FTIR microspectroscopy of Ediacaran phosphatized microfossils from the Doushantuo Formation, Weng’an, South China

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Phosphatized microfossils from ca. 580 Ma from the Doushantuo Formation in the Weng’an region of South China were analyzed by Fourier transform infrared (FTIR) microspectroscopy for their chemical characterization. Two morpho-types of phosphatized embryo-like fossils (Megasphaera and Megacolonophycus) were analyzed, together with algal fossils. Transmission IR spectra of the microfossils have absorption bands of around 2960 cm⁻¹ and 2925 cm⁻¹, indicating the presence of aliphatic hydrocarbon (anti-symmetric aliphatic CH₃ and aliphatic CH₂), and have an additional band of around 1595 cm⁻¹, probably derived from aromatic moieties (aromatic C=C). In addition, IR microscopic mapping shows that aliphatic hydrocarbon and aromatics are distributed inside the embryo-like fossils. The embryo-like fossils appear to show three types of CH₂/CH₃ peak height ratios (R3/2) and aromatic C=C/CH₃ peak height ratios (RC=,C₂): (1) high-R3/2/low-RC=,C₂ type (RC=,C₂ = -0.2–1.0 and RC=,C₂ = -0–2), (2) low-R3/2/medium-RC=,C₂ type (RC=,C₂ = -0.2–0.6 and RC=,C₂ = -1–4); and (3) low-R3/2/high-RC=,C₂ type (RC=,C₂ = -0.2–0.6 and RC=,C₂ = -1–8). All three types are contained in both Megasphaera and Megacolonophycus. Raman spectra for the carbonaceous matter within the rock sample show a similar degree of thermal alteration, indicating that the organics were embedded in situ prior to thermal matura-

1. Introduction

Fossilized animal embryos and metazoan fossils from the Ediacaran and Cambrian periods provide early records of the metazoan radiation (e.g., Bengtson and Zhao, 1997; Xiao et al., 1998; Xiao and Knoll, 2000). Fossil records suggest that all animal lineages (phyla) appeared in the Early Cambrian period (e.g., Knoll and Carroll, 1999). Recent paleontological studies point to the existence of some metazoan organisms, including sponges, cnidarians, molluscs and small bilaterians in the early Ediacaran period (Cao and Zhu, 2001; Chen et al., 2002, 2004, 2006). Extremely well-preserved spherical microfossils from the Ediacaran Doushantuo Formation (ca. 580 Ma) have been interpreted as being the oldest animal embryos (Xiao et al., 1998) and thus provide key information of animal evolution. The Doushantuo microfossils have been extensively studied by petrographic investigation of thin sections and by scanning electron microscopic examination (e.g., Xue et al., 1995; Xiao et al., 1998; Xiao and Knoll, 1999a;b; Yin et al., 2004, 2007). Recently, a few new non-destructive technologies such as Synchrotron X-ray Microtomography (SRXMT) observations have been applied to Cambrian and Ediacaran microfossils (Chen et al., 2006; Chi et al., 2006; Donoghue et al., 2006; Hagadorn et al., 2006; Chi et al., 2008; Chen et al., 2009a,b; Huldgren et al., 2011; Cunningham et al., 2012a; Yin et al., 2013, in press). Based on a...
morphological analogy of the microfossils with equivalent modern organisms, the spherical microfossils were first interpreted as algae (Xue et al., 1995), but later reinterpreted as being metazoan embryos (Xiao et al., 1998). They were also interpreted alternatively as giant vacuolated sulfur-oxidizing bacteria (Bailey et al., 2007a,b) and recently as nonmetazoan holozoans (Huldtgren et al., 2011). In addition, taphonomic experiments were conducted on extant microorganisms to test the hypothesis of their origin (Raff et al., 2008; Cunningham et al., 2012b).

However, the actual morphology of the microfossils has often been insufficient to make a detailed phylogenetic classification. It has not yet been firmly established whether the spherical microfossils are animals, algae, bacteria, or nonmetazoan holozoans (Xue et al., 1995; Xiao et al., 1998; Xue et al., 1999; Bailey et al., 2007a,b; Xiao et al., 2007; Butterfield, 2011; Huldtgren et al., 2011, 2012; Xiao et al., 2012). Different disciplines of data and evidences to address the origin of the spherical microfossils as an exact definition is very important in decoding the early evolution of the Metazoa. The use of micro-scale spectroscopic and geochemical analyses are possible ways to approach the origins of individual microfossils (Arouri et al., 1999, 2000; House et al., 2000; Kudryavtsev et al., 2001; Ueno et al., 2001; Schopf et al., 2002; Marshall et al., 2005; Schopf et al., 2005; Igisu et al., 2006; Ueno et al., 2006; Chen et al., 2007; Komiya et al., 2008; Igisu et al., 2009; Kremer et al., 2012).

Fourier transform infrared (FTIR) spectroscopy is a powerful tool for the characterization of chemical components derived from biochemical constituents in microorganisms (lipids, proteins, sugars, nucleic acids, etc.) (e.g., Helm et al., 1991a,b; Naumann et al., 1991a,b). FTIR microspectroscopy has previously been applied to Proterozoic organic-walled acritarchs and bacterial fossils as well as to Phanerozoic miospores (e.g. Arouri et al., 1999, 2000; Marshall et al., 2005; Igisu et al., 2006, 2009; Steemans et al., 2010). These studies have demonstrated that IR signatures can be useful in characterizing organic-walled microfossils, including unknown affinity such as acritarchs. However, FTIR microspectroscopy has not yet been applied to phosphatized embryo-like fossils.

Here we report the first use of FTIR microspectroscopy of the Doushantuo embryo-like fossils, together with algal fossils from the Weng’an region, South China, in order to examine the chemical signatures retained in the microfossils. The obtained IR features of the embryo-like fossils are compared with those of the algal fossils and those of extant microorganisms.

