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Laboratory goals and considerations for multiple microfossil extraction in archaeology

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Abstract

We discuss some recent trends in archaeological microfossil research, including approaches using multiple microfossil data sets (e.g. pollen, phytoliths, starches, and microscopic charcoal). We review eight types of microfossils, with reference to their physical characteristics, their uses in archaeology, and specific requirements for laboratory extraction and microscope viewing. Rather than presenting any single processing protocol, in order to assist individual researchers or laboratories in developing safe, effective, and economical procedures for extracting their microfossils of interest, we provide guidelines based on previous studies from various fields of microfossil research. We articulate the various general goals of extraction and slide-mounting protocols, and tabulate the potentially destructive effects of discrete methodological procedures on individual microfossil types, including raw sample preparation, disaggregation, deflocculation, clay removal, organic and mineral destruction, heavy-liquid flotation, slide-mounting, and light microscopy. Finally, we present two illustrative archaeological case studies: (1) an example of developing a microfossil extraction protocol to accommodate refractory volcanic soils from the Hawaiian Islands, and (2) a discussion of the development of a low-chemical laboratory approach for extraction and interpretation of multiple microfossil types from agricultural and pastoral archaeological sites in an Argentinean high valley.

Keywords: Phytoliths; Pollen; Spherulites; Charcoal; Starch; Paleoethnobotany; Geoarchaeology

1. Introduction

Microfossils, herein defined as biogenic particles invisible to the naked eye, and deposited in any type of soil or sediment context, are familiar to scientists in fields such as paleontology, limnology, paleoecology, and, increasingly, archaeology. Although some microfossil types have well-established histories of use in archaeology, such as pollen or silica phytoliths [11,23,71,86], results obtained by research projects can be constrained by the interpretive limitations inherent in individual microfossil data sets.

Several microfossil researchers in archaeology have begun to practice more pluralistic or conjunctive approaches towards the incorporation of microfossil data in their research designs, based on the combined recovery and interpretation of more than one type of microfossil from individual soil and sediment samples, or in other cases, from the same sediment cores [10,30,41,43,50,69,72,73,79,92,99,100]. We see these approaches as recent manifestations of a long tradition in archaeological theory, that promotes the epistemological advantages of conjunctive research, and suggests that efforts to combine multiple lines of evidence can help overcome limitations or biases in individual data sets [12,35,36,46,65,94]. Microfossils are presented in this study as an ideal domain of mutually reinforcing data, and we stress the potential for studies involving multiple microfossil types to strengthen archaeological interpretations.

While aspects of microfossil research, such as field sampling, taxonomic systematics, quantification methods,

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and anthropological and ecological interpretation, are important, our focus in this study is on issues related to laboratory extraction and viewing of microfossils. For researchers using multiple types of microfossil data in archaeological studies, the idea of developing efficient laboratory extraction protocols that might allow simultaneous recovery of more than one type of microfossil is compelling, as extraction procedures tend to be both time-consuming and labor-intensive. However, while such goals are attainable to a degree, they can also be fraught with difficulties [30]. In this study, in lieu of advocating a panacea protocol for extraction of single or multiple microfossil types, we present considerations of: (1) physical qualities of several organic and inorganic microfossil types, (2) the general goals of microfossil extraction protocols, including the destructive or limiting factors inherent in many commonly used procedures, and (3) two case studies that demonstrate both the practical challenges and archaeological benefits of methodological experimentation.

While some researchers may not agree that the development of conjunctive approaches is the proper direction for microfossil research to take, we believe that the information compiled in this study will be useful even to those who advocate a more traditional focus on single microfossil types. The information we present in this article has been assembled from a wide range of published and unpublished literature, experiments both formal and informal conducted by ourselves and others, and conversations with helpful colleagues.

2. Microfossils in archaeology

Archaeological researchers whose laboratory work is focused on microscopic plant remains are increasingly associating their work with the larger subject of microfossils [53,74,75,87]. In this study, we wish to adapt the paleontological definition offered by Brasier [8, p. 1], that any microscopic biogenic substance 'that is vulnerable to the natural processes of sedimentation and erosion may be called a [micro]fossil, irrespective of the way it is preserved or of how recently it died'.

The contexts from which microfossils can be recovered may be divided into four groups, each of which requires different sample collection and microfossil extraction considerations:

1. 'Dry' soils and sediment samples from sites of archaeological or environmental study.
2. 'Wet' sediment cores, taken from lake or ocean floors, swamps, bogs, marshes, etc.
3. Residues adhering to artifacts, such as stone cutting or grinding tools.
4. Samples from sedimentary rocks, which are dissolved away from their microscopic contents.

We restrict our discussions in this article primarily to the first of these microfossil contexts, especially in our following discussion of extraction protocol development. Samples from archaeological sites often come from developed surface soil horizons, buried soils, or contexts enriched by anthropogenic midden. Archaeological samples can therefore require extraction methods which differ significantly from those used by, for example, pollen core analysts.

2.1. Microfossil types

We begin with a brief review of eight microfossil types, which have histories of use in archaeology, focusing mainly on physical characteristics, which affect microfossil preservation in the ground and during laboratory extraction. The microfossils we subsequently discuss are either remains of entire biological organisms (such as diatoms), portions or fragments of such organisms (such as pollen), or their organic and inorganic byproducts (such as faunal spherulites). Fig. 1 contains sample images of these microfossils, all of which have been extracted from archaeological contexts. We divide our microfossil discussion below into organic (numbers 1–4) and biogenic mineral (numbers 5–8) groups, because microfossils within each of these categories share some fundamental characteristics relevant to preservation and extraction.

2.1.1. Organic microfossils

2.1.1.1. Pollen and spores. Pollen grains are the reproductive male gametes of seed-producing plants. Spores, on the other hand, are produced in fungi and non-flowering plants, such as lower pteridophytes (ferns and fern allies), mosses, and algae [17,24]. The decay-resistant portion of both pollen and spores is composed of sporopollenin, an organic compound. Because of their distinctive morphologies and surfaces, fossil pollen and spores can often be taxonomically assigned to their plants of origin with a high degree of precision, often to species level.

Preservation of pollen and spores over time is affected by characteristics related to both taxonomic origin and depositional environment. Pollen preservation suffers the most in the conditions of high soil-redox potential, since oxidation is the natural process that is most destructive to pollen [11]. On-site archaeological pollen studies from dry soil and sedimentary contexts, where preservation and contextual integrity is much more variable than wet contexts, and where selective preservation of only the most resistant pollen and spore types may have occurred, have had decidedly mixed, but nonetheless often interesting results [21,34,44,97]. Methods for improvements in dry context pollen extractions have been discussed by Fish [26] and Kelso et al. [44], who suggest that sampling from beneath artifacts in

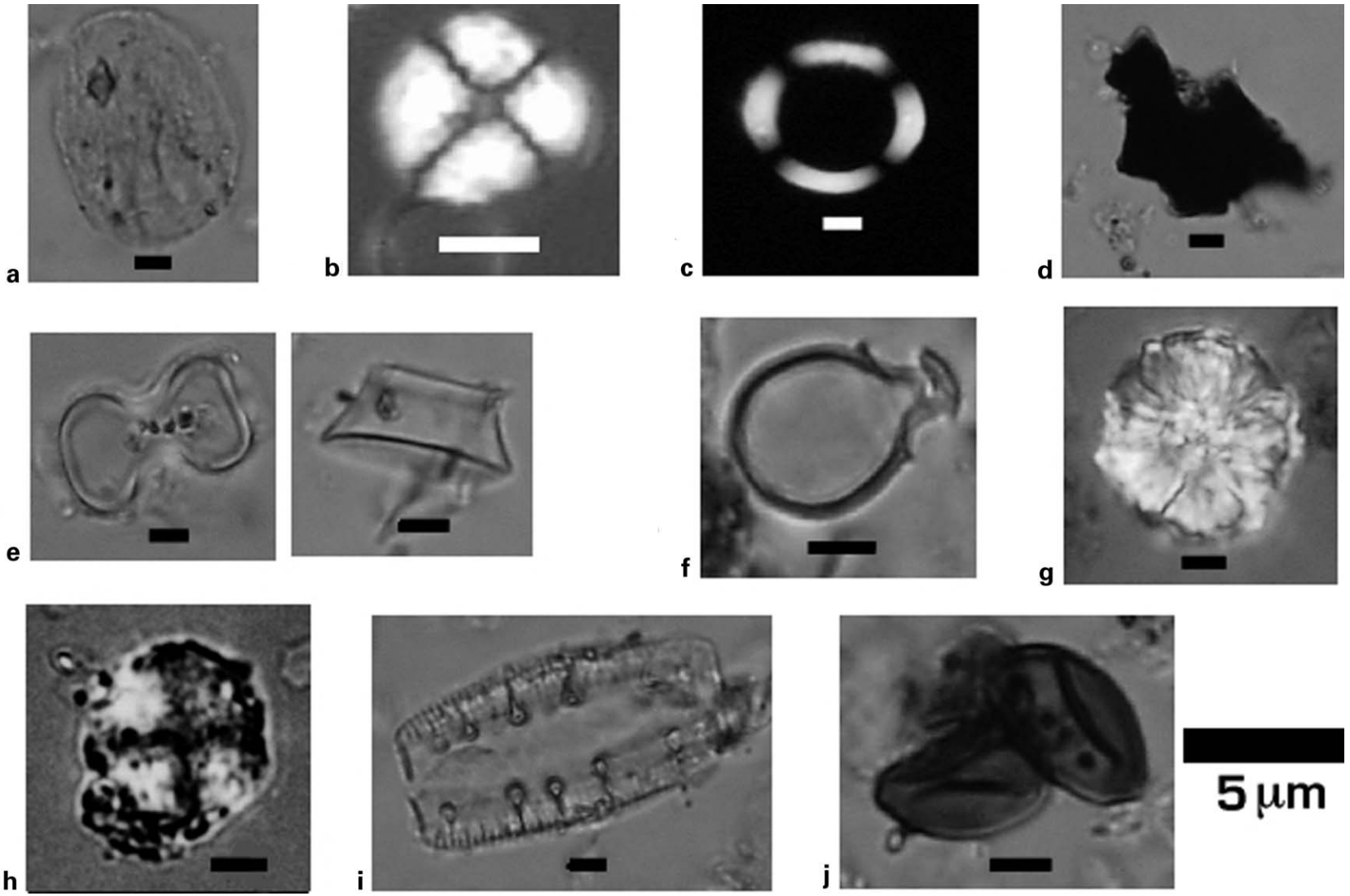

Fig. 1. Photomicrographs of the microfossil types discussed in this article—all of these examples have been extracted from archaeological soils and sediments: (a) pollen grain; (b) starch; (c) cellulose ring (plant tissue); (d) microscopic charcoal; (e) silica phytoliths; (f) chrysophyte; (g) calcium phytolith; (h) faunal spherulite; (i) diatom; (j) spores.

stratigraphic profiles may help to recover pollen sheltered from wetting and drying processes. Horowitz [39] also suggests that processing larger samples, and using low-chemical methods, can often yield substantial pollen counts from contexts with poor preservation conditions or low-pollen concentrations.

