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Deciphering the fossil record of early bilaterian embryonic development in light of experimental taphonomy

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SUMMARY Experimental analyses of decay in a tunicate deuterostome and three lophotrochozoans indicate that the controls on decay and preservation of embryos, identified previously based on echinoids, are more generally applicable. Four stages of decay are identified regardless of the environment of death and decay. Embryos decay rapidly in oxic and anoxic conditions, although the gross morphology of embryos is maintained for longer under anoxic conditions. Under anoxic reducing conditions, the gross morphology of the embryos is maintained for the longest period of time, compatible with the timescale required for bacterially mediated mineralization of soft tissues. All four stages of decay were encountered under all environmental conditions, matching the spectrum of preservational qualities encountered in all fossil embryo assemblages. The preservation potential of embryos of deuterostomes and lophotrochozoans is at odds with the lack of such embryos in the fossil record. Rather, the fossil record of embryos, as sparse as it is, is dominated by forms interpreted as ecdysozoans, cnidarians, and stem-metazoans. The dearth of deuterostome and lophotrochozoan embryos may be explained by the fact that ecdysozoans, at least, tend to deposit their eggs in the sediment rather than through broadcast spawning. However, fossil embryos remain very rare and the main controlling factor on their fossilization may be the unique conspiracy of environmental conditions at a couple of sites. The preponderance of fossilized embryos of direct developers should not be used in evidence against the existence of indirect development at this time in animal evolutionary history.

INTRODUCTION

The study of fossil embryos from the late Proterozoic and early Paleozoic has become of significance to evolutionary developmental biology because fossil embryos and larvae can give direct evidence on early metazoan developmental evolution among early metazoans. Such fossils also have the potential to reveal information on the timing and modes of origin of planktotrophic larval forms (Nützel et al. 2006; Raff in press). We here address experimentally the ways that embryos can be preserved in early steps of fossilization and test the possible biases against the known embryo fossil record.

A fossil record of diverse complex bilaterian animals appears within the Early Cambrian (c. 530 Ma) and becomes visible in detail 10 million years later in the soft-bodied faunas of the late Early Cambrian (Benton and Donoghue 2007). The origin of bilaterians lies in the late Precambrian, with recent molecular clock estimates suggesting a time in the range of 580–600 Ma (Peterson et al. 2004; Peterson 2005), although inferences nearing 1000 Ma have also been reported (Blair and Hedges 2005). There are strong reasons to propose that the early metazoans were direct developers with large eggs and no feeding larvae (Valentine and Collins 2000; Raff in press; Sly et al. 2003). Ancestral bilaterians are thus hypothesized to be small worm-like creatures, perhaps acoelomorph-like.

It has been suggested that the evolution of planktonic feeding larvae took place some 500 Ma in the Late Cambrian to Early Ordovician, driven by an expansion of plankton and sanctuary from predation (Signor and Vermeij 1994; Peterson 2005). Fossil evidence for larval evolution in Late Precambrian and Cambrian metazoans comes from exquisite phosphoritic preservation of late cleavage stage embryos of unknown taxa, larval forms of cnidarians, and small ecdysozoans (Donoghue et al. 2006b). The fossil embryos so

far described are large, lying in the range seen with living direct developing marine embryos. The size ranges of the fossils are from 350 to 1100 mm for Late Precambrian embryos (Xiao and Knoll 2000; Hagadorn et al. 2006) and from 350 to 750 mm for Early to Mid Cambrian embryos (Steiner et al. 2004b; Donoghue et al. 2006b). Thus, in the absence of evidence of indirect developers, it has been proposed that classical arguments for the plesiomorphy of larval development among metazoans (Haeckel 1874; Nielsen and Nørrevang 1985; Nielsen 1994, 1995, 1998, 2005) are unfounded, and that direct development is primitive (Bengtson and Yue 1997; Conway Morris 1997, 1998, 2004; Donoghue and Dong 2005).

However, the fossil record should not be read literally, and the fossil record of embryonic development is no exception (Xiao et al. 2000). It is possible to improve our understanding of what is preserved—and the significance of what is not—through taphonomy experiments in which the conditions of death and decay are manipulated experimentally to uncover their effects on likely fossil remains (Briggs 1996, 2003). The first such experiments were conducted by Raff et al. (2006) on embryos and larvae of echinoids, attempting to uncover the pattern and rate of decay of embryos under environmental conditions compatible, and incompatible, with authigenic mineralization, and controlling for factors such as embryo size and developmental mode. This study revealed that although embryos decay quickly under oxic conditions, under reducing conditions the morphology of embryos can be maintained for weeks to months. Both the conditions and timescale are compatible with the establishment of conditions necessary for the bacterially mediated replacement of soft tissues with calcium phosphate mineral (Briggs et al. 1993).

