SUPPLEMENTARY INFORMATION

Materials and Methods

Sample Description

A calcareous concretion containing a well preserved invertebrate fossil was collected from Paddy's Valley, Canning Basin, north-west Western Australia by Prof. Tim Sendon (Australian National University). These bedded carbonate concretions occur in clayey soils -derived from the rarely exposed soft shale (Playford et al., 2009). The carbonate concretion was found weathering out of the basinal black shales of the Frasnian-aged Gogo Formation, the oldest inter-reef deposited in the region. The nodules comprise thinly laminated carbonate muds.

The concretion was divided into part and counterpart and only one half used for the experiments described below. From the inner surface of the counterpart a thin slice (ca. 5 mm thick) containing most of the fossilized tissue was separated from the carbonate matrix. . Concentrically away from the fossil nucleus additional samples were taken from the carbonate matrix. All the samples were analyzed using the same techniques. Procedure blanks were performed to ensure no contaminants were incorporated in the samples.

Extractions

Both fossil and carbonate concretions were weighed into a pre-extracted cellulose thimble. The extraction was performed using a pre-extracted Soxhlet extractor using a combination of dichloromethane (DCM) and methanol (CH3OH, 9:1). Activated copper turnings were added to remove elemental sulfur. The extraction was allowed to proceed for 72 hrs until the solvent was clear. Excess solvent was removed from the extracts by rotary evaporation.

After the Soxhlet extraction, all samples residues were treated with Hydrochloric Acid (15% v/v) to remove the carbonates mineral. The de-carbonated residue was washed (x3) with pre-extracted deionized water and dry in an oven (40 ºC) overnight. After the residue was dry, each sample was extracted with a mixture of dichloromethane (DCM) and methanol (CH3OH, 9:1).using an ultrasonic bath (4Hr). The OM released after decarbonation was further separated by column chromatography as described below.

Column chromatography

The extract was separated by small columns (5.5 cm x 0.5 cm i.d.) filled with activated silica gel (120°C, 8 hr). Five fractions were separated using an elution scheme of solvents of increasing polarity. Aliphatic hydrocarbons were eluted in the first fraction with hexane (1⅜ column dead volume (DV) determined empirically for each silica bed) followed by aromatic hydrocarbons in 2 DV of 4:1 hexane: DCM, ketones and fatty acid methyl esters (FAME) in 2DV of DCM, alcohols in 2 DV of 4:1 DCM: ethyl acetate and the last polar fraction was eluted with 2 DV of DCM: methanol (7:3). The saturated and aromatic hydrocarbon fractions were reduced to near dryness with a N_2 purge and the fractions analyzed by gas chromatography - mass spectrometry (GC-MS). In addition the saturated hydrocarbon fractions were analyzed by gas chromatography – isotope ratio monitoring - mass spectrometry (GC-irMS).

Raney Nickel desulfurisation

The polar fraction (ca. 5 mg) of the fossil extract was desulfurised with Raney nickel. The fraction was dissolved in a minimum amount of toluene. Raney nickel was washed with double distilled water and dry ethanol and then added to the polar fraction from the fossil extract. The mixture was stirred and refluxed under a N2 stream for 2 h. The desulfurisation product was obtained by subsequent extraction with DCM and filtered over anhydrous MgSO4. The desulfurised polar fraction was further separated by column chromatography as described above. The saturated and aromatic hydrocarbon fractions were reduced to near dryness with a N_2 purge and the fractions analyzed by gas chromatography - mass spectrometry (GC-MS).

GC-MS

GC-MS analyses were performed with a Hewlett Packard 6890 gas chromatograph (GC) interfaced to a Hewlett Packard 5973 mass selective detector (MSD). The aromatic and saturated hydrocarbon fractions, dissolved in n-hexane, were introduced via the Hewlett Packard 6890 Series Injector into the electronically pressure controlled (EPC) split/splitless injector (320 ºC) which was operated in the pulsed splitless mode. The GC was fitted with a 60 m x 0.25 mm i.d. WCOT fused silica capillary column coated with a 0.25 μm phenyl arylene polymer stationary phase (DB-5MS, J&W Scientific). The oven temperature was programmed from 40 °C to 325 °C (at 3 °C/min) with the initial and final hold times of 1 and 50 min, respectively. Ultra high purity helium was used as the carrier gas and maintained at a constant flow of 1.1ml/min. The MSD was operated at 70 eV and the mass spectra were acquired in full scan mode, m/z 50-600 at ~ 4 scans per second and a source temperature of 230 °C. The aromatic hydrocarbon fractions were analyzed in full scan and selected ion-monitoring (SIM) modes. Diagenetic products of the C40 carotenoids of Chlorobi were analyzed by GC-MS to determine their relative

