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A possible chlorophycean affinity of some Neoproterozoic acritarchs

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Abstract

Two taxa of Neoproterozoic acritarchs of unknown affinity, *Multifronsphaeridium pelorium* and Species A, are analysed by electron microscopic (SEM, TEM) and chemical (micro-FTIR, pyrolysis GC–MS, thermal desorption–MS) methods. Both acritarch species are characterised by multi-branched processes and a remnant trilaminar sheath (TLS) structure. The TLS-bearing wall structures in these acritarchs suggest a possible biological affinity to chlorophyte algaenan. The molecular data obtained from the two acritarchs were generally similar and also consistent with a chlorophycean affinity. A significant aliphatic moiety is evident in these acritarchs as a short-chain series of *n*-alkene/alkane pyrolysates and prominent aliphatic IR bands. The restricted molecular-weight range ($<C_{20}$) of the *n*-alkene/alkane doublets and the lack of isoprenoid and other branched alkanes in the pyrolysates suggest a low degree of branching in the aliphatic component of these acritarch macromolecules. The significant methyl (CH_3) IR signal was attributed to the terminal groups of short *n*-alkyl moieties. Alkylbenzenes, alkylphenols and alkylindoles were also significant pyrolysis products, indicating an aromatic component, although the latter two components may be attributed to artificially- and/or diagenetically-formed melanoidin moieties. The macromolecular structure of *Multifronsphaeridium* sp. and Species A consists of short *n*-alkylpolymethylenic chains, probably linked via ether/ester bonds, with possibly a small aromatic content. This study presents ultrastructural and molecular evidence of a genetic relationship between Neoproterozoic acritarchs and Chlorophyceae.

Keywords: Neoproterozoic acritarchs; Chlorophyceae; Algaenan; Electron microscopy; Trilaminar sheath cell walls; Micro-FTIR; Laser micropyrolysis GC–MS; Thermal desorption–MS

1. Introduction

The evolution of eukaryotic algae and the affinity of acritarchs are still a matter of considerable debate. In their recent review of the biological affinities of Paleozoic acid-resistant, organic-walled algal microfossils, including acritarchs, Colbath and Grenfell (1995) indicated that only the Prasinophyceae had a record extending back to the Proterozoic, whereas most other algal groups first appeared in the Silurian or later. On the other hand, molecular phylogeny indicates a much earlier (~1000 Ma) algal radiation (e.g. Knoll and Butterfield, 1989; Knoll, 1996). In the most recent palynological study of Neoproterozoic acritarchs from the Australian Centralian Superbasin (Grey, 1998), a prasinophycean affinity has, indeed, been demonstrated for a few species such as *Tasmanites* sp., some

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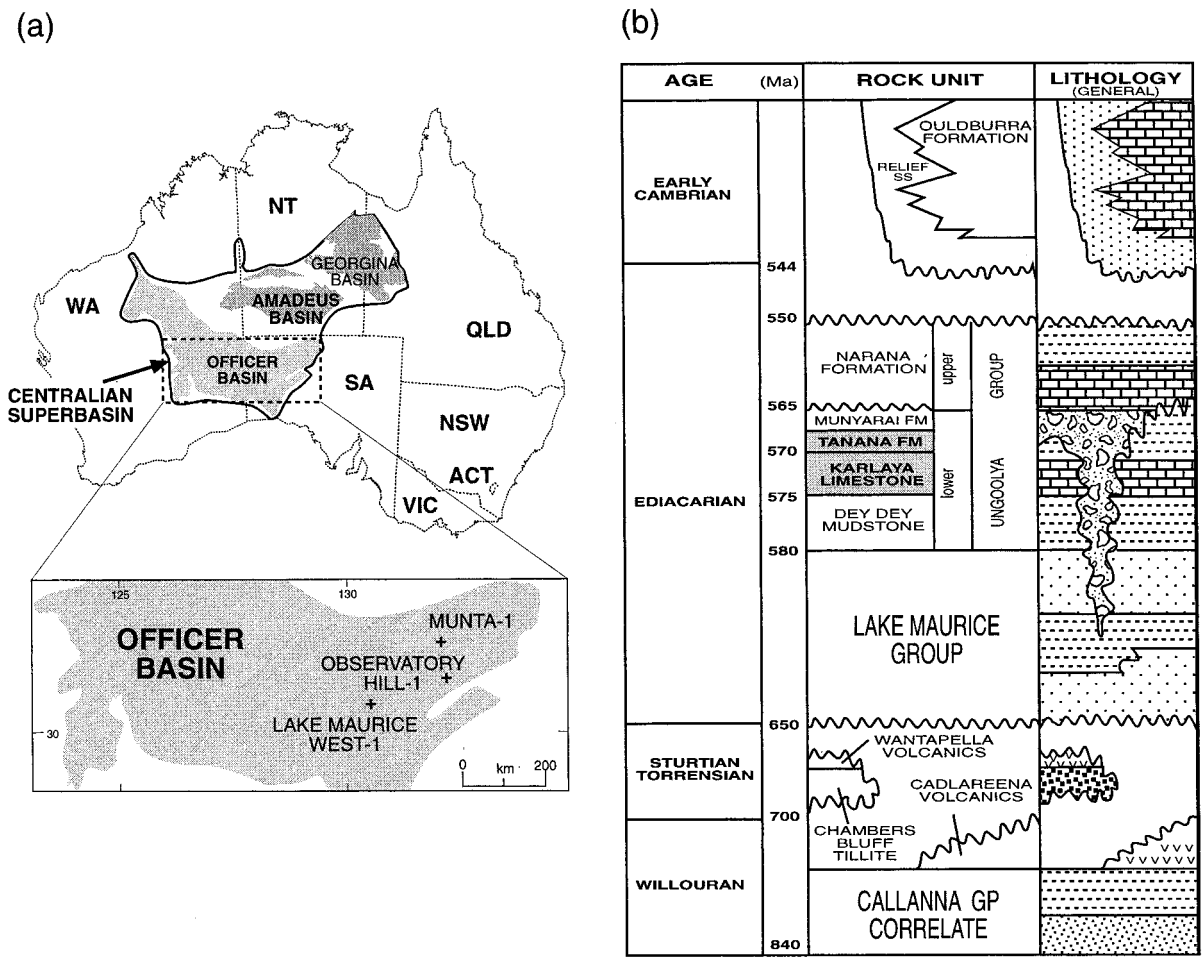

Fig. 1. (a) Location of the Officer Basin within the Australian Centralian Superbasin showing the boreholes sampled for acritarchs. (b) Simplified Neoproterozoic to Early Cambrian stratigraphy of the eastern Officer Basin showing the Ediacarian formations (shaded) sampled for acritarchs.

Leiosphaeridia sp. and *Appendisphaera* species. A few other acritarch species were also assigned to other classes of the Division Chlorophyta (*viz*. Chlorophyceae and Zygnemaphyceae) and the Division Pyrrhophyta (*viz*. Dinophyceae). These assignments are based solely on preserved morphological similarities and, therefore, remain largely uncertain and speculative. For example, although fossilised dinoflagellate-like cysts are not uncommon in pre-Triassic (Colbath and Grenfell, 1995), including even Neoproterozoic (Grey, 1998; Butterfield and Rainbird, 1998) sediments, their true relationships are generally indecisive due to the lack of paratabulation, paracingulum and archaeopyle, considered diagnostic for dinoflagellates in post-Permian sediments.