2.1.1.2. Starches. Starches are sub-cellular food storage units formed in all parts of plants. Of greatest interest to archaeologists are the starches formed in plant storage organs used as human food sources, such as seeds, roots, and tubers. Such structures tend to produce starches in greater abundance, and frequently in greater size and morphological distinctiveness, than the 'transient' starches found in other plant parts, such as leaf tissue or wood [96]. Starches are composed of two organic polymers, amylose and amylopectin, which form a series of laminated layers around a central hilum [80]. Like other organic microfossils, preservation of starches over time seems to depend on microcontextual conditions, although the specific conditions most amenable to starch preservation are not yet well understood. However, recent research has clearly demonstrated that starches can survive long periods of time in a wide range of contexts. Starch research in archaeology has focused on residues extracted from tools [4,42,56,57,76,78], as well as sediments and soils [19,92,95].

2.1.1.3. Plant cellular tissues. This category includes undecomposed plant cells that can form a significant component of a soil's non-mineral fraction, and archaeological samples may contain any of the several types of cellular material. For example, cellulose rings from spiral thickenings in primary xylem cell walls [104] appear commonly in the samples from Argentina discussed subsequently in case study 2. Palynologists can sometimes use the presence of undecayed plant tissues in their samples, such as trichomes or stomata cells, to derive supplemental information on pollen transport or origins [24], and paleobotanists have also used preserved plant tissues to interpret the environmental context of fossil plants [93]. In archaeological tool residue studies, microfossils are sometimes found still articulated within their cellular contexts, such as epidermal tissues or parenchyma cells, providing further information on the taxonomic origins of identified microfossils [3,19].

2.1.1.4. Microscopic charcoal and carbon. Composed mainly of elemental carbon, charcoal cannot strictly be said to be either organic or mineral. We include this microfossil type with the organics both for convenience, and because the study of microscopic charcoal has largely been taken place by palynologists, who, following the pioneering work of Iversen [40] (cited in Ref. [83]), began counting charcoal particles that commonly appeared on their pollen extraction slides. Microscopic charcoal extracted from archaeological samples from cultivated soils or off-site comparative sediments may be useful as an indication of field clearance by burning, natural fire regimes, or post-burning erosion.

The literature on microscopic charcoal is growing, as researchers conduct further experiments for more precise taxonomic identification of microscopic charcoal particles [98], and improved extraction methods [83]. A newly utilized class of microscopic carbon is also represented by the carbonaceous soot particles [82,84,102], which sediment core researchers have begun to recognize as airborne byproducts produced during the burning of peat, coal, and oil.

2.1.2. Biogenic mineral microfossils

2.1.2.1. Silica phytoliths. Silica phytoliths are microscopic casts of cells, aggregates of cells, or intercellular spaces in silica-accumulating plants, which include some ferns, as well as gymnosperms and angiosperms [37,71]. Composed of non-crystalline silicon dioxide (SiO_2), silica phytoliths are the most commonly studied biogenic mineral microfossil in archaeology. Their silica composition gives them an enhanced degree of durability in soils and sediments compared to many other microfossil types, although dissolution of silica phytoliths does take place in some contexts, such as in highly alkaline conditions [64], or over longer spans of geological time [31].

The ability to correlate individual phytolith forms with taxonomic groups is highly variable. However, phytolith literature is extensive and much is now understood about silica accumulation patterns and the nature of soil phytolith assemblages [6,65,66,70,71,81,85,87].

2.1.2.2. Other biogenic silica. Other types of organisms produce biogenic silica particles that share many physical properties with silica phytoliths. Therefore, they can be extracted from soils using similar methodologies, and possess similar optical qualities under the microscope. Diatoms are non-flagellate, single-celled algae that produce opal silica frustules, or 'shells', with taxonomically distinctive morphologies. Diatoms occur in two main groups: (1) radially symmetrical forms (centric), and (2) bilaterally symmetrical forms (pinnate). Diatoms exist in both fresh and salt water, or in nearly any condition where moisture is present, including soils, tree trunks, brick walls, and clay deposits [38]. Also, like silica phytoliths, diatoms can have different degrees of silicification, and can therefore be differentially preserved in deposits [63].

Archer and Bartoy [1] found that using a very simple distinction in diatom morphology could be useful in a study of archaeological microfossils from plowzone assemblages, as centric diatoms tended to correlate only with sub-surface features, while the pennate diatoms were ubiquitous. Another archaeological case study with

diatoms used in conjunction with phytolith evidence is found in the study by Brochier et al. [10].

Other algae of the order Chrysophyta produce two types of durable silica 'shells' known as scales and cysts. While these preserved microfossil particles are less taxonomically specific than the entire living organism, some researchers have begun to classify these siliceous remnants [20]. Sponge spicules represent another type of biogenic silica microfossil. Although sponges are commonly thought of as marine animals, scores of species occur in freshwater habitats. Spicules are inorganic support structures in these sponges [90], and are found even in soils far removed from oceanic resources [103]. Various types of chrysophytes and sponge spicules may potentially serve, like diatoms, as indicators of general environmental conditions or as evidence of flooding, hydrological change, or soil transport.

2.1.2.3. Calcium phytoliths. Calcium phytoliths, like their siliceous brethren, are produced and deposited in living plant cells and tissues, and are released into the soil following the decomposition of the plants. Calcium phytoliths are made up of either calcium carbonate or calcium oxylate. Calcium phytoliths, which have been found in comparative reference material, are less likely to be recovered and identified archaeologically, because of these microfossils' susceptibility to destruction in acidic depositional or laboratory conditions. Because of their crystalline growth patterns, there is also a limited range of forms in which calcium phytoliths tend to occur [15,29,61]. Archaeological case studies involving calcium phytoliths can be found in the studies of Scott-Cummings [91] and Loy et al. [57].

2.1.2.4. Faunal spherulites. Spherulite is a geological term referring to a 'small crystalline body with a radial fabric' [27p. 284]. Spherulites of interest to archaeologists are spherical or semi-spherical calcium carbonate particles produced in the digestive tracts of certain mammals, such as sheep, goats, cattle [9,14–16], and camelids including llama and alpaca [49]. These microfossils, because they become incorporated in animal dung, have been called 'faecal spherulites' in some sources [14,15], although we use the term 'faunal spherulites' because it gives a more general indication of the faunal origin of these particles. The calcium carbonate composition of microfossils suggests that faunal spherulites may be preserved in only a limited range of depositional conditions, and will be destroyed by most standard extraction procedures incorporating acid-based processing steps. In archaeology, spherulites have been reported in studies of microstratigraphic thin-sections [59], while Brochier et al. [10] have also described and analyzed soil-extracted spherulites.

3. Extraction goals and practical choices

3.1. Methodological concerns

In many archaeological projects, combining data from more than one type of microfossil may appear to be an attractive option. The microfossil types reviewed in the previous discussion have much in common in terms of the methods used in sample collection, as well as in laboratory extraction, identification, and interpretation. Most microfossils fall within a similar size range, have specific gravities below that of most non-biogenic minerals (which aids in separation by gravity sedimentation or heavy-liquid flotation), and behave similarly in their reaction to post-depositional movement or mixing. The wide areas of overlap in the basic knowledge required of pedology, chemistry, biology, ecology, and microscopy greatly facilitate the ability of a single researcher to incorporate recovery and interpretation of multiple microfossil types into field and laboratory research designs.

However, the microfossil types previously discussed also differ in significant ways. One critical aspect of microfossil variation is differential susceptibility to damage or destruction during laboratory extraction procedures. When extraction goals involve simultaneous extraction of multiple types, difficulties can increase [30]. Performing the juggling act of retaining a variety of chemically diverse microfossils, while destroying a similarly chemically diverse set of unwanted soil compounds, is the single most problematic task in designing microfossil extractions. Heat (including exothermic reactions), excessive exposure to extreme pH levels (either basic or acidic), and other chemical reactions are the main agents of damage or destruction for different microfossil types.

During our various research efforts on microfossil extractions from archaeological soils and sediments, we have come to appreciate the oft-cited warnings that there can be no single ideal protocol for every data set or every site [32,65]. In lieu of advocating one overarching protocol, experienced researchers tend to suggest the development of customized protocols, specific to the research goals and conditions of individual projects or sample characteristics. To this end, we summarize the main goals that successful extraction protocols must achieve, as well as alternate ways of accomplishing these required tasks (Table 1). We also review many of the more specific points in extraction procedures where each type of microfossil becomes most susceptible to loss, damage, or destruction (Tables 4 and 5). The content of these tables is discussed further in subsequent sections.