2. Geological setting

Neoproterozoic to Cambrian successions are widely distributed in South China (Han et al., 2008; Zhang et al., 2008). In particular, many phosphorite deposits from the early Ediacaran period occur in shallow carbonate platforms distributed within the Yangtze platform, including the areas of Mianxian, Shaanxi Province, Weng’an, Kaiyuan and Zunyi, Guizhou Province, Huaibei, Hunan Province, Shimen and Xiangfan, Hubei Province, and Shangrao, Jiangxi Province (Xiao and Knoll, 2000; Zhou et al., 2001). The Weng’an area (Fig. 1) is one of the most famous areas for fossils, in particular the Weng’an Lagerstätte, which yielded an animal embryo and egg, as well as for other multicellular algal fossils, acritarchs, sponge spicules, cnidarian fossils and putative small bilaterian fossils (e.g., Xiao et al., 1998; Zhang et al., 1998; Xiao and Knoll, 1999a,b, 2000; Yin and Gao, 2000; Cao and Zhu, 2001; Zhou et al., 2001; Chen et al., 2002, 2004, 2006; Zhou et al., 2007).

In the Neoproterozoic period, the Weng’an region was situated near a persistent paleotopographic high, the Upper Yangtze Oldland (Wang, 1985) on a marginal shelf bank (Jiang et al., 2008, 2011). The Neoproterozoic successions in Weng’an comprise the Marinoan Nantuo Tillite and the Doushantuo and Dengying Formations in ascending order (Fig. 1). The Doushantuo Formation unconformably overlies the older metamorphic Qingshuijiang Formation of the Banxi Group, or the Marinoan Nantuo Tillite (Xiao et al., 1998; Zhang et al., 1998; Yin et al., 2004; Zhou et al., 2004). It also lies under the thick dolostone layer of the Dengying Formation, in which dolostone is found in the bottom layer and small Cambrian shellbed fossils are found in the top layer (Wang et al., 1984).

The Doushantuo Formation at Weng’an is ca. 40 m thick, and composed of two (Zhou, 1997; Zhang et al., 1998; Xiao and Knoll, 2000; Zhou et al., 2001) or three sequences (Jiang et al., 2011), bounded by unconformities at the top of the dolostone layers. The lower Doushantuo sequence begins with ca. 5–10 m of dolostone, corresponding with the Cap dolomite on the Marinoan glacial deposit. This is then succeeded by an alternation of black shale and phosphorite, an alternation of phosphorite and dolostone, and then thick dolostone ca. 5 m thick, in ascending order (Zhang et al., 1998; Xiao and Knoll, 2000; Zhou et al., 2001; Jiang et al., 2008; Komiya et al., 2008; Jiang et al., 2011). The upper Doushantuo sequence begins with silica nodule-bearing phosphorite, followed by bedded phosphorite then dolostone. Towards the top of the section, phosphate becomes less abundant and dolostone becomes increasingly common. The two sequences represent shallow-upward sequences, separated by a laterally continuous karstic, subaerial exposure surface (e.g. Xiao and Knoll, 1999b; Fig. 1c), commonly interpreted as having been deposited in a shallow-marine environment during the highstand system tract (HST) for the lower sequence, and above storm wave base for the upper sequence, respectively (e.g. Zhang et al., 1998).

Based on correlations of stable carbon isotopes and diverse acritarchs, the age of the Doushantuo Formation has been suggested as being between 600 and 550 Ma; probably around 570 ± 20 Ma (Xiao et al., 1998; Zhang et al., 1998; Knoll and Xiao, 1999; Xiao and Knoll, 1999a,b). However, the presence of large acanthomorphic acritarchs (Tianzhushania and Papilionembrana) in the Doushantuo Formation suggests that they are older than the diverse Ediacaran assemblages (Zhang et al., 1998; Yin et al., 2001). Barford et al. (2002) obtained dates of 584 ± 26 Ma (Lu–Hf) and 599.3 ± 4.2 Ma (Pb–Pb) from Doushantuo phosphorites. The Pb–Pb isochron age of the upper part of phosphorite in the upper Doushantuo sequence reveals the age as being 576 ± 14 Ma (Chen et al., 2004).

3. Materials and methods

3.1. Samples

We analyzed phosphorite rock samples (WY13 and WY24) collected from phosphoritic dolostones in the upper sequence of the Doushantuo Formation (Fig. 1c). Thin doubly-polished petrographic sections of the samples were prepared to obtain IR spectra of the microfossils, as previously described in Igisu et al. (2006) (except for the exclusion of alumina paste so as to avoid interference fringes). Based on petrographic investigation of the thin sections, the phosphorite is composed mainly of dolomite and phosphate. The grain size of the dolomite varies up to ~200 μm, and that of phosphate varies up to ~850 μm. In descending order of abundance, the following was observed: dolomite, phosphate, brown to dark amber carbonaceous matter, and microbial fossils (same color as carbonaceous matter). Three types of microfossils were analyzed in this study (Fig. 2 and Table 1): (1) globular microfossils (670–850 μm in diameter) consisting of one internal body (~540–700 μm in diameter) enclosed/unclosed in an envelope; (2) globular microfossils (~600–670 μm in diameter) consisting of hundreds of small spherical internal bodies (~20–30 μm in diameter) enclosed/unclosed in an envelope; and (3) algal microfossils consisting of tens or hundreds of rectangular or polygonal shaped cells (2.5–10 μm on a side). The globular microfossils were assigned to Megaphaera sp., and Megalomplus sp., respectively, based on their sizes, internal cell numbers, and overall appearances (Xiao and Knoll, 2000).
where $T_0$ represents the transmitted intensity of infrared light at each wavenumber for the air background and $T$ represents that for a sample. An IR spectrum was obtained by collecting 64 scans in the spectral range of 4000 to 1000 cm$^{-1}$ at a 4 cm$^{-1}$ resolution. The spatial resolution was 12.5 $\times$ 12.5 $\mu$m$^2$ corresponding to a pixel area of the IR linear array detector. More than ten thousand of IR spectra were obtained. Distributions of organic and inorganic signatures were obtained at the following peak heights with linear baseline corrections: 3000–2800 cm$^{-1}$ for 2960 and 2925 cm$^{-1}$ bands (aliphatic CH$_3$ and CH$_2$, respectively); 1720–1570 cm$^{-1}$ for 1595 cm$^{-1}$ band (aromatic C=C); 2700–2400 cm$^{-1}$ for 2630 cm$^{-1}$ band (dolomite); 2230–1950 cm$^{-1}$ for 2000 cm$^{-1}$ band (phosphate). Analytical error was determined by duplicate measurements in the same position as the background spectrum and was better than the 0.003 absorbance unit (AU) in the 3000–1900 cm$^{-1}$ range, except in the CO$_2$ absorption region, and an error in the range of 1700–1550 cm$^{-1}$ was better than 0.007. All the IR spectral data were processed with Spectra Manager (JASCO).

