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Experimental mineralization of crustacean eggs leads to surprising tissue conservation: new implications for the fossilization of Precambrian-Cambrian embryos

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Abstract

Phosphatized globular microfossils from the Ediacaran and Lower Cambrian of South China represent an impressive record of early animal evolution and development, however their affinity based on putative embryonic metazoan, bacterial and inorganic features is strongly debated. Understanding key processes and conditions that cause exceptional egg and embryo preservation and fossilization are therefore crucial for a reliable interpretation of their phylogenetic position. Taphonomic experiments on eggs of the marbled crayfish indicate a close link between early mineralization and rapid anaerobic decay of the endochorional envelope, producing different preservational stages of degradation resembling the various decay stages observed in the fossil record. Stabilization of the spherical morphology was achieved by pre-heating of the eggs. Complete surface mineralization occurred under reduced conditions within one to two weeks, with fine-grained brushite ($CaHPO_4 \cdot 2H_2O$) over calcite as the dominating mineral phase. Although the endochorional envelope was not preserved, experiments resulted in exceptional preservation of the embryonic tissue at the cellular level. Thus our findings suggest that the mechanisms of decay, preservation of surface structures, and mineral replacement in the experiment and during fossilization of Cambrian embryos were likely operating at a similar rationale.

1 Introduction

Exceptional three-dimensional preservation of fossilized eggs and embryos from the Neoproterozoic, the Cambrian and the Ordovician provide a tantalizingly direct insight into embryology during the eve of animal radiation (Bengtson and Zhao, 1997; Li et al., 1998; Xiao et al., 1998, 2000; Chen et al., 2004; Steiner et al., 2004a,b; Donoghue and Dong, 2005; Donoghue et al., 2006; Liu et al., 2007; Dong et al., 2010). In particular, the terminal Neoproterozoic Doushantuo Formation (635 to 551 Ma; Condon et al., 2005) of South China has attracted considerable attention, first because fossilized

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algae and embryos occur in nearly rock-forming abundance, and second because radiometric ages of the Doushantuo Formation imply that the putative animal embryos predate the Precambrian-Cambrian boundary (542 Ma) for more than 20 million years. The latter finding in turn would rather confirm hypotheses based on molecular clocks indicating that the last common ancestor of Metazoa appeared in the Neoproterozoic between 676 and 766 Ma (Peterson et al., 2005, 2008).

Fossilized animal eggs and embryos, particularly of the Ediacaran Weng'an and Cambrian Kuanchuanpu biota (Fig. 1), are mainly preserved as diagenetically phosphatized replacements and encrustations in dolostone or limestone (Steiner et al., 2004a; Hubert et al., 2005) and perhaps exhibit the most remarkable variety of early biodiversity with extraordinary preservation of cell geometry and cytological structure. However, the classification and preservational context (taphonomy) of most of these fossils is still the subject of extensive research. To date for the Weng'an embryos, primarily the very earliest cleavage stages have been recovered, leading to the suggestion that the paleo-embryological record may have strong biases on developmental stages preserved (Dornbos et al., 2005; Raff et al., 2006). Furthermore, an alternative interpretation of these microfossils was introduced suggesting that some putative fossil animal eggs and embryos, such as *Megasphaera inornata* and *Parapandorina*, represent solitary and reductively dividing giant vacuolated sulphur bacteria (Bailey et al., 2007a). This intriguing reinterpretation and a recent publication describing helical spheroidal fossils as prehatchling forms (Xiao et al., 2007), has launched an "embryonic identity crisis" debate (Donoghue, 2007; Gostling et al., 2007). Additional data are therefore highly desirable to constrain the proposed interpretations (cf. Hagadorn et al., 2006; Bailey et al., 2007b; Chen et al., 2009).

Understanding the mechanism of decay and soft-tissue preservation through experimental taphonomic analyses, in which the conditions of decay and mineralization are explored experimentally (Briggs, 1995, 2003), is crucial to interpreting the fossil record of early metazoan evolution. Previous experimental studies investigating the controls on the formation of authigenic minerals in association with decaying organic matter

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reported phosphatization of muscle tissue within weeks to few months, associated with a drop in the oxygen level and pH, after the introduction of an anaerobic bacterial community and/or sea floor sediment to shrimp carcasses (Briggs and Kear, 1993a, 1994; Sagemann et al., 1999). The precipitation of either calcium phosphate or calcium carbonate is thereby related to local pH changes, with calcium phosphate being favored as pH decreases from neutral to acidic (Briggs and Wilby, 1996). Only a few studies have noted the experimental mineralization of invertebrate eggs. Experimental phosphatization of eggs was first reported from the modern shrimp *Palaemon elegans*, which was subjected to decay as a complete animal for 20 weeks (Briggs and Kear, 1993a). Later studies, which focused only on the eggs, demonstrated that it was possible to maintain the external shape of *Homarus gammarus*, *Limulus polyphemus*, and *Nephrops norvegicus* egg cases for at least a year, including surface mineralization mainly as calcium carbonate or as a combination of calcium carbonate and calcium phosphate (Martin et al., 2003, 2005). Laboratory decay experiments also achieved the replication of the outer morphology of *H. gammarus* eggs by coating the eggs with pre-existing clay minerals in the presence of metabolizing bacteria (Martin et al., 2004).

None of these studies, however, considered the effects of inherent fragility on the internal morphology and histology of invertebrate eggs. In normal seawater, freshly killed embryos are generally prone to rapid decomposition within a few hours. Embryo preservation for a prolonged time prior to mineralization is thus rather unlikely. Recent studies thus focused on the factors, which affect the decay and preservation of the earliest stages on embryonic and larvae development (Gostling et al., 2008, 2009; Raff et al., 2006, 2008). Four stages of decay can be distinguished (Gostling et al., 2008): (a) The onset of autolysis, (b) some shrinkage within the fertilization envelope, enzymatic breakdown of the cyctoplasm and coalescence of sub-cellular lipids, often associated with the degradation of cell structures through the activity of endogenous and exogenous microbes (cf. Raff et al., 2006), (c) continued degradation of cell structures and breakdown of the fertilization envelope, (d) generation of amorphous organic matter due to the total breakdown of subcellular material. Embryo fossilization can

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therefore be attributed to three processes (Raff et al., 2008): (a) The rapid blockage of autolysis within an anaerobic environment, (b) bacterial invasion and consumption of the embryo, forming a replica that retains cell organization and morphology, and (c) bacterial development of microenvironments inducing changes in embryo chemistry and mineralization potential.

