Infrared mapping resolves soft tissue preservation in 50 million year-old reptile skin

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Non-destructive Fourier Transform InfraRed (FTIR) mapping of Eocene aged fossil reptile skin shows that biological control on the distribution of endogenous organic components within fossilized soft tissue can be resolved. Mapped organic functional units within this approximately 50 Myr old specimen from the Green River Formation (USA) include amide and sulphur compounds. These compounds are most probably derived from the original beta keratin present in the skin because fossil leaf- and other non-kin-derived organic matter from the same geological formation do not show intense amide or thiol absorption bands. Maps and spectra from the fossil are directly comparable to extant reptile skin. Furthermore, infrared results are corroborated by several additional quantitative methods including Synchrotron Rapid Scanning X-Ray Fluorescence (SRS-XRF) and Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS). All results combine to clearly show that the organic compound inventory of the fossil skin is different from the embedding sedimentary matrix and fossil plant material. A new taphonomic model involving ternary complexation between keratin-derived organic molecules, divalent trace metals and silicate surfaces is presented to explain the survival of the observed compounds. X-ray diffraction shows that suitable minerals for complex formation are present. Previously, this study would only have been possible with major destructive sampling. Non-destructive FTIR imaging methods are thus shown to be a valuable tool for understanding the taphonomy of high-fidelity preservation, and furthermore, may provide insight into the biochemistry of extinct organisms.

Keywords: keratin; amide; taphonomy; Synchrotron Rapid Scanning X-Ray Fluorescence; Pyrolysis-Gas Chromatography/Mass Spectrometry; Green River

1. INTRODUCTION

Recent palaeontological research has concluded that physical structures within soft tissue may be preserved during fossilization [1–3]. Related attempts to detect organic compounds preserved over geological time have also shown progress. Pioneering work has shown the survival of plant-derived matter from sediments as old as 1 Gyr [4] and that protein moieties derived from invertebrate material may survive over at least 25 Myr [5]. Several recent studies that apparently show preservation of biological structures [1,6] and biomolecular residue [7,8] are still under active scientific debate [9–11]. Thus, it may be concluded that the results from current studies showing structural control on biomolecular preservation point towards the need for advances in analytical methods.

A recent multi-technique study of an exceptionally well-preserved Upper Cretaceous (approx. 65 Myr old) hadrosaurine dinosaur (specimen MRF-03) unequivocally showed differences between the organic compounds present within the fossilized skin envelope and compounds present within the enclosing sedimentary matrix; this difference was interpreted as resulting from the presence of endogenous organic compounds within soft tissue [12]. This interpretation was verified by combining the results from Fourier Transform InfraRed spectrometry (FTIR), Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS) and amino-acid composition analyses [12]. Infrared methods especially can fingerprint chemical units [13–15] and map their distribution. FTIR analysis of fossilized skin demonstrated the presence of characteristic amide I and II bands that were comparable to those measured in extant beta keratin samples. However, mapping of the beta keratin distribution in the skin of MRF-03 was not possible because of the friable nature of the available samples. The study suggested that using modern infrared mapping of organic functional groups within more suitable specimens may provide important new constraints on interpreting fossil preservation without requiring destructive sampling.

A related advance in chemical imaging applied to palaeontology has involved the use of non-destructive
FTIR mapping of Eocene reptile skin

2 METHODS

(a) Sample handling and storage

Attempts to resolve trace organic residue in soft tissue over 50 Myr old must take extreme caution with respect to adventitious contamination. Many samples, which have been handled and curated using traditional methods, are no longer suitable for the types of analyses described here. This is a key reason why we selected BHI-102B as a suitable specimen for FTIR mapping; a member of our team sampled it and we know how it has been handled and stored. Most importantly, the sample surface has, to the best of our knowledge, never been exposed to any type of organic or inorganic contamination. All samples handled by our research team for analysis follow a strict protocol to minimize any contamination, see electronic supplementary material, note S3 for further sample handling details.

