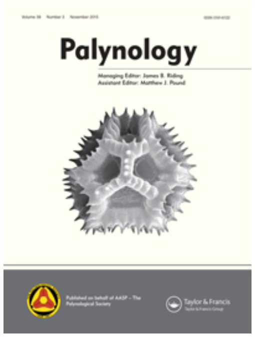

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An effective method for the observation and documentation of highly mature palynomorphs using reflected light microscopy

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Palynomorphs undergo progressive darkening and loss of translucency as temperature increases over geological time periods. The use of oxidising agents that lighten highly mature palynomorphs enables their observation and documentation using transmitted light microscopes, but in certain cases, oxidation destroys part of or the entire organic residue. A new method was tested using palynological residues derived from Devonian and Carboniferous metasediments that allows the microscopic observation and documentation of highly mature palynomorphs without loss of specimens. In its simplest form, it uses non-oxidized, air-dried 20-μm-sieved palynological residues mounted on acrylic slides without the use of cover slips. Observation and documentation were performed routinely using reflected light petrographic microscopes. The resulting photomicrographs are scanning electron micrograph (SEM)-like in the sense that the observed objects are opaque. Depth-of-field issues can be easily overcome by editing the partially focussed images with z-stack image software. The method was found to be useful up to estimated peak rock temperatures of 290°C and over a wide range of tectonic deformation degrees. The application of this method may allow the biostratigraphic and paleaeoenvironmental study of metasediments in areas where this kind of information was assumed to be unattainable.

Keywords: palynomorphs; metasediments; reflect light microscopy; vitrinite reflectance

1. Introduction

Palynomorphs and most organic matter lose volatiles with the increase of temperature over geological time periods (e.g. Bostick 1971; 1979; Marshall & Yule 1999). This is a gradual and irreversible process which renders palynomorphs progressively darker, from yellow through brown to grey or black (Marshall & Yule 1999; Yule et al. 1998, 1999, 2000) and also involves a decrease in transparency. This gradual darkening obscures many features and distinctive characteristics of palynomorphs and, in extreme cases, only black opaque particles are observed. While many acritarchs retain transparency in higher maturity stages (Duggan & Clayton 2008), miospores become dark brown and black with temperatures higher than ca. 200°C (see for example Staplin 1977 for spore colour and temperature ranges). Fragmentation of palynomorphs is a common feature in thermally mature organic residues.

Several chemicals can be used to lighten palynomorphs and render them translucent so that their morphological characteristics can be observed under transmitted light microscopes. Mild products such as hydrogen peroxide, bleach (low-concentrated sodium hypochlorite, NaOCl) and stronger ones such as nitric acid (HNO_3) alone or as Schultze's solution (nitric acid 1:3 potassium chlorate) are commonly used (Gray 1965; Marshall 1980; Colbath 1985; Eshet & Hoek 1996; Jones 1998). These methods are effective in non-metamorphosed rocks, but were found to be ineffective in deformed low-grade metasediments, resulting in only partial lightening of spores and/or the destruction of the majority of palynomorphs (Machado et al. 2011).

Methods using binocular reflected light microscopic observation of miospores are seldom described in the literature (Reitz 1992; Steemans 1989). The previously described methods allowed the observation of spores (Reitz 1992; Steemans 1989), but photographic documentation invariably resulted in blurred images, which were difficult to relate with transmitted light photomicrographs. One of the major problems is the very shallow depth of field obtained by most reflected light microscopes. Here, we describe a simple and effective method to mount non-oxidized sieved organic residues, observe miospores and obtain good-quality

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photomicrographs. This method is an adaptation of another used to mount organic particles in the Organic Petrology and Geochemistry laboratory of the Faculty of Sciences of the University of Porto, Portugal.

2. Materials and methods

In this work, the organic residues obtained from Frasnian–Mississippian thermally-mature (anchizone range, peak rock temperatures above 200°C–Vázquez et al. 2007) and considerably deformed metasediments of the Albergaria-a-Velha Unit in Central Western Portugal were initially tested (see Chaminé et al. 2003 and Machado et al. 2011 for details). Organic residues were extracted using standard palynological methods without oxidation (Batten 1999). Miospores were found to be frequent to abundant, but almost invariably black and opaque. Several oxidation methods were tested but all failed to lighten the palynomorphs, and the ones using nitric acid completely destroyed the organic residues.

A small amount of a 20-μm-sieved organic residue was pipetted onto a thin 7 × 2-cm colourless and totally transparent acrylic slide and left to dry. It is important that no debris (organic or mineral) smaller than 20 μm remain as its presence can seriously decrease the quality of the results. The size of the slide can be changed, depending on the amount of residue to be mounted and the microscope's mechanical stage. Likewise, if the minimum and/or maximum size of the palynomorphs to be observed is different, other mesh sizes can be used.