Here, we report a succession of simplified taphonomic experiments on eggs of the parthenogenetic crayfish *Procambarus fallax* f. *virginalis* (Martin et al., 2010), in order to elucidate alternative scenarios for embryo fossilization. We investigated the conditions that promote both the mineralization of invertebrate eggs as well as decay and preservation of organic tissue over a prolonged period of time under non-marine laboratory conditions. Our results show that experimental mineralization of invertebrate eggs can produce different qualities of preservation, which were similar in appearance to what has been documented from the fossil record.

2 Material and methods

2.1 Sample material

Eggs of the parthenogenetic crayfish *Procambarus fallax* f. *virginalis* (Fig. 2; Martin et al., 2010) were taken from living animals that were kept in culture at the Department of Comparative Zoology, Humboldt-University Berlin. Its capability, high fertility, fast growth, unisexuality and isogenic progeny (Scholtz et al., 2003; Martin et al., 2007) have made this species useful for physiological, ecological, evolutionary and genetic studies. Crayfish eggs, as those of decapod crustaceans in general, are considered as being encased by an inner vitelline membrane and a chorion (Winnicki et al., 2004) although the exact sequence and number of layers and their origin is debated (Cheung, 1966). The eggs are rich in yolk and reach diameters up to approximately 1000 µm. Ten defined stages from zygote to hatching (Alwes and Scholtz, 2006) were determined for most of the specimens used in the experiments.

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2.2 Experimental design

Taphonomy experiments (E1 to E7, Table 1) were conducted under different redox and pH conditions. In all experiments, crustacean eggs were embedded in a gel-like medium and transferred to an exsiccator at room temperature and atmospheric pressure. The embedding medium was prepared using analytically-grade synthetic calcite ($CaCO_3$), phosphoric acid (H_3PO_4, 85 %), distilled water and powdered activated carbon. According to Eq. (1) brushite crystals ($CaHPO_4 \cdot 2H_2O$) formed in the gel, which was verified by X-ray powder diffraction (Table 2).

$$CaCO_3 + H_3PO_4 + H_2O \rightarrow CaHPO_4 \cdot 2H_2O + CO_2 \uparrow \quad (1)$$

Prior to the experiments, the eggs were stored in tapwater-filled glass vials at $9 \pm 1\,^{\circ}C$. Experimental series E1 and E2 were conducted to explore the effect of differences in the composition of the embedding medium on embryo preservation and mineralization. Phosphate was supplied in the form of phosphoric acid (H_3PO_4), and brushite. Experiment E1 took place under normal air, whereas experiment E2 proceeded under an N_2-atmosphere. Experimental series E3 to E7 were performed to study the temporal progress of preservation and mineralization. For this purpose, one to four embryos were removed from the experiment every week. After removal of the sampled specimens the exsiccator was refilled with nitrogen. The duration of these experiments was generally between seven and 44 days. The latter experiments were designed applying the excess $CaCO_3$ medium under the N_2-atmosphere. In order to reduce oxidation processes, and thus the decay process of the embryo tissue, powdered activated carbon was added to the sample surfaces in experimental series E3. To explore whether and how initial heating might stabilize the eggs and prevent them from collapsing during fossilization, crustacean embryos were initially heated in distilled water at $80 \pm 1\,^{\circ}C$ for approximately five minutes in experiments E6 and E7.

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2.3 Sample preparation

For scanning electron microscopy analyses, the treated crayfish eggs were first dehydrated in ethanol. Then they were either dried at the critical point (Balzer) and mounted on stubs with adhesive tape or prepared in histological blocs. They were infiltrated overnight in a basic solution of 100 ml Technovit® 7100 and a hardener and subsequently filled in histo-moulds. Histoblocs were then prepared with Technovit® 3040 and coated with silver and carbon.

For histological sections, the ethanol-dehydrated samples of experimental series E7 were infiltrated with a 1 : 1 mixture of araldite epoxy resin and epoxy propane overnight and subsequently transformed to solely araldite for one hour. Samples were then transferred to an araldite-loaded embedding mould and stationed till polymerisation at 70 °C overnight. Histological sections were trimmed applying a rotation microscope (HM 350, Micron), cut into semi-thin sections (2 µm) and transferred to slides. Histological sections of experimental series E7 were stained with toluidin blue (0.5 %) solution.

2.4 Analytical techniques

The mineralogical phase composition of the embedding medium was determined with a Philips PW1729 X-ray diffractometer at the Department of Mineralogy and Petrology of the Technical University Berlin. The device is equipped with a PW1050 goniometer, a Cu-tube with a Ni-filter, a secondary monochromator unit, and a scintillation counter (LiF doped with Tl), operated at 40 kV/20 mA. Measurements were conducted with the mean K_{α}-beam ($[K_{\alpha 1} + K_{\alpha 2}]/2$) at 1.5419 Å between 5–80° (2θ). Measurement time per scan (step size 0.02°) was 2 s. Samples were ground in an agate mortar by adding acetone and spread on a silica-sampling holder. Data were evaluated with the software packages X'Pert High Score and Origin.

The mineralogical phase composition of samples of experimental series E7 was analyzed with a fully-automated STOE STADI P X-ray powder diffractometer, equipped with

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a primary monochromator unit and a 7°-position detector (Gottschalk et al., 1998) at the GeoForschungsZentrum Potsdam, operated at 40 kV/40 mA. Measurements were conducted with a $K_{\alpha 1}$-beam between 5–125° (2θ). The step size was 0.1° and the measurement time was 5–20 s. Samples were ground in an agate mortar and mixed with Elmers white glue, superimposed on a plastic film, which was transferred to a transmission-sampling holder. Mineralized eggs were directly glued on the plastic film. Structural parameters were refined using the software package GSAS for Rietveld analyses.

