BIOPETROLOGY

Research Article

The generality of preservation of organic remains in body fossils by FTIR spectra

Timothy D. Huang,[1,2] Yao-Chang Lee,[3] Cheng-Cheng Chiang[3], Robert R. Reisz[1,2,4]

[1]College of Life Sciences, National Chung Hsing University, Taichung, Taiwan, China
[2]DERC and ICFS of Jilin University, Changchun, Jilin 130012, China
[3]National Synchrotron Radiation Research Center, Hsinchu 30076, Taiwan, China
[4]Department of Biology, University of Toronto Mississauga, Mississauga, Ontario, L5L 1C6, Canada
[*]Correspondence and request for materials should be addressed to email: timd_huang@yahoo.com.

Abstract

Each chemical bond has unique vibration energy, which yields a certain characteristic peak(s) in Fourier-Transform Infrared Spectroscopy (FTIR). The alkyl (CH_{3-}, CH_{2-}) functional group, which exists in almost all organic compounds, will show two or more distinct absorption peaks in the 2800-3000 cm^{-1} range. Thus, if a given spectrum shows these peaks, we can safely say that the specimen contains the alkyl structure(s), which means organic compounds. Alkyl peaks can be used as a positive screening indicator without diving into the other IR spectra section. The overtone peaks, shapes, and intensity of carbonates in the 2800-3000cm^{-1} range are different from the alkyl group. This paper uses FTIR, including synchrotron radiation sr-FTIR, to examine 63 fossil specimens spanning across a long geological time and different taphonomic conditions and 15 extant specimens and nine matrices for comparisons. Each specimen was FTIR scanned multiple times. A total of 107 spectra were selected from 525 scans. The results indicate that preservation of organic remains in body fossils is a common phenomenon, not a particular case.

Key words: **Organic Remains, FTIR, Organic Remains, Body Fossils.**

1 Introduction

We reported preserved organic remains, collagen type I, inside the 195 Ma old *Lufengosaurus* dinosaur and embryonic bones from Lufeng (Reisz et al., 2013; Lee et al., 2017). Was it just a lucky find or a representative of a common phenomenon? In order to check the extent of the preservation, a

Funding: This research project was funded by NSC 100-2116-M-008-016.

Cite it as: Huang, T.D., Lee, Y.C., Chiang, C.C., Reisz, R.R. 2022. The generality of preservation of organic remains in body fossils by FTIR spectra. Biopetrology, 1(1): 48-60. http://biopetrology.com/tdhtgo

variety of fossils from the Precambrian (Ediacaran body fossil), Paleozoic (reptiles or fishes), Mesozoic (dinosaurs), and Cenozoic (mammals) eras were tested and compared with extant animal bones and their matrices (Supplemental Figure S1 Complete Spectra, Supplemental Table S1-S8). If fossils from various geological times and different taphonomic conditions show preserved organic remains indicated by IR alkyl functional group peaks, the preservation of organic remains inside fossils is a common phenomenon, not a unique case of just one particular dinosaur fossil. The original organic material has been modified by various geological interactions related with factors such as pressure, temperature, bacteria, and pH and constituents of ground-water, and should have certain degrees of degradation, such as protein to peptides or even to amino acid level. Furthermore, the degradation products could undergo secondary polymerization by catalytic trace metal elements under the proper geological conditions. Thus, this study also explores the composition of the preserved organic remains.

During the study of the preservation of organic remains in fossils, several points have to be determined. First, is the fossil a body fossil or cast/trace/imprint fossil? For the cast/trace/imprint fossils, their chance to preserve native organic remains is uncertain. If organic remains were preserved, they were not likely from the ancient organisms but the surrounding or fill-in matrix. On the other hand, body fossils are the fossilized products of ancient organisms. Finding what kind of organic remains were preserved can reveal much information. The second issue is whether the preserved organic remains are native (endogenous) or from outside (exogenous), such as invading microbes or other organic matter into the body fossil. It is essential to distinguish these two. Endogenous organic remains tell us the fossil constituents, while the exogenous ones show more on the taphonomy conditions.

With the objectives stated above, three steps are designed for this study. The first step is to explore the generality of preservation of organic remains in fossils. Then, the second is to find out what compounds/components are the preserved organic remains. Finally, the third step is to propose some hypotheses for the preservation mechanism of the organic remains. After the data obtained from the former two steps, we might propose some hypothetical mechanism on why/how the organic remains were preserved.

2 Materials and Methods

Various body fossils spanning a long geological time were chosen, including the fan parts of Ediacaran *Chinglian huangyoung* (Huang et al., 2010), Paleozoic reptile bones and teeth of the same species, Mesozoic dinosaur embryonic, adult, claw, tendon, and teeth, Late Cretaceous hadrosaur and ceratopsian ribs and teeth, Cenozoic mammals from the Taiwan Strait of Penghu, and the centrum and femur of the *Lufengosaurus* embryonic bones. Various common extant animal bones and eggshells were also used for comparison. Based on their taphonomic variations, the selected fossil specimens were divided into three types: (1) marine-terrestrial, (2) terrestrial-terrestrial, and (3) terrestrial-marine/river. The word before " - " means the environment where the fossil lived/formed. The word after "- " indicates where the fossil was found. Furthermore, when available, the surrounding rock of the fossils was analyzed.

No glue was used during the preparation of the slides. Organic contaminants were avoided. Slices with a thickness of 15-30 μm were made for FTIR. Sr-XRF was used to scan the *Lufengosaurus* embryonic jaw to determine the distribution of 14 chemical elements to explore the taphonomic features. Organic sulfur was used as crucial evidence for organic remains.