In our experience, we have found many published or otherwise shared protocols to be difficult to follow because of both vagueness on one hand and overly detailed exactitude on the other. While cryptic

Table 1
Summary of overarching goals of microfossil extraction, and various means of accomplishing them

Goals	Laboratory considerations and options
Sample preparation	Quantity to use depends on microfossil density and on soil and sediment qualities (e.g. sandy/silty/clay-rich/humic/O-horizon?). Usually 1–100 g
	Initial soaking in water may improve the effectiveness of later steps
Sample disaggregation and deflocculation	Mechanical disaggregation (e.g. crushing, grinding, stirring), breaks up larger aggregates, but may damage some microfossils
	Chemical disaggregation through destruction of humic and/or carbonate components. May be necessary for some soils, but may harm some microfossils
	Deflocculation—for clay dispersal—check for Brownian motion of individual clay particles under microscope to determine state of deflocculation. Different clay types respond best to different deflocculants
Removing unwanted organic or mineral components	Chemical destruction (oxidation for organics, dissolving of clays with strong, hot acids)
	Size/density separation is achieved by gravity or centrifugation, with decantation used to remove unwanted particles
	Sieving/filtration can trap and remove particles of almost any size
	Microwave digestion can destroy unwanted organic and mineral components and preserve silica microfossils [62]. The effects of this method on other microfossil types remain unknown
Separating and concentrating particles of interest	Size/density separation (as previously described)
	Sieving: single or nested sieves can capture microfossils, while smaller particles are flushed through, or vice versa, with larger particles being removed. Such methods are common in micropaleontology [8]
	Heavy-liquid flotation: lower specific gravity microfossils float, heavier mineral particles sink
Slide-mounting and microscopic-viewing issues	Staining can improve visibility of some microfossils [2,88]
	Factors for choosing a mounting medium include preserving mobility (by using a viscous liquid mounting medium), and using a medium with a refractive index that allows clear, high-resolution viewing of microfossils of interest
	Petrographic, transmitted-light compound microscopes provide best routine microfossil viewing. SEM use can also be valuable, but is generally too time-consuming or costly, for routine use

procedural statements like 'organics were removed by oxidation' can be frustratingly vague, detailed specifics can also be confusing. For example, 'use a Whitman number 42 filter paper to rinse your sample' can be too precise—is it the Whitman filter paper that is crucial, or the size 42 filter paper, or are other brands or sizes of filter paper also acceptable? Further, one might wonder, 'How do I *rinse* phytoliths? Do we time the centrifuge from when it reaches 1000 RPM, or from when it starts rotating? Do we have to pay $50 for *ultra-pure water*?'

We also recognize that successful extractions must not only attain the overarching goals subsequently discussed, but also do so safely, economically, and efficiently. While space restrictions prevent an in-depth discussion of field sampling, laboratory equipment, and safety in this study, we note that many pieces of costly laboratory equipment can be regarded as luxuries rather than necessities. On the other hand, use of proper safety equipment, such as gloves, goggles, and fume hoods, must always be considered essential, and researchers new to the subject should understand basic laboratory safety procedures and be fully aware of hazardous properties of any chemicals they are using (see Ref. [50] for an extensive summary), before proceeding with extraction and slide-mounting work.

4. Physical requirements for microfossil extraction

Understanding the underlying physical goals of microfossil extraction is the key to adapting and developing detailed, specific laboratory protocols. We therefore have structured our discussion of laboratory extraction methods in three parts. First, what are the main goals that must be accomplished in a successful microfossil extraction? These include: (1) preparing a sub-sample of the field sample for processing, (2) disaggregating and deflocculating the sample so that individual mineral and organic particles are free to move independently, (3) removing unwanted particles, which can interfere with viewing of microfossils, from the sample, (4) isolating (separating and concentrating) microfossils from unwanted soil particles, and (5) slide mounting and microscope viewing. Table 1 summarizes these general extraction goals and some of the various ways they can be accomplished.

Next, we more specifically discuss issues related to the performance of these general procedural steps, such as sieving, destruction of organic or mineral components, heavy-liquid flotation, and microscope slide mounting. Finally, we briefly discuss the final stage, which is the construction of specific, step-by-step written protocols that allow standardization of extraction procedures, and which can be tailored to suit the preferences and laboratory conditions of individual researchers.

4.1. Procedural steps

The following discussion is included to provide more details related to the procedures presented in Table 1.

4.1.1. Raw soil and sediment samples

Firstly, using proper technique and making meaningful choices in field sampling are essential considerations—less experienced researchers can consult textbook sources, such as by Pearsall [65]. When sub-sampling the field samples, care should be taken to obtain representative 'splits' by using a riffle box, cone-and-quarter method, or by thoroughly mixing the sample [54]. Gravel-sized particles (>2 μm) are often removed by sieving before further processing begins. It is advisable to record the dry weight and/or volume of a given sub-sample before processing, as this can be important in later determinations of microfossil concentrations. The appropriate amount of sub-sample with which to begin will depend on the concentration of the microfossil(s) of interest, preservation conditions, and the desire to generate additional extract for archiving or re-analysis. As there is no general rule for 'how much' soil to use, testing small samples for their content before standardizing protocols is advisable. Sample quantities used by microfossil researchers tend to range from 1 to 100 g. Most microfossils fall in the size range of silts and fine sands (5–150 μm), so samples which are particularly clay-rich, or which contain mostly sand or gravel, will often require increased initial amounts of sample. Protocols that split samples at various points for extracting different types of microfossils, or into size-based fractions for facilitating visibility [13,71], may require larger amounts of raw sample, as different microfossil classes will invariably be present in different concentrations. Introduction of an artificial marker to samples, such as prepared spore tablets or manufactured glass microspheres [5], can facilitate calculation of microfossil counts and concentrations.

4.1.2. Dehydration

In some protocols, samples are initially dried for a variety of reasons, such as to calculate standardized dry weight. Heat sterilization of imported soil samples may also be required by law. This can have a destructive effect on certain microfossil types, and finding options for complying with regulations that will not harm organic microfossils may be necessary. If heat is used for sterilization or dehydration, starch grains are particularly vulnerable, since they may begin to gelatinize in the presence of water at 40–50 °C [96]. Oven-drying wet samples may also cause clay particles to bake together, making later deflocculation and removal of these difficult [28]. Air-drying in sealed paper bags is another approach, which allows moisture to leave the sample, while preventing airborne contaminants from entering. Finally, rinsing out water from samples with a volatile liquid, such as alcohol or acetone, then evaporating under a fume hood, can also accomplish the process of dehydration without the use of heat.

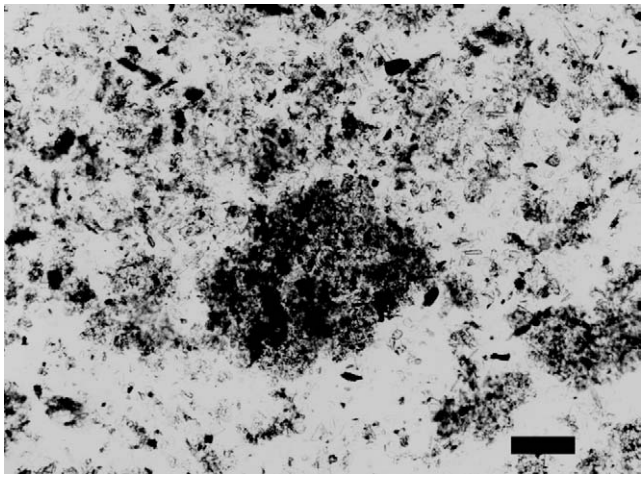

Fig. 2. Partially disaggregated sample during processing. Opaque black microscopic charcoal fragments clearly show a duality of states, either aggregated with other particles near center of photo, or as individual disaggregated particles towards edges of photo. Scale bar=100 μm, photographed in transmitted light at 100×.

4.1.3. Disaggregation and deflocculation

Disaggregation and deflocculation are closely allied processes that both address the need to break up the organic and mineral constituents of samples into individual particles, before attempting to separate or concentrate them further. In most soils and sediments, particles are bound together by organic, mineral, and/or electrostatic 'glues'. These bonds must be broken and/or the bonding substances dissolved in order to separate individual particles from each other. Failure to adequately disaggregate and deflocculate samples before attempting to separate microfossils can lead to a low or biased yield of some particles [30,71]. Disaggregation refers to the process of breaking apart the soil aggregates with chemical or mechanical actions, such as crushing in a mortar and pestle for larger soil aggregates or shaking/stirring for finer ones. Chemical steps to dissolve binding agents such as humic substances or soil carbonates are often necessary to achieve adequate sample disaggregation. These procedures are discussed in more detail in subsequent paragraphs. Aggregation (or lack thereof) is easily monitored beneath the microscope. After soaking a sample in water, place a single drop on a microscope slide and observe. With only a little practice, it is easy to distinguish soil aggregates from individual particles, as seen in Fig. 2.

Deflocculation refers to the process of inducing a state of electrical charge repulsion between clay-sized (<2–4 μm) mineral particles, so that these can be separated from larger particles and ultimately removed from the samples. Clay can be defined in two ways: as mineral particles of a particular size class (usually <2 μm), or more technically by soil scientists as the products of rock weathering, which have undergone mineralogical metamorphosis [28]. While the first definition is in many ways

sufficient for our purposes, it is also important to realize that clay minerals vary greatly in their molecular structures and electrical charge properties [25,28,105]. This variability can affect deflocculation of clays, and can necessitate flexibility in deflocculation methods for diverse samples. Deflocculation is commonly accomplished via a combination of agitation (hand or mechanical stirring with the sample either dry or soaked in water) and/or the addition of a chemical deflocculant, which allows positively charged cations to bond with negatively charged clay particles and causes clay particles to repel one another [28]. If deflocculation is not properly achieved, clays will continue to stick together and will be difficult to remove from samples. Deflocculation can be checked under the microscope by observing the phenomenon of Brownian motion. At 400× magnification, clays appear as small dots, the size of a typed period or so. If they are well deflocculated, they will appear to vibrate energetically, and individually, on a water-mounted microscope slide.

Folk [28] reviews several chemicals commonly used to deflocculate clays, and recommends a side-by-side deflocculation test among them to determine the best deflocculating agent for any given set of samples. Most of the commonly used deflocculants are sodium phosphates, and are generally non-destructive to microfossils, as well as are non-toxic to humans. Deflocculants should be chosen with regard to the ionic properties of samples. Some clays actually carry variable or weak negative charges and even positive charge, and such samples will not react well to standard deflocculation methods [28]. For some samples, deflocculation may be most effectively achieved with nothing more than plain water. Also, some clays from volcanic or tropical soils may be forced into a state of loose aggregation during acid-based steps in protocols, and will be difficult to remove from samples if this has occurred. Solutions to this problem may be found by using a very strong acid treatment suggested by Zhao and Pearsall [105] to help remove these clays, or by avoiding protocol steps inducing an acidic pH in the sample until after clays have been removed.