Calcium and phosphorus content of fresh crayfish eggs were determined on a Varian (Vista-MPX) inductively coupled plasma-atomic emission spectrometer (ICP-AES) at the GeoForschungsZentrum Potsdam. Eggs were taken from mother animals during cell devision stage 5–6 (after Alwes and Scholtz, 2006). They were oven-dried at 105 °C for 24 h. Dried embryos were put in Savillex® beakers which were filled with 10 ml of quartz-distilled ultra pure water, for five hours at room temperature. After rinsing of the embryos two times with ultra pure water, they were dried on a hot plate at 120 °C overnight for dry weight determination. About 2 ml of 7 N HNO_3 was added to the dry mass, which was then vaporized on a hot plate at 160 °C overnight. Since parts of the dried residues were not completely dissolvable, the 7 N HNO_3 step was repeated three times. For chemical analysis, another 2 ml of 1 N HNO_3 was finally added to the yellowish solution.

A Zeiss stereoscope (Stemi SV11) equipped with a Zeiss digital camera (AxioCam HRc) was used to examine the surfaces of the samples. In order to map the topography and determine the chemical composition of the samples, eggs were investigated using scanning electron microscopy (Hitachi S-2700, equipped with a tungsten cathode, operated at 20 kV/500 nA) at ZELMI, Technical University Berlin. Element determination was done applying an EDX system. The detection limit for measured elements was 0.1 weight-%.

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3 Results

We both determined and localized the initial calcium and phosphorus content of fresh crayfish eggs (Table 3). The dry mass contained 120 ppm Ca and 1.3 wt.-% P, comparable to concentrations described for other invertebrate eggs (Martin et al., 2005). Scanning electron microscopic (SEM) mapping of P of an untreated egg in cross-section furthermore revealed an almost uniform distribution of P, with higher initial P concentrations only in the germ area (Fig. 2).

Treatment with excess phosphoric acid (H_3PO_4; Table 1) preserved fresh crayfish eggs neither in an oxygenated (air) nor in a reduced atmosphere (N_2). A few minutes after embedding the dark brown to black embryos became red colored, and tiny blebs occurred on the egg's envelope. After two weeks, the eggs decomposed and orange lipid droplets leached into the embedding medium.

Fresh crayfish eggs exposed to excess $CaCO_3$ (experiment E1b, E2b, E3 and E4; Table 1) in an oxygenated or a reduced atmosphere, in contrast were preserved over several weeks. The crayfish eggs, however, only partially retained their original spherical shape (Fig. 1). Collapse structures of the outer membrane were observed at least on one side. Commonly, polygonal ridges formed which we interpret as shrinkage patterns, resulting from atrophy of the internal body. Subtle mineralization of fine-grained flaky crystals (size range 10 µm), mostly along these polygonal structures, was evident after six days. The addition of activated carbon (experiment E3; Table 1) did not affect the tendency to mineralization. After two weeks, crystal envelopes covered the complete egg surface. In places, dense crystal accumulations up to 150 µm in diameter formed (Fig. 1). The SEM X-ray mappings highlight the post-experimental distribution of P and Ca with clear enrichment of these elements in surface crystallites (Fig. 3).

Crayfish eggs that were heated prior to embedding (experiments E6 and E7; Table 1), exhibit complete preservation of the spherical morphology and complete mineralization of the outer membrane after one week (Fig. 1). After two weeks, the crystal layer had accumulated to a thickness of approximately 100 µm. Continuous mineralization

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occurred as fine-grained botryoidal or kidney-shaped crystallites (Fig. 4). X-ray maps show that these are composed of P- and Ca-bearing phases (Fig. 3).

Stained histological sections showed that the embryonic tissues of the embryo, i.e. various cell types and components, e.g. the cell nuclei, the segmented caudal papillae, and muscle cells were exceptionally well preserved after two and three weeks, respectively (Fig. 5). The endochorional envelope was not preserved. A fine-grained crystal rind completely covered the outer surface of the embryo replacing the endochorional envelope.

X-ray diffraction and Rietveld refinement on the embedding medium and on surface crystallites of the mineralized layer of crayfish eggs of experimental series E7 confirmed the occurrence of brushite and calcite in approximately equal proportions (Table 2). However, the refinement of XRD data obtained from sample material of experiment E7.3 proved to be difficult, because the Durbin Watson factor (DW) and χ^2 (Table 2) were unsatisfactory and the background values of the X-ray spectra were elevated. The ambiguous results of the Rietveld refinement might also reflect the low crystallinity of the sampled material (see Briggs and Kear, 1993b).

4 Discussion

4.1 Mineralization

Our experiments show that the outer surface of crayfish eggs can be mineralized within one or two weeks under laboratory conditions in a manner that retains morphological detail similar to that preserved in fossil metazoan eggs (cf. Fig. 1). This finding holds true for all experiments applying the excess $CaCO_3$ medium and anoxic conditions. The crayfish specimens in our experiments became mineralized by a combination of fine-grained brushite and calcite, and showed no explicit signs of mineralized bacteria. The ratio of calcium phosphate to $CaCO_3$ of the crystallites was measured to be 55 : 45 and 62 : 38 (in wt.-%), respectively (Table 2).

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Preferred calcium phosphate precipitation over calcium carbonate was previously reported for laboratory-mineralized body tissue of decapod crustaceans (Briggs and Kear, 1993a, 1994; Sagemann et al., 1999) as well as for fossil specimens (Bengtson and Zhao, 1997; Steiner et al., 2004a). Our phosphate to carbonate ratios and the ratios of laboratory-mineralized body tissue of decapod crustaceans are, however, higher than the ratio obtained for laboratory-mineralized eggs of decapod crustaceans. Initially, laboratory mineralization of metazoan eggs had been thought to be closely associated with closed-system conditions and the presence of the decaying mother animal carrying the egg mass (Briggs and Kear, 1993a). In this case, decomposition of *Palaemon elegans* occurred over a period of up to 25 weeks and the organism itself acted as the source of phosphate. Mineralization of the body tissue and the eggs occurred within a few weeks, either as pure calcium carbonate or a mix of calcium carbonate and phosphate. The eggs were, however, less mineralized than the other tissue and with a much lower proportion of phosphate-to-carbonate (Briggs and Kear, 1993a). Whether changes in pH, the concentration of P, or a combination of both caused the observed differences in *Palaemon elegans*, however, remained unclear. Partial mineralization was also described for another range of decay experiments on invertebrate eggs of the European and Norwegian lobsters, *Homarus gammarus* and *Nephrops norvegicus* (Martin et al., 2005), in which experimental mineralization of invertebrate eggs has been closely associated with the presence of bacteria generating anoxic conditions and reduced pH. Mineralization of eggs occurred most commonly as calcium carbonate, or as a combination of calcium carbonate and calcium phosphate. The only study reporting complete mineralization of the egg surface was performed on *H. gammarus*, where a quantity of eggs equivalent to 520 mg dry mass was decomposed in artificial seawater (ASW) mixed with sediment over one month under anoxic conditions (Martin et al., 2003). In this experiment, the eggs were encrusted on the outside of the preserved egg envelope with a 50 µm thick layer of mostly amorphous calcium carbonate and mineralized bacteria, with the latter result providing further evidence that bacteria may promote mineralization. Replicate experiments, with the same

