



**HAL**  
open science

## Microfossils with tail-like structures in the 3.4 Gyr old Strelley Pool Formation Authors

Frédéric Delarue, Sylvain Bernard, Kenichiro Sugitani, François Robert,  
Romain Tartèse, Sonja-Verena Albers, Rémi Duhamel, Sylvain Pont, Sylvie  
Derenne

► **To cite this version:**

Frédéric Delarue, Sylvain Bernard, Kenichiro Sugitani, François Robert, Romain Tartèse, et al.. Microfossils with tail-like structures in the 3.4 Gyr old Strelley Pool Formation Authors. *Precambrian Research*, 2021, 358, pp.106187. 10.1016/j.precamres.2021.106187 . hal-03349816

**HAL Id: hal-03349816**

**<https://hal.sorbonne-universite.fr/hal-03349816>**

Submitted on 20 Sep 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# 1           **Microfossils with tail-like structures in the 3.4 Gyr old Strelley Pool Formation**

2

## 3   **Authors**

4   Frédéric Delarue<sup>a\*</sup>, Sylvain Bernard<sup>b</sup>, Kenichiro Sugitani<sup>c</sup>, François Robert<sup>b</sup>, Romain Tartèse<sup>d</sup>,  
5   Sonja-Verena Albers<sup>e,f</sup>, Rémi Duhamel<sup>b</sup>, Sylvain Pont<sup>b</sup>, Sylvie Derenne<sup>a</sup>

6

## 7   **Affiliations**

8   <sup>a</sup>Sorbonne Université, CNRS, EPHE, PSL, UMR 7619 METIS, 4 place Jussieu, F-75005 Paris,  
9   France

10   <sup>b</sup>Muséum National d'Histoire Naturelle, Sorbonne Université, UMR CNRS 7590, IRD, Institut  
11   de Minéralogie, de Physique des Matériaux et de Cosmochimie, IMPMC, 75005 Paris, France

12   <sup>c</sup>Department of Earth and Environmental Sciences, Graduate School of Environmental Studies,  
13   Nagoya University, Nagoya, Japan

14   <sup>d</sup>Department of Earth and Environmental Sciences, The University of Manchester, Manchester  
15   M13 9PL, United Kingdom

16   <sup>e</sup>Molecular Biology of Archaea, Institute of Biology II, Faculty of Biology, University of  
17   Freiburg, Freiburg, Germany.

18   <sup>f</sup>Spemann Graduate School of Biology and Medicine, University of Freiburg, Freiburg,  
19   Germany.

20

21   \*Correspondence to: [frederic.delarue@upmc.fr](mailto:frederic.delarue@upmc.fr)

22

23

24

25

## 26 **Abstract**

27 Some of the oldest traces for planktonic lifestyle have been reported in ca. 3.4 billion years old  
28 silicified sediments from the Strelley Pool Formation in Western Australia. Observation of  
29 flange appendages suggests that Archean life motility was passive and driven by drifting of  
30 microorganisms in their surrounding environment. Until now, the oldest traces for active  
31 motility are ca. 2.1 billion years old. Whether or not active motility already existed during the  
32 Archean eon remains an open question. In this study, we report the discovery of new 3.4 billion  
33 years old microfossils exhibiting a tail-like structure isolated from the Strelley Pool Formation.  
34 Exhibiting Raman spectra typically observed in organic-walled microfossils from the Strelley  
35 Pool Formation, these microfossils exhibiting a tail-like structure are syngenetic with their host  
36 rock. Composed of carbon, nitrogen, and, for one specimen, phosphorus, some of these organic-  
37 walled microfossils also exhibit significant level of aliphatic and amide moieties supporting  
38 their biogenicity. In addition, these microfossils exhibit a tail-like appendage sharing similar  
39 morphological features with locomotory organelles in modern microorganisms such as  
40 archaella, flagella, and cilia. This suggests that this observed appendage likely provided them  
41 with movement capabilities. If correct, with the ability to move, these microorganisms were  
42 capable of escaping from harsh environments and/or colonizing new ecological niches as early  
43 as 3.4 billion years ago.

44

## 45 **Keywords**

46 Archean – Early life - Morphology - NanoSIMS - Raman spectroscopy - Strelley Pool

47

## 48 **1. Introduction**

49 Archean carbonaceous microfossils testify for the widespread presence of life on Earth as early  
50 as ca. 3.4 billion years ago (Westall et al., 2006; Sugitani et al., 2010; Wacey et al., 2011; Alleon  
51 et al., 2018). However, the interpretation of the Archean palaeobiological record is fraught with

52 difficulties pertaining to fossilization and burial-induced degradation processes, as exemplified  
53 by intense debates over the past couple of decades (e.g., Schopf et al., 2002; Brasier et al., 2002;  
54 Wacey et al., 2016). Remnants of early life forms have experienced burial and thermal alteration  
55 for billions of years, which led to the degradation of many pristine biological traits (Javaux et  
56 al., 2019). Archean putative microfossils tend to exhibit simple morphological shapes at the  
57 micrometric scale (e.g., spheroidal, filamentous, film, and lenticular forms) that can also be  
58 abiotically produced (Garcia-Ruiz et al., 2003; Cosmidis et al., 2016), precluding, in turn, any  
59 simple morphological distinction between genuine biological remnants and mineral/organic  
60 biomorphs. Because of the lack of taxonomically informative features (Javaux et al., 2019),  
61 morphological criteria alone are generally considered as insufficient to assess the biological  
62 nature of ancient traces of life in the Archean geological record (Brasier et al., 2006). As a  
63 result, the ancient fossil record has not yet conveyed a complete picture of ancient biodiversity.  
64 Here, we report the discovery of 3.4 billion years old organic microfossils from the Strelley  
65 Pool Formation (SPF) exhibiting exceptionally preserved morphological traits possibly  
66 indicative of active motility.

67

68

## 69 **2. Material and methods**

### 70 **2.1. Studied sample**

71 For this study, we selected a 3.4 billion year-old black chert sampled from the Panorama locality  
72 (PANX1-1) situated in the SPF (Western Australia), and which displays abundant microfossils  
73 and microscopically identifiable parallel laminations (see Sugitani et al., 2010, 2013, and 2015  
74 for detailed description of the geological background).

75

### 76 **2.2. Chemical isolation of microfossils**

77 Organic-walled microfossils were isolated from the SPF carbonaceous black chert sample using  
78 a modified version of the classical acid maceration procedure (Delarue et al., 2020). A ‘soft’  
79 acid maceration procedure was applied in order to minimize both potential physical and  
80 chemical degradation of organic microstructures. Prior to acid maceration, about 30 g of rock  
81 samples were fragmented into ~3 g rock chips rather than crushed into finer grains. Rock chips  
82 were cleaned using ultrapure water and a mixture of dichloromethane/methanol (v/v: 2/1), and  
83 were then directly placed in a Teflon vessel filled with a mixture of HF (40%, reagent grade) /  
84 HCl (37%; reagent grade; v/v: 9/1) at room temperature. After 48 hours, successive  
85 centrifugation and rinsing steps using ultrapure water were performed until reaching neutrality.  
86 The residual material was suspended in ethanol and filtered on polycarbonate filters (pore  $\varnothing$  =  
87 10  $\mu$ m). After ethanol evaporation, polycarbonate filters were fixed on carbon tape and coated  
88 with 20 nm of gold to prevent further contamination by atmospheric deposits and for further  
89 analyses.

90

### 91 **2.3. Scanning electron microscopy and Energy Dispersive X-Ray Spectroscopy (SEM-** 92 **EDXS)**

93 SEM-EDXS imaging and analysis were performed on gold-coated filters using a TESCAN  
94 VEGA II at the French National Museum of Natural History (MNHN) operated with an  
95 accelerating voltage of 15 kV.

96

#### 97 **2.4. Raman spectroscopy**

98 Raman microspectroscopy was carried out using a Renishaw InVIA microspectrometer  
99 equipped with a 532 nm green laser. The laser was focused on the sample by using a DMLM  
100 Leica microscope with a 50× objective. The spectrometer was first calibrated with a silicon  
101 standard before the analytical session (matching at 520.5 cm<sup>-1</sup>). For each target, we determined  
102 the Raman shift intensity in the 1000 to 2000 cm<sup>-1</sup> spectral window that includes the first-order  
103 defect (D) and graphite (G) peaks. A laser power below 1 mW was used to prevent any thermal  
104 alteration during spectrum acquisition. Spectrum acquisition was achieved after three iterations  
105 using a time exposure of 10 seconds (spectral resolution of 1.5 cm<sup>-1</sup>). Raman  
106 microspectroscopy was performed on gold-coated organic surfaces, implying a slight lowering  
107 of the relative intensity of the D band with respect to the G one (Delarue et al. 2020).

108

#### 109 **2.5. Nanoscale secondary ion mass spectrometry**

110 Isolated microfossils were analyzed using a CAMECA NanoSIMS 50 ion probe using a Cs<sup>+</sup>  
111 primary ion beam. Before measurements, pre-analysis sputtering was performed over 30 × 30  
112 μm<sup>2</sup> areas for ca. 8 minutes using a 500 pA primary current (750 μm aperture diaphragm) to  
113 remove surficial contamination, and achieve Cs<sup>+</sup> saturation fluence and constant secondary ion  
114 count rates. Analyses were then carried out using a 10 pA primary current (200 μm aperture  
115 diaphragm) on smaller areas to avoid pre-analysis sputtering edge artifacts. The secondary  
116 molecular species <sup>12</sup>C<sup>14</sup>N<sup>-</sup> and <sup>31</sup>P<sup>-</sup> were collected simultaneously in electron multipliers. The

117 NanoSIMS raw data were corrected for a 44 ns dead time on each electron multiplier and  
118 processed using the Limage software.

119

## 120 **2.6. Focused ion beam (FIB)**

121 FIB ultrathin sections were extracted from the organic microfossils using an FEI Strata DB 235  
122 (IEMN, Lille, France). Milling at low gallium ion currents minimizes common artefacts,  
123 including local gallium implantation, mixing of components, creation of vacancies or  
124 interstitials, creation of amorphous layers, redeposition of the sputtered material on the sample  
125 surface, and significant changes in the speciation of carbon-based polymers.

126

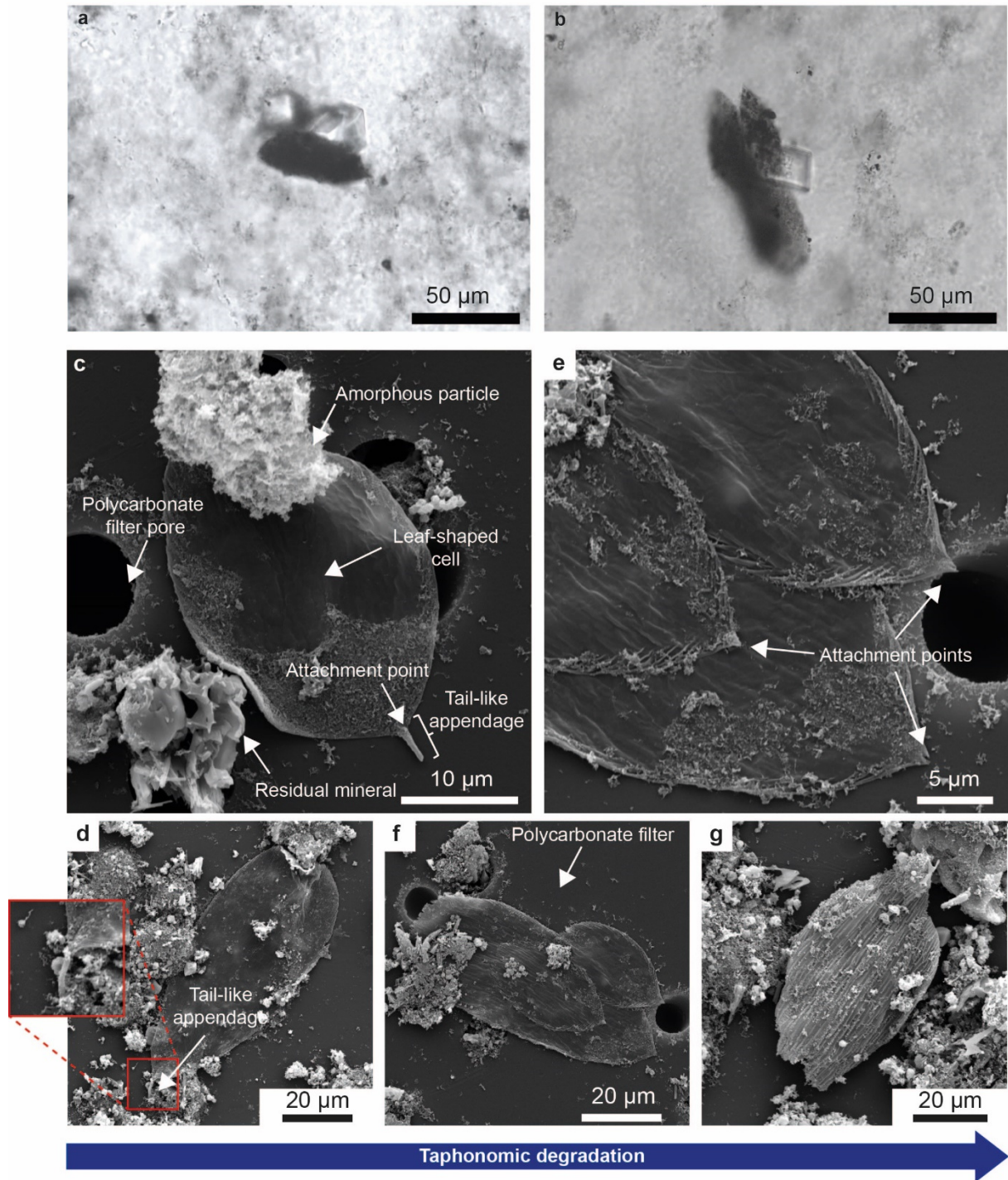
## 127 **2.7. Scanning transmission X-ray microscopy (STXM)**

