15 July 2015

A tough egg to crack: Oldest lizard embryos discovered in fossil eggs

Synchrotron X-ray scanning has solved the mystery surrounding the 125 million-year-old tiny fossil eggs discovered in Thailand in 2003. A first analysis of the eggshells concluded that they belonged to a small theropod dinosaur, or possibly a primitive bird.
Recent experiments carried out at the ESRF, the European Synchrotron, Grenoble, France, by an international team of scientists have swept aside this conclusion and found evidence to solve this palaeontological mystery. Synchrotron imaging of the embryonic skeletons preserved in these eggs revealed that they are in fact anguimorph lizards. They are the oldest lizard embryos ever discovered in fossil eggs.
Aguimorph lizards also include slow-worms, komodo dragons and extinct mosasaurs.
The discovery of anguimorphs in hard-shelled eggs comes as a considerable surprise. So far, only geckos were known to lay hard-shell eggs and most lizards lay soft-shelled eggs. The identification of the Thai embryos has an important scientific impact, providing essential information for our understanding of the evolution of reproduction in lizards and the diversity of eggs produced by different lineages of lizards. The results are published 15 July 2015 in PLOS ONE.

Synchrotron X-ray scanning of fossils is playing an increasingly important part in palaeontological research, helping to visualise hidden inner structures, with no-destructive solutions, or to virtually extract bones from surrounding rock. Recently, an international team used this technique at the European Synchrotron (ESRF) in Grenoble, France, to solve the mystery of small fossil eggs excavated in Thailand.

The sedimentary rock strata of the Sao Khua Formation (north-eastern Thailand) were deposited in a flood plain during the Early Cretaceous, some 125 million years ago. From one of the numerous localities surveyed by the international team that was led by the Thai palaeontologist Varavudh Suteethorn, a batch of minute eggs were unearthed. “We discovered these eggs over the course of several fieldtrips, between 2003 and 2005”, **says Varavudth Suteethorn**, **(Mahasarakham University, Thailand)** “they were very tiny eggs, I had never seen anything like them”.

These eggs, which are the size of a sparrow’s egg, consist of a solid calcareous eggshell covered by tiny knobs. A preliminary study of the eggshell structure suggested that these eggs may have been laid by a small carnivorous dinosaur, or a very primitive bird. However, minute embryonic bones were visible in the rocky matrix filling some of the eggs. It was clear that if the bones could be identified the question of what animal had laid the eggs could be answered.

To study these embryos, the team applied cutting-edge synchrotron X-ray methods such as X-ray synchrotron tomography. This scanning technique offers non-destructive probing of fragile or valuable objects and samples. The fossil eggs were flown from the Sirindhorn Museum at Sahatsakhan in Thailand to Grenoble (France) to be scanned using the ultra-bright X-rays produced at the European Synchrotron (ESRF). Each egg was scanned at very high resolution (5 microns or 0.005 mm, the width of a strand of spider silk) allowing the research team to observe the finest details of the fossil bones inside the eggs.

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As always in palaeontology, the analysis of these results was a long process. Using 3D software, each bone or fragment of bone, in each embryo, was analysed individually to create a 3D reconstruction. **Dr. Vincent Fernandez, (ESRF/Wits University), head of the research team,** who led this tedious task explains: "*each time we had a new bone virtually extracted, we had a new clue about the nature of these embryos. We created a 3D print-out of each piece of the skeleton, and it took us several months of work before we could solve this scientific enigma*."

The eureka moment came during another unrelated experiment at the ESRF. **Dr. Paul Tafforeau (ESRF) recalls** "*a team came to study the inner ear of modern lizards and after their experiment they were looking at a 3D reconstruction of a common wall lizard. I immediately recognised one of the bones that we also had seen in the fossil embryos from Thailand: it was the quadrate, a bone for the articulation of the jaw*." After finding that cornerstone piece, all the elements of the skeleton fitted together like a jigsaw puzzle.

