Biogeosciences Discuss., doi:10.5194/bg-2016-391, 2016
Manuscript under review for journal Biogeosciences
Published: 24 October 2016

Biogeosciences Discussions
Open Access
EGU

Identification of reworking in Eocene to Miocene pollen records from offshore Antarctica: a new approach using red fluorescence

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Abstract. Antarctic palaeoclimate evolution and vegetation history after the formation of a continent-scale cryosphere at the Eocene/Oligocene boundary, 33.9 million years ago, has remained a matter of controversy. In particular, the reconstruction of terrestrial climate and vegetation has been strongly hampered by uncertainties in unambiguously identifying *in situ* as opposed to reworked sporomorphs that have been transported into Antarctic marine sedimentary records by waxing and waning ice sheets. Whereas reworked sporomorph grains over longer non-successive geological time scales are easily identifiable within younger sporomorph assemblages (e.g., Permian sporomorphs in Pliocene sediments), distinguishing *in situ* from reworked material in palynological assemblages over successive geological time periods (e.g., Eocene sporomorphs in Oligocene sediments) has remained problematic. This study presents a new quantitative approach to identifying *in situ* grains from a marine sediment core from circum-Antarctic waters. We measured the fluorescence signature and mean red, green and blue, brightness, intensity and saturation values of selected pollen and spore taxa from Eocene, Oligocene and Miocene sediments from the Wilkes Land margin Site U1356 (East Antarctica) recovered during Integrated Ocean Drilling Program (IODP) Expedition 318. Our study identified statistically significant differences in mean red fluorescence values of *in situ* sporomorph taxa against age. We conclude that red fluorescence is a reliable parameter to identify the presence of *in situ* pollen and spores in Antarctic marine sediment records from the circum-Antarctic realm that are influenced by glaciation and extensive reworking. Our study provides an essential new tool required to accurately reconstruct Cenozoic terrestrial climate change on Antarctica using fossil pollen and spores.

Keywords: Fluorescence, pollen, spores, Antarctica, reworking, vegetation, climate reconstruction

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1 Introduction

Antarctica plays a key role in understanding past and future global climate change due to the impact its large ice sheets exert on sea level as well as on oceanic and atmospheric circulation. Throughout the last 65 million years, the Antarctic continent has undergone a drastic change from a greenhouse environment in the early Paleogene towards an icehouse world in the late Paleogene and Neogene (e.g., Askin and Raine, 2000; Prebble et al., 2006; Bijl et al., 2009; Anderson et al., 2011; Pross et al., 2012; Passchier et al., 2013). The analysis of fossil pollen and spores is one of the most important tools for reconstructing and quantifying past vegetation and terrestrial climate change. For Antarctica the lack of long and well-dated sediment records puts considerable constraints on a detailed spatial and temporal reconstruction of terrestrial environmental change. *In situ* macro- and microfossil evidence for vegetation cover from continental sections of Antarctica is often difficult to date and in general sparse due to ice cover (e.g., Birkenmajer and Zastawniak, 1989; Pole et al., 2000; Lewis et al., 2008; Warny et al., 2016). Therefore, most reconstructions of climate and vegetation on the Antarctic continent are based on palynological records from marine, circum-Antarctic sediment cores. However, the waxing and waning of Antarctic ice sheets throughout the Oligocene and Miocene caused reworking of terrestrial material into marine sediments, ultimately leading to a combination of *in situ* and reworked palynomorphs in palaeorecords that are difficult to differentiate especially over short geological time scales (e.g., Askin and Raine, 2000; Raine and Askin, 2001; Prebble et al., 2006; Salzmann et al., 2011; Griener et al., 2015).

The unambiguous differentiation between *in situ* and reworked palynomorphs in palaeorecords is essential to establish reliable climate and vegetation reconstructions for the Antarctic continent. However, a quantitative approach to differentiate *in situ* from reworked sporomorphs over shorter geological time scales (e.g. Oligocene to Miocene) has not yet been established. Previous palynological studies in Antarctica have identified reworked Cenozoic sporomorphs based on approaches using the thermal alteration of grains (e.g., Askin and Raine, 2000; Raine and Askin, 2001; Prebble et al., 2006, Griener et al., 2015; Warny et al., 2016). These approaches only take into account reworked pollen grains that have been exposed to strongly different taphonomical conditions than the *in situ* material. However, submarine reworking of shelf material can only have small impacts on preservation quality, hampering the unambiguous identification of reworked palynomorphs using light microscopy (e.g., Salzmann et al. 2011).

Subjective fluorescence microscopy has been applied in Antarctic pollen studies to help remedy the issue of reworking. Raine (1998) and Salzmann et al. (2011) used autofluorescence to identify reworked Permian and Mesozoic sporomorphs within Cenozoic sediments from the Cape Roberts cores in the Ross Sea and James Ross Island, Antarctica. Qualitative attempts to separate reworked and *in situ* sporomorphs based on their fluorescence colours through geological time have been shown to work (Phillips, 1972; Bujak and Davies, 1982). However, these methods are highly subjective; being dependent upon the observer and difficult to reproduce. The fluorescence signal from fossil pollen and spores comes from the sporopollenin in the exine, which contains heteroatomic compounds (Yeloff and Hunt, 2005). Over geological timescales pollen and spores in sediments are confronted with elevated temperatures and pressures after burial, and the less resistant compounds of the sporopollenin shift to the

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red end of the colour spectrum and ultimately towards no fluorescence (Van Gijzel, 1967; Bujak and Davies, 1982; Yeloff and Hunt, 2005). This suggests that the amount of fluorescence changes with burial time: sporomorphs from old sediments show little to no fluorescence, and pollen and spores from the oldest section of a core show fluorescence predominantly on the red end of the spectrum. Critically, the process of fluorescence loss is irreversible, meaning that fluorescence cannot be re-gained by the sporomorphs at any time. This behaviour provides an opportunity to assess whether sporomorphs are *in situ* or reworked from older strata.