(b) Fourier Transform InfraRed

Absorption spectra and maps were recorded with a Perkin Elmer Spotlight 400 FTIR Imaging System. The gecko skin map was collected in transmission mode with a 100 × 100 μm aperture and consists of 33 × 23 points with two spectral scans per point. The BHI-102B map and BHI-7032 point analysis were collected using Attenuated Total Reflectance (ATR) mode also with a 100 × 100 μm aperture. The BHI-102B map consists of 26 × 26 points with 10 spectral scans per point, resulting in a spatially resolved dataset of 676 individual point analyses. Every point on these maps records a full range infrared spectrum (4000–800 cm⁻¹ with 4 cm⁻¹ resolution). Maps are produced by selecting a specific absorption band and then displaying the intensity of absorption (or transmission) within each spectrum recorded at each point in the area mapped. All spectra and maps were background subtracted. Organic peak assignments were made using the Bio-Rad KnowItAll Informatics System 8.2 Multi-Technique database. Absorption bands for the inorganic phases are assigned based on mineralogical reference values [19]. The modern and fossil specimens have never come in contact with each other and they have furthermore never even come in contact with the same surface, therefore transference of material is a virtual impossibility. See electronic supplementary material, note S3 for further FTIR details.

(c) Synchrotron Rapid Scanning X-Ray Fluorescence

Maps of Cu and Fe were obtained at the Stanford Synchrotron Radiation Lightsource (SSRL) wiggler beamline 6-2 under ambient conditions with an incident beam energy of
either 12 or 13.5 keV, and a flux varying between approximately $10^{15}$ and $10^{11}$ photons s$^{-1}$. For sulphur mapping at the SSRRL, specimens were placed inside a helium-purged sample chamber. Two different methods were used. Method 1 mapped total sulphur, while Method 2 was used for BHI-102B to either map total sulphur or to exclude sulphate and map only organic sulphur. See electronic supplementary material, note S3 for further details of SRS-XRF maps and point analyses.

(d) Pyrolysis-Gas Chromatography/Mass Spectrometry analysis

Residues of the skin (BHI-102B), surrounding sedimentary matrix (BHI-102B), and another leaf from the Green River Formation (MGSF312; see electronic supplementary material, note S1) were analysed by normal flash Py-GC/MS. See electronic supplementary material, note S3 for further details of Py-GC/MS details.

(e) Other destructive methods

As an additional stage in characterizing the organic component of this fossil, amino-acid analysis was attempted as a precursor to proteomics. There is simply not enough material to proceed with this type of destructive analytical pathway for BHI-102B given current technology. This underlines the importance of the chemical information that can be acquired using non-destructive FTIR and SRS-XRF mapping for samples such as this. See electronic supplementary material, note S3 for further details of other destructive analyses.

(f) Image analysis

Spatial correlations of the optical, FTIR and SRS-XRF images were calculated with ImageJ [20] using the Image CorrelationJ plugin [21]. See electronic supplementary material, note S3 for further image analysis information.

(g) X-ray powder diffraction

X-ray powder diffraction was completed on a bulk sample of sediment from BHI-102B and also on a sample scraped from the bedding plane surface where the fossil reptile skin was deposited. A Bruker D8-Advance diffractometer was used to scan the 2$\theta$ range from 5° to 70° with step size of 0.020° at a scan rate of 2 s per step. Quartz was present and used as an internal standard to check that alignment gave accurate peak positions. Incident beam was Cu Kα radiation. Patterns were interpreted using the EVA software.

