Collagen Remains in Palaeotherium Bone from the Isle of Wight (UK)

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A QUESTION OF COLLAGEN

Analysis of original biomolecules in ancient bone, including fossils, continues to grow. Once thought of as soft parts preserved as mere impressions, new techniques reveal original biomaterials including whole tissues that persist in ancient and fossil samples of various taxa. A range of techniques are used including LC-MS/MS, microscopy, RAMAN and FTIR being prominent. There are now over 85 publications that report soft tissue (including collagen remnants) being present in ancient fossil bone.[1]

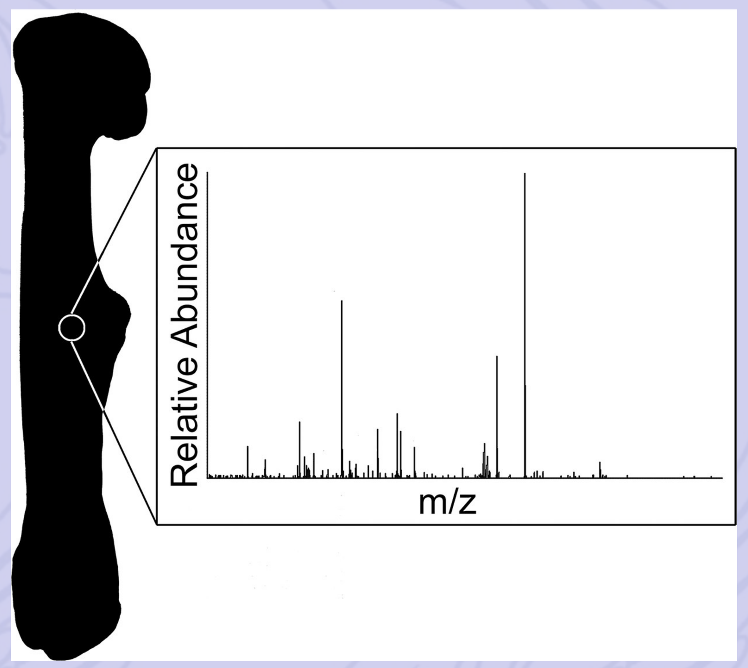


Fig 1. High-resolution MS results from Brachylophosaurus canadensis bone, showing the apparent presence of collagen.[5]

The question of whether collagen is present in ancient bone can be controversial, as it calls into question either the ages assigned to ancient bone or the current understanding of collagen decay rates. The presence of collagen in ancient bone is therefore both contended and contested by researchers.[2, 3] Some, such as Schweitzer, continue to maintain that Type 1 collagen has been identified in ancient bone, up to the Cretaceous,[4,5] while others claim that the results are contamination from modern organics, particularly from microbial communities, and that only 1% of bone collagen will remain after less than one million years even in *"an optimal burial environment*.".[6,7]

However, one noticeable area that lacks data is the sedimentological aspect of the ancient bone specimens used, e.g. location, excavation details, geological sequence context, matrix context, and specimen preservation details. This study uses LC/MS following trypsin digestion to extract collagen remains in a sample of Palaeotheriid jawbone that was excavated from the Isle of Wight (UK), containing sedimentological data sets and 3D Scan/model of the fossil. LC/MS for collagen analysis in ancient bone, compared with optical techniques provides the ability to see trends in original biomolecule preservation when comparing geological context.[8]

PALAEOTHERIUM JAWBONE FROM THE ISLE OF WIGHT, UK

A *Palaeotherium* jawbone was excavated from the Hamstead Member Clays, Bouldnor Formation, Isle of Wight (IoW), UK. The location is significant as it borders the *Grande Coupure*, a major terrestrial faunal turnover recorded in Eurasia associated with the overall climate shift at the Eocene–Oligocene transition.[9] This makes the jawbone relevant to many branches of science, including studies in palaeoclimatology, sedimentology, mass extinctions, and taxonomy.

Location & Geological Context

Fig 2. Location context. The British Isles, with the IoW (A). The IoW, with the Hamstead area (B). The precise location of the jawbone (C).

The South Coast of the UK is famous for its fossil deposits, such as Dorset's 'Jurassic Coast', home of famed Victorian fossil hunter, Mary Anning. The Isle of Wight has wide geological diversity, including deposits from the dinosaurian Cretaceous, Eocene, and Oligocene. The North-West of the island contains some of the most abundant Palaeogene fossil deposits in the UK.

