A guide to preparation protocols in palynology

James B. Riding
British Geological Survey, Keyworth, Nottingham NG12 5GG, UK

CONTACT James B. Riding jbri@bgs.ac.uk
Orcid: http://orcid.org/0000-0002-5529-8989

ABSTRACT

A comprehensive, illustrated guide to to the preparation (i.e. extraction, concentration and microscope slide production) of palynomorphs from samples of sediments, sedimentary rocks and other materials is presented. The traditional technique, based upon mineral acid digestion of the sample matrix, is subdivided into four phases. These are: sampling and pre-preparation; acid digestion; palynomorph concentration; and presentation of palynomorphs for study and archiving of materials. Modifications for preparing Quaternary and modern materials such as acetolysis are outlined, as are methods of preparation which do not use hazardous acids. One of the most effective non-acid preparation techniques uses sodium hexametaphosphate as a clay deflocculant and works well on clay-rich samples which are not intensely lithified. Hydrogen peroxide is another reagent which can be used. The contamination of samples by material from other samples or modern pollen can lead to spurious data and interpretations. Strenuous efforts to avoid contamination should be made. Modifications of the traditional preparation technique are described for 14 specific sample materials. For example, many pure limestones only require digestion with hydrochloric acid. Moreover, coal is typically simply oxidised using nitric acid or Schulze's solution then reacted with dilute potassium hydroxide solution to produce organic substances which are then rinsed away using water. Traditional preparation techniques are used for all palynomorph groups irrespective of their biological affinity, however certain of these require some specific modifications. For example chitinozoa and megaspores are substantially larger than acritarchs, dinoflagellate cysts, miospores and pollen, therefore modifications to the technique must be used, principally in the sieve sizes used. Some attempts have been made to automate

palynomorph processing. The equipment for this is discussed, together with other technological solutions such as microwave digestion. Eight techniques closely associated with palynological processing and the microscopical observation of palynomorphs such as scanning electron microscopy are also reviewed.

KEYWORDS laboratory preparation; methods; palynology; palynomorphs; review; techniques

1. Preface by the author

When I was a postgraduate student in the Department of Geology of the University of Sheffield, UK during the early 1980s, I was taught palynological preparation techniques by the academic and technical staff there. All Sheffield palynologists at that time learned how to process palynomorphs from the virtuoso technicians Steve Ellin and Paul Higham. The University of Sheffield remains a major centre in palynology and, over the years, has inarguably been among the leading schools of this topic in the world (Wellman 2005). Sheffield postgraduate students are taught extremely well in every aspect of palynomorph processing from sampling all the way through to microscope slide production. I enjoyed my early learning curve in palynomorph preparation at Sheffield immensely, and I vividly recall those sessions in the old laboratory facilities upstairs in the Applied Sciences building on Mappin Street as if they were yesterday (Figure 1). In particular, it was a great feeling to begin to process the samples for one's own research project.

Upon joining the British Geological Survey (BGS), my contact with day-to-day, hands-on laboratory operations inevitably diminished somewhat. However, I have always taken a keen interest in preparation techniques and, in the early 2000s, my interest in palynomorph extraction and concentration was substantially rekindled. This was due to the rather urgent need to develop effective procedures which are less reliant on hazardous mineral acids and hence are safer and more environmentally friendly. The driver for this came from both within BGS, and from our many external clients and stakeholders. So, during 2002, Jane Kyffin-Hughes and I began to develop several ideas in this area. We looked at the rather few mentions in the literature of non-acid palynomorph preparation,

and talked to colleagues who worked on the extraction of calcareous, phosphatic and silicious microfossils. Jane and I then began several experiments on non-acid physico-chemical palynomorph extraction and processing. By far the best results were obtained using sodium hexametaphosphate as a clay deflocculant (section 12). The first of several papers describing the new method, and the results obtained using this reagent, was Riding and Kyffin-Hughes (2004). In this paper, we included a section entitled 'A review of the traditional methods of laboratory preparation of palynomorphs'. I found this evaluation extremely interesting to research and to write, and realised that the scientific literature on this topic was relatively extensive. Palynological preparation has generated a substantial body of contributions, the majority of which are post-1950. These items range from substantial generic accounts to briefer and more focussed articles describing a specific aspect of processing and/or techniques. This made me realise that there was a need for a comprehensive review of this subject. In fact, it was evident that a wide-ranging synthesis would be a useful guide for both palynologists and palynology laboratory technicians alike. Hence, this contribution aims to provide a comprehensive, extensive, illustrated, user-friendly and wide-ranging guide to the laboratory preparation of palynomorphs of all ages, and closely-related topics.

2. Introduction

Palynological preparation (or processing) techniques aim to extract the indigenous palynomorphs from sedimentary rocks and sediments, and then to isolate, concentrate, and present fully representative assemblages for microscopical study in as perfect preservational condition and density on the slide as possible (Plate 1). Note that there is a hierarchy of terms; a technique may require one or more phases (or procedures), that are performed using a variety of methods and which in turn comprise numerous stages or steps (Figure 2; Green 2001a).

In the traditional preparation technique, the mineral fabric (or matrix) of the rock or sediment is removed by separate treatments with hydrochloric and hydrofluoric acids. Then the palynomorphs are concentrated from the resulting organic residue and resistant minerals by procedures such as acetolysis, alkali treatment, density separation, oxidation,

sieving and ultrasonic treatment. Finally, microscope slides are produced and the excess palynomorph concentrate is archived (Figure 2). However it should be noted that terms such as ‘standard’ and ‘traditional’ (the latter is used herein), frequently seen in the literature, are misnomers because every technician adopts their own variations.

High quality palynological preparations are critical to both economically important operational scenarios and excellent science projects. A prerequisite for an effective palynological study is good quality palynomorph concentrates and microscope slides. If the sample processing is flawed, this will severely impact on the data quality and subsequent interpretations, irrespective of the competence and experience of the analyst(s). To obtain good preparations, the techniques used should be tailored to the nature of the samples being prepared; for example the diagenetic change, geological age, level of induration, lithotype, mineralogy and thermal maturity must all be taken into account. Furthermore, the time-urgency of the project is also an important factor. Critical judgements by the preparator are required at every stage. Although there is a relatively straightforward generalised technique for the processing of the majority of carbonate and siliciclastic sedimentary rocks and sediments (Appendix 1), many modifications and variations may be used. There are virtually as many preparatory techniques as there are laboratory personnel, and no single methodology fits every scenario. This is a major factor behind the extensive literature on this topic, and palynomorph preparation is constantly evolving.

The present contribution aims to provide a wide-ranging review of techniques in palynology based upon the author’s experience and a comprehensive synthesis of the literature on this subject. The principal emphasis is on laboratory procedures associated with the extraction, isolation and concentration of all types of Proterozoic and Phanerozoic palynomorphs from sedimentary rocks and sediments (Plates 1–3).

The present section sets the scene and, following it and three other brief introductory chapters, the ‘mainstream’ mineral acid-based palynological preparation from sampling to microscope slide production is described and illustrated in sections 6 to 10. These first-order sections are themselves subdivided into lower order subsections as appropriate. Adaptations for Quaternary and modern material such as acetolysis, non-acid preparation techniques, and contamination are reviewed in sections 11, 12 and 13

125 respectively. Sections 14 through 17 are more specifically focussed. These are pertaining
126 to specific materials, individual palynomorph groups, specialist equipment and
127 miscellaneous techniques closely related to palynomorph preparation respectively. The
128 latter include the determination of the absolute concentration of palynomorphs and the
129 use of electron microscopy.
130 The layout, ordering and subdivision of the topics considered herein has been
131 carefully planned so as to make this contribution as coherent and user-friendly as possible
132 as a reference text and training guide. This article therefore has a broad scope, and has
133 attempted to cover all aspects of laboratory and practical work associated with pre-
134 Quaternary and Quaternary palynology. The variety of topics covered makes it an ideal
135 companion to major generic texts on palynology such as Tschudy and Scott (1969),
136 Traverse (1988; 2007), Moore et al. (1991) and Jansonius and McGregor (1996). It will
137 hopefully be a useful text for experienced and inexperienced laboratory personnel alike,
138 as well as operators wishing to establish a new palynology laboratory.
139 The need for a comprehensive review of all the various techniques pertaining to
140 the laboratory preparation of palynomorphs is clear. This interest is evidenced by
141 relatively recent work on non-acid preparation techniques for safer laboratory and rigsite
142 operations (Williams et al. 2005, Riding and Kyffin-Hughes 2004; 2006; 2010; 2011,
143 Riding et al. 2007a; O'Keefe and Eble 2012) and on the continuing development of new,
144 more efficient laboratory equipment (e.g. Jones 2003). It is anticipated that this article
145 will effectively review modern laboratory procedures in palynology, and expedite both
146 the search for key references and the time-consuming task of synthesising the widely-
147 disseminated literature on this topic. Many items of pertinent literature are summarised in
148 the Supplementary Data. Here, 407 items of pertinent literature are summarised in seven
149 appendices. If any proprietary laboratory products are inadvertently mentioned in this
150 contribution, these do not constitute endorsements or recommendations for its use by the
151 author or his employers.
152
153
154 **3. Palynology – a brief overview**

Palynology is a subdiscipline of, or extremely pertinent to, agriculture, anthropology, archaeology, biology, forensic science, geography and geology. It is the study of microfossils and their living counterparts which are composed of resistant organic materials, principally chitin, dinosporin, pseudochitin and sporopollenin. These substances are complex organic macromolecules; they are among the most resistant biological materials known to science in part due to the unique molecular structure of the exine (Brooks and Shaw 1978; Li et al. 2019; Barrier 2008; Stephen Stukins personal communication 2020). They are resistant to the majority of aggressive chemicals including hydrochloric acid, hydrofluoric acid and concentrated alkalis, and can survive heating to ~300°C. This resistance is in large part due to the macromolecules containing acetyl and ester cross linkages which protect against alkalis and acids respectively (Li et al. 2019). By contrast they are, however, highly susceptible to oxidation (Hopkins and McCarthy 2002).

Actuopalynology and palaeopalynology began to diverge in the 1890s, and paleopalynology was reasonably active in the early part of the 20th century. At this time actuopalynology, often referred to as 'pollen analysis', was confined to the study of Quaternary and modern pollen and spores. Hyde and Williams (1944) proposed the word palynology for the analysis of microfossil groups that are apparently unaffected by aggressive substances such as hydrochloric and hydrofluoric acids (Edwards and Pardoe 2018). The word palynology is derived from the Greek words *palé* (πάλη) and *paluno* (παλύνω), meaning fine meal and to strew or sprinkle respectively. It hence acknowledges the essence of the subject.

Organic microfossils are termed palynomorphs; they may be marine or terrestrially-derived and can be of algal, animal, bacterial, fungal, plant or protistan affinity. Palynomorphs range in size between ~5 μm and 500 μm, although most are <100 μm in maximum dimension. They comprise acritarchs, arthropod organs, chitinozoa, dinoflagellate cysts, a wide array of eggs, fungal spores, microforaminiferal linings, plant spores, pollen grains, prasinophytes, rotifers, scolecodonts, tardigrades and testate amoebae (Plates 1–3). Non-microfossil organic elements such as amorphous organic material, plant cuticle and wood fragments are not palynomorphs (Figure 3). Phytoclasts is the generic term for these various kerogen fragments (and palynomorphs).

Palynomorphs have a diverse, long and rich geological record which is used in integrated studies from the Proterozoic and throughout the Phanerozoic for providing detailed biostratigraphical, climatic and ecological information (Figure 4; Jansonius and McGregor 1996). Their utility is enhanced by their abundance, wide distribution, high preservation potential and small size. These factors make them virtually ubiquitous in sediments and sedimentary rocks. In particular, palynology is a reliable method of relative geological age assessment used extensively in oil/gas exploration and production operations (e.g. Stover et al. 1996; Jones 2004). Key marker palynomorphs are used to determine the relative ages of intermediate and terminal depths in boreholes/wells, and in the biosteering of horizontal and directional drilling operations (Powell and Riding 2005). These commercial applications have greatly stimulated the study of both marine and terrestrially-derived palynomorphs. Palynology is also used extensively in research on palaeobiology and palaeoecology (e.g. Sluijs et al. 2005, Pound et al. 2011, Woods et al. 2014, Boyd et al. 2018). Comprehensive overviews of the subject were given by Manten (1966), Tschudy and Scott (1969), Traverse (1988; 2007) and Jansonius and McGregor (1996). The science of palynology was revolutionised by four key breakthroughs. The first was the invention of the microscope itself by Antoni Philips van Leeuwenhoek FRS (1632–1723) in the 17th century (Snyder 2015). The second and third were the development of compound achromatic and apochromatic lenses by Giovanni Battista Amici (1786–1863) in 1827 and Ernst Karl Abbe (1840–1905) in 1884 respectively (Manten 1969). The fourth major development was the development of chemical preparation techniques that allowed palynomorphs to be extracted from sediments and sedimentary rocks in the middle of the 19th century (see section 4).

4. The historical development of palynological preparation techniques

The discovery and refinement of chemical methods for the extraction of palynomorphs was a watershed in the science of palynology. Prior to the mid 19th century, fossil palynomorphs were occasionally documented from thin sections and slivers of rock. The first known report of pre-Quaternary palynomorphs was in 1833 by Henry T.M.S. Witham (1779–1844), who observed Carboniferous spores in petrological thin sections of

bituminous coals from Lancashire, UK (Witham 1833). These were erroneously interpreted as xylem vessels which supported the vegetable origin of coal. Three years later, Heinrich R. Göppert (1800–1884) reported pollen grains from the Miocene brown coals of northern Germany (Göppert 1836). Plant spores in thin section were not intensively studied during this phase of the development of palynology, but they were illustrated relatively frequently (e.g. Wethered 1886; Thiessen and Wilson 1924). Translucent flakes of chert and flint were first studied by Christian Gottfried Ehrenberg (1795–1876), and this method persisted into first half of the 20th century (subsection 14.6; Ehrenberg 1837; Wetzel 1933a, 1933b).

The first documented use of mineral acid to isolate palynomorphs was by Heinrich Göppert, who used dilute hydrochloric acid to extract pollen grains from Paleogene limestones from Croatia (Göppert 1848). This strategy of chemical preparation was highly unusual at this time. Another huge step forward was made by the German geologist Franz Schulze, who successfully used a mixture of nitric acid and potassium chlorate to macerate coals so that the spores are released (subsections 9.3, 14.3.3; Schulze 1855). This mixture is still used, and it is unsurprisingly known as Schulze's solution (Tschudy 1958; Manum 1956). Schulze's solution was used by the German geologist Paulus Friedrich Reinsch (1836–1914) in the late 19th century. Reinsch (1881, 1884) described the extraction of spores from Carboniferous, Permian and Triassic coals from Germany and Russia by sequentially using concentrated potassium hydroxide solution, hydrofluoric acid, and a mixture of potassium chlorate and nitric acid. Bennie and Kidston (1886) published a highly influential paper on Scottish Carboniferous megaspores, which were extracted by washing and sieving naturally-weathered low grade coals and shales. This find was made purely by accident, through the washing and sieving of the overlying Quaternary sediments for seeds and other plant fossils (Chaloner 1968).

These procedures represented massive breakthroughs because they allowed the detailed microscopical study of palynomorphs from sedimentary rocks for the first time. Most previous studies were confined to modern pollen and spores collected from plants, and palynomorphs in thin sections. The groundbreaking work of scientists such as Heinrich R. Göppert, Paulus Friedrich Reinsch and Franz Schulze on palynomorph extraction therefore laid the foundations for the development of modern palynology. This

era also provided a landmark study of the pollen and spores from Scandinavian peats by Lennart von Post (1884–1951) who pioneered the diagrammatic quantitative (percentage) analysis of palynomorphs. The study of von Post (1916) marked the beginning of modern analytical/interpretative palynology, as opposed to merely observational data several decades after the groundbreaking work on preparation of Göppert, Schulze and Reinsch (Manten 1967). The quantitative techniques developed by von Post were taken up by coal geologists such as Robert Potonié (1889–1974), Isabel C. Cookson (1893–1973), Arthur Raistrick (1896–1991), James M. Schopf (1911–1978) and Leonard R. Wilson (1906–1998) in order to correlate coal seams (e.g. Raistrick and Simpson 1933; Wilson and Brokaw 1937; Schopf et al. 1944; Grebe 1974; Cross and Kosanke 1995; Marshall 2005; Riding and Dettmann 2013).

Several years after the groundbreaking paper of von Post (1916), Assarsson and Granlund (1924) first used hydrofluoric acid to digest the matrix of silicious material for the extraction of sub-fossil pollen. This pioneering discovery laid the ground for the development of traditional palynological preparation techniques thereafter (subsection 8.3). The first reported use of hydrofluoric acid in pre-Quaternary palynology was by Lang (1925), who used this reagent to extract Middle Devonian spores from Scotland. However, hydrofluoric acid was not used extensively in pre-Quaternary palynology during the early years of the 20th century. Likewise, the use of hydrochloric acid at this time was limited because the major breakthrough of Göppert (1848) was also not widely recognised. Some years later, however, Alfred Eisenack (1891–1982) began to use hydrochloric acid to liberate fossil palynomorphs from the Palaeozoic and Mesozoic limestone erratics of the Baltic region (subsection 8.2; Eisenack 1930, 1931; Sarjeant 1985). By contrast, this work was well circulated and hence was highly influential in the development of the acid digestion technique for preparing fossil palynomorphs. A contemporary of Alfred Eisenack, the French palynologist Georges Deflandre (1897–1973), simply washed the Upper Jurassic (Oxfordian) mudstones of Villers-sur-Mer, northern France in water to isolate the abundant palynomorphs (subsection 12.2; Deflandre 1938; Riding and Schmitt 2009).

Palynology as a science expanded greatly after World War II; this reflects the large number of scientific disciplines where it is applicable. In particular, between the late

1930s and the 1970s, the application of stratigraphical palynology by the oil industry hugely stimulated research on fossil palynomorphs (Wilson 1961). At the same time, a generalised technique of preparing pre-Quaternary samples rapidly evolved. In broad terms this is: (1) pre-treatment (cleaning/crushing); (2) demineralisation using hydrochloric and hydrofluoric acids; (3) oxidation/alkali treatment; (4) density separation to remove resistant minerals; (5) staining; (6) sieving/concentration; and (7) slide production (sections 6–10; Figure 2; Appendix 1). In commercial palynology, typically many samples require preparing simultaneously. Consequently, it was found that the most efficient way of processing is to treat batches of several samples together. The numbers within these batches depends upon the capacity of the laboratory and the nature of the work. A comprehensive review of commercial micropalaeontological laboratory operations, with the emphasis on calcareous microfaunas, was given by Schenck and Adams (1943). Quaternary palynology also expanded significantly at the same time, due to its utility for providing detailed palaeoenvironmental interpretations. The preparation protocols for Quaternary sediments are broadly similar, however generally the residues are normally acetolysed and some procedures including acid digestion are not always necessary (section 11).

There are five books relevant to the preparation of palynomorphs. The most focussed is Brown (1960; 2008), and is entitled *Palynological Techniques.* This volume is briefly and selectively summarised in Supplementary Data Appendix 1. The other four books pertinent to this topic are Kummel and Raup (1965), Bryant and Wrenn (1998), Jones and Rowe (1999) and Green (2001a); these all have more than one chapter on palynomorph extraction.

5. The palynology laboratory

A modern, safe, well-equipped, well-maintained and well-ventilated laboratory is a prerequisite for palynological processing (Figure 5). The laboratory should be designed around three principal considerations. These are: 1) the health and safety of the laboratory personnel; 2) the protection of the environment; and 3) the effectiveness of the laboratory consumables, equipment and infrastructure to allow the extraction palynomorph

assemblages from a wide variety of sample materials (Freeman and Whitehead 1982; Nemchin and Brusick 1985).

Because all palynology laboratories use several hazardous substances, including concentrated mineral acids, oxidising agents and bases, health and safety considerations are of paramount importance. Safety features such as the eye-wash, the fire extinguisher and the safety shower should be in appropriate positions (Figure 6). Thomas (1989) gave a review of health hazards in generic palaeontology laboratories including key safety parameters for 21 of the commonest laboratory reagents. It is clearly imperative that all procedures involving hazardous chemicals are performed in an efficient fume hood or fume cupboard (Figures 7, 8), and that all the necessary personal protective equipment is worn by the laboratory staff (Figure 9; Thomas 1989 and references therein). Hydrofluoric acid is by far the most hazardous substance used in a palynology laboratory. The specific dangers of this reagent have been documented by Shewmake and Anderson (1979), Head (1995a, Head 1995b), Riding and Kyffin-Hughes (2004) and Ohtani et al. (2007). Personal protective clothing comprises acid/chemical-resistant aprons, boots, cap/hairnet, eye protection, face shields, gloves, laboratory coats and long trousers (Figure 9). All these items should be clean to avoid any potential contamination of samples. Any items that potentially could trap chemicals against skin or other tissues, such as contact lenses, jewellery or wristwatches should not be worn. Laboratory technicians should be comprehensively trained in all aspects of safety, including the safe handing of chemicals. For example, if any strong acid needs diluting, or when mixing reagents and residues, it should always be remembered to add acid to a larger volume of water, and never the other way around. Mineral acids may react vigorously with water in a highly exothermic reaction which may cause boiling and spitting. Barss and Williams (1973) recommended that squeeze bottles of acetone and distilled or reverse osmosis (RO) water be available at all times in the laboratory for dampening down any unexpected violent chemical reactions. A dilute mixture of ethanol and RO water is also effective for supressing intense reactions.

All large items of equipment such as centrifuges and fume hoods should be regularly serviced by qualified engineers. Fume hoods should be effective, and have efficient chimney filters (‘scrubbers’) so that exhaust pollution is eliminated or minimised

(Nemchin and Brusick 1985). Furthermore it is essential that all spent chemicals are disposed of safely. For example the spent hydrofluoric acid should be decanted into a large vessel partially filled with a solution of calcium chloride or calcium hydroxide (slaked lime) prior to disposal (Litwin and Traverse 1989). This neutralises the acid by sequestering the fluorine ions as calcium fluoride. Calcium carbonate and orthoboric acid are also suitable neutralising agents. It is absolutely imperative that all laboratory procedures conform to the relevant health and safety legislation of the respective jurisdiction.

This contribution reviews some literature which pre-dates contemporary health and safety regulations. Readers of the older items on this topic should bear this in mind, and adapt all procedures to conform to modern health and safety practices. Laboratory personnel should never compromise health and safety considerations in order, for example, to save energy, materials or time. Where any authors clearly describe procedures which are hazardous, these are highlighted and strenuously discouraged herein. An example of this is Franks (1965); this author advocated macerating samples with hydrofluoric acid in closed, screw top vessels outside a fume hood. Of course, this is potentially highly dangerous; pressure may build up in the vessel with the attendant risk of an explosive burst.

6. Mineral acid digestion-based palynological preparation techniques

6.1. Introduction and basic principles

In this and the following five sections, the traditional technique for extracting and concentrating palynomorphs from sedimentary rocks and sediments is described. However, it must be borne in mind that terms such as ‘standard’ and ‘traditional’ when referred to palynomorph preparation are somewhat misleading. Acid digestion followed by palynomorph concentration procedures is practiced worldwide, but every laboratory technician has their own unique way of executing this technique with countless variations (O’Keefe and Eble 2012). In a generic sense, it is generally assumed that the sedimentary sample material is a clay-rich lithotype which is moderately organic-rich. It is also

assumed that all the laboratory equipment is effective, scrupulously clean, and all reagents are pure and of the highest quality.

This technique has not changed fundamentally since the mid-late 1950s and potentially includes many procedures, not all of which will be consistently required. These steps range from the field collection of samples, through all the laboratory procedures, to the archiving of excess sample materials following the preparation of microscope slides (sections 7–11). The entire technique can be conveniently subdivided into four separate phases. These main stages all have culinary analogies, which are admittedly not perfect, but will serve as both readily-understandable metaphors and *aide-mémoires* (Figure 2). The first phase is the collection of samples and pre-preparation (section 7). Next is the dissolution of the carbonate and silicate mineral matrix of the sample material using hydrochloric and hydrofluoric acids, effectively leaving a mixture of sedimentary organic material (i.e. kerogen including palynomorphs) and acid-resistant mineral grains (section 8). This means that the overwhelming mass of the sample is destroyed chemically. The third part of the process is the concentration of the palynomorphs (section 9). This concerns the separation of the palynomorphs from the refractory minerals and as much as the non-palynomorph kerogen as possible. It comprises acetolysis, oxidation, alkali treatment, density separation, ultrasonic treatment and sieving as appropriate and necessary. Finally, microscope slides are produced and the excess raw samples and palynomorph residues are archived and curated (section 10). Section 11 discusses specific procedures used in the preparation of Quaternary and modern material. Note that the sample residues should be thoroughly washed between the various chemical phases in order to avoid chemical reactions that may damage the palynomorphs, or produce unwanted precipitates. The technique for pre-Quaternary material is illustrated diagramatically in Figure 2, and summarised in Appendix 1.

It should be stressed here that the technique and procedures decribed herein should absolutely not be regarded as inflexible. Clearly certain steps are universal, for example sampling has to take place, and mineral acid digestion is normally undertaken. A mineral acid is an one derived from one or more inorganic compounds, and ionises when dissolved in water. However, not all the palynomorph concentration procedures are necessarily performed on every sample during the third phase. For example if the sample

material is free of amorphous organic material and wood, oxidation and/or ultrasonic treatment may be completely unnecessary. The procedures should be varied whenever appropriate depending on factors such as the precise nature of the individual sample material and the urgency of the project. For example in a commercial environment, speed is the prime factor so some shortcuts can be taken which would not be appropriate in an academic or strategic research study. The latter require complete, fully representative assemblages of pristine specimens. Furthermore, accurate record-keeping is essential at all times. Comprehensive notes should be always made on the preparation history of each sample. If the technique was varied significantly, for example to take into account of an unusual lithology, the notes will be invaluable for the processing of future samples of this type.

This, and the following five sections are based on the author's experience and the literature. There are numerous major papers on this topic, notably Norem (1953; 1956), de Jekhowsky (1959), Delcourt et al. (1959), Funkhouser and Evitt (1959), Staplin et al. (1960), Rigby (1963), Caro et al. (1964), Delcourt (1964), Schopf (1964), Gray (1965a; 1965b), Lennie (1968), Barss and Williams (1973), Sarjeant (1974), Doher (1980), Batten and Morrison (1983), Herngreen (1983), Phipps and Playford (1984), Traverse (1988; 2007), Litwin and Traverse (1989), Wood et al. (1996), Batten (1999), Green (2001b; 2001c), Riding and Kyffin-Hughes (2004) and Pound et al. (2021). The majority of these items are summarised in Supplementary Data Appendix 1. Many laboratories have their own in-house manual on palynological techniques (e.g. Hennissen et al. 2018).

6.2. Cleanliness is next to godliness?

During palynological processing all laboratory equipment, especially the vessels used for procedures involving liquids such as acid digestion and oxidative macerations, should be scrupulously clean in order to prevent any cross contamination (section 13). It is impractical and uneconomic to expect a laboratory to use brand new labware such as beakers, centrifuge tubes, sieves, sinter glass filters, stirring rods etc. for every sample.

Clearly these items must be thoroughly washed after use. Prolonged soaking in a 3.7% solution of sodium hypochlorite is a cheap and relatively safe cleaning strategy for all laboratory items. However, some laboratories use chromic acid, a solution of

potassium dichromate in concentrated sulphuric acid, as a laboratory equipment cleaner (Evitt 1951, p. 695). Chromic acid effectively removes any extraneous organic matter because it is an extremely strong oxidising agent (Traverse 1988; 2007). If chromic acid is used, the cleaning should of course be undertaken in a fume hood. Alternatively, all suitable items can be effectively cleaned using a laboratory grade dishwasher, and/or manually washed using a strong detergent and a stiff-bristled brush. Small to medium-size labware items can also be cleaned using ultrasound (subsection 9.5). When clean and dry, the laboratory equipment should be stored in clean, dust-free cupboards with close-fitting doors to prevent any ingress of airbourne contaminants (Figure 10).

Irrespective of cost issues, it is recommended that laboratories do not use equipment such as vessels for too long a period of time. For example, plastic beakers are prone to scratching during stirring and these defects can retain vestiges of residues which are difficult to completely remove however assiduous the cleaning procedures. These residues of previous preparations can obviously contaminate subsequent samples.

7. Phase one of the traditional technique – sampling and pre-preparation

7.1. Introduction

Phase one comprises the collection of sample material for palynology, and preliminary procedures such as cleaning and crushing in the laboratory prior to acid digestion and the concentration of palynomorphs (Figure 2; Appendix 1). It is particularly vital that the samples be fresh, uncontaminated, and well-constrained both geographically and stratigraphically. Before preparation, study and interpretation can commence, sample material hopefully containing palynomorphs must be collected. This material is normally samples of sedimentary rock or modern sediment. One overarching factor in the sampling stage is that extreme care must be taken to avoid the contamination of samples. Clearly if any extraneous matter such as fragments from other samples, or modern material, are introduced into the sample the small size of palynomorphs (typically <100 μm) means that, even if a minute amount of material is allowed to contaminate a sample, significantly spurious data may be generated. Contamination can also be caused by

impure laboratory reagents and even blackboard chalk (section 13; Fisher 1962; Jung Echols and Levin 1964). The procedures involved in the collection of sedimentary rock and sediment samples is applicable to all groups of palynomorphs and these have been previously described by many authors, for example, Wood and Segroves (1963), Adam and Mehringer (1975), Mildenhall et al. (1975), Wiltshire (1988); Horowitz (1992), Rowe and Jones (1999) and Green (2001a, p. 20–61), and is described further in section 13. Supplementary Data Appendix 1.2 includes commentaries on four relevant contributions. For the culinary analogy, this first phase of the traditional palynological preparation technique can be envisaged as 'acquiring the ingredients, and washing and chopping them' (Figure 2).

7.2. Sampling

7.2.1. Introduction

When collecting samples for palynomorphs, the ultimate aim is to obtain adequate amounts of well-constrained material that will yield rich, uncontaminated and well-preserved palynomorph associations. This subsection is an guide to how to sample sedimentary rock, unconsolidated sediments, the atmosphere and water for palynomorphs.

Most sedimentary rocks and sediments will yield palynomorphs but as a general rule dark coloured, fine grained (mud/silt) material is the most palyniferous. Normally, the darker and more fine-grained the lithology, the better the palynomorph productivity will be, hence these lithotypes should be targeted in heterolithic successions. Red or red/brown material is highly likely to have been oxidised and hence should be avoided. If fine-grained siliciclastic lithotypes are absent, coals, clayey/silty sandstones, limestones and marls will frequently be productive. The most common rock types which are likely to be entirely barren of palynomorphs are very pure limestones and clean (i.e. winnowed), highly quartzose sandstones. The five main aspects to bear in mind when sampling are: adequate numbers of samples of sufficient mass (Table 1); the avoidance of contamination; comprehensive documentation; effective bagging/packaging; and site conservation.

A comprehensive account of field collecting procedures in palaeontology was given by Green (2001a, p. 20–26). This author stressed the importance of the formulation of a collecting strategy, the maintenance of good practice including adhering to the geological fieldwork code and the importance of site conservation. It begins with an excellent description of good practice in collecting, and goes on to discuss aspects such as the field collection of micropalaeontological samples, specimen stabilisation, field staining of calcite, dolomite and phosphate, and field documentation, packing and transportation. Green (2001a, p. 27–34) also described sample collecting techniques in micropalaeontology more broadly, and this is an excellent account of how to collect material from cores, marine sediments and outcrops. This author also gave valuable advice on the documentation of samples in the field, sample packaging and transportation (Green 2001a, p. 59–61). Robust, sealable plastic sample bags are are strongly recommended (Figure 11). By contrast, cloth and paper bags may allow the ingress of contaminants and are susceptible to fungi and mould (mildew) infestation and degradation, especially if they are stored or transported in damp and/or hot conditions.

The following three subsections are on coherent rock and unconsolidated sediments. When collecting these materials, especially if the rock is friable, samples can be preserved intact by embedding a large block in Plaster of Paris at the collecting site. When cured, the block can be sawn and individual horizons sampled. This strategy is especially useful when sampling plant beds, and aims to prevent cross-horizon contamination (Pearson and Scott 1999). The final two subsections are on airbourne and waterbourne palynomorphs respectively.

7.2.2. Sampling from coherent sedimentary rock successions

In summary, when collecting material from successions of sedimentary rock, the samples should be taken at suitable intervals from fresh surfaces of unweathered strata (Figure 12). If the lithotypes being sampled are relatively friable and soft, it may be possible to sample using a spatula, trowel or similar tools (Figure 13). For most types of sedimentary rock however, a geological hammer and a set of suitable chisels are required. Great care should be taken to collect relatively large (>100 g) fragments of fresh rock. A single piece of rock makes an ideal sample because this will help to minimise cross

526 contamination. Sharp edges should be avoided if at all possible because these can
527 puncture and tear sample bags in transit. However if highly indurated rock, which tends
528 to fracture into sharp fragments, is being sampled, the material can be covered in material
529 such as aluminium foil, bubble wrap or plastic wrap before placing it into the sample bag
530 for extra protection (Figure 11). It is important not to completely pulverise a hard
531 lithotype when sampling and collect fine rock flour unless this is absolutely the only
532 possible sampling method. Palynomorphs are small, but many specimens will inevitably
533 be mechanically damaged if the sample material comprises very fine powdered rock
534 flour. Despite this, it is feasible to collect rock material by drilling provided that the
535 fragments are not too small (Wood and Segroves 1963) and the drill bit does not become
536 too hot. If sampling a specific horizon is problematic, a 'channel' (i.e. averaged) sample
537 of the entire bed can be taken to ensure the acquisition of productive material.
538 Sampling tools such as augers, chisels, hammers and trowels should be
539 thoroughly cleaned immediately before and after collecting a sample so that cross-
540 contamination between adjacent samples is avoided. The sample material should be
541 immediately placed in brand new, clean, clearly-labelled, robust and securely sealable
542 plastic bags to protect them from contamination during transportation and storage. All
543 notes (date, depth, geographical coordinates, lithostratigraphical unit, location etc.)
544 should be unambiguous and made in bold, neat handwriting and using indelible,
545 waterproof ink directly onto the bag (Figure 11). Comprehensive notes should also be
546 made in a suitable field notebook on all samples (Coe et al. 2010). It is imperative that the
547 geographical locality and the depth from a datum are recorded with the highest level of
548 accuracy so that precise recollecting is possible. It is also good practice to place a card
549 with notes on the sample in the appropriate sample bag (Figure 11). This should only be
550 done if the sample material is absolutely dry. Placing material such as card, fabric or
551 paper in the sample bag can be a vector for introduction of fungi, which can contaminate
552 the sample and, in extreme cases, result in the destruction of the *in situ* palynomorphs.
553 However, the strategy of writing sample details on the sample bag itself, on a card placed
554 in the sample bag and in a field notebook means that the chances of getting the samples
555 back from the field lacking key details is minimised.

By far the most important aspect in collecting palynology sample material from outcrop sections is to obtain fresh, unweathered material (Funkhouser 1969). Sporopollenin is susceptible to oxidation (Hopkins and McCarthy 2002) hence intensely weathered strata will normally be severely depleted in, or largely devoid of, sedimentary organic material and may be also contaminated by modern palynomorphs (typically windbourne pollen). Therefore the weathered outer layer of sedimentary rock should be completely removed by cleaning the section, and only fresh material collected (Figures 12, 13). This means that the sample material should be collected from holes in the 'cleaned' section which ideally are ~10 cm deep (Figure 14). The depth of the weathering crust is dependent upon climate and lithology. For example, hard rocks such as chert, concretions and indurated sandstone are more resistant to weathering than softer lithotypes such as mudstone. In very hot and tropical climates the weathering crust is relatively deep, and in these regions palynomorphs can only be collected from borehole samples (Wilson 1964; Riding and Dettmann 2013).

Fissures should be avoided as the surfaces will be weathered and the infills will risk considerable contamination. Similarly material which is highly permeable and porous, for example loosely cemented conglomerates or gravelly sediments, should be avoided because contaminating fine-grained sediment and/or allochthonous palynomorphs themselves ('reverse reworking' or 'washdown pollen') can be washed into the interstices. Care should also be taken when sampling from river beds in order to avoid waterbourne modern pollen. Excessively cemented/indurated, contorted, faulted, oxidised (red), recrystallised, thermally altered, sheared and slickensided horizons should also not be sampled unless absolutely unavoidable (Mildenhall et al. 1975). Likewise any lithotype which has been winnowed, typically these are sand-rich beds, are highly unlikely to be palynomorph-bearing (Table 1; Traverse 1988; 2007).

As mentioned above, it is vital to make detailed lithological notes of samples for future reference, and to ensure that the precise stratigraphical positions of samples are located against reliable datums and a detailed lithological log ideally with sedimentological interpretations. As an *aide-mémoire*, and for future reference, it is good practice to photograph sample bags *in situ* at their respective horizons, in addition to the overall succession sampled (Figure 14). If there are no constraints on sample size,

587 significantly more sample material than is needed for a single palynological preparation
588 (i.e. ~20–30 g for fine-grained material) should be collected so that repeat preparations
589 can be made if necessary and that the sample can be used for other analyses such as
590 calcareous micropalaeontology and geochemistry (Table 1).
591 In boreholes, conventional core, drill cuttings and sidewall core samples should
592 normally of course be in unweathered material. However, the circulation of drilling mud
593 in the well environment may have caused potentially contaminating materials to coat and
594 penetrate the core, sidewall cores and drill cuttings (Figures 15, 16; Traverse et al. 1961).
595 Hence all borehole material should be extremely thoroughly cleaned before the rock is
596 processed further. In extreme cases, drilling mud may have penetrated any pores and
597 cracks thus impermeable lithotypes should be collected in preference to porous material
598 wherever possible.
599 The mass of sample required is inversely proportional to the amount of
600 sedimentary organic material in the rock. The ideal lithotype is of silt/clay grade and is
601 relatively organic-rich. Normally, ~20–25 g of rock/sediment is an ideal weight for
602 processing. However more than this is needed for more organic-lean lithotypes and less
603 for organic-rich lithotypes (Table 1). Informal, individual campaign based, sample
604 numbers can be assigned in the field. However, the formal/museum/laboratory sample
605 numbers can be either allocated in the field or back in the office. It is normal curatorial
606 practice to use a sequential list of formal sample registration numbers, with a
607 comprehensive archive of sample and preparation procedure data.
608
609 *7.2.3. Sampling drill cuttings from boreholes*
610 Borehole samples are not normally affected by weathering, and it is relatively
611 straightforward to sample from conventional core, sidewall cores and drill (or ditch)
612 cuttings. Drill cuttings are the broken rock fragments of solid material produced by a
613 destructive percussion or rotary drill bit in non-cored (‘open hole’) boreholes (Figure 17).
614 The cuttings are carried to the surface by the circulating drilling mud (Figures 15, 16).
615 Drilling mud, or drilling fluid, is a heavy and viscous liquid mixture used in borehole
616 drilling operations principally to carry drill cuttings to the surface, to control hydrostatic
617 pressure in the well to prevent ingress by formation waters, and to ensure that the drill bit

618 is cooled, clean and lubricated (ASME Shale Shaker Committee 2004). The drilling mud
619 is pumped through the centre of the drill string or pipe. It returns to the surface in the
620 annulus, i.e. the void between the wall of the well and the drill string, bringing up the drill
621 cuttings with it. They are removed from the drilling mud by screening in the shale shaker,
622 or by centrifugation, at the well head then the sieved drilling mud is recycled down the
623 well (Figure 15; Nguyen 1996).
624 Drill cuttings may be subject to contamination by a phenomenon known as
625 caving. This is where fragments of friable lithostratigraphical units higher in the
626 succession fall down the well towards the drill bit in uncased wells, or are simply
627 suspended and transported to the well head by the flow of drilling mud. This therefore
628 potentially mixes anomalously young palynomorphs with the *in situ* forms. If the well has
629 been carefully steel-cased throughout, caved fragments clearly should not be present
630 (Figure 15). The potential presence of caving makes range bases unreliable in cuttings
631 samples, therefore range tops are used exclusively in this situation (Riding 1984).
632 Barss and Williams (1973) advocated sieving cuttings samples in order to remove
633 much of the caved fragments. The drill bit should produce cuttings of a reasonably
634 consistent size, i.e. ~1 mm in diameter. By contrast, caved fragments are normally
635 substantially larger. Barss and Williams (1973) washed cuttings through a series of nested
636 sieves with mesh sizes 1.630 mm, 0.250 mm and 0.106 mm. The material remaining on
637 the 0.250 mm screen is retained for processing. The fraction on the 1.630 mm screen is
638 considered to contain the majority of the caved material. By contrast, the sample
639 remaining on the 0.106 mm screen, and the material washed away, contains potential
640 contaminants from the drilling mud. Burgess et al. (2020) recommended that, if the
641 cuttings are coated in tenacious drilling mud, they are soaked in dichloromethane solvent
642 to help clean them. This is very important as the drilling mud may contain contaminants
643 and it will impede effective acid digestion (section 8).
644
645 *7.2.4. Sampling unconsolidated sediment*
646 The points made in subsection 7.2.2 pertaining to the avoidance of weathered material,
647 constraining the samples, labelling etc. are equally relevant to the sampling of unlithified
648 sediment, and hence are not repeated here. Under normal circumstances, modern or

649 unlithified deep time sediments are relatively straightforward to sample for palynological
650 analysis. Sections can easily be cleaned for logging and sampling using a spade or trowel
651 by removing the outermost layer (Figure 13). Ideally, clay-rich, undisturbed material
652 should be sampled and ~20 grams of clay-rich sediment are usually sufficient to obtain a
653 representative assemblage (Table 1). Material can be collected using tools like spatulas or
654 trowels. Because these types of material are relatively soft, hand augers can be used to
655 take samples (e.g. Böhm 1979). A sidewall corer fitted to an auger was described by
656 Klaus (1975); this can be used to obtain small samples from shallow auger holes.
657 Alternatively, continuous vertical profiles of soft sediment samples (monoliths)
658 can be collected using Kubiena tins (Mangili et al. 2005). These are metal boxes of a
659 suitable size which can be inserted into a section, and then cut around to remove an
660 undisturbed block of the sediment. Specifically, Kubiena tins are open boxes with
661 removable bases and lids made of ~1.2 mm gauge sheet aluminium (Figure 18). In some
662 designs, two sides of the tin are not attached so that they can be folded back to easily
663 extract the intact sediment sample in the laboratory, which can be stabilised with resin if
664 necessary (Goldberg and Macphail 2003). The outcrop is cleaned to give a smooth,
665 vertical surface. Then blocks of sediment are collected by cautiously hammering or
666 pushing the Kubiena tins into the outcrop and carefully removing them. The thin
667 aluminium sheet is sharp and strong enough to be relatively easily worked into
668 successions of unconsolidated sediments. The samples can be taken with overlaps to
669 ensure that the entire succession is collected. Kubiena tins are not always necessary, and
670 soft sediment sampling can often be achieved using any suitable small plastic tube or box.
671 A device for obtaining samples at 2 mm intervals was devised by Wiltshire
672 (1988). Here a series of razor blades were mounted in a perspex block which can be
673 pressed into a core monolith or exposed *in situ* sediment in order to recover closely
674 adjacent samples. This technique is unsuitable for coarse-grained or fibrous materials.
675 There may be challenges should lake bottom or sea bottom sediments be required.
676 The simplest method of sampling from shallow waters is to safely wade into the water
677 body and sample the bottom sediment manually. Similarly, it is possible to sample
678 bottom sediments in deeper waters by scuba diving. Most offshore marine samples are
679 taken from ships using various methods such as box coring, conventional coring, gravity

680 coring, piston coring, vibrocoring, dredge sampling and grab sampling. Marine sampling
681 devices were reviewed by, for example, Hopkins (1964) and Jonasson and Olausson
682 (1966). The amount of sample available is often related factors such as the method of
683 sampling and the prevailing weather conditions, and these may vary significantly.
684
685 *7.2.5. Collecting airbourne palynomorphs*
686 Aeropalynology is the study of pollen and spores collected from the atmosphere (Hyde
687 1969; Biesboer 1977). Obviously, the overwhelming majority of pollen grains in the air
688 are from anemophilous (wind-pollinated) plants. Many airbourne spores are fungal in
689 origin. Much of the contemporary literature on this topic is published in *Grana*, which is
690 an international journal of palynology and aerobiology (Königsson 1975;
691 https://www.tandfonline.com/toc/sgra20/current). Aeropalynology helps our
692 understanding of the distribution patterns of modern pollen and spores, the development
693 of pollen calendars, and in the monitoring of airborne pollen levels which relate to
694 allergies, principally hay fever (Wodehouse 1945; Colldahl and Carlson 1968).
695 There are several varieties of air-samplers currently in use to measure the pollen
696 rain. These vary in sophistication from simple passive devices to mechanised units which
697 are directional and filter specific volumes of air (Tomas et al. 1997, p. 122). Either the
698 pollen rain is sampled passively, or airborne particles including pollen and spores come
699 into contact with an adhesive surface which is examined microscopically. Some of these
700 are briefly described below. Chemical treatment is normally not necessary, however the
701 palynomorphs can be stained.
702 The simplest method is the gravity slide device, which is frequently referred to as
703 the Durham sampler (Durham 1946; Kapp et al. 2000, fig. 8). A microscope slide, or part
704 thereof, is smeared with a thin film of an adhesive. The latter is normally glycerine or
705 silicone grease/oil. The slide is exposed horizontally at the sampling site for a specified
706 time interval. A hood is normally placed over the slide to protect it from rain. One variant
707 of the Durham sampler is the aeroscope, where an adhesive-laden microscope slide is
708 placed and held obliquely (~45º). A wind-vane ensures that the slide is always facing into
709 the wind, in order to maximise exposure to the air (Srivastava and Wadhwani 1992, fig.
710 1).

711 The Tauber Trap is a passive method for collecting pollen (Tauber 1967, 1974). It
712 is a non-volumetric sedimentary sampler that relies on gravity-fall to assess the pollen
713 rain. This device is extensively used in long-term studies of airborne pollen, especially
714 where mains electricity is not available. It consists of a curved lid with a central hole ~5
715 cm wide. The lid is aerodynamic in shape in order to minimise turbulence and hence
716 maximise the pollen collected (Tauber 1974, fig. 1). A collecting jar is placed below the
717 lid to store the captured pollen.
718 The Cour Trap is also passive and was described by Cour (1974). It comprises
719 two vertical filters supported by a post three metres high. These can be protected from the
720 rain by a cowl in wet climates and continuously face into the wind due to a vane placed at
721 the rear (Figure 19). The airbourne particles are pressed by the wind into the filter mesh.
722 Each of the filters consists of five overlain/stacked gauzes which are impregnated with a
723 silicone-based adhesive and fitted into a plastic frame with a surface area of 400 cm^2. The
724 filters are normally exposed for one week (Kiared et al. 2017). Other passive devices
725 include Behling, Oldfield, and reference traps (Jantz et al. 2013, fig. 1). Behling traps are
726 now used extensively for sampling pollen rain because they are easy to deploy and cause
727 virtually no environmental impact.
728 There are two types of mechanical airborne particle samplers collectors in use;
729 these are the Burkard (or Hirst) and Rotorod samplers. The Burkard seven day recording
730 volumetric spore trap was adapted from the device originally described by Hirst (1952). It
731 is an accurate, compact and portable continuous air-sampler device, with an intregral
732 vacuum pump and motor drive (Kapp et al. 2000, fig. 10). Airborne particles can be
733 continuously sampled for periods of up to one week without attention. The pollen, spores
734 and other particles are impacted onto clear plastic tape which is coated with adhesive and
735 supported on a clockwork-driven drum. The plastic tape is secured around the drum using
736 double-sided adhesive tape. The standard orifice is 2 x 14 mm, but it has interchangeable
737 orifices which improve the trapping efficiency for particles 1–10 μm in diameter. The
738 drum revolves once in seven days at two millimetres per hour, and the air throughput is
739 10 litres per minute. After seven days, the pollen/spore-laden adhesive tape is removed
740 from the drum and carefully cut into daily or hourly segments which can them be stained
741 and mounted onto microscope slides for examination. The Burkard seven day recording

742 volumetric spore trap has an alternative head/lid assembly for the 24 hour sampling of
743 airborne particles directly onto a standard microscope slide if data are needed quickly.
744 The Rotorod sampler is a relatively simple rotation-impaction device which
745 collects pollen from the atmosphere. It consists of a metal rod with two small plastic arms
746 attached to an electric motor which is set to 2,400 revolutions per minute (RPM) (Kapp et
747 al. 2000, fig. 9). The arms swing out from protective sheaths in a retracting head due to
748 centrifugal force, and are exposed to ambient air. The pollen and spores are impacted
749 onto the plastic arms which are lightly coated on one side with an adhesive, typically
750 silicone grease or double-sided adhesive tape. After the sampling cycle is complete, the
751 arms are removed from the sampler, lightly stained and examined microscopically. The
752 number of pollen and spores on the arm is related to the volume of air sampled, which is
753 3.12 m^3 per 24 hours. This means that pollen/spores per cubic metre of air can be easily
754 determined (Frenz et al. 1996). Noll (1970) demonstrated that rotating impactors such as
755 the Rotorod sampler recover pollen-sized particles with <85% efficiency.
756 Gagnon and Comtois (1992) undertook a comparative study of the performances
757 of the Burkard, Durham and Rotorod samplers. The Burkard and Durham samplers gave
758 the highest (99.7 per m^3 of air) and lowest (17.0 per m^3 of air) mean concentrations of
759 pollen and spores respectively. The Rotorod sampler gave an intermediate mean figure of
760 35.7 palynomorphs per cubic metre of air. Gagnon and Comtois (1992) found that
761 meteorological conditions (principally wind speed and precipitation), and the amount and
762 smoothness of the adhesive used, can affect the efficiency of pollen and spore capture.
763 For example, the Durham sampler was more effective than the Rotorod when the wind
764 speed was greater than 12 km/hr, or when daily precipitation was over 18 mm.
765
766 *7.2.6. Collecting waterbourne palynomorphs*
767 Some projects require waterbourne palynomorphs to be sampled. Dinoflagellate cysts,
768 pollen, spores and other palynomorphs can be collected from water bodies at the margin
769 of the water or from a small boat using a plankton net or a plankton tow with a mesh size
770 of ~25 μm (e.g. Figures 20, 21; Dale 1979; Traverse 1988, fig. 12.3a; Traverse 2007, fig.
771 12.4a; Evitt 1984). It is also possible to install a plankton net, a suitable sieve or a Tauber

772 Trap in flowing terrestrial water courses in order to sample suspended pollen and spores
773 (Peck 1972).
774 Modern dinoflagellate cysts, pollen and spores can also be collected from specific
775 lake or marine sites using sediment traps. These are devices used in limnology and
776 oceanography to measure the amount and type of inorganic and organic particulate matter
777 (aquatic/marine snow) which sinks through the water column to the sediment-water
778 interface (e.g. Buesseler et al. 2007; Giesecke and Fontana 2008). Sediment traps are
779 upward-facing cylinders or funnels which collect aquatic/marine snow, including
780 dinoflagellate cysts, pollen and spores, and conserves it in collecting vessels at regular
781 intervals for collection during a deployment that typically lasts several months. The traps
782 are normally moored by cables at specific depths in the water column which is usually
783 below the euphotic zone, and may be close to the sediment-water interface. Individual
784 palynomorphs can be manually picked out of sediment trap sample material, thereby
785 retaining the remaining sample for other analyses.
786
787 ***7.3. Pre-preparation, i.e. the subsampling, cleaning and crushing of samples***
788 Many field sampling campaigns collect relatively large samples because multiple
789 preparations may be necessary, and other analyses such as calcareous micropalaeontology
790 and geochemistry may be needed. Irrespective of the mass of sample collected, normally,
791 around 20–25 g of fine-grained sample material is used for a single palynology
792 preparation. Considerably less can be used if the sample material is limited (Wood et al.
793 1996, fig. 5). This weight assumes the aforementioned clayey and moderately organic-
794 rich lithology. More will be needed if the lithotype is relatively organic-poor because the
795 mass of sample required is inversely proportional to the amount of clay/silt-sized
796 particles present. This means that, for example, if the mudstone/siltstone being sampled is
797 sandy, it is probably prudent to use ~50–60 g (Table 1). Therefore careful subsampling of
798 the main sample is frequently required.
799 Prior to the next, acid digestion, phase of the technique, it is essential that the
800 sample material is thoroughly cleaned and suitably fragmented. Precautions should of
801 course be taken during field sampling, but further cleaning in the laboratory is necessary
802 because any extraneous surface materials can yield contaminants. The outer part of the

803 sample also may be organic-lean. Assuming that the sample material is large enough, all
804 the surface material (~2–5 mm) should be stripped off using a scalpel, spatula or other
805 suitable tool. If the samples are relatively small, they should be methodically scrubbed
806 using a brush with stiff bristles in a flow of running water. Wire brushes are ideal for this
807 purpose. If it is suspected that modern plant matter is adhering to the surface of the
808 sample, the exterior of the sample can be briefly and carefully flamed using a small
809 domestic blowtorch to remove this material by burning. Samples should not be
810 overheated because palynomorphs can easily be burnt or singed, and it may be difficult to
811 remove small modern rootlets which have penetrated the material.
812 The next stage is digestion with hydrochloric acid (section 8.2), and it is hence
813 essential that the surface area available to this reagent is as large as possible so that the
814 breakdown of the sediment matrix is efficient. Therefore the sample material is gently
815 crushed as gently as possible. It can, for example, be placed between two (or more) thick
816 sheets of heavy duty aluminium foil (or similar disposable material), and crushed using a
817 geological hammer to ~0.5–1.0 cm^3 ('pea-size') fragments (Figure 22). Alternatively, an
818 agate, glass or metal pestle and mortar can be used for this. Crushing using a pestle and
819 mortar should be done using a vertical ('up-and-down') motion, because grinding and
820 twisting can damage palynomorphs. Fragmentation using a ceramic pestle and mortar is
821 not recommended because these surfaces are porous and/or uneven, hence could cause
822 contamination (Phipps and Playford 1984). It is also possible to use a ball mill to crush
823 sample material. This apparatus is a simple mechanical grinder comprising a hollow,
824 rotating cylinder partially filled with metal balls. The action of the latter as they drop in
825 the rotation cylinder crushes the rock. Clearly the timing of ball milling is critical to
826 avoid palynomorph damage, and both the cylinder and the balls must be cleaned
827 scrupulously between samples to prevent cross-contamination. Additionally, disc grinders
828 can be used to mill coal samples.
829 In reference to the ideal size of the pieces of sample, many publications refer to
830 'pea-sized fragments'. Great care should be taken to avoid producing small (<1–2 mm)
831 fragments because, despite their very small size, palynomorphs can be damaged during
832 this step. On a related note, if sample material has been powdered, for example prior to
833 geochemical analysis, it is of very limited use in palynology and should only be used if

834 nothing else is available. If the rock being prepared is highly indurated and hard, it may
835 be necessary to pre-fragment samples using a geological hammer and a small anvil before
836 crushing with the pestle and mortar. By contrast, if the samples are unlithified it may be
837 possible to fragment them by hand.
838 It is very important to accurately weigh the sample which is going to be prepared.
839 This will help to assess the organic richness of the sample, and the concentration
840 (absolute numbers of grains per gram) of palynomorphs can be worked out if the weight
841 is known (subsection 17.3). Finally any remaining raw sample material should be
842 carefully archived in a clean, dark, dry, secure, temperature-controlled storage facility
843 (subsection 10.4).
844
845
846 **8. Phase two of the traditional technique – mineral acid digestion**
847
848 ***8.1. Introduction***
849 The second phase of the traditional palynomorph processing technique is the separate
850 dissolution (digestion) of the carbonate and silicate minerals in the matrix of the sample
851 material by using hydrochloric acid and then hydrofluoric acid. The culinary analogy for
852 this phase of the preparation technique, also referred to as demineralisation, is ‘soaking
853 the pulses’ (Figure 2; Appendix 1). First the calcite and dolomite must all be removed
854 with hydrochloric acid and washed away, otherwise insoluble fluorides (mainly calcium
855 fluoride) will form when the hydrofluoric acid is added (Grayson 1956). Barss and
856 Williams (1973) recommended the use of 600 ml polypropylene beakers for this in order
857 to allow for violent reactions, for ease of washing, to enable rapid and effective dilution
858 of reagents, and to allow extraneous materials to be floated off.
859 Assarsson and Granlund (1924) first used hydrofluoric acid to prepare Quaternary
860 pollen, and Alfred Eisenack successfully extracted palynomorphs from Palaeozoic and
861 Mesozoic limestones with hydrochloric acid (Eisenack 1930; 1931). These key
862 breakthroughs revolutionised palynological preparation techniques (section 4).
863 Palynomorphs and kerogen are apparently unaffected by these acids, despite some
864 assertions that they may be corrosive (e.g. Staplin et al. 1960; Doher 1980; Reid and John

1981; Johnson and Fredlund 1985; Clarke 1994; Van Geel 2001). More specifically, Durand and Nicaise (1980) reported that hydrochloric acid and hydrofluoric acid may chlorinate, fluorinate, hydrolyse and oxidise sedimentary organic matter including palynomorphs. Young, immature organic material is particularly prone to hydrolysis, while relatively old kerogen is far less prone to chemical alteration. However, despite the concerns of Durand and Nicaise (1980), these reagents rapidly dissolve the mineral fabric of the material, leaving a largely organic residue from which the palynomorph fraction can be concentrated.

Staplin et al. (1960) discussed the pre-treatment of samples prior to the addition of mineral acids in order to disaggregate the material. These authors advocated treating dry, fragmented unconsolidated sample material with a mixture of 'Soltrol C' (a core analysis fluid) and detergent. This mixture is left to disaggregate and this procedure preceded acid digestion. Staplin et al. (1960) found that some samples break down fully, and that further chemical treatment is superfluous. However, if the samples require acid treatment following pre-treatment disaggregation, Staplin et al. (1960) stated that this pre-treatment makes the hydrochloric acid and hydrofluoric acid digestion substantially faster due to the increased surface area, and that it requires less volumes of reagents. Raistrick (1934) pre-treated high rank coals with pyridine prior to oxidation, and Riding and Kyffin-Hughes (2010) found that pre-treatment with detergent or white spirit (paint thinner) increased the effectiveness of their non-acid preparation method (section 12). Burgess et al. (2020) used industrial strength detergents/degreasers, specifically ARCO cleaner, to help clean up and concentrate the structured organic material following acid maceration.

8.2. Hydrochloric acid treatment

The first step of phase two is the treatment of the cleaned and crushed fragments of the sample material with hydrochloric acid to remove any carbonate minerals, dominantly aragonite, dolomite, calcite and siderite (Figure 23). Calcite is the principal mineral of interest here and, for the sake of conciseness, these minerals are normally referred to as calcite hereafter. Assuming the carbonate mineral present is calcite, the chemical reaction with hydrochloric acid is thus:

$$2HCl + CaCO_3 \rightarrow 2Cl^- + Ca^{2+} + CO_2 + H_2O$$

This step helps to break down the matrix of the sample. Perhaps more importantly, it is imperative to remove all carbonates during this stage in order to prevent the formation of insoluble fluorides during the subsequent hydrofluoric acid treatment. In addition to carbonates, hydrochloric acid can dissolve most hydroxide, oxide, sulphate and sulphide minerals.

There are alternative reagents for this procedure; acetic, carbonic, formic, orthophosphoric and oxalic acids can be used for carbonate dissolution. These are all considerably higher in pH than hydrochloric acid, therefore the reaction will be slower. The concentration of the hydrochloric acid is not imperative, but ~30–40% is normally used. The ranges of concentrations recommended in the literature varies from 10% to 60%. Some authors advocate heating and stirring the vessel using an oscillating/rotary hotplate or a hot water bath (e.g. Staplin et al. 1960; Schopf 1964; Sarjeant 1974), but this should only be considered if the reaction proceeds very slowly and the speed of preparation is an issue. Phipps and Playford (1984) suggested heating to 50°C, and that dolomite normally needs heat to react. Dolomite reacts significantly slower than calcite when treated with hydrochloric acid, but even very large samples of intensely dolomitised limestone will dissolve completely in cold acid over several days. Very few sedimentary rocks or sediments are entirely devoid of carbonate minerals. Even if there are none in the cement or matrix, there will normally be some shelly material present. This means that only on rare occasions, when the sample material entirely lacks calcite, can this step be omitted.

The sample material should be tested for calcite by pipetting a few drops of dilute (~10%) hydrochloric acid onto it (Figure 23A). If the sample is calcareous, the calcite reacts vigorously with hydrochloric acid and produces prodigious amounts of bubbles of carbon dioxide. Assuming the sample is calcareous, the fragmented material is placed in a suitably-sized glass or plastic container; 600 ml is normally adequate, but larger capacities (1000 or 2000 ml) can be used. It is best to use a plastic container because glass vessels are unsuitable for the subsequent hydrofluoric acid treatment (subsection 8.3). Next hydrochloric acid is carefully and slowly added to the sample plus any tablets of exotic markers (subsection 17.3) in a fume hood. It is far safer to add the hydrochloric acid using a manual chemical dispenser which fits onto the top of the vessel containing

927 the acid (Figure 23B), as opposed to simply pouring the acid into the sample vessel. The
928 sample material can be dry, or it may be covered in water to reduce effervescence
929 (Herngreen 1983).
930 Because of the risk of high levels of excessive effervescence of highly acidic
931 liquid, this step should be performed with great caution. The bubbles of carbon dioxide
932 can be extremely abundant and they can easily flow out the sample vessel (Figure 23C).
933 Tests have convincingly shown that this effervescence can frequently be rich in
934 palynomorphs so it is important not to lose any of this foamy material. In addition to the
935 risk of palynomorph loss, a spill of hydrochloric acid effervescence in the fume hood can
936 be arkward and difficult to clean up, plus there is the danger of cross-contamination. This
937 means that a very highly calcareous lithotype such as pure limestone should be treated
938 with hydrochloric acid in a very large container, such as a plastic bucket which can
939 contain any vigorous effervescence.
940 The effervescence can be suppressed by using a narrow jet of acetone, ethanol,
941 isopropyl alcohol or water (or suitable mixtures thereof) from a plastic mister-bottle or a
942 wash-bottle (Figure 23D). Care must be taken if acetone or alcohol is used because these
943 substances are volatile and flammable. The liquid jet bursts large bubbles and somewhat
944 dilutes the hydrochloric acid. If acetone and/or ethanol are used, the surface tension is
945 substantially reduced thereby suppressing further bubbles (Wilson 1971a). This foam-
946 suppressing method can be extremely laborious because it only subdue foam production
947 for a very short time. An alternative is to add 100–200 ml of paraffin (kerosene) to the
948 limestone sample prior to the addition of the hydrochloric acid. The paraffin prevents
949 vigorous foam production by producing a ~1 cm thick layer above the reacting
950 hydrochloric acid (Nørgaard et al. 1991). However, if excessive effervescence is a
951 persistent issue, less concentrated hydrochloric acid should be used.
952 Normally, the sample material should be fully covered with the hydrochloric acid,
953 and an excess of one or two centimetres added. When a suitable volume of hydrochloric
954 acid has been added, the reaction should be allowed to proceed. The reaction time is
955 extremely variable; it can be completed in around one hour, but can take up to a one or
956 two days. It is finished when no more bubbles appear, even after stirring the sample.

Next the spent hydrochloric acid liquor, which is typically clear and yellow in colour, can be carefully removed. Typically, the hydrochloric acid treatment disaggregates the sample material so a sludgy, clay-rich material remains at the bottom of the vessel (Figure 23E, Schopf 1965). Obviously, none of this sludge should be decanted away with the spent hydrochloric acid liquor because it will contain palynomorphs. Next, the residue should be tested to check if there is any remaining calcite by adding further hydrochloric acid. If effervescence is observed, the residue should be given further hydrochloric acid treatments; up to four of these may prove to be necessary. When the residue is entirely calcite-free, it can then be decant-washed to neutrality so that fluoride minerals do not form during the next step (subsection 8.4; Figure 23F). After an initial decant, water is added to the residue, and the vessel is left to settle. After several decants, the supernatant is tested for acidity using pH-indicator paper. When the liquid is absolutely neutral (seven), a final decantation should be done, removing as much liquid as possible, before moving on to the next stage. Neutralisation can also be done using a centrifuge to speed up the separation of the supernatant and the residue. If the sample is urgent, the residue can be carefully sieve-washed in a fume hood. Here the residue is sieved with a jet of water or ethanol through a suitable acid-resistant mesh (15–25 μm) to quickly wash away the remaining hydrochloric acid. This method has the advantage of saving time, but there is a risk that palynomorphs may be lost through the sieve. Needless to say, extreme care must be taken to avoid splashes of acid caused by the jet of water or ethanol.

If the sample material contains evaporite minerals such as anhydrite, gypsum and halite, these can generally simply be removed by dissolving them in water (subsection 14.5; Brown 1960; 2008) This step should ideally done prior to hydrochloric acid treatment. More on hydrochloric acid treatment can be found in subsection 14.2 on carbonates.