In our previous microscopic and chemical investigation of several Neoproterozoic acritarchs, a possible link was suggested between some acanthomorph taxa and dinoflagellates based on ultrastructural and molecular similarities such as a distinct fibrillar multilayered wall and a condensed polyaromatic macromolecular structure (paper currently in review). This data has several parallels with structural data obtained independently from the resting cysts of the marine dinoflagellate *Lingulodinium poleydra* (Kokinos et al., 1998). This conclusion is also consistent with previous biomarker (Summons et al., 1988; Summons and Walter, 1990; McKirdy and Imbus, 1992; Zang and McKirdy, 1994; Moldowan and Talyzina, 1998) and palynological (Mendelson and Schopf, 1992; Martin, 1993; Grey, 1998; Butterfield and Rainbird, 1998) speculations that some dinoflagellates had become established by the Neoproterozoic. However, the only direct biomarker evidence of an intimate re-

lationship between acritarchs and a certain algal group (in this case dinoflagellates) was the detection of dinoflagellate-specific biomarkers dinosteranes and 4α-methyl-24-ethylsteranes in the pyrolysates of Early Cambrian acritarch concentrates (Moldowan and Talyzina, 1998). No reports are known of these biomarkers from older, individually-analysed, acritarchs.

Despite the recent findings regarding the biological affinities of acritarchs, a direct molecular relationship between Neoproterozoic acritarchs and known algae has not yet been identified, and the majority of these acritarchs remain of unknown affinity. Here, we examine two further acritarch taxa from the late Neoproterozoic (Ediacarian, 575 to ~567 Ma) sedimentary succession. These acritarchs are: (1) the genus *M. pelorium* Zang *in* Zang and Walter (1992, p. 81); and (2) Species A, a new genus and species of acritarch with multistranded processes described by Grey (1998, p. 403, pl. 8.48, A–D; pl. 8.49, A–C) and is herein referred to as Species A, pending formal systematic description. These species show no morphological similarity with known algae (Grey, 1998) and no inference of their biological affinity has yet been made. These 'biomacromolecules' have been analysed by both microscopic and chemical methods to gain insights into their ultrastructural and molecular compositions.

2. Experimental methods

2.1. Samples

A collection of superbly-preserved Neoproterozoic acritarchs have been obtained from the Ediacarian (575 to ~567 Ma) lower Ungoolya Group in the Observatory Hill-1, Munta-1 and Lake Maurice West-1 boreholes in the eastern Officer Basin, South Australia. The location of the boreholes and the stratigraphic horizons sampled for acritarchs are shown in Fig. 1a, b. *Multifronsphaeridium* sp. samples were collected from kerogen concentrates of the Tanana Formation of the lower Ungoolya Group (Observatory Hill-1, 232.2–233.2 m; Munta-1, 1198.6–1443.6 m), whereas Species A specimens were sampled from the Karlaya Limestone (Lake Maurice West-1, 310.4–314.6 m). The *Multifronsphaeridium* sp. is common in the Ediacarian lower Ungoolya Group (Officer Basin) and the Pertatataka Formation (Amadeus Basin), whereas Species A is known from the Karlaya Limestone and basal Wilari Dolomite Member, Officer Basin (Grey, 1998). Individual acritarch specimens were hand-picked from the >25 μm fractions of the kerogen concentrates, under a stereomicroscope. Acritarch-containing sediments of the Tanana Formation and the underlying Karlaya Limestone in eastern Officer Basin comprise rhythmically-laminated and graded-bedded calcareous mudstone and dolomitic siltstones which deposited variously in hemipelagic, chemogenic or shallow-water (peritidal) environments. The marginal thermal maturity of these formations is discussed in section 3.1 below.

2.2. Microscopy

An Olympus BH2 microscope was used for initial white-light and UV-excitation observations and photography. Detailed ultrastructural microscopy was carried out on a Jeol JSM scanning electron microscope (SEM) and a CM10 Philips transmission electron microscope (TEM). Hand-picked acritarch samples were dehydrated using the CO_2 critical-point technique prior to gold-coating and subsequent SEM observations. For TEM, the samples were fixed for 4 h in 1% glutaraldehyde and 4% paraformaldehyde mixture buffered at pH 7.2 with 0.1 M phosphate buffer and post-fixed in a 1% O_sO_4 for 1 h in the same buffer. Block-staining with 2% aqueous uranyl acetate for 30 min was followed by dehydration with methanol, infiltration with resin/ethanol mix for 1 h, infiltration in LR White pure resin for ~18 h, and then embedding and polymerisation at 60°C for 24 h, before sectioning (1 μm) on a Reichert ultra-microtome and staining with 1% methylene blue/2% aqueous uranyl acetate/Reynolds lead citrate.

2.3. FT-IR spectroscopy

Fourier transform infrared (FT-IR) spectra were acquired with a Magna-IR 760 spectrometer equipped with a Nicolet, Nic-Plan IR microscope. The microscope was fitted with a 250-μm diameter narrow-band MCT (mercury–cadmium–telluride) detector cooled to 77°C, and both 20 and 40× objectives were used. Transmission spectra were acquired at a resolution of 8 cm^{-1} over a frequency interval of 650–4000 cm^{-1}.

2.4. Thermal desorption–mass spectrometry (TD–MS)

TD–MS was performed on 10 μg (~50 specimens) of acritarchs using the solids-probe of an AutoSpec-UltimaQ double focusing mass spectrometer. The sample was heated from 200 to 650°C at 20°C/min. Mass spectra (EI) were obtained with 16 eV ionisation energy over the range m/z 20–750 amu at a scan rate of 1 scan/s. These mass spectrometric parameters were selected to mimic the acquisition parameters used in a recent direct temperature-resolved MS analysis of marine dinoflagellate resting cysts (Kokinos et al., 1998).