retention times for the given GC conditions. Data processing was performed with the Hewlett Packard Chemstation software. Semi quantitative analyses were performed in the saturated fraction in order to estimate the relative concentration within the concretion of diagnostic compounds for this study (n-alkanes, Pristane, phytane and staranes). Calibration curves were prepared using different standards (C17, C25, Squalane and Cholestane) ensuring a linear range within 0.2 ppm and 40 ppm. All the concentrations reported in the publication are in ppm (mg/kg) of subsample.

GC-irMS

A Micromass IsoPrime isotope ratio monitoring - mass spectrometer (irm-MS) coupled to a Hewlett Packard HP6890 gas chromatograph (60 m x 0.25 mm i.d., 0.25 μm thick DB-1 phase) was used to determine the $\delta^{13}C$ of the selected compounds in the saturated hydrocarbon fractions from the extracts obtained from the fossil and calcareous matrix. The samples were injected using pulsed splitless mode (30 seconds hold time at 15 psi above the head pressure of the column and 35 seconds for purge). The GC oven was programmed with the same temperature ramp as the GC-MS analysis. The $\delta^{13}C$ compositions of the compounds were determined by integrating the 44, 45 and 46 mass ion currents, and are reported in parts per mil (‰) relative to the international Vienna Peedee belemnite (VPDB) standard. Reported values are the average of at least two analyses.

Supplementary Figure Captions

Figure DR1. GC-MS ion chromatogram of *m/z* 133 of standards compounds of authentic Chlorobi carotenoids (isorenieratane, Rt: 113.27 minutes and renieratane Rt: 114.13 minutes) were analyzed and their retention time and mass spectra were compared with the free and sulfur bound aromatic hydrocarbon fractions extracted from the fossil.

Figure DR2. Identification of steranes was performed by GC-MS selected ion chromatogram of the typical ions (*m/z* 217, 218) and molecular ions of the C_{27}, C_{28} and C_{29} steranes (*m/z* 372, 386 and 400). GC-MS ion chromatogram of *m/z* 217 of: A. Saturated hydrocarbons from the fossil extract B. S-bound saturated hydrocarbons released by Raney Nickel desulfurisation from the polar fraction of the fossil extract.

Figure DR3. Mineralogy of the fossil layer of the sample by X-Ray Diffraction analysis (XRD). Mineral as calcite and fluorapatite are part of the replacing minerals of the fossil tissue.

Figure DR4. Elemental mapping of the fossil surface by Scanning Electron Microscopy (SEM). An association of carbon, oxygen and calcium is dominant, consistent with calcite in the fossil and concretion. Elements as Si, Al, K, Fe, Mg, Ti, P, F, and S, where also identified and corresponds with the minerals identified by XRD. Additional Fe and S where associated in aggregated from 4 to 20 μm in diameter, corresponding to pyrite.