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or modified parameters (e.g. egg mass, volume of ASW, various additives), however, did not show complete surface mineralization with reasons for the observed variability in mineralization between treatments being unidentified.

A lack of mineralization and rapid decomposition of crayfish eggs was only observed in treatments with excess phosphoric acid, independent of the redox conditions. In this case crayfish eggs rapidly degrade through the process of autolysis, an internal cell destruction process caused by endogenous enzymes resulting in the coalescence of pigments and internal lipids or proteins, and finally the breakup of the endochorional envelope.

4.2 Preservation of organic tissue

Histological sections of treated crayfish eggs showed exceptional preservation of the embryonic tissue during the experiment. Our study is to our knowledge, the first investigation of artificially fossilized embryos at the tissue and cellular level, and is in general line with results of recent taphonomic experiments demonstrating that the morphology of metazoan embryos could be well preserved during prolonged periods for mineralization to occur (Raff et al., 2006; Gostling et al., 2008). These taphonomic experiments particularly showed that reducing conditions are a key factor preventing autolysis and precluding the activity of aerobic microbes.

Even though the embryonic tissue is well preserved, SEM images and histological sections clearly indicated that the endochorional envelope of crayfish eggs showed degradation. A thin sheat of fine-grained authigenic minerals replaces the endochorional envelope. This indicates that rapid decomposition of the endochorional envelope is attended by the rapid growth of authigenic minerals. Further evidence for this "replacement hypothesis" is provided by completely mineralized crayfish eggs showing different degrees of preservation, such as polygonal shrinkage patterns and collapse structures. Here, progressive mineralization must have occurred simultaneously to the decay of the endochorional envelope and the egg's internal content, thus preserving different stages of decay (after Gostling et al., 2008). This is commonly observed in the

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fossil record (cf. Fig. 1). Several studies on fossil metazoan eggs and embryos have shown that their organic envelopes are not originally preserved, but that their original composition and structure is altered diagenetically by phosphatic replacements or encrustations of the envelope (Steiner et al., 2004a). Particularly, the detailed petrographic and geochemical studies on some organic-walled microfossils of the Doushantuo fossil Lagerstätte suggested that phosphatization was an early diagenetic process in shallow burial depth which occurred after rapid burial of the microorganisms and prior to the total decay of the organic matter and intergranular compaction (Hubert et al., 2005). All findings thus suggest that the mechanisms of decay and mineral replacement were likely similar in both this study and the fossil material.

Mineralization did not occur in the interior of the eggs even when the embryonic tissue had been exceptionally preserved, resembling results of similar experiments using eggs of *Homarus gammarus* (Martin et al., 2003). There are at least two possible explanations for this phenomenon: the newly formed mineral layer may either have acted as a barrier to microbial degradation of the internal cellular material or the dense structure of the yolk slowed or prevented the migration of substances necessary for mineralization. This "hollow egg" phenomenon is also known from Ediacaran and Cambrian fossil eggs (Steiner et al., 2004b).

4.3 Preservation of the spherical egg-morphology

Our experiments show that initial pre-heating of the crayfish eggs can stabilize their spherical morphology (Figs. 1 and 4). This is achieved both through pre-preservation of the yolk and the embryonic tissues and through prevention of internal decay, creating a stable template for mineralization. This result stands in marked contrast to the observations of all non-heated experiments, in which complete mineralization occurred but the original spherical shape was only partly retained. We therefore hypothesize that a moderate supply of heat, possibly through the proximity of a hydrothermal vent system, might assist the preservation and fossilization of yolk-bearing eggs and embryos, larvae and small animals. This is not a prerequisite for preservation, because

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spherically-preserved eggs and embryos are rare in the fossil record, whereas most show collapsed structures (Fig. 1). If eggs and embryos are preserved at all in Cambrian rock sequences only 9.5 % of them at best indicate a pristine external preservation without collapse structures. Several geochemical studies have demonstrated widespread hydrothermal venting on the Yangtze Platform in Early Cambrian times (Steiner, 2001). We therefore suggest that their emergence not only constitute a unique ecological niche for metazoan evolution, but also a potential environment for enhanced preservation and fossilization.

5 Conclusions

Our laboratory experiments show that complete surface mineralization of crayfish eggs, which were exposed to excess $CaCO_3$, may occur under anoxic conditions after one or two weeks, producing different grades of preservation in a manner similar to those described from the fossil record. While we found exceptional preservation of embryonic tissue at the cellular and morphological level after the time of the experiment, the endochorional envelope was not preserved, but was replaced by a thin rind of fine-grained phosphate and carbonate minerals. This indicates a close link between rapid anaerobic decay of the endochorional envelope and mineral authigenesis, possibly microbially mediated. Pre-heating of the crayfish eggs stabilized the egg's spherical shape without changing the environment so that endochorional decay and mineralization could occur. Our simplified experiments provide further insights on the processes and conditions that might lead to exceptional egg and embryo fossilization, notably those at the Precambrian-Cambrian transition.

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References

Alwes, F. and Scholtz, G.: Stages and other aspects of the embryology of the parthenogenetic Marmorkrebs (Decapoda, Reptanis, Astacida), Dev. Gen. Rev., 216, 169–184, 2006.

Bailey, J. V., Joye, S. B., Kalanetra, K. M., Flood, B. E., and Corsetti, F. A.: Evidence of giant sulphur bacteria in Neoproterozoic phosphorites, Nature, 445, 198–201, 2007a.