3. RESULTS

(a) X-ray diffraction

Diffraction analysis of the bulk sedimentary matrix of sample BHI-102B shows the presence of several silicate and carbonate minerals, dominated by quartz and ankerite, respectively (see electronic supplementary material, figure S2 for the full diffraction patterns and peak assignments). Inorganic carbonate and silica functional groups are infrared active. Notably, both analcime (a feldspathoid mineral with zeolite-type cavities) and smectite (a phyllosilicate clay mineral group) are present. Ankerite (a rhombohedral calcium-iron carbonate) was most probably precipitated within pore space after sediments were deposited [22]. Along with ankerite, calcite (hexagonal calcium carbonate) is also detected. In the sample taken from the bedding plane surface, the mineralogy is nearly identical to that of the bulk, except for the emergence of an additional single strong diffraction peak at relatively low angle (approx. 19.2°±2°). Unambiguous mineral assignment is not possible based on a single peak; however, this peak may indicate the presence of an evaporite sulphate mineral (thenardite, Na$_2$SO$_4$) that has been preferentially deposited along the bedding plane surface. Evaporite minerals are well-documented and abundant in the Green River Formation [23], and therefore, the presence of thenardite or a related sulphate phase is consistent with what is known about the geochemistry of this location. The diffraction patterns taken from the sedimentary matrix of BHI-102B do not detect any common iron sulphide, iron oxide or manganese oxide minerals.

(b) Fourier Transform InfraRed imaging and spectroscopy

Figure 2a presents an optical image of the extant gecko skin moult, clearly illustrating a physical structure dominated by distinct (approx. 1500 μm) scales. The infrared spectrum of beta keratin, the dominant component of reptile skin [24], shows strong absorption at wavenumbers corresponding to the characteristic amide functional group (–CO–NH–C) present in the beta keratin molecular structure. A map of the FTIR absorption intensity of the amide I band (C=O at 1653 cm$^{-1}$) for the region shown in figure 2a is presented in figure 2b. The amide I map clearly resolves the physical structure of the extant skin, in particular it shows higher absorption in the scales when compared with the inter-scale integument or hinge regions. Figure 2 also shows an optical image (figure 2c) and an FTIR amide I map (1653 cm$^{-1}$; figure 2d) of the fossil skin. The amide I absorption band intensity map in the fossil displays a spatial distribution pattern that is comparable with the structure of extant tissue.

Full FTIR point spectra within the extant and fossil skin are compared in figure 3. Spectra obtained in this study (black lines) are compared with reference peaks (blue regions). A full spectrum from a point on the extant reptile skin (crosshair 1 in figure 2a) is presented in figure 3a, and shows that infrared absorption is dominated by the characteristic amide functional group (amides I, II and III). Several other expected peaks corresponding to C–H groups from the lipid portion of the skin are also present [25]. Full spectral analysis of a point on the fossil skin (crosshair 2 in figure 2c) is presented in figure 3b, and also clearly shows the presence of amide I, II and III absorption bands (along with the C–H peaks). Although attenuated, these diagnostic organic absorption bands are easily resolved in the fossil and are directly comparable to extant skin. Amide I peak analysis [26] shows strong similarities between the fossil and extant skin (see electronic supplementary material, figure S3). A comparison of the distribution of C–H (2931 cm$^{-1}$) and amide I groups within the fossil skin shows good correlation between these two different functional units which were both present in and thus may be derived from the original organism (see electronic supplementary material, figure S4). Note that carbonate and silicate peaks are present in the fossil infrared scans because the evanescent wave of the infrared beam penetrates through the organic material and interacts in part with the
underlying inorganic sedimentary matrix. Full FTIR band assignments are given in electronic supplementary material, table S2.

In contrast, an FTIR spectrum (figure 3c) produced exclusively from the sedimentary matrix of BHI-102B (crosshair 3 on figure 2c) shows only the inorganic carbonate (green box) and silicate (grey box) peaks. A representative FTIR spectrum of fossil leaf material from the Green River formation (BHI-7032) is also shown for comparison (figure 3d) and although it clearly shows the presence of organics, amide peaks are not resolvable. Importantly, a thiol stretch (S–H) is also apparent in the skin of BHI-102B, weak, but clearly visible at approximately 2535 cm$^{-1}$. Maps of thiol distribution (not shown) replicate the amide and CH maps.

