USING LASER MICROPYROLYSIS TO ASSESS POTENTIAL RELATIONSHIPS BETWEEN CAMBRIAN TOMMOTIIDS AND ORGANOPHOSPHATIC BRACHIOPODS

Bronwyn L Teece\textsuperscript{1,2*}, Glenn A. Brock\textsuperscript{1,6}, John R. Paterson\textsuperscript{3}, Christian B. Skovsted\textsuperscript{4}, Lars E. Holmer\textsuperscript{5,6}, Simon C. George\textsuperscript{2}

\textsuperscript{1} Department of Biological Sciences, Macquarie University, Sydney, NSW, 2109, Australia, b.teece@unsw.edu.au, +61420905613, corresponding author
glenn.brock@mq.edu.au

\textsuperscript{2} Department of Earth and Environmental Sciences, Macquarie University, Sydney, NSW, 2109, Australia simon.george@mq.edu.au

\textsuperscript{3} Palaeoscience Research Centre, School of Environmental and Rural Science, University of New England, Armidale, NSW, 2351, Australia, jpater20@une.edu.au

\textsuperscript{4} Department of Palaeobiology, Swedish Museum of Natural History, Box 5007, SE-104 05, Stockholm, Christian.skovsted@nrm.se

\textsuperscript{5} Department of Earth Sciences, Palaeobiology, SE-752 36, Uppsala, Sweden; lars.holmer@pal.uu.se

\textsuperscript{6} Early Life Institute and Department of Geology, State Key Laboratory for Continental Dynamics, Northwest University, Xi’an 710069, China.

*Corresponding author current address: Australian Centre for Astrobiology, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, NSW, 2052, Australia
Abstract

Laser micropyrolysis gas chromatography-mass spectrometry enables researchers to selectively obtain chemical information about the organic matter in specific parts of a variety of specimens, such as coals and fossils in order to elucidate chemical composition. This paper briefly reviews the history of this type of pyrolysis and examines whether the technique can be used to isolate and recover biogeochemical signatures directly from the mineralised organophosphatic sclerites of Cambrian tommotiids—a group of enigmatic lophotrochozoans—and potentially related organophosphatic brachiopods. We analysed specimens of two tommotiids (*Micrina etheridgei* and *Dailyatia* sp.) and the paterinate brachiopod *Askepasma toddense* from the lower Cambrian of South Australia. Pyrolysate hydrocarbons from the sclerites of these species were detected and compared. Results indicate that *A. toddense* is more chemically complex than either of the two tommotiid taxa, but that *M. etheridgei* is compositionally more similar to *A. toddense*. Importantly, this study has demonstrated that laser micropyrolysis gas chromatography-mass spectrometry of Cambrian organophosphatic small shelly fossils yields detectable pyrolsates that have geochemical significance. It will be analytically possible and useful in the future to apply this technique to a larger sample set to elucidate deep time biogeochemical homologies, and to test intra-shell heterogeneity.

Keywords – brachiopod, laser, Lophotrochozoa, metazoans, pyrolysis, tommotiid,

Highlights

- Short review of laser micropyrolysis gas chromatography-mass spectrometry history
- Demonstrates the use of analytical pyrolysis on Cambrian brachiopods and tommotiids
- Biogeochemical signatures support phylogenetic interpretations in the fossil record.

Author contributions

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1. Introduction

Organic geochemical analysis of fossils is multidisciplinary in nature, complex, and time consuming, yet has huge potential in both palaeontology and the petroleum geochemistry [1]. When organic matter (OM) is buried through sedimentary or tectonic processes, it undergoes a series of physical and chemical changes via the processes of diagenesis, thermal maturation, and metamorphism, which eventually leads to a radically transformed chemical structure. Ongoing advances in instrumentation and analytical methodologies have led to a greater understanding of how these processes occur and have aided in the reconstruction of the original chemical compositions of kerogen in a variety of fossils, such as microfossils and amber [1]. Gas chromatography-mass spectrometry (GC-MS) of bulk rock samples, either by liquid injection of extracted and fractionated hydrocarbons or by pyrolysis of kerogen, has traditionally been utilized in this field [2].

Typically, extraction and fractionation techniques for analysing OM have necessitated bulk analyses, with experimental methodologies that can be overly time consuming [3,4]. Furthermore, when these methods are applied to organically-lean samples, the potential risks of contamination are high, but
can be somewhat mitigated when stringent controls such as slice experiments and blanks are utilized [5-7]. The most apparent drawback is the averaging effect of bulk geochemical techniques. Samples that are known from microscopy to be morphologically heterogenous and thus of likely variable OM content are typically analysed in bulk, leading to averaging information that refers to the total composition of the bulk samples (typically 1 to 40 g weight). This can lead to a lack of appreciation of the diverse chemistry of heterogenous OM, which can provide geochemically-useful information about its diagenetic history and biological origins [4].