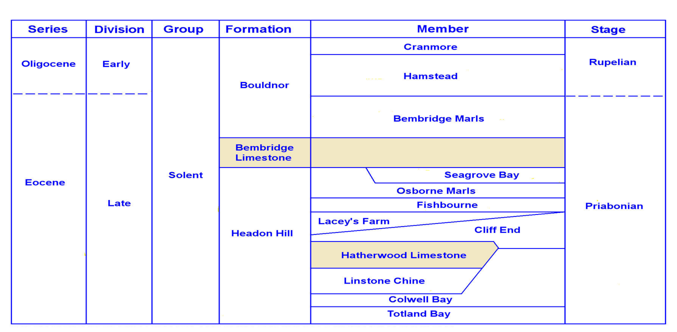


Fig 3. Late Palaeocene Sequence Stratigraphy of the Isle of Wight.[10] Note the Eocene/Oligocene crossover at the Hamstead Member. This marks a major ecological change and extinction in the fossil record, making this location significant in palaeoclimatological studies.

Sedimentological Data

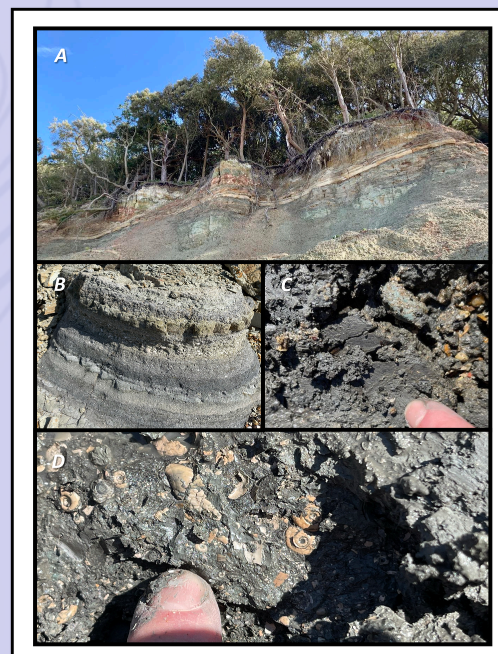


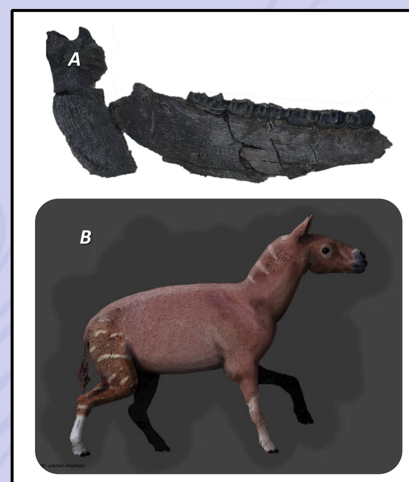
Fig 3. Photographic details of excavation.

The jawbone was excavated from foreshore-level deposits, having been exposed during the previous tide. This is common along the Hamstead area of the IoW, as the Bouldnor formation is predominately soft clay (A). The formation features multiple fossiliferous seams, usually containing compacted aquatic shells (B).

The Bouldnor formation is well known for its diversity of fossils. These include turtle (*Trionyx* and *Emys* sp.), crocodile (*Diplocynodon* sp.), various mammals, and multiple forms of aquatic gastropods and bivalves. Fossil plants are also common.

During excavation, full sedimentological details were recorded. The jawbone was found in 9 separate pieces, but still in original form and location, indicating breakage after death and burial, and was oriented in a NE/SW direction (C). Note also the presence of *Planorbina* shells, an aquatic gastropod. This indicates a water-based burial for the *Palaeotherium* (D).

Palaeotherium Species



There is relatively little published material on *Palaeotherium*, mostly by 18th & 19th century scientists Cuvier and Owen. The lack of material on these creatures means there is still debate over their place in taxonomy.[9] Once believed to be related to modern-day tapirs, they are now viewed as a sister taxon to Equidae (B).

By working with the Dinosaur Isle Museum (IoW) and the Natural History Museum, London, the excavated jawbone (A) was confirmed to be *Palaeotherium sp.*, with a strong likelihood of it being *P. muehbergi*, although this research is ongoing.

***Fig 4.** Excavated & reconstructed jawbone (A), and Palaeotherium reconstruction (B), Humberto Sorrano, 2008 .*

3D SCANNING & SAMPLE PREPARATION

The jawbone had minor repairs performed on major breakages, before being kept at a low temperature and sealed environment, to both aid preservation, and avoid contamination. In addition, using new technology from Augmented Reality programs, a 3D scan was made of the jawbone. This aids in analysis, as well as research outreach, both allowing for the jawbone to be studied and shared around without any risk to the original specimen. The 3D scan also allows for easier collaboration between institutions involved with this research, and models of the jawbone can be passed around collections with ease.

***Fig 5.** 3D scan of Palaeotherium jawbone, with scale for reference.*

A 5g sample was taken from areas uncontaminated by repairs and stabilisation on the lower ramus of the jawbone. The sample was ground into a fine powder (<50 microns) in a laboratory environment using instruments sterilised with high purity ethanol alcohol. Masks, gloves and coats were worn to avoid any potential contamination to the sample.