Figure DR1

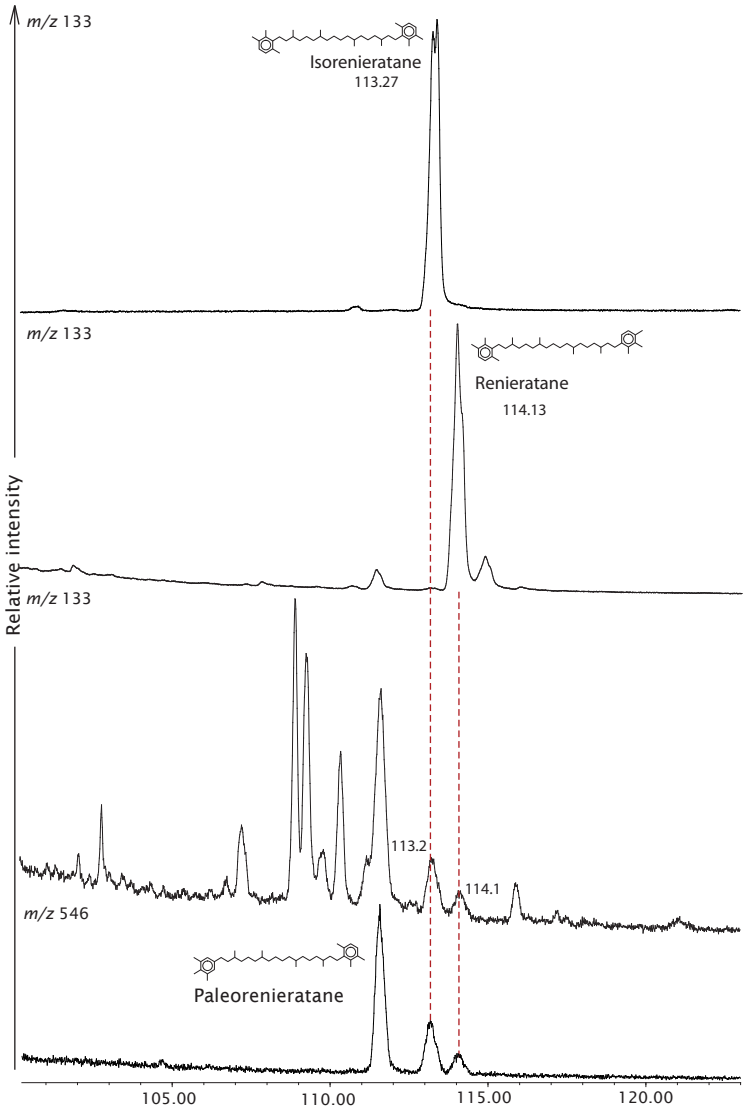
m/z 133
Isorenieratane
113.27
m/z 133
Renieratane
114.13
Relative intensity
m/z 133
113.2
114.1
m/z 546
Paleorenieratane
105.00
110.00
115.00
120.00

Figure DR2

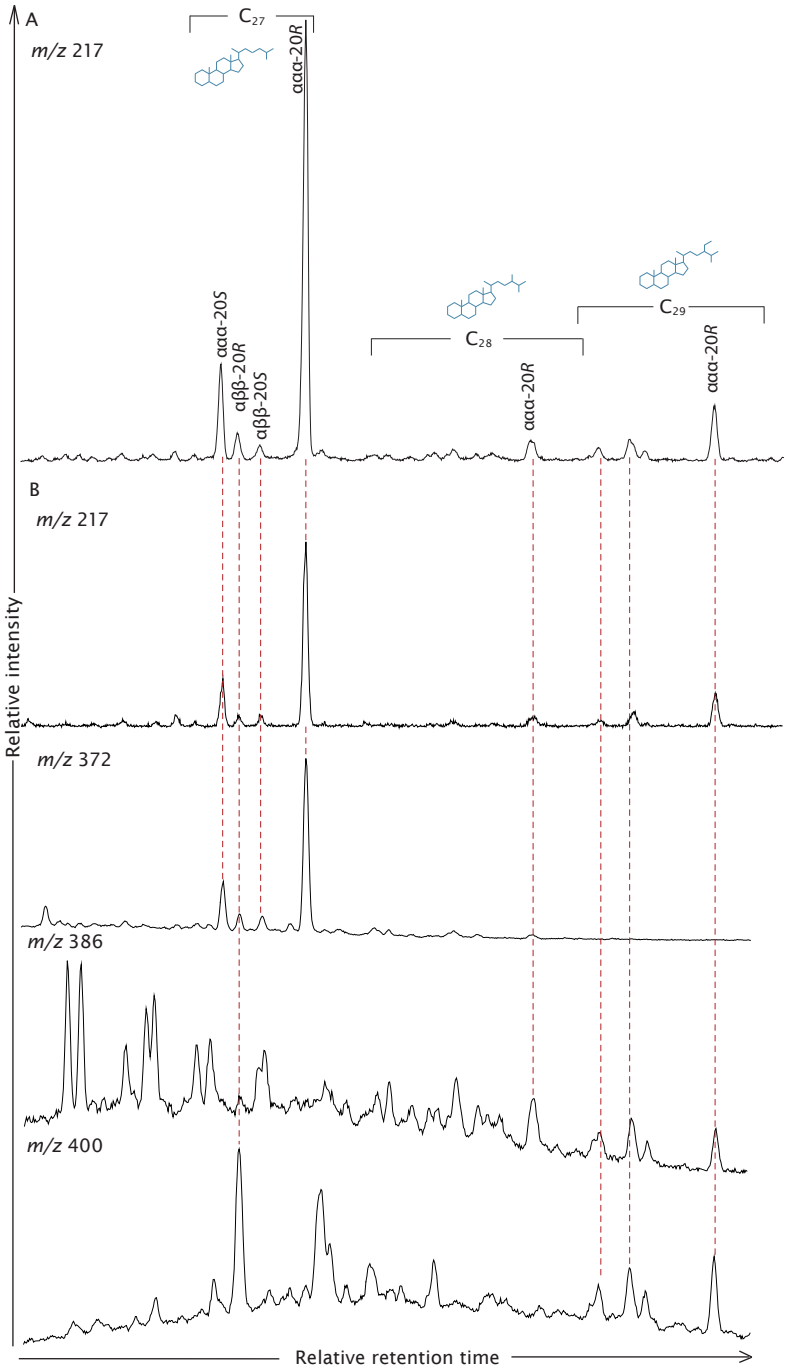
A
m/z 217
C27
αααα-20R
αααα-20S
αββ-20R
αββ-20S
C28
αααα-20R
C29
αααα-20R
B
m/z 217
m/z 372
m/z 386
m/z 400
Relative intensity
Relative retention time