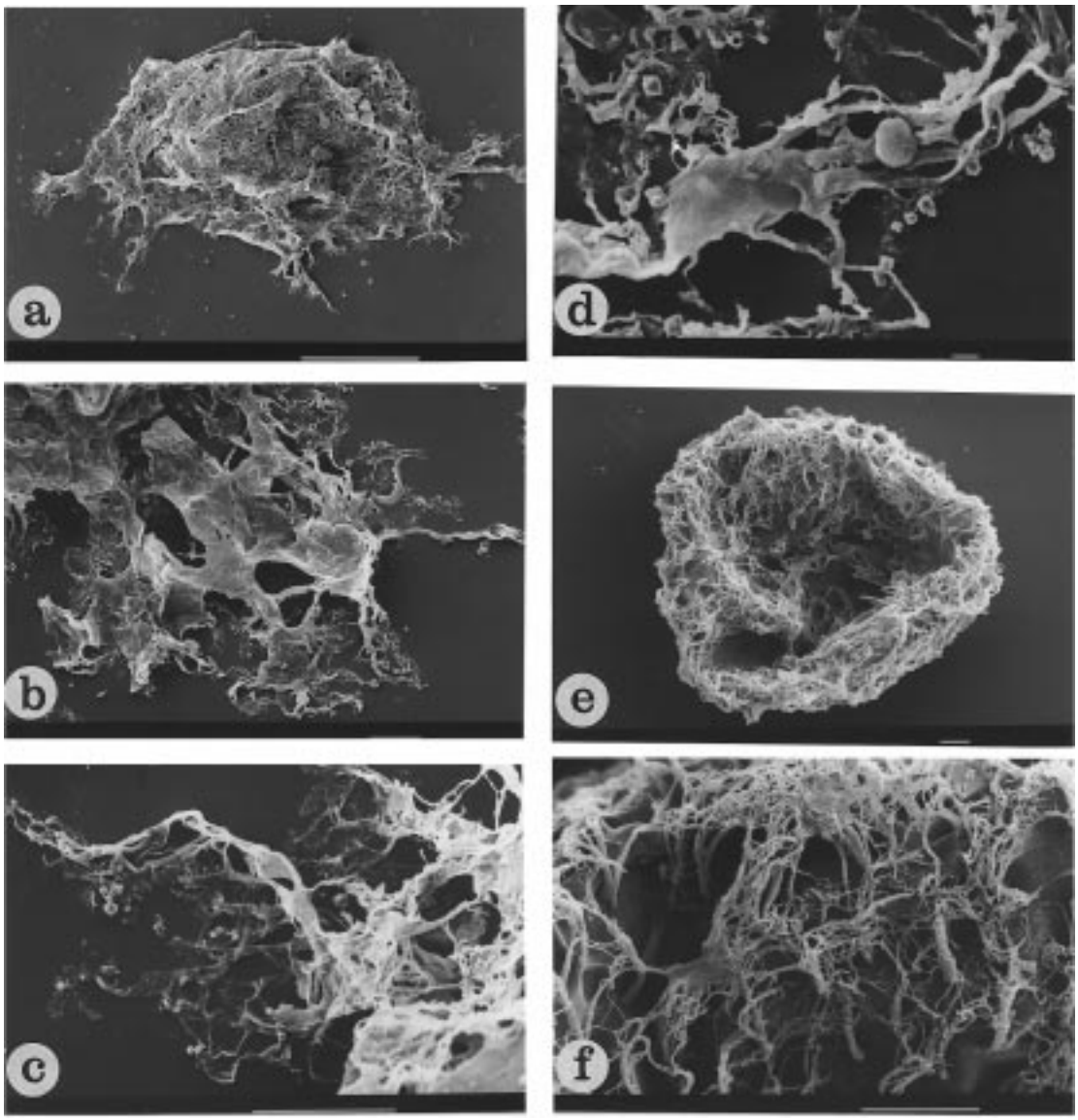

Fig. 2. Scanning electron micrographs of acritarchs: (a–d) the genus *M. pelorium*; and (e, f) Species A, showing their multi-stranded processes. *M. pelorium* is a large (~0.1–1 mm) ellipsoidal central body surrounded by complex processes and multiple branching (b–d), whereas Species A is a smaller (200–300 μm) subspherical mass of finely-divided and dense processes with no distinct central outline (f). Scale bar: (a) = 100 μm; (b, c, e, f) = 10 μm; d = 1 μm.

2.5. Laser micropyrolysis gas chromatography-mass spectrometry

Laser micropyrolysis GC–MS was performed on small populations (≤ 10) of acritarch specimens. Details of the laser technique have been outlined previously (Greenwood et al., 1996, 1998) and a brief description is given here. Pyrolysis was achieved by 30 s of ~19 W/s of 1064 nm irradiation. GC–MS detection of the laser pyrolysates was performed with a HP 5890/Series II gas chromatograph interfaced to a Micromass-Autospec UltimaQ mass spectrometer. Chromatography was carried out on a 25-m DB-5 column (5% phenyl polysiloxane, 0.32 mm i.d., 0.52 μm film thickness). The GC was typically temperature-programmed for an initial 40°C, held for 2 min, then increased at 4°C/min to a final temperature of 300°C, held for 25 min. Two mass spectral analytical modes were used: (1) full scan from m/z 50 to 550, and (2) selected ion monitoring (SIM) of m/z 91 (toluene), 94 (phenol), 97 (*n*-alkenes), 99 (*n*-alkanes), 105 and 106 (alkylbenzenes), 107 (alkylphenols) and 110 (benzene-

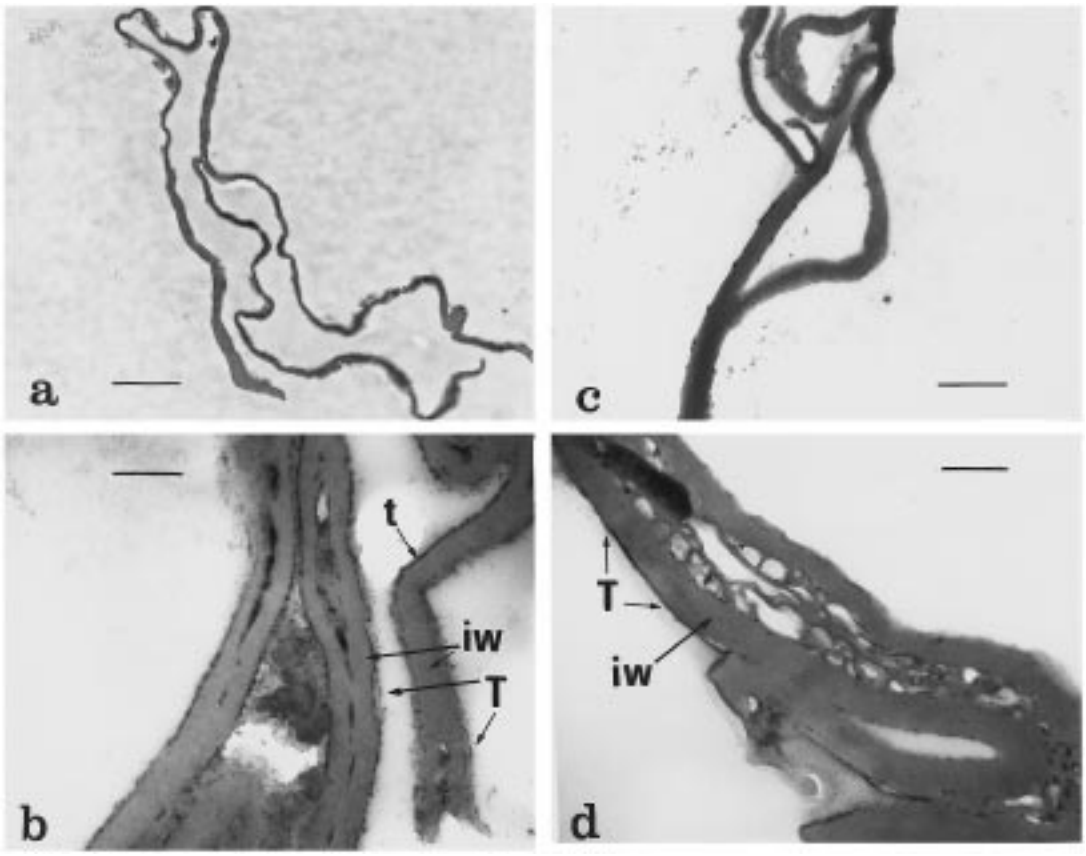

Fig. 3. Transmission electron micrographs of ultra-thin sections of: (a, b) *M. pelorium*; and (c, d) Species A acritarchs, showing the ultrastructural details of their walls. The electron-lucent, thick, inner wall (iw) is enveloped by a thin external layer (T) with a possible trilaminar sheath organisation present either intact (i.e. showing the characteristic TLS trilaminar organisation (T)) or as remnant (t), where the middle sheath of the TLS structure is not visible. Scale bar: (a, c) = 1 μm; (b, d) = 150 nm.

diol). The mass resolution was 1000 for both modes of analysis. Mass spectra were obtained using standard detection parameters (electron energy = 70 eV; filament current = 200 μA; source temperature = 250°C; electron multiplier = 200 V).