It remains unclear to what extent the Raff et al. (2006) study is representative of the decay and likely preservation of the embryos of marine metazoans more broadly. Our study has three aims: (i) to extend the experimental approach of Raff et al. (2006) to other metazoan phyla to determine the extent to which the pattern and tempo of decay and preservation identified in echinoids are representative of metazoan embryos in general; (ii) to expand the range of experimental conditions of decay; and, (iii) formalize the stages and pathways of decay seen in experimental studies so that they can be better compared with assemblages of fossil embryos.

We studied the decay of embryos of an additional deuterostome—the ascidian tunicate *Herdmania momus*, as well as three lophotrochozoans—the annelid *Pomatoceros lamarckii* (keel worm), the gastropod mollusk *Haliotis asinina* (abalone) and the bivalve mollusk *Crassostrea gigas* (an oyster). These organisms were each allowed to decay in three environmental conditions: (i) oxic water within open diffusion, (ii) anoxic water in a closed atmospheric system, and (iii) anoxic reducing conditions simulated by a solution of 100 mM β-mercaptoethanol (β-ME) in ASW.

Under both oxic and anoxic conditions, all four taxa exhibited a common pattern of decay, comparable to that observed in echinoids by Raff et al. (2006), although the lophotrochozoans decayed slightly faster than the echinoids. Under reducing conditions, the morphology of the embryos of all taxa was maintained for the lifetime of the experiments, a span of time compatible with the authigenic mineral replacement of labile soft tissues mediated by bacteria (Briggs et al. 1993). However, under all environmental conditions, the rate of decay was not uniform within the population of embryos in each sample, and so at any one-time interval, a spectrum of decay was observed. Although some "pristine" specimens (specimens that appear structurally intact under light microscopy) were present in the last weeks of the experiment, the numbers were in many cases $<5\%$. The spectrum of decay seen here might go some way to explaining the variability in preservation seen in even the most spectacular assemblages of fossil embryos.

Although it has been demonstrated that the original morphology of deuterostome and lophotrochozoan embryos can be maintained over a timescale, and in conditions compatible with authigenic mineralization of soft tissues, the fact remains that there are no fossil embryos of deuterostomes and only one possible instance of a lophotrochozoan, a fact that may be explained by the fertilization strategies seen in members of these clades. In conclusion, there appears to be a strong taphonomic bias against the fossilization of larvae as well as the embryonic developmental stages of metazoans that exhibit indirect development. It has been speculated that an absence of indirect developers among Ediacaran, Cambrian, and Ordovician embryo assemblages indicates that direct development is primitive for metazoans (Conway Morris 1998, 2004; Donoghue and Dong 2005). However, these experimental results indicate that nothing should be read into the absence of indirect developers from these fossil assemblages other than the fact that they were not fossilized.

MATERIALS AND METHODS

Embryo culture

The serpulid annelid *Pomatoceros lamarkii* (Quatrefages, 1865) was collected from Tinside, Plymouth, UK. Gametes were obtained from individuals by removing the worm from its cast with a mounted needle, causing the adult to spawn. Eggs from different females were kept separately in 40 ml of filtered artificial seawater (ASW), while the sperm was pooled. Eggs were fertilized by applying 200 µl of diluted sperm (from 10 to 12 males, in 30 ml of filtered ASW) for 30 min. Fertilized eggs were washed into fresh ASW to prevent polyspermy and maintain the oxygen levels in the water, depleted by the presence of the sperm. The resulting embryos were allowed to develop over 48 h to obtain a spectrum of embryological stages.

The bivalve mollusk *Crassostrea gigas* (Thunberg, 1793) was supplied in mating pairs by Guernsey Sea Farms ⟨www.guernsey-seafarms.com⟩. They were kept at 12°C in a matured ASW system until needed. Adults were opened along the hinge, exposing the gut and, dorsal to it, the gamete tissue. Gametes were gently removed from the gonad to prevent contamination from the gut and collected into petri dishes with fresh, well-oxygenated ASW. Sperm was diluted in 40 ml of ASW, and a 200 µl aliquot was added to the eggs. After 30 min the fertilized eggs were washed into fresh ASW, and concentrated, using a sieve with a 20 µm mesh. A range of developmental stages, from cleavage embryo to larva, were raised.

Haliotis asinina, a vetigastropod mollusk, was collected from Heron Island Reef, Great Barrier Reef, Queensland, Australia, and maintained in an ambient flow-through seawater system at Heron Island Research Station, with males and females in separate aquaria. Gametes were procured from natural spawnings as described in Jackson et al. (2005). Spawned eggs were collected and washed in seawater and then concentrated using 100 µm mesh sieve. Eggs were back washed into a 1-l beaker and fertilized for 5 min with sperm collected from males spawning in aquaria. Fertilized eggs were thoroughly washed with 0.2 µm Millipore-filtered seawater and left to develop at 25°C. Different cleavage and early gastrula stages were then collected over a period of 4 h.

Herdmania momus, a self-sterile hermaphroditic ascidian tunicate, was also collected from Heron Island Reef and maintained under constant light at Heron Island Research Station. Gametes were removed from dissected gonads as described in Degnan et al. (1996). Sperm was siphoned away and stored separately, then added to the egg batch of another adult for a maximum of 10 min, to avoid polyspermy. Fertilized eggs were then concentrated down using a 200 µm mesh sieve, and a range of cleavage-stage embryos were collected.