Figure DR3

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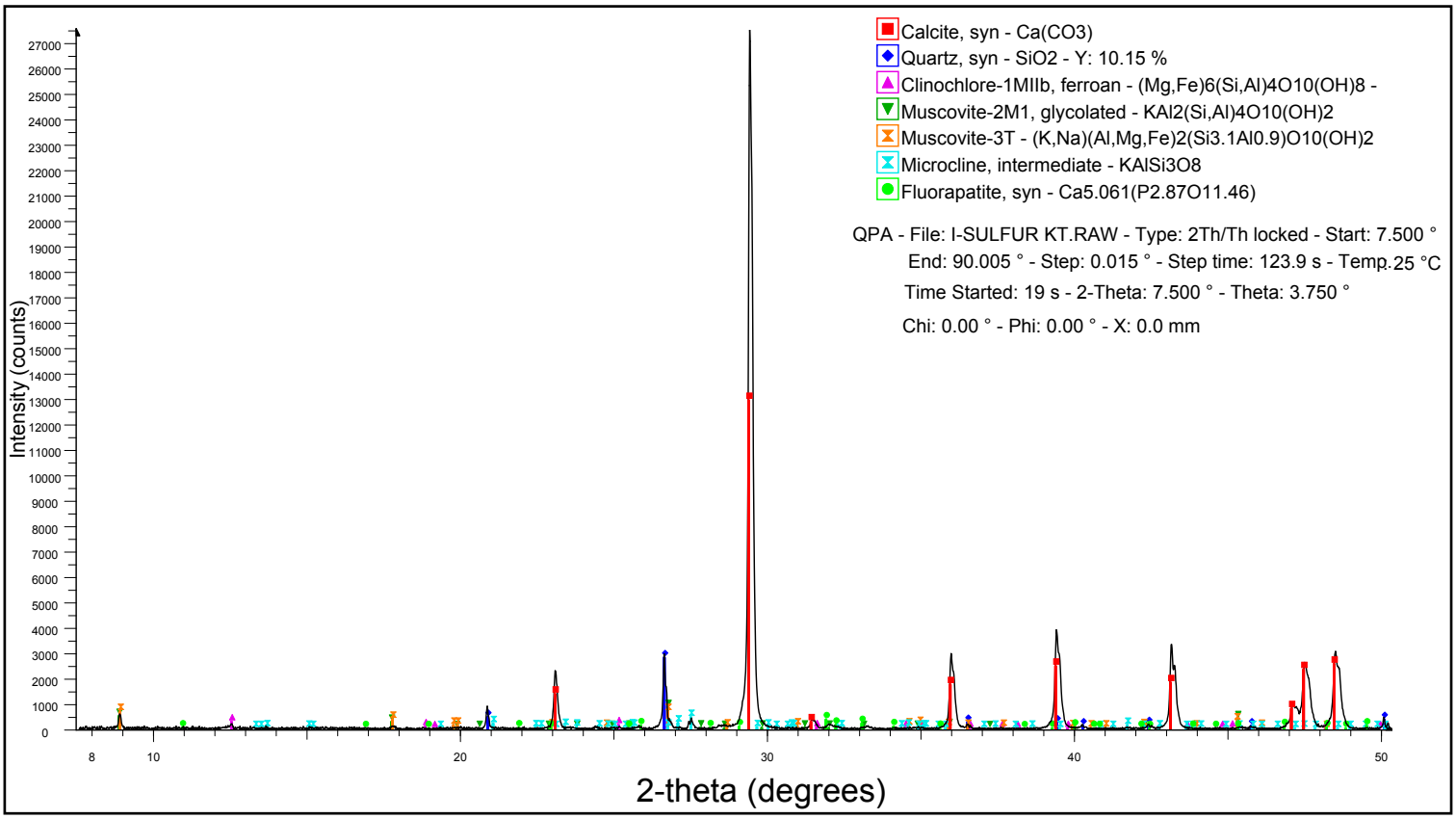