The ground sample was transferred to a sealed sample aliquot and kept at a low temperature (< 0 °C) until use.

FTIR

Fourier-Transform Infrared Spectroscopy

Attenuated Total Reflectance – Fourier-Transform Infrared Spectroscopy (ATR-FTIR) was performed on powdered samples of the *Palaeotherium* jawbone, taken from the lower ramus. The tests were done on a Bruker Vertex 70© equipped with Deuterated Lanthanum α Alanine doped TriGlycine Sulphate (DLaTGS) detector. Spectra in the range 4,000 to 600 cm^{-1} were collected and analysed with OPUS software.

The inorganic component of the bone is largely comprised of calcium phosphate (Ca_3PO_4) Antisymmetric stretching of PO_4 occurs between approximate wavenumbers 1,000 to 1,100 cm-1. The organic component comprises mostly type I collagen in fresh bone. The triple right-handed helix consists of three left-handed α-polypeptide chains linked by repetitive interstrand amide–amide hydrogen bonds, and this gives collagen its durability. An FTIR spectrum shows a peak for amide I group (carbonyl, C=O) absorption around 1650 cm^{-1}.[11] Peak intensities correspond to the part of the molecule absorbing the energy at a certain frequency. The peak ratio for carbonyl over phosphate (indicated herein by "CO/P") is usually used as a proxy for collagen presence.[12] Our results show just such a peak for amide I group (carbonyl, C=O) absorption around 1650 cm^{-1}.

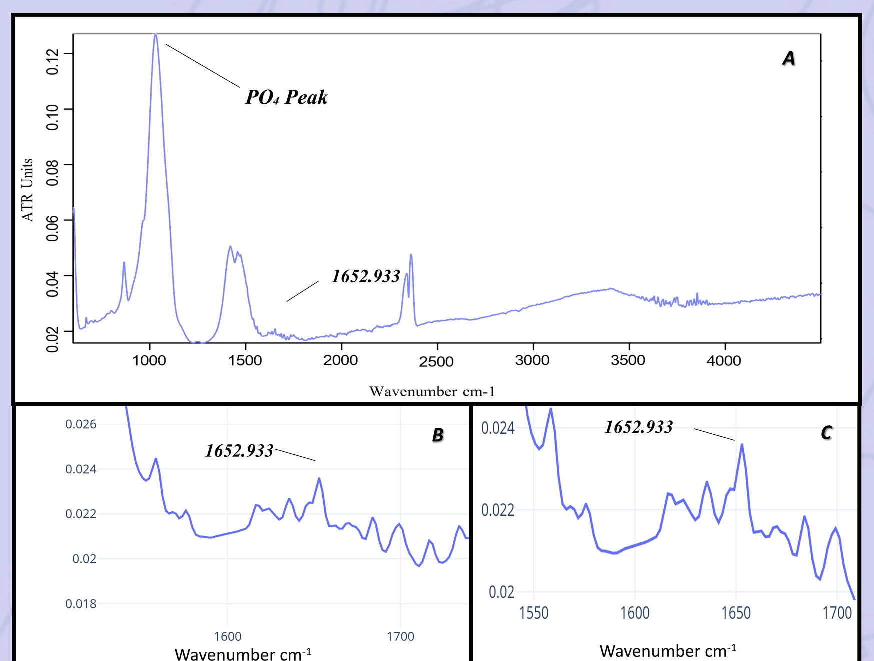


***Fig 6.** Graphical results of ATR-FTIR performed on the Palaeotherium jawbone. 16 sample scans were performed to achieve this data. (A) shows the full spectra, including the antisymmetric stretching of PO_4 with a peak at around 1650 cm^{-1}. When scaled (B/C), this peak ratio (at 1652.933) for carbonyl over phosphate strongly indicates the presence of original biomolecules within the bone sample.*

LC-MS

Liquid Chromatography – Mass Spectrometry

LC-MS analysis of the *Palaeotherium* jawbone was completed in the Materials Innovation Factory (University of Liverpool). Approximately 1 mg of lyophilized trypsin powder was transferred to labelled glass vials and dissolved in 200 µl of 25 mM ammonium bicarbonate at 35 °C. Approximately 20 mg of each bone sample was added, and the samples were bath-sonicated for 30 minutes. Formic acid was added for acidification, and the samples were mixed and centrifuged to pellet undissolved material. Aliquots of the resulting solutions were injected onto a reversed phase UPLC column (Waters™ Acquity UPLC BEH, 100 x 2.1 mm, 1.7 µm particle size) at 37 °C and the column effluent was directed at an electrospray ionization source connected to QToF mass spectrometer (Waters™ Xevo G2-XS) scanning in the positive mode with a 3.3 kV capillary voltage.