8.3. Hydrofluoric acid treatment

The next step is by far the most hazardous of the entire technique due to the extremely toxic nature of hydrofluoric acid. This is a colourless liquid which is highly corrosive and rapidly causes deep burns if it comes into contact with human skin. Because of its highly

ionised nature, it quickly penetrates tissue, and the burns may not be initially painful because hydrofluoric acid affects nerve function (Head 1995a; 1995b; Riding and Kyffin-Hughes 2004, p. 18–19). Any splashes of hydrofluoric acid onto skin, no matter how small, should be fully and urgently medically evaluated. In these cases, the affected area should be copiously irrigated with water and treated with a water-based calcium gluconate gel as first aid. The latter is effective as it neutralises the fluoride ions.

The corrosive nature of this substance means that this procedure must always be undertaken using plastic beakers and other laboratory equipment such as stirring rods because hydrofluoric acid attacks and dissolves glass. Polypropylene, teflon and other synthetic plastics are ideal for this.

The use of hydrofluoric acid in palynological processing is necessary where the sample material is cemented by silica and/or there are substantial proportions of clay and silicate minerals present. Due to the virtual ubiquity of silicate minerals, the vast majority of samples require this procedure. Therefore, following hydrochloric acid treatment, hydrofluoric acid is added to the residue in order to dissolve the readily soluble silicate minerals, i.e. clay minerals and silicious cement (Figure 24). The hydrofluoric acid simultaneously converts silica and silicate minerals to silicon tetrafluoride (SiF_4) and hexafluorosilicic acid (H_2SiF_6). The two relevant chemical reactions are:

$$SiO_2 + 4HF \rightarrow SiF_4 + 2H_2O$$

$$SiO_2 + 6HF \rightarrow H_2SiF_6 + 2H_2O$$

Moreover, some metal oxides may be converted to fluorides (Phipps and Playford 1984). Therefore, for most siliciclastic sample material, this is this stage that releases the sedimentary organic material from the mineral matrix.

During the final decantation of the post-hydrochloric acid neutralised wet residue from a ~600 ml plastic vessel, as much of the supernatant is removed as possible. Then 40% hydrofluoric acid is carefully and slowly added until the vessel is filled to ~50% capacity (Figure 24A). The precise concentration of the hydrofluoric acid is not critical; 40% is normally recommended and this seems to be effective for the majority of lithotypes. However hydrofluoric acid concentrations of 25% to 70% have been mentioned in the literature.

In certain cases, an exothermic reaction can occur when the hydrofluoric acid is added and occasionally this can be violent. These reactions appear to be most prevalent if the sample is rich in pyrite or volcanic ash. Obviously, this situation can be potentially highly hazardous so it is stressed that great care should be taken when adding the hydrofluoric acid, even where apparently similar lithologies are being prepared. The addition of crushed ice to the sample prior to hydrofluoric acid treatment can help to avoid a violent exothermic reaction (Taggart and Cross 1980). A vigourous reaction can also be avoided or subdued by saturating the residue with 95% ethanol before the hydrofluoric acid is added (Lennie 1968).

The hydrofluoric acid normally digests the clay minerals relatively rapidly, in many cases within one day. Norem (1956) stated that 12–16 hours is normally sufficient. However, some zeolite minerals are unaffected by hydrofluoric acid (Jen O'Keefe, personal communication 2021). Clays are the most readily soluble silicate minerals, however complete silicate dissolution is rarely achieved (Schopf 1964). Small quartz grains may be destroyed, but the denser silicate mineral species such as garnet, tourmaline and zircon are rarely fully degraded. Oxide and sulphide minerals such as anatase, brookite, pyrite and rutile are similarly relatively unaffected by hydrofluoric acid. Pyrite is the most problematic mineral species as it can be abundant (subsection 9.3.2.3); others such as zircon are relatively rare.

The sample residue should ideally be left in hydrofluoric acid for around three days to ensure maximum mineral digestion, but this depends upon the effectiveness of the dissolution of the mineral material and the urgency of the project being undertaken. Barss and Williams (1973) recommended that residues are left in hydrofluoric acid for at least 18 hours. The vessels should be carefully stirred at least daily (Figure 24B), or an oscillating/rotary hotplate with a magnetic stirrer used. The reaction will be complete when all the 'grittiness' has disappeared, and is not felt when stirring. This means that the matrix has broken down and the reaction can be stopped (Schopf 1964). In some cases, the hydrofluoric acid will need replacing to achieve the maximum mineral digestion.

When any initial heat has fully dissipated, the reaction can be accelerated by heating the plastic vessel to ~50–80°C on a hotplate with a magnetic stirrer. If a preparation is extremely urgent, the silicate digestion can be achieved more rapidly by

boiling the hydrofluoric acid-residue mixture in a copper beaker or nickel crucible for around 30 minutes (Herngreen 1983; Phipps and Playford 1984). The latter authors advocated placing a sand tray between the crucible and the heat source to ensure even heat dispersal. West (1977) suggested mounting the crucible in a clay triangle to keep it firmly held in place. It should be stressed again that this procedure is highly hazardous and should be undertaken only where absolutely necessary, and with extreme caution by a highly experienced technician.

When the sediment matrix has broken down, and as much mineral content as possible has been digested, the residue can be decant-washed, centrifuge-washed or sieve-washed to neutrality (subsection 8.2; Figure 24C). The supernatant is normally clear and colourless (Figure 24D). When the residue is neutral, it should be examined under the microscope to check if it needs further hydrofluoric acid treatment. If the sediment matrix has not broken down sufficiently, it should be retreated with hydrofluoric acid. However, in most cases at this stage, kerogen macerals such as wood fragments and palynomorphs will be clearly visible even using low-power microscope objectives. Cridland (1966) described a specialised technique for efficient hydrofluoric acid treatment.

8.4. Removal of fluoride minerals

Following the treatment with hydrochloric acid, it is imperative that the residue should be thoroughly cleaned of all residual chemicals. The reason for this is that, when the hydrofluoric acid is added, any remaining calcium, magnesium, potassium and/or sodium cations will react with the fluorine anions from this extremely highly ionised acid to form highly insoluble crystals of, for example, calcium fluoride (Grayson 1956). The chemical reaction, assuming that calcium is the cation, is:

$$Ca^{2+} + 2F^{-} \rightarrow CaF_2$$

If these grey, opaque crystals of neoformed fluorides are present, these can be removed by carefully boiling the residue in hydrochloric acid (Norem 1953). Hydrochloric acid is the most effective reagent for dissolving these crystals, but alternatives include ammonium carbonate, boric acid and sodium carbonate. The concentration of the

hydrochloric acid is not critical, but the stronger it is, the faster it will dissolve the fluoride crystals.

Similarly, if the sample material is especially clay-rich, insoluble fluorides may also form during the hydrofluoric acid treatment. These form from metal cations released from the lattices of the clay reacting with the hydrofluoric acid. In these cases, Phipps and Playford (1984) recommended a pre-treatment with a clay deflocculant such as EDTA (ethylenediaminetetraacetic acid) or sodium hexametaphosphate prior to the addition of hydrofluoric acid (subsections 9.2, 12.4). EDTA is an organic chelating agent that removes free cations such as calcium and magnesium from solution.

9. Phase three of the traditional technique – palynomorph concentration

9.1. Introduction

Normally the neutral post-hydrofluoric acid sample residue will be a black/brown sludge which readily settles out from suspension in a clear aqueous residue (Figure 24D). It will generally comprise a poorly-sorted mixture of various kerogen macerals, residual/resistant mineral grains and palynomorphs. Unless a kerogen/palynofacies slide is required (Figure 3), as much of the kerogen and mineral fractions as possible should be removed in order to concentrate the palynomorphs so that they will be easier to examine when mounted onto microscope slides.

This concentration phase comprises the removal of dispersed clay, oxidation and alkali treatment, density separation, ultrasonic treatment, and sieving. Because this part of the technique is the ‘cleaning up’ of the raw post-acid residues, the culinary analogy applied to it is ‘culinary alchemy, i.e. flavourings, herbs, seasonings, spices etc.’ (Figure 2; Appendix 1). Of these various steps, oxidation and alkali treatment, density separation, and sieving are needed for most samples. By contrast, many samples will not need dispersed clay removal and/or ultrasonic treatment. Throughout phase three, the neutral post-acid residue should frequently be checked using a microscope to determine which of these procedures to apply and to check the efficacy of them. It is strongly recommended to split the post-acid residue so that more material is available if the first split is

overexposed to potentially destructive procedures such as oxidation or ultrasonic treatment, and the palynomorphs are damaged or degraded. In particular, a split of the pre-oxidised residue should be retained in case spore colour/thermal maturation analyses are needed because oxidation causes loss of natural body colour or bleaching (subsection 17.9).

9.2. Removal of dispersed clay

This is not a mainstream step in palynological processing, however, there are certain instances when the aqueous residues include abundant small clay fragments which can occlude the palynomorphs, before and after acid digestion. For example, O'Keefe and Eble (2012, p. 121) commented that hydrofluoric acid did not remove all the clay mineral fraction on some of their Eocene material from the USA. This finely disseminated particulate clay, which causes the residue to appear milky or muddy, can be removed by deflocculation (i.e. dispersing the clay) followed by decantation, centrifugation and/or sieving; this can be done either before or after hydrofluoric acid digestion. Most authors advocated the use of a dilute (~5%) solution of sodium pyrophosphate as a deflocculant (e.g. Bates et al. 1978; Cwynar et al. 1979; Heusser and Stock 1984). However, surfactant detergents such as Alcojet, Alconox, Liquinox and Teepol can also be used for this.

Bates et al. (1978) stated that this clay-rich material can take a long time to digest in hydrofluoric acid because the clay particles tend to form tenacious aggregates which are difficult to break down. Therefore if it can be removed, the acid digestion step will be much more efficient. Similarly, Batten (1999), in a description of preparing Quaternary material, recommended removing clay by adding 30 ml of 5% sodium pyrophosphate solution, and heating the mixture in a water bath at 90°C for 10–20 minutes. The deflocculated clay and the sodium pyrophosphate solution can then be separated from the palynomorphs and kerogen by centrifugation. If there are residual mineral grains present, these are then removed using mineral acid digestion.

By contrast, Traverse (1988; 2007) and Litwin and Traverse (1989) stated that the optimum juncture to perform clay deflocculation is following hydrofluoric acid digestion. These authors removed the remaining finely dispersed clay in the residue using the now discontinued detergent Darvan No. 4 mixed with formalin and water. The mixture is

stirred, then short centrifuged to concentrate the palynomorphs, which accumulate at the bottom of the tube, leaving the clay in suspension (subsection 9.4.2.4). Jackson (1999) also advocated the removal of residual clay after hydrofluoric acid digestion. The residue is treated with a 5% solution of sodium pyrophosphate, then rinsed with water two or three times. If dispersed clay persists, the sample is retreated with sodium pyrophosphate and the dispersed clay sieved away using 7 or 8 μm mesh.

9.3. Oxidation and alkali treatment

9.3.1. Introduction

Phase three of palynomorph preparation normally begins with controlled oxidation, followed by alkali treatment. The oxidation stage depolymerises lignins and similar macromolecules, making them more liable to form soluble salts when treated with conjugate alkalis which can then be rinsed away with water. Oxidation also bleaches and fragments finely disseminated amorphous organic material (AOM) so that this chemically altered material can be removed by dissolution using a weak alkaline solution. The removal of AOM and lignins thereby concentrates the palynomorphs. If the samples are highly carbonaceous (e.g. carbon-rich mudstone or coal), oxidative maceration is the first step in processing because this will achieve the structural breakdown of the rock. Therefore this topic is also highly relevant to peat and coal (section 14.3). Supplementary Data Appendix 1.4 includes commentaries on 10 contributions on oxidation in palynological preparation. There are very few consistently effective alternatives to oxidation in palynological preparation. However Riding and Kyffin-Hughes (2004; 2006) and Eble (2017) successfully used sodium hexametaphosphate and glycol ethers respectively to remove AOM (subsection 12.4).

9.3.2. Oxidation

9.3.2.1. General. The concentration of palynomorphs can be substantially increased by the destructive oxidation of extraneous organic material. Typically, post-hydrofluoric acid digestion organic concentrates include complex organic materials such as clumped and finely-disseminated humic material (AOM), huminite/vitrinite, non-woody plant tissues and wood in various stages of chemical transformation i.e.

1172 coalification and decomposition (Figure 3). Huminite/vitrinite, wood and AOM can all
1173 contain palynomorphs. Batten (1996) commented that AOM derived from land plants is
1174 significantly more susceptible to removal via oxidation than the AOM produced by the
1175 microbial degradation of aquatic algae. All these constituents can be abundant, therefore
1176 they dilute and occlude palynomorphs on microscope slides. Hence it is desirable to
1177 minimise these, or entirely remove them. It is possible to sieve away much of the finely-
1178 disseminated organic material if it is <10 μm in maximum diameter, but the most
1179 effective method of removing the >10 μm extraneous organic materials is oxidation.
1180 The oxidation stage aims to transform insoluble extraneous organic debris into
1181 humic acids that can be dissolved and removed using a dilute alkaline solution. The
1182 method of oxidation selected should be as brief and mild as possible in order to avoid
1183 damage to palynomorphs; oxidation is aggressive to all organic materials and is
1184 irreversible. If possible, the oxidation reaction should be as slow as possible to avoid
1185 degradation (Schopf 1964). If the palynomorphs are dark and the residue is rich in
1186 extraneous organic material, a very strong reagent will be needed. By contrast, if the
1187 palynomorphs are light in colour and the organic debris is negligible or already alkali-
1188 soluble, this step may be entirely omitted. Furthermore, if palynomorphs have been
1189 darkened post-mortem during thermal maturation, the body colour can be lightened in
1190 order to enhance their appearance by oxidising the residue. The oxidant acts as a
1191 bleaching agent. Buratti and Cirilli (2011) advocated the use of sodium hypochlorite
1192 solution to lighten the colour of highly oxidised palynomorphs. In certain cases, typically
1193 with carbonised Palaeozoic material, oxidised palynomorphs can later redarken and crack
1194 (Marshall 1980; Harvey 2001).
1195 Excessive oxidation, however, can selectively damage or destroy palynomorphs
1196 (e.g. Schrank 1988; Dodsworth 1995; Eshet and Hoek 1996; Jardine et al. 2015). For
1197 example, Kuyl (1960) found that oxidation of residues derived from pure limestone using
1198 Schulze's solution destroyed all the palynomorphs. It therefore appears that
1199 palynomorphs preserved in clay-rich lithologies are substantially more resistant to
1200 oxidation than those from carbonates. Furthermore, oxidation and other harsh chemical
1201 treatments can rapidly destroy certain dinoflagellate cyst types, for example
1202 representatives of the family Congruentidiaceae (see Dale 1976; Head 1996; Hopkins and

McCarthy 2002; Riding et al. 2007a). This differential destruction means that samples of marine Neogene and Quaternary material are normally never oxidised in order to avoid damage to the congruentidioidean dinoflagellate cysts. However AOM, coalified material and non-woody plant tissue are normally destroyed or depolymerised before the inherently more resistant palynomorphs are adversely affected. Hence, a balance should be sought whereby extraneous organic material is removed without damage to, or the destruction of, palynomorphs.

9.3.2.2. Oxidising agents used in the palynology laboratory. The most common oxidising agents used in palynological preparation are ~15–30% hydrogen peroxide, hypochlorous acid, concentrated (70%) nitric acid, fuming (90–100%) nitric acid, Schulze's solution, sodium chlorite, and sodium hypochlorite or household bleach (e.g. Hoffmeister 1960; Wood et al. 1996; O'Keefe and Eble 2012). These reagents can be combined, for example nitric acid and sodium hypochlorite (Varma 1964). Other oxidants which have been advocated are aqua regia (a mixture of hydrochloric acid and nitric acid), a saturated solution of chromium trioxide in concentrated nitric acid, clove oil, ozone, a solution of potassium chloride in nitric acid, a saturated solution of potassium dichromate in concentrated nitric acid, a solution of potassium permanganate in sulphuric acid and an acidified aqueous solution of sodium chlorate (Godwin 1934; Funkhouser and Evitt 1959; Staplin et al. 1960; Dettmann 1961; West 1977; Woolsley 1978). The chromium trioxide-concentrated nitric acid mixture is a particularly fast-acting oxidant according to Funkhouser and Evitt (1959). Schulze's solution is also a very strong reagent, and comprises a solution of concentrated nitric acid and potassium chlorate in a 1:5 ratio. The nitric acid is the oxidant and the potassium chlorate is the accelerator for the reaction. It was first developed by Schulze (1855), who found that this mixture bleached coalified plants so as to make their structure visible (Manum 1956). The ratio of nitric acid and potassium chlorate can be varied; for example if a stronger oxidant is required, the proportion of nitric acid is increased. By contrast, oxidation using hydrogen peroxide, sodium chlorite or sodium hypochlorite can normally be safely observed under a microscope.

Because the oxidants involving hydrochloric, nitric and sulphuric acids are highly hazardous, oxidation using them should always be undertaken in a fume hood. Fuming nitric acid is an extremely strong oxidant, and its use is potentially very hazardous because it may decompose explosively. Nitric acid and Schulze's solution produces toxic nitrogenous gases. They also can react violently with pyrite (section 9.3.2.3), therefore pyrite-bearing sample material should be initially treated with dilute nitric acid to test for this.

9.3.2.3. Pyrite. Pyrite is the most common sulphide mineral in crustal rocks. It occurs as cubic, isometric crystals and has a specific gravity of 4.95 to 5.10. Pyrite is often common in anoxic mudstones and coal. Small crystals may be present inside palynomorphs, typically between the walls; these are easily recognisable as small opaque cubes (Plate 3.3). Naturally, any pyrite crystals will significantly increase the specific gravity of palynomorphs, and this must be borne in mind during density separation (subsection 9.4). Clearly, anything that compromises the disparity in density between minerals and organic material will render the density separation process ineffective.

Pyrite is unaffected by hydrochloric and hydrofluoric acids, however it can be eliminated chemically by oxidation or reduction (Saxby 1970; Durand and Nicaise 1980; Merrill 1980; Gelsthorpe 2002). If a sample is rich in pyrite, there may be a violent reaction with strong oxidants such as nitric acid and Schulze's solution. Pyrite-bearing material should therefore be initially treated with dilute nitric acid to test for this. This mineral can be removed by treatment with most oxidising agents. Acidified ferric chloride, acidified ferric sulphate, concentrated nitric acid, sodium hydroxide solution or sodium hypochlorite solution are normally used for this (Batten 1999). The use of the reducing agents hydrogen gas, lithium aluminium hydride and sodium borohydrite, or a mixture of powdered zinc in concentrated hydrochloric acid to remove pyrite was recommended by Saxby (1970) and Durand and Nicaise (1980). The latter authors stated that the most effective reagents are acidified ferric sulphate and lithium aluminium hydride. The sample residue being oxidised or reduced should normally be heated to 100°C. For example, Durand and Nicaise (1980) advocated reflux boiling the sample with 150 g of hydrated ferric sulphate dissolved in one litre of concentrated hydrochloric

acid. It can be extremely difficult to eliminate all the pyrite in a sample because it is frequently intimately associated with sedimentary organic material; kerogen frequently forms a protective coating around pyrite crystals.

9.3.2.4. Laboratory procedures in oxidation. The first step in this procedure is to test whether or not a sample requires oxidation. If a sample is naturally oxidised (i.e. weathered) at outcrop, it will probably not need treatment with an oxidant, but it will need treatment with a base. Specifically, if the raw sample, or the sample residue, reacts with dilute alkali (e.g. potassium hydroxide solution) to give a strong dark brown colouration, the sample has been naturally oxidised and should require little or no oxidation in the laboratory. This was termed the 'base test' by Funkhouser and Evitt (1959).

If the sample/residue is unreactive with a dilute base solution, strong consideration should be given to oxidation. Most naturally unoxidised residues benefit from even a brief treatment with a mild oxidising agent; this will clean it of finely disseminated organic material. Conversely if a residue is rich in clumps of tenacious AOM, it will need substantial oxidation to release most of the palynomorphs. Thus a residue is assessed at this juncture for which oxidant to use, and for how long. It is best to achieve a relatively slow oxidation reaction in order to prevent and reduce any oxidative degradation of the palynomorphs. If the palynomorphs have been fully released by the acid digestion, and there is little extraneous organic material, a relatively mild oxidant such as hydrogen peroxide, nitric acid or sodium hypochlorite should be used for a short time (~one minute). Nitric acid is often used for this, and the residue receives what is colloquially known as a 'nitric wash'. Conversely, if it is clear that substantial oxidation is required, the residue should be treated with Schulze's solution or fuming nitric acid for around ten minutes or longer. By contrast, extremely tenacious and unreactive residues may need treating with a very strong oxidant such as Schulze's solution for up to a few days to two or three weeks (Wood et al. 1996; Harvey 2001). There is no formalised predicated strategy for this, however, the higher the thermal maturity, the stronger the oxidant should be as a general rule (O'Keefe et al. 2011; Jen O'Keefe, personal

communication 2021). Despite this. the oxidant used and the duration of the reaction should be determined by trial and error, sample-by-sample.

The residue can be oxidised in a small vessel such as a beaker (Figure 25), or oxidation can be done using a sinter glass funnel (Figure 26) in a fume hood. The oxidant should be added with great care as this reaction can be exothermic. If a beaker is used, it can be gently agitated and heated in order to speed up the reaction. Using a sinter glass Büchner funnel allows the timing of the oxidation reaction to be extremely carefully controlled using, for example, a reversible aspirating rubber bulb ('hand bellows') on the sidearm of the Büchner flask (Figure 26).

During the oxidation procedure, small portions of the mixture should be regularly removed and tested with an alkali (the base test). This is because when the oxidation is complete, the residue is highly reactive with a weak base such as potassium hydroxide solution which turns the liquid a distinctive brown/red/yellow colour. Alternatively, when the oxidation is ended, the supernatant naturally becomes a golden colour. After the reaction, or when it needs to be stopped, the residue should be diluted then washed/centrifuged to neutrality. If the oxidising agent is not entirely washed from the residue, oxidation can continue for long after the initial treatment.

The non-acid processing technique using sodium hexametaphosphate frequently produces relatively clean palynomorph associations which do not apparently require oxidation (subsection 12.4). Possibly the sodium hexametaphosphate disaggregates AOM as well as clay, or makes some organic matter soluble (Riding and Kyffin-Hughes 2006; Jen O'Keefe, personal communication 2021). This phenomenon requires further investigation, but may significantly reduce the need to oxidise the residue using hazardous reagents such as nitric acid.

9.3.3. Alkali treatment

Humic compounds are produced during oxidation, and these are dissolved by using weak alkaline solutions. These should be as weak as possible (ideally ~5%) in order to avoid damage to palynomorphs. Following oxidation, the oxidising agent is thoroughly washed away. Then the humic components which have been produced are dispersed by dissolving them in a weak alkali, such as 5% or 10% ammonium hydroxide or potassium hydroxide

solutions. Suitable alkalis are, in order of decreasing reactivity, solutions of potassium hydroxide, sodium hydroxide, ammonium hydroxide and potassium carbonate (Bruch and Pross 1999). Other alkali solutions which can also be used include those of sodium carbonate, sodium perborate and sodium silicate, but these are generally less effective. An alternative is to wash the oxidised residue with organic solvents such as ethanol, or an acetone-water solution to remove the oxidised humic acids particularly if the residue has been overoxidised (Bruch and Pross 1999).

However alkalis are able to disperse soluble humic acids in mature peats without pre-oxidation. Phipps and Playford (1984) found that 5% potassium hydroxide solution is the most effective reagent. Typically, ~2 ml of 5% ammonium hydroxide or potassium hydroxide solution is added to the aqueous residue. If there is no observable reaction, a stronger solution should be used. According to Phipps and Playford (1984), moderate heating and/or ultrasonic treatment can help this procedure and a few drops of a non-ionic detergent will help avoid clumping of the palynomorphs (subsection 9.5).

The humic material is highly soluble in weak bases and dispersal of it normally proceeds very rapidly; this treatment should not normally be for over two minutes. It is important not to overexpose the residue to the alkaline solution, or to use too strong a reagent, because this can cause the palynomorphs to swell (e.g. Large and Braggins 1990; Bruch and Pross 1999). Alkali treatment can be undertaken in a small vessel such as a beaker, or in a sinter glass funnel (Figures 25, 26). It is good practice to consistently undertake alkali treatment, although this is not always essential. The supernatant can be separated by centrifuge-washing or decant-washing the residue after the reaction is complete. The palynomorph concentrate should be thoroughly washed until the brown humic material and any remaining alkali are entirely removed. It is very important that all traces of the alkali are eliminated, especially if alkali treatment is the final step, for example in a peat preparation (subsection 14.3.2). The residue can also be neutralised using hydrochloric acid. If the residue is not neutralised, any remaining alkali will continue to react. This can even happen following slide mounting; the mounting medium will turn brown, and the palynomorphs may eventually be destroyed (Bruch and Pross 1999).

9.4. Density separation

9.4.1. Introduction

Generally, density separation is undertaken following oxidation (Barss and Williams 1973; Doher 1980; Phipps and Playford 1984; Batten 1999). Density separation, which is also refered to as gravity separation, aims to separate the remaining organic fraction from the extraneous heavier elements, principally resistant mineral grains. This dense material comprises hydrofluoric acid-resistant mineral grains such as pyrite, garnet, rutile, tourmaline and zircon, and some wood fragments. The dense fraction can be substantial and its removal, therefore, significantly concentrates the palynomorphs. This can be achieved by centrifugation, flotation, passive settling or swirling. These four methods are described below, and are all predicated by the relatively low density of palynomorphs; sporopollenin has a specific gravity of 1.168 to 1.96 (Flenley 1971; Barrier, 2008; Stephen Stukins personal communiction 2020).

There is no overall concensus in the literature as to whether oxidation and alkali treatment should be undertaken prior to density separation. For example Traverse (1988; 2007) recommended oxidising after density separation. The main reason for oxidising the residue first is that pyrite is removed by treatment with an oxidising agent, for example nitric acid (subsection 9.3.2.3). Pyrite crystals often develop within the sacci of bisaccate pollen grains and inside the cavities of acritarchs, dinoflagellate cysts and spores, making them substantially denser (e.g. Plate 3.3). If this mineral is not dissolved away, the pyrite-bearing palynomorphs would be prone to be being physically separated into the dense mineral concentrate during density separation and thus lost (Gelsthorpe 2002). Pyrite can be removed by treating the organic residue with ~10% nitric acid, heated for 1–2 minutes in a water bath at 90°C, then washing to neutral (subsection 9.3.2.5; Batten 1999). Another reason for undertaking oxidation and alkali treatment first is that, if organic-rich/mineral-poor residues are centrifuged prior to oxidation, everything will float and a large and unwieldy amount of organic sludge mixed with heavy liquid will ensue. Supplementary Data Appendix 1.5 includes commentaries on 14 contributions on density separation.

9.4.2. Centrifugation

9.4.2.1. Introduction. A laboratory centrifuge rotates customised glass or plastic tubes in holders around a fixed axis, thereby applying a strong outwards force (Figure 27). Centrifugal acceleration causes the denser material to move outwards. By contrast, fragments that are less dense move to the centre. This means that the denser mineral grains to settle at the bottom of the tube, while the dark, low-density organic material rises to the top (Figure 28; Mikkelsen and Cortón 2016). Centrifuge units with swinging bucket rotors are strongly preferred, as opposed to those with fixed angle rotors. The former allow effective density fractionation by rapidly pushing the heavy materials into the distal tips of the centrifuge tubes. Centrifuges with fixed-angle rotors give imperfect density separations.

It is very important that the centrifuge unit is balanced during use. This means that either all the tube holders are full with centrifuge tubes of a similar weight, or that opposite tube holders are similarly loaded. A balanced centrifuge unit will give optimum density separation, and should not vibrate during the centrifugation process. Regular maintenance of centrifuge units will also ensure minimal vibration and optimum density separation.

9.4.2.2. Centrifugation using heavy liquids. If a palynomorph residue is rich in refractory mineral grains and heavy wood fragments, density centrifugation is an essential step. It may take several cycles of centrifugation to completely eliminate the mineral grains and hence concentrate the palynomorphs. Specifically, small palynomorphs can be efficiently concentrated by centrifuging the demineralised and oxidised organic residue, normally in a heavy liquid (i.e. one with a specific gravity of >1). The optimum specific gravity for heavy liquids is 2.0 to 2.2. The palynomorphs, charcoal/inertinite and light plant tissues (specific gravity 1.168–1.96) float, whereas the substantially denser resistant minerals (specific gravity >2.6) and much of the heavier wood fragments settle to the base of the centrifuge tube (Figure 28). It should be borne in mind that the density of palynomorphs increases with thermal maturity, and this will affect the choice of the specific gravity of the heavy liquid used in centrifugation (Wood et al. 1996). Specifically, if the palynomorphs are highly aromatised , the heavy liquid used should have a specific gravity of 2.3 (Deunff 1977; Cody et al. 1996). However,

palynomorphs from indurated, thermally mature strata are often opaque and brittle; these ideally should not be centrifuged due to potential damage during this procedure (Grey 1999, 2000). Similarly, larger palynomorphs such as chitinozoa and megaspores are normally degraded or destroyed by centrifugation (subsections 15.2 and 15.6).

Heavy liquids used in palynology laboratories include bromoform, cadmium iodide solution, carbon tetrachloride, lithium hetropolytungstate solution, potassium iodide solution, sodium polytungstate solution, stannic chloride solution, tetrabromoethane, Thoulet's solution (an aqueous solution of cadmium iodide and potassium iodide, see Goeury and de Beaulieu 1979), zinc bromide solution, zinc chloride solution and zinc iodide solution. The most common media used are aqueous solutions of zinc bromide or zinc chloride, lithium hetropolytungstate solution and sodium polytungstate solution. Bromoform mixed with ethanol can be effective, but is currently not used extensively due to the toxicity of the former reagent. Zinc chloride and zinc bromide have specific gravities of 2.9 and 4.2 respectively, and must therefore be diluted to achieve the ideal specific gravity of 2.0–2.2. Zinc chloride is relatively viscous, which is a disadvantage of this substance relative to zinc bromide (e.g. Staplin et al. 1960). Zinc bromide and zinc chloride solutions should be acidified with a few drops of 10% hydrochloric acid to prevent the formation of precipitates of zinc hydroxide during this procedure. Bromoform and tetrabromoethane cannot be used with water hence the residue must be dehydrated before mixing with these substances. All the substances mentioned above except lithium hetropolytungstate and sodium polytungstate are hazardous. Bromoform, cadmium iodide, zinc bromide and zinc chloride are toxic. Hence these should all be used with extreme care. Krukowski (1988), Munstermann and Kerstholt (1996), Bolch (1997); Six et al. (1999), Zabenskie et al. (2006), O'Keefe and Eble (2012) and Campbell et al. (2016) recommended the use of sodium polytungstate (or sodium metatungstate) because it is effective and non-toxic, and can be recycled. Caffrey and Horn (2013) successfully used lithium heteropolytungstate solutions of specific gravities between 2.0 and 2.2 to concentrate pollen from palynologically sparse sediments. Lithium heteropolytungstate solution is less dense than sodium polytungstate, so may need less centrifuging time, and is non-toxic. This substance was further tested by Leipe et al. (2019). Non-toxic heavy liquids such as lithium heteropolytungstate and

sodium polytungstate solutions are non-corrosive, safe to use, and are relatively straightforward to recycle (Van Ness et al. 2017).

The concentrated, dehydrated post-oxidation organic residue is mixed with a heavy liquid and placed in a centrifuge tube. Centrifuge units have many tube holders so several samples can be centrifuged simultaneously (Figure 27). The optimum duration, heavy liquid and its precise specific gravity, and the relative centrifugal force (RCF) should be determined by trial and error, sample-by-sample. Many authors quote the speed of centrifugation in RPM. However, RPM data are not helpful unless the dimensions (specifically the radius) of the centrifuge unit are known. Rickwood (1984) and Pendleton (2006) described, using a simple equation, how to determine the RCF from RPM and the centrifuge rotor radius. The RCF is a parameter that can be easily replicated between centrifuge units. If the RCF is too high, overcompaction ('caking') can occur and, if it is too low, the palynomorphs will not be effectively separated. Jones and Bryant (2004) stated that if a RCF of 10,388 is not reached, the palynomorphs would not all be efficiently partitioned. This means that the RPM needed to reach 10,388 RCF should be calculated for any given centrifuge. Relatively few authors quote RCF data, however, there are a very wide variety of durations and speeds mentioned in the literature. However most relevant papers advocate centrifuging for 5–15 minutes at 500–2,500 RPM. Normally, 5 to 10 minutes of centrifugation is sufficient to achieve full separation of the light organic and heavy mineral fractions. Phipps and Playford (1984) recommended centrifuging in acidified zinc bromide solution at 1,000 RPM for about two minutes. They stated that a longer time and/or higher speeds may cause compaction of the organic float, which makes the organics difficult to separate. If palynomorph-mineral separation is not being effectively achieved, one or more of these variables should be altered. The simplest variable to change is the specific gravity of the heavy liquid being used.

Slowly increasing the speed of the unit, centrifuging at top speed, then relatively slowly coasting to a stop is a widely used procedure. This is used when, for example, some palynomorphs have been physically prevented from floating due to the presence of abundant mineral grains. This phenomenon is minimised if the centrifuge unit is accelerated to its maximum speed slowly. If this is done, mineral grains do not tend to

physically drag palynomorphs down into the heavy fraction (Traverse 1988; 2007). Alternatively, despite oxidation, there may be some residual dense mineral crystals, typically pyrite, within the palynomorphs that will cause them to sink down into the mineral fraction (Gelsthorpe 2002).

After the centrifuge has been stopped, either by braking or being left to coast to a stop, the dark brown/black palynomorph concentrate floating at the top of the tube (Figure 28), is pipetted off and the heavy liquid sieve-washed away. The residue can also be centrifuge-washed in water. The mineral concentrate in the bottom of the centrifuge tube should be thoroughly checked for palynomorphs, for example those which are pyrite-bearing. Separation may not have been complete, and in these cases the mineral concentrate should be re-centrifuged. However, if effective separation has been achieved, the heavy liquid in the centre of the liquid column can be recycled, provided it is filtered so that it is entirely devoid of palynomorphs. It should be noted that organic material is present from above the base of the lowest visible organic layer. In some cases the 'float' may be present virtually everywhere above the 'sink' and not merely concentrated into a thin, discrete layer at the top of the tube. This can often be remedied by a second high-speed centrifugation.

Forster and Flenley (1993) described a density gradient centrifugation procedure for separating different types of organic particles. A residue is suspended in potassium iodide solution and the different organic materials, including different palynomorph taxa groups, are separated by their respective buoyancies. Therefore specific organic types can be located and separated.

9.4.2.3. The Bostick tube method. Funkhouser and Evitt (1959) described using a U-shaped plastic tube for heavy liquid centrifugation. This protocol was emended by Evitt (1984) who used a straight cylinder which he called a Bostick tube. A rubber bung is placed into one end of a 10 cm length of flexible, narrow gauge (~1.6 cm) plastic tubing and the bung is secured with a metal ring. Approximately 5 ml of the organic residue is thoroughly mixed with an aqueous solution of zinc bromide (specific gravity 2.0), and placed in the tube. A drop of concentrated hydrochloric acid is added. The tube is then placed in a 50 ml centrifuge tube, and centrifuged for about 20 minutes at full speed.

Following centrifugation, the plastic tube is removed and the tube pinched immediately below the floating organic fraction using narrow-width pliers and the organics decanted off (Evitt 1984, fig. 1A). The residue is then diluted, homogenised, and repeatedly centrifuge-washed to remove the zinc bromide solution. Funkhouser and Evitt (1959, fig. 1) folded the plastic tube into a U-shape before placing it inside the centrifuge tube.

9.4.2.4. Centrifugation using light liquids. A procedure termed short (or differential) centrifuging was described by Funkhouser and Evitt (1959, p. 371) to remove extraneous fine debris, largely clay (subsection 9.2). The aqueous residue with a detergent is centrifuged for ~45 seconds at ~15,000 RPM. Surfactant detergents such as Alcojet, Alconox and Liquinox deflocculate and suspend clays during short centrifugation. The centrifuge is turned off, and allowed to coast to a stop naturally; no braking is applied. The palynomorphs are concentrated at the bottom of the tube and the suspended fine clay-rich debris can be decanted away. The process should be repeated, at lower speeds. The decanted liquor should be checked for palynomorphs and centrifuged if any are present in the fine fraction. Short centrifuging is unsuitable for residues when some palynomorphs are extremely small (<10 μm). This procedure was discussed by Phipps and Playford (1984) and Brown (1960; 2008). Bond (1964) described a similar method using a dilute detergent solution in water to remove extraneous colloidal material.

As mentioned above, centrifugation in light liquids is also frequently used to wash an organic residue of a chemical following a specific procedure such as acetolysis, alkali treatment or oxidation (subsections 9.3, 11.3). Here the residue is simply concentrated in water, by rotating the residue until it descends to the botton of the centrifuge tube. Similarly, light liquids can be used to separate different palynomorph fractions in a pure palynomorph concentrate. Using a light liquid, the larger and heavier palynomorphs such as chitinozoa are ‘thrown down’ during centrifugation much more readily than the smaller, lighter ones such as acritarchs which remain in suspension.

9.4.2.5. Extraction from honey and hydrocarbons by centrifugation. Centrifugation can be used to extract pollen from honey (subsection 14.10). Both centrifugation and filtration were reviewed by Lutier and Vaissière (1993), and a summary flow chart of

various procedures in melissopalynology was presented by Wood et al. (1996, fig. 6). Early studies on this topic advocated dilution, filtration and acetolysis, however some melissopalynologists do not filter, but simply centrifuge after dilution of the honey sample. The recommended sample size for honey is no lower than 10 g, but the endorsed duration, RCFs and speed of centrifugation vary considerably. The reported RCFs used are normally ~1350–2355, partly due to the type of honey examined. Lower RCF's are generally used for honey expected to yield larger pollen grains. Jones and Bryant (1994a) stated that some honey pollen has a specific gravity of ~1.0, so centrifuging honey in water may be ineffective. Instead, Jones and Bryant (1996) dissolved the honey in ethanol (specific gravity 0.8) prior to short-spin centrifugation. A liquid with a specific gravity of <1 means that fresh pollen will rapidly settle at the base of the tube during centrifugation. Using light liquids such as ethanol for centrifugation means that no pollen is lost in suspension, and that this procedure is relatively rapid.

Palynomorphs can also be isolated from hydrocarbons by centrifugation (subsection 14.4; Sittler 1955; Horowitz and Langozky 1965). The asphalt/bitumen or oil is dissolved or diluted respectively with organic solvents such as acetone, benzene, methanol, xylene or various mixtures thereof prior to centrifugation. When the relatively heavy palynomorph-mineral residue has been separated, the supernatant hydrocarbon liquor is decanted off, and the residue repeatedly centrifuge-washed until all the residual hydrocarbons are removed.

9.4.3. Flotation

Palynomorphs can be separated from the heavier elements in aqueous residues by flotation using liquids which are less dense than water (i.e. specific gravity <1). Both ethanol (specific gravity 0.789) and oil have been used for this. Ledingham and Chinn (1955) and Kurtz and Turner (1957) described a method of extracting pollen and spores from soils and other unconsolidated sediments. A light liquid acts as a carrier to float palynomorphs to the surface of an aqueous suspension of a sample. Specifically, the sediment is combined with oil and water in a test tube and the mixture vigorously agitated. The palynomorphs are brought to the top of the tube in the buoyant oil emulsion, which can then be removed using a pipette.

Hansen and Gudmundsson (1979) described a cheap and safe method of separating palynomorphs from extraneous phytoclasts, which avoids heavy liquid separation which they believed may damage palynomorphs. Their protocol is particularly effective on carbonised material and/or sparsely productive material. It is based on the fact that palynomorphs are hollow particles. If the residue is treated with ethanol to fill all the cavities, it dilutes any water present. The palynomorphs will be reduced in density in comparison to the solid phytoclasts such as wood, and the two fractions can be separated (Hansen and Gudmundsson 1979, fig. 1). The organic residue was treated with ethanol and transferred to a separation tube filled with water (Hansen and Gudmundsson 1979, fig. 2). After ~30 minutes, the phytoclasts have settled into the water and the palynomorphs float in the ethanol fraction and can be removed.

A method of separating palynomorphs from the denser mineral fraction by floatation in a mixture of bromoform and toluene with a specific gravity of 2.3 was outlined by Felix (1963). Brasier (1980) described the separation of microfossils, including palynomorphs, by flotation using carbon tetrachloride which has a specific gravity of 1.58. The aqueous residue is dehydrated using acetone, treated with carbon tetrachloride, mixed and left to stand for two hours. The organic fraction floats and can be decanted off. Similar methods were outlined by Frey (1955) and Lentfer and Boyd (2000).

9.4.4. Passive settling

Doher (1980, p. 15–18) outlined four methods using passive settling to separate the palynomorphs from heavy particles and any fine clay debris, and this can be effective if the residues are rich in the latter. These all rely on the different settling rates of palynomorphs and extraneous elements. In settling procedures A and B, a wetting agent such as Darvan No. 4 is added to the residue in a tube that is agitated. It is allowed to settle for 30 seconds and the suspended palynomorph-rich material is decanted or pipetted off, leaving the heavy fraction at the bottom of the tube. If palynomorphs remain in the heavy fraction, the procedure is repeated until they are all extracted.

Settling procedure C requires four adjacent 15 ml tubes in a stand (Doher 1980, fig. 3). The residue is placed in the left hand tube, is agitated and left to settle for 10

minutes. Then the top half of the tube contents is removed using a dropper and placed in the second tube. Both tubes are then topped up with water, agitated and left to settle. The top half of tube two is then transferred to tube three, and the top of tube one placed in tube two (Doher 1980, fig. 3D). This 'fractionation' process is continued until all tubes are used (Doher 1980, fig. 3F). Theoretically, the fourth tube should contain fine debris and, if it is free of palynomorphs, can be discarded. The remaining three fractions can then be amalgamated, and the palynomorphs concentrated.

Settling procedure D also aims to eliminate the fine debris, and uses multiple washes with methanol. This procedure aims to break up the fine organic material so as to enable the more efficient removal of this element using settling procedure C. The first nine steps involve the centrifugation of the residue in methanol, followed by decantation. The concentration of the methanol solution in the first step is 10%. This is increased to 25%, 50%, 75% and 95% in steps two, three, four and five respectively. It is then reduced to 75%, 50%, 25% and 10% (steps two to nine). The residue is then washed of methanol and settling procedure C is performed.

Another passive settling methodology to remove fine debris and residual clay was described by Colbath (1985). The residue is 'passively settled' in a solution of sodium hexametaphosphate. This reagent prevents the clay from flocculating and settling out with the palynomorphs (subsection 9.2). The upper half of the mixture, containing small, suspended clay particles, was decanted off after one hour. The vessel is refilled, and this process repeated until the supernatant is clear. However, perhaps unsurprisingly, this procedure caused significant loss of small palynomorphs (Colbath 1985, tables 3, 4). If this scenario is to be avoided, the supernatant should be centrifuged and examined for palynomorphs at regular intervals.

9.4.5. Swirling

Swirling is a very simple procedure, based on gold panning, whereby the aqueous palynomorph concentrate is gently rotated (swirled) in a large watch glass. It was first described by Funkhouser and Evitt (1959, p. 373–374), and is an effective, rapid and safe procedure for concentrating palynomorphs. It relies on the fact that palynomorphs are less dense, and hence more buoyant, than mineral grains and wood. This means that the

palynomorphs can be made to move into suspension, leaving the denser material in the bottom of the watch glass. The residue should be repeatedly swirled in order to attain maximum separation. Swirling can rapidly concentrate palynomorphs in sparsely fossiliferous residues.

The organic residue is placed into a large watch glass, ~20 cm in diameter or a tri-corner plastic beaker. A small amount of dispersing agent can be added at this stage. The watch glass is then filled to about two thirds capacity with water and the residue left to settle for about one minute (Figure 29A). Then the watch glass is gently rocked using the pressure of the index finger and thumb of each hand, or otherwise slowly rotated in a circular motion, so that the residue is gently agitated (Figure 29B). The aim of this is to initiate a slow circular flow of water, the gentle turbulence of which suspends the light palynomorphs in a plume in the centre of the watch glass. By contrast, the heavy carbonised plant material and mineral grains sink, and become concentrated at the bottom of the centre of the watch glass. This procedure is very rapid; normally the two density fractions are separated after around 30 seconds to one minute of gentle swirling. The separation is clearly observable because the heavy fraction, which is usually very dark in colour, is visible in the centre of the watch glass. Visual checks on the progress of the separation are easier if the watch glass is placed on a sheet of white card or paper (Figure 29A–C).

The next step is to physically separate the heavy and light fractions. This can be achieved in two ways. Doher (1980, figs 4–6) recommended that the watch glass be tilted and the heavy material in the centre removed using a pipette, thereby leaving the palynomorphs in suspension. If any mineral grains and wood remain, the residue can be re-swirled. When full separation is believed to be complete, the heavy fraction can be swirled again to check for any palynomorphs. An alternative strategy is to pipette off the the palynomorphs in suspension with or without tilting the watch glass after swirling (Figure 29C; Funkhouser and Evitt 1959, fig. 2c). This can be done repeatedly if necessary, and care must be taken not to lose certain palynomorph fractions. Pipetting the suspension can be done in several steps, each after a swirling. Another extremely effective method of separating the post-swirled fractions is simply to pour off the suspended palynomorphs into a vessel such as a small beaker directly from the watch

glass, leaving the dense fraction in the watch glass (Figure 29D). Again, this step can be repeated several times in order to ensure that all the palynomorphs have been separated.

Swirling generally works best for highly palynologically productive samples. The heavy fraction should be tested to see if any palynomorphs are present. If this is the case, the residue should be swirled again. It is good practice to archive the heavy fraction and the residual liquid in the watch glass, which can easily be concentrated by sieving. This is because it is difficult to pipette or pour off all the palynomorphs from the watch glass, so this residue can be re-swirled if more slides need to be produced. Furthermore, it is possible that the heavy minerals may prove useful for future geological investigations such as clast provenance analysis (e.g. Morton et al. 2002).

Because swirling is a relatively simple procedure, it can be undertaken at any stage of phase three. For example, the residue may have been centrifuged and oxidised. After oxidation, the neutral aqueous residue can be swirled to ensure that as much dense extraneous material has been removed as possible.

9.5. Ultrasonic treatment

9.5.1. Introduction

Ultrasound comprises cyclic sound waves with a frequency above the human audible range, which is 20 to 20,000 Hz. Hence ultrasound is not physically different from audible sound, it is simply inaudible to humans. It is used in many different fields and ultrasound devices use frequencies from 20 kHz up to several gigahertz; typically they are operate between 20 and 60 kHz. This equipment principally supplies focussed energy, to penetrate a medium and measure the reflection signatures for imaging and distance-measuring purposes. The most well known application of ultrasound is in medical sonography to produce images of, for example, the human foetus. It is also used in the nondestructive testing of items, to detect invisible flaws and for accelerating chemical processes, cleaning and mixing (Kundu 2014).

9.5.2. Ultrasound cleaning in general

The ability of ultrasound to deliver focussed energy makes it ideal for cleaning many delicate and small items such as electronic parts, jewellery, medical instruments, optical

equipment and wristwatches. Specifically, it can rapidly and safely clear away relatively soft materials from hard objects. In palaeontology, ultrasonic treatment can help to clear extraneous material from fossils (Stevens et al. 1960).

Ultrasound cleaning works by creating alternating high and low pressure waves in a liquid to form millions of microscopic bubbles which expand during low pressure waves, and collapse during high pressure waves. This collapse of the bubbles, or cavitation, causes a mechanical scrubbing action via jets of water which loosens any soft, adherent material on the surfaces of the hard objects. Because of the frequency, this scrubbing action can take place ~55,000 times per second, hence is extremely effective. Most objects will be cleaned after 30 seconds to two minutes. However, ultrasonic treatment should be used judiciously because if the energy levels are too high, they can damage the items being cleaned. It is known that high power ultrasound between 20 and 40 kHz can cause particles, for example biological cells, to disintegrate (Mason 2016).

9.5.3. The use of ultrasound in palynomorph preparation

Palynomorphs can be effectively concentrated from disaggregated organic residues by the use of ultrasonic treatment. Ultrasonification at suitable frequencies can deflocculate or fragment certain elements that occlude and/or obscure palynomorphs on microscope slides. It also can clear extraneous debris adhering to individual palynomorphs. The material most prone to removal using ultrasonic treatment is clumped amorphous organic material (AOM). The ultrasonic irradiation of the aqueous organic residue mixed with a detergent or a dispersing/wetting agent causes the break up of the fluffy AOM into small fragments that can then be sieved away. Alternatively, a tunable ultrasonic probe can be used (e.g. Sheenan 1992). This is placed into the aqueous residue in a suitable vessel such as a small beaker and switched on for a short interval.

Ultrasonification must, however, be used extremely carefully because it can badly damage or destroy palynomorphs if used for too long and at too high a frequency. Palynomorphs which are already brittle, damaged and fragile, or have been subjected to prolonged alkali treatment or oxidation, are especially susceptible to further degradation. McIntyre and Norris (1964) suggested that this is because ultrasound accelerates chemical reactions and that therefore any ultrasonifiction should be done prior to

oxidation. This was also recommended by Lennie (1968), who suggested 10 minutes ultrasonification prior to the oxidation stage, and Sheenan (1992). By contrast, if ultrasonic treatment is undertaken following the oxidation stage, the duration should be reduced to one minute to minimise any damage according to Lennie (1968).

Irrespective of preservation levels, it is known that certain palynomorphs are more resistant than others, and that certain ultrasonic frequencies are more destructive than others (Caratini 1980). The ideal frequency of the ultrasound and the optimal duration of the treatment are dependent on factors such as lithology, thermal maturation, nature of the organic material to be removed and the palynomorph groups in the sample. Therefore the intensity and length of time of the ultrasonic treatment for each sample is determined solely by the experience and judgement of the laboratory technician, and by trial and error sample-by-sample. Caution should be used and any sample should be given the absolute minimum level of ultrasound that it requires.

Dumait (1962a) reported no damage to acetolysed modern pollen after up to 15 minutes of ultrasound, however, some degradation was noted following 30 minutes of treatment. This author suggested that pollen grains may be damaged during ultrasonic treatment due to physical damage from mineral grains. This was subsequently disputed by McIntyre and Norris (1964), who recommended 10 minutes ultrasound as a being optimal. These authors noted that palynomorph morphology is an important factor. For example small pollen grains with thick exine and robust spores like *Densosporites* are, unsurprisingly, more resistant to to ultrasonic treatment than large, delicate pollen taxa such as *Pinus radiata* D.Don. Marceau (1969) recommended subjecting diluted aqueous palynomorph residues to relatively high power of ultrasound for what he described as a ‘short time’, and that the vessel should be constantly shaken. Sheenan (1992) found that after 60 seconds of ultrasound, the sacci of bisaccate pollen were removed and that, by contrast, angiosperm pollen remained undamaged after 180 seconds of treatment. The lowest possible output settings on the sonificator were recommended by Perrotti et al. (2018) in order to avoid damage to fragile palynomorphs. It is also important to use tuned, as opposed to standard, sonification because some wavelengths are much more damaging to palynomorphs than others (Jen O’Keefe, personal communication 2021).

Wire sieves and other fine-mesh instruments that may become clogged with debris during palynological preparation are easily cleaned by treatment in a standard ultrasonic tank (Kidson and Williams 1969). Detergents are usually added to the water in the bath so that maximum disaggregation of the clogging materials is effected (Barrs and Williams 1973).

Occupational exposure to ultrasound devices which emit >120 decibels can cause hearing loss, hence technicians undertaking ultrasonic treatment should always wear effective ear defenders. Supplementary Data Appendix 1.6 includes commentaries on 11 contributions on ultrasonic treatment in palynological preparation.

9.5.4. Ultrasonic cleaning baths/units and their use in palynological preparation

Normally, ultrasonification of the aqueous organic residue mixed with a small amount of detergent and/or dispersing agent takes place in a beaker or tube suspended in an ultrasonic cleaning bath (Figure 30). These ultrasonic cleaning units comprise a transducer and a bath which is filled with liquid (normally water plus a small amount of anionic detergent) during cleaning. The transducer converts electrical energy into mechanical energy as vibrations. These are transmitted to the water bath, where the liquid develops zones of compression and rarefaction which produce cavitation ('cold boiling') thereby effecting the palynomorph concentration/cleaning process (Mason 2016). After ultrasonification, the residue is sieved using a 10–20 μm mesh to remove the small fragments of extraneous debris.

Pojeta and Balanc (1989) outlined a five stage procedure for the ultrasonic cleaning of palynomorph residues. The cleaning tank is filled almost to the top with warm water, and a few drops of household detergent are added. Vessels containing residues should not be allowed to rest on the base of the tank. These should be suspended in the water column in the tank using the insert tray (Figure 30). The duration and intensity of the ultrasonic treatment should be carefully determined by trial and error, because palynomorphs can be damaged by excessive sonification or treatment with the wrong wavelength(s). The tank should be cleaned and dried after use and fingers, hands etc. should never be placed into the tank during operation.

9.5.5. Ultrasonic sieving

Palynomorph residues can be sieved and subjected to ultrasonification simultaneously (subsection 9.6.3). The ultrasound fragments extraneous material which is then immediately sieved away. This is done using a ultrasonic probe, the size of a pen. It is inserted into the residue and it disseminates ultrasonic vibrations. This can be done in any sieving device; Batten (1999) recommended that this be done in a sinter glass funnel (Figure 26).

Caratini (1980) described a small ultrasonic sieve where the post-acid residue is screened with ultrasound using nylon mesh in a ultrasonic water bath. This prevents the sieve becoming blocked or clogged with fine material, and simultaneously breaking up organic material and mineral aggregates. An ultrasonic filtration technique for samples with high levels of clay and silt was outlined by Tomlinson (1984). The residues were sieve-washed in a filled water bath which has a suction filter pump. An ultrasonic shaking device is housed in the water bath. The combined action of the ultrasonic vibration and the mesh effectively sieves the residue, the pollen remaining on the screen, while the dispersed clay and silt pass through. Jemmett and Owen (1990) subjected suspensions of *Alnus incana* (L.) Moench pollen in a one μm mesh bag in a water-filled beaker to ultrasonification. This was found to eliminate fine organic particles, but pollen damage was noted if the treatment was carried out for over five minutes. A sieving technique using nested sieves and an ultrasonic probe (or sonifier) was outlined by Wood et al. (1996). This can be used to fragment extraneous organic material such as wood fragments and amorphous material which can then be sieved away. Perrotti et al. (2018) recommended using a sonicating disruptor horn to help sieve away very fine (<10 μm) extraneous debris.

9.6. Sieving

9.6.1. Introduction

During phases three and four of the traditional processing technique the sample residue must be repeatedly sieved or filtered. This screening is normally done more than once, in order to remove coarse fragments, fine extraneous organic materials and mineral grains so as to further concentrate the palynomorphs (Araújo et al. 2011). Sieving is also used to

rinse away chemicals, detergent, excess stain, heavy liquid residues etc., and sort the palynomorphs into different size fractions. Nested sieves can be used to separate out the smaller acritarchs from the much larger chitinozoa, or the bigger megaspores from the more diminuitive miospores. For example, a 60 µm mesh will concentrate chitinozoans, and a 5–7 µm weave will retain the smallest palynomorphs (Figures 31–33). Urban et al. (2018) described the use of nested (stackable) cell strainers. Sieving is undertaken following the post-hydrofluoric acid neutralised phase, density separation, oxidation, ultrasonic treatment and staining stages.

Normally, following oxidation and/or ultrasound, the fragmented fine organic material is eliminated by sieving. A small amount of non-ionic detergent, dispersant or ethanol can help speed up this procedure. However, sieving is absolutely essential at the end of phase three, irrespective of which previous steps have been performed. Sieving concentrates the palynomorphs in the aqueous residue. Normally 10–15 ml of the aqueous residue is placed in a small vial in readiness for microscope slide production (section 10). A comprehensive review of sieving in palynological processing, and the equipment used, was given by Ediger (1986, p. 256–257, table 1); a newer method, using small nested microsieves was described by Urban et al. (2018).

Sieving the fines out of the organic palynomorph residue was first introduced by Reissinger (1939; 1950). Up to that time, filtration in palynology was mainly used to remove coarse fragments from coal macerations (e.g. Bennie and Kidston 1886), and to separate megaspores from smaller palynomorphs. The use of sieving increased markedly during the late 1960s, when metal sieves with mesh sizes down to 5 µm (± 2 µm) became available for the first time (Figure 34; Kidson and Williams 1969). Prior to this, the smallest mesh size was 37 µm, which was too large to prevent small palynomorphs passing through. Supplementary Data Appendix 1.7 comprises summaries of 15 contributions on sieving palynomorph residues.

9.6.2. Sieve materials and mesh sizes

Metal sieves, which were mainly used up to the 1960s, are delicate, expensive and are predisposed to clogging (Figure 34). Likewise, glass fibre and paper filters easily become blocked and are prone to contaminating the residue with fibres. Synthetic polymer cloth

sieves such as nylon, polyester and polypropelene are markedly superior to glass fibre, metal and paper; these became widely available during the 1960s and 1970s (Figures 31–33; Cwynar et al. 1979; Ediger 1986). Polyester is more resistant than nylon to chemical degradation, and the weave in polyester mesh is more regular than that of nylon (Lignum et al. 2008, pl. 1). However, nylon sieves, made from high tensile strength nylon bolting cloth, are most commonly used. This is because nylon mesh is inexpensive, and has a very high abrasion resistance, some elasticity, and will not deform under normal laboratory temperature conditions. It is woven into cloth with a regular weave, providing square holes of a consistent size, and hence is ideal as a filter. Nylon is relatively alkali-resistant, but degrades upon contact with strong acids. The latter means that it is essential that these screens are only used with palynomorph residues which have been thoroughly neutralised following acid digestion and oxidation.

Nylon sieves are especially good for eliminating fine particulate clay fragments and organic materials (Figures 31–33). If there are large levels of fine particulates in the residue, sieving times can be protracted because the mesh becomes clogged, especially if the mesh size is small. Tapping the sieve gently during sieving will increase the amount of liquid flowing through the sieve (Figure 32B). Moreover, the mechanical flexibility of nylon is an advantage because, *in extremis*, the mesh can be gently moved from below during the sieving process in order to prevent blockage. However, a disadvantage of doing this is that the holes in the mesh may enlarge, allowing small palynomorphs to pass through the sieve. This enlargement can be permanent if the cloth is subjected to prolonged manual manipulation. Furthermore nylon is difficult to use in vacuum systems and, as mentioned above, is degraded by many aggressive chemicals. Because of the risk of cross-contamination and mesh size increase, unless they can be cleaned effectively, they should only be used once.

Naturally, the mesh size is critically important. To remove coarse mineral grains and plant materials from the palynomorph concentrate, a sieve mesh size of ~150–200 µm is suitable and a metal screen is ideal for this (Figures 33, 34). The nature of the palynomorphs being studied dictates the mesh size of the fine nylon sieve used to remove the fine extraneous material. The aim is to remove as much fine particulate material as possible without the risk, or minimising the danger, of palynomorph loss. Sieving at 10

μm or 15 μm should ensure that the majority of palynomorphs are retained. However, many authors such as Raine and Tremain (1992) recommended using 7 μm mesh. Some taxa of, for example, acritarchs and angiosperm pollen are <20 μm in maximum diameter, hence a mesh size of 20 μm may allow the smaller forms to be lost. Clearly the differential loss of the smaller palynomorphs can potentially seriously bias the palynological data, and should be avoided. Certain acritarchs are extremely small (Habib and Knapp 1982). Also, Schrank (2003) reported Upper Cretaceous acritarchs that vary in maximum diameter between 4 and 17 μm, with the majority being about 10 μm. For this reason, Schrank (2003) did not sieve these residues. It is therefore recommended that, if possible, fine sieving is not undertaken if the smallest palynomorphs are critical to the study.

Lignum et al. (2008) studied the size and type of sieve mesh used following the acid digestion of limestone samples. New and used meshes were studied using the scanning electron microscope (SEM) to investigate aperture diameters and the nature of the weave. The true aperture size of 20 μm nylon mesh was found to be 28.3 μm when measured diagonally, meaning that objects >28 μm can easily pass through. Significant losses occurred through the 20 μm polyester (45%) and 20 μm nylon (20%) meshes. Both the 15 μm polyester and nylon meshes proved to be much more effective. Lignum et al. (2008) concluded that sieve mesh of ~15 μm is far more effective in preventing the loss of palynomorphs during sieving than 20 μm meshes. Sieve meshes of 20 μm are prone to allow some palynomorphs to pass through, and 10 μm mesh is susceptible to clogging with fine particulate matter (Lignum et al. 2008).

9.6.3. Conventional sieving with nylon mesh

Despite some authors, for example Vidal (1988), Heunisch and Muntzos (1990) and Ashraf and Hartkopf-Fröder (1996), advocating the use of automated sieving devices using features such as compressors and suction pumps, most laboratories utilise simple two-piece plastic sieves which house a square of nylon or polyester mesh (Figures 31–33). The first mention of these types of sieves was by Cwynar et al. (1979). These authors constructed nylon sieve holders using plastic food/kitchen receptacles with close-fitting and strong snap-on lids. The bottom of the container and the centre of the lid were

removed, then the nylon screen was placed between the newly-configured lid and body (Cwynar et al. 1979, fig. 2). If the size and robustness of the receptacle is suitable, this method is very effective.

Evitt (1984) described constructing a sieve using sections of glass or rigid plastic tubing cut to lengths of ~3–4 cm. Rubber or plastic rings that fit tightly over the tubing are positioned. The sieve is assembled by placing the square of nylon over the smaller piece of tubing. It is completed by pressing the larger tube over the smaller one such that the nylon is stretched across the end of the tube, forming the screen of the sieve (Evitt, 1984, fig. 1E). Zippi (1986) constructed a sieve cloth holder constructed from cut lengths of polypropylene centrifuge tubes. Bowler and Hall (1989) described a similar configuration, i.e. an outer sleeve and an inner tube. They placed a square of synthetic cloth mesh between two tightly interlocking sections of plastic pipe. The plastic pipes should be of suitable diameter, i.e. ~10–20 cm (Figures 31–33). Provided the sections of pipe fit together securely, the circular shape is highly ergonomic for sieving. Sheenan (1992) described a similar design, which was five cm in diameter. Several types of proprietary brands of sieve holders which would be suitable for use in palynological processing are currently available. These include polypropylene sieve holders and single sieves. Regarding the latter, standard mesh sizes between 500 (25 μm) and 1250 (10 μm) are suitable for sieving palynomorph residues.

The sieving method is extremely straightforward. The pH-neutral aqueous residue to be screened is poured into the sieve and the unit is either left to drain passively or is gently shaken and/or tapped in order to expedite the liquid passing through the mesh (Figures 32, 33). If the mesh is prone to clogging, the residue can be washed through using a gentle, narrow jet of water. This sluicing with water and shaking and tapping the sieve housing during this step helps the fines to be filtered out, and prevents the cloth mesh becoming blocked. Water can be mixed with detergent or 10% ethanol and in a wash bottle, and a stream of this can also help the fines pass through the sieve mesh (Sheenan 1992). Alternatively a small amount of detergent is simply added to the liquid in the sieve to prevent clogging of the mesh (Evitt 1984). The latter author also recommended using a medicine dropper used to pump the residue onto the sieve in order to expedite sieving. It is good practice to retain the filtrate in order to check for small

palynomorphs which may have passed through the sieve cloth. The fraction which remains on the mesh is then carefully decanted and washed into a suitable receptacle (e.g. a small beaker or a watch glass). It can then be examined to determine if the residue needs further density separation, oxidation etc.

Zippi (1986) recommended using the needle-tip of a vibrating engraving tool, covered by a rubber sheath, to speed up the sieving process. The rubber sheath is placed against the outside of the sieve holder with the engraver switched on. The vibrations thus caused disaggregate and suspend any clumps of particulates (e.g. AOM), hence speeding up the sieving process. In extreme cases an ultrasonic probe may be used, with care, to expedite the sieving process (subsection 9.5.5).

9.6.4. Sinter glass sieving

The first reports of the sieving of fine particulates in palynomorph preparation were by Reissinger (1939; 1950), who used discs of fritted or sintered glass to filter the aqueous residues. This technique, albeit somewhat modified, is still used extensively today to concentrate palynomorphs by allowing the alkali-soluble ulmins produced during the oxidation process to be sieved away (e.g. Neves and Dale 1963; Ediger 1986). The filter discs in question are made of fritted glass, and positioned in Büchner funnels (subsection 9.3.2.4). Sintered glass is very finely porous (the pore sizes vary from 1 μm to 500 μm) and allows gases and liquids to pass through. For palynology, pore sizes of 10–16 μm or 16–40 μm are recommended. Fritted glass is made by sintering (i.e. heating and pressuring) glass particles into a solid but porous material. The funnels are generally termed sinter glass funnels (as opposed to fritted glass funnels). The sinter glass funnels are then placed in Büchner (sidearm) flasks to keep them in position, and to collect the filtrate (Figure 26).