3.3. Absorbance ratio estimation

In order to evaluate the relative length and degree of branching of the aliphatic hydrocarbon chain in the microfossils, we used the aliphatic CH$_3$/CH$_2$ absorbance ratio ($R_{3/2}$):

$$R_{3/2} = \left[\frac{\nu_{as} \text{CH}_3}{\nu_{as} \text{CH}_2}\right]$$

where $\nu_{as} \text{CH}_3$ and $\nu_{as} \text{CH}_2$ represent peak heights of anti-symmetric stretching bands for aliphatic CH$_3$ (end-methyl; ~2960 cm$^{-1}$) and CH$_2$ (chain-methylene; ~2925 cm$^{-1}$) after linear baseline correction, respectively (Igisu et al., 2009). We also calculated the absorbance ratio of aromatic C=C to aliphatic CH$_2$ ($R_{C=C/2}$) to evaluate the relative amount of aromatics in the microfossils:

$$R_{C=C/2} = \left[\frac{\nu \text{C}}{\nu_{as} \text{CH}_2}\right]$$

In this study, five specimens of *Megaphaera* sp. and three specimens of *Megaclonophycus* sp., together with three specimens of algal fossils, were analyzed (Table 1 and Fig. 2). All the specimens except one (WY13) are from the same sample (WY24).

3.2. Micro FTIR measurements and mapping

FTIR microspectroscopic analysis and IR mapping were conducted on the thin sections using an automated XYZ stage set in an FTIR micro-spectrometer (JASCO FTIR6200 + IRT7000) with a 16-channel linear array mercury cadmium telluride (MCT) detector. The thin section was placed over a hole in a sample holder, and measured in the following manner.

A reference background spectrum was first measured from a place away from the mounted sample (air), and then a transmission IR spectrum of the sample was measured. The IR spectrum is described as IR absorbance as a function of the wavenumber (cm$^{-1}$):

$$\text{absorbance} = -\log_{10}\frac{T}{T_0}$$

where $T_0$ represents the transmitted intensity of infrared light at each wavenumber for the air background and $T$ represents that for a sample.
where νC–C represents peak height of the stretching band for the aromatic ring C=C (~1595 cm$^{-1}$) after a linear baseline correction.

3.4. Micro Raman measurements

Laser Raman micro-spectrometer (JASCO NRS-2000) was used to characterize the alteration degree of the carbonaceous matter. The thin sections were twice exposed to an Ar laser (514.5 nm) for 5 s at a laser power of 20 mW to obtain Raman spectra in a range of 1750 to 200 cm$^{-1}$ at a 1 cm$^{-1}$ resolution. One measurement obtained a Raman spectrum with a range of about 800 cm$^{-1}$ and spectra in the 1750–200 cm$^{-1}$ were obtained by combining the two spectra. A 50× objective lens (NA = 0.80) was used, so the spatial resolution of the Raman analysis was about 1.5 μm.

We only analyzed the carbonaceous matter embedded within the rocks below the surface of the thin-section to avoid the effect of polishing, which can induce deformation of carbonaceous matter during sample preparation and thus possibly induce artificial modification of the Raman spectroscopic feature (Pasteris, 1989). Because of a strong structural anisotropy, orientation of individual graphite and carbonaceous matter grains affects their Raman spectra (e.g., Katagiri et al., 1988; Pasteris, 1989; Wang et al., 1989). The orientation of the graphite planes can affect the intensity ratio of disorder band (D band; ~1340 cm$^{-1}$) to graphite band (G band; ~1600 cm$^{-1}$) (e.g., Katagiri...
4. Results

4.1. IR spectral features

Typical infrared transmission spectra of thin sections of the embryo-like fossils and the matrices are shown in Fig. 3. The embryo-like fossils show IR absorption bands of aliphatic C–H moieties (∼2960, ∼2925 and ∼2850 cm\(^{-1}\)) in the range of >2500 cm\(^{-1}\) (M in Fig. 3A). The bands around 2960 and 2925 cm\(^{-1}\) are derived from anti-symmetric stretching of end-methyl aliphatic CH\(_3\) and chain-methylene CH\(_2\), respectively (Bellamy, 1954). A weaker band from the symmetric stretching of CH\(_2\) is also seen around 2850 cm\(^{-1}\). In the range of ∼2000 cm\(^{-1}\), IR spectra of the embryo-like fossils are similar to those of the phosphate matrix but a weak shoulder around 1595 cm\(^{-1}\) seems to be due to \(\text{C} = \text{C}\) bond in aromatic ring (dotted arrow in Fig. 3A) (Bellamy, 1954). IR spectra of the algal fossils show the same IR absorption bands as the embryo-like fossils.

