GeoArabia, Vol. 13, No. 3, 2008
Gulf PetroLink, Bahrain

Colour change in the acritarch *Veryhachium* as an indicator of thermal maturity

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ABSTRACT

Veryhachium is an acritarch genus that occurs commonly in marine shales from the Ordovician to the Tertiary, with morphologically closely related forms appearing in the Cambrian. Its simple morphology together with its long stratigraphic range and abundance makes it attractive as a potential thermal maturity indicator. The colour of *Veryhachium* is characterised in terms of red, green and blue intensity of transmitted white light. With increasing thermal maturity, *Veryhachium* changes from almost colourless to black, with the most rapid change taking place in rocks that are marginally postmature with respect to the oil window. The cut-off point for *Veryhachium* fluorescence lies between 1.5% and 2.0% in terms of vitrinite reflectance (R_r), providing another useful tie-point in terms of thermal maturity. This technique is simple, inexpensive and has potential for establishing the thermal maturity of both marine Lower Palaeozoic rocks and younger marine sections deficient in vitrinite.

INTRODUCTION

Mean random vitrinite reflectance (VR) is the industry standard for assessing thermal maturity. However, vitrinite is absent from most Lower Palaeozoic successions, only appearing from the Upper Silurian onwards (Tricker et al., 1992). Because vitrinite precursors are derived from terrestrial sources, it is also sometimes rare or absent in post Silurian marine successions.

The assessment of microfossil colour is perhaps the most common tool used to assess thermal maturity of sediments after VR. Thermal alteration of microscopic organic material within sediments affects both its chemical and physical properties, typically resulting in colour change. Conodonts, spores and pollen are the most frequently used microfossils for assessing thermal maturity based on colour (Marshall, 1990).

The five point Conodont Alteration Index (CAI) of Epstein et al. (1977) was correlated with specific temperature ranges based on experimental heating. CAI is determined by visual estimation of colour and comparison with a representative series of photographs. This technique is widely used because of the range of temperatures that can be estimated and the relatively long (Cambrian to Triassic) fossil record of conodonts (Marshall, 1990). However, it is rarely applicable to lithologies other than limestones.

Many workers have explored techniques for describing palynomorph colour. Two different approaches have been attempted, the first based on spectral analysis of light transmitted through palynomorphs (Gutjahr, 1966; Grayson; 1975; Smith, 1983) and the second utilising image analysis to characterise colour in terms of red, green and blue intensity values (RGB) (Yule et al., 1998; Ujiié, 2001; McCaughan, 2002).

Early attempts to utilise spectral analysis included those of Gutjahr (1966) and Grayson (1975) who demonstrated a systematic decrease in spore transmittance with increasing depth of burial. Smith (1983) used spectral analysis data to correlate 'standard' palynomorph material from Staplin's TAI scale (1977), the Robertson Research International SCI scale and Batten's seven-point scale (1981). Lo (1988) measured the transmittance spectra of spores at a wavelength of 546 nm and compared the results with visually estimated TAI and vitrinite reflectance data. Marshall (1991) also used spectral analysis to describe spore colour change and was the first to use the *Commission Internationale de l'Eclairage* (CIE) colour system. This system defines colour in terms of three variables; *X* and *Y*, the chromaticity co-ordinates, and *L*, luminance; the total amount of light as defined by the transmittance or absorbance of a substance. The advantage of this technique is that inter-laboratory variation can be reduced significantly but the disadvantage is that micro-spectrophotometers required for this work are expensive and not readily available in most laboratories.

An alternative approach for the determination of palynomorph colour utilises colour image analysis (CIA). This involves digitising images of palynomorphs, which are usually displayed on a VDU and consist of an array of pixels each having specific RGB values (Ujiié, 2001). Using the RGB system, colours are described in terms of three variables; red, green and blue intensities. The intensity of each colour can vary between 0 and 255, and 16.7 million colours can be described using this scheme. The equipment necessary to make RGB colour determinations is inexpensive and readily available, comprising only a transmitted light microscope, a colour video or digital camera, and an image analysis system. However, unlike the CIE colour system, determinations of RGB intensities are device-dependant with the choice of microscope, camera and software all influencing the RGB values determined.

We discuss herein the determination of the colour of specimens of a single common and long-ranging acritarch genus (Plate 1), *Veryhachium,* using image analysis techniques. These results are correlated with VR determined from the same samples, enabling the relationship between the two variables to be defined.

ACRITARCHS AND ACRITARCH COLOUR ALTERATION

Acritarchs are considered to be the resting cysts of single-celled or apparently single-celled eukaryotic, predominantly marine, organic walled microfossils (Martin, 1993; Wicander, 2002). They vary in size from <10 µm to more than 1 mm, but the majority of species range from c. 15 µm to 80 µm (Wicander, 2002). First appearing in the Proterozoic, they are most abundant and diverse between the early Cambrian and late Devonian (Martin, 1993; Wicander, 2002; Playford, 2003).