4.1.4. Sieving

In many protocols, larger particles are removed at some point using a fine-meshed sieve. This removes larger silt and sand-sized particles, and helps eliminate modern contaminants, such as rootlets or leaf fragments. However, if particles are not sufficiently disaggregated and deflocculated first, soil aggregates may also be inadvertently removed from the sample. Again, testing a drop of suspended sediment under the microscope can reveal what the sieve is actually removing. The majority of microfossils are found in the silt and finer sand fractions of sediments (2–150 μm), although there are some larger classes of diatoms and dicotyledon phytoliths [71]. It is generally desirable to remove particles both larger (by sieving or settling) and smaller (see clay removal in subsequent discussion) than this target size range. Many protocols use 250 μm as the upper limit for recovery, i.e. soil is at some point sieved through a 250 μm mesh to remove large particles. If these larger forms are of interest, it may be necessary to either separately process a >100 μm sample, or examine the particles removed by the sieve to ensure that larger microfossil forms are recorded. A 125–150 μm sieving step is a compromise solution, allowing recovery of some large class phytoliths while removing larger sand particles. Sieving procedures can also be used in other stages of the extraction process, such as to separate larger diatoms from smaller phytoliths where overabundance of diatoms is a problem (D. Pearsall, personal communication, 2000).

4.1.5. Clay removal

Clay removal is essential to microfossil extractions, as unremoved clay particles can interfere with heavy-liquid flotation attempts and obscure visibility of slide mounted microfossils. Clay removal steps in most protocols are based on Stokes' Law, which establishes that tiny clay particles will settle most slowly in a column of water (or under centrifugation in an analogous approach). Larger particles are allowed to settle to the bottom, and clays which remain in suspension are poured or siphoned off.

Deflocculation is a prerequisite to clay removal, since Stokes' Law assumes that clays are behaving as individual small particles, rather than as clumps or chains. Both gravity and centrifuge approaches to clay removal have their proponents, although the main difference between the two methods is one of time economy. Both methods require removal steps to be repeated several times (in some cases even 20 or more). Gravity sedimentation without a centrifuge requires samples to be attended for a few minutes, with long periods of undisturbed settling taking place in-between. Centrifugation accelerates the settling process to about 1–3 min per cycle, but requires constant attention. Centrifugation may also be problematic for larger volumes of soil, possibly requiring samples to be split among several centrifuge tubes.

Because settling times determined by Stokes' Law are based on particle density as well as size, light organic microfossil types, such as starches or pollen, are subject to slower settling than similarly sized mineral particles. One must therefore decide where to 'draw the line' between the sizes of smaller clay mineral particles to be removed, and larger organic particles to be retained. Lentfer [50] has produced a series of tables based on Stokes' Law for determining both gravity and centrifuge settling times required for retention of specific size/density particle classes. While such tables are helpful in

establishing target times for settling or centrifugation, it is advisable to check samples under the microscope at various points to ensure that only the desired particles are being removed, and that microfossils of interest are not also being inadvertently lost. Single-drop samples taken with a pipette from near the bottom of the water column or centrifuge tube before pouring or siphoning can reveal exactly what types of particles are being removed with a given process. This is especially important when developing centrifuge-based clay removal procedures, as different centrifuges accelerate and brake at different rates, affecting the total centrifugal force exerted on individual samples.

Because clay removal is often the most time and labor-intensive part of the microfossil extraction process, some researchers have experimented with other approaches. Hydrofluoric acid (HF) can be used, as in traditional pollen preparations, to dissolve silicate clays, although this also destroys silica-based microfossils and is especially hazardous. Zhao and Pearsall [105] have developed 'the fractionator', a device used to reduce the number of repetitions of gravity settling needed to remove clays from samples. Finally, use of vacuum filtration to remove clay particles has been attempted by some researchers as a faster alternative to settling-based approaches, although difficulties with filter clogging and loss of particles larger than the mesh size have both been reported [52,105], indicating a need for further refinements in these potentially more efficient approaches.

4.1.6. Organic destruction

Organic components of soil include both living and dead flora and fauna, as well as various products of the decomposition process. As organic tissues naturally decompose, a diverse array of chemical constituents colloquially grouped as humic acids or humic colloids are produced [25]. While samples very high in undecomposed organic tissues (such as pollen samples from peat bogs or surface soil samples containing leaf litter) may require treatments to destroy undecomposed plant tissues, it is the partially decomposed humic colloids, which bind mineral particles in terrestrial samples and cause the majority of disaggregation problems. Therefore, even when the microfossils of interest are organic, some type of 'organic destruction' step, designed to remove humic binding agents, can be critical for unbiased recovery.

An oxidation reaction, such as that produced by hydrogen peroxide, "either dissolves the organic matter directly, or converts it into a form (humic acids) that is soluble in a base" [33, p. 369]. Both reactions usually occur simultaneously. It is this reaction and its products that help determine how organic destruction procedures can best be incorporated into an extraction protocol. Another potential approach (Ref. [71]; case study 1 subsequently discussed) is the use of strong bases such as KOH (potassium hydroxide) or NaOH (sodium hydroxide), in lieu of or in addition to the more commonly used acids. The base dissolves alkali-soluble humic components while other organic tissues may remain more intact compared to the treatments with aggressive acid. Hot alkaline treatments are carried out for dissolving humic compounds, and must be applied cautiously (i.e. for short periods of time) since such treatments may be damaging to silica or organic microfossils [39,65,89].

4.1.7. Mineral destruction

Procedures for isolating organic microfossils often specify destruction of mineral particles with chemical treatments. Extensive exposure to strong bases, or HF, will dissolve or damage silica phytoliths, diatoms, and other silica-based particles. Protocols designed for soils from arid environments, or with carbonate-rich parent material, often include an acid-based step to remove soil carbonates, which can precipitate from groundwater and bind particles together as aggregates. However, acids may also destroy calcium phytoliths and faunal spherulites. Acidic treatments to destroy carbonates can be less crucial in cases where soil pH measurements, or even a simple visual test for an HCl reaction (Scott-Cummings, personal communication, 2001), indicate low soil carbonate content.

Glacial acetic acid may represent an underutilized alternative to HCl for carbonate removal, since it will dissolve carbonates, but it does not dissolve calcium oxalates including many calcium-based phytoliths [61,91]. In situations where mineral or organic content of soils does not inordinately interfere with the extraction or viewing of microfossils, some protocols dispense with both organic and inorganic destruction, and by doing so may avoid destruction of calcium-based microfossils. 'Gentle' protocols do risk insufficient disaggregation of individual particles, so microscope monitoring of actual results is again advisable to ensure that microfossils do not remain aggregated with other clastic particles.

Finally, Parr's [62] method of phytolith extraction using laboratory microwave digestion may represent a more time-efficient approach to extracting silica phytoliths and possibly other microfossil types as well. By subjecting samples to digestion with nitric and hydrochloric acids in microwave conditions of high pressure and temperature, most unwanted organic and inorganic components of soil and sediment samples are destroyed, with only silicates remaining. However, the acids used in Parr's protocol would also be destructive to some of the less-durable microfossil types discussed in this article. Further experimentation with microwave digestion processing should help determine this new technique's most appropriate applications in microfossil research.

4.1.8. Heavy-liquid flotation

Although microfossils can be separated by size using gravity-settling principles similar to those previously discussed in the clay removal section, most microfossil researchers now use heavy-liquid flotation. To separate microfossils from the disaggregated sample, a high-density heavy liquid is adjusted to a density above the specific gravity of the microfossil, but below that of other inorganic particles. Under centrifugation, lighter microfossils float or remain in suspension, while heavier mineral particles sink. Microfossils can then be recovered with a pipette and put into a new tube for rinsing, or the entire heavy liquid can be poured off along with the suspended microfossils. Most analysts recommend repeating the flotation process more than once to help ensure full microfossil recovery [30]. One reason for this is that the density of a heavy liquid will be reduced when added to a wet or damp sample. The effects of this heavy-liquid dilution can be avoided by either drying the sample thoroughly before adding heavy liquid, or repeating the addition of new heavy liquid.

The dry chemicals used to make heavy-liquid solutions can vary greatly in cost, toxicity, as well as unforeseen reactions with various soils. Perhaps the heavy liquid most commonly used today is sodium polytungstate ($Na_6(H_2W_{12}O_{40})$, also known as sodium metatungstate, which is relatively expensive, but reportedly less toxic than many other media. Aqueous sodium polytungstate solutions have an acidic pH, however, and this is perhaps why some researchers have experienced problems with the formation of 'sludge' during flotation of certain soil types ([105]; see also case study 1 in subsequent discussions). This could represent aggregation of humic materials upon exposure to acidic conditions.

Zinc iodide (ZnI_2) is another heavy liquid recently introduced into the field. It has been shown to be an effective flotation solution for silica phytoliths [105] and has a low-toxicity level [22]. One problem with zinc iodide is its tendency to precipitate a hydroxide byproduct when diluted, although addition of a small amount of dilute hydrochloric acid or glacial acetic acid quickly re-dissolves this 'snow' (Ref. [22]; K. Ezell, personal communication, 2000). Ezell also recommends using warm water to mix the heavy liquid and to rinse samples after flotation to help avoid this problem.

Zinc bromide ($ZnBr_2$), mixed with water or HCl [65, p. 423] is a less expensive, but more toxic heavy liquid still favored by some researchers. Other heavy liquids used in the past by microfossil researchers include cadmium iodide and potassium iodide, tetrabromoethane and absolute ethanol, tetrabromoethane and nitrobenzene, and bromoform and nitrobenzene [71]. Most of these have fallen out of favor because of their hazardous properties. Disposal and recycling considerations should be considered as well when choosing a heavy liquid. Recycling involves distilling and vacuum filtering used heavy liquid (see Ref. [105] for a detailed description of this process). After rinsing the heavy liquid from floated microfossil extracts, the extraction process is essentially complete (see Ref. [65], for descriptions of these final steps).

4.1.9. Storage and slide mounting

After separating microfossils and obtaining a quantity of 'pure' microfossil extract, at least one microscope slide is usually made for viewing, and a small vial is filled with the remaining extract for later use. Extracts can be stored dry, in water, or in other media such as alcohol or oil. Silica phytoliths are quite resistant to any storage system, while calcium carbonate microfossils, such as spherulites, may dissolve over time during storage in distilled water, which can be naturally acidic [14]. Many permanent slide mounts begin with dried extract, and it may be advisable to test particular microfossils' susceptibility to quickly evaporating drying agents, such as alcohol or acetone, before using regularly. Extracts containing pollen should not be allowed to dry, since dried pollen grains can collapse and stick together (R. Byrne, personal communication, 2001).