Emerging enhancements to analytical technologies in organic geochemistry have been met with varying success at decoding ancient molecular compositions. Laser micropyrolysis GC-MS (La-Py-GC-MS) is one such method. La-Py-GC-MS enables pyrolysis of spatially-selected parts of samples using a laser to 'crack' macromolecules into smaller GC-amenable compounds, affording researchers an alternate and in situ methodology for understanding the organic makeup of sedimentary rocks [8]. Current La-Py-GC-MS instruments permit analyses of areas as small as ~20 µm, providing a way to obtain geochemical information at a resolution similar to microscopy [9]. al Sandouk-Lincke et al. [1] compared La-Py-GC-MS with Curie-point (flash) pyrolysis GC-MS and found that La-Py-GC-MS is more appropriate for mature samples (see their table 2). Immature samples of scolecodonts (biomineralised jaws of polychaete annelids) tended to produce short aliphatic chains, creating unspecific compounds, which meant that the temperature of the pyrolysis of the La-Py-GC-MS was too high, thus producing no useable structural information [1]. Additional challenges occurred in analysing scolecodonts because the laser reflected off the surface and the laser energy had to be maximised for products to be detected [1]. Analysis of amber was similarly challenged and only worked after the sample was covered with a thin layer of higher photon-adsorbing graphite; even then the analysis produced only minor aromatic hydrocarbons and no diterpenoids [1]. Problems typically associated with
La-Py-GC-MS relate to small sample size, discrimination against heavy hydrocarbons, and laser-produced artefacts, although it has generally provided reliable analyses of high maturity samples [1,10].

La-Py-GC-MS has been used to analyse a wide variety of geological materials, including coal macerals [1,11-13], shale [4,14,15], solid bitumens [11], oil-bearing fluid inclusions [9,15,16], microfossils [1,10,14,17,18], and stalagmites [19]. Other applications of La-Py-GC-MS have been on environmental samples [3] and forensic samples [20]. For example, La-Py-GC-MS was used to examine the structure of different kerogens and confirmed the hypothesis that lignin contains abundant phenolic and methoxy-phenolic compounds [12]. Arouri et al. [17] utilised La-Py-GC-MS to demonstrate the biological affinities of two species of acritarchs and established that some forms of acritarchs can be entirely geochemically distinct from each other. Although some geochemical signatures of acritarchs were consistent with dinoflagellates, incompatible thermal maturity results hindered a clear conclusion to be drawn [17].

In this study, La-Py-GC-MS is used to examine biominerals secreted by early lophotrochozoans that co-occur in the same lower Cambrian strata of the Flinders Ranges, South Australia. The aim of the study is to determine whether La-Py-GC-MS is a suitable method for identifying and comparing original geochemical signals in shelly fossils in order to assess if they potentially share homologous biogeochemical traits.

2. Laser micropyrolysis gas chromatography-mass spectrometry

2.1. Background to laser micropyrolysis gas chromatography-mass spectrometry

La-Py-GC-MS is a versatile technique that has multidisciplinary applications for the analysis of OM [8]. La-Py-GC-MS can provide information similar to results from flash pyrolysis [8], but at much higher spatial resolutions, as small as a few microns [9]. Furthermore, the use of observational and
refined output directional capabilities, through microscopic optical systems, allows for the systematic study of heterogenous OM [21].

The ability to separately analyse macerals has potential for determining not only biogenicity in samples, but also syngeneity[11,13]. The addition of a laser microprobe in analytical pyrolysis studies is beneficial in many ways. In particular, the high spatial resolution enables small samples to be analysed in situ, or specific areas of heterogeneous fossils to be targeted [1,8,15,22]. Traditional bulk pyrolysis methods [22,23] are not capable of distinguishing heterogeneous material [1]. Hand-picking of heterogeneous organic matter (e.g. of separated macerals or microfossils) is possible, but it is laborious and can lead to contamination [1,15]. In addition to speed, ease, specificity, and purity of analyses, La-Py-GC-MS benefits from its customisability e.g. adjustment of laser power, sample spot size, and the potential to amalgamate multiple laser spot analyses [11]. Custom-specific systems can be designed to maximise the potential for successful pyrolysis of a given material [11]. Finally, data obtained by La-Py-GC-MS have been thoroughly compared to previously established methods of analysis, and produces comparable results [8,15,21].

La-Py-GC-MS targets small, spatially-constrained areas for pyrolysis (typically 50–1000 μm), so typically only yields small amounts of analytes, which can challenge the sensitivity of GC-MS instruments [25]. Increasing the pyrolysis temperature can lead to a greater yield, but leads to larger craters, thus reducing selectivity, and may also create more pyrolysate artefacts, which are problematic in analyses [1,8]. Additionally, the pyrolysis temperature is reliant on laser energy, which can be difficult to regulate at the sample surface [8]. Sample-specific properties can also impede the usefulness of this technology, because colour and surface properties affect the extent to which the laser energy is adsorbed and the sample heated/pyrolysed [8]. The La-Py-GC-MS technology is complicated—for example, the elaborate interface of the laser microprobe to the microscope and GC-MS—and is not
available “off-the-shelf” from any instrument manufacturer. Thus, specific multidisciplinary expertise must be obtained in order to design new La-Py-GC-MS instruments and appropriately use the technique, involving organic geochemists, organic petrographers, laser physicists, and engineers [8,26].