Synchrotron FTIR was used for mapping and spectroscopic analysis to resolve a 5,000-year-old

mammalian bone (Bergmann et al., 2012). That study sought to use the relatively high spatial resolution of the synchrotron IR beam to resolve the distribution of chemical functional groups with the fine structure of bone. The details of the phosphate groups' antisymmetric stretch were analyzed because the intensity ratio of the peak at 1,030 cm^{-1}, relative to that at 1,020 cm^{-1}, can indicate apatite crystallinity. For modern samples, this ratio tends to be approximately equal to 1. With aging, crystallinity tends to improve, and the value of this ratio increases. It was calculated that the 1030/1020 cm^{-1} ratio of the *Lufengosaurus* embryonic specimen of 195 Ma to be 1.72. Thus, this ratio can be a new way to date fossils.

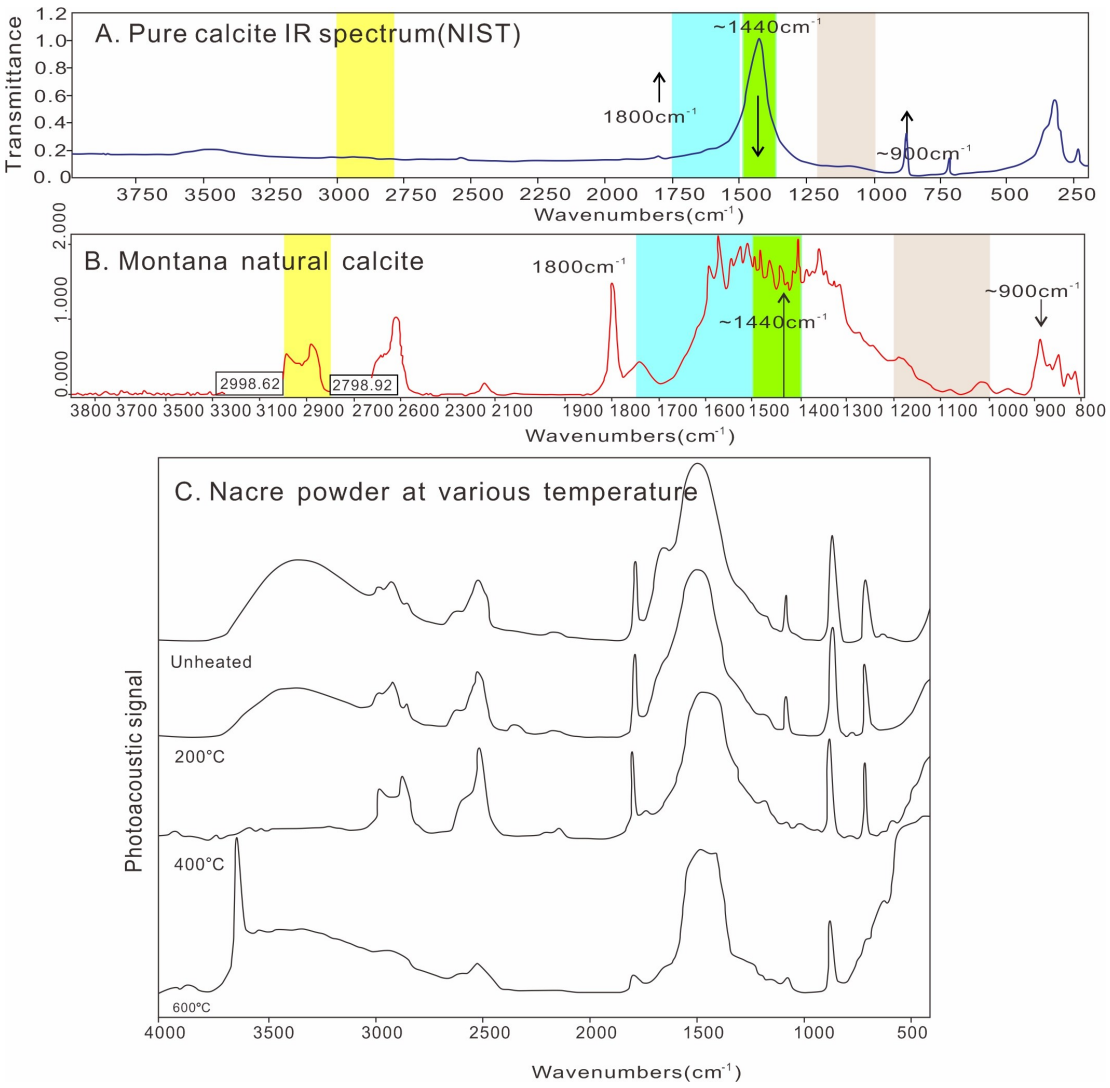

Fig. 1 Infrared Spectrums of pure calcite and natural calcite of nacre. **A**: Pure calcite (calcium carbonate) showing standard IR spectrum from NIST. **B:** Natural calcite collected in Montana, USA. **C:** Nacre powder (Verma et al., 2006) (calcite) showing diminished alkyl as temperature increased. Yellow boxes indicate the alkyl peak regions from 2800-3000 cm^{-1}, the most substantial IR peaks at ~1440 cm^{-1} pointed by the black arrow inside the green boxes. The apatite regions (1000-1200 cm^{-1}) marked as brown boxes. In the region from 1500 cm^{-1} to 1750 cm^{-1} (cyan box), many non-carbonate spectral contributions of impurities from organic matters are seen, including amide-I and amide-II asymmetric and symmetric stretching protonated carboxylic group of protein.

3 Results and Discussions

(1) FTIR case studies, just calcite in the fossil spectra?