128 X-ray Absorption Near Edge Structure (XANES) investigations were conducted using the  
129 HERMES STXM beamline at the synchrotron SOLEIL (Gif-sur-Yvette, France). Carbon  
130 contamination on beamline optics was constantly removed thanks to a continuous flow of pure  
131 O<sub>2</sub>. The well-resolved 3p Rydberg peak of gaseous CO<sub>2</sub> at 294.96 eV was used for energy  
132 calibration. Collecting image stacks at energy increments of 0.1 eV with a dwell time of  $\leq 1$  ms  
133 per pixel prevented irradiation damage. The estimations of N/C values and the normalization  
134 of the C-XANES spectra shown here were done using QUANTORXS (Le Guillou et al., 2018).

135

## 136 **3. Results and Discussion**

137 Microscope observations of the studied SPF sample thin sections revealed the presence of  
138 organic-walled microfossils exhibiting a tail-like structure (Fig. 1a, b). These microfossils are  
139 exclusively observed within the siliceous sedimentary matrix, precluding their introduction  
140 during hydrothermal fluid circulation post 3.4 Ga.



141

142 **Figure 1: Thin section micrographs and scanning electron microscopy images of organic**  
 143 **microfossils** exhibiting or not a tail-like structure. (a) Micrograph presenting a microfossil  
 144 exhibiting a tail-like structure embedded in the main siliceous matrix of the studied SPF chert.  
 145 (b) Micrograph presenting a microfossil exhibiting no tail-like structure. (c-f) SEM images of  
 146 organic microfossils exhibiting or not a tail-like structure isolated by acid maceration. (c,d)  
 147 Exceptionally-well preserved leaf-shaped cells presenting a locomotory organelle composed of  
 148 an attachment point and of a tail; (e-g) Corresponding degraded organic-walled microfossils  
 149 lacking a tail but (e, f) exhibiting an attachment point. Comparison between microfossils

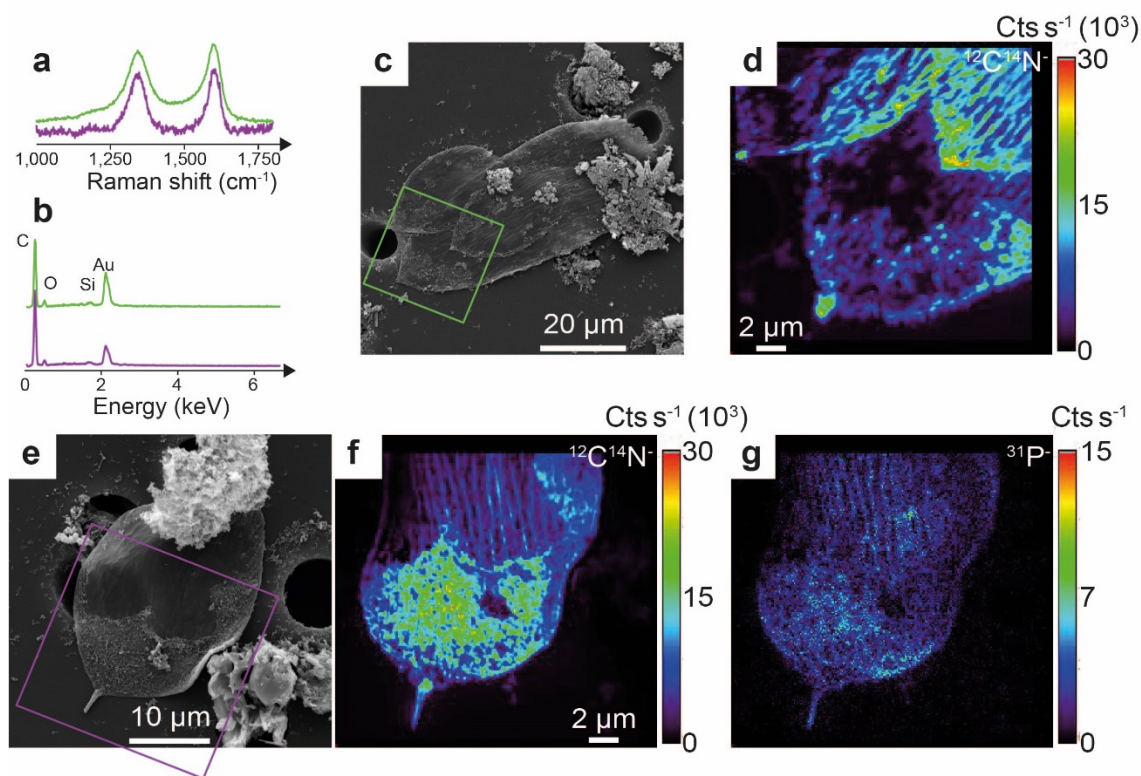


150 exhibiting a tail-like structure and microfossils presenting an attachment point suggests that  
151 tails may be lost during taphonomy. A taphonomic degradation gradient is observed from the  
152 left to the right. Classic taphonomical degradation features, including folds and tears, are  
153 observed.

154  
155 Raman spectra of specimens exhibiting a tail-like structure chemically isolated from the  
156 siliceous matrix are typical of those of disordered carbonaceous materials having undergone a  
157 low-grade metamorphism (Fig. 2a; Pasteris and Wopenka, 2003). Their Raman line shapes  
158 suggest that these microfossils experienced hydrothermal and/or diagenetic peak temperatures  
159 of approximately 250-300 °C (Lahfid et al., 2010; Kouketsu et al., 2014). Raman first-order  
160 spectra of the studied SPF microfossils exhibiting a tail-like structure are similar to those  
161 previously determined on syngenetic microfossils from the same geological formation observed  
162 in thin sections (Lepot et al., 2013; Sugitani et al., 2013), on freshly fractured faces (Alleon et  
163 al., 2018), and in acid maceration residue (Delarue et al., 2020). Therefore, these organic-walled  
164 microfossils exhibiting a tail-like structure should be regarded as syngenetic as they were  
165 subjected to the maximum metamorphic temperature registered by their host rock.

166  
167 Although Raman spectroscopy is a useful tool to assess syngeneity, it is not sufficient to  
168 determine the biogenicity of putative remains of ancient life (Pasteris and Wopenka, 2003).  
169 Energy-dispersive X-ray spectroscopy data show that the studied specimens essentially contain  
170 C and O (Fig. 2b), confirming their organic nature, while nanoscale secondary ion mass  
171 spectrometry reveals the presence of nitrogen and, in one specimen, phosphorus (Figs. 2d, f-g).  
172 The presence of these key elements of cell walls, proteins, and nucleic acids are consistent with  
173 a biological origin.

174



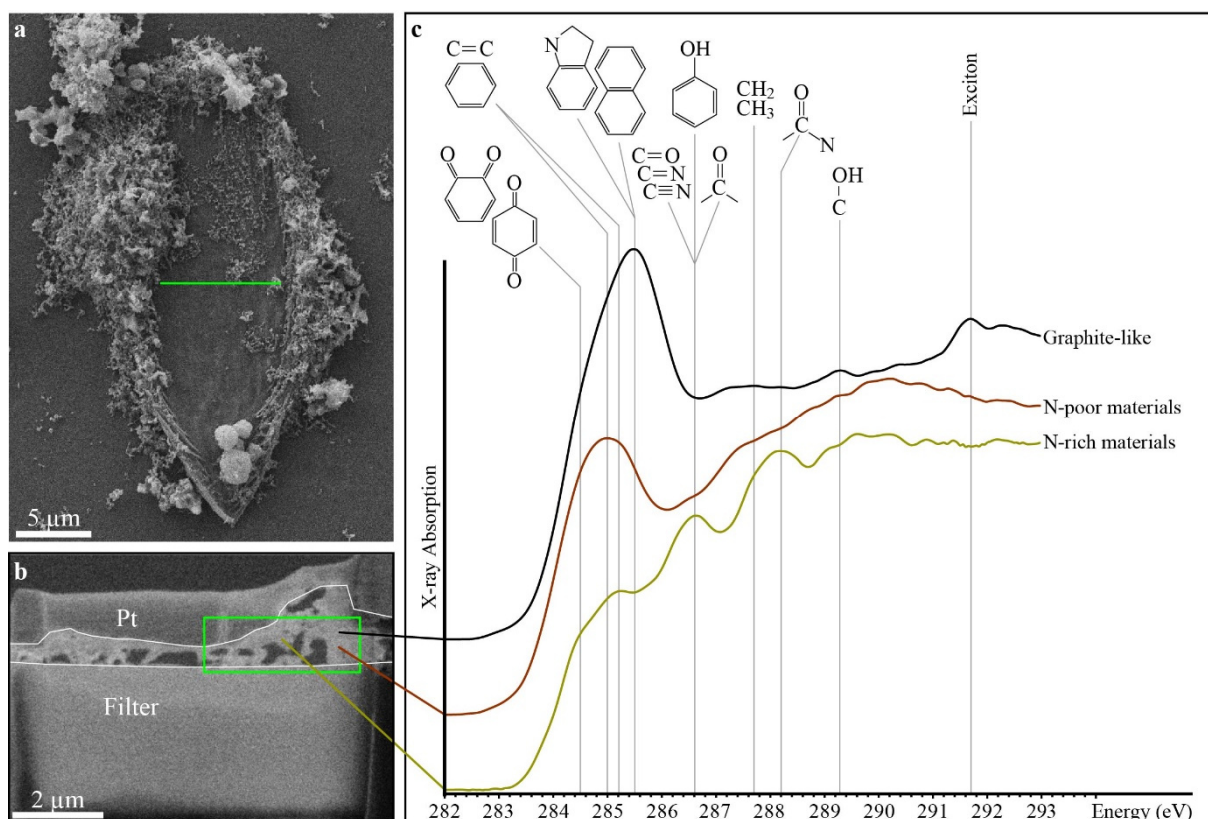
175

176 **Figure 2: Raman spectra, energy-dispersive X-ray spectra, and nanoscale secondary ion**  
 177 **mass spectrometry images.** (a) First-order Raman spectra determined on isolated organic  
 178 microfossils exhibiting a tail-like structure and (b) corresponding energy-dispersive X-ray  
 179 spectra. Green and purple lines indicate that spectra were acquired on specimens shown in  
 180 panels c and e, respectively. (c, e) SEM images of organic-walled microfossils investigated by  
 181 EDXS, Raman spectroscopy and NanoSIMS. Green and purple squares indicate areas probed  
 182 by NanoSIMS. (d, f) The  $^{12}\text{C}^{14}\text{N}^-$  ion images illustrate the presence of nitrogen. (g) The  $^{31}\text{P}^-$   
 183 ion image illustrates the presence of phosphorus. No  $^{31}\text{P}^-$  was recorded on the second specimen  
 184 shown in panel c. Variations in  $^{31}\text{P}^-$  emission intensity between the smooth (e.g., microfossil  
 185 itself) and rough (e.g., coating by amorphous submicrometric organic matter) surfaces cannot  
 186 be used to depict any P enrichment because of microtopographic features biasing ion emissions  
 187 (Delarue et al., 2017).