Taphonomy conditions

Three environmental conditions were simulated in the taphonomy experiments: (i) oxic seawater, (ii) anoxic seawater, and (iii) anoxic reducing conditions. Experiments were conducted in 500 µl eppendorpf tubes and maintained at 15°C in a refrigerated incubator for the lifetime of the experiment, which was a maximum of 6 weeks; in exception to this, the experiments involving *Haliotis* and *Herdmania* were subject to varying temperatures for the first 2 weeks as they were transported from Australia, where the experiments were established, to the UK, where the results of the experiments were analyzed. ASW (25 ppt) was used in the experiments involving *Pomatoceros* and *Crassostrea*, whereas filtered seawater was used with *Haliotis* and *Herdmania*.

For the oxic and anoxic systems, embryos and larvae were killed through exposure to anoxic water prepared by autoclaving and then bubbling nitrogen through it as it cooled; in the third experiment, embryos and larvae were killed by their introduction to the reducing system. The oxic system was established using oxygenated water and oxic conditions were maintained by allowing open diffusion with the atmosphere. The anoxic system was established using anoxic water prepared as per embryo euthanasia. The system was closed with a rubber seal to prevent oxygenation through diffusion with the atmosphere. Reducing conditions were simulated through addition of β-ME to seawater to a concentration of 100 mM, and the system closed with a rubber seal. The concentration of β-ME will have diminished through the life of the experiment as it slowly oxidized; no attempt was made to replenish β-ME as it would have required opening the sealed system. For each taxon and environmental system, parallel experiments were established so that the effects of decay could be determined at weekly intervals.

β-ME was previously used as a simulant of naturally occurring reducing conditions by Raff et al. (2006) who demonstrated not only that it diminished decay, but that this effect was reversible such that embryos subsequently returned to oxic or anoxic water lacking β-ME continued to decay as normal. Our experiments show that although the progress of decay was diminished, it was not suppressed entirely, and so although a proportion of the sample population remained pristine, the majority underwent progressive decay, albeit at a slower rate. We conclude that the effect of 100 mM β-ME is to strongly reduce both microbial activity and autolysis, but that it does not fully block decay processes.

Although through autoclaving media we were able to preclude an exogenous source of microbes that might mediate decay, we could not preclude the possibility that exogenous bacteria entered the experimental systems in the small volume of water that accompanied the embryos. Furthermore, the animals from which the eggs and sperm were collected were not sterile and so our experiments do not control for an endogenous source of microbes. Thus, in the decay patterns observed it has not been possible to distinguish the effects of autolytic and microbial activity.

Analysis

Experimental products were fixed overnight in 4% paraformaldehyde, mounted on glass slides in 80% glycerol, and observed on a Zeiss Photomicroscope III (Zeiss, Germany) fitted with Nomarski DIC (Zeiss, Germany). Photomicrographs were obtained using a 5.0 megapixel Q-Imaging Micropublisher Firewire camera (Q-Imaging, Surrey, British Columbia, Canada) attached to the microscope.

Tomography

Fossil specimens were scanned using the X04SA beamline at the Swiss Light Source, Paul Scherrer Institute, Villigen, Switzerland. Rendering was undertaken using the software package AMIRA. Further details can be found in Donoghue et al. (2006a).

Repositories

Figured fossil specimens have been deposited in the following institutions: Geological Museum of Peking University, Beijing, China (GMPKU), Museum of Earth Science, Institute of Geology, Beijing, China (MESIG), and Swedish Museum of Natural History, Stockholm, Sweden (SMNH).

RESULTS

Pomatoceras lamarckii

Under oxic conditions, for the first 24 h (postmortem), embryos showed no noticeable signs of autolysis. However, after 36 h blastomeres had shrunk to approximately a quarter of

their original volume. The chorion still appeared to be intact, but showed signs of a loss of sphericity in a majority of specimens. After 48 h of oxic decay the fertilization envelope was highly distorted, containing embryos that were little more than shrivelled masses, with no distinguishable blastomeres within the mass. Most fertilization envelopes were absent after 48 h, and at this time, embryos began to aggregate. After 60 h, the shrivelled embryos were almost indistinguishable from the amorphous decayed organic matter that made up the majority of any material found. The majority of oxic decay takes place over a few hours.

After 7 days under oxic conditions, a spectrum of decay was apparent (Fig. 1A). Pristine embryos (Fig. 2A) coexisted in approximately equal numbers with embryos in which blastomeres (Fig. 2B) were shrunken within chorions, collapsed embryos (Fig. 2C), and indeterminate organic matter (Fig. 2D). After 14 days all the embryos had undergone extensive shrinkage within collapsed chorions. After 21 days all that remained of the embryos was indeterminate organic matter.