Various carbonates (CO_3^{2-}) are very common inside fossils. The pure calcite ($CaCO_3$) (NIST, IR spectrum) shows the most substantial IR peaks at ~1440 cm^{-1} pointed by the black arrow inside the green box of Figure 1a. It is broadband with slightly more tailing toward higher wavenumbers in the 1500-1750 cm^{-1} range (cyan box). In the normal IR range (800-4000 cm^{-1}) of pure calcium carbonate, the next noticeable peak is at ~900 cm^{-1} and there is a tiny overtone kink at ~1800 cm^{-1}. However, for the carbonates inside fossils, organic impurities can be seen clearly as for the Montana natural calcite in Figure 1b. In this spectrum, carbonate peaks show up at ~1450 cm^{-1} as expected, along with many other even stronger or not negligible peaks/bands. The calcium carbonate peak at ~900 cm^{-1} shows pure form and natural calcite. However, the intensity is not as strong as pure calcium carbonate in the naturally formed carbonates. In the region from 1500 cm^{-1} to 1750 cm^{-1} (cyan box), many non-carbonate spectral contributions of impurities from organic matters are seen, including amide-I and amide-II asymmetric and symmetric stretching protonated carboxylic group of protein (Sasaki et al., 1994). The yellow box marked in the range of 2800-3000 cm^{-1} is the alkyl peaks. It is noticed that the pure calcium carbonate has no peak in this region. This is understandable from the taphonomic point of view. When the carbonate anions (CO_3^{2-}) participated with various cations (such as Ca^{2+}, Mg^{2+}, Fe^{2+}) to form various carbonate crystals (calcite, aragonite, magnesite, siderite, etc.), some decomposed organic debris from macromolecules were incorporated inside the crystals. Thus, naturally formed carbonate minerals containing these organics will show various organic peaks in the IR spectra. Verma reported that when powdered and undisturbed nacre was heated for 6 hours, these organic peaks gradually diminished along with an increase in temperature and were almost totally gone (Verma et al., 2006) at 600 ^oC as shown in the yellow box of Figure 1c.

The broadband at around 2920 cm^{-1} of Figure 1b is also assigned to the organic impurities, i.e., organic remains containing the alkyl functional group. The sharp peak at 1800 cm^{-1} is the stretching of carbonyl of acidic protein, while 2920 cm^{-1} stretching C-H bonds of alkyl. The 1800 cm^{-1} peak could also come from acyl halides (R-COX), which could be formed by the replacing of amine functional group ($-NH^2$) such as amino acids from the degradation of proteins with the halide ions (Cl^-, Br^-, and I^-) from the groundwater or seawater. So, when interpreting the IR spectra of fossils, the existence of carbonates should not be neglected. Carbonates are parts of fossil constituents. Fossils contain more than just simple carbonates. They contain preserved organic remains.

(2) Taiwan mammals

Figures 2a, b, and c are spectra of mammal fossils from different taphonomic environments, with an age of 30,000 years ago, i.e., the last global ice age of the late Pleistocene. At that time, the sea level was 130-180 meters below the current level. The deepest site of the current Taiwan Strait is about 70 meters deep, and the mainland of China and Taiwan were connected by a land bridge, allowing mammals to roam freely between the mainland and Taiwan (He, 2011, in Chinese).

This set of FTIR spectra shows the taphonomic influence on the preservation of organic remains. Figure 2a is the spectra of a mammal excavated from the Tainan Formation at Guanmiao of Tainan city. Its taphonomic type is terrestrial-terrestrial. Figure 2b is the spectra of a mammal believed to be in the same Tainan Formation but was washed out into the river bed of Tsailiao Creek. It is a transition

from terrestrial-fresh river water type taphonomically. This fossil was being washed by fresh river water before it was collected, and how long it has been in the water is unknown. However, certain degrees of decomposition of organic remains should be expected. Figure 2c is a fossil recovered in a fish-net from the Taiwan Strait near Penghu islets. It is terrestrial-marine type taphonomically. It was subjected to seawater washing for an unknown period of time, estimated to be up to 30,000 years or more, and should be considered the most influenced by water.

As shown by the alkyl regions (2800-3000 cm^{-1}, yellow boxes), the amount of the preserved alkyl material was the greatest in the terrestrial-terrestrial (Figure 2a) fossils, that in Figure 2b is less probably because some alkyl material was decomposed due to soaking by river water, and that in Figure 2c is almost absent due to a long time in seawater. A similar situation can be observed from the region of organic functional groups (1500-1750 cm^{-1}, cyan boxes). In Figure 2a, intense and unresolved mix peaks/bands can be seen. However, in Figure 2b, the absorbance of amide peaks was reduced to two unresolved humps, while in Figure 2c, many components have decomposed, and only two main peaks at ~1580 cm^{-1} (amide-II) and ~1620 cm^{-1} (amide-I) were preserved. The apatite region (1000-120 cm^{-1}, brown boxes) of the three different taphonomic fossils show about the same strong apatite peaks/bands, indicating that the apatite in these fossilized bones has not been influenced by seawater or freshwater. Figure 2c shows that apatite, amide-I, and amide-II were all well preserved. Amide-I and amide-II are two prominent peaks for collagenous proteins intermixed with apatite during bone formation. The organic remains were likely native (endogenous).