By using fluorescence microscopy this study aims to develop a new systematic and quantitative approach to identify *in situ* pollen and spore assemblages in marine sediments from Antarctica. We measured the fluorescence signature and mean red, green, blue, brightness, intensity and saturation values of the most common pollen and spore taxa under ultra-violet (UV) light in Eocene, Oligocene and Miocene sediments. All samples were taken from the Wilkes Land margin sediment record at IODP Site U1356 (Figure 1), and cover the early Eocene through the mid-Miocene with two hiatuses from the mid- Eocene to the early Oligocene (~47 – 33.6 Ma) and from the latest Oligocene to early Miocene (~23.12 – 16.7 Ma) (Escutia et al., 2011; Tauxe et al., 2012). This provides us with three timeintervals , all yielding abundant sporomorphs (Escutia et al., 2011; Pross et al., 2012, Contreras et al., 2013; Sangiorgi et al., in review) in which each should come with different fluorescence behaviour. The Cenozoic sediment record of Site U1356 provides a unique opportunity to compare the fluorescence of the same pollen taxa through the Eocene to Miocene, i.e., before and during the impact of large-scale glaciation in order to unambiguously identify reworked palynomorphs.

2 Materials and Methods

2.1 Sampling and sedimentology

Pollen and spores were examined from Eocene, Oligocene and Miocene sediments from IODP Site U1356 located ~ 300 km off Wilkes Land, East Antarctica (63°18.6138'S, 135°59.9376'E) taken at the transition between the continental rise and the abyssal plain (Figure 1; Escutia et al., 2014). The Wilkes Land margin formed during the late Cretaceous during a non-volcanic rift, with Oligocene-Eocene shelf sediments exposed today on the continental shelf proximal to Site U1356 (Close et al., 2009; Expedition 318 Scientists, 2011). Pollen taxa were analysed from 28 samples between 106.62 and 998.99 mbsf. The early (53.9 – 51.9 Ma) to mid-Eocene (49.3 – 46 Ma) sediments covered depths between approximately 1000.08 and 893 mbsf (Expedition 318 Scientists, 2011). The lowermost Eocene interval consists of clay mineral assemblages mainly containing smectite and kaolinite (Expedition 318 Scientists, 2011). This lithology points to chemical weathering under warm and humid conditions and a shallow-water depositional environment (Expedition 318 Scientists, 2011). Graded sandstones units suggesting mass transports comprise the upper Eocene sediments (Expedition 318 Scientists, 2011). The WL-U3 unconformity separated the non-glacial Eocene strata from the Oligocene strata influenced by glacial deposition (Escutia et al., 2005). Approximately 455 m of sedimentary strata were recovered from Site U1356 (440.7 – 895.41 mbsf) and dated as early to late Oligocene (~33 – 23 Ma) (Escutia et al., 2011; Tauxe et al., 2012). The Wilkes Land Oligocene

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sedimentary deposits reside in a distal setting (lowermost rise abyssal plain) with evidence of iceberg activity indicated by dropstones suggesting a glaciated environment (Escutia et al., 2011).

The lithology of the Lower Oligocene shows evidence of terrestrial sediment influence from contorted diamictites along with contorted and convoluted bedded mudstones (Expedition 318 Scientists, 2011). The Upper Oligocene indicates a further increase in terrigenous sediment with bioturbated claystones, siltstones, sandstones, and contorted diamictites (Expedition 318 Scientists, 2011). The Upper Oligocene and Lower to Middle Miocene (23.12 – 16.7 Ma) are separated by a ~6 m.y. long hiatus (Escutia et al., 2011). The Lower to Middle/Upper Miocene encompassed depths of ~459.4 mbsf to 3 mbsf and Miocene lithologies document fine-grained terrestrial input with bioturbated claystones, siltstones and sandstones (Expedition 318 Scientists, 2011). The Upper Miocene shows evidence of ice rafting with gravel in diatom-rich silty clays and oozes (Expedition 318 Scientists, 2011). The Oligocene to Miocene sediments are indicative of relatively deep-water, sea-ice-influenced setting (Escutia et al., 2011). The lithologies recognised at Site U1356 suggest a large contribution of transported sediment from the shelf, indicating a strong likelihood of reworked palynomorphs in the record.