Bailey, J. V., Joye, S. B., Kalanetra, K. M., Flood, B. E., and Corsetti, F. A.: Reply, Nature, 446, E10–E11, doi:10.1038nature/05754, 2007b.

Bengtson, S. and Zhao, Y.: Fossilized metazoan embryos from the earliest Cambrian, Science, 277, 1645–1648, 1997.

Briggs, D. E. G.: Experimental taphonomy, Palaios, 10, 539–550, 1995.

Briggs, D. E. G.: The role of decay and mineralization in the preservation of soft-bodied fossils, Annu. Rev. Earth Planet. Sci., 31, 275–301, 2003.

Briggs, D. E. G. and Kear, A. J.: Fossilization of soft tissue in the laboratory, Science, 259, 1439–1442, 1993a.

Briggs, D. E. G. and Kear, A. J.: Decay and preservation of polychaetes: taphonomic thresholds in soft-bodied organisms, Paleobiology, 19, 107–135, 1993b.

Briggs, D. E. G. and Kear, A. J.: Decay and mineralization of shrimps, Palaios, 9, 431–456, 1994.

Briggs, D. E. G. and Wilby, P. R.: The role of the calcium carbonate-calcium phosphate switch in the mineralization of soft-bodied fossils, J. Geol. Soc. London, 153, 665–668, 1996.

Chen, J.-Y., Braun, A., Waloszek, D., and Qingqiang, P.: A lower Cambrian yolk pyramid embryos from Southern Shaanxi, China, Progr. Nat. Sc., 14, 167–172, 2004.

Chen, J.-Y., Bottjer, D. J., Li, G., Hadfield, M. G., Gao, F., Cameron, A. R., Zhang, C.-Y., Xian, D.-C., Tafforeau, P., Liao, X., and Yin, Z.-J.: Complex embryos displaying bilaterian characters from Precambrian Doushantuo phosphate deposits, Weng'an, Guizhou, China, Proc. Natl. Acad. Sci. USA, 106, 19056–19060, 2009.

Cheung, T. S.: The development of egg-membranes and egg attachment in the shore crab, *Carcinus maenas*, and some related decapods, J. Mar. Biol. Ass. UK, 46, 373–400, 1966.

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Condon, D., Zhu, M., Bowring, S., Wang, W., Yang, A., and Jin, Y.: U-Pb ages from the Neoproterozoic Doushantuo Formation, China, Science, 308, 95–98, 2005.
Dong, X.-P., Bengtson, S., Gostlings, N. J., Cunningham, J. A., Harvey, T. H. P., Kouchinsky, A., Val'kov, A. K., Repetski, J. E., Stampanoni, M., Marone, F., and Donoghue, P. C. J.: The anatomy, taphonomy, taxonomy and systematic affinity of *Markuelia*: early Cambrian to early Ordovician scalidophorans, Palaeontology, 53, 1291–1314, 2010.
Donoghue, P. C. J.: Embryonic identy crisis, Nature, 445, 155–156, 2007.
Donoghue, P. C. J. and Dong, X.: Embryos and ancestors, in: Evolving form and Function: Fossils and Development, edited by: Briggs, D. E. G., Yale Peabody Museum, New Haven, 81–99, 2005.
Donoghue, P. C. J., Kouchinsky, A., Waloszek, D., Bengtson, S., Dong, X., Val'kov, A. K., Cunningham, J. A., and Repetski, J. E.: Fossilized embryos are widespread but the record is temporally and taxonomically biased, Evol. Dev., 8, 232–238, 2006.
Dornbos, S. Q., Bottjer, D. J., Chen, J.-Y., Oliveri, P., Gao, F., Li, C.-W.: Precambrian animal life: taphonomy of phosphatized metazoan embryos from Southwest China, Lethaia, 38, 101–109, 2005.
Gostling, N. J., Donoghue, P. C. J., and Bengtson, S.: The earliest fossil embryos begin to mature, Evol. Dev., 9, 206–207, 2007.
Gostling, N. J., Thomas, C., Greenwood, J. M., Dong, X., Bengtson, S., Raff, E. C., Raff, R. A., Degnan, B. M., Stampanoni, M., and Donoghue, P. C. J.: Deciphering the fossil record of early bilaterian embryonic development in light of experimental taphonomy, Evol. Dev., 10, 339–349, 2008.
Gostling, N. J., Dong, X., and Donoghue, P. C. J.: Ontogeny and taphonomy: an experimental taphonomy study of the development of the brine shrimp *Artemia salina*, Palaeontology, 52, 169–186, 2009.
Gottschalk, M., Najorka, J., and Andrut, M.: Structural and compositional characterization of synthetic (Ca, Sr)-tremolite and (Ca, Sr)-diopside solid solutions, Phys. Chem. Miner., 25, 415–428, 1998.
Hagadorn, J. W., Xiao, S., Donoghue, P. C. J., Bengtson, S., Gostling, N. J., Pawlowska, M., Raff, E. C., Raff, R. A., Turner, F. R., Chongyu, Y., Zhou, C., Yuan, X., McFeely, M. B., Stampanoni, M., and Nealson, K. H.: Cellular and subcellular structure of Neoproterozoic animal embryos, Science, 314, 291–294, 2006.
Hubert, B., Alvaro, J. J., and Chen, J.-Y.: Microbially mediated phosphatization in the Neopro-

