Preservation of organic matter in sponge fossils: a case study of ‘round sponge fossils’ from the Cambrian Chengjiang Biota with Raman spectroscopy

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Understanding the taphonomy of organic matter of sponges will be helpful in reconstructing a more exhaustive picture of the evolutionary history of these organisms from fossil records. The so-called ‘round sponge fossils’ (RSF) from the Burgess Shale-type (BST) Chengjiang Lagerstätte predominantly yield explicit organic remains, which seem more durable than the carbonaceous components of other fossils in the same Lagerstätte. In order to characterize these carbonaceous remains with Raman spectroscopy, a quick and non-destructive technique with the ability of analyzing the molecular composition and crystal structure in high resolution, 5 RSF specimens were examined in this study. Another Cambrian sponge fossil from the Xiaoyanxi Formation and a few algal remains from the Ediacaran Wenghui Biota were also measured for comparison.

The resulting Raman spectra of the macroscopic fossils confirmed previous observations on microfossils by Bower et al. (2013) that carbonaceous material with compositionally complex precursor material and low diagenetic thermal affection will plot in a certain region in a $\Gamma_D$ over $R_1$ diagram. The results also successfully differentiated the sponge material from the algal material, as well as the fossil-derived signal from the background. However, it is still uncertain whether the different clustering of the RSF data and the algal data reflects the variance of precursor material or only the diagenetic and geological history. The variance within the RSF data appears to be larger than that within the algal data. Considering the similar diagenetic history of the RSF, this is possibly reflecting the difference in precursor material. Nonetheless, further measurements on other fossil algal and poriferan material must be involved in the future, in order to improve and testify the current interpretation.

Despite the properties revealed by Raman spectroscopy, the taphonomy of carbonaceous material in RSF has not been investigated. According to our observation, as well as the phenomenon described in previous studies, the preservation of the carbonaceous material in RSF does not show obvious taxonomical preferences. Because the RSF are polyphyletic and currently lack evidence to indicate that they represent any special development stage of sponges, we infer that this unusual carbonaceous preservation is due to diagenetic bias relating to their specific morphology, which in turn is possibly controlled by similar living environments. Again, to test these inferences, more detailed taxonomical and paleoecological studies are necessary.

Received: 19 August 2013
Subject Areas: Palaeontology, Geobiology
Keywords: Porifera, Cambrian, Burgess Shale-type preservation, Raman spectroscopy, China

Accepted: 01 December 2013
Porifera is the known most primitive lineage of Metazoa (e.g., Philippe et al. 2009; Sperling et al. 2009) and is now suspected having originated as early as Cryogenian (Petersen et al. 2008; Sperling et al. 2010). However, the commonly acknowledged fossil record of this lineage is not older than the Early Cambrian, although a group of unusual structures from Precambrian rocks have been proposed as candidates of early sponges (e.g., Maloof et al. 2010; Brain et al. 2012). This is partly because spicules, the traditionally adopted criteria for setting up a poriferan affinity, may have not been evolved in Precambrian, or the taphonomical windows at that time were not favorable for the spicular material (Sperling et al. 2010). Therefore, a revaluation of the taphonomical potential of sponges may lead to a new understanding on the early evolutionary history of Porifera.

One aspect of this question is the preservation of the sponge-derived organic matter. It was generally believed that the soft part is of low potential to be fossilized as macroscopic fossils, and the poriferan fossil record has therefore a strong bias toward mineral skeletons (Pisera 2006). However, at least the Burgess Shale-type (BST) Lagerstätten yield exceptions. From the Middle Cambrian Burgess Shale (Rigby & Collins 2004), V. aucia has been identified as the earliest fossil of ceractinomorph demosponge due to the preservation of carbon remains in the coring fibers of these fossils. Li et al. (1998) also mentioned an observation of a single specimen of keratose sponge in their collection from Chengjiang fauna.