In most cases, the air-dried residue was conveniently affixed to the slide to allow manipulation and observation without loss of material. When this was not the case, a few drops of ethyl acetate were added, just enough to cover the entire slide surface, and left to dry (Figure 1). In cases where some humidity was left, water droplets formed under the dried acetate film and distorted the surface, making the observation and documentation much harder. When ethyl acetate was used, the mounted residues were checked under the microscope to evaluate if miospores could be properly observed. The acetate film was occasionally thick enough to blur the images. When this was the case, a very smooth polishing of the slide with #1200 silicon carbide wet powder on a smooth

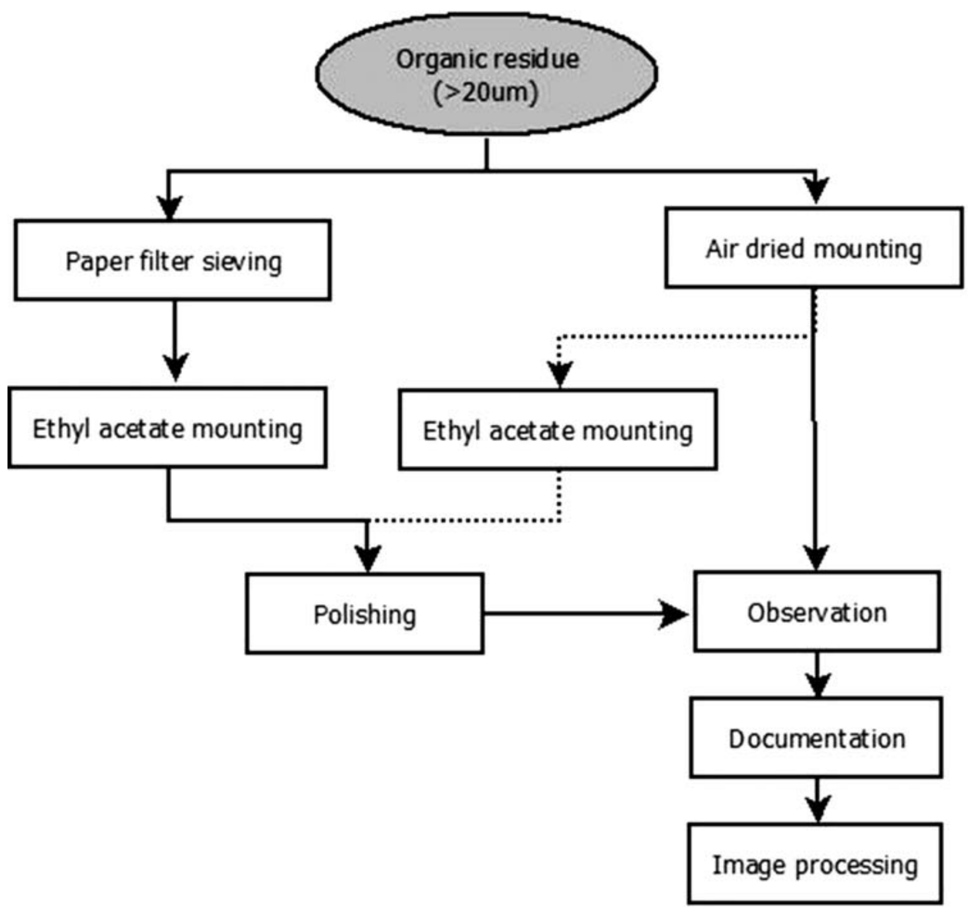

Figure 1. Flow chart of the procedure used to observe and document highly mature miospores using reflected light microscopy.