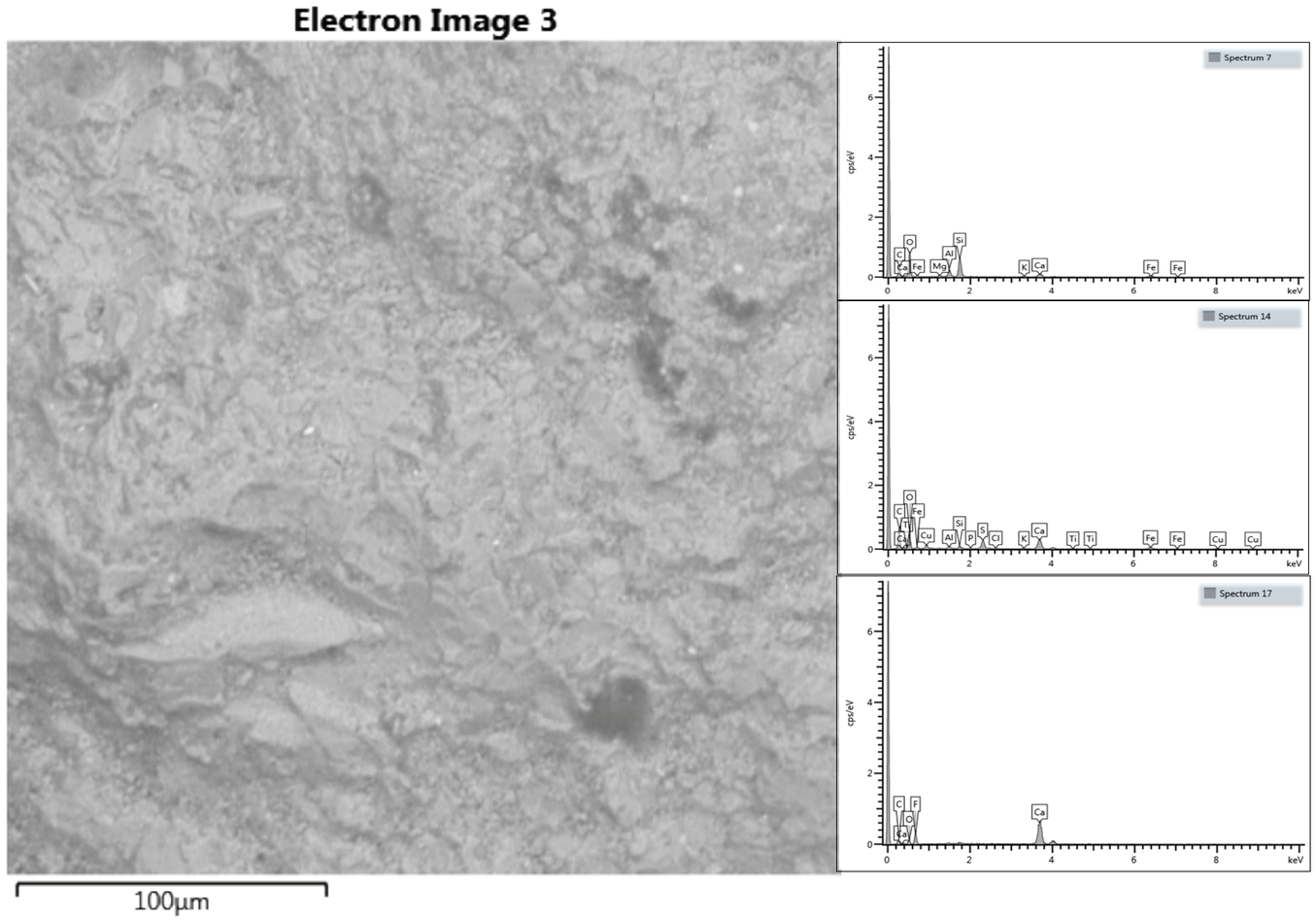


Figure DR4


Electron Image 3
100µm
Spectrum 7
Spectrum 14
Spectrum 17
cps/eV
keV