The so-called ‘round sponge fossils’ (RSF) from the Chengjiang Biota are another example. They are always circular to sub-circular in shape, small in size (3–40 mm in diameter, mostly <10 mm) and exhibiting an intensive or even continuous carbonaceous cover on the surface. Because of these morphological similarities, they were loosely mentioned as a group under the name RSF by Wu (2004), although they seem polyphyletic and the affinity is still unresolved. Compared to most other fossils from the same locality, which are strongly weathered and exhibit very few organic remains (e.g., Zhu et al. 2005), the dense carbonaceous cover of RSF appears quite unique.

Raman spectroscopy is a valuable tool for almost non-destructive high resolution analysis of the molecular composition and crystal structure of samples. It does not need extensive sample preparation and can give comparatively quick information. However, when applying this method in fossil studies, the interpretation of the data is not always clear and often critically discussed (e.g. in case of putative microfossils in Archean rocks; Kudryavtsev et al. 2001; Brasier et al. 2002; Pasteris & Wopenka 2002; Schopf et al. 2002; Schopf et al. 2005). Already Marshall et al. (2012) made an approach for investigating BST type preservation on a fossil from the Cambrian Spence Shale with Raman spectroscopy. They focused on mineral replacement on different parts of the fossil and the associated thermal history. Only recently Bower et al. (2013) published comprehensive results concerning the interpretation of carbon signals from microfossils, in regard of tracing the differences in the putative precursor material. The most promising parameters turned out to be the full width at half height of the D-band (Γ D) and the intensity ratio of the D- and the G-band (Γ 1). As in our case the biogenicity of the sponge fossils is not questionable, the application of Raman spectroscopy serves the purpose of revealing the nature of the carbon cover of the RSF and at the same time extending the results to macroscopic fossils following the work of Bower et al. (2013).

In this study, a few specimens of RSF were examined with a short description and discussion on their taphonomical and taxonomical affinity. The Raman spectra of their carbonaceous cover were illustrated and analyzed. In order to identify the characters of these RSF-derived spectra, a sponge fossil from Cambrian Xiaoyanxi Formation and several algal remains from the Ediacaran Wenghui Biota were also examined for comparison.

Material and Methods

All the specimens studied in this paper are from the collection of Professor Zhu Mao-Yan’s group in the Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences, including five RSF from the Cambrian Chengjiang Biota (inventory numbers no. 41047, no. 42436, no. 42446, no. 42952 and no. 42982; Fig. 2), one sponge fossil from the Cambrian Xiaoyanxi Formation (inventory name XXX) and several algal fossils from the Wenghui Biota (inventory name WH). These fossils were observed with a JEISS Stemi 2000-C microscope and photographed with a CANON EOS 50D. For Raman spectroscopic analysis a confocal Horiba Jobin-Yvon LabRam-HR 800 UV Raman spectrometer attached to an Olympus BX41 microscope was used. The excitation wavelength for the Raman spectra was the 488 nm line of an Argon Ion Laser (Melles Griot IMA 106020B0S) with a laser power of 20 mW. A detailed description of the spectrometer is given in Beimforde et al. (2011). All spectra were recorded and processed using LabSpec™ version 5.19.17 (Horiba Jobin-Yvon, Villeneuve d’Ascq, France). Mineral identification was performed on the basis of the Horiba Jobin-Yvon database for minerals.

The RSF were collected from fossil sites near Chengjiang County, mainly from Maotianshan and Xiaolantian (Wu 2004). They are preserved in the yellowish-green shale from the Maotianshan Shale Member of the Yu’anshan Formation (Fig. 1). The age of the fossiliferous layer has been estimated as ca. 520 Ma (Hu 2005). Previous research distinguished two types of sediments from the Maotianshan Shale: the slowly deposited background.
beds and the rapidly deposited event beds which probably represent storm-induced distal tempestites (Hu 2005). Because the collection of these RSF is contributed by several workers over a long period, it is now impossible to know the exact type of sediments in which each RSF was collected. However, it has been confirmed by quantitative analysis that the fossils in the two taphonomical facies originated from a single local community, because the two types of beds exhibit similar recurrent and abundant species, as well as similar temporal trends in evenness and richness (Zhao et al. 2009).