Following trypsin digestion and analysis by LC-MS, the results from the *Palaeotherium* jawbone were compared with LC-MS/MS tests performed on modern turkey bone (*Meleagris gallopavo*), dating from 2022 with high collagen content, dried in oven prior to digestion and MS analysis.

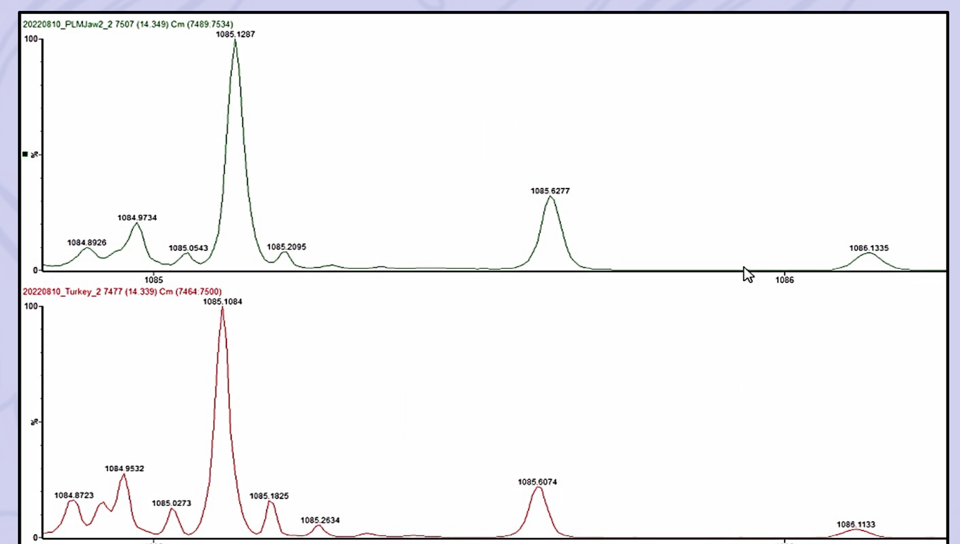


***Fig 7.** Results from HP-LC MS showing Palaeotherium jawbone peaks (green) compared to modern turkey bone peaks (red). The y-axis shows relative concentration, and the x-axis shows mass to charge (m/z) numbers.*

The results show strong correlation between two samples, with 6 – 8 peaks in the mass range of 1084 – 1086 occurring at the same m/z value as those in modern bone. The relative heights of the corresponding peaks from both sample results (Palaeotherium sp. and Meleagris gallopavo) are also in a similar ratio, howbeit at a much-reduced signal intensity. The MS peaks from the modern turkey bone results correspond to type 1 collagen, strongly indicating the presence of collagen in the Palaeotherium jawbone sample also.[13]

CONCLUSIONS

Collagen is a principal component of animal and human bone, being the main structural protein in extracellular matrix.[14] The presence of collagen in ancient bone (particularly fossil bone) is of particular current interest and has been both claimed and contested. This study provides additional evidence from ATR-FTIR and LC/MS following protein digestion of the presence of type 1 collagen in palaeontological bone. The apparent presence of collagen in the *Palaeotherium* jawbone makes this the first experimental observation of original biomolecules within fossil bone from the UK. The additional sedimentological data now allows for comparison with other results from ancient (fossil) bone from different geological sequences, including the Cretaceous.

REFERENCES

1. Thomas B, Taylor S, *Expert Rev Proteomics*. 16 (11-12): 881-895, 2019
2. Avci, et. al. *Langmuir*, 21(8): 3584-3590, 2005
3. Buckley, M, Herman J, *Int Journal of Osteoarchaeology*. 29 (6): 1051-1059, 2019
4. Schweitzer, M. H., et al. *Journal of Vertebrate Paleontology*. 14 (3): 45-46, 1994
5. Schroeter, Elena R., et al. *Journal of Proteome Research*. 16 (2): 920–32, 2017
6. Saitta, Evan T., et al. *eLife*. (8): 2019
7. Buckley M, Collins M, *Antiqua*. 1 (1), 2011
8. Cappellini E, et. al. *Annual Review of Biochemistry*. 87 (1): 1029–1060, 2018
9. Hooker, J, *The Micropalaeontological Society,Special Publications*: 147–215, 2010
10. Insole A, Hutt, S, *Zoological Journal of the Linnean Society*. (112) 1998
11. de Campos Vidal, & Mello, M. L. S. *Micron*. 42 (3): 283–289, 2011
12. Paschalis, et. al. *Clinical Orthopaedics and Related Research*. 469 (8): 2170–2178, 2011
13. Hill R, et. al. *Molecular & Cellular Proteomics*. 14(7): 1946–1958, 2015
14. B. Thomas et al. *Bone Reports*. (7): 137-144, 2017

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