Natural gravity filtration through sinter glass Büchner funnels can be very slow because the filter discs of sinter glass are reasonably thick (~3 mm), and the pores are prone to clogging. Sieving small amounts of organic residue at a time, and dilution with water, mitigates this situation. However, this process can be speeded up by changing the pressure in the Büchner flask. Specifically, the direction of flow of liquid/air through the sinter glass filter disc can be changed from downwards to upwards in order to free the

pores that had become occluded by backflushing. This can be done using a reversible aspirating rubber bulb, tapwater pump or vacuum pump connected by rubber tube via the sidearm (Figure 26). Neves and Dale (1963, fig. 1) used a small compressor, a valve-controlled air system and a time switch to change the direction of flow. The time switch is very useful if filtration takes a long time. However, a much more pragmatic variation of this procedure is to use the aforemetioned manually-operated reversible suction/pressure device mounted on the sidearm of the Büchner flask (Figure 26; Sarjeant 1974, fig. 45). Ediger (1986) described a similar system that was termed his 'Modified Reissinger Apparatus' (MRA). Here the flask is partially evacuated of air by flowing tapwater. After use, the sinter glass filter disc is cleaned using chromic acid (subsection 6.2). Alternatives to the sinter glass filter disc include the Sartorius Cellulose Nitrate Membrane, which can be used for the automated mass production of sieved organic residues (e.g. Vidal 1988).

10. Phase four of the traditional technique – presentation of palynomorphs for microscopical study and sample material archiving

10.1. Introduction

After the aqueous palynomorph residue is sieve-concentrated to ~10–15 ml at the end of the previous procedures, the final phase in palynological processing can begin. Phase four comprises procedures for preparing the concentrate for microscopical study and archiving, and this account is subdivided into three further subsections. If the palynomorphs are pale in colour, they can be stained. Then microscope slides are prepared; these can be strew mounts or single/multiple grain slides. Finally the sample materials should be stored in a dedicated repository. In terms of the culinary analogy, this final phase of the traditional palynological preparation technique can be thought of as 'plating up and replacing the unused ingredients in the larder' (Figure 2). A comprehensive, pan-scientific review of all aspects pertaining to microscope slides, including curatorial procedures, deterioration, long-term storage, preparation, restoration and study, was given by Neuhaus et al. (2017).

10.2. Staining

10.2.1. Introduction

Despite the availability of specialist microscope illumination techniques such as differential interference contrast and phase contrast (Pluta 1989), which enhance the contrast of light coloured palynomorphs, staining the palynomorph residue is often necessary for effective observation and photography (Plate 1.3; 1.4; Riding and Head 2018). If palynomorphs are either primarily pale, or have been bleached of body colour via oxidation during diagenesis, preparation or weathering, they may be stained using a variety of proprietary dyes. This is done immediately prior to microscope slide production. Staining expedites observation/study, highlights micromorphology, provides enhanced contrast for photography and can help to differentiate certain palynomorph groups. The darker, stained, palynomorphs are normally substantially easier to study and to photograph; this is especially the case for those with delicate/thin structures such as spines etc. This technique can be used for other plant macrofossils (Wilson 1971; Krings 2000).

10.2.2. Practical aspects of staining palynomorphs

Suitable stains for palynomorphs include powdered dyes, which are soluble in water or alcohol, and liquid stains. Both of these types include Bismarck Brown, Calberla's Solution, Crystal/Gentian Violet, fuchsin (acidic and basic), Lactophenol Aniline Blue, Malachite Green, Methyl Blue, Green or Violet, Neutral Red, Ruthenium Red, Safranin O, Toluidine Blue, Vert Green and various food colourings. Safranin O, or basic red 2, is a cationic dye widely used in cytology and histology (Rosenberg 1971), and this red stain is the most widely used dye in palynology (Plate 1.3; 1.4; Casas-Gallego et al. 2020). Brown (1960; 2008) and Litwin and Traverse (1989) recommended Bismarck Brown; the former author stated that this stain does not cause overdarkening.

The final palynomorph concentrate is divided into two portions in case of overstaining and the potential need to retain some original residue if thermal maturation studies need to be undertaken; staining clearly masks the true palynomorph colour. Stains are normally made up to ~1% solutions in organic solvents or water as appropriate. One

or two drops of the stain solution are normally mixed with a portion of the aqueous palynomorph concentrate following processing in a small vessel, immediately prior to slide production, and left for a short time (Figure 35). The optimum length of treatment with the stain must be determined by trial and error, residue-by-residue. The mixing can be done in a small beaker or vial, or in a sinter glass funnel. The excess stain then should be washed, or centrifuged, away. If a sinter glass funnel is used, the removal of the excess stain is easy and quick to achieve using an reversible aspirating rubber bulb or 'hand bellows' (Figure 26).

Brown (1960; 2008) suggested that two or three drops of a 0.5%/99.5% mixture of Bismarck Brown solution and ethanol should be mixed with the palynomorph concentrate and left for 10–15 minutes. The mixture is then centrifuged, decanted, washed and mixed with mounting medium prior to slide production. Doher (1980, p. 20) recommended that Bismarck Brown Y or Safranin O is dissolved in ethanol or 90% methanol, then the residue is dehydrated using methanol prior to staining. Similarly, several drops of a concentrated solution of Safranin O in 90% ethanol and 5% hydrochloric acid mixed with the residue and left for 5–10 minutes before centrifuge-washing is also very effective (Jen O'Keefe, personal communication 2021). Barss and Williams (1973), Moore et al. (1991) and Green (2001b) stated that several drops of a dilute alkaline solution (<10% ammonium hydroxide or <10% potassium hydroxide) will help the stain to be absorbed by the palynomorphs much more effectively. Van Cleave and Ross (1947) noted that pre-treatment with a suitable detergent may help the penetration of stain.

It is very important that palynomorphs are not overstained, or key morphological features such as fine exine structure or low-relief ornamentation may be obscured. Casas-Gallego et al. (2020, pls 1, 2) is an example where the staning is very intense. It is difficult to remove some stains although, in general, it is possible that palynomorphs can be rebleached or reoxidised. Barss and Williams (1973) and Wood et al. (1996) suggested that overstained palynomorphs can be lightened by using a wash of 10% hydrochloric acid. Slightly acidified ethanol can also be used for this. For example, Safranin O can be removed by one or two washes with ethanol (Jen O'Keefe, personal communication

2021). Eshet and Hoek (1996) stated that brief ultrasonic treatment can reduce overstaining. However, Stevens et al. (1960) reported that this is not the case.

It is possible that the stain will run or 'bleed' into the mounting medium. For example, basic fuchsin stain 'bleeds' into glycerine jelly according to Litwin and Traverse (1989). Basic fuchsin only coats the surface of palynomorphs and,in a short time (~1-3 months), it will fall away, forming pooled accumulations around specimens and/or dissolving into most liquid or semi-liquid mounting media. By contrast, basic fuchsin works well with solid mounting media (Jen O'Keefe, personal communication 2021). Clarke (1963) used sodium hypochlorite to prevent the 'bleeding' of Safranin O stain from palynomorphs into Clearcol mountant.

The stain can be mixed with the mounting medium prior to slide production (Sanders 1966; Green 2001b). Next, several drops of residue are pipetted into the stain/mountant mixture and mounted on microscope slides. An example of this is 1% Safranin O solution in glycerine jelly. The palynomorphs then take up the stain from the mountant following slide production. It is absolutely vital that the stain and the mounting medium are compatible, so that the colour does not 'bleed' from the palynomorphs into the mountant. Supplementary Data Appendix 1.8 provides commentaries on six contributions on various stains and their use.

10.2.3. Differential takeup of stain by palynomorphs

Certain palynomorphs absorb dyes differently, hence staining can help in identification. An example of this phenomenon is that modern pollen grains stain to a variable degree, but they take up dye far more readily than spores. Fern spores stain relatively lightly and *Sphagnum* moss spores do not take up stain according to Brown (1960; 2008). Similarly, certain dinoflagellate cyst taxa take up stain far more readily than others (Plate 1.3). Another instance of this is that Safranin O appears to be especially sensitive to palynomorphs of different ages. Normally, the less thermally altered and/or younger the palynomorphs, the brighter the shade of red they are stained (Wilson 1964). The factors involved here include aspects such as depth of burial, tectonism and the level of thermal maturation. Wilson (1964) reported that allochthonous Carboniferous spores from the Paleocene of Arkansas, USA are dark-brown/black, and do not absorb stain. By contrast,

the indigenous palynomorphs are much lighter and absorb stain readily. Furthermore, some morphological features of palynomorphs, take up stain differently to others (Wood et al. 1996). Staining can also help to identify allochthonous palynomorphs, because caved or reworked specimens may stain differently to *in situ* forms (Wilson 1965).

10.3. Preparation of microscope slides

10.3.1. Introduction

When the final palynomorph-rich organic residue has been satisfactorily prepared and concentrated, microscope slides are produced. Normally strew slides are prepared for study. However, if detailed morphological study is needed, single/multiple grain slides can also be made (Figure 36). The production of palynomorph slides requires great care to present the palynomorphs for microscopical study effectively, and also of course to prevent contamination. The density of palynomorphs mounted on microscope slides should not be too dense or too sparse; the precise concentration is however subject to the preference of the individual microscopist and/or the nature of the task in hand (Plate 1). Wilson (1971a) stated that the optimum number of palynomorphs per slide is between 3,000 and 20,000.

The unique sample registration number plus, if possible, other relevant data (e.g. age, depth, lithostratigraphical unit, locality etc.) should be written on a paper label affixed to the slide. However, some institutions may prefer to keep details such as depth and locality confidential on secure corporate databases, and only give the sample number on the slide itself (Figure 36). In addition, the registration number should be indicated directly on the glass slide in indelible ink or using a diamond marker; this is a safeguard against the paper label becoming damaged or detached.

It is essential that the final palynomorph residue has been concentrated and washed to neutrality so that no chemical reagents from the various preparation procedures, which could adversely affect the microscope slides, remain. If the aqueous residues are contaminated, the optical properties of the coverslip and slide may be substantially compromised. It is also vital that the concentrated palynomorph residue be thoroughly homogenised by agitation prior to being drawn off for slide production. This means that the residue that is withdrawn using a pipette will be taxonomically

representative, and that no biases due to, for example, differential settling will occur (Doher 1980). Furthermore, slide production should always be done in a clean, dust-free environment to prevent contamination.

The slides used for palynomorph mounts should be of high quality, i.e. made from clear, optical quality material such as borosilicate or soda lime glass, and with ground smooth edges for safety (Figure 36). Standard microscope slides are 75 mm x 25 mm, and 1 mm thick. Normally, number 1 coverslips (or cover glasses) are used, which are 0.13–0.17 mm thick. This is because modern microscope objectives are normally designed for use with coverslips up to 0.17 mm thick (Wood et al. 1996). Coverslips are available in various sizes. Four-sided ones are normally ~20 mm in width. These can either be square or rectangular, with the latter being ~30 mm in length or even longer (Figure 36). Circular coverslips are also available. The use of 20 mm circular or square coverslips allow the use of two per slide, for example for oxidised and unoxidised residues, or for different size fractions (Figure 36C). Good quality coverslips and slides should of course be in pristine condition upon purchase. However, it is important to thoroughly clean all of these items with ethanol or soap and water prior to use in order to remove any residual dust or lubricants (Wood et al. 1996). In Supplementary Data Appendices 1.9. to 1.11, 29 commentaries on relevant contributions on all aspects of microscope slide production are provided. This subsection is subdivided into three more subsections. Firstly the materials used, i.e. embedding and mounting media are described. This is followed by subsections on strew slides and single/multiple grain mounts.

10.3.2. Embedding and mounting media

10.3.2.1. Introduction. Transparent embedding and mounting media accommodate the palynomorphs in suspension and attach the coverslip to the microscope slide respectively (Figure 37; Singer 1967; Ravikumar et al. 2014). A wide variety of products and substances have been used for embedding and mounting during the production of palynomorph slides. These media should be robust, have good clarity, not degrade and have suitable refractive indices for microscopy (Table 2; Herngreen 1983, p. 24–26). If palynomorphs are mounted in a medium which has a refractive index either too different or too similar to the sporopollenin being studied, they will have poor optical definition in

transmitted light (Berglund et al. 1959). For example if one mounts unacetolysed modern pollen in water (refractive index 1.33), the significant difference in the refractive indices means that the pollen grains have a dark outline and excessive contrast (Traverse 1988; 2007). The refractive indices of acetolysed/fossilised and fresh sporopollenin are substantially different, i.e. 1.48 and 1.55–1.62 respectively (Table 2; Christensen 1954; Jones 1984). This means that the refractive indices of the embedding and mounting media should ideally not be within 1.55–1.62, the refractive index of fresh sporopollenin, in order for effective microscopy if one is working with modern material which has not been acetolysed. Media with refractive indices of 1.40–1.46 are hence ideal for acetolysed and fossil material. Those of 1.55–1.60 and above may give too little contrast or produce confusing images (Andersen 1965). Canada balsam (refractive index 1.54) and glycerine jelly (refractive index 1.43) are suitable for mounting palynomorphs, but both these media are prone to long term degradation. Despite the similarity in the refractive indices of deep time sporopollenin and Elvacite (Table 2), the latter is an effective mounting medium. The most widely-used embedding media reported n the literature are Cellosize and polyvinyl alcohol. By contrast there is a much wider range of mounting media, but most contemporary practitioners use Canada balsam, clear glass adhesive, Elvacite, glycerine USP, glycerine jelly and silicone oil. However, numerous other media have been used including albumen, Amman's Lactophenol, Castolite, corn syrup, Diaphane, Entellan, Eukitt, Euparal, Euparol Green, Glue4Glass, glycerol, Gum Arabic, Gum Damar, Histoclad, Hoyer's Solution, Hyrax, lactic acid, Lurifax, Malinol, MeltMount, Neo-Mount, Okol, Permount, Petropoxy 154, Piccolyte, Pleurax, polystyrene, Realgar, Sirex, Styrax, Tanglefoot, Vinylite and Viscol. Some of these are mentioned in relatively old literature, hence certain of them may not be currently commercially available (e.g. Brown 1960, 2008; Green 2001b). These media differ, for example, in their durability, effects on palynomorphs and optical properties. It is also possible to make temporary mounts of palynomorphs in distilled or RO water (Berglund et al. 1959; Harland and Sutherland 1972).

It is possible that the embedding/mounting medium type and thickness may increase palynomorph size (e.g. Reitsma 1969; Large and Braggins 1990; Meltsov et al. 2008). Glycerine jelly and silicone oil are prime examples of this. This is due to swelling

because of osmotic effects, and/or overpressure from the coverslip. To avoid the crushing and distortion of delicate palynomorphs by the coverslip, tiny cover glass supports can be used. These can be small pieces of clay, small shards of coverslip glass, strands of fibreglass wool, small plastic spheres or fine sand grains (Figure 37A; Cushing 1961; Moore et al. 1991; Miller 1996). Elvacite and clear glass adhesive slides can be stored vertically, however Canada balsam, glycerine USP, glycerine jelly and silicone oil slides should be stored horizontally in order to avoid slippage of the coverslips (Figure 38). Four of the most commonly used mounting media are discussed in the following subsections.

10.3.2.2. Canada balsam. This is a mountant made from the resin of the balsam fir tree (*Abies balsamea* (L.) Mill.) of Arctic Canada. It is prone to darkening and deterioration, and has a fairly low melting point. Canada balsam also has a relatively high refractive index (1.54) that may make the study of pale modern palynomorphs difficult. Neuhaus et al. (2017) stated that the expected lifetime of this mounting medium is 150 years.

10.3.2.3. Elvacite. This mounting medium is a high molecular weight iso-butyl methacrylate resin widely used as an adhesive, as a coating and in the printing industry. It is a plastic derivative that is dissolved in a solvent, typically xylene, to produce a viscous liquid that dries to give a clear, permanent mount which is extremely durable. The refractive index (1.48) is identical, or very similar, than that of sporopollenin (Table 2). Despite this, Elvacite has proved eminently suitable as a mountant. Elvacite is very useful for fluorescence microscopy because it does not fluoresce (subsection 17.5). If unusually large grains, such as intertinite (fossil charcoal) or mineral crystals, are present on a coverslip mounted using Elvacite, dendritic air bubbles may form. Furthermore, if the Elvacite did not cure properly, or the coverslip was moved during the curing process, dendritic air bubbles tend to form in the centre of the coverslip (Dunn 2003, fig. 6c). Particular care should be taken to use a pure palynomorph residue because Elvacite can be prone to some crystallisation initiated by residual chemicals from processing (Dunn 2003, fig. 6b).

2222

2223 *10.3.2.4. Glycerine USP*. Glycerine (glycerin or glycerol) is a sugar alcohol
2224 derived from animal products, petroleum or plants. Today, most glycerine USP (i.e.
2225 pharmaceutical grade) is derived from plant (typically palm) triglycerides or is
2226 synthesised. It is a clear, colourless, hygroscopic, non-toxic, odourless, sweet tasting,
2227 viscous, water soluble liquid with a high boiling point (SDA 1990). Its refractive index is
2228 1.47 (Table 2). Like silicone oil (subsection 10.3.2.6), glycerine USP is used as a 'wet'
2229 mounting medium, and the dehydrated residue is mixed with silicone oil and spacers
2230 before being mounted on microscope slides beneath coverslips which are sealed with
2231 varnish or paraffin wax (Figure 37A). Many practitioners, especially in North America,
2232 store palynomorph residues in glycerine USP. Typically, several crystals of phenol are
2233 added to the phial in order to prevent fungal growth (subsection 10.4.3).
2234

2235 *10.3.2.5. Glycerine jelly*. Sometimes termed glycerine gelatin, glycerine jelly
2236 is a water soluble mounting medium with a low melting temperature. It is a mixture of
2237 gelatin, glycerine, phenol and water, and has a suitable refractive index (1.43, especially
2238 for fossil palynomorphs. The phenol is to inhibit fungal growth. Dunn (2003) quoted a
2239 recipe for glycerine jelly of 70 ml of glycerine, 10 g of gelatine, 0.2 g of phenol and 60
2240 ml of water. The ingredients are warmed in a water bath until a homogeneous solution is
2241 formed. This reduces the water content, and thus minimises desiccation of the glycerine
2242 jelly in future.
2243 Unfortunately, as mentioned above, glycerine jelly is prone to cracking and
2244 dessication (e.g. Woessner 2005). This deterioration commences at the periphery of the
2245 coverslip, and moves into the centre of the slide (Figure 39; Dunn 2003, fig. 6d). It can
2246 also autooxidise the palynomorphs, and is susceptible to fungal infection despite the
2247 addition of phenol. To prevent drying out, the coverslips must be thoroughly sealed at the
2248 periphery using clear varnish or molten paraffin wax (Figures 37E, F; Barghoorn 1947).
2249 The use of glycerine jelly was described, for example by Traverse (1965) and Zander
2250 (1997; 2014 and references therein). If a specimen in a glycerine jelly slide needs to be
2251 repositioned, heat from a gently-applied soldering iron will also allow reorientation by

partially melting the medium. Wilson (1971b) described how to extract and remount palynomorphs from existing slides that were originally mounted using glycerine jelly.

Glycerine jelly is unsuitable for use in tropical climates due to its low melting temperature (~40°C). Traverse (1965, p. 607) confirmed the observation of Cushing (1961) that most pollen grains mounted in glycerine jelly increase in size. Cushing (1961) believed that, in the mounted slide, glycerine jelly absorbs water and this causes palynomorphs to swell. However, because pollen residues stored in glycerine in vials remain fresh and undeformed, Traverse (1965) felt that the swelling and/or degradation of pollen in glycerine jelly slides is entirely related to compression by the coverslip, contact with glass and/or exposure to air. Glycerine jelly exhibits some autofluorescence, and hence is not ideal for use in fluouresence microscopy because it can obscure the potential fluorescence of the palynomorphs (subsection 17.5).

10.3.2.6. Silicone oil. This mounting medium has a refractive index of 1.39 and is used extensively in modern pollen research, but can be used for any palynomorphs. The dehydrated palynomorph residue is simply mixed with silicone oil and spacers then mounted on slides under coverslips which are securely sealed with clear varnish or paraffin wax (Figure 37A). It is important that the palynomorph concentrate is dehydrated as silicone oil is immiscible with water and benzene or tertiary-butyl alcohol has been used for this (Moore et al. 1991). Recently however, Whitney and Needham (2014) demonstrated that isopropyl alcohol is a better dehydrating agent as it is miscible with silicone oil and water, and does not freeze at room temperature.

The silicone oil method gives a palynomorph suspension, and the high viscocity of the silicone oil allows the grains to be rotated by applying gentle pressure to the coverslip (Flenley 1980). This means that a pollen grain can be studied and photographed in equatorial/lateral and polar views. For example the colpi, exine and pores, which are vital for reliable pollen identification, can be studied from virtually any angle. Dinoflagellate cysts also often need to be manipulated in this way for comprehensive study (Evitt 1984). However grains may of course move position under the coverslip in these mounts, even if they are stored horizontally, meaning that key specimens cannot be reliably relocated. This means that silicone oil mounts are unsuitable for type slides, and

in studies where relocation is absolutely essential for example in forensic palynology (subsection 14.15). Cushing (2011) warned that if slides mounted with silicone oil are sealed to hold the coverslip in place, chemicals from the sealant can seep into the oil and cause degradation of the palynomorphs. One of the conditions noted by Cushing (2011) was termed pollen pox turns pollen grains into featureless spheres. This phenomenon was also discussed by Caffrey and Horn (2012).

10.3.3. Strew slides

10.3.3.1. Introduction. A strew slide comprises a glass coverslip, which is permanently attached to a clearly-labelled glass microscope slide (Figures 36A, B; 37B–D). There are two, somewhat similar, procedures used for producing these. The most widely-practiced strategy is where several drops of aqueous residue, with or without an embedding medium, are placed onto a coverslip which, when dry, is then attached to the microscope slide using a mounting medium. The other methodology involves mixing the palynomorphs with a mountant or mounting medium, then covering a suitable amount of this mixture with a coverslip on a microscope slide. Both procedures are described below. Wilson and Goodman (1964, fig. 1) is an excellent pictorial account of this procedure.

10.3.3.2. The embedding and mounting media method. The most widely practiced method of producing strew slides is to use both embedding and mounting media (Wood et al. 1996). Here, the embedding medium entirely surrounds the palynomorphs and cements them to the coverslip, whereas the mounting medium simply permanently attaches the dry coverslip to the glass microscope slide (Figure 37B). This strategy was termed ‘double mounting’ by Traverse (1988; 2007, p. 629–631). In the older literature, the embedding medium is sometimes erroneously referred to as the mounting medium.

The earliest description of this method was outlined by Norem (1956) who placed a thin film of albumen (egg white) onto the area of the slide where the coverslip is to be placed and left to dry. This film of albumen acts as an adhesive embedding medium, and prevents the palynomorphs being floated away by the mounting medium. A drop of the palynomorph residue is placed onto the area with the albumen film and left to dry. Next a

drop of mounting medium is placed onto the dry residue and the coverslip is carefully placed on top.

The method of Norem (1956) is effective but, since the late 1950s, the residue is generally mixed directly with a chemical embedding medium (Figure 37A). A portion of the aqueous residue is mixed with a small amount of an embedding medium such as a 2% hydroxyethyl cellulose (Cellosize) solution (Jeffords and Jones 1959; Jenkins 1967; Smith and Butterworth 1967) and/or polyvinyl alcohol (Clearcol or Elmer's clear school glue), and several drops of this are carefully dropped onto a coverslip (Figure 40A, B). Cellosize and polyvinyl alcohol are also dispersants, and they prevent the palynomorphs forming clumps on the coverslip. The Cellosize/polyvinyl alcohol and palynomorph concentrate mixture is hence an embedding medium because it permanently affixes the palynomorphs to the coverslip in a single optical plane (Figure 37B). Slow drying of the coverslip will helped to avoid clumping of the palynomorphs. The dry coverslip is then carefully turned onto a mounting medium such as Canada balsam or Elvacite to complete the slide making process as described above (Figures 40C, D).

By contrast, some laboratories simply allow the aqueous paynomorph concentrate to evaporate on the coverslip prior to carefully and slowly inverting the dry coverslip onto a small portion of a mounting medium on a slide (Figure 37D). This can be effective, but there is a possibility that the palynomorphs will not all being in the same optical plane when the water has all evaporated. To avoid this, it is strongly recommended that at least a dispersant such as Cellosize, or another non-dispersing film-former, be used.

In certain instances, small crystals may grow around the palynomorphs and other grains, causing haloes to form in the embedding and mounting media (Dunn 2003, fig. 6b). This phenomenon was attributed to the recrystallisation of dispersants (Cellosize or polyvinyl alcohol solution). Similarly, in samples that are prone to producing petrofilaments, it is recommended that the embedding and mounting media used are relatively chemically similar and do not react with asphaltene (Graham et al. 2000).

10.3.3.3. The palynomorph and mountant mix method (Figure 37C). Using this strategy, a small amount of Canada balsam or glycerine jelly is placed in the centre of a

microscope slide on a hotplate which has been heated to ~100°C and left for 1–2 minutes. When the mounting medium is suitably liquid, one or two drops of the homogenised palynomorph concentrate are added, and the two components thoroughly mixed using a thin glass rod, needle or wooden toothpick. This mixture can be done in reverse, with the drops of palynomorph residue placed on the heated slide, then heated Canada balsam or molten glycerine jelly added. Alternatively the mixture does not need to be done directly on the slide, and can be effected in a vial or similar vessel, then transferred to the slide using a pipette. The latter strategy may save time if several slides need making up. Funkhouser and Evitt (1959) described using water-miscible polyvinyl alcohol solution as a mountant in this way. This is mixed with the palynomorph residue, but there is no need to use a hotplate.

After mixing the mountant and residue, a coverslip is then carefully placed onto the mixture. Specifically one edge of the coverslip is placed onto the slide immediately adjacent to the mixture, and the other side gently and slowly lowered onto it using a mounted needle, scalpel blade or delicate tweezers, avoiding the incorporation of air bubbles (Figure 40D; Wilson and Goodman 1964, fig. 1.9). The mixture is allowed to flow to the periphery of the coverslip, hence it is important to place an appropriate amount of mounting medium onto the slide. If any air bubbles are observed, these can be eliminated by gentle pressure on the coverslip using a wooden toothpick. Some delicate palynomorphs may be crushed or otherwise deformed by the weight of the coverslip. To prevent this, tiny spacers can be used (subsection 10.3.2.1; Figure 37A).

Then the slide is left so that the mountant can cool and cure. This can be done with the slide inverted on a hotplate heated to 40–50°C for up to 48 hours depending on the mounting medium (Phipps and Playford 1984; Litwin and Traverse 1989). The slide being inverted in this way allows the palynomorphs to settle into one optical plane, close to the surface of the coverslip, so that examination with high power objectives is made easier. Alternatively, the slide is left to cure the right way up. In this case the palynomorphs settle to the base of the mounting medium in a single optical plane (Figure 37C).

When the mountant has hardened, the slides are carefully cleaned using acetone or ethanol. The coverslips should be carefully sealed to prevent dessication if glycerine jelly

has been used and finally labelled. If a liquid or low viscocity mounting medium is used the coverslip needs a highly effective sealant to hold it in place, and to prevent dessication. This is normally achieved using clear nail polish/varnish or paraffin wax. Caffrey and Horn (2012) recommended using three-in-one nail laquer that is less than 36 months old as a sealant. If paraffin wax is used as a sealant, it should have a melting point of >64°C (Chanda and Ganguly 1980). A continuous, narrow strip of sealant is placed around the coverslip (Figures 37A, E, F). Alternatively, the residue can be mounted between two coverslips, then attached to the microscope slide using transparent adhesive tape (Schopf 1960; Traverse 1988, 2007).

10.3.3.4. The clear glass adhesive method. In recent decades, high quality clear (invisible-when-dry) glass adhesives, normally acrylate gels, have become widely available (Noetinger et al. 2017). This product, an example of which is Norland optical adhesive, which can be bought in most hardware stores is ideal for attaching dried palynomorph coverslips to microscope slides. Clear glass adhesive forms high strength bonds with very good optical clarity. The dry coverslip with dispersant, embedding medium and palynomorphs is gently and slowly inverted onto a small amount of clear glass adhesive on a microscope slide (Figure 40D). It may need to have air bubbles removed using a toothpick. When the coverslip has been carefully positioned, the slide is simply placed in direct sunlight or under a ultraviolet lamp for a short time (normally less than one minute). The ultraviolet light quickly cures the adhesive via a photochemical process, thereby making a cured, useable microscope slide in minutes rather than hours. It is recommended that laboratories test several of these products in order to find the the most suitable clear glass adhesive in terms of, for example, optical clarity, refractive index and robustness. Several well-known and widely-available optical adhesives have been used successfully for this purpose. It is possible to obtain these products with refractive indices of between 1.48 and 1.50 (Table 2; Tennent and Townsend 1984). A review of these ultraviolet-curable acrylate gels as a mountant for palynomorphs was given by Noetinger et al. (2017).

10.3.4. Single and multiple grain mounts

10.3.4.1. Introduction. This subsection describes the production of single and multiple grain mounts. Strew mounts have certain disadvantages if key specimens, such as nomenclatural types, need to be studied in great detail and photographed for publication. Palynomorph specimens can be obscured, or partially occluded, by extraneous palynodebris in strew mounts due to the random nature of the configuration of kerogen and palynomorphs on these slides. Alternatively, other grains or phytoclasts can be adjacent to, or very close to, the key specimen, thereby detracting from its appearance on a photographic plate (e.g. Plate 3.7). Separate objects can easily be removed digitally, but the editing of the palynomorph specimen itself to remove a piece of kerogen or a mineral grain is extremely bad practice (Riding and Head 2018). Furthermore, relocating key specimens on densely-mounted strew slides can be problematical (subsection 17.7).

10.3.4.2. The Faegri Method. To avoid the problems caused by overcrowded microscope slides, the technique of mounting single palynomorph specimens was first developed by Faegri (1936, 1939), Erdtman (1943), Klaus (1953) and Mädler (1956). Grains may be picked directly from aqueous residues and individually remounted. However, the more usual methodology is that a microscope slide is heated to ~150°C on a hotplate. A drop of glycerine jelly is placed on the slide, and mixed with one drop of the aqueous residue then allowed to cool. Next a suitable specimen is located microscopically, and its position marked on the underside of the slide. The grain is then cleaned of any adherent debris with a fine needle, the point of which has been sharpened. When it has been fully isolated of debris and medium it is moved using the needle and placed on a new slide. A piece of the original glycerine jelly around 200 μm across can, with practice, be relatively easily picked out. Alternatively, the grain can be picked from the original slide using a tiny piece of glycerine jelly which is attached to the fine needle. The selected grain readily sticks to the glycerine jelly fragment. The specimen and glycerine jelly fragment are then placed on another heated slide, which melts the surrounding jelly. The droplet is then carefully covered with a circular coverslip, which is then encircled by shavings of paraffin wax. The heat melts the wax, which then flows underneath the coverslip, surrounding the specimen and attaching the coverslip to the slide. When the slide has cooled, the specimen should be within a small, clear area of

glycerine jelly, surrounded by opaque wax (Figures 36D, 37E; Plate 4.1). The excess wax is removed with an organic solvent and the coverslip is thoroughly sealed using a suitable clear varnish. An alternative method is to fully remove all the kerogen and palynomorphs from the original slide using a scalpel then more pure glycerine jelly is added and the specimen remounted and sealed as described above (Doher 1980).

One coverslip can accommodate many more than just one palynomorph specimen. Frequently practitioners place more than one grain per slide, as multiple grain mounts (Plate 4.2). Examples of the use of single/multiple grain mounts are the figured specimens in the nine papers in Laurie and Foster (2001). The overwhelming majority of the photomicrographs in this major taxonomic work are of specimens on single/multiple grain mounts.

10.3.4.3. The Micropipette Method. A second technique for producing single/multiple grain mounts is the micropipette method. This was first described by Anderson (1958), and is a length of narrow (4–5 mm) glass capillary tube which has been heated, extended, bent to ~90° and carefully cut with a diamond scribe when cool so that the distal end is ~100 µm in diameter (Evitt 1984, fig. 1B,C). The proximal end is attached to some rubber tubing. The apparatus is attached to a mechanical microscope stage so that picking can be easily done using the microscope (Anderson (1958, figs 1, 2). A palynomorph residue is mixed into a thin film of glycerine, molten glycerine jelly or oil on a microscope slide. Suitable specimens are extracted by lowering the micropipette onto the grain. If necessary, gentle suction can be applied to the proximal end so that the specimen is drawn into the distal end and deposited onto a new slide and a single grain mount made as outlined above.

This technique was also used by Gocht (1972) and Damassa (1979). The latter author individually picked dinoflagellate cyst specimens from the aqueous residue using a micropipette attached to a syringe with rubber tubing under a microscope. Traverse (1988, 2007) also described using the micropipette. This author used glass tubing 3 mm in diameter and attached it to a syringe as described by Damassa (1979). The palynomorph residue is spread out on a slide in a mixture of glycerine, molten glycerine jelly or oil and water. A selected specimen is cleaned of palynodebris using a fine needle

then picked up using the capillary tube. It is then blown out onto a piece of glycerine jelly on another slide, acetolysed or oxidised if neccessary and mounted as described above.

Evitt (1984) also commented on this apparatus. He recommended that the syringe be used only for major movements; delicate operations are most normal, and are performed by gently squeezing the plastic tubing with the fingers. The distal tip is inserted into the medium and the plastic tube squeezed; the pressure is released and, as soon as fluid flows into the micropipette, the tip is withdrawn. One micropipette can pick up several specimens before discharging them.

10.3.4.4. The glass-cutting technique. Wilson (1971a, p. 32–33) described another method of producing single grain mounts. Specimens can be extracted from strew slides using a slide marking objective, which is used to cut through the glass coverslip above the grain. The slide is then heated and the glass fragment lifted off using a fine needle and placed onto another heated slide. The specimen is then picked up with a needle, and placed onto a drop of gycerine jelly on the second slide. It is then preserved under a circular coverslip, when the glycerine jelly has partially hardened. This partially cured glycerine jelly prevents crushing of the specimen by the coverslip. The specimen can be oriented, and any extraneous material dispersed before the glycerine jelly fully sets. The single grain mount is then sealed with paraffin wax as described above. The hole(s) in the coverslip of the strew slide should be sealed with laquer. However, if many specimens from a single strew mount are required, it may be best to entirely remove the glass coverslip. This is done by removing the varnish sealant and heating the slide. The palynomorph-bearing glycerine jelly is scraped off, water added and the mixture spread out on a clean, heated slide. Specimens can then be removed with a fine needle in the normal way. Hill (1983) described a similar procedure.

10.4. Storage and recovery of samples, slides and palynomorph concentrates

10.4.1. Introduction and the storage of excess sample material

This subsection describes how palynology sample materials are curated. Good practice in slide/residue storage is essential if the materials are to have long-term stability. Moreover, it is very important that a palynology sample can be reprepared if, for

example, more slides are needed or it is suspected that the original preparation was in some way compromised or suboptimal. Therefore relatively large samples should be collected if possible so that repreparations can be made or that other tests can be carried out (subsection 7.2.2). The excess sample material should be retained, preferably in its original packaging, and curated in a dark, secure, temperature-controlled storage facility. In Supplementary Data Appendix 1.12, eight commentaries on articles on all aspects of this topic are given.

10.4.2. Storage of microscope slides

As with the raw sample material, palynomorph slides require effective and safe curation and storage (subsection 7.3). Like with the raw samples, a clean, dark, dry, secure, air-conditioned room is the best type of repository. Chanda and Ganguly (1980) recommended that slides should be stored in an air-conditioned room maintained at 15–20°C. There are several slide storage solutions, principally cabinets and slide boxes. The latter are widely available in different sizes and capacities (Figure 41; Dunn 2003, fig. 3). Plastic or wooden slide boxes are very useful for temporary storage, or for sending slides through the mail. However, they are not necessarily the best solution if the overall collection of which they are a part runs to many thousands of slides. For example, horizontally stacked slide boxes are highly susceptible to falling over and they can frequently be misfiled if stored in this way. However, because of the possibility of coverslips moving in vertical storage solutions, slide boxes should ideally be stored vertically so that the slides are horizontal (Figure 37). Furthermore, it is frequently difficult to locate and retrieve specific slides or collections of slides if the collection is housed in tens or hundreds of stacked slide boxes.

Large steel and wood cabinets are available which allow the horizontal and vertical storage of palynomorph slides. If a liquid mountant or one with a low melting point have been used, the slides must be stored horizontally with the coverslips facing upwards, so that movement of both coverslips and palynomorphs is minimised. Additionally, all type slides should ideally be curated horizontally in bespoke cabinets so that they can be easily monitored for degradation and retrieved for study. These type of

cabinets can be large (Figure 38). However, bespoke cabinets like this are not necessarily the most space-efficient.

By contrast, if the mounting medium used is permanent one such as Elvacite, slides may be safely stored vertically. Many styles are available and many have drawers with slots specifically designed to store glass microscope slides vertically, i.e. ~78 mm deep and ~28 mm wide. These cabinets are typically 40–50 cm deep hence each drawer can store hundered of slides. Units such as this are extremely space-efficient, capable of storing many thousands of slides in a small space (Dunn 2003, fig. 2). However, individual slides can be difficult to relocate and retrieve, especially if the slides are tightly packed.

Another strategy is to house slides in thin sheet metal holders which can be archived in metal or wood cabinets (Figures 42, 43). These cabinets are identical to ones which were used to store index cards (Riding et al. 2012). Typically, each slide holder houses four slides and they are 12.7 x 7.7 cm in size (Figure 43).

10.4.3. Storage of aqueous palynomorph residues

Following processing and initial slide production, the excess aqueous palynomorph concentrate/residue should be stored in case, for example, further slides are needed or that scanning electron microscopy is required. Organic residues can also be used for various geochemical analyses. Most laboratories store the palynomorph residues in small, labelled glass or plastic vials with effective (i.e. airtight) seals to prevent contamination, bacterial/fungal infection and dessication (Figure 44; Dunn 2003, figs 4, 5). Plastic is, of course, much less fragile than glass. ‘Click on/off’ vial caps made of plastic are recommended. Push-in stoppers (‘corks’) or screw-tops are not as reliable according to Dunn (2003). If push-in stoppers are used, the edge of the cork should be sealed with paraffin wax. It is important that the sample number be etched, scratched or written directly onto the vial and the stopper because adhesive labels and adhesive taped labels can degrade and detach if stored for long periods (Dunn 2003).

The organic residues, and sometimes palynomorph slides, may become infected by bacteria and fungi while in long-term storage. Palynomorphs are attacked and destroyed by fungal growth, and this can eventually swamp the residue. Contamination,

dessication and bacterial/fungal infection are considerably worse in hot and humid climates. However these issues can normally be avoided. Firstly high-quality vials should be used with close-fitting closures, preferentially click on/off or screw-top caps, which make an effective hermetic seal (Doher 1980). This will prevent dessication and the ingress of bacteria, contaminants and fungal spores. In order to prevent bacterial and/or fungal infestation of the aqueous organic residues, several drops of a preservative should be added to each tube. Examples of these anti-bacterial/anti-fungal agents include dilute copper sulphate solution, ethanol, formaldehyde, formalin, glutaraldehyde, dilute hydrochloric acid, methanol, phenol and thymol. Several laboratories use a mixture of methanol and glycerine, or phenol and glycerine to preserve palynomorph residues (e.g. Doher 1980). Phipps and Playford (1984) recommended an equal mixture of 3% copper sulphate solution and glycerine, plus a few drops of the antiseptic thiomersal. Evitt (1984) advocated using 'FAA', which is a mixture of equal parts of ethanol, formalin and glacial acetic acid. Traverse (1988; 2007) and Litwin and Traverse (1989) stored residues in glycerine USP. Lennie (1968) was in favour of floating silicone oil on the surface of the aqueous residue to prevent dessication and infection.

Some practitioners, notably Dempsey and Urban (1965) and Felix and Burbridge (1985), advocated evaporating off the liquid and storing the residues when they are dry. This method is undoubtedly space-efficient and may prevent deterioration due to bacteria and/or fungi. Another benefit is that dried residues can be easily and safely transported by post. One potential downside to this mode of storage is that dehydrated residues are prone to clumping when remixed with water (subsection 10.4.4; Dunn 2003).

10.4.4. Recovery of aqueous palynomorph residues

There is no reason why palynomorph residues cannot be satisfactorily archived for many years in suitable vessels by storage in a clean, dark, dry, temperature-controlled facility. However, if aqueous residues have been in storage for some time, they should be thoroughly checked before more slides are produced. Residues should first be sieved and meticulously rinsed to remove any preservatives. If the palynomorphs have clumped together, they can be dissagregated by brief and mild ultrasonic treatment. The phials or vessels should be given five to 30 seconds of ultrasound. This also breaks up any residual

AOM which then can be sieved away. The palynomorphs should then be concentrated, a small amount of dispersant such as Cellosize added, and mounted on slides.

Dunn (2003) described how to restore palynomorph residues which have completely dehydrated. Several drops of 10% hydrochloric acid were added, left overnight, then flooded with water. This effectively rehydrates the dried residue and new slides can be made. If some of the residue adheres to the container, this can be released by using brief ultrasonic treatment. Unfortunately, it was found that the rehydrated residues are prone to clumping. This can be remedied by adding small amounts of Cellosolve, detergent or dispersant.

11. The preparation of palynomorphs from Quaternary and modern materials

11.1. Introduction

The Quaternary Period covers the past 2.58 million years (Gradstein et al. 2021). For some Quaternary and modern carbonate and siliciclastic sedimentary rocks and sediments, the methods of palynomorph preparation are virtually identical to those described above in sections 6–10. However there are certain differences between palynomorph extraction techniques for many other Quaternary and modern materials. The most important of these dissimilarities are that: 1) acid digestion is often unnecessary; 2) acetolysis is used virtually exclusively to remove cellulose and related non-fossilisable materials; and 3) pollen grains are frequently mounted in glycerine USP or silicone oil so that their orientations can be adjusted. Furthermore, the removal and concentration of pollen and spores from modern plants is fundamentally different to extraction from sedimentary rocks and sediments. This is frequently undertaken to produce pollen/spore reference slides which are unequivocally from confidently-identified plant taxa. The three principal departures from the traditional technique are described below in subsections 11.2 to 11.4. Finally, subsection 11.5 is on the extractions from modern plant material and the production of pollen/spore reference slides.

11.2. Acid digestion in Quaternary and modern material and related aspects

If Quaternary and modern samples are substantially minerogenic, the preparation procedure is considerably similar to the traditional hydrochloric-hydrofluoric acid digestion-based technique, even if the material is unconsolidated. However if the samples lack substantial levels of carbonate and silicate minerals, mineral acid treatment is normally unnecessary. A flowchart depicting the preparation of Quaternary and modern sedimentary rock and sediments is given as Figure 45.

Organic matter in non-minerogenic (i.e. humic or peaty) Quaternary and modern sediments predominantly comprises alkali-soluble substances (humic acids etc.), cellulose and refractory materials. The latter category includes palynomorphs, together with charcoal, chitin and lignins. The aim of the processing procedure is therefore to isolate the refractory material, especially the palynomorphs.

Even if acid treatment is not required, normally the first step is to disaggregate (deflocculate) the clay minerals using a surfactant such as a strong detergent, sodium hexametaphosphate or sodium pyrophosphate. This step is especially important in clay-rich samples (subsection 9.2). Next the sample is briefly (~10–15 minutes) boiled in 10% potassium hydroxide solution to remove the soluble humic acids (i.e. unsaturated organic soil colloids) as described in subsections 9.3.3 and 14.3.2. Dilute sodium hydroxide solution can also be used. If the sample is rich in humic acids, the mixture turns dark brown in colour. In very highly humified material, this alkali treatment may be all that is required to extract the palynomorphs (Figure 45).

If the material is calcareous, hydrochloric acid treatment is needed, the potassium hydroxide solution step should always be undertaken preceding this, unless the sample is vey highly calcareous when the reverse should apply (Faegri et al. 1989; Moore et al. 1991; Jackson 1999). Should hydrofluoric acid be needed, this is done following the hydrochloric acid treatment (Figure 45). Sieving to eliminate large organic particles, density separation and ultrasonification can be carried out after the alkali and acid treatments as necessary. Next the residue is subjected to acetolysis and alkali treatment (subsection 11.3). If any highly resistant lignin remains following acetolysis, the residue may be oxidised (subsection 9.3.2). Finally, the residue can be filtered, stained and mounted on microscope slides (Figure 45).

11.3. Acetolysis

11.3.1. Introduction

Acetolysis is used in most preparations of sub-fossil palynomorphs to remove the non-fossilisable materials. This procedure is more properly termed acetylation or ethanoylation (Guthrie and McCarthy 1967), was first described pertaining to the preparation of palynomorphs by Erdtman (1934). This is a chemical reaction that replaces hydroxyl groups with acetyl groups in complex organic compounds. Specifically this is the substitution of an acetyl group for an active hydrogen atom. Acetic anhydride is typically used for this procedure because it reacts with free hydroxyl groups in the substrate being acetolysed. Three items on acetolysis are summarised in Supplementary Data Appendix 1.13.

11.3.2. Why acetolysis is used in palynology

In palynology, acetolysis is principally used to remove the extraneous cellulose, hemicellulose and other non-sporopollenin materials from pollen grains, spores, dinoflagellate cysts and the residue more generally (e.g. Erdtman 1936; 1960; Traverse 1965; 1988; 2007; Wood et al. 1996; Jones 2014; Jardine et al. 2015). Unsurprisingly, acetolysis is at the heart of protocols to extract pollen grains from honey and insects (e.g. Jones and Bryant 1998; Jones 2012, fig. 5). Hydrogen-oxygen bonds in the cellulose/hemicellulose are destroyed via the introduction of acetyl groups during acetolysis, which is achieved by the addition of acetic anhydride, with sulphuric acid as a catalyst. This reaction anhydrously depolymerises (esterifies) the celluloses into cellulose triacetate, which is soluble in glacial acetic acid hence it can be easily removed. Glacial acetic acid is also a by-product of the acetylation reaction. Acetolysis is a routine procedure for Quaternary and modern samples because these normally contain significant levels of cellulose. It is not normally used on deep time palynomorph assemblages because cellulose decomposes naturally relatively quickly. On sub-fossil and modern material, acetolysis effectively cleans up the residue, by breaking apart molecular cross-linkages and replacing them with larger moieties. This causes palynomorphs to expand and fractures the delicate nanofoam structure (Stephen Stukins personal communication 2020). This fracturing of the nanofoam weakens the grains, resulting in fragmentation

when dehydrated for long periods. Acetolysis removes the external coating of pollen grains (pollenkitt) and the internal contents or protoplasm (Pacini and Hesse 2005). These materials make the palynomorphs much more difficult to study. For example pollenkitt can obscure the ornamentation of the exine in pollen grains. Acetolysis can also extract pre-Quaternary palynomorphs from amorphous organic material, perhaps in combination wth oxidation (Phipps and Playford 1984). Acetolysis also helps to darken pale pre-Quaternary palynomorphs as an aid to observation and study (e.g. Lennie 1968; Wilson 1971a; Sarjeant 1974; Lieux 1980; Herngreen 1983; Faegri et al. 1989). If the palynomorphs become overdarkened, they can be bleached using 5% sodium hypochlorite solution for 2–3 minutes (Traverse 2007). This reagent should be used only with extreme care on relatively robust palynomorphs (Riding and Kyffin-Hughes 2010).

11.3.3. How acetolysis is used in palynology

Acetolysis in palynology is a procedure where the dehydrated samples are treated with freshly made acetolysis mixture. The material to be acetolysed may be prepared and concentrated palynomorph residues, or modern anthers, flowers etc. Acetolysis mixture is a 9:1 combination of acetic anhydride and concentrated sulphuric acid. Acetic anhydride is a colourless liquid that smells of acetic acid, and is widely used in organic synthesis. Because the acetolysis mixture rapidly loses efficacy after it is first mixed, it should not be stored for more than a few hours. The two constituents can be combined in the correct proportions in the sample vessel itself. For example, 5 ml of acetic anhydride can be added, followed by 0.5 ml of sulphuric acid. The reaction between acetic anhydride and sulphuric acid is highly exothermic, and these reagents should be mixed very carefully and slowly. Note that all the utensils used should be absolutely dry because acetic anhydride is explosive in the presence of water. Hence all the acetolysis procedure must be done in a fume hood. Similarly, the palynomorph residue/pollen sample must firstly be completely dehydrated by successive, repeated washings with glacial acetic acid. Three of these washes normally suffice, and they can be done using a centrifuge. However, Higgins and Spinner (1968) advocated the use of a sinter glass Büchner funnel housed in a Büchner Flask for this step (Figure 26).

If the acetolysis mixture is pre-mixed, it should be added to the palynomorph residue extremely carefully because the acetylation reaction is exothermic. The mixture should be gently stirred; the liquid will normally quickly turn reddish brown, and heat is produced. The sample vessel (a small beaker is ideal for this) can simply be left to react at room temperature for about 30 minutes, or it can be briefly heated to around boiling point in a water bath or aluminium block to accelerate the reaction. The recommended reaction times for acetolysis vary substantially. Most authors advocate between one and five minutes (Charman 1992). If the residue is acetolysed for nine minutes, the palynomorphs attain a golden-brown colour similar to that achieved through staining with Bismarck Brown (Jen O'Keefe, personal communication 2021). However, Bigelow (1980) recommended a maximum of 15 seconds and West (1977) advocated 30 minutes. The acetolysis reaction can be stopped by adding glacial acetic acid. When the acetolysis reaction is complete, the sample residue is repeatedly washed with glacial acetic acid to remove all acetic anhydride and cellulose triacetate. Following the final glacial acetic acid rinse, the residue is washed to neutrality with water. These steps can be done in a centrifuge or in a sinter glass funnel. If any cellulose triacetate and/or acetic anhydride remains, the residue can be retreated with glacial acetic acid. Erdtman (1935) added several drops of dilute sodium hydroxide solution prior to the first water wash. Similarly, Wilson and Goodman (1963) and Higgins and Spinner (1968) treated the neutralised residue with dilute ammonium hydroxide and potassium hydroxide solutions respectively. Prior to acetolysis, Litwin and Traverse (1989) recommended pre-treatment by heating the residue in a 7% solution of sodium hydroxide for 30–60 minutes. The latter treatment is somewhat harsh, and may cause some damage to the palynomorphs.

11.3.4. The potential harmful effects of acetolysis on palynomorphs

Acetolysis is a relatively harsh chemical treatment, and the reaction time should be kept to a minimum because significant damage and/or loss of certain palynomorph groups can occur (e.g. Southworth 1974; Lieux 1980; Wolter and Schill 1985; Hesse and Waha 1989; Charman 1992; Schols et al. 2004; van Asperen et al. 2016; Riddick et al. 2017; Shumilovskikh et al. 2019). Susceptible palynomorphs include highly-textured sporomorphs such as bisaccate conifer pollen and thin-walled dinoflagellate cysts,

principally the families Polykrikaceae and Protoperidiniaceae (Hafsten 1959; Marret 1993). Furthermore, Large and Braggins (1990) and Moore et al. (1991) reported that acetolysis selectively degrades the perine of spores, and the colpi and pori of pollen grains respectively. The connecting elastoviscin threads of zoophilous angiosperm pollen are also highly susceptible to degradation by acetolysis (Wolter and Schill 1985). Moreover, the sporopollenin of immature pollen is frequently not completely polymerised, and these grains may therefore be destroyed or badly damaged by acetolysis (Hesse and Waha 1989). It is also possible that acetolysis can effect the size of palynomorphs. Most reports of size effects have found that overacetolysis can cause expansion of pollen and spores (Traverse 1965; Lennie 1968; Reitsma 1969; Large and Braggins 1990; Bruch and Pross 1999; Meltsov et al. 2008).

11.3.5. Alternatves to acetolysis

Because acetolysis can be a relatively harsh treatment, less severe alternatives have been proposed. Shane and Clarke (1981) used dimethylsulfoxide to remove the intine and cytoplasm from modern pollen. Another procedure involving the filtration of pollen using acetone and ethanol was outlined by Bredenkamp and Hamilton-Atwell (1988). This method can remove the pollenkitt, and other organic solvents such as chloroform and methanol can be used. The extraneous organic material on the surface of palynomorphs can also be removed from modern material by boiling in concentrated hydrochloric acid then washing in 10% potassium hydroxide solution according to Hesse and Waha (1989). However, the hydrolysis procedure with dilute alkali is less effective than acetolysis (Traverse 2007). Schols et al. (2004) introduced an enzyme-based method using cellulase and pectinase for preparing fragile pollen grains which are susceptible to damage by acetolysis. This procedure produced good results and was also successfully used by O'Keefe and Wymer (2017) on a bee pollen capsule, fresh pollen and honey. Gonzalez-Cruz et al. (2018) described cleaning pollen grains from a diverse array of plant taxa using a sequential treatment with acetone, phosphoric acid and potassium hydroxide to remove the extraneous materials.

11.4. Microscope slide mounting

Many practitioners prefer to use glycerine USP or silicone oil as ‘wet’ mounting media when producing microscope slides of Quaternary and modern material. These highly viscous mountants enable the orientation of pollen grains to be adjusted using gentle pressure on the coverslip with a needle, seeker, toothpick or similar implement so that they can be effectively identified using a botanical key (subsections 10.3.2.4 and 10.3.2.6; Figure 37A; Moore et al. 1991, p. 48–49).

11.5. The production of pollen/spore reference slides and the preparation of modern plant material

It is extremely important to extract pollen and spores from confidently identified modern plant material. This enables practitioners to know with certainty which plants produce specific pollen/spore types. A cognizance of this is a prerequisite for ecological reconstructions using palynomorphs alone. This technique also allows the production of reference slides. The latter are used to compile a collection of mounts containing pollen or spores which are from unambiguous plant taxa (Plate 2.2, 2.5, 2.6). Material used to make reference slides are taken carefully and sparingly from plant specimens preserved on herbarium voucher sheets, and are clearly essential to allow reliable identifications of dispersed modern pollen and spores (e.g. Andrew 1970; Jarzen and Jarzen 2006; Martin and Harvey 2017). Plant specimens on herbarium voucher sheets are pressed, dried, mounted on card and stored in an insect-proof herbarium case (e.g. Wilson and Goodman 1963, fig. 1). If palynologists do not have access to herbarium collections, plant specimens collected in the field have to be identified with advice from specialist plant taxonomists or using the botanical literature. It is desirable to have several type slides of individual taxa from different locations in order to assess the levels of morphological variability of the pollen/spores.

The catkins, cones and flowers collected should ideally be on the point of maturing or opening so that the pollen is abundant and fully developed. The pollen from open flowers may have been contaminated by insects. Also, if young flower buds are sampled, some of the pollen may be immature and hence may not be morphologically representative (Moore et al. 1991). For plants with separate male and female flowers, only the male flowers should be collected. Similarly, for spore-bearing plants such as

ferns, parts of leaves where the sporangia are unopened should be collected. If the sporangia have already opened, they will be virtually devoid of spores (Traverse 1965)

As much as possible of the obviously extraneous leaves, sepals etc. should be carefully removed from the pollen/spore sample, which should be stored in glacial acetic acid or, if this is not possible, air-dried. Pollen from anthers or spores from sporangia can be transferred directly onto a microscope slide with a mounted needle (Figure 46). However much better results are produced by concentrating the pollen/spore content, then subjecting it to acetolysis (Figure 47). This procedure is executed by treating the dissected pollen/spore-bearing parts of the catkins, cones, flowers, flower buds or sporangia with glacial acetic acid, crushing them lightly and wet-sieving in acetone or ethanol using a brass or stainless steel screen or screens. Specifically the separated anthers, pollen sacs or sporangia are soaked in glacial acetic acid, then very gently crushed using a pestle and mortar to loosen the pollen grains or spores. Next the material is lightly rubbed and washed with acetone or ethanol through a metal screen with a ~400 μm mesh using a thumb or forefinger to physically separate out the pollen or spores. The relatively large mesh size ensures that any extraneous plant tissues are left on the screen. Alternatively, a suitable nest of sieves can be used. The separated pollen or spores are collected and concentrated by floating them off in water. The pollen/spore concentrate should then be acetolysed to remove the extraneous cellulose, protoplasm etc. and treated with dilute alkali before mounting on slides (Figure 47; Wilson and Goodman 1963; Traverse 1965; 1988; 2007; West 1977; Moore et al. 1991).

A gentler alternative to acetylation is alkali-maceration and this should be used for delicate modern pollen types. The pollen is briefly boiled in 5–10% potassium hydroxide solution. This removes the intine and the cell contents but affects the exine less than acetylation, provided the alkali treatment is relatively brief. The pollen and spores normally need staining following alkali maceration (Traverse 1965; Schols et al. 2004). Alternative methods of producing microscope slides of modern pollen and spores by dehydration using glycerine and castor oil were described by Chitaley (1966–1967) and Chitaley and Deshpande (1969). Supplementary Data Appendix 1.14 includes four relevant items on this topic.

12. Non-acid palynomorph preparation techniques

12.1. Introduction

This section is on palynological preparation techniques which do not use hydrochloric and hydrofluoric acids to dissolve the carbonate and silicate minerals respectively. Mineral acid dissolution and oxidation with nitric acid and other strong oxidants are by far the most hazardous steps in the traditional preparation technique. The disadvantages of mineral acid treatment include significant health and safety risks, the potential of environmental damage from any spills and waste materials, the high cost of these reagents, and the need for sophisticated laboratory facilities (notably fume hoods) and personal protective equipment (PPE). The fiscal cost of these highly hazardous chemicals lies in their initial purchase, safe storage and in the responsible disposal of the spent acidic residues. These financial burdens tend to increase beyond background price inflation due to the ever stricter regulatory frameworks for hazardous substances justifiably legislated by governments worldwide. This means that any palynomorph preparation techniques which do not use mineral acids will be both safer, more environmentally friendly and cheaper than the traditional methods; they also tend to be simpler and faster to execute. Non-acid techniques are also ideal for use in remote locations such as a field-based laboratory or at a rigsite.

Because palynomorphs are acid-resistant, they can clearly be extracted from sediment/rock matrices by simply dissolving the mineral fraction using mineral acids. The same paradigm applies to the extraction of conodonts, which are phosphatic microfossils (Lindstrom 1964; Higgins and Austin 1985). By contrast, calcareous and silicious microfossils require much gentler preparation techniques. If they cannot simply be washed out of the matrix with water, foraminifera, ostracods and silicofossils are typically freed from the sediment/rock matrix using substances such as detergent, hydrogen peroxide, paraffin (kerosene), petrol (gasoline), sodium carbonate, sodium pyrophosphate and white spirit, then are physically picked out from the residues (e.g. Armstrong and Brasier 2005). Due to their extremely small size, calcareous nannofossils are normally simply washed out of the rock from scrapings with a sharp knife which are

smeared onto a microscope slide (Bown and Young 1998, p. 17). There is no known successful methodology of extracting palynomorphs using freeze drying (Kennedy and Coe 2014).

Stimulated by the clear environmental, financial and health/safety benefits of non-acid processing in palynology, since 2004, there has been an upsurge of interest in this topic. The first publication during this time frame was Riding and Kyffin-Hughes (2004) who reviewed preparation techniques in palynology, and went on to describe an effective method using sodium hexametaphosphate and hydrogen peroxide (subsection 12.4). These authors subsequently refined their technique (e.g. Riding and Kyffin-Hughes 2006; Riding et al. 2007a). Other relatively recent significant papers on this subject are Williams et al. (2005), O'Keefe and Eble (2012) and Wheeler at al. (2020). Supplementary Data Appendix 2 gives summaries of 25 papers on this topic.

12.2. Early work on non-acid palynomorph preparation (1930s to 1970s)

Between the 1930s and the 1970s, non-acid palynomorph preparation was mentioned sporadically, but these techniques never became widespread. The earliest mention of this topic was Deflandre (1938), who simply sieve-washed and swirled water-soaked samples of Upper Jurassic (Oxfordian) mudstones from Villers-sur-Mer, northern France with water to extract palynomorphs (Riding and Schmitt 2009). This extremely simple methodology has apparently only been used rarely since. However, in a generic textbook on micropalaeontology, Armstrong and Brasier (2005, p. 274) briefly described a method of palynomorph extraction by simply pounding and water-washing rock samples.

Other early methods include Knox (1942) who separated palynomorphs by deflocculation using acetone or bromoform followed by centrifugation. Caro et al. (1964) commented that marls can be disaggregated by treatment with ammonium nitrate, bromine, detergent, 10–15% hydrogen peroxide, magnesium sulphate, petrol (gasoline), potassium hydroxide, sodium carbonate, sodium hydroxide or sodium sulphate. In some cases, combinations of these substances can be effective, for example petrol and sodium carbonate. Occurrences of dinoflagellate cyst clumps in calcareous microfossil preparations from the London Clay Formation were also reported by Williams and Downie (1966, p. 20). These had been pointed out to these authors in 1958 by Murray J.

Hughes of the British Geological Survey (Riding and Kyffin-Hughes 2004; Riding et al. 2006), and palynomorphs from this unit had also been extracted using non-acid techniques by Eagar and Sarjeant (1963). Megaspores and scolecodonts have been extracted and concentrated without the use of mineral acids (e.g. Dijkstra 1951; Hughes 1955; Dettmann 1965). Also Goldman (1952) prepared anhydrite and gypsum samples simply by dissolution in dilute sodium thiosulphate solution (subsection 14.5).

There are many papers describing the disaggregation of sedimentary rocks in relation to the preparation of calcareous and silicious microfossils (e.g. Layne 1950; Crowley 1952) and all these have potential relevance to palynology. Specifically, clay deflocculants/dispersants such as Darvan 4 and Quaternary O (both now unavailable) have both been used to extract calcareous microfossils (e.g. Zingula 1968; Snyder et al. 1983). These products have also been utilised to remove finely-disseminated clay in palynological preparations (e.g. Funkhouser and Evitt 1959). Furthermore Quaternary O, a highly surface-active, but low sudsing detergent, was used by Snead (1969) to extract megaspores from Upper Cretaceous and Palaeogene material of western Canada. It was also used as a pre-treatment in the acid digestion method by Hills and Sweet (1972). This product is no longer available but Miramine is a suitable alternative substitute, as are other coco betaine sulphates.

12.3. Hydrogen peroxide

Hydrogen peroxide can be used to disaggregate clay rich sediments and sedimentary rocks physico-chemically, and is often used to extract calcareous microfossils and plant fossils (e.g. Oldham 1976, Penny 1999, Worobiec 2003 and references therein). The action of the hydrogen peroxide is twofold. Because it spontaneously dissociates into oxygen and water at atmospheric pressure, hydrogen peroxide causes the physical disintegration of the clay-rich material by ‘deposit swelling’. This phenomenon results from the action of oxygen bubbles generated in the matrix/pores of the sample material during dissociation. The expansion pressure of the dissociated hydrogen peroxide physically breaks up the rock or sediment, and the effectiveness of this is directly proportional to the concentration of this reagent.

However, hydrogen peroxide is also a powerful oxidising reagent and it will rapidly destroy organic matter in sediments and sedimentary rocks. It is especially effective at removing AOM. This reagent should therefore be used extremely carefully because, if it is too concentrated, hydrogen peroxide will degrade and destroy palynomorphs, often selectively (Hopkins and McCarthy 2002). Furthermore, hydrogen peroxide is a hazardous reagent because it gives off oxygen when it dissociates hence increasing the risk of combustion. This means that it should always be used in a fume hood.

Leschik (1956) and Brown (1960; 2008) described a method of using hydrogen peroxide to prepare brown coal. However, one of the first studies to use hydrogen peroxide on clay-rich lithotypes is Lund and Ecke (1988) who studied Middle and Upper Jurassic claystones from southeast Germany. If the material is calcareous, the samples were pretreated with hydrochloric acid. The samples were then disintegrated using hydrogen peroxide, and the disaggregated residues subjected to ultrasound if necessary. Finally the fine clay/fine organic fraction was separated from the palynomorphs by repeatedly sieving.

Riding and Kyffin-Hughes (2004) outlined the use of hydrogen peroxide to break down rock fragments that resisted disaggregation using sodium hexametaphosphate (subsection 12.4). To ensure that all the rock is broken down, any remaining material is repeatedly treated with hydrogen peroxide for 15–20 minutes to attempt to achieve full disaggregation of the sample, thereby maximising the palynomorph yield.