2.2 Palynology

All samples were processed at the Laboratory of Palaeobotany and Palynology, Utrecht University, The Netherlands, using their standard palynological processing method for marine sediments (e.g., Bijl et al., 2013). Samples were treated with 10% HCl and cold 38% HF to dissolve carbonates and silicates, respectively, and again with 10% HCl to eliminate silica gel and sieved with a 10-micrometre mesh. Residues were mounted on glass microscope slides using glycerine jelly, and the edges were sealed with nail polish. The nail polish seems to limit the fluorescence of the underlying palynomorphs due to the additional medium diminishing the intensity of the brightness. Therefore, we chose to consider only those palynomorphs that were away from the edges of the slide. The use of acids such as HF and HCl can alter the fluorescence of grains towards the red end of the spectra (Van Gijzel, 1971; Waterhouse, 1998). However, the same palynological processing techniques were uniformly used for all samples. For each sample 30 pollen and spore grains with no obvious signs of reworking (e.g., colour, corrosion) were randomly selected for the Miocene, late Oligocene, early Oligocene and Eocene to determine the fluorescence signatures through geological time. Five common pollen and spore taxa, which are abundant in most Antarctic pollen records and also found in the majority of the Wilkes Land samples, were selected. Dependent on availability the number of different taxa per time slice varied. These taxa include (name in brackets indicate potential nearest living relative after Raine et al., 2011 and Contreras et al., 2013) *Cyathidites minor* (*Cyathea*), *Myricipites harrisii* (Casuarinaceae or Myricaceae), *Nothofagidites fleminigii* (*Nothofagus*), *N. lachlaniae* (*Nothofagus*), and *Podocarpidites ellipticus* (*Podocarpus*).

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135 **2.3 Fluorescence microscopy**

Various factors such as burial depth and geological age contribute to the fluorescence emission of sporomorphs. However, these factors reflect the ultimate determining factor of fluorescence alteration, which is heat flow and the length of time the sporomorphs are exposed to this heat (Waterhouse et al., 1998). The biochemical fluorescence emitted in Cenozoic sporomorphs ranges through the red, green and blue light intensity spectrum (Bujak and Davies,
140 1982). Factors such as intensity, saturation and brightness also affect the fluorescence emission and were measured to test whether these variables changed with age and depth.

Sporomorphs were examined under light and UV-fluorescence using an Olympus BX40F microscope with a high-pressure mercury burner, dichronic mirror with a 330 – 385nm exciter filter and 420nm long-pass barrier filter. Pollen and spores emit fluorescence ranging from blue (400nm) to red (700nm), and the preservation of the exine
145 helps to determine the fluorescence colour (Van Gijzel, 1971). For an initial qualitative colour classification of the investigated pollen and spores, the colour chart based on UV-fluorescence by Yeloff and Hunt (2005) was used. When correlating the UV-fluorescence signal of sporomorph grains, only comparison between the same sporomorph taxa can be done. This is due to variations in the chemical composition of the exine that affects the fluorescence colour of the grains (Hunt et al., 2007). The gain and exposures were standardised for all measured grains
150 throughout the analysis to allow for accurate representation of the mean red, green and blue (RGB) values measured. For the light microscope the gain was 1.00x, and the exposure 20 ms (+2.0 EV), while under fluorescence the gain stayed at 1.70x and the exposure was 100 ms (+2.0 EV). The white balance (1.30, 1.00, 2.00) was constant through the entire process. Pictures were taken using a Nikon DS-Fil camera and analysed in image processing software (NIS-Elements Basic Research 3.0 program). A pre-selection was performed removing obviously reworked, older-
155 than-Eocene sporomorphs that were extremely dark to almost opaque under a light microscope and with very little to no fluorescence under UV excitation. This allowed for the investigation of the fluorescence signature of grains that are *in situ* or not identified as obviously reworked. This examination was done on all slides studied from the Eocene, early Oligocene, late Oligocene and Miocene. The mean RGB, intensity, saturation and brightness values were measured for each grain under light and UV-fluorescence. This was in relation to a greyscale from 0 (no light) to
160 256. The mean values were taken from each grain through an autodetect tool, which draws a contour around the grain, and the fluorescence values were only measured from this contour image.

2.4 Statistical analyses: factors influencing the fluorescence signature of the sporomorphs

To quantitatively assess the fluorescence behaviour of the five taxa from Site U1356, three different statistical approaches were used:

165 (i) A Pearson's correlation coefficient (r) was calculated to determine whether the fluorescence measurements of sporomorphs correlate with age. This correlation coefficient shows the strength of the linear relationship from independently measured fluorescence variables against age through the Eocene to the Miocene. Coefficient values that are closer to 1 or -1 show a better linear agreement. The most

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statistically significant fluorescence value, mean red, was then measured for each taxon to indicate the correspondence of taxa mean red values against age. This was undertaken in IBM SPSS Statistic version 22, and the significance threshold was set at 0.01 (99%) for all measured p-values.

(ii) The Mann-Whitney *U* test was performed in PAST (Hammer et al., 2001) to compare whether two datasets that are not normally distributed are statistically different from one another. The datasets being compared are sporomorph fluorescence measurements (mean red, mean brightness and mean intensity) of successive geological time slices (e.g., late Oligocene vs. Miocene mean red values). This tests if a statistically significant fluorescence signature can be identified to separate sporomorphs over subsequent geological epochs in the Wilkes Land core. The fluorescence variables mean red, mean brightness and mean intensity were chosen because these values had the strongest linear relationship against age (high r values) from the Pearson correlation tests.

