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1 **Chemical preservation of tail feathers from *Anchiornis huxleyi*, a theropod dinosaur from the**
2 **Tiaojishan Formation (Upper Jurassic, China)**

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28 **Abstract:** A panel of geochemical techniques is used here to investigate the taphonomy of fossil
29 feathers preserved in association with the skeleton of the Jurassic theropod *Anchiornis huxleyi*. Extant
30 feathers were analysed in parallel to test whether the soft tissues morphologically preserved in the fossil
31 also exhibit a high degree of chemical preservation. Scanning electron microscopy (SEM) and energy
32 dispersive spectroscopy (EDS) indicate that clays and iron oxide pseudomorphs occur in the surrounding
33 sediment and also reveal the preservation of melanosome-like microbodies in the fossil. Carbon gradient
34 along a depth profile and co-occurrence of carbon and sulphur are shown in the fossil by elastic
35 backscattering (EBS) and particle-induced X-ray emission (PIXE), which are promising techniques for
36 the elemental analysis of fossil soft tissues. The molecular composition of modern and fossil soft tissues
37 was assessed from micro-Attenuated Total Reflectance Fourier Transform Infrared spectroscopy (micro-
38 ATR FTIR), solid-state ^{13}C nuclear magnetic resonance (^{13}C CP-MAS NMR) and pyrolysis- gas
39 chromatography-mass spectrometry in the presence of TMAH (TMAH-Py-GC-MS). Results indicate
40 that the proteinaceous material that comprises the modern feathers is not present in the fossil feathers.
41 The latter and the embedding sediment exhibit a highly aliphatic character. However, substantial
42 differences exist between these samples, revealing that the organic matter of the fossil feathers is, at
43 least partially, derived from original constituents of the feathers. Our results suggest that, despite the
44 morphological preservation of *Anchiornis* feathers, original proteins, i.e. keratin, were probably not
45 preserved in the 160-Ma-old feathers.

46 **Key words:** *Anchiornis*; fossil feathers; taphonomy; soft tissue preservation; dinosaur.

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49 INTRODUCTION

50 Preservation of soft-bodied animals – with non-mineralized tissues – is relatively rare, when considering
51 the whole geological record. Soft parts of organisms are usually lost during the diverse degradation
52 processes occurring during fossilization. Their constitutive labile organic compounds are usually too
53 fragile to be preserved, compared to the ‘hard’ – biomineralized – parts, which are generally better
54 preserved. However, some important fossil-bearing sites yield not only exquisitely preserved skeletons
55 but also remains of soft tissues, such as skin, scales, hair or feathers (e.g. Allison and Briggs 1993; Zhu
56 *et al.* 2005; Pan *et al.* 2013). Feathers, the epidermal appendages that form the external covering of
57 modern birds, have been discovered preserved in close association with fossils of theropod dinosaurs in
58 Konservat-Lagerstätten – localities that are characterized by the unusual quality of the fossils – from the
59 Upper Jurassic and Lower Cretaceous of China (Hu *et al.* 2009; Xu *et al.* 2012; Xu *et al.* 1999; Xu *et al.*
60 2009; Chu *et al.* 2016; Godefroit *et al.* 2013) and Germany (Rauhut *et al.* 2012). During the last twenty
61 years, Liaoning Province, in north-eastern China, has yielded well-preserved vertebrate fossils with soft
62 parts (e.g. Benton *et al.* 2008; Kellner *et al.* 2010; Li *et al.* 2012). The most striking discoveries were
63 exquisitely well-preserved feathered theropod dinosaurs, evidencing their relationship with modern
64 birds. Since the discovery of the Early Cretaceous *Sinosauropteryx prima* in 1996 (Ji and Ji 1996), many
65 other feathered specimens have been found (Hu *et al.* 2009; Li *et al.* 2012; Xu *et al.* 2012; Xu *et al.*
66 2015). In the same way, the discovery of one of the most primitive birds, *Archaeopteryx lithographica*,
67 associated with well-preserved feathers, constitutes a gigantic step in the comprehension of bird – and
68 feather – evolution (Christiansen and Bonde 2004). Interestingly, elongate filaments interpreted as
69 primitive feathers were observed in ornithischian – non-theropod – dinosaurs (Mayr *et al.* 2002; Zheng
70 *et al.* 2009). Recently, both ‘feather-like’ structures and scales were discovered together with remains of

71 the middle Jurassic neornithischian *Kulindadromeus zabaikalicus* collected in volcanoclastic deposits
72 from Siberia (Godefroit *et al.* 2014; Cincotta *et al.* 2019; Godefroit *et al.* 2020). Recently, a small
73 theropod dinosaur, the scansoriopterygid *Ambopteryx longibrachium* (Wang *et al.* 2019) from the Upper
74 Jurassic of China, was described with membranous wings instead of feathered ones. This wing
75 configuration was probably lost during evolution in favour of the feathered wing configuration that
76 occurs in modern birds.

77 In a recent study, Zhao *et al.* (2020) observed the structure of experimentally matured feathers and
78 reported the fusion of barbules in the matured feathers. This result has implications in terms of feather
79 taphonomy and evolution, for the absence of barbules in fossil feathers could be due to their fusion
80 during diagenesis instead of their true absence in the specimen. This is unfortunate that no chemical
81 analyses have been performed to better understand how maturation affects the preservation of
82 biomolecules in feathers.

83 Fossil feathers show a wide range of preservation degrees (e.g. Schweitzer 2011; Xing *et al.* 2016). The
84 study of these diversely preserved structures is crucial for a better understanding of the taphonomic
85 processes leading to their preservation. In most cases, feathers and other types of preserved soft-tissues
86 were deposited in calm, low-energy environments (e.g. Kellner and de Almeida Campos 2002). They are
87 found in diverse environmental settings such as shallow-marine (e.g. Martill and Heimhofer 2007;
88 Barthel 1964; Heimhofer and Martill 2007), lacustrine (e.g. Harms 2002; Sullivan *et al.* 2014; Zhou and
89 Wang 2010), or terrestrial (Manning *et al.* 2013). Different modes of preservation occur for ancient soft
90 tissues: carbonaceous films (e.g. Li *et al.* 2010; Lindgren *et al.* 2015), phosphate (Allison and Briggs
91 1993; Briggs *et al.* 1993), pyrite (Briggs *et al.* 1991; Farrell *et al.* 2013; Leng and Yang 2003), clay

92 minerals (Gabbott *et al.* 2001; Martin *et al.* 2004), aluminosilicates (Butterfield *et al.* 2007), or a
93 combination of these (Wilby *et al.* 1996).

94 Feathers are epidermal appendages mainly composed of keratin (Lucas & Stettenheim 1972), which is
95 present as two secondary structures, alpha-helices and beta-sheets, corresponding to alpha- and beta-
96 keratin, respectively (e.g. Fraser and MacRae 2012). Alpha-keratin plays a hydrophobic role in avoiding
97 water loss, whereas beta-keratin increases skin hardness (Fraser and Parry 1996; Gregg and Rogers
98 1986). According to Lucas and Stettenheim (1972), the amino acid content of keratin in modern bird
99 feathers is rather homogenous in identical parts of a feather (e.g. in rachis of feathers belonging to the
100 same species), although it varies from one species to another. Nonetheless, feather keratin always
101 comprises high amounts of serine, glycine, proline, and lower quantities of valine, leucine, alanine and
102 cysteine (Arai *et al.* 1983, 1986; Gregg & Rogers 1986; Murphy *et al.* 1990; O'Donnell and Inglis 1974;
103 Saravanan and Dhurai 2012a; Staroń *et al.* 2011).

104 The potential of keratin to resist diagenetic processes is still poorly known. Saitta *et al.* (2017)
105 performed decay and maturation experiments of various keratinous structures, whose conclusions
106 suggest that feather keratin would not survive diagenesis. Although the ultrastructure of feather keratin,
107 i.e. fibrils, can be preserved (e.g. Lindgren *et al.* 2015), there is no direct evidence for the preservation of
108 its proteinaceous compounds. Several immunohistological studies have suggested that keratin could be
109 preserved (Schweitzer *et al.* 1999; Moyer *et al.* 2016; Pan *et al.* 2016), although this method remains
110 highly controversial. By contrast, melanin – the natural pigment present in a variety of soft tissues
111 including hair, skin and feathers – is considered to be more resistant to degradation and fossilization
112 processes. Melanin has been unequivocally identified in various types of fossil tissues, such as fish eyes

113 (Lindgren *et al.* 2012), bird feathers (Colleary *et al.* 2015), non-avian dinosaur feathers (Lindgren *et al.*
114 2015), mammal hair (Colleary *et al.* 2015), or frog skin (McNamara *et al.* 2016).

115 Here, we investigate the ultrastructure and chemical composition of fossil feathers of a theropod
116 dinosaur, *Anchiornis huxleyi* (YFGP-T5199), collected from Upper Jurassic deposits of the Tiaojishan
117 Formation (Liaoning Province, China). Previous study of the same specimen focused on the
118 identification of pigment remains (eumelanin), and evidenced the preservation of melanosomes in the
119 feathers (Lindgren *et al.* 2015). We report new and complementary geochemical information about the
120 preservation of macromolecular compounds in the fossil feathers using a range of analytical tools. The
121 surrounding sediment and modern feathers were analysed in parallel to ascribe pristine constituents.

122 Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) were used to
123 identify and characterize the elemental composition of preserved pigment organelles and the
124 sedimentary matrix. X-ray diffraction (XRD) was used to analyse the mineralogical composition of the
125 samples in an attempt to understand the role of sediment mineralogy in the preservation of soft tissues.

126 Ion beam analysis (IBA), is recognised as a promising archaeometric tool (Jeynes and Colaoux 2016) and
127 has recently been successfully applied to human bone analyses (Beck 2014). In this work, Particle-
128 Induced X-ray Emission (PIXE) and Elastic Backscattering Spectrometry (EBS) were used for the first
129 time on fossil soft tissues to get insights into the heavy (PIXE) and light (EBS) in-depth elemental
130 composition of the samples and demonstrate the co-occurrence of sulphur and carbon. This approach is
131 innovative in the study of organic materials. Organic geochemistry techniques, micro-Attenuated Total
132 Reflectance Fourier Transform Infrared Spectroscopy (micro-ATR FTIR), ¹³C-Nuclear Magnetic
133 Resonance (NMR), Pyrolysis Gas Chromatography-Mass Spectrometry in the presence of TMAH

134 (TMAH-Py-GC-MS), were applied to characterize the functional groups and other biomolecular
135 components present in the studied samples. To our knowledge, the detailed chemical characterization by
136 ^{13}C NMR and Py-GC-MS of fossil feathers from a non-avian dinosaur has not been done elsewhere.