3. Results

3.1. Thermal maturity of the acritarchs and the host-rock

The present set of acritarch species are yellow–golden brown in colour in transmitted light, and exhibit yellow fluorescence under UV-excitation, a result indicative of thermally-immature or only marginally mature organic matter (cf. Staplin, 1977; Traverse, 1988, for example). This is similar to the maturity status of other acritarch samples analysed previously from the eastern Officer Basin (paper in review). It is also consistent with previous regional thermal maturation studies (McKirdy and Michaelsen, 1994; Gravestock and Hill, 1997) on the Ediacarian sedimentary sequences of the eastern Officer Basin, including the Karlaya Limestone and Tanana Formation, which testify the marginal maturity of organic matter ($T_{max} \leq 438$°C) in the vicinity of Munta-1, Observatory Hill-1 and Lake Maurice West-1 boreholes. The low thermal maturity of these acritarchs, therefore, makes them a pertinent target for the present study aimed at addressing their biological affinities. Nevertheless, at this age (575 to ~567 Ma), acritarchs — although morphologically well-preserved — must have undergone some diagenesis, whereby the more labile (less resistant) components may have been removed, leaving behind the more robust and selectively-preserved macromolecular structure.

3.2. White light and scanning electron microscopy

Both *M. pelorium* (Fig. 2a–d) and Species A (Fig. 2e, f) are characterised by multi-branched processes.

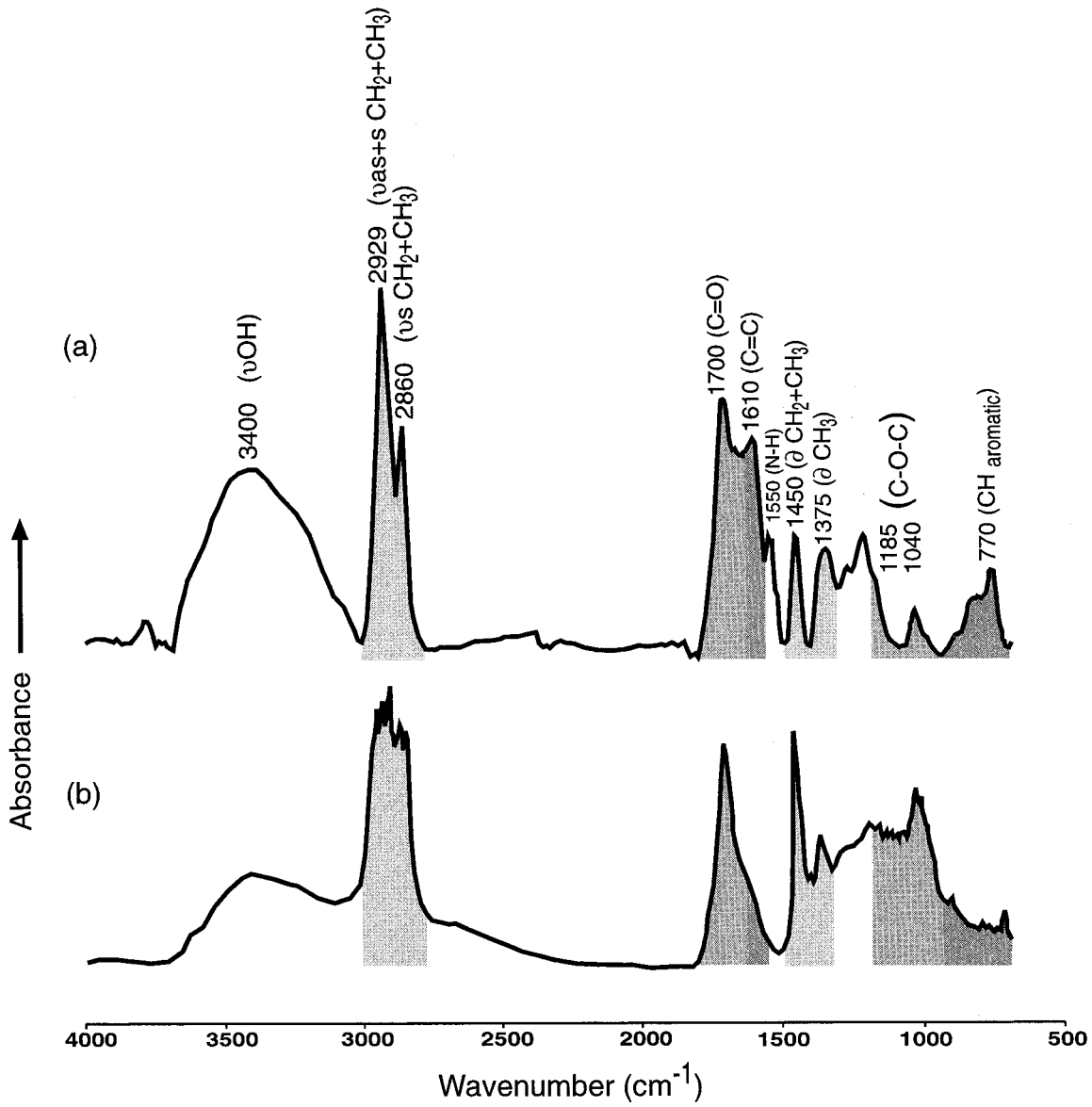

Fig. 4. FT-IR spectra of (a) *M. pelorium*, and (b) Species A acritarchs. Major aliphatic signals are observed at 2800–3000 and 1300–1500 cm^{-1}. Assignments of absorption bands and vibration modes (∂ = deformation; υ = stretching; s = symmetric; as = asymmetric) are indicated in parentheses.

The following morphological/anatomical description is partly extracted from Zang and Walter (1992) and Grey (1998). The genus *M. pelorium* is a large ellipsoidal microfossil (~0.1–1 mm in diameter) surrounded by variably-complex cylindrical processes and widely-spaced multiple branching. The wall is very thin with no obvious excystment structure (Grey, 1998). Species A is a smaller (200–300 μm), subspherical to ovoid organic body of primarily non-reticulate 'numerous, densely-crowded and finely-divided processes', and no distinct central outline and excystment structure is visible (Grey, 1998).

Superficial resemblance of *Multifronsphaeridium* to *Multiplicisphaeridium* Staplin 1961 emend. Eisenack et al., 1973, and of Species A to *Fimriaglomerella* Loeblich and Drugg 1968 has been suggested (Grey, 1998), although the Australian species are generally larger and have less regular processes. No further biological affinities could be demonstrated at normal white light microscopic levels.