188

189 Spatially resolved chemical investigations exploiting X-ray absorption confirm the  
 190 heterogeneous chemical nature of the investigated organic-walled microfossils: at least three  
 191 different types of chemical structures could be distinguished within a given specimen (Fig. 3).  
 192 Specimens contain some highly graphitic organic materials with almost no nitrogen as revealed  
 193 by X-ray absorption spectra exhibiting a broad peak of conjugated aromatic groups (285.5 eV)  
 194 and the excitonic absorption feature of planar domains of highly conjugated  $\pi$  systems (291.7

195 eV; Bernard et al., 2010). Closely associated are N-poor materials with XANES spectra similar  
 196 to those of thermally-altered kerogen with an intense absorption peak at 285 eV (aromatic or  
 197 olefinic groups), a relatively broad absorption feature at 287.5 eV (aliphatic carbons), and an  
 198 absorption feature at 286.6 eV (imine, nitrile, carbonyl and/or phenol groups; Bernard et al.,  
 199 2010; Le Guillou et al., 2018). Specimens also contain N-rich compounds (N/C ~ 0.22) with  
 200 XANES spectra that exhibit clear contributions of quinones or cyclic amides (284.5 eV),  
 201 aromatic or olefinic carbons (285.1 eV), imine, nitrile, carbonyl and/or phenol groups (286.6  
 202 eV), aliphatics (287.7 eV) and amides (288.2 eV). Altogether, the chemical structure of the SPF  
 203 specimen investigated here is consistent with the preservation of partially degraded  
 204 biomolecules.



206 **Figure 3: Scanning transmission X-ray microscopy-based X-ray absorption near edge**  
 207 **structure characterization.** (a) SEM image of the specimen from which a focused ion beam  
 208 foil has been extracted (green line). (b) SEM image of the focused ion beam foil evidencing the  
 209 limited thickness of the specimen. The green square indicates the area investigated using

210 STXM. (c) Carbon-X-ray absorption near edge structure spectra of the organic materials  
211 composing the investigated specimen.  
212

213 From a morphological point of view, the organic-walled microfossils are leaf-shaped cells  
214 ranging from 30 to 84  $\mu\text{m}$  in length and from 16 to 37  $\mu\text{m}$  in width (Fig. 1). They exhibit classic  
215 taphonomical degradation features, including folds and tears (Figs. 1c-g). The preparation of  
216 ultrathin foils using focused ion beam illustrates their relative limited thickness, ranging from  
217 200 to 500 nm (Fig. 3). Four specimens also exhibit a specific morphological feature: a tail-like  
218 appendage protruding from the leaf-shaped cell (Figs. 1c, d).

219  
220 From the comparison with modern microorganisms, we can assume that this tail-like appendage  
221 is a remnant of an ancient prostheca or of a locomotory organelle. The tail-like appendages  
222 observed in SPF microfossils are between 0.7 and 1.2  $\mu\text{m}$  in diameter, which is, by far, larger  
223 than those reported for modern archaella, flagella, and cilia, reaching ca. 10, 20, and 200 nm,  
224 respectively (Jarell and McBride, 2008; Beeby et al., 2020). This would be forgetting that  
225 Precambrian organic-walled microfossils exhibit very large cell dimensions ( $\text{\O} > 10 \mu\text{m}$ )  
226 compared to modern microorganisms (Javaux et al., 2010; Sugitani et al., 2010; 2015;  
227 Balidukay et al., 2016; Loron et al., 2019).

228  
229 In order to take this difference into account, we propose to use the Appendage Shape Index  
230 (ASI), which is based on the ratio between the width of the tail-like appendage and that of the  
231 parent cell (Fig. 4). Compilation of morphometric data from extant microorganisms shows that  
232 prosthecae and locomotory organelles are characterized by different ASI values. Prosthecae  
233 display ASI values ranging from 13 to 39 % while they range from 1 to 10 % for modern  
234 archaella, flagella, and cilia (Fig. 4; see supplementary information for detailed values). In the

235 present study, the tail-like appendages are characterized by ASI values ranging from 2 to 6 %,  
236 that is values falling within the domain of modern archaella, flagella, and cilia (Fig. 4).

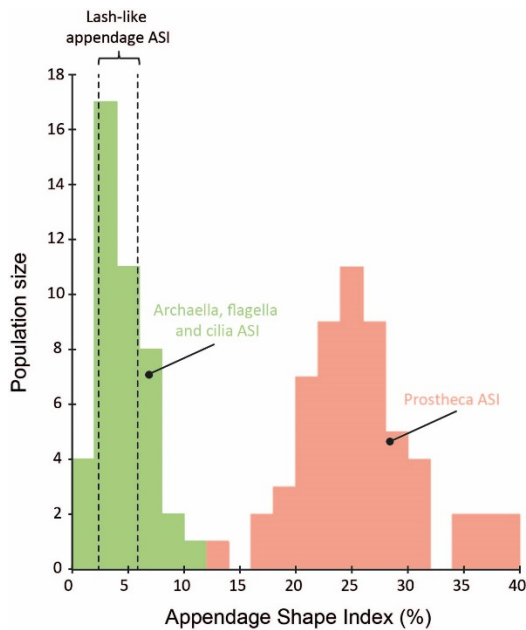
237

238 Involved in anchoring cells to organic and mineral surfaces, in nutrient uptake, or in asexual  
239 reproduction by budding at its tip (Curtis, 2017), a prostheca is a micrometric tube-like  
240 appendage consisting in an extension of the cellular membrane. This implies a structural  
241 continuity between the microorganism body and the base of the prostheca (Javaux *et al.*, 2003).  
242 Here, the SPF specimens investigated exhibit an anchoring attachment point and a filament-like  
243 appendage, indicating two distinct structural subunits (Fig. 1) at odds with any extension of the  
244 cellular membrane. Because of their ASI values and their two structural subunits, the tail-like  
245 appendages observed in SPF microfossils cannot be considered as remnants of prosthecae.

246

247 As far as we are aware, occurrence of distinct external and functional subunits can only be  
248 assigned to locomotory organelles, in good agreement with ASI values. However, these  
249 subunits do not meet standard structural features (for instance, a curved hook connecting the  
250 filament to the basal body in flagella) observed on locomotory organelles from any organism  
251 of the three extant domains of life (see Khan and Scholey, 2018) implying in turn, that the  
252 observed structural features do not allow to depict the biological affinity of these remnants of  
253 tailed microorganisms..

254



255

256 **Figure 4: Compilation of Appendage Shape Indices determined on extant microorganisms**

257 **and on studied microfossils.** ASI was computed according to the ratio between the width of

258 appendage (archaellum, flagellum, cilium and prostheca) and that of its parent cell ( $\times 100$ ). Each

259 width of appendage and of its parent cell was determined graphically based on micrographs and

260 images published in Southam et al. (1990), Poindexter and Staley (1996), Furuno et al. (1997),

261 Wustman et al. (1997), Quintero et al. (1998), Wang et al. (2000), Miller et al. (2004), Bergholtz

262 et al. (2006), Vasilyeva et al. (2006), Wagner et al. (2006), Kanbe et al. (2007), Abraham et al.

263 (2008), Nge et al. (2008), Pyatibratov et al. (2008), Siano et al. (2009), Craveiro et al. (2010),

264 Wang et al. (2011), Abraham and Rohde (2014), Lim et al. (2014), Albers and Jarrell (2015),

265 Deng et al. (2016), Kinoshita and Nishizaka (2016), Sugitomo et al. (2016), Curtis (2017), and

266 Leander et al. (2017; (see supplementary information)). ASI determined on archaella, flagella,

267 and cilia are indicated in green while those determined on prosthecae are indicated in pink. The

268 area delimited by dotted lines indicate ASI determined on four tail-like appendages observed

269 on SPF organic-walled microfossils. ASI ranges from 4.8 to 5.8 % and from 2.2 to 3.3 % in

270 organic-walled microfossil observed in thin sections ( $n = 2$ ) and in the acid maceration residue

271 ( $n = 2$ ), respectively. ASI is likely overestimated in thin sections as a consequence of shadows

272 occurring at the edge of microfossils.

273

274 Previous reports of 3.4-3.0 Ga lenticular microfossils exhibiting a flange were interpreted as

275 demonstrating passive motility of microbial planktons drifting depending on their surrounding

276 environment (House et al., 2013; Sugitani et al., 2015; Oehler et al., 2017; Kozawa et al., 2019).

277 To date, the oldest evidence for active motility was recorded as tubular sedimentary structures

278 in 2.1 Ga Francevillian sedimentary series in Gabon (El Albani et al., 2019). The preservation

279 of tail-like structures by some SPF microfossils suggests that some microorganisms could have

280 been capable of active motility – a mechanism whereby microorganisms can direct their  
281 movement – as early as 3.4 Gyr ago. Since it likely provided them with the ability to move in  
282 the water column or at the surface of organic and/or mineral surfaces, this finding suggests that  
283 microorganisms were possibly able to escape harsh environments, adapt their feeding strategies  
284 moving towards more favorable nutrient sources, and colonize new ecological niches less than  
285 a billion years after the Earth became habitable.

286

## 287 **Acknowledgments**

288 We thank D. Troadec (IEMN) for FIB extraction. We also acknowledge The National  
289 NanoSIMS Facility at the MNHN, supported by MNHN, CNRS, Région Ile de France, and  
290 Ministère de l'Enseignement Supérieur et de la Recherche. Special thanks go to Stefan Stanescu  
291 and Sufal Swaraj for their expert support with the HERMES STXM beamline at SOLEIL. The  
292 HERMES beamline (SOLEIL) is supported by the CNRS, the CEA, the Region Ile de France,  
293 the Departmental Council of Essonne and the Region Centre. This work was supported by the  
294 Programme National de Planétologie (PNP) of CNRS/INSU, co-funded by CNES R.T. also  
295 acknowledges the UK Science and Technology Facilities Council (grant ST/P005225/1) for  
296 financial support. Authors are grateful to the associate editor and the two anonymous reviewers  
297 for their constructive comments.

298

## 299 **References**

300

301 Abraham, W.-R., Macedo, A.J., Lunsdorf, H., Fischer, R., Pawelczyk, S., Smit, J., Vancanneyt,  
302 M., 2008. Phylogeny by a polyphasic approach of the order Caulobacterales, proposal of  
303 *Caulobacter mirabilis* sp. nov., *Phenylobacterium haematophilum* sp. nov. and

304 *Phenylobacterium conjunctum* sp. nov., and emendation of the genus *Phenylobacterium*.  
305 *International Journal of Systematic and Evolutionary Microbiology* 58, 1939–1949.

306 Abraham, W.-R., Rohde, M., 2014. The Family Hyphomonadaceae, in: Rosenberg, E., DeLong,  
307 E.F., Lory, S., Stackebrandt, E., Thompson, F. (Eds.), *The Prokaryotes*. Springer Berlin  
308 Heidelberg, Berlin, Heidelberg, pp. 283–299.

309 Albers, S.-V., Jarrell, K.F., 2015. The archaellum: how archaea swim. *Frontiers in*  
310 *Microbiology* 6. doi:10.3389/fmicb.2015.00023

311 Alleon, J., Bernard, S., Le Guillou, C., Beyssac, O., Sugitani, K., Robert, F., 2018. Chemical  
312 nature of the 3.4 Ga Strelley Pool microfossils. *Geochemical Perspectives Letters* 37–42.

313 Baludikay, B.K., Storme, J.-Y., François, C., Baudet, D., Javaux, E.J., 2016. A diverse and  
314 exquisitely preserved organic-walled microfossil assemblage from the Meso–Neoproterozoic  
315 Mbuji-Mayi Supergroup (Democratic Republic of Congo) and implications for Proterozoic  
316 biostratigraphy. *Precambrian Research* 281, 166–184.