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terozoic Doushantuo Lagerstätte, South China. Bull. Soc. Geol. Fr., 176, 355–361, 2005.
Li, C.-W., Chen, J.-Y., and Hua, T.-E.: Precambrian sponges with cellular structures, Science, 279, 879–882, 1998.
Liu, Y., Li, Y., Shao, T., Zhu, Z., Yu, B., Wang, Z., Zhang, W., and Li, R.: Phosphatized Punctatus with mouthparts and its embryo fossils from the lower Cambrian of Ningqiang, South Shaanxi, China, Front. Earth Sci. China, 1, 1–11, 2007.
Martin, D., Briggs, D. E. G., and Parkes, R. J.: Experimental mineralization of invertebrate eggs and the preservation of Neoproterozoic embryos, Geology, 31, 39–42, 2003.
Martin, D., Briggs, D. E. G., and Parkes, R. J.: Experimental attachment of sediment particles to invertebrate eggs and the preservation of soft-bodied fossils, J. Geol. Soc. London, 161, 735–738, 2004.
Martin, D., Briggs, D. E. G., and Parkes, R. J.: Decay and mineralization of invertebrate eggs, Palaois, 20, 562–572, 2005.
Martin, P., Kohlmann, K., and Scholtz, G.: The parthenogenetic Marmorkrebs (marbled crayfish) produces genetically uniform offspring, Naturwissenschaften, 94, 843–846, 2007.
Martin, P., Dorn, N. J., Kawai, T., van der Heiden, C., and Scholtz, G.: The enigmatic Marmorkrebs (Marbled crayfish) is the parthenogenetic form of *Procambarus fallax* (Hagen, 1870), Contrib. Zool., 79, 107–118, 2010.
Peterson, K. J., McPeek, M. A., and Evans, D. A. D.: Tempo and mode of early animal evolution: inferences from rocks, Hox, and molecular clocks, Paleobiology, 31, 36–55, 2005.
Peterson, K. J., Cotton, J. A., Gehling, J. G., and Pisani, D.: The Ediacaran emergence of bilaterians: congruence between the genetic and the geological fossil records, Philos. Tr. R. Soc. B, 363, 1435–1443, 2008.
Raff, E. C., Villinski, J. T., Turner, F. R., Donoghue, P. C. J., Raff, R. A.: Experimental taphonomy shows the feasibility of fossil embryos, Proc. Natl. Acad. Sci. USA, 103, 5846–5851, 2006.
Raff, E. C., Schollaert, K. L., Nelson, D. E., Donoghue, P. C. J., Thomas, C., Turner, F. R., Stein, B. D., and Dong, X., Bengtson, S., Huldtgren, T., Stampanoni, M., Chongyu, Y., Raff, R. A.: Embryo fossilization is a biological process mediated by microbial biofilms, Proc. Natl. Acad. Sci. USA, 105, 19360–19365, 2008.
Sagemann, J., Bale, S. J., Briggs, D. E. G., and Parkes, R. J.: Controls on the formation of authigenic minerals in association with decaying organic matter: an experimental approach, Geochim. Cosmochim. Acta, 63, 1083–1095, 1999.
Scholtz, G., Braband, A., Tolley, L., Reimann, A., Mittmann, B., Lukhaup, C., Steuerwald, F.,

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and Vogt, G.: Parthenogenesis in an outsider crayfish, Nature, 421, 806, 2003.
Steiner, M.: The facies development and fossil distribution of the Yangtze Platform (South China) in the Neoproterozoic/earliest Cambrian, Freiberger Forschungshefte, C 492, 1–26, 2001 (in German).
Steiner, M., Li, G., Qian, Y., and Zhu, M.: Lower Cambrian small shelly fossils of Northern Sichuan and Southern Shaanxi (China), and their biostratigraphic importance, Geobios, 37, 259–275, 2004a.
Steiner, M., Zhu, M., Li, G., Qian, Y., and Erdtmann, B. D.: New early Cambrian bilaterian embryos and larvae from China, Geology, 32, 833–836, 2004b.
Winnicki, A., Pawlos, D., Formicki, K., and Smietana, P.: On selected morphomechanical characteristics of signal crayfish (*Pacifastacus leniusculus* Dana) eggs during incubation, Bull. Fr. Pêche. Piscic., 372–373, 431–438, 2004.
Xiao, S., Zhang, Y., and Knoll, A. H.: Three-dimensional preservation of algae and animal embryos in a Neoproterozoic phosphorite, Nature, 391, 553–558, 1998.
Xiao, S., Yuan, X., and Knoll, A. H.: Eumetazoan fossils in terminal Proterozoic phosphorites? Proc. Natl. Acad. Sci. USA, 97, 13684–13689, 2000.
Xiao, S., Hagadorn, J. W., Zhou, C., and Yuan, X.: Rare helical spheroidal fossils from the Doushantuo Lagerstätte: Ediacaran animal embryos come of age?, Geology, 35, 115–118, 2007.

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Table 1. Taphonomy experiments performed and corresponding conditions and duration.

Species	Experiment	Number of embryos	$CaCO_3$ (g)	H_3PO_4 (ml)	H_2O (ml)	C_{act} (g)	Atmosphere	Pre-heating at 80 ± 1 °C	Time (days)	EDS peaks of minerals
Procambarus fallax f. *virginalis*	E1a	1	2.75	17.0	2.5		Air		30	
	E1b	1	2.80	0.5	2.5		Air		6	
	E2a	1	2.75	17.0	2.5		Air		15	
	E2b	1	2.80	0.5	2.5		N_2		15	P, Ca
	E3.1	2	2.80	0.5	2.5	0.02	N_2		7	
	E3.2	1	2.80	0.5	2.5	0.02	N_2		14	
	E3.3	1	2.80	0.5	2.5	0.02	N_2		21	
	E3.4	2	2.80	0.5	2.5	0.02	N_2		28	
	E3.5	1	2.80	0.5	2.5	0.02	N_2		35	
	E3.6	1	2.80	0.5	2.5	0.02	N_2		44	
	E4.1	1	2.80	0.5	2.5		N_2		7	
	E4.2	2	2.80	0.5	2.5		N_2		14	
	E4.3	1	2.80	0.5	2.5		N_2		21	
	E4.4	1	2.80	0.5	2.5		N_2		28	
	E4.5	2	2.80	0.5	2.5		N_2		35	
	E4.6	4	2.80	0.5	2.5		N_2		44	
	E6.1	4	2.80	0.5	2.5		N_2	Yes	7	
	E7.1	2	2.80	0.5	2.5		N_2	Yes	8	P, Ca
	E7.2	3	2.80	0.5	2.5		N_2	Yes	14	
	E7.3	3	2.80	0.5	2.5		N_2	Yes	21	P, Ca

Experiment E6 was discontinued after one week because of a leaky exsiccator. All experiments were performed at room temperature and atmospheric pressure.

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Table 2. Phase fractions, lattice constants and statistical parameters of XRD-Rietveld refinement of experimental series E7.