2.2. Laser micropyrolysis gas chromatography-mass spectrometry as a phylogenetic tool

La-Py-GC-MS has rarely been employed as a tool in an attempt to uncover information about phylogenetic relationships, especially the affinities of problematic taxa. Arouri et al. [17] used La-Py-GC-MS with other molecular methods to try to elucidate the phylogeny of acritarchs, which may be related to some algal species. The La-Py-GC-MS data from acritarchs included prominent short chain n-alkane/alkene doublets (similar to those found in algaenan), which decreased in intensity with increasing number and had no odd or even carbon number preference [17]. Arouri et al. [17] also detected alkylbenzenes, which are the major aromatic pyrolysates found in algaenan, and concluded that acritarchs and algaenan-bearing groups had a close structural and biological affinity. In a similar study using a different pyrolysis technique, Dutta et al. [27] used Curie-point pyrolysis of an enigmatic Precambrian fossil, *Churia circularis*, to try to shed some light on its affinity. They found a mainly aliphatic pyrolysate, with a bimodal distribution of n-alkene/n-alkane doublets with a maxima at C7, and concluded that the organic remains were probably from early eukaryotic algae that appeared during the early Mesoproterozoic [27].

2.3. Typical instrument configurations

La-Py-GC-MS instruments are not commercially available from manufacturers, so have to be designed and constructed in science laboratories from separate components. The typical La-Py-GC-MS configurations usually include a laser, microscope, pyrolysis chamber, gas inlet system and GC-MS
The microscope enables positioning of the targeted part of the sample while it is hosted within the pyrolysis chamber [8], as well as observation of the laser event (using a video camera). The fundamental laser specifications relate to energy and duration. Energy directly affects the size of the crater, with higher energy creating a larger crater [8,28]. Objectives can alter the size of the area directly exposed to pyrolysis. Laser beams are typically around 5 mm in diameter, but with microscope or other optics can be focused to a higher magnification to selectively pyrolyse smaller areas [8]. Conversely, a defocused beam may be preferable for homogeneous samples to produce more pyrolysates [8].

Several studies have used Nd:YAG (1.064 \( \mu \text{m} \)) or Ar ion (0.514 \( \mu \text{m} \)) lasers for La-Py-GC-MS, especially when obtaining data from small samples [8,13,15]. However, these early studies showed that the pyrolysis products were composed of a multitude of volatile degradation compounds, and relatively high-molecular-weight molecules such as long chain (>C25) \( n \)-alkanes, triterpenoids, and steranes were not necessarily preserved under these conditions, possibly due to high photon energies [4]. Yoshioka & Ishiwatari [4] used a CO\textsubscript{2} laser with a longer wavelength (infrared, 10.6 \( \mu \text{m} \)) and found that although the distributions of \( n \)-alkanes slightly depended on the laser power, the distributions of isoprenoids, triterpenoids and steranes were similar under all experimental conditions, indicating that La-Py-GC-MS with an infrared laser may be an alternate method for undertaking these analyses. A study by Greenwood [28] tested a Sydney torbanite standard with different laser sources in a La-Py-GC-MS. A continuous wave 0.532 \( \mu \text{m} \) laser gave better results than a Q-Switched pulsed 10.64 \( \mu \text{m} \) laser and a Q-Switched pulsed 0.266 \( \mu \text{m} \) laser. This was attributed to the exceptionally high power densities of pulsed lasers, which led to the almost complete disintegration of organic molecules, rather than any specific difference in wavelength [28].

Microscope objectives used are typically 10\times, 20\times, and 50\times [8,11,12]. The 20\times and 50\times objectives are used for higher resolution studies and have to be ultra-long distance to provide sufficient distance to
accommodate the sample in the pyrolysis chamber below the microscope objective [8]. The glass window of the pyrolysis chamber allows observation of the sample and is the avenue for the laser to access it [8]. The sample chamber is typically kept at a small size (e.g., internal diameter = ~7 mm, depth ~10 mm) so as to reduce the dead volume [8]. An inert metal (e.g. nickel; anodised, coated, or glass-lined stainless steel) is characteristically used for the lining of tubes to decrease secondary reactions between pyrolysates [8]. A cold trap can be used for cryogenic focusing of the pyrolysates over the duration that the laser is being used [8]. Partial vacuums may be used to enhance entrapment of the pyrolysates [8].

The GC-MS should be equipped for high sensitivity and mass resolution, but fairly standard GC-MS operating conditions are often used, such as a fused silica capillary column (25 m × 0.22 mm i.d.) coated with BPX5 phase (1.0 µm film thickness) [8,11,12]. Typically, the GC oven temperature starts at ~40º C, at which it is held for 10–15 minutes during cryogenic focusing. If the first cryogenic focusing event is external to the GC, then a second cryogenic focusing event internal to the GC oven can be used to concentrate the pyrolysates in a tight band at the front of the GC column [8]. Then the GC oven temperature is programmed to increase at a rate of 3–6º C/min until a final temperature of ~300º C, where it is held for a period of 10–15 minutes [17,28].