(c) Elemental maps using Synchrotron Rapid Scanning X-Ray Fluorescence

SRS-XRF maps of total sulphur distribution using Method 1 described above (see electronic supplementary material, figure S5) confirm that sulphur is correlated with fossil skin and also show that the sulphur distribution in extant and fossil skin is comparable. Beta keratin is high in cystine moieties (up to 6% by weight [27]), which contain cross-linking S–S bonds. Degradation of keratin breaks these S–S bonds and protonation produces a cysteine-like moiety. Products would then contain an –SH group, thus explaining why thiol appears in the FTIR spectrum from the fossil but not in extant skin. As a further test of the hypothesis that the thiol groups may be skin residue, we employed the second method of sulphur mapping in order to try to distinguish organic from inorganic sulphur. Figure 4a shows total sulphur in a detailed area of the fossil. (Spatial resolution is a factor of five poorer than in electronic supplementary material figure S5 because the pinhole was removed in order to increase X-ray flux on the sample.) The region where part of the sample was removed for Py-GC/MS analysis is obvious. Figure 4b reveals the organic sulphur distribution mapped by setting the incident beam energy below the sulphate absorption edge. Comparison of figure 4a with b shows that there is a clear presence of inorganic sulphur on this bedding plane (consistent with the XRD analyses), but that the organic sulphur signal is strongly correlated with the fossil reptile skin. Figure 4c shows the X-ray Absorption Near Edge Structure (XANES) spectrum produced from the organic material within the fossil skin. The position of the sulphate absorption energy as determined from the K$_2$SO$_4$ reference material is also indicated (vertical dash-dotted line at 2481.3 keV). Inorganic sulphate is associated with the fossilized skin as shown by the large peak coincident with the reference sulphate energy. However, to the low energy side of the dominant peak a region of lower intensity absorption peaks is discernable. Peak energies here correspond to cystine (a pair of vertical dashed lines at 2471.4 and 2473.1 eV), cysteine (thick solid line at 2472.3 eV) and methionine sulfoxide (2475.1 eV). Energies of possible sulphur species are taken from Sandström et al. [28] and adjusted for comparison to our data relative to the sulphate peak energy reported herein. The sulphur XANES spectrum agrees with the FTIR spectroscopy, and shows that along with the thiol-bearing organic compounds (cysteine),
apparently there are also traces of intact disulphide bonds (cystine) associated with the organic matter in the skin. The fact that organic sulphur compounds do not coat the
bedding plane but rather are confined to the fossil skin region supports the interpretation of the organic components as being endogenous to the organism.

Trace metals are present in skin owing to chelation by melanin. Ca, Mn, Fe, Cu and Zn were mapped in both skin samples. Best results for metals were obtained for Fe in the extant skin and Cu in the fossil (high Fe levels in the sedimentary matrix interfered with mapping Fe in the fossil skin). Trace metal fluorescence for both the extant and fossil skins is higher within scales and lower in hinges (figure 5). Image correlation analysis of the fossil optical image (as a proxy for organic carbon) with the Cu map indicates that the best correlation between organic carbon and Cu occurs within scales and not in hinge regions (electronic supplementary material, figure S6). Thus, both FTIR and SRS-XRF maps both reveal biological structure in this fossil.

Point analyses were completed on the gecko skin, fossil skin and sedimentary matrix at both high and low incident energies in order to further quantify the chemistry of the fossil residue and also to constrain the concentration range represented within the chemical maps. Sulphur concentrations in the gecko skin are approximately 1.65 wt%, about a factor of seven higher than sulphur concentrations in the fossil skin (0.24 wt%). Iron in the gecko skin ranges from 110 to 7 ppm, comparable to the copper concentration in the fossil skin of 177 ± 70 ppm (see electronic supplementary material, table S3 for further point analysis details).