The sponge fossil from the carbonaceous black shale of Xiaoanyxi Formation in Yuanling, Hunan Province has not yet been taxonomically described (Figs. 1, 3a). Similar to other Lower Cambrian sequences in South China, the fossiliferous black shale is successively underlain by a layer of Ni–Mo ore, a phosphorite layer and then Precambrian sedimentary rocks. The absolute age of the fossil horizon was evaluated as younger than 532 Ma (Jiang et al. 2012).

The algal remains are of Ediacaran age and were collected from the Wenghui Biota in Guizhou Province (Zhao et al. 2004; Wang et al. 2007). This fossil assemblage is composed of dominantly algal organisms preserved in the black shale of the upper Doushantuo Formation (Figs. 1, 3b–d). These algae appear to be benthic and buried in situ, therefore considering the paleogeography of the Doushantuo Formation, the sedimentary environment of these rocks was believed as on the slope, below the storm wave base but still within the photic zone (Jiang et al. 2011; Zhu et al. 2012).

**Results**

*Preservation of carbonaceous remains in studied fossils*

Except one incomplete specimen (no. 41047), the other four RSF studied in this paper have an elliptical outline and a diameter of 0.6–0.8 cm. No. 42952 maintains the thickest carbonaceous remains. Polygonal cracks are developed on the upper surface of the carbonaceous cover while traces of spicules are absent (Figs. 2a–c). However, the carbonaceous cover on this fossil can be removed quite easily, and where the cover is absent, impressions of a hexactinellid skeleton similar to that of *Triticispongia diagonata* turns out to be quite clear (Fig. 2b). By comparison, no. 42436 (Figs. 2d–f) and no. 42446 (Figs. 2g–i) show a thinner but also continuous carbonaceous cover, which is more tightly compacted to the siliciclastic matrix and not easy to remove. Some small and faint marks, resembling moulds of hexactinellid spicules, are distributed on parts of the fossil surface (Figs. 2e, 2h–i). The carbonaceous cover of no. 41047 (Fig. 2j) is not continuous as those of the aforementioned three specimens, although it looks also intensive. The siliceous skeleton of this specimen is distinctly preserved as mould and shows characters of a hexactinellid, whose skeleton seem denser and better interconnected than *T. diagonata*. No. 42982 exhibits a reddish surface with weakly preserved moulds of spicules and only scattered carbonaceous remains (Figs. 2k–l), generally resembling the surface of no. 42952 after removal of the carbonaceous cover. In the research of Wu (2004) on ca. 270 RSF specimens, it has been described that in these fossils the conspicuousness of the spicules decreases with increasing density of the carbonaceous cover.
However, our observations indicate there may not be any definite relationship between the preservational qualities of the carbonaceous remains and the spicules.

In contrast to the RSF, the sponge specimen from Xiaoianxi Formation does not have an obvious carbonaceous cover. Though, the fossil region appears generally darker than the background, the boundary between them can be quite obscure in many places (Fig. 3a).

The Ediacaran algal specimens show high morphological diversity within only square-decimeter scale. Although the thickness of carbonaceous remains of these fossils varies between different morphological taxa (Figs. 3b–d) and between different parts of a single individual (Fig. 3e), the boundary between fossil and background is mostly distinct.
In some individuals, the carbonaceous remains are extremely thick and form polygonal fractures on the surface like those in RSF no. 42952 (Figs. 3d, f).

