional observation of the phytolith is required for its correct identification. Therefore, it is very important that the mounting media used allows the rotation of phytoliths so that they can be observed from different angles.



**Fig. 1** Phytoliths in archaeological contexts. **(a)** Fiber-like phytolith accumulation associated with a basalt grinding implement in Area A at Tell es-Safi/Gath, Iron Age I, ca. 1000 BCE. Israel. The white fibers are approximately 4 mm length and 1–2 mm width and were visible to the naked eye immediately below and around the implement. **(b)** Detail of a phytolith-rich layer in Area Q at Tel Megiddo, Israel (Iron Age IIA period, ca. ninth century BCE). The phytolith-rich layers were visible to the naked eye as thin and compacted white sediment layers. Individual white fibers a few millimeters in length were identified using a binocular microscope. These fibers are remains of vegetal matter decayed in situ. **(c)** Magnified view of one of the fibers associated with the grinding implement at Tell es-Safi/Gath formed by phytoliths in anatomical connection. The fibers contain highly decorated phytoliths, including elongate dendritic (previously known as “dendritic long cells”) and papillae, which are common in cereal husks (Fig. 2a, b). The image was collected using a reflected light microscope and no preparation was required due to the excellent preservation of the sample. **(d)** Expanded detail of one of the phytolith-rich layers at Megiddo. Individual bilobate short cell phytoliths (1) can be identified in anatomical connection with elongate entries (previously known as “psilate long cells”) (2) and acute bulbosus (previously known

The number of identified phytoliths per sample might affect the interpretation of the fossil assemblages (Strömberg 2009). A minimum of 200 individual phytoliths identified per sample produces an error of 20% in the assemblage interpretation (Albert et al. 2003; Albert and Weiner 2001). Thus, the most accepted standard minimum count size is between 250 and 300 individual phytoliths, regardless of their type (Zurro 2017).

The foundations for phytolith identification are the reference collections from modern plants. Several exhaustive works in different parts of the world have provided data on phytolith variability across plant species (Albert et al. 2009, 2006; Carnelli et al. 2004; Esteban et al. 2017b, c; Fernández Honaine et al. 2006; Gallego and Distel 2004; Iriarte and Paz 2009; Kerns 2001; Mercader et al. 2009, 2011; Morris et al. 2009; Novello et al. 2018; Thorn 2004b; Watling and Iriarte 2013). Moreover, with the development of the internet and database technology, reference collections are now available online. These online reference collections are extremely useful and they are continuously growing in a collaborative effort to include phytoliths from modern plants and soils, as well as fossil phytoliths, allowing researchers to observe the effects of weathering on different morphotypes (Albert et al. 2016).

Phytoliths from soils and sediments can be present as isolated elements or in groups. When phytoliths appear in groups, they have been traditionally labeled as silica skeletons, multicellular structures, or conjoined phytoliths (Fig. 2a, b). In fact, these are nothing but phytoliths in anatomical connection; therefore, I believe that they should be referred to as anatomically connected phytoliths. The percentage of phytoliths in anatomical connection can give an idea of the preservation state of the phytolith assemblage (Cabanes et al. 2009, 2011); consequently, each one of the phytoliths forming the group should be identified and counted, and the total number of phytoliths in anatomical connection should be reported. A quite misleading practice has been counting phytoliths in anatomical connection as a single phytolith when calculating the concentrations of phytoliths per gram of sediment. This could lead to lower concentrations of phytoliths in the samples that have better retained the anatomical structure of the plant, and that are actually better preserved.

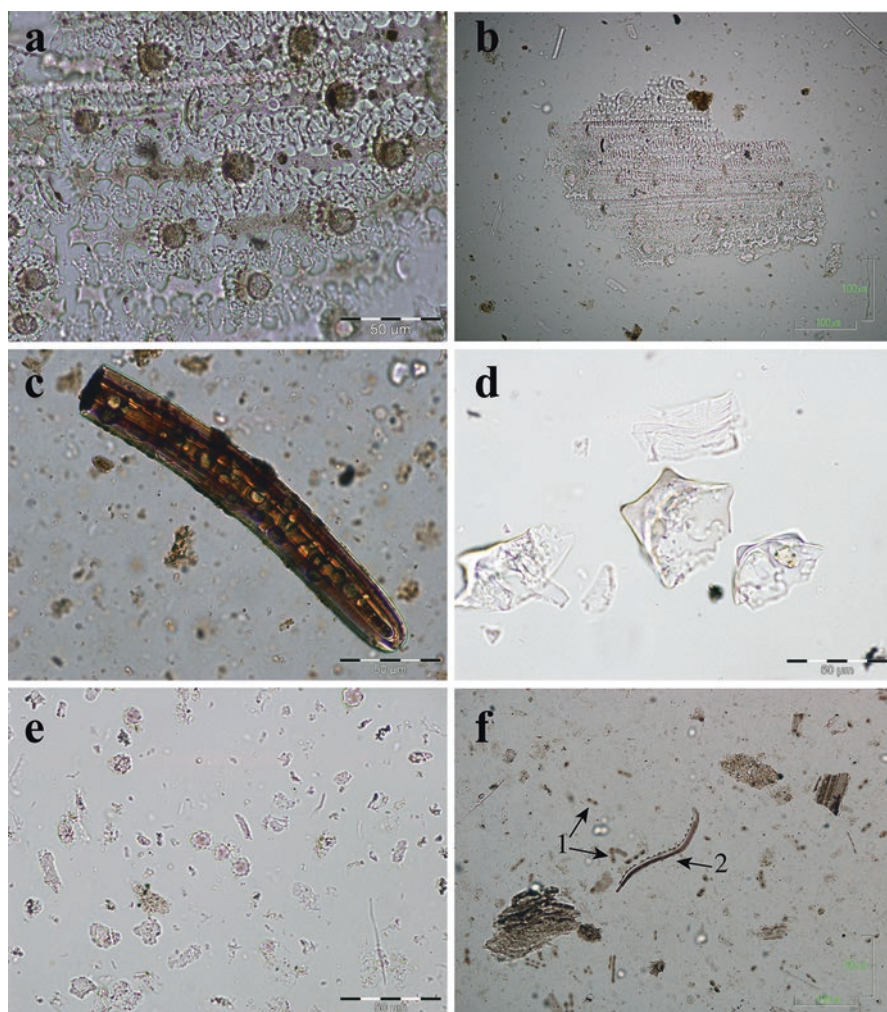
Another important aspect of phytolith studies is morphometric analyses. Morphometric analyses are used to differentiate plant species that share similar cell structure. Using computer-assisted photography, different dimensions of an individual phytolith are measured in the fossil assemblage. To identify the original