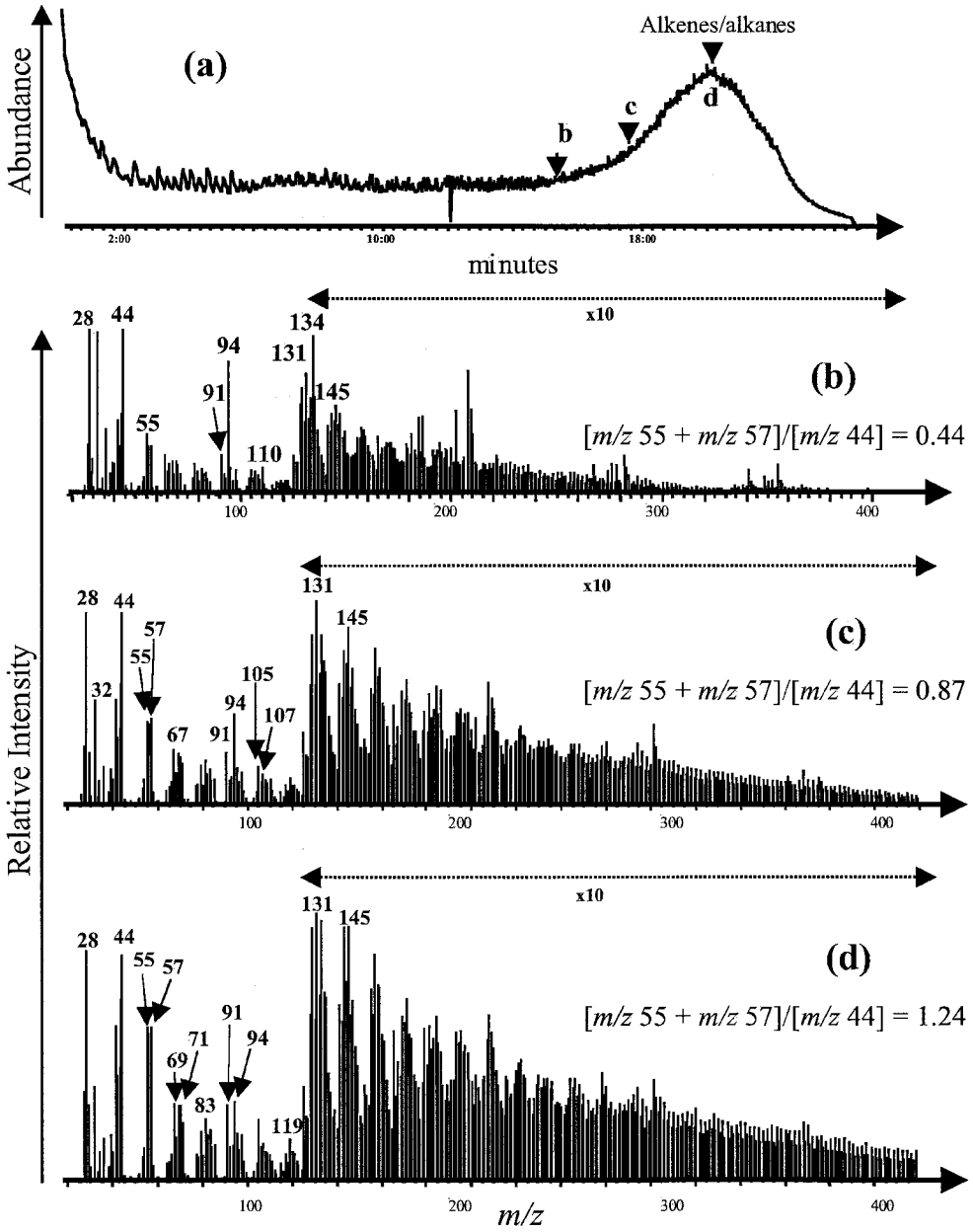

Fig. 5. Direct thermal desorption–mass spectrometry of Species A acritarchs. (a) Total-ion-chromatogram (TIC) with an apex at ca. 600°C representing the maximum generation of pyrolysis product (predominantly *n*-alkene/alkanes) from the acritarch macromolecule. (b–d) Mass spectra at three different desorption temperatures. A greater amount of aliphatic ions were progressively produced with the increase in desorption temperature as exemplified by the values 0.44, 0.87 and 1.24 for the ratio (m/z 55 + 57)/(m/z 44) for (b–d), respectively.

3.3. Transmission electron microscopy (ultrastructures)

When observed by TEM, the two species of acritarchs showed a cell wall up to ~300 nm thick (Fig. 3a, c). At higher magnifications, this wall exhibits an electron-lucent inner sublayer (marked as 'iw' in Fig. 3b, d) enveloped by a relatively thin ($\leq$30 nm) electron-dense outer covering (T). The outer wall typically shows an 'intact' trilaminar organisation (T) reminiscent of the so-called trilaminar sheath (TLS) structure

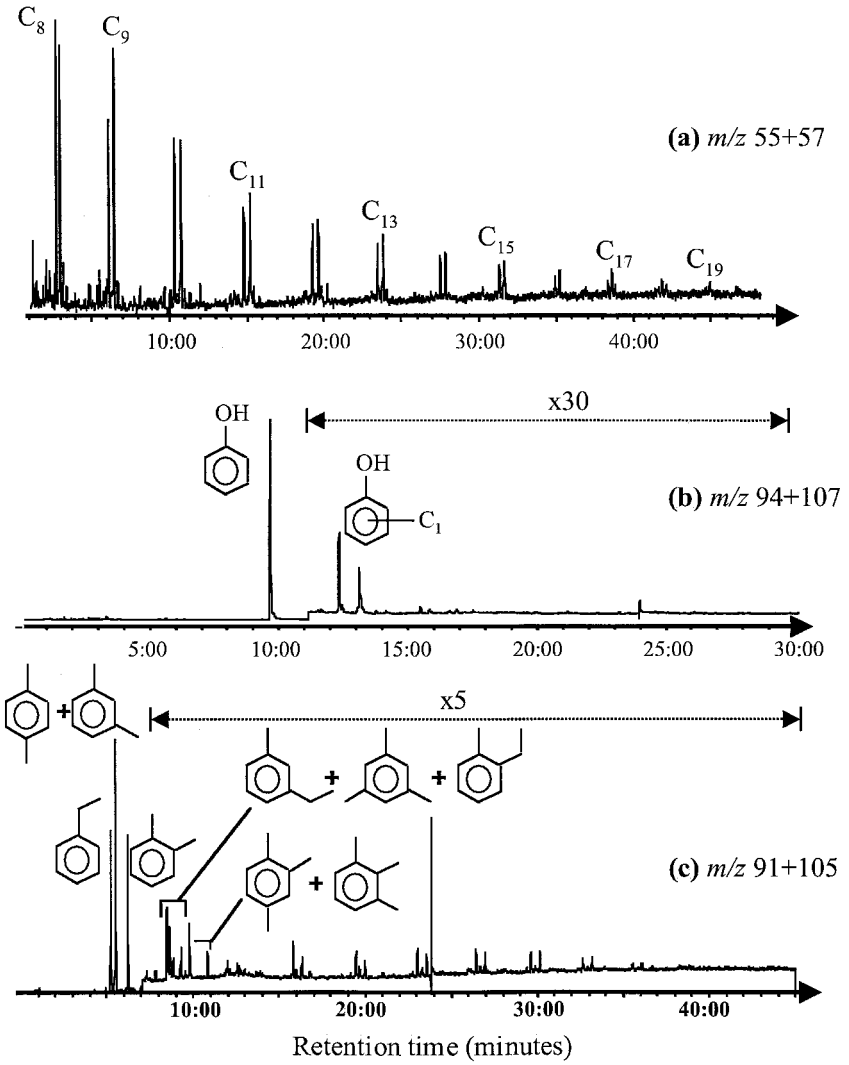

Fig. 6. Partial mass chromatograms from the laser micropyrolysis GC–MS analysis of *M. pelorium* acritarchs: (a) m/z 55 + 57 — from full scan analysis, C_n = *n*-alkene/alkane; (b) m/z 94 + 107 — selected ion analysis; and (c) m/z 91 + 105 — selected ion analysis.

of several species of green algae (cf. Tegelaar et al., 1989). However, in some places along the outer walls, an apparently single layer is visible (t) probably representing a less intact TLS structure with only its two peripheral sublayers (without the inner core) preserved. Such a structure may result from partial degradation of the less resistant central core, and selective preservation of the peripheral sublayers (cf. Brunner and Honneger, 1985) during the rupture and release of the mother cell wall.