In the matrices, two types of spectra were observed (P and D in Fig. 3A). The saturated absorption band at around 1100 cm\(^{-1}\) is due to the stretching vibrations of phosphate anions PO\(_4^{3-}\) (Rehman and Bonfield, 1997; Elliot, 2002) (P in Fig. 3A). Bands at around 1640, 1440 and 870 cm\(^{-1}\) are considered to be due to the vibrations of carbonate anions CO\(_3^{2-}\), possibly replacing PO\(_4^{3-}\) in phosphate (Rehman and Bonfield, 1997; Elliot, 2002). Bands at 2150, 2080 and 2000 cm\(^{-1}\) are due to overtones and combinations of \(\text{P} – \text{O}\) vibrations and also seem to be characteristic of phosphates including HPO\(_4^{2-}\) components (Elliot, 2002). A sharp band at around 3535 cm\(^{-1}\) is assigned to the stretching of O–H (Rehman and Bonfield, 1997; Elliot, 2002). A broad band at around...
3400 cm$^{-1}$ originates from the liquid-like molecular H$_2$O. This band may reflect H$_2$O present at the grain boundary of phosphates as is observed in cherts composed of cryptocrystalline quartz (Nakashima et al., 1995; Ito and Nakashima, 2002; Igisu et al., 2006, 2009).

Another sample from the matrices shows IR absorption bands at around 3020, 2900, 2630 and 2525 cm$^{-1}$ in the >2500 cm$^{-1}$ region together with more saturated bands around 1820, 1500, and 880 cm$^{-1}$ (D in Fig. 3A). These are typical bands for carbonates (RRUFF Data base, ...
Fig. 4 (continued).

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Fig. 4 (continued).
Fig. 4 (continued).
Based on the bands near 2600–2500 cm$^{-1}$, which are diagnostic features for the identification of calcite and dolomite (Nguyen et al., 1991), the matrices are composed of dolomite rather than calcite.

4.2. Peak height ratios and spatial distributions of functional groups

In order to clarify the distribution of the organic components, dolomite, and phosphate, IR mapping analysis against the air background was conducted on the microfossils (Fig. 4). In the lower wavenumber regions of less than 2500 cm$^{-1}$, many intense bands of the phosphate matrix would have masked any small signals from organic matter (Fig. 3). Hence, we focused on IR bands originating from the aliphatic C–H and the aromatic C=C bands as being the organic components. We chose the band at 2960 cm$^{-1}$ as a representative peak of the aliphatic C–H bonds. This is because a baseline correction of between 3000 and 2800 cm$^{-1}$ can preclude the peak height derived from dolomite, while the IR absorption bands of dolomite in the 3000–2800 cm$^{-1}$ region could overlap and mask those of dolomite.
the aliphatic C–H bonds (Schenk et al., 1985) (Fig. 3A). It should also be noted that the 1595 cm$^{-1}$ peak height can be affected not only by the band of the aromatic C=C bond, but also by the saturated band of dolomite (Fig. 3A). For a better understanding of the distribution of the organic components, distributions of functional groups in the mineral matrix were examined. We do not use the data in which the dolomite peak (2630 cm$^{-1}$) was detected.

We classify the area analyzed by petrographic investigation in the embryo-like fossils into two types, as shown in Fig. 4a2–g2: (1) the inner part, which consists of an internal body (bodies); and (2) an envelope surrounding the internal body (bodies) of the embryo-like fossil. Among the 7 specimens, five have an envelope structure (Fig. 4). Mapping images are shown in Fig. 4 and the $R_{3/2}$ and $R_{C=C/2}$ values obtained from the embryo-like fossils together with the algal fossils are shown in Fig. 5. We do not use the data detected by the 16th channel in the MCT linear array detector because its signal-to-noise ratio is at least one order larger than that of the other fifteen channels.

In all the specimens, the distributions of the intensities of two bands at 2630 and 2000 cm$^{-1}$ due to dolomite and phosphate, respectively, show that the main mineral matrix hosting the studied microfossil is considered to be phosphate, not dolomite (Fig. 4).
distribution of the phosphate roughly agrees with those of aliphatic
CH₃ and aromatic C=C (Fig. 4), indicating that the phosphate matrix
contains organic matter.

4.2.1. Megasphaera sp.
Megasphaera is classified into two types by the absence or presence
of an envelope structure (Fig. 4A and B–D, respectively). In the
specimen without an envelope structure (WY24-1-5: Fig. 4A), the 2960 cm$^{-1}$ (aliphatic CH$_3$) and the 1595 cm$^{-1}$ peak heights (aromatic C=C) are higher in the dark part than in the light part (Fig. 4a1, a3, a5). The peak height ratio of the R$_{3\alpha\beta}$ value ranges from 0.36 to 0.86 (average = 0.73, SD = 0.07, n = 1377), and that of the R$_{c\alpha\beta}$ value ranges from 0.10 to 1.98 (average = 1.02,
In the two specimens with an envelope structure, one (WY13) shows lower intensities of 2960 cm$^{-1}$ (aliphatic CH$_2$) and 1595 cm$^{-1}$ peaks (aromatic C=C) in the rim in the inner part (Fig. 4F, 5). The R$_{3/2}$ values for the inner part in specimen WY13 range from 0.25 to 0.47 (average = 0.38, SD = 0.05, n = 79), and those for the envelope range from 0.20 to 0.47 (average = 0.33, SD = 0.17, n = 70) (Fig. 4G). The R$_{8-2}$ values for the inner part range from 0.84 to 3.72 (average = 1.77, SD = 0.62, n = 79), and those for the envelope range from 0.91 to 3.89 (average = 2.24, SD = 0.94, n = 70) (Fig. 4H). The R$_{2}$ and R$_{8-2}$ values show a homogeneous distribution within the microfossil (Fig. 4G, H).