Microfossil extract is most commonly suspended in a slide-mounting medium for examination with a transmitted light microscope. Use of a microscope with a polarizer/analyzer set is preferable, and somewhat indispensable for some microfossil types, such as starches or faunal spherulites. Various degrees of cross-polarization can be used to partially illuminate birefringent particles, to scan slides for 'illuminated' starches or faunal spherulites while also seeing non-birefringent microfossils, or to photograph a particle's surface and birefringence pattern simultaneously (e.g. Fig. 1h).

Proper viewing of microfossils requires that they be suspended in a mounting medium of appropriate refractive index, generally higher than that of the microfossil. A mounting medium with a refractive index too near that of the microfossil will impart a faint, blurred appearance, although this can be mediated by staining. Diatom analysts tend to use media of very high refractive indices (RI) (>1.65) to highlight minute ornamentations on otherwise translucent silica. However, many media of very high RI are also highly toxic [60]. Cronberg [20] has reported a 'double edge' effect produced on chrysophytes in some media, giving the false appearance of extra layers in a microfossil. This can be problematic for morphological classification where evaluation of fine laminations or layering is critical. Most microfossils described in this article can successfully be viewed using media with RI in the more common 1.53–1.60 range. Tables 2 and 3 provide summaries of the RI of common mounting media as well as the microfossils discussed, respectively.

Table 2
RI (at 20–25 °C) and general properties of several common mounting media for microfossils

Mountant	RI[a]	Viscosity	Properties
Water	1.33	High	Evaporates, impermanent, dissolves $CaCo_3$, good contrast for phytoliths (e.g. photos)
Permount	1.52	Solidifies	Yellowing, clouding in slide mounts over time is possible
Entellan	1.49–1.50	Solidifies	
Silicone oil	~1.4	High	Low RI very close to phytoliths, commonly used for pollen mounting
Immersion oil	1.52	High	Highly viscous, poor for starches, good for phytoliths
Clove oil	1.53	High	Viscous
Naphrax	1.70	Solidifies	Recommended by McLaughlin [60] for diatoms

[a] information provided by manufacturers, see also White [101] and Loveland and Centifanto [55].

Table 3
RI of discussed microfossil types as known

Microfossil	RI
Pollen/spores	Variable
Starch	1.52 [96] to 1.53 [101]
Plant cellular tissues	Variable
Microscopic charcoal	N/A (opaque)
SiO_2 phytoliths	1.42 [71]
Diatoms	1.43 [101]
Chrysophyte statospores, scales	Similar to phytoliths and diatoms
Sponge spicules	Similar to phytoliths and diatoms

There are literally hundreds of agents that have been used as mountants for microfossils—see the study by Loveland and Centifanto [55] for an extensive historical review. Many commercially available mountants are preparations of resins dissolved in xylenes or toluenes, and solidify upon the eventual evaporation of the thinning agent over a period of days or weeks. Such solidified slide mounts may not be ideal for microfossil analysts, who often need to roll particles to locate diagnostic features or evaluate three-dimensional (3-D) morphology. Some analysts take advantage of the 1–2 week drying period required by some media, and view their slides 'fresh', before the mountant solidifies.

An alternative to resinous media is the use of permanently liquid or viscous media for slide mounting. Glycerine mixed with water can be a good medium for observing fine detail in starches (L. Perry, personal communication, 2000). Oils, such as essential clove oil, silicone oil, or microscope immersion oil, can be used successfully if cover slips are properly sealed (often with fingernail polish) to prevent leaking. Temporary water mounts are useful for monitoring results at various points in extraction protocols or taking photographs of microfossil assemblages (e.g. Fig. 3), but can be too ephemeral for anything but quick checks. Although water mounts can be too ephemeral for other purposes, R. Evett (personal communication, 2001) suggests that petroleum jelly applied to the edges of a cover slip before it is lowered onto a drop of water-suspended extract can help delay evaporation for 1 or 2 days. Because of variability in mountant and microfossil optical properties, some extracts containing multiple types of microfossils may benefit from a series of slides prepared with different media.

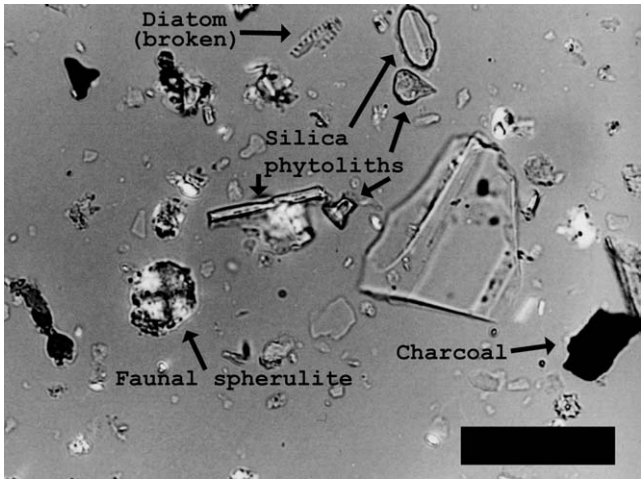

Fig. 3. Example of extracted microfossil assemblage from Valle del Bolsón, Argentina. Photographed in partially cross-polarized, transmitted light at 400×. Scale bar=50 μm.

4.2. Summary of procedural steps

Tables 4 and 5 summarize laboratory considerations for successful extraction and viewing of individual organic and biogenic mineral microfossil types, respectively. We note that some of these assertions are tentative, and have yet to be empirically verified, or have been established by relatively informal laboratory experiments by others and ourselves. We therefore recommend treating these as guidelines.

4.3. Writing a protocol

Once a series of procedures is decided upon to adequately address the extraction goals summarized in Table 1, a detailed 'protocol' should be written. A protocol consists of a step-by-step list of sequential

Table 4
Summary of possible effects of various processing treatments on organic microfossil types

	Pollen and spores	Starches	Plant cell tissue	Microscopic charcoal
Raw sample preparation	Samples should be dried or treated with fungicide	Sterilization with heat should be avoided	Samples should be dried or treated with alcohol	No problems known
Deflocculation	No harm from common deflocculants known	No harm from common deflocculants known	No harm from common deflocculants known	No harm from common deflocculants known
Mechanical disaggregation	Surface ornamentation may be abraded	No known problems	Articulated cells or their contents may by separated	May fragment charcoal and affect size distributions
Clay removal	Low specific gravity causes slow settling relative to size, see Lentfer [50]	Smallest forms (1–5 μm) may be lost during clay pour-offs	Probably no concerns	
Organic destruction/inorganic destruction	Partially oxidized palynomorphs may be damaged by common methods [39]	Still largely unstudied, but starches probably are damaged by acid/alkali organic destruction steps	Will be destroyed by many methods, including acid and oxidation (e.g. H_2O_2) treatments	Probably not harmed at all
Heavy-liquid floatation	No problems known	Therin has reported starch damage from $CsCl_2$[96]	No problems known	No problems likely
Slide mounting/storage	Preserving mobility essential for rotating forms	Preserving mobility essential for rotating forms	Preserving mobility useful for rotating forms	Opaque, mobility not really necessary

Table 5
Summary of possible effects of various processing treatments on biogenic mineral microfossil types

	Silica phytoliths	Other biogenic silica	Calcium phytoliths	Faunal spherulites
Raw sample preparation	Starting sample quantity can be adjusted for silica microfossil concentrations after initial testing		Subject to dissolution in acidic pH conditions, which may include soaking in distilled H_2O (which can be naturally acidic)	
Deflocculation	Deflocculants generally harmless, long-term soaking in high-pH conditions could harm silica		Most common deflocculants are basic in pH, so these should not be damaged during deflocculation	
Mechanical disaggregation	Lightly silicified forms, or fragile forms or surface details, could be damaged		Probably subject to fragmentation (e.g. druses or raphides)	Effects unstudied
Clay removal	Most forms large enough not to overlap with clay particle settling rates, and specific gravities are relatively high, so pour-off loss during clay removal is probably not a major concern. See Lentfer [50]			
Organic destruction/inorganic destruction	These are unharmed by most organic destruction methods. Damage is possible with prolonged alkali treatments. Dissolved by HF (see Section 4.1.7)		Dissolved by hydrochloric acid (but not glacial acetic acid), alkali treatments probably OK	Dissolved by hydrochloric acid and glacial acetic acid, alkali treatments probably OK
Heavy-liquid flotation	Specific gravities range from 1.5 to 2.3, unharmed by common heavy-liquid solutions		Effects of various flotation agents unstudied. However, the ones which are acidic in solution may damage these types of microfossils	
Slide mounting and viewing	Preserving mobility is useful for viewing 3-D morphology and surface decoration		No special mounting concerns. Viewing of both of these types of birefringent minerals may be enhanced with polarized light microscopy	

activities that begins with raw samples and ends with isolated microfossils mounted on microscope slides. The order in which the various procedural steps previously discussed should be arranged is generally flexible, although experimental re-ordering of steps may help dispel certain problems, which can arise. Also it is important to thoroughly rinse samples between chemical treatments to avoid mixing incompatible chemicals.

While exact adherence to a published or shared protocol may be desirable in some cases, deviations will usually become necessary while adapting to a different laboratory setting. Protocol modifications should be carried out with the overarching goals of microfossil extraction in mind. Many of the details found in extraction protocols reflect the availability of particular equipment, as well as personal preferences for certain types of tools, supplies and chemicals. Eventually, explicit enumeration and description of protocol steps is necessary to maintain consistency between the sample runs, between multiple laboratory workers involved in sample processing, and to allow accurate replication of extractions.

5. Case studies

Finally, we briefly discuss two archaeological case studies where conjunctive approaches to microfossil research are being developed with encouraging results. In the spirit of our overall subject matter, we focus on laboratory-related issues from these projects. Our development of laboratory procedures has benefited from both formally designed experiments [51,105] as well as from more expedient tests and trials. We also wish to emphasize that specific archaeological questions should be formulated to guide decisions about how or whether to attempt recovery of specific microfossil types.

In the first case study, Coil describes his efforts to extract multiple types of microfossils from archaeological samples from Maui, Hawaii, where extraction is complicated by the unusual properties of humus-rich, volcanic soils. In the second case study, Korstanje describes her recent efforts to apply a wider range of microfossil evidence to address research questions of agricultural and pastoral production in Argentina's Valle del Bolsón.