(iii) To determine if similar fluorescence values of palynomorphs can be grouped by age, burial depth, taxonomy or fluorescence colour, a series of 1-way Analysis of Similarities tests (ANOSIM) with 999 permutations were conducted using PRIMER 6 (Clarke and Gorley, 2006). The raw fluorescence data for the 120 measured palynomorphs was first pre-treated with a square root transformation and then a resemblance matrix was constructed using the Bray-Curtis similarity algorithm. Using ANOSIM tested if palynomorphs with similar fluorescence values (mean intensity, mean RGB, mean saturation and mean brightness) could be grouped into categorical factors: Age – Eocene, Early Oligocene, Late Oligocene, Miocene; Burial Depth; Taxonomy (*Cyathidites minor*, *Myricipites harrisii*, *Nothofagidites flemingii*, *N. lachlaniae*, *Podocarpidites ellipticus*); fluorescence light colour (yellow, orange and red). ANOSIM tests the null hypothesis that there are no fluorescence differences between samples grouped by the levels of a factor (e.g. fluorescence values for an Eocene sample would be distinct from a Miocene sample). If the Global R is close to 0 then fluorescence values characterised by different levels of a factor (e.g. Eocene, Early Oligocene, Late Oligocene, Miocene) are similar and the hypothesis that the age of the sample determines the fluorescence (in this example) can be rejected. Conversely, the closer the Global R value is to 1, the more strongly that factor explains the separation of the similar fluorescence values (Clarke and Gorley, 2006).

3 Results

3.1 Subjective assessment of sporomorphs through fluorescence colours

A subjective assessment of the sporomorphs fluorescence signature revealed that a purely visual assessment of fluorescence colour only allows a limited identification of reworking and separation of geological ages. Following the colour chart classification of Yeloff and Hunt (2005), Eocene pollen and spores generally graded from an orange/red (46 – 49) while Oligocene grains fluoresced an orange/yellow colour (43 – 46) and Miocene grains showed similar yellow/light orange fluorescence (42 – 45). The visible red colour fluorescence clearly distinguishes Eocene sporomorphs from Oligocene and Miocene grains in the Site U1356 material, shown from the contrast

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between pollen and spore grains and the slide background under red filter (Figure 2). However, the subjective colour comparison of fluorescence alone could not distinguish between Oligocene and Miocene grains (Figure 2).

3.2 Variation of fluorescence values through the Eocene to Miocene

The Pearson's correlation (r) coefficient indicates a moderate to strong relationship between fluorescence values mean red, mean intensity, mean brightness and geological age. Mean red values showed the strongest statistical correlation with age values, r = -0.459 ($p < 0.0001$) (Table 1a; Figure 3a). Due to the moderate to very strong relationship between total mean red values and age, a Pearson's correlation was also performed on each taxon's mean red value (Table 1b; Figure 3b). A very strong agreement includes *Cyathidites minor* (r = -0.687, p = 0.004) and *Nothofagidites lachlaniae* (r = -0.663, p = 0.001), with *Podocarpidites ellipticus* (r = -0.503, p = 0.0007) showing a moderate correlation between mean red values and age (Table 1b; Figure 3b). Brightness and intensity had a moderate correlation with age, r = -0.323, but high significance (p = 0.0003). The mean saturation values had no relationship with age, r = -0.22 and was not significant (p = 0.118). Mean green (r = -0.308, p = 0.0006) and mean blue values (r = -0.269, p = 0.003) had a weak relationship to age with high significance. Pearson's correlation indicates mean red, mean intensity and mean brightness as the most statistically significant fluorescence signatures to separate *in situ* sporomorphs in the Wilkes Land core.

The Mann-Whitney *U* tests show statistically highly significant ($p < 0.0001$) changes of fluorescence mean red, mean intensity and mean brightness values from the Eocene to late Oligocene and the Eocene to Miocene (Table 2). However, there is no statistically considerable difference between the Eocene to early Oligocene mean red values (p = 0.2772), whereas significant differences exist between the mean intensity (p = 0.0014) and brightness (p = 0.0014) (Table 2). The early Oligocene to Miocene (p = 0.0009) and early Oligocene to late Oligocene (p = 0.0067) mean red signals can be distinguished from one another (Table 2), however the intensity and brightness cannot. The late Oligocene to Miocene mean red (p = 0.2772), mean intensity (p = 0.0451) and mean brightness (p = 0.0459) cannot be differentiated from each other. This indicates that measuring the mean red fluorescence values from the Wilkes Land samples can distinctively separate the fluorescence signal from sporomorphs in the Eocene, Oligocene and Miocene. The Mann-Whitney *U* test indicates that non-successive intervals in geological time, e.g., Eocene to Miocene and Eocene to late Oligocene, show more distinctive differences in fluorescence mean red, mean intensity and mean brightness values. However, unlike the subjective fluorescence colour comparison, the Mann-Whitney *U* test shows Oligocene and Miocene grains can now be separated based on the quantitative measurement of their mean red fluorescence signature.

3.3 Factors potentially influencing fluorescence sporomorph signals

To understand if certain factors: stage (e.g., Eocene, Oligocene and Miocene), fluorescence colour of palynomorphs, burial depth and number of taxa had any influence on the similarity of fluorescence values an ANOSIM analysis was done. The ANOSIM tests demonstrated that the age of a sample (Global R = 0.145, P = 0.001) and the depth (Global R = 0.315, P = 0.001) could explain the separation of samples with similar fluorescence into factors. This

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shows that both age and depth (age being a function of depth in this study) influence the fluorescence of palynomorphs. The taxonomy of the samples could not explain any similarity in fluorescence values (Global R = 0.006, P = 0.385) and neither could the fluorescence colour of the palynomorph (Global R = 0.085 P = 0.011).