Because melanin chelation is a likely origin for copper in the fossil skin, we note that there also may be a contribution from melanin-derived organic residue to the FTIR spectrum displayed in figure 3b. Carboxylate groups in melanin tend to dominate its FTIR absorption spectrum [29], and therefore, we label the asymmetric (1600–1560 cm⁻¹) and symmetric (1420–1400 cm⁻¹) C = O carboxylate stretch positions in figure 3b which could potentially resolve the presence of such material but are obscured by the strong amide II and inorganic absorption bands.

4. DISCUSSION

(a) Origins of the organic compounds and trace metals in the fossil skin

The Green River Formation is universally recognized as a major hydrocarbon reservoir and has been studied in detail for over 80 years [31,32]. These hydrocarbons are derived from the degradation of ancient organisms (higher plants being the likely dominant contributor) and not from modern sources. Therefore, the survival of organic compounds for 50 Myr within the Green River Formation is
not problematic. Py-GC/MS shows the presence of plant-derived organics within the BHI-102B sample matrix, but also clearly shows that there has been little or no transfer of this component into the fossil skin because the skin shows a distinct inventory of organic matter. Furthermore, all our multiple-technique analyses strongly indicate that contamination cannot cause our observations. Modern human contamination is unlikely as no amide peaks are seen in the matrix or on any of the other fossil samples we have examined except for two other specimens of fossilized keratinous skin including MRF-03 [12] and a second reptile sample also from the Green River Formation (BHI-045A). BHI-045A also displays an FTIR spectrum containing a strong amide I band (see electronic supplementary material, figure S8) consistent with our findings from BHI-102B. In comparison, fish integument from the Green River displays a significantly different FTIR spectrum with organic peaks apparently dominated by carboxylate (see electronic supplementary material, figure S9). Out of nine non-reptilian Green River Formation samples analysed via FTIR including fish, feathers, leaves and invertebrates, with over 50 maps reviewed, only skin from BHI-102B and BHI-045A has so far produced clearly resolvable intense amide absorption peaks. In all, over 20 fossils from various formations have been examined and over 100 maps reviewed with only BHI-102B, BHI-045A and MRF-03 displaying intense amide bands.

It is not just the fact that amide bands are only resolved in fossil keratinous skin, however, that argues against contamination. We must also consider the fact that skin patterns in amide, thiol, hydrocarbons and copper are also preserved. FTIR maps consist of a two-dimensional grid of multiple data points, with each point recording a full range infrared spectrum. The map of the fossil skin consists of 676 individual point analyses. Over 200 points, or approximately 30 per cent, of the individual point analyses that are combined to form the image presented in figure 2d are taken from matrix material and none of these show amide peaks. Amide I and II absorption is confined to the skin region as determined optically. For the observed amide distribution pattern to be caused

![Figure 6. Partial Py-GC/MS m/z 55 and 57 mass chromatograms of (a) fossil leaf MGSF312, (b) BHI-102B matrix and (c) BHI-102B organic residue of lizard skin, revealing the distribution of n-alkane/alkene moieties with number indicating the carbon chain length. Open circles indicate straight chain n-alkane/n-alkenes; closed circles indicated branched n-alkane/n-alkenes.](http://rspb.royalsocietypublishing.org/Downloaded from)
by contamination, there would need to be a process whereby contamination intermittently missed over 200 points in the course of scanning, affected only points within the skin, and somehow managed to reproduce the scale/hinge pattern that is visible in both infrared and visual light. This scenario has vanishingly small probability.