5.1. Case study 1: troubleshooting extraction problems, Kahikinui, Maui, Hawaiian islands

In this study, microfossil research began with the goal of integrating phytolith data with plant macrofossil evidence excavated from a wide range of pre-contact residential and agricultural sites in Maui, Hawaii [18,45]. Efforts to extract microfossils from these organic-rich soils, derived from basaltic lava and pyroclastic tephra parent materials, began with following published phytolith protocols (such as Ref. [58]). In addition to the standard problems with attaining supplies and equipment, which confront new microfossil researchers, processing problems soon arose similar in nature to those reported by Pearsall and Trimble [67,68] in an earlier Hawaiian phytolith study. Attempts to apply standard extraction protocols to these samples failed in several ways, with problems including inadequate disaggregation with oxidation and HCl steps, difficulty in deflocculating clays with sodium salts, such as sodium hexametaphosphate, especially after steps involving acids, and the problem with sodium polytungstate flotation first described in Ref. [105], where floated fractions consisted of a thick band of mysterious 'sludge', rather than a clean separation of microfossils. Another problem encountered with these samples was the inclusion of low-density pumice sand fragments with floated microfossils in some samples. When these were large, they interfered with slide mounting as cover slips were held aloft by these particles. Introducing a 150 μm sieving step into the protocol largely eliminated this last problem.

Other difficulties were eventually resolved through exploration of the literature, consultation with other microfossil researchers, and experimentation. Soil pH levels tend to be slightly acidic in these samples, indicating little need for carbonate removal (Scott-Cummings, personal communication, 2001). Owing to the acidic soil, only one chemical treatment is employed, using hot KOH (potassium hydroxide 10%) to dissolve the soils' alkali-soluble humic components [65]. Humic colloids appear to be the major source of particle aggregation in these samples. Also, clays are deflocculated with plain water because sodium-based deflocculants have little effect on these volcanic clays. After clay removal is complete, a drop of waterborne sediment reveals thousands of individual, non-aggregated organic and mineral particles ready to be separated. Separation is then performed using heavy-liquid flotation with zinc iodide, which produces clean extractions including a range of organic and mineral microfossils—spores, pollen, silica phytoliths, microscopic charcoal, and diatoms. Starches do not seem to survive this protocol, possibly because of their susceptibility to destruction during the hot KOH step.

Some of these extraction problems could have been surmounted in other ways, including strong acid oxidation, as used by Zhao and Pearsall [105] to process samples containing oxide clays. This may also have prevented the problems arising with using sodium polytungstate as the heavy-liquid floatation medium. Kealhofer, who routinely uses a strong acid oxidation step in her extraction protocols, has not experienced the 'sludge' problem when floating phytolith samples with sodium polytungstate (personal communication, 2000).

Extractions containing silica phytoliths, pollen, microscopic charcoal, and diatoms are now being analyzed. These data will be used to infer vegetation types contributing to microfossil assemblages from both on- and off-site contexts, as well as adding to our understanding of our study area's geological and geomorphological complexities. While interpretive challenges remain, it is gratifying to know that samples that were once considered highly refractory are now

Table 6
Summary of microfossil-related research design considerations at Valle de Bolsón, Argentina

Phytoliths	Of the probable crop taxa, only maize, beans and cucurbits have diagnostic phytoliths. But frequencies and diversity of phytolith assemblages could also help to indicate dung deposition as fertilizer, since grass phytoliths in dung are found to be much more diverse, and appear in higher concentrations, than in other site sediments
Starches	Starches seem to represent the best possibility for identifying the remains of cultivated tuber crops in these sites. Also, the variety in forms and frequencies might be taken into account as an estimation of variability between the different sites, structures and layers
Diatoms and algal remains	When identification is possible (especially in diatoms), these may help in the characterization of the conditions of microfossil deposition, especially as signals of changing moisture conditions or the introduction of irrigation practices
Pollen	Maize pollen is diagnostic to species level and tends to have a limited range of natural transport. We didn't know if pollen would survive in the semi-arid environmental conditions of the valley, but we now know that some does.
Microscopic charcoal	Both animal husbandry and agricultural practices may have involved burning episodes as they do presently. The occurrence of microscopic charcoal fragments in the samples, as well as their taxonomic identities (e.g. grass vs. dicot.), size distributions, and concentrations, could be used as evidence suggesting various types of anthropogenic fire regimes
Faunal spherulites	The occurrence of faunal spherulites in the samples can be taken as evidence of camelid dung deposition, with concentrations and archaeological contexts used to determine whether dung deposition at a given site may reflect, for example, a site's use as a corral, addition of dung to cultivated fields as fertilizer, or natural deposition events by non-domestic camelid populations (such as in rock shelters)

effectively processed with only potassium hydroxide, water, and zinc iodide.

5.2. Case study 2: low-destruction approaches for maximum microfossil diversity, Valle del Bolsón, Catamarca, Argentina

In this study, we are attempting to identify early husbandry practices from archaeological open-air and rock shelter contexts in an arid, high-valley study area. We assume that economic strategies involving camelid herding, dry-farmed tuber/chenopod cultivation, and intensive, maize-focused irrigated agriculture, were all potentially practiced by the area's pre-historic populations. We are attempting to track the chronological and spatial distributions of these three types of economic activities.

Phytolith analysis was initially recognized as a potential avenue for contributing to this investigation [47], inspired in part by low levels of macrobotanical recovery from open-air fields. As our processing of phytolith reference materials and archaeological sediment samples progressed, the limitations of a phytolith-centered approach became more apparent. Few of the indigenous tuber taxa potentially cultivated during the early phases of these occupations produce diagnostic or abundant phytoliths while others do not, nor do other important local crop plants such as *Chenopodium* sp. or *Amaranthus* sp. The only regional crops seemingly amenable to producing phytolith evidence are maize, beans, and cucurbits [7,75,77,78], but none of these would be expected during the early phases of occupation at these high-altitude sites (3000–3200 m.a.s.l). Eventually, our extraction and examination of additional microfossil types not only enhanced the interpretive potential of the phytolith data, but also produced data sets that were more relevant to the given research questions [49].

A specific example reveals as to how the analysis of multiple microfossil types is aiding our interpretations of site formation as well as function. Microfossil assemblages extracted from a well-preserved camelid dung layer in a rock shelter site suggest that domesticated camelids may have been fed with some of the same crops that humans consumed, since *Cucurbita* sp. and *Zea mays* phytoliths were found in the preserved dung. These results imply that crop phytoliths might have been deposited in open-air site contexts in the form of animal dung, rather than only as human-food remains. However, as was the case in the study of Brochier et al. [10], we realized that analyzing several types of microfossils together, especially examination of the relationship between phytoliths, diatoms, and faunal spherulites, could improve interpretation of the depositional origins of these assemblages. After realizing the advantages of incorporating multiple types of microfossil data in our study, we evaluated the potential contribution of these types for our research problem, as summarized in Table 6 .

In the laboratory, we first used a low-chemical, low-tech protocol based mainly on gravity separation, at the UNCIEP's laboratory (Uruguay), under the guidance of del Puerto and Inda. Later refinements made at UC Berkeley included the addition of a heavy-liquid flotation step, which both concentrated microfossils and improved their visibility. Because we developed our protocol mainly to preserve relatively fragile microfossils (e.g. starch and faunal spherulites), we conducted tests with modern starch to determine whether the zinc iodide flotation step was damaging and destroying starch during sample processing. Out of four different tests controlling time, temperature, and different concentrations of the product, we found zinc iodide causes no damage to starch at room temperature, but may destroy some starches when heated in an aqueous medium [48].

After establishing the possibility of using faunal spherulites as evidence for the deposition of camelid dung, we conducted experiments with modern dung samples from species *Lama pacos* and *L. glama* to determine the susceptibility of these microfossils to destruction by some common extraction chemicals [49]. An entirely chemical-free protocol might be the best choice to extract only the spherulite assemblage, but from our experiments, we can conclude that the low-chemical protocol we used for our multiple-microfossil extractions was suitable for spherulite recovery.

Besides starch and faunal spherulites, samples from Valle del Bolsón contain a wide range of other organic and mineral microfossils, including silica and calcium phytoliths, diatoms, chrysophytes, cellulose tissue, palynomorphs, and microscopic charcoal (Fig. 3). An added benefit of developing low-chemical and low-tech extraction protocols is the lower hazards and costs associated with such approaches.

Our initial results suggest that a range of microfossil assemblages may most effectively address our research questions. When these lines of microfossil-based evidence are combined with information from more traditional archaeological avenues, such as architectural interpretation of site function, spatial distribution of material evidence for economic activities, and geomorphological signs of field irrigation, the possible interpretive advantages of extracting and conjunctively interpreting several types of microfossils become apparent.

6. Conclusions

Microfossil research in archaeology continues to develop on a truly international scale. New advances and old wisdom can both be found well beyond the mainstream of recent anglophone literature. Our own experience with microfossil research in archaeology is insufficient to justify any programmatic statements as to how microfossil research should be done, although we believe that focusing more conjunctively on a wider range of microfossil types can contribute to archaeological problem solving. The further development of low-destruction laboratory protocols is a direction that should help microfossil research in archaeology to further flourish and advance, and also to become accessible in a practical way to more archaeologists. Such experimentation must be balanced, however, with a recognition that the basic standards of microfossil recovery must be maintained in newly developed protocols.

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Finally, we note that this article was in preparation during the tragic world events of September 11, 2001, and was submitted during the continuing world violence of these events' aftermath. In this context, the authors wish to acknowledge their collective thankfulness for the spirit of international cooperation and collaboration that exists among members of the scientific community, and for the fact that such collegiality continues to provide a working alternative model for how world relations can be conducted.

