SOFT TISSUES, HARD EVIDENCE

Paleontology is the study of fossils. Although most people think of fossils as only being bones and shells, this research field also focuses on fossilized soft tissues, such as blood vessels. Using fine-scale techniques allows us to investigate the internal anatomy of Triassic vertebrates.

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Fossilized soft structures such as blood vessels, cells, or even whole internal organs are occasionally discovered in the fossil record alongside bone remains. They are a fascinating resource for research, since they can tell us far more about the biology of extinct animals than their bones or shells. Although such finds are exceptionally rare, in the last two decades far more discoveries of mineralized soft tissues have been reported than in the entire two-hundred-year-long history of paleontology. This is almost certainly due to the rapid development of sophisticated analytical methods making it possible to conduct unprecedentedly intricate studies of fossils, going down as far as the nanoscale. Now scientists may look inside the bones and get to know how they grew, decayed, and were fossilized. In certain conditions, like rapid mineralization with iron access, the remnants of soft tissues may be preserved.

Whence the iron?

Iron is one of the most abundant elements on Earth, comprising around 6% of the Earth's crust. It is also commonly found in many living organisms, and it

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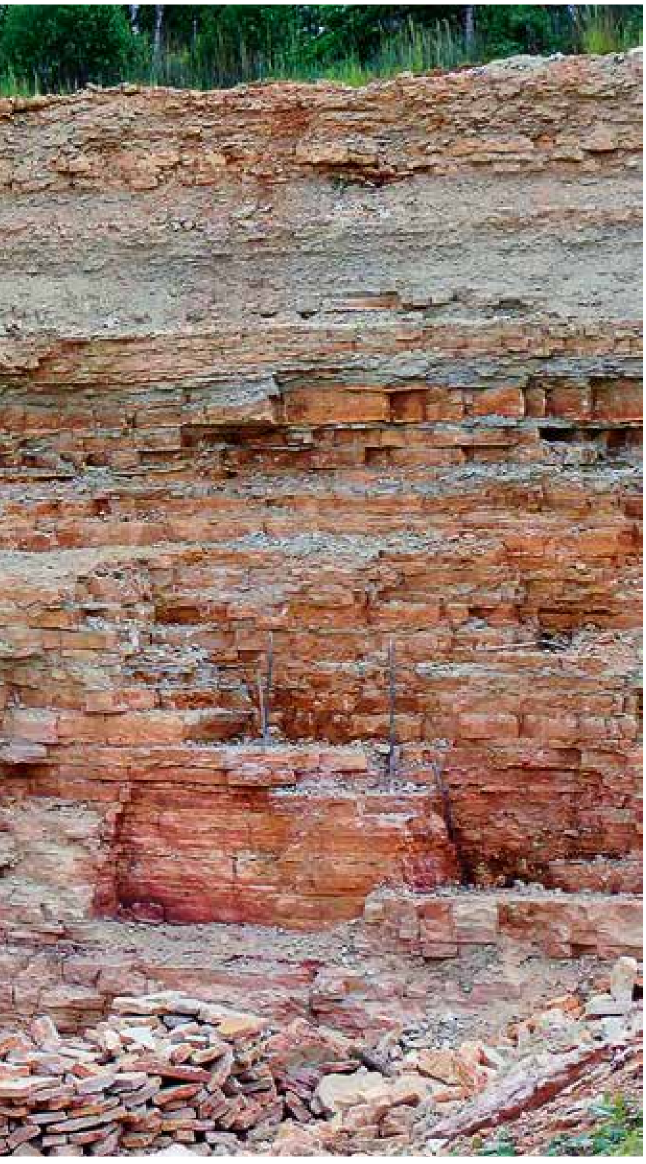
The restoration of nothosaurs at the GEOsfera Nature and Geology Education Centre in Jaworzno (left) and blood vessels extracted from a nothosaur fossil (right); the vessels are 10×20 micrometers thick.