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**Fig. 1** (continued) as “prickles”) morphotypes (3). The sample was collected from a series of thin superimposed layers showing discrete phytolith assemblages formed by sedges (Fig. 2f), reeds, and cereal inflorescence, probably resulting from the collapse of a thatched roof on the activity surface (Regev et al. 2015). (e) Grass leaf phytolith in thin section (1) in the Middle Paleolithic layers at El Esquilleu, Spain. Some of the phytoliths are still in anatomical connection (2) and are related to bedding areas produced by Neanderthals (Cabanes et al. 2010; Mallol et al. 2010) (Picture: Carolina Mallol). (f) Elongate phytoliths from grass leaf in anatomical connection after being extracted from the Middle Paleolithic sediments at El Esquilleu, Spain (Cabanes et al. 2010)





**Fig. 2** A few typical phytolith types and sponge spicules. (a) Anatomically connected phytoliths from the inflorescence of *Triticum aestivum* showing elongate dendritic (dendritic long cells) and papillae in their original position in the plant. Modern plant reference collection extracted by dry ashing. (b) Phytoliths in anatomical connection related to a grinding implement from Tell es-Safi/Gath, in Israel, showing similar features to those of the reference collection in (a). (c) Fragment of sponge spicule showing the central hollow canal from the Middle Paleolithic layers in el Salt site, Spain. (d) Double-peaked phytolith characteristic of rice inflorescence (*Oryza sativa* L. cv. *Gleba*). Modern plant reference collection extracted by dry ashing. (e) Spheroid echinate typically present in palms. Modern plant reference collection extracted by dry ashing leaves of *Phoenix dactylifera*. (f) Hat-shaped sedge phytoliths in anatomical connection from Area Q in Tel Megiddo. Phytoliths are randomly oriented and can be observed from the top (1) or from the side (2)



plant, the results are then compared to the modern reference collection using various statistics (Albert et al. 2009; Ball et al. 2017, 2006, 1992, 2016b, 2009; Evett and Cuthrell 2016; Out and Madella 2016, 2017; Portillo et al. 2006; Whang et al. 1998).

When studying archaeological and paleoecological records, there are other elements in the sediments that can be confused with phytoliths. Diatoms, sponge spicules, radiolaria, and some volcanic ashes are also made of amorphous silica and have a similar aspect under the microscope. These elements have clear, differentiated morphologies, but they still can be confounding factors if some of these morphologies are incomplete. Sponge spicules can be especially challenging because some have a cylindroid morphology similar to some grass cells, but they can be distinguished from phytoliths since spicules have an organic axial filament in their central hollow canal (Müller et al. 2005) (Fig. 2c).

Phytolith concentrations in soils and archaeological sediments can range from a few hundreds to tens of millions per gram of sediment. In soils, this variability is the result of the different phytolith productivity in plants and the taphonomical processes affecting phytoliths (Cabanès and Shahack-Gross 2015). In archaeological sediments, the differences in the concentration of phytoliths can be an indicator of the intensity of human activities (Cabanès et al. 2012); therefore, it is essential to report phytolith concentrations in archaeology.

In addition, phytolith concentrations can change if the sediment matrix is affected by diagenesis. Some diagenetic processes involving phosphates are common in caves and can reduce the volume of calcitic sediments after burial (Karkanas et al. 2000). Diagenesis, then, can artificially increase the concentration of phytolith per gram of sediment. Hence, it is necessary to assess the preservation state of the sediments studied before analyzing its phytolith content. When diagenesis has affected the sediments, the concentration of phytoliths is given by gram of AIF (Albert et al. 2003, 1999; Albert and Weiner 2001), as a way to normalize the counts between altered and unaltered sediments. A good practice can be to provide the percentage of AIF in each sample analyzed regardless of the preservation state or the extraction method used.