3. A Cambrian case study

3.1. The tommotiids Micrina and Dailyatia

*Micrina* (Fig.1a) and *Dailyatia* (Fig. 1b) belong to a problematic group called the tommotiids, which represent some of the first biomineralizing animals to arise during the early Cambrian [29-31]. These taxa have a complex scleritome exoskeleton composed of morphologically diverse organophosphatic sclerites. Although tommotiids likely represent a polyphyletic group, they are
generally considered to be members of the Lophotrochozoa, with some taxa having a probable affinity to organophosphatic brachiopods [29,31-33]. Cambrian small shelly fossils, such as tommotiiids, are generally recovered from the rock record as isolated (or disarticulated) sclerites, so understanding their overall morphology is often very difficult [34-36]. Additionally, tommotiid sclerites do not have modern analogues, so determining phylogenetic affinity is a considerable challenge [31,37]. Tommotiid sclerites are highly variable, including a range of symmetrical and asymmetrical (sinistral and dextral), cone-shaped forms [31,38]. The configuration of the scleritome has only been revealed in three taxa—Eccentrotheca [38], Micrina [28], and Paterimitra [39]—with other taxa having only hypothetical scleritome reconstructions. No previous studies have examined the biogeochemistry of tommotiid sclerites. Dailyatia is a speciose camenellan tommotiid [31,40] that exhibits a large range of sclerite morphotypes, suggesting that this taxon possessed a complex, bilaterally symmetrical scleritome that protected its slug-like body [31]. Micrina is a tannuolinid tommotiid composed of only two sclerites and can be confidently reconstructed as a fixed-sessile bivalved animal, very similar to (and probably homologous with) the bivalved body plan of early organophosphatic brachiopods, such as the paterinates [29,36].

3.2. Askepasma

Askepasma (Fig. 1c) belongs to the paterinate brachiopods—one of the earliest known groups—and occurs in the lower Cambrian of southern and central Australia [36,41]. The phylogenetic placement of Askepasma was problematic until a detailed study of its shell ultrastructure revealed a remarkable similarity to that of certain tommotiiids, specifically Eccentrotheca and Paterimitra [32]. Paterimitra pyramidalis in particular has remarkably similar shell ultrastructure to Askepasma toddense, with both exhibiting first and second order laminations composed of polygonal compartments [32,36,42]. This
suggests that *Askepasma* and paterinates in general have a more basal position within the Brachiopoda, and that at least some tommotiids occupy close stem group positions. The very similar shell morphology, composition and ultrastructure of tannuolinids (*Micrina*), eccentrothecimorphs (*Eccentrotheca* and *Paterimitra*) and paterinate brachiopods (such as *Askepasma*) have since been used to support close relationships between all of these groups [36,38,39]. Conversely, the various sclerite morphotypes and therefore complex multi-element scleritomes of camenellan tommotiids such as *Camenella* and *Dailyatia* led Skovsted et al. [30,31] suggest the Tommotiida is probably polyphyletic (or paraphyletic at best).

In order to elucidate potential relationships of these pivotal early lophotrochozoans, La-Py-GC-MS was performed on sclerite samples of *A. toddense*, *M. etheridgei*, and *Dailyatia* sp. to assess and compare biomarker signals from these three key taxa.

### 3.3. Selection and analysis of Cambrian fossils by laser micropyrolysis gas chromatography-mass spectrometry

The experiments were carried out on chemically- and physically-isolated sclerites of two tommotiid taxa, *M. etheridgei* and *Dailyatia* sp., and hand-picked valves of the paterinate brachiopod *A. toddense* [29-31,43]. All samples were recovered from a single stratigraphic horizon, MMF/0.0 in the uppermost outcropping Wilkawillina Limestone (Winnitinny Creek Member), located c. 1 km south of Balcoracana Creek, Flinders Ranges, South Australia [31°11'38.4"S, 138°52'28.7"E; map datum: WGS84]. Sample horizon MMF/0.0 is also the base of the MMF section [44], which occurs in the recently established lower Cambrian *M. etheridgei* Zone of Betts et al. [45,46]. All sclerites were chemically isolated from their rock matrix by dissolving the limestone samples in weak (7%) glacial
acetic acid within sterilised glass beakers at the Macquarie University Acid Leaching Facility. The calcium phosphate shells were ultrasonicated in dichloromethane prior to analysis.

A detailed description of the La-Py-GC-MS instrument used can be found elsewhere [8,11,15,19]. Briefly, a continuous wave laser (Laser Applications 9500, Nd:YAG laser, $\lambda = 1064$ nm) was tightly focused onto selected areas of the sample through an Olympus BPX60M microscope equipped with reflected light illumination and using a long distance working objective (10 $\times$). The sample was located in a purpose-built pyrolysis chamber (100–110°C, 100 mL helium flow) which was interfaced to a GC-MS system (Hewlett Packard 6890 GC interfaced to a 5973 mass selective detector, electron energy 70 eV) via a gas inlet system designed for maximum transfer efficiency of the gaseous products. The products of the pyrolysis process were cryogenically trapped in a coiled nickel loop using a liquid nitrogen bath. After trapping the products, a 6 port transfer valve was rotated to transfer 1 mL/min helium through the trap and the contents were then desorbed by heating to 320°C. The products were cryo-focused again in a loop of GC column immersed in a liquid nitrogen bath. The full scan GC-MS analysis ($m/z$ 50–550) of the pyrolysates was performed on a DB-5 column (J&W, 25 m, 0.32 mm I.D., 0.52 μm film thickness) with helium as the carrier gas with a constant pressure of 25 psi. The GC oven was programmed for an initial temperature of 40°C (2 min hold) followed by heating at 10°C/min to 320°C (4 min hold).