One of the most significant papers on the use of hydrogen peroxide is Williams et al. (2005). These authors described a method of non-acid palynomorph preparation using hydrogen peroxide, and demonstrated its utility in five North Sea rigsite case histories. It was stated that trial and error are required to find the optimum strength of hydrogen peroxide, and the timing of the treatment. Williams et al. (2005) outlined their methodology for the post hydrogen peroxide treatment of the residue only in the broadest of terms. The residues are sieved, gravity separated then cleaned using detergents and sieves.

The non acid technique described by Riding and Kyffin-Hughes (2004) was further trialled by Riding and Kyffin-Hughes (2006), who prepared three samples with

hydrogen peroxide. Here the crushed sample material was repeatedly heated to ~70˚C, covered in hydrogen peroxide for around five minutes, then diluted with water and left until any reaction had ceased and all the sample had broken down. Unfortunately this method proved ineffective on one Ordovician sample, and proved of limited effectiveness on the two Carboniferous samples which were tested.

Riding et al. (2007a) continued this work, and focused on a technique using hot hydrogen peroxide. The samples were prepared quantitatively using hydrogen peroxide and also with hydrochloric acid/hydrofluoric acid and/or sodium hexametaphosphate to provide absolute frequency data. In the Lower Carboniferous sample 1, the hydrogen peroxide method was more effective than the sodium hexametaphosphate procedure. Sample 2 (Upper Carboniferous) was prepared effectively using both hydrogen peroxide and mineral acids, however the palynomorph concentration is higher with the latter technique. The hydrogen peroxide method produced a residue virtually clear of AOM. Samples 3 and 4 are of Middle Jurassic age and the palynofloras are comparable with both the hydrogen peroxide and the acid preparations. As with sample 2, the hydrogen peroxide method produced a palynomorph assemblage that was cleaner in terms of the levels of extraneous AOM than the residue produced by the acid technique for sample 3. The former thus has the marked advantage of its capability to simultaneously macerate the matrix and to oxidise the AOM. Two samples, 6 and 7, are Lower Paleocene and represent two dinoflagellate cyst acmes. Treatment with 30% hydrogen peroxide totally destroyed *Palaeoperidinium pyrophorum* (Ehrenberg 1837 ex Wetzel 1933) Sarjeant 1967. Emendations in sample 6, but did not affect *Spinidinium* sp. in sample 7. Finally, the hydrogen peroxide and mineral acid methods produced similar palynofloras in sample 7, which is a Quaternary clay. In most cases, the acid technique produced a significantly greater concentration of palynomorphs than either of the non-acid protocols. However, in most of the Mesozoic and Cenozoic samples, the palynomorph yields of the hydrogen peroxide and mineral acid methods are broadly comparable.

Two samples of palynomorph-rich Upper Jurassic mudstones from northwest Scotland were prepared quantitatively using acid digestion, sodium hexametaphosphate and hydrogen peroxide by Riding and Kyffin-Hughes (2011).The sodium hexametaphosphate method proved ~50% as efficient as acid digestion. By contrast, the

hydrogen peroxide technique proved to be significantly less effective, at approximately 10% of the extraction level of acid digestion; this appears to be largely due to losses caused by oxidation. This disparity means that some of the rarer forms may not be extracted using this method, hence the diversities of palynofloras may appear to be lower than they actually are.

It is clear that hydrogen peroxide is an important reagent in preparing palynomorphs, especially when and where the use of mineral acids is precluded. However, Lund and Ecke (1988) demonstrated that a pre-treatment with hydrochloric acid can be very effective on calcareous samples. For certain ages of material and lithotypes, hydrogen peroxide can be highly effective. It frequently is highly effective in breaking down relatively indurated lithotypes. However, because of the oxidising effects of this substance, the concentration/strength and the duration of the reaction should be kept as low and as short as possible respectively.

12.4. Sodium hexametaphosphate

Sodium hexametaphosphate, or Graham's Salt, is relatively non-hazardous (Lanigan 2001) and can be used to disaggregate sediments due to the high ionic charges of phosphates in solution. This substance was the active ingredient in Calgon or Calgon S, marketed as a detergent, dispersant and/or water softener. However, the formulation of these products were recently changed. Phosphates reduce the coherence of clay because these ions are strongly adsorbed onto the clay particles. The ions adsorbed onto the clay particles produce a strong electrostatic charge that causes the particles to repel each other, hence breaking up or dispersing the clay (Bates et al. 1978). Furthermore, the adsorbed sodium hexametaphosphate also displaces some of the bound water in the clay, resulting in further disaggregation. The surface charges then prevent any reflocculation of the clay. Sodium hexametaphosphate has been used to extract calcareous microfossils from clay-rich sediments, however it has been found to cause damage to calcite by corrosion (Oda et al. 1975; Hodgkinson 1991; Kontrovitz et al. 1991). This substance has also been successfully used to extract palynomorphs. Traverse (1978; 1988; 2007) used it as part of an impromptu technique to prepare samples on a drilling ship when hydrofluoric acid was unavailable.

Riding and Kyffin-Hughes (2004) recently developed an apparently reliable method for preparing palynomorphs from clay-rich lithologies of Early Jurassic to Quaternary age. This method was further investigated by Riding and Kyffin-Hughes (2006; 2010; 2011) and is summarised in Figure 48 and Appendix 2. The developmental approach taken by these authors was to test reagents that disaggregate relatively unindurated sedimentary rocks and unconsolidated sediments. The inspiration for this procedure were the deflocculating/sieving protocols of Eagar and Sarjeant (1963) and Traverse (1978; 1988; 2007). Sodium hexametaphosphate was chosen as a disaggregating agent because of its proven use in preparing calcareous microfossils, and its non-hazardous nature.

The method of Riding and Kyffin-Hughes (2004) also involves hydrogen peroxide. Basically, the sample material is treated with detergent and warm water overnight to soften it in a large (~2000 ml) beaker. Following decantation of the pretreatment supernatant, more warm water is poured into the beaker, a small amount (~5 g) of sodium hexametaphosphate flakes added and the mixture agitated for 15–20 minutes (Figure 49). This should deflocculate the clay particles so that they can be sieved away. The use of a large-diameter (~30 cm) sieve is best for this due to the large amount of residue that has to be screened. Because there has been no mineral digestion, there is much clay to remove, and this procedure is usually relatively protracted. Because of this, it is likely that palynomorphs may be lost during this step. If any of the sample has not broken down, the coarse fraction is repeatedly treated with hydrogen peroxide, again for 15–20 minutes. When all the sample has broken down, and the palynomorph-rich residue concentrated, it can be subjected to heavy liquid separation and oxidation as necessary prior to mounting. The methodology was outlined in detail by Riding and Kyffin-Hughes (2004, appendix 2), Riding and Kyffin-Hughes (2006, appendix 3) and Riding and Kyffin-Hughes (2011, appendix 1), and is summarised in Appendix 2.

Riding and Kyffin-Hughes (2004) found that in most cases that they tested, i.e. their Lower Jurassic, Lower Cretaceous, Upper Cretaceous and Quaternary siliciclastic samples, the final palynomorph concentrates were found to be as rich and as well preserved as those produced using acid digestion. However, the sodium hexametaphosphate method proved ineffective on limestones. It is therefore clear that,

unsurprisingly, this reagent disaggregates clays and claystones far more effectively than limestones. Riding and Kyffin-Hughes (2006) undertook further testing of the sodium hexametaphosphate technique on samples of Ordovician, Carboniferous, Jurassic and Paleogene age from the UK. The results were somewhat mixed on the Palaeozoic material. The sodium hexametaphosphate method failed to break down the sample from the Lower Ordovician Shineton Shale Formation. One of the Carboniferous samples proved a success with the sodium hexametaphosphate method; the other one failed to disaggregate. By contrast, the sodium hexametaphosphate technique was consistently successful on the Jurassic and Eocene material tested by these authors. In several of these younger samples, it outperformed acid digestion in terms of absolute numbers extracted and produced clean (AOM-free) residues which do not require further oxidation.

The effectiveness of the use of pre-treatments prior to the sodium hexametaphosphate technique were investigated by Riding and Kyffin-Hughes (2010) using a sample of palynomorph-rich Upper Carboniferous mudstone. The sample was separately pre-treated overnight by soaking in acetone, two detergent solutions, formic acid, household bleach (two methods), methylated spirits and white spirit prior to preparation using sodium hexametaphosphate. Generally, the pre-treatments increased the mass of sample that was eventually broken down by the susequent sodium hexametaphosphate treatment. The pre-treatment softens the sample material, thereby allowing the sodium hexametaphosphate to act on an increased surface area, thereby releasing more palynomorphs. Five of the others (acetone, both detergents, methylated spirits and white spirit) produced substantially higher concentrations of miospores from the sample studied than with no pre-treatment. Of these, the detergents and white spirit approximately doubled the palynomorph yield.

The latest paper by these authors is Riding and Kyffin-Hughes (2011) who quantitatively prepared two samples of palynomorph-rich Upper Jurassic mudstones from northwest Scotland using acid digestion and their non-acid protocols using sodium hexametaphosphate and hydrogen peroxide. This was to compare the three techniques in terms of both the numbers of palynomorphs extracted, and the numbers of the individual taxa present to test for any taxonomic biases. The sodium hexametaphosphate method proved ~50% as efficient as acid digestion in terms of absolute numbers of palynomorphs

extracted. It is clear that the effectiveness of the sodium hexametaphosphate technique is indirectly proportional to the levels of lithification/induration of the material studied. The shortfall in palynomorph yield is probably as a result of the imperfect dissagregation of the rock/sediment matrix and losses during sieving. Despite this, the sodium hexametaphosphate method produces relatively clean (i.e. largely AOM-free) residues which frequently do not require further oxidation. The majority of the taxa present were recovered in similar relative proportions throughout.

Wheeler et al. (2020) reported on a test between mineral acid digestion and non-acid preparation using sodium hexametaphosphate on some Upper Permian material from the southern part of the Galilee Basin, central Queensland, northeastern Australia. These authors documented their results comprehensively, and both the preparation methods used proved very successful. The non-acid protocol provided higher species richnesses, but it was clear that acid digestion normally provides higher palynomorph yields. The lower yields for the non-acid method are, at least partially, due to the abundant phytoclasts occluding palynomorphs, and the reduced effectiveness of centrifugation and density separation because of the larger proportions of residual clay. Despite these issues, overall the associations proved broadly comparable for both methods. The authors suggested that further refinement and testing of their non-acid methodology should be undertaken.

It is clear that the sodium hexametaphosphate technique can be highly effective on low to moderately indurated siliciclastic sedimentary rocks and unconsolidated clay-rich material. However, there may be substantial palynomorph shortfalls, probably due to the partial dissagregation of the clay minerals and depredations in the relatively lengthy sieving procedure. Moreover, the effectiveness of this technique is indirectly proportional to the levels of lithification/induration of the material studied; it does not fully disaggregate some older and more mechanically robust lithotypes. It is clear that this and other non-acid methods are ineffective on siliciclastic sedimentary rocks which are cemented by quartz (O'Keefe and Eble 2012). There are other disadvantages, for example it is ineffective on limestones and that any silicofossils are not destroyed. If the sample is very rich in diatoms or radiolaria, these can dilute, or even swamp, the smaller and less abundant palynomorphs (Riding and Kyffin-Hughes 2004, figs 6E, F). Another potential

problem is that the waste supernatant is rich in dissolved phosphates. If many samples are being prepared with sodium hexametaphosphate, the disposal of the surplus liquor may be difficult in areas where phosphates are regulated. Phosphate discharge can cause the eutrophication of surface waters (Correll 1999). Dissolved phosphates can be removed biologically or by chemical precipitation.

12.5. Tetrasodium pyrophosphate

A method of deflocculating Quaternary siliciclastic sediments using tetrasodium pyrophosphate (or sodium pyrophosphate or tetrasodium phosphate) was first developed by van der Kaars (1991). Tetrasodium pyrophosphate is a non-hazardous, highly soluble salt used, for example, as a food additive, dispersing agent, emulsifier and thickener. van der Kaars (1991, p. 246) did not provide much detail regarding his methodology. However this technique was later used and further developed by Moss et al. (2005; 2016), Moss and Kershaw (2007) and Moss (2013) for Eocene and Quaternary age siliciclastic sedimentary rocks and sediments. The sample material was crushed to small fragments and soaked in a 10% solution of tetrasodium pyrophosphate then sieved to remove large (>180 μm) fragments. The deflocculation is more effective if the vessel is heated to ~40°C for ~40 minutes and stirred. The >8 μm fraction was retained and any calcareous material was dissolved in hydrochloric acid. The residues were then subjected to acetolysis, heavy liquid separation and oxidation as necessary. If substantial amounts of silicate minerals remain following the tetrasodium pyrophosphate treatment, the residue can be subjected to hydrofluoric acid digestion (Moss 2013, p. 317).

12.6. O'Keefe and Eble (2012)

O'Keefe and Eble (2012) is a landmark paper on both acid digestion and non-acid palynomorph preparation techniques. These authors worked on samples of clay-rich and organic-rich lignite samples of the Claiborne Group of middle Eocene age from Kentucky, USA. This material is known to be rich in palynomorphs but is problematical to prepare. O'Keefe and Eble (2012) processed their material using six standard and non-standard techniques, and compared the results. The techniques used in this study include both acid-based and non-acid procedures.

Firstly, the ‘Modified Traverse’, ‘Unmodified Eble’, ‘Colombian’ and ‘Unmodified Riding and Kyffin-Hughes’ techniques were tested by O’Keefe and Eble (2012). The ‘Colombian method’ proved most effective. However, in two out of the five samples prepared by this method, the palynomorphs were damaged and the alkaline depolymerisation step was dangerously explosive. The ‘Unmodified Riding and Kyffin-Hughes’ technique proved effective but was found to be excessively time-consuming in the view of O’Keefe and Eble (2012). Furthermore, abundant finely-disseminated clay particles remain in the residue following the ‘Modified Traverse technique’, despite the use of hydrofluoric acid.

The second phase of this study was using the ‘Modified Eble’, ‘New Heard’, ‘KOH’ and ‘Modified Riding and Kyffin-Hughes’ techniques. These four procedures markedly outperformed the previous four in terms of palynomorph concentration (O’Keefe and Eble 2012, fig. 9). In overall terms, the ‘KOH’ and ‘New Heard’ techniques were the most optimal. The ‘Modified Riding and Kyffin-Hughes’ technique again proved time consuming.

Based on the results produced in the two previous phases, a new technique was conceived. The ‘O’Keefe technique’ draws on the others used here. It is based on dissagregation with a dilute detergent (Liquinox) solution followed by alkali treatment and heavy liquid separation. Organic-rich samples were treated with hypochlorous acid after soaking in detergent (O’Keefe and Eble 2012, fig. 13). Importantly, the residue is sieved to remove clay and fine organic debris prior to heavy liquid separation as in the ‘Riding and Kyffin-Hughes technique’. Care must be taken to remove finely disseminated clay by short intervals of centrifugation following the detergent step (Funkhouser and Evitt 1959, p. 371).

The ‘O’Keefe technique’ was modified so that it is suitable for a wider variety of lithotypes. Calcareous samples are first treated with dilute hydrochloric acid. The hypochlorous acid step is ideal for low maturity material (e.g. lignite). However much stronger oxidants, such as nitric acid and Schulze’s solution (depending on the rank), should be used on material of higher thermal maturities i.e. sub-bituminous and bituminous coal (O’Keefe and Eble 2012, fig. 15). This technique, was termed the

'refined O'Keefe technique'. If the sample material is indurated, the addition of sodium hexametaphosphate speeds up the dissagregation (Riding and Kyffin-Hughes 2004).

12.7. The effectiveness of non-acid palynomorph preparation and future work

It is clear that non-acid palynomorph preparation has considerable practical advantages over the more traditional acid digestion procedure. However, it is readily conceded that the various non-acid techniques are not always as effective in terms of absolute palynomorph extraction. Reagents such as hydrogen peroxide, sodium hexametaphosphate or tetrasodium pyrophosphate will not break down carbonates or sedimentary rocks tightly cemented by quartz. However, if the sample material is relatively soft, non-acid techniques can be just as effective as acid digestion. For many projects, a hybrid solution might be used. For example non-acid techniques might be used in the field, at rigsite or on ships to provide initial biostratigraphical and palaeoenvironental assessments, prior to full acid digestion in the laboratory.

Clearly there is much further scope for experimentation in order to make non-acid palynomorph preparation more efficient and faster. Potential new avenues to explore include more effective pre-treatment procedures, boiling the sample material and reagent mixture, and automated sieving perhaps with nested sieves. Hydrochloric acid is far less hazardous than hydrofluoric acid so the former may prove to be a highly effective reagent for pre-treatment.

13. Contamination

This relatively short section focuses one of the major inherent problem areas in palynological processing, i.e. the contamination of samples with extraneous palynomorphs. It is possible for modern pollen or fragments from other samples to contaminate either sample material or palynomorph concentrates during sampling, transport, preparation or storage (Wilson 1964; Funkhouser 1969). Naturally the presence of allochthonous palynomorphs could seriously compromise the palynological data and interpretations. This is especially important in areas such as forensic palynology

(subsection 14.15; Horrocks 2004; Wiltshire 2016). Hence strenuous efforts should be made to maintain the absolute integrity of individual palynomorph associations.

During sampling from outcrops, the weathered surface of the rock or sediment should be thoroughly cleaned away, thereby exposing fresh material (subsection 7.2.2; Figures 12–14). The palynomorph yield will be greater because the organic fraction in weathered material may have been oxidised, and any adherent modern pollen and spores will be avoided. Any sampling tools such as chisels, hammers etc. should be thoroughly cleaned after collecting a sample so that cross-contamination between adjacent samples is avoided. Samples should be immediately placed in brand new clean, clearly labelled, securely sealable bags to protect them from contamination during transportation and storage (subsection 7.2; Figure 11). In the laboratory, each sample should be opened and resealed sequentially, one at a time to minimise the risk of cross-sample contamination. Ideally, a new pair of disposable rubber gloves should be worn for the handling of each sample. It is important to store the samples in conditions that are not conducive to fungal growth (i.e. clean, dark, dry and temperature-controlled). Sampling cores, sidewall cores and cuttings should also be undertaken extremely carefully to avoid the introduction of contaminants.

Tireless efforts should be made to avoid contamination in the laboratory. Ideally, the laboratory should be semi-sealed, and the air conditioned and filtered to avoid the ingress of airborne modern pollen and spores. Clearly, any windows should remain closed, and draughts and dust should be minimised (section 5; Jung Echols and Levin 1964). The latter will prevent any disturbance of pollen and spores that have settled on flat surfaces. Nonetheless, it is recommended that air ducts, counter tops etc. are regularly and thoroughly cleaned. If any of these measures are not possible, air quality can be monitored by leaving out adhesive-laden microscope slides (adhesive side uppermost) around the laboratory to check for any airbourne contaminants. Periodically, coverslips are placed on the slides so that they can be examined for any air-transported materials. Alternatively, several *Lycopodium* tablets can be processed in the normal way and any exotic grains monitored in the resultant slides. If other palynomorphs are observed, this indicates that wind-blown sporomorphs are present in the laboratory

Laboratory air quality is most important if Quaternary/modern material is being prepared. If the laboratory is working exclusively on Palaeozoic and Mesozoic material, any modern/subfossil contaminants will be very easily spotted. It should go without saying that ornamental plants should never be placed in a palynology laboratory. Samples should be thoroughly cleaned before the preparation process is commenced. This can be done by brushing, flaming, scraping or washing (subsection 7.3). In particular core, sidewall core and cuttings samples should be thoroughly washed to remove all traces of drilling mud because this can contain organic 'thinners' which can be rich in potential contaminants (Traverse et al. 1961). All laboratory surfaces and equipment should be scrupulously clean. The highest purity and quality reagents should be used because suboptimal ('technical grade') chemical supplies may not be filtered and hence can include contaminants (Fisher 1962). However, since the work of Fisher (1962), the quality of technical grade reagents has unequivocally improved markedly. Today the principal contaminants in laboratory chemicals is microbial DNA (e.g. Salter et al. 2014). Plastic vessels should be discarded after a short time because these are susceptible to scratching and these imperfections can harbour contaminating materials. The sample material and any aqueous residues should not be left uncovered for any significant length of time (Figure, 23E, 24D). Furthermore the local water supply can contain impurities and contaminants including modern palynomorphs, hence distilled, filtered or RO water should ideally be used throughout preparation rather than tap water. Hence, whenever water is mentioned throughout this article, this should ideally be pure water.

Many authors warn against contamination in robust terms, however Traverse (2007, p. 624) was relatively relaxed about this phenomenon. He reasoned that indigenous palynomorphs should numerically overwhelm isolated contaminants. For example Traverse (2007) stated that airborne pollen contaminants should be negligible, even during the flowering season. This author went on to state that even if one is studying Quaternary/modern material, provided acetolysis is used during preparation (subsection 11.3), any contaminating pollen will be obvious because the grains will be coated with pollenkitt and contain protoplasm. An account of sample contamination and reliability in micropalaeontology *sensu lato* was given in Green (2001a, p. 138–145). Supplementary Data Appendix 3 includes syntheses of five relevant contributions on contamination.

14. Palynological preparation techniques for specific materials

14.1. Introduction

In this section, the palynological preparation of 14 non-siliciclastic sedimentary rocks, sediments and various other materials of both pre-Quaternary and Quaternary age are reviewed. Specific materials frequently require substantial modification of the traditional palynological preparation procedure. These materials are not arranged alphabetically; firstly specific sediment/rock types are treated, followed by a variety of other materials. The major sediment and sedimentary rock types are presented first in a logical order. These comprise carbonates, carbon-rich sediments (e.g. coal and peat), hydrocarbons, evaporites, chert/flint, pyroclastics/volcaniclastics, coprolites/faecal pellets/faeces and amber/copal. Siliciclastic sedimentary rocks and sediments are not included here because, due to their relative ubiquity, the traditional acid-based preparation techniques treated in sections 6 to 11, by default, pertain to these materials. Next, the preparation of four natural modern materials, honey, beeswax, ice/snow and soil are documented. Finally the laboratory treatment of ceramics and materials analysed in forensic investigations are reviewed.

14.2. Carbonates

Carbonates, dominated by limestone and dolomite, make up ~15% of the sedimentary rocks at the surface of the Earth (Tucker 2001). Limestones, particularly the virtually pure carbonate rock chalk, are generally very organic-poor. This means that some limestones are either sparse in palynomorphs, or are entirely palynologically barren. Because of the organic-lean nature of these lithotypes, larger samples than normal should be collected and processed. If large samples (up to ~200–400 g) are used, potentially abundant assemblages of well-preserved palynomorphs may be obtained (Table 1). Many limestones were precipitated chemically and became lithified relatively quickly, hence may have excellent (i.e. three dimensional) preservation potential (Munneke and Servais 1996). Generally speaking the darker (or ‘marlier’) the limestone, the better the potential

palynomorph yield (Wilson 1971a). Limestones are generally prepared using the traditional hydrochloric acid-hydrofluoric acid acid digestion technique. Because of the preponderance of calcite, the hydrochloric acid phase is frequently accompanied by violent effervescence and the associated foam is often rich in palynomorphs (section 8.2; Figure 23C). Unsurprisingly, the use of deflocculating agents such as sodium hexametaphosphate appears to be relatively ineffective on carbonate rocks (Riding and Kyffin-Hughes 2004).

In Supplementary Data Appendix 4.1, six relevant papers are reviewed. Kuyl (1960), in part, and Traverse and Ginsburg (1966) described the preparation of modern carbonate sediments. Traverse and Ginsburg (1966) described their methodology of preparing quantitative slides from the Holocene carbonate sediments of the Great Bahama Bank, in the West Atlantic. The remainder of the papers are on pre-Quaternary limestones, principally the Upper Cretaceous Chalk Group. It was pointed out by Kuyl (1960) that organic residues obtained from carbonates normally do not need as much oxidation as ones prepared from silicious materials. Kuyl (1960), Clarke and Verdier (1967), and Wilson (1971a) used the traditional palynological procedure involving both hydrochloric and hydrofluoric acids. However, Schopf (1965) and Traverse and Ginsburg (1966) reported that palynomorphs can be effectively released from carbonate sediments by simply using hydrochloric acid. Similarly, Nørgaard et al. (1991) and Riding and Kyffin-Hughes (2004) reported that the use of hydrofluoric acid in the preparation of chalks is frequently superfluous because palynomorphs are fully extracted by the action of hydrochloric acid only. Wilson (1971a) is an extremely detailed processing protocol for chalk and other limestones, and this method can be used and/or adapted for any carbonate lithotypes. Glover (1961) described the use of the relatively gentle organic acid ethylenediaminetetraacetic acid (EDTA) for palynological processing. The method of studying *in situ* palynomorphs from polished, slightly etched limestone surfaces described by Munnecke and Servais (1996) is an extremely novel one.

14.3. Carbon-rich sediments and sedimentary rocks

14.3.1. Introduction

This subsection pertains to the palynological preparation of carbon-rich sediments and sedimentary rocks, and is is subdivided into three subsections on peat, coal and charcoal It largely comprises a synthesis of how to extract and concentrate palynomorphs from all types and ranks of coals and peat. This aspect is summarised in Figure 50.

14.3.2. Peat

Peat is an accumulation of partially decayed vegetation; it forms in wetlands such as bogs, marshes, mires and moors. It is preserved when plant material cannot fully decay due to acidic and anaerobic conditions. It may contain insects and animals, but is dominantly composed of the remains of fungi, grasses, herbs, shrubs and trees (Rydin and Jeglum 2013). Clay and sand are normally also present, but only in relatively minor quantities. Peat forms in very wet conditions will accumulate significantly faster, and be less decomposed (i.e. humified), than peat deposits in drier localities. The composition of peat is used to reconstruct palaeoecologies by examining the types and quantities of its organic elements (Moore 1987). Peats are classified as, in order of increasing plant decomposition, fibric, hemic or sapric, and peat is the precursor to the formation of coal.

Pollen and spores are normally extracted from Quaternary and fossil peats by alkali treatment followed by acetolysis (subsections 9.3.3 and 11.3). The alkali most often used is 5–10% potassium hydroxide solution, but ammonium hydroxide and sodium hydroxide solution are also suitable. If the peat is mature such that the cellulose is decomposed, alkali digestion alone may be sufficient. The alkali should be thoroughly washed from the residue after the reaction is complete. Supplementary Data Appendix 4.2 summarises seven publications on the preparation of peat.

Bruch and Pross (1999, p. 29) outlined a four-stage procedure for alkali treatment, and a seven-stage protocol for acetolysis. This procedure was also described by Moore et al. (1991). In the alkali treatment, ~3–5 g of dried peat is boiled in a test tube or small beaker for five to 15 minutes in ~10 ml of <10 % potassium hydroxide solution and agitated, then washed. This both fragments the matrix of the peat and dissolves the humic materials. Some tenacious peats need up to 60 minutes of alkali treatment, but great care must be taken because prolonged alkali treatment causes the differential destruction of pollen membranes (Godwin 1934). The residue is then sieved using a 100–120 μm mesh

to separate the pollen from the coarse plant debris and sand. The aqueous pollen concentrate is then centrifuged. The supernatant in the centrifuge tube is decanted such that none of the pollen 'pellet' is disturbed and lost. The centrifugation procedure is then repeated successively until the liquid is clear of dark organic colloids and any remaining alkali.

If cellulose, pollenkitt or protoplasm is present, the residue is acetolysed. If this is the case, it is dehydrated in a centrifuge tube, then fresh acetolysis mixture (a 9:1 combination of acetic anhydride and concentrated sulphuric acid) is added, and the mixture stirred. The tube is boiled in a water bath for one to 15 minutes. It is then centrifuged and the supernatant decanted. The residue is washed with glacial acetic acid, recentrifuged and decanted before being washed with water several times. This procedure is repeated if any cellulose remains. Finally, any remaining coarse material can be removed by sieving with a 150–200 μm mesh to concentrate the palynomorphs.

Some variations on this traditional procedure have been described. For example the method of Churchill (1957) was modified by Lennie (1968). Gauze tubes with 177 μm apertures are loosely filled with peat. These are stoppered and placed in polypropylene tubes containing 5% potasium hydroxide solution, and heated in a water bath for 10 minutes. When the tubes are stirred, the pollen and spores move from the gauze tube by convection and the coarse material remains within the gauze. The pollen/spore concentrate is washed, and may be acetolysed and/or stained prior to slide mounting.

Erdtman and Erdtman (1933) discussed biases which may occur in the pollen residue if peats are boiled in dilute alkali solutions. They described an alternative method using acid hydrolysis. Another alternative procedure was described by Geisler (1935, p. 143) who boiled peat samples in 95% ethanol to liberate the pollen and spores; this author commented that this technique is quicker than other methods. Benninghoff (1947, p. 325) used a dilute (0.25–0.50%) solution of trisodium phosphate to deflocculate peat, to expand and soften the plant tissue, and hence release the pollen and spores. The peat and trisodium phosphate solution mixture is heated and the residue washed. However, this procedure may expand the pollen grains. Hendon and Charman (1997) found that various alkali treatments used routinely in pollen analysis cause substantial corrosion of testate

amoebae. A dense-media separation technique for the effective extraction of pollen assemblages from peat and gyttja was outlined by Nakagawa et al. (1998). Bending (2005) undertook experiments on the extraction of plant materials from peat using alkali digestion. He discovered that a 1% sodium carbonate solution is the most gentle in isolating plant debris from peat, but that disaggregation is relatively slow. In the view of Bending (2005), stronger alkalis such as 5% potassium hydroxide solution may cause significant damage to all plant fossils.

14.3.3. Coal

14.3.3.1. Background. Coal is a highly combustible black or brownish-black rock, composed primarily of carbon with low proportions of other elements such as hydrogen, oxygen and sulphur. It is peat which has been matured in the crust of the Earth. Coal is therefore is a fossil fuel which originally formed in settings where vascular plant remains were not biodegraded or oxidised because they were rapidly covered by sediments and water. The classification of coal is generally based on the content of volatile constituents. As peat ages and is overpressured, under suitable conditions, it is successively transformed to lignite, bituminous coal, anthracite and eventually graphite. Following the extremely complex diagenesis of this plant-dominated material, peat and coal consist of many compounds such as alcohols, carbohydrates and waxes (Francis 1954; Mazor et al. 1979; Stach et al. 1982; Osborne 2013; Dai et al. 2020).

14.3.3.2. Introduction to coal preparation. Plant spores were first noted in coals in thin sections (Witham 1833). However, the extraction of palynomorphs from coals and related sediments is fundamentally different to that of siliciclastic and carbonate rocks in that the acid digestion phase is normally not required. Generally, the procedure falls into two phases. These are partial oxidation of vitrinite and insoluble humic material using a strong oxidising agent such as Schulze's solution, followed by alkali treatment to dissolve the humic acids produced during oxidation. Schulze's solution is a mixture of concentrated nitric acid and potassium chlorate, often in a 1:5 ratio; the former is the oxidising agent, and the latter accelerates the reaction (Manum 1956). The oxygen liberated by this reagent partially oxidises the coal. This two-step procedure is termed

maceration, and was first described by Schulze (1855); it is used on coal and for the maceration of modern plant tissues. Coals are typically rich in palynomorphs. For example, sporinitic coals (i.e. cannel coal) are extremely rich in spores and pollen; these coals may be phenomenally palynologically productive. By contrast fusinitic coal, the precursor of which is burned woody tissue, is normally non-palyniferous (Stach et al. 1982). There is an extensive literature on the palynology of coal and Supplementary Data Appendix 4.3 comprises commentaries on 14 relevant items. Two especially comprehensive accounts on coal preparation are Smith and Butterworth (1967) and Bruch and Pross (1999).

Coal is a highly variable lithotype in terms of both inorganic mineral content and rank, therefore preparation procedures need to be similarly varied in order to produce optimum results. Frequent inspection of the residue is needed, especially during oxidation. To extract palynomorphs from this chemically complex material, a variety of methods are used. These all rely on the fact that sporopollenin is more robust than all the other constituents of coal. The basis of coal preparation is that the extraneous materials, principally bituminous substances (e.g. lipids), cellulose/hemicellulose, humic acids and lignins are removed by using organic solvents, acetolysis, alkali treatment and oxidation respectively (Bruch and Pross 1999). The latter three procedures are by far the most important; organic solvents are rarely used. Following each stage except acetolysis, the residue should be cleaned with water to remove residual traces of the reagents used. This can be achieved by centrifugation, decantation, sieving or sieving with ultrasonic treatment. Some practitioners strongly recommend that coal samples are not acetolysed. Acetolysis normally makes the preparations ‘cleaner’, but this procedure typically destroys or degrades some palynomorphs, especially smooth, thin-walled forms and fungal spores (Jen O’Keefe, personal communication 2021).

It is imperative that insoluble organic materials, for example those derived from lignin, are removed by oxidation. These materials are formed during the process of coalification (Bruch and Pross 1999, fig. 6.1). The most common oxidising agents used are nitric acid, fuming (100%) nitric acid and Schulze’s solution (subsection 9.3.2.2). Normally the strength of the oxidant is proportional to the rank (i.e. maturity) of the coal (O’Keefe et al. 2011). It is imperative not to overoxidise a residue to avoid damaging or

destroying palynomorphs. Excessive maceration leads to the overrepresentation of the more resistant spores such as *Densosporites* spp. It is best to obtain a relatively slow oxidation reaction in order to reduce oxidative degradation of the palynomorphs. Dilute alkali solutions break the humic acids produced during oxidation into soluble salts, which can be rinsed away (subsection 9.3.3). Acetolysis is normally used on modern or sub-fossil material. This is a depolymerisation reaction which converts the celluloses and similar materials into soluble cellulose triacetate which can easily be removed (subsection 11.3).

14.3.3.3. Methods of coal preparation. If the coal includes significant proportions of inorganic minerals, these may need removing using hydrochloric and hydrofluoric acid digestion. Alternatively, these constituents can be removed using swirling and sieving, as noted above. Likewise, any pyrite should be removed using dilute nitric acid prior to the main oxidation if nitric acid is to be used as the oxidant because this mineral may react violently with strong nitric acid (subsection 9.3.2.3). However, most coal samples do not require these steps, and a sample size of between one to 15 g is normally sufficient (Table 1). Rather than using a vessel such as a beaker, Higgins and Spinner (1968) recommended undertaking the partial oxidation and alkali treatment of coal in a sinter glass Büchner funnel placed in a Büchner flask (Figure 26). This allows reactions to be controlled via efficient flushing and washing of the funnel contents.

The key stage in the preparation of coal is partial oxidation. If the sample material is lignite (i.e. low in rank), or the coal is weathered, it may be possible to entirely omit the oxidation stage, or to use dilute hydrogen peroxide, hypochlorus acid, nitrogenated hypochlorous acid or relatively weak nitric acid as an oxidant (Zetzsche and Kälin 1932a; Kosanke 1950, fig. 1; Smith and Butterworth 1967; O'Keefe et al. 2011; O'Keefe and Eble 2012). At the other extreme, if the coal is highly mature (i.e. anthracite), fuming nitric acid is the best reagent to use (Zetzsche and Kälin 1932b). This is because the chemistry of individual macerals becomes increasingly aromatic, and the molecular structures of woody and non-woody macerals are increasingly similar with increasing rank. Fuming nitric acid is an extremely powerful oxidant and can readily cleave the remanent humic gels, making them susceptible to reaction with their conjugate bases,

producing soluble salts, which are then washed away. Smith and Butterworth (1967) recommended a reaction time of at least 16 hours, but the duration can be decreased if the vessel is heated in a water bath. Zetzsche and Kälin (1932b) advocated pretreating the coal with bromine to increase the resistance of spores to oxidation. This is termed the Zetzsche method; bromine attaches to certain valences of the organic material of palynomorphs, and stops the oxidation of the organic fraction by the nitric acid. Paradoxically, fuming nitric acid apparently does not damage and/or bleach the spores as can be the case with Schulze's solution. Unlike the Schulze's solution method, this technique does not require alkali treatment, which is also potentially damaging to the spores. Caro et al. (1964) described a similar method of halogenation which they termed the Erdtman method. Here chlorination and acetolysis removes the extraneous material. The material is dehydrated using glacial acetic acid then treated with a mixture of glacial acetic acid, hydrochloric acid and potassium chlorate. The reaction should be stopped as this mixture bleaches palynomorphs. Finally the halogenised residue is acetolysed. However, most coals are medium or high rank, and Schulze's solution should be used in one of two ways for these; great care must be exercised when using both of these.

Raistrick (1934) found that high rank coals macerate better in Schulze's solution if the sample is pretreated by soaking in cold pyridine for 24 hours, or boiling in pyridine or 16 hours. The pretreated residue is then mixed with one gram of potassium chlorate and 25 ml of fuming nitric acid and left for 16 hours. Other methods have been described using aqua regia, a mixture of chromium trioxide and concentrated nitric acid, a mixture of solid periodic acid and 50% perchloric acid, and sodium hypochlorite (Supplementary Data Appendix 4.3; Funkhouser and Evitt 1959; Hoffmeister 1960; Spielholtz et al. 1962; Lee 1964). Smith and Butterworth (1967) stated that these methods are inferior to partial oxidation using hydrogen peroxide or nitric acid.

14.3.3.4. The 'wet' method. The first technique is the 'wet' method, and Smith and Butterworth (1967) used a saturated solution of potassium chlorate and cold concentrated or fuming nitric acid in a 1:2 or a 1:3 ratio to macerate the coal. Alternatively, potassium chlorate can be added to fuming nitric acid until the acid is saturated; this gives a stronger oxidant. Bruch and Pross (1999, p. 30) advocated mixing

100 ml of Schulze's solution with 10–20 g of coal. If the reaction is violent, for example with pyrite-rich coals, water is carefully added. The reaction time is highly variable, and can be less than five minutes and up to eight days. If the reaction takes several days, the Schulze's solution should be replaced daily. A portion of the residue should be tested periodically with alkali to check the progress of the reaction because overoxidation should be assiduously avoided. If the liquid turns brown, the reaction is complete. A less reliable test of this is that the oxidation reaction is complete when the coal feels soft when stirred. If this procedure is not effective, Bruch and Pross (1999) suggested that the proportion of nitric acid in the Schulze's solution can be increased, the reaction time can be lengthened and/or the mixture can be carefully heated. Alternatively, a stronger oxidising agent such as fuming nitric acid can be used. It cannot be overstated that these variations should be used extremely carefully because overoxidation can destroy palynomorphs. Smith and Butterworth (1967) reported that they failed to extract spores from very high rank coals (i.e. >90% carbon) using this method.

14.3.3.5. The 'dry' method. By contrast, the 'dry' (or Schulze) method outlined by Raistrick (1934), is where the crushed coal sample is mixed with an equal amount of dry potassium chlorate or sodium chlorate crystals. Next, twice to three times the volume of concentrated nitric acid is added. The 'dry' method may react faster, but the chloric acid generated may explode spontaneously if the concentration of this exceeds 30%. Smith and Butterworth (1967) favoured the 'dry' Schulze's method because it is normally quicker and does not normally damage the miospores. These authors used 10 ml of concentrated nitric acid which is added to one gram each of coal and potassium chlorate. This volume of concentrated nitric acid should dissolve the potassium chlorate. If there is more potassium chlorate than coal, the spores are prone to damage. For most coals, the reaction is left for ~16 hours. If excess heat or effervescence occurs during the reaction, these can be mitigated by slowly adding solid potassium chlorate (Green 2001b). When the oxidation reaction is complete, the liquid turns brown; the coal particles should have broken down and the spores will have been released.

14.3.3.6. Alkali treatment. Bruch and Pross (1999, p. 30) described the next stage of this procedure, which is alkali treatment (subsection 9.3.3). The washed residue is treated with 10% potassium hydroxide solution, and heated to close to boiling point. The heat should be removed if the liquid turns brown. The residue is thoroughly washed when the reaction is complete. Several drops of 30% hydrochloric acid are added to neutralise any remaining potassium hydroxide, and the residue washed again. The alkali treatment is repeated if palynomorphs remain surrounded by AOM. Blandon et al. (2008) adapted the normal coal preparation by using two alkali treatments, separately involving sodium hydroxide and ammonium hydroxide, following Schulze's solution for preparing their Paleogene bituminous and sub-bituminous coals from Colombia.

14.3.4. Charcoal

Charcoal is an inertinite maceral that is a frequent component of kerogen, and not a palynomorph *sensu stricto*. It is a black, brittle porous material resembling coal, and comprises 85–98% carbon. Charcoal is a residue of carbon formed by the slow and incomplete combustion of wood, bone or other organic materials in an anaerobic environent, causing the removal of various volatiles and water (Antal and Grønli 2003). Charcoal fragments frequently retain their structure down to the cellular level and can help to indentify phenomena such as anthropogenic cooking fire sites and natural wildfire horizons (Whitlock and Larsen 2001; Scott 2018).

During combustion, differential shrinking due to dehydration results in fracturing into a wide range of fragment sizes. This means that significant levels of charcoal may be present in palynology samples. Small charcoal fragments may be identified and studied in normal palynological preparations. Clark (1984) investigated the effects of various laboratory preparation techniques on the recovery of charcoal of the principal procedures employed during palynological preparation e.g. acetolysis, acid digestion, alkali treatment, density separation, oxidation and ultrasonification. This author recommended that the use of strong oxidising agents should be avoided, or used with care, wherever possible (Supplementary Data Appendix 4.4). Heinz and Barbaza (1998) demonstrated that charcoal fragments can be separated by sieving from unconsolidated sediments. Bryant and Holloway (2000) outlined a method of separating charcoal from the

remainder of the organic residue using multiple heavy liquid separation using zinc bromide of several specific gravities. Subsequently, Urban et al. (2018) described a method of using a system of nested sieves for preparing charcoal. These authors found that this protocol produced substantially less fragmentation of the pieces of charcoal than techniques which include centrifugation and traditional sieving.

14.4. Hydrocarbons

Hydrocarbons in a geological context refer to accumulations of mixtures of gaseous, liquid and solid complex compounds reservoired in crustal rocks. These were derived from the slow, geothermal maturation of fossil material and are of huge economic importance (e.g. Bjørlykke 2015). Palynomorphs can be extracted from liquid, semi-solid and solid hydrocarbon deposits such as crude oil, bitumen, ozokerite and tar (Cross and Wood 1976). Since the pioneering work of Sanders (1937), there have been many investigations of palynomorphs contained in liquid and solid hydrocarbons. The palynology of oil can help determine the age of the source zone, and/or the migration path of hydrocarbons. During the 1950s and 1960s, research on this topic was dominated by Russian and French workers (e.g. Timofeyev and Karimov 1953; Sittler 1955; Brown 1960; 2008; Chepikov and Medvedeva 1961). More recently, the Chinese researcher De-Xin Jiang has published much on this topic (e.g. Jiang 1990; Jiang et al. 2008, Jiang et al. 2016 and references therein).

Palynomorphs are normally isolated from hydrocarbons by dilution and filtration, or dilution and centrifugation/filtration prior to any treatments such as acetolysis or oxidation. First, large samples of bitumen, oil or tar are dissolved or diluted respectively with an organic solvent such as acetone, benzene, chloroform, ethanol, methanol, paraffin (kerosene) or xylene, or various mixtures (Supplementary Data Appendix 4.5). Centrifugation can help to concentrate the palynomorphs, and a large capacity centrifuge is necessary for this procedure (e.g. Sittler 1954; 1955; De Jersey 1965). Alternatively the dissolved bitumen or diluted oil can be left to settle, and the hydrocarbon supernatant decanted away to leave the heavy organic/mineral residue. The latter can be repeatedly centrifuge-washed until all the residual hydrocarbons are removed. However, centrifugation is optional in this overall procedure and the next essential stage following

dilution/dissolution of the hydrocarbon samples is filtration. This is the by far the most effective way of concentrating the palynomorphs. Various filter media have been used including mesh and powder filters. Specifically, loose, oil-insoluble crystalline filters such as ammonium chloride, sodium chloride and fine-grained quartz sand were advocated by Sanders (1937), Tomor (1950) and Artamonova and Medvedeva (1963), however these may allow some palynomorphs to pass through. Paper and membrane filters appear to be significantly superior to using loose crystals (e.g. Timofeyev and Karimov 1953). In particular, the method of Shkrebta (1966), where membrane filters which are dissolved in acetone is especially effective in terms of minimising palynomorph losses. The partially-automated filtration method of Dolgayeva (1968) avoids problems associated with the manual filling of filter funnels, and the clogging of filters. To expedite the filtration process by making the oil flow more easily, the liquid being filtered may need to be carefully heated to 30–80°C.

Later, Jiang (1990) and Jiang et al. (2016) recommended that at least five litres of crude oil per sample is used. The procedure used by this researcher is oil dilution with benzene or gasoline, heating the liquid to 70–75°C, filtration to collect the palynomorph residue, kerogen extraction in a Soxhlet apparatus using benzene, ethanol, ether and ketone, and finally palynomorph concentration by heavy liquid flotation. It should be noted that this procedure can be time-consuming, it involves potentially hazardous organic substances and the loss of palynomorphs is possible during centrifugation, decantation and filtering. Syntheses of 13 relevant papers on the extraction of palynomorphs from hydrocarbons are given in Supplementary Data Appendix 4.5.

Oil-bearing sediment or rock samples are difficult to process in the normal way because the hydrocarbon fraction must be removed in addition to the extraction of the palynomorphs from the matrix. If oil contamination is not tackled, the hydrocarbon residue makes the sieving of palynomorphs difficult or impossible due to clogging effects. Normally, oil is removed by dissolving it in an organic solvent or an emulgating agent (e.g. Nørgaard et al. 1991). The latter study is on the extraction of palynomorphs from tar and oil-stained Chalk Group material (Upper Cretaceous to lowermost Paleocene) of the North Sea. Drill cuttings are also frequently contaminated with oil-based drilling mud, which must be removed prior to preparation (subsection 7.2.3). This

can be achieved by sieving away the hydrocarbon-rich mud using a strong industrial detergent.

14.5. Evaporites

Evaporites are sedimentary rocks comprised almost entirely of water-soluble minerals formed by the evaporation of bodies of surface marine and lacustrine water. Marine evaporites are by far more common than non-marine deposits of this type. For evaporation to occur, the water has to have entered a restricted environment where water input is below the rate of evaporation. This is normally in an arid setting and supplied by a limited input of water. Upon evaporation, the remaining water is enriched in salts, and these begin to precipitate when the water becomes oversaturated with them. In other words when their aqueous concentration reaches a level where they can no longer exist as solutes. The depositional environments of evaporites include grabens in continental and oceanic rift situations fed by limited aqueous input, internal drainage basins fed by ephemeral drainage, and restricted coastal plains in regressive sea environments (sabkhas). Evaporite deposits are dominated by halite, and most evaporite units also inclue non-evaporitic, detrital silicious minerals. The major groups of evaporite minerals are borates, carbonates, halides, nitrates and sulphates. Because carbonates are so abundant, they are normally considered separately from borates, halides, nitrates and sulphates (section 14.2). Evaporitic minerals precipitate from solution in reverse solubility order. Hence the order of precipitation from seawater is calcite and dolomite, gypsum and anhydrite, halite, and potassium and magnesium salts. The relative abundances are in the same order as the succession of precipitation. Hence, limestone is more common than gypsum, which is more common than halite, which is in turn more common than potassium/magnesium salts (Melvin 1991; Warren 2016).

Because evaporites are normally deposited relatively rapidly, and in an arid climate, the concentration of organic material is likely to be low. Hence, large amounts of sample material will normally be needed in order to obtain workable palynomorph associations (Table 1). The conventional hydrochloric acid/hydrofluoric acid maceration procedure is normally ineffective on evaporites because these mineral acids cannot remove significant levels of sulphate minerals (Clay-Poole 1990). Specifically, gypsum is

insoluble in hydrochloric acid, and only weakly soluble in hydrofluoric acid. This mineral also frequently reacts violently with hydrochloric acid and hydrofluoric acid, and calcium fluoride colloids may also form. This situation can present significant problems in operational scenarios as there are several major oilfields, including in the Middle East, that are in evaporitic facies. The traditional hydrochloric and hydrofluoric acid method cannot be adequately modified to mitigate this phenomenon (Sittler 1955). This means that, if the traditional preparation technique is applied to evaporites, any palynomorphs cannot be concentrated effectively due to the abundance of the resistant sulphate mineral grains. The seven contributions described in Supplemental Data Appendix 4.6 outline several procedures for eliminating evaporitic minerals from palynology samples.

Most publications on this topic are on the elimination of anhydrite and gypsum. Various reagents are used for the elimination of these sulphate minerals, and all of these appear to be effective in certain scenarios. These include EDTA, organic solvents, potassium hydroxide, sodium carbonate, sodium chloride and sodium thiosulphate. The specific nature of the samples will depend upon which procedure works best, hence, experimentation with these methods is strongly recommended. Nelson (1950) advocated heating the sample in a supersaturated solution of sodium chloride. This reagent increases the solubility of the anhydrite and gypsum by decreasing the activity coefficient. The residue is then sieve-washed or treated with hydrochloric acid. The use of heated dilute sodium thiosulphate (or ammonium sulphate and sodium hyposulphite) solution to dissolve anhydrite and gypsum was described by Goldman (1952). Similarly, Deák (1959) and Caro et al. (1964) outlined how hot alkaline solutions can remove anhydrite and gypsum. Both these authors boiled samples in 50% potassium hydroxide solution or 50% sodium hydroxide solution for 10–15 minutes prior to washing, hydrochloric acid treatment, heavy liquid separation and oxidation. Caro et al. (1964) also stated that anhydrite and gypsum can be prepared by soaking the sample in fuming nitric acid. Brown (1960; 2008) described the use of dry heated hydrated sodium carbonate to disaggregate anhydrite. After the water of crystallization is driven off, water is then added and the residue left to evaporate. Then the residue is subjected to the traditional preparation method used. However, Brown (1960; 2008) reported that he did not obtain satisfactory results using the latter procedure, or the sodium thiosulphate method of

Goldman (1952). In a short contribution, Mapes and Mapes (1982) described the removal of gypsum from claystone samples by soaking them in an organic solvent, such as paraffin (kerosene) followed by sieve-washing and treating with Stoddard solvent and washed again.Currently, the most well-used method for extracting carbonate and sulphate minerals such as anhydrite and gypsum is by using EDTA. This reagent is a chelating agent that removes calcium and is also known as Versene and was first used for this purpose by Bodine and Fernalld (1973). Rueger (1986) boiled samples for four hours in a solution of tetrasodium EDTA. This removes all the anhydrite, aragonite, calcite, dolomite, gypsum and magnesite, and does not adversely affect the palynomorphs. Following this, the traditional acid-based procedure can be used but this is normally unnecessary because the fabric of the rock should have been broken down by the EDTA. The latter method was modified by Clay-Poole (1990) who boiled samples in a solution of EDTA. If the ph is too high, it may destroy the palynomorphs, hence the ph of the solution is adjusted to to 10 using a solution of sodium hydroxide. Then the residues were centrifuge-washed and treated with a dilute solution of tetrasodium pyrophosphate to deflocculate any remaining clay (subsection 12.5). The samples were then treated with dilute potassium hydroxide solution to remove humic acids, prior to hydrochloric acid and hydrofluoric acid digestion and acetylation.

Leschik (1956), Brown (1960; 2008) and Caro et al. (1964) described several methodologies for the eliminating halite (rock salt) by dissolution in water. In most cases, simple aqueous dissolution should extract the majority of the palynomorphs. However, if there are substantial proportions of insoluble mineral fragments, the residues may need subjecting to hydrochloric and hydrofluoric acid digestion.

14.6. Chert and flint

Chert is a fine-grained, cryptocrystalline, microcrystalline or microfibrous silicious sedimentary rock that may be fossiliferous (e.g. Boggs 2006). Flint is a nodular variety of chert which occurs in chalks and marly limestones. This variety was extensively used for the manufacture of tools during the Stone Age, because flint readily breaks into thin, sharp splinters when struck by another hard object (Luedtke 1992). There are many other varieties of chert including agate, chalcedony, jasper, onyx and radiolarite. Chert and flint

should not be confused with metaquartzite or orthoquartzite. The latter are metamorphosed (recrystallised) sandstone and pure quartz sandstone robustly cemented by silica respectively (Tucker 2001). Chert and flint are hard and hence are relatively resistant to metamorphism, recrystallation and weathering. They exhibit a great variety of colours, and may be black, brown, grey, green, red or white depending on the trace elements present.

Chert and flint normally occur as irregular to oval nodules in carbonate rocks, dominantly chalks, dolomites and limestones. The precise mode of formation of nodular chert and flint is not fully understood. However, it is believed to have formed due to chemical changes in overpressured sedimentary rocks during diagenesis (Knauth 1979). One theory is that gelatinous material filled cavities such as the borings of invertebrates, and that this substance silicified due to replacement by percolating waters. This hypothesis is consistent with the shapes of most flint nodules. The dissolved silica may have derived from silicious sponge spicules and/or silicofossils such as radiolaria (Bromley and Ekdale 1984; 1986). By contrast, the varieties jasper and radiolarite are formed as primary deposits (De Wever 1989).

Prior to the discovery of chemical preparation methods, dinoflagellate cysts and other palynomorphs were first illustrated from translucent flakes of chert and flint. This pioneering work was by Ehrenberg (1837) on the Upper Cretaceous flints of Poland. Sarjeant (1974, p. 49–54), Lejeune-Carpentier and Sarjeant (1981, p. 1–2), and Evitt (1985, p. 31–35) gave accounts of the history of study of palynomorphs from these silicious sedimentary rocks. Chert and flint flakes were also studied by several prominent workers at the beginning of the modern era of palynology such as Walter Wetzel (1922), Otto Wetzel (1933a; 1933b), Deflandre (1935; 1936; 1937) and Lejeune-Carpentier (e.g. 1938; 1940). Maria Lejeune-Carpentier published a long series of ‘notes’ on dinoflagellate cysts from flakes of Upper Cretaceous flints from Belgium and the Baltic region between 1936 (as Maria Lejeune) and 1951 (Sarjeant and Vanguestaine 1999). The study of dinoflagellate cysts and acritarchs in chert and flint was subsequently continued by Valensi (1953; 1955), Gocht (1970a) and Foucher (1975; 1976). The Upper Cretaceous dinoflagellate cysts from flint flakes in the early papers of Deflandre were largely collected from nodules in the pathways, pavements and roads of central Paris.

Hence this material had no stratigraphical calibration whatsoever. By contrast, Jean-Claude Foucher examined dinoflagellate cysts from well-dated flint nodules from the Paris Basin and published a comprehensive biostratigraphical synthesis (Foucher 1979).

The opaline texture of chert and flint means that a powerful light source can pass through delicate flakes, thereby allowing the microfossils to be studied. If the chert or flint contains abundant and well-preserved material, and thin flakes can be readily obtained, this method can give adequate results. However the optical resolution of detailed morphological features is substantially inferior to palynomorphs that have been fully extracted from rock because of the light interference effects of the surrounding silica. A direct comparison is possible in Foucher (1983) on the dinoflagellate cysts from the Upper Cretaceous of the Paris Basin, northern France. In Foucher (1983), plates 1 and 2 are of dinoflagellate cysts extracted chemically, and plate 3 is of forms embedded in flint flakes. The specimens in plates 1 and 2 are significantly superior in appearance to those in plate 3 in this study.

In this mechanical isolation technique that is now not normally used, thin flakes were broken from nodules of chert or flint nodules, and examined using a low power microscope under xylene. If the flakes were fossiliferous, they were trimmed to a suitable size and mounted onto a microscope slide using Canada balsam. These could then be studied and photographed using a high power microscope. The chert/flint may be oiled to reduce optical interference. Good specimens in these flakes are relatively rare; the majority of grains are broken, unfavourably oriented or too deeply imbedded for detailed study.

Only one paper is reviewed in detail in Supplementary Data Appendix 4.7. This is Gocht (1970a) who described dissolving flint using hydrofluoric acid. This study recognised the apparently normal preservation of dinosporin dinoflagellate cysts and two types of inorganic pseudomorphs after dinosporin. Gocht (1970a) referred to three substances. These are the original dinosporin (substance A), a primary inorganic substance which gradually replaced the original (or filled the cavity) over geological timescales (substance B) and a secondary inorganic substance which is formed from the primary material on reaction with hydrofluoric acid during processing (substance C). It is probable that substance B is calcite infilling the dinoflagellate cyst cavity, left by the

destruction of substance A. There would certainly have been sufficient calcium ions available for this scenario in the Upper Cretaceous chalk. During laboratory processing with hydrofluoric acid, in some specimens, the calcite was possibly transformed to calcium fluoride (subsection 8.2; Grayson 1956; Stancliffe and Matsuoka 1991). However this was not proved by Gocht (1970); for example, the refractive index of substance C is higher than that of calcium fluoride.

The eminent dinoflagellate cyst researcher Bill Evitt from the USA was aware of the research undertaken in Belgium and France by Deflandre, Foucher and Lejeune-Carpentier on dinoflagellate cysts from Upper Cretaceous flints. Georges Deflandre had given Bill several flakes of palyniferous flint so he prepared them in the normal way to extract the dinoflagellate cyst assemblages. To Bill's surprise, he found these samples all to be entirely devoid of palynomorphs. Because of this apparent paradox he then undertook an experiment, described by Riding and Lucas Clark (2016, p. 55, 56), where he dissolved a small flake of the French flint with a clearly observable dinoflagellate cyst embedded in it using dilute hydrofluoric acid in a shallow vessel under a stereomicroscope. It should be stated that the use of hydrofluoric acid outside a fume hood as described above is emphatically not recommended by the present author. The hydrofluoric acid slowly dissolved the flint and, to Bill's astonishment, the dinoflagellate cyst simply disappeared. Therefore these dinoflagellate cyst specimens in at least some of Deflandre's flint material are simply well preserved cavities/moulds with a thin dark coating of organic residue with no mechanical competency and which simply disintegrate upon dissolution. This scenario is clearly inconsistent with the findings of Gocht (1970a) hence the preservation of palynomorphs in flint appears to be extremely variable, and not fully understood. More recently, Jones et al. (1995) briefly described the acid digestion of flints using a microwave unit, strongly implying that dinosporin/sporopollenin palynomorphs can be extracted from flint.

14.7. Pyroclastic and volcaniclastic rocks and sediments

Pyroclastic rocks and sediments are dominantly comprised of volcanic material such as airbourne ash and lapilli which have been accumulated directly. However, in situations where the volcanic constituents have been mobilised, transported and resedimented by

aeolian or fluvial action, the materials thus formed are termed volcaniclastic (e.g. Heiken and Wohletz 1985). Typically, pyroclastic and volcaniclastic sediments and sedimentary rocks are relatively organic lean due to the overwhelming presence of volcanic material. Furthermore, any pollen and spores incorporated into these volcanically-generated material will probably have been destroyed by the inherently high temperatures.

There are very few publications on the palynology of pyroclastics and volcaniclastics, and there is no overarching reason why normal palynological preparation procedures will not be suitable for them. However, Taggart and Cross (1980) reported that they used traditional preparation techniques in a study of Miocene volcaniclastic rocks from the USA. They found that a strong exothermic reaction occurred when hydrofluoric acid was added to their samples. This is due to chemical reactions between the hydrofluoric acid, the volcanic ash and the altered volcanic ash minerals. Substantial proportions of pollen appeared to be destroyed by oxidation during this exothermic reaction. This situation was avoided mixing the sample with crushed ice prior to adding the hydrofluoric acid. The ice cools the sample material so that boiling point is not reached. Taggart and Cross (1980) also did not use strong oxidising reagents on the post-acid residues to avoid the degradation and destruction of palynomorphs (Supplementary Data Appendix 4.8).

14.8. Coprolites, faecal pellets and faeces

A coprolite is a piece of fossilised invertebrate or vertebrate animal dung. The name is derived from the Greek *kopros* and *lithos*, meaning dung and stone respectively (e.g. Amstutz 1958; Häntzschel et al. 1968; Wood 1980; Hunt et al. 2012). Coprolites are trace fossils because they provide evidence of, for example, diet and size as opposed to being part of the body of the producer. They range in size from several millimetres to ~50 cm in maximum diameter. The smaller individuals are termed faecal pellets, and many limestone are very rich in these allochems (Tucker 2001). Coprolites are recognised by structural patterns, for example annular or spiral markings, undigested food and associated fossils. Most coprolites are largely composed of calcium phosphate, with subordinate levels of organic matter. The producer is rarely unequivocally indentified, especially for Palaeozoic and Mesozoic coprolites. By contrast other aspects, for example

whether the producer was a carnivore or a herbivore, can normally be determined. Similarly, if the producer was a terrestrial herbivore, coprolites could potentially be very rich in pollen and spores (Scott 1977). Likewise, the coprolites of marine organisms may yield abundant marine microplankton (Eisenack 1938; Evitt 2001).

There are relatively few contributions on the palynomorph content of coprolites and faecal pellets; five items on this topic are reviewed in Supplementary Data Appendix 4.9. Most authors found that macerating the material with hydrochloric acid and/or hydrofluoric acid was effective. Eisenack (1938) simply dissolved phosphatic nodules of Eocene age using hydrochloric acid. However, Scott (1977) and Edwards et al. (1995) used the traditional method of digestion with hydrochloric and hydrofluoric acids. By contrast, Srivastava (1984) did not obtain good results for some Miocene organic-rich phosphatic mudstones using mineral acid maceration. This author found that oxidising the material by boiling in concentrated aqua regia to be the best method of extracting the palynomorphs. It therefore appears that, if the coprolites are highly organic-rich and/or relatively unindurated, simply oxidising them is potentially a better method than mineral acid digestion.

Compared to pre-Quaternary coprolites, there is a considerable literature on the palynology of Quaternary coprolites, faecal pellets and subfossil faeces. Twelve representative papers are reviewed in Supplementary Data Appendix 4.9. There are a very wide variety of preparation methods used on Quaternary and modern faecal samples. This material can be processed using the traditional procedure of acid digestion, followed by acetolysis or alkali treatment (e.g. Mehringer and Wigand 1990; Carrión 2002; Reinhard et al. 2006). However, most researchers in this field have found that the lack of mineral grains and/or matrix makes hydrochloric and hydrofluoric acid digestion superfluous (Supplementary Data Appendix 4.9). Maher (2006) commented that, because guano is normally too acidic to contain significant levels of carbonates, hydrochloric acid treatment is uneccessary. It is therefore recommended that non-acid techniques are attempted before the use of hydrochloric and hydrofluoric acids are considered.

Several authors advocated the use of a strong detergent, normally trisodium phosphate, to rehydrate and dissagregate Quaternary/modern coprolites and faecal materials such as guano (e.g. Sobolik 1988). Maher (2006) recommended that the

detergent concentration should be low in order to avoid excessive foaming. The rehydrated and dissagregated aqueous residue generally needs various combinations of acetolysis, alkali treatment with potassium hydroxide solution, heavy liquid separation, sieving, and washing with detergent to be adequate for the extraction of pollen and spores (e.g. Sobolik 1988; Akeret et al. 1999). Dettmann et al. (1995) studied faecal and intestinal samples from the mahogany glider or *Petaurus gracilis* (de Vis 1883) of Australia. These authors simply mounted crushed, untreated material on microscope slides. However a second, more refined, slide was produced from residue treated with warm 5% potassium hydroxide solution, washed with water and sieved. Sodium pyrophosphate solution was successfully used to dissagregate samples of subfossil bat guano from karst systems in the UK by Leroy and Sims (2006). The residues were then subjected to alkali treatment, acetolysed and sieved. Horrocks et al. (2008) found that only acetolysis was necessary to extract palynomorphs from modern and subfossil parrot coprolites from New Zealand.

14.9. Amber and copal

Terpenoid resins are produced by many angiosperms and most conifers. These sticky, viscous subsances protect plants by discouraging herbivores, repelling insects and sealing any wounds. The terpenoid compounds in tree resin rapidly polymerise upon exposure to air and sunlight, and this causes the resin to harden (Langenheim 2003). Because exposed resin is hard and has antimicrobial properties, the masses observed on trees are relatively resistant to biological and physical degradation, and consequently they are commonly preserved in sediments. Heat and overburden pressure in successions of sedimentary rock cause molecular polymerisation of the resin and transforms it firstly to copal, and then to amber, via the expulsion of volatiles, for example turpenes. Amber is classified on the chemical nature of the polymerised terpenoids which comprise the macromolecular structure (Anderson and Winans 1991).

Amber is is a soft (2.0–2.5 on the Mohs scale) gemstone and is used to manufacture jewellery and ornaments (Grimaldi 1996). It is a macromolecule formed by the polymerisation of certain organic precursors in the resin such as labdanes, and is largely soluble in organic solvents such as chloroform, ethanol and ether. The process of

molecular polymerisation continues as amber matures. It has a fossil record of Late Carboniferous to Quaternary, but is most abundant in the Late Cretaceous to Paleogene interval (Bray and Anderson 2009). Because of the nature of amber it frequently contains abundant animal and plant material, typically insect remains.