317 Beeby, M., Ferreira, J.L., Tripp, P., Albers, S.-V., Mitchell, D.R., 2020. Propulsive  
318 nanomachines: the convergent evolution of archaella, flagella, and cilia. *FEMS Microbiology*  
319 *Reviews* fuaa006.

320 Bergholtz, T., Daugbjerg, N., Moestrup, O., Fernandez-Tejedor, M., 2006. On the identity of  
321 *karlodium veneficum* and description of *karlodium armiger* sp. nov. (dinophyceae), based  
322 on light and electron microscopy, nuclear-encoded *lsu rDNA*, and pigment composition. *Journal*  
323 *of Phycology* 42, 170–193.

324 Bernard, S., Beyssac, O., Benzerara, K., Findling, N., Tzvetkov, G., Brown, G.E., 2010.  
325 XANES, Raman and XRD study of anthracene-based cokes and saccharose-based chars  
326 submitted to high-temperature pyrolysis. *Carbon* 48, 2506–2516.



327 Brasier, M., McLoughlin, N., Green, O., Wacey, D., 2006. A fresh look at the fossil evidence  
328 for early Archaean cellular life. *Philosophical Transactions of the Royal Society B: Biological*  
329 *Sciences* 361, 887–902.

330 Brasier, M.D., Green, O.R., Jephcoat, A.P., Kleppe, A.K., Van Kranendonk, M.J., Lindsay,  
331 J.F., Steele, A., Grassineau, N.V., 2002. Questioning the evidence for Earth’s oldest fossils.  
332 *Nature* 416, 76–81.

333 Cosmidis, J., Templeton, A.S., 2016. Self-assembly of biomorphic carbon/sulfur  
334 microstructures in sulfidic environments. *Nature Communications* 7, 12812.

335 Craveiro, S.C., Moestrup, Ø., Daugbjerg, N., Calado, A.J., 2010. Ultrastructure and Large  
336 Subunit rDNA-Based Phylogeny of *Sphaerodinium cracoviense*, an Unusual Freshwater  
337 Dinoflagellate with a Novel Type of Eyespot: *Sphaerodinium* ultrastructure and phylogeny.  
338 *Journal of Eukaryotic Microbiology* 57, 568–585.

339 Curtis, P.D., 2017. Stalk formation of *Brevundimonas* and how it compares to *Caulobacter*  
340 *crescentus*. *PLOS ONE* 12, e0184063.

341 Delarue, F., Robert, F., Derenne, S., Tartèse, R., Jauvion, C., Bernard, S., Pont, S., Gonzalez-  
342 Cano, A., Duhamel, R., Sugitani, K., 2020. Out of rock: A new look at the morphological and  
343 geochemical preservation of microfossils from the 3.46 Gyr-old Strelley Pool Formation.  
344 *Precambrian Research* 336, 105472.

345 Delarue, F., Robert, F., Sugitani, K., Tartèse, R., Duhamel, R., Derenne, S., 2017. Investigation  
346 of the Geochemical Preservation of ca. 3.0 Ga Permineralized and Encapsulated Microfossils  
347 by Nanoscale Secondary Ion Mass Spectrometry. *Astrobiology* 17, 1192–1202.

348 Deng, Y., Chen, C., Zhao, Z., Zhao, J., Jacq, A., Huang, X., Yang, Y., 2016. The RNA  
349 Chaperone Hfq Is Involved in Colony Morphology, Nutrient Utilization and Oxidative and  
350 Envelope Stress Response in *Vibrio alginolyticus*. *PLOS ONE* 11, e0163689.

351 El Albani, A., Mangano, M.G., Buatois, L.A., Bengtson, S., Riboulleau, A., Bekker, A.,  
352 Konhauser, K., Lyons, T., Rollion-Bard, C., Bankole, O., Lekele Baghekema, S.G., Meunier,  
353 A., Trentesaux, A., Mazurier, A., Aubineau, J., Laforest, C., Fontaine, C., Recourt, P., Chi Fru,  
354 E., Macchiarelli, R., Reynaud, J.Y., Gauthier-Lafaye, F., Canfield, D.E., 2019. Organism  
355 motility in an oxygenated shallow-marine environment 2.1 billion years ago. *Proceedings of*  
356 *the National Academy of Sciences* 116, 3431–3436.

357 Furuno, M., Atsumi, T., Yamada, T., Kojima, S., Nishioka, N., Kawagishi, I., Homma, M.,  
358 1997. Characterization of polar-flagellar-length mutants in *Vibrio alginolyticus*. *Microbiology*  
359 143, 1615–1621.

360 Garcia-Ruiz, J.M., 2003. Self-Assembled Silica-Carbonate Structures and Detection of Ancient  
361 Microfossils. *Science* 302, 1194–1197.

362 House, C.H., Oehler, D.Z., Sugitani, K., Mimura, K., 2013. Carbon isotopic analyses of ca. 3.0  
363 Ga microstructures imply planktonic autotrophs inhabited Earth's early oceans. *Geology* 41,  
364 651–654.

365 Jarrell, K.F., McBride, M.J., 2008. The surprisingly diverse ways that prokaryotes move. *Nature*  
366 *Reviews Microbiology* 6, 466–476.

367 Javaux, E.J., 2003. Recognizing and interpreting the fossils of early eukaryotes. *Origins of Life*  
368 *and Evolution of the Biosphere* 33, 75–94.

369 Javaux, E.J., 2019. Challenges in evidencing the earliest traces of life. *Nature* 572, 451–460.

370 Javaux, E.J., Marshall, C.P., Bekker, A., 2010. Organic-walled microfossils in 3.2-billion-year-  
371 old shallow-marine siliciclastic deposits. *Nature* 463, 934–938.

372 Kanbe, M., Yagasaki, J., Zehner, S., Göttfert, M., Aizawa, S.-I., 2007. Characterization of Two  
373 Sets of Subpolar Flagella in *Bradyrhizobium japonicum*. *Journal of Bacteriology* 189, 1083–  
374 1089.

375 Khan, S., Scholey, J.M., 2018. Assembly, Functions and Evolution of Archaeella, Flagella and  
376 Cilia. *Current Biology* 28, R278–R292.

377 Kinoshita, Y., Uchida, N., Nakane, D., Nishizaka, T., 2016. Direct observation of rotation and  
378 steps of the archaeellum in the swimming halophilic archaeon *Halobacterium salinarum*. *Nature*  
379 *Microbiology* 1, 16148.

380 Kouketsu, Y., Mizukami, T., Mori, H., Endo, S., Aoya, M., Hara, H., Nakamura, D., Wallis, S.,  
381 2014. A new approach to develop the Raman carbonaceous material geothermometer for low-  
382 grade metamorphism using peak width. *Island Arc* 23, 33–50.

383 Kozawa, T., Sugitani, K., Oehler, D.Z., House, C.H., Saito, I., Watanabe, T., Gotoh, T., 2019.  
384 Early Archean planktonic mode of life: Implications from fluid dynamics of lenticular  
385 microfossils. *Geobiology* 17, 113–126.

386 Lahfid, A., Beyssac, O., Deville, E., Negro, F., Chopin, C., Goffé, B., 2010. Evolution of the  
387 Raman spectrum of carbonaceous material in low-grade metasediments of the Glarus Alps  
388 (Switzerland): RSCM in low-grade metasediments. *Terra Nova* 22, 354–360.

389 Le Guillou, C., Bernard, S., De la Pena, F., Le Brech, Y., 2018. XANES-Based Quantification  
390 of Carbon Functional Group Concentrations. *Analytical Chemistry* 90, 8379–8386.

391 Leander, B.S., Lax, G., Karnkowska, A., Simpson, A.G.B., 2017. Euglenida, in: Archibald,  
392 J.M., Simpson, A.G.B., Slamovits, C.H. (Eds.), *Handbook of the Protists*. Springer International  
393 Publishing, Cham, pp. 1047–1088.

394 Lepot, K., Williford, K.H., Ushikubo, T., Sugitani, K., Mimura, K., Spicuzza, M.J., Valley,  
395 J.W., 2013. Texture-specific isotopic compositions in 3.4Gyr old organic matter support  
396 selective preservation in cell-like structures. *Geochimica et Cosmochimica Acta* 112, 66–86.

397 Lim, H.C., Leaw, C.P., Tan, T.H., Kon, N.F., Yek, L.H., Hii, K.S., Teng, S.T., Razali, R.M.,  
398 Usup, G., Iwataki, M., Lim, P.T., 2014. A bloom of *Karlodinium australe* (Gymnodiniales,

399 Dinophyceae) associated with mass mortality of cage-cultured fishes in West Johor Strait,  
400 Malaysia. *Harmful Algae* 40, 51–62.

401 Loron, C.C., Rainbird, R.H., Turner, E.C., Greenman, J.W., Javaux, E.J., 2019. Organic-walled  
402 microfossils from the late Mesoproterozoic to early Neoproterozoic lower Shaler Supergroup  
403 (Arctic Canada): Diversity and biostratigraphic significance. *Precambrian Research* 321, 349–  
404 374.

405 Miller, T.R., Hnilicka, K., Dziedzic, A., Desplats, P., Belas, R., 2004. Chemotaxis of  
406 *Silicibacter* sp. Strain TM1040 toward Dinoflagellate Products. *Applied and Environmental*  
407 *Microbiology* 70, 4692–4701.

408 Ng, S.Y.M., Zolghadr, B., Driessen, A.J.M., Albers, S.-V., Jarrell, K.F., 2008. Cell Surface  
409 Structures of Archaea. *Journal of Bacteriology* 190, 6039–6047.

410 Oehler, D.Z., Walsh, M.M., Sugitani, K., Liu, M.-C., House, C.H., 2017. Large and robust  
411 lenticular microorganisms on the young Earth. *Precambrian Research* 296, 112–119.

412 Pasteris, J.D., Wopenka, B., 2003. Necessary, but Not Sufficient: Raman Identification of  
413 Disordered Carbon as a Signature of Ancient Life. *Astrobiology* 3, 727–738.

414 Poindexter, J.S., Staley, J.T., 1996. *Caulobacter* and *Asticcacaulis* stalk bands as indicators of  
415 stalk age. *Journal of bacteriology* 178, 3939–3948.

416 Pyatibratov, M.G., Beznosov, S.N., Rachel, R., Tiktopulo, E.I., Surin, A.K., Syutkin, A.S.,  
417 Fedorov, O.V., 2008. Alternative flagellar filament types in the haloarchaeon *Haloarcula*  
418 *marismortui*. *Canadian Journal of Microbiology* 54, 835–844.

419 Quintero, E.J., Busch, K., Weiner, R.M., 1998. Spatial and Temporal Deposition of Adhesive  
420 Extracellular Polysaccharide Capsule and Fimbriae by *Hyphomonas* Strain MHS-3. *Applied*  
421 *and Environmental Microbiology* 64, 1246–1255.

422 Schopf, J.W., Kudryavtsev, A.B., Agresti, D.G., Wdowiak, T.J., Czaja, A.D., 2002. Laser–  
423 Raman imagery of Earth’s earliest fossils. *Nature* 416, 73–76.

424 Siano, R., Kooistra, W.H.C.F., Montresor, M., Zingone, A., 2009. Unarmoured and thin-walled  
425 dinoflagellates from the Gulf of Naples, with the description of *Woloszynskia cincta* sp. nov.  
426 (Dinophyceae, Suessiales). *Phycologia* 48, 44–65.

427 Southam, G., Kalmokoff, M.L., Jarrell, K.F., Koval, S.F., Beveridge, T.J., 1990. Isolation,  
428 characterization, and cellular insertion of the flagella from two strains of the archaeobacterium  
429 *Methanospirillum hungatei*. *Journal of Bacteriology* 172, 3221–3228.

430 Sugimoto, S., Okuda, K., Miyakawa, R., Sato, M., Arita-Morioka, K., Chiba, A., Yamanaka,  
431 K., Ogura, T., Mizunoe, Y., Sato, C., 2016. Imaging of bacterial multicellular behaviour in  
432 biofilms in liquid by atmospheric scanning electron microscopy. *Scientific Reports* 6, 25889.

433 Sugitani, K., Lepot, K., Nagaoka, T., Mimura, K., Van Kranendonk, M., Oehler, D.Z., Walter,  
434 M.R., 2010. Biogenicity of Morphologically Diverse Carbonaceous Microstructures from the  
435 ca. 3400 Ma Strelley Pool Formation, in the Pilbara Craton, Western Australia. *Astrobiology*  
436 10, 899–920.