Comparable results were seen in decay experiments conducted under anoxic conditions (Fig. 1B). After 7 and 14 days, an entire spectrum of decay was represented with the relative stages represented in approximately equal number (Fig. 2, A–D). By 21 days approximately a third of the embryos had degraded to amorphous organic matter, and very few pristine embryos remained. After 28 days no pristine specimens remained. Amorphous organic matter accounted for half of the material recovered, the remaining material was accounted for by shrunken embryos in which the original blastomere arrangement was difficult to discern, contained within damaged or collapsed chorions. After 35 days only indeterminate organic matter remained.

In 100 mM β-ME (Fig. 1C), a spectrum of decay stages was apparent by 7 days but the majority of embryos were still pristine (Fig. 2A). Shrinkage of the embryos within their fertilization envelope was apparent in a minority of specimens at this early stage, but this proportion increased over the lifetime of the experiment (Fig. 2B). The next stage of decay, the loss of sphericity, and damage to the chorion, was observed in approximately half of the embryos by 3 weeks, and by 4 weeks, the majority of embryos had reached this stage (Fig. 2C). By 5 weeks approximately three-quarters of the embryos had degraded to indeterminate organic matter, but all stages of decay were still represented, including pristine embryos (Fig. 2D).

Haliotis asinina

At 7 days approximately half of the specimens displayed an intact vitelline envelope in oxic seawater (Fig. 1A). In these specimens, the appearance of blastomeres and internal cellular structures still reflected the in vivo condition (Fig. 2E). In the remaining "oxic" specimens the vitelline envelope had begun to break down (Fig. 2F). In these specimens, blastomere boundaries started to appear diffuse, and lipid droplets coalesced. At 14 days, all vitelline envelopes had broken down, the original blastomere arrangements were difficult to discern, and embryos had begun to aggregate (Fig. 2G). By 28 days specimens had degraded to indeterminate organic matter (Fig. 2H).

In anoxic seawater (Fig. 1B) approximately half of specimens at day 7 displayed a robust fertilization envelope and the internal morphology faithfully reflected the in vivo condition. In the remaining specimens the boundaries between blastomeres had begun to look diffuse yet, although their original arrangement was still discernible, the internal lipid droplets had begun to coalesce. By 14 days no specimens displayed any discernible vitelline envelope and blastomeres had begun to separate from one another although the original three-dimensional morphology was still discernible. In some cases, blastomere membranes had broken down completely and aggregates of lipid droplets were all that remained. This was also the case at 21 days after death; however, regions of amorphous organic material were also present. At the close of the experiment at 28 days, a few clumps of lipids remained, but the majority of the material had broken down completely.

In β-ME (Fig. 1C), over half of those embryos maintained their gross in vivo morphology to the close of the experiment. Among the remaining embryos, a spectrum of degradation was observed, ranging from distorted vitelline envelopes, to embryos in which vestigial blastomere arrangements were still discernible but vitelline envelopes had degraded to a halo of amorphous matter (Fig. 2, E–H).

Crassostrea gigas

Under oxic conditions (Fig. 1A), an approximately equal distribution of all decay stages were present by 7 days (Fig. 2, I–L), but by 14 days the majority of embryos had degraded to indeterminate organic matter (Fig. 2I), and by 21–35 days few recognizable remains of embryos were seen and these had undergone dramatic shrinkage within chorions that showed evidence of damage and collapse. By 42 days only indeterminate organic matter remained.

Under anoxic conditions (Fig. 1B), embryo morphology was maintained for approximately 1 week longer than under oxic conditions. At 7 days the majority of embryos were pristine, but approximately one-third of embryos showed some evidence of decay including embryo shrinkage, damaged or entirely degraded chorions, embryos at an advanced stage of autolysis, as well as embryos that had degraded to indeterminate organic matter. By 14 days, approximately two-thirds of embryos had degraded to indeterminate organic matter and very few pristine embryos remained. By 21 days, the proportion of completely degraded embryos had increased to roughly three-quarters, increasing in proportion to the end