Organic-rich rocks such as the Sydney Basin torbanite laboratory standard pyrolyse at relatively low powers (e.g. 10 $\times$ objective, 10 A for 1 sec: 0.5 W). It was found that the Cambrian sclerites had to be pyrolysed at higher powers to obtain good data. Two laser conditions were used. For the A and B runs of all samples, the laser power was 12.5 A for 0.2 sec (3.17 W). However, for the *M. etherigdei* and *A. toddense* samples C-E the laser power was higher (12.5 A for 1 sec: 7.2 W), and multiple shots (5 to 10) were aggregated on the cold-trap. Typical laser micropyrolysis craters were
around 50 μm (Fig. 2). Under these conditions, La-Py-GC-MS results were obtained that are significantly above the system blank for the instrument. In this paper five laser runs are reported for each of *M. etheridgei* and *A. toddense*, and two laser runs are reported for *Dailyatia*.

### 4. Results and discussion

The total ion chromatograms from La-Py-GC-MS reveal products such as alkylbenzenes, alkylphenols and *n*-alkenes/*n*-alkanes present to differing degrees in the fossil samples (Fig. 3). The products of laser micropyrolysis isolated in each species have subtle but noticeable differences, with different relative proportions of naphthalene, benzene, phenols, *n*-alkenes, and *n*-alkanes, suggesting variability in specific structures within each fossil group (Fig. 3; Tables 1, 2). Within the fossil groups, the overall trends of the detected compounds found are relatively similar. No pyrolysates with a molecular weight >C21 were detected with the lower laser power, but for the samples analysed at 7.2 W *n*-alkanes up to C29 were detected, as were phenanthrene and some methylphenanthrenes. A few compounds in the pyrolysates that were detected (2-ethylhexanol, a phthalate and siloxanes; Figs 3, 4) were attributed to artefacts from a pyrolysis background signal, and were not used in any interpretation.

#### 4.1. Alkanes and alkenes

*n*-Alkane and *n*-alkene doublet peaks were detected in the pyrolysates of five *M. etheridgei* specimens, four *A. toddense* specimens, and two *Dailyatia* specimens (Fig. 4). The *n*-alkanes were detected in a limited carbon number range (C7–C20) for the lower laser power, but over a broader range (C6-C29) for the higher laser power. However, the chain length of the *n*-alkenes was unaffected by laser power (C6-C21; Fig. 5) suggesting a relatively short average chain length in the kerogen in the fossils. The *M. etheridgei* pyrolysates have greater amounts of *n*-alkanes and *n*-alkenes relative to other
compounds (Figs. 2 and 3; Table 1), suggesting that the kerogen in *M. etheridgei* is more aliphatic than in *A. toddense* and *Dailyatia*. The mean of the n-alkane maxima for the *Dailyatia* pyrolysates is at C\textsubscript{17} (Fig. 5). The n-alkane distributions of *M. etheridgei* and *A. toddense* are trimodal and similar, with a short chain maxima around C\textsubscript{9-10}, a secondary maxima around C\textsubscript{21-22}, and a third maxima at C\textsubscript{25-36} (Fig. 5). The *M. etheridgei* pyrolysates have a strong n-alkane odd-over-even predominance (OEP2) at C\textsubscript{13}, C\textsubscript{15}, and C\textsubscript{17} (Table 1; Fig. 5), but not for the n-alkenes. The *Dailyatia* pyrolysates have a particularly high relative abundance of C\textsubscript{16} and C\textsubscript{17} n-alkanes, but the distribution of n-alkenes do not follow the same trend (Fig. 5). All *Dailyatia* pyrolysates contain high relative abundances of the C\textsubscript{10} and C\textsubscript{14} n-alkenes (Fig. 5), and there is a slight odd predominance in the C\textsubscript{9}-C\textsubscript{13} molecular weight range for most of the *A. toddense* pyrolysates and both of the *Dailyatia* pyrolysates (OEP1; Table 1). Both of the *Dailyatia* pyrolysates contain C\textsubscript{16} and C\textsubscript{17} n-alkanes, but not C\textsubscript{16} and C\textsubscript{17} n-alkenes, and additionally one of the *Dailyatia* pyrolysates is missing the C\textsubscript{15} n-alkene.

For the shorter chain lengths, the samples have a predominance of n-alkenes over n-alkanes (n-alkane/n-alkene ratios <1), but at longer chain lengths the predominance changes to n-alkanes (Fig. 5). This is somewhat species dependent, with n-alkanes > n-alkenes > C\textsubscript{16} for *M. etheridgei* and *A. toddense*, whereas for *Dailyatia* n-alkanes > n-alkenes for C\textsubscript{13}, C\textsubscript{15} and C\textsubscript{19} (Fig. 5). n-Alkanes and n-alkenes in pyrolysates can be the product of carbon-carbon bond cracking in alkyl chains of the kerogen, leading to the products of alkyl chain cracking typically exhibiting similar distributions [47,48]. The outliers with high n-alkane/n-alkene ratios may be the result of other (non-pyrolysis) OM contributions, for example formerly trapped moieties that were released during laser pyrolysis, but by a thermal desorption (heating) process. The occurrence of n-alkanes trapped in a macromolecular structure and freed by heating has been observed in previous studies [49]. For example, Quénéa *et al.* [50] analysed the OM in forest soil using Curie-point pyrolysis and reported radically different distributions of n-alkanes relative
to n-alkenes. Plant lipids are characterised by long chain n-alkanes with high odd-over-even carbon number predominance, whereas Quénéa et al. [50] suggested that n-alkenes are uncommon in higher plant lipids. Thus, the trapped n-alkenes were more likely to be related to secondary compounds formed by degradation, rather than the same n-alkane source.