137

138

139 MATERIAL AND METHODS

140

141 *Specimen information*

142 The studied specimen, *Anchiornis huxleyi* (YFGP- T5199) (Fig.1), is a basal Avialan (the description of
143 the specimen is available in the Supplementary Information of Lindgren *et al.* 2015: pp. 18-23) that was
144 collected from the Tiaojishan Formation in the Yaolugou locality (Liaoning Province, China), and
145 belongs to the Yizhou Fossil and Geology Park in Liaoning. The Tiaojishan Formation consists of
146 hundreds of meters of alternating sedimentary and volcanic beds (Liu *et al.* 2012; Yang *et al.* 2006;
147 Yuan *et al.* 2005). Absolute dating on a laterally equivalent formation – the Lanqi Formation – indicates
148 an age ranging between 165.0 ± 1.2 Ma and 153.0 ± 2.0 Ma (Chang *et al.* 2009; Zhang *et al.* 2008),
149 which spans the Callovian-Kimmeridgian interval (Middle-Late Jurassic; Gradstein *et al.* 2012). YFGP-
150 T5199 is embedded in thinly laminated carbonate sediments, corresponding to alternation of very thin
151 marl and thicker clay laminae. These sediments were deposited in the context of a lake affected by
152 episodic volcanic eruptions (Nan *et al.* 2012). Recent U-Pb radiochronological analyses on zircons from
153 the Jianchang locality indicate that the Yanliao Biota, that includes *Anchiornis* as well as pterosaurs and

154 eutherian mammals, is Oxfordian in age (Chu *et al.* 2016). The plumage of the specimen studied herein
155 is morphologically preserved as dark brown residues around the skeleton, especially around the tail and
156 the forelimbs, and on the skull.

157 *Sample description*

158 The studied samples consist of fossil feather fragments dissected from the posterior end of the tail (Fig.
159 1, the dark area in the white box, top right) of YFGP- T5199, as well as fragments of the host sediment
160 (Fig. 1, the light area in the white box). To test for possible chemical contamination of the fossil feathers
161 by sediment, the sediment samples were analysed using the same methodology as for the fossil feathers.
162 Two types of sediment samples were studied: (1) ‘host’ sediment directly in contact with the feathers
163 from the tail (light area in white box on Fig. 1); and (2) ‘remote’ sediment located > 100 mm from the
164 fossil on the same slab (yellow box bottom left on Fig. 1).

165 *Sample preparation*

166 Two modern brown wing feathers of *Buteo buteo* (buzzard, Aves: Accipitriformes; RBINS collection
167 number: A4011A01; Supplementary Fig. 1) were analysed for comparative purposes. Different parts of
168 the feathers, rachis and barbs, were analysed with IBA. The modern feathers come from a specimen that
169 died naturally and was stored at -18°C at the Royal Belgian Institute of Natural Sciences prior to
170 analysis.

171 Two types of samples were collected on the fossil specimen: millimetre-sized samples for SEM and
172 EDS, as well as centimetre-sized samples for the other analytical approaches. We took 12 millimetre-
173 sized samples (Fig. 1) from different regions of the body of YFGP-T5199 with a sterile scalpel. The

174 samples were mounted on double-sided carbon tape and sputter-coated with gold (Baltec SCD 050).
175 Centimetre-sized fragments of approximately 5 mm² and 2 mm thick, from fossil feathers (white box in
176 Fig. 1) and sediment (yellow box in Fig. 1) were dissected with a sterile scalpel. Samples were cleaned
177 with distilled water without any additional preparation prior to analysis.

178 Several points were analysed with IBA and micro-ATR FTIR on two centimetre-sized fragments
179 containing both fossil feathers and their 'host' sediment, and one fragment of 'remote' sediment
180 (Supplementary Figs. 1B and 2). Other centimetric samples from the same region were collected for
181 NMR and Py-GC-MS (white and yellow boxes on Fig. 1). These samples were crushed and lipids were
182 extracted in order to (1) eliminate potential contaminants related to sample manipulation; and (2)
183 concentrate macromolecular organic matter which mainly corresponds to proteins in modern feathers.
184 Samples were ground to a fine homogeneous powder in an agate mortar. Lipid extraction involved three
185 successive ultrasonications (ten minutes) in 15 ml of dichloromethane/methanol (2:1, v/v), at room
186 temperature and centrifugation at 3500 rpm (ten minutes). The supernatant was removed and the pellet
187 was dried under nitrogen and stored in the dark at 5 °C prior to analysis.

188 *Analytical methods*

189 Samples were imaged under low vacuum with an environmental QUANTA 200 (FEI) scanning electron
190 microscope (at an acceleration voltage ranging from 20 to 30 kV and working distances of 8 to 15 mm).
191 Subsequent semi-quantitative EDS analyses (single point and mapping) were performed using either an
192 environmental QUANTA 200 (acceleration voltage of 30 kV and working distance of 10 mm) or a field-
193 emission JEOL 7500F (acceleration voltage of 15 kV and a working distance of 8 mm).

194 XRD analyses were carried out on both bulk rock and clay mineral with a Philips diffractometer using
195 Cu K α radiation. A tube voltage of 40 kV and a tube current of 30 mA were used. The goniometer
196 scanned from 3 to 70 degrees 2 θ for the bulk rock and from 3 to 30 degrees 2 θ for clay minerals. The
197 clay minerals (< 2 μ m fraction) were isolated by successive centrifuging after decarbonation of the
198 crushed rock with HCl 1N. The preparation was mounted on glass slides and treated according to the
199 three following protocols: (1) natural (air-dried); (2) ethylene-glycol solvation; and (3) heated at 490 °C
200 for two hours. Clay minerals were identified according to the position of the (001), or (0001), series of
201 basal reflections on the X-ray diffractograms.

202 Elastic Backscattering Spectrometry (EBS) and Particle-Induced X-ray Emission (PIXE) measurements
203 were performed using a 3 MeV proton (1 H) beam from the Tandetron linear accelerator ALTAÏS
204 (University of Namur). PIXE is highly sensitive to Na to U elements whereas EBS signals are enhanced
205 for light elements (e.g. C, N and O) due to strong non-Rutherford cross-sections. These two integrative
206 methods, together, can identify almost all elements of the periodic table. The beam spot size was
207 reduced to 0.5 mm in diameter to minimize topographic effects. Backscattered particles were detected
208 using two detectors mounted at scattering angles of 170° and 165°, whereas the emitted X-rays were
209 collected with an Ultra-LEGe (Ultra Low Energy Germanium) detector mounted at 135°. Angles are
210 given relative to the incident beam direction. A selective filter (6 μ m of Al) was mounted in front of the
211 Ultra-LEGe detector to lower the strong Si signal and therefore enhance the rather weak S signal
212 observed in the fossil feathers. The modern brown feathers were analysed at two different locations
213 (barb and rachis; Supplementary Figs. 1 and 2). Two locations were analysed in the fossil feathers. The
214 'host' sediment was analyzed at three different locations (at 1.7, 3.2, and 4.8 mm away from the fossil)
215 and the 'remote' sediment at one location (Supplementary Fig.2). All the samples were analysed using

216 the same experimental settings. A certified reference material (BCR-126A lead glass from NIST) was
217 analysed to (1) calibrate the detectors (both EBS and PIXE); and (2) estimate the accuracy of the PIXE
218 measurements. The EBS spectra were analysed with DataFurnace software (Jeynes *et al.* 2003) together
219 with the cross-sections generated by SigmaCalc (Gurbich 2016) to derive the depth profiles of the major
220 elements (see Supplementary Fig. 4 for carbon). The integral of C, O and Si depth profiles (integration
221 limits set to 0 – 25 000 TFU¹, or 0 – 3 μm considering a density of 2.65 g/cm³) yields the C, O and Si
222 equivalent thicknesses given in TFU (details on the global uncertainty calculations can be found in
223 Supplementary Table 1). The PIXE spectra were manipulated with GUPIX software (Campbell *et al.*
224 2010). The matrix composition to be used in GUPIX was determined by integrating the depth profiles of
225 the main components observed by EBS (i.e. C, O and Si) on a given interval (0 – 100 000 TFU).

226

227 Micro-Attenuated Total Reflectance Fourier Transform Infrared spectroscopy (micro-ATR FTIR) was
228 performed on modern and fossil feathers using a Bruker Vertex 70 FTIR spectrometer (University of
229 Hasselt, Belgium) equipped with a Hyperion 2000 microscope and MCT detector. The infrared spectra
230 were collected in the mid-IR range, from 4000 to 600 cm^{-1} , and 32 scans were acquired in Attenuated
231 Total Reflectance (ATR) mode (Ge –ATR crystal) with a resolution of 4 cm^{-1} . FTIR spectroscopy was
232 used on a modern buzzard feather (dark regions), one sample of fossil feather from the tail of *A. huxleyi*
233 and the surrounding sediment, to identify the presence or absence of functional groups in their molecular
234 composition.

235 ¹³C Nuclear Magnetic Resonance is a spectroscopic method that documents the chemical environment of
236 carbon in organic compounds. Solid state ¹³C NMR spectra of the lipid-free samples were obtained at

237 125 MHz (Bruker Avance 500 spectrometer) using a 4 mm zirconium rotor, with a cross-polarization
238 (CP) sequence and magic angle spinning (MAS) at 14 kHz. CP-MAS ^{13}C NMR spectra were acquired
239 with contact time of 1 ms and recycle time of 1s (fossil and sediment) or 3s (modern feathers). The use
240 of a single contact time does not allow precise quantification of the identified chemical functional
241 groups. Each spectrum was the result of 6 000 (modern samples) to 400 000 (sediments) scans.

242

243 Curie point Pyrolysis-Gas Chromatography- Mass Spectrometry (Py-GC-MS) gives insight into the
244 molecular composition of organic macromolecular materials through their thermal degradation into
245 molecular building blocks that can be separated by gas chromatography (GC) and further identified by
246 mass spectrometry (MS). Tetramethylammonium hydroxide (TMAH) was used to enhance the thermal
247 breakdown of macromolecules and induce *in situ* methylation of pyrolysis products, which, in turn,
248 enhance their detection and identification in GC-MS. The samples were mixed with an excess of TMAH
249 (25 wt % in methanol) in a 1:1 (wt/wt) ratio before loading in ferromagnetic tubes with Curie
250 temperature of 650 °C. Two mg were used for the modern samples, 6 mg for the fossil feathers, and 16
251 mg for the sediment. Curie point pyrolysis was carried out with a Pilodist Curie flash pyrolyser. Samples
252 were heated at their Curie temperature for 10 s under a He flow of 1 ml/min. The instrument was
253 coupled directly to a GC-MS system. The pyrolysis products were separated using a Trace Thermo gas
254 chromatograph equipped with a Rxi5SilMS column (30 m × 0.25 mm i. d., 0.5 μm film thickness).
255 Helium was used as carrier gas at constant pressure of 15 psi. The injector temperature was 280 °C in
256 spitless mode. The oven temperature was maintained at 50 °C for ten minutes and was progressively
257 increased to 310 °C at 2 °C/min. Coupled to the gas chromatograph was a DSQ Thermo mass

258 spectrometer with a heated interface (310 °C), electron energy of 70 eV and ion source at 220 °C,
259 scanning from m/z 35 to 800 at 2 scans/s. Compounds were assigned on the basis of their mass spectra,
260 comparison with the NIST library mass spectra, published mass spectra (e.g. Gallois *et al.* 2007;
261 Templier *et al.* 2013) and GC retention times. The molecular structure of all compounds present in
262 substantial amount was investigated without any ion selection that could have biased interpretations.