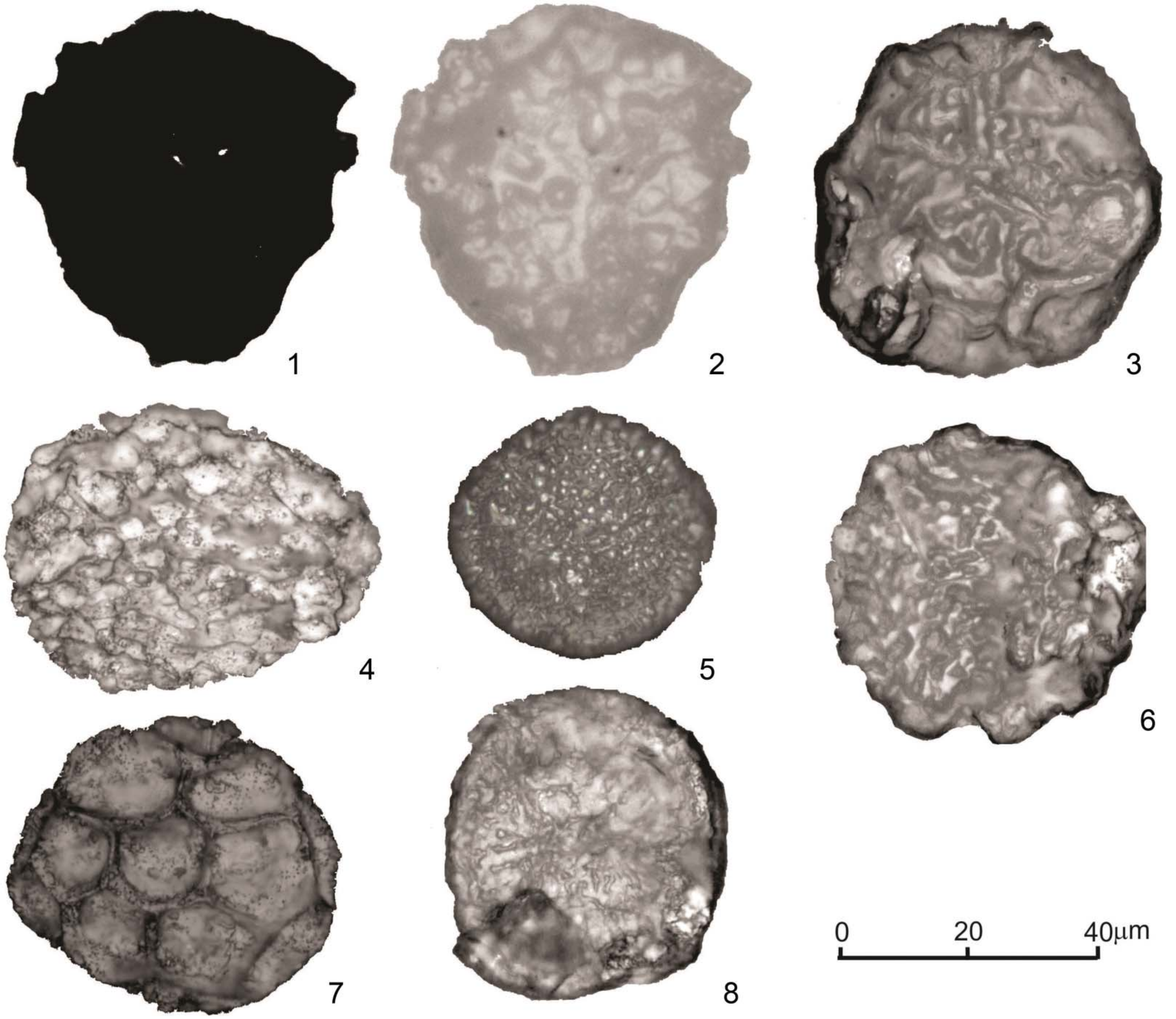

Plate 1. Examples of reflected light photomicrographs of Devonian and Mississippian miospores from the Albergaria-a-Velha Unit. 1. *Raistrickia* sp. (transmitted light); 2. same specimen in reflected light; 3. *Corbulispora cancellata* (Waltz) Bharadwaj & Venkatachala, 1961; 4. *Dictyotriletes* cf. *densoreticulatus* Potonié & Kremp, 1955; 5. *Convolutispora* cf. *ampla* Hoffmeister, Staplin & Malloy, 1955; 6. *Lophozonotriletes tuberosus* Sullivan, 1964; 7. *Dictyotriletes* sp.; 8. *Rugospora* sp.

surface was necessary to expose miospore surfaces. Alternatively, the residue can be sieved using a millipore paper filter with appropriate mesh size. The paper filter with the residue was carefully placed over the acrylic slide and left to dry completely. In this case, the use of ethyl acetate was mandatory to dissolve the paper filter and affix the residue to the slide.

The slides were observed using an Olympus BX60 petrographic reflected light microscope, and documentation was performed with an Olympus C3030 camera. The method was also tested on a petrographic reflected light Leitz ORTHOPLAN microscope. The 40× objective can be used to scan the slide, but the 90× or 100× objectives were required to obtain good-quality photomicrographs. Both oil immersion and air objectives were suitable. If the microscope allows both reflected and transmitted light observation, the two can be used as complementary methods of observation and documentation. This method was used essentially to observe and document miospores, but acritarchs can also be observed.