The other specimen (WY24-4-6) has open spaces between the inner structure and the envelope where small spheroids are not observed (Fig. 4I, J). These void portions do not include organic components (Fig. 5J, K). Specimen WY24-4-6 shows a homogeneous distribution of 2960 cm$^{-1}$ peak (aliphatic CH$_2$) within the microfossil, while 1595 cm$^{-1}$ peak height (aromatic C=C) is higher in the inner part than within the envelope (Fig. 4J, K). In specimen WY24-4-6, the R$_{2}$ values for the inner part vary from 0.29 to 0.66 (average = 0.45, SD = 0.06, n = 205), and those for the envelope vary from 0.20 to 0.71 (average = 0.44, SD = 0.07, n = 225) (Fig. 4J). The R$_{8-2}$ values for the inner part range from 1.29 to 7.88 (average = 4.02, SD = 1.40, n = 205), and those for the envelope range from 0.41 to 4.15 (average = 2.40, SD = 0.76, n = 225) (Fig. 4K). The R$_{2}$ values are fairly homogeneously distributed within the microfossil, while the R$_{8-2}$ values are larger in the inner part than within the envelope (Fig. 4J, K).

FTIR observation of three specimens of Megaglonocephalus sp. shows that in the individual fossils, the averages of R$_{3/2}$ and R$_{8-2}$ values for the inner part do not significantly differ from those for the envelope (Fig. 5E–G and L). However, the R$_{2}$ and R$_{8-2}$ values ranges and their averages for specimens WY24-1-1, WY13 and WY24-4-6 are somewhat different (Fig. 5E, F, G and L). The R$_{2}$ values ranges and averages for specimen WY24-1-1 are larger than for the other two specimens, while the R$_{8-2}$ values ranges and averages are larger in the following order: WY24-4-6 > WY13 > WY24-1-1 (Fig. 5E, F, G and L).

4.2.3. Algal fossils

Three specimens of algal fossils were also analyzed. The R$_{2}$ values for specimen WY24-2-3 range from 0.30 to 0.51 (average = 0.41, SD = 0.03, n = 337, Fig. 5H). The R$_{8-2}$ values range from 1.75 to 5.20 (average = 2.73, SD = 0.49, n = 337, Fig. 5H). In specimen WY24-4-9, the R$_{2}$ values vary from 0.32 to 0.62 (average = 0.45, SD = 0.04, n = 200, Fig. 5I). The R$_{8-2}$ values vary from 1.86 to 3.58 (average = 2.61, SD = 0.30, n = 200, Fig. 5I). Specimen WY24-5-5 shows the R$_{2}$ values from 0.34 to 0.56 (average = 0.47, SD = 0.03, n = 1006, Fig. 5J) and the R$_{8-2}$ values from 1.96 to 5.84 (average = 3.05, SD = 0.70, n = 1006, Fig. 5J).

The R$_{2}$ and R$_{8-2}$ values for the three specimens of algal fossils show similar ranges and averages (Fig. 5J–k and m). Their R$_{2}$ values are consistent with those for whole cell and extracted lipid of extant microeukaryotes (average: 0.58 ± 0.09 for cells and 0.48 ± 0.07 for lipids; Igisu et al., 2009, 2012).

4.3. Raman spectral features

A representative Raman spectrum of carbonaceous matter in the thin sections is shown in Fig. 6. It shows two bands of G band (~1600 cm$^{-1}$) and D band (1340 cm$^{-1}$). These are characteristic of the C=C bond of disordered (disorganized) aromatic carbonaceous matter (Kudryavtsev et al., 2001; Ueno et al., 2001; Brasier et al., 2002; Pasteris and Wopenka, 2002; Schopf et al., 2002; Pasteris and Wopenka, 2003; Brasier et al., 2004; Schopf et al., 2005), which supports the possible presence of the aromatic C=C group observed in the IR spectra of the microfossils (Fig. 3). The Raman spectra of the
carbonaceous matter in thin section WY24 are characterized by first order bands; D/G intensity ratios from 0.75 to 0.87 (ave. 0.81, SD = 0.05, n = 9); FWHM of G band from 70.1 to 79.7 (ave. 74.4 cm\(^{-1}\)), those in thin section WY13 are characterized by first order bands; D/G intensity ratios from 1.01 to 1.22 (ave. 1.10, SD = 0.09, n = 4); FWHM of G band from 65.7 to 82.3 (ave. 73.1 cm\(^{-1}\), SD = 6.9 cm\(^{-1}\)). There is a no clear correlation between the D/G intensity ratios and FWHM of G band. The observed variations of the D/G intensity ratios are probably due to orientation effect and due to alteration degree of the carbonaceous matter. Although orientation of graphite planes may affect FWHM of G band, the FWHM of G band for the thin sections WY13 and WY24 are similar and are corresponding to chitinozoan and vitrinite re-

5. Discussion

In order to better understand the chemical characteristics of the Doushantuo embryo-like fossils, we discuss their IR signatures. We then discuss the possible origin of the fossils as implicated by the IR characteristics.