Sample	Mineral phase	wt.-%	a (Å)	2σ	b (Å)	2σ	c (Å)	2σ	α (°)	β (°)	2σ	γ (°)	V (Å^3)	2σ	DW	χ^2
E 7.1 ebm	Brushite	52	5.810	1	15.184	4	6.238	1	90	116.3	1	90	493.1	2	1.279	1.267
	Calcite	48	4.995	1	4.995	1	17.042	4	90	90		120	368.3	1		
E 7.2 ebm	Brushite	50	5.812	1	15.189	3	6.241	1	90	116.3	1	90	493.6	7	1.162	1.394
	Calcite	50	4.997	1	4.997	1	17.047	3	90	90		120	368.6	1		
E 7.2 sc	Brushite	55	5.812	1	15.173	4	6.241	1	90	116.3	1	90	493.1	2	1.201	1.360
	Calcite	45	4.996	4	4.996	4	17.044	2	90	90		120	368.4	7		
E 7.3 ebm	Brushite	47	5.812	1	15.187	3	6.240	1	90	116.3	1	90	493.4	7	1.319	1.204
	Calcite	53	4.997	1	4.997	1	17.046	3	90	90		120	368.6	1		
E 7.3 sc	Brushite	62	5.810	11	15.192	4	6.234	9	90	116.4	1	90	492.7	16		
	Calcite	38	4.996	4	4.996	4	17.046	9	90	90		120	368.4	6	0.056	2.956

ebm = embedding medium
sc = surface crystallites
V = volume
DW = Durbin Watson factor

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Table 3. Comparison of initial phosphorus and calcium concentrations, occurrence of mineralization and embryo preservation in this and previous studies.

Species	Reference	P (wt-%) dry mass	Ca (wt-%) dry mass	Mineralization	Soft-tissue preservation
Procambarus fallax* f. *virginalis	**This study**	**1.30**	**0.01**	**Yes**	**Yes**
Pomatoceras lamarckii eggs	Gostling et al. (2008)				Yes
Haliotis asinina eggs	Gostling et al. (2008)				Yes
Crassostrea gigas eggs	Gostling et al. (2008)				Yes
Herdmania momus eggs	Gostling et al. (2008)				Yes
Heliocidaris erythrogramma eggs	Raff et al. (2006)				Yes
Homarus gammarus eggs	Martin et al. (2005)	1.08	0.17	Yes	n.d.
Limulus polyphemus eggs	Martin et al. (2005)	0.32	0.21	No	n.d.
Nephrops norvegicus eggs	Martin et al. (2005)	1.13	0.10	Yes	n.d.
Palaemon elegans and eggs	Briggs and Kear (1994)	0.67	7.90	Yes	Yes*

* Soft-tissue preservation was mainly studied on the shrimp itself.

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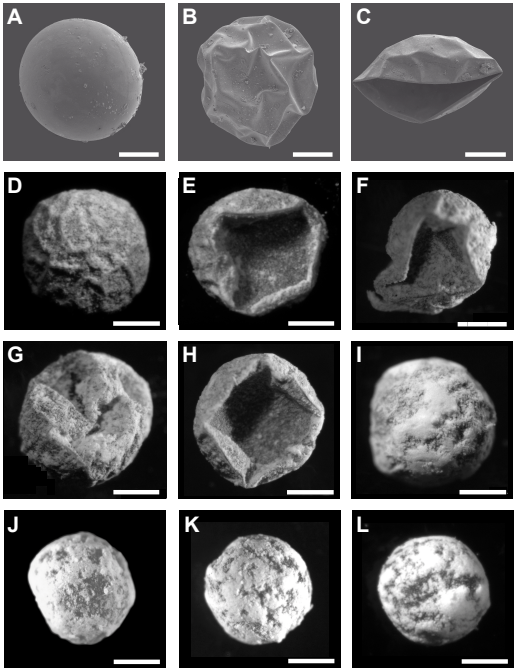

Fig. 1. Morphology preservation and mineralization of embryos. **(A–C)** Scanning electron micrographs of phosphatized embryo fossils from the Lower Cambrian Kuanchuanpu fauna, China, and **(D–L)** light microscope illustrations of experimentally mineralized embryos of the marbled crayfish. **(A)** Smooth egg of *Olivooides multisulcatus*. **(B)** Fossil egg showing polygonal shrinkage pattern. **(C)** Fossil embryo displaying physical degradation. **(D, E)** Experimentally-mineralized embryo after 1 week, showing subtle surface mineralization, polygonal shrinkage pattern, and hemispherical internal collapse. **(F)** Completely mineralized and shrunken embryos after 3 weeks, and **(G, H)** after 4 weeks. **(I)** Dense crystal accumulations appeared after 5 weeks. **(J–L)** Pre-heated crayfish embryos retained their globular morphology and exhibited complete mineralization of the outer membrane after 1, 2 and 3 weeks (Scale bar: **A** and **B** = 200 µm; **C** = 300 µm; **D**–**L** = 500 µm).

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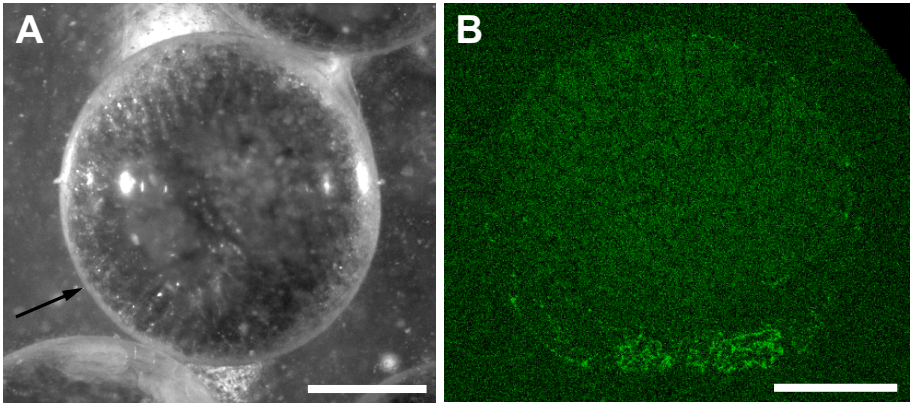

Fig. 2. (A) Fresh egg of the crayfish *Procambarus fallax* f. *virginalis*. Black arrow points to the translucent endochorion covering the yolk and the embryonic tissue. **(B)** SEM X-ray mapping of phosphorus of a sliced untreated crayfish egg illustrating the original P distribution. An almost uniform distribution of P is documented for the yolk, whereas the germ area shows elevated P content (Scale bar: **A** = 500 µm; **B** = 400 µm).