Although much work has been carried out on spore colour and its use as a thermal maturity indicator, acritarchs have, for the most part, been neglected. Legall et al. (1981) first published an 'Acritarch

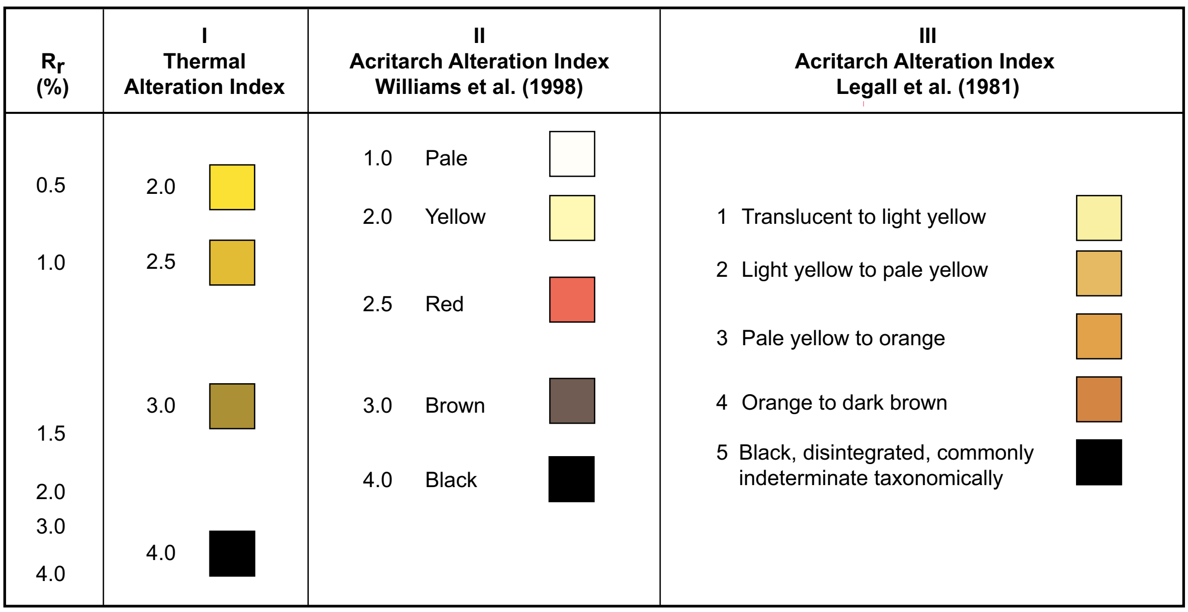


Figure 1: Correlation of various thermal maturity indicators after Legall et al. (1981) and Williams et al. (1998). I: Thermal Alteration Index from Staplin (1977) in Williams et al. (1998), colour values from Pearson (1984). II: Acritarch Alteration Index from Williams et al. (1998). Colour values are based on their description as no colour chart was published. III: Acritarch Alteration Index from Legall et al. (1981). Colour values based on their description (1, 5) and colour photographs (2, 3 and 4).

Alteration Index' (AAI) (Figure 1) based on the thermal maturation of Palaeozoic strata in Southern Ontario, Canada. In this study, they calibrated the colour of the sphaeromorph (leiosphere) acritarch genus *Leiosphaeridia* Eisenack 1958 emend Downie and Sarjeant, 1963 against CAI.

Legall et al. (1981) considered *Leiosphaeridia* to be the most suitable acritarch for colour determinations because of its simple morphology and large size. They visually estimated the colour of leiospheres and, to ensure consistency, compared them to colour photographs representing each point on their scale. Williams et al. (1998) studied Palaeozoic strata in western Newfoundland, Canada. Unlike Legall et al. (1981), they used acritarch assemblages rather than a single genus as a thermal maturity indicator. Their acritarch assemblage colours from Cambro-Ordovician rocks were correlated with TAI based on miospores extracted from nearby Carboniferous rocks (Figure 1).

Veryhachium Deunff 1954 ex Downie emend Sarjeant and Stancliffe is a common, long ranging (Ordovician-Tertiary, with morphologically closely related forms appearing in the Cambrian)