5.1. IR characteristics of the embryo-like fossils

According to the relationship between the R\(_{3/2}\) and R\(_{C=C/2}\) values for the inner part (Fig. 5), we classified the embryo-like fossils roughly into three types: (1) high-R\(_{3/2}\)/low-R\(_{C=C/2}\) type (R\(_{3/2}\) = -0.2 to 1.0 and R\(_{C=C/2}\) = 0–2), (2) low-R\(_{3/2}\)/medium-R\(_{C=C/2}\) type (R\(_{3/2}\) = -0.2–0.6 and R\(_{C=C/2}\) = -1–4); and (3) low-R\(_{3/2}\)/high-R\(_{C=C/2}\) type (R\(_{3/2}\) = -0.2–0.6 and R\(_{C=C/2}\) = 1–8). All these three types were observed in both Megasphaera and Megacolonophyus.

5.1.1. High-R\(_{3/2}\)/low-R\(_{C=C/2}\) type

Two specimens of Megasphaera (WY24-1-5 and WY24-1-2) and one of Megacolonophyus (WY24-1-1) were classified in the high-R\(_{3/2}\)/low-R\(_{C=C/2}\) type (Fig. 5A, B, E). Specimen WY24-1-2 had an envelope structure but the other two specimens did not. The R\(_{3/2}\) values of their inner parts varied from 0.23 to 0.95 and the R\(_{C=C/2}\) values varied from 0.10 to 2.65 (Fig. 5). Both two specimens of Megasphaera showed smaller R\(_{3/2}\) values in the rim of the inner part (Fig. 4a4, b4), while R\(_{C=C/2}\) values showed relatively homogeneous distributions. Regardless of presence or absence of the envelope structure, similar ranges of R\(_{3/2}\) and R\(_{C=C/2}\) values for two specimens of Megasphaera (Fig. 5A, B) suggest that these two specimens might belong to the same kind of microorganism. This is consistent with the suggestion by Xiao et al. (2007) that Megasphaera inornata without the envelope originates from Megasphaera ornata with the envelope. In one specimen of Megacolonophyus, the R\(_{3/2}\) values were larger in the rim portions of the inner part where small spheroids were morphologically preserved (Fig. 4e4), while R\(_{C=C/2}\) values show a relatively homogeneous distribution (Fig. 4e6).

5.1.2. Low-R\(_{3/2}\)/medium-R\(_{C=C/2}\) type

One specimen of Megasphaera (WY24-5-1) and one of Megacolonophyus (WY13) belonged to the low-R\(_{3/2}\)/medium-R\(_{C=C/2}\) type (Fig. 5C, F). Both specimens had an envelope structure. The R\(_{3/2}\) values of their inner parts ranged from 0.25 to 0.62 and the R\(_{C=C/2}\) values ranged from 0.84 to 3.72 (Fig. 5). The R\(_{3/2}\) values of their envelopes varied from 0.20 to 0.47 and the R\(_{C=C/2}\) values varied from 0.91 to 3.89 (Fig. 5). The envelope of Megasphaera showed somewhat smaller R\(_{C=C/2}\) values than did its inner part (Figs. 4f6, 5F), while that of Megacolonophyus showed somewhat larger R\(_{C=C/2}\) values than did its inner part (Figs. 4c6 and 5C). Both specimens showed relatively homogeneous distributions of the R\(_{3/2}\) values and R\(_{C=C/2}\) values within their inner parts (Figs. 4c4, f4).

5.1.3. Low-R\(_{3/2}\)/high-R\(_{C=C/2}\) type

One specimen of Megasphaera (WY24-2-4) and one of Megacolonophyus (WY24-4-6) were classified in the low-R\(_{3/2}\)/high-R\(_{C=C/2}\) type (Fig. 5D, G). Both specimens had an envelope structure. The R\(_{3/2}\) values of their inner parts varied from 0.25 to 0.66 and the R\(_{C=C/2}\) values varied from 1.29 to 8.84 (Fig. 5D, G). The R\(_{3/2}\) values of their envelopes varied from 0.20 to 0.71 and the R\(_{C=C/2}\) values varied from 0.41 to 7.58 (Fig. 5D, G). Their envelopes showed R\(_{3/2}\) values similar to their inner parts (Fig. 4d4, g4). Both specimens showed relatively homogeneous distributions of the R\(_{3/2}\) values within their inner parts (Fig. 4d4, g4). The R\(_{C=C/2}\) values of both were larger in the inner part than in the envelope (Fig. 4d6, g6). Within the inner part, the R\(_{C=C/2}\) values were variable and some portions of the inner part showed larger R\(_{C=C/2}\) values (Fig. 4d6, g6). The resemblance of average R\(_{3/2}\) and R\(_{C=C/2}\) values between the specimens might imply that they have the same origin.

The variations in R\(_{3/2}\) and R\(_{C=C/2}\) values could possibly have been produced by post-mortem alteration. Previous experimental studies on the thermal alteration of organic matter reported that the R\(_{3/2}\) value of organic matter can increase during thermal alteration (Huang and Otten, 1998; Igisu et al., 2009). Therefore, the observed difference in R\(_{3/2}\) values between the individual microfossils could be produced by post-depositional thermal alteration. In addition, the R\(_{C=C/2}\) value can change during post-depositional thermal alteration. It is known that the IR spectra of kerogen during the diagenesis stage generally show aliphatic C–H bands as well as aromatic C–C bands (Tissot and Welte, 1984). It has been indicated that there are less aliphatic C–H bands and that aromatic C–C bands become a dominant feature of IR spectra as kerogen alteration proceeds (Tissot and Welte, 1984). This implies that R\(_{C=C/2}\) value of organic matter increases during thermal alteration. Thus the variances between R\(_{3/2}\) values as well as the R\(_{C=C/2}\) values can be produced by the thermal alteration of microorganisms, and more thermally degraded microfossils would have larger R\(_{3/2}\) and R\(_{C=C/2}\) values.
In order to evaluate the degree of thermal alteration of carbonaceous matter, we estimated the FWHM of G band for carbonaceous matter using Raman spectroscopy. Based on the FWHM of G band for each sample, the carbonaceous matter in one thin section seems to be degraded to an approximately similar degree. There are also no significant differences in FWHM of G band between the thin sections WY24 and WY13. Raman spectroscopic feature indicates the organics were embedded in situ prior to thermal maturation. Moreover, all specimens (except WY13) are preserved in one hand specimen (WY24). It is unlikely that degree of thermal alteration of organic matters is different within the individual microfossils, and is different between the individual microfossils in the same sample. The previous study showed that experimental thermal alteration increased $R_{C/2}$ values for cyanobacterial whole cells and extracted membrane fractions, but did not significantly change those for extracted lipids (Igisu et al., 2009). Each source organic matter can undergo different thermal maturation pathways. It was also shown that the same starting material has a similar thermal maturation trend in $R_{C/2}$ values (Igisu et al., 2009). Thus the carbonaceous matter with different $R_{C/2}$ values and similar FWHM of G band implies that the different organics existed at least before thermal maturation. The variations of the $R_{C/2}$ and $R_{C=c=C/2}$ values for the microfossils seem to reflect original compositions of microorganisms and/or immediately occurred post-mortem alteration.