4 Discussion

4.1 Importance and application of mean red fluorescence

Our study demonstrates that a qualitative, subjective assessment of fluorescence colour alone cannot be used to separate *in situ* and reworked pollen and spores in the Wilkes Land core over successive geological time scales. Our quantitative approach identified the mean red fluorescence colour of pollen and spores as the most reliable indicator for reworking, showing a strong linear correlation with age (Figure 3). This clear statistical grouping of red fluorescence values over consecutive time scales indicates a considerable portion of the measured pollen and spore taxa are *in situ*. For Site U1356, the record is expected to have significant reworking in the Neogene section due to the submarine exposure of Eocene sediments close to the site. However, the mean red fluorescence values from this approach clearly separate the Eocene to Miocene grains (Table 2) indicating with some certainty a major influence of reworking is not present in the Wilkes Land record. This does not completely disregard the influence of reworking in a sample, but ensures a sample has enough *in situ* pollen of a taxa to be used for environmental reconstruction. Our study therefore provides a new essential and simple tool to distinguish *in situ* palynological assemblages in marine sediment records from the high latitudes that are influenced by glaciation and extensive reworking. However, it must be noted that the red fluorescence values in our new approach are not absolute values, which can be transferred between cores. Because Palynology uses the entire assemblage of taxa and never a single grain to reconstruct vegetation communities, our approach has not been designed to determine if a single grain is *in situ* or reworked. In order to successfully identify if a sample contains an *in situ* pollen taxa assemblage for environmental reconstructions, the measurement of red fluorescence colour needs to be adhered to individual coring sites covering successive geological epochs.

Our approach offers an opportunity to resolve difficulties in differentiating *in situ* and reworked palynomorphs in Antarctic palynological assemblages from the Ross and Weddell Sea (e.g., Raine, 1998; Askin, 2000; Askin and Raine, 2000; Warny and Askin, 2011). Subjective fluorescence microscopy has been use to separate *in situ* grains from recycled Permian-lower Mesozoic sporomorphs in the early Miocene Cape Roberts Project (CRP) 1 core (Raine, 1998). However, both transmitted light (yellow to yellow-brown) and fluorescence colour (yellow to orange) comparisons could not discern Cenozoic pollen and spores (Raine, 1998; Askin and Raine, 2000; Raine and Askin, 2001). There is no apparent pattern of variation found in the fluorescence colour of sporomorphs in assemblages from the Ross Sea emphasizing the importance of taking quantitative fluorescence measurements.

4.2 Influence of heat flow, burial depth and hiatuses on fluorescence

In order for a distinct fluorescence signature to be unambiguously identified in a palynological assemblage an ample amount of geological time between samples is needed (e.g. Van Gijzel, 1967; Bujak and Davies, 1982). It is

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important to discern whether fluorescence values can be distinguished over successive geological intervals and how factors such as hiatuses and burial depth can possibly affect fluorescence. The largest differences in depth and intervals of geological time in the Wilkes Land core are between the Eocene to late Oligocene and the Eocene to Miocene and these intervals show the highest significance of mean red, mean intensity and mean brightness values ($p < 0.0001$; Table 2). However, this does not always mean geological age directly determines the fluorescence signal of sporomorphs. The amount and length of exposure to burial heat ultimately establishes the fluorescence alteration of sporomorphs (Waterhouse et al., 1998). Mean red fluorescence values are still statistically significant ($p = 0.0067$; Table 2) when comparing the early and late Oligocene samples. The oldest early Oligocene sample analysed was taken at 795.58 mbsf and the youngest late Oligocene sample was analysed at 555.19 mbsf. This difference in burial depth could contribute to the differentiation of mean red values between the early and late Oligocene. Burial depth is shown to play a role in the fluorescence of sporomorphs as indicated by the Global R value in the ANOSIM analysis (Section 3.3).

Disruption of sporomorph exposure to burial heat is shown to have an effect on fluorescence mean red values as well. Between the mid-Eocene to early Oligocene and the late Oligocene to Miocene, the differences in mean red values are insignificant ($p = 0.2772$; Table 2). These mean red values correlate with two major hiatuses observed from the Eocene – Miocene sediment record from Site U1356 off Wilkes Land, East Antarctica (Escutia et al., 2011). A ~13 m.y. hiatus is found between the middle Eocene and the early Oligocene (Escutia et al., 2014). This unconformity represents extensive erosion correlating with the onset of glaciation at the Eocene-Oligocene boundary (Escutia et al., 2011; 2014; Stocchi et al., 2013). Another hiatus correlates with a regional unconformity at Wilkes Land that coincides with the Mi-1 event and extends from ~23.12 to 16.7 Ma (Escutia et al., 2005; Escutia et al., 2011). The hiatuses in the Wilkes Land core could have potentially disrupted the sporomorphs exposure to burial heat causing a less distinctive fluorescence signature between the mid-Eocene to early Oligocene and late Oligocene to Miocene. However, it is important to reiterate that this fluorescence approach can still separate the mean red fluorescence values between the entire Oligocene and Miocene ($p = 0.0083$; Table 2).