Pristane was detected in pyrolysates of both *M. etheridgei* and *A. toddense* (Fig. 4; Table 1), and phytane was detected in one of the *M. etheridgei* pyrolysates and one of the *A. toddense* pyrolysates. No unsaturated equivalents such as prist-1-ene were detected, consistent with these compounds being released by thermal desorption and not by cracking. Pr/Ph ratios are less than 1 for both samples, which when detected in solvent extracted or thermally desorbed samples is typical of reducing or hypersaline depositional environments [51]. The depositional environment of MMF/0.0 is reconstructed as a shallow, wave-swept, nearshore carbonate platform dominated by well-bedded to nodular, clean grey-white bioclastic limestone with generally well-preserved, abundant and diverse shelly faunas [44]. High energy conditions are evinced by occasional shell hash dominated by sclerites of *M. etheridgei* in neptunian dykes at many levels, and laminate and low domical stromatolites plus archaeocyath-calcimicrobe bioherms are also prevalent [45,46].

The low Pr/\(n-C_{17}\) ratios (<0.5) and Ph/\(n-C_{18}\) ratios (<1.0) are typical of extracted samples in the oil generation window. Teece et al. [52] reported similar Pr/\(n-C_{17}\) (<0.55) and Ph/\(n-C_{18}\) (<0.7) ratios from GC-MS of solvent extracted microbialite samples from younger Cambrian strata in the Flinders Ranges. That study included samples from the Wilkawillina Limestone and the coeval Wirrapowie Limestone, but these were overmature and did not return diagnostic biomarker information using extract chemistry [52]. Through Raman spectrometry and the methylphenanthrene index (MPI) obtained through GC-MS, it was concluded that the least thermally mature samples in their study had burial temperatures of \(~200^\circ\text{C}\), with thermal maturity decreasing through younger strata above the Flinders Unconformity.
Wirrapowie > Wilkawillina > Wirrealpa limestones). The sample analysed from the Wilkawillina Limestone has a burial temperature of approximately 201-210°C [52]. While little is known about the thermal maturation of Cambrian small shelly fossils, many early-mid Palaeozoic organophosphatic fossils (e.g. conodonts) have a similar apatitic composition and are commonly used as geothermometers up to temperatures of 600°C [53], suggesting they retain palaeontological integrity at relatively high burial and metamorphic temperatures.

4.2. Aromatic hydrocarbons and polar compounds

Dailyatia has the most aromatic character, followed by A. toddense and lastly M. etheridgei (Table 1). The pyrolysates of all specimens contain ethylbenzene, the three dimethylbenzene (xylene) isomers, naphthalene, methylnaphthalenes and biphenyl (Fig. 3; Tables 1, 2). The pyrolysates of two Dailyatia specimens also contain phenanthrene, while the pyrolysates of A. toddense and M. etheridgei C–E contain phenanthrene and methylphenanthrenes. The MPI values that Teece et al. [52] reported were <0.44, whereas the values reported here are between 0.42 and 0.73. The pyrolysates from each species have similar peak distributions relative to one another, and there are consistent differences between species. For example, the ratio of naphthalene to the C12 alkane/alkene doublet is <6 for A. toddense, <3 for M. etheridgei, but >8 for Dailyatia (Fig. 6). Phenol was detected in all the samples. 2-methylphenol and 3-methylphenol were also detected in all the A. toddense and M. etheridgei specimens. Various aromatic ratios were calculated (Table 1), but mostly show no interpretable trends. The abundance of aromatic hydrocarbons are compared to aliphatic hydrocarbons in Table 2, from which it is evident that M. etheridgei is the most aliphatic species, and Dailyatia is the most aromatic (Fig. 6).

Pyrolysis techniques often yield simple aromatics such as benzene and alkylbenzenes, due to the fragmentation of larger moieties in the analyte (e.g. the kerogen in the fossils) by a high energy
pyrolysis event [8,23,54]. Simple aromatics may also be derived from aliphatic moieties via thermally-promoted condensation reactions during pyrolysis, e.g. Rushdi et al. [55]. Benzene was the most abundant aromatic hydrocarbon detected in the total ion chromatograms, except the SO$_2$ peak, in all samples (Fig. 3). Benzene and alkylbenzenes can be specifically derived from algal-derived lacustrine kerogens [56], but are present in many different types of samples because they are common secondary products, so they are not strong indicators of kerogen type [57].

The *Dailyatia* specimens reveal the largest abundances of naphthalene relative to aliphatic hydrocarbons, followed by *A. toddense* and lastly *M. etheridgei* (Table 2). Naphthalene is present as both bound bio-geomacromolecules and as a free compound in solvent extracted rocks [57]. Alkynaphthalenes are not specific biomarkers, but have been found in analyses of both fossilised animal and plant materials [57]. The samples mostly have methylnaphthalene ratios (MNR) of ~1 (Table 1), except for one of the *M. etheridgei* specimens which has an MNR of 2.1. The MNR in solvent extracted rocks typically increases with increasing maturity, but in pyrolysates could be influenced by the pyrolysis conditions.