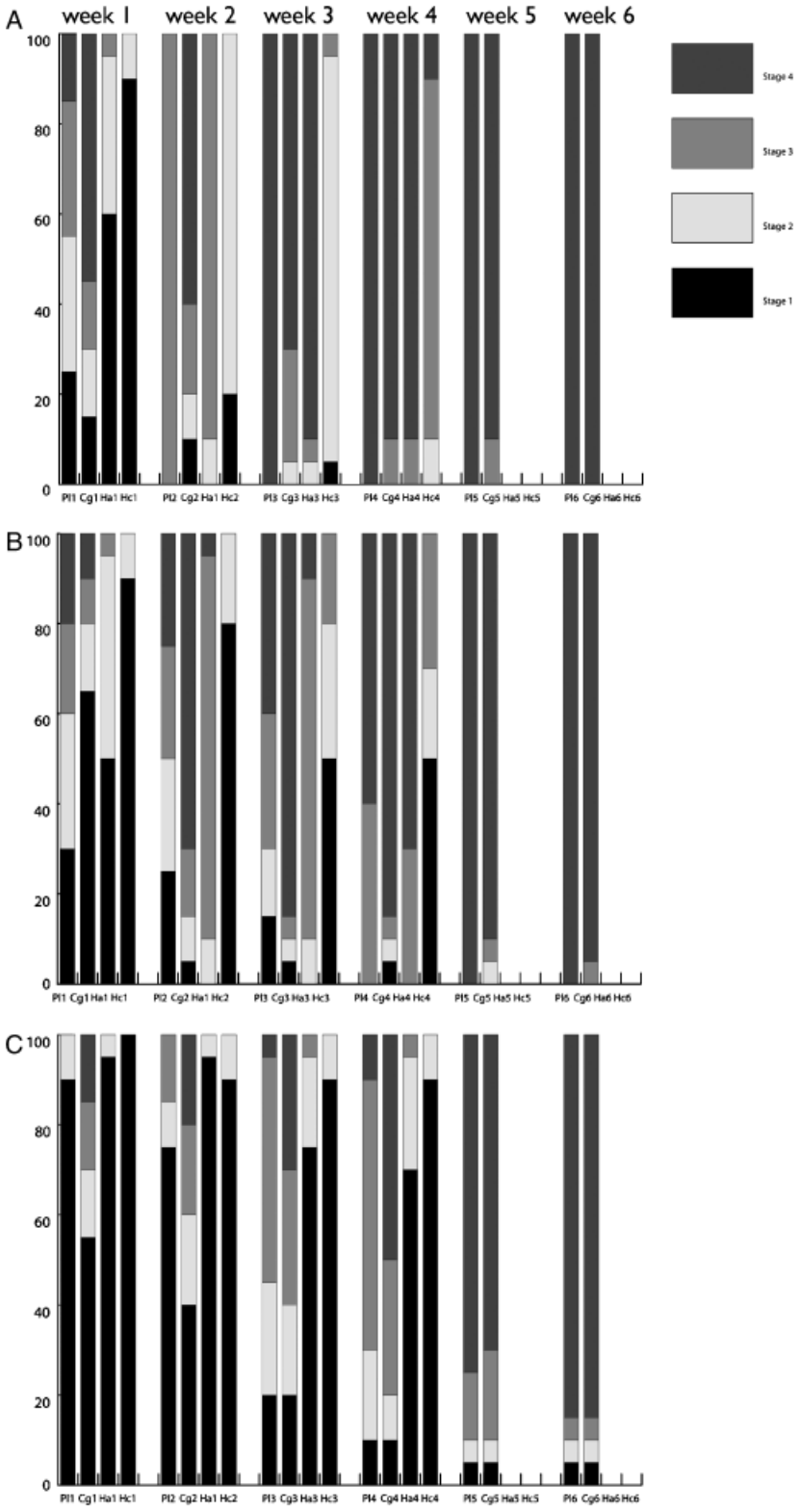

Fig. 1. Graphs showing the proportions of different decay morphologies over time, and, under the different decay environments, (A) oxic, (B) anoxic, and (C) β-mercaptoethanol (β-ME). Note progression to stage 4 was most rapid for all taxa under oxic conditions, and slowest under reducing conditions. However, it should also be noted that all of the time points show a spectrum of morphologies, with the most decayed stage (stage 4) present after the first week, but some relatively undecayed specimens present in the later stages of the experiment. Pl, *Pomatoceros lamarckii*; Cg, *Crassostrea gigas*; Ha, *Haliotis asinina*; Hm, *Herdmania momus*.