4.3 Fluorescence variation between taxa

In order to produce comparable values for each geological time interval, our fluorescence approach requires sediment cores spanning successive geological epochs with similar palynological assemblages. The Paleogene and Neogene Antarctic palaeovegetation are unique with common taxa (e.g., *Nothofagidites* and *Podocarpidites*) still present in palynological assemblages through a major climatic change from a greenhouse to icehouse world (e.g., Truswell and Macphail, 2009; Pross et al., 2012; Griener et al., 2015). When comparing the fluorescence signature between geological time slices the same taxa must be used. Differences between the fluorescence signatures of individual taxa in the Wilkes Land assemblage are apparent (Figure 3; Table 1). Factors such as exine composition and differential sensitivity to thermal alteration can cause variation in fluorescence measurements between sporomorph taxa (Waterhouse et al., 1998). The differences in mean red fluorescence signature could also have occurred due to variation in the number of taxa measured for each geological time slice. The only spore taxa in this study, *Cyathidites minor* showed the strongest correlation between fluorescence mean red values ($r = -0.687$, $p <$

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0.004) through geological time. This could be due to the chemical composition of *Cyathidites minor*, the thickened and complex perispore (Marquez and Morbelli, 2014) or how this spore chemistry reacts to degradation over geological time.

5 Conclusions

Reworking has long been a factor hindering a full appreciation of Cenozoic Antarctic palynology. The impact of ice-sheet fluctuations since the Oligocene makes identifying *in situ* pollen grains in Neogene Antarctic assemblages particularly challenging (e.g., Francis and Hill, 1996; Wilson et al., 2002; Salzmann et al., 2011). However, the unambiguous identification of *in situ* palynomorphs is a prerequisite to fully understand the terrestrial vegetation response to periods of extensive cooling and environmental changes on Antarctica. By using fluorescence microscopy this study identifies mean red fluorescence as the most reliable parameter to statistically identify reworking on million-year time scales during the Paleogene and Neogene. For Site U1356 the mean red fluorescence values measured clearly separate the Eocene to Miocene grains despite that proximal to the site, submarine exposure of Eocene sediments is present. It is important to emphasize that the red fluorescence values from this study are not absolute and are specific to the Wilkes Land core. Fluorescence variation between taxa is apparent, but the mean red fluorescence measurements for each taxon (*Cyathidites minor*, *Myricipites harrisii*, *Nothofagidites flemingii*, *N. lachlaniae*, and *Podocarpidites ellipticus*) still show a strong linear relationship against age. Our study offers a new approach to identify if a sample contains an *in situ* pollen taxa assemblage to reconstruct with high confidence Cenozoic climate change andvegetation pre- and post-Antarctic cryosphere formation. Additional studies are needed to systematically explore the wider use of our red fluorescence approach for Antarctic palynology. These studies should include forthcoming IODP drilling expeditions and possibly existing sites such as ANDRILL (AND-2A) where several taxa such as *Nothofagidites brassii* group, Proteaceae and podocarp conifers were denoted with uncertainty because it is unknown when these taxa disappeared from Antarctica (Griener et al., 2015).

Data availability

The raw data analysed in this study is available as supplementary material.

Competing interests

The authors declare that they have no conflict of interest.

Acknowledgements

This study was funded by the Faculty of Engineering and Environment Research Studentship from Northumbria University. The samples and data used for this research were provided by the Integrated Ocean Drilling Program (IODP). Ulrich Salzmann acknowledges funding received from the Natural Environment Research Council (NERC Grant NE/H000984/1). Francesca Sangiorgi thanks the Netherlands Organization for Scientific research (NWO) for NNPP Polar 866.10.110. Peter K. Bijl acknowledges the Dutch National Organisation for Scientific Research

(NWO) for VENI grant no 863.13.002. Jörg Pross acknowledges support through the German Science Foundation (DFG; grant PR 651/10).

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Published: 24 October 2016

Table 1. Pearson's correlation coefficient values of the (a) mean fluorescence variables (RGB, intensity, brightness and saturation), and (b) mean red values of each taxon measured, along with sample size, mean, standard deviation (SD) and p-values.

Mean fluorescent variables	N	Mean	SD	Pearson correlation coefficient (r)	p-value
Red	120	29.18	10.08	-0.459	0.0001
Green	120	103.51	35.45	-0.308	0.0006
Blue	120	180.52	50.14	-0.269	0.0030
Intensity	120	108	29.94	-0.323	0.0003
Brightness	120	42.36	11.74	-0.323	0.0003
Saturation	120	184.34	19.24	0.220	0.0159

Species mean red values	N	Mean	SD	Pearson correlation coefficient (r)	p-value
Podocarpus ellipticus	41	30.44	9.29	-0.503	0.0007
Nothofagidites lachlaniae	19	28.17	8.03	-0.663	0.0010
Nothofagidites flemingii	32	27.81	8.82	-0.387	0.0290
Myricipites harrisii	13	41.72	12.03	-0.471	0.1050
Cyathidites minor	15	26.29	8.97	-0.687	0.0040

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Published: 24 October 2016

Table 2. Mann-Whitney *U* test values correlating the mean red, mean intensity and mean brightness throughout the Eocene to the Miocene.