5.2. Implication of IR signatures for possible origin of the embryo-like fossils

The Doushantuo spherical microfossils were first interpreted as being volvocalean green algae (Xue et al., 1995), but later reinterpreted as being metazoan embryos (Xiao et al., 1998) based on their size and the geometry of cell division, with apparently little or no intervening growth. Embryo-like fossils, previously interpreted as being animal eggs, were documented by Yin et al. (2007) as preserved inside an anachorontic acritarch (Tianzhushania). This suggested that the Tianzhushania could be an early cleavage stage embryo preserved within a diapause egg cyst (Yin et al., 2007). SRXTM observations revealed inner structures of the embryo-like fossils to be consistent with sponge, cnidarian or bilaterian affinities (Chen et al., 2006; Hagadorn et al., 2006; Chen et al., 2009a,b). However, Xue et al. (1999) refuted some of the evidence for the spherical microfossils being animal embryos given by Xiao et al. (1998), and emphasized that volvocalean green algae had originated from the spherical microfossils. In addition, Bailey et al. (2007a, 2007b) reported that, based on their size, morphologies and cell division geometries, some microfossils, previously interpreted as being animal embryos, resembled giant vacuolated sulfur-oxidizing bacteria. In contrast, Bengtson et al. (2010) and Huldtgren et al. (2011) noted that the presence of nuclei was inconsistent with sulfur-oxidizing bacteria, but that this did not imply that the organisms were animals. Finally, they concluded that close relatives of the microfossils were neither animals nor embryos, but that they possibly belonged to holozoans, based on the similarity of the developmental pattern of an embryo-like fossil with the life style of holozoans (Huldtgren et al., 2011). Because the Doushantuo embryo-like fossils have been variously morphologically interpreted as algae, animal eggs, giant sulfur oxidizing bacteria, or nonmetazoan holozoans (Xiao et al., 1998; Xiao and Knoll, 2000; Bailey et al., 2007a,b; Huldtgren et al., 2011), we now discuss the possible origins of these fossils.

The variations of $R_{C/2}$ values and $R_{C=c=C/2}$ values within/between the individual microfossils can then be explained as follows: the specimens with different $R_{C/2}$ and $R_{C=c=C/2}$ values belong to different kinds of microorganisms; and/or the variations have been caused by post-mortem alteration.

Firstly, the IR signatures of CH2 and CH3 functional group ratios generally reflect the degree of branching and the chain length of the aliphatic hydrocarbon moiety (e.g., Lin and Ritz, 1993; Marshall et al., 2005). This concept has been used to provide an indicator for the chemical classification of organic-walled fossils (Marshall et al., 2005; Igisu et al., 2009), and can be used as a “domain-specific” indicator for extant prokaryotes (Igisu et al., 2012). The $R_{C/2}$ value can then be considered to be a clue for the chemical identification of the Doushantuo microfossils.

In contrast, there have been very few reports of highly aromatic C=C biomacromolecules (Arouri et al., 2000). Chlorophyte Chlorella marina (Derenne et al., 1996) and the resting cyst of dinoflagellate Kokinos et al., 1998) have been reported as microorganisms possessing highly aromatic C=C biomacromolecules. Their sizes and morphologies, however, do not resemble the microfossils studied here.

5.2.1. Algal origin

To test the potential algal origin, we discuss the IR characteristic of the algal fossils analyzed in this study. It is suggested that the $R_{C/2}$ values for the algal fossils are consistent with those for whole cell and extracted lipids of extant microeukaryotes (Igisu et al., 2009, 2012). After considering thermal alteration which could increase the $R_{C/2}$ values (Huang and Otten, 1998; Igisu et al., 2009), the $R_{C/2}$ values for the algal fossils are comparable to those for degraded microeukaryotes. Although the presence of highly aromatic molecules have not been understood (as mentioned above), similarities in trend of the obtained $R_{C=c=C/2}$ values (~2 to ~6) and of the minimum $R_{C=c=C/2}$ values (~2) seem to be one of the chemical characteristics of algal fossils, because the variations in $R_{C/2}$ and $R_{C=c=C/2}$ values are unlikely to be produced by thermal alteration of microorganisms. It is, however, unclear whether biological/chemical alteration occurring immediately after death and before phosphatization might have produced the variations in $R_{C/2}$ and $R_{C=c=C/2}$ values. Assuming that post-mortem biological/chemical alteration caused the variations in the $R_{C=c=C/2}$ values for the algal fossils, the obtained relatively high $R_{C=c=C/2}$ values might represent the selective preservation of a carbonized ring-structured component in cell walls, such as cellulose.