437 Sugitani, K., Mimura, K., Nagaoka, T., Lepot, K., Takeuchi, M., 2013. Microfossil assemblage  
438 from the 3400Ma Strelley Pool Formation in the Pilbara Craton, Western Australia: Results  
439 form a new locality. *Precambrian Research* 226, 59–74.

440 Sugitani, K., Mimura, K., Takeuchi, M., Lepot, K., Ito, S., Javaux, E.J., 2015. Early evolution  
441 of large micro-organisms with cytological complexity revealed by microanalyses of 3.4 Ga  
442 organic-walled microfossils. *Geobiology* 13, 507–521.

443 Vasilyeva, L.V., Omelchenko, M.V., Berestovskaya, Y.Y., Lysenko, A.M., Abraham, W.-R.,  
444 Dedysh, S.N., Zavarzin, G.A., 2006. *Asticcacaulis benevestitus* sp. nov., a psychrotolerant,  
445 dimorphic, prosthecate bacterium from tundra wetland soil. *International Journal of Systematic  
446 and Evolutionary Microbiology* 56, 2083–2088.

447 Wacey, D., Kilburn, M.R., Saunders, M., Cliff, J., Brasier, M.D., 2011. Microfossils of sulphur-  
448 metabolizing cells in 3.4-billion-year-old rocks of Western Australia. *Nature Geoscience* 4,  
449 698–702.

450 Wacey, D., Saunders, M., Kong, C., Brasier, A., Brasier, M., 2016. 3.46 Ga Apex chert  
451 ‘microfossils’ reinterpreted as mineral artefacts produced during phyllosilicate exfoliation.  
452 *Gondwana Research* 36, 296–313.

453 Wagner, J.K., Setayeshgar, S., Sharon, L.A., Reilly, J.P., Brun, Y.V., 2006. A nutrient uptake  
454 role for bacterial cell envelope extensions. *Proceedings of the National Academy of Sciences*  
455 103, 11772–11777.

456 Wang, H., Lu, D., Huang, H., Göbel, J., Dai, X., Xia, P., 2011. First observation of *Karlotinium*  
457 *veneficum* from the East China Sea and the coastal waters of Germany. *Acta Oceanologica*  
458 *Sinica* 30, 112–121.

459 Wang, Y., Chen, Y., Lavin, C., Gretz, M.R., 2000. Extracellular matrix assembly in diatoms  
460 (Bacillariophyceae). iv. ultrastructure of *Achnanthes longipes* and *Cymbella cistula* as revealed  
461 by high-pressure freezing/freeze substitution and cryo-field emission scanning electron  
462 microscopy. *Journal of Phycology* 36, 367–378.

463 Westall, F., de Ronde, C.E.J., Southam, G., Grassineau, N., Colas, M., Cockell, C., Lammer,  
464 H., 2006. Implications of a 3.472–3.333 Gyr-old subaerial microbial mat from the Barberton  
465 greenstone belt, South Africa for the UV environmental conditions on the early Earth.  
466 *Philosophical Transactions of the Royal Society B: Biological Sciences* 361, 1857–1876.

467 Wustman, B.A., Gretz, M.R., Hoagland, K.D., 1997. Extracellular Matrix Assembly in Diatoms  
468 (Bacillariophyceae) (I. A Model of Adhesives Based on Chemical Characterization and  
469 Localization of Polysaccharides from the Marine Diatom *Achnanthes longipes* and Other  
470 Diatoms). *Plant Physiology* 113, 1059–1069.

471

1 **Chemical degradation of thermally altered silicified organic matter during acid**  
2 **maceration: a case study from the Lower Devonian Rhynie chert**

3

4 Frédéric Delarue <sup>a\*</sup>, Thanh Thuy Nguyen Tu <sup>a</sup>, Rémi Duhamel <sup>b</sup>, Céline Paris <sup>c</sup>, François  
5 Baudin <sup>d</sup>

6

7 <sup>a</sup> *Sorbonne Université, CNRS, EPHE, PSL, UMR 7619 METIS, 4 place Jussieu, F-75005*  
8 *Paris, France*

9 <sup>b</sup> *Muséum National d'Histoire Naturelle, Sorbonne Université, UMR 7590, IRD, IMPMC, F-*  
10 *75005 Paris, France*

11 <sup>c</sup> *Sorbonne Université, CNRS, UMR 8233 MONARIS, 75005, 4 place Jussieu, F-75005 Paris,*  
12 *Paris, France*

13 <sup>d</sup> *Sorbonne Université, CNRS, UMR 7193 IStEP, 4 place Jussieu, F-75005 Paris, France*

14

15 \*Correspondence to: frederic.delarue@sorbonne-universite.fr

16

## 17 **ABSTRACT**

18 The effect of standard acid maceration on organic matter (OM) from ancient silicified  
19 sediments remains undocumented. Early silicification favours preservation of organic  
20 moieties against thermal alteration over time. In this study, we investigated the effects of acid  
21 maceration on the structure of OM isolated from the Lower Devonian Rhynie chert. The  
22 structure of OM was investigated by combining Rock-Eval pyrolysis and Raman  
23 spectroscopy. Besides a loss of thermolabile organic matter owing to solvent extraction,  
24 Rock-Eval pyrolysis showed that standard acid maceration also causes a loss of C-H  
25 emissions at high pyrolysis temperature ( $> 500\text{ }^{\circ}\text{C}$ ). The standard acid maceration procedure  
26 was also associated with the disappearance of the D4 and D5 Raman spectrum shoulders  
27 assigned to C–H bonds in aliphatics and bitumens, respectively, entrapped in the  
28 macromolecular network. Taken together, Rock-Eval pyrolysis and Raman spectroscopy  
29 indicate that standard acid maceration can lead to the chemical degradation of syngenetic  
30 hydro-carbonaceous moieties of OM isolated from ancient silicified and thermally altered  
31 sediments. In sediments having experienced early silicification, which hampers bitumen  
32 migration and favours pyrobitumen formation, we suggest that novel in situ molecular  
33 analytical techniques are required to provide a thorough examination of the syngenetic  
34 molecular content independent of the soluble/insoluble operational definition.

35

## 36 **1. Introduction**

37 The chemical composition of ancient organic matter (OM) attests to turning points in  
38 the evolution of life during the history of the Earth (Summons et al., 1988; Edwards et al.,  
39 1997; Love et al., 2008; Duda et al., 2016; Nguyen et al., 2019; Love et al., 2020). However,  
40 investigating the structural and chemical compositions of ancient OM is still a challenging  
41 issue as thermal alteration during geological times results in losses of pristine molecular and



42 elemental content. Since ancient sediments and metasediments are generally depleted in OM,  
43 isolation procedures have typically been employed before applying analytical techniques to  
44 investigate the chemical composition of ancient OM, notably at the molecular scale.  
45 Historically, in the field of organic geochemistry, isolation of organic-insoluble and mineral-  
46 free OM is performed through a standard acid maceration procedure involving maceration  
47 followed by successive organic solvent extractions (e.g., dichloromethane [DCM] and  
48 methanol [MeOH]) and demineralisation with acid (e.g., hydrochloric [HCl] and hydrofluoric  
49 [HF]). This physical and chemical maceration procedure (HMM) yields kerogen, which is  
50 defined as insoluble macromolecular OM (Durand, 1980; Vandenbroucke and Largeau,  
51 2007). Several investigations have suggested that acid maceration does not significantly  
52 modify the structure of kerogens from ancient rocks (Larsen et al., 1989; Vandenbroucke and  
53 Largeau, 2007; Aboulkas and El Harfi, 2009). However, there is contrasting evidence that  
54 acid maceration of coals can lead to either a rise in carbon structural order (Zhang et al., 2016)  
55 or a decrease in hydrocarbon content (Tekely et al., 1987). Moreover, Kebukawa et al. (2019)  
56 demonstrated the contrasting effects of acid maceration by comparing the chemical structure  
57 of bulk chondrites to their corresponding insoluble OM fractions. Insoluble OM from type 3  
58 chondrite was depleted in aliphatic moieties in comparison to the bulk type 3 chondrite  
59 starting material. However, the effect of acid maceration varied among the different chondrite  
60 groups studied (Kebukawa et al., 2019). Thus, according to current literature, it therefore  
61 seems that the chemical stability of thermally altered OM to standard acid maceration cannot  
62 simply be assumed.

63         Among ancient sedimentary rocks, silicified sediments are of interest as they are  
64 essential geological archives of biological evolution throughout Earth's history. However,  
65 there is often very little OM remaining in these sediments and acid maceration procedures are  
66 required to concentrate it before studying its molecular content. Occurring prior to cell lysis

67 and early degradation of organic matter, rapid silicification leads to a closed chemical system  
68 by reducing sediment porosity (Boyce et al., 2002; Ledevin et al., 2014), which favours  
69 preservation of organic remnants against thermal alteration (Alleon et al., 2016). Using  
70 geochemical micrometre-scale analysis tools, it was demonstrated that some silicified  
71 Precambrian organic-walled microfossils are composed of significant amounts of carbonyl,  
72 phenolic, carboxylic, hydroxyl, and amide functional groups, despite being subjected to peak  
73 temperatures of up to 300 °C (Alleon et al., 2016). However, standard acid maceration  
74 procedures applied to ancient silicified sediments have the potential to degrade syngenetic  
75 OM through solvent extraction and acid hydrolysis of bitumens and chemically labile organic  
76 moieties, respectively. Such degradation, if it occurs, implies that a substantial amount of the  
77 initial molecular content depicting the evolution of life, past environmental conditions, and/or  
78 thermal maturation may be lost in the process. Further investigations on the effect of standard  
79 acid maceration on the chemical structure of ancient thermally altered silicified OM are  
80 therefore required.

81         Our aim in this study was to investigate the effect of the standard acid maceration  
82 procedure on the chemical structure of ancient silicified OM. We selected the iconic Lower  
83 Devonian Rhynie chert, the earliest preserved terrestrial ecosystem owing to a rapid and  
84 complete silicification through siliceous hot-spring deposits (Trewin, 2003; Preston and  
85 Genge, 2010). Despite the exceptional preservation of fossil plants, fungi, insects, and other  
86 organisms, the overall organic carbon content of Rhynie chert is very low (Summons et al.,  
87 1996). It therefore typically requires acid maceration to access the molecular signature(s). The  
88 Rhynie chert therefore constitutes a case study to evaluate the impact of standard acid  
89 maceration on the chemical structure of ancient thermally altered silicified OM. To this end,  
90 OM was isolated according to two acid maceration procedures: (1) a high-manipulation  
91 maceration (HMM), which followed the standard treatments generally applied to geological

92 samples, and (2) a low-manipulation maceration (LMM), which minimized chemical  
93 degradation by avoiding organic solvents and HCl. Rock-Eval pyrolysis and Raman  
94 spectroscopy were used to assess the chemical structure of the OM isolated by each  
95 procedure. Comparison of the so-obtained chemical structures allowed us to estimate the  
96 minimum amount of chemical degradation imparted by HMM maceration.

97

## 98 **2. Material and methods**

### 99 *2.1. The Rhynie chert*

100 Situated in Aberdeenshire (Scotland), the Rhynie chert is a Konservat-Lagerstätte. It is  
101 hosted by the Dryden Flags Formation, which is characterised by a succession of Lower  
102 Devonian sedimentary and volcanic rocks (Rice et al., 2002). Pragian–earliest Emsian in age  
103 ( $407.1 \pm 2.2$  Ma; Mark et al., 2011), the Rhynie chert is composed primarily of  
104 microcrystalline silica and was deposited as siliceous sinter from subaerial hot springs  
105 systems (Rice et al., 1995).