Baltic amber from Kaliningrad Oblast, Russia, is the largest deposit of this material and it is mined extensively in this region. It is found in nodular form in glauconitic sand of Oligocene age. Some may have been reworked from Eocene strata, and Baltic amber also is an allochthonous constituent of Quaternary glaciogenic sediments in and around the Baltic Sea coast. Baltic amber was derived from conifers of the family Sciadopityaceae (Wolfe et al. 2009)

Three papers which described processing protocols for amber and copal are reviewed in Supplementary Data Appendix 4.10. Saunders et al. (1974) macerated Eocene amber in hydrofluoric acid. The residue was then treated twice with concentrated ammonium hydroxide solution, before and after oxidation with nitric acid.

By contrast, Arobba (1976) dissolved subfossil resin in benzene. The residue was then washed, hydrated, treated with hydrochloric acid then boiled in dilute potassium hydroxide solution. Recently, Halbwachs (2020) sourced eight different samples of amber and tested their solubilities using eleven organic solvents. Ten of these proved effective and are acetone, chloroform, dichloromethane, ethyl acetate, isobutyl acetate, nitro thinner, tetrahydrofuran, turpentine, white spirit and xylene. This author thoroughly cleaned the samples to avoid contamination prior to dissolution using a eight stage procedure involving ultrasound, ethanol, bleach and hydrochloric acid. For the dissolution, one gram of sample was treated with 10 ml of solvent and agitated for 6–20 hours. The residue is then sieved, centrifuged and mounted on microscope slides.

14.10. Honey

Honey is a sweet, viscous fluid derived from the collection of nectar from flowers by social honey bees such as the western honey bee (*Apis mellifera* Linnaeus 1758). Another effect of bees collecting nectar to make honey is cross pollination, which is critical for flowering plants. Honey is a mixture of sugars and other compounds with trace amounts of minerals, protein and vitamins. In cold weather, or when food is sparse, bees use their

store of honey as a source of food. The 20,000–60,000 worker bees per hive or swarm collect sugar-rich nectar and raise the bee larvae. Once collected, the nectar is ingested and repeatedly regurgitated by the worker bees until it is partially digested; then it is stored in the honeycomb. Nectar is high in natural yeasts and water which cause the sugars to ferment. The worker bees dry the honeycomb by fanning their wings to evaporate most of the water. This reduction in water content increases the sugar concentration, thereby preventing fermentation of the honey (e.g. Doner 1977; White 1978; Bogdanov et al. 2008).

Pollen is the major source of fat, minerals, protein and vitamins of honey bees, and it is essential in the rearing of worker bees. During the nectar foraging process, pollen grains from entomophilous plants become mixed with nectar while the bee is in the flower. Additionally, pollen can fall into the honeycombs within the hive as a result of airborne contamination or bee grooming (Winston 1987).

Pollen grains may be extracted from samples of raw honey, and the study of the palynology of honey is termed melissopalynology. This spelling is preferred to melittopalynology, mellissopalynology and mellittopalynology by the International Commmission for Bee Research (Jones and Bryant 1996). The pollen types represented will therefore indicate the plants that the bees visited in search of nectar and pollen, i.e. the floral source(s). This means that the specific composition of any batch of honey will depend largely on the mix of flowers available to the bees that produced the honey. It is therefore possible to test the claims of honey producers that their honeys are plant-specific, or to check the country of origin of the honey. Honeys with certain dominant floral types from specific areas can be sold at a premium prices. According to the United States National Honey Board and various other international food regulations, "honey stipulates a pure product that does not allow for the addition of any other substance…this includes, but is not limited to, water or other sweeteners". Melissopalynology can therefore help in the detection of fraudulent practices such as marketing cheap imported honey as a premium local product, or the blending/adulteration of honeys (Vorwohl 1967; Lieux 1981; White et al. 1991). Furthermore it is possible to determine at what time or season of year the honey was produced from the pollen because different types are indicative of specific intervals because of differences in flowering intervals.

Melissopalynology can also be potentially applied to archaeology (Zander 1941; Sowunmi 1976).

Melissopalynology was originally developed via baseline studies in Europe, for example the series of papers by Erdtman (1935; 1943) and Zander (1935; 1937). Since then, melissopalynology has been practiced worldwide and there is an extensive literature on this topic (e.g. Lieux 1972; Jones and Bryant 1992; 1996; 1998; 2001; 2004; Bryant 2014). Prior to Jones and Bryant (2004), there was no standard pollen extraction method for honey samples. The principal difference being that some practitioners acetolyse the pollen residues, and some do not. Wood et al. (1996, fig. 6) is a summary flow chart of the various procedures documented in the literature and based on the work of Lutier and Vaissière (1993). This flow chart clearly shows that the first stage used by all workers prior to 1996 is dilution with heated water. Some researchers add substantial amounts of dilute sulphuric acid to the mixture (e.g. Lieux 1980). It is important to remove all the sugars from the honey by dissolving them and washing them away. This is because any remaining soluble substances will crystallise during slide production and therefore potentially obscure the pollen grains. Furthermore, if the sample residues are acetolysed, any remaining sugars will produce dark, amorphous masses during acetolysis that obscure pollen, and are extremely difficult to remove. The early studies on this topic recommended a three-pronged technique of water dilution, filtration and acetolysis (Erdtman 1935). However some workers do not filter, but simply centrifuge the insoluble residues after water dilution (e.g. Barth 1990). The sample size and the duration and speed of centrifugation vary considerably across the literature. Until recently, the melissopalynological method of Lutier and Vaissière (1993) was used, because it was a highly effective technique. The water diluted honey sample is sieved through a 0.8 μm membrane made from cellulose acetate. Following sieving, the membrane, including the pollen, is acetolysed.

Jones and Bryant (2004) is the definitive paper on techniques in melissopalynology. Many honey pollen taxa have specific gravities of ~1.0, therefore centrifuging in water may be highly ineffective. These authors noted that the techniques for extracting pollen from honey samples vary substantially across this important subdiscipline, and that these differences produce anomalies in pollen recovery. These

variations are the weight of honey prepared, the volume of water for dilution and the speed/duration of centrifugation. Jones and Bryant (2004) used ethanol to dilute honey samples. This technique had already been discussed earlier by Jones and Bryant (2004). These authors added 10 g of honey to 10 ml of water and agitated the mixture to dissolve the honey. Ten grams of honey per sample is the minimum to ensure that the entire pollen assemblage is sampled, and that statistically useful pollen data can be recorded. Next, 100 ml of 95% ethanol was added, the mixture homogenised and then centrifuged for three minutes at 4,000 RPM. This methodology was then compared to two water dilution techniques, with centrifugation times of one and ten minutes, again at 4,000 RPM. It was found that the ethanol diluted samples produced substantially higher pollen concentrations and diversities than all the water diluted samples. Jones and Bryant (2004) recommended that the ethanol dilution technique should be used as the standard technique for the extraction of pollen from honey due to the markedly better pollen recovery compared to water dilution methods. Nine papers on melissopalynology are summarised in Supplementary Data Appendix 4.11.

14.11. Beeswax

Beeswax, or *cera alba* meaning white wax, is an edible natural wax which is produced by honey bees. Worker bees produce scales of this mixture of esters of fatty acids and complex alcohols from their abdominal glands. These scales are discarded, where they are used by the hive worker bees to make the distinctive hexagonal prismatic wax cells for housing honey, larvae and pupae. Beeswax has been used in candlemaking and cosmetics, for casting glass and metals, and as a lubricant, plastic, polish and waterproofing agent. It has also been utilised as an artistic medium, for example in sculpture (Winston 1987; Brown 2015).

Furness (1994) described a relatively sparse pollen flora from beeswax extracted from a statue of a horse and rider which was possibly made by Leonardo da Vinci. This author centrifuged molten wax and attempted to dissolve the beeswax using a proprietary wax solvent; both these methods proved unsuccessful. However, treatment with glacial acetic acid followed by acetolysis enabled the effective extraction of pollen grains (Furness 1994, fig. 2). Eight cored beeswax samples from the statue were processed.

Each one was cut into small pieces and placed in a glass tube then treated with 5 ml of glacial acetic acid. The fragments were crushed using a mounted needle and left for 10 minutes to dissolve the wax. The cloudy mixture was then centrifuged, the supernatant decanted away and the residue acetolysed for six minutes. The resultant pollen concentrate proved relatively sparse, compared to the control sample of moden beeswax (Supplementary Data Appendix 4.12).

14.12. Ice and snow

The pollen and spore content of glacier ice and snow can be extremely informative. Pollen and spores from the near hinterland can provide information on the vegetation of the surrounding areas, and any far-travelled pollen can help reconstruct palaeoclimates and wind regimes. Snow deposited during autumn, spring and summer should contain pollen grains and spores characteristic of the particular season. By contrast, winter snow should be virtually devoid of plant remains so seasonal stratigraphy and annual ablation surfaces should be recognisable. For example, Ambach et al. (1966) demonstrated that summer and winter ice layers can be distinguished based upon their pollen spectra and quantities. These authors determined the pollen content of glacier ice from the Ötzal Alps, Austria can vary profoundly. From these data, the absolute pollen influxes can be worked out. Furthermore, the nature of the pollen/spore assemblages and other elements of insoluble residues from ice can also indicate the onset and evolution of anthropological activity.

Vareschi (1935) first recorded pollen and spores from ice from the Grindelwald Glacier, Switzerland, and published a series of papers on the palynology of several European glaciers (e.g. Vareschi 1937). The method used by Volkmar Vareschi, the pioneer of this topic, was to clean the ice surface and the faces of a crevasse, before cutting out 2–10 litres of representative (i.e. homogenous) ice. The ice samples were later melted using a Primus stove in the field and the meltwater hand-centrifuged to concentrate the insoluble residue. The concentrated residues were preserved with thymol and transported to the laboratory. The mineral fraction was removed by hydrofluoric acid digestion. If necessary, the organic concentrates were also aceolysed. Microscope slides are made using glycerine; these can be produced quantitatively thereby allowing pollen

concentration in grains per litre to be determined. Volkmar Vareschi found that European glacier ice normally yields pollen and spores, and that the preservation is generally satisfactory. However, the bisaccate pollen grains are sometimes damaged. The substantial body of research of Vareschi was summarised by Godwin (1949).

Five papers on this topic are summarised in Supplementary Data Appendix 4.13. There are substantial differences in the methodologies used to concentrate the pollen assemblages from samples of glacier ice and snow. Obviously, in all cases, the ice and snow is melted. The meltwater can be left to settle and the the insoluble residue concentrated by centrifugation and/or decantation. Basically the residue is then generally treated like a normal sub-fossil or modern sample. The pollen and spores are subjected to acetolysis or alkali treatment with potassium hydroxide solution prior to slide production (e.g. Ambach et al. 1966). However, if significant levels of mineral grains are present, the residue should be treated with hydrochloric acid and/or hydrofluoric acid prior to acetolysis (Heusser 1954; Fredskild and Wagner 1974).

A successful method of concentrating pollen grains and plant spores from ice based on filtration was developed and used by, for example, Lichti-Federovich (1974; 1975), Bourgeois et al. (1985) and Bourgeois (1990). The meltwater is filtered through 5 or 8 μm mesh 25 mm cellulose triacetate or cellulose nitrate filters. The filters is then treated with hydrofluoric acid for one day to remove any silicate mineral grains. The residue is then refiltered using a Nuclepore filter with a mesh size of 8 μm to remove the partly-dissolved cellulose filter. Finally the sieved residue is acetolysed to dissolve any remaining cellulose and to darken the pollen grains.

14.13. Soil

Soil is the naturally occurring, unconsolidated covering of broken mineral and rock particles, and decaying organic matter or humus which covers much of the surface of the Earth and supports life. It is a loosely packed mixture of mineral/rock grains, organic matter, and void spaces containing air and water. Soil formation, which is termed pedogenesis, is the combined effect of the anthropogenic, biological, chemical and physical processes on parent material (van Breemen and Buurman 2002). Pedogenesis results in the formation of the various soil horizons (Ruhe and Daniels 1958). Soil texture

is dependent on the clay, sand and silt content. Clay is the product of the chemical weathering of crystalline minerals such as feldspar, whereas sand and silt are produced by physical weathering (Shirazi and Boersma 1984). The clay content is the principal influence on the nutrient and water retention capacity of a soil. By contrast, the sand content of soils controls their ability to allow liquids to drain through them. The colour of soils is a reflection of biological and chemical weathering. For example, iron forms red or yellow secondary minerals and organic matter decomposes into brown compounds (Black 1993). The life cycle from soil genesis to degradation is typically long and productive. The principal factors in soil formation are biotic potential, parent material, regional climate, time and topography. Soil chemistry, colour and texture normally reflect the underlying geological parent material, and soil types often change at geological boundaries. (White 2006; Eash et al. 2016).

Because soils contain decomposing organic material, pollen and spores derived from from local plants and the airborne pollen load are normally present (Dimbleby 1957). Additionally, soils may contain fossil palynomorphs derived from the parent materials. The palynology of soils is clearly of interest to Quaternary palynologists. Other applications include archaeology and forensic geoscience (e.g. Coil et al. 2003; Horrocks 2004; Macphail et al. 2004; Riding et al. 2007b).

As soil contains substantial amounts of organic matter, and peat contains significant proportions of clay and sand, there is much crossover in the methods of extracting palynomorphs from soil and peat using alkaline solutions. This extraction is achieved by disintegrating the material, mineral dissolution if necessary and the removal of any extraneous materials. Some soils, however, can be disaggregated by simply boiling them in water. Dimbleby (1957) treated his soil samples with a 10% solution of sodium hydroxide. By contrast, Doher (1980), Moore et al. (1991) and Bruch and Pross (1999) outlined digestion procedures for peats and modern soils using potassium hydroxide solution. This was described in subsection 14.3.2.

However, if the soil is mineral rich, the silt/sand fraction can be removed using hydrochloric and hydrofluoric acids (Smith 1966). Next the soil sample is boiled in a 5–10% aqueous solution of potassium hydroxide for 1–60 minutes and agitated. Typically, this takes between five and ten minutes. This procedure helps to disaggregate the material

and dissolve the humic materials, then the residue is centrifuged to remove these dark organic colloids. Dilute (5–10%) sodium hydroxide solution can also be used for this. Any coarse plant materials can be separated by sieving and the residue centrifuge-washed. The palynomorphs can be further concentrated using heavy liquids (Dimbleby 1957; Funkhouser and Evitt 1959; Holloway et al. 1995) or light liquids (Ledingham and Chinn 1955; Kurtz and Turner 1957), then acetolysed. Sodium pyrophosphate can be used as a first and last step in order to achieve an initial and final dissagregation of the clay fraction. Furthermore, the acid digestion steps can be done following the alkali treatment (Bates et al. 1978; Wood et al. 1996, figs 3, 4). Two relevant articles on this topic are summarised in Supplementary Data Appendix 4.14.

14.14. Ceramics

Ceramics are man made inorganic non-metallic materials formed by the action of intense heat. They include clay objects, such as bricks, pipes, pottery and tiles, together with cements and glass. Most ceramic materials are brittle, hard and porous. There are a very small number of papers which mention the palynology of ceramic items, two of which are summarised in Supplementary Data Appendix 4.15. These papers both describe the palynology of potsherds. These are fragments of pottery, and are normally archaeological relics. Normally, the firing process will completely destroy any sedimentary organic material in the original clay by heat and vitrification. However, palynomorphs can survive heating to temperatures of ~1,000°C according to Quinn and Day (2007). This is below the middle of the range for most industrial kilns and means that, if the clay is only partially burnt, some sedimentary organic material, including palynomorphs, may remain. The palynology of poorly-fired pottery items has great relevance to archaeological studies. Palynomorphs may help to identify the age and therefore the provenance of the clay that was used. Fedorova (1964) extracted palynomorphs from a poorly-baked potsherd from Russia by simply crushing the sample and subjecting it to acetolysis. By contrast, Ghosh et al. (2006) were able to prepare material from half-fired potsherds from West Bengal, India by separate treatments with hydrochloric acid, with the addition of dilute potassium hydroxide solution between these, followed by density separation.

14.15. Materials in forensic investigations

Forensic palynology is by now a mature subdisipline (e.g. Horrocks 2004; Wiltshire 2016). Pollen and spore assemblages are used to help link a person to a specific locality, typically a crime scene (e.g. Riding et al. 2007b; Warny 2013; Warny et al. 2020). This means items seized by the authorities from suspects should be examined for palynomorphs. Hence, palynomorphs must be collected and concentrated from articles such as car air filters, fabrics, footwear, firearms/tools, feathers/fur/hair and rope. In the cases of these materials, the sample material is simply treated with warm/hot 10% potassium hydroxide solution and the pollen concentrate collected. Large items such as rifles can be simply washed down or brushed with warm potassium hydroxide solution. Fabrics, leather etc. can be heated (to ~70°C) in potassium hydroxide solution for 10–30 minutes, stirring the vessel occasionally (Horrocks 2004). The potassium hydroxide solution frees the pollen and spores, which can then be subjected to acetolysis if necessary prior to mounting on slides.

A practical example of this is a study of some raw sheeps' wool suspected of being 'overmarketed' as premium quality from the UK was analysed to establish its geographical provenance (Riding 2006). The wool was gently rinsed in dilute (~3–5%) potassium hydroxide solution and the pollen concentrated using a 15 μm sieve. This allowed the recovery of a relatively abundant palynoflora dominated by the pollen of shrubs and trees of distinctly Australasian affinity for one of the samples investigated.

Crystals, powders and other granulated materials, including illicit drugs in these forms, faecal material, mucous, plant material and stomach contents are prepared using destructive methods such as acetolysis and mineral acid digestion (Horrocks 2004).

15. Preparation techniques for specific palynomorph groups

15.1. Introduction

Although generic (or 'traditional') preparation techniques will normally extract all the organic microfossils from sediments and sedimentary rocks, certain palynomorph groups may require specialised preparation procedures. These are documented in the following

six subsections. The groups treated here are chitinozoa, dinoflagellates and dinoflagellate cysts, microforaminiferal linings and microforaminifera, scolecodonts, megaspores, and sedimentary organic matter. Acritarchs, fungal spores, plant pollen and microspores, and prasinophytes are not specifically reviewed in this section because these groups are extracted effectively by the techniques reviewed above. Sedimentary organic matter is not a palynomorph group *sensu stricto* but, because of the importance of the analysis of phytoclasts, it is included here. The four marine palynomorph groups (i.e. chitinozoa, dinoflagellates and dinoflagellate cysts, microforaminiferal linings and microforaminifera, and scolecodonts) are reviewed first, followed by the single terrestrially-derived group (megaspores), and sedimentary organic matter. Supplementary Data Appendix 5 includes summaries of 74 published items on these palynomorph groups.

15.2. Chitinozoa

The chitinozoa are an important group of Early Ordovician to Late Devonian extinct marine palynomorphs with uncertain biological affinities (Figure 4). They were first discovered in the Ordovician and Silurian rocks of the Baltic region by Alfred Eisenack, who worked extensively on their chemistry, classification and taxomony (e.g. Eisenack 1931). Chitinozoans are found singly or in aggregates; they probably represent the egg cases of an extinct metazoan group (Kozłowski 1963; Laufeld 1974; Riding 1980; Taugourdeau 1981; Paris and Nõlvak 1999). In terms of biological affinities, Gabbott et al. (1998) ruled out gastropods and graptolites, and suggested either conodonts or orthocone cephalopods. Recently Liang et al. (2020) suggested a protistan affinity based upon ultrastructural analysis. Chitinozoans are elongate, tend to be urn- or bottle-shaped and are relatively large (Figure 51; Plate 3.8). The maximum diameter ranges from 50 to 2,000 μm. The chitinozoa evolved rapidly and are valuable biostratigraphical markers. The distributions of some taxa were controlled by biogeography and/or ecology. They can be used, for example, to track climate belts and reconstruct palaeoclimates (Vandenbroucke et al. 2010), and analyse carbon and redox cycling (Vandenbroucke et al. 2013; 2015).

Chitinozoa were reviewed by Jansonius (1970), Jenkins (1970), Jansonius and Jenkins (1978) and Miller (1996). Eisenack (1931) experimented with acid and alkali treatments to attempt to determine the chemical composition of the chitinozoa. He used hot concentrated hydrochloric acid, hot 40% hydrofluoric acid, hot 20% and 50% potassium hydroxide solution, and hot 90% sulphuric acid. Eisenack (1931) found that all these treatments have no discernible effect on chitinozoans. Jacob et al. (2007) used laser pyrolysis GC-MS on individual specimens and found no direct evidence for the preservation of chitin-related molecules. These authors concluded that chitinozoans are made from a kerogen type, perhaps a variety of sporopollenin, dominated by aromatics, and with few aliphatics. This may be due to the loss of chitin during diagenesis or during preparation (Stankiewicz et al. 1997).

This palynomorph group is normally processed using the traditional acid digestion palynological technique, however certain modifications are necessary. Chitinozoans are generally extremely brittle and fragile, and hence great care must be taken at all stages in order to avoid chemical and/or mechanical damage, for example during sieving. They tend to be relatively low in abundance/concentration (normally <20 specimens per gram of rock) hence relatively large samples (~200–300 g) must be processed in order to obtain representative associations (Jenkins 1970). However sample sizes of 25 g and up to 3 kg have been recommended (Laufeld 1967; Miller 1996). Prior to the acid digestion phase, the rock should be crushed to about 1.0–2.5 cm^3 fragments in order to minimise mechanical damage. The neutralised post-acid residue is than sieved to remove the fine (<30–50 μm) fraction (Laufeld 1974). Chitinozoans are frequently dark, and hence can be difficult to study. They can be made translucent by bleaching using any of the normal oxidising agents. Several authors have recommended the use of sodium hypochlorite solution for this. The action of this reagent can be safely monitored under the microscope, and the reaction stopped using sodium sulphite solution so that the chitinozoans are not overoxidised (Jenkins 1967; 1970). Because chitinozoans are relatively large, they can easily be manually picked from the neutral, oxidised and sieved residue using a micropipette or a fine brush under the microscope and single/multiple mounts produced. Miller (1967) is exclusively on the preparation and study of the chitinozoa. This is

summarised in Supplementary Data Appendix 5.1, together with the sections on the processing of chitinozoans in eleven other papers.

15.3. Dinoflagellate cysts and dinoflagellates

The dinoflagellates are an important group of unicellular flagellate protists (Fensome et al. 1996a; Medlin and Fensome 2013). Most representatives are marine, planktonic cells called thecae, but dinoflagellates are also present in freshwater ecosystems. A significant proportion of the dinoflagellates are photosynthetic. Others are endosymbionts and a small number are parasitic (Spector 1984; Taylor 1987). The photosynthetic dinoflagellates are hence primary producers, comprising a significant part of the aquatic food chain (Falkowski et al. 2004).

Dinoflagellates are a diverse group, and many forms predate other protozoa (Taylor 1980). They have an unusual nucleus, termed a dinokaryon, and the outer (thecal) membrane is cellulosic and hence is not fossilisable. The motile dinoflagellate thecae are usually in the haploid condition, and reproduce primarily through simple vegetative fission. However, sexual reproduction also takes place in certain taxa by the fusion of two haploid individuals to form a zygote. The diploid zygote may form a resting cyst (Evitt 1985, fig. 1.3). Following the resting phase, the dinoflagellate cytoplasm exudes from the ruptured cyst and starts a new generation of dinoflagellates. Cyst-theca relationships are normally determined by incubating living cysts which have been extracted from sediment, and identifying the emergent thecate stage (e.g. Wall and Dale 1966). Most resting cysts are made from dinosporin, which is a resistant organic macromolecule similar to sporopollenin (Plate 3.1–3). This means that dinoflagellate cysts are eminently fossilisable, and they have a rich and diverse Triassic to Quaternary fossil record (Figure 4). Comprehensive reviews of the dinoflagellates and their cysts were given by Evitt (1985), Fensome et al. (1996b) and Riding and Lucas-Clark (2016).

The procedures for the extraction and concentration of fossil and subfossil dinoflagellate cysts from sedimentary rocks and sediments are virtually identical to those for the other principal palynomorph groups such as acritarchs, pollen and spores due to the similarities in composition and size. The traditional acid digestion technique is used virtually universally (Sarjeant 1974). However, it should be noted that certain

dinoflagellate cysts are especially prone to aggressive chemical treatment such as acetolysis, mineral acid treatment and oxidation. This is particularly the case with geologically young material. Samples of Neogene and Quaternary age are normally not oxidised in order to avoid damage to congruentidioidean dinoflagellate cysts (Head 1996; Hopkins and McCarthy 2002). These forms, predominantly modern specimens, are so susceptible to oxidation that they can be selectively destroyed during the natural weathering process. These forms, and the cysts of *Gonyaulax tamarensis*, may also be harmed by prolonged treatment with concentrated acids. Specifically, the removal of neoformed fluorides after hydrofluoric acid treatment using hot hydrochloric acid is especially damaging (subsection 8.4; Dale 1976). Furthermore, acetolysis or treatment with hydrogen peroxide can partially or totally destroy some dinoflagellate cysts, principally the families Congruentidiaceae, Polykrikaceae and Protoperidiniaceae (subsections 11.3; 12.3; Marret 1993; Head 1996; Riding et al. 2007a).

There is an extensive literature on the collection, culturing and preparation of modern dinoflagellates and dinoflagellate cysts, and this topic was reviewed by Dale (1979). Hence the papers on this topic summarised in Supplemental Data Appendix 5.2 are a highly selective representation of this substantial body of work. Some of these papers (e.g. Wall et al. 1977) are concerned with the study of modern dinoflagellate cysts. In these cases, it is recommended that the processing procedure is as chemically gentle as possible.

Modern natural marine shelf sediment should contain dinoflagellate cysts in varying concentrations. This material can be collected using standard coring tools, or sinking cysts can be trapped in the water column. Modern dinoflagellate cysts are collected from marine humus on the sea floor, or from the bottoms of large settling tanks (Wall and Dale 1966). Normally the surface or floculative layer, from the surface down to ~2 cm is sampled (Dale 1979). Direct plankton sampling from the water column is also used to collect dinoflagellates and their cysts. This is achieved by plankton netting or towing; this is the simple extraction by filtration, normally from surface waters (Figures 20, 21; Evitt 1984). The use of Nansen bottles to collect modern dinoflagellates and dinoflagellate cysts in the water column was advocated by Dale (1976).

Following collection, the dinoflagellate cysts are cleaned and concentrated by washing with filtered sea water and sieving, sometimes with ultrasound treatment (Wall and Dale 1966). A nest of 250 μm, 125 μm and 20 μm sieves is ideal; the dinoflagellate cysts should accumulate on the 20 μm sieve (Dale 1979). Wall et al. (1967) collected the 37–74 μm fraction, which is especially rich in dinoflagellate cysts. If sand particles are present, these can be separated by swirling the residue and decanting off the lighter cysts.

Many papers, for example Lewis et al. (1984), describe the incubation of living cysts in order to determine cyst-theca relationships. The concentrated cyst residue should be stored at a temperature lower than that at the seawater at the time of collection. The incubation should be commenced as soon as possible following collection. Incubation experiments are achieved by gently and gradually heating the water to 15–30 °C in a suitable medium for several days (e.g. Wall and Dale 1966; Wall et al. 1967; Head 1996). The incubation vessel (a glass tube or a culture chamber) is subjected to artificial lighting set to a light-dark cycle. These hatching experiments can unequivocally prove that a specific dinoflagellate theca emerges from a given cyst type. If the cysts are stored long term, they should be fixed using neutralised formalin or glutaraldehyde. Cyst-theca relationships can also be investigated by studying the cysts produced by a monoclonal culture of thecate dinoflagellates, and observations of cysts within thecae collected from plankton tows (Head 1996).

There are several publications on the laboratory preparation of dinoflagellate cysts, for example Sarjeant (1974) and Green (2001c). However, the most comprehensive publication on laboratory techniques pertaining to the study of fossil dinoflagellate cysts and modern dinoflagellate cysts and thecae is Evitt (1984). This paper, and 12 others on the collection, culturing and laboratory preparation of fossil and modern dinoflagellate cysts and modern dinoflagellates are summarised in Supplementary Data Appendix 5.2.

15.4. Microforaminiferal linings and microforaminifera

Foraminifera are an important group of marine unicellular protozoa which emerged during the Early Cambrian. They are benthic or planktonic, and their cytoplasm is largely enclosed in a shell or test. The shells are mainly calcareous, but can also be agglutinated

where the test comprises small pieces of sediment cemented together, and may rarely be silicious (e.g. Haynes 1981; Sen Gupta 2003).

Many marine and brackish water palynomorph associations include the acid-resistant inner linings of certain foraminifera. Most of these were produced by benthic foraminifera (Tyson 1995; Monga et al. 2015). These are not a mainstream palynomorph group, and have been termed foraminiferal test linings and Scytinascia. However, the term microforaminiferal linings was established by Stancliffe (1989). Microforaminiferal linings are generally <150 μm in size. These organic-walled remains of microforaminifera are thin layers which formed inside the inner whorls of some foraminifera (Plate 3.6). If certain calcareous microforaminiferal tests are dissolved, their organic linings are liberated and these linings closely reflect the internal test morphology of the original foraminifers (e.g. Wetzel, 1957; Muller 1959; Echols and Schaeffer 1960; Monga et al. 2015, fig. 2). Traverse and Ginsburg (1966) and Cohen and Guber (1968) demonstrated that the numbers of species of foraminifera which yield microforaminiferal linings are a small proportion of the overall microfauna. The biostratigraphy and taxonomy of microforaminiferal linings has not been exhaustively researched, however there are some important papers on these palynomorphs. They were reviewed, for example, by Tappan and Loeblich (1965) and Stancliffe (1989; 1996).

It is very important to understand the difference between the terms microforaminiferal linings and microforaminifera. Wilson and Hoffmeister (1952) treated samples with hydrofluoric acid and recovered small translucent mineralised tests which had been converted to calcium fluoride by the action of the hydrofluoric acid (Grayson 1956). These are termed microforaminifera, and are resistant to the action of hydrochloric acid. Microforaminifera are therefore significantly different to the organic-walled inner linings of foraminifera (i.e. microforaminiferal linings). Because the term microforaminiferal linings was only introduced in 1989, there is a degree of confusion in the earlier literature. For example Wilson and Hoffmeister (1952) illustrated both microforaminifera and microforaminiferal linings. The former group have not been significantly researched.

The procedures for the extraction of microforaminiferal linings are generally identical to those for the main palynomorph groups (e.g. Terasmae 1958; Stancliffe

1989). Supplementary Data Appendix 5.3 gives summaries of 13 papers on both microforaminiferal linings and microforaminifera. This is a selective representation of the literature on this topic, and it includes accounts of both these groups.

15.5. Scolecodonts

Scolecodonts are not one of the more mainstream palynomorph groups, and they are not often used routinely in biostratigraphical investigations. They have been studied most intensively since the 1960s (Hints and Eriksson 2007), but are still much less well known than most other palynomorph groups. Scolecodonts are the jaws or teeth of marine polychaete (annelid) worms (Plate 5). Hence they are most abundant in shallow water marine sediments, but they may also occur in deeper water settings. Scolecodonts appeared in the latest Cambrian, but became common from the Ordovician onwards. Like the acritarchs, they were most abundant and diverse in the Palaeozoic, especially during the Ordovician, Silurian and Devonian (Szaniawski 1996; Eriksson et al. 2004; Armstrong and Brasier 2005, p. 101–103).

Scolecodonts are organic, usually opaque, and brown to black in colour. For palynomorphs they are relatively large; normally scolecodonts are ~100–2000 μm in length, and occasionally may be >10,000 μm long (Plates 3.9, 5; Eriksson et al. 2017). Scolecodonts are composed mainly of scleroprotein; they are not apparently chitinous. Their detailed chemistry, and the degree of sclerotisation of the proteins, are apparently highly variable. Certain taxa are significantly mineralised, for example with aragonite, calcite, calcium apatite or fluoroapatite (Schwab 1966; Colbath 1986). Certain modern forms may have calcite mandibular pads, which would not survive if they were subjected to acid digestion during preparation (Boyer 1980a). It is possible that some palynomorphs resembling scolecodonts represent the jaw apparatus of other organisms (Schwab 1966).

Scolecodonts are typically present in relatively low concentrations in sedimentary rocks. Usually this is <1 specimen per gram of rock, but occasionally abundances of ~10 scolecodonts per gram have been recorded. This means that large samples (>200 g) are needed for their effective study (Hints et al. 2015). They may be brittle and fragile, so great care should be taken during procedures such as sieving (Eller 1941). They can be extracted using the generic mineral acid-based palynological processing procedures (e.g.

Higgins and Spinner 1968; Green 2001b, fig. 26.3). For example, Kozłowski (1956) and Taugourdeau (1971) successfully extracted scolecodonts from Ordovician and Late Devonian (Frasnian) material respectively using both hydrochloric and hydrofluoric acids. Basically, the rock matrix is dissolved using hydrochloric acid and hydrofluoric acid, and the scolecodonts are concentrated by delicate washing and sieving. However, if the sample material is limestone, scolecodonts can be released using dilute acetic or hydrochloric acids (Eller 1941; Kielan-Jaworowska 1966; Bergman 1989). Earlier researchers (e.g. Stauffer 1933; 1939) obtained scolecodonts directly from bedding planes, or by washing shales and dissolving limestones using hydrochloric acid. Charletta and Boyer (1974) and Boyer (1980b) also simply washed the scolecodonts out of the rock matrix.

Following extraction, the scolecodont residues should be sieved using a 32–63 μm mesh prior to flotation in a heavy liquid and/or bleaching if necessary, picking using a fine brush or micropipette and mounting (Eller 1941; Traverse 1988, 2007). If the specimens are opaque, they can be lightened by oxidation. Tasch and Shaffer (1961) used dilute sodium hypochlorite solution for this, but Schwab (1966) recommended stronger reagents such as Schulze's solution. If the residues are allowed to dessicate, this will significantly decrease the possibility of finding articulated scolecodont apparatuses (Szaniawski 1996). To preserve articulated forms, and allow for the effective examination of individual jaws from different sides, the specimens are picked from water and then stored in glycerine (Kielan-Jaworowska 1966; Hints 2000). An alternative method to store scolecodonts is to dry the material and mount specimens on microfossil slides using the same technique as for conodonts (Lindstrom 1964). Both the 'dry' and 'wet' methods allow the extraction of specimens from prepared residues for mounting on scanning electron microscope stubs. Scanning electron microscopy is the preferred imaging technique for scolecodonts, although light microscopy with focus stacking may also be used for photographing small and fragile specimens. Scolecodonts stored in permanent palynological slides are often difficult to identify, describe and photograph; thus this method should normally be avoided. Seven accounts of the preparation of scolecodonts are summarised in Supplementary Data Appendix 5.4. These contributions are highly varied, and deal with extraction with and without using mineral acids and oxidation.

15.6. Megaspores

Megaspores or macrospores (Figure 52; Plate 2.8) are the relatively large spores of heterosporous plants, which produce two spore types, megaspores and microspores. Normally, the megaspore germinates into a multicellular female (i.e. egg-producing) gametophyte. This is fertilised by sperm produced by the male gametophyte, which develops from the microspore (Bateman and DiMichele 1994, fig. 1; Willis and McElwain 2013). Seed plants (i.e. angiosperms and gymnosperms) are heterosporous, but the megaspores are produced inside the embryo sac or nucellus of the ovule and are not released. However heterosporous, free-sporing plants do release megaspores from the megasporangium and these develop into the megagametophyte. Today only a few heterosporous pteridophytes (e.g. the lycopsid *Selaginella*), and heterosporous ferns (e.g. *Azolla* and *Salvinia*) produce free-sporing megaspores (e.g. Wagner 1997).

When heterospory first developed during the Devonian, free-sporing megaspores were abundant until seed plants became dominant in the Carboniferous (Chaloner 1970; DiMichele et al. 1989). Following the Carboniferous, megaspores became less common, but are nevertheless still a significant palynomorph group (Batten and Kovach 1990). In deep geological time, it can sometimes be somewhat difficult to determine unequivocally whether or not a large dispersed spore is a true megaspore. An arbitrary lower size limit of 200 μm has been set for megaspores (Guennel 1952). Small spores (microspores or miospores) were defined by Guennel (1952) as those <200 μm in maximum diameter. It should be stressed that this is a purely artificial distinction, because functional megaspores are known which are <200 μm in diameter.

Sixteen papers on megaspores are reviewed in Supplementary Data Subppendix 5.5. These provide a comprehensive and representative assessment of preparation techniques used for this group. Dettmann (1965) and Pearson and Scott (1999) are excellent review papers on this topic. Additionally, references to the preparation of megaspores can be found in several generic accounts including Traverse (2007, p. 647–649).

Megaspores can be prepared in conjunction with smaller palynomorphs, although they are significantly less abundant than miospores. Hills and Sweet (1972) stated that

palynologically productive mudstones and siltstones typically yield 3–5 specimens per gram. However, the mass of sample of siliciclastic lithologies needed for megaspore preparation varies greatly; between 50 g and 1 kg have been advocated (Hughes 1955; Pearson and Scott 1999). Coals are relatively rich in megaspores and 5–10 g per sample is generally sufficient (Dettmann 1965).

In order to derive workable assemblages of megaspores, special techniques have been developed. Relatively unconsolidated material can simply be wet-sieved or treated with disaggregating agents such as hydrogen peroxide, Quaternary O or sodium carbonate solution (e.g. Dijkstra 1951; Collinson 1980; Wilde and Hemsley 2000). However if the sample material is coherent and well-indurated, hydrochloric and hydrofluoric acid digestion is used (Cookson and Dettmann 1958; Chaloner 1959). Megaspores are released from coals by oxidation (e.g. Arnold 1950), and Darrah (1968) and Zimmerman and Taylor (1970) described the extraction of megaspore membranes from calcareous coal balls using dilute hydrochloric acid. The body colour of specimens can be lightened using the normal range of oxidants and Dettmann (1961; 1965) used clove oil for this purpose. Dettmann (1965) described the sectioning of megaspores in some detail. Because megaspores are relatively large, they can be concentrated from a processed residue by sieving. Hughes (1955) advocated using a nest of three sieves with 375 μm, 190 μm and 150 μm mesh and found that most megaspores are present on the 190 μm sieve. Other authors found that megaspores could be effectively concentrated on screens with a mesh size of between 150 μm and 200 μm (e.g. Dettmann 1965; Wilde and Hemsley 2000). Collinson et al. (1985) used a simple aqueous flotation to concentrate magaspores. Because megaspores are relatively large, they can be easily picked out from the final residue using a fine brush, micropipette or needle under the microscope (Chaloner 1959).

15.7. Sedimentary organic matter

Studies of insoluble sedimentary organic matter (SOM) or kerogen (Figure 3; Hutton et al. 1994) can provide valuable palaeoenvironmental data, and indicate the potential of a stratal succession to have generated hydrocarbons. Traverse (2007, p. 563) preferred the use of the term SOM because kerogen has not been used in a consistent way and, in his

4502 view, is not defined adequately. The range of types of SOM and their relative proportions
4503 allows the study of palynological facies (or palynofacies of Combaz 1964), and this area
4504 has expanded greatly during recent decades (e.g. Traverse 1994; Tyson 1995).
4505 In a palynofacies study, the individual components of SOM, such as amorphous
4506 organic material, charcoal, cuticle, fungal material, palynomorphs, resin and wood are
4507 identified and recorded (Figure 3). Therefore it is essential to have a comprehensive
4508 appreciation of the affinity, classification and environmental significance of all the many
4509 components of SOM. An inherent problem associated with palynofacies studies is that
4510 different workers have not classified SOM consistently. This has meant that there is
4511 significant subjectivity and variation in the literature with regard to the nomenclature of
4512 organic particles. In detailed, local/field-based studies, characteristic SOM assemblages
4513 (i.e. distinctive palynofacies) can be used as marker horizons. This scenario is especially
4514 useful if the unit is sparse in palynomorphs. It is vital that palynofacies analysis be done
4515 in conjunction with lithofacies. For example if several samples are virtually identical
4516 using sedimentological criteria, palynofacies may help differentiate their respective
4517 subenvironments of deposition. Palynofacies cycles can be discerned in rhythmic
4518 successions; these may or may not be in step with the lithological cycles (Waterhouse
4519 1995). Computer-based numerical/statistical methods may help in the detailed analysis of
4520 large palynofacies datasets. The utility of palynofacies analysis has been reviewed by, for
4521 example, Batten (1981; 1982; 1996), Traverse (1994), and Tyson (1995). An example of
4522 a specific palynofacies study is Parry et al. (1981).
4523 It is critical that sample processing and information handling are undertaken
4524 consistently so that variations in laboratory procedure do not result in data anomalies (e.g.
4525 Batten and Morrison 1983). Furthermore, because palynofacies analysis depends on the
4526 relative proportions and the body colour of types of SOM, it is vital that none of the
4527 components are lost or altered during preparation. Preparation procedures should be
4528 controlled, gentle, minimalist and routine. Ideally, a known mass of sample is
4529 demineralised by using hydrochloric acid and hydrofluoric acid prior to mounting an
4530 aliquot of the raw unsieved, unoxidised SOM on microscope slides. Additional
4531 preparation steps such as oxidation, sieving and ultrasonic treatment will potentially
4532 remove and/or destroy certain types of SOM and their use should be avoided altogether

or extremely carefully monitored (e.g. Farr 1989; Tyson 1995). Batten (1981; 1996) strongly advocated the production of two microscope slides per sample. The first is the raw SOM. The next is a slide made up from the split of the residue which has been sieved at between 5 and 10 μm to remove the fine fraction (small humic fragments and/or finely-divided AOM) which can dominate the assemblage (Figure 3). In some cases, it may be necessary to administer a brief oxidation followed by ultrasonic treatment on this split. The sieved SOM normally provides a substantially cleaner and clearer residue. Moreover, the combination of the two different slides allows an accurate assessment of the depositional environment and the hydrocarbon source potential. Thirteen relevant accounts on this topic are summarised in Supplementary Data Appendix 5.6.

16. Palynological preparation using specialist equipment

16.1. Introduction

This section comprises four novel methods of processing palynomorphs using specialist equipment. These are dialysis, the Maceration Tank, microwave digestion, and the Vibraflute and *le Vibroséparateur*. These are treated in turn below in four subsections. The developers of these methodologies are to be enthusiastically congratulated for attempting to automate and mechanise what is an expensive, hazardous and relatively laborious endeavour. However, for various reasons, none of these specialised techniques have become routinely used in palynology laboratories. Ten articles on these items of specialist equipment are synthesised in Supplementary Data Appendix 6.

16.2. Dialysis equipment

The use of kidney dialysis equipment for the neutralisation of sample residues following hydrofluoric acid treatment was described by Jackson et al. (1974) and McKee (1977). In the overwhelming majority of palynology laboratories, the spent hydrofluoric acid is merely repeatedly decanted from open vessels and diluted to neutrality (subsection 8.3). Palynomorphs can be lost during decantation, and a closed vessel neutralisation method such as the use of dialysis equipment may eliminate this problem. In this method, the

residue and the spent hydrofluoric acid is transfered into a dialysis bag and dialysed against water in a large vessel or a sink which is continuously flushed with water. Because of the osmotic properties of the dialysis membrane, the hydrofluoric acid passes through the film into the water and is washed away by the circulating water. This process effectively neutralises the sample in around one to two days. However, this method is not currently extensively used. This may be due to the large amount of space needed in the fume hood for the dialysis vessel (up to 100 litres), the risk of flooding and the relatively long neutralisation times.

16.3. The Maceration Tank

The Maceration Tank was developed during the early 1980s at the Geological Survey of Denmark and Greenland (GEUS), Copenhagen, Denmark (Figure 53). It is a closed vessel for undertaking multiple sample hydrofluoric acid macerations, and these units are still in use in the palynological laboratories of GEUS. It is well known to be effective, however the author is not aware of any other laboratories which use this apparatus. The Maceration Tank has never been made available commercially. This piece of equipment was first described by Gudmundsson (1985), however the major paper on this topic is Poulsen at al. (1990).

This equipment is a custom-built closed polypropylene vessel which can hold up to 40 samples. Decalcified and neutralised residues are placed in small bags made from 10 µm filter cloth and put into the Maceration Tank. Then hydrofluoric acid is piped into the unit. Following the reaction, the spent hydrofluoric acid is pumped away and the Maceration Tank is flushed with water to neutralise the samples. The main advantage of this apparatus is that it allows the safe use of hydrofluoric acid because it is not used in open vessels. Furthermore, it is economical in the use of this expensive reagent because of its multiple sample capability.

16.4. Microwave digestion

During the 1990s, it was contended that microwave digestion is an effective method of extracting palynomorphs from sedimentary rocks. Using this technique, crushed samples are placed in closed vessels and treated with hydrochloric acid followed by hydrofluoric

acid inside an industrial microwave unit. Specific lithologies can be prepared (Jones et al. 1995) and the oxidation of organic residues can also be carried out in a microwave unit (Jones 1994). The microwave energy makes the acid digestion and oxidation processes significantly faster than in the traditional open-vessel method because the speed of the chemical reactions is increased due to the elevated temperatures. Additionally, the acid procedures in microwave units were claimed to be significantly safer than conventional preparations because the acids are piped in and out of the vessels. Hence there is negligible risk of splashing accidents or the inhalation of acidic fumes. Further advantages are that smaller volumes of acids are used in the microwave procedure, thereby cutting costs, and that the microwave units are relatively small hence are space-efficient. Microwave units are also beneficial if the sample material is limited since smaller sample sizes, typically 10–16 g, are required for microwave processing as compared to traditional preparation techniques. Initially, the vessels in microwave units were sealed and pressurised (Ellin and McLean 1994); later models have a focussed microwave beam that negates the need to have pressurised sample vessels that is considerably safer (Jones 1998; Jones and Ellin 1998). Following the mineral acid digestion process, the organic residues are prepared for study in the normal way.

Microwave-assisted extraction is used in other scientific fields such as chemistry, phytolith studies and organic geochemistry. Microwave energy has also been used to dry biological and herbarium specimens (e.g. Arens and Traverse 1989). The use of this technique in palynology was pioneered at the University of Sheffield, UK, and several contributions were published on this topic, the first major works being Ellin and McLean (1994) and Jones (1994). Other laboratories have used microwave digestion, for example Simes and Wrenn (1998).

Despite the apparent effectiveness of microwave technology in palynological preparation, this method has not become widely established in laboratories. This may be due in part to the relatively high cost of industrial microwave units, and considerable health and safety concerns. Furthermore, Wood et al. (1996, p. 33) stated that this system may be useful at the rigsite, but that their experience with microwave preparation was ‘not encouraging’; these authors also mentioned safety problems with pressurised microwave systems (Jones 1998). However, self-contained, computerised microwave

systems encourage their use in areas where non-hazardous processing is necessary, such as Antarctica (Simes and Wrenn 1998).

16.5. The vibraflute and Vibroséparateur

This short subsection reviews two pieces of equipment, the Vibraflute and *le Vibroséparateur* designed by Tschudy (1960) and Dumait (1962b) respectively, to separate the organic and mineral fractions using vibration. In both these units, the residues are placed in elongate subhorizontal containers and vibrated. The Vibraflute uses a glass tube, and the aqueous residue is vibrated using a vibrotool to effect separation (Tschudy 1960). *Le Vibroséparateur* comprises a steel trough which is moved using an electromagnet, and the organic residue mixed with zinc chloride solution is vibrated. *Le Vibroséparateur* was claimed to be effective in separating relatively sparse organic material from mineral-rich residues (Dumait 1962b). Both these units have never been manufactured commercially, and are not extensively used.

17. Miscellaneous techniques related to palynomorph preparation

17.1. Introduction

This section is on eight techniques associated with the preparation and microscopical observation of palynomorphs. These are: elutriation; determination of the absolute concentration of palynomorphs in sediments and sedimentary rocks; electron microscopy; fluorescence microscopy; photomicroscopy; recording the location of palynomorphs on slides; the measurement of palynomorphs; and spore colour measurement. Supplementary Data Appendix 7 includes commentaries on 50 contributions pertaining to these procedures.

17.2. Elutriation

Elutriation is a process used in biology, chemistry and metallurgy to separate small particles from larger ones based on their physical properties (i.e. shape, size and density), using a stream of gas bubbles or liquid which flows upwards. In a mixture of different

constituents, the lighter and smaller particles move upwards if the buoyancy and the friction of the water are greater than the weight of the particle (Monazam et al. 2017).

Clearly therefore, if one needed to extract palynomorphs from an unconsolidated sand, elutriation could be used. Arms (1960) described what he termed a 'silica depressant' method for extracting palynomorphs from organic lean sediments from Mexico and the southwestern USA. The sample material is placed in a centrifuge tube with detergent, pine oil, Quebracho extract solution and water. The tube is then placed in a beaker and a gas jet at a low pressure is inserted and elutriated (i.e. 'bubbled') for ~15 minutes and the tube agitated. The rising bubbles should contain the palynomorphs and these are collected in the beaker; the sand grains should remain in the centrifuge tube due to the Quebracho extract solution acting as a depressant.

Juvigné (1975) published a short note summarising three methods of extracting pollen from unconsolidated coarse-grained siliciclastic sediments developed two years earlier (Juvigné 1973a; 1973b). These include separation using an upward-directed stream of water (i.e. elutriation). The samples are elutriated in a water column, and the water that flows out of the elutriator tube is extracted using a centrifuge and concentrated on a filter (Juvigné 1975, fig. 1). Juvigné (1975) asserted that if the largest pollen grains, in this case *Abies*, are moved upwards, all the pollen will be separated from the mineral fraction. Arms (1960) and Juvigné (1975) are both summarised in Supplementary Data Appendix 7.1. A similar technique, the siphoning of buoyant spores, was described by Bhutta (1974).

17.3. Determination of the absolute concentration of palynomorphs

It is frequently the case that the absolute concentration of palynomorph specimens per unit volume or weight of sediment is critical to the interpretations being sought. This parameter can help investigate aspects such as anomalous abundances of certain taxa, bioproductivity, rate of palynomorph destruction in the sediment, ecology, eutrophication levels and the rate of sedimentation. Simple counts of palynomorphs are susceptible to introducing biases emanating from processing techniques such as centrifugation and sieving (Colbath 1985). This may lead, for example, to the spurious assessments of relative abundances. Hence, it is far better to determine the absolute concentration of

palynomorphs in a sample. Quantitative palynological studies are most prevalent in studies of Quaternary material, but this technique is also extensively applied to samples from deep geological time. Palynomorph concentration can be worked out using three methods. The first is the addition of a known quantity of exotic markers to the sample at the onset of preparation. The alternative techniques use a carefully measured volumetric aliquot of the sample, and the weight of a known aliquot of the palynomorph residue on a slide. These three procedures are documented below. It should be noted that other methods have been described for this, but have not been adopted by most palynologists. These include a procedure where all palynomorphs from a sample are embedded in a film of polyvinyl alcohol and a representative sector counted introduced by Middeldorp and Mijzen (1986).

17.3.1. The use of exotic markers

The concentration of palynomorphs in sediments can be determined by the addition of exotic pollen, spores or microspheres to the sample (Benninghoff 1962; Kirkland 1967; Stockmarr 1971; 1972; Ogden 1986). The most frequently used exotic markers are tablets of known numbers of *Lycopodium clavatum* L. spores, and these are commercially available. The *Lycopodium clavatum* spore tablets are produced by the Department of Quaternary Geology, University of Lund, Sweden (Figure 54; https://www.geology.lu.se/services/pollen-tablets). The numbers of spores per tablet are known with very great accuracy and this figure is ~10,000 (Berglund and Persson 1995). The *Lycopodium clavatum* spores in the tabets have been darkened by acetolysis in order to distinguish them from any similar *in situ* forms. Obviously similar tablets widely marketed as homeopathic medicines and treatments are numerically uncalibrated and therefore should never be used. Other exotic palynomorphs which have been used for this purpose is the pollen of *Eucalyptus globulus* Labill. and *Nyssa sylvatica* Marshall (e.g. Matthews 1969; Cwynar et al. 1979).

A known quantity of exotic ('tracer') palynomorphs is added to a known volume or weight of sample before the processing procedure begins, normally prior to hydrochloric acid treatment. The binding agent in the *Lycopodium clavatum* spore tablets is calcareous so the hydrochloric acid effectively releases the exotic spores into the

sample residue. The aim should be to obtain similar proportions of exotic and indigenous grains (Maher 1981). The tablets dissolve during hydrochloric acid treatment (subsection 8.2). It is very important that the exotic palynomorphs are entirely absent from the sample material being prepared or significant anomalies will potentially be introduced. Following preparation, the ratio of the numbers of exotic and *in situ* palynomorphs allows the calculation of the number of authochthonous grains per unit volume or weight of the sample. This is done using the equation of Benninghoff (1962), i.e.:

$$c = \frac{m_c \times L_t \times t}{L_c \times w}$$

This is where:

c = the number of indigenous palynomorphs per gram of dry rock (= concentration)

m_c = the number of indigenous palynomorphs counted

L_t = the number of *Lycopodium clavatum* spores in each tablet; this is given for each batch

t = the number of tablets added to the sample

L_c = the number of *Lycopodium clavatum* spores counted

w = the weight of dry sediment processed in grams

Routine 'spiking' of samples with exotic marker particles helps to determine if the sample preparation method is effective. If the final residue is barren of both exotic and *in situ* grains, the preparation process is causing the loss and/or the destruction of all palynomorphs. Likewise, exotic marker particles can confirm a sample is barren of *in situ* palynomorphs; this is where the final residue only contains the markers. Similarly, the processing of samples which only contain exotic markers can monitor the contamination of modern pollen in the laboratory.

Mertens et al. (2009) was an inter-laboratory calibration exercise to test the comparability of results obtained by 23 institutions using four Quaternary samples. The relative abundances proved to be broadly reproducible. However, by contrast, substantial loss of *Lycopodium clavatum* spores during sample preparation resulted in the non-

reproducibility of absolute abundances. These authors recommended that procedures such as acetolysis, heated acids, alkali treatment, oxidation, sieving using mesh sizes >15 µm and sustained ultrasonification should be avoided to determine reproducible absolute abundances. Further work on this topic was undertaken by Mertens et al. (2012) and Price et al. (2016).

17.3.2. The aliquot method

An alternative method of the derivation of palynomorph concentration is to process a known dry weight of the sample material (e.g. Muller 1959; Jorgensen 1967; Battarbee and McCallan 1974; Harland 1989). Following the processing procedure, the organic residue is made up to (for example) 50 ml. The residue is homogenised and several 0.05 ml aliquots are taken and mounted on individual coverslips/slides. The palynomorphs are counted and, by a simple calculation, the average palynomorph concentration can be worked out. The calculation is the original weight of the dry sample, divided by the grains per aliquot multiplied by 100 (0.05 ml is one hundredth of 5 ml). This method is reliable providing the weighing, measuring of aliquots and counting are executed with the greatest precision. This method will of course be inaccurate if there are any losses of palynomorphs during preparation. Furthermore, Davis (1965) stated that the aliquot method is prone to errors in accurately measuring the small aliquots. He recommended that the errors can be reduced by diluting the palynomorph residue with a volatile liquid such as an organic solvent prior to withdrawing the aliquots.

17.3.3. The sample/residue weight method

This procedure was described by Traverse and Ginsburg (1966). The original sample is carefuly weighed, then processed in the normal way. At the end of the preparation procedure, the final palynomorph residue is washed into a weighed graduated vial, and mixed with an equal volume of molten glycerine jelly such that the concentration of palynomorphs is suitable for slide production. This mixture is then weighed. Next a microscope slide and coverslip are weighed accurately. A small drop of the residue is then mounted using the weighed slide and coverslip. The slide is then reweighed to ascertain the weight of the glycerine jelly/residue mixture, and the numbers of

palynomorphs on the slide carefully counted. Because the weights of the original sample, the macerated residue and glycerine jelly mixture, and the slide/coverslip are known, the numbers of palynomorphs per gram can be calculated (Traverse and Ginsburg 1966, p. 427).

This method is subject to substantial levels of error. Firstly in ensuring that the volumes of the glycerine jelly and palynomorph residue are equal. However, more importantly, that the weights of the slide/coverslip combination before and after slide mounting are recorded with scrupulous accuracy. The gain in mass by mounting up the slide with the mixture is absolutely miniscule, and consequently very difficult to measure with sufficient precision for a repeatable assessment of palynomorph concentration.

17.4. The electron microscopy in palynology

The traditional transmitted light microscope has certain limitations in palynology. For example it cannot accurately optically resolve ultrafine morphological detail (i.e. at the nanometer scale). If extremely small and subtle morphological detail must be studied, one or both types of electron microscopy should be undertaken. The scanning electron microscope (SEM) images the sample surface and the transmission electron microscope (TEM) can resolve the internal structure by imaging an ultrathin (20–100 nm), electron-transparent section using ultramicrotomy. These two techniques are documented below.

It should be noted that it is virtually certain that the use of electron microscopy will always be confined to the investigation of the detailed morphology of well-preserved palynomorphs. The preparation procedures can be laborious and time consuming, and it takes far longer to scan a strew mount under the SEM than using a transmitted light microscope. Nonetheless, several prominent palynologists such as Norman F. Hughes (1918–1994) and Raimond Below have enthusiastically advocated routinely using the SEM (e.g. Hughes et al. 1979; Batten 1986; Below 1987a; Below 1987b; Below 1990; Hughes and McDougall 1990).

17.4.1. The Scanning Electron Microscope

The Scanning Electron Microscope (SEM) was pioneered by Manfred von Ardenne in 1937 (Figure 55; von Ardenne 1938). The instrument was further developed by Charles

Oatley, and first produced commercially in 1965. It rapidly became extensively used in biology, geology, medicine and material sciences. This instrument offers the opportunity to study the detailed morphology of objects such as microfossils at extremely high levels of magnification (e.g. Hay and Sandberg 1967; Sandberg and Hay 1967; Leffingwell and Hodgkin 1971; Hill and Dilcher 1990). The SEM can produce sharp images of very fine surface detail at different relative levels because the depth of field is substantially higher than of the light microscope. The optical resolution is a function of the diameter of the electron beam. The large depth of field is a function of the high working distance in an SEM (up to ~5 cm). For example, at x 20,000 magnification, an SEM can retain a depth of field of 10 μm with a resolution of ~100 Å. By contrast, a light microscope at x1,200, the depth of field is ~0.08 μm. In summary, in the case of palynomorphs, and other microfossil groups, the ultramicroscopical resolution of the SEM, produced by the the diameter of the electron beam, is significantly higher than that achievable with conventional light microscopy.

The principle behind the SEM is that an electron beam scans the surface of the object under study in a vacuum. The stream of electrons is generated by heating a filament of tungsten or another suitable source. Electromagnetic lenses focus the beam to ~100 Å in diameter before the beam scans the specimen. A significant proportion of the electrons in the beam cause the specimens to emit secondary electrons. It is these secondary electrons which produce the SEM images which are displayed on a monitor. The level of secondary electrons generated depends on the chemistry and topography of the area being scanned. A modern textbook on the SEM is Goldstein et al. (2003).

The material to be examined should be between several microns to 1 cm in diameter and must be securely attached to a small aluminum stub 12.5 mm in diameter. A stub is a circular aluminium plate with a perpendicular shaft which fits into the SEM unit (Figure 56). In palynology, single specimens can be micro-manipulated onto a SEM stub and secured onto an ultrathin layer of a suitable adhesive, or placed on double-sided adhesive tape (e.g. Kidston and Williams 1971; Tomb 1982). Alternatively, small squares of photographic film can be glued to SEM stubs with the emulsion side facing upwards. Then single palynomorph specimens in water (or a small amount of aqueous residue) can be placed onto the exposed film. The water slightly softens the emulsion and allows the

specimens to become securely affixed, without sinking into the surface. This method can still be used as it is, even now, possible to obtain photographic film despite the digital revolution. When using this method to examine strew mounts it is possible to carefully engrave an oriented ('way up') grid pattern into the photographic emulsion with a sharp pin, in order to help relocate significant specimens (Ian C. Harding, personal communication 2020). Strew mounts on circular glass coverslips can be made in the usual way (subsection 10.3.3) and affixed to the SEM stub (Finch 1974). The specimens must be securely affixed to the stub because of the vacuum conditions in the SEM (Dayanandan 1979). To prevent a surface charge build-up on electrically insulating materials like palynomorphs, and to promote the emission of secondary electrons, the specimens are coated with an ultrathin layer of conducting material, commonly carbon, gold, gold-palladium alloy or silver before being placed in the SEM (Leffingwell and Hodgkin 1971). Metal coatings are normally most effective for high resolution electron imaging; gold is ideal because it does not oxidise. This thin layer is produced using vacuum evaporation or sputter coating. Alternatively, electrically insulating material can be examined without a conductive coating in an SEM capable of low vacuum operation. Most modern SEM units have this facility.

Using the SEM in palynology is relatively time-consuming and it is virtually never used in routine analyses (Kidson and Williams 1971). The utility of the SEM in palynology is solely for obtaining high quality, high resolution images of palynomorphs (Figures 51, 52). If a new taxon is being described, the micromorphology of the outer surface can be analysed most effectively with the SEM. Examples of the effective use of the SEM for illustrating palynomorphs include Gocht (1970b), Piel and Evitt (1980), Dodge (1985), Below (1987a,b, 1990), Harding (1990) and Evitt et al. (1998) for dinoflagellates and dinoflagellate cysts, and Leffingwell and Hodgkin (1971) and Hughes et al. (1979) for pollen and spores. The first SEM image of a dinoflagellate was that of *Peridinium limbatum* by Evitt and Wall (1968, pl. 1, figs 2, 3). Below (1987a,b, 1990) developed a groundbreaking technique for mounting dinoflagellate cysts on SEM stubs on their pointed extremities or single processes, but he has never outlined his methodology for doing this. Stereopairs of SEM photographs can provide three dimensional images of palynomorphs. These are obtained by re-photographing a

specimen after tilting it ~7°. The two images are then suitably mounted and studied using a stereo viewer (Dayanandan 1979).

Despite the many advantages of the SEM in terms of optical resolution, it can only image the outer surface of the palynomorph. Any internal structures are not fully resolved unless they are exposed due to deliberate dissection, mechanical damage or natural openings (Figure 57). However in complete (i.e. unbroken) specimens, if they are compressed, the inner layer is often visible topographically by distinct lineations where the walls meet. Also, when palynomorphs are mounted on SEM stubs, they are difficult to restudy using the conventional light microscope. However Jacobson and Schopf (1979) described a method for transferring specimens from SEM stubs to glass microscope slides. Miller et al. (2004) and Leslie and Mitchell (2007) outlined how to remove the gold-palladium coating from specimens which have been studied using the SEM. Moreover, palynomorph specimens on SEM stubs are exposed and vulnerable, thereby presenting problems in terms of long term curation. The permanence and stability of these specimens is not assured. Furthermore, specimens on stubs may be difficult to relocate, however Norman F. Hughes and John F. Laing devised a nickel grid with coordinates to help with this (Laing 1974; Hughes 1994). Whittaker and Hodgkinson (1991) gave an account of techniques for the preparation of calcareous microfossils and conodonts for SEM study, some of which may be applicable to palynomorphs.

17.4.2. The transmission electron microscope and ultramicrotomy

The transmission electron microscope (TEM) uses a beam of electrons transmitted through a specimen and is used in all the practical scientific disciplines. Normally, the specimen is an ultrathin section <100 nm thick. The image is formed by the interaction of the electrons with the sample material as the electron beam is transmitted through the specimen. This specialist equipment is capable of very high optical resolution and extremely fine morphological detail can be imaged (Carter and Williams 2016). The first TEM was developed by the German scientists Max Knoll (1897–1969) and Ernst A.F. Ruska (1906–1988) in 1931. Ernst Ruska received the Nobel Prize in physics for the development of the TEM in 1986. Transmission electron microscopy is therefore an excellent method for elucidating the detailed wall structure of palynomorphs (Kempf

1973). A comprehensive knowledge of palynomorph wall, and the relationships of the various layers enhances descriptions and hence can contribute significantly to taxonomy. The German researcher Ulrich Jux pioneered the use of the TEM to study the structure of the walls of acritarchs, dinoflagellate cysts and prasinophytes from throughout the Phanerozoic although he never described the techniques he used to prepare his material for examination (e.g. Jux 1968a; Jux 1968b; Jux 1971a; Jux 1971b).

Because of their size, palynomorphs are embedded in a small block of a suitable medium, typically resin, then cut into <100 nm sections using ultramicrotomy. Other embedding media such as agar or hard wax can be used. An ultramicrotome is an instrument which cuts specimens of animals, film, magnetic tape, soft metals and rock, plants, plastics etc. into ultra-thin slices that can be studied using the TEM. The sections must be extremely thin because the 50–120 kV electrons produced by normal TEM units cannot pass through biological material which is >150 nm. For best resolution, the ultrathin sections should be 20–100 nm.

The ultramicrotome can be fitted with either a diamond knife for most cuts, or a glass knife, normally for the initial cuts. In order to select the precise area of the specimen block to be sectioned, survey sections are made with the glass knife which are 0.5–2 μm. These can be viewed under a light microscope in order to determine if the desired area of the specimen is in position for ultrathin sectioning with the diamond blade. After the ultrathin sections have been cut, they are left floating on water in a small receptacle, prior to mounting on a metal grid. When the resin block is cut using the diamond or glass knife, the ultrathin sections should not be deformed, and will allow the ultrastructure of the palynomorphs to be resolved (Supplementary Data Appendix 7.4; Dettmann 1965; Skvarla and Pyle 1968; Kempf 1970; Leffingwell and Hodgkin 1971; Griffin 1972; Blackmore and Dickinson 1981). Microtomy was developed prior to the use of the TEM in palynology and the ultrathin sections can also be studied with the transmitted light microscope (e.g. Christensen 1946; Hughes et al. 1962; Evitt 1965).