263 *Institutional Abbreviation*

264 YFGP: Yizhou Fossil and Geology Park; RBINS: Royal Belgian Institute of Natural Sciences; NIST:
265 National Institute of Standards and Technology.

266

267 **RESULTS**

268

269 *SEM/EDS and XRD*

270 SEM of the fossil feathers revealed that they are embedded in a sedimentary matrix containing mainly
271 quartz, carbonates, and phyllosilicates. The latter are organized in thin platelets oriented parallel to each
272 other (Figs. 2A-B). A feather sample from the right wing of *Anchiornis* (sample 1 on Fig. 1) showed
273 abundant rounded crystals that are present only beneath the surface. They occur mainly as framboids
274 (Fig. 2C), but also as individual microcrystallites (Fig. 2D) and, in some cases, are associated with
275 voids. Framboids are spheroidal or ovoid, 6 to 9 µm in diameter and contain dozens of euhedral crystals,
276 750 nm in diameter. In contrast, individual cubic crystals are much smaller (about 500 nm³) and contain
277 micro-crystallites (Fig. 2D). EDS analyses indicate that the sediment is composed of Fe, Si, O, Al, C, Ca

278 (and Mn, K, Mg), probably indicating the presence of quartz, calcite, and various phyllosilicates. XRD
279 analyses confirmed the presence of these minerals in the sediment (Fig. 3A). In addition, the XRD
280 spectrum of the < 2 µm phase shows that expansive material, such as illite and interstratified
281 illite/smectite, is present in the sediment (Fig. 3B). Due to their characteristic framboidal shape and
282 elemental composition, the crystal clusters observed beneath the fossil feather surface are attributed to
283 diagenetic iron oxides or hydroxides. Indeed, although the framboidal habit is common for iron
284 sulphides, the lack of sulphur here shows they are rather iron oxide pseudomorphs probably resulting
285 from the *in situ* weathering of pyrite framboids (Blanco *et al.* 2013; Kaye *et al.* 2008; Nordstrom 1982;
286 Wang *et al.* 2012). These structures are associated with thin clay overgrowths (arrow in Figs. 2C, E),
287 indicating that the iron oxides (or the preceding pyrites) precipitated first. Tiny stellate minerals were
288 also observed and identified as probable iron oxides by X-ray spectroscopy (Fig. 2F). The presence of
289 calcium carbonates, feldspars (Fig. 2G) and quartz (Fig. 2H) in the sedimentary matrix was confirmed
290 by X-ray spectroscopy.

291 Elongate microbodies, 650 to 950 µm, and their associated imprints were observed in three samples (6,
292 9, and 12, on Fig. 1) collected from the anterior and posterior parts of the dinosaur tail (Fig. 4).
293 Microbody imprints are abundant, tightly packed together and randomly oriented (Figs. 4A–B). They
294 likely represent traces of melanosomes. Similar microbodies interpreted as melanosomes have
295 previously been observed in feathers from the crest of this specimen (YFGP-T5199; Lindgren *et al.*
296 2015). Here, isolated elongate structures were observed (Fig. 4C). These fossil organelles are preserved
297 within the thin clay-rich sediment.

298

299 *Ion Beam Analysis (IBA): EBS and PIXE*

300 The great virtue of EBS is to be capable of yielding the elemental depth profiles non-destructively from
301 the outermost microns of the sample with good sensitivity and depth resolution (Jeynes and Colaux
302 2016). A typical EBS spectrum obtained from the fossil feathers is shown in Supplementary Figure 3
303 together with its best fit. The experimental spectrum was inverted to recover the elemental depth profiles
304 (examples are shown for carbon in Supplementary Fig. 4). Integration of these elemental depth profiles
305 allows derivation of the concentration of each element at a given depth. Figure 5A clearly shows that the
306 carbon enrichment in the near surface region (ca. 60%) decreases at increasing distance from the fossil
307 feathers, reaching a minimum in the ‘remote’ sediment (ca. 14%). Sample concentrations of oxygen and
308 silicon (although less obvious) follow an opposite trend. The nitrogen content in the modern buzzard
309 feathers is ca. 20-26 %. The very low content of nitrogen in the fossil (ca. 5%) and even less (under the
310 limit of detection) in the remote sediment (Supplementary Fig.3) precluded its depth profiling.

311 In contrast, EBS analysis of modern buzzard feathers shows homogeneous concentrations with depth
312 (Supplementary Fig. 5). Carbon content in the buzzard feathers is about 60 at. %, while nitrogen and
313 oxygen are both around 20 at. % for the rachis and around 25 and 15 at. % respectively for the barbs.
314 Typical PIXE spectra acquired from the fossil feathers, ‘host’ sediment and ‘remote’ sediment are
315 shown in Figure 5B. The samples differ in the amounts of several elements present (Supplementary
316 Tables 1–2). Of particular interest is the sulphur content: there are elevated concentrations of sulphur in
317 the fossil feathers (average $1,842 \pm 208.5$ ppm), less in the ‘host’ sediment ($1,162 \pm 143$ ppm), and much
318 lower concentrations in the ‘remote’ sediment (98 ± 35 ppm). The concentration of S in the fossil
319 feathers is roughly twenty times lower (ca. 0.2 wt. %) than in the modern bird feathers (ca. 3.7 – 4.3 wt.

320 %). The co-occurrence between sulphur and carbon is also highlighted (Table 1). Both concentrations
321 decrease with depth within the fossil feathers whereas the reverse situation is observed for Si and O.

322

323 ^{13}C NMR

324 The ^{13}C NMR spectrum of buzzard feathers (Fig. 6A) shows a complex signal in the aliphatic region,
325 with well-resolved peaks between 10 and 65 ppm and a narrow peak at 173 ppm, due to carboxyl
326 carbons, i.e. carboxylic groups and esters and amides. Two additional, less intense, signals can be seen
327 at 129 ppm and 158 ppm in the unsaturated/aromatic carbon region. In comparison to the spectrum of
328 modern feathers, the ^{13}C NMR spectra of the fossil feathers and their surrounding sediment show much
329 simpler patterns (Figs. 6B–C). The spectra are similar to each other and both are dominated by a broad
330 peak in the aliphatic region, maximizing at 30 ppm and thus indicative of long alkyl chains. Two
331 additional broad signals contribute to the spectra. The first one occurs as a broad shoulder between 68
332 and 80 ppm, in the O-alkyl C and N-alkyl C range, and the second one is a broad peak at 129 ppm,
333 attributed to aromatic carbons.

334

335 *Micro-ATR FTIR spectroscopy*

336 The micro-ATR FTIR spectra of the theropod feathers, the embedding sediment and the modern buzzard
337 feather (dark regions) are shown in figure 7.

338 The spectrum of the dark region of a modern buzzard feather shows characteristic bands of secondary
339 amides – as in proteins and polypeptides – at 1628 cm^{-1} (C=O stretch of Amide I), 1531 cm^{-1} (C-N
340 stretch, Amide II) and 1239 cm^{-1} (N-H in plane bending coupled with C-N stretch, Amide III). Broad

341 bands around 3274 and 3125 cm^{-1} can be attributed to the N-H stretching of secondary amides. The
342 bands at 2961, 2922 and 2852 cm^{-1} are assigned to the C-H stretching of methylene and methyl groups.
343 These two spectra are very similar and no significant differences could be found between IR response of
344 the dark and white regions of the same feather.

345 The spectrum of the fossil feathers has a different pattern but some similarities with the modern buzzard
346 feather appear. The distinct bands at 2920 and 2851 cm^{-1} can also be attributed to C-H stretching of
347 methylene and methyl groups. These associated bands are also present in the IR spectrum of the
348 surrounding matrix but with different relative intensities. A broad region around 3300 cm^{-1} is present,
349 although much less marked, and is indicative for O-H stretching as found in carboxylic groups and
350 alcohols. In the spectra of the fossil feathers, a broad band at about 1560 cm^{-1} can be attributed to
351 carboxylate. This band is not present in IR spectrum of the sediment. Another broad band around 1412
352 cm^{-1} is found in the spectrum of the fossil feathers and might be related to the presence of CaCO_3 in
353 overlap with C-H bending vibrations at 1460 and 1380 cm^{-1} (Andersen and Brečević 1991; Kroner et al.
354 2010; Kiros et al. 2013) . This is also confirmed by the presence of weak bands at 873 and 718 cm^{-1} . The
355 IR spectrum of the sediment shows a similar, but less defined, absorption band between 1415 and 1463
356 cm^{-1} . The IR spectrum of the fossil feathers and its surrounding matrix both show a narrow band at 1260
357 cm^{-1} that could be attributed to the Si- CH_3 vibrations. Both spectra show an intense broad band at 1013
358 cm^{-1} together with the weaker bands at 873 and 797 cm^{-1} related to the sedimentary matrix (clay
359 minerals, quartz, silicates, Si-O stretching). The IR spectrum of the sedimentary matrix shows an
360 additional band at 720 cm^{-1} that is also present, although very weak, in the fossil, indicating long-chain
361 alkyl groups (CH_2 rocking vibrations). All the peaks mentioned above are absent in the IR spectra of the
362 modern feather.