106

### 107 *2.2. High- and low-manipulation maceration procedures*

108 HMM and LMM procedures were performed on similar portions of the same rock  
109 sample. HMM was performed on ~30 g of crushed rock. Solvent extraction was first  
110 performed on rock powder using a mixture of DCM and MeOH (2:1; v:v). Carbonates were  
111 then removed at room temperature using HCl (37%; reagent grade) to minimise the formation  
112 of fluorides during subsequent HF/HCl maceration. Samples were then centrifuged and  
113 washed with ultrapure water until reaching neutrality. Concentration of OM was achieved  
114 through acid maceration at room temperature in a mixture of HF (40%, reagent grade) and  
115 HCl (2:1, v/v; reagent grade). Samples were centrifuged and washed with ultrapure water to  
116 reach neutrality. Finally, HCl (37%; reagent grade) at 60 °C (24 h) was used to degrade

117 neoformed fluorides. After an additional step of solvent extraction using a mixture of DCM  
118 and MeOH (2:1, v/v), the isolated OM was again centrifuged/washed with ultrapure water  
119 until reaching neutrality. Samples were then air-dried at 60 °C after final rinsing in acetone.  
120 Hereafter, OM isolated by the HMM procedure is referred to as OHMM.

121 LMM was performed by first fragmenting ~30 g of rock samples into ~3 g rock chips.  
122 Rock chips were cleaned using ultrapure water and a mixture of DCM and MeOH (2:1, v/v)  
123 and were then placed directly in a Teflon vessel filled with HF (40%, reagent grade) at room  
124 temperature. After 48 h, successive centrifugation and rinsing steps using ultrapure water  
125 were performed until reaching neutrality. Samples were then air-dried at 60 °C. Hereafter,  
126 OM isolated by the LMM procedure is referred to as OLMM.

127

### 128 *2.3. Rock-Eval pyrolysis*

129 OHMM and OLMM were analysed using Rock-Eval 6 (Vinci Technologies) following  
130 the standard pyrolysis protocol described in Behar et al. (2001). Performed in a N<sub>2</sub>  
131 atmosphere, Rock-Eval pyrolysis comprises two steps: an isothermal phase held for 3 minutes  
132 followed by a rise in pyrolysis temperature from 300 to 650 °C at a rate of 25 °C/min. After  
133 pyrolysis, the residual material was then heated from 300 °C to 850 °C under purified air in an  
134 oxidation oven in order to calculate total organic carbon (TOC) value (see Behar et al., 2001  
135 for further details about calculation procedure). Released hydrocarbons (HC) were  
136 continuously quantified by a flame ionisation detector (S1 and S2, for the first and second  
137 pyrolysis steps, respectively, in mg HC/g of sample) while released CO and CO<sub>2</sub> were  
138 continuously and simultaneously monitored by infrared detectors during both pyrolysis  
139 (S3CO and S3CO<sub>2</sub>) and combustion (S4CO and S4CO<sub>2</sub>). Quantification of the amount of  
140 effluents led to the determination of the TOC (wt%), of the Hydrogen Index (HI, defined as  
141  $S2 \times 100 / \text{TOC}$ , in mg HC/g of TOC) and of the Oxygen Index (OI; defined as  $S3 \times 100 / \text{TOC}$ , in

142 mg CO<sub>2</sub>/g of TOC). The pyrolysis temperature associated with the maximum release of  
143 hydrocarbons, called “TpKs2”, was also determined.

144

#### 145 *2.4. Raman spectroscopy*

146 Raman spectroscopy analysis was performed using a Renishaw inVia micro-  
147 spectrometer equipped with a 532 nm argon laser. The spectrometer was first calibrated using  
148 a silicon standard before each session. For each sample analysis, the laser was focused using a  
149 Leica microscope with a ×50 objective and the spectra were recorded in the 1000–1900 cm<sup>-1</sup>  
150 first order spectral window including the defect (D) and graphite (G) peaks (Fig. 1). The laser  
151 power at the sample surface was kept below 1 mW to prevent thermal alteration of isolated  
152 OM (Everall et al., 1991). Spectra acquisition was achieved after two iterations using a time  
153 exposure of 40 s. After linear correction of the baseline between 1000 and 1900 cm<sup>-1</sup> and  
154 spectra normalization, we identified Raman peaks according to the nomenclature defined in  
155 Romero-Sarmiento et al. (2014).

156 The heights of the sub-bands D1 (~1365 cm<sup>-1</sup>), D4 (~1285 cm<sup>-1</sup>), D5 (~1445 cm<sup>-1</sup>) and  
157 G+D2 (~1600 cm<sup>-1</sup>; Fig. 1) were then determined to compute the  $I_{D1}/I_{G+D2}$ ,  $I_{D4}/I_{G+D2}$  and  
158  $I_{D5}/I_{G+D2}$ , ratios. Slopes  $\alpha_{D4}$  (determined between 1265 and 1300 cm<sup>-1</sup> Raman shift) and  $\alpha_{D5}$   
159 (determined between 1415 and 1445 cm<sup>-1</sup> Raman shift) were determined to geometrically  
160 evaluate the expression of D4 and D5 shoulders on the D band.

161

### 162 **3. Results and discussion**

163 The TOC of OHMM and OLMM samples was measured to determine the efficiency  
164 of rock mineralization through HMM and LMM, respectively. The TOC in all OHMM and  
165 OLMM samples was low (25.4% and 27.5% respectively; Table 1) suggesting that the  
166 preservation and/or neoformation of substantial mineral content had occurred during both

167 procedures. The OI was approximately 15 mg and 59 mg CO<sub>2</sub>/g TOC in OHMM and OLMM,  
168 respectively (Table 1). The higher OI in OLMM is partially explained by the higher level of  
169 CO<sub>2</sub> emissions during the first isothermal step, showing that they are mostly related to  
170 desorbable OM (Fig. 2). Such a result is in line with previous investigations on coals  
171 suggesting that acid maceration can lead to a rise in oxygen content resulting possibly from  
172 the neoformation of carboxylic groups replacing carboxylate through ion exchange (Larsen et  
173 al., 1989). Higher levels of CO<sub>2</sub> emissions were also recorded for OLMM during the rise in  
174 pyrolysis temperature from 300 to 650 °C (Fig. 2). In this pyrolysis temperature range, CO<sub>2</sub>  
175 emissions appeared to be independent of hydrocarbon emissions suggesting that they do not  
176 correspond to the thermal cracking of the macromolecular network.

177 OHMM and OLMM isolated from the Rhynie chert were characterised by a TpkS2 of  
178 481 °C and 504 °C, respectively (Table 1). In every type of kerogen, these pyrolysis  
179 temperatures are commonly assigned to OM that has been subjected to high thermal  
180 alteration. Consistent with high TpkS2 values, pyrolysis of OHMM and of OLMM released  
181 low amounts of HC as evidenced by HI of 46 HC/g TOC and 68 mg HC/g TOC, respectively.  
182 Together, high TpkS2 and low HI imply that OM from the Rhynie chert falls at the transition  
183 between the oil and gas windows registered by TpkS2 ranging between 470 °C and 505 °C in  
184 analogous Type III kerogens (Espitalié et al., 1986). Hydrocarbon release during Rock-Eval  
185 pyrolysis was higher in OLMM than in OHMM (Table 1). The S1 value for OLMM was  
186 about 320% higher than for OHMM (Table 1), which suggests that thermolabile OM was  
187 more abundant in OLMM than in OHMM. This is consistent with the fact that OLMM was  
188 not subjected to organic solvent extractions, which have been shown to dramatically reduce  
189 S1 values (Delvaux et al., 1990). Between 300 and 350 °C, no release of hydrocarbons was  
190 observed suggesting a virtual absence of residual heavy oil and/or of pyrobitumen in both  
191 OHMM and OLMM (Fig. 2; Clementz, 1979; Sanei et al., 2015). The S2 curves for OHMM

192 and OLMM differ at high pyrolysis temperature ( $> 500\text{ }^{\circ}\text{C}$ ), at which point a broad shoulder  
193 is only observed in OLMM (Fig. 2). The occurrence of this thermorecalcitrant OM in OLMM  
194 mostly explains its higher HI, in the absence of solvent extractions and/or hydrochloric acid  
195 hydrolysis (Table 1). In turn, this suggests that standard acid maceration can lead to a  
196 modification of the macromolecular chemical structure in this ancient silicified OM by  
197 promoting a loss of hydrocarbonaceous moieties.

198 Determined on 15 random targets in both OHMM and OLMM, Raman spectra  
199 exhibited two broad D and G+D2 bands at around  $1365\text{ cm}^{-1}$  and  $1600\text{ cm}^{-1}$  in each  
200 preparation (Fig. 1). The D band presents a complex structure resulting from the presence of  
201 several sub-bands including here, the D4, D1, and D5 bands. In addition to the contribution of  
202 amorphous carbon, the D band is dominated by the D1 sub-band corresponding to the  
203 breathing mode of the  $\text{sp}^2$  aromatic ring within polyaromatic clusters and is attributed to  
204 defects in these aromatic structures (Ferrari and Robertson, 2000). The D2 band corresponds  
205 to defects in aromatic structure but in contrast to the D1 band, does not include amorphous  
206 carbon. A distinct D2 peak (usually centered at about  $1620\text{ cm}^{-1}$ ) is mainly observed in highly  
207 mature OM (Kouketsu et al. 2014) and is not observed in OM studied here. Therefore, the  
208 G+D2 band is mostly related to the G band corresponding to in-plane C-C bond stretching in  
209 polyaromatic layers from thermally altered materials (Marshall and Marshall, 2014). As the  
210 height of the D1 band increases with temperature, the  $I_{\text{D1}}/I_{\text{G+D2}}$  ratio is often used to probe the  
211 structural order of OM in the course of carbonization (Table 2; Wopenka and Pasteris, 1993;  
212 Quirico et al., 2005; Kouketsu et al., 2014; Delarue et al., 2016). In this study, OHMM and  
213 OLMM are characterized by  $I_{\text{D1}}/I_{\text{G+D2}}$  ratios of  $0.58 \pm 0.02$  and  $0.52 \pm 0.02$ , respectively  
214 (Table 2). This result would imply a higher carbon structural order in OHMM compared to  
215 OLMM, which is in agreement with previous findings indicating that standard acid  
216 maceration can yield a rise in carbon structural order (Zhang et al., 2016).

217           The D band also exhibits two shoulders corresponding to the D4 (1285 cm<sup>-1</sup>) and D5  
218 (1445 cm<sup>-1</sup>) bands (Fig. 1). The D4 peak is generally assigned to C–H bonds in aliphatics as  
219 C-H in aromatics does not seem to directly contribute to the D4 region (Ferralis et al., 2016).  
220 The D5 band indicates the presence of hydrocarbons trapped within the organic porosity and  
221 has been detected in a few oil and gas shale samples (Romero-Sarmiento et al., 2014;  
222 Rouzaud et al., 2015). In the current study,  $\alpha_{D4}$  and  $\alpha_{D5}$  slopes in Raman spectra were lower in  
223 OLMM than in OHMM, consistent with the more pronounced D4 and D5 shoulders in the  
224 Raman line of OLMM (Fig. 1; Table 2). However, the more pronounced D4 and D5 shoulders  
225 in OLMM were not associated with higher  $I_{D4}/I_{G+D2}$  and  $I_{D5}/I_{G+D2}$  ratios (Table 2). This could  
226 be explained by the higher carbon structural order in OHMM implying a bias in the  
227 determination of  $I_{D4}/I_{G+D2}$  and  $I_{D5}/I_{G+D2}$  ratios. The occurrence of more pronounced D4 and D5  
228 shoulders in OLMM indicate that OLMM is indeed enriched in hydrocarbonaceous moieties,  
229 due to the C-H bonds in aliphatics and other entrapped hydrocarbons, relative to OHMM. The  
230 presence of entrapped hydrocarbons in the macromolecular network of OLMM likely explains  
231 the broad shoulder cracking at high temperature (> 500 °C) as revealed by Rock-Eval  
232 pyrolysis (Fig. 2). Both Raman spectroscopy and Rock-Eval pyrolysis suggest that standard  
233 acid maceration results in a loss of hydrocarbons in OM isolated from the Rhynie chert. As  
234 LMM does not involve the organic solvent extraction used in HMM, part of this hydrocarbon  
235 loss is likely due to solvent-extractible OM, which is reflected by the respective S1 values. In  
236 contrast to OHMM, OLMM did not involve extraction with HCl, which when mixed with HF  
237 has been demonstrated to favour hydrolysis leading to a loss of aliphatic CH and/or CH<sub>2</sub>  
238 functional groups in coal (Tekely et al., 1987). Even though our experimental design did not  
239 allow us to test the singular effect of HCl hydrolysis, such an effect can be hypothesized to  
240 partly explain the loss in aliphatic moieties with HMM treatment.



