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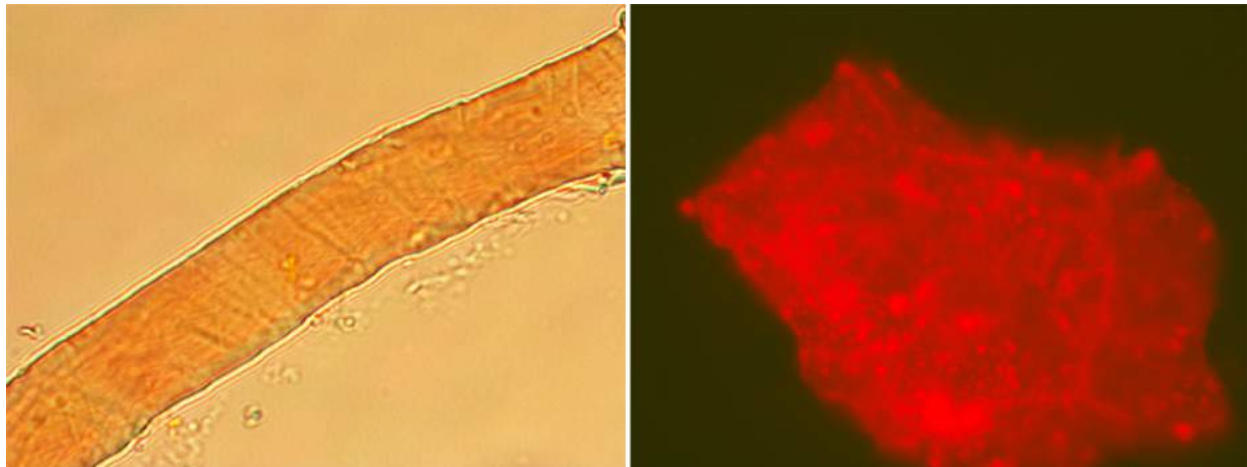
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Long-dead dinosaurs support new life

Abstract: --- Some researchers think that ancient proteins and other soft tissues can be preserved in fossilized dinosaur bones. Our recent work using updated methods failed to detect evidence for ancient protein, but did discover a unique microbial community living within buried fossils.



*Image credits: A vessel-like structure (left) and DNA-stained fibrous mass (right) from demineralized dinosaur bone.
Image credit: Evan T. Saitta.*

Modern scientific equipment has revolutionized the study hidden life. Advances genetic sequencing allows us to discover mysterious worlds of diverse microbes in Earth's harshest environments or within our own bodies.

Life is also hidden to us through time. Studying long extinct creatures can be challenging. Speculation must be separated from evidence-based assertions. Modern chemical analyses have opened new lines of evidence and research. Protein and DNA sequences can resolve evolutionary relationships, and detecting fossil pigments allows us to reconstruct dinosaur colors.

Some organic molecules break down faster than others. DNA is the least stable, the oldest coming from 750,000-year-old bones in permafrost. However, steroids can persist for hundreds-of-millions of years. Some have suggested that biological structures and molecules, thought to quickly degrade, can preserve in dinosaur fossils over 66 million years old, including collagen protein and blood vessels in bone.

These dinosaur soft tissue reports were based on some mass spectrometry protein sequences and the use of antibodies, immune system proteins that can bind to specific proteins

of interest. Although these reports garnered much scientific and popular interest, other scientists were skeptical of such miraculous preservation. Some suggested that the sequence data was statistically insignificant or possibly laboratory contamination. Others, like myself, suggest that the antibodies were not specifically binding to the purported proteins of interest, but to other components of the fossils or even glues used to prepare them.

One alternate explanation for these soft tissues is that they represent a biofilm, a structure produced by a bacterial colony and its secretions, forming within cavities of the bone mineral. Accordingly, we tested whether the organic material inside dinosaur bone was ancient and whether it was deriving from within the bone itself.

To do this, I had to excavate fresh fossil bone. I went to Dinosaur Provincial Park in Canada to a dense bone bed of the horned *Centrosaurus*. After removing overlying sediment, I used sterilized equipment to collect the fossils and surrounding sediment in as aseptic of a manner as possible to limit any alteration of the specimens – dig techniques have changed little in the last century and any environmental sample cannot be collected in the sorts of sterile conditions of the lab. The bones were kept refrigerated as much as possible until they could be analyzed in several different labs.

Our team then applied a battery of chemical analyses. We found recent organic material in the bone at elevated concentrations compared to the surrounding sediment, consistent with a modern microbial community, but found no evidence for ancient dinosaur protein.

When the mineral component of the bone is dissolved in acid, the resulting structures had some mineralization typical of a biofilm and lacked the elemental composition of collagen in modern or thousands-of-year-old bone under X-ray spectroscopy, and some vessels showed infilling. The chemical fingerprint observed using pyrolysis-gas chromatography-mass spectrometry lacked clear markers of protein-rich material. The organic carbon was not radiocarbon dead when studied with accelerator mass spectrometry, unlike the expectation if the organic material was dinosaurian, and was in elevated concentrations in the bone. Liquid chromatography revealed that the amino acid (i.e., the constituents making up a protein sequence) profile not match that from collagen and the amino acids were not altered in their structure as in ancient proteins.

A lack of evidence for dinosaur protein and multiple signs of recent organic material at elevated levels in the bone compared to the surrounding sediment meant that detailed study of any potential microbial community was warranted. Not only was DNA concentrated in the soft tissue, but RNA sequencing revealed a unique bacterial community in the bones unlike any described before.

This unique community, likely drawn to the phosphate nutrients of bones, might explain reports of dinosaur soft tissues and is helping us understand subsurface life. There is a sort of awe in thinking about how a dinosaur that died 75 million years ago is providing a home to modern life under our feet.