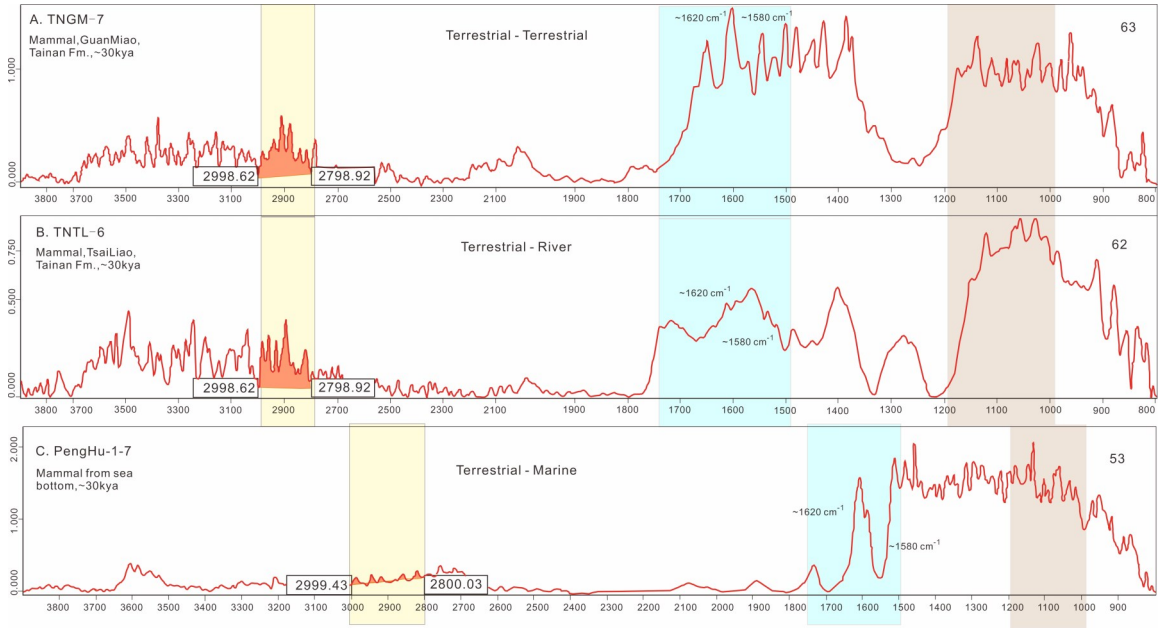

Fig. 2 Taiwan mammal fossils in different burials and found taphonomic environments. **A:** Mammal fossils formed and excavated in the terrestrial-terrestrial environment. **B:** A mammal fossil formed in terrestrial and found in a river environment. **C:** A mammal fossil formed in terrestrial and collected from sea bottom environment. The significant peak strength of alkyl groups can be seen in **A** and **C**. Also, apparent differences can be seen in the organic functional groups' region. Yellow boxes indicate the alkyl peak regions from 2800-3000 cm^{-1}. In the region from 1500 cm^{-1} to 1750 cm^{-1} (cyan box), many non-carbonate spectral contributions of impurities from organic matters are seen, including amide-I and amide-II asymmetric and symmetric stretching protonated carboxylic group of protein.

(3) Paleozoic Permian Dolese fossils

Fossil materials were collected from the early Permian infill in the Ordovician limestone and dolostone karst fissures in the cave system at Dolese Richards Spur, Oklahoma, USA. The fossils were often disarticulated, although articulated materials were not uncommon (MacDougall et al., 2017). The white bones are consistent with the surrounding clay/calcite-rich sediments and have the typical type of Permian preservation, but groundwater does not appear to have deposited colored minerals (iron, copper, manganese, etc.). The brown-black bones have concentrated hydrocarbons in their surficial layer, while the surrounding sediments were not similarly affected.

The graphs of the two fossils in Figure 3 show very significant differences in their alkyl ranges (2800-3000 cm^{-1}). The low peak in Figure 3b is attributed to the decomposition of organic remains in the groundwater, leaving the bones white. The black color of the backbones was due to being soaked in hydrocarbons. Significant differences are also present in the other two wavenumber regions: apatites (1000-1200 cm^{-1}) and organic functional groups (1500-1750 cm^{-1}), meaning that the permeating water and hydrocarbons have significantly changed the composition of the organic remains in the fossils.

(4) Wide geological time span, various taxa and taphonomy

The fossils of Figures 4a-j include various taxa from land animals to marine creatures of a long age ranging from ~30 kya to an estimated 565 Ma, from many different taphonomic environments, such as marine-terrestrial and terrestrial-terrestrial. A terrestrial-marine specimen (Figure 4j) was also included. Many originally marine animal and terrestrial animal fossils were scanned and show the preservation of organic remains evidenced by the strong alkyl peaks. Different taphonomic environments were included. Some fossils went through the change from the marine environment to the terrestrial. The Mesozoic dinosaurs were buried in terrestrial conditions and found in terrestrial conditions. Seawater and groundwater likely played a key role during and after the fossilization process.

All spectra of the fossil specimens (Supplemental Table S1-S6) show clear evidence of preserved organic remains, such as the relatively high alkyl functional group peaks/bands (2800-3000 cm^{-1}, yellow boxes) and the organic functional group peaks/bands at the 1500-1750 cm^{-1} (cyan boxes). The preserved organic remains are most likely the endogenous proteins and their degradation products. These proteins bond inorganic mineral apatite for bone and inorganic mineral carbonates and could be seen for Brachiopoda and Echinoidea shells. After the animal died, the soft body parts started to decompose by bacteria. However, the tightly bonded organics (proteins) with inorganic apatite/carbonates would be less affected by bacterial attacks. During the fossilization process, pressure, temperature, and groundwater could break down the complex macromolecular proteins. The breakdown products from the macromolecular proteins, such as peptide fragments and even amino acids, could react with each other or other chemicals brought in by groundwater. SRS-XRF (Synchrotron Rapid Scan X-Ray Fluorescence) of a Dawa embryonic jaw bone containing un-erupted teeth clearly shows the distribution and presence of organic sulfur, as shown in Figure 5.

(5) Preservation of complex organic residues in Dawa *Lufengosaurus*

Thin transversal section (Fig. 6a) of a Dawa embryonic limb bone was examined with Synchrotron Radiation Fourier Transformation Infrared (SR-FTIR) spectroscope. Organic residues, very likely original or minor likely degradation products of complex proteins, were observed in both the cortex region's fast-growing fibrous bone and the vascular canals (Fig. 6b) (Jackson et al., 2010).