## 4 Advantages and Limitations in Phytolith Analyses

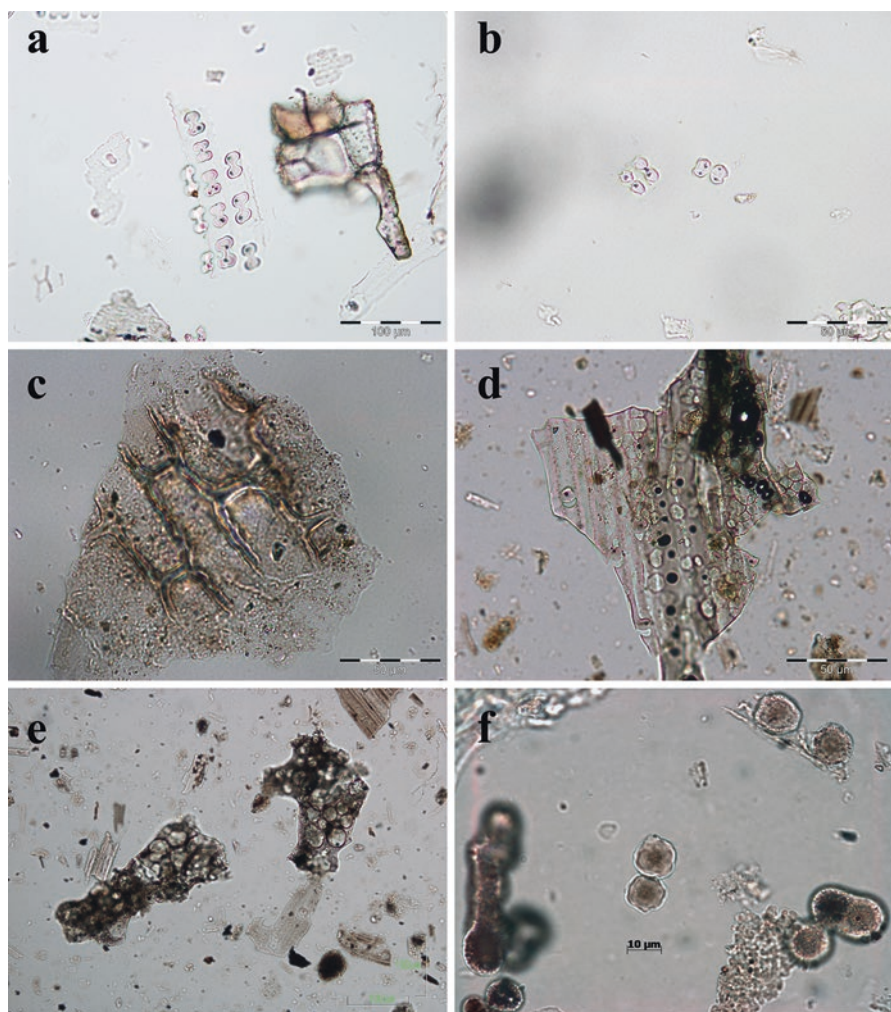
Throughout this chapter, we have seen a series of major advantages when using phytolith as a proxy for past vegetation. Certain phytolith morphotypes can be easily assignable to a specific plant (Ball et al. 2006; Bozarth 1987; Iriarte 2003; Ollendorf 1992; Ollendorf et al. 1987; Piperno 2009; Zhao et al. 1998), and some of them can be traced back to their anatomical origin within the plant (Ball et al. 1996; Hart et al. 2011; Lu et al. 2009b; Novello and Barboni 2015; Piperno 1989; Portillo et al. 2006) (Fig. 2d–f). Once deposited in the soils and sediments, they suffer little vertical or horizontal movement and, therefore, phytolith assemblages are considered to be *in situ* and, thus, can be used to produce high-resolution data of past human activities. Nevertheless, any post-depositional process affecting the sedi-

ment matrix will affect the phytolith assemblages and, in special circumstances, long-distance transportation and translocation through the profile can occur. Perhaps the most interesting advantage in phytoliths is their inorganic composition. Phytoliths are extremely resistant to time, acidic pH that can affect the preservation of organic matter and temperatures up to 700 °C without significant changes in their morphologies. Because they can be quantified, the different concentrations of phytoliths can give an idea of the intensity of human activities.

However, phytolith analyses have a series of intrinsic problems which are multiplicity and redundancy, differential production, and differential preservation. Because phytolith shape depends on the shape of the cell where it is formed, plants can produce different types of phytoliths in the same tissue (multiplicity). Redundancy happens when the same type of phytolith is produced by different plant species. In most cases, redundancy occurs between plants that are closely genetically related, but it can also happen with plants that are more distantly related. Morphometric analyses of the redundant morphotypes can help to identify the original plant (Out and Madella 2016, 2017) (Fig. 3a, b).

One of the major problems in phytolith analysis is the differential production of phytoliths in plants. Not all the plants accumulate the same amount of silica and certain plants do not accumulate silica at all, making these taxa invisible in the fossil record. In addition to these invisible taxa, there are also overrepresented species (Tsartsidou et al. 2007). For instance, grasses can produce 20 times more phytoliths than wood and bark of woody dicotyledonous plants (Albert and Weiner 2001). Moreover, differences in phytolith production can also be found in different parts of the plant (Piperno 2006). Plants with an annual cycle of life or deciduous trees will deposit phytoliths in a yearly basis and they might be more represented in natural fossil assemblages than perennial plants or plants with longer life cycles. In archaeology, one way to approach the differential production problem is to integrate phytolith analysis with pollen and charred micro- and macro-remains analyses (Cabanes et al. 2009). To calibrate the differences of production within the same plant, modern reference collections must be prepared both from the whole plant and the different organs of the plant.

Finally, not all the phytolith morphotypes have the same chances of being preserved. There is a systematic difference between the phytolith assemblage produced by a group of plants and the phytolith assemblage recovered in the soils where these plants grow (Albert et al. 2006). In addition, in pH above 8 phytoliths start to dissolve and not all the morphotypes are dissolved at the same rate (Cabanes et al. 2011). There is an apparent correlation between the phytolith shape and their dissolution rate. Those with a more compact shape and, therefore, lower surface/volume ratio tend to dissolve less than those with complex decorations or a higher surface/volume ratio (Cabanes and Shahack-Gross 2015). Therefore, there are a series of delicate morphotypes that will be more prone to disappear, eventually becoming the so-called weathered morphotypes (Fig. 3c). Unfortunately, these highly decorated phytoliths are often used to identify the original plant or the anatomical origin of the phytolith. When losing their decoration, these phytoliths can be confounded with other morphotypes and additional analyses like morphometrics