Having discounted contamination, we then need to consider whether the mapped chemistry is endogenous or not. In the section on Py-GC/MS, we explain why the patterns we see are extremely unlikely to be caused entirely by microbial processes, and hence we concluded that at least part of the chemical inventory is residual from the original organism. However, it would be possible for geochemical fluids to transfer a range of components into the system and potentially replace the original chemistry. In particular, the processes of sulphurization, phosphatization and pyritization have been identified as important pathways to preserve biological structures over geological time. Pyrite is not identified here via XRD and the sulphur XANES are also inconsistent over geological time. Pyrite is not identified here via XRD and the sulphur XANES are also inconsistent with sulphide mineralogy, and so pyritization is not probable. Sulphurization remains as the last potential analysis in BHI-102B and so phosphatization is also not probable. Phosphate levels are below detection by point analysis in BHI-102B and so phosphatization is also not probable. Sulphurization remains as the last potential process, but this process is also not consistent with our data. First of all, sulphur containing moieties such as thiophenes, which could be indicative of sulphurization as a mode of preservation were not detected. Secondly, there is no need for sulphur to be added to account for the sulphur inventory we see in the skin; quantification reveals a negative mass transfer vector of sulphur from the original skin (several weight per cent) to produce the much lower levels we measure in the fossil skin (approx. 0.24 wt%). Sulphurization apparently acts to stabilize organic compounds by adding disulphide bonds, but the keratin structure is already rich in disulphide crosslinks. Finally, even if sulphur was added from the inorganic sulphate present on the bedding plane to the fossil skin, this does not explain the presence of amide groups and unique organic inventory in the skin relative to the matrix, and so we are confident in concluding that at least part of the imaged organic chemical distribution is original to the fossil.

In a similar way, we can consider whether the trace metal distribution that correlates strongly with the organic components of the skin reflects an original pattern in the organism or if copper has been added via geochemical activity. Unfortunately, we are not able to resolve whether two other typical melanin chelates, calcium and iron, show patterns similar to copper because the background levels of these two elements are too high in the matrix. Zinc, another chelate, is slightly higher in the skin relative to the matrix (SRS-XRF point analyses give approx. 116 versus 68 ppm, respectively), suggesting that at least two melanin chelates are present in the fossil skin. Interference from the copper emission line and the smaller difference in matrix versus skin concentrations obscures zonation in the zinc map. However, there is no evidence of copper or zinc fluid transport such as enrichment at zones of high permeability (fractures or along the bedding plane) and there are virtually no copper- or zinc-enriched oxide precipitates within the fossil skin. In fact, if the evaporitic sulphates on the bedding plane occurred subaerially or soon after burial, then the persistence of highly soluble sulphate along the bedding plane implies that the fossil environment may have been extremely dry for most of the geological history of the specimen. In that case explaining addition of copper by aqueous fluid movement would be difficult.

It is also worth noting that transfer of copper into the skin is not required in terms of mass balance. Just as for sulphur, the maximum copper levels that we map here of approximately 180 ppm are not problematic. Iron levels in the extant gecko skin moult are as high as 110 ppm, and so trace metal concentrations on the order of a few hundred ppm in skin do not require mass transfer into the fossil. Note also that volatile loss will tend to concentrate metals and raise their concentration in residual material: based on sulphur loss approximately 85 per cent of the original skin mass may have been lost as volatiles. If we reasonably assume that trace metals remain concentrated in the solid residue, this would mean that original copper concentration in the skin could have been as low as 30 ppm. Melanin within the original skin could act as a sink for divalent trace metals, and so it is of course possible that copper and other metals were added to the skin posthumously from evaporating fluids or from other soft body tissues as they desiccated. But a scenario involving large amounts of mass transfer from large volumes of geochemical fluids is not required, and in fact, is contrary to several pieces of evidence as described above. In any case, the copper maps do correlate with original biochemical structure in the fossil. We conclude that at least part of the mapped copper, in addition to the organic residue, is original to the fossil.