363

364 *TMAH Py-GC-MS*

365 Pyrochromatograms were obtained for the three following samples: the modern and fossil feathers, and
366 the ‘host’ sediment. In agreement with previous studies on bird feathers (Brebou and Spiridon 2011;
367 Saitta *et al.* 2017), the pyrochromatogram of the modern feathers is dominated by cyclic molecules
368 containing nitrogen, along with methylbenzene **1**, methylbutane nitriles **2, 3** and cyclohexanedione **11**
369 derivatives (Fig. 8A). Detailed interpretation of mass spectral fragmentation patterns allowed
370 identification of the major pyrolysis products (Table 2) and further assignment to possible source.
371 Molecular structures are given in Appendix, with methyl groups added by TMAH indicated in bold.
372 Products **6, 8, 10** result from direct methylation of alanine, valine and proline, thus pointing to a
373 proteinaceous origin for the feathers. This is further supported by the occurrence in substantial amounts
374 of alkylnitriles **2, 3** resulting from dehydration of amides involving isoleucine and leucine, respectively,
375 and of methoxybenzenes **7, 9** released through homolysis of the side chain of tyrosine (Ratcliff *et al.*,
376 1974). Methylbenzene **1**, pyrrole **4** and ethylbenzene **5** are rather ubiquitous pyrolysis products in
377 sedimentary organic matter. However, they can also be released upon pyrolysis of phenylalanine and
378 serine (Gallois *et al.* 2007). Mass spectral fragmentation pattern (base peak at m/z 82) suggests an origin
379 from the side chain of histidine for compound **16**. Similarly, compound **17** probably corresponds to a
380 valine derivative as its mass spectrum is characterized by the loss of 42 amu (i.e. valine side chain).
381 Dimethylcyclohexanedione **11** was reported as pyrolysis product of glycine (Moldoveanu 2009).
382 Glycine is also present as its diketopiperazine **15** resulting from combined dehydration and cyclisation
383 (Simmonds *et al.* 1972). The same mechanism involving two different amino acids (isoleucine-glycine)

384 leads to another diketopiperazine **18** (Hendricker and Voorhees 1996). The formation of more complex
385 diketopiperazines was proposed by Templier *et al.* (2013) from tripeptide units. Similar mechanism can
386 be invoked for the formation of compound **19** from valine, as well as compounds **20** and **21** from serine
387 and leucine (Table 2; Supplementary Fig. 6). Imidazolidinedione **12** probably results from the internal
388 cyclisation of tripeptide comprising an alanine unit as reported by Templier *et al.* (2013; Supplementary
389 Fig. 6). The formation of imidazolidinone **13** can be related to the decomposition of bicyclic amidine
390 derived from valine as suggested by Basiuk and Navarro-González (1997; Supplementary Fig. 6).
391 Another decomposition pathway of bicyclic amidine is probably responsible for the formation of
392 imidazolidinone **14** from valine and possibly glycine (Templier *et al.* 2013). As far as we know, this is
393 the first identification of such complex molecules (diketopiperazines from tripeptide and
394 imidazolidinone from bicyclic amidine) in the pyrolysate of a natural sample.

395 By comparison, pyrochromatograms of the fossil feathers and their ‘host’ sediment are simpler. They are
396 dominated by *n*-alkane/*n*-alkene doublets (Figs. 8B–C), resulting from the homolytic cleavage of long
397 alkyl chains. In the fossil feathers, these doublets comprise from 8 to 30 carbon atoms, and exhibit a
398 smooth distribution except an intense C₁₈ doublet. An additional series of fatty acid methyl esters with
399 alkyl chain ranging from C₈ to C₃₀ and maximizing at C₁₆ is also identified (Table 2). It results from the
400 release upon pyrolysis of a series of fatty acids that are methylated thanks to TMAH. In addition to these
401 series, a methoxybenzene substituted by two methyl groups or an ethyl group **22** is detected in minor
402 amounts, at the beginning of the pyrochromatogram. A trimethylbenzene and a methylated derivative of
403 methoxyaniline **23** also contribute to this part of the pyrochromatogram. However, the most prominent
404 pyrolysis product **24** corresponds to the C₁₈ alcohol methylated through TMAH pyrolysis.

405 The pyrochromatogram of the “host” sediment shares several similarities with that of the fossil feathers.
406 Indeed, it is dominated by series of alkane/alkene doublets and fatty acid methyl esters. Although the
407 distribution of the fatty acid methyl esters is similar in both samples, that of the doublets differs. Indeed,
408 whereas their range (C₈ – C₃₀) is similar, the maximum of the series appears at C₁₅ in the sediment,
409 instead of a marked predominance of the C₁₈ in the fossil (Table 2). Moreover, when comparing the
410 minor compounds eluting at the beginning of the pyrochromatogram, compounds **22** and **23** are common
411 in both samples, whereas a higher number of alkylbenzene homologues occurs in the sediment. Finally,
412 the contribution of octadecanol **24** is much lower in the sediment pyrolysate.

413

414 **DISCUSSION**

415 *Ultrastructure*

416 Microbodies and elongate moulds are observed in feather samples collected at three different locations
417 on *Anchiornis* tail. The elongate shape, parallel orientation and location of the microbodies within the
418 feathers strongly suggest that they correspond to eumelanosomes. These pigment organelles are
419 associated to brown, grey and black hues in modern bird feathers. The preservation of melanosomes, and
420 especially eumelanosomes, in dinosaur feathers is not uncommon. Such microscopic melanin-bearing
421 structures have been described in other theropod dinosaurs, basal birds and isolated feathers (Li *et al.*
422 2010, 2012; Zhang *et al.* 2010; Carney *et al.* 2012; Colleary *et al.* 2015; Pan *et al.* 2016; Hu *et al.* 2018).
423 The chemical composition of these microbodies has been assessed in a previous study, confirming a
424 melanosome origin (Lindgren *et al.* 2015).

425 Recent taphonomic experiments in abiotic conditions suggest that the preservation of mouldic
426 melanosomes requires interaction with an oxidant prior to maturation, and that the preservation of
427 melanosomes is probably less frequent than the preservation of keratinous structures in fossil feathers
428 (Slater *et al.* 2020). It is interesting to see that *Anchiornis* feathers contain both melanosomes and
429 moulds, given their – anoxic – depositional setting. The abiotic nature of the former experiments does
430 however not reflect the depositional and fossilization conditions of *Anchiornis*.

431

432 *Depth profiling, light and heavy element composition*

433 The carbon and nitrogen concentrations determined by EBS led to N/C ratios of 0.33 to 0.42 in modern
434 buzzard feathers, and of 0.08 for the fossil, suggesting a marked relative decrease in nitrogen. The
435 carbon concentration gradient observed by EBS in the fossil feathers of *A. huxleyi* strongly suggests that
436 they are preserved as carbonaceous layers located at the uppermost part of the sample (i.e. 0 – 3 μm
437 depth, given a rock density of 2.65 g/cm^3) and suggests that fossil organic matter could have
438 impregnated the sediment only in a nearby area.

439 The PIXE spectra show elevated concentrations of sulphur in the fossil feathers and, to a lesser extent, in
440 the ‘host’ sediment, together with very low concentrations in the ‘remote’ sediment. This suggests that S
441 is associated with the soft tissues. The fossil feathers are therefore preserved as a S-rich carbonaceous
442 film. Substantial quantities of sulphur are present in the modern buzzard feathers ($43,070 \pm 4,236$ ppm
443 in the brown barbs and $37,142 \pm 3,652$ ppm in the rachis, Table 1). This is not surprising due to the
444 presence of sulphur-containing biomacromolecules, such as the pigment phaeomelanin and cysteine- or
445 methionine-containing proteins (i.e. keratins) in bird feathers (Bortolotti 2010; Cesarini 1996; Harrap

446 and Woods 1964; Murphy *et al.* 1990; Riley 1997; Saravanan and Dhurai 2012b). Important studies on
447 the chemical composition of feathers have shown that S is a major element of bird feathers (Harrap and
448 Wood, 1964, 1967; King and Murphy 1987; Murphy *et al.* 1990; Edwards *et al.* 2016). Some authors
449 could even discriminate between organic S originating from keratin and phaeomelanin based on its
450 speciation (Edwards *et al.* 2016). Previous *in situ* chemical analysis (TOF-SIMS) of the melanosomes
451 from the present fossil revealed their enrichment in sulphur with respect to the surrounding sediment,
452 but it could not determine whether it reflects the occurrence of phaeomelanin or diagenetic incorporation
453 of sulphur in eumelanin (Lindgren *et al.* 2015). In a Cretaceous early bird, divalent elements (Cu, Ca,
454 Zn) were suggested to form chelates with melanin (Wogelius *et al.* 2011). Such a complexation may
455 have played a role in sulphur preservation in fossil soft tissues. Alternatively, the presence of sulphur in
456 the fossil feathers can be attributed to natural sulphurization of the organic matter, i.e. the abiogenic
457 intra-molecular incorporation of sulphur from the depositional environment during early diagenesis. The
458 incorporation of sulphur into organic matter was interpreted as a way to enhance the preservation
459 potential of certain labile substances through cross-linking (Sinninghe-Damsté and De Leeuw 1990;
460 Sinninghe-Damsté *et al.* 1989; Sinninghe-Damsté *et al.* 1988; McNamara *et al.* 2016). Indeed, organic
461 matter has the ability to form complexes with inorganic elements, including sulphur, which was traced
462 in fossil soft tissues (e.g. Wogelius *et al.* 2011).

463 *Functional groups in the organic matter*

464 On the whole, the ¹³C NMR and IR spectra of buzzard feathers (Fig. 6A and 7A) are comparable to that
465 of several keratinous materials, such as feather keratin (Barone *et al.* 2005; Kricheldorf and Müller
466 1984; Wang and Cao 2012, Sharma *et al.* 2018), wool keratin (Yoshimizu and Ando 1990;

467 Wojciechowska *et al.* 2004) or gecko setae keratin (Jain *et al.* 2015). Indeed, the peak at 173 ppm should
468 mainly correspond to the signal of secondary amide (O=C-NH) groups involved in the peptidic bonds.
469 This is confirmed by the presence of characteristic bands of secondary amides in the IR spectrum of the
470 brown feathers (Fig. 7A). This includes a broad band around 3277 cm⁻¹ attributed to the N-H stretching
471 band of amides, a narrow band at 1628 cm⁻¹ related to the C=O stretch of Amide I, a band at 1518 cm⁻¹
472 attributed to Amide II and a band at 1237 cm⁻¹ related to Amide III (e.g. Bendit 1966; Yu *et al.* 2004;
473 Wang and Cao 2012; Giraldo *et al.* 2013; Tesfaye *et al.* 2017).

474 Carbon atoms bearing both COOH and NH₂ groups (termed C_α) in the amino acids (except glycine)
475 resonate between 50 and 60 ppm in ¹³C NMR. They account for the peaks at 52.9 and 60.2 ppm in the
476 broad aliphatic signal, whereas the signal at 42.6 ppm is assigned to the C_α of glycine. The other peaks
477 are mainly associated with the amino acid side chains, that at 30.8 ppm being assigned to C_β along with
478 C in long alkyl chains, and those at 19.8 and 25.7 ppm to C_γ and C_δ. The three bands located in the
479 2961-2850 cm⁻¹ range in the IR spectra confirms the presence of aliphatic moieties in the modern
480 feathers, although their precise assignment to dedicated compounds is uncertain. In the ¹³C NMR
481 spectrum, the 129 ppm peak is typical for aromatic carbons, including those from phenylalanine and
482 tyrosine (Jain *et al.* 2015; Yoshimizu and Ando 1990). Finally, the peak at 158 ppm can be ascribed to
483 the O-alkyl C of tyrosine and/or the C of the guanidino group (N-C=N) of arginine (Jain *et al.* 2015;
484 Yoshimizu and Ando 1990). This spectrum is in agreement with previous reports indicating that keratin
485 is a major constituent of feathers (Lucas and Stettenheim 1972). The ¹³C NMR and IR spectra of the
486 modern feathers also shares some similarities with various types of melanins (Adhyaru *et al.* 2003; Duff
487 *et al.* 1988; Centeno and Shamir 2008; Ito and Nicol 1974).