**Fig. 3** Examples of some of the problems with phytolith analyses, including redundancy, weathering, and melting. (a, b) Bilobate short cells from (a) *Arundo donax* leaf, and (b) *Oryza sativa* leaf. The type of short cells is similar in the two species from different subfamilies. Morphometric analyses could help to identify the original species. Modern plant reference collection extracted by dry ashing. (c) Sedge phytoliths during the weathering process in alkaline pH. Further dissolution will produce a weathered morphotype, which will be no longer identifiable. Modern plant reference collection, in vitro dissolution experiment. (d, e) Phytoliths melted at high temperature. (d) Anatomically connected phytoliths in the process of melting from Tell es-Safi/Gath, Area E, Early Bronze Age levels. On the left side of the picture, individual grass short cells and elongate phytoliths can still be identified, whereas the right side shows the bubble-like pattern of melted phytolith. (e) Completely melted phytoliths from Tel Megiddo area Q. (f) Spheroid phytolith showing spiraling ornamentation characteristic of restionaceae plants. Modern plant reference collection extracted by dry ashing from *Hypodiscus laevigatus*, from Fynbos biome, South Africa. (Picture: Irene Esteban)

will be misleading (Cabanes et al. 2011). An additional identification problem might be the presence of incompletely formed phytoliths that resemble weathered phytoliths. Since phytoliths can be present in plants at different stages of formation, some of the incomplete phytoliths may cause identification issues (Cabanes et al. 2011; Madella and Lancelotti 2012).

In addition to chemical weathering, there are other factors that can affect phytolith preservation. Sediment reworking either by bioturbation or anthropogenic activities will separate phytoliths in anatomical connection, with the consequent loss of information, but also making them more vulnerable to chemical weathering (Cabanes et al. 2009, 2012). Phytoliths can also be wind or water transported and during this transport damage on the phytolith surface may occur (Madella and Lancelotti 2012; Osterrieth et al. 2009).

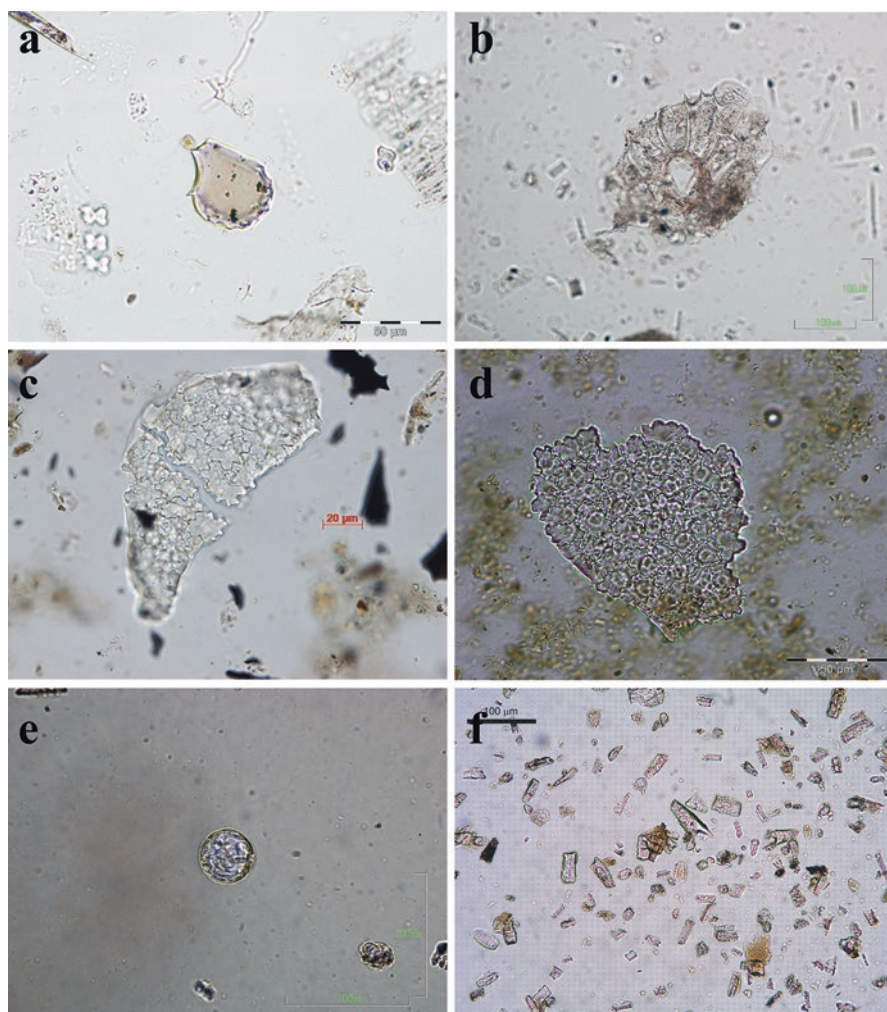
High temperatures can also affect phytolith integrity. According to Jenkins (2009), dry ashing produces the fusion of phytoliths in anatomical connection due to the dehydration process. Melted phytoliths might appear before reaching the melting temperatures of silica (Fig. 3d, e). During the preparation of a modern reference collection with green *Festuca eskia* inflorescence, melted phytoliths appeared around 700 °C and they became the vast majority above 800 °C. A plausible explanation for this phenomenon is the presence of some salt within the organic material that might reduce the melting point of silica.