3.4. FT-IR microspectroscopy

The FT-IR signals from both species include a major aliphatic component (Fig. 4). The high aliphaticity is indicated by prominent alkyl group bands between 2800–3000 and 1300–1500 cm^{-1}, peaking at 2929 cm^{-1} (due to symmetric and asymmetric stretching vibration of primarily CH_2 but possibly also CH_3), 2860 cm^{-1} (symmetric stretching vibration of primarily CH_3 but possibly also CH_2), 1450 cm^{-1} (CH_2/CH_3 deformational asymmetric bending), and 1375 cm^{-1} (deformational vibration of CH_3 with possible contribution from $[CH_2]_n$ bending). The particularly high methylene (CH_2) abundance suggests a major contribution of polymethylenic chains. Whilst the methyl (CH_3) signal is significant, a C—H aliphatic band at 720 cm^{-1} was not detected suggesting a low level of

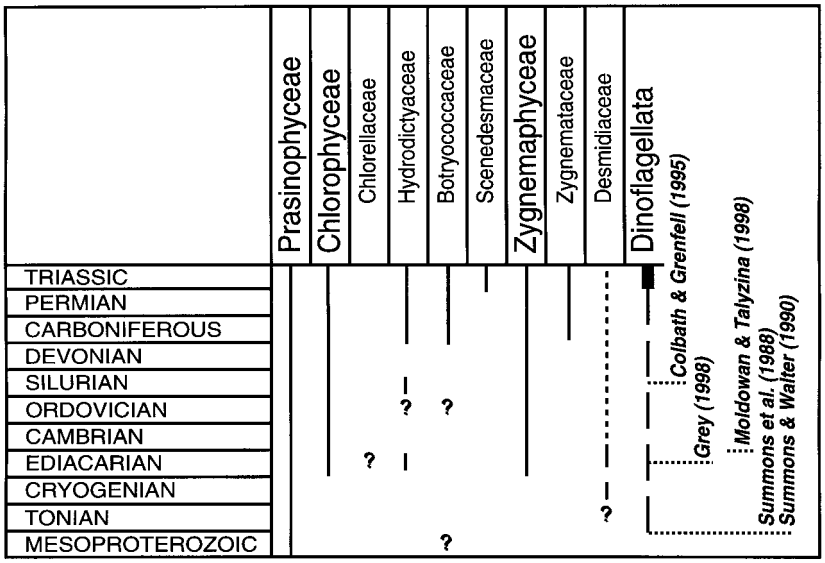

Fig. 7. The extension in time of prasinophyte, chlorophyte, zygnemaphyte and dinoflagellate algal families, as established by microfossil (adapted from Colbath and Grenfell, 1995; Grey, 1998) and biomarker (Summons et al., 1988; Summons and Walter, 1990) data. A biological affinity of some Australian Neoproterozoic acritarchs with these four algal classes has been further suggested from recent palynological observations (Grey, 1998). (The ultrastructural and molecular analyses of two new species of Neoproterozoic acritarchs in the present study provide further evidence of a possible chlorophyte ancestor in the latest Neoproterozoic, i.e. Ediacarian.)

branching. Therefore, the CH_3 signal probably corresponds to terminal CH_3 groups of the alkyl chains (confirmed by the 1375 cm^{-1} band).

Oxygenated functions (ester, ether) from carboxylic acid groups are also abundant (1625–1800, 1185, 1040 cm^{-1}). A narrow amide N—H deformation band centred at 1550 cm^{-1} is also evident in the case of *M. pelorium* (Fig. 4a). Hydroxyl groups (alcoholic, phenolic and/or carboxylic hydroxides) are represented by the broad 3100–3700 cm^{-1} band centred at ~3400 cm^{-1}. However, this latter band may also include contribution from NH groups or adsorbed water.

Subordinate absorption signals attributed to aromatic skeletal (C=C) vibrational modes (1590–1625 cm^{-1}, peaking at 1610 cm^{-1}) and an aromatic C—H out-of-plane deformation band (720–920 cm^{-1}, maximum at 770 cm^{-1}) were detected.

3.5. Thermal desorption-mass spectrometry

Direct thermal desorption-mass spectrometry of the *M. pelorium* and Species A produced similar results. The single-peaked total-ion-chromatogram (TIC) profile from the thermal-desorption of Species A is shown in Fig. 5a. The apex of the TIC corresponds to a temperature of ~600°C. At this temperature, the macromolecule is pyrolysed giving rise to a number of structural fragments. Several mass spectra, corresponding to different desorption temperatures along the TIC profile, are shown in Fig. 5b–d. In all the spectra, peaks from background air (i.e. m/z 44 = CO_2; m/z 28 = CO; m/z 18 = H_2O) predominate. Nevertheless, extensive hydrocarbon ion distributions are observed over a relatively modest molecular-weight range and ions of various m/z ratios can be tentatively attributed to certain compound classes. The complex mass spectra shown in Fig. 5b–d actually represent the superimposed mass spectra from a number of different compounds. Therefore, product assignments remain equivocal as more than one compound may contribute to a given m/z value. Nevertheless, several series of ions typical of *n*-alkene (e.g. m/z 55, 69, 83, ...) and *n*-alkanes (e.g. m/z 57, 71, 85 ...) are prominent in the TIC profile (refer to mass spectral libraries — e.g. Wiley, NIST — for classical 70 eV mass spectra of an extensive range of acyclic aliphatic products including *n*-alkenes and *n*-alkanes). As the desorption temperature increased, a relatively greater amount of aliphatic ions was produced. For example, the ratio of [m/z 55 + 57] to [m/z 44] is 0.44, 0.87 and 1.24 in Fig. 5b–d, respectively. Aliphatic entities obviously form a major thermally-labile constituent of the Species A (and *M. pelorium*) macromolecule.

Several ions attributed to the parent ion — the present mass spectra were acquired with low ionization energy (viz 16 eV) to preserve molecular ion formation — and major fragment ions of aromatic compounds

are also conspicuous. Alkylbenzenes (e.g. m/z 91, 105, 119), phenol (m/z 94), alkylphenols (m/z 107), benzenediol (m/z 110) and indole/alkylindoles (e.g. m/z 117, 131, 145) are all detected in considerable abundance.

3.6. Laser micropyrolysis GC–MS

Representative chromatograms extracted from the laser micropyrolysis GC–MS analysis of Species A are shown in Fig. 6. A series of *n*-alkene/alkanes in the range C_8–C_{19} is indicated by the m/z 55 + 57 summed chromatogram (Fig. 6a); phenol/methylphenol by m/z 94 + 107 (Fig. 6b); and a homologous series of alkylbenzenes by the m/z 91 + 105 chromatogram (Fig. 6c). The limited range of *n*-alkene/alkane pyrolysates ($< C_{20}$) suggests a relatively short average chain-length. Neither alkylcyclohexanes nor branched alkanes (including isoprenoids) were detected upon pyrolysis. The absence of branched alkanes supports the earlier inference that the significant CH_3 signal measured by FT-IR analysis is due to the terminal CH_3 of short *n*-alkyl moieties rather than to a highly branched aliphatic moiety. These laser pyrolysates reflect a significant aliphatic and aromatic content of the studied acritarchs, a result consistent with the TD–MS data.