The embryos were neither dinosaurs nor birds, but they were something exciting: the oldest lizard embryos ever discovered in fossil eggs. On the basis of their skeletal anatomy, it could be determined what kind of lizards they were. As the embryos died close to hatching, their skeletons were already quite developed. From the close inspection of the morphology of each bone, the team was able to recognize numerous diagnostic features. These embryos were neither dinosaur, nor birds but lizards from a group called anguimorph**.** This group is nowadays represented by several species including alligator lizards, glass lizards and monitor lizards, but surprisingly none of these animals lay eggs similar to those from the Cretaceous of Thailand. "In fact, hard-shelled eggs like those from Thailand are only known in geckos", explains **Vincent Fernandez.**

Left: Photograph of one of the eggs (SK1-1) from Phu Phok (Thailand). The cracked eggshell is in black. Credit E. Buffetaut
Right: Artist's impression of the anguimorph lizard embryo in its egg. Credit Vladimir Rimbala

The hard-shelled eggs of geckos were thought to be an evolutionary novelty within modern lizards. Consequently, all fossil eggshells resembling those of modern geckos were tentatively attributed to this group of wall-climbing lizards.

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This new discovery, however, shows that the evolution of lizard reproduction is more complicated than previously thought. **Jean-Claude Rage (CNRS/MNHN, Paris)** adds "the first attested case of lizard embryos fossilised inside their eggs has led to quite surprising conclusions."

Dr Tafforeau concludes "*thanks to the unique possibilities of the ESRF for high quality imaging of fossils developed during the last decade, these remarkable specimens still remain intact , protected by their mineralised matrix*."

Reference: Evidence of egg diversity in squamate evolution from Cretaceous anguimorpha embryos, Vincent Fernandez, Eric Buffetaut, Varavudh Suteethorn, Jean-Claude Rage, Paul Tafforeau and Martin Kundrát, PLOS ONE 15 July 2015
DOI: http://dx.plos.org/10.1371/journal.pone.0128610

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Multimedia:
A zipped folder with still images, animations and captions is online under:
http://ftp.esrf.fr/tmp/PhuPhok_embryos/

A B-roll with video footage in HD broadcast quality is online under:
http://ftp.esrf.fr/tmp/PhuPhok_embryos/

About the ESRF:
The ESRF – the European Synchrotron – is a large scale international research instrument. It is the world's most intense source of X-rays. The extremely bright light that the ESRF provides to scientists from around the globe enables them to explore matter in many disciplines. Founded in 1988, the ESRF is a model of European and International cooperation with 21 partner countries, of which 13 are Members, and 8 are Scientific Associates. In 2009, the ESRF embarked upon an ambitious renovation programme worth 330 M€, the Upgrade Programme phases I and II. This programme has devised a new generation of synchrotrons and will enable its users to push the limits of scientific exploration of matter.

Multimedia credits:

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Image_01: Photograph of one of the eggs (SK1-1) from Phu Phok (Thailand). The cracked eggshell is in black. Credit E. Buffetaut

Image_02: Photograph of the team looking for fossils at Phu Phok (Thailand). Most specimens are quite small (less than a centimetre) and visible, scattered on the ground, when the sediment is washed out by the rain. Credit R. Amiot

Image_03: Photograph of four fossil eggs from Phu Phok (Thailand). The two eggs that have been intensively investigated using synchrotron imaging are the two larger ones in the middle. Credit V. Fernandez

Image_04: 3D reconstruction of the skull of the anguimorph lizard embryo from Phu Phok. The reconstruction was made using the best preserved bones from two eggs (SK1-1 in yellow; SK1-2 in green) and complemented by generating symmetrical bones when missing. Credit ESRF/V. Fernandez

Image_05: Artist's impression of the anguimorph lizard embryo in its egg. Credit Vladimir Rimbala

Animated_gif: Animation revealing the embryonic bones preserved in the egg. Credit E. Buffetaut/V. Fernandez
(Still version of the animated Gif: Images_01-b; Images_01-c)

Sequence: 3D reconstruction of the anguimorph lizard embryonic skull using bones from the two best preserved eggs. Credit ESRF/V. Fernandez

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