## 5 Applications in Paleoecology and Archaeology

### 5.1 Paleoecology

Phytolith analyses are a powerful tool to reconstruct past paleoenvironments (Blinnikov et al. 2002; Fredlund and Tieszen 1997; Rovner 1971; Stromberg 2002, 2004). Despite that plants recycle actively phytoliths, some phytoliths deposited in paleosoils, lakes, and other natural sedimentary deposits survived until today (Piperno 2006). Paleoecological studies focused on phytolith analyses base their approach on the identification of the type of phytoliths produced by certain groups of ecologically significant plants. Therefore, many works in the field of paleoecology have been aimed to produce a modern reference collection from plants or groups of plants that allow the identification of ecological patterns (Figs. 3f and 4a, b) (Carnelli et al. 2004; Gallego and Distel 2004; Krishnan et al. 2000; Mercader et al. 2009; Mulholland et al. 1988; Ollendorf et al. 1987; Rapp Jr. and Mulholland 1992; Tsartsidou et al. 2007; Wallis 2003). Because not all the phytoliths produced by plants will be preserved in the sediments studied, modern reference collections have been combined with the study of the phytolith assemblages in the soils where these plants grow (Albert et al. 2006; Blinnikov 2005; Esteban et al. 2017b; Fredlund and Tieszen 1997; Mercader et al. 2011; Runge 1999; Thorn 2004b). The preservation of phytoliths in tropical environments, where other organic botanical remains





**Fig. 4** Phytoliths as indicators of environment and behavior. (a) Bulliform flabellate (previously known as “bulliform cuneiform cell”) phytolith from rice leaf. The proportion of bulliform phytoliths in the phytolith assemblage is an indicator of water availability in the environment. Modern plant reference collection extracted by dry ashing. (b) A polyhedral hair base from a dicotyledonous leaf in Middle Paleolithic layers at El Esquilleu. The presence of wood and bark phytoliths and dicotyledonous leaf phytoliths indicates a forested environment when the Neanderthals inhabited the area. (c, d) Phytoliths in anatomical connection from *Celtis* seed coats. (c) Phytoliths recovered from modern experimental fires (Picture: Aitor Burguet). (d) Phytolith recovered from the layer Xb in the Middle Paleolithic site of El Salt (Picture: Ágata Rodríguez-Cintas). (e) Spheroid rugose phytolith from wood and bark recovered from a Middle Paleolithic hearth in El Esquilleu. (f) Occupation floor phytolith assemblage at the Talaotic site of Torre de'n Gaumes, Minorca, Spain

do not survive, encouraged innovative studies using phytolith analyses in the American tropics (Bush and Colinvaux 1994; Bush et al. 1992; Piperno 1989, 1991), which built upon the pioneering studies carried out in the temperate areas of the continent (Twiss et al. 1969). Currently, paleoecological studies including phytolith analysis have extended around the globe including most of the possible chronologies (Albert et al. 2009, 2006; Alexandre et al. 1997b; Bamford et al. 2006; Blinnikov et al. 2002; Bremond et al. 2004; Carter and Lian 2000; Fisher et al. 1995; Fredlund and Tieszen 1997; Prebble and Shulmeister 2002; Stromberg 2002, 2004; Thorn 2004a; Zucol et al. 2005). Most of these works are based on the different photosynthetic pathways in plants (C3 vs. C4) (Barboni et al. 1999; Thorn 2004a) and the differentiation of this process in grass short cells following the classical ecological division proposed by Twiss among pooid, panicoid, and chloridoid short cells (Twiss 1987, 1992; Twiss et al. 1969). In addition, a series of indexes can be applied to phytolith assemblages to calculate hydric stress (Bremond et al. 2005b), tree density cover vs. grass cover (Alexandre et al. 1997b; Bremond et al. 2005a; Delhon et al. 2003; Stromberg 2002), and the type grass subfamilies (Bremond et al. 2008).

Since phytoliths are mostly made of silicon and oxygen, and can include occluded carbon, the isotopic composition of these elements has been studied to perform paleoecological reconstructions with different grades of success. Phytolith silicon isotope composition exhibits a high variability in plants due to fractionation processes, which seems to accumulate the heavier isotopes in the higher parts of the plants (Ding et al. 2008; Hodson et al. 2008; Opfergelt et al. 2006). The isotopic composition of oxygen in phytoliths appears to be derived from environmental factors (Shahack-Gross et al. 1996; Webb and Longstaffe 2002, 2006, 2000, 2003). However, the process by which plants incorporate oxygen isotopes into the phytoliths is extremely complex and not fully understood (Hodson et al. 2008). The most promising isotope in phytoliths so far seems to be  $^{13}\text{C}$ , which can be used to differentiate between C3 and C4 photosynthetic pathways in plants and, therefore, provide paleoecological interpretations of the fossil phytoliths assemblages (Hodson et al. 2008; Kelly et al. 1991, 1998; Lu et al. 2000; Smith and White 2004).

## 5.2 Archaeology

As in the case of the modern plants and soils reference collection, phytolith analyses have benefited substantially from the development of ethnoarchaeological and experimental research projects (Fig. 4c, d). Ethnoarchaeology has contributed to a better understanding of how phytolith assemblages are formed in archaeological sites (Friesem 2016; Friesem et al. 2016, 2014b; Gur-Arieh et al. 2013; Harvey and Fuller 2005; Portillo et al. 2014; Shahack-Gross et al. 2004, 2003; Tsartsidou et al. 2008; Zurro et al. 2017) and experimentation allows one to simulate the accumulation of phytolith assemblages under more controlled conditions (Albert and Cabanes 2007; Jenkins et al. 2016; Miller and Sievers 2012; Portillo et al. 2017; Raviele 2011).