4. Discussion

4.1. Background

Direct microfossil evidence suggests the extension of certain families of green algae only as far back in time as the Silurian (Colbath and Grenfell, 1995). However, indirect evidence from molecular phylogeny (Woese, 1987; Sogin et al., 1989; Knoll and Butterfield, 1989; Knoll, 1996) and biomarkers (Summons et al., 1988; Summons and Walter, 1990; Summons and Powell, 1991; Arouri et al., 1999; Hill et al., 1999) are both suggestive of an earlier algal radiation at, or slightly before, Mesoproterozoic. This controversy has recently been scrutinised in an extensive palynological study of Neoproterozoic acritarchs from Australia (Grey, 1998). Based on morphological similarities, and as depicted in Fig. 7, Grey (1998) suggested that some of these acritarch species might, in fact, represent prasinophyte ancestors, whereas a few other examples have been assigned to either Chlorophyceae, Zygnemaphyceae, or Dinophyceae (Fig. 7).

It has therefore proven difficult to attribute the majority of Proterozoic acritarchs to a particular group of algae on the basis of palynological observations. The two species examined in the present study (viz. *M. pelorium* and Species A) represent two previously unassigned Neoproterozoic acritarchs.

4.2. Morphological evidence

Most previous studies on acritarch morphological features were limited to light microscopy (e.g. Grey, 1998; Butterfield and Rainbird, 1998), or occasionally SEM (e.g. Zang and Walter, 1992) techniques allowing only bulk features to be observed. Some green algae (chlorophytes) possess trilaminar outer walls (trilaminar sheaths: TLS) composed of two electron-dense layers enclosing an electron-lucent layer (e.g. Brunner and Honneger, 1985; Largeau et al., 1990) which can only be identified by transmission electron microscopy. These TLS structures are commonly thin (< 30 nm), like those occurring in the green microalgae *Scenedesmus* sp. (Largeau et al., 1990), but can also be thicker (ca. 1000 nm) like those in *Botryococcus braunii* (Berkaloff et al., 1983; Kadouri et al., 1988; Derenne et al., 1989), *Tetraedron minimum* (Goth et al., 1988) and in *Gloeocapsomorpha prisca* (Largeau et al., 1990).

These TLS-containing walls form a minor percentage (< 4%) of the original biomass (Derenne et al., 1992a; de Leeuw and Largeau, 1993), and are solvent-insoluble and highly resistant to chemical degradation and diagenesis. These components play an important protective role in the life cycle of such organisms and during later fossilisation, leading to their subsequent selective-preservation in the geological fossil record (Largeau et al., 1990). Remnant TLS structures are visible in the outer walls of the *M. pelorium* (Fig. 3b) and Species A (Fig. 3d) acritarchs. In some places along the external wall, a less intact TLS structure missing the inner, electron-lucent, core is visible ('t' in Fig. 3b). The presence of a remnant TLS suggests a possible relationship between these acritarchs and chlorophytes. The multiple acritarch walls shown in Fig. 3 are undulations of the same acritarch wall which can give rise to bundles of ultralaminae like those common in green algal kerogens (cf. Largeau et al., 1990; Derenne et al., 1992b). The preservation of the inner wall (iw, Fig. 3b, d) of these Neoproterozoic acritarchs supports an earlier suggestion (Afi et al., 1996) that superb preservation of algal remains is not confined to their TLS entities.

4.3. Molecular evidence

Distinct FT-IR and pyrolysis GC–MS signals detected in *M. pelorium* and Species A acritarchs contrast the essentially featureless spectra recently obtained from a separate class of Neoproterozoic acanthomorph acritarchs of essentially similar thermal maturity (unpublished results). The intractability of those acanthomorph acritarchs was suggestive of a highly ordered, polyaromatic biomacromolecule, resembling the resistant biopolymer of the dinoflagellate resting cyst wall.

The prominent methylene group bands in the *M. pelorium* and Species A acritarchs observed by FT-IR spectroscopy (Fig. 4) indicate the high aliphatic nature of these organisms. The TD–MS (Fig. 5) and laser micropyrolysis GC–MS (Fig. 6) results reflect significant both aliphatic and aromatic contributions. In the TD–MS experiment, the aliphatic compounds such as alkenes/alkanes appear to be in higher relative proportions at higher temperatures (~600°C). This may indicate that the more intractable kerogen macromolecule is enriched in aliphatics relative to the bulk acritarch body. The prominent *n*-alkene/alkane series (up to C_{19}) in the pyrolysates detected in these acritarchs is also similar to, albeit shorter than, those typically obtained from the highly aliphatic biomacromolecules 'algaenan' of many species of Chlorophyceae (Tegelaar et al., 1989). Although no carbon chains longer than C_{19} are detected in the acritarch pyrolysates, this does not exclude a biopolymer/algaenan relationship. Known examples of algaenan-containing chlorophytes include the freshwater green algae *Botryococcus braunii* (Berkaloff et al., 1983; Largeau et al., 1984, 1986; Kadouri et al., 1988; Derenne et al., 1989, 1990), *Chlorella fusca* (Derenne et al., 1992a), *Scenedesmus obliquus* (Burczyk and Dworzanski, 1988), *S. quadricauda* (Derenne et al., 1991), and *Tetraedron minimum* (Goth et al., 1988), and the marine chlorophytes *Gloeocapsomorpha prisca* (Douglas et al., 1991) and *Nanochlorum eucaryotum* (Derenne et al., 1992a). The pronounced *n*-alkane series in these algae have often been attributed to a macromolecular structure based on polymethylenic chains, linked primarily via ether bonds.

The *n*-alkene/alkane series detected in the laser micropyrolysis GC–MS analyses of the acritarchs studied show a regular decrease in intensity with increasing carbon number (Fig. 6). Such a feature, particularly when associated with the lack of an odd- or even-carbon-number predominance, suggests that these hydrocarbon products are derived from fragmentation of longer hydrocarbon chains (e.g. Kadouri et al., 1988). These hydrocarbon chains are probably cross-linked by ester/ether bridges and hydroxyl groups, which would account for the high carbonyl/carboxyl and hydroxyl infrared absorptions (Fig. 4).

Alkylbenzenes and alkylphenols were other pyrolysates detected in significant concentrations (Figs. 5 and 6). The corresponding FT-IR analyses of both samples showed a broad –OH signal and a smaller aromatic C—H signal. Alkylbenzenes are the major aromatic pyrolysates typically detected in algaenan, however, they usually occur in low concentrations relative to aliphatic hydrocarbons (e.g. Derenne et al., 1992a). Only one of the marine chlorophytes, *Chlorella marina* (Derenne et al., 1996), has been shown to be aromatic-rich with only minimal *n*-alkene/alkane chains. The *M. pelorium* and Species A acritarchs may comprise a new class of biopolymer containing significant aliphatic and aromatic molecular constituents.