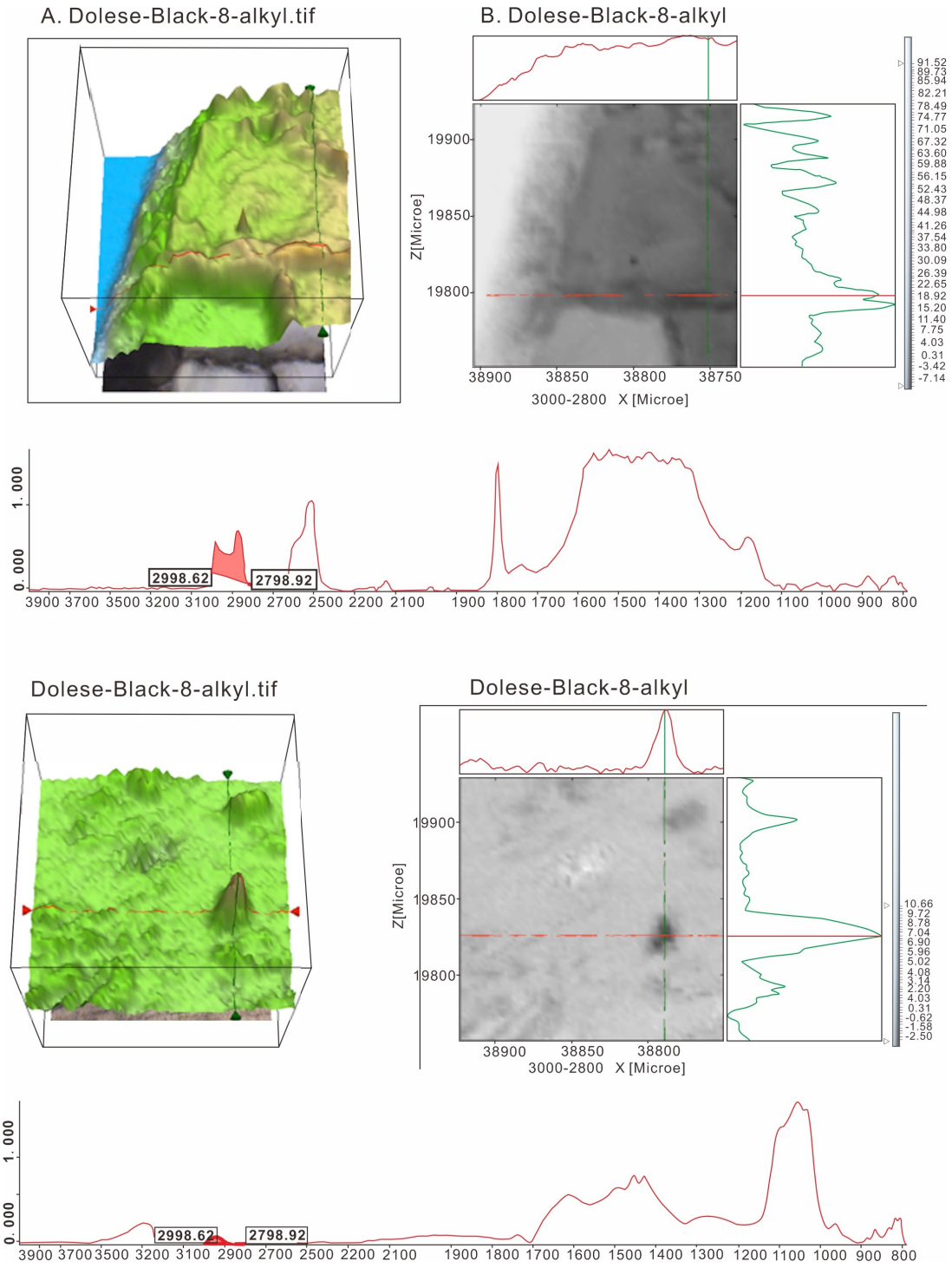

Fig. 3 Spectra of Dolese fossils. **A:** Black Dolese fossil impregnated with hydrocarbons. **B:** White Dolese fossil by the influence of groundwater. Significant spectra are showing different degrees of preservation of chemical compounds. Spectra of early Permian tetrapod bones from the Dolese Brothers Limestone Quarry, near Richards Spur, Oklahoma. In both **A** and **B**, the top left images are the 3D color mapping of the FTIR scan area, with green and red marking lines intercepted at the selected point. The top right image shows the greyscale image corresponding to the 3D color image at the top left. The bottom spectrum shows the complete FTIR spectrum of the intersection with the alkyl region (2800-3000 cm^{-1}) high-lighted.