Phytolith analyses in Paleolithic sites have contributed to a better understanding of the exploitation of plant resources by Neanderthals and Modern Humans (Fig. 4e), including spatial organization, fuel, bedding, and food (Albert et al. 2012; Albert and Marean 2012; Cabanes et al. 2010; Esteban et al. 2017a; Goldberg et al. 2009; Henry et al. 2004; Madella et al. 2002; Rodríguez-Cintas and Cabanes 2017; Schiegl et al. 2004; Wadley et al. 2011). The emergence of the first Neolithic societies has been a key issue for phytolith analyses applied to archaeological sites (Ball et al. 2016a). Studying the origins of agriculture in the New World has been a major incentive for the development of phytolith studies, especially in tropical regions where preservation conditions favored phytolith analyses over other botanical remains (Piperno 1988; Piperno et al. 2000; Piperno and Stothert 2003). In the Americas, maize domestication has been thoughtfully studied and debated using phytolith analysis (Boyd et al. 2006; Holst et al. 2007; Iriarte 2003; Pearsall 2002; Pearsall et al. 2003, 2004a, b; Pohl et al. 2007; Rovner 2004). In Asia, rice and millet domestication has been the main focus of phytolith analysis, especially in China (Bestel et al. 2014; Itzstein-Davey et al. 2007; Jiang 1995; Li et al. 2007; Lu et al. 2009a; Zhang et al. 2012), Thailand (Kealhofer and Piperno 1994), and India (Saxena et al. 2006). Phytolith analysis also helped to identify the earliest record of millet noodle preparation and an ancient recipe for beer (Lu et al. 2005; Wang et al. 2016). In the Middle East, phytolith analysis has focused on the identification of domestic wheat and barley in Neolithic contexts (Albert and Henry 2004; Baird et al. 2018; Portillo et al. 2009; Rosen 1992, 1989, 1997, 1993).

Among many other applications, phytolith analysis has been also applied to study site formation processes (Cabanes et al. 2009; Devos et al. 2009; Matthews 2010; Namdar et al. 2011), funerary practices (Cabanes and Albert 2011; Nadel et al. 2013; Out et al. 2016; Power et al. 2014), storage pit construction and fills (Balbo et al. 2015), pottery contents (Peto et al. 2013), activity areas (Regev et al. 2015; Sullivan and Kealhofer 2004; Tsartsidou et al. 2009) (Fig. 4f), and to define the integrity of the archaeological context for radiometric dating (Asscher et al. 2015; Toffolo et al. 2012).

Finally, the presence of small amounts of organic material within the phytolith matrix can be a source of carbon for  $^{14}\text{C}$  dating (Wilding 1967). Carbon is trapped during phytolith formation; therefore, it should represent the atmospheric composition at the time when the silica was deposited in the plant (Piperno 2016; Santos et al. 2012). Phytolith assemblages have been dated in a series of environments (Asscher et al. 2017; Piperno 2016). However, some old dates have been obtained for modern samples and some young dates have been obtained in fossil samples (see Asscher et al. 2017 and references therein). The reason behind these inconsistencies can be the uptake, transport, and reallocation of soil organic carbon into phytoliths (Santos et al. 2010, 2012). In addition, the phytolith surface can contain voids where dissolved carbon could have been introduced after the deposition of the phytoliths (Asscher et al. 2017). The complexities of using phytoliths for  $^{14}\text{C}$  dating are still a matter of debate, but hopefully, the prodigious amount of effort applied to this field will soon produce a widely accepted approach to the subject.

## 6 Future Challenges in Phytolith Analysis

One of the major challenges in phytolith analysis is to understand the level of confidence when identifying phytolith assemblages. The development of new extraction techniques allows sampling in a centimetric scale, which improves our resolution. In addition, this high-resolution sampling should give us the opportunity to test the reproducibility of our results by comparing multiple samples from the same feature/layer. Experiment replication is essential in sciences. However, this is a major factor that weighs against archaeology where an excavation process is a destructive event that could prevent the replication of the results. Thus, it is critical to record at the maximum detail the origin of each sample, yet it is more important to validate the interpretation of our assemblages using different lines of evidence, such as pollen, starches, charred remains, FTIR, or soil micromorphology. Whereas integrating the results of different micro-remains has been a common practice, a pending subject has been the integration of phytolith analysis with the rest of the archaeological remains on a larger scale. This integration should be accompanied by statistical models capable of making predictions and inferences, and determining the degree of confidence of our hypothesis. The error in the interpretation of our assemblages can come as well via the differential preservation of phytoliths in archaeological sites and natural deposits. In this sense, I believe that phytolith results should be accompanied by a section describing the integrity of the assemblage. Moreover, I think that we have only seen the tip of the iceberg on phytolith taphonomy and that more experimental and field work is required to fully understand all its implications.

The increase of synthetic works at regional scales could open many possibilities. As in any other scientific discipline, phytolith studies should move toward the standardization of the results to allow inter-site comparison between different authors (Zurro et al. 2016). The developing of accessible online databases is a major step forward to develop studies at a bigger scale, but it should be accompanied by all the necessary information required to reinterpret the data if necessary. Lastly, a vast amount of socialization and diffusion work is needed from all of us. Phytoliths remain a quite obscure micro-remain to the general public, and sometimes their possibilities are really unknown to some archaeologists. I believe that the future of our discipline lies in knowing the limits of our capabilities, producing reproducible results, and being able to explain to the general public the real potential of phytolith analysis.

**Acknowledgments** I would like to acknowledge Amanda Henry for organizing a magnificent meeting, giving me the opportunity to write this chapter, and especially, for being a great colleague. I would like to thank also Carolina Mallol, Irene Esteban, Aitor Burguet, and Ágata Rodríguez-Cintas for contributing with their own pictures to this chapter. I am grateful for the comments of the anonymous reviewers that helped to improve the earlier versions of the manuscript.

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# Other Microparticles: Volcanic Glass, Minerals, Insect Remains, Feathers, and Other Plant Parts



Amanda G. Henry

## 1 Volcanic Glass

Microscopic particles of volcanic glass, called tephra, are occasionally found in archaeological sediments. Tephra can be correlated to volcanic eruptions of known ages, based on morphology and chemical signatures, and can thus provide a means of dating an archaeological layer. Tephra particles may be identified based on their color, shape, isotropy, and size and shape of their gas inclusions (also called vesicles). They are generally glassy and can range from clear to uneven translucent brown (Fig. 1). They can appear as irregularly broken shards of glass, but can also be flat/platy, or with abundant linear gas inclusions (sometimes called pulled or fluted), or with many small bubbles (sometimes called bubbly or vesicular) (Stevenson et al. 2015; Swindles et al. 2010). Because they are isotropic, tephra do not glow under cross-polarized light. This feature may help differentiate them from other minerals. Once identified using light microscopy, the tephra are usually further analyzed using a variety of methods to determine the relative proportions of minerals that provide the unique fingerprint for each volcanic eruption (Swindles et al. 2010).