Plate 1

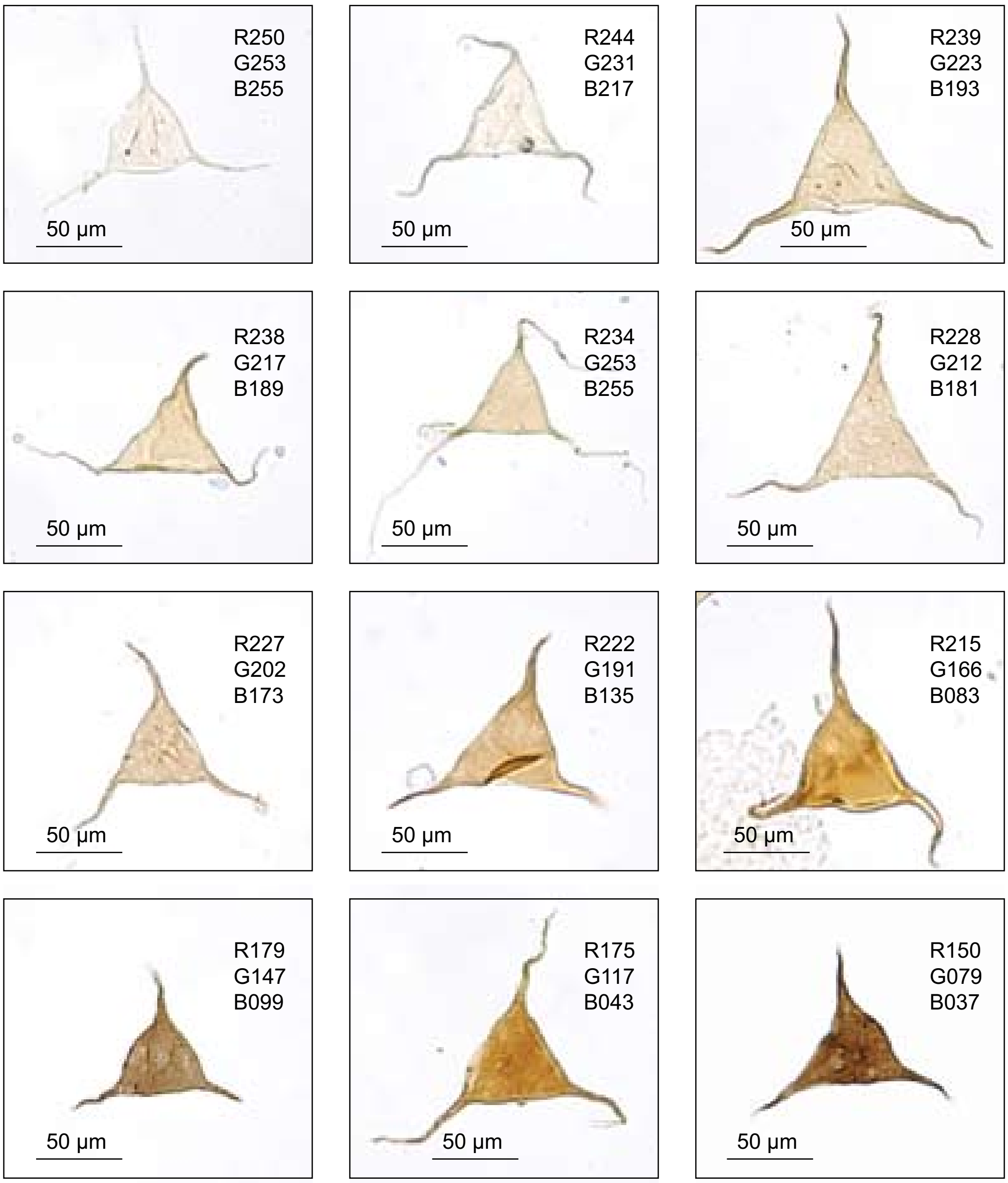


Plate 1: Representative specimens of *Veryhachium* together with their RGB intensities. Measured background R, G and B values were all with the range 249–254.

acritarch with a simple and smooth-walled morphology. Numerous species have been erected but they are all very similar to each other in terms of size and wall thickness so that inter-specific variation in colour is negligible. Only the triangular form of *Veryhachium* with one process at each apex was used. *Veryhachium* was selected for this study because it occurs commonly in muddy marine Lower Palaeozoic strata whose thermal maturity has often been extremely difficult to establish.

MATERIALS AND METHODS

Sampling

Silurian, Devonian and Lower Carboniferous samples were investigated in order to obtain residues that contained both acritarchs and vitrinite. Outcrop samples, cuttings and cores were investigated from several regions, including Jordan, Belgium, the United Kingdom, Venezuela, Canada and the USA, details of which are given in Table 1. The Jordanian samples contained chitinozoans but no vitrinite, so chitinozoan reflectance was measured and the equivalent VR was calculated using the relationship derived by Tricker et al. (1992).

Sample preparation

All samples were processed using standard HF extraction techniques (Wood et al., 1996) with no oxidation. Once processed, sieved organic residue was pippetted onto No. 1 thickness 22 x 40 mm coverslips and allowed to dry overnight. These were then mounted onto 1–1.2 mm thick, 76 x 26 mm slides using *Elvacite* mounting medium.

Acritarch colour determination

Slides were studied using a Leitz Dialux 20 transmitted light microscope with a Leitz NPL Fluotar L25/0.55 objective. Images of the selected acritarchs were captured using a JVC TK-C1380 colour video camera attached to the microscope's phototube. Illumination was provided by a Leitz Wild Heerbrugg power supply set to 9 V and a 100 W halogen light bulb. Equipment settings were not altered from one session to the next and the camera's white balance remained the same. Images of suitable palynomorphs were captured by the colour video camera and converted to a digital image by an analogue-to-digital converter (ADC) within the Leica Q500IW imaging workstation.

In making RGB colour determinations, pixels were only selected where the wall (eilyma) of *Veryhachium* was intact; thinned portions of the wall associated with the excystment structure or where the vesicle had been damaged were avoided, as were surface blemishes, pyrite inclusions, and the yellow 'halo' around the pyrite. Furthermore, the three processes of *Veryhachium* were excluded from measurement because of their limited surface area.

Vitrinite reflectance determination

Polished palynological sections offer an alternative to resin blocks. Polished slides were used in this study, based on a technique adapted from that described by Hillier and Marshall (1988). Vitrinite and chitinozoan reflectance measurements were made using standard techniques (Tricker et al., 1992; Taylor et al., 1998).

Fluorescence

Qualitative fluorescence properties were determined using the same palynological slides and microscope used for colour determinations. The microscope was fitted with a Leitz PLOEMOPAK 2.4 high-powered mercury vapour fluorescence illuminator and a Leitz H2 filter block (BP 390–490 excitation filter, RKP 510 dichroic mirror, LP 515 barrier filter). Using this system, violet-blue radiation (390–490 nm) was produced. Palynomorphs were viewed using a Leitz NPL Fluotar L25/0.55 objective in a darkened room. After approximately 1-minute excitation of the organic matter, the presence or absence of fluorescence was recorded.

Table 1

Details of samples used in this project. dc indicates cuttings sample; c denotes core; all other samples are from outcrop. R, G and B are *Veryhachium* mean RGB intensities; VR is vitrinite relectance (% R_r); sd is standard deviation and n is number of data. Samples are classified as containing well-preserved or poorly-preserved specimens. Those samples containing well-preserved specimens are further divided into mature (0.5–1.3%R_r) or postmature (>1.3% R_r) with respect to the oil window (Selley, 1998).