References

[1] S. Archer, K. Bartoy, Plants in the plowzone?: Archaeology and paleoethnobotany at Carter's Grove Site 10, Williamsburg, VA,

paper presented at the 65th Annual Meeting of the Society for American Archaeology, Philadelphia, PA, 2000.
[2] T.n.d. Ball, Staining phytoliths. Unpublished handout distributed at Phytolith Workshop during SAA meetings in Seattle, 1997.
[3] H. Barton, Raw material and tool function: a residue and use—wear analysis of artefacts from a Melanesian rock shelter, B.A. (Hons.) thesis, Department of Prehistory and Historical Archaeology, University of Sydney, 1990.
[4] H. Barton, R. Torrence, R. Fullagar, Clues to stone tool function re-examined: comparing starch grain frequencies on used and unused obsidian artefacts, Journal of Archaeological Science 25 (1998) 1231–1238.
[5] R.W. Batterbee, M.J. Kneen, The use of electronically counted microspheres in absolute diatom analysis, Limnology and Oceanography 27 (1982) 184–188.
[6] H. Bertoldi de Pomar, Los silicofitolitos: sinópsis de su conocimiento, Darwiniana 19 (1975) 173–206.
[7] S. Bozarth, Classification of opal phytoliths formed in selected dicotyledons native to the Great Plains, in: G. Rapp Jr., S. Mulholland (Eds.), Phytolith Systematics: Emerging Issues, Plenum Press, New York, 1992, pp. 193–214.
[8] M.D. Brasier, Microfossils, Unwin Hyman, Ltd, London, 1980.
[9] J. Brochier, Feuilles ou fumiers? Observations sur le rôle des poussièeres sphérolitiques dans l'interprétations des dépôts archéologiques holocènes, Anthropozoologica 24 (1996) 19–30.
[10] J. Brochier, P. Villa, M. Giacomarra, A. Tagliacozzo, Shepherds and sediments: geo-ethnoarchaeology of pastoral sites, Journal of Anthropological Archaeology 11 (1992) 47–102.
[11] V.M. Bryant Jr., R.G. Holloway, The role of palynology in archaeology, Advances in Archaeological Method and Theory 6 (1983) 191–223.
[12] K.W. Butzer, Archaeology as Human Ecology: Method and Theory for a Contextual approach, Cambridge University Press, Cambridge, 1982.
[13] S. Campos, L. del Puerto, H. Inda, Opal phytolith analysis: its application to the archaeobotanical record in the Eastern Uruguay, in: J. Meunier, F. Collin (Eds.), Phytoliths: Applications in Earth Science and Human History, Balkema, Rotterdam, 2001, pp. 129–142.
[14] M.G. Canti, An investigation of microscopic calcareous spherulites from herbivore dungs, Journal of Archaeological Science 24 (1997) 219–231.
[15] M.G. Canti, The micromorphological identification of faecal spherulites from archaeological and modern materials, Journal of Archaeological Science 25 (1998) 435–444.
[16] M.G. Canti, The production and preservation of faecal spherulites: animals, environment and taphonomy, Journal of Archeological Science 26 (1999) 251–258.
[17] J. Cochrane, The World of Plants Without Flowers, Castle Books/B.P.C. Publishing, London, 1972.
[18] J. Coil, Design, methods, and initial results of microfossil research in Kahikinui, Maui, Hawaiian Islands, in: D. Hart, L. Wallis (Eds.), The State of the Art in Phytolith and Starch Research in the Australian/Pacific/Asian Regions, Pandanus Books, Canberra (in press).
[19] J. Coil, H. Barton, Microbotanical analyses of artifacts and sediment sample 1, in: B. Dixon, D.J. Welch, C.M. Magnuson (Eds.), Archaeological Data Recovery of Site TN-1-691, West Tinian Airport Improvement Area, Island of Tinian, International Archaeological Research Institute Inc., Honolulu, 2001, pp. 125–144.
[20] G. Cronberg, Chrysophycean cysts and scales in lake sediments: a review, in: J. Kristiansen, R. Andersen (Eds.), Chrysophytes: Aspects and Problems, Cambridge University Press, Cambridge, 1986, pp. 281–315.
[21] O.K. Davis, J.A. Overs (Eds.), Aspects of Archaeological Palynology: Methodology and Applications, AASP Contributions Series No. 29, American Association of Stratigraphic Palynologists Foundation, Cambridge, 1994.
[22] S. DeMeo, Synthesis and decomposition of zinc iodide, Journal of Chemical Education 72 (1995) 836–839.
[23] G. Dimbleby, The Palynology of Archaeological Sites, Academic Press, London, 1985.
[24] K. Faegri, J. Iversen, P.E. Kaland, K. Krzywinski, Textbook of Pollen Analysis, Wiley, New York, 1989.
[25] G. Filep, Soil Chemistry: Processes and Constituents, Akadémiai Kiadó, Budapest, 1999.
[26] S. Fish, Archaeological palynology of gardens and fields, in: N.F. Miller, K.L. Gleason (Eds.), The Archaeology of Garden and Field, University of Pennsylvania Press, Philadelphia, 1994, pp. 44–69.
[27] E.A. Fitzpatrick, Soil Microscopy and Micromorphology, Wiley, London, 1993.
[28] R.L. Folk, Petrology of Sedimentary Rocks, Hemphill Publishing Company, Austin, 1964.
[29] V.R. Franceschi, H.T. Horner Jr., Calcium oxalate crystals in plants, The Botanical Review 46 (1980) 361–416.
[30] G. Fredlund, Problems in the simultaneous extraction of pollen and phytoliths from clastic sediments, in: I. Rovner (Ed.), Plant Opal Phytolith Analysis in Archaeology and Paleoecology, Occasional Papers of the Phytolitharean, No 1, North Carolina State University, Raleigh, 1986, pp. 102–110.
[31] G. Fredlund, L. Tieszen, Calibrating grass phytolith assemblages in climatic terms: application to late Pleistocene assemblages from Kansas and Nebraska, Paleogeography, Paleoclimatology, Paleoecology 136 (1997) 199–211.
[32] G. Fredlund, C.B. Bousman, D.K. Boyd, The Holocene phytolith record from Morgan Playa in the rolling plains of Texas, Plains Anthropologist 43 (164) (1998) 187–200.
[33] J.W. Funkhouser, W.R. Evitt, Preparation techniques for acid-insoluble microfossils, Micropaleontology 5 (3) (1959) 369–375.
[34] S.A. Hall, T.J. Ferguson, Pollen, stratigraphy and chronology of the North edge of Zuni Pueblo, Kiva 61 (1996) 225–239.
[35] C.A. Hastorf, Gender, space and food in prehistory, in: J. Gero, M. Conkey (Eds.), Engendering Archaeology: Women and Prehistory, Blackwell, Oxford, 1991, pp. 132–159.
[36] I. Hodder, Reading the Past, Cambridge University Press, Cambridge, 1986.
[37] M.J. Hodson, S.E. Williams, A.G. Sangster, Silica deposition in the needles of the gymnosperms. I. Chemical analysis and light microscopy, in: A. Pinella, J. Juan-Tresserras, M.J. Machado (Eds.), Estado Actual de los Estudios de Fitolitos en Suelos y Plantas, CSIC, No. 4, Monografías del Centro de Ciencas Medioambientales, Madrid, 1997, pp. 113–122.
[38] R.B. Hoover, Those marvelous, myriad diatoms, National Geographic 155 (1979) 870–878.
[39] A. Horowitz, Palynology of Arid Lands, Elsevier, Amsterdam, 1992.
[40] J. Iversen, Land occupation in Denmark's Stone Age, Danmarks Geologiske Undersogelse II 66 (1941) 1–68.
[41] J. Juan-Tresserras, Procesado y preparación de alimentos vegetales para consumo humano. Aportaciones del estudio de fitolitos, almidones y lípidos en yacimientos arqueológicos prehistóricos del cuadrante en de la Península Ibérica, Ph.D. thesis, Facultad de Prehistoria, Historia Antigua y Arqueología, Universidad de Barcelona, Spain, 1992.
[42] J. Juan-Tresserras, La cerveza prehistórica: investigaciones arqueobotánicas y experimentales, in: J.L. Maya, F. Cuesta, J. López Cachero (Eds.), Genó: un poblado del Bronce Final en le Bajo Segre (Lleida), Universidad de Barcelona, Barcelona, 1998, pp. 239–252.

[43] L. Kealhofer, D. Penny, A combined pollen and phytolith record for fourteen thousand years of vegetation change in northeastern Thailand, Review of Palaeobotany and Palynology 103 (1998) 83–93.

[44] G.K. Kelso, S.A. Mrozowski, D. Currie, A.C. Edwards, M.R. Brown III, A.J. Horning, G.J. Brown, J.R. Dandoy, Differential pollen preservation in a seventeenth-century refuse pit, Jamestown Island, Virginia, Historical Archaeology 29 (1995) 43–54.

[45] P.V. Kirch (Ed.), Na Mea Kahiko o Kahikinui: Studies in the Archaeology of Kahikinui, Maui, Oceanic Archaeology Laboratory Special Publication No. 1, UC Berkeley Archaeological Research Facility, Berkeley, 1997.

[46] P.V. Kirch, R.C. Green, Hawaiki, Ancestral Polynesia: An essay in historical anthropology, Cambridge University Press, Cambridge, 2001.

[47] M.A. Korstanje, Sobre la producción agrícolo-ganadera en el Formativo: Reflexiones para el camino, Actas del I Congreso de Investigación Social: 'Región y Sociedad en el NOA', Facultad de Filosofía y Letras, Universidad Nacional de Tucumán, Tucumán, 1996, pp. 402–411.

[48] M.A. Korstanje, Taphonomy in the lab: considerations on starch damage from multiple microfossil recovery in sediments, in: D. Hart, L. Wallis (Eds.), The State of the Art in Phytolith and Starch Research in the Australian/Pacific/Asian Regions, Pandanus Books, Canberra (in press).

[49] M.A. Korstanje, Microfossils in Camelid Dung: Taphonomical Considerations for the Archaeological Study of Agriculture and Pastoralism, Oxbow book series for the International Council For Archaeozoology, Durham (submitted for publication).

[50] C.J. Lentfer, Extraction of phytoliths and other palynomorphs from sediments, M.Sc. thesis, School of Resource Science and Management, Southern Cross University, New South Wales, 1998.

[51] C.J. Lentfer, W.E. Boyd, A comparison of three methods for the extraction of phytoliths from sediments, Journal of Archaeological Science 25 (1998) 1159–1183.

[52] C.J. Lentfer, W.E. Boyd, An assessment of techniques for the deflocculation and removal of clays from sediments used in phytolith analysis, Journal of Archaeological Science 26 (1999) 31–44.

[53] C.J. Lentfer, W.E. Boyd, Simultaneous extraction of phytoliths, pollen and spores from sediments, Journal of Archaeological Sciences 27 (2000) 363–372.

[54] S. Limbrey, Soil Science and Archaeology, Academic Press, San Diego, 1975.

[55] R.P. Loveland, Y.M. Centifanto, Mounting media for microscopy, Microscope 34 (1986) 181–242.