241           Although the Raman spectroscopy results support the notion that hydrocarbons are lost  
242 during HMM, it does not directly allow the extent of this loss to be determined. Following the  
243 relationship between H/C and HI (Espitalié et al., 1977), we determined that H/C atomic  
244 ratios were 0.53 and 0.57 in OHMM and OLMM, respectively (Table 1). Standard acid  
245 maceration therefore led to a reduction of at least about 7–8% of the hydrocarbon content of  
246 Rhynie OM. We use “at least” because chemical degradation of hydrocarbons occurring  
247 during the isolation of OLMM cannot be straightforwardly excluded. To evaluate this point,  
248 Raman spectra were also acquired on in situ OM from thin sections of Rhynie chert  
249 (Supplementary information). These in situ Raman spectra were acquired with another Raman  
250 spectroscope and on polished thin sections, which can modify the apparent carbon structural  
251 order by yielding an increase in the D band (Ammar et al., 2011; Maslova et al., 2012). These  
252 issues therefore prevent a straightforward comparison between Raman-derived ratios from in  
253 situ OM and from OLMM/OHMM. Nonetheless, the  $\alpha_{D4}$  and  $\alpha_{D5}$  parameters are independent  
254 of the height of the D band and can be used to track a potential effect of LMM procedure. In  
255 situ OM presents a mean  $\alpha_{D4}$  and  $\alpha_{D5}$  of about  $31 \pm 4$  and  $-24 \pm 23$ , respectively ( $n = 10$ ),  
256 indicating more pronounced D4 and D5 shoulders in the in situ OM compared to OLMM  
257 (Supplementary information). This suggests that simple treatment with HF and water also led  
258 to a slight loss in aliphatic content. In addition to these hydrocarbon losses related to acid  
259 maceration procedures, the estimated 7–8% loss in hydrocarbons can also be considered as  
260 minimum estimation because of thermolabile hydrocarbons (S1 parameter), which are not  
261 taken into account in the calculation of HI and were more abundant in OLMM than in  
262 OHMM.

263           Thermolabile hydrocarbons are tightly linked to soluble OM (Delvaux et al., 1990).  
264 However, this soluble OM in ancient sediments is often ignored because its syngeneity can be  
265 difficult to prove due to the potential for post-deposit contamination (Brocks et al., 2003a,

266 2003b; Derenne et al., 2008). However, silicified sediments can be seen as unconventional  
267 dual source/reservoir rocks. Indeed, rapid silicification drastically reduces porosity leading to  
268 a closed chemical system (Boyce et al., 2002; Ledevin et al., 2014). In such source/reservoir  
269 rocks, migration of bitumens was very restricted implying that bitumens and/or pyrobitumens  
270 coexisted in close proximity with residual kerogens (Vandenbroucke and Largeau, 2007).  
271 Hence, in ancient silicified sediments, bitumens and pyrobitumens can be considered  
272 syngenetic.

273 In contrast to soluble OM, insoluble OM is often considered less prone to late  
274 contamination (Derenne et al., 2008). Involving no chemical treatment or low-manipulation  
275 procedures, numerous studies have investigated the molecular structure of OM – at the scale  
276 of plant fossils – to assess the effects of fossilization or to depict plant evolution and affinities  
277 (Ewbank et al., 1996; Edwards et al., 1997; Abbott et al., 1998; Czaja et al., 2009; Quijada et  
278 al., 2016). Because a large proportion of the hydrocarbon content of these samples is  
279 preserved, these investigations minimized secondary hydrothermal and endolithic  
280 contaminations – which can also yield insoluble OM – by studying insoluble OM directly  
281 from plant fossils. To avoid contamination and to optimize the study of syngenetic  
282 hydrocarbons that can be degraded in the course of acid maceration, the development of in  
283 situ molecular micrometre-scale analyses techniques (e.g., time-of-flight secondary ion mass  
284 spectrometry and laser micropyrolysis) are of interest. Such techniques have the potential to  
285 directly probe the molecular structure of organic fossils independent of the soluble/insoluble  
286 operational definition. In addition to insightfully assessing chemical heterogeneity within  
287 macrofossils (Boyce et al., 2002; Abbott et al., 2018), in situ molecular micrometre-scale  
288 analyses also offers the possibility to study the molecular composition of micrometric to sub-  
289 micrometric diffuse particulate OM (Stout, 1993; Greenwood et al., 2001; Yoshioka and

290 Takeda, 2004; Silva et al., 2016). This includes remnants of microorganisms, such as organic-  
291 walled microfossils, which are essential organic components of the early geological record.  
292 In the specific context of silicified rocks – where silicification prevented migration of  
293 bitumens and favoured formation of pyrobitumen in the close vicinity of the residual kerogen  
294 – the use of in situ molecular micrometre-scale analyses offers promise in the investigation of  
295 the molecular composition of early life found within Archean silicified sediments. Indeed, the  
296 emergence of micrometre-scale *in situ* analyses have provided compelling evidence  
297 supporting an unexpected and wide chemical heterogeneity among Archean putative organic-  
298 walled microfossils (Delarue et al., 2017, 2020; Hickman-Lewis et al., 2020). Overlooked by  
299 bulk extraction approaches, this chemical heterogeneity defines a chemically-well preserved  
300 end product among the earliest traces of life. Therefore, the precise study of such geological  
301 archives requires further development of in situ molecular micrometre-scale analyses. Such  
302 techniques may pave the way to document biological and abiotic processes and affinities of  
303 the earliest putative remnants of life by: (i) focussing on chemically well preserved  
304 specimens, (ii) without the use of chemical extraction and maceration techniques, which can  
305 degrade aliphatic moieties preserved in sediments subjected to early and prompt silicification.

306

#### 307 **4. Conclusions**

308 In this study, we investigated the impact of standard acid maceration on the chemical  
309 structure of silicified OM from the Lower Devonian Rhynie chert. Using Rock-Eval pyrolysis  
310 and Raman spectroscopy, we assessed the chemical structure of OM isolated by two different  
311 physical and chemical manipulation procedures. Results from Rock-Eval pyrolysis and  
312 Raman spectroscopy converged to demonstrate that the standard procedure of OM isolation  
313 led to a significant and substantial degradation of hydrocarbons. These chemical alterations  
314 indicate that a significant amount of molecular content is lost as a result of standard acid

315 maceration procedures, thus preventing a thorough examination of the molecular content of  
316 organic remnants in thermally altered silicified sediments.

317

### 318 **Acknowledgements**

319 Authors are grateful to Andrew Ross (Department of Natural Sciences, National  
320 Museum of Scotland, Edinburgh) for providing the Rhynie chert sample. We also thank F.  
321 Savignac (ISTeP) for Rock-Eval pyrolysis and O. Belhadj (CRCC) for Raman spectroscopy.  
322 We also acknowledge K. Liitschwager for proofreading the English writing of this  
323 manuscript. J.K. Volkman, G.D. Abbott, anonymous reviewers and C.K. Boyce are  
324 acknowledged for their constructive comments on the former versions of the manuscript.

325

### 326 **References**

- 327 Abbott, G.D., Ewbank, G., Edwards, D., Wang, G.-Y., 1998. Molecular characterization of  
328 some enigmatic Lower Devonian fossils. *Geochimica et Cosmochimica Acta* 62,  
329 1407–1418.
- 330 Abbott, G.D., Fletcher, I.W., Tardio, S., Hack, E., 2018. Exploring the geochemical  
331 distribution of organic carbon in early land plants: a novel approach. *Philosophical  
332 Transactions of the Royal Society B: Biological Sciences* 373, 20160499.
- 333 Aboulkas, A., El Harfi, K., 2009. Effects of acid treatments on Moroccan Tarfaya oil shale  
334 and pyrolysis of oil shale and their kerogen. *Journal of Fuel Chemistry and  
335 Technology* 37, 659–667.
- 336 Alleon, J., Bernard, S., Le Guillou, C., Daval, D., Skouri-Panet, F., Pont, S., Delbes, L.,  
337 Robert, F., 2016. Early entombment within silica minimizes the molecular degradation  
338 of microorganisms during advanced diagenesis. *Chemical Geology* 437, 98–108.
- 339 Ammar, M.R., Charon, E., Rouzaud, J.-N., Aleon, J., Guimbretière, G., Simon, P., 2011. On a

340 reliable structural characterization of polished carbons in meteorites by Raman  
341 microspectroscopy. *Spectroscopy Letters* 44, 535–538.

342 Behar, F., Beaumont, V., De B. Pentead, H.L., 2001. Rock-Eval 6 Technology:  
343 Performances and Developments. *Oil & Gas Science and Technology* 56, 111–134.

344 Boyce, C.K., Cody, G.D., Feser, M., Jacobsen, C., Knoll, A.H., Wirick, S., 2002. Organic  
345 chemical differentiation within fossil plant cell walls detected with X-ray  
346 spectromicroscopy. *Geology* 30, 1039-1042.

347 Brocks, J.J., Buick, R., Logan, G.A., Summons, R.E., 2003a. Composition and syngeneity of  
348 molecular fossils from the 2.78 to 2.45 billion-year-old Mount Bruce Supergroup,  
349 Pilbara Craton, Western Australia. *Geochimica et Cosmochimica Acta* 67, 4289–4319.

350 Brocks, J.J., Love, G.D., Snape, C.E., Logan, G.A., Summons, R.E., Buick, R., 2003b.  
351 Release of bound aromatic hydrocarbons from late Archean and Mesoproterozoic  
352 kerogens via hydrolysis. *Geochimica et Cosmochimica Acta* 67, 1521–1530.

353 Czaja, A.D., Kudryavtsev, A.B., Cody, G.D., Schopf, J.W., 2009. Characterization of  
354 permineralized kerogen from an Eocene fossil fern. *Organic Geochemistry* 40, 353–  
355 364.

356 Clementz, D., 1979. Effect of oil and bitumen saturation on source-rock pyrolysis: Geologic  
357 notes. *AAPG Bulletin* 63. doi:10.1306/2F918919-16CE-11D7-8645000102C1865D

358 Delarue, F., Robert, F., Derenne, S., Tartèse, R., Jauvion, C., Bernard, S., Pont, S., Gonzalez-  
359 Cano, A., Duhamel, R., Sugitani, K., 2020. Out of rock: A new look at the  
360 morphological and geochemical preservation of microfossils from the 3.46 Gyr-old  
361 Strelley Pool Formation. *Precambrian Research* 336, 105472.

362 Delarue, F., Robert, F., Sugitani, K., Tartèse, R., Duhamel, R., Derenne, S., 2017.  
363 Investigation of the geochemical preservation of ca. 3.0 Ga permineralized and  
364 encapsulated microfossils by nanoscale secondary ion mass spectrometry.

365           Astrobiology 17, 1192–1202.

366 Delarue, F., Rouzaud, J.-N., Derenne, S., Bourbin, M., Westall, F., Kremer, B., Sugitani, K.,  
367           Deldicque, D., Robert, F., 2016. The Raman-derived carbonization continuum: A tool  
368           to select the best preserved molecular structures in Archean kerogens. *Astrobiology*  
369           16, 407–417.

370 Delvaux, D., Martin, H., Leplat, P., Paulet, J., 1990. Comparative Rock-Eval pyrolysis as an  
371           improved tool for sedimentary organic matter analysis. *Organic Geochemistry* 16,  
372           1221–1229.

373 Derenne, S., Robert, F., Skrzypczak-Bonduelle, A., Gourier, D., Binet, L., Rouzaud, J.-N.,  
374           2008. Molecular evidence for life in the 3.5 billion year old Warrawoona chert. *Earth*  
375           and Planetary Science Letters 272, 476–480.

376 Duda, J.-P., Thiel, V., Reitner, J., Grazhdankin, D., 2016. Opening up a window into  
377           ecosystems with Ediacara-type organisms: preservation of molecular fossils in the  
378           Khatyspyt Lagerstätte (Arctic Siberia). *PalZ* 90, 659–671.

379 Durand, B. (Ed.), 1980. *Kerogen, Insoluble Organic Matter from Sedimentary Rocks*.  
380           Editions Technip 27, Paris, pp. 519.

381 Edwards, D., Ewbank, G., Abbott, G.D., 1997. Flash pyrolysis of the outer cortical tissues in  
382           Lower Devonian *Psilophyton dawsonii*. *Botanical Journal of the Linnean Society* 124,  
383           345–360.