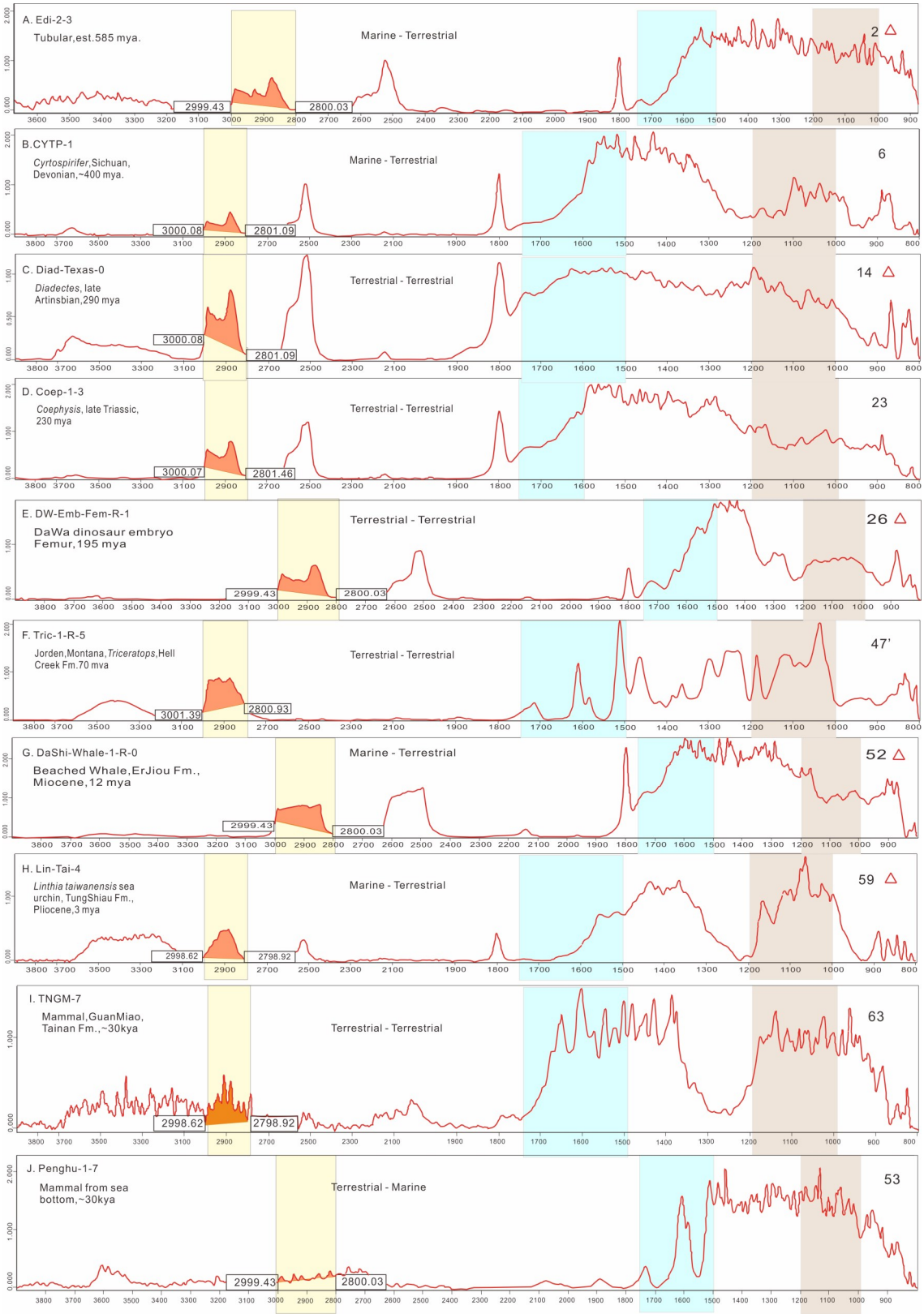

Fig. 4 Wide geological times span, various taxa and taphonomic types. Brief fossil description is at the left side of each spectrum for each one from **A** to **J**. Burial and found conditions are around the center. Alkyl region in yellow boxes, amide region in blue boxes, and apatite in brown boxes.

The SR-FTIR spectrum of the fibrous bone clearly shows organic functional group amide peaks (deconvoluted 1500-1700 cm^{-1} strong band from amide I & II in Figure 6d); and 1200-1300 cm^{-1} weak band from amide III (Lindgren et al., 2011). The Raman spectroscopy shows a strong amide A peak at 3264 cm^{-1} in Figure 6e. So, three significant peaks for positive amide (I, II, and A) identification are all clearly present. A further study from the Fourier self-deconvolution of the amide I peaks (Fig. 6d) shows secondary protein structural peaks indicating presence of complex organic residues. These deconvoluted peaks match very well with previously reported data (Warren et al., 2009; Gallagher, 2009).

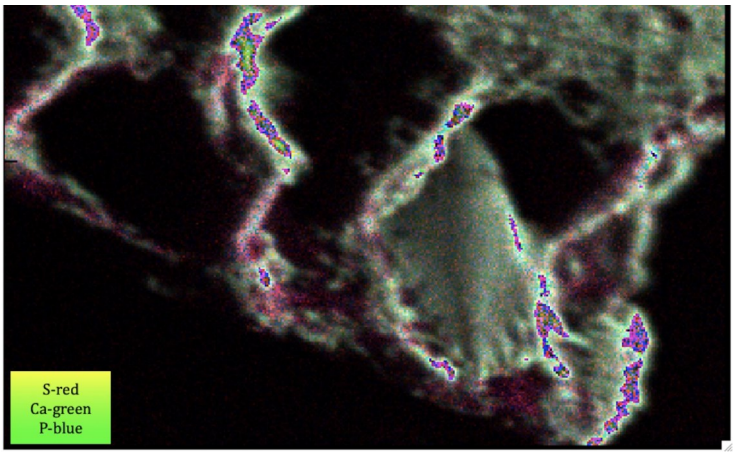

Fig. 5 SRS-XRF scan image of organic sulfur from S-containing amino acids inside a Dawa embryonic jaw bone.
Color assignment: **Ca** in green and **P** in blue as apatite bone matrix. Organic **S** in red to show the S-containing amino acids from the preserved collagen remains. SRS-XRF were conducted at SLAC of Stanford University, California, USA.

Complex organic matter preservation inside dinosaur bones have been reported (Schweitzer et al., 1997; Schweitzer & Horner, 1999; Bern et al., 2009; Asara et al., 2007; Martill & Unwin, 1997; Schweitzer et al., 1997, 2005, 2007, 2009). However, it was also interpreted as possible microbial biofilms (Peterson et al., 2010) and was also rebutted (Lindgren et al., 2011). Here we provide further information to support the complex organic preservation. It can be argued that during the very long period after the animal died, the microbes might enter into the spaces of vascular canals and cause the preservation of the organic residue of the invading microbes. However, it is unlikely that the microbes could enter into the fibrous bone's tightly woven apatite and collagen. The fibrous bone area's FTIR spectra clearly show both apatite and amide peaks, suggesting strongly that the preserved organic residues were not introduced postmortem. Our analyses also exclude the possibility of contamination from handling or from the surrounding matrix.

4 Conclusions

The representative spectra from more than 550 FTIR scans of numerous fossils from a long geological time and different taphonomic conditions show that preservation of the original organic remains in fossils is a common phenomenon, not an exceptional event. If the proper method and sensitive enough instrumentation are used, most, if not all, body fossils could be found to contain preserved organic remains. This suggests that future work using various methods for identifying what was preserved in fossils and the mechanisms of preservation will yield valuable results.

Supplemental Information

Supplemental Information includes 107 figures and 8 tables.