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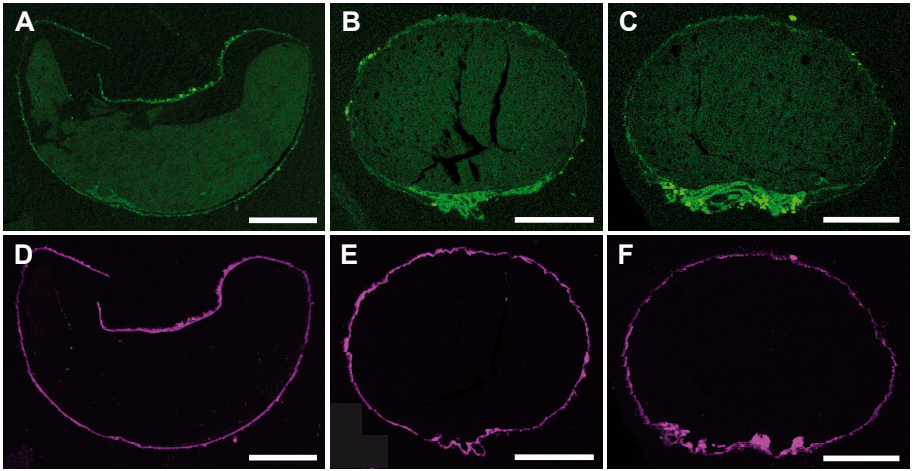

Fig. 3. Post-experimental distribution of phosphorus and calcium of sliced crayfish eggs. X-ray mappings of **(A–C)** phosphorus and **(D–F)** calcium highlight both the degree of morphology preservation and of continuous phosphate and carbonate mineralization (Scale bar: 400 µm).

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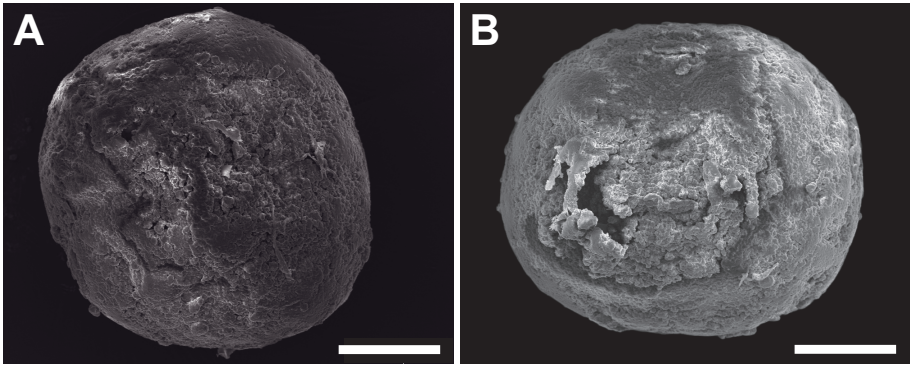

Fig. 4. SEM illustrations of pre-heated crayfish eggs incubated under the same experimental conditions conditions. **(A)** After 1 week. **(B)** After 3 weeks. Complete preservation of the spherical morphology is evident and continuous mineralization occurs as fine-grained botryoidal or kidney-shaped crystallites (Scale bar: 400 µm).

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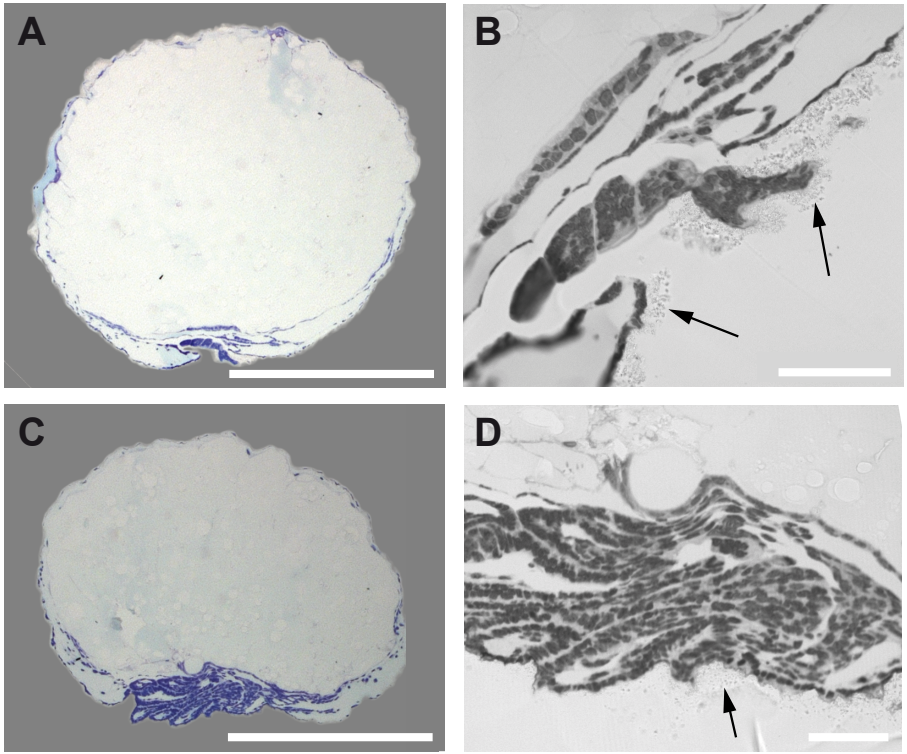

Fig. 5. Preservation of embryonic tissue. Histological sections of mineralized preheated crayfish eggs. **(A, B)** After two weeks. **(C, D)** After three weeks. Various cell types and components (e.g., cell nuclei of the segmented caudal papillae) are exceptionally well preserved with fine-grained authigenic brushite and calcite crystals (arrows) completely replacing the degraded endochorional envelope (Scale bars: **A** and **C** = 500 µm; **B** and **D** = 50 µm).

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