384 Espitalié, J., 1986. Use of Tmax as a maturation index for different types of organic matter:  
385           comparison with vitrinite reflectance. *Collection colloques et séminaires - Institut*  
386           français du pétrole pp. 475–496.

387 Espitalié, J., Laporte, J.L., Madec, M., Marquis, F., Leplat, P., Paulet, J., Boutefeu, A., 1977.  
388           Méthode rapide de caractérisation des roches mères, de leur potentiel pétrolier et de  
389           leur degré d'évolution. *Revue de l'Institut Français du Pétrole* 32, 23–42.

390 Overall, N.J., Lumsdon, J., Christopher, D.J., 1991. The effect of laser-induced heating upon  
391 the vibrational Raman spectra of graphites and carbon fibres. *Carbon* 29, 133–137.

392 Ewbank, G., Edwards, D., Abbott, G.D., 1996. Chemical characterization of Lower Devonian  
393 vascular plants. *Organic Geochemistry* 25, 461–473.

394 Ferralis, N., Matys, E.D., Knoll, A.H., Hallmann, C., Summons, R.E., 2016. Rapid, direct and  
395 non-destructive assessment of fossil organic matter via microRaman spectroscopy.  
396 *Carbon* 108, 440–449.

397 Ferrari, A.C., Robertson, J., 2000. Interpretation of Raman spectra of disordered and  
398 amorphous carbon. *Physical Review B* 61, 14095–14107.

399 Greenwood, P.F., George, S.C., Pickel, W., Zhu, Y., Zhong, N., 2001. In situ analytical  
400 pyrolysis of coal macerals and solid bitumens by laser micropyrolysis GC–MS.  
401 *Journal of Analytical and Applied Pyrolysis* 58–59, 237–253.

402 Hickman-Lewis, K., Westall, F., Cavalazzi, B., 2020. Diverse communities of Bacteria and  
403 Archaea flourished in Palaeoarchean (3.5–3.3 Ga) microbial mats. *Palaeontology* 63,  
404 1007–1033.

405 Kebukawa, Y., Alexander, C.M.O., Cody, G.D., 2019. Comparison of FT-IR spectra of bulk  
406 and acid insoluble organic matter in chondritic meteorites: An implication for missing  
407 carbon during demineralization. *Meteoritics & Planetary Science* 54, 1632–1641.

408 Kouketsu, Y., Mizukami, T., Mori, H., Endo, S., Aoya, M., Hara, H., Nakamura, D., Wallis,  
409 S., 2014. A new approach to develop the Raman carbonaceous material  
410 geothermometer for low-grade metamorphism using peak width: Raman CM  
411 geothermometer using FWHM. *Island Arc* 23, 33–50.

412 Larsen, J.W., Pan, C.S., Shawver, S., 1989. Effect of demineralization on the macromolecular  
413 structure of coals. *Energy & Fuels* 3, 557–561.

414 Ledevin, M., Arndt, N., Simionovici, A., Jaillard, E., Ulrich, M., 2014. Silica precipitation

415 triggered by clastic sedimentation in the Archean: New petrographic evidence from  
416 cherts of the Kromberg type section, South Africa. *Precambrian Research* 255, 316–  
417 334.

418 Love, G.D., Stalvies, C., Grosjean, E., Meredith, W., Snape, C.E., 2008. Analysis of  
419 molecular biomarkers covalently bound within Neoproterozoic sedimentary kerogen.  
420 *The Paleontological Society Papers* 14, 67–83.

421 Love, G.D., Zumberge, J.A., Cárdenas, P., Sperling, E.A., Rohrssen, M., Grosjean, E.,  
422 Grotzinger, J.P., Summons, R.E., 2020. Sources of C<sub>30</sub> steroid biomarkers in  
423 Neoproterozoic–Cambrian rocks and oils. *Nature Ecology & Evolution* 4, 34–36.

424 Mark, D.F., Rice, C.M., Fallick, A.E., Trewin, N.H., Lee, M.R., Boyce, A., Lee, J.K.W.,  
425 2011. <sup>40</sup>Ar/<sup>39</sup>Ar dating of hydrothermal activity, biota and gold mineralization in the  
426 Rhynie hot-spring system, Aberdeenshire, Scotland. *Geochimica et Cosmochimica*  
427 *Acta* 75, 555–569.

428 Marshall, C.P., Marshall, A.O., 2014. Raman spectroscopy as a screening tool for ancient life  
429 detection on Mars. *Philosophical Transactions of the Royal Society A: Mathematical,*  
430 *Physical and Engineering Sciences* 372, 20140195.

431 Maslova, O.A., Ammar, M.R., Guimbretière, G., Rouzaud, J.-N., Simon, P., 2012.  
432 Determination of crystallite size in polished graphitized carbon by Raman  
433 spectroscopy. *Physical Review B* 86, 134205.

434 Nguyen, K., Love, G.D., Zumberge, J.A., Kelly, A.E., Owens, J.D., Rohrssen, M.K., Bates,  
435 S.M., Cai, C., Lyons, T.W., 2019. Absence of biomarker evidence for early eukaryotic  
436 life from the Mesoproterozoic Roper Group: Searching across a marine redox gradient  
437 in mid-Proterozoic habitability. *Geobiology* 17, 247–260.

438 Preston, L.J., Genge, M.J., 2010. The Rhynie Chert, Scotland, and the search for life on Mars.  
439 *Astrobiology* 10, 549–560.



440 Quijada, M., Riboulleau, A., Strother, P., Taylor, W., Mezzetti, A., Versteegh, G.J.M., 2016.  
441 Protosalvinia revisited, new evidence for a land plant affinity. Review of Palaeobotany  
442 and Palynology 227, 52–64.

443 Quirico, E., Rouzaud, J.-N., Bonal, L., Montagnac, G., 2005. Maturation grade of coals as  
444 revealed by Raman spectroscopy: Progress and problems. Spectrochimica Acta Part A:  
445 Molecular and Biomolecular Spectroscopy 61, 2368–2377.

446 Rice, C.M., Ashcroft, W.A., Batten, D.J., Boyce, A.J., Caulfield, J.B.D., Fallick, A.E., Hole,  
447 M.J., Jones, E., Pearson, M.J., Rogers, G., Saxton, J.M., Stuart, F.M., Trewin, N.H.,  
448 Turner, G., 1995. A Devonian auriferous hot spring system, Rhynie, Scotland. Journal  
449 of the Geological Society 152, 229–250.

450 Rice, C.M., Trewin, N.H., Anderson, L.I., 2002. Geological setting of the Early Devonian  
451 Rhynie cherts, Aberdeenshire, Scotland: an early terrestrial hot spring system. Journal  
452 of the Geological Society 159, 203–214.

453 Romero-Sarmiento, M.-F., Rouzaud, J.-N., Bernard, S., Deldicque, D., Thomas, M., Littke,  
454 R., 2014. Evolution of Barnett Shale organic carbon structure and nanostructure with  
455 increasing maturation. Organic Geochemistry 71, 7–16.

456 Rouzaud, J.-N., Deldicque, D., Charon, É., Pageot, J., 2015. Carbons at the heart of questions  
457 on energy and environment: A nanostructural approach. Comptes Rendus Geoscience  
458 347, 124–133.

459 Sanei, H., Wood, J.M., Ardakani, O.H., Clarkson, C.R., Jiang, C., 2015. Characterization of  
460 organic matter fractions in an unconventional tight gas siltstone reservoir.  
461 International Journal of Coal Geology 150–151, 296–305.

462 Silva, T.F. da, Mendonça Filho, J.G., da Silva, M.C., de Oliveira, A.D., de Souza, J.T.,  
463 Rondon, N.F., 2016. Botryococcus braunii versus *Gloeocapsomorpha prisca* :  
464 Chemical composition correlation using laser micropyrolysis-gas

465 chromatography/mass spectrometer (LmPy-GCMSMS). *International Journal of Coal*  
466 *Geology* 168, 71–79.

467 Stout, S.A., 1993. Lasers in organic petrology and organic geochemistry, II. In-situ laser  
468 micropyrolysis-GCMS of coal macerals. *International Journal of Coal Geology* 24,  
469 309–331.

470 Summons, R.E., Jahnke, L.L., Simoneit, B.R.T., 1996. Lipid biomarkers for bacterial  
471 ecosystems: studies of cultured organisms, hydrothermal environments and ancient  
472 sediments. In: *Evolution of Hydrothermal Ecosystems on Earth (and Mars?)*. Wiley,  
473 Chichester, pp. 174–194.

474 Summons, R.E., Brassell, S.C., Eglinton, G., Evans, E., Horodyski, R.J., Robinson, N., Ward,  
475 D.M., 1988. Distinctive hydrocarbon biomarkers from fossiliferous sediment of the  
476 Late Proterozoic Walcott Member, Chuar Group, Grand Canyon, Arizona.  
477 *Geochimica et Cosmochimica Acta* 52, 2625–2637.

478 Tekely, P., Nicole, D., Delpuech, J. -j., Totino, E., Muller, J.F., 1987. Chemical structure  
479 changes in coals after low-temperature oxidation and demineralization by acid  
480 treatment as revealed by high resolution solid state <sup>13</sup>C NMR. *Fuel Processing*  
481 *Technology* 15, 225–231.

482 Trewin, N.H., 2003. History of research on the geology and palaeontology of the Rhynie area,  
483 Aberdeenshire, Scotland. *Transactions of the Royal Society of Edinburgh: Earth*  
484 *Sciences* 94, 285–297.

485 Vandenbroucke, M., Largeau, C., 2007. Kerogen origin, evolution and structure. *Organic*  
486 *Geochemistry* 38, 719–833.

487 Wopenka, B., Pasteris, J.D., 1993. Structural characterization of kerogens to granulite-facies  
488 graphite: Applicability of Raman microprobe spectroscopy. *American Mineralogist*  
489 78, 533–557.

490 Yoshioka, H., Takeda, N., 2004. Analysis of organic compounds in coal macerals by infrared  
491 laser micropyrolysis. *Journal of Analytical and Applied Pyrolysis* 71, 137–149.  
492 Zhang, L., Li, Z., Yang, Y., Zhou, Y., Kong, B., Li, J., Si, L., 2016. Effect of acid treatment  
493 on the characteristics and structures of high-sulfur bituminous coal. *Fuel* 184, 418–  
494 429.

495

496

#### 497 **Figure captions**

498

499 **Fig. 1.** (a) Typical Raman spectra measured on OHMM (black line) and OLMM (grey line).

500

501 **Fig. 2.** Emissions of (a) hydrocarbons and (b) CO<sub>2</sub> during Rock-Eval pyrolysis of OHMM

502 (black line) and OLMM (grey line). The red curve indicates the pyrolysis temperature

503 program. The green area in (b) indicates the temperature interval, for which CO<sub>2</sub> emissions

504 are included in the calculation of OI.

505

Table 1: Rock-Eval parameters determined in OHMM and OLMM. \*H/C atomic ratio was estimated using the relationship between H/C atomic ratio and HI ( $H/C = 0.0017 \times HI + 0.453$ ) published by Espitalié et al. (1977).

	<b>S1</b> (mg HC/g)	<b>S2</b> (mg HC/g)	<b>TpkS2</b> (°C)	<b>TOC</b> (%)	<b>HI</b> (mg HC/g TOC)	<b>OI</b> (mg CO <sub>2</sub> /g TOC)	<b>H/C</b> <b>atomic ratio*</b>
OHMM	0.59	11.76	481	25.4	46	59	0.53
OLMM	2.48	18.83	504	27.5	68	15	0.57

**Table 2:** Raman-derived parameters (mean value  $\pm$  S.D.) determined in OHMM and OLMM (n= 15; Wilcoxon rank test).

	$I_{D1}/I_{G+D2}$	$I_{D4}/I_{G+D2}$	$I_{D5}/I_{G+D2}$	$\alpha_{D4}$	$\alpha_{D5}$
OHMM	$0.58 \pm 0.02$	$0.38 \pm 0.02$	$0.43 \pm 0.04$	$359 \pm 35$	$-288 \pm 39$
OLMM	$0.52 \pm 0.02$	$0.36 \pm 0.02$	$0.31 \pm 0.01$	$202 \pm 37$	$-240 \pm 28$
<i>p</i> -value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