The TEM has been used extensively in palynology and has proved ideal for analysing the ultrastructure of cavate, spine-bearing or thick-walled taxa (e.g. Benedek 1972; Jux 1980; Eaton 1984). Kennaway et al. (2008) studied the relationships between wall layers in dinoflagellate cysts using transmission electron microscopy. These authors

isolated and embedded specimens in stained agarose. The medium was coloured so that individual specimens, are visible to the naked eye, and hence can be oriented and tracked through their preparation protocol (Kennaway et al. 2008, pls 1, 2). Superb TEM images of the dinoflagellate cyst genus *Hystrichosphaeropsis* were obtained (Kennaway et al. 2008, pls 3–5).

17.5. Fluorescence microscopy in palynology

Fluorescence is a form of luminescence, and is the emission of light by a substance that has absorbed electromagnetic radiation and/or light. The best practical example is where the absorbed radiation is in the ultraviolet region, hence it is invisible, whereas the emitted light is in the visible region of the spectrum. This imparts a characteristic colour to the fluorescent substance that can be seen only when exposed to ultraviolet light (Jameson 2014).

Fluorescence microscopy (or spectroscopy) uses fluorescence to study the properties of both inorganic and organic substances. The specimens are illuminated with light of a specific relatively short wavelength, which is absorbed by them, causing the emission of light of longer wavelength. The light source of a fluorescence microscope is typically either a mercury vapour lamp, a xenon arc lamp or, more recently, a light emitting diode (LED) source. Mercury vapour lamps are currently being phased out due to health and safety concerns. Incident light fluorescence microscopy is used widely for example in coal petrology, forensics, medicine, metallurgy and mineralogy (Lakovicz 2006). It can also be used in palynology, and its use was pioneered by the Dutch researcher Pieter van Gijzel (e.g. van Gijzel 1967 and references therein). As a general rule, bright fluorescence is indicative of aquatic algal, rather than terrestrial plant, affinities. For example, some freshwater algae and prasinophytes typically fluoresce very brightly and stand out from the rest of the palynomorphs. Marine amorphous organic material fluoresces more strongly that its freshwater counterpart. Furthermore, inertinite and vitrinite does not fluoresce, however by contrast immature liptinite (i.e. cuticle and palynomorphs) does (McPhilemy 1988).

Geologically young material, i.e. of Quaternary age, emits intense natural biofluorescence colours throughout the entire visible spectrum. However in older

material, the shorter wavelength fluorescence diminishes markedly and rapidly. During the early and middle Cenozoic, the fluorescence weakens in intensity and moves to the orange, red and yellow areas of the spectrum. By the latest Cretaceous, any natural fluorescence is a dull red colour. This relatively rapid diminution in natural biofluorescence means that pre-Cenozoic reworking into for example Neogene and Quaternary strata can be rapidly recognised (Phillips 1972; Bujak and Davies 1982; Strother et al. 2017). Likewise, Quaternary caving into older successions can be efficiently pinpointed. Palaeozoic and Mesozoic fluorescent material emits thermal fluorescence at the red-yellow end of the spectrum. This phemonenon occurs in sedimentary successions which are thermally mature, thus has relevance to petroleum prospectivity. Specifically the presence of orange-red-yellow thermochemical fluorescence can quickly identifiy hydrocarbon-prone intervals (Bujak and Davies 1982).

Kerogen/palynology residues to be studied using fluorescence microscopy should not be oxidised because this significantly affects the levels of fluorescence. Furthermore, the residues should not be mounted using a medium which fluoresces. Elvacite and glycerine jelly are non-fluorescent and are eminently appropriate. By contrast, Canada balsam is fluorescent and this medium is therefore unsuitable (subsection 10.3.2; Wood et al. 1996).

17.6. The photography of palynomorphs

Riding and Head (2018) is a comprehensive account of the photomicrography of palynomorphs, and the production of photographic plates in the digital era. A similar tract on the photography of plant fossils was published by Kerp and Bomfleur (2011). Obviously, analog/film photography is now very rarely practiced; digital photography is now standard in all scientific endeavours. Flenley (1980) described the difficulty of photographing a pollen grain mounted in silicone oil due to problems holding it in a fixed position during a typical exposure time. To overcome this problem, he designed an aluminium microscope micromanipulator which is attached directly to a photomicroscope (Supplemental Data Appendix 7.5). Another micromanipulator design was outlined by Leffingwell and Hodgkin (1971). Bercovici et al. (2009) discussed a digital method for

reconstructing the depth of field of a photomicroscope which reduces the need for multiple photographs of the same specimen.

17.7. Recording the location of palynomorphs on microscope slides

It is critical to be able to record the precise location of a palynomorph in a strew slide, or a single/multiple grain mount so that they can be easily relocated. This is most important in strew mounts, especially those with large coverslips. An example of this scenario is the location of a holotype in a large, densely-mounted strew mount. Another would be recording the locations of suitable specimens for measurement and/or photography during a systematic scan of a slide.

There are three methods of recording the locations of palynomorphs on microscope slides. The first is simply to physically mark the location on the slide by scratching the coverslip, or using permanent ink or pencil on both surfaces of the slide (Figure 58A; Nelson 1962). If ink is used, the ink rings can be protected from deterioration by using clear nail polish or varnish. It can be somewhat physically arkward to mark the slide while the slide is on the mechanical stage, although inking and marking tools can be obtained which fit into the microscope nosepiece in the place of an objective that will do this. Furthermore, the marked circles can be inaccurate, enclose many specimens, the circles themselves can obscure important palynomorphs and 'overcrowding' problems can occur if there are too many circles on a single slide (Figure 58B). Moreover the ink or pencil circles can be erased. Variations of this method are to use small adhesive pointer arrows, or superimpose a grid system on the microscope slide itself that enables a palynomorph to be relocated within a specified small area (Laing 1974; Zippi 1991).

The second method is to use the coordinates of the millimetre x (horizontal) and y (vertical) vernier scales on the mechanical stage of the microscope. A significant problem here is that the settings of mechanical stages can easily be changed. More importantly, the coordinates of mechanical stages are normally not interchangeable between different microscopes, thus are not unique. Lohmann (1933) and Wodehouse (1933) first proposed simple systems of using carefully measured reference points on a microscope slide to allow the field point coordinates to be converted for use with other microscopes. During

5027 the mid 1950s and early 1960s, this method was intensely debated (Supplementary Data
5028 Appendix 7.6; Pierce 1959; Traverse 1960). Later, Frederiksen (1978) and Cockbain
5029 (1980) attempted to fully resolve this methodology.
5030 The third method is to use a gridded calibrated reference slide and this is the most
5031 frequently used strategy. These were pioneered by Maltwood (1858) and Loose (1934),
5032 but the one which is virtually universally used currently is the England Finder (Graticules
5033 Ltd. 1962). This calibrated slide allows the same point on a slide to be relocated on any
5034 microscope irrespective of the configuration of the mechanical stage. The England Finder
5035 consists of a 2.7 x 7.6 cm glass slide accurately marked on the top surface with a
5036 coordinate style reference system (González 2012, fig. 1). This comprises 25 columns
5037 (A–Z, omitting I) and 75 rows of squares (Figure 59). Each alphanumeric cell typically
5038 appears upside down. They may be reversed in some microscopes due to optical effects
5039 inherent to certain manufacturers and models (Berland 1982; Jansonius 1984, 1985;
5040 Edwards 1985; Michoux 1988). The cells are each subdivided into five sectors. The
5041 principal reference/coordinate, for example T60, is in a circle within one square, and the
5042 remaining peripheral area of the square is divided into four sectors marked, 1, 2, 3 and 4
5043 (Figure 59; Traverse 2007, fig. A.6). Microscope slides should always be placed in the
5044 mechanical stage with the label on the same side; the manufacturers of the England
5045 Finder recommend that it should be on the left. When a significant palynomorph is found
5046 on a slide, it is centered underneath the cross-hairs. The microscope slide is then carefully
5047 removed, and the England Finder placed on the microscope stage the right way up and so
5048 that the three most accurately milled edges (which are clearly marked by black arrows)
5049 are all in contact with the metal sides of the mechanical stage. The manufaturer's label on
5050 the England Finder should hence always appear at the bottom left corner depending on
5051 the orientation of the mechanical stage. The reference, for example T60/3, for the centre
5052 of the field of view is read and recorded. If the specimen is within the inner circle, the
5053 coordinate would therefore be T60/0. When the slide is being studied using a different
5054 microscope, the England Finder is first used to set the respective coordinates. When the
5055 slide is placed on the stage, the specimen of interest should be relocated beneath the
5056 crosshairs. This system works only if the microscope slides used are well cut and are the
5057 same size as the England Finder, and the mechanical stages of the two microscopes are of

5058 good quality and are broadly similar in layout. If any of these situations do not pertain,
5059 conversion factors can be worked out relatively easily.
5060 González (2012) described the England Finder Calculator (EFC) to mitigate the
5061 constant manual manipulation of slides on the microscope stage. The EFC is a software
5062 tool which allows microscopists to digitally transform the x/y coordinates on their
5063 microscope into England Finder references. The user must enter the x/y coordinates of
5064 several predefined control points taken from a standard microscope slide or directly from
5065 an England Finder into the EFC software. A microscope-specific calibration file is
5066 created that will digitally convert the x/y coordinates into England Finder references and
5067 vice versa. It could be argued that, with the EFC, the operator still has to obtain the x/y
5068 coordinates from the vernier scales on the microscope which is just as time-comsuming
5069 as taking out the object slide, inserting and removing the England Finder, then replacing
5070 the object slide. An alternative to using an England Finder is to scribe a cross on the
5071 microscope slide and to measure the location of specimens with respect to this point.
5072 Summaries of 13 relevant items are given in Supplementary Data Appendix 7.6.
5073
5074 ***17.8. The measurement of palynomorphs***
5075 It is vital that the key dimensions of palynomorphs can be measured accurately and
5076 expediently. For most palynomorph taxa, the size range is an integral indentificational
5077 feature. In some cases, species within a genus are morphologically similar, differing only
5078 in their overall size. An example of this is the Middle Jurassic dinoflagellate cyst species
5079 *Chytroeisphaeridia hyalina* (Raynaud 1978) Lentin & Williams 1981. This species is
5080 unusually large (~100 μm), compared to other, much smaller, species in this
5081 morphologically simple genus which are typically much less than half this size (Plate 3.3;
5082 Riding 1990; Riding and Thomas 1997, fig. 4; Riding et al. 1999, pls NS7, TP3).
5083 Traditionally palynologists have used simple calibrated graticules (‘rulers’) in the
5084 eyepieces of their microscopes to measure specimens. The specimen is placed under the
5085 graticule and the number of units read off. For each objective, the number of micrometres
5086 per graticule unit is known, so the dimension in question can easily be worked out. A
5087 wide ranging review of microscope graticules was given by, for example Bovey (1962).
5088 Many palynologists still use this method. Traverse (1988; 2007) commented that if one

can obtain a photographic image of a specimen with an accurate magnification factor of x1000, 1 mm on the photograph equates to 1 μm actual size.

However, digital imaging has revolutionised measurements in palynology. Practitioners can rapidly and accurately obtain dimensions of entire specimens or individual morphological features such as length of processes or wall thickness using proprietary software associated with their photomicroscope. Curved surfaces, overall surface area and density can also be easily measured in addition to length and width. Schols et al. (2002) described 'Carnoy', a shareware package which was designed to enable biologists and palynologists to measure specimens (Supplementary Data Appendix 7.7).

17.9. Spore colour measurement

The sporopollenin which forms the walls of pollen and plant spores matures thermally in sediments during diagenesis and especially burial. This process involves the progressive loss of hydrogen and oxygen (as 'volatiles'), relative to carbon. The sporopollenin changes in both colour and reflectivity, which become darker and higher respectively. The colour of spore walls changes from initially being virtually transparent, through yellow, orange and brown to black with increasing thermal maturity (Supplementary Data Appendix 7.8; Marshall 1991; Yule et al. 1998; Marshall and Yule 1999; Yule et al. 2000; Machado and Flores 2015). Spores also reduce slightly in size during thermal maturation. The colour of spores therefore is an indication of the burial and thermal history of a sedimentary rock, which has clear significance in the evaluation of hydrocarbon prospectivity (Gutjahr 1966). This is because spore colour can indicate whether or not a rock is thermally mature enough to have generated hydrocarbons.

A closely related topic is that of vitrinite reflectance, which lies within the field of organic petrology. Any palynomorph group can be used for colour determination, but plant spores are typically used because of their relative ubiquity, and uniformity in size and wall thickness. Furthermore, different palynomorphs do not exhibit identical colour changes with increasing temperature (Traverse 2007, p. 581–590, fig. 19.1).

The most recent contribution on this topic is Goodhue and Clayton (2010) who proposed a novel measurement, the Palynomorph Darkness Index (PDI). This is a

numerical scale from 0% (white) to 100% (black) using a simple formula involving the adjusted greyscale value (Y_a). Modern digital cameras can be used to measure the red, green and blue intensities of palynomorphs. These data are converted to greyscale in order to derive the numerical PDI. If a correlation between PDI and vitrinite reflectance, the former can be used to determine thermal maturity.

18. Conclusions

This contribution aims to be a comprehensive and illustrated guide to the laboratory processing of a wide variety of sample ages and types for palynomorphs. It is envisioned as a reference and training manual. But however extensive the range of this contribution is, it cannot possibly take into consideration the huge number of variables involved in palynological processing. An item such as this cannot possibly furnish all the answers. However, it is fervently hoped that it will guide practitioners to develop the best methodology for their operational needs and sample materials. Each sample is different, and the use of inflexible protocols will emphatically not give uniformly good results. As a laboratory technician gains in experience in this endeavour it will become clear that, like a chef constantly tasting the food that is being prepared, a good preparator will observe the palynomorph residue under the microscope at all stages in order to decide on how best to proceed next. The advice must be not to be constrained by convention; if a novel procedure, or a new twist, gets the job done then that is absolutely acceptable.

An important axiom in the palynology laboratory is that samples should not be overprepared, for example subjected to excessive acetolysis, heating, heavy liquid separation, oxidation, sieving, swirling or ultrasonic treatment. Most laboratory procedures during processing are potentially damaging and hence should be minimised wherever possible (Hodgkinson 1991). Palynomorphs can be damaged or lost during any stage of processing so the maxim that ‘less is more’ is highly relevant here. It should be remembered at all times that the complete elimination of certain palynomorphs, or palynomorph groups, during processing may occur in certain instances. The most obvious loss is via chemical destruction, especially during acetolysis, bleaching or oxidation. Degradations may also occur during centrifuging, decanting, sieving, washing and in the

course of the storage of residues. That said, a balance must be struck and one must not be overly fearful. If the palynomorphs in a residue are diluted or occluded by superfluous organic material it can often pay dividends to be bold with the use of a strong oxidant on a split of the residue. A good technician will, over time, develop an instinct for assessing the benfits and risks of specific procedures, reagents, timings etc.

If one is processing a moderately organic-rich, sand-free, soft, thermally immature mudstone or siltstone, the stong likelihood is that it will be very easy to process, and will be highly palynologically productive. On the other hand, if the material being processed is a highly indurated, palynomorph-lean, thermally mature, silicious lithotype which is rich in tenacious amorphous organic material, it will probably need severe processing comprising many procedures to extract and concentrate as many palynomorphs as possible despite some potential damage and losses.

The advice therefore is to be creative and flexible throughout the preparation of a sample or batch of samples. A good starting point is to undertake a thorough lithological examination of the sample and make a draft plan for preparation. For example, pyrite-bearing samples should be identified so that they can be pre-treated with dilute nitric acid to dissolve this mineral and other sulphides, which can react violently when mixed with concentrated nitric acid and other strong oxidants (subsection 9.3.2.3). Furthermore, the importance of record keeping cannot be overstated. If, while processing a particular sample, a significant breakthrough is made. The details in the laboratory notebook or spreadsheet will record what was done so that the protocol can be easily recreated (Dunn 2003, fig. 1).

One final piece of advice for palynology technicians is to read around the literature for tips. The comprehensive and extensive bibliography and the Supplementary Data in this contribution should be good starting points for this endeavour, and should help to guide readers to the relevant literature.

Disclaimer

In the text, several trade names are mentioned. This is for information only; these do not constitute endorsements of the respective manufacturers and products.

Acknowledgements

The author would like to acknowledge all the palynology technicians he has worked with in the past, notably Steve Ellin and Paul Higham (University of Sheffield), Linda Orange, Janet Marshall and Jane Sharp (British Geological Survey [BGS], Leeds), and Jane Kyffin-Hughes, Max Z.A. Page and Stephanie Wood (BGS, Nottingham). Furthermore my thanks are extended to all the many fellow palynologists whom I have discussed palynological techniques with over the years. Max Z.A. Page helped with, and posed for, many of the laboratory photographs taken in the BGS laboratory. Simon J. Harris of BGS also undertook some imaging. The BGS graphics team, i.e. Lesley M. Oliver, Debbie C. Rayner and James I. Rayner, expertly drew the line drawings and made up the Plates. Many colleagues have provided advice, help, images and literature to the author on specific topics. These include Allison K. Barbato, Peter K. Bijl, David Bodman, Vaughn M. Bryant, Robert A. Fensome, Lorraine P. Field, Ian C. Harding, Jan A.I. Hennissen, Olle Hints, R. Alison Hunter, Ghania Kiared, Tony Loy, George MacLeod, Duncan McLean, Stewart G. Molyneux, Jen O'Keefe, Roy Starkey, Wilson A. Taylor, Thijs R.A. Vandenbroucke, Sophie Warny, Charles H. Wellman, Graham L. Williams and Will Young. This contribution has benefitted substantially from extremely helpful, incisive and positive reviews from Jan A.I. Hennissen, Jen O'Keefe, and Sophie Warny and editorial comments from Encarni Montoya. The paper is published with the approval of the Executive Director, British Geological Survey (NERC).

Dedication

This contribution is respectfully dedicated to those among the second generation of palynologists in the modern era (i.e. born in the first half of the 20th century), who have contributed substantially to palynomorph preparation techniques. These include David J. Batten (1943–2019); William R. Evitt (1923–2009), Jane Gray (1929–2000), Alfred Traverse (1925–2015), Leonard R. ('Doc') Wilson (1906–1998) and Gordon D. Wood (1949–2015).

Disclosure statement

The author has no potential conflict of interest.

Notes on contributor

JAMES B. RIDING is a geologist/palynologist working at the British Geological Survey in Nottingham, UK. He undertook the MSc in palynology at the University of Sheffield and, several years later, Jim was awarded a PhD by the same institution. During 2004, Jim gained a DSc from the University of Leicester, where he did his Bachelor's degree in geology. His interests include the Mesozoic and Cenozoic palynology of the world, palaeoenvironmental palynology, palynomorph floral provinces, forensic palynology, palynomorph preparation techniques, the history of palynology, the biostratigraphy, morphology, systematics and taxonomy of dinoflagellate cysts, and the geology of the Peak District, UK. Jim is a past Director-at-Large and President of AASP – The Palynological Society, and became Managing Editor in 2004. He was awarded the John Phillips Medal by the Yorkshire Geological Society in 2019 and is currently President-Elect of the International Federation of Palynological Societies.

References

Adam DP, Mehringer PJ. 1975. Modern pollen surface samples – an analysis of subsamples. US Geological Survey Journal of Research. 3:733–736.

Akeret Ö, Haas J-N, Leuzinger U, Jacomet S. 1999. Plant macrofossils and pollen in goat/sheep faeces from the Neolithic lake-shore settlement Arbon Bleiche 3, Switzerland. The Holocene. 9:175–182.

Ambach W, Bortenschlager S, Eisner H. 1966. Pollen-analysis investigation of a 20m firn pit on the Kessclwandferner (Ötztal Alps). Journal of Glaciology. 6:233–236.

Amstutz GC. 1958. Coprolites: A review of the literature and a study of speciens from southern Washington. Journal of Sedimentary Petrology. 28:498–508.

Andersen S.T. 1965. Mounting media and mounting techniques. In: Kummel B, Raup D (editors). Handbook of Paleontological Techniques. W.H. Freeman and Company, San Francisco, 587–598.

Anderson KB, Winans RE. 1991. Nature and fate of natural resins in the geosphere. 1. Evaluation of pyrolysis-gas chromatography/mass spectrometry for the analysis of natural resins and resinites. Analytical Chemistry. 63:2901–2908.

Anderson RY. 1958. A micromanipulator for single-mounting microfossils. Micropaleontology. 4:205–206.

Andrew R. 1970. The Cambridge pollen reference collection. In: Walker D, West RG (editors). Studies in the Vegetational History of the British Isles. Cambridge University Press, Cambridge, UK, 225–232.

Antal MJ, Grønli M. 2003. The art, science, and technology of charcoal production. Industrial and Engineering Chemistry Research. 42:1619–1640.

Araújo R, Natário C, Pound M. 2011. How to mount an inexpensive sieving lab. Journal of Paleontological Techniques. 9:1–8.

Arens NA, Traverse A. 1989. The effect of microwave oven-drying on the integrity of spore and pollen exines in herbarium specimens. Taxon. 38:394–403.

Arms BC. 1960. A silica depressant method for concentrating fossil pollen and spores. Micropaleontology. 6:327–328.

Armstrong HA, Brasier MD. 2005. Microfossils. Second Edition. Blackwell Publishing, Oxford, 296 p.

Arnold CA. 1950. Megaspores from the Michigan coal basin. Contributions from the Museum of Paleontology, University of Michigan. 8:59–111.

Arobba D. 1976. Analisi pollinica di una resina fossile rinvenuta in un dolio romano. Pollen et Spores. 18:385–393.

Artamonova SV, Medvedeva AM. 1963. Methods of extracting spores and pollen from oils and the waters of oil deposits. International Geology Review. 5:1510–1511.

Ashraf AR, Hartkopf-Fröder C. 1996. Die Siebverfahren bei der Aufbereitung palynologischer Proben. Neues Jahrbuch für Geologie und Paläontologie Abhandlungen. 200:221–235.

ASME (American Society of Mechanical Engineers) Shale Shaker Committee. 2004. Drilling Fluids Processing Handbook. First Edition. Gulf Professional Publishing. Elsevier, Amsterdam, The Netherlands, 700 p.

Assarsson G, Granlund E. 1924. En metod för pollenanalys av minerogena jordater. Geologiska Föreningens i Stockholm Förhandlingar. 46:76–82.

Barghoorn ES. 1947. Use of phenol formaldehyde and vinyl resins in sealing liquid mounting media on microscope slides. Science. 106:299–300.

Barrier S. 2008. Physical and chemical properties of sporopollenin exine particles. Unpublished PhD Thesis, University of Hull, Hull, UK, 348 p. (https://hydra.hull.ac.uk/assets/hull:6412a/content).

5303 Barss M.S, Williams GL. 1973. Palynology and nannofossil processing techniques.
5304 Geological Survey of Canada Paper. 73-26, 25 p.
5305
5306 Barth OM. 1990. Pollen in monofloral honeys from Brazil. Journal of Apicultural
5307 Research 29:89–94.
5308
5309 Bateman RM, DiMichele WA. 1994. Heterospory: the most iterative key innovation in
5310 the evolutionary history of the plant kingdon. Biological Reviews. 69:345-417.
5311
5312 Bates CD, Coxon P, Gibbard PL. 1978. A new method for the preparation of clay-rich
5313 sediment samples for palynological analysis. New Phytologist. 81:459–463.
5314
5315 Battarbee RW, McCallan ME. 1974. An evaporation tray technique for estimating
5316 absolute pollen numbers. Pollen et Spores. 16:143–150.
5317
5318 Batten DJ. 1981. Palynofacies, organic maturation and source potential for petroleum. In:
5319 Brooks J (editor). Organic maturation studies and fossil fuel exploration. Academic Press,
5320 London, 201–223.
5321
5322 Batten DJ. 1982. Palynofacies, palaeoenvironments and petroleum. Journal of
5323 Micropalaeontology. 1:107–114.
5324
5325 Batten DJ. 1986. N. F. Hughes – biography and bibliography. In: Batten DJ, Briggs DEG
5326 (editors). Studies in palaeobotany and palynology in honour of N. F. Hughes. Special
5327 Papers in Palaeontology. 35:1–5.
5328
5329 Batten DJ. 1996. Chapter 26A. Palynofacies and palaeoenvironmental interpretation. In:
5330 Jansonius J, McGregor DC (editors). Palynology: principles and applications. American
5331 Association of Stratigraphic Palynologists Foundation, Dallas. 3:1011–1064.
5332

Batten DJ. 1999. Small palynomorphs. In: Jones TP, Rowe NP. (editors). Fossil plants and spores: modern techniques. Geological Society, London, p. 15–19.

Batten DJ, Morrison L. 1983. Methods of palynological preparation for palaeoenvironmental, source potential and organic maturation studies. In: Costa LI (editor). Palynology – Micropalaeontology: Laboratories, Equipment and Methods. Norwegian Petroleum Directorate Bulletin. 2:35–53.

Batten DJ, Kovach WL. 1990. Catalog of Mesozoic and Tertiary Megaspores. American Association of Stratigraphic Palynologists Contributions Series. 24, 227 p.

Below R. 1987a. Evolution und Systematik von Dinoflagellaten–Zysten aus der Ordnung Peridiniales. I. Allgemeine Grundlagen und Subfamilie Rhaetogonyaulacoideae (Familie Peridiniaceae). Palaeontographica Abteilung B. 205:1–164.

Below R. 1987b. Evolution und Systematik von Dinoflagellaten–Zysten aus der Ordnung Peridiniales. II. Cladopyxiaceae und Valvaeodiniaceae. Palaeontographica Abteilung B. 206:1–115.

Below R. 1990. Evolution und Systematik von Dinoflagellaten–Zysten aus der Ordnung Peridiniales. III. Familie Pareodiniaceae. Palaeontographica Abteilung B. 220:1–96.

Bending J. 2005. An experimental approach to the disaggregation of samples from peat deposits. Environmental Archaeology. 10:83–90.

Benedek PN. 1972. Phytoplankton aus dem Mittel- und Ober-Oligozän von Tönisberg (Niederrheingebiet). Palaeontographica Abteilung B. 137:1–71.

Bennie J, Kidston R. 1886. On the occurrences of spores in the Carboniferous Formation of Scotland. Proceedings of the Royal Physical Society, Edinburgh. 9:82–117.

Benninghoff WS. 1947. Use of trisodium phosphate with herbarium material and microfossils in peat. Science. 106:325–326.

Benninghoff WS. 1962. Calculation of pollen and spore density in sediments by addition of exotic pollen in known quantities. Pollen et Spores. 4:332–333.

Bercovici A, Hadley A, Villanueva-Amadoz U. 2009. Improving depth of field resolution for palynological photomicrography. Palaeontologia Electronica. 12.2, 12 p. (http://palaeo-electronica.org/2009_2/170/index.html).

Berglund B, Erdtman G, Praglowski J. 1959. Några ord om Betydelsen av Inbäddningsmediets Brytningsindex vid Palynologiska Undersökningar. Svensk Botanisk Tidskrift. 53:462–468.

Berglund B, Persson T. 1995. Information on spore tablets. AASP Newsletter. 28.1: 17–18.

Bergman CF. 1989. Silurian paulinitid polychaetes from Gotland. Fossils and Strata 25:1–128.

Berland B. 1982. Image reversal in microscopy. Transactions of the American Microscopical Society. 101:174–180.

Bhutta AA. 1974. Siphon technique for isolating buoyant spores. Pakistan Journal of Botany. 6:87–88.

Biesboer D. 1977. Aeropalynology. The American Biology Teacher. 39:88–92.

Bigelow JH. 1980. Improved palynographic technique. Micropaleontology. 26:193–195.

5394 Bjørlykke K. 2015. Chapter 14. Source rocks and petroleum geochemistry. In: Bjørlykke
5395 K (editor). Petroleum Geoscience: From Sedimentary Environments to Rock Physics.
5396 Springer-Verlag, Berlin Heidelberg, 361–371.
5397
5398 Black CA. 1993. Soil Fertility Evaluation and Control. CRC Press, Boca Raton, Florida,
5399 USA, 768 p.
5400
5401 Blackmore S, Dickinson HG. 1981. A simple technique for sectioning pollen grains.
5402 Pollen et Spores. 23:282–285.
5403
5404 Blandon A, Parra N, Gorin GE, Arango F. 2008. Adapting palynological preparation
5405 methods in subbituminous and bituminous coals from Colombia to improve palynofacies
5406 and hydrocarbon source rock evaluations. International Journal of Coal Geology. 73:99–
5407 114.
5408
5409 Bodine MW Jr., Fernalld T.H. 1973. EDTA dissolution of gypsum, anhydrite, and Ca-Mg
5410 carbonates. Journal of Sedimentary Petrology. 43:1152–1156.
5411
5412 Bogdanov S, Jurendic T, Sieber R, Gallmann P. 2008. Honey for nutrition and health: A
5413 review. Journal of the American Colege of Nutrition. 27:677–689.
5414
5415 Boggs, S. Jr. 2006. Principles of Sedimentology and Stratigraphy. Fourth Edition.
5416 Pearson Prentice Hall, New Jersey, USA, 662 p.
5417
5418 Böhm W. 1979. Auger methods. In: Methods of Studying Root Systems. Ecological
5419 Studies 33. Springer Verlag, Berlin, Heidelberg, New York, 39–47.
5420
5421 Bolch CJS. 1997. The use of sodium polytungstate for the separation and concentration of
5422 living dinoflagellate cysts from marine sediments. Phycologia. 36:472–478.
5423

Bond TA. 1964. Removal of colloidal material from palynological preparations. Oklahoma Geology Notes. 24:212–213.

Bourgeois JC. 1990. Seasonal and annual variation of pollen content in the snow of a Canadian high Arctic ice cap. Boreas. 19:313–322.

Bourgeois JC, Koerner KM, Alt, BT. 1985. Airborne pollcn: a unique air mass tracer, its influx to the Canadian high Arctic. Annals of Glaciology. 7:109–116.

Bovey E. 1962. Graticules and fine scales: their production and application in modern measuring systems. Journal of Scientific Instruments. 39:405–413.

Bowler M, Hall VA. 1989. The use of sieving during standard pollen pre-treatment of samples of fossil deposits to enhance the concentration of large pollen grains. New Phytologist. 111:511–515.

Bown PR, Young JR. 1998. Techniques. In: Bown PR (editor). Calcareous Nannofossil Biostratigraphy. British Micropalaeontological Society Publications Series. Chapman and Hall, London, UK, 16–28.

Boyd JL, Riding JB, Pound MJ, De Schepper S, Ivanovic RF, Haywood AM, Wood SEL. 2018. The relationship between Neogene dinoflagellate cysts and global climate dynamics. Earth-Science Reviews. 177: 366–385.

Boyer PS. 1980a. Calcite in the mandibles of a marine polychaete. In: Chaloner WG, Sheerin A (editors). Fifth International Palynological Conference, Cambridge 1980, Abstracts volume, 54.

Boyer PS. 1980b. Polychaete jaw apparatuses obtained without acid leaching from the Devonian of Iowa, USA. In: Chaloner WG, Sheerin A (editors). Fifth International Palynological Conference, Cambridge 1980, Abstracts volume, 55.

Brasier MD. 1980. Microfossils. George Allen and Unwin, London, 193 p.

Bray PS, Anderson KB. 2009. Identification of Carboniferous (320 million years old) class Ic amber. 326:132–134.

Bredenkamp CL, Hamilton-Atwell VL. 1988. A filter technique for preparing pollen for scanning electron microscopy. Pollen et Spores. 30:89-94.

Bromley RG, Ekdale AA. 1984. Trace fossil preservation in flint in the European Chalk. Journal of Paleontology. 58:298–311.

Bromley RG, Ekdale AA. 1986. Flint and fabric in the European Chalk. In: de G Sieveking G, Hart MB (editors). The Scientific Study of Flint and Chert. Cambridge University Press, Cambridge, UK, p. 71–82.

Brooks J, Shaw G. 1978. Sporopollenin: a review of its chemistry, palaeochemistry and geochemistry, Grana. 17:91–97.

Brown CA. 1960. Palynological Techniques. Privately published, 1180 Stanford Avenue, Baton Rouge, Louisiana, USA, 188 p.

Brown CA. 2008. Palynological Techniques. Second Edition. Riding JB, Warny S (editors). American Association of Stratigraphic Palynologists, Dallas, USA, 137 p.

Brown RH. 2015. Beeswax. Fourth (Revised) Edition. Northern Bee Books, Hebden Bridge, UK, 88 p.

Bruch AA, Pross J. 1999. Palynomorph extraction from peat, lignite and coal. In: Jones TP, Rowe NP (editors). Fossil Plants and Spores: modern techniques. Geological Society, London, 26–30.

Bryant V. 2014. Truth in labelling: Testing Honey. Bee Culture. August 2014:29–33.

Bryant VM Jr, Wrenn JH (editors). 1998. New developments in palynomorph sampling, extraction, and analysis. American Association of Stratigraphic Palynologists Contributions Series. 33, 155 p.

Bryant VM, Holloway RG. 2000. Reducing charcoal abundance in archaeological samples. Palynology. 33:63–72.

Buesseler KO, Antia AN, Chen M, Fowler SW, Gardner WD, Gustafsson O, Harada K, Michaels AF, van der Loeff MR, Sarin M, Steinberg DK, Trull T. 2007. An assessment of the use of sediment traps for estimating upper ocean particle fluxes. Journal of Marine Research. 65:345–416.

Bujak JP, Davies EH. 1982. Fluorescence and the search for petroleum. Bedford Instituite of Oceangraphy Annual Review. 1982:54–57.

Buratti N, Cirilli S. 2011. A new bleaching method for strongly oxidized palynomorphs. Micropaleontology. 57:263–267.

Burgess R, Jolley D, Hartley A. 2020. Stratigraphic palynology of the Middle–Late Triassic successions of the Central North Sea. Petroleum Geoscience. doi: 10.1144/petgeo2019-128.

Caffrey MA, Horn SP. 2012. Buying and maintaining nail lacquer for laboratory use: a practical guide for palynologists. AASP – The Palynological Society Newsletter. 45.1:24–26.

Caffrey MA, Horn SP. 2013. The use of lithium heteropolytungstate in the heavy liquid separation of samples which are sparse in pollen. Palynology. 37:143–150.

Campbell JFE, Fletcher WJ, Hughes PD, Shuttleworth EL. 2016. A comparison of pollen extraction methods confirms dense-media separation as a reliable method of pollen preparation. Journal of Quaternary Science. 31:631–640.

Caratini C. 1980. Ultrasonic sieving to improve palynological processing of sediments: a new device. International Commission for Palynology Newsletter. 31:4.

Caro Y, Caye JP, Etienne J, Glintzboeckel Ch, Oertli M, Ragot JP, Vincent E. 1964. Techniques de Laboratoire en Geologie Pétrolière. Chambre Syndicale de la Recherche et de la Production du Pétrole et du Gaz Naturel. Comité des Techniciens. Éditions Technip, Paris, 190 p.

Carrión JS. 2002. A taphonomic study of modern pollen assemblages from dung and surface sediments in arid environments of Spain. Review of Palaeobotany and Palynology. 120:217–232.

Carter CB, Williams DB (editors). 2016. Transmission Electron Microscopy: Diffraction, Imaging, and Spectrometry. Springer, Switzerland, 518 p.

Casas-Gallego M, Gogin I, Vieira, M. 2020. Two new dinoflagellate cyst species and their biostratigraphical application in the Eocene and Oligocene of the North Sea. Palynology, https://doi.org/10.1080/01916122.2020.1819457.

Chaloner WG. 1959. Devonian megaspores from Arctic Canada. Palaeontology. 1:321–332.

Chaloner WG. 1968. British pre-Quaternary palynology: A historical review. Review of Palaeobotany and Palynology. 6:21–40.

Chaloner WG. 1970. The evolution of miospore polarity. Geoscience and Man. 1:47–56.

Chanda C, Ganguly P. 1980. On the problem of oxidation of pollen and preservation of reference pollen slides in tropical climate. Fourth International Palynological Conference, Lucknow (1976–1977) Proceedings. 2:595–596.

Charletta AC, Boyer PS. 1974. Scolecodonts from Cretaceous greensand of the New Jersey coastal plain. Micropaleontology. 20:354–366.

Charman DJ. 1992. The effects of acetylating on fossil *Pinus* pollen and *Sphagnum* spores discovered during routine pollen analysis. Review of Palaeobotany and Palynology. 72:159–164.

Chepikov KR, Medvedeva AM. 1961. Organic remains of ancient appearance in oil of Tertiary, Mesozoic and Palaeozoic deposits. Dokladi an Akad. Nauk an SSSR. 140:339–340 (in Russian).

Chitaley SD. 1966–1967. Preservation of natural colours in pollen and spores – a new technique and its utility. Palynological Bulletin. 2–3:99–100.

Chitaley SD, Deshpande SU. 1969. A new method of pollen preparation. Journal of Palynology. 2:129–131.

Christensen BB. 1946. Om mikrotomsnit af pollenexiner. Meddelelser fra Dansk Geologisk Forening. 11:441–448.

Christensen BB. 1954. New mounting media for pollen grains. Danmarks Geologiske Undersøgelse. 2:7–11.

Churchill DM. 1957. A method for concentrating pollen grains and small fossil remains from fibrous peats and moss pollsters. Nature. 180:1437.

Clark RL. 1984. Effects on charcoal of pollen preparation procedures. Pollen et Spores. 26:559–576.

Clarke CM. 1994. Differential recovery of fungal and algal palynomorphs versus embryophyte pollen and spores by three processing techniques. In: Davis OK (editor). Aspects of Palynology: Methodology and Applications. American Association of Stratigraphic Palynologists Contributions Series. 29:53–62.

Clarke RT. 1963. Elimination of bleeding of Safranine O stain in Clearcol. Oklahoma Geology Notes. 23:259.

Clarke RFA, Verdier J-P. 1967. An investigation of microplankton assemblages from the Chalk of the Isle of Wight, England. Verhandelingen der Koninklijke Nederlandsche Akademie van Wetenschappen, Afdeeling Natuurkunde, Eerste Reeks. 24:1–96.

Clay-Poole ST. 1990. Calcium dissolution and clay flocculant removal in pollen extractions from sediments of the arid southwest, USA. Journal of Paleontology. 64:483–484.

Cockbain AE. 1980. Conversion of microscope-stage coordinates. Micropaleontology. 26:95–96.

Cody GD, Botto RE, Ade H, Wirick S. 1996. The application of soft X-ray microscopy to the in-situ analysis of sporinite in coal. International Journal of Coal Geology. 32:69–86.

Coe AL (editor), Argles TW, Rothery DA, Spicer RA. 2010. Geological Field Techniques. Wiley-Blackwell, Chichester, 323 p.

Cohen A, Guber AL. 1968. Production of pollen-sized “microforaminifera” from “normal” foraminifera. Micropaleontology. 14:361–362.

Coil J, Korstanje MA, Archer S, Hastorf CA. 2003. Laboratory goals and considerations for multiple microfossil extraction in archaeology. Journal of Archaeological Science. 30:991–1008.

Colbath GK. 1985. A comparison of palynological extraction techniques using samples from the Silurian Bainbridge Formation, Missouri, U.S.A. Review of Palaeobotany and Palynology 44:153–164.

Colbath GK. 1986. Jaw mineralogy in Eunicean polychaetes (Annelida). Micropaleontology. 32:186–189.

Colldahl H, Carlson G. 1968. Allergens in pollen: Allergic reactions in mucous membranes (eye and nose) and in the skin provoked by conventional pollen extracts and by extracts derived from micro-organisms derived from pollen. Acta Allergologia. 23:387–395.

Collinson ME. 1980. A new multiple-floated *Azolla* from the Eocene of Britain with a brief review of the genus. Palaeontology. 23:213–229.

Collinson ME, Batten DJ, Scott AC, Ayonghe SN. 1985. Palaeozoic, Mesozoic and contemporaneous megaspores from the Tertiary of southern England: indicators of sedimentary provenance and ancient vegetation. Journal of the Geological Society of London. 142:375–395.

Combaz A. 1964. Les palynofaciès. Revue de Micropaléontologie. 7:205–218.

Cookson IC, Dettmann ME. 1958. Cretaceous "megaspores" and a closely associated microspore from the Australian region. Micropaleontology. 4:39–49.

Correll DL. Phosphorus: a rate limiting nutrient in surface waters. Poultry Science. 78:674–682.

5641
5642 Cour P. 1974. Nouvelles techniques de detection des flux et des retombées polliniques:
5643 étude de la sedimentation á la surface du sol. Pollen et Spores. 16:103–141.
5644
5645 Cridland AA. 1966. An apparatus for hydrofluoric acid macerations. Micropaleontology.
5646 12:510.
5647
5648 Cross AT, Wood GD. 1976. Palynology and petrography of some solid hydrocarbons of
5649 Utah. Brigham Young University Geology Studies. 22:157–173.
5650
5651 Cross AT, Kosanke RT. 1995. History and development of Carboniferous palynology in
5652 North America during the early and middle twentieth century. In: Lyons PC, Morey ED,
5653 Wagner RH (editors). Historical perspective of early twentieth century Carboniferous
5654 paleobotany. Geological Society of America Memoir. 185:353–388.
5655
5656 Crowley AJ. 1952. Method of extracting foraminifera from refractory shale. Bulletin of
5657 the American Association of Petroleum Geologists. 36:2185.
5658
5659 Cushing EJ. 1961. Size increase in pollen grains mounted in thin slides. Pollen et Spores.
5660 3:265–275.
5661
5662 Cushing EJ. 2011. Longevity of reference slides of pollen mounted in silicone oil.
5663 Review of Palaeobotany and Palynology 164:121–131.
5664
5665 Cwynar LC, Burden E, McAndrews JH. 1979. An inexpensive sieving method for
5666 concentrating pollen and spores from fine-grained sediments. Canadian Journal of Earth
5667 Sciences. 16:1115–1120.
5668
5669 Dai S, Bechtel A, Eble CF, Flores RM, French D, Graham IT, Hood MM, Hower JC,
5670 Korasidis VA, Moore TA, Pütmann W, Wei Q, Zhao L, O'Keefe JMK. 2020.

Recognition of peat depositional environments in coal: A review. International Journal of Coal Geology. 219: 103383.

Dale B. 1976. Cyst formation, sedimentation, and preservation: factors affecting dinoflagellate assemblages in Recent sediments from Trondheimsfjord, Norway. Review of Palaeobotany and Palynology. 22:39–60.

Dale B. 1979. Collection, preparation and identification of dinoflagellate resting cysts. In: Taylor DL, Seliger HH (editors). Toxic dinoflagellate blooms. Elsevier, Amsterdam, 137–152.

Damassa SP. 1979. Eocene dinoflagellates from the coastal belt of the Franciscan complex, northern California. Journal of Paleontology. 53:815–840.

Darrah EL. 1968. The microstructure of some Pennsylvanian seeds and megaspores studied by maceration. Micropaleontology. 14:87–104.

Davis MB. 1965. A method of determination of absolute pollen frequency. In: Kummel B, Raup D. Handbook of Paleontological Techniques. WH Freeman and Co., San Francisco and London, p. 674–686.

Dayanandan P. 1979. Scanning electron microscopy of pollen and spores. Journal of Palynology 15:111–119.

Deák HM. 1959. Experimental palynological investigations of gypsum from the Messek mountain range. Bulletin of the Hungarian Geological Society. 89:170–173.

Deflandre G. 1935. Considérations biologiques sur les microorganisms d'origine planctonique conservés dans les silex de la craie. Bulletin biologique de la France et de la Belgique. 69:213–244.

Deflandre G. 1936. Microfossiles des Silex Crétacés. Première Partie. Généralités. Flagellés. Annales de Paléontologie. 25:151–191.

Deflandre G. 1937. Microfossiles des Silex Crétacés. Deuxième Partie. Flagellés incertae sedis. Hystrichosphaeridés. Sarcodinés. Organismes divers. Annales de Paléontologie. 26:51–103.

Deflandre G. 1938. Microplancton des mers Jurassiques conservé dans les marnes de Villers-sur-Mer (Calvados). Étude liminaire et considérations générales. Travaux de la Station zoologique de Wimereux. 13:147–200.

de Jekhowsky B. 1959. Une technique standard de préparation des roches pour l'étude des microfossiles organiques. Revue de l'Institut Francais du Pétrole. 14:315–320.

De Jersey NJ. 1965. Plant microfossils in some Queensland crude oil samples. Geological Survey of Queensland Publication. 329, 9 p.

Delcourt A. 1964. Techniques simples pour la préparation des spores et des grains de pollen des gisements préhistoriques. Bulletin de la Société d'Études et de Recherches Préhistoriques les Eyzies. 13:146–152.

Delcourt A, Mullenders W, Piérart P. 1959. La préparation des spores et des grains de pollen, actuels et fossils. Les Naturalistes Belges. 40:90–120.

Dempsey JE, Urban LL. 1965. Dry storage of processed palynological residues. Pollen et Spores 7:577–579.

Dettmann ME. 1961. Lower Mesozoic megaspores from Tasmania and South Australia. Micropaleontology. 7:71–86.

Dettmann ME. 1965. Techniques used in the study of megaspores. In: Kummel B, Raup D (editors). Handbook of Paleontological Techniques. W.H. Freeman and Company, San Francisco, p. 699–706.

Dettmann ME, Jarzen DM, Jarzen SA. 1995. Feeding habits of the mahogany glider: palynological evidence. Palynology. 19:137–142.

Deunff J. 1977. Sur une méthode complémentaire de traitement et d'éclaircissement du microplancton paléozoïque carbonifié. Bulletin du B.R.G.M. (Bureau de Recherches Geologiques et Minieres), deuxième série, section 1. No. 1:51–54.

De Wever P. 1989. Radiolarians, radiolarites, and Mesozoic paleogeography of the circum-Mediterranean Alpine Belts. In: Hein JR, Obradović J. (editors). Siliceous Deposits of the Tethys and Pacific Regions. Springer, New York, USA, p. 31–49.

Dijkstra SJ. 1951. Wealden megaspores and their stratigraphical value. Mededelingen van de Geologische Stichting, Nieuwe Serie. 5:7–21.

Dimbleby GW. 1957. Pollen analysis of terrestrial soils. New Phytologist. 56:11–28.

DiMichele WA, Davis JI, Olmstead RG. 1989. Origins of heterospory and the seed habit: the role of heterochrony. Taxon. 38:1–11.

Dodge JD. 1985. Atlas of dinoflagellates. A scanning electron microscope survey. Farrand Press, London, UK, 119 p.

Dodsworth P. 1995. A note of caution concerning the application of quantitative palynological data from oxidized preparations. Journal of Micropalaeontology. 14:6.

Doher LI. 1980. Palynomorph preparation procedures currently used in the paleontology and stratigraphy laboratories, U.S. Geological Survey. United States Geological Survey Circular. 830, 29 p.

Dolgayeva ZN. 1968. Advances in extraction of plant microremains from oil. Paleontological Journal. 3:387–389.

Dumait P. 1962a. L'action des ultrason sur les pollens. Note Préliminaire. Pollen et Spores. 4:175–180.

Dumait P. 1962b. Le Vibroséparateur. Pollen et Spores. 4:311–316.

Doner LW. 1977. The sugars of honey - a review. Journal of the Science of Food and Agriculture. 28,443–456.

Dunn J. 2003. Curation of palynological material: a case study on the British Petroleum Micropalaeontological Collection. The Geological Curator. 7:365–372.

Durand B, Nicaise G. 1980. Procedures for kerogen isolation. In: Durand B. (editor). Kerogen. Insoluble organic matter from sedimentary rocks. Éditions Technip, Paris, 35–53.

Durham OC. 1946. The volumetric incidence of atmospheric allergens. IV. A proposed standard method of gravity sampling, counting and volumetric interpolation of results. The Journal of Allergy and Clinical Immunology. 17:79–86.

Eagar SH, Sarjeant WAS. 1963. Fossil hystrichospheres concentrated by sieving techniques. Nature. 198:81.

Eash NS, Sauer TJ, O'Dell D, Odoi E. 2016. Soil Science Simplified. Sixth Edition. John Wiley and Sons Incorporated, Hoboken, New Jersey, USA, 276 p.

Eaton GL. 1984. Structure and encystment in some fossil cavate dinoflagellate cysts. Journal of Micropalaeontology. 3:53–64.

Eble CF. 2017. The use of glycol ethers to help reduce amorphous organic matter (AOM) in palynological preparations. Palynology. 41:180–182.

Echols DJ, Schaeffer KMM. 1960. Microforaminifera of the Marianna limestone (Oligocene), from Little Stave Creek, Alabama. Micropaleontology. 6:399–415.

Edwards D, Selden PA, Richardson JB, Axe L. 1995. Coprolites as evidence for plant–animal interaction in Siluro–Devonian terrestrial ecosystems. Nature. 377:329–331.

Edwards KJ, Pardoe HS. 2018. How palynology could have been paepalology: the naming of a discipline. Palynology. 42:4–19.

Edwards LE. 1985. Comment on "Disoriented dinoflagellates?" by Jan Jansonius. American Association of Stratigraphic Palynologists Newsletter. 18.1:9.

Ehrenberg CG. 1837. Über das Massenverhältniss der jetzt lebenden Kiesel-Infusorien und über ein neues Infusorien-Conglomerat als Polirschiefer von Jastraba in Ungarn. Abhandlungen der Königlichen Akademie der Wissenschaften zu Berlin, aus dem Jahre 1836, Physikalische Klasse. 1:109–135.

Ediger VS. 1986. Sieving techniques in palynological sample processing with special reference to the MRA system. Micropaleontology. 32:256–270.

Eisenack A. 1930. Neue Mikrofossilien des baltischen Silurs (Vorläufige Mitteilung). Naturwissenschaften. 18:889–881.

Eisenack A. 1931. Neue Mikrofossilien des baltischen Silurs 1. Paläontologische Zeitschrift. 13:74–118.

Eisenack A. 1938. Die Phosphoritknollen der Bernsteinformation als Überlieferer tertiären Planktons (Vorläufige Mitteilung). Schriften der physikalisch-ökonomischen Gesellschaft zu Königsberg (Prussia). 70:181–188.

Eller ER. 1941. Removal of scolecodonts from the matrix. Proceedings of the Pennsylvania Academy of Science. 15:119–120.

Ellin S, McLean D. 1994. The use of microwave heating in hydrofluoric acid digestions for palynological preparations. Palynology. 18:23–31.

Erdtman G. 1934. Über die Verwendung Essigsäurenhydrid bei Pollenuntersuchungen. Svensk Botanisk Tidskrift. 28:354–358.

Erdtman G. 1935. Investigation of honey pollen. Svensk Botanisk Tidskrift. 29:79–80.

Erdtman G. 1936. New methods in pollen analysis. Svensk Botanisk Tidskrift. 30:154–164.

Erdtman G. 1943. An introduction to pollen analysis. Chronica Botanica Company, Waltham, Massachusetts, 239 p.

Erdtman G. 1960. The acetolysis method: a revised description. Svensk Botanisk Tidskrift. 54:561–564.

Erdtman G, Erdtman H. 1933. The improvement of pollen analysis technique. Svensk Botanisk Tidskrift. 27:347–357.

5852 Eriksson ME, Bergman CF, Jeppsson L. 2004. Silurian scolecodonts. Review of
5853 Palaeobotany and Palynology. 3:269–300.
5854
5855 Eriksson ME, Parry LA, Rudkin DM. 2017: Earth's oldest 'Bobbit worm' – gigantism in
5856 a Devonian eunicidan polychaete. Scientific Reports. 7:43061.
5857
5858 Eshet Y, Hoek R. 1996. Palynological processing of organic-rich rocks, or: How many
5859 times have you called a palyniferous sample "barren"? Review of Palaeobotany and
5860 Palynology. 94:101–109.
5861
5862 Evitt WR. 1951. Paleontologic techniques. Journal of Paleontology. 25:693–695.
5863
5864 Evitt WR. 1965. A method for making serial sections of pollen and other organic
5865 microfossils. In: Kummel B, Raup, D (editors). Handbook of Paleontological Techniques.
5866 WH Freeman and Company, San Francisco, p. 696–699.
5867
5868 Evitt WR. 1984. Some techniques for preparing, manipulating and mounting
5869 dinoflagellates. Journal of Micropalaeontology. 3.2:11–18.
5870
5871 Evitt WR. 1985. Sporopollenin dinoflagellate cysts. Their morphology and interpretation.
5872 American Association of Stratigraphic Palynologists Foundation, Dallas, 333 p.
5873
5874 Evitt WR. 2001. Eisenack's dinoflagellates from the amber-bearing beds of East Prussia.
5875 Neues Jahrbuch für Geologie und Paläontologie Abhandlungen. 219:3–14.
5876
5877 Evitt WR, Wall D. 1968. Dinoflagellate studies IV. Theca and cyst of Recent freshwater
5878 *Peridinium limbatum* (Stokes) Lemmerman. Stanford University Publications, Geological
5879 Sciences. 12, 15 p.
5880

Evitt WR, Damassa SP, Albert NR. 1998. A tiger by the tail: the exophragm of the Cretaceous–Paleocene dinoflagellate *Palaeoperidinum* and its implications. Palynology. 22. 1–55.

Faegri K. 1936. Einige Worte über Färbung der für die Pollen-analyse hergestellten Präparate. Geologiska Föreningen i Stockholm Förhandlingar. 58:439–443.

Faegri K. 1939. Single-grain pollen preparations; a practical suggestion. Geologiska Föreningen i Stockholm Förhandlingar. 61:513–514.

Faegri K, Kaland PE, Krzywinski K. 1989. Textbook of Pollen Analysis, by Knut Faegri and Johs. Iversen. Fourth edition. John Wiley and Sons, Chichester, UK, 328 p.

Falkowski PG, Katz ME, Knoll AH, Quigg A, Raven JA, Schofield O, Taylor FJR. 2004. The evolution of modern eukaryotic phytoplankton. Science. 305:354–360.

Farr KM. 1989. Palynomorph and palynofacies distributions in modern British and Irish estuarine sediments. In: Batten DJ, Keen MC (editors). Northwest European micropalaeontology and palynology. British Micropalaeontological Society Series. Ellis Horwood Limited, Chichester, 193–213.

Fedorova RV. 1964. Occurrence of pollen grains of synanthropic and cultured plants in archaeological monuments. Pollen et Spores. 6:141–146.

Felix CJ. 1963. Mechanical sample disaggregation in palynology. Micropaleontology. 9:337–339.

Felix CJ, Burbridge P. 1985. Reappraisal of a palynological storage technique. Pollen et Spores 27:491–492.

Fensome RA, Riding JB, Taylor FJR. 1996a. Chapter 6. Dinoflagellates. In: Jansonius J, McGregor D.C (editors). Palynology: principles and applications. American Association of Stratigraphic Palynologists Foundation, Dallas, 1:107–169.

Fensome RA, MacRae RA, Moldowan JM, Taylor FJR, Williams GL. 1996b. The early Mesozoic radiation of dinoflagellates. Paleobiology. 22:329–338.

Finch EM. 1974. An improved method of mounting palaeontological specimens for SEM examination. Palaeontology. 17:431–434.

Fisher JC. 1962. Laboratory reagents as a possible source of microfossil contamination. Micropaleontology. 8:508.

Flenley JR. 1971. Measurements of the specific gravity of the pollen exine. Pollen et Spores. 13:179–186.

Flenley JR. 1980. A micro-manipulator for pollen photography. Pollen et Spores. 22:257–259.

Forster M, Flenley JR. 1993. Pollen purification and fractionation by equilibrium density gradient centrifugation. Palynology. 17:137–155.

Foucher, J.-C. 1975. Dinoflagellés et acritarches des Silex Crétacés du Bassin de Paris: une synthèse stratigraphique. Annales scientifique de l'Université de Reims et de l'Association régionale pour l'étude et la recherche scientifiques. 13:8–10.

Foucher, J.-C. 1976. Les dinoflagellés des silex et la stratigraphie du Crétacé supérieur français. Revue de Micropaléontologie. 18:213–220.

Foucher, J.-C. 1979. Distribution stratigraphique des kystes de dinoflagellés et des acritarches dans le Crétacé supérieur du Bassin de Paris et de l`Europe septentrionale. Palaeontographica Abteilung B. 169:78–105.

Foucher J.-C. 1983. Distribution des kystes de dinoflagellés dans le Crétacé Moyen et Supérieur du Bassin de Paris. Cahiers de Micropaléontologie. 1983-4:23–41.

Francis W. 1954. Coal: Its Formation and Composition. Edward Arnold, London, UK. 567 p.

Franks JW. 1965. The preparation of samples for pollen analysis. Pollen et Spores. 7:573–575.

Frederiksen NO. 1978. Note on the conversion of microscope stage coordinates. Micropaleontology. 24:222–223.

Fredskild B, Wagner P. 1974. Pollen and fragments of plant tissue in core samples from the Greenland Ice Cap. Boreas. 3:105–108.

Freeman NT, Whitehead J. 1982. Introduction to Safety in the Chemical Laboratory. Academic Press, London, 244 p.

Frenz DA, Scamehorn RT, Hokanson JM, Murray LW. 1996. A brief method for analyzing Rotorod® samples for pollen content. Aerobiologia. 12:51–54.

Frey DG. 1955. A differential flotation technique for recovering microfossils from inorganic sediments. New Phytologist. 54:257–258.

Funkhouser JW. 1969. Factors that affect sample reliability. In: Tschudy R, Scott RT (editors). Aspects of Palynology. Wiley-Interscience, New York, p. 97–102.

5971 Funkhouser JW, Evitt WR. 1959. Preparation techniques for acid-insoluble microfossils.
5972 Micropaleontology. 5:369–375.
5973
5974 Furness CA. 1994. The extraction and identification of pollen from a beeswax statue.
5975 Grana. 33:49–52.
5976
5977 Gabbott SE, Aldridge RJ, Theron JN. 1998. Chitinozoan chains and cocoons from the
5978 Upper Ordovician Soom Shale lagerstätte, South Africa: implications for affinity. Journal
5979 of the Geological Society. 155:447–452.
5980
5981 Gagnon L, Comtois P. 1992. Peut-on comparer les résultats de différents types de
5982 capteurs polliniques? Grana. 31:125–130.
5983
5984 Geisler F. 1935. A new method of separation of fossil pollen from peat. Butler University
5985 Studies. 3:141–145.
5986
5987 Gelsthorpe DN. 2002. Testing of palynological processing techniques: an example using
5988 Silurian palynomorphs from Gotland. Journal of Micropalaeontology. 21:81–86.
5989
5990 Ghosh R, D'Rozario A, Bera S. 2006. Can palynomorphs occur in burnt ancient
5991 potsherds? An experimental proof. Journal of Archaeological Science. 33:1445–1451.
5992
5993 Giesecke T, Fontana SL. 2008. Revisiting pollen accumulation rates from Swedish lake
5994 sediments. The Holocene. 18:293–305.
5995
5996 Glover ED. 1961. Method of solution of calcareous materials using the complexing agent,
5997 EDTA. Journal of Sedimentary Research. 31:622–626.
5998
5999 Gocht H. 1970a. Dinoflagellaten-Zysten aus einem Geschiebefeuerstein und ihr
6000 Erhaltungszustand Neues Jahrbuch für Geologie und Paläontologie Monatshefte.
6001 1970:129–140.

6002
6003 Gocht H. 1970b. Dinoflagellaten-Zysten aus dem Bathonium des Erdölfeldes Aldorf
6004 (NW-Deutschland). Palaeontographica Abteilung B. 129:125–165.
6005
6006 Gocht H. 1972. “Grouping” preparation of fossil dinoflagellates. Micropaleontology.
6007 18:235–239.
6008
6009 Godwin H. 1934. Pollen analysis. An outline of the problems and potentialities of the
6010 method. Part 1. Technique and interpretation. New Phytologist. 33:278–305.
6011
6012 Godwin H. 1949. Pollen analysis of glaciers in special relation to the formation of various
6013 types of glacier bands. Journal of Glaciology. 1:325–332.
6014
6015 Goeury C, de Beaulieu J-L. 1979. A propos de la concentration du pollen a l’aide de la
6016 liqueur de Thoulet dans les sédiments minéraux. Pollen et Spores. 21:230–251.
6017
6018 Goldberg P, Macphail RI. 2003. Short Contribution: Strategies and techniques in
6019 collecting micromorphology samples. Geoarchaeology. 18: 571–578.
6020
6021 Goldman ML. 1952. Deformation, metamorphism, and mineralization in gypsum-
6022 anhydrite cap rock, Sulphur Salt Dome, Louisiana. Geological Society of America
6023 Memoir. 50, 169 p.
6024
6025 Goldstein J, Newbury DE, Joy DC, Lyman CE, Echlin P, Lifshin E, Sawyer L, Michael
6026 JR. 2003. Scanning Electron Microscopy and X-Ray Microanalysis. Third Edition.
6027 Kluwer Academic, Plenum Publishers, New York, 688 p.
6028
6029 González F. 2012. Software for universally relocating specific points of interest on
6030 microscope slides. Marine Micropaleontology. 96–97:63–65.
6031

Gonzalez-Cruz P, Uddin MD, Atwe SU, Abidi N, Gill HS. 2018. Chemical treatment method for obtaining clean and intact pollen shells of different species. ACS Biomaterials Science and Engineering. 4:2319–2329.

Goodhue R, Clayton G. 2010. Palynomorph Darkness Index (PDI) – a new technique for assessing thermal maturity. Palynology. 34:147–156.

Göppert HR. 1836. De floribus in statu fossili commentatio. Nova Acta Academiae Caesareae Leopoldino Carolinae Germanicae Naturae Curiosorum. 18:547–572.

Göppert HR. 1848. Über das Vorkommen von Pollen im fossilen Zustande. Neues Jahrbuch für Mineralogie, Geognosie, Geologie und Petrefaktenkunde. 11:338–340.

Gradstein FM, Ogg JG, Schmitz MB, Ogg GM (editors). 2021. The Geologic Time Scale 2020. Second Edition. Elsevier, Amsterdam, The Netherlands, 1176 p.

Graham A, Wood GD, Elsik WC, Speed, RC. 2000. Petrofilaments in palynological preparations. American Journal of Botany. 87:752–753.

Grahn Y, Paris F. 2011. Emergence, biodiversification and extinction of the chitinozoan group. Geological Magazine. 148:226–236.

Graticules Ltd. 1962. New instruments, materials and methods. Journal of Scientific Instruments. 39:250.

Gray J. 1965a. Palynological techniques. In: Kummel B, Raup D (editors). Handbook of Paleontological Techniques. WH Freeman and Company, San Francisco, p. 471–481.

Gray J. 1965b. Extraction techniques. In: Kummel B, Raup D (editors). Handbook of Paleontological Techniques. WH Freeman and Company, San Francisco, p. 530–587.

6063 Grayson JF. 1956. The conversion of calcite to fluorite. Micropaleontology. 2:71–78.
6064
6065 Grebe H. 1974. In memoriam Robert Potonié (1889–1974). Review of Palaeobotany and
6066 Palynology. 17:217–220.
6067
6068 Green OR. 2001a. A manual of practical laboratory and field techniques in
6069 palaeobiology. Kluwer Academic Publishers, Dordrecht, The Netherlands, 538 p.
6070
6071 Green OR. 2001b. Extraction techniques for palaeobotanical and palynological material.
6072 In: Green OR. A manual of practical laboratory and field techniques in palaeobiology.
6073 Kluwer Academic Publishers, Dordrecht, The Netherlands, 256–287.
6074
6075 Green OR. 2001c. Extraction techniques for acid insoluble microfossils. In: Green OR. A
6076 manual of practical laboratory and field techniques in palaeobiology. Kluwer Academic
6077 Publishers, Dordrecht, The Netherlands, 288–317.
6078
6079 Grey K. 1999. A modified palynological preparation technique for the extraction of large
6080 Neoproterozoic acanthomorph acritarchs and other acid insoluble microfossils. Record-
6081 Geological Survey of Western Australia. 199:1–23.
6082
6083 Grey K. 2000. An interesting new Palynological processing technique from Australia.
6084 Acritarch Newsletter. 16:14–15.
6085
6086 Griffin RL. 1972. Ultramicrotomy (Laboratory Monograph). Bailliere Tindall, London,
6087 93 p.
6088
6089 Grimaldi DA. 1996. Amber: Window to the Past. American Museum of Natural History
6090 Press/Harry N. Abrams Inc., New York, USA, 216 p.
6091
6092 Gudmundssonn L. 1985. Nedsyring af uorganiske materialer ved hjaelp af
6093 massenedsyringsmetoden (macerationstank-metoden). Palyno-Nytt. 2:3–6.

Guennel GK. 1952. Fossil spores of the Alleghenian Coals in Indiana. Indiana Geological Survey Report of Progress. 4, 40 p.