Sample	Location of outcrop, well, or borehole	Latitude / Longitude	Formation	Age	R	sd	G	sd	B	sd	n	R_r	sd	n	Preservation
TCD56736[c]	Ontario Dept of Mines Campbell Lake b.h, 880-881ft, Canada	50°14'30"N, 80°09'30"W	Kwataboahegan Formation	M. Devonian	224	4	231	4	202	10	4	0.5	0.09	12	Well-preserved specimens, mature with respect to the oil window
TCD56739[dc]	Well RH-3, 1320 - 1333m, Risha, Jordan	32°35'39"N, 39°2'36"E	Mudawwara Formation	Silurian	226	2	232	2	198	16	9	0.68	0.09	22	
TCD56742[dc]	Well RH-3, 1260m, Risha, Jordan,	32°35'39"N, 39°2'36"E	Khishsha Formation	Silurian	223	4	228	6	200	22	7	0.68	0.09	37	
TCD 56765[c]	Williams Co. Core b.h, 359ft, Superior Township, Ohio, USA	41°33' 32"N, 84°39'22"W	Bedford Shale	L. Devonian	227	4	233	3	196	24	21	0.79	0.24	100	
TCD 56737	Clay City Quarry, Powell County, Kentucky, USA.	37°52'09"N, 83°56'48"W	Chattanooga Shale	L. Devonian	224	5	225	6	166	20	6	0.85	0.21	90	
TCD56744[dc]	Well WS-6, 1208m, Wadi Sirhan, Jordan	30°42' 05"N, 37°29'33"W	Mudawwara Formation	Silurian	228	3	228	4	162	24	48	0.91	0.1	44	
TCD56743[dc]	Well WS-6, 1118m, Wadi Sirhan, Jordan	30°42' 05"N, 37°29'33"W	Mudawwara Formation	Silurian	229	6	216	15	135	31	32	0.92	0.15	28	
TCD56767[c]	Williams Co. Core b.h., 347ft, Superior Township, Ohio, USA	41°33'32"N, 84°39'22"W	Bedford Shale	L. Devonian	225	2	229	3	168	27	3	0.94	0.32	94	
TCD56756	Three Lock Quarry, Ross Co., Ohio, USA	39°17'26"N, 82°56'36"W	Bedford Shale	L. Devonian	226	3	230	2	184	25	25	1.03	0.27	40	
TCD 56751	Walnut Creek, Sunbury, Delaware Co. Ohio, USA	40°14'46"N, 82°50'60"W	Bedford Shale	L. Devonian	225	4	230	3	188	25	38	1.08	0.32	85	
TCD56766[c]	Williams Co. Core b.h, 315ft, Superior Township, Ohio, USA	41°33'32"N, 84°39'22"W	Bedford Shale	L. Devonian	227	3	231	2	182	21	6	1.08	0.26	32	
TCD56740[dc]	Well RH-3 , 520m, Risha, Jordan	32°35'39"N, 39°2'36"E	Khishsha Formation	Silurian	227	4	228	3	178	26	11	1.17	0.25	6	
TCD56752	Walnut Creek, Sunbury, Delaware Co. Ohio, USA	40°14'46"N, 82°50'60"W	Bedford Shale	L. Devonian	223	7	232	4	196	22	15	1.28	0.25	40	
TCD56753	Three Lock Quarry, Ross Co., Ohio, USA	39°17'26"N, 82°56'36"W	Bedford Shale	L. Devonian	225	3	218	16	156	48	5	1.28	0.17	34	
TCD56755	Three Lock Quarry, Ross Co., Ohio, USA	39°17'26"N, 82°56'36"W	Bedford Shale	L. Devonian	226	5	229	7	182	32	24	1.28	0.3	51	
TCD56754	Three Lock Quarry, Ross Co., Ohio, USA	39°17'26"N, 82°56'36"W	Bedford Shale	L. Devonian	227	4	219	13	154	40	21	1.49	0.19	52	Well-preserved specimens, postmature with respect to the oil window
TCD56764[c]	Cwrt-yr-ala borehole, 28.5m - 29.44m, South Wales, UK	51°27'10"N, 3°14'16"W	Tongwynlais Formation	Mississippian	199	23	184	37	121	54	6	1.97	0.42	27	
TCD56758	Villers Sur Lesse, Belgium	50°09'40"N, 05°06'58"W	Assise de Mariembourg	L. Devonian	124	58	105	41	64	15	3	2.13	0.21	13	
TCD56757	Villers Sur Lesse, Belgium	50°09'40"N, 05°06'58"W	Assise de Mariembourg	L. Devonian	154	26	121	22	66	11	16	2.27	0.29	12	
TCD56759	Villers Sur Lesse, Belgium	50°09'40"N, 05°06'58"W	Assise de Mariembourg	L. Devonian	156	23	123	21	65	12	24	2.31	0.3	11	
TCD56760	Villers Sur Lesse, Belgium	50°09'40"N, 05°06'58"W	Assise de Mariembourg	L. Devonian	149	18	117	16	61	10	11	2.45	0.5	27	
TCD56763	Caño Colorado, Sierra de Perijá, Venuzuela	10°41'78"N, 72°26'28"W	Campo Chico Formation	L. Devonian	176	11	145	11	79	11	7	2.72	0.26	10	
TCD56746[dc]	Well WS-6, 1357m, Wadi Sirhan, Jordan	30°42' 05"N, 37°29'33"W	Mudawwara Formation	Silurian	84	29	75	20	51	9	4	1.09	0.15	26	Poorly-preserved specimens
TCD56750[dc]	Well RH19, 1390-1400m, Risha, Jordan	31°49' 46"N, 38°9'45"E	Mudawwara Formation	Silurian	83	40	70	22	40	5	12	1.6	0.22	21	
TCD56762	Caño Colorado, Sierra de Perijá, Venuzuela	10°41'78"N, 72°26'28"W	Campo Chico Formation	L. Devonian	106	19	89	15	44	9	11	2.08	0.3	6	
TCD56761	Caño Colorado, Sierra de Perijá, Venuzuela	10°41'78"N, 72°26'28"W	Campo Chico Formation	L. Devonian	118	26	95	20	45	12	6	2.11	0.25	14	