488 The much simpler ^{13}C NMR spectra of the fossil feathers and their surrounding sediment are dominated
489 by long alkyl chains with a low contribution of aromatic carbons. This is also consistent with the IR
490 spectra (Fig. 7B), mainly showing contributions of aliphatics and silicate and carbonate minerals. When
491 compared to the ^{13}C spectrum of the modern feathers, the aliphatic signal in the fossil is poorly resolved;
492 the aromatic peak is broad and no resonance signal could be detected in the carboxylic region. These
493 features suggest that the proteinaceous contribution identified in the buzzard feathers is no longer
494 present in the fossil sample. The comparison between the FTIR spectrum of the modern feather and that
495 of the fossil feathers (Fig. 7A) shows that the characteristic bands of amides are absent in the IR spectrum
496 of the fossil feathers. However, a more precise comparison can be achieved at the molecular level thanks
497 to pyrolysis in the presence of TMAH coupled with GC-MS.

498 *Molecular building blocks of organic matter*

499 TMAH Py-GC-MS analysis of modern feathers thus highlights the presence of glycine, serine, leucine,
500 alanine, valine and proline moieties in buzzard feather keratin, in agreement with previous studies on
501 feather keratin (Fig. 8A; Arai *et al.* 1983, 1986; Murphy *et al.* 1990; O'Donnell and Inglis 1974;
502 Saravanan and Dhurai 2012b; Staroń *et al.* 2011). Additionally, pyrolysis products derived from
503 isoleucine, phenylalanine and tyrosine occurred in substantial amounts although they are often
504 considered as minor constituents of feather keratin. However, homolysis of the side chain of
505 phenylalanine and tyrosine favours high yields in TMAH pyrolysis (Gallois *et al.* 2007). Despite its
506 acknowledged high abundance in feather keratin, cysteine is absent in the pyrochromatogram of buzzard
507 feathers, probably because it mainly releases H_2S upon pyrolysis (Moldoveanu 2009) not detected in the
508 presently used analytical conditions. Alternatively, some of the identified products (methylbenzene **1**,

509 methyl, methoxybenzene **9**) as well as glycine derivatives may originate from melanin, although they are
510 poorly diagnostic compounds (Stępień *et al.* 2009). However, the melanin signal was reported to be
511 overwhelmed by protein-derived products upon pyrolysis of bulk feathers (Barden *et al.* 2011).

512 The pyrochromatograms of the fossil feathers and their ‘host’ sediment are comparable as they are both
513 dominated by series of alkane/alkene doublets and fatty acid methyl esters as well as minor compounds
514 eluting at the beginning of the pyrochromatogram. The latter include a methoxybenzene substituted by
515 two methyl groups or an ethyl group **22** which originates from lignin or polysaccharides such as
516 cellulose, depending on its substitution pattern (Choi *et al.* 2013; Seitz and Ram 2000). Despite these
517 similarities, differences are observed, including the much weaker abundance of octadecanol **24** in the
518 sediment pyrolysate. These differences clearly show that even though some imprint from the sediment
519 may have contributed to the fossil feather pyrolysate, at least some features are typical for the fossil
520 feathers. They notably include the C₁₈ doublet and octadecanol **24**. The predominance of the
521 alkane/alkene doublets in the pyrolysate is in agreement with the strong aliphatic signal observed in
522 NMR and FTIR (bands at 2690, 2920 and 2851 cm⁻¹) (Figs. 6–7). A similar highly aliphatic character
523 has been reported in Eocene bird feathers (O’Reilly *et al.* 2017) but also in soft tissues from other fossil
524 organisms, such as in cuticles from Carboniferous arthropods (Baas *et al.* 1995; Stankiewicz *et al.*
525 1998), skin from a Cretaceous mummified hadrosaur (Manning *et al.* 2009), and Cretaceous fish scales
526 (Gupta *et al.* 2008). C₁ to C₃ alkylbenzenes were also identified in pyrolysates of Oligocene weevil and
527 tadpole, and associated matrix (Barden *et al.* 2015; Gupta *et al.* 2007).

528 The aliphatic series dominating the pyrolysate of the fossil feathers reflect either selective preservation
529 of macromolecular aliphatic matter pre-existing in the extant organism (Tegelaar *et al.* 1989) or *in situ*

530 polymerization of aliphatic lipids (Gupta *et al.* 2007; Stankiewicz *et al.* 2000). No such aliphatic series
531 could be detected in the pyrolysate of modern feathers, likely precluding the first hypothesis. In contrast,
532 several aliphatic series were identified in the lipid extract of the modern feathers (*n*-alkanes, *n*-acids, *n*-
533 alcohols; data not shown). Indeed, modern bird feathers are coated by lipids as protection against
534 adverse environmental factors. Such lipids, secreted by the uropygial gland, were recently shown to be
535 preserved through geopolymerisation in an Eocene bird (O'Reilly *et al.* 2017). A similar
536 geopolymerisation can be put forward to account for the occurrence of aliphatic moieties of the present
537 fossil feathers. Endogenous lipids may be transformed into more stable geopolymers, composed of
538 alkane/alkene doublets, and can therefore be 'preserved' and traced in vertebrate fossils (O'Reilly *et al.*
539 2018).

540 The absence of signals typical for proteinaceous material in the NMR and IR spectra and
541 pyrochromatogram of the fossil feathers is noteworthy. It is further suggested by the weak N/C ratio in
542 the fossil when compared with modern feathers. The lack of proteinaceous components consistent with
543 keratin was previously suggested on the same fossil based on TOF-SIMS and IR analyses (Lindgren *et al.*
544 *al.*, 2015). In agreement with the commonly accepted lability of proteins, this feature suggests their
545 extensive degradation in our specimen upon diagenesis. Recent taphonomy experiments on extant
546 feathers demonstrated substantial degradation of keratin upon microbial and thermal decay (Saitta *et al.*
547 2017). Moreover, it must be noted that diagenetic degradation of proteinaceous moieties was previously
548 put forward for Eocene birds (Saitta *et al.* 2017; O'Reilly *et al.* 2017) and Palaeozoic annelid fossils
549 (Dutta *et al.* 2010). However, even if no proteinaceous compounds were detected in *Anchiornis* feathers,
550 one cannot exclude the possibility of finding similar biomolecules preserved in other fossils in the
551 future.

552 Although PIXE analyses showed that carbon and sulphur are closely associated in the fossil feathers, no
553 organosulphur compound could be detected in the pyrolysate. Altogether, the lack of organosulphur
554 compounds (such as thiophenes) in the fossil feather pyrolysates and the lack of C=S/C-S species in
555 their IR spectrum strongly suggest a lack of organic-S species in this sample. In contrast, sulphur
556 incorporation was evidenced through FTIR and TMAH-Py-GC-MS in melanosomes of Miocene frogs,
557 thus demonstrating involvement of natural sulphurization in the preservation of the fossil organic matter
558 (McNamara *et al.*, 2016). The lack of organosulphur compounds in *Anchiornis* pyrolysate should thus
559 reflect diagenetic conditions that prevented such natural sulphurization of organic matter. In the
560 sedimentary environment, the sulphurization of organic matter to form organosulphur compounds
561 requires the presence of reactive organic matter and inorganic sulphides (i.e. anoxic conditions), with
562 sufficient, but not excessive, reactive iron. If reactive iron exceeds a certain quantity, iron sulphides
563 (pyrite) would precipitate instead (Werne *et al.* 2000; Canfield 1989). Here, our results suggest that the
564 concentration of sulphur and iron in the environment was high enough to form iron sulphides (i.e. pyrite
565 framboids and microcrystallites). The occurrence of iron oxides or hydroxides as framboid crystals
566 (SEM and EDX characterization) suggests that sulphur may have been preferentially used for the
567 formation of iron sulphides (such as pyrite) during early diagenesis (Sinninghe-Damsté and De Leeuw
568 1990). During later diagenesis, pyrite framboids were probably *in situ* weathered into the iron oxides and
569 hydroxides observed beneath the carbonaceous surface of feathers, thus releasing sulphur that may have
570 been further associated with organic compounds, e.g. through the formation of chelate with melanin
571 (Wogelius *et al.* 2011). Such associations may have favoured/enhanced organic matter preservation and
572 are consistent with the interrelation between sulphur and carbon highlighted by PIXE analyses.

573 Recently, a mechanism of nitrogen preservation based on lipoxidation and glycoxidation of protein was
574 proposed in biomineralized tissues of diverse Mesozoic and Cenozoic vertebrates, fossilized in oxidative
575 settings (Wiemann *et al.* 2018). Such a process, which can be catalysed by transition metal such as iron,
576 may have led to the preservation of the small amount of nitrogen in YFGP-T5199. However, the present
577 study deals with soft tissues and not biomineralized one. Additionally, so far this preservation
578 mechanism could only be evidenced in oxidizing environment (Wiemann *et al.* 2018). The occurrence of
579 iron oxide framboids in the studied *Anchiornis* fossil probably resulting from pyrite weathering, rather
580 attests for the reducing conditions of fossilisation, thus making unlikely the involvement of this
581 preservation pathway (i.e. lipoxidation).

582 Further experimental studies on modern feathers and comparisons with the fossil record are required to
583 explain why keratin is not preserved in *Anchiornis* feathers although melanin has been detected, and
584 melanosomes and moulds have been observed.

585

586 **CONCLUSIONS**

587

588 The methods used in this study provide new and complementary information on how the plumage of
589 *Anchiornis huxleyi* (YFGP-T5199) is preserved. SEM and EDS reveal that fossil feathers are preserved
590 in a fine-grained material constituted of K-rich phyllosilicates, illite and interstratified illite/smectite.
591 PIXE analyses show that both light (C, N, O) and heavy (S, Na, Ca, etc.) elements are present in the
592 fossil samples, even at very low concentrations. The presence of iron oxide pseudomorphs after pyrite
593 likely indicates a reduced depositional environment for *Anchiornis*. Carbon is the dominant element in

594 the fossil feathers, which are also enriched in sulphur with respect to the ‘host’ sediment. EBS mapping
595 of the interior of the samples revealed a decrease in carbon concentration with depth. Our analysis
596 therefore shows that the fossil feathers are preserved in the uppermost part of the sample, as a thin – ca.
597 3 µm-thick – S-bearing carbonaceous layer. High resolution imaging of the feather microstructure
598 revealed the presence of elongated microbodies (650-950 nm), likely corresponding to eumelanosomes.
599 Molecular characterization of the organic matter in the ‘host’ sediment, fossil feathers and modern
600 feathers by ¹³C-NMR, micro-ATR FTIR and Py-GC-MS shows that the fossil does not display the
601 complex amino-acid signature typical for keratin, the main constituent of modern feathers. Although the
602 organic matter of the fossil feathers and their ‘host’ sediment are both dominated by aliphatic moieties,
603 they exhibit substantial differences (distribution pattern of series, occurrence of components specific to
604 the feathers) suggesting that the organic matter of the fossil feathers is derived, at least partially, from
605 original constituents of the feathers.