Guthrie RD, McCarthy JF. 1967. Acetolysis. Advances in Carbohydrate Chemistry. 22:11–23.

Gutjahr CCM. 1966. Carbonization measurements of pollen-grains and spores and their application. Leidse Geologische Mededelingen. 38:1–29.

Habib D, Knapp SD. 1982. Stratigraphic utility of Cretaceous small acritarchs. Micropaleontology. 28:335–371.

Hafsten U. 1959. Bleaching + HF + acetolysis a hazardous preparation process. Pollen et Spores. 1:77–79.

Halbwachs H. 2020. Detecting fungal spores and other palynomorphs in amber and copal by solvent treatment. Palynology. 44:521–528.

Hansen JM, Gudmundsson L. 1979. A method for separating acid-insoluble microfossils from organic debris. Micropaleontology. 25:113–117.

Häntzschel W, El-Baz F, Amstutz GC. 1968. Coprolites, an annotated bibliography. Geological Society of America Memior. 108, 132 p.

Harding IC. 1990. A dinocyst calibration of the European Boreal Barremian. Palaeontographica Abteilung B. 218:1–76.

Harland R. 1989. A dinoflagellate cyst record for the last 0.7 Ma from the Rockall Plateau, northeast Atlantic Ocean. Journal of the Geological Society. 146:945–951.

Harland R, Sutherland G. 1972. A technique for the water mounting of microfossils for study and photography. Micropaleontology. 18:119–121.

Harvey C. 2001. An oxidation and stable mounting technique for geothermally altered Upper Devonian palynomorphs from western Venezuela. Journal of Micropalaeontology. 20:123–125.

Hay WW, Sandberg PA. 1967. The scanning electron microscope, a major break-through for micropaleontology. Micropaleontology. 13:407–418.

Haynes JR. 1981. Foraminifera. Springer, London, 467 p.

Head MJ (editor). 1995a. HF fatality. American Association of Stratigraphic Palynologists Newsletter. 281:14–15.

Head MJ (editor). 1995b. Treatment of HF burns. American Association of Stratigraphic Palynologists Newsletter. 281:15.

Head MJ. 1996. Chapter 30. Modern dinoflagellate cysts and their biological affinities. In: Jansonius J, McGregor DC. (editors). Palynology: principles and applications. American Association of Stratigraphic Palynologists Foundation, Dallas. 3:1197–1248.

Heiken G, Wohletz K. 1985. Volcanic Ash. University of California Press, Berkeley, USA, 246 p.

Heinz C, Barbaza, M. 1998. Environmental changes during the Late Glacial and Post-Glacial in the central Pyrenees (France): new charcoal analysis and archaeological data. Review of Palaeobotany and Palynology. 104:1–17.

Hendon D, Charman DJ. 1997. The preparation of testate amoebae (Protozoa: Rhizopoda) samples from peat. The Holocene. 7:199–205.

Hennissen JAI, Hough E, Vane CH, Leng MJ, Kemp SJ, Stephenson MH. 2017. The prospectivity of a potential shale gas play: An example from the southern Pennine Basin (central England, UK). Marine and Petroleum Geology. 86:1047–1066.

Hennissen JAI, Wood SEL, Flint J. 2018. The standard palynological preparation protocol used in the Biostratigraphy and Palaeontology Laboratories at the British Geological Survey. British Geological Survey Internal Report. IR/18/43, 18 p. (unpublished).

Herngreen GFW. 1983. Palynological preparation techniques. In: Costa LI (editor). Palynology – Micropalaeontology: Laboratories, Equipment and Methods. Norwegian Petroleum Directorate Bulletin. 2:13–34.

Hesse M, Waha M. 1989. A new look at the acetolysis method. Plant Systematics and Evolution. 163:147–152.

Hesselbo SP, Bjerrum CJ, Hinnov LA, MacNiocaill C, Miller KG, Riding JB, van de Schootbrugge B and the Mochras Revisited Science Team. 2013. Mochras borehole revisited: a new global standard for Early Jurassic earth history. Scientific Drilling. 16:81–91.

Heunisch C, Muntzos T. 1990. Zwei neue automatisierte Siebverfahren zur Anreicherung von Palynomorphen. Review of Palaeobotany and Palynology. 66:159–162.

Heusser CJ. 1954. Palynology of the Taku Glacier snow cover, Alaska and its significance in the determination of glacier regimen. American Journal of Science. 252:291–308.

Heusser LE, Stock CE. 1984. Preparation techniques for concentrating pollen from marine sediments and other sediments with low pollen density. Palynology. 8:225–227.

Higgins AC, Austin RL (editors). 1985. A Stratigraphical Index of Conodonts. British Micropalaeontological Society Series. Ellis Horwood Limited, Chichester, UK, 263 p.

Higgins AC, Spinner EG. 1968. Techniques for the extraction of selected microfossils. Welsh Geological Quarterly. 4:25–36.

Hill CR. 1983. Glycerin jelly mounts. One man's receipe for re-mounting glycerine jelly mounts. American Association of Stratigraphic Palynologists Newsletter. 16:3.

Hill CR, Dilcher DL. 1990. Scanning electron microscopy of the internal ultrastructure of plant cuticle. In: Claugher D (editor). Scanning Electron Microscopy in Taxonomy and Functional Morphology. Systematics Association Special Volume 41, 95–124. Clarendon Press, Oxford.

Hills LV, Sweet AR. 1972. The use of "Quaternary O" in megaspore palynological preparations. Review of Palaeobotany and Palynology. 13:229–231.

Hints O. 2000. Ordovician eunicid polychaetes of Estonia and surrounding areas: review of their distribution and diversification. Review of Palaeobotany and Palynology. 113:41–55.

Hints O, Eriksson ME. 2007. Diversification and biogeography of scolecodont-bearing polychaetes in the Ordovician. Palaeogeography, Palaeoclimatology, Palaeoecology. 245:95–114.

Hints O, Paris F, Al-Hajri S. 2015. Late Ordovician scolecodonts from the Qusaiba-1 core hole, central Saudi Arabia, and their paleogeographical affinities. Review of Palaeobotany and Palynology 212:85–96.

Hirst JM. 1952. An automatic volumetric spore trap. Annals of Applied Biology. 39:257–265.

Hodgkinson RL. 1991. Microfossil processing: a damage report. Micropaleontology. 37:320–326.

Hoffmeister WS. 1960. Sodium hypochlorite, a new oxidising agent for the preparation of microfossils. Oklahoma Geology Notes. 20:34–35.

Holloway RG, Dering P, Bryant VM Jr. 1995. The use of bleach for extraction of arid southwestern pollen samples. Palynology. 19:239.

Hopkins JA, McCarthy FMG. 2002. Post-depositional palynomorph degradation in Quaternary shelf sediments: A laboratory experiment studying the effects of progressive oxidation. Palynology. 26:167–184.

Hopkins TL. 1964. A survey of marine bottom samplers. Progress in Oceanography. 2:213–256.

Horowitz A. 1992. Sampling. In: Palynology of Arid Lands. Elsevier, Amsterdam, 149–167.

Horowitz A, Langozky Y. 1965. Preliminary palynological study of hydrocarbons in Israel. Geologie en Mijnbouw. 44:59–62.

Horrocks M. 2004. Sub-sampling and preparing forensic samples for pollen analysis. Journal of Forensic Science. 49:1024–1027.

Horrocks M, Salter J, Braggins J, Nichol S, Moorhouse R, Elliot G. 2008. Plant microfossil analysis of coprolites of the critically endangered kakapo (*Strigops*

habroptilus) parrot from New Zealand. Review of Palaeobotany and Palynology. 149:229–245.

Hughes NF. 1955. Wealden plant microfossils. Geological Magazine. 92:210–217.

Hughes NF, Dettmann ME, Playford G. 1962. Sections of some Carboniferous dispersed spores. Palaeontology. 5:247–252.

Hughes NF, Drewry GE, Laing JF. 1979. Barremian earliest angiosperm pollen. Palaeontology. 22:513–535.

Hughes NF, McDougall AB. 1990. Barremian-Aptian angiospermid pollen records from southern England. Review of Palaeobotany and Palynology. 65:145–151.

Hughes NF. 1994. The enigma of angiosperm origins. Cambridge Paleobiology Series 1. Cambridge University Press, UK, 303 p.

Hunt AP, Lucas SG, Milàn J, Spielmann JA. 2012. Vertebrate Coprolite Studies: Status and Prospectus. New Mexico Museum of Natural History and Science Bulletin. 57:5–24.

Hutton A, Bharati S, Robl T. 1994. Chemical and petrographic classification of kerogen macerals. Energy and Fuels. 8:1478–1488.

Hyde HA. 1969. Aeropalynology in Britain – an outline. New Phytologist. 68:579–590.

Hyde HA, Williams DA. 1944. The right word. Pollen Analysis Circular. 8:6.

Jackson ST. 1999. Techniques for analysing unconsolidated lake sediments. In: Jones TP, Rowe NP (editors). Fossil plants and spores: modern techniques. Geological Society, London, p. 274–278.

6278 Jackson TA, Germs A, Moorman M. 1974. An improved method for the chemical
6279 maceration of sedimentary rocks. Journal of Paleontology. 48:844–859.
6280
6281 Jacob J, Paris F, Monod O, Miller MA, Tang P, George SC, Bény J-M. 2007. New
6282 insights into the chemical composition of chitinozoans. Organic Geochemistry. 38:1782–
6283 1788.
6284
6285 Jacobson SR, Schopf JM. 1979. Preservation of palynological specimens examined by
6286 SEM for later microscopical study and reference. Journal of Paleontology. 53:744–746.
6287
6288 Jameson DM. 2014. Introduction to Fluorescence. CRC Press, Boca Raton, Florida, USA,
6289 313 p.
6290
6291 Jansonius J. 1970. Classification and stratigraphic application of Chitinozoa. Proceedings
6292 of the North American Paleontological Convention 1969. 789–808.
6293
6294 Jansonius J, Jenkins WAM. 1978. Chitinozoa. In: Haq BU, Boersma A (editors).
6295 Introduction to Marine Micropaleontology. Elsevier Science Publishing Company, New
6296 York, p. 341–355.
6297
6298 Jansonius J. 1984. Disoriented dinoflagellates? American Association of Stratigraphic
6299 Palynologists Newsletter. 17.4:8–9.
6300
6301 Jansonius J. 1985. Image reversal in microscope optics: one more flip. American
6302 Association of Stratigraphic Palynologists Newsletter. 18.2:8.
6303
6304 Jansonius J, McGregor DC (editors). 1996. Palynology: principles and applications.
6305 American Association of Stratigraphic Palynologists Foundation, Dallas, three volumes,
6306 1330 p.
6307

Jantz N, Homeier J, León-Yánez S, Moscoso A, Behling H. 2013. Trapping pollen in the tropics — Comparing modern pollen rain spectra of different pollen traps and surface samples across Andean vegetation zones. Review of Palaeobotany and Palynology. 193:57–69.

Jardine PE, Fraser WT, Lomax BH, Gosling WD. 2015. The impact of oxidation on spore and pollen chemistry. Journal of Micropalaeontology. 34:139–149.

Jarzen DM, Jarzen SA. 2006. Collecting pollen and spore samples from herbaria. Palynology. 30:111–119.

Jeffords RM, Jones D.H. 1959. Preparation of slides for spores and other microfossils. Journal of Paleontology. 33:344–350.

Jemmett G, Owen JAK. 1990. Where has all the pollen gone? Review of Palaeobotany and Palynology. 64:205–211.

Jenkins WAM. 1967. Ordovician chitinozoa from Shropshire. Palaeontology. 10:436–488.

Jenkins WAM. 1970. Chitinozoa. Geoscience and Man. 1:1–21.

Jiang D-X. 1990. Palynological evidence for identification of nonmarine petroleum source rocks, China. Ore Geology Reviews. 5:553–575.

Jiang D-X, Wang Y-D, Robbins EI, Wei J, Tian N. 2008. Mesozoic non-marine petroleum source rocks determined by palynomorphs in the Tarim Basin, Xinjiang, northwestern China. Geological Magazine. 145:868–885.

Jiang D-X, Robbins EI, Wang Y-D, Yang H. 2016. Petrolipalynology. Science Press, Beijing and Springer-Verlag Berlin, Heidelberg, 263 p.

Johnson WC, Fredlund GG. 1985. A procedure for extracting palynomorphs (pollen and spores) from clastic sediments. Transactions of the Kansas Academy of Science. 88:51–58.

Jonasson A, Olausson E. 1966. New devices for sediment sampling. Marine Geology. 4:365–372.

Jones GD. 2012. Pollen extraction from insects. Palynology. 36:86–109.

Jones GD. 2014. Pollen analyses for pollination research, acetolysis. Journal of Pollination Ecology. 13:203–217.

Jones GD, Bryant VM Jr. 1992. Melissopalynology in the United States: a review and critique. Palynology. 16:63–71.

Jones GD, Bryant VM Jr. 1996. Chapter 23D. Melissopalynology. In: Jansonius J, McGregor DC (editors). Palynology: principles and applications. American Association of Stratigraphic Palynologists Foundation, Dallas. 3:933–938.

Jones GD, Bryant VM Jr. 1998. Pollen recovery from honey. In: Bryant VM, Wrenn JH (editors). New developments in palynomorph sampling, extraction, and analysis. American Association of Stratigraphic Palynologists Contributions Series. 33:107–114.

Jones GD, Bryant VM Jr. 2001. Alcohol dilution of honey. In: Goodman, D.K. and Clarke, R.T. (editors). Proceedings of the IX International Palynological Congress, Houston, Texas, USA, 1996. American Association of Stratigraphic Palynologists Foundation, Dallas, Texas, 453–458.

Jones GD, Bryant VM Jr. 2004. The use of ETOH for the dilution of honey. Grana. 43:174–182.

Jones P. 1984. A note on D.P.X. mountant. American Association of Stratigraphic Palynologists Newsletter. 17.4:7–8.

Jones RA. 1994. The application of microwave technology to the oxidation of kerogen for use in palynology. Review of Palaeobotany and Palynology. 80:333–338.

Jones RA. 1998. Focused microwave digestion and oxidation of palynological samples. Review of Palaeobotany and Palynology. 103:17–22.

Jones RA, Ellin SJ. 1998. Improved palynological sample preparation using an automated focused microwave digestion system. In: Bryant VM, Wrenn JH (editors). New developments in palynomorph sampling, extraction, and analysis. American Association of Stratigraphic Palynologists Contributions Series. 33:23–28.

Jones RA, Dorning KJ, Ellin SJ, Brooks IP. 1995. Microwave preparation techniques for the palynological analysis of flint and chert. Seventh International Flint Symposium, Institute of Archaeology and Ethnology, Polish Academy of Science, Al Solidarnosd, Warsaw, Poland, 105:140 (abstract).

Jones RA. 2003. The Palytech “Sieve-O-Matic” - an automated system for sieving and washing palynological samples. Palynology. 27:259.

Jones RW. 2004. Micropalaeontology in Petroleum Exploration. Clarendon Press, Oxford, 416 p.

Jones TP, Rowe NP (editors). 1999. Fossil plants and spores: modern techniques. Geological Society, London, 396 p.

Jørgensen S. 1967. A method of absolute pollen counting. New Phytologist. 66:489–493.

6401 Jung Echols D, Levin HL. 1964. Chalk crayons and microfossil contamination.
6402 Micropaleontology. 10:80.
6403
6404 Juvigné E. 1973a. Une méthode de séparation des pollens applicable aux sédiments
6405 minéraux. Annales de la Société Géologique de Belgique. 96:253–262.
6406
6407 Juvigné E. 1973b. Densité des exines de quelques espèces de pollens et spores fossiles.
6408 Annales de la Société Géologique de Belgique. 96:363–374.
6409
6410 Juvigné E. 1975. Note on pollen extraction from coarse sediments. Quaternary Research.
6411 5:121–123.
6412
6413 Jux U. 1968a. Über den Feinbau der Wandung bei *Cordosphaeridium inodes* (Klumpp
6414 1953). Palaeontographica Abteilung B. 122:48–54.
6415
6416 Jux U. 1968b. Über den Feinbau der Wandung bei *Tasmanites* Newton.
6417 Palaeontographica Abteilung B. 124:112–124.
6418
6419 Jux U. 1971a. Über den Feinbau der Wandungen einiger Tertiärer Dinophyceen-Zysten
6420 und Acritarcha. *Hystrichosphaeridium, Impletosphaeridium, Lingulodinium.*
6421 Palaeontographica Abteilung B. 132:165–174.
6422
6423 Jux U. 1971b. Über den Feinbau der Wandungen einiger paläozoischer
6424 Baltisphaeridiaceen. Palaeontographica Abteilung B. 136:115–128.
6425
6426 Jux U. 1980. Über den Feinbau der Wandung bei *Oligosphaeridium abaculum* Davey
6427 1979. Palaeontographica Abteilung B. 174:1–6.
6428
6429 Kapp RO, Davis OK, King JE. 2000. Ronald O. Kapp's Pollen and Spores. Second
6430 Edition. American Association of Stratigraphic Palynologists Foundation, Dallas, USA,
6431 279 p.

Kielan-Jaworowska Z. 1966. Polychaete jaw apparatuses from the Ordovician and Silurian of Poland and a comparison with modern forms. Palaeontologica Polonica. 16:1–152.

Kempf EK. 1970. Electron microscopy of the megaspore *Horstisporites semireticulatus* from Liassic strata of Germany. Grana. 10:18–22.

Kempf EK. 1973. Transmission electron microscopy of fossil spores. Palaeontology. 16:787–797.

Kennaway GM, Eaton GL, Feist-Burkhardt S. 2008. A detailed protocol for the preparation and orientation of single fossil dinoflagellate cysts for transmission electron microscopy. Palynology. 32:1–15.

Kennedy AE, Coe AL. 2014. Development of the freeze–thaw processing technique for disaggregation of indurated mudrocks and enhanced recovery of calcareous microfossils. Journal of Micropalaeontology. 33:193–203.

Kerp H, Bomfleur B. 2011. Photography of plant fossils – New techniques, old tricks. Review of Palaeobotany and Palynology. 166:117–151.

Kiared G, Bessedik M, Riding JB. 2017. The aeropalynology of Es-Sénia airport, Oran, northwest Algeria. Palynology. 41:121–131.

Kidson EJ, Williams GL. 1969. Concentration of palynomorphs by use of sieves. Oklahoma Geology Notes. 29:117-119.

Kidson EJ, Williams GL. 1971. Device for the manipulation of microfossils. Pollen et Spores. 13:359–364.

Kirkland DW. 1967. Method of calculating absolute spore and pollen frequency Oklahoma Geology Notes. 27:98–100.

Klaus W. 1953. Zur Einzelpräparation fossiler Sporomorphen. Mikroskopie. 8:1–14.

Klaus W. 1975. Ein neues Handbohrgerät zur Gewinnung verfestigter Sedimentproben für Pollenanalysen. Jahrbuch des Oberösterreichischen Musealvereines. 120:345–350.

Knauth, LP. 1979. A model for the origin of chert in limestone. Geology. 7:274–277.

Knox AS. 1942. The use of bromoform in the separation of non-calcareous microfossils. Science. 95:307–308.

Königsson L-K. 1975. Editorial: Writing in GRANA. Grana. 15:1–5.

Kontrovitz M, Slack JM, Yuhong Z. 1991. On the use of some phosphates in the preparation of ostracod shells. Journal of Micropalaeontology. 10:121–126.

Kosanke RM. 1950. Pennsylvanian spores of Illinois and their use in correlation. Illinois State Geological Survey Bulletin. 74, 128 p.

Kozłowski R. 1956. Sur quelques appareils masticateurs des Annélides Polychètes ordoviciens. Acta Palaeontologica Polonica 1:165–210.

Kozłowski R. 1963. Sur la nature des Chitinozoaires. Acta Palaeontologica Polonica. 8:425–449.

Krings M. 2000. The use of biological stains in the analysis of late Palaeozoic pteridosperm cuticles. Review of Palaeobotany and Palynology. 108:143–150.

6493 Krukowski ST. 1988. Sodium metatungstate: a new heavy mineral separation medium for
6494 extraction of conodonts from insoluble residues. Journal of Paleontology. 62:314–316.
6495
6496 Kummel B, Raup D (editors). 1965. Handbook of Paleontological Techniques. WH
6497 Freeman and Company, San Francisco, 852 p.
6498
6499 Kundu T. 2014. Ultrasonic and electromagnetic waves for nondestructive evaluation and
6500 structural health monitoring. Procedia Engineering. 86:395–405.
6501
6502 Kurtz EB, Turner RM. 1957. An oil-flotation method for the recovery of pollen from
6503 inorganic sediments. Micropaleontology. 3:67–68.
6504
6505 Kuyl OS. 1960. The pollen preparation of calcareous sediments. Mededelingen van de
6506 Geologische Stichting, Nieuwe Serie. 13:27–28.
6507
6508 Laing JF. 1974. A specimen location technique for SEM strew mounts. Palaeontology.
6509 17:435–436.
6510
6511 Lakovicz JR. 2006. Principles of Fluorescence Spectroscopy. Third revised edition.
6512 Springer-Verlag New York Inc., New York, USA, 954 p.
6513
6514 Lang WH. 1925. Contributions to the study of the Old Red Sandstone flora of Scotland.
6515 Transactions of the Royal Society of Edinburgh. 54:253–272.
6516
6517 Langenheim JH. 2003. Plant Resins: Chemistry, Evolution, Ecology, and Ethnobotany.
6518 Timber Press Inc., Portland, Oregon, USA, 589 p.
6519
6520 Lanigan RS. 2001. Final report on the safety assessment of sodium metaphosphate,
6521 sodium trimetaphosphate, and sodium hexametaphosphate. International Journal of
6522 Toxicology. 20, Supplement 3:75–89.
6523

6524 Large MF, Braggins JE. 1990. Effect of different treatments on the morphology and size
6525 of fern spores. Review of Palynology and Palaeobotany. 64:213–221.
6526
6527 Laufeld S. 1967. Caradocian chitinozoa from Dalarna, Sweden. Geologiska Föreningens i
6528 Stockholm Förhandlingar. 89:275–349.
6529
6530 Laufeld S. 1974. Silurian chitinozoa from Gotland. Fossils and Strata. 5, 130 p.
6531
6532 Laurie JR, Foster CB (editors) 2001. Studies in Australian Mesozoic Palynology II.
6533 Memoir of the Association of Australasian Palaeontologists. 24, 235 p.
6534
6535 Layne NM Jr. 1950. A procedure for shale disintegration The Micropaleontologist. 4:21.
6536
6537 Ledingham RJ, Chinn SHF. 1955. A flotation method for obtaining spores of
6538 *Helminthosporium sativum* from soil. Canadian Journal of Botany. 33:298–303.
6539
6540 Lee HW. 1964. A modified method of coal maceration and a simple technique for slide
6541 preparation. Micropaleontology. 10:486–490.
6542
6543 Leffingwell HA, Hodgkin N. 1971. Techniques for preparing fossil palynomorphs for
6544 study with the scanning and transmission electron microscopes. Review of Palaeobotany
6545 and Palynology. 11:177–199.
6546
6547 Leipe C, Kobe F, Müller S. 2019. Testing the performance of sodium polytungstate and
6548 lithium heteropolytungstate as non-toxic dense media for pollen extraction from lake and
6549 peat sediment samples. Quaternary International. 516:207–214.
6550
6551 Lejeune-Carpentier M. 1938. L'étude microscopique des silex. *Areoligera*: nouveau
6552 genre d'Hystrichosphaeridée (Sixième note) Annales de la Société géologique de
6553 Belgique. 62:B163–B174.
6554

Lejeune-Carpentier M. 1940. L’étude microscopique des silex. Systématique et morphologie des "tubifères" (Huitième note). Annales de la Société géologique de Belgique. 63:B216–B236.

Lejeune-Carpentier M, Sarjeant WAS. 1981. Restudy of some larger dinoflagellate cysts and an acritarch from the Upper Cretaceous of Belgium and Germany. Annales de la Société géologique de Belgique. 104:1–39.

Lennie CR. 1968. Palynological techniques used in New Zealand. New Zealand Journal of Geology and Geophysics. 11:1211–1221.

Lentfer CJ, Boyd WE. 2000. Simultaneous extraction of phytoliths, pollen and spores from sediments. Journal of Archaeological Science. 27:363–372.

Leroy SAG, Simms MJ. 2006. Iron age to medieval entomogamous vegetation and *Rhinolophus hipposideros* roost in South-Eastern Wales (UK). Palaeogeography, Palaeoclimatology, Palaeoecology. 237:4–18.

Leschik G. 1956. Sporen aus dem Salzton des Zechsteins von Neuhof (Bei Fulda). Palaeontographica Abteilung B. 100:122–142.

Leslie SA, Mitchell JC. 2007. Removing gold coating from SEM samples. Palaeontology. 50:1459–1461.

Lewis J, Dodge JD, Tett P. 1984. Cyst-theca relationships in some *Protoperidinium* species (Peridiniales) from Scottish sea lochs. Journal of Micropalaeontology. 3:25–34.

Li F-S, Phyo P, Jacobowitz J, Hong M, Weng J-K. 2019. The molecular structure of plant sporopollenin. Nature Plants. 5:41–46.

Liang Y, Hints O, Tang P, Cai C, Goldman D, Nõlvak J, Tihelka E, Pang K, Bernardo J, Wang W. 2020. Fossilized reproductive modes reveal a protistan affinity of Chitinozoa. Geology. 48:1200–1204.

Lichti-Federovich S. 1974. Pollen analysis of surface snow from the Devon Ice Cap. Geological Survey of Canada Paper. 74-1A:197–199.

Lichti-Federovich S. 1975. Pollen analysis of ice core samples from the Devon Island Ice Cap. Geological Survey of Canada Paper. 75-1A:441–444.

Lieux MH. 1972. A melissopalynological study of 54 Louisiana (U.S.A.) honeys. Review of Palaeobotany and Palynology. 13:95–124.

Lieux MH. 1980. Acetolysis applied to microscopical honey analysis. Grana. 19:57–61.

Lieux MH. 1981. An analysis of Mississippi (U.S.A.) honey: pollen, color and moisture. Apidologie. 12:137–158.

Lignum J, Jarvis I, Pearce MA. 2008. A critical assessment of standard processing methods for the preparation of palynological samples. Review of Palaeobotany and Palynology. 149:133–149.

Lindstrom M. 1964. Conodonts. Elsevier, Amsterdam, The Netherlands, 196 p.

Litwin RJ, Traverse A. 1989. Basic guidelines for palynomorph extraction and preparation from sedimentary rocks. In: Feldmann RM, Chapman RE, Hannibal JT (editors). Paleotechniques. The Paleontological Society Special Publication. 4:87–98.

Lohmann KE. 1933. A method for permanently recording the locations of objects on microscope slides. Science. 78:214–215.

Loose F. 1934. Sporenformen aus dem Floz Bismarck des Ruhrgebietes. Arbeiten zur der Institut Paleobotaniken und Petrografischen Brennsteine Berlin. 4:127–164.

Luedtke BE. 1992. An Archaeologist's Guide to Chert and Flint. Archaeological Research Tools 7, University of California, Los Angeles, USA, 154 p.

Lund JJ, Ecke H-H. 1988. Dinoflagellate cyst stratigraphy applied to the Middle to Late Jurassic of the Regensburg-Passau area, Bavaria. Bulletin des Centres de Recherches Exploration-Production Elf-Aquitaine. 12:345–359.

Lutier PM, Vaissière BE. 1993. An improved method for pollen analysis of honey. Review of Palaeobotany and Palynology. 78:129–144.

Machado G, Flores D. 2015. An effective method for the observation and documentation of highly mature palynomorphs using reflected light microscopy. Palynology. 39:345–349.

Macphail RI, Cruise GM, Allen MJ, Linderholm J, Reynolds P. 2004. Archaeological soil and pollen analysis of experimental floor deposits; with special reference to Butser Ancient Farm, Hampshire, UK. Journal of Archaeological Science. 31:175–191.

MacRae RA, Fensome RA, Williams GL. 1996. Fossil dinoflagellate diversity, originations, and extinctions and their significance. Canadian Journal of Botany. 74:1687–1694.

Mädler KA. 1956. A technique for the preparation of multi-grain palynological slides. Micropaleontology. 2:399–401.

Maher LJ Jr. 1981. Statistics for microfossil concentration measurements employing samples spiked with marker grains. Review of Palaeobotany and Palynology. 32:153–191.

6647
6648 Maher LJ Jr. 2006. Environmental information from guano palynology of insectivorous
6649 bats of the central part of the United States of America. Palaeogeography,
6650 Palaeoclimatology, Palaeoecology. 237:19–31.
6651
6652 Maltwood T. 1858. On a finder for registering the position of microscopic object.
6653 Transactions of the American Microscopical Society. 6:59–62.
6654
6655 Mangili C, Brauer A, Moscariello A, Naumann R. 2005. Microfacies of detrital event
6656 layers deposited in Quaternary varved lake sediments of the Piànico-Sèllere Basin
6657 (northern Italy). Sedimentology. 52:927–943.
6658
6659 Manten AA. 1966. Half a century of modern palynology. Earth-Science Reviews. 2:277–
6660 316.
6661
6662 Manten AA. 1967. Lennart Von Post and the foundation of modern palynology. Review
6663 of Palaeobotany and Palynology. 1:11–22.
6664
6665 Manten AA. 1969. The history of the microscope and its impact on the development of
6666 palynology. Review of Palaeobotany and Palynology. 9:137–148.
6667
6668 Manum S. 1956. Schulzes maserasjonsblanding. Et hundreårs-minne. Blyttia. 14:126–
6669 130.
6670
6671 Mapes RH, Mapes G. 1982. Removal of gypsum from microfossiliferous shales.
6672 Micropaleontology. 28:218–219.
6673
6674 Marceau L. 1969. Effets, sur le pollen, des ultra-sons de basse fréquence. Pollen et
6675 Spores. 11:147–164.
6676

6677 Marret F. 1993. Les effets de l'acétolyse sur les assemblages des kystes de dinoflagellés.
6678 Palynosciences. 2:267–272.
6679
6680 Marshall JEA. 1980. A method for the successful oxidation and subsequent stabilization
6681 of highly carbonized spore assemblages. Review of Palaeobotany and Palynology.
6682 29:313–319.
6683
6684 Marshall JEA. 1991. Quantitative spore colour. Journal of the Geological Society.
6685 148:223–233.
6686
6687 Marshall JEA, Yule BL. 1999. Spore colour measurement. In: Jones TP, Rowe NP
6688 (editors). Fossil plants and spores: modern techniques. Geological Society, London, p.
6689 165–168.
6690
6691 Marshall JEA. 2005. Arthur Raistrick: Britain's premier palynologist. In: Bowden AJ,
6692 Burek CV, Wilding R. (editors). History of Palaeobotany: Selected Essays. Geological
6693 Society of London Special Publications. 241:161–179.
6694
6695 Martin AC, Harvey WJ. 2017. The Global Pollen Project: a new tool for pollen
6696 identification and the dissemination of physical reference collections. Methods in
6697 Ecology and Evolution. 8:892–897.
6698
6699 Mason TJ. 2016. Ultrasonic cleaning: An historical perspective. Ultrasonics
6700 Sonochemistry. 29:519–523.
6701
6702 Matthews J. 1969. The assessment of a method for the determination of absolute pollen
6703 frequencies. New Phytologist. 68:161–166.
6704
6705 Mazor JR, Ammosov II, Bogoljubova LI, Golitsyn MV, Eryomin IV. 1979. Petrography
6706 and genesis of coal. Huitième Congrès International de Stratigraphie et de Géologie

Carbonifère, Moscow, September 8–13 1975, Compte Rendu Volume 4. Nauka, Moscow, Russia, 200 p.

McIntyre DJ, Norris G. 1964. Effect of ultrasound on Recent spores and pollen. New Zealand Journal of Science. 7:242–257.

McKee K. 1977. A dialysis unit in palynological sample preparation. The British Micropalaeontologist. 5:12–13.

McPhilemy B. 1988. The value of fluorescence microscopy in routine palynofacies analysis: Lower Carboniferous successions from Counties Armagh and Roscommon, Ireland. Review of Palaeobotany and Palynology. 56:345–359.

Medlin LK, Fensome RA. 2013. Dinoflagellate macroevolution: some considerations based on an integration of molecular, morphological and fossil evidence. In: Lewis JM, Marret F, Bradley L. (editors). Biological and Geological Perspectives of Dinoflagellates. The Micropalaeontological Society, Special Publications. Geological Society, London, 263–274.

Mehringer PJ Jr, Wigand PE. 1990. Comparison of Late Holocene environments from woodrat middens and pollen: Diamond Craters, Oregon. In: Betancourt JL, Van Devender TR, Martin PS (editors). Packrat middens. The last 40,00 years of biotic change. The University of Arizona Press, Tucson, Arizona, USA, 294–325.

Meltsov V, Poska A, Saar M. 2008. Pollen size in *Carex*: The effect of different chemical treatments and mounting media. Grana. 47:220–233.

Melvin JL (editor). 1991. Evaporites, petroleum and mineral resources. Elsevier, Amsterdam, 555 p.

Merrill GK. 1980. Removal of pyrite from microfossil samples by means of sodium hypochlorite. Journal of Paleontology. 54:633–634.

Mertens KN, Verhoeven K, Verleye T, Louwye S, Amorim A, Ribeiro S, Deaf AS, Harding IC, De Schepper S, González C, Kodrans-Nsiah M, De Vernal A, Henry M, Radi T, Dybkjaer K, Poulsen NE, Feist-Burkhardt S, Chitolie J, Heilmann-Clausen C, Londeix L, Turon J-L, Marret F, Matthiessen J, McCarthy FMG, Prasad V, Pospelova V, Kyffin Hughes JE, Riding JB, Rochon A, Sangiorgi F, Welters N, Sinclair N, Thun C, Soliman A, Van Nieuwenhove N, Vink A, Young M. 2009. Determining the absolute abundance of dinoflagellate cysts in recent marine sediments: The *Lycopodium* marker-grain method put to the test. Review of Palaeobotany and Palynology. 157:238–252.

Mertens KN, Price AM, Pospelova V. 2012. Determining the absolute abundance of dinoflagellate cysts in recent marine sediments II: Further tests of the *Lycopodium* marker-grain method. Review of Palaeobotany and Palynology. 184:74–81.

Michoux D. 1988. Dinoflagellate cysts of the *Wetzeliella*-complex from Eocene sediments of the Aquitaine Basin, southwestern France. Palynology. 12:11–41.

Middeldorp AA, Mijzen P. 1986. Embedding pollen in a film of polyvinyl alcohol, a method for establishing pollen concentration. Pollen et Spores. 28:451–456.

Mikkelsen SR, Cortón E. 2016. Centrifugation Methods. Bioanalytical Chemistry. Second Edition. John Wiley and Sons, p. 325–348.

Mildenhall DC, Raine JI, Wilson GJ. 1975. Collection of palynological samples. Geological Society of New Zealand Newsletter, 39:48–52.

Millay MA, Taylor TN. 1976. Evolutionary trends in fossil gymnosperm pollen. Review of Palaeobotany and Palynology. 21:65–91.

Miller CG, Cornish L, Jones C, Jones CG, Henderson AS. 2004. A new laser method for cleaning micropalaeontological specimens. Journal of Micropalaeontology. 23:165–169.

Miller MA. 1996. Chapter 11. Chitinozoa. In: Jansonius J, McGregor DC (editors). Palynology: principles and applications. American Association of Stratigraphic Palynologists Foundation, Dallas. 1:307–336.

Miller TH. 1967. Techniques for processing and photographing chitinozoans. The University of Kansas Paleontological Contributions Paper. 21, 10 p.

Monazam, ER, Breault RW, Weber J, Layfield K. 2017. Elutriation of fines from binary particle mixtures in bubbling fluidized bed cold model. Powder Technology. 305:340–346.

Monga P, Kumar M, Joshi Y. 2015. Morphological variations and depositional processes of microforaminiferal linings in the early Tertiary sediments of northeastern and northwestern India. The Palaeobotanist. 64:129–138.

Moore PD. 1987. Ecological and hydrological aspects of peat formation. In: Scott AC (editor). Coal and Coal-bearing Strata: Recent Advances. Geological Society Special Publication. 32:7–15.

Moore PD, Webb JA, Collinson ME. 1991. Pollen Analysis. Second Edition. Blackwell Scientific Publications, Oxford, 216 p.

Morton A, Knox RWO'B, Hallsworth C. 2002. Correlation of reservoir sandstones using quantitative heavy mineral analysis. Petroleum Geoscience. 8:251–262.

Moss PT, Greenwood DR, Archibald SB. 2005. Regional and local vegetation community dynamics of the Eocene Okanagan Highlands (British Columbia - Washington State) from palynology. Canadian Journal of Earth Sciences. 42:187–204.

Moss PT, Kershaw AP. 2007. A late Quaternary marine palynological record (oxygen isotope stages 1 to 7) for the humid tropics of northeastern Australia based on ODP Site 820. Palaeogeography, Palaeoclimatology, Palaeoecology. 251:4–22.

Moss PT. 2013. Palynology and its application to geomorphology. In: Shroder JF, Switzer AD, Kennedy DM (editors). Treatise on Geomorphology. Academic Press, San Diego, USA. Volume 14, Methods in Geomorphology, p. 315–325.

Moss PT, Smith RY, Greenwood DR. 2016. A window into mid-latitudinal Early Eocene environmental variability: a high-resolution palynological analysis of the Falkland site, Okanagan Highlands, British Columbia, Canada. Canadian Journal of Earth Sciences. 53:605–613.

Muller J. 1959. Palynology of Recent Orinoco delta and shelf sediments: reports of the Orinoco Shelf Expedition; volume 5. Micropaleontology. 5:1–32.

Munnecke A, Servais T. 1996. Scanning electron microscopy of polished, slightly etched rock surfaces: a method to observe palynomorphs *in situ.* Palynology. 20:163–176.

Munsterman D, Kerstholt S. 1996. Sodium polytungstate, a new non-toxic alternative to bromoform in heavy liquid separation. Review of Palaeobotany and Palynology. 91:417–422.

Nakagawa T, Brugiapaglia E, Digerfeldt G, Reille M, de Beaulieu J-L, Yasuda Y. 1998. Dense-media separation as a more efficient pollen extraction method for use with organic sediment/deposit samples: comparison with the conventional method. Boreas. 27:15–24.

Nelson DO. 1950. Method of eliminating gypsum from samples. The Micropaleontologist. 4(3): 21.

Nelson DO. 1962. A new slide-marking ink. Oklahoma Geology Notes. 22:223.

Nemchin RG, Brusick DJ. 1985. Basic principles of laboratory safety. Environmental and Molecular Mutagenesis. 7:947–971.

Neuhaus B, Schmid T, Riedel J. 2017. Collection management and study of microscope slides: Storage, profiling, deterioration, restoration procedures, and general recommendations. Zootaxa. 4322, 173 p.

Neves R, Dale B. 1963. A modified filtration system for palynological preparations. Nature. 198:775–776.

Nguyen J-P. 1996. Drilling: Oil and Gas Field Development Techniques. Editions Technip, Paris, France, 384 p.

Noetinger S, Pujana RR, Burrieza A, Burrieza HP. 2017. Use of UV-curable acrylates gels as mounting media for palynological samples. Revista del Museo Argentino de Ciencias Naturales. 19:19–23.

Noll KE. 1970. A rotary inertial impactor for sampling giant particles in the atmosphere. Atmospheric Environment. 4:9–19.

Norem WL. 1953. Separation of spores and pollen from siliceous rocks. Journal of Paleontology. 27:881–883.

Norem WL. 1956. An improved method for separating fossil spores and pollen from siliceous rocks. Journal of Paleontology. 30:1258–1260.

Nørgaard I, Rasmussen A-M, Schiøler P, Stouge S. 1991. A new method for bulk palynological processing of oil-contaminated chalks. Journal of Micropalaeontology. 10:202.

Oda M, Kitazato H, Hasagawa S. 1975. Some problems using sodium hexametaphosphate in treatment of rocks for microfossil analysis. Journal of the Japanese Association of Petroleum Technologists. 50:1–8.

Ogden JG III. 1986. An alternative to exotic spore or pollen addition in quantitative microfossil studies. Canadian Journal of Earth Sciences. 23:102–106.

Ohtani M, Nishida N, Chiba T, Muto H, Yoshioka N. 2007. Pathological demonstration of rapid involvement into the subcutaneous tissue in a case of fatal hydrofluoric acid burns. Forensic Science International. 167:49-52.

O'Keefe J, Hower J, Hatch R, Bartley, R, Bartley S. 2011. Fungal forms in Miocene Eel River coals: correlating between reflected light petrography and palynology. Annual Meeting of the Geological Society of America, Abstracts with Program. 43:501.

O'Keefe JMK, Eble CF. 2012. A comparison of HF-based and non-HF-based palynology processing techniques in clay-rich lignites from the Claiborne Group, upper Mississippi Embayment, United States. Palynology. 36:116–130.

O'Keefe JMK, Wymer CL. 2017. An alternative to acetolysis: application of an enzyme-based method for the palynological preparation of fresh pollen, honey samples and bee capsules. Palynology. 41:117–120.

Oldham TCB. 1976. Flora of the Wealden plant debris beds of England. Palaeontology. 19: 437–502.

Osborne D (editor). 2013. The Coal Handbook. Towards Cleaner Production. Volume 1. Coal Production. Woodhead Publishers, Cambridge, UK, 776 p.

6891 Pacini E, Hesse M. 2005. Pollenkitt – its composition, forms and functions. Flora.
6892 200:399–415.
6893
6894 Paris F, Nõlvak J. 1999. Biological interpretationand paleobiodiversity of a cryptic fossil
6895 group: The “chitinozoan animal”. Geobios. 32:315–324.
6896
6897 Parry CC, Whitley PKJ, Simpson RDH. 1981. Integration of palynological and
6898 sedimentological methods in facies analysis of the Brent Formation. In: Illing LV,
6899 Hobson GD. (editors). Petroleum Geology of the Continental Shelf of north-west Europe.
6900 Institute of Petroleum, London, 205–215.
6901
6902 Pearson T, Scott AC. 1999. Large palynomorphs and debris. In: Jones TP, Rowe NP
6903 (editors). Fossil plants and spores: modern techniques. Geological Society, London, 20–
6904 25.
6905
6906 Peck RM 1972. Efficiency tests on the Tauber Trap used as a pollen sampler in turbulent
6907 water flow. New Phytologist. 71:187–198.
6908
6909 Pendleton M. 2006. Descriptions of melissopalynological methods involving
6910 centrifugation should include data for calculating Relative Centrifugal Force (RCF) or
6911 should express data in units of RCF or gravities (*g*). Grana. 45:71–72.
6912
6913 Penny JHJ. 1999. Extraction of lignitic and fusainized plant fragments from
6914 unconsolidated sandy and clay-rich sediments. In: Jones TP, Rowe NP (editors). Fossil
6915 Plants and Spores: Modern Techniques. Geological Society, London, 9–10.
6916
6917 Perrotti AG, Siskind T, Bryant MK, Bryant, VM. 2018. Efficacy of sonification-assisted
6918 sieving on Quaternary pollen samples. Palynology. 42:466–474.
6919
6920 Phillips L. 1972. An application of fluorescence microscopy to the problem of derived
6921 pollen in British Pleistocene deposits. New Phytologist. 71:755–762.

Phipps D, Playford G. 1984. Laboratory techniques for extraction of palynomorphs from sediments. Papers of the Department of Geology, University of Queensland. 11.1, 23 p.

Piel KM, Evitt WR. 1980. Paratabulation in the Jurassic dinoflagellate genus *Nannoceratopsis* and a comparison with modern taxa. Palynology. 4:79–104.

Pierce RL. 1959. Converting coordinates for microscope-stage scales Micropaleontology. 5:377–378.

Pluta M. 1989. Advanced Light Microscopy. Volume 2. Specialized Methods. Elsevier, Amsterdam, The Netherlands, 494 p.

Pojeta J, Balanc M. 1989. Uses of ultrasonic cleaners in paleontological laboratories. In: Feldmann RM, Chapman RE, Hannibal JT (editors). Paleotechniques. The Paleontological Society Special Publication. 4:213–217.

Poulsen NE, Gudmundssonn L, Morten Hansen J, Husfeldt Y. 1990. Palynological preparation techniques, a new Macerationtank-method and other modifications. Geological Survey of Denmark, DGU Series C, No. 10, 23 p.

Pound, MJ, Haywood AM, Salzmann U, Riding JB, Lunt DJ, Hunter SJ. 2011. A Tortonian (Late Miocene, 11.61–7.25 Ma) global vegetation reconstruction. Palaeogeography, Palaeoclimatology, Palaeoecology. 300:29–45.

Pound MJ, O'Keefe JMK, Nuñez Otaño NB, Riding JB. 2019. Three new Miocene fungal palynomorphs from the Brassington Formation, Derbyshire, UK. Palynology. 43:596–607.

Pound MJ, O'Keefe JMK, Marret F. 2021. An overview of techniques applied to the extraction of Non-Pollen Palynomorphs, their known taphonomic issues, and

recommendations to maximise recovery. In: Marret F, O'Keefe JMK, Osterloff PL, Pound MJ, Shumilovskikh L (editors). Applications of Non-Pollen Palynomorphs: from palaeoenvironmental reconstructions to biostratigraphy. The Micropalaeontological Society, Special Publications. The Geological Society, London, doi: 10.1144/SP511-2020-40.

Powell AJ, Riding JB (editors). 2005. Recent Developments in Applied Biostratigraphy. The Micropalaeontological Society, Special Publications. The Geological Society, London, 256 p.

Price AM, Gurdebeke PR, Mertens KN, Pospelova V. 2016. Determining the absolute abundance of dinoflagellate cysts in recent marine sediments III: Identifying the source of *Lycopodium* loss during palynological processing and further testing of the *Lycopodium* marker-grain method. Review of Palaeobotany and Palynology. 226:78–90.

Quinn PS, Day PM. 2007. Ceramic micropalaeontology: the analysis of microfossils in ancient ceramics. Journal of Micropalaeontology. 26:159–168.

Raine JI, Tremain R. 1992. Apparatus for rapid sieving of palynological residues. New Zealand Geological Survey Report. PAL 151, 14 p.

Raistrick A. 1934. The correlation of coal seams by microspore content. Part I. – The seams of Northumberland. Transactions of the Institution of Mining Engineers. 88:142–153 and 259–264.

Raistrick A, Simpson J. 1933. The microspores of some Northumberland coals, and their use in the correlation of coal-seams. Transactions of the Institution of Mining Engineers. 85:225–235.

Ravikumar S, Surekha R, Thavarajah R. 2014. Mounting media: An overview. Journal of Dr. NTR University of Health Sciences. 3(Supplement 1):S1–S8.

Reid PC, John AWG. 1981. A possible relationship between chitinozoa and tintinnids. Review of Palaeobotany and Palynology. 34:251–262.

Reinhard KJ, Edwards S, Damon TR, Meier DK. 2006. Pollen concentration analysis of Ancestral Pueblo dietary variation. Palaeogeography, Palaeoclimatology, Palaeoecology. 237:92–109.

Reinsch PF. 1881. Neue Untersuchungen iiber die Mikrostruktur der Steinkohle des Carbon des Dyas und Trias. Weigel, Leipzig, Germany, 1241 p.

Reinsch PF. 1884. Micro-Palaeophytologia Formationis Carboniferae 1 Continens Trileteas et Stelideas . Theo Krische, Erlangen, Germany, 80 p.

Reissinger A. 1939. Die “Pollenanalyse” ausgedehnt auf alle Sedimentgesteine der geologischen Vergangenheit. Part I. Palaeontographica Abteilung B. 84:1–20.

Reissinger A. 1950. Die “Pollenanalyse” ausgedehnt auf alle Sedimentgesteine der geologischen Vergangenheit. Part II. Palaeontographica Abteilung B. 90:99–126.

Reitsma Tj. 1969. Size modification of recent pollen grains under different treatments. Review of Palaeobotany and Palynology. 9:175–202.

Rickwood D (editor). 1984. Centrifugation: A practical approach. Second Edition. IRL Press, Oxford, UK, 350 p.

Riddick NL, Volik O, McCarthy FMG, Danesh DC. 2017. The effect of acetolysis on desmids. Palynology. 41:171–179.

Riding JB. 1980. The nature and affinity of the Chitinozoa – a review. Journal of the University of Sheffield Geological Society. 7.5:262–268.

Riding JB. 1984. Dinoflagellate cyst range-top biostratigraphy of the uppermost Triassic to lowermost Cretaceous of northwest Europe. Palynology. 8:195–210.

Riding JB, Penn IE, Woollam R. 1985. Dinoflagellate cysts from the type area of the Bathonian Stage (Middle Jurassic; southwest England). Review of Palaeobotany and Palynology. 45:149–169.

Riding JB. 1990. On *Chytroeisphaeridia hyalina* (Raynaud) Lentin & Williams (Pyrrhophyta, Dinophyceae). Taxon. 39:311–312.

Riding JB, Thomas JE. 1997. Marine palynomorphs from the Staffin Bay and Staffin Shale formations (Middle–Upper Jurassic) of the Trotternish Peninsula, NW Skye. Scottish Journal of Geology. 33:59–74.

Riding JB, Fedorova VA, Ilyina VI. 1999. Jurassic and lowermost Cretaceous dinoflagellate cyst biostratigraphy of the Russian Platform and northern Siberia, Russia. American Association of Stratigraphic Palynologists Contributions Series. 36, 179 p.

Riding JB, Kyffin-Hughes JE. 2004. A review of the laboratory preparation of palynomorphs with a description of an effective non-acid technique. Revista Brasileira de Paleontologia. 7:13–44.

Riding JB. 2006. A palynological investigation of five wool samples. British Geological Survey Technical Report. CR/06/057, 10 p.

Riding JB, Kyffin-Hughes JE. 2006. Further testing of a non-acid palynological preparation procedure. Palynology. 30:69–87.

Riding JB, Wilkinson IP, Jones LD, Freeborough K. 2006. The occurrence of dinoflagellate cysts in calcareous/silicious microfossil preparations from the Eocene of southeast England. Journal of Micropalaeontology. 25:35–36.

Riding JB, Kyffin-Hughes JE, Owens B. 2007a. An effective palynological preparation procedure using hydrogen peroxide. Palynology. 31:19–36.

Riding JB, Rawlins BG, Coley KH. 2007b. Changes in soil pollen assemblages on footwear worn at different sites. Palynology. 31:135–151.

Riding JB, Schmitt FG. 2009. Deflandre (1938) – a palynological classic. AASP – The Palynological Society Newsletter. 42.4:16–18.

Riding JB, Kyffin-Hughes JE. 2010. The use of pre-treatments in palynological processing. Review of Palaeobotany and Palynology. 158:281–290.

Riding JB, Kyffin-Hughes JE. 2011. A direct comparison of three palynological preparation techniques. Review of Palaeobotany and Palynology. 167:212–221.

Riding JB, Pound MJ, Hill TCB, Stukins S, Feist-Burkhardt S. 2012. The John Williams Index of Palaeopalynology. Palynology. 36:224–233.

Riding JB, Dettmann ME. 2013. The first Australian palynologist: Isabel Clifton Cookson (1893–1973) and her scientific work. Alcheringa. 38:97–129.

Riding JB, Lucas-Clark J. 2016. The life and scientific work of William R. Evitt (1923-2009). Palynology. 40 S1: 2–131.

Riding JB, Head MJ. 2018. Preparing photographic plates of palynomorphs in the digital age. Palynology. 42:354–365.

Rigby JF. 1963. Application of palynological techniques to palaeobotany. Annals of Botany. 27:371–372.

Rosenberg L. 1971. Chemical basis for the histological use of Safranin O in the study of articular cartilage. The Journal of Bone and Joint Surgery. 53:69–82.

Rowe NP, Jones TP. 1999. Locating and collecting. In: Jones TP, Rowe NP (editors). Fossil plants and spores: modern techniques. Geological Society, London, 5–8.

Rueger BF. 1986. Use of EDTA for palynomorph extraction from evaporites. Journal of Paleontology. 60:189–190.

Ruhe RV, Daniels RB. 1958. Soils, paleosols, and soil-horizon nomenclature. Soil Science Society of America Journal. 22:66–69.

Rydin H, Jeglum JK. 2013. The Biology of Peatlands. Second Edition. Oxford University Press, Oxford, UK, 398 p.

Salter SJ, Cox MJ, Turek EM, Calus ST, Cookson WO, Moffatt MF, Turner P, Parkhill J, Loman NJ, Walker AW. 2014. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biology. 12: 87.

Sandberg PA, Hay WW. 1967. Study of microfossils by means of the scanning electron microscope. Journal of Paleontology. 41:999–1001.

Sanders JM. 1937. The microscopical examination of crude petroleum. Journal of the Institute of Petroleum Technology. 23:525–573.

Sanders RB. 1966. Technique for mounting saccate pollen grains. Oklahoma Geology Notes. 26:257–258.

Sarjeant WAS. 1974. Fossil and living dinoflagellates. Academic Press, London, 182 p.

Sarjeant WAS, Vanguestaine M. 1999. Maria Lejeune-Carpentier (1910–1995): a memorial. Journal of Micropaleontology. 18:137–142.

Sarjeant WAS. 1985. Alfred Eisenack (1891–1982) and his contributions to palynology. Review of Palaeobotany and Palynology. 45:3–15.

Saunders WB, Mapes RH, Carpenter FM, Elsik WC. 1974. Fossiliferous amber from the Eocene (Claiborne) of the Gulf Coastal Plain. Geological Society of America Bulletin. 85:979–984.

Saxby JD. 1970. Isolation of kerogen in sediments by chemical methods. Chemical Geology. 6:173–184.

Schenck HG, Adams BC. 1943. Operations of commercial micropaleontologic laboratories. Journal of Paleontology. 17:554–583.

Schols P, Dessein S, D'hondt C, Huysmans S, Smets E. 2002. Carnoy: a new digital measurement tool for palynology. Grana. 41:124–126.

Schols P, Es K, D'hondt C, Merckx V, Smets E, Huysmans S. 2004. A new enzyme-based method for the treatment of fragile pollen grains collected from herbarium material. Taxon. 53:777–782.

Schopf JM. 1960. Double cover-glass slides for plant microfossils. Micropaleontology. 6:237–240.

Schopf JM. 1964. Practical problems and principles in study of plant microfossils. In: Cross AT (editor). Palynology in oil exploration. A symposium. Society of Economic Paleontologists and Mineralogists Special Publication.11:29–57.

Schopf JM. 1965. A method for obtaining small acid-resistant fossils from ordinary solution residues. In: Kummel B, Raup D. (editors). Handbook of Paleontological Techniques. WH Freeman and Company, San Francisco, 301–304.

Schopf JM, Wilson LR, Bentall R. 1944. An annotated synopsis of Paleozoic fossil spores and the definition of generic groups. Illinois State Geological Survey Report of Investigations. 91, 73 p.

Schrank E. 1988. Effects of chemical processing on the preservation of peridinioid dinoflagellates: a case from the Late Cretaceous of NE Africa. Review of Palaeobotany and Palynology. 56:123–140.

Schrank E. 2003. Small acritarchs from the Upper Cretaceous: taxonomy, biological affinities and palaeoecology. Review of Palaeobotany and Palynology. 123:199–235.

Schulze F. 1855. Über das Vorkommen wohlerhaltener Zellulose in Braunkohle und Steinkohle. Königlichen Akademie der Wissenschaften zu Berlin. 21:676–678.

Schwab KW. 1966. Microstructure of some fossil and recent scolecodonts. Journal of Paleontology. 40:416–423.

Scott AC. 1977. Coprolites containing plant material from the Carboniferous of Britain. Palaeontology. 20:59–68.

Scott AC. 2018. Burning Planet: The Story of Fire Through Time. Oxford University Press, Oxford, UK, 224 p.

SDA (The Soap and Detergent Association). 1990. Glycerine: an overview. New York, 27 p.

Sen Gupta KB (editor). 2003. Modern foraminifera. Springer, Dordrecht, The Netherlands, 371 p.

Shane JD, Clarke WD. 1981. Recovering polymerized fossil pollen residues. Micropaleontology. 27:109-110.

Sheenan TP. 1992. Modifications of two palynological processing techniques: ultrasonic processing and early-stage sieving. United States Geological Survey Open File Report. 92-564, 7 p.

Shewmake SW, Anderson BG. 1979. Hydrofluoric acid burnsa report of a case and review of the literature. Archives of Dermatology. 115:593–596.

Shirazi MA, Boersma L. 1984. A unifying quantitative analysis of soil texture. Soil Science Society of America Journal. 48:142–147.

Shkrebta GP. 1966. Separation of plant remains from oils by filtration. International Geology Review. 8:1118–1122.

Shumilovskikh LS, Schlütz F, Lorenz M, Tomaselli MB. 2019. Non-pollen palynomorphs notes: 3. Phototrophic loricate euglenoids in paleoecology and the effect of acetolysis on *Trachelomonas* loricae. Review of Palaeobotany and Palynology. 270:1–7.

Simes J, Wrenn J. 1998. Palynologic processing in Antarctica. Terra Antarctica. 5:549–552.

Singer G. 1967. A comparison between different mounting techniques commonly employed in acarology. Acarologia. 9:475–484.

Sittler C. 1954. Principe et application de l'analyse des pollens aux études de recherches du pétrole. Revue de l'Institut Français du Pétrole. 9:367–375.

Sittler C. 1955. Méthodes et techniques physico-chimiques de préparation des sédiments en vue de leur analyse pollinique. Revue de l'Institut Français du Pétrole et Annales des Combustibles Liquides. 10:103–114.

Six J, Schultz PA, Jastrow JD, Merck R. 1999. Recycling of sodium polytungstate used in soil organic matter studies. Soil Biology and Biochemistry. 31:1193–1196.

Skvarla JJ, Pyle CC. 1968. Techniques of pollen and spore electron microscopy. Part II. Ultramicrotomy and associated techniques. Grana Palynologica. 8:255–270.

Sluijs A, Pross J, Brinkhuis H. 2005. From greenhouse to icehouse; organic-walled dinoflagellate cysts as paleoenvironmental indicators in the Paleogene. Earth-Science Reviews. 68:281–315.

Smith C, Warny S, Shevenell AE, Gulick SPS, Leventer A. 2019. New species from the Sabrina Flora: an early Paleogene pollen and spore assemblage from the Sabrina Coast, East Antarctica, Palynology. 43:650–659.

Smith AVH, Butterworth MA. 1967. Miospores in the coal seams of the Carboniferous of Great Britain. Special Papers in Palaeontology. 1, 324 p.

Smith RT. 1966. Sone refinements in the technique of pollen and spore extraction from soil. Laboratory Practice. 15:1120–1123.

Snead RG. 1969. Microfloral diagnosis of the Cretaceous–Tertiary boundary, central Alberta. Alberta Research Council Bulletin. 25, 148 p.

Snyder LJ. 2015. Eye of the Beholder: Johannes Vermeer, Antoni van Leeuwenhoek, and the Reinvention of Seeing. WW Norton and Company, New York, 448 p.

7230 Snyder SW, Mauger LL, Akers WH. 1983. Planktonic foraminifera and biostratigraphy
7231 of the Yorktown Formation, Lee Creek, North Carolina. Smithsonian Contributions to
7232 Paleobiology. 53:455–482.
7233
7234 Sobolik KD. 1988. The importance of pollen concentration values from coprolites: an
7235 analysis of southwest Texas samples. Palynology. 12:201–214.
7236
7237 Southworth D. 1974. Solubility of pollen exines. American Journal of Botany. 61:36–44.
7238
7239 Sowunmi MA. 1976. The potential value of honey in palaeopalynology and archaeology.
7240 Review of Palaeobotany and Palynology. 21:171-185.
7241
7242 Spector, DL (editor). 1984. Dinoflagellates. Academic Press, Orlando, 545 p.
7243
7244 Spielholtz G, Thomas LA, Diehl H. 1962. Isolation of spores by wet oxidation.
7245 Micropaleontology. 8: 109–110.
7246
7247 Srivastava AK, Wadhwani K. 1992. Dispersion and allergenic manifestations of
7248 *Alternaria* airspora. Grana. 31:61–66.
7249
7250 Srivastava SK. 1984. Palynology of the Monterey formation (Miocene) phosphatic facies
7251 at Lions head, Santa Maria area, California. Palynology. 8:33–49.
7252
7253 Stach E, Mackowsky MT, Teichmüller M, Taylor GH, Chandra D, Teichmüller R. 1982.
7254 Stach's Textbook of Coal Petrology. Third Edition. Gebrüder Borntraeger, Berlin-
7255 Stuttgart, Germany, 109 p.
7256
7257 Stancliffe RPW. 1989. Microforaminiferal linings: their classification, biostratigraphy
7258 and paleoecology, with special reference to specimens from British Oxfordian sediments.
7259 Micropaleontology. 35:337–352.
7260

Stancliffe RPW. 1996. Chapter 13D. Microforaminiferal linings. In: Jansonius J, McGregor DC (editors). Palynology: principles and applications. American Association of Stratigraphic Palynologists Foundation, Dallas. 1:373–380.

Stancliffe RPW, Matsuoka K. 1991. Note on the preparation of small calcareous tests and shells using hydrofluoric acid. Micropaleontology. 37:419–421.

Stankiewicz BA, Briggs DEG, Evershed RP. 1997. Chemical composition of Paleozoic and Mesozoic fossil invertebrate cuticles as revealed by pyrolysis-gas chromatography/mass spectrometry. Energy and Fuels. 11:515–521.

Staplin FL, Pocock SJ, Jansonius J, Oliphant EM. 1960. Palynological techniques for sediments. Micropaleontology. 6:329–331.

Stauffer CR. 1933. Middle Ordovician Polychaeta from Minnesota. Bulletin of the Geological Sociery of America. 44:1173–1218.

Stauffer CR. 1939. Middle Devonian Polychaeta from the Lake Erie District. Journal of Paleontology. 13:500–511.

Stephenson MH, Owens B. 2006. Taxonomy Online 2: The ‘Bernard Owens Collection’ of single grain mount palynological slides: Carboniferous spores part I. British Geological Survey Research Report. RR/06/05, 80 p.

Stevens CH, Jones DH, Todd RG. 1960. Ultrasonic vibrations as a cleaning agent for fossils. Journal of Paleontology. 34:727–730.

Stockmarr J. 1971. Tablets with spores used in absolute pollen analysis. Pollen et Spores. 13:615–621.

Stockmarr J. 1972. Determination of spore concentration with an electronic particle counter. Danmarks Geologiske Undersøgelse Årbok. 1972:87–92.

Stover LE, Brinkhuis H, Damassa SP, de Verteuil L, Helby RJ, Monteil E, Partridge AD, Powell AJ, Riding JB, Smelror M, Williams GL. 1996. Chapter 19. Mesozoic–Tertiary dinoflagellates, acritarchs and prasinophytes. In: Jansonius J, McGregor DC (editors). Palynology: principles and applications. American Association of Stratigraphic Palynologists Foundation, Dallas. 2:641–750.

Strother SL, Salzmann U, Sangiorgi F, Bijl PK, Pross J, Escutia C, Salabarnada A, Pound MJ, Voss J, Woodward J. 2017. A new quantitative approach to identify reworking in Eocene to Miocene pollen records from offshore Antarctica using red fluorescence and digital imaging. Biogeosciences. 14:2089–2100.

Szaniawski H. 1996. Scolecodonts. In: Jansonius J, McGregor DC (editors). Palynology: principles and applications. American Association of Stratigraphic Palynologists Foundation, Dallas. 1:337–354.

Taggart RE, Cross AT. 1980. Vegetation change in the Miocene Succor Creek flora of Oregon and Idaho: A case study in paleosuccession. In: Dilcher DL, Taylor TN (editors). Biostratigraphy of Fossil Plants. Dowden, Hutchinson, and Ross Incorporated, Stroudsburg, Pennsylvania, 185–210.

Tappan, H, Loeblich AR. 1965. Foraminiferal remains in palynological preparations. Revue de Micropaléontologie. 8:61–63.

Tasch P, Shaffer BL. 1961. Study of scolecodonts by transmitted light. Micropaleontology. 7:369–371.

Tauber H. 1967. Investigations of the mode of pollen transfer in forested areas. Review of Palaeobotany and Palynology. 3:277–286.