[56] T.H. Loy, Methods in the analysis of starch residues on prehistoric stone tools, in: J.G. Hather (Ed.), Tropical Archaeobotany: Applications and New Developments, Routledge, London, 1994, pp. 86–114.

[57] T.H. Loy, M. Spriggs, S. Wickler, Direct evidence for human use of plants 28,000 years ago: starch residues on stone artefacts from the Northern Solomon Islands, Antiquity 66 (1992) 898–912.

[58] M. Madella, A.H. Powers-Jones, M.K. Jones, A simple method of extraction of opal phytoliths from sediments using a non-toxic heavy liquid, Journal of Archaeological Science 25 (1998) 801–803.

[59] W. Matthews, C.A.I. French, T. Lawrence, D.F. Cutler, M.K. Jones, Multiple surfaces: the micromorphology, in: I. Hodder (Ed.), On the Surface: Çatalhöyük 1993–95, McDonald Institute for Archaeological Research and British Institute of Archaeology at Ankara, Cambridge, 1996, pp. 301–342.

[60] R.B. McLaughlin, Diatom microscopy, Microscope 42 (1994) 75–79.

[61] C.R. Metcalfe, L. Chalk, Anatomy of the Dicotyledons, Clarendon Press, Oxford, 1950.

[62] J.F. Parr, A comparison of heavy liquid flotation and microwave digestion techniques for the extraction of fossil phytoliths from sediments, Review of Palaeobotany and Palynology 120 (3–4) (2002) 315–336.

[63] M.L. Parsons, Q. Dortch, R.E. Turner, Sedimentological evidence of an increase in *Pseudo-nitzschia* (Bacellariophyceae) abundance in response to coastal eutrophication, Limnology and Oceanography 47 (2) (2002) 551–558.

[64] D.M. Pearsall, Application of phytolith analysis to reconstruction of past environments and subsistence: recent research in the Pacific, Micronesica Suppl. 2 (1990) 65–74.

[65] D.M. Pearsall, Paleoethnobotany: A Handbook of Procedures, second ed., Academic Press, New York, 2000.

[66] D.M. Pearsall, D.R. Piperno (Eds.), Current Research in Phytolith Analysis: Applications in Archaeology and Paleoecology, MASCA, The University Museum of Archaeology and Anthropology, University of Pennsylvania, Philadelphia, 1993.

[67] D.M. Pearsall, M.K. Trimble, Report 18: phytolith analysis of soil samples, in: J.T. Clark, P.V. Kirch (Eds.), Archaeological Investigations of the Mudlane-Waimea-Kawaihae Road Corridor, Island of Hawai'i: An Interdisciplinary Study of an Environmental Transect. B. P, Bishop Museum Department of Anthropology, Honolulu, 1983, pp. 472–497.

[68] D.M. Pearsall, M.K. Trimble, Identifying past agricultural activity through soil phytolith analysis: a case study from the Hawaiian Islands, Journal of Archaeological Science 11 (1984) 119–133.

[69] D. Penny, J. Grindrod, P. Bishop, Preliminary microfossil analysis of a lake sediment core: Nong Han Kumphawapi, Udon Thani, Northeast Thailand, Asian Perspectives 35 (1996) 209–228.

[70] A. Pinilla, J. Juan-Tresserras, M.J. Machado (Eds.), Estado Actual de los Estudios de Fitolitos en Suelos y Plantas, CSIC, No. 4, Monografias del Centro de Ciencas Medioambientales, Madrid, 1997.

[71] D.R. Piperno, Phytolith Analysis: An Archaeological and Geological Perspective, Academic Press, San Diego, 1988.

[72] D.R. Piperno, Phytolith and charcoal records from deep lake cores in the American Tropics, in: D.M. Pearsall, D.R. Piperno (Eds.), Current Research in Phytolith Analysis: Applications in Archaeology and Paleoecology, MASCA, The University Museum of Archaeology and Anthropology, University of Pennsylvania, Philadelphia, 1993, pp. 58–72.

[73] D.R. Piperno, Phytolith and charcoal evidence for prehistoric slash-and-burn agriculture in the Darien rain forest of Panama, Holocene 4 (1994) 321–325.

[74] D.R. Piperno, Plant microfossils and their application in the New World tropics, in: P.W. Stahl (Ed.), Archaeology in the Lowland American Tropics: Current Analytical Methods and Recent Applications, Cambridge University Press, Cambridge, 1995, pp. 130–153.

[75] D.R. Piperno, Paleoethnobotany in the Neotropics from microfossils: new insights into ancient plant use and agricultural origins in the tropical forest, Journal of World Prehistory 12 (1998) 393–449.

[76] D.R. Piperno, I. Holst, The presence of starch grains on prehistoric stone tools from the humid Neotropics: indications of early tuber use and agriculture in Panama, Journal of Archaeological Science 25 (1998) 765–776.

[77] D.R. Piperno, I. Holst, L. Wessel-Beaver, T.C. Andres, Evidence for the control of phytolith formation in *Cucurbita* fruits by the hard rind (*Hr*) genetic locus: Archaeological and ecological implications, Proceedings of the National Academy of Sciences 99 (2002) 10923–10928.

[78] D.R. Piperno, A.J. Ranere, I. Holst, P. Hansell, Starch grains reveal early root crop horticulture in the Panamanian tropical forest, Nature 407 (2000) 894–897.
[79] L. del Puerto, S. Campos, Silicofitolitos: Un abordaje alternativo de la problemática arqueobotánica del este de Uruguay, in: A. Aschero, A. Korstanje, P. Vuoto (Eds.), En los Tres Reinos: Prácticas de Recolección en el Cono Sur de América, Instituto de Arqueología y Museo (UNT), Tucumán, 1999, pp. 141–150.
[80] J.A. Radley, Starch and its Derivatives, second ed., Chapman and Hall, London, 1943.
[81] G. Rapp Jr., S.C. Mulholland, Phytolith Systematics: Emerging Issues, Plenum Press, New York, 1992.
[82] I. Renberg, M. Wik, Soot particle counting in recent lake sediments: an indirect dating method, Ecological Bulletin 37 (1985) 53–57.
[83] A.N. Rhodes, A method for the preparation and quantification of microscopic charcoal from terrestrial and lacustrine sediment cores, The Holocene 8 (1998) 113–117.
[84] N.L. Rose, A method for the extraction of carbonaceous particles from lake sediment, Journal of Paleolimnology 3 (1990) 45–53.
[85] I. Rovner, Potential of opal phytoliths for use in paleoecological reconstruction, Quaternary Research 1 (1971) 343–359.
[86] I. Rovner, Plant opal phytolith analysis: Major advances in archaeobotanical research, in: M. Schiffer (Ed.), Advances in Archaeological Method and Theory 6, Academic Press, New York, 1983, pp. 225–266.
[87] I. Rovner, J.C. Russ, Darwin and design in phytolith systematics: morphometric methods for mitigating redundancy, in: G. Rapp Jr., S.C. Mullholand (Eds.), Phytolith Systematics: Emerging Issues, Plenum Press, New York, 1992, pp. 253–276.
[88] S.E. Ruzin, Plant Microtechnique. UC Berkeley–NSF Center of Plant Development Biology, Berkeley, 1996.
[89] L.P. Schwandes, Environmental durability of biogenic opal, Soil and Crop Science Society of Florida Proceedings 57 (1998) 36–39.
[90] L.P. Schwandes, M.E. Collins, Distribution and significance of freshwater sponge spicules in selected Florida soils, Transactions of the American Microscopical Society 113 (1994) 242–257.
[91] L. Scott-Cummings, Illustrated phytoliths from assorted food plants, in: G. Rapp Jr., S.C. Mullholand (Eds.), Phytolith Systematics: Emerging Issues, Plenum Press, New York, 1992, pp. 175–192.
[92] L. Scott-Cummings, Phytolith Analysis at Keokea Ahupua'a, Kula District, Maui, PaleoResearch Laboratories, Denver, CO, 1995 (unpublished laboratory report).
[93] W.N. Stewart, Paleobotany and the Evolution of Plants, Cambridge University Press, Cambridge, 1983.
[94] W.W. Taylor, A Study of Archaeology, Southern Illinois University Press, Carbondale, 1948.
[95] M. Therin, R. Fullagar, R. Torrence, Starch in sediments: a new approach to the study of subsistence and land use in Papua New Guinea, in: C. Gosden, J. Hather (Eds.), The Prehistory of Food: Appetites for Change, Routledge, London, 1999, pp. 438–461.
[96] M.J. Therin, Subsistence through starch: the examination of subsistence changes on Garua Island, West New Britain, Papua New Guinea, through the extraction and identification of starch from sediments, B.A. (Hons) thesis, Department of Prehistoric and Historic Archaeology, University of Sydney, 1994.
[97] R. Tipping, 'Ritual' floral tributes in the Scottish Bronze Age: palynological evidence, Journal of Archaeological Science 21 (1994) 133–139.
[98] C.E. Umbanhowar Jr., M.J. McGrath, Experimental production and analysis of microscopic charcoal from wood, leaves and grasses, The Holocene 8 (1998) 341–346.
[99] P. Verdin, J.-F. Berder, J.A. López-Saez, Contribution of phytolith analyses to the understanding of historical agrosystems in the Rhône Mid-Valley (Southern France), in: J. Meunier, F. Colin (Eds.), Phytoliths: Applications in Earth Science and Human History, Balkema, Rotterdam, 2001, pp. 155–172.
[100] P.J. Warnock, K.J. Reinhard, Methods for extracting pollen and parasite eggs from latrine soils, Journal of Archaeological Science 19 (1992) 261–264.
[101] G.W. White, A survey of refractive indices, Microscopy 31 (1970) 257–267.
[102] M. Wik, I. Renberg, Distribution in forest soils of carbonaceous particles from fossil fuel combustion, Water, Air, and Soil Pollution 33 (1987) 127–129.
[103] L.P. Wilding, L.R. Drees, Biogenic opal in Ohio soils, Proceedings of the Soil Science Society of America 35 (1971) 1004–1010.
[104] C.L. Wilson, W.E. Loomis, T.A. Steeves, Botany, fifth ed., Holt, Rinehart and Winston, New York, 1971.
[105] Z. Zhao, D.M. Pearsall, Experiments for improving phytolith extraction from Soils, Journal of Archaeological Science 25 (1998) 587–598.