

Keratin, proteins from 54 million-year-old sea turtle show survival trait evolution

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Researchers from North Carolina State University, Lund University in Sweden and the University of Hyogo in Japan have retrieved original pigment, beta-keratin and muscle proteins from a 54 million-year-old sea turtle hatchling. The work adds to the growing body of evidence supporting persistence of original molecules over millions of years and also provides direct evidence that a pigment-based survival trait common to modern sea turtles evolved at least 54 million years ago.

Tasbacka danica is a species of sea turtle that lived during the Eocene period, between 56 and 34 million years ago. In 2008 an extremely well-preserved *T. danica* hatchling was recovered from the Für formation in Jutland, Denmark. The specimen was less than 3 inches (74 millimeters) long. In 2013 paleontologist Johan Lindgren of Lund University uncovered soft tissue residues from an area located near the sea turtle's left "shoulder." He collected five small samples for biomolecular analysis.

The shells of modern sea turtle hatchlings are dark colored - this pigmentation gives them protection from aerial predators (such as seagulls) as they float on the ocean surface to breathe. Since <u>turtles</u> are reptiles, and therefore cold-blooded, the dark coloration also allows them to absorb heat from sunlight and regulate their body temperature. This elevated body temperature also allows more rapid growth, reducing the time they are vulnerable at the <u>ocean surface</u>.

The *T. danica* hatchling specimen appeared to share this coloration with



its living counterparts. The researchers observed round organelles in the fossil that could be melanosomes, pigment-containing structures in the skin (or epidermis) that give turtle shells their dark color.

To determine the structural and chemical composition of the soft tissues Lindgren collected and see if the fossil sea turtle did have a dark colored shell, the researchers subjected the sample to a selection of highresolution analytical techniques, including field emission gun scanning electron microscopy (FEG-SEM), transmission electron microscopy (TEM), in situ immunohistochemistry, time-of-flight secondary ion mass spectrometry (ToF-SIMS), and infrared (IR) microspectroscopy.

Lindgren performed ToF-SIMS on the samples to confirm the presence of heme, eumelanin and proteinaceous molecules - the components of blood, pigment and protein.

Co-author Mary Schweitzer, professor of biological sciences at NC State with a joint appointment at the North Carolina Museum of Natural Sciences, performed histochemical analyses of the sample, finding that it tested positive against antibodies for both alpha and beta-keratin, hemoglobin and tropomyosin, a muscle protein. TEM, performed by University of Hyogo evolutionary biologist Takeo Kuriyama, and Schweitzer's immunogold testing further confirmed the findings.

In the end, the evidence pointed to these molecules as being original to the specimen, confirming that these ancient turtles shared a pigmentationbased survival trait with their modern-day brethren.

"The presence of eukaryotic melanin within a melanosome embedded in a keratin matrix rules out contamination by microbes, because microbes cannot make eukaryotic melanin or keratin," Schweitzer says. "So we know that these hatchlings had the dark coloration common to modern <u>sea turtles</u>.



"The data not only support the preservation of multiple proteins, but also suggest that coloration was used for physiology as far back as the Eocene, in the same manner as it is today."

More information: Johan Lindgren et al, Biochemistry and adaptive colouration of an exceptionally preserved juvenile fossil sea turtle, *Scientific Reports* (2017). DOI: 10.1038/s41598-017-13187-5

Provided by North Carolina State University

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