606 Altogether, these results show that the fossil feathers can be described as compression fossils, as
607 described in Schweitzer (2011: p. 192). The finely grained (clay-rich) host sediment contributed to the
608 morphological preservation of *Anchiornis* soft tissues. As stressed by Schweitzer (2011: p. 192), the
609 very fine grain size of the sediments might have prevented the degradation of soft tissues by microbes,
610 and subsequent loss of degraded organic matter in the environment before and during diagenesis.
611 However, the lack of protein-derived moieties in the fossil organic matter shows that the latter has been
612 significantly altered during diagenesis. The excellent morphological preservation of the fossil soft tissue
613 is not associated here with a high preservation level of organic matter. Hence, the fossil feathers have
614 likely undergone a complex diagenetic history including several steps affecting differentially their
615 morphology and chemistry. *In situ* polymerization of lipids into more stable aliphatic compounds during

616 early diagenesis was likely the main process responsible for organic matter preservation in the fossil
617 feathers. Additionally, sulphur was probably involved in several steps of the fossil preservation although
618 no natural sulphurization took place.

619 Our results are therefore unique by combining different analytical techniques on Jurassic fossil feathers.

620 This integrative multidisciplinary study appears as a powerful approach to decipher morphological,
621 mineralogical, structural and chemical features of fossil soft tissues and their fossilization processes.

622 This study provides new insights into the taphonomy of labile compounds, suggesting that keratin,
623 unlike the pigment melanin, is not present in the feathers of YFGP- T5199. Our results question the
624 preservation potential of keratin (and melanin) in anoxic conditions. Further analyses of fossil feathers
625 of different ages and deposited in different environmental settings, are required to better understand the
626 preservation potential of melanin and keratin.

627

628 Finally, we used here for the first time on a Jurassic fossil Ion Beam Analysis (IBA), a non-destructive
629 analytical technique providing an in-depth profiling of C to U elements. Further developments of this
630 technique to palaeontological samples might help at identifying the precise location of fossil soft tissues
631 within the sediment and then characterizing metal elements that are directly associated with the
632 fossilised tissues.

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641

642 **DATA ARCHIVING STATEMENT**

643 Additional data for this study are available in the [Dryad Digital Repository]:

644 <https://datadryad.org/review?doi=doi:10.5061/dryad.XXXX>

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997 **FIGURE CAPTIONS**

998 Figure 1. *Anchiornis huxleyi* (YFGP-T5199). Photograph of the Jurassic feathered theropod with
999 location of sampled areas. The white box indicates locations of fossil feather and ‘host’ sediment
1000 sampling, while the yellow box indicates location of ‘remote’ sediment sampling, for NMR, Py-GC-MS,
1001 and IBA analyses. White dots are samples used for SEM imaging and EDS. Scale bar = 5 cm.

1002 Photograph by Thierry Hubin (IRSNB).

1003 Figure 2. Scanning electron microscopy (SEM) images of minerals observed in the plumage of
1004 *Anchiornis huxleyi*. (A), (B) thin platelets of phyllosilicates observed in sample 7 (see Fig. 1 for
1005 location); scale bar = 5 μm (A) and 3 μm (B), (C) Pyrite framboids (sample 1); scale bar = 10 μm , (D)
1006 Pyrite crystallites (sample 1); scale bar = 5 μm , (E) Clayed pores containing small star-shaped iron-
1007 oxide crystals (sample 8); scale bar = 2 μm , (F) Star-shaped iron oxides (sample 1); scale bar = 2 μm ,
1008 (G) Feldspar crystal surrounded by clay sheets (sample 5); scale bar = 5 μm , (H) Quartz crystals
1009 embedded in a clayed matrix (sample 9); scale bar = 5 μm . Arrowheads point towards the
1010 aforementioned minerals. Abbreviations: Phy: phyllosilicate; Py: pyrite; Fd: feldspar; Qz: quartz.

1011 Figure 3. X-ray powder diffraction patterns of the ‘host’ sediment. Spectrum of (A) the bulk rock; qz,
1012 quartz; ca, calcite, (B) the fraction < 2 μm , with spectra of natural (N), glycolated (G), and heated (H)
1013 sample. The expansive illite/smectite interstratifications are identified in the spectrum of the glycolated
1014 (G) material.

1015 Figure 4. SEM images of the ultrastructure of *Anchiornis* plumage. (A), (B) elongated microbodies
1016 observed in sample 6 (see Fig. 1 for location), (C) Elongated microbodies (arrows) observed in sample
1017 9, and (D) imprints observed in samples 6. Scale bars: 2 μm .

1018 Figure 5. Results of Elastic Backscattering Spectrometry (EBS) and Particle-Induced X-ray Emission
1019 (PIXE) analyses on the fossil feathers, the ‘host’ sediment and the ‘remote’ sediment. (A) Global content
1020 of C, O and Si obtained by EBS, in integrating the C, O and Si depth profiles over the 0 – 25 000 TFU
1021 interval. The error bars give an estimate of the uncertainties considering the counting statistics as well as
1022 the cross-section and stopping power uncertainties. Analysis have been performed in duplicate on the
1023 same area of the fossil feather (Fossil #1, Fossil #2), three points of analysis have been taken at three
1024 different locations in the ‘host’ sediment (at 1.7, 3.2, and 4.8 mm away from the fossil), and one in the
1025 remote sediment (B) PIXE spectra obtained for one point of analysis in the fossil feather (red), the ‘host’
1026 sediment (black), and the remote sediment (blue). The spectra are normalized to the Al signal (which is
1027 mainly coming from the selective filter) to allow for a direct comparison.

1028 Figure 6. Cross polarization/magic angle spinning ^{13}C nuclear magnetic resonance (CP-MAS ^{13}C -NMR)
1029 spectra of (A) the modern bird feather, (B) the fossil feathers, and (C) the ‘host’ sediment. Major
1030 chemical functions are indicated on each peak.

1031 Figure 7. IR spectra showing the comparison between of (A) a modern buzzard feather and *Anchiornis*
1032 feathers, and (B) *Anchiornis* feathers and the sedimentary matrix (‘remote’ sediment). See the text for
1033 peak assignment. The spectra are normalized to 100 % transmittance.

1034 Figure 8. Chromatograms of the products formed during the pyrolysis of (A) modern buzzard feathers,
1035 (B) fossil feathers, and (C) remote sediment. Peak identifications are given in Tables 2 and 3. C_n : carbon
1036 chain, with n indicating the length of the chain.

1037 Table 1. Sulphur content derived from PIXE using GUPIX for the data reduction. The global uncertainty
1038 is obtained by combining the counting statistics and the fit error (both calculated by GUPIX) with the

1039 estimated accuracy of our PIXE measurements. The latest contribution is obtained by direct comparison
1040 to a BCR-126A certified reference material. The global uncertainty is obtained by summing these three
1041 contributions in quadrature.

1042 Table 2. Significance of the peaks observed on the pyrochromatogram of the modern bird feather. Peaks
1043 are listed by increasing retention times. The table shows the complete name of each identified
1044 compound, the origin of these compounds, and whether they are observed in the modern feather, the
1045 fossil plumage, and the sediment. Peaks 1-18 and a-e were observed in the spectrum of the modern
1046 feathers, peaks f-g were observed in the spectrum of the fossil feather.

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Appendix

1049 Molecular structures of compounds identified in the modern bird feathers. The correspondence with the numbers appears in Table 2
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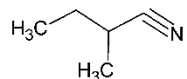
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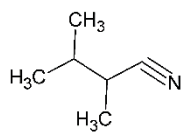
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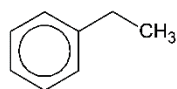
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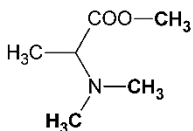
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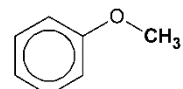
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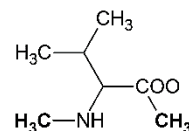
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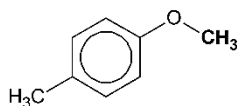
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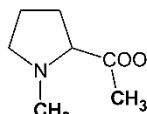
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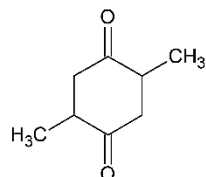
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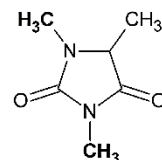
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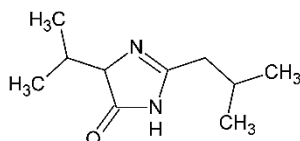