RESULTS

Mean RGB intensities and VR values from the same samples are shown in Table 1 and in Figure 2. Obviously caved or reworked *Veryhachium* specimens were excluded from the calculation of mean values for each sample.

Mean RGB values from samples containing degraded *Veryhachium* specimens plot off the trend defined by the well-preserved samples. The colour of well-preserved samples represents the thermal alteration of the original eilyma, which has essentially remained intact. The walls of degraded specimens appear to have been thinned in some cases, counteracting the darkening caused by thermal alteration of the vesicle. Therefore, the thermal alteration scale proposed herein is based on only well preserved specimens of *Veryhachium*. Degraded and poorly preserved specimens were identified but excluded from the correlation with VR.

The cut-off point for *Veryhachium* fluorescence lies between 1.5 and 2.0% in terms of VR (R_r). This is at a significantly higher level of maturity than the VR level of c. 1.35% for spores (Van Gijzel, 1982).

DISCUSSION

RGB intensities as Thermal Maturity Indicators

Samples were classified as either mature (R_r 0.6 - 1.3%) or postmature (R_r > 1.3%) with respect to the oil window (Selley, 1998). The relationship between R, G, and B intensities and VR are summarised in Figures 2a to 2c and Table 2. It is clear that two discrete populations can be identified. This is particularly obvious for R vs. G mean intensities (Figure 3c) where mature samples cluster tightly and postmature samples show a large amount of scatter. There is little change in the colour of *Veryhachium* specimens until the floor of the oil window is reached. The lack of significant colour change in *Veryhachium* specimens in mature samples means a simple linear equation is an inadequate method for describing the darkening of the palynomorph colour with increasing maturity.

Comparison with Previously Published Acritarch Alteration Indices

In their study of thermal maturation of Palaeozoic strata in Southern Ontario based on *Leiosphaeridia*, Legall et al. (1981) described degraded leiospheres from strata with CAI values of 2–2.5 (indicating peak temperatures of 50°C to 90°C). They proposed a maximum peak palaeotemperature of 90°C for their Acritarch Alteration Index and suggested that leiospheres could not survive much higher temperatures. They also inferred a 60°C minimum palaeotemperature for the strata studied, based on a range of data collected in their study area. Results from the present study suggest that *Veryhachium* is useful as a maturity indicator for much higher palaeotemperatures, with well preserved specimens observed in samples with VR values of 2.45% indicative of peak palaeotemperatures of c. 207°C (Barker and Pawlewicz, 1994).

Table 2
Summary of correlation of VR (% R_r) with *Veryhachium* mean RGB intensities (I_r, I_g, I_b).

Intensity	Equation	R^2	95% Confidence Interval
Red	$R_r = -0.017 I_r + 4.8762$	0.72	± 0.72% R_r
Green	$R_r = -0.0128 I_g + 3.926$	0.8	± 0.61% R_r
Blue	$R_r = -0.0117 I_b + 3.118$	0.831	± 0.57% R_r