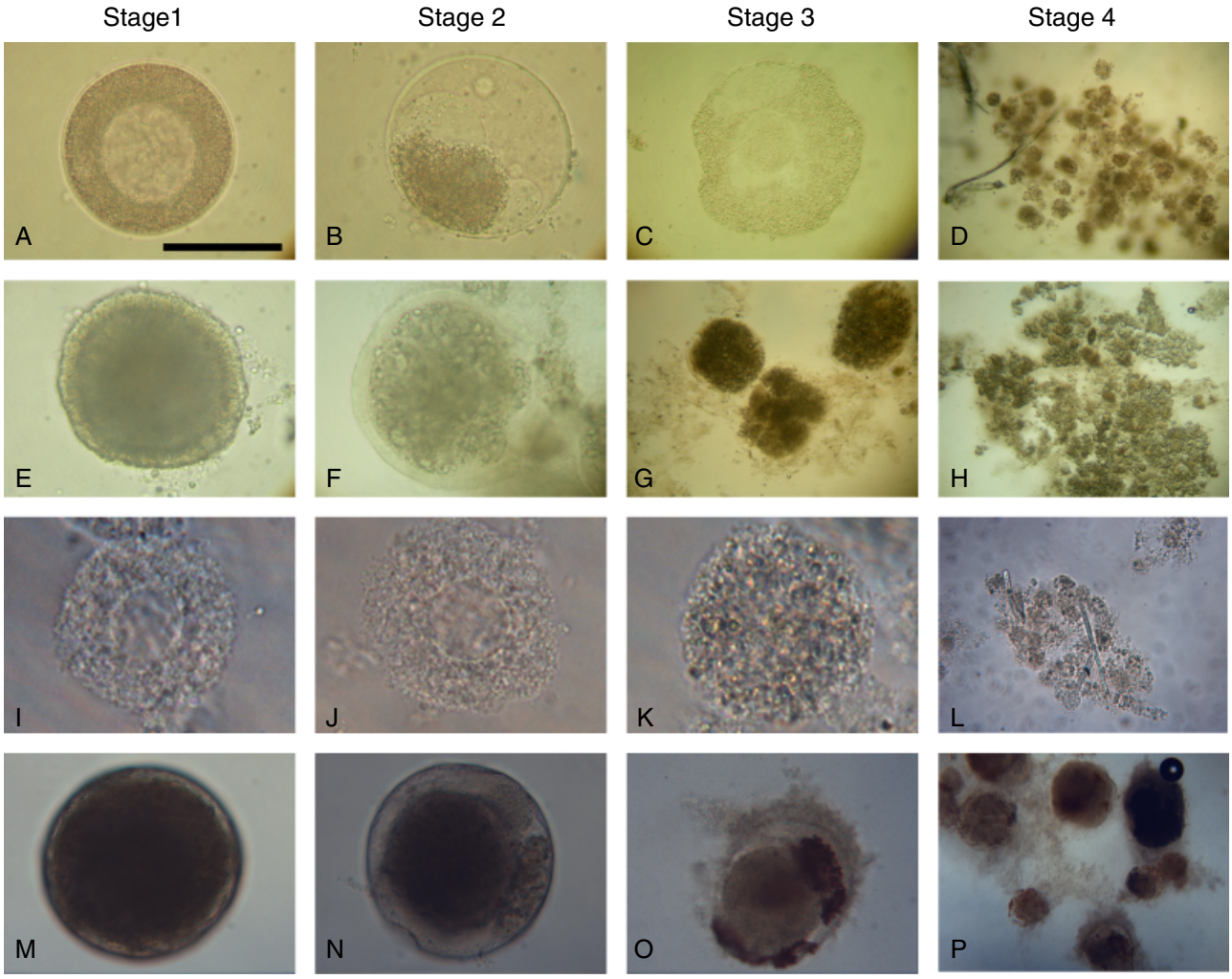

Fig. 2. Outline of the four stages of decay demonstrated by each taxa. (A–D) *Pomatoceras lamarkii* (keel worm), (E–H) *Haliotis asinina* (abalone), (I–L) *Crassostrea gigas* (oyster), (M–P) *Herdmania momus* (tunicate). Specimens of each taxon come from different decay environments. The decay pattern transcends the decay conditions. Relative scale bar: A–C 50 μm; D 300 μm; E–F 50 μm; G 100 μm; H 200 μm; I–K 25 μm; L 100 μm; M–O 50 μm; P 150 μm.

of the experiment. However, a small proportion of pristine embryos persisted up to 28 days; by 35 days the best specimens showed only a vestigium of in vivo morphology, and by 42 days only material lacking a robust chorion was observed, among a mass of amorphous organic material.

Under reducing conditions (Fig. 1C) approximately half of the embryos remained intact and "pristine" after 7 days (Fig. 2I), whereas the remainder showed a spectrum of decay stages, from loss of the chorion, through degradation of blastomere boundaries to indeterminate organic matter. After 2 weeks the chorion was absent in approximately two-thirds of specimens and, of these, half showed evidence of cellular degradation (Fig. 2, G and H). After 3 weeks, more than three-quarters of the embryos had lost their spherical shape and the cell contents had begun to homogenize. By the fourth week, recognizable embryos had small droplets (likely subcellular lipids) and particles attached to their surfaces. In the fifth week, internal cell–cell boundaries had broken down in most, and the remaining embryos had begun to aggregate. Finally, by 6 weeks, only fragments of decayed embryos were observed among a mass of amorphous organic matter (Fig. 2L)

Herdmania momus

Under oxic conditions (Fig. 1A), the chorion was lost within 7 days in the majority of specimens, and the external follicle cells (Degnan et al. 1996) were lost in all cases. Where the chorion was absent, the in vivo blastomere arrangements were still present at 7 days but these broke down by 14 days. In those specimens where the chorion was present at 7 days, specimens still exhibited their in vivo morphology in oxic conditions (Fig. 2M). Bright orange test cells were visible on

the surface of the blastomeres membranes in all specimens regardless of whether the chorion was present or not. At 21 days, where the chorion was present, the embryos had shrunk to approximately half their original size (Fig. 2N). Chorions continued to break down (Fig. 2O) and there were none present by the close of the experiment at 28 days under the oxic environment. At this stage the original in vivo blastomere arrangements had broken down completely and internal lipids had begun to coalesce and become rounded (Fig. 2P).