Alkylphenols are not very source-specific compounds. Originally, the brown algae were thought to be a unique biosynthetic source of polyphenolic macromolecules (e.g. Ragan and Glombitza, 1986). However, van Heemst et al. (1995, 1996) later showed that phenols and alkylphenols are not exclusive to brown algae, but are also present in the pyrolysates of green, red and other microalgae, although they occur in low relative concentration in red algae compared to brown and green algae. It was suggested that these polyphenolic polymers were related to phlorotannin-type macromolecules (van Heemst et al., 1996). Abundant alkylphenols reported by Gelin et al. (1996) from algaenans of several species of marine green microalgae (*Chlorella spaerckii, Chlorococcum* sp. and *Nannochloris* sp.) were, however, non-reproducible when a different isolation method was used (Gelin et al., 1997). A further non-reproducibility relating to alkylphenol detection was also observed when the same enigmatic Upper Devonian microfossil, *Protosalvinia*, was analysed by two different groups of researchers (Romankiw et al., 1988; Mastalerz et al., 1998). Abundant lignin-derived alkylphenol concentrations were reported from *Protosalvinia* by Romankiw et al. (1988), whereas only minor amounts of alkylphenols were observed by Mastalerz et al. (1998) who, hence, suggested a non-lignin composition for the same fossil. Alkylphenols were also reported to be abundant in the pyrolysates of enigmatic Lower Devonian nonvascular plant remains (*Prototaxites* Dawson, *Pachytheca* Hooker, and *Parka* Fleming: Abbott et al., 1998). Alkylphenols were also prominent products in the Curie-point pyrolysate of the dinoflagellate *L. polyedrum* resting-cyst cell wall (Kokinos et al., 1998). It is now well-established that alkylphenols in kerogen pyrolysates are not necessarily indicative of lignin (van Heemst et al., 1996).

Alkylphenols can also be derived from melanoidin-like compounds. Melanoidins have been recently shown to form at very early stages in the water-column and in the sediment upper layers (Zegouagh et al., 1999) by the classical degradation–recondensation pathway of proteinaceous organic matter (Tissot and Welte, 1984). Therefore, the presence of melanoidin compounds in the acritarch does not necessarily imply an indigenous macromolecular origin. Furthermore, melanoidin-like compounds can also be formed artificially during laboratory preparation procedures (Allard et al., 1997). Since the acritarch samples were not solvent-extracted prior to HCl/HF treatment, it is difficult to discriminate between naturally and artificially formed melanoidin products. Given the variety of sources for alkylphenols, a genetic conclusion based

on their distribution in the acritarch species studied here cannot be made.

N-containing compounds (e.g. indoles and alkylindoles) may also be indicated by ions of m/z 131 and 145 in the thermal-desorption experiment (Fig. 5). Such proteinaceous-derived compounds were previously detected in the pyrolysates of brown and green algae, and to a lesser extent in red algal residues (van Heemst et al., 1996; Gelin et al., 1997). Proteins and tetrapyrrole pigments are the two possible sources of nitrogen in algae (Gelin et al., 1997). The hydrolysability of alkylindoles in HCl led van Heemst et al. (1996) to suggest an algal proteinaceous (tryptophan amino acid; Tsuge and Matsubara, 1985) origin of indoles. However, these compounds were later shown to be non-hydrolysable in $HCl/KOH/H_2SO_4$ acids but hydrolysable in trifluoroacetic acid (Gelin et al., 1997), suggesting derivation from melanoidin-like artefacts (see also Allard et al., 1997). Similar to phenols, nitrogen-containing products with a proteinaceous origin may result from natural condensations of amino-acids and sugars at an early stage of diagenesis (Zegouagh et al., 1999). The source of alkylindoles in the acritarch samples may therefore be protein and/or chlorophyll (pigment) probably present in the loosely-bound components or free-lipids (as opposed to the macromolecular biopolymer). Although the acritarch samples were treated with HCl/HF acids prior to pyrolysis, they were not solvent-extracted, so chlorophyll (the main source of tetrapyrroles) has not been extracted (cf. Gelin et al., 1997).

In summary, the *Multifronsphaeridium* sp. and Species A acritarchs comprise both a selectively preserved highly aliphatic biomacromolecule and a melanoidin-like component. Collectively, the FT-IR, TD–MS and laser micropyrolysis GC–MS results indicate that these two species of acritarchs represent a new class of highly resistant biopolymers, distinct from other acritarchs and most known biomacromolecules. For example, the presence of *n*-alkene/alkane pyrolysates distinguishes the present set of acritarchs from the dinoflagellate *L. polyedrum* resting-cyst cell wall (Kokinos et al., 1998) and the recently proposed dinoflagellate-related acritarchs (unpublished results), which are both of a highly-condensed polyaromatic structure. In particular, saturated hydrocarbon chains are an integral part of the parent macromolecule of both the *M. pelorium* and Species A. Because no aliphatic products were detected from the pyrolysis of the sporopollenin of the *L. clavatum* spore (Burczyk and Dworzanski, 1988) for example, it can be concluded that they are also quite different from the present acritarch macromolecules. Furthermore, sporopollenins or sporopollenin-like materials are a heterogenous group of chemically-different substances that are expected to generate a complex array of pyrolysates (e.g. Burczyk, 1987a, b).

Whilst the presence of aliphatic hydrocarbons and alkylphenols is not inconsistent with a brown algal affinity (cf. Ragan and Glombitza, 1986) for these two acritarch species, a distinctive TLS-bearing wall structure could argue against such a classification. The absence of methoxyalkylphenols in the acritarch pyrolysates does not support a lignin or a lignin-type macromolecule present in the younger fossil and extant vascular plants. Also, the absence of isoprenoids in the acritarch pyrolysates suggests a complete lack of carotenoid contribution in the formation of these non-lignin acritarch polymers.

Species A and *Multifronsphaeridium* sp. are perhaps most similar in nature to the chlorophyte algaenan. For example, both are characterised morphologically by the TLS-bearing wall structures and molecularly by an abundance of aliphatic hydrocarbons. Polymethylene chains are probably cross-linked by ester/ether bridges and hydroxyl groups. An apparent dissimilarity, however, is the significant abundance of aromatic (alkylbenzene, alkylphenol and alkylindole) hydrocarbons in these acritarch species; a feature uncommon to most known examples of modern green algae (e.g. Derenne et al., 1992a, b). While alkylphenols and alkylindoles are probably loosely-bound to the highly aliphatic biomacromolecule, the high relative abundance of alkylbenzenes in these Neoproterozoic microfossils may also be a function of their 'long-term residence' (575 to ~567 Ma) in these sedimentary sequences.

5. Conclusions

Two newly discovered multi-branched acritarchs, *M. pelorium* and Species A, were examined ultrastructurally and chemically to investigate their biological affinities.These acritarch macromolecules may represent a new class of highly resistant biopolymers. Their organic walls are characterised by trilaminar sheath (TLS) structures, similar in many aspects to those of green algae. A major aliphatic component is evident from the FT-IR, TD–MS and laser micropyrolysis GC–MS data.

Alkylbenzene, alkylphenol and alkylindole pyrolysates are also detected. FT-IR bands attributed to hydroxyl and amide groups as well as ester and ether groups further confirm the functionalised nature of the acritarch organic matter. However, it has not been established if all of these aromatic products are indigenous to the acritarch macromolecule.

The macromolecular make-up of these samples may consist mainly of short ($< C_{20}$) *n*-alkylpolymethylenic chains, probably linked via ether/ester bonds, with a

lesser amount of alkyl/methylbenzenes and/or alkylphenols. This is largely similar to algaenan biomacromolecules of many species of Chlorophyceae, suggesting a close structural and biological affinity. Conversely, affinities to lignin-, sporopollenin-, cyanobacteran- or dinosporin-type macromolecules are rather unlikely.

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