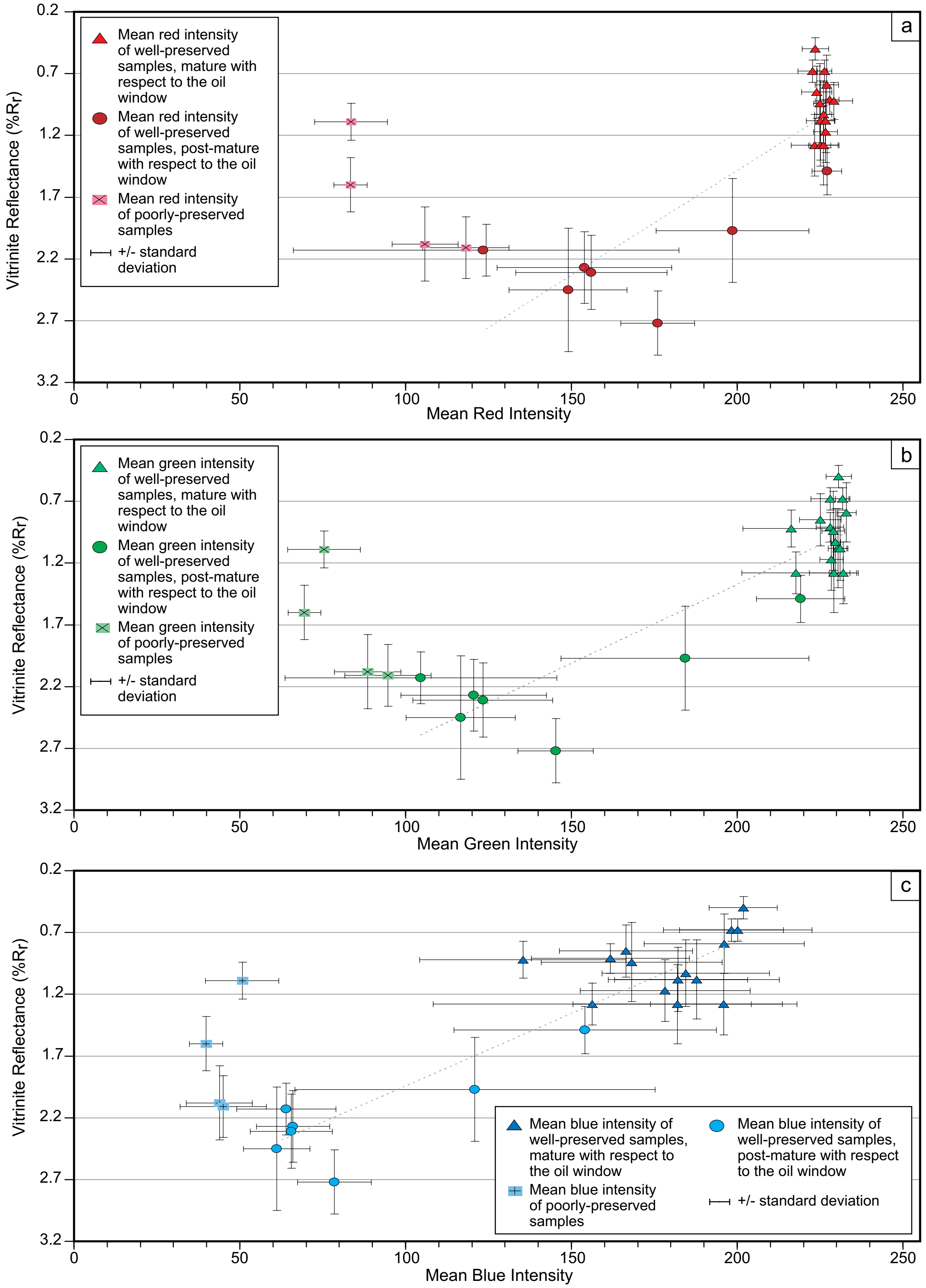
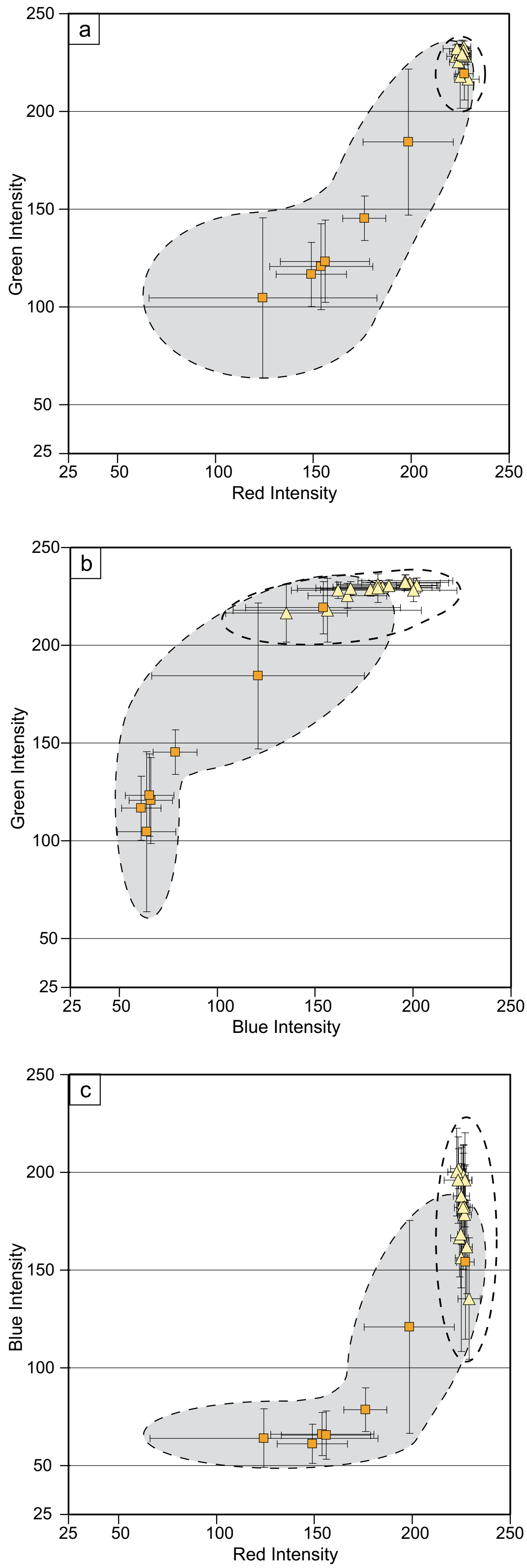


Figure 2: For each of the above plots, correlation between mean vitrinite reflectance (R_r) and mean RGB intensity is based on samples containing well-preserved *Veryhachium* specimens only.

(a) Mean vitrinite reflectance (R_r) relative to mean red *Veryhachium* intensity with both well-preserved and poorly-preserved samples shown.

(b) Mean vitrinite reflectance (R_r) relative to mean green *Veryhachium* intensity with both well-preserved and poorly-preserved samples shown.