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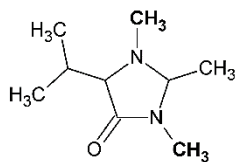
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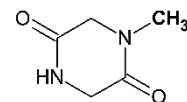
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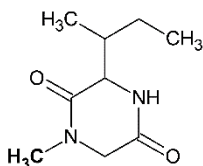
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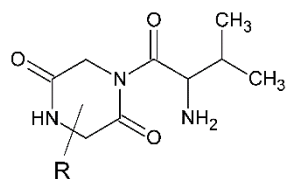
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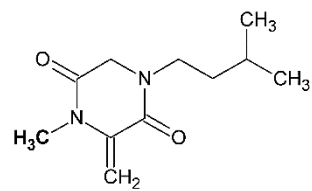
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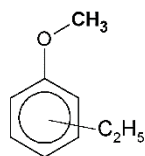
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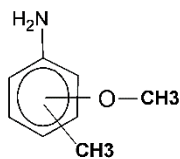
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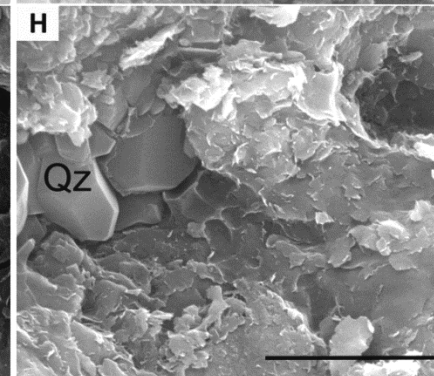
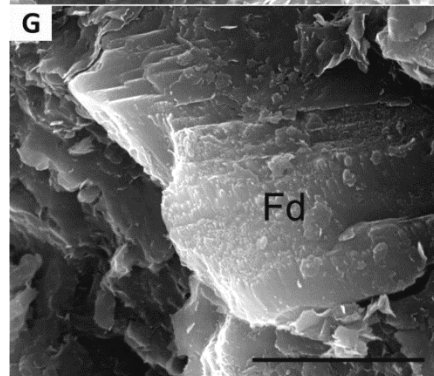
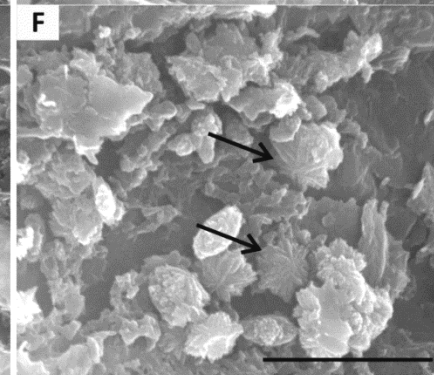
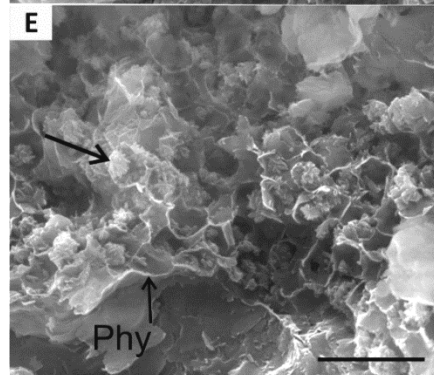
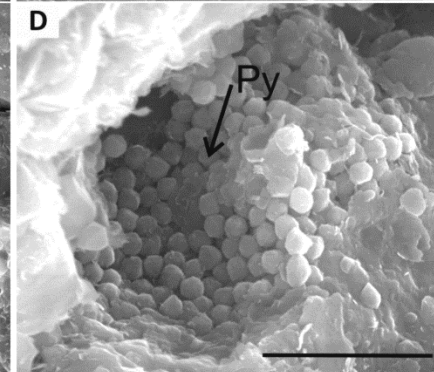
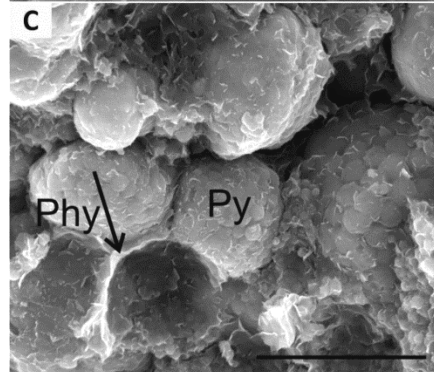
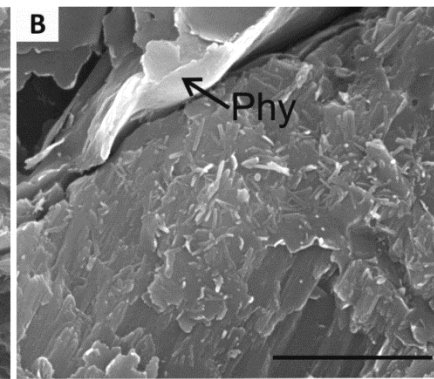
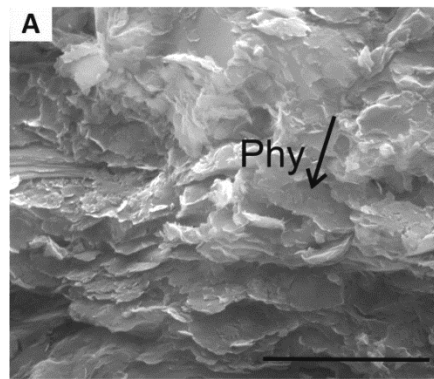


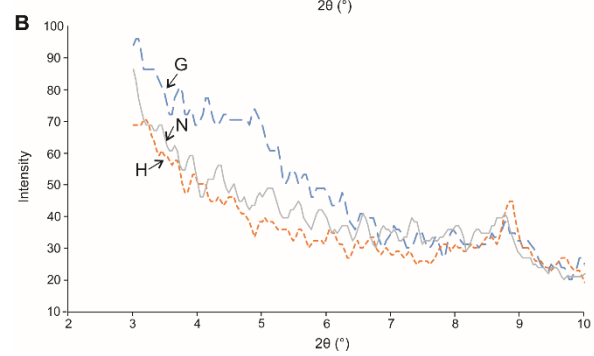
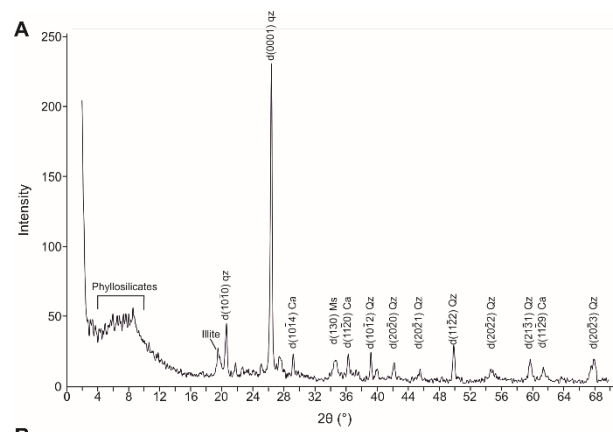
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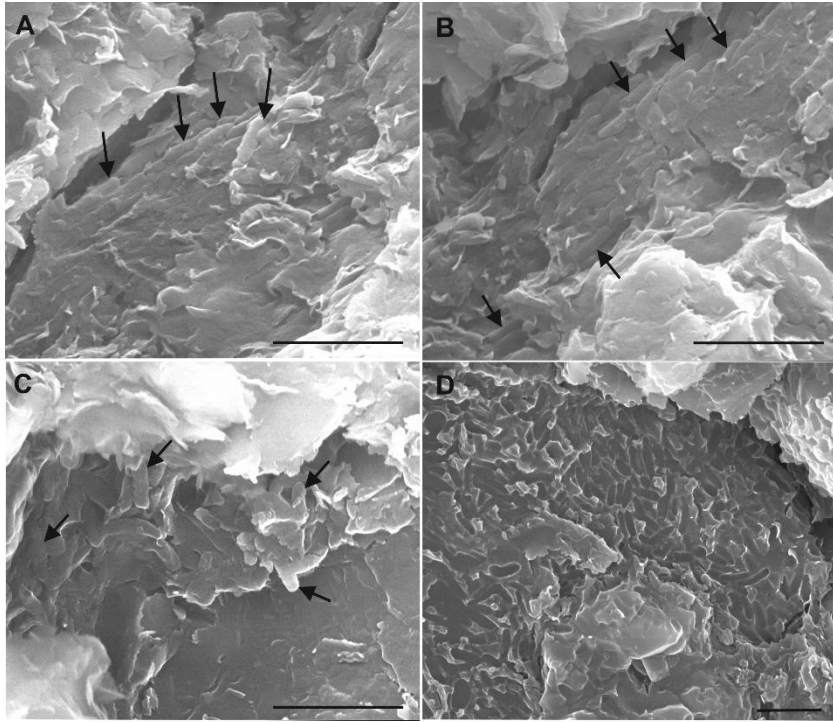
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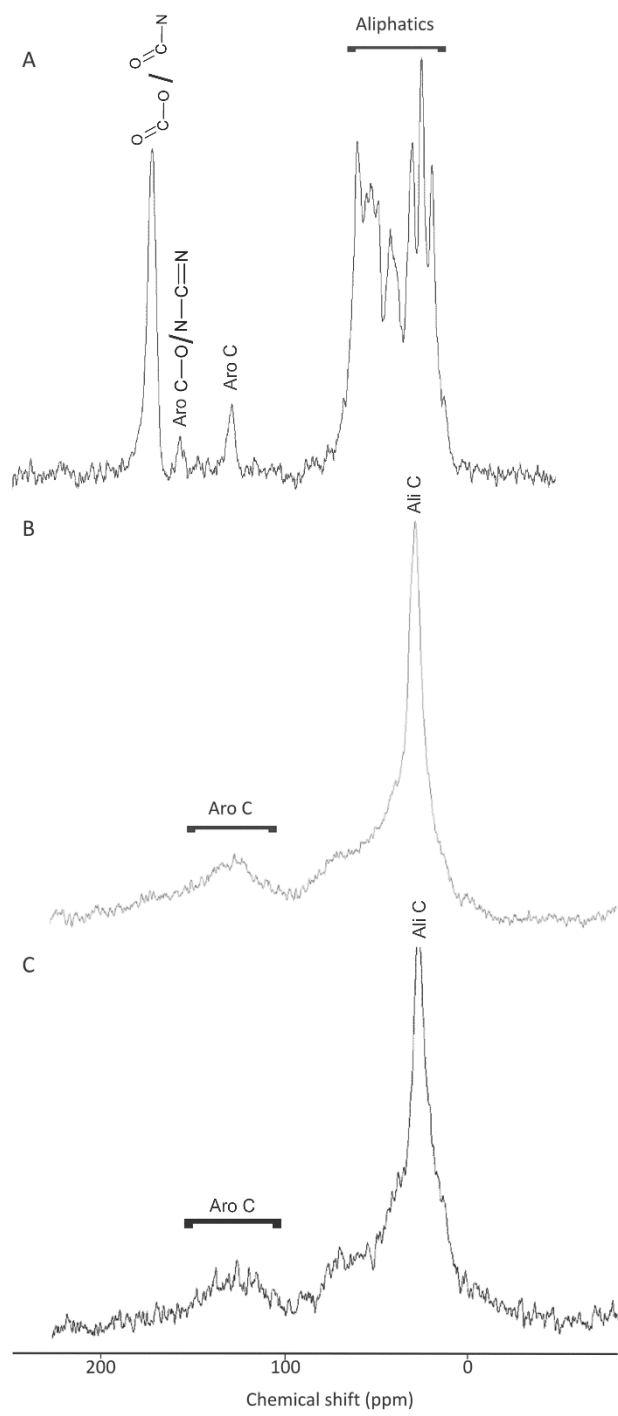