(b) Taphonomy of exceptional skin preservation

Interestingly, experimental work shows that the cysteine–SH group may deprotonate and strongly adsorb to Cu\(^{2+}\) substituted smectite minerals, thus decreasing compound mobility and thereby preserve an original biochemical distribution. Cysteine (or thiol) is shown by both FTIR and XANES to be present in the fossil skin, and XANES further indicates that precursor disulphide (cystine) is also present. These spectroscopic results support the postulated breakdown pathway for keratin derived from the original reptile skin. Other groups like melanin-derived compounds within skin residue may also bond to trace metals. Chelate complex formation may act to stabilize these organic compounds over time. An additional bonding mode for cysteine has also been documented, which does not involve deprotonation; in this mode cysteine adsorbs onto phyllosilicate basal plane surfaces while retaining the thiol group. A combination of edge and basal plane adsorption modes for cysteine-terminated degradation products would result in: (i) stabilization of amide groups, (ii) preservation of amide, CH, sulphur and copper distributions, (iii) presence of a weak thiol infrared peak, and (iv) presence of a cysteine band in the XANES spectrum. XRD shows that silicates, such as analcime and smectite, which display cation-exchange capabilities suitable for the formation of ternary complexes are indeed present. Furthermore, copper is shown by SRS-XRF to be present in the skin and thus is proximately available for complexation. Precipitation of carbonate minerals, such as ankerite and calcite detected here, may also act to preserve organic material. It has been suggested that rapid carbonate
precipitation could encapsulate cellular material and thus reduce rates of organic degradation [12]. That process was proposed to explain the exceptional skin preservation observed in specimen MRF-03. Carbonate precipitation could act in parallel with ternary complexation as an additional process preserving beta keratin-derived degradation products, or it could act to enhance preservation of ternary complexes by acting subsequently to complex formation and encapsulating adsorbates post-attachment. Precipitation could also aid preservation simply by decreasing local permeability. Extremely dry conditions, either evaporitic before the specimen was buried or imposed after burial as implied by the presence of highly soluble sulphate minerals, would also serve to inhibit microbial degradation and solvent attack (hydrolysis) of the organic compounds present in the skin. This proposed taphonomic model explains all of the FTIR, SRS-XRF, XRD and Py-GC/MS data.

5. CONCLUSIONS
We have used FTIR mapping to show that the chemistry of this fossilized reptile skin is different from the embedding sedimentary matrix and retains important compositional features in common with extant skin. SRS-XRF mapping, another non-destructive method, supports these findings. Previously, results such as those presented here could only be achieved at the cost of major damage to the specimen. Reluctantly, we decided to carry out an additional test on this specimen and performed destructive sampling for Py-GC/MS analysis, whereby our original interpretation of the infrared images was indeed confirmed.

Taken together, all the analyses performed in this study strongly suggest that the fossilized reptile skin in BHI-102B is not a simple impression, mineralized replacement or an amorphous organic carbon film, but contains a partial remnant of the living organism’s original chemistry, in this case derived from proteinaceous skin. On the basis of residual chemistry, we have proposed a cleavage/adsorption model to explain the taphonomy of beta keratin breakdown and preservation. Our data, therefore, suggest that the presence of transition metal substituted silicates may aid in the preservation of beta keratin degradation products through the formation of stable ternary surface complexes. This process may act in sequence with or in parallel to carbonate mineralization processes previously postulated to aid in the preservation of skin-derived organic matter [12]. The observed spatial correlations, combined with the precise composition and structure of the organic and inorganic phases of the specimens robustly supports the mode of preservation proposed.

We conclude that it is now possible to non-destructively map organic compounds and reveal the chemistry of biological structures preserved from fossil organisms up to at least 50 Myr old. This opens up new applications for FTIR imaging in the science of palaeontology such as: performing organic analyses of rare specimens where destructive sampling is not possible, targeting ‘hotspots’ and thereby minimizing destructive sampling required for proteomics and mass spectrometry, and providing spatially resolved comparative organic analyses to improve our understanding of taphonomic processes.

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