Age comparison		Mean Red				Mean Intensity				Mean Brightness			
		U	n_1	n_2	p-value	U	n_1	n_2	p-value	U	n_1	n_2	p-value
Miocene	late Oligocene	376	30	30	0.2772	314	30	30	0.0451	315	30	30	0.0459
late Oligocene	early Oligocene	266	30	30	0.0067	357	30	30	0.1715	357	30	30	0.1714
early Oligocene	Eocene	376	30	30	0.2772	234	30	30	0.0014	234	30	30	0.0014
late Oligocene	Eocene	166	30	30	0.0001	126	30	30	0.0001	126	30	30	0.0001
Miocene	Eocene	121	30	30	0.0001	200	30	30	0.0001	200	30	30	0.0001
early Oligocene	Miocene	224	30	30	0.0009	419	30	30	0.6520	419	30	30	0.6520
Oligocene	Eocene	293	30	30	0.0207	145	30	30	0.0001	145	30	30	0.0001
Oligocene	Miocene	271	30	30	0.0083	326	30	30	0.0679	326	30	30	0.0679

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Figure 1. Location of IODP Expedition 318 Site U1356 during the Eocene-Oligocene transition adapted from Houben et al. (2013). Pale blue areas indicate shelf environments and green areas show lowland regions in Antarctica. The black circle is located at 60° S. Antarctic topography and palaeoceanography modified after Lawver and Gahagan (2003) and Wilson et al. (2012).