**Raman spectra**

Totally 46 Raman spectra have been obtained from four of the Chengjiang fossils, the sponge fossil from the Xiaoyanxi Formation and a few algal remains from Wenghui Biota (Fig. 4). The most prominent signals from all the samples are typical for amorphous carbon, characterized by two prominent bands in the lower wavenumber region around 1600 cm\(^{-1}\) (G-band; graphite-band) and around 1350 cm\(^{-1}\) (D-band; disorder-band) (cf. Tuinstra & Koenig 1970; Wopenka & Pasteris 1993; Quirico et al. 2009). Sometimes additional bands for minerals also occur, which are a good sign for influence of the background material. Furthermore, especially the background shale material exhibits a high fluorescence, which can be caused by the extremely fine grained clay minerals, resulting in a reduction of the Raman signal (Wang & Valentine 2002). Since this study is focused on the analysis of the carbon signal, the mineral- and fluorescence-influenced spectra are not shown in this paper.

On first sight, the results for all samples look quite similar, with the exception of XYX (Fig. 4), in which the D-band is always higher than the G-band. The differences between the fossil and background material are also difficult to recognize. However, it is well known that peak intensities of the two bands can vary in a small range due to several independent factors, e.g., thermal alteration, the original carbonaceous material, and crystallinity of the carbon (Robertson 1986; Pasteris & Wopenka 2003; Busemann et al. 2007; Marshall et al. 2010). Therefore, geologically valuable information was extracted by calculating the relative intensity ratio between the D- and the G-band (R1) and the full width at half height of the D-band (\(\Gamma_D\)). Both parameters seem to be suitable to differentiate between different samples as well as between fossil and background material (Fig. 5; Table 1). The ‘round sponge fossils’ from the Chengjiang Biota show mean R1 values of 0.79 (no. 42952), 0.69 (no. 42436), 0.81 (no. 42981) and 0.74 (no. 41407). The sample XYX from the Xiaoyanxi Formation is characterized by signals with higher D- than G-band, with an average R1 value of 1.08. The different algal fossils from the Wenghui Biota have mean R1 values of 0.90 (algae 1), 0.88 (algae 2), 0.91 (algae 3) and 0.97 (algae 4). In all samples except XYX, there is a clear difference between the R1 values of the fossils and those of the background material. When plotting the average \(\Gamma_D\) values over the average R1 values becomes even clearer (Fig. 5a). The carbonaceous remains of XYX seem too thin and discontinuous, so that the carbon signals of the fossil do not differ from those of the background. Additionally, this sample is the only one with a mean R1
value greater than 1, suggesting intensive diagenesis and/or metamorphism (cf. Wopenka & Pasteris 1993; Rahl et al. 2005; Bower et al. 2013; Foucher & Westall 2013), which may have caused an equalization between the fossil-derived carbon and the carbon from the matrix. Because the signals from this sample cannot reflect the nature of sponge-derived carbon, it is not considered in the further discussion of the Raman signals.

Discussion

BST preservation was firstly defined as ‘exceptional organic preservation of non-mineralizing organisms in fully marine siliciclastic sediments’, with ‘some degree of early diagenetic mineralization’ (Butterfield 1995). Following this definition, the algal fossils studied in this paper should also be regarded as a representative of BST preservation, as what has been figured out by Xiao et al. (2002) for the algal fossils from the equivalent Miaobe Biota. Although the mechanisms causing exceptional BST preservation are still under debate (e.g., Butterfield 1990; Petrovich 2001; Gaines et al. 2005), it is obvious that materials of different taphonomical resistance are preserved in different states in BST Lagerstätten. It has been summarized by Butterfield et al. (2007) that the two-dimensional carbonaceous compressions represent relatively recalcitrant extracellular components (e.g., cuticles and chaetae), while former labile soft-tissues may result in three-dimensional mineralization of either carbonates or phosphates, and the early diagenetic pyrite incidentally distributes in all Burgess Shale fossils.