Southern wall of the quarry in Żyglin.

forms a part of animal proteins such as hemoglobin and myoglobin. When structures reminiscent of blood vessels or cells were for the first time observed within fossilized bones, they were noted for their reddish-brown coloring, clearly suggestive of blood residuum. Further research determined that the color is associated with iron oxides, mainly hematite and goethite. Scientists speculating about the origins of this iron expressed divergent opinions. Some claimed that the iron originated directly from decomposed blood proteins, while others countered that it permeated into the bones from outside as a result of secondary decay processes. The Polish physician and acclaimed researcher Professor Roman Pawlicki, who studied vertebrate fossils from the Gobi Desert, was the first scientist who utilized molecular methods to study fossilized soft tissues. The American paleontologist Dr. Mary Schweitzer from the University of North California bases her studies on methods developed by Prof. Pawlicki. She and her team noted that organic matter can be "fixed" in a crystalline lattice of iron oxides, which acts like a time capsule. On the basis of her own experimental research, Dr. Schweitzer concluded that the compounds clearly preserve original organic residues which comprise soft tissues.

Acid reveals all

Prior to the publication of the results of our research, many examples of fossilized soft tissues had been described from bones of fossilized animals (mainly dinosaurs) of different geological ages, stretching from almost present times as far back as the Early Jurassic. The mineralized soft tissues that have been found at fossil sites in Upper Silesia document preserved organic matter in bones of vertebrates dating back even 50 million years further.



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The research I have conducted as part of my PhD work has involved studies of bone remains of nothosaurs (*Nothosaurus marchicus*) from the Middle Triassic, dating back nearly 250 million years and commonly found in quarries of Upper Silesia. I observed high amounts of iron oxides in the marrow cavities and vascular channels. Investigation of the conditions in which the nothosaur bones were preserved was one of the aims of my research project financed by the National Science Centre. Being aware of how blood vessels can survive in fossilized bones, I decided to investigate further, and dissolved a few bone fragments in a weak acid to see if any residue would remain. During the digestion process, I observed an increasingly visible network reminiscent of blood capillaries emerging from the bones. At first, it was not clear whether they were original vessels or secondary, iron-mineral fillings of empty vascular channels, which mimic real blood vessels. Although the size and location of the structures within bones pointed towards their origins as blood vessels, their walls were highly blurred and lacked any clear structure which I might have expected. To confirm that I was in fact dealing with fossilized soft tissues, I needed proof in form of their original chemical composition – assuming it had been preserved.

Quarter of a billion years – and more?

To help solve this, we set up an interdisciplinary team to study the composition of samples and the degree of molecular preservation of proteins. A significant part of the research was conducted at the

Silesian Centre for Education and Interdisciplinary Research in Chorzów, where we used sophisticated equipment such as a Time-of-Flight Secondary Ion Mass Spectrometer (ToF-SIMS). The method determines precise ion mass, which in turn helped us to identify amino acids in the blood vessels isolated from the fossilized bone. These particular amino acids are rarely found in bacterial and fungal proteins, but they are the main component of type I collagen, which form the organic part of bones. The presence of the compounds confirmed that we in fact had the blood vessels of a marine reptile living in Silesia around 250 million years ago.

Such positive identification of the remains of ancient organic matter, existing in fossils dating back so many years, serves as encouragement for even more advanced research into the structure and function of proteins in fossil vertebrates.

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Further reading:

Schweitzer M.H. et al. (2014). A role for iron and oxygen chemistry in preserving soft tissues, cells and molecules from deep time. *Proceedings of the Royal Society of London B: Biological Sciences* 281, 20132741. (doi: 10.1098/rspb.2013.2741).

Surmik D. et al. (2016). Spectroscopic Studies on Organic Matter from Triassic Reptile Bones, Upper Silesia, Poland. *PLOS ONE* 11, e0151143. (doi: 10.1371/journal.pone.0151143).

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