## 2 Mineral Particles

In rare cases, visually identifiable mineral particles have been recovered from dental calculus samples. The notable example was the identification of blue particles seen in a preparation of calculus from a tenth-century German woman who lived in a

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A. G. Henry (ed.), *Handbook for the Analysis of Micro-Particles in Archaeological Samples*, Interdisciplinary Contributions to Archaeology, [https://doi.org/10.1007/978-3-030-42622-4\\_12](https://doi.org/10.1007/978-3-030-42622-4_12)

**Fig. 1** An example tephra particle, of the light brown “bubbly” type. This particle was recovered from faunal dental calculus. The box is 50  $\mu\text{m}$  on a side

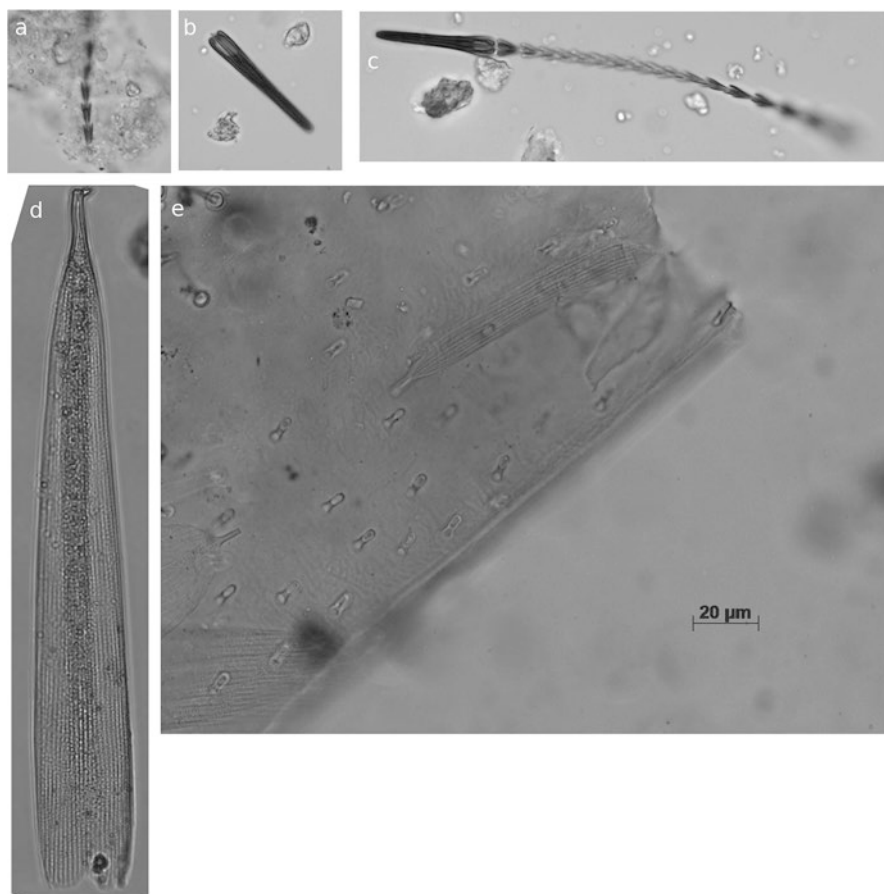


religious community (Radini et al. 2019). The particles were first viewed in the calculus during a preparation for plant microremains analysis and subsequently confirmed as coming from lapis lazuli using scanning electron microscopy with energy-dispersive X-ray spectroscopy and micro-Raman spectroscopy.

### 3 Insect Remains

Insect remains are common on archaeological materials. Larger body parts such as heads, thoraxes, wings, legs, and mandibles often retain morphology that allows the identification of taxa. Some insects have known preferred habitats, foods, and temperature ranges, and have been used for environmental reconstruction [e.g., insect remains helped establish the lack of cold temperatures of a late-glacial deposit in the Netherlands (Van Geel et al. 1989)]. Similarly, the presence or absence of insect parts in human occupations provides information about past behaviors, particularly about animal husbandry, fur and leather use, and hygiene [e.g., the spatial patterning of human- and dog-lice remains in Inuit homes in Greenland reflected where dogs were allowed and where human parasites were removed (Forbes et al. 2013)]. Not all insect parts can be so successfully used for reconstructing past behavior or environments, however. In the author’s laboratory, other microscopic parts of insects, namely, hairs from the larvae of dermestid beetles (Fig. 2a–c), and the wing scales from moths or butterflies (Fig. 2d, e), have been recovered from a variety of sample types, including dental calculus, stone tools, and controls. These taxa are common pests of museum collections (Querner 2015) and therefore likely indicate post-exca-