Under anoxic conditions (Fig. 1B), the chorion was still present in approximately half of the specimens at the close of the experiment (28 days). Where the chorion was present, the in vivo blastomere morphologies were maintained and this was the case for every time stage in the experiment. At 21 days, where the chorion was present, embryos had shrunk to approximately half their original size (Fig. 2N). The chorion was absent in approximately half of the specimens viewed and in these specimens the blastomere morphologies had broken down so that the boundaries between individual blastomeres had become diffuse and cells had begun to move away from one another and the internal lipid droplets had begun to coalesce.

At 28 days approximately half of the specimens exhibited the in vivo morphology (Fig. 2M) whereas the remaining specimens had broken down and only amorphous organic matter was present (Fig. 2P). In reducing conditions with 100 mM β-ME (Fig. 1C) the in vivo morphology (Fig. 2M) was retained in the majority (approximately 90%) of specimens up until the end of the experiment at 28 days. In the few specimens where the chorion was absent, blastomere arrangements were still visible at 28 days and membranes were still intact.

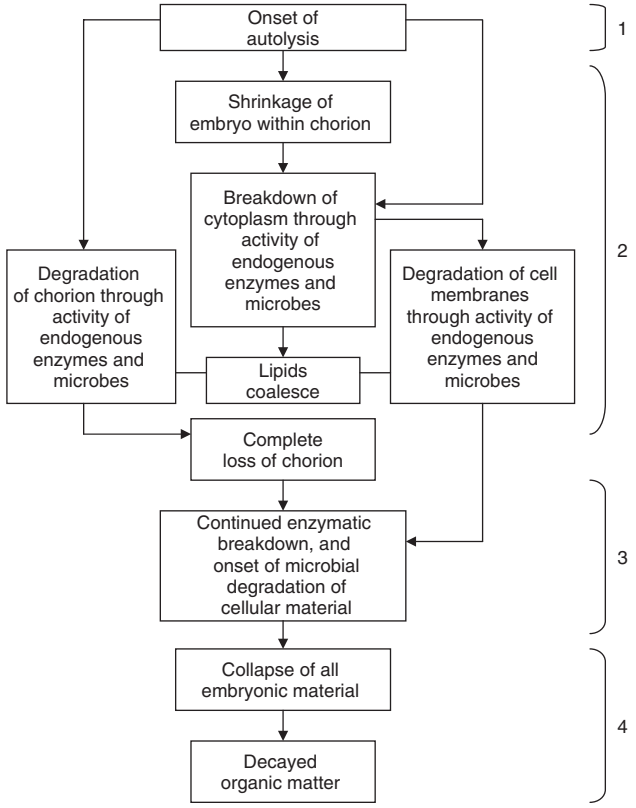

Fig. 3. Flow diagram demonstrates the general mode of decay observed among lophotrochozoan and deuterostome taxa. After breakdown of the chorion, lipids are subject to bacterial attack, which may induce the onset of the complete collapse of material in the final stages of decay. Numbered divisions correspond to the stages of decay we describe. The onset and the length of these phases are variable among the four taxa, therefore the diagram is not scaled to time.

Generalized stages of decay

The pattern of autolysis seen in the experiments can be summarized into four stages: (1) Embryo morphology faithfully reflects the original in vivo condition. (2) Some shrinkage is observed in the embryos within their fertilization envelopes. This is followed by enzymatic breakdown of the cytoplasm by endogenous proteases and other lytic enzymes. Following the breakdown of the cytoplasm, subcellular lipids begin to coalesce. Degradation due to the activity of endogenous and exogenous microbes has also been observed (Raff et al. 2006). This co-occurs with the degradation of the chorion and cell membranes through the activity of endogenous enzymes and microbial activity. (3) Lipid particles continue to degrade and blastomere boundaries become more diffuse, and the original arrangement of blastomeres begins to degrade so that only gross, vestigial arrangements can be seen. There is also continued breakdown of the fertilization envelope leaving the internal material subject to microbial attack. (4) Amorphous organic matter: total breakdown of subcellular material, with a loss of blastomere membranes and fertilization envelopes. Although, in general, there appears to be a strict ordering of these events, the relative timing of degradation of the fertilization envelope varies considerably. The progression of these events is expressed in Fig. 3.

The main difference observed, between organisms and between environmental conditions, was in rate. Where examined, the decay of larvae was seen to take place over a period of hours to days, even under reducing conditions. The autolysis of embryos took place over an extended time period, shortest under oxic conditions, although the results of oxic and anoxic experimental conditions were in some instances difficult to distinguish. Reducing conditions, simulated using β-ME, showed the lowest rate of autolysis, in some instances maintaining the original morphology of the embryos (Phase 1) to the lifetime of the entire experiment. There was a marginal difference in rate between the deuterostome (slowest) and the three lophotrochozoans. Any artefacts that may have been introduced through transport of the experiments involving *Herdmania* and *Haliotis*, were not discernible.