7322
7323 Tauber H. 1974. A static non-overload pollen collector. New Phytologist. 73:359–369.
7324
7325 Taugourdeau PH. 1971. Scolécodontes du Frasnien du Boulonnais. Revista Española de
7326 Micropaleontologia. 3:71–108.
7327
7328 Taugourdeau P. 1981. Les diverses attributions systematiques proposees pour les
7329 chitinozoaires. Cahires de Micropaleontologie. 17–28.
7330 Check this one
7331
7332 Taylor FRJ. 1980. On dinoflagellate evolution. BioSystems. 13:65–108.
7333
7334 Taylor FRJ. 1987. The biology of dinoflagellates. Blackwell Scientific Publications,
7335 Oxford, 785 p.
7336
7337 Taylor WA, Taylor TN. 1988. Ultrastructural analysis of selected Cretaceous megaspores
7338 from Argentina, Journal of Micropalaeontology. 7:73–87.
7339
7340 Tennent NH, Townsend JH. 1984. The significance of the refractive index of adhesives
7341 for glass repair. Studies in Conservation. 29 supplement 1: 205–212.
7342
7343 Terasmae J. 1958. "Microforaminifera" from Pleistocene deposits, Prince Edward Island,
7344 Canada. Micropaleontology. 4:429–430.
7345
7346 Thiessen R. Wilson FE. 1924. Correlation of coal beds of the Allegheny Formation of
7347 Western Pennsylvania and Eastern Ohio. Carnegie Institute for Technology, Coal-Mining
7348 Investigation Bulletin. 10, 56 p.
7349
7350 Thomas KM. 1989. 5. Health hazards in the paleontology laboratory. In: Feldmann RM,
7351 Chapman RE, Hannibal JT (editors). Paleotechniques. The Paleontological Society
7352 Special Publication. 4:30–36.

Timofeyev BV, Karimov AK. 1953. Spory i pyltsa v neftyakh. Doklady an Akademii Nauk an SSSR. 92:151–152.

Tomas C, Candau P, Gonzalez Minero FJ. 1997. A comparative study of atmospheric pollen collected with Burkard and Cour samplers, Seville (Spain), 1992–1994. Grana. 36:122–128.

Tomb AS. 1982. A new method for isolating pollen for scanning electron microscopy. Micropaleontology. 28:215–217.

Tomlinson P. 1984. Ultrasonic filtration as an aid in pollen analysis of archaeological deposits. Circaea. 2:139–140.

Tomor J. 1950. Szerves maradvány-vizsgálatok magyarországi kőolajokban. Földtani Közlöny. 80:335–360.

Traverse A. 1960. Still more on conversion of microscope coordinates. Micropaleontology. 6:424.

Traverse A. 1965. Preparation of modern pollen and spores for palynological reference collections. In: Kummel B, Raup D (editors). Handbook of Paleontological Techniques. W.H. Freeman and Company, San Francisco, 598–613.

Traverse A. 1978. Palynological analysis of DSDP Leg 42B (1975): cores from the Black Sea. Initial Reports of the Deep Sea Drilling Project. 42:993–1015.

Traverse A. 1988. Paleopalynology. Unwin Hyman, Boston, USA, 600 p.

Traverse A (editor). 1994. Sedimentation of organic particles. Cambridge University Press, Cambridge, UK, 544 p.

Traverse A. 2007. Paleopalynology. Second Edition. Springer, The Netherlands, 813 p.

Traverse A, Clisby KH, Foreman F. 1961. Pollen in drilling-mud “thinners,” a source of palynological contamination. Micropaleontology. 7:375–377.

Traverse A, Ginsburg RN. 1966. Palynology of the surface sediments of Great Bahama Bank, as related to water movement and sedimentation. Marine Geology. 4:417–459.

Tschudy RH. 1958. A modification of the Schulze digestion method of possible value in studying oxidised coals. Grana Palynologica. 1:34–38.

Tschudy RH. 1960. “Vibraflute”. Micropaleontology. 6:325–326.

Tschudy RH, Scott RA (editors). 1969. Aspects of Palynology. Wiley-Interscience, New York, USA, 510 p.

Tucker ME. 2001. Sedimentary Petrology: an Introduction to the Origin of Sedimentary Rocks. Third Edition. Blackwell, Science Limited, Oxford, UK, 272 p.

Tyson RV. 1995. Sedimentary organic matter. Organic facies and palynofacies. Chapman and Hall, London, 615 p.

Urban MA, Romero IC, Sivaguru M, Punyasena SW. 2018. Nested cell strainers: An alternative method of preparing palynomorphs and charcoal. Review of Palaeobotany and Palynology. 253:101–109.

Valensi L. 1953. Microfossiles des silex du Jurassique moyen. Remarques pétrographiques. Mémoire de la Société Géologique de France. 68, 100 p.

Valensi L. 1955. Etude micropaléontologique des silex du magdalénien de Saint-Amand (Cher). Bulletin de la Société Préhistorique Française. 52:584–596.

van Asperen EN, Kirby JR, Hunt CO. 2016. The effect of preparation methods on dung fungal spores: Implications for recognition of megafaunal populations. Review of Palaeobotany and Palynology. 229:1–8.

van Breemen N, Buurman P. 2002. Soil Formation. Second Edition. Kluwer Academic Publishers, Dordrecht, The Netherlands, 424 p.

Van Cleave HJ, Ross JA. 1947. Use of trisodium phosphate in microscopical technic. Science. 106:194.

Vandenbroucke TRA, Armstrong HA, Williams M, Paris F, Zalasiewicz JA, Sabbe K, Nõlvak J, Challands TJ, Verniers J, Servais T. 2010. Polar front shift and atmospheric CO_2 during the glacial maximum of the Early Paleozoic Icehouse. PNAS. 107:14983–14986.

Vandenbroucke TRA, Munnecke, A, Leng MJ, Bickert T, Hints O, Gelsthorpe D, Maier G, Servais T. 2013. Reconstructing the environmental conditions around the Silurian Ireviken Event using the carbon isotope composition of bulk and palynomorph organic matter. Geochemistry, Geophysics, Geosystems. 14:86–101.

Vandenbroucke TRA, Emsbo P, Munnecke A, Nuns N, Duponchel L, Lepot K, Quijada M, Paris F, Servais T, Kiessling W. 2015. Metal-induced malformations in early Palaeozoic plankton are harbingers of mass extinction. Nature Communications. 6:7966.

van der Kaars WA. 1991. Palynology of eastern Indonesian marine piston-cores:A Late Quaternary vegetational and climatic record for Australasia. Palaeogeography, Palaeoclimatology, Palaeoecology. 85:239–302.

Van Geel B. 2001. Non-pollen palynomorphs. In: Smol JP, Birks HJB, Last WM (editors). Tracking environmental change using lake sediments. Terrestrial, algal and silicious indicators. 3:99–119. Kluwer, Dordrecht, The Netherlands.

van Gijzel P. 1967. Palynology and fluorescence microscopy. Review of Palaeobotany and Palynology. 2:49–79.

Van Ness BG, Black MK, Gullett CR, O'Keefe JMK. 2017. A recycling method for LST® contaminated during heavy liquid separation in palynological processing. Palynology. 41:498–503.

Vareschi V. 1935. Pollenanalysen aus Gletschereis. Bericht über das Geobotanische Forschungsinstitut Rubel in Zürich für dar Jahr 1934:81–99.

Vareschi V. 1937. Pröfung der neuen pollenanalytischen Methode der Gletscherforschung an Hand eines Firnprofils von bekannter Enstehungszeit. Zeitschrift für Gletscherkunde. 25:17–35.

Varma CP. 1964. Palynology in oil exploration. In: Nair PKK (editor). Advances in palynology. National Botanic Gardens, Lucknow, India, 378–403.

Vidal G. 1988. A palynological preparation method. Palynology 12:215–220.

von Ardenne. M. 1938. Das Elektronen-Rastermikroskop. Theoretische Grundlagen. Zeitschrift fur Physik. 108:553–572.

von Post L. 1916. Einige siidschwedischen Quellmoore. Bulletin of the Geological Institutes of Upsala. 15:219–278.

Vorwohl G. 1967. The microscopic analysis of honey, a comparison of its methods with those of the other branches of palynology. Review of Palaeobotany and Palynology. 3:287–290.

Wagner GM. 1997. *Azolla*: A review of its biology and utilization. The Botanical Review. 63:1–26.

Wall D, Dale B. 1966. “Living fossils” in western Atlantic plankton. Nature. 211:1025–1026.

Wall D, Guillard RRL, Dale B. 1967. Marine dinoflagellate cultures from resting spores. Phycologia. 6:83–86.

Wall D, Dale B, Lohman GP, Smith WK. 1977. The environmental and climatic distribution of dinoflagellate cysts in modern marine sediments from regions in the North and South Atlantic Oceans and adjacent seas. Marine Micropaleontology. 2:121–200.

Warny S. 2013. Museums’ role: Pollen and forensic science. Science. 339:1149–1149.

Warny S, Ferguson S, Hafner MS, Escarguel G. 2020. Using museum pelt collections to generate pollen prints from high-risk regions: A new palynological forensic strategy for geolocation. Forensic Science International. 306, 11006.

Warren JK. 2016. Evaporites: A Geological Compendium. Second Edition. Springer, Switzerland, 1813 p.

Waterhouse H. 1995. High-resolution palynofacies investigation of Kimmeridgian sedimentary cycles. In: House MR, Gale AS (editors). Orbital forcing timescales and cyclostratigraphy. Geological Society Special Publication. 85:75–114.

Wellman CH. 2005. Half a century of palynology at the University of Sheffield. In: Bowden AJ, Burek CV, Wilding R. (editors). History of Palaeobotany: Selected Essays. Geological Society of London Special Publications. 241:259–279.

West RG. 1977. Pleistocene geology and biology, with especial reference to the British Isles. Second edition. Longman Group Limited, London, UK, 440 p.

Wethered E. 1886. On the occurrence of spores of plants in the Lower Limestone Shales of the Forest of Dean Coalfield and in the black shales of Ohio, United States. Proceedings of the Cotteswold Naturalists' Field Club. 8:167–173.

Wetzel W. 1922. Sediment-petrographische Studien. I. Feuerstein. Neues Jahrbuch für Mineralogie, Geologie und Paläontologie, Beilagebände. 47:39–92.

Wetzel O. 1933a. Die in organischer Substanz erhaltenen Mikrofossilien des baltischen Kreide-Feuersteins mit einem sedimentpetrographischen und stratigraphischen Anhang. Palaeontographica Abteilung A. 77:141–188.

Wetzel O. 1933b. Die in organischer Substanz erhaltenen Mikrofossilien des baltischen Kreide-Feuersteins mit einem sedimentpetrographischen und stratigraphischen Anhang. Palaeontographica Abteilung A. 78:1–110.

Wetzel O. 1957. Fossil "microforaminifera" in various sediments and their reaction to acid treatment. Micropaleontology. 3:61–64.

Wheeler A, Moss PT, Götz AE, Esterle JS, Mantle D. 2020. Acid-free palynological processing: A Permian case study. Review of Palaeobotany and Palynology. doi 10.1016/j.revpalbo.2020.104343.

White JW Jr. 1978. Honey. Advances in Food Research. 24:287–374.

White JW Jr, Bryant VM Jr, Jones JG. 1991. Adulteration testing of southwestern desert honeys. American Bee Journal. 131:123–126.

White RE 2006. Principles and Practice of Soil Science: The Soil as a Natural Resource. Fourth Edition. Wiley-Blackwell, Oxford, UK, 376 p.

Whitlock C, Larsen C. 2001. Chapter 5. Charcoal as a fire proxy. In: Smol JP, Birks HJB, Last WM, Bradley RS, Alverson K. (editors). Tracking Environmental Change Using Lake Sediments. Developments in Paleoenvironmental Research. Volume 3. Terrestrial, Algal, and Siliceous Indicators. Kluwer Academic Publishers, Dordrecht, The Netherlands, 75–97.

Whitney BS, Needham T. 2014. Isopropyl alcohol: A replacement for tertiary-butyl alcohol in pollen preparations. Review of Palaeobotany and Palynology. 203:9–11.

Whittaker JE, Hodgkinson, R.L. 1991. On the preparation of specimens for scanning electron microscopy and a simple technique for plate making, using a black background. Journal of Micropalaeontology. 9:219–220.

Wilde V, Hemsley AR. 2000. Morphology, ultrastructure and affinity of Barremian (Lower Cretaceous) megaspores *Dijkstraisporites* and *Paxillitriletes* from Brilonnehden, Germany. Palynology. 24:217–230.

Williams G, Payne SNJ, Dyer R, Ewen DF, Patrick N, Watson P. 2005. Non-acid wellsite palynology: widening opportunities. In: Powell AJ, Riding JB (editors). Recent Developments in Applied Biostratigraphy. The Micropalaeontological Society, Special Publications. The Geological Society, London, 219–235.

Williams GL, Downie C. 1966. The London Clay. In: Davey RJ, Downie C, Sarjeant WAS, Williams GL (editors). Studies on Mesozoic and Cainozoic dinoflagellate cysts. Bulletin of the British Museum (Natural History) Geology. Supplement 3:20–27.

Williams GL, Damassa SP, Fensome RA, Guerstein GR. 2015. *Wetzeliella* and its allies – the 'hole' story: a taxonomic revision of the Paleogene dinoflagellate cyst subfamily Wetzelielloideae. Palynology. 39:289–344.

Willis KJ, McElwain JC. 2013. The evolution of plants. Second edition. Oxford University Press, Oxford, UK, 408 p.

Wilson GJ. 1971a. A chemical method for the palynological processing of Chalk. Mercian Geologist. 4:29–36.

Wilson GJ. 1971b. A method for the recovery of mounted palynological residues. Mercian Geologist. 4:139–141.

Wilson LR. 1961. Palynology as a tool for economic geology. Micropaleontology. 2:1–6.

Wilson LR. 1964. Recycling, stratigraphic leakage, and faulty techniques in palynology. Grana. 5:425–436.

Wilson LR. 1965. Stained mounting medium for palynological fossils. Oklahoma Geology Notes. 25:130–131.

Wilson LR. 1971. Note on the use of stains for colorless silicified wood. Journal of Paleontology. 45:912–913.

Wilson LR, Brokaw AL. 1937. Plant microfossils of an Iowa coal deposit. Proceedings of the Iowa Academy of Science. 44:127–130.

Wilson LR, Hoffmeister WS. 1952. Small foraminifera. The Micropaleontologist. 6:26–28.

Wilson LR, Goodman GJ. 1963. Techniques of palynology – Part I. Collection and preparation of modern spores and pollen. Oklahoma Geology Notes. 23:167–171.

Wilson LR, Goodman GJ. 1964. Techniques of palynology – Part II. Microscope-slide preparation of modern spores and pollen. *Oklahoma Geology Notes*, 24: 277–280.

Wiltshire PEJ. 1988. A simple device for obtaining contiguous peat samples of small volume for pollen analysis. Circaea. 5:97–99.

Wiltshire PEJ. 2016. Protocols for forensic palynology. Palynology. 40:4–24.

Winston ML. 1987. The Biology of the Honey Bee. Havard University Press, Cambridge, Massachusetts, USA, 294 p.

Witham HTM. 1833. The internal structure of fossil vegetables found in the Carboniferous and oolitic deposits of Great Britain. Adam and Charles Black, Edinburgh, and Longman, Rees, Orme, Grown, Green and Longman, London, 84 p.

Wodehouse RP. 1933. Tertiary Pollen II. The Oil Shales of the Eocene Green River Formation. Bulletin of the Torrey Botanical Club. 60:479–524.

Wodehouse RP. 1945. Hayfever Plants. Their appearance, distribution, time of flowering, and their role in hayfever with special reference to North America. Chronica Botanica Company, Waltham, Massachusetts, USA, 278 p.

Woessner E. 2005. Alt - Uralt - Antiquität? Der Freizeit-Mikroskopiker als Restaurator von Dauerpräparaten. Mikrokosmos. 94:215–217.

Wolfe AP, Tappert R, Muehlenbachs K, Boudreau M, McKellar RC, Basinger JF, Garrett A. 2009. A new proposal concerning the botanical origin of Baltic amber. Proceedings of the Royal Society B. 276:3403–3412.

7628
7629 Wolter M, Schill R. 1985. On acetolysis resistant structures in the Orchidaceae – why
7630 fossil record of orchid pollen is so rare. Grana. 24:139–143.
7631
7632 Wood GD. 1980. Coprolite, urolite, and "vomite". Maledicta. 4:109–115.
7633
7634 Wood GD, Gabriel AM, Lawson JC. 1996. Palynological techniques – processing and
7635 microscopy. In: Jansonius J, McGregor DC (editors). Palynology: principles and
7636 applications. American Association of Stratigraphic Palynologists Foundation, Dallas.
7637 1:29–50.
7638
7639 Wood JM, Segroves KL. 1963. Method for collecting coal and shale samples.
7640 Micropaleontology. 9:340.
7641
7642 Woods MA, Vandenbroucke TRA, Williams M, Riding JB, De Schepper S, Sabbe K.
7643 2014. Complex response of dinoflagellate cyst distribution patterns to cooler early
7644 Oligocene oceans. Earth-Science Reviews. 138:215–230.
7645
7646 Woolsley AI. 1978. Pollen extraction for arid land sediments. Journal of Field
7647 Archaeology. 5:349–355.
7648
7649 Worobiec G. 2003. An improved technique for separation, bleaching and preparation of
7650 slides from fossil leaf compressions. Review of Palaeobotany and Palynology. 126:1–5.
7651
7652 Yule B, Roberts S, Marshall JEA, Milton JA. 1998. Quantitative spore colour
7653 measurement using colour image analysis. Organic Geochemistry. 28:139–149.
7654
7655 Yule BL, Roberts S, Marshall JEA. 2000. The thermal evolution of sporopollenin.
7656 Organic Geochemistry. 31:857–870.
7657

Zabenskie S, Peros M, Gajewski K. 2006. The use of heavy-liquid in the separation of pollen from Arctic lake sediments. Canadian Association of Palynologists Newsletter. 29.2:5–7.

Zander E. 1935. I. Pollengestaltung und Herkunftsbestimmung bei Blütenhonig. Reichsfachgruppe Imker, Berlin und Liepzig.

Zander E. 1937. II. Pollengestaltung und Herkunftsbestimmung bei Blütenhonig. Reichsfachgruppe Imker, Berlin und Liepzig.

Zander E. 1941. III. Pollengestaltung und Herkunftsbestimmung bei Blütenhonig. Reichsfachgruppe Imker, Berlin und Liepzig.

Zander RH. 1997. On mounting delicate bryophytes in glycerol. Bryologist. 100:380–382.

Zander RH. 2014. Four water-soluble mounting media for microslides. Phytoneuron. 32:1–4.

Zetzsche F, Kälin O. 1932a. Untersuchungen über die Membran der Sporen und Pollen VIII. Pollenin aus der Braunkohle des Geiseltales b. Halle a.S. Helvetica Chimica Acta. 15:437–464.

Zetzsche F, Kälin O. 1932b. Untersuchungen über die Membran der Sporen und Pollen VII. Eine Methode zur Isolierung des Polymerbitumens (Sporenmembranen Kutikulne, usw) aus Kohlen. Braunkohle. 20:345–351; 363–366.

Zimmerman RP, Taylor TN. 1970. Ultrastructure of Paleozoic megaspore membranes. Pollen et Spores. 12:451–468.

Zingula RP. 1968. A new breakthrough in sample washing. Journal of Paleontology. 42:1092.

Zippi PA. 1986. Vibration sieving. American Association of Stratigraphic Palynologists Newsletter. 19.1:9.

Zippi PA. 1991. SEM and light microscope mounting and specimen location technique for same-specimen study of palynological strew mounts. Micropaleontology. 37:407–413.

Appendix 1 – Summary of the palynological preparation technique based around acid digestion

This appendix aims to provide a concise guide to the preparation of palynomorphs from a typical sample of pre-Quaternary siliciclastic sedimentary rock or sediment based around the acid digestion of the mineral matrix (sections 6–10). There are, of course, alternative strategies for some of these 13 procedures which are fully detailed in the main text. If the material being prepared is Quaternary or modern, it should be acetolysed (subsection 11.3) and steps such as acid digestion, oxidation and ultrasonification may not be necessary. See also Figure 2.

PHASE 1 – SAMPLING AND PRE-PREPARATION (*ACQUIRING THE INGREDIENTS, THEN WASHING AND CHOPPING THEM*)

1. Collect clay/silt-rich, fresh (unweathered), uncontaminated samples which are well geographically and stratigraphically constrained.
2. Thoroughly clean the sample material, and carefully fragment a suitable weight of material to ~0.5–1.0 cm fragments.

PHASE 2 – MINERAL ACID DIGESTION/DEMINERALISATION (*SOAKING THE PULSES*)

3. If the sample is calcareous, carefully treat it with ~40% hydrochloric acid to dissolve the carbonate minerals; when the reaction is complete, decant wash the residue to neutrality.
4. Very carefully treat the neutralised post-hydrochloric acid residue with ~40% hydrofluoric acid to digest as much of the silicate minerals present as possible and stir at least daily; when the sediment matrix has broken down, decant wash the residue to neutrality.
5. If opaque crystals of neoformed metal flourides (typically calcium fluoride) have formed, boil the residue in hydrochloric acid.

PHASE 3 – CONCENTRATION OF PALYNOMORPHS
(*CULINARY ALCHEMY*)

This phase aims to remove as much extraneous material as possible in order to provide a final residue which is as concentrated in palynomorphs as possible; for most samples, not all of these procedures will be necessary.

6. Should the post hydrofluoric acid residue be rich in small fragments of clay, the residue can be treated with a deflocculant such as sodium hexametaphosphate then removed, for example, by sieving; this step can also be executed prior to hydrofluoric acid treatment.
7. Extraneous organic material such as amorphous matter and vitrinite, if present, is removed by careful, controlled oxidation with, for example, nitric acid; the humic substances produced by oxidation are then removed by treatment with a dilute alkaline solution.
8. Residual mineral grains and heavy fragments of woody material are much denser than palynomorphs; these fractions are normally separated by centrifugation using a heavy liquid or swirling in a large watch glass.
9. If any amorphous organic material is still present, it can be broken up by subjecting the aqueous residue to brief ultrasonic treatment then sieving the small fragments away.

10. Finally both coarse and fine extraneous materials can be separated from the palynomorphs by sieving, then the palynomorphs are concentrated into a small volume (10–15 ml) of aqueous residue.

PHASE 4 – MICROSCOPE SLIDE PRODUCTION AND SAMPLE MATERIAL ARCHIVING
(*PLATING UP AND REPLACING THE INGREDIENTS IN THE LARDER*)

11. If the palynomorphs are pale, they can be darkened by staining with a proprietary dye or stain such as Safranin O.
12. Microscope slides are produced; typically these are strew mounts. Several droplets of aqueous residue are either are mixed with an embedding medium and placed onto a coverslip or simply evaporated directly onto a coverslip. When dry, the coverslip is carefully inverted onto a small amount of mounting medium on a microscope slide. Alternatively some residue is premixed with a mounting medium and this is mounted. It is possible to pick out selected specimens and mount them separately on single (or multiple) grain slides.
13. The unused aqueous residue should be carefully archived in small vials with some preservative to prevent fungal growth.

Appendix 2 – Summary of an effective non-acid palynological preparation technique

The preparation of palynomorphs not using mineral acid digestion is fully discussed in section 12. There is no concensus on the most effective protocol for this. In this Appendix, the method described by Riding and Kyffin-Hughes (2004; 2006; 2011) is adapted and summarised. Note that this protocol is flexible, for example pre-treatment may not be necessary, especially if the sample is urgent. Steps 1 and 2 are best undertaken using a hotplate with a magnetic stirrer. See also Figure 48.

1. Place ~50–100 g of clean, fragmented sample material into a beaker and pre-treat, for example with white spirit (Riding and Kyffin-Hughes 2010), or mix with ~500 ml of warm water and detergent, stir thoroughly and leave overnight; the beaker may be heated or simmer-boiled on a hotplate

2. After decanting the clear supernatant, add ~500 ml of warm water and a small amount (~5 g) of sodium hexametaphosphate flakes to the sample material and agitate the mixture, which can be heated or simmer-boiled on a hotplate, for ~20–40 minutes
3. Sieve off any coarse (>500 μm) material and retain; this may be broken down using hydrogen peroxide if desired as described by Riding and Kyffin-Hughes (2004) and Riding et al. (2007a)
4. Sieve the mixture using 10 μm or 15 μm cloth (e.g. polyester) mesh to remove the <10 μm deflocculated clay particles; this step may be somewhat time-consuming and, if the potential loss of small palynomorphs is unimportant (e.g. in a real time drilling scenario), 20 μm mesh can be used.
5. Subject the palynomorph-rich residue to oxidation, density separation, ultrasonic treatment and final sieving as necessary
6. Mount the palynomorphs on microscope slides as described in subsection 10.3 and Appendix 1

Display material captions:

Figure 1. A photograph of the Applied Science Building of the University of Sheffield on Mappin Street directly opposite St George's Church taken in 1975. The Department of Geology was housed here on the first and second floors to the left of the main entrance. Part of the Department moved to the main campus on Brookhill in 1978, with the remainder, including palynology, relocating in 1996. The palynology laboratory was located in the northwest corner of the first floor. This building is now called the Sir Frederick Mappin Building. This image was taken by Roy Starkey and is used with permission.

Figure 2. A summary of the traditional, mineral acid-based palynological preparation technique for typical pre-Quaternary siliciclastic sedimentary rocks and sediments presented as a flowchart. The left-hand column illustrates the four phases (or procedures)

of this technique, with their respective culinary analogies, as described in sections 6 to 10 herein. The right-hand column lists the 13 main stages in chronological order. This scheme of stages should not be viewed as inflexible, for example if a sample is devoid of carbonate minerals then hydrochloric acid treatment (3) is uneccessary. This scenario is, however, very unusual. Likewise, the majority of samples require oxidation and alkali treatment (7) and density separation (8). By contrast, the four stages which are only undertaken where necessary (5, 6, 9 and 11) are asterisked. See also Appendix 1.

Figure 3. A low magnification image of an assemblage of kerogen macerals with a 200 µm scale bar. Note the prominent elongate subangular fragment of brown wood tissue in the centre, the abundant pale amorphous organic material and the small dark pieces of dark woody material. This residue has not been oxidised in order to accurately assess the relative proportions of the various kerogen types. This is BGS sample SSK 46363 from 55.46 m depth in the Carsington Dam Reconstruction C3 borehole, drilled northeast of Ashbourne, Derbyshire, UK (Hennissen et al. 2017). This horizon is within the Morridge Formation (Serpukhovian/Arnsbergian, Upper Mississippian). Photograph taken by Jan A.I. Hennissen and is reproduced with permission.

Figure 4. The stratigraphical extents of the six principal palynomorph groups. The relative widths of the lines indicate major trends in taxonomic diversity. These diversity variations are strictly indicative; the breadths of the lines are not precisely calibrated to absolute numbers of taxa. Dashed lines indicate that the respective palynomorph group is relatively sparse. Relatively minor palynomorph groups such as fungal spores, microforaminiferal linings, prasinophytes and scolecodonts are not included here. The ranges and diversity trends are taken from key papers such as Millay and Taylor (1976), MacRae et al. (1996) and Grahn and Paris (2011).

Figure 5. Photographs of modern palynology laboratories from three world-leading centres in the subject.
A – The palynology laboratory at the Center for Excellence in Palynology (CENEX) Louisiana State University, Baton Rouge, Louisiana, USA. Note the fume hood on the

back wall with a safety shower, PPE and a fire extinguisher to the right. There is ample table space for non-hazardous procedures such as swirling and staining, in addition to space for laying out samples. Photograph taken by Allison K. Barbato, and reproduced with permission.
B – The palynology laboratory at the University of Sheffield, UK. Note the cluster of three fume hoods, the neat general layout, the prominent signage ('Acid' etc.) and the careful record keeping on the left. Image reproduced with the permission of Charles Wellman and David Bodman (University of Sheffield).
C – Part of the laboratory of the Palynology section in the Geolab, Geosciences faculty, Utrecht University, The Netherlands. Note the good lighting and the clean, spacious benches. The white stand with steeply-angled blue plastic rods for storing beakers (to the right of the sink) keeps these vessels inverted hence they are unlikely to accumulate airbourne contaminants. The apparatus connected to the ceiling in the bottom right is an adjustable ventilator which can be used whenever there is a need to locally extract chemical fumes when, for example, making up microscope slides. Photograph taken by Peter Bijl and reproduced with permission.
D – A more general view of the laboratory of the Palynology section in the Geolab, Geosciences faculty, Utrecht University, The Netherlands. Note the bank of three sinks in the centre of the image and the clean, wide benches with adjustable ventilators adjacent to the windows to the left. Photograph taken by Peter Bijl and reproduced with permission.

Figure 6. The safety shower in the British Geological Survey palynology laboratory. This simple pipe and shower head operated by a pull-chain is capable of delivering a substantial volume of water in a short time should a person need to wash off a chemical spill. Should an inadvertent chemical spill occur anywhere in the laboratory, and someone is splashed, they only have to move the short distance to the centre of the room and pull the shower cord. Therefore the chemicals can be quickly and effectively irrigated. Photograph taken by the author.

Figure 7. One of the two main fume hoods in the British Geological Survey palynology laboratory; note the sink unit on the left. Photograph taken by the author.

Figure 8. A laboratory technician working in one of the fume hoods at the Palynology section in the Geolab, Geosciences faculty, Utrecht University, The Netherlands. Note that the sash can be raised to allow appropriate procedures to be carried out. Photograph taken by Peter Bijl and reproduced with permission.

Figure 9. A cartoon of a laboratory technician wearing all eight items of personal protective equipment (PPE) for palynology preparation, specifically the mineral acid digestion phase and oxidation.

Figure 10. The storage of clean and dry laboratory vessels such as beakers and other labware in the BGS palynology laboratory. This is a clean, dust-free cupboard with close-fitting doors to prevent the ingress of airbourne contaminants. Note the beakers are inverted to ensure that they are not contaminated. Photograph taken by the author.

Figure 11. Two palynology samples in strong, sealable plastic bags which have been labelled. Note the sample details are written both on the bag and on a card inside the bag. On the left, the sample has been wrapped in aluminium foil for extra protection. Plastic wrap or bubble-wrap can also be used for this purpose. In the sample on the right, the sample material has simply been placed in the bag. Photograph taken by Simon Harris (BGS).

Figure 12. Removing the weathered outer layer of rock ('cleaning the section') using a trowel and a spade, and preparing for graphic logging and sample collection in order to be able to procure fresh, unweathered material. This succession is the Mercia Mudstone Group (Triassic) [aka the Bees Nest Member of the Brassington Formation] at Bees Nest Pit, Brassington, Derbyshire, UK (NGR SK 24115 54580). Photograph by Peter F. Jones and used with permission.

Figure 13. Cleaning a succession of unconsolidated sediments with a spade and trowel in preparation for graphic logging, photography and sampling. Ryder Point Quarry, near

Brassington, Derbyshire, UK (NGR SK 25501 54916). The succession exposed is highly weathered Sherwood Sandstone Group (Lower–Middle Triassic) [aka the Kirkham Member of the Brassington Formation]. Photograph taken by Peter F. Jones and reproduced with permission.

Figure 14. Sample cavities in two cleaned sections from where palynomorph samples have been collected, with the full sample bags in them. This strategy acts as a reference in case the sample notes are compromised or lost.
A – One sample hole in a cleaned section at Ryder Point Quarry, near Brassington, Derbyshire, UK (NGR SK 25501 54916). Spade, geological hammer and trowel for scale. Photograph taken by Peter F. Jones and reproduced with permission.
B – A trench sample transect in the Brassington Formation (Miocene) at Kenslow Top Pit, near Friden, Derbyshire (NGR SK 18287 61411) with five sample bags *in situ* at their respective horizons. Photograph taken by the author.

Figure 15. A highly simplified diagram of a drilling rig with a destructive percussion rotary drill bit which produces drill cuttings as it penetrates the rock succession. The drill cuttings are brought to the surface by the circulating drilling mud and sieved out by the shale shaker. At the start of every borehole operation fresh drilling mud is mixed at the rigsite in the mud pit. As drilling begins, drilling mud is injected down the centre of the drill pipe (string) and recirculates back to the surface in the annulus, i.e. the space between the drill string and the well. Caving potentially occurs in the lower (uncased) part of the well indicated by the vertical arrow to the right of the borehole. This is the phenomenon whereby friable horizons slough off the wall of the borehole above the drill bit and hence contaminate the drill cuttings.

Figure 16. A cartoon of the distal part of the drill string in a borehole illustrating the destructive percussion drill bit. Recirculated drilling mud is pumped down the hollow centre of the steel drill pipe. It lubricates and cools the drill bit and circulates back up to the surface in the annulus. This upward flow of drilling mud brings to the surface cuttings from the bit/rock interface together with caved fragments of wall rock from higher in the

succession. The latter are contaminants and all these rock fragments are screened out of the drilling mud by the shale shaker.

Figure 17. Cleaned and dried drill cuttings. On the left is a glass tube of cuttings, and on the right another sample has been poured into a small metal tray. Note the imperfect size sorting, and the clear mixing of lithotypes which indicates that some caving has occurred. The scale bar on the left represents one centimetre. The well concerned is a relatively old one. Samples of drill cuttings have, for some time, been stored in robust paper envelopes with stout wire closures. Photograph by Simon Harris (BGS).

Figure 18. A Kubiena tin with the detachable base in place, and the lid removed, with dimensions. The tin, made from sheet aluminium, is hammered into position into a succession of unconsolidated sediments in order to obtain an undisturbed sample, then carefully removed. This image was supplied by Thin Section and Micromorphology at the University of Stirling (http://www.thin.stir.ac.uk/consultancy/) and is reproduced with permission.

Figure 19. A Cour Trap for the continuous sampling of airbourne pollen and spores. Note the two vertical mesh filters which have been impregnated by an adhesive to trap the pollen and spores on the right. The wind vane on the left keeps the filters facing directly into the oncoming wind. The image was supplied by Ghania Kiared, and is used with permission.

Figure 20. A simple plankton net. The net is pulled through the water manually using a rope, or is towed behind a boat to collect a representative sample of plankton. The organisms are concentrated into the white plastic vessel at the narrow distal (or cod) end. This image was supplpied by Aquatic Research Instruments, Hope, Idaho, USA and is reproduced with their kind permission.

Figure 21. The author using a plankton net to attempt to collect modern plankton including living dinoflagellates from a small lake in Dhahran, eastern Saudi Arabia

during January 2020. An abundant and diverse assemblage was collected, which included occasional thecae of the dinoflagellate species *Ceratium hirundinella* (Müller 1773) Dujardin 1841. Photograph taken by Patrice Brenac and reproduced with permission.

Figure 22. Crushing a palynology sample to 'pea-size fragments' (i.e. ~0.5–1.0 cm^3) between two sheets of pristine heavy duty aluminium foil, to avoid contamination, using a geological hammer. The bench top is thoroughly cleaned prior to crushing every sample, and the lowermost sheet of aluminium foil is placed on a thick metal plate to provide a solid base. Photograph by the author.

Figure 23. Various aspects of the hydrochloric acid digestion procedure. All photographs taken by the author.
A – testing a sample for calcite and other carbonate minerals by carefully dropping some hydrochloric acid onto it; note the vigourous effervescence (bubbles of carbon dioxide).
B – carefully adding concentrated hydrochloric acid to a sample in a beaker using a liquid bottle-top liquid dispenser in a fume hood.
C – the resulting effervescence can be vigourous, and can easily flow out the sample vessel. Note that the beaker is placed in a plastic washing up bowl to protect the fume hood from spillages.
D – excessive effervescence can be suppressed by carefully using a narrow jet of acetone, ethanol or water (or suitable mixtures thereof) from a plastic mister-bottle or wash-bottle.
E – a sample treated with hydrochloric acid where the dissolution of carbonate minerals is complete, and the insoluble residue has settled to the bottom of the beaker. The supernatant is clear and light yellow in colour. Note the square of polyester sieve cloth which prevents the ingress of contaminants, but does not allow pressure to build up in the vessel.
F – carefully decanting off the supernatant in order to neutralise the sample residue. Specifically, the spent hydrochloric acid liquor is carefully and slowly poured away from the clay-rich 'sludge' at the bottom of the beaker. Great care must be taken to avoid losing any of the solids, which will contain palynomorphs. The beaker is then refilled with water; this procedure is termed decant-washing. This will need to be undertaken

several times before the pH is increased to seven. The liquid in the sink is a solution of sodium carbonate which immediately neutralises the acidic liquor, hence the slight effervescence.

Figure 24. Four aspects of the hydrofluoric acid digestion procedure. Note the use of full PPE for this procedure. All photographs taken by the author.
A – carefully adding hydrofluoric acid to a sample residue in a plastic beaker from a smaller beaker which has been calibrated to measure specific volumes of hydrofluoric acid in a fume hood. In this case, 30 ml of hydrofluoric acid is being added.
B – stirring the hydrofluoric acid using a plastic stirring rod to stimulate the dissolution of silicate minerals; this agitation of the mixture should be undertaken at least daily during this treatment. The squares of polyester sieve cloth placed firmly over the tops of the beakers prevents the ingress of contaminants, but does not allow pressure to build up.
C – very carefully decanting off the clear supernatant in order to neutralise the post hydrofluoric acid digestion residue. Specifically, the spent acidic liquor is cautiously and slowly poured away from the dark 'sludge' at the bottom of the beaker. Great care must be taken to avoid losing any of the solids, which will contain palynomorphs. The beaker is then refilled with water; this procedure is termed decant-washing; this will need to be undertaken at least six times before neutrality is achieved.
D – a sample treated with hydrofluoric acid where the dissolution of silicate minerals is complete and the insoluble residue has been decant-washed five times (see the five 'tick-counts' to the right of the 300 ml mark). Note the dark settled-out insoluble residue and the relatively clear supernatant.

Figure 25. The oxidation procedure. Here a neutralised, post hydrofluoric acid organic residue is being treated with concentrated nitric acid in a beaker in a fume hood. Note how the liquid is dark brown in colour due to the humic acids produced during the oxidation reaction. Photograph taken by the author.

Figure 26. A line drawing to illustrate the use of a Büchner funnel fitted with a sinter glass disk (porosity 2) housed in a Büchner flask with a rubber bung in order to oxidise

an aqueous palynomorph residue. This apparatus can also be used for any filtration in palynological processing. The reversible aspirating rubber bulb (or 'hand bellows') allows the reaction to be stimulated or stopped by respectively increasing or decreasing the internal air pressure in the Büchner flask. In this example the pressure is being reduced hence the drops of filtrate below the funnel.

Figure 27. A laboratory centrifuge unit with swinging bucket rotors in use for density separation in a palynology laboratory. The lid is open to show the housings for multiple centrifuge tubes. The tubes in the unit are filled with a mixture of the palynomorph residue and the heavy or light liquid as appropriate. It is essential that the centrifuge is balanced. This means that all the housings are full, or the housings are symmetrical in terms of loading in both 'north-south' and 'west-east' directions. If the centrifuge is operated when the tube housings are unbalanced (in an extreme example, all at one side), the unit will not operate properly. The unit may vibrate excessively and/or the tubes may break. The operating instructions pertaining to centrifuges must be rigidly adhered to in order to avoid problems such as these. If only one tube needs centrifugation, a 'dummy' tube filled with an identical amount of the same liquid is placed in the opposite tube housing. Both photographs were supplied by Jen O'Keefe and are reproduced with permission.
A – a general view of the open centrifuge unit with 10 tubes in a balanced arrangement. The settings on the front panel indicate the speed and duration of centrifugation, and acceleration/decelaration.
B – a closeup of the open centrifuge unit with thirty 15 ml tubes in a balanced arrangement.

Figure 28. Two palynomorph residues in tubes housed in a plastic rack following centrifugation. Note the very dark brown organic-rich 'float' which contains the palynomorphs at the top of the tubes, the clear liquid in the centre and the dense, mineral grain-rich 'sink' at the the base of the tubes. The organic fraction can be separated either using a pipette or carefully poured off. The photograph was supplied by Jen O'Keefe and is reproduced with permission.

Figure 29. Various aspects of the swirling procedure to separate palynomorphs from the dense fraction. All photographs taken by the author

A – a watch glass, 20 cm in diameter, with a mixture of the organic residue and reverse osmosis (RO) water prior to swirling. Note the dark residue in the centre.

B – gently rocking the watch glass with both hands in order to agitate the residue. The resultant slow circular motion suspends the palynomorphs in the water column in the centre of the watch glass and the dark, dense, extraneous materials sink.

C – separating the palynomorphs from the heavy fraction. Here the watch glass is slightly tilted and the plume of water containing the suspended palynomorphs in the centre (i.e. above the settled, dense fraction) is pipetted off. This procedure may have to be repeated to effect full separation of the two fractions. An alternative to this strategy is to pipette off the heavy material at the base, thereby leaving the palynomorphs in suspension.

D – another method to separate the palynomorphs. Here the plume of water in the centre of the watch glass containing the palynomorphs is carefully poured off into a small beaker, leaving a tear-shaped slick of heavy material behind in the watch glass.

Figure 30. An aqueous organic residue in a small beaker undergoing ultrasonification in a water-filled ultrasonic cleaning unit. Note the metal basket which holds the receptacle in the water bath. Photograph taken by the author.

Figure 31. A simple two-piece plastic sieve which, when one part is placed inside the other, tightly house a square piece of nylon or polyester mesh. It is used to sieve palynomorph residues to remove the extraneous fine materials. Photographs taken by the author.

A - The three component pieces of this sieve. It comprises two tightly interlocking pieces of carefully chamfered (smoothed) plastic pipe (above), which house the square of fabric mesh (below). Pen for scale.

B – The sieve in A when assembled; it is 11 cm in diameter.

Figure 32. A plastic sieve with nylon mesh for fine sieving in use. This step aims to sieve away the fine fraction from the palynomorph concentrate. Both photographs taken by the author.
A – passive sieving of the residue with the filled plastic sieve placed onto a beaker of suitable size. The filtrate passes through the mesh into the beaker under the influence of gravity, and can be concentrated and checked for palynomorphs.
B – active sieving of the residue; the sieve housing is being gently shaken and tapped in order to further expedite the flow of filtrate through the sieve mesh. Note the dark filtrate, which is rich in organic fines, can be clearly seen in the beaker.

Figure 33. A typical stack of sieves in action; the residue is being sieved passively. The large watch glass at the top prevents the ingress of any airbourne contamination. Immediately below the watch glass is a brass sieve with a 250 μm mesh to remove the extraneous coarse fraction (plant debris etc.). The plastic/nylon sieve above the beaker removes the fine fraction. This stack has sieved almost one litre of aqueous residue; the sieves are topped up several times when the plastic sieve has drained. Photograph taken by the author.

Figure 34. Seven sieves with metal screens with a mesh size of 60–250 μm which are used to remove coarse extraneous debris such as mineral grains and plant materials from the palynomorph concentrate, or to concentrate large palynomorphs such as chitinozoa and megaspores. The large sieve in the front centre is 21 cm in diameter. The remaining smaller sieves are 11 cm in diameter. Photograph taken by the author.

Figure 35. Staining a palynomorph residue. A split of the residue is in the small beaker on the bench top, and a one or two drops of a very dilute aqueous solution of Safranin O dye are added. The mixture is left for around one minute, then the excess stain is sieve-washed away and the residue thoroughly cleaned of the dye. Note the lurid red colour of the Safranin O solution in both beakers. Photograph taken by the author.

Figure 36. Four microscope slides with palynomorphs mounted on them. All the slides are 75 x 25 mm in size. Photographs all by Simon Harris (BGS).
A – a strew mount with a paper label; the sample mumber is MPA 28390.
B – a strew mount with the sample number (MPA 64602) written in indelible black ink on a portion of the slide which is etched glass.
C – a strew mount with two coverslips. The uppermost coverslip is oxidised and sieved residue and the lowermost coverslip is raw (i.e. oxidised and unsieved) kerogen. The sample number is MPZ 5309.
D - a single grain mount of the Carboniferous miospore *Raistrickia nigra* Love 1960. This mount is part of the 'Bernard Owens Collection' (Stephenson and Owens 2006).

Figure 37. Six vertical section line drawings illustrating the detailed configurations of permanent palynomorph microscope slides. The individual drawings are lateral cross sections of microscope slides. Note that the drawings are for illustrative purposes only and the dimensions, especially the thicknesses of the horizontal layers, are emphatically not to scale. The abbreviations are: CS – coverslip; CSS – coverslip sealant; MM – mounting medium; and MS – microscope slide. The palynomorphs are depicted by the small dots.
A – the microscope slide, silicone oil and palynomorph concentrate mixture with spacers, the coverslip and the coverslip sealant (typically clear varnish). The robust spacers prevent any crushing or distortion of the palynomorphs by the weight of the coverslip. Frequently smaller, circular coverslips are used in these mounts.
B – the microscope slide (MS), mounting medium (MM), embedding medium with the palynomorphs within it and coverslip (CS). The palynomorphs are the line of dots immediately beneath the coverslip. Several drops of the aqueous palynomorph concentrate are mixed with an embedding medium such as polyvinyl alcohol and left to dry. The polyvinyl alcohol coats (embeds) the palynomorphs which settle by gravity to the coverslip/liquid interface so are all in a single optical plane.
C – the microscope slide (MS), cured mounting medium and palynomorph concentrate mixture and coverslip (CS). Note that the line of palynomorphs (dots) at the base of the mixture in a single optical plane.

D – the microscope slide (MS), mounting medium (MM), layer of palynomorphs adhering to the surface of the coverslip in one optical lane and coverslip (CS). Here an embedding medium has not been used; several drops of the aqueous palynomorph concentrate are placed onto the coverslip and simply left to evaporate.
E – a single grain mount. This is where a single palynomorph is placed in a small fragment of glycerine jelly on a hotplate. When the glycerine jelly has cured, a small circular coverslip is placed on top, and the void surrounding the glycerine jelly filled using molten paraffin wax. When the wax has entirely solidified, the coverslip is sealed, usually with clear varnish.
F – a multiple grain mount. This type of mount is identical to the single grain mount (E), except that the fragment of glycerine jelly contains several palynomorphs.

Figure 38. The storage of permanent palynomorph slides horizontally in a large bespoke darkwood microscope slide cabinet with multiple drawers. This example houses part of the palynomorph type and figured slide collection of the British Geological Survey. Photographs taken by the author.
A – the cabinet with the doors closed to occlude dust and light.
B – the cabinet with its doors open. Note that there are four banks of slide drawers and two of these have been opened, exposing the rows of palynology slides in each one.
C – a closeup image of the cabinet with two drawers open.
D – a closeup image of a single slide drawer; note the neat serial numbering of the drawers.

Figure 39. Part of a microscope slide made using glycerine jelly which has undergone dessication. The drying out of the glycerine jelly causes these prominent, irregularly branching bubbles to form which can obscure, or partially occlude, the palynomorphs. Unsurprisingly, the bubbles tend to affect the pure glycerine jelly and not objects such as palynomorphs. This deterioration commences at the periphery of the coverslip and moves into the centre of the slide. The chorate (spinose) dinoflagellate cyst in the centre is *Diphyes colligerum* (Deflandre & Cookson 1955) Cookson 1965 and is 69 μm in maximum length including the processes. Note how most of the specimen remains visible

despite the dessication around it. BGS specimen registration number PK 44, from the Paleogene of the Hadleigh Borehole, Suffolk, southern England. Image taken using differential interference contrast; photograph by the author.

Figure 40. The production of palynomorph strew mounts. In this example, the palynomorph residue is mixed with a dispersant/embedding medium before being pipetted onto the coverslip. Note that producing a high quality strew slide demands substantial dexterity, a steady hand, considerable patience and practice. All photographs taken by the author.
A – mixing a small amount of dispersant/embedding medium, in this case polyvinyl chloride (the clear liquid), with a mixture of one or two drops of palynomorph concentrate and reverse osmosis (RO) water (the dark liquid).
B – carefully pipetting the mixture of palynomorph concentrate and dispersant/embedding medium onto a new coverslip on a hotplate. When the coverslip has been covered in sufficient of this mixture to produce the desired density of palynomorphs, it is normally left to evaporate naturally so that the palynomorphs will settle onto a single optical plane. However, if the slide is required urgently, evaporation can be speeded up by switching on the hotplate. Note the labelled slide behind the coverslip; this ensures that the correct coverslip is attached to the appropriate slide.
C – carefully pipetting uncured mounting medium, in this case Elvacite, onto a new microscope slide so that the dried coverslip (seen on the left) can be attached. It is important to cover an area slightly smaller than the coverslip to be used so that the mountant will not be too thick. The mountant should be pipetted systematically; here the technician is working in an anticlockwise direction.
D - completing the strew mount production step. One of the narrow edges of the dried coverslip is placed adjacent to the uncured mountant on the slide, then it is carefully and gently lowered onto the mountant using a scalpel blade. This procedure in particular needs considerable dexterity in order to avoid or minimise the incorporation of air bubbles between the coverslip and mountant. Any air bubbles that form can be eliminated by gentle pressure onto the coverslip applied using, for example, a wooden toothpick before the mountant cures.

Figure 41. A selection of slide storage boxes of various capacities. These items are constructed from plastic or wood, and have slots for storing microscope slides and space for inserting sample details. They are ideal for slide storage in remote locations such as at rigsite, and are also useful for sending slides through the post. If they are stacked vertically, the slides are in the horizontal position. All the boxes illustrated here are ~3.3 cm deep. Pen and slides for scale. All photographs taken by the author.
A – a relatively small capacity plastic one-row slide box 14 cm long and 8.5 cm wide which can store up to 25 slides.
B – a wooden one-row slide box 28 cm long and 10.5 cm wide which can store up to 50 slides.
C – a relatively large capacity plastic two-row slide box 25.5 cm long and 17.5 cm wide which can store up to 100 slides.
D – six large capacity plastic two row slide boxes (as in C) stored vertically in the micropalaeontology microscope facility of the British Geological Survey. This method, for example, enables a practitioner to temporarily store several hundreds of microscope slides close to the microscope during the completion of a major project.

Figure 42. The storage of permanent palynomorph slides vertically. An example of a large bank of wood index card cabinets used to store palynomorph microscope slides acommodated in thin metal metal slide holders (Figure 43). This forms part of the palynology collections of the British Geological Survey. Photograph taken by the author.

Figure 43. The storage of permanent palynomorph slides vertically 2. Four palynomorph microscope slides housed in a thin metal slide holder (above) and an empty slide holder (below). Photograph by Simon Harris (BGS).

Figure 44. A glass vial containing an aqueous palynomorph residue in readiness for archiving. Note the snap-on plastic cap and that the palynomorph residue has settled to the bottom of the vessel. Photograph taken by the author.

Figure 45. A flowchart summarising the 10 potential steps involved in the preparation of Quaternary and modern sedimentary rock and sediments. This protocol is very flexible and not all the steps except both alkali treatments, acetolysis, filtering and microscope slide production are universally required. The other steps depend on the nature of the material being prepared.

Figure 46. A class of students at CENEX, Department of Geology and Geophysics, Louisiana State University (LSU) mechanically extracting pollen from modern plant samples directly from the anthers to microscope slides; this method enables reference slides to be produced rapidly. Image provided by Sophie Warny (LSU).

Figure 47. A flowchart summarising eight steps in the preparation of pollen and spores from modern plant material.

Figure 48. A flowchart summarising the non-acid preparation technique using sodium hexametaphosphate originally published by Riding and Kyffin-Hughes (2004) in six steps. Note that step 1, pre-treatment, is desireable but not essential. See also Appendix 2.

Figure 49. The sodium hexametaphosphate (non-acid) preparation technique of Riding and Kyffin-Hughes (2004) in action. Photographs taken by the author.
A - the sample material has been pre-treated with detergent and warm water in a large beaker overnight (Appendix 2). In this photograph, a small amount (~5 g) of sodium hexametaphosphate flakes which have been ground to powder in a pestle and mortar are being added to the sample.
B - The mixture is being stirred. The sodium hexametaphosphate deflocculates the clay fraction, and this should normally be complete after 15–20 minutes. This procedure can be done on a hotplate to speed up the deflocculation. Next the deflocculated residue will be sieved to remove the small clay particles using a sieve with a 10 μm, 15 μm or 20 μm nylon or polyester cloth mesh.

Figure 50. A flowchart depicting the preparation of palynomorphs from carbon-rich sediments, i.e. peat and coal. This assumes that the materials are highly carbonaceous and mineral-free. For the oxidation of coals, the strength of the oxidant and the rank of the coal are directly proportional. This means that concentrated nitric acid, Schulze's solution and fuming nitric acid are normally suitable for lignite, bituminous coal and anthracite respectively. However, this should not be regarded as a fixed rule; the best oxidant for any given coal should be determined using trial and error, sample-by-sample. Note that, if pollen and spores are not effectively released after one phase of acetolysis (peat) or one phase of oxidation and alkali treatment (coal), these steps should be repeated as necessary. Abbreviation: bit. coal = bituminous coal.

Figure 51. A scanning electron microscope image of a specimen of the Silurian (Llandovery/Wenlock) chitinozoan *Angochitina longicollis* Eisenack 1959 from the Visby Formation of the Lusklint 1 section, Gotland, eastern Sweden (Vandenbroucke et al. 2013, fig. 5F). The specimen is 225 μm in length. Imaged by Thijs R.A. Vandenbroucke and reproduced with permission.

Figure 52. A scanning electron microscope image of two specimens of the Pennsylvanian megaspore *Valvisisporites auritus* (Zerndt 1930) Potonié & Kremp 1956. Note the substantial intraspecific morphological variability. This image was taken by Wilson A. Taylor and is reproduced with permission.

Figure 53. The Maceration Tank, an automated closed vessel device for undertaking batches of hydrofluoric acid macerations. It was developed during the 1980s at the Geological Survey of Denmark and Greenland (GEUS), Copenhagen, Denmark (Poulsen et al. 1990). In this photograph it is being operated by Yvonne Husfeldt. Photograph kindly supplied by Karen Dybkjær of GEUS, and used with permission.

Figure 54. A bottle of 250 *Lycopodium clavatum* L. tablets used as exotic markers for determining the concentration of palynomorphs. Photograph taken by the author.

Figure 55. A Scanning Electron Microscope (SEM). This is one of the SEM units used by the British Geological Survey. The main unit is on the right and includes the sample chamber at the base below the cylindrical vacuum chamber which houses the electron gun. This is BGS image P875917 and is used with permission.

Figure 56. An aluminium stub for use with the Scanning Electron Microscope. The palynomorphs are mounted on the flat top of the stub, which is 12.5 mm in diameter. They can either be single specimens attached to the stub using adhesive, or as strew mounts on circular coverslips which are glued to the stub. Photograph by Simon Harris (BGS).

Figure 57. A specimen of the dinoflagellate cyst genus *Stenodinium* in dorsal view imaged using the scanning electron microscope (SEM). This genus has a distinct internal structure, and is part of the Paleogene subfamily Wetzelielloideae which was recently revised by Williams et al. (2015). Specifically, *Stenodinium* is a cavate genus and the two cyst layers, the inner endophragm and the outer periphragm, are separated by a prominent cavity or pericoel (Williams et al. 2015, pl. 2.13, 14). Note that in the SEM image here only the outer periphragm and its spines are visible, except through the archaeopyle, an excystment opening, clearly seen in the upper-mid-centre of the specimen. The polygonal opening formed by the detachment of an intercalary plate provides a window through which the apical portion of the endocyst can be seen. If the archaeopyle on this specimen had not operated, the inner body of the cyst would not be discernible. This specimen, which is ~150 μm long, is likely from the Lower Eocene of offshore eastern Canada (Williams et al. 2015, p. 344) and is reproduced with permission.

Figure 58. Recording the locations of palynomorph specimens on microscope slides using permanent ink rings. The rings should be inked on both sides of the slide in case some of the ink is rubbed off. Photographs by Simon Harris (BGS).
A – a microscope slide with a single ringed specimen. The British Geological Survey (BGS) figured specimen number is MPK 5190 and the BGS sample number is MPA 12423.

B – a microscope slide with nine ringed specimens. If too many rings are inked onto a slide, it can be somewhat confusing to use, and many palynomorphs will be obscured by the ink. However, in this case all nine specimen are clearly marked. The figured specimen numbers are non-sequential between MPK 5083 and MPK 5098; the sample number is MPA 13736.
C – A card map of the microscope slide in B (MPA 13736/1). This guide helps the user to quickly locate the specimen of interest, and obviates the need to write the specimen numbers anywhere on the slide.

Figure 59. The most extensively used gridded calibrated reference slide used to record the location of a palynomorph on a slide, the England Finder. This reference slide is the same size as a regular microscope slide, i.e. 2.7 x 7.6 cm. Photographs by the author.
A – photograph of the England Finder under the microscope at low magnification. Note the three rows of cells or squares, i.e. S59–S61 to U59–U61.
B – photograph of the England Finder under the microscope at a higher magnification than in A. The image is centered on the T60 cell/square of the England Finder. Note the five sectors of each cell/square, comprising a central circle and four surrounding subtriangular areas numbered 1–4. The overall height and width of one entire cell/square is 850 μm.

Table 1. The ideal sample weights in grams for eleven of the major types of sedimentary rock. Note that the mass of sample that is needed is inversely proportional to the level of clay/silt-sized particles present.

Table 2. The refractive indices of nine embedding and mounting media, plus five related materals arranged from low to high. The related materals are air/vacuum, borosilicate glass, pure glass, acetolysed or fossil sporopollenin and fresh sporopollenin. The refractive index of a vacuum is 1.00 by definition. STP = standard temperature and pressure.

Plate 1. Four selected very rich and well-preserved palynomorph assemblages which were all prepared using acid digestion, photographed at low magnification in normal (brightfield) illumination (1 and 2) and differential interference contrast (3 and 4). Asemblages 1 and 2 have not been stained, however 3 and 4 were stained using Safranin O.

1. An assemblage of Silurian acritarchs from Wales, UK, largely the acanthomorph (spine-bearing) genus *Micrhystridium*. Note the very dark body colour of the acritarchs caused by relatively high levels of thermal maturity. The vesicle (body) of the spinose acritarch in the bottom left corner is 24 μm in diameter.
2. A sample of Middle Jurassic (Aalenian) pollen and spores from North Yorkshire, northern England dominated by the smooth plant spore genus *Cyathidites*. The association represents a damp, low-lying terrestrial depositional environment. The well-preserved spore in the top centre is 44 μm in diameter.
3. An association of Upper Cretaceous (Maastrichtian) dinoflagellate cysts dominated by process-bearing (chorate) taxa from ENCI Quarry, near Maastricht, southeast Netherlands. This slide was prepared by Graeme J. Wilson using the techniques outlined in Wilson (1971a). The cyst body of the large, thick-walled specimen in the centre right is 82μm in diameter. Note the differential takeup of the stain by the dinoflagellate cysts.
4. A shallow subsurface sample from the North Sea extremely rich in Quaternary dinoflagellate cysts, dominantly the spinose species *Operculodinium centrocarpum* (Deflandre & Cookson 1955) Wall 1967. Note that this residue has been stained using Safranin O. The cyst body of the specimen in the centre of this field of view is 38 μm in diameter.

Plate 2. A representative selection of nine specimens of terrestrially-derived palynomorphs to demonstrate some of the biological, ecological and morphological diversity of pollen and spores. Photographs 1 to 6 inclusive and 9 were taken using differential interference contrast. Photographs 7 and 8 were taken using the scanning electron microscope.

1. The cryptogam spore *Concavissimisporites* sp. BGS specimen MPK 14717 from the lowermost Cretaceous (Berriasian) of the Wealden Group of southeast England. The maximum overall diameter is 62 μm.
2. The modern moss spore *Sphagnum* sp. from a reference collection of modern pollen and spores held by the British Geological Survey. The maximum overall diameter is 38 μm.
3. The gymnosperm pollen *Callialasporites trilobatus* (Balme 1957) Sukh Dev 1961. BGS specimen MPK 14718 from the Middle Jurassic Brent Group of the northern North Sea. The maximum overall diameter is 51 μm.
4. *Alisporites* sp., a bisaccate pollen grain of gymnospermous affinity. BGS specimen number MPK 14719 from the Middle Jurassic Brent Group of the northern North Sea. The overall width and height of the specimen are 87 μm x 62 μm respectively.
5. The angiosperm herb pollen *Chenopodium album* L. (Lamb's Quarters) from a reference collection of modern pollen and spores held by the British Geological Survey. The maximum overall diameter is 29 μm.
6. The angiosperm tree pollen *Tilia* x *europaea* L. (common lime) from a reference collection of modern pollen and spores held by the British Geological Survey. The maximum overall diameter is 31 μm.
7. A scanning electron microscope image of the angiosperm pollen *Gambierina askiniae* Smith et al. 2019 from the Paleogene of the Sabrina Coast, East Antarctica. The width of this specimen is 33 μm (Smith et al. 2019, pl.2.2).
8. The Early Cretaceous megaspore *Horstisporites iridodea* Taylor & Taylor 1988 from the Baqueró Formation of Argentina; note the elaborately patterned wall. The specimen is ~1.2 mm in diameter (Taylor and Taylor 1988). This image was taken by Wilson A. Taylor and is reproduced with permission.
9. The fungal spore *Rhexoampullifera stogieana* Pound et al. 2019 from the Miocene of Derbyshire, central England (Pound et al. 2019). The specimen is 68 μm in length. The image was made by Jennifer M.K. O'Keefe and is reproduced with permission.

Plate 3. A representative selection of specimens of six marine palynomorph groups to demonstrate some of the biological, ecological and morphological diversity of indigenous aquatic organic-walled microfossils. Photographs 1 and 3 to 7 inclusive were taken using differential interference contrast. Photograph 2 was taken in normal (brightfield) illumination. Photographs 8 and 9 were taken using the scanning electron microscope.

1. The dinoflagellate cyst *Wetzeliella articulata* Wetzel in Eisenack 1938 in a single grain mount. BGS specimen PK 197 from the Upper Paleocene Thanet Formation (Thanetian) of Stanford-le-Hope, Essex, southeast England. The overall length and width of the specimen are 127 µm x 129 µm respectively.
2. The dinoflagellate cyst *Oligosphaeridium complex* (White 1842) Davey & Williams 1966. BGS specimen MPK 14720 from the Lower Cretaceous of the North Sea. The equatorial width of the cyst body is 56 µm.
3. The dinoflagellate cyst *Chytroeisphaeridia hyalina* (Raynaud 1978) Lentin & Williams 1981. BGS specimen MPK 14721 from the Middle Jurassic (Lower Callovian), Isle of Skye, northwest Scotland (Riding and Thomas 1997, fig. 2). The irregular, small black mass immediately posterior of the archaeopyle near the midline is probably pyrite. The overall length and width of the specimen are 96 µm x 84 µm respectively.
4. The acritarch *Domasia elongata* Downie 1960. BGS Specimen MPK 14722 from the Silurian of Wales. The overall length is 58 µm.
5. The acritarch *Dorsennidium europaeum* forma *wenlockianum* (Downie 1959 ex Wall & Downie 1963) Sarjeant & Stancliffe 1994. BGS Specimen MPK 14723 from the Silurian of Wales. The overall length is 56 µm.
6. A microforaminiferal lining. Note the dark brown colour and the tightly coiled chambers. BGS specimen MPK 14724 from the Lower Jurassic (Toarcian) of the Mochras Borehole, west Wales (Hesselbo et al. 2013). The maximum diameter is 78 µm.
7. The prasinophyte *Tasmanites* sp.; mid/low focus Note the striking mid brown colour and the thick wall. BGS specimen MPK 14725 from the Lower Jurassic (Toarcian) of the Mochras Borehole, west Wales. The maximum diameter is 100 µm.

8. A chitinozoan, *Ancyrochitina* sp., from the Visby Formation (Silurian Llandovery/Wenlock) of the Lusklint 1 section, Gotland, eastern Sweden (Vandenbroucke et al. 2013, fig. 5D). Imaged using a scanning electron microscope by Thijs R.A. Vandenbroucke and reproduced with permission. The combined length of the body and neck is 100 μm.
9. A scolecodont, *Kettnerites* sp. from the uppermost Ordovician/Hirnantian (Porkuni Regional Stage) Kuldiga Formation at 319.5 m in the Valga 10 borehole, central southern Estonia. Specimen number GIT 433-31 (https://geocollections.info/specimen/125717). Photograph taken using the scanning electron microscope by Olle Hints and reproduced with permission. See Hints and Eriksson (2007) and http://geocollections.info/file/36875. The overall height is ~850 μm.

Plate 4. Examples of dinoflagellate cysts in single and multiple grain mounts. Both photographs were taken using differential interference contrast.

1. Two specimens of the dinoflagellate cyst genus *Deflandrea* in a multiple grain mount. Note the surrounding paraffin wax at the top of the image. BGS specimen registration number PK 183, from the Paleogene of Denmark. The overall length and width of the specimen on the left are 98 μm x 80 μm respectively.
2. The dinoflagellate cyst species *Thalassiphora pelagica* (Eisenack 1954) Eisenack & Gocht 1960 in a single grain mount. Note the surrounding paraffin wax at the top right. BGS specimen registration number PK 133, from the Paleogene of the Hadleigh Borehole, Suffolk, southern England. The overall length and width of the specimen are 160 μm x 111 μm respectively. It is in left lateral view and oriented obliquely, with the antapical end to the top left.

Plate 5. An assemblage of 12 scolecodonts photographed using the scanning electron microscope. This is an apparently complete reconstructed polychaete jaw apparatus of the genus *Ramphoprion* from the Middle Ordovician of Estonia. The largest elements are ~1 mm long. Image by Olle Hints and reproduced with permission; see: https://et.wikipedia.org/wiki/Skolekodondid.

8486