Reflected light microscopes frequently have a shallow depth of field, so several photomicrographs needed to be taken to ensure the entire surface of each specimen is properly documented. In this work, a completely focussed image was obtained from the several partially focussed images using z-stack software. Helicon Focus was used.

3. Results and further testing

The above-described method allows the documentation of virtually all specimens mounted, provided they are in appropriate orientation and free of organic or mineral debris. The quality seemed to depend essentially on the original preservation of the palynomorphs (see Plate 1) and the size and distribution of the residue particles. Small mineral grains, having greater reflectivity, blurred images when they were on or close to palynomorphs. Similarly, palynomorphs overlapping each other or in close proximity rendered observation and documentation difficult. Experience showed that the ideal situation is to have palynomorphs distributed as isolated grains with a few dozen microns between them.

The best results were obtained with the simplest variation of the method: air-dried residue on an acrylic slide with no ethyl acetate. The classical glass slide and glass cover slip mounts, when observed under reflected light microscopes, produced significant light diffraction, which precludes proper observation. Thus, drying the residue on a glass cover slip and mounting it over a slide is not recommended for this kind of microscopic observation. Generally, the best results were obtained when the least materials lay between the palynomorphs and the microscope objective.

When more than one photomicrograph per specimen was taken, two to four partially focussed images worked best to obtain a completely focussed image using z-stack software.

Residues obtained by microwave-assisted Hydrofluoric acid (HF) dissolution (Ellin & McLean 1994) were also tested. No differences were observed when mounting, observing or photographing.

After initial testing, over 60 different samples from the same Albergaria-a-Velha Unit were observed and photographed (Machado et al. 2011) using the same method. The quality of results seems to be independent of age for these metasediments (bracketed between Frasnian and Serpukovian). Increasing deformation decreases the overall quality of the palynological residues, but even highly deformed black and grey shales with original bedding obliterated and extremely penetrative sub-millimetrical foliation yielded observable spores.

A few samples from the slates of the Mississippian Toca da Moura complex from the Ossa–Morena Zone in Southern Portugal (Pereira et al. 2006) were also tested. The regional peak rock temperature of this unit is estimated to be around 290°C (from unpublished data on vitrinite reflectance). The complex is mildly deformed, except along major regional faults. The method was successful, although fewer specimens were suitable for observation. Standard palynological methods with oxidation also have been successful in this unit (see Pereira et al. 2006 and Lopes et al. 2014 for details).

The method was also used with organic residues derived from Middle Devonian slates and silicylites interbedded with calciturbidites of the Western Ossa–Morena zone in Southern Portugal (see Machado et al. 2010 for details). The estimated Conodont Alteration Index is 5, corresponding to peak rock temperatures above 300°C. The unit is mildly deformed. The results show rounded organic opaque black particles (in transmitted light) which resemble spores with a featureless surface when observed under reflected light microscopes. Although it is uncertain if these rounded graphitic particles are indeed spores, rock temperatures above 300°C may be an upper limit for the usage of this method.

4. Discussion and conclusions

This method allows all palynomorphs in residues derived from metasediment samples can be mounted, observed and documented. This capability is particularly useful in residues which are totally or significantly destroyed by laboratory oxidation methods. The destruction of more fragile taxa and over- or underestimation of certain organic-walled fossil groups are known problems for palynologists when using oxidation methods. The method can thus be used as quality check when working with sedimentary rocks that require oxidation to study their palynological content.

An additional advantage, provided that microwave HF digestion is not used, is that, with ethyl acetate covering and gentle polishing, the same slides can be used for vitrinite reflectance measurements (when the maceral is present).

The observation and documentation seem to be independent of the microscope system used. The results between the two systems used in this work are very similar.

Although the number of tested sample sets is limited, the results suggest that palynomorphs derived from metasediments with a higher deformation degree are more susceptible to breakage and destruction when exposed to strong oxidising agents.

The most significant disadvantage is that only one side of a palynomorph is observable (either distal or proximal) and internal features are not discernible. In this sense, the resulting images resemble scanning electron photomicrographs and may be difficult, in some cases, to relate with transmitted light images (the standard used for documentation).

Most of the materials and equipment needed are already present in most palynology laboratories, or can be easily and inexpensively obtained. Reflected light microscopes are available in many geoscience departments. The conversion of petrographic transmitted light microscopes to dual systems is fairly straightforward, but biological microscopes will probably require major rebuilding.

This method allows the effective observation and documentation of miospores and other palynomorphs from highly thermally mature rocks, which would, in some cases, be otherwise unobservable. The method has the potential to unlock biostratigraphic and palaeoenvironmental information of metasediments, especially tectonically deformed ones, which were previously assumed to be barren.

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