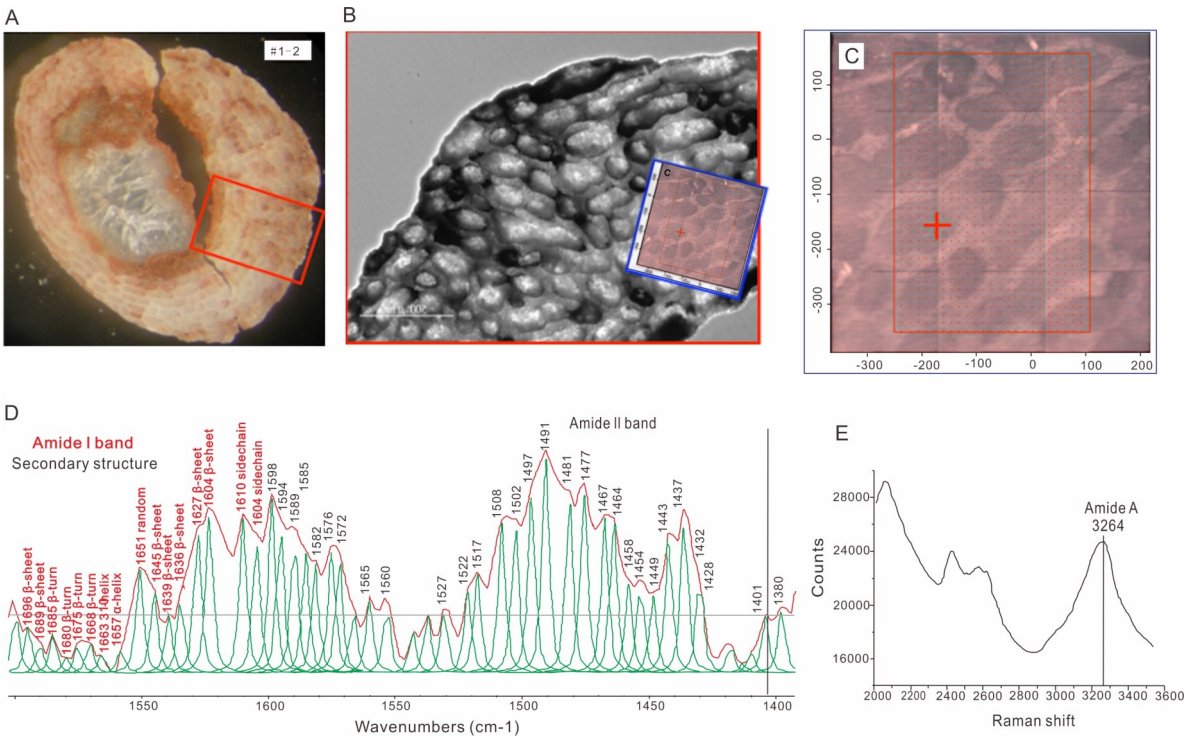

Fig. 6 Optical, microscopic and infrared microscopic images and deconvolution of FTIR spectra. **A:** Optical image of the Dawa embryonic limb bone, transversal cut. **B:** Magnified section of the boxed area of cortical bone in **A**, showing fast-growing fibrous bone tissue and vascular canals. (scale bar: 500μm). **C:** Infrared microscopic image of the boxed area in **B**, clearly showing both fast-growing fibrous bone tissues and vascular canals. The distance between each adjacent red dot in FTIR scan **C** inside the red box is 15 μm. The photo was taken from the NSRRC FTIR instrument at 300X. **D:** Further Fourier self-deconvolution of Amide I peaks (around 1,537 cm^{-1}). The wavenumbers marked in red show the decomposed peaks of protein secondary structures. **E:** Raman spectrum of Dawa embryonic limb bone clearly shows the Amide A peak at 3264 cm^{-1}.

Declaration of Interests

The authors declare no competing interests.

Acknowledgement

We want to thank Mr. David Chin-Ning Chu and Ms. Tina Xiang-Jun Lin for their donations to support this research. We would also like to thank Director Jiang-Rong Liu and Chuan-Wei Yang of the Lufeng County Dinosaur Museum, Director Shi-Ming Zhong of Chuxiong Prefecture Museum for helping with the fieldwork. Thanks to Chun-Chieh Wang, Hwo-Shuenn Sheu, and many others of the National Synchrotron Radiation Research Center for their help on various experiments.

References

Asara, J.M., Schweitzer, M.H., Freimark, L.M., Phillips, M., Cantley, L.C. 2007. Protein Sequences from Mastodon and Tyrannosaurus Rex Revealed by Mass Spectrometry. Science, 316: 280-285.

Bergmann, U., Manning, P.L., Wogelius, R.A. 2012. Chemical mapping of paleontological and archeological artifacts with Synchrotron X-Rays. Annual Review of Analytical Chemistry 2012. 5: 361-89.

Bern, M., Phinney, B.S., Goldberg, D. 2009. Reanalysis of *Tyrannosaurus rex* Mass Spectra. Journal of proteome research, 8(9): 4328–4332. doi:10.1021/pr900349r.

Brink, K.S., Reisz, R.R., LeBlanc, A. R. H., Chang, R.S., Lee, Y.C. Chiang, C.C., Huang, T., Evans, D.C. 2015. Developmental and evolutionary novelty in the serrated teeth of theropod dinosaurs. Scientific Reports, 5: 12338. DOi: 10.1038/srep12338.

Gallagher, W. 2009. FTIR Analysis of protein structures. https://www.chem.uwec.edu/chem455_S05/Pages/Manuals/FTIR_of_proteins.pdf.

He, Z.K. 2011. Underwater archaeology and landbridge Fauna of Taiwan, To discuss and evaluate the importance of Taiwan's underwater assets based on the paleontological fossils retrieved from Penghu Trench in the past 10 years. Scientific Development, 2011(458). (In Chinese). (何傳坤. 2011. 水下考古與台灣陸橋動物群，依據近10年來在澎湖海溝撈獲的古生物化石，探討和評估台灣水下資產的重要性. 科學發展, 2011年2月，458期.）

Huang, T.D., Shaw, J.F., Zheng, L., Huang, C.L., Chang, Y.L., Yang, C.W. 2010. Ediacaran macro body fossils. Nature Precedings, Published: 07 May 2010. https://doi.org/10.1038/npre.2010.4423.1

Jackson, F.D., Horner, J.R., Varricchio, D.J. 2010. A study of a Troodon egg containing embryonic remains using epifluorescence microscopy and other techniques. Cretaceous Research, 31: 255–262.