Comparing the $R_{C/2}$ and $R_{C=c=C/2}$ values of the embryo-like fossils with those of the algal fossils, low $R_{C/2}/$medium $R_{C=c=C/2}$ type and some of the low $R_{C/2}/$high $R_{C=c=C/2}$ type of the embryo-like fossils seem to show similar $R_{C/2}$ and $R_{C=c=C/2}$ values to those of the algal fossils (Fig. 5). This indicates that they are of algal origin. However, embryo-like fossils of the high $R_{C/2}/$medium $R_{C=c=C/2}$ type show different $R_{C/2}$ and $R_{C=c=C/2}$ values from those of the algal fossils, suggesting that they do not belong to algae.

5.2.2. Animal origin

We analyzed eukaryotic cells including the resting eggs of Artemia and Branchinella, which have been morphologically compared to the Doushantuo microfossils (Cohen et al., 2009; Gostling et al., 2009). $R_{C/2}$ values for the resting eggs without outer wall structure are 0.70 ± 0.07 for Artemia and 0.60 ± 0.04 for Branchinella. Those for the eukaryotic cells of algae, plant and fungi range from 0.30 ± 0.02 to 0.67 ± 0.02 (Igisu et al., 2009, 2012). The variation in $R_{C/2}$ values of whole cells would reflect the differences in composition of the cellular components (lipids, protein, etc.) and their abundance ratios. Although $R_{C/2}$ values for lipids and water-soluble substances of animal eggs have not been analyzed, the $R_{C/2}$ value for the water-soluble substances is expected to be larger (~0.9) than that of whole cells, based on a calculation using published IR spectra of cell fractions of extant bovine liver cells (Lasch et al., 2002). Using linear correlation between the $R_{C/2}$ values and CH2/CH3 functional group ratios (Igisu et al., 2009), the $R_{C/2}$ value of lipids is predicted to be 0.3–0.4 because C14–C20 fatty acids are major components in lipids extracted from animal embryos such as Artemia and sea urchin (Kozhina et al., 1978; Navarro et al., 1993).

If thermal alteration had increased the $R_{C/2}$ value of organic matter (Huang and Otten, 1998; Igisu et al., 2009), the possible $R_{C/2}$ values of
the thermally degraded animal cells would be more than 0.3. Comparing the \( R_{3/2} \) values of the embryo-like fossils with those of the expected thermally-degraded animal cells, the \( R_{3/2} \) values of all the embryo-like fossils range from those belonging to the thermally-degraded eukaryotic lipids to those of soluble substances. This can therefore be explained by the presence of a mixture of animal cellular components. Thus there is no evidence to contradict the interpretation that the embryo-like fossils are of animal origin. It is, however, unclear which process produces the variation in the \( R_{C=C/C} \) values.

5.2.3. Bacterial origin

\( R_{3/2} \) values of extant bacteria are 0.66 for the whole cell, 1.16 for soluble substances and 0.36 for lipids, respectively (Igisu et al., 2009, 2012). The \( R_{3/2} \) values of the embryo-like fossils mostly range from those belonging to bacterial lipids to those of soluble substances; indicating that they can be explained by a mixture of degraded bacterial cellular substances (lipids and soluble substances). The inner part of giant sulfur oxidizing bacteria *Thiomargarita* is, however, mainly composed of a liquid vacuole in which nitrate accumulates to a concentration of 0.1–0.8 M (Schulz et al., 1999). Its cytoplasm is restricted to a thin outer layer of 0.5 to 2 \( \mu m \) thickness (Schulz et al., 1999). Thus, the IR characteristics of lipids are unlikely to be observed in the inner part of fossilized giant sulfur oxidizing bacteria. Moreover, the IR spectra of the inner part of *Megasphaera* do not show any bands characteristic of nitrate (1410–1340 cm\(^{-1}\) and 860–800 cm\(^{-1}\) bands; Bellamy, 1954) (Fig. 3). There does not seem to be a difference in the IR spectra between the microfossils and the matrix (Fig. 3) in the region of these wavenumbers. These results do not, therefore, support the interpretation that *Megasphaera* is of bacterial origin.

Cunningham et al. (2012b) conducted an experimental degradation of *Thiomargarita* and reported that the inner part of *Thiomargarita* contains diffuse and amorphous material, possibly representing a degraded cytoplasm as decay progressed, and that as such, the degraded *Thiomargarita* has a distorted sheath but does not show an inner structure. Every embryo-like fossil studied here has a spherical appearance. Although the degraded cytoplasm of bacteria might show the various ranges of \( R_{3/2} \) and \( R_{C=C/C} \) values consistent with those of the embryo-like fossils, the absence of any distorted sheaths in any of the specimens studied here is incomparable to the results of the experimental degradation of *Thiomargarita*.

Consequently, these results seem to support the interpretation that *Megasphaera* and *Megasclonophycus* are of animal origin, and are not of bacterial origin. If the variation in the \( R_{C=C/C} \) values can be explained by the presence or absence of cell walls and/or the degree of alteration of organic molecules, a likely explanation at this stage is that the four embryo-like fossil specimens (WY24-5-1, WY24-2-4, WY13 and WY24-4-6) belong to algae, and the other three (WY24-1-1, WY24-1-2 and WY24-1-5) belong to the animal kingdom.

FTIR microspectroscopy is able to detect some organic signatures in the embryo-like fossils, and the IR signatures of the microfossils can differentiate specimens within a morphologically same taxon. Chemical characterization of the Doushantuo microfossils together with a morphological analysis will provide new biological information on embryo-like fossils, while the chemical signatures for the taxonomy of eukaryotes need to be further explored.

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**References**


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