*A. toddense* contains the largest proportion of phenol relative to aliphatic hydrocarbons, followed by *Dailyatia* (Table 2). It is not clear what role phenol plays in the structure of extinct marine organisms, but it has been detected in younger microfossils such as chitinozoans [57]. Phenolic moieties have been found in a variety of plant matter and were imperative compounds in the success of the first land plants [57]. Dutta *et al.* [57] suggested that phenol in pyrolysates may come from precursor amino acids, which could explain the presence of phenol in both chitinozoans and *A. toddense*, which is more morphologically complex than the other analysed species [57,58]. Few pyrolysate compounds containing N were detected in the samples, making an amino acid source unlikely. However, phenols have also been known to be produced by diagenesis of aromatic structures, and may not be derived from
the fossil itself [56]. Although the compounds present are regulated by selective preservation and resistance to degradation, diagenetic reactions of both the OM in the fossils and the inorganic constituents can greatly affect kerogen, especially at the inferred maximum temperatures of ~200°C. How heavily the OM preserved in the shells of these specimens was affected by diagenetic processes is unquantifiable from chemical studies alone.

Toluene is a major pyrolysis product in A. toddense and Dailyatia (Fig. 3), where it is present in many times the abundance of the other predominant aromatic compounds (e.g. phenol and naphthalene). However, toluene is only slightly more abundant than the aliphatic compounds in Micrina, reflecting the greater aliphatic nature of this species (Table 2). Toluene is a common pyrolysate product for marine samples and can either be present as an indicator of the aromaticity of the original sample matter or may be formed from the thermal cracking of alkylbenzenes [59].

5. Conclusions

The success of La-Py-GC-MS analyses of fossils is dependent on many factors, including surface condition, colour, and thermal maturity [1]. However, the benefits of specificity and the ability to carry out high resolution studies far outweigh the shortcomings of using this technique [1]. The development of this technique has eliminated the laborious picking of fossils for bulk analysis [15].

Here we report, for the first time, differences in the composition of kerogen pyrolysates from Cambrian brachiopods and tommotids. The major outcomes are summarised below:

1. Pyrolysates that contain benzene, toluene, phenol, other aromatic compounds and n-alkyl chains were produced from the laser pyrolysis of all of the fossil samples. These laser pyrolysates are similar to those expected from conventional Curie-point (flash) pyrolysis, but were obtained from
selective laser ablation from small areas of the shell material. This preliminary study shows that OM was preserved in these shell materials since the Cambrian, despite geological heating to ~200°C.

2. *Askepasma toddense* contains the highest relative amount of phenol to \( n \)-alkane/\( n \)-alkene doublets. *Micrina etheridgei* produced more aliphatic pyrolysates, so is interpreted to contain kerogen that is more dominated by alkyl chains than in the other species.

3. *Askepasma toddense* and *M. etheridgei* have more similar compound suites than *A. toddense* and *Dailyatia*, particularly with regard to aromatic content. This supports the view of Skovsted et al. [31], interpreting *Dailyatia* as a mobile epibenthic bilaterian with complex, cataphract external armour that does not appear to be closely related to the fixed sessile, filter-feeding forms such as the paterinate brachiopod *Askepasma* and the tannuolinid tommotiid *Micrina*, both of which lie close to the root of the phylum Brachiopoda [31].

4. The experiments yielded pyrolysates of analysable abundance, but source diagnostic biomarkers were not detected in this suite of fossils, perhaps due to the temperatures that the host rocks were exposed to.

5. Further analysis of metazoan biominerals by La-Py-GC-MS using a set of samples from less thermally matured rocks may provide important supplementary biogeochemical character traits to help resolve relationships between some of the earliest and most enigmatic animal clades that emerged during the Cambrian radiation.

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We thank Ian, Di and Alice Fargher (Angorichina Station) for continued support of our Cambrian research and permitting access to the sample locality. Acid leaching and sample processing was carried
with great efficiency by David Mathieson. We thank David Fuentes for help with the operation of the laser micropyrolysis gas chromatography-mass spectrometry at CSIRO. We show our respect and acknowledge the Adnyamathanha people, the Traditional Custodians of the Land from which our samples come, and the Wattamattagal Clan of the Darug Nation, where this research took place. We thank Paul Greenwood, Herbert Volk and an anonymous reviewer for their helpful comments that substantially improved the final version of this paper.

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Figures

**Fig. 1** Scanning electron microscope images of a) *Micrina etheridgei*, b) *Dailyatia* sp., and c) *Askepasma toddense*. Scale bars are 100 μm in a; 200 μm in b; and 500 μm in c.

**Fig. 2** Typical laser micropyrolysis craters in *Askepasma* shells. The crater size is around 50 μm.

**Fig. 3** Representative total ion chromatograms from laser micropyrolysis gas chromatography-mass spectrometry of samples of *M. etheridgei*, *Dailyatia* and *A. toddense*. Δ = n-alkenes, x = n-alkanes, EB = ethylbenzene, M+P X = meta- and para-xylene, OX = ortho-xylene, N = naphthalene, MN = methylnaphthalene.

**Fig. 4** Representative added partial m/z 55+57 mass chromatograms from laser micropyrolysis gas chromatography-mass spectrometry of samples of *M. etheridgei*, *Dailyatia*, and *A. toddense*.