Lee, Y.C., Chiang, C.C., Huang, P.Y., Chung, C.Y., Huang, T.D., Wang, C.C., Chen, C.L., Chang, R.S., Liao, C.H., Reisz, R.R. 2017. Evidence of preserved collagen in an Early Jurassic sauropodomorph dinosaur revealed by synchrotron FTIR microspectroscopy. Nature Communications, 8,14220. DOI: 10.1038/ncomms14220.

Lindgren J., Uvdal P., Engdahl A., Lee, A.H., Alwmark, C., Bergquist, K.E., Nilsson, E., Ekstro¨m, P., Rasmussen, M., Douglas, D.A., Polcyn, M.J., Jacobs, L.L. 2011. Microspectroscopic evidence of Cretaceous bone proteins. PLoS ONE, 6, e19445.

MacDougall, M.J., Tabor, N.J., Woodhead, J., Daoust, A.R., Reisz, R.R. 2017. The unique preservational environment of the Early Permian (Cisuralian) fossiliferous cave deposits of the Richards Spur locality, Oklahoma. Palaeogeography, Palaeoclimatology, Palaeoecology, 475: 1–11.

Martill, D.M., Unwin, D.M. 1997. Small spheres in fossil bones: blood corpuscles or diagenetic products? Palaeontology, 40(3): 619-624.

NIST Calcium Carbonate (Calcite), Infrared Spectrum, https://webbook.nist.gov/cgi/cbook.cgi?ID=B6004659&Mask=80#IR-Spec

Peterson, J.E., Lenczewski, M.E., Scherer, R.P. 2010. Influence of microbial biofilms on the preservation of primary soft tissue in fossil and extant Archosaurs. PLoS ONE, 5(10): e13334. https://doi.org/10.1371/journal.pone.0013334.

Reisz, R.R., Huang, T.D., Roberts, E.M., Peng, S.R., Corwin, S., Stein, K., LeBlanc, A. R. H., Shieh, D.B., Chang, R.S., Chiang, C.C., Yang, C.W., Zhon, S.M. 2013, Embryology of Early Jurassic dinosaur from China with evidence of preserved organic remains. Nature, 496: 210-214. doi:10.1038/

nature11978.

Sasaki, J., Lanyi, J.K., Needleman, R., Yoshizawa, T., Maeda, A. 1994. Complete identification of C=O stretching vibrational bands of protonated aspartic acid residues in the Difference Infrared Spectra of M and N intermediates versus bacteriorhodopsin. Biochemistry, 33: 3178-3184.

Schweitzer, M.H., Horner, J.R. 1999. Intravascular microstructures in trabecular bone tissues of *Tyrannosaurus rex*. Annales de Paleontologie, 85(3): 179-192.

Schweitzer, M.H., Marshall, M., Carron, K., Bohle, D.S., Busse, S.C., Arnold, E.V., Barnard, D., Horner, J.R., Starkey, J.R. 197. Heme compounds in dinosaur trabecular bone. Proceedings of the National Academy of Sciences of the United States of America, 94: 6291–6296.

Schweitzer, M.H., Suo, Z.Y., Avci, R., Asara, J.M., Allen, M.A., Arce, F.T., Horner, J.R. 2007. Analyses of soft tissue from *Tyrannosaurus rex* suggest the presence of protein. Science, 316(5822): 277-280. DOI: 10.1126/science.1138709.

Schweitzer, M.H., Wittmeyer, J.L., Horner, J.R. 2007. Soft tissue and cellular preservation in vertebrate skeletal elements from the Cretaceous to the Present. Proceedings of the Royal Society B, 274: 183-197.

Schweitzer, M.H., Wittmeyer, J.L., Horner, J.R., Toporski, J.K. 2005. Soft-tissue vessels and cellular preservation in *Tyrannosaurus rex.* Science, 307(5717): 1952-1955

Schweitzer, M.H., Zheng, W.X., Organ, C.L., Avci, R., Suo, Z.Y., Freimark, L.M., Lebleu, V.S., Duncan, .B., Heiden, M.G.V., Neveu, J.M., Lane, W.S., Cottrell, J.S., Horner, J.R., Cantley, L.C., Kalluri, R., Asara, J.M. 2009. Biomolecular characterization and protein sequences of the Campanian Hadrosaur B. canadensis. Science, 324(5927): 626-631. DOI: 10.1126/science.1165069

Schweitzer, M.H., Johnson, C., Zocco, T.G., Horner, J.R., Starkey, J.R. 1997. Preservation of biomolecules in cancellous bone of *Tyrannosaurus rex.* Journal of Vertebrate Paleontology, 17(2): 349-359. https://doi.org/10.1080/02724634.1997.10010979

Verma, D., Katti, K., Katti, D. 2006. Photoacoustic FTIR spectroscopic study of undisturbed nacre from red abalone. Spectrochimica Acta Part A, 64: 1051-1057.

Comments by Robert Burne:

The field of Organic Geochemistry presents overwhelming evidence for organic materials being preserved in seemingly "lifeless inorganic rock".

I recommend the authors review the publications of organic geochemists such as Roger Summons or Jochen Brocks and redraft their paper to highlight the use of FTIR Spectra as an alternative method for the identification of fossilized organic material.

Comment by Ya-Sheng Wu:

What is the probability that organic matter is preserved in body fossils, what is the composition of the preserved organic matters, and what is the relationship between the composition and preservation conditions? These are questions that need further study.

Innovation scored by: Robert Burne, Hua-Xiao Yan, Dong-Jie Tang, GW Hugheson, Fritz Neuweiler, Adrita Choudhuri, Ya-Sheng Wu.

Innovation score (0-5): 0+5+5+5+1.5+0+5=3.1

Detailed reviewed by: Ya-Sheng Wu, Santanu Banerjee, Giorgio Bianciardi, Anonymous reviewer 1, Hua-Xiao Yan, G. Wyn Hughes.

Published on: 6 January, 2022