(c) Mean vitrinite reflectance (R_r) relative to mean blue *Veryhachium* intensity with both well-preserved and poorly-preserved samples shown.



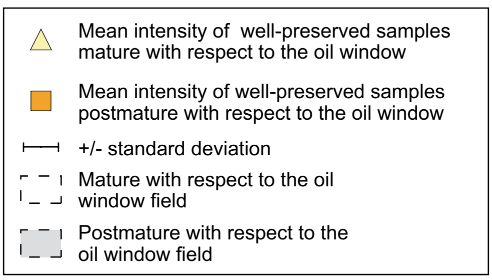
Comparison of *Veryhachium* Colour and Spore Colour

Bertrand and Heroux (1987) stated that for samples of equal rank, acritarch colour was lighter than spore colour. They also found that acritarchs were better preserved than spores in samples of higher maturity. Observations made during this study concur with these results.

Using multi-taxon acritarch assemblages, Williams et al. (1998) determined that, while there were different maturation paths for spores and acritarchs in immature strata, there was little difference within the oil window. They also found that AAI values in the upper part of the oil window were lower than corresponding (spore-based) TAI but by the time the floor of the oil window was reached, spore and acritarch colours were essentially the same. Our results show that marked differences in colour between *Veryhachium* and miospores still persist at the floor of the oil window. However, in many samples examined for this study, specimens belonging to other acritarch genera such as *Unellium* were observed to be darker in colour than those of *Veryhachium.* This observed variation in colour between genera probably accounts for the different conclusions reached.

A crude comparison of the change in colour of spores and *Veryhachium* specimens with increasing thermal maturity can be made. It is clear that whereas most spore colour change

Figure 3: *Veryhachium* mean RGB intensities relative to each other for all samples containing well-preserved specimens investigated in this study. In each of the three plots the mature and postmature fields with respect to the oil window are also shown.
(a) *Veryhachium* mean green intensity (G) v mean red intensity (R).
(b) *Veryhachium* mean green intensity (G) v mean blue intensity (B).
(c) *Veryhachium* mean blue intensity (B) v mean red intensity (R).



occurs within the oil window, *Veryhachium* colour appears to remain almost constant through this interval (Figure 4). It is not until after the floor of the oil window has been reached that significant *Veryhachium* colour change occurs.

In their CIA study of spores, Yule et al. (1998) found that most spore colour change occurred rapidly over a small temperature interval and interpreted this event as being directly related to the chemical breakdown of the spore wall during hydrocarbon generation. This represented the 'mature phase' of their samples where spores changed from a stable yellow colour to a stable brown/black colour. Our V*eryhachium* specimens did not change significantly in colour until after the floor of the oil window had been reached, suggesting that the chemical breakdown of their walls occurs at a different stage relative to that of spores, probably reflecting differences in chemical composition.

Choice of R, G or B as the Preferred Parameter

Based on R^2 value and calculation of 95% confidence intervals (Table 2), it appears that blue intensity (B) is more sensitive to changes in thermal maturity than red (R) or green (G) for our *Veryhachium* dataset. This contrasts with results obtained by Yule et al. (1998) who used CIE to quantify the colours of spores from a series of boreholes and from artificially matured samples and who found that average green intensity of spores within a sample was the most effective indicator of maturity. It also contrasts