**Fig. 5** Line diagrams of the n-alkane (A) and n-alkene (B) distribution from laser micropyrolysis gas chromatography-mass spectrometry of the samples of *M. etheridgei*, *Dailyatia*, and *A. toddense*. (C) Column chart of the n-alkane/n-alkene ratio of the C₈–C₂₀ homologues. The ratio for C₁₉ in *Dailyatia* is cut off as it is 42.6. The error bars report the standard deviations.

**Fig. 6** Cross plot showing the relationship of aromatic compound abundances to aliphatic compound abundances in samples from this study. n=C₁₂ = n-dodec-1-ene, n=C₁₃ = n-tridec-1-ene, Tol = toluene, EB = ethylbenzene.
Table 1. Aliphatic and aromatic hydrocarbon ratios from laser micropyrolysates of fossil samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pr/Ph</th>
<th>Pr/n-C17</th>
<th>Ph/n-C18</th>
<th>OEP1 (n-C9–C13)</th>
<th>OEP2 (n-C13–C17)</th>
<th>MPI</th>
<th>MNR</th>
<th>M+P-xylene/EB</th>
<th>N/EB</th>
<th>N/M+P-xylene</th>
<th>N/O-xylene</th>
<th>N/2-MN</th>
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<tbody>
<tr>
<td>A. toddense A</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.98</td>
<td>2.0</td>
<td>4.9</td>
<td>2.47</td>
<td>5.1</td>
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<td></td>
</tr>
<tr>
<td>A. toddense B</td>
<td>0.76</td>
<td>0.37</td>
<td>0.94</td>
<td>1.2</td>
<td>1.3</td>
<td>nd</td>
<td>2.0</td>
<td>5.4</td>
<td>2.46</td>
<td>5.2</td>
<td>7.7</td>
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<tr>
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<td>nd</td>
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<td>0.99</td>
<td>0.42</td>
<td>1.25</td>
<td>2.9</td>
<td>29</td>
<td>10</td>
<td>22</td>
<td>14</td>
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<td>14</td>
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<td>11</td>
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<td>0.67</td>
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<td>nd</td>
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<tr>
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<td>nd</td>
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<td>0.63</td>
<td>nd</td>
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<td>nd</td>
<td>1.05</td>
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<td>nd</td>
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<td>12</td>
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<td>M. etheridgei B</td>
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<td>0.46</td>
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<tr>
<td>M. etheridgei C</td>
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<td>nd</td>
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<td>0.73</td>
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<td>M. etheridgei D</td>
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<td>nd</td>
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<td>0.54</td>
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<td>3.3</td>
<td>5.9</td>
<td>3.3</td>
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nd = not detected. Alkane and isoprenoid ratios were calculated from area integration in the m/z 57 trace. Pr = pristane; Ph = phytane; OEP1 = (C9+6×C11+C13)/(4×C10+4×C12). OEP2 = (C13+6×C15+C17)/(4×C14+4×C16) Ratios were calculated from area integration in the following m/z traces. m= meta xylene, m/z 106; p = para xylene, m/z 106; EB = ethylbenzene, m/z 106; o= ortho xylene, m/z 106; N = naphthalene, m/z 128; MN = methylnaphthalene, m/z 142. MNR = methylnaphthalene ratio (2-MN/1-MN); MPI = Methylphenanthrene index (1.5×(3+2)/(P+9+1), measured in m/z 178 + 192.
Table 2. Aromatic/aliphatic hydrocarbon ratios for laser micropyrolysates of fossil samples.

<table>
<thead>
<tr>
<th></th>
<th>B/(n-C7+n=C7)</th>
<th>Tol/(n-C8+n=C8)</th>
<th>(EB+3 x xylene)/(n-C9+n=C9)</th>
<th>Phenol/(n-C10+n=C10)</th>
<th>N/(n-C12+n=C12)</th>
<th>(2-MN+1-MN)/(n-C13+n=C13)</th>
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<tbody>
<tr>
<td>A. toddense B</td>
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<td>0.62</td>
<td>8.4</td>
<td>3.03</td>
<td>0.58</td>
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<td>A. toddense C</td>
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<td>3.9</td>
<td>5.4</td>
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<td>3.8</td>
<td>6.0</td>
<td>0.67</td>
</tr>
<tr>
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<td>1.8</td>
<td>1.2</td>
<td>7.9</td>
<td>3.0</td>
<td>1.6</td>
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<tr>
<td>Dailyatia A</td>
<td>nd</td>
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<td>nd</td>
<td>2.6</td>
<td>8.3</td>
<td>4.0</td>
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<td>Dailyatia B</td>
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<td>nd</td>
<td>3.7</td>
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<td>5.1</td>
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<td>0.57</td>
<td>0.94</td>
<td>0.22</td>
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<tr>
<td>M. etheridgei B</td>
<td>8.1</td>
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<td>M. etheridei D</td>
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<td>M. etheridei E</td>
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<td>1.7</td>
<td>6.5</td>
<td>2.4</td>
<td>1.4</td>
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</table>

Ratios were calculated from area integration in the following m/z traces. n-alkanes = m/z 57; n-alkenes = m/z 55; B = benzene, m/z 78; Tol = toluene, m/z 91; EB = ethylbenzene, m/z 106; N= naphthalene m/z 128; MN= methylnaphthalene m/z 142.
References


Fig. 3
Fig. 4
Fig. 5
Fig. 6