In the Chengjiang Biota, most 2-D soft-bodied fossils are originally preserved in organic carbon, although due to intensive weathering the carbon material is largely diminished (Zhu et al. 2005). Compared with these fossils, the durability of carbonaceous material in the RSF appears unusual. As examples for diagenetically recalcitrant organic material, the taphonomy of collagenous periderm of Rhabdopleura (an extant close relative of graptolites) and the cuticle of arthropods composed of wax and chitin have been comprehensively studied (Gupta & Briggs 2011). Although received much less attention and having not been studied by taphonomical experiments, sponges possess diagenetic-recalcitrant organic material as well. Collagen distribute pervasively in sponges as either supplementary or main components in the skeletal frame (Bergquist 1978). Furthermore, chitin was recently detected in the skeleton of some hexactinellids (Ehrlich et al. 2007) and as a scaffold material tangling with collagen in the spongin skeleton of Verongida (Ehrlich et al. 2010).

In the case of algae, algaenan has been identified as a material with high fossilizing potential. However, the known occurrence of this material is restricted to Chlorophyta and some unicellular algae (Collinson 2011). Because the phylogeny of these Ediacaran macroscopic algae is uncertain (Xiao & Dong 2006), it is currently impossible to postulate the original material of the algal carbonaceous remains from Wenghui Biota.

The Raman signals from these recalcitrant carbonaceous materials are clearly distinguishable from those of their backgrounds in the $\Gamma_D - R_1$ diagram (Fig. 5a), supporting the fossil-derived nature of these carbon remains. When the data points of the background material are removed, it is obvious that the signals from Chengjiang sponge fossils and those from Wenghui algal fossils do cluster separately (Fig. 5b).
Comparing with the data published by Bower et al. (2013), all our fossil signals fall into the region in which other samples containing compositionally complex precursor material plot (Γ_D between 100 and 250 cm^{-1}; R1 0.5 to 1.4). Bower et al. (2013) suggested that an extensive analysis of the carbon signature can reveal information on the precursor material, when other influences like a high thermal maturity can be ruled out. In our data, the Γ_D and R1 values of the algal fossils are very similar to each other, while the carbon of the sponge fossils shows a greater variability. Since the sponge fossils are from the same age and region, as well as preserved in similar yellowish-green shales, indicating a similar thermal diagenetic impact, it could be possible that a bigger variance of precursor material is responsible for the higher variance in the Raman signal of the carbonaceous remains. The values of the sponge sample no. 42952 show a higher similarity to those of the algae, with a slightly narrower D-band. The narrowing of the D-band could mean that this sample was either influenced by a thermal event (Wopenka & Pasteris 1993; Bower et al. 2013) or that the thick carbon cover has a different origin than the thinner covers of the other sponge fossils. Although these data show a clear differentiation between the carbon cover of the BST sponge fossils and the Ediacaran algal fossils, it is still hard to judge whether this phenomenon is caused by a divergent diagenetic history or the different source of organic carbon (sponge vs. algae).

Fig. 5: Cross plots of the average values of the full width at half maximum of the D-band (Γ_D) over the intensity ratio of the D- and the G-band (R1) (cf. Table 1). (a) Values of the sponge fossils from Chengjiang and the Xiaoyanxi Formations (XYX), the algal fossils from the Wenghui Biota (WH), as well as the associated background materials. Fossil points are marked with circles and the associated background materials with diamonds. Except for samples from the Xiaoyanxi Formation, fossil points are clearly distinguishable from the respective background values. (b) Exclusively values of sponges from the Chengjiang Biota (blue symbols) and algae from the Wenghui Biota (red symbols); note the apparent clustering of the data (see squares).
Wu (2004) regarded the RSF as sponge gemmules because of the exceptionally recalcitrant organic matter and the round shape. But this interpretation is not in line with the character of modern and confirmed fossil sponge gemmules, which are at largest only about 1 mm in diameter (Fell 1993; Petit & Charbonnier 2012) and thus much smaller than the RSF. When erecting some RSF from Sancha as genera (Triticispongia and Saetaspongia), Steiner et al. (1993) interpreted T. diagonata as juveniles because their size hardly exceeds 10 mm, although adult forms have not been discovered then. But the juvenile theory cannot contribute directly to the interpretation of the thick carbon cover, because to our knowledge, the juvenile sponges do not necessarily have enriched spongin as in the case of gemmules.