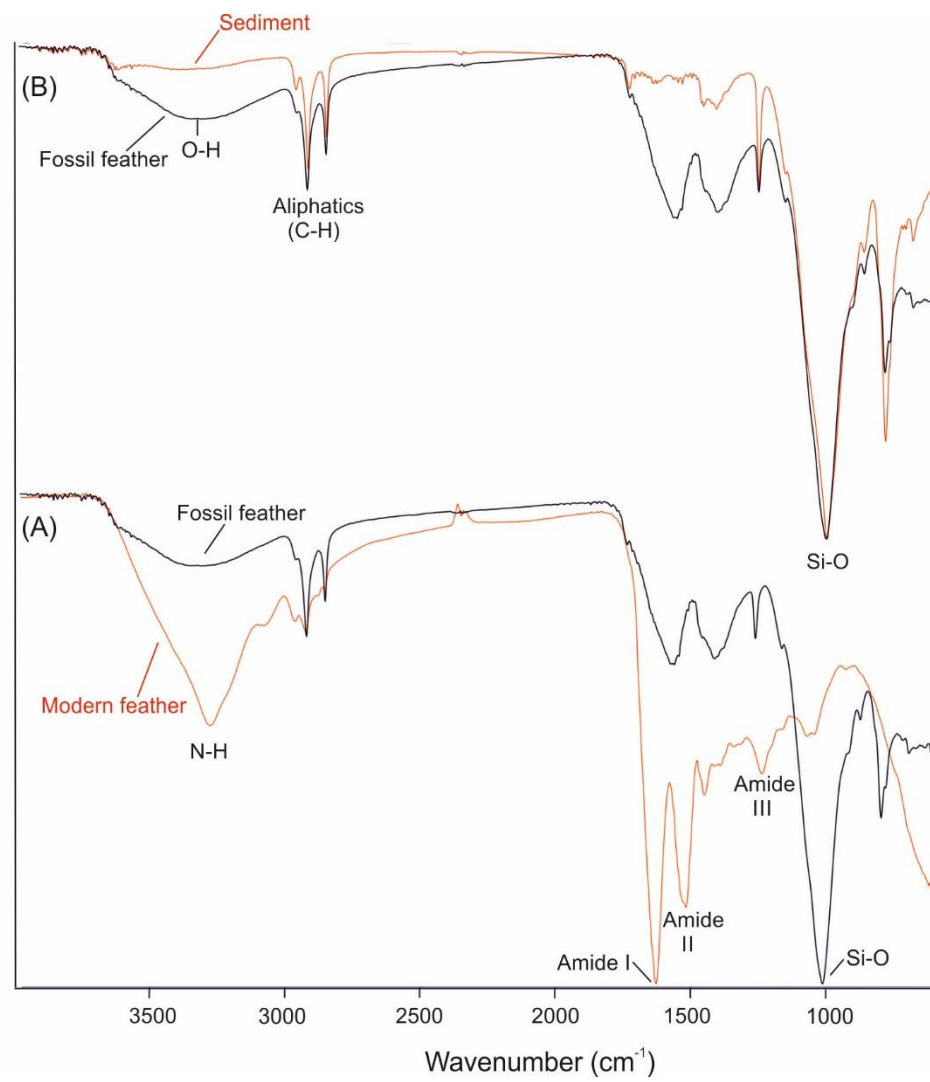
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- *n*-alkanes
- *n*-alkenes
- △ fatty acid methyl esters
- alkyl benzenes
- * TMAH and column phase derivatives

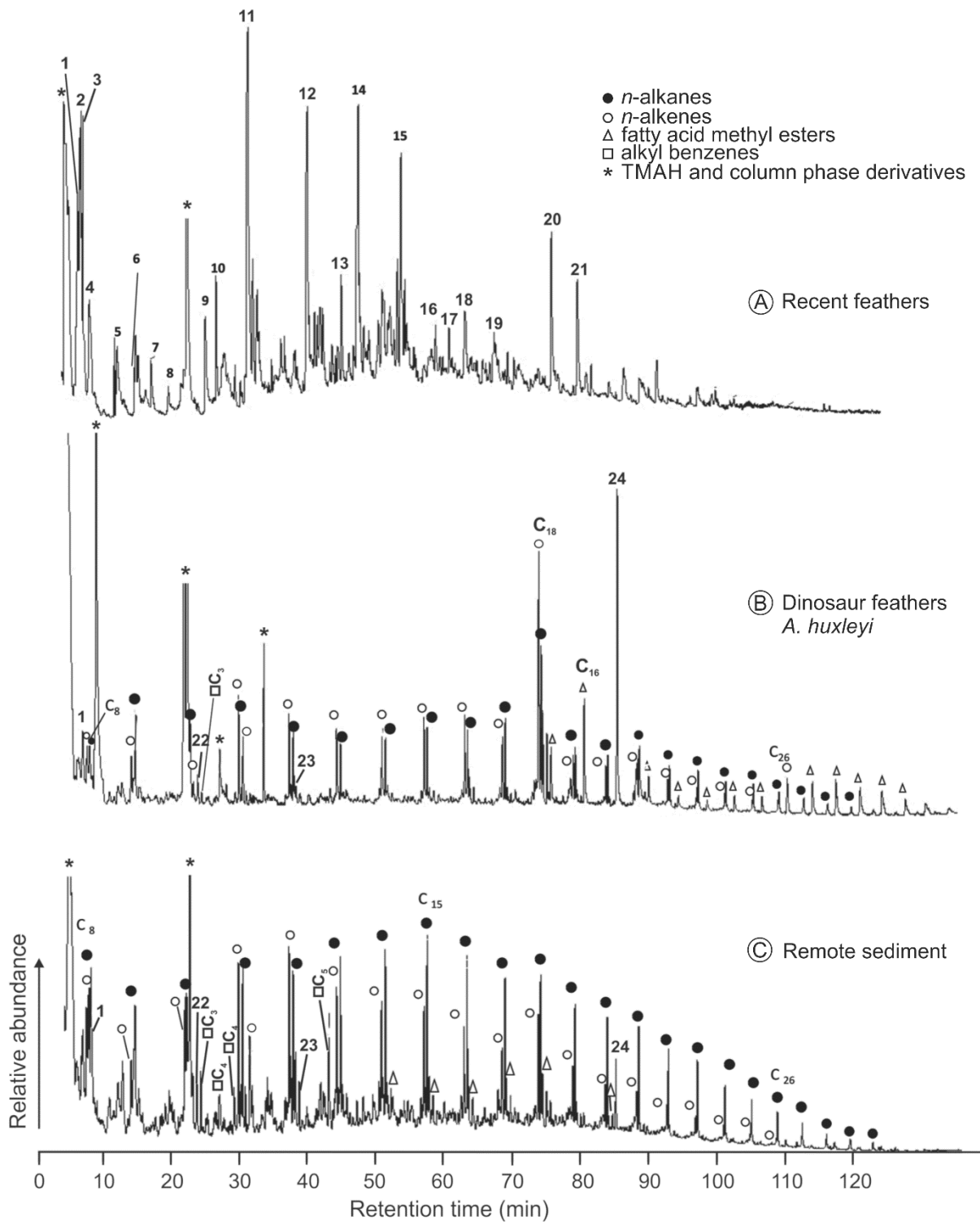


Table 1. PIXE derived concentration data for S and C in the fossil and modern feathers, and “host” sediment. The numbers in the left row represent the distance of the analyzed spots, away from the feather/sediment boundary. See Supplementary Table 1 for details on the global uncertainty calculations, Figure 1 and Supplementary Figure 2 for the location of the analyses.

	[S] (ppm)	[C] (ppm)
Fossil Feather #1	1739	80,385
Fossil Feather #2	1946	82,171
+1.7 mm	1162	72,012
+3.2 mm	801	49,213
+4.8 mm	893	41,835
Remote sediment	98	20,331
Rachis	37,142	547,174
Barbs	43,070	314,962

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Peak	Retention time (min)	Major characteristic ions ^a (m/z)	Molecular ion	Compound	Possible origin ^b	Modern feather	Fossil feather ^c	Embedding sediment ^c
1	6.7	<u>91</u> ; 92; 39; 65	92	Methylbenzene	(Phe)	X	X	X
2	6.9	<u>55</u> , 54, 42	83	2-methylbutanenitrile	Ileu	X		
3	7.1	<u>43</u> , 41, 39, 68	83	3-methylbutanenitrile	Leu	X		
4	8.0	<u>67</u> ; 41; 39; 40	67	1 <i>H</i> -Pyrrole	(Ser)	X		
5	12.1	<u>91</u> ; 55; 106; 65	106	Ethylbenzene	(Phe)	X		
6	14.8	<u>72</u> ; 42; 56; 131	131	N,N- Dimethylalanine methylester	Ala	X		
7	17.0	<u>108</u> ; 78; 65; 39	108	Methoxybenzene	Tyr	X		
8	19.5	<u>86</u> ; 102; 42; 55	145	N-Methyl-valine Methyl Ester	Val	X		
9	25.0	<u>122</u> ; 77; 107; 91	122	1-Methoxy-4-methylbenzene	Tyr	X		
10	26.5	<u>84</u> ; 42; 100; 58	143	N-Methyl-proline Methyl Ester	Pro	X		
11	31.2	<u>56</u> ; 140; 42; 112; 83	140	2,5-Dimethylcyclohexane-1,4-Dione	Gly	X		
12	39.7	<u>42</u> ; 127; 142; 56	142	1,3,5-Trimethylimidazolidine-2,4-Dione	Ala?	X		
13	44.8	<u>56</u> ; 126; 139; 41	182	1-Isobutyl-4-isopropylimidazolinone	Val	X		
14	47.2	<u>128</u> ; 42; 71; 113	170	5-Isopropyl-1,2,3-trimethylimidazolidinone	Val-(Gly)	X		
15	53.6	<u>128</u> ; 42; 57; 71	128	1-Methylpiperazine-2,5-dione	Gly	X		
16	58.6	<u>82</u> ; 167; 182; 110	182	?	His?	X		
17	60.5	<u>152</u> ; 41; 55; 137; 179	194	?	Val?	X		
18	62.9	<u>142</u> ; 113; 42; 71	198	1-Methyl-3-(1-methylpropyl)piperazine-2,5-dione	Ileu-Gly	X		
19	67.1	<u>142</u> ; 113; 42; 98; 212	?	N-(1-oxo-2-amino-3-methyl-butyl)piperazine-2,5-dione derivative	Val	X		
20	75.5	<u>139</u> ; 70; 42; 168	210	N-Methyl-3-methylidene-6-(3-methylbutyl)piperazine-2,5-dione	Ser-Leu	X		
21	79.2	<u>168</u> ; 139; 70; 42	210	Isomer of compound 20	Ser-Leu	X		
22	23.7	<u>136</u> , 121, 122, 91	136	1-Methoxy-2,3-dimethylbenzene or 1-Ethyl-2-methoxybenzene	Lignin or cellulose		X	X
23	38.1	<u>136</u> , 122, 137	137	Methoxy-methylaniline	?		X	X
24	86.3	<u>45</u> ; 57; 97; 224; 252	284	1-Methoxyoctadecane	?		X	X
•		<u>43</u> ; 57; 71; 85		<i>n</i> -alkanes	Aliphatic chains		C ₈ -C ₃₀ (C ₁₈ , C ₁₁)	C ₈ -C ₂₉ (C ₁₅)
○		<u>55</u> ; 43; 69; 83, 97		<i>n</i> -alk-1-enes	Aliphatic chains		C ₈ -C ₂₆ (C ₁₁ , C ₁₈)	C ₈ -C ₂₉ (C ₁₁ , C ₁₈)
Δ		<u>87</u> ; 74; 43; 55		Fatty acid methyl esters	Aliphatic chains		C ₈ -C ₃₀ (C ₁₆)	C ₈ -C ₃₀ (C ₁₆)
□		91, 105		Alkyl benzenes	?		C ₃	C ₃ -C ₅ (C ₅)

1102 Table 2. Main products released from pyrolysis of modern feathers, fossil feathers and embedding sediment in the presence of TMAH.

1103 ^a MS fragments are in order of decreasing abundance, with base peak underlined.

1104 ^b Compounds in brackets indicate possible origin that is not univocal; Ala, alanine; Gly, glycine; His, histidine; Phe, phenylalanine; Pro, proline;
1105 Ser, serine; Tyr, tyrosine; Val, valine; ? Tentative origin
1106 ^c C_{range} (C_{max}, C_{submax})
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1108

1109