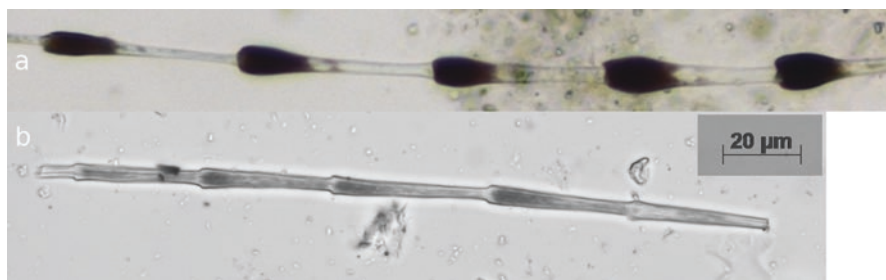




**Fig. 2** Insect parts that are likely the result of post-excavation contamination during storage in the museum. (a) Fragment of a hair from a dermestid beetle larva. Unlike feather barbules, the segments are very fine, roughly 5  $\mu\text{m}$ . (b) Fragment of the end of a hair from a dermestid beetle larva. (c) More complete hair from a dermestid beetle larva. (d) Large wing scale from a member of the Lepidoptera (moths and butterflies). (e) Section of the wing of a member of the Lepidoptera, with the attachment marks for the (mostly missing) wing scales in focus. Two folded scales are visible: one center top and the other lower left. The scale bar in this sub-figure applies to the entire image

## 4 Feather Barbules

Fragments of bird feathers can occasionally be found in archaeological samples. These feather barbules vary in length (though complete barbules are rare in archaeological samples), node morphology, internode length, pigment location and distribution, and surface texture (Fig. 3), and can in some cases be identified to the bird taxa that produced them (Dove and Koch 2011). The barbules are made of keratin, and thus of moderate survivability in the archaeological record (Bertrand et al.



**Fig. 3** (a, b) Feather barbules. These two barbules were recovered from human dental calculus. The scale bar applies to both sub-figures

2014). Barbules have been recovered from fabrics (Sibley et al. 1992), stone tools (Robertson 2002; Robertson et al. 2009), and dental calculus (Gismondi et al. 2018; Juhola et al. 2019).

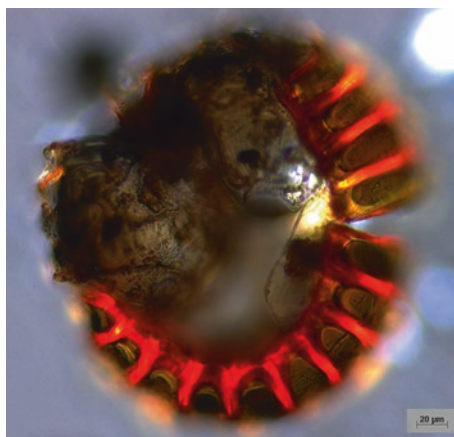
## 5 Other Plant Markers

In addition to charcoal, ash pseudomorphs, pollen, phytoliths, and starch, other plant remains may also be preserved in the archaeological record and have the potential to provide information about the environment and behavior.

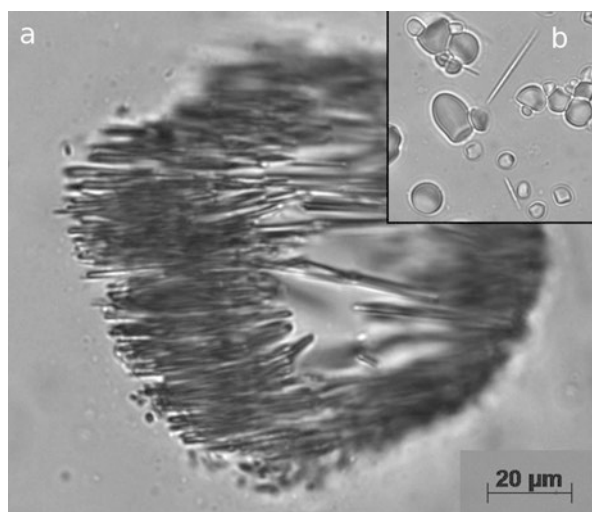
As part of their reproductive system, ferns produce sporangia on the underside of their leaves. Sporangia have been recovered in sediments (author, unpublished data, Fig. 4) and dental calculus (Fiorin et al. 2019). The large, colorful, and radially-grooved margins of a sporangium (called the annellus) can sometimes be identified to fern taxa and may provide information about the dietary or medicinal use of these plants.

Many plants deposit calcium oxalate in their tissues, often in the form of needle-like raphides, in addition to druses and rhombs (see chapter “Ash and Dung Calcitic Micro-Remains” by Gur-Arieh and Shahack-Gross, this volume). These raphides are produced as a means of defense against herbivory, as their shape irritates the mouths and throats of consumers (Bradbury and Nixon 1998). They are particularly abundant in the Araceae (Crowther 2009a), but are also found in Oxalidaceae and in other archaeologically-relevant taxa such as *Vitis* (grapes) (Arnott and Webb 2000). Raphides can form singly or in bundles, and can have distinctive morphology (e.g., varying shapes of the points, barbs, cross sections, or crystal structures) (Fig. 5) depending on the plant in which they formed (Crowther 2009a). When recovered from stone tools and other samples, raphides can help identify the use of particular plants (e.g., Horrocks and Bedford 2005). However, calcium oxalate is not always preserved in archaeological contexts (see chapter “Ash and Dung Calcitic Micro-Remains”), and raphide-like needle-fiber calcite crystals form readily on archaeological materials (Crowther 2009b), so care must be taken when interpreting these

**Fig. 4** Fern sporangium. This sporangium was found in a modern sediment sample



**Fig. 5** Raphides. The long, needle-like calcium oxalate crystals found in some taxa. Both of these examples come from modern reference material. (a) A large ring-shaped cluster of raphides from a member of the Vitaceae. (b) Starch and isolated raphides found in *Arum maculatum*. The scale bar in the lower right applies to both sub-figures



objects. Good identification relies on a deep and broad reference collection of raphides, the use of SEM in addition to light microscopy, and potentially chemical tests that would dissolve calcite while leaving calcium oxalate unharmed (Crowther 2009a, b).

Other plant remains such as xylem vessel elements and leaf epidermal tissues have been reported in studies of coprolites and sediments (e.g., Horrocks et al. 2008; Horrocks and Lawlor 2006). Further research into these less common plant remains is needed.

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