On the other hand, based on the material from Chengjiang Biota, some other researchers tended to interpret T. diagonata as adult precursor of reticulosid sponges, considering the missing adults and its well-organized skeletal structures (Rigby & Hou 1995). Larger specimens of T. diagonata (2.5–3 cm) were further discovered in the Niutitang Formation in Guizhou (Zhao et al. 2006). They are more probably giant individuals relating to certain environment or evolutionary stage than adult forms of the Hunan and Chengjiang analogues, because none of these three fossil communities contain big and small specimens at the same time. However, interpreting T. diagonata as a specific evolutionary stage of hexactinellids also could not help to explain the intensive carbon preservation of RSF, because RSF are polyphylogenetic. Wu (2004) has described sponges which possess only diactinal spicules in the RSF, indicating a demosponge population in them. And in this study, the observed specimens appear to belong to different hexactinellid morphotypes (Fig. 2).

For these reasons, we prefer to suggest that the unusual preservation of the organic carbon in RSF is probably due to some specific processes during early diagenesis, e.g., more effective adsorption of clays relating to the specific size and shape of these fossils. Furthermore, we attribute the exclusively small and round shape of these sponges to similar environmental controls. It has been observed in modern examples, that the exterior morphology of sponges can be strongly affected by environment, while the skeletal construction is still controlled by gene expression. The juvenile theory cannot be completely excluded as an interpretation of the specific morphology of RSF. However, the difficulty here is that the RSF are composed of various biological taxa, and it seems unlikely to expect that the juvenile of all the sponges possessed a similar morphology when the influence from the environment is absent. Furthermore, there has not been any reconstruction on the ontogenies of Chengjiang sponges, which could relate RSF to any potential adult forms. Nevertheless, to test proofing these postulations further investigations on the taxonomy of the RSF and precise observations in the outcrops are required.

Conclusions

Our observations show that the recalcitrant organic material from sponges, most probably being collagen and chitin according to current knowledge, can be preserved as dense carbonaceous remains in macroscopic fossils in BST Lagerstätten. The present application of Raman spectroscopy with the interpretation of the \( \Gamma_D \) and \( R_1 \) values of the carbon signal still cannot directly reveal the nature of the precursor of the carbonaceous remains. However, our results confirmed suggestions by Bower et al. (2013) that carbonaceous material from complex precursor material with low thermal affection cluster in a certain area in the \( \Gamma_D-R_1 \) plot (\( \Gamma_D \) between 100 and 250 cm\(^{-1}\); \( R_1 \) 0.5 to 1.4). As the study of Bower et al. (2013) deals with microfossils, our findings represent first evidence that this approach could also be applied to macrofossils. This method also appears to be able to differentiate several sources of carbon in macroscopic fossil samples: between the fossil and the background, between the fossil material from Cambrian RSF and Ediacaran algal fossils, and between different specimens of RSF. It is still uncertain whether the separate clustering of the RSF data and the algal data reflects the variance of precursor material or only the diagenetic or geological history. However, the larger variance within the RSF data compared to the algal data probably is caused by different precursor material, because the RSF derive from the same setting and thus have a similar diagenetic and geological history. Nonetheless, further measurements on other fossil algal and poriferan material must be involved in the future, in order to improve and testify the current interpretations.

The taphonomical mechanism of the dense carbonaceous material in RSF is also interesting. Because the RSF are polyphylogenetic and there is currently no evidence to support their nature as any special development stage of sponges, we infer that this unusual carbonaceous preservation is due to a diagenetic bias relating to their specific morphology, which in turn is possibly controlled by similar living environments. Again, to test these inferences, more detailed taxonomical and paleoecological studies are necessary.

Acknowledgements

We appreciate Zhu Mao-yan (NIGPAS) for providing the fossil material, and Michael Steiner (Freie Universität Berlin) for his beneficial review and precious suggestions for this text. We are also grateful for the help from Wu Wen (NIGPAS), Miao Lan-Yun (NIGPAS) and Zeng Han (Nanjing University) with the literature collecting.


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