DISCUSSION

In general, the results of our experiments, based on a diversity of lophotrochozoan and deuterostome taxa, are directly compatible with those presented by Raff et al. (2006), based on echinoids alone. Reducing conditions are integral to the maintenance of in vivo gross morphology of embryos, halting or slowing the rate of autolysis and precluding the activity of aerobic microbes. These are the conditions under which microbially mediated authigenic mineralization of soft tissues is known to occur (Briggs et al. 1993). Given the combined dataset, which is taxonomically sparse, but nevertheless covers the great breadth of bilaterian animal diversity, it is likely that the observed effects of differing environmental conditions on the tempo and mode of decay of embryos are representative of Bilateria as a whole.

However, our data also provide additional insight in that they reveal that the rate of decay can vary considerably within any one population of embryos, regardless of whether the ambient environmental conditions are oxic, anoxic, or anoxic reducing. Although it has been shown mainly for the Ediacaran Doushantuo embryo assemblage (Xiao and Knoll 1999; Dornbos et al. 2005), all assemblages of fossil embryos show a broad range of preservational quality (Donoghue et al. 2006a) (Fig. 4, H–K). Dornbos et al. (2005) showed that this variation was most obvious in comparing embryos representative of the first few rounds of cleavage, where two-cell embryos were invariably very well preserved and 16-cell embryos were invariably very poorly preserved. We found no evidence for such a bias in our experiments, although the broad pattern of decay that they, and Xiao and Knoll (1999), describe, compares well with the stages of decay that we observed, including the loss of distinct blastomere boundaries (Fig. 4, H–K) and the shrinkage of the embryo within its fertilization envelope.

The broad pattern of decay that we observe in our experiments also resembles that of other assemblages of fossil embryos, including the Lower Cambrian Kuanchuanpu assemblage from Shaanxi Province, South China, where a range of preservation qualities is observed (Fig. 4, A–G). There is evidence for the breakdown of adjacent blastomere boundaries within the embryos (Fig. 4E), but none have shown evidence of breakdown of the boundaries between blastomeres on the surface of the embryo (Donoghue et al. 2006a). The basis for this difference in preservation between the Doushantuo and Kuanchuanpu embryo assemblages is unclear but it may reflect differences in endogenous and exogenous mineralization, respectively.

At Kuanchuanpu, there is also a bias in favor of specimens in which only the chorion is preserved, which may be compared with the presence of chorion-only specimens in certain stages of our experimental results (Fig. 4, B–D). We further show that complete loss of the fertilization envelope can occur through autolysis (although we cannot exclude microbial action), before the complete degradation of the embryo. Thus, the absence of fertilization envelopes in embryo assemblages, including the Doushantuo, need not be explained through sedimentary abrasion of fossilized embryos soon after their mineralization (Xiao et al. 2007), nor through the re-interpretation of these fossils as bacteria (Donoghue 2007; Bailey et al. 2007a, b). The results of our experiments suggest that the range of preservational quality seen in the Doushantuo assemblage is compatible with preservation under a single suite of environmental conditions and, assuming that reducing conditions prevailed—given that authigenic phosphatization has occurred and given the abundance of pyrite in the deposit (Xiao and Knoll 1999; Dornbos et al. 2005; Raff et al. 2006)—the mineralization of embryos need not have been so rapid, on a timescale of hours or days, as has been suggested previously (Dornbos et al. 2005). Indeed, the preservational quality of the embryos may not provide any insight into the temporal scale of the mineralization process.

Given the evidence that the morphology of deuterostome and lophotrochozoan protostome embryos can be maintained under reducing conditions compatible with microbially mediated authigenic phosphatization, and on a timescale compatible with the establishment of such mineralization, it is surprising that there are no unequivocal records of deuterostome or lophotrochozoan protostome fossil embryos. Some embryos from the Doushantuo assemblage have been attributed to lophotrochozoans (spiralians) and deuterostomes by Chen et al. (2000). However, these fossils are known only from whole-rock thin sections and, in the absence of knowledge of their three-dimensional morphology, they are just as readily interpreted as phosphatized, but otherwise unremarkable acritarchs or fertilization envelopes, some of which contain indeterminate decay residues or have undergone mechanical deformation before mineralization (Xiao et al. 2000). Chen et al. (2006) have also described what they interpret as polar-lobe bearing embryos from the same fossil assemblage, drawing comparison with lophotrochozoans (spiralians), but they refrain from concluding anything other than an animal affinity for the embryos. Kouchinsky et al. (1999) described possible embryos from the Lower Cambrian of Siberia, which they compared with annelids and mollusks because of the presence of a tetraradial structure in the fossils which bore a passing resemblance to the annelid or molluskan "cross" or micromeres. However, the comparison is vague and the authors settled on a cnidarian affinity for the fossils, interpreting the cross-shaped structure as incipient tentacles of actinula larvae. Finally, it was originally suggested that the worm-like embryo of *Markuelia* could be of lophotrochozoan affinity (Bengtson and Yue 1997; Conway Morris 1998), but further resolution of the anatomy and embryology of this organism indicates that it is an ecdysozoan (Dong et al. 2004, 2005; Donoghue