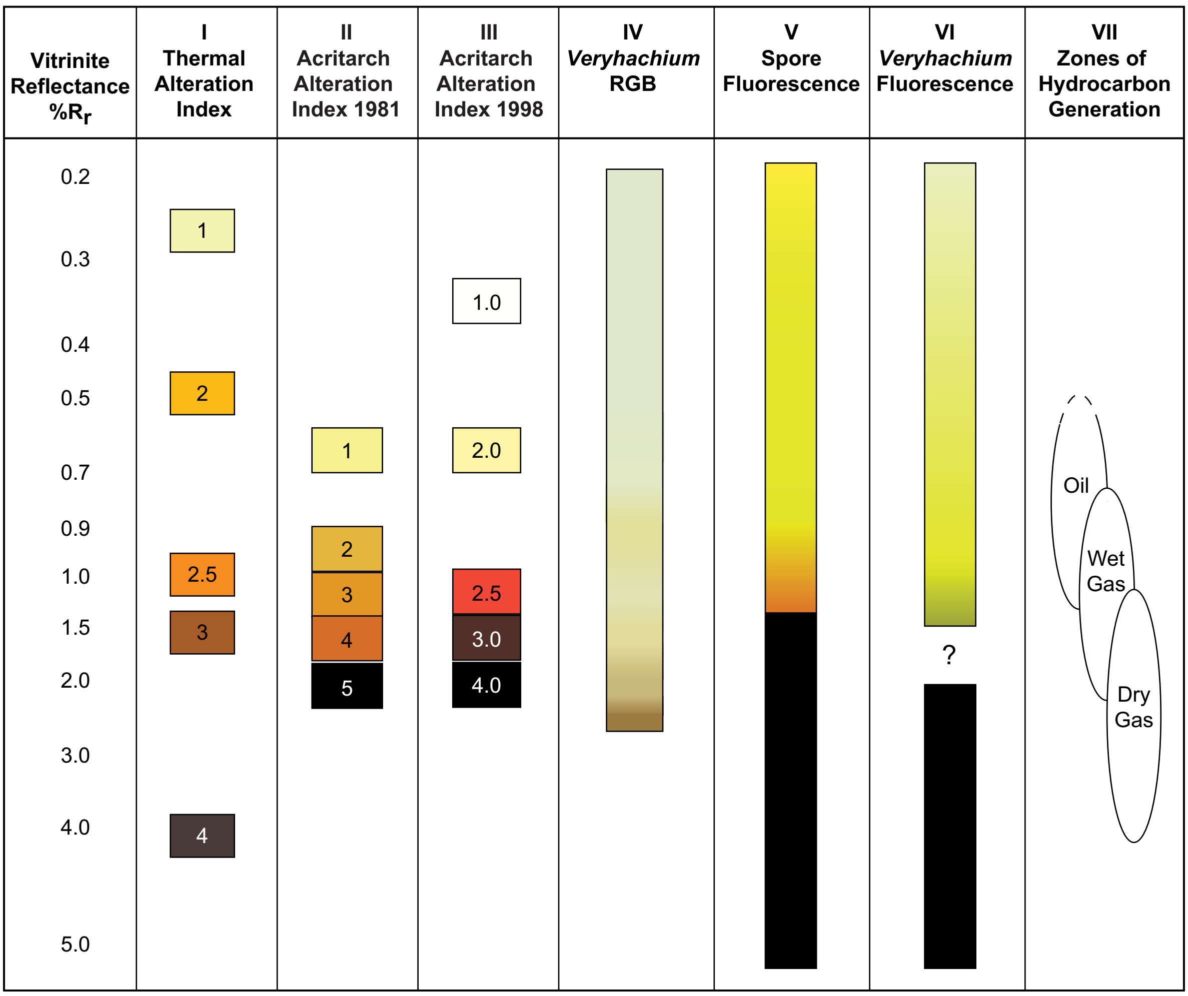


Figure 4: Tentative correlation of selected thermal maturity indicators. I: Staplin's Thermal Alteration Scale (TAI), correlation with vitrinite VR from Williams et al., 1998; colours from Pearson (1984). II: Acritarch Alteration Index (Legall et al., 1981). III: Acritarch Alteration Index (Williams et al., 1998). IV: *Veryhachium* RGB (this study). V: Spore fluorescence range (Van Gijzel, 1982). VI: *Veryhachium* fluorescence range (this study). VII: Zones of hydrocarbon generation (Taylor et al., 1998, figure 3.40).

with the conclusion of Van de Laar and David (1998), that red intensity (R) was the most sensitive parameter in terms of changing thermal maturity, based on their study of Carboniferous miospores.

Calibration

Several spore and pollen colour scales have been published, with Staplin's (1969, 1977) five point 'Thermal Alteration Index' (TAI) perhaps the most widely used. Other schemes include The 'Spore Colouration Index' (SCI), a ten-point scale produced by Robertson Research International (Barnard et al., 1976) and Batten's (1976, 1996) seven-point spore colour scale. Attempts to ensure inter-laboratory consistency have included published visual references such as the Pollen/Spore Colour 'Standard' of Pearson (1984) and sets of representative spore and pollen specimens, though neither of these has proved particularly successful.

Calibration of any formal *Veryhachium* Alteration Index would ideally utilize several readily-available standards consisting of thin sections of readily-available synthetic materials with colours closely corresponding to actual *Veryhachium* colours. Each standard would be allocated a nominal RGB value against which a microscope and camera could be calibrated. Preliminary attempts to identify suitable standards have included investigation of photographic filters but these have proved unsuccessful due to large variations in thickness and colour. A '*Veryhachium* Colour Index' based on relative R, G and B intensities would largely overcome the problem of device dependence. However, the darkening of *Veryhachium* with increasing thermal maturity represents broadly similar reductions in R, G and B intensities (Figure 3), so that an index based on a comparative measure such as $B(R+G+B)^{-1}$ would not be a satisfactory discriminant.

CONCLUSIONS

Determination of the colour of *Veryhachium* offers a partial solution to determining the thermal maturity of Lower Palaeozoic rocks and certain younger marine rocks deficient in other organic maturity indicators. Unlike spore colour, significant change in the colour of *Veryhachium* due to thermal alteration takes place only after the oil window floor has been reached. *Veryhachium* colour is most useful in distinguishing between samples that are mature, marginally postmature, and postmature with respect to the oil window.

The cut-off point for *Veryhachium* fluorescence lies between 1.5 and 2.0% in terms of VR (R_r). This is at a significantly higher level of maturity than the VR level of c. 1.35% for spores (Van Gijzel, 1982). Further work is needed to devise a system of calibration so that comparable *Veryhachium* RGB results can be obtained from different laboratories.

ACKNOWLEDGMENTS

This study was funded by a Petroleum Technology Scholarship from the Petroleum Affairs Division of the Irish Government in association with the Occidental Petroleum Corporation, Marathon Oil Corporation and ConocoPhillips. Additional funding was provided by Petrel Resources plc. and Trinity College Dublin. The authors are grateful to the following for assistance in collecting samples and data for the investigation; Craig Harvey, Mike Howe, Dave Naylor, Aideen McNestry, Munim Al-Rawi, Ron Rea, Thomas Servais and Ann Wilson. Thanks also to Robbie Goodhue, James Killen, John Marshall and Reed Wicander for their advice and support. The Jordanian Natural Resources Authority (NRA), the British Geological Survey, the Ohio Geological Survey, the Ontario Geological Survey and Petrel Resources plc. kindly provided access to samples and data for the study and have granted permission to publish. The final design and drafting by Nestor Buhay is appreciated.

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Manuscript received October 3, 2007

Revised December 17, 2007

Accepted December 22, 2007

Press version proofread by the authors March 24, 2008