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Published: 24 October 2016

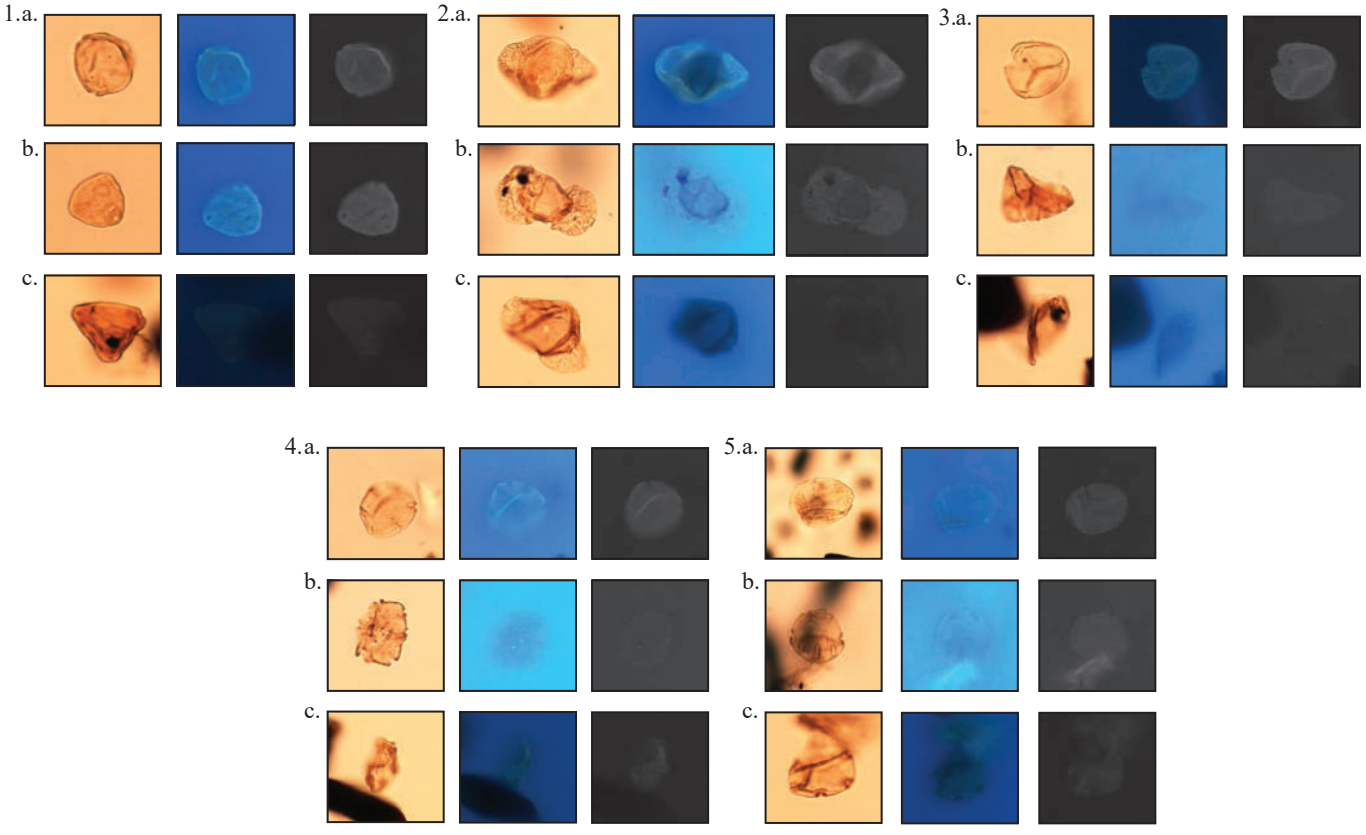

Figure 2. Images of Eocene, Oligocene and Miocene pollen and spore taxa analysed under white light, UV-fluorescence and red filter. The red filter shows better contrast between the grain and the background. 1. *Myricipites harrisii* (a) Miocene, Slide 15R-6W, 20-22 cm (b) Oligocene, Slide 84R-1W, 44-48 cm (c) Eocene, Slide 106R-2W, 80-83 cm, 2. *Podocarpidites ellipticus* (a) Miocene, Slide 35R-2W, 20-22 cm (b) Oligocene, 85R-3W, 20-24 cm (c) Eocene, 103R-4W, 120-124 cm, 3. *Cyathidites minor* (a) Miocene, Slide 15R-6W, 20-22 cm (b) Oligocene, Slide 50R-1W, 24-30 cm (c) Eocene, Slide 103R-4W, 120-121 cm, 4. *Nothofagidites lachlaniae* (a) Miocene, Slide 22R-2W, 20-22 cm (b) Oligocene, Slide 87R-5W, 40-44 cm (c) Eocene, Slide 97R-1W, 60-63 cm, 5. *Nothofagidites flemingii* (a) Miocene, Slide 12R-2W, 20-22 cm (b) Oligocene, Slide 59R-1W, 17-19 cm (c) Eocene, Slide 99R-2W, 40-43 cm.

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Published: 24 October 2016

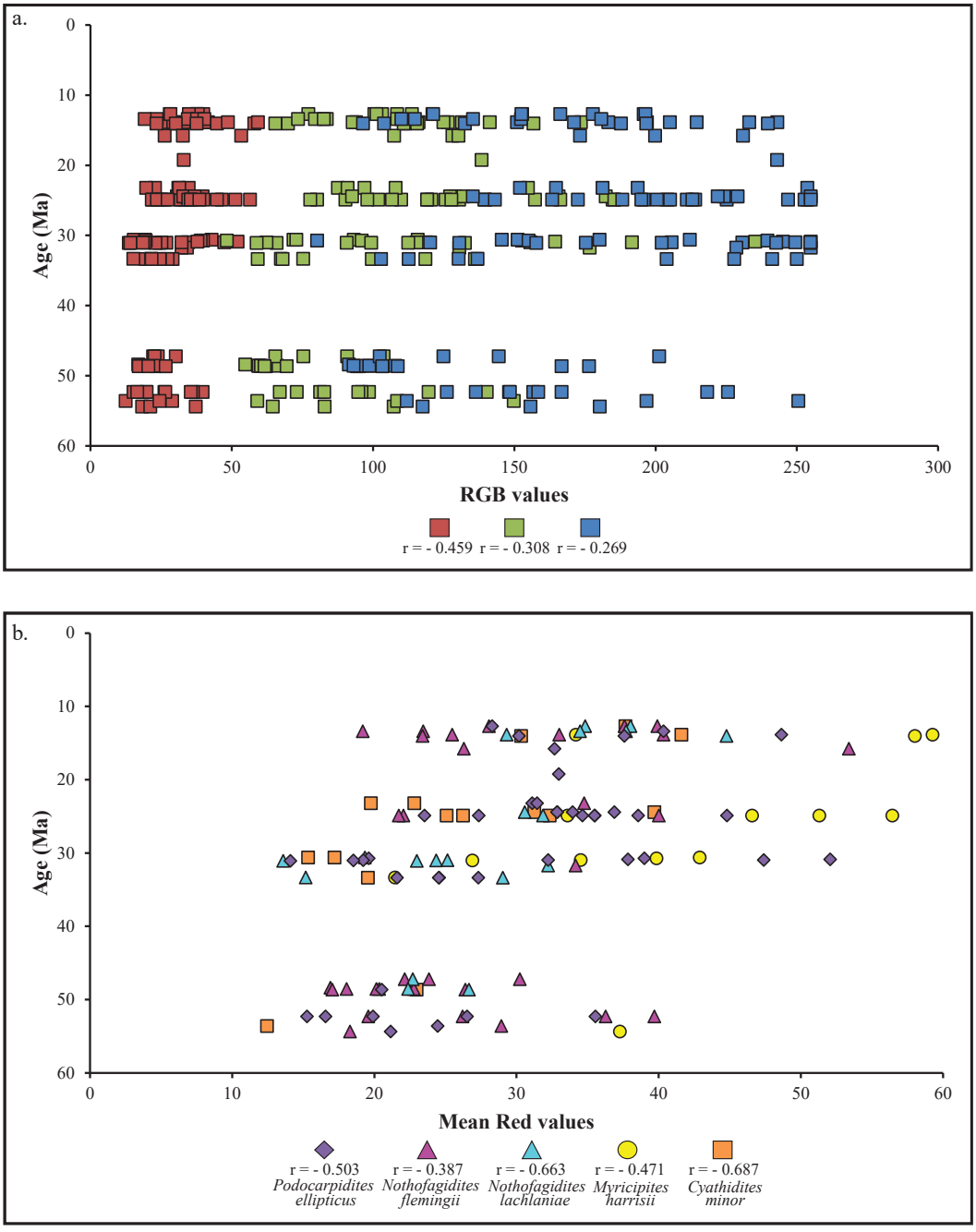

Figure 3. (a) Mean RGB values for each grain sample plotted against age with Pearson's correlation coefficient to show the relationship between the fluorescence colour and age. (b) Mean red values for each taxon (*Cyathidites minor*, *Myricipites harrisii*, *Nothofagidites flemingii*, *N. lachlaniae*, *Podocarpidites ellipticus*) plotted versus age along with Pearson's correlation coefficient to indicate the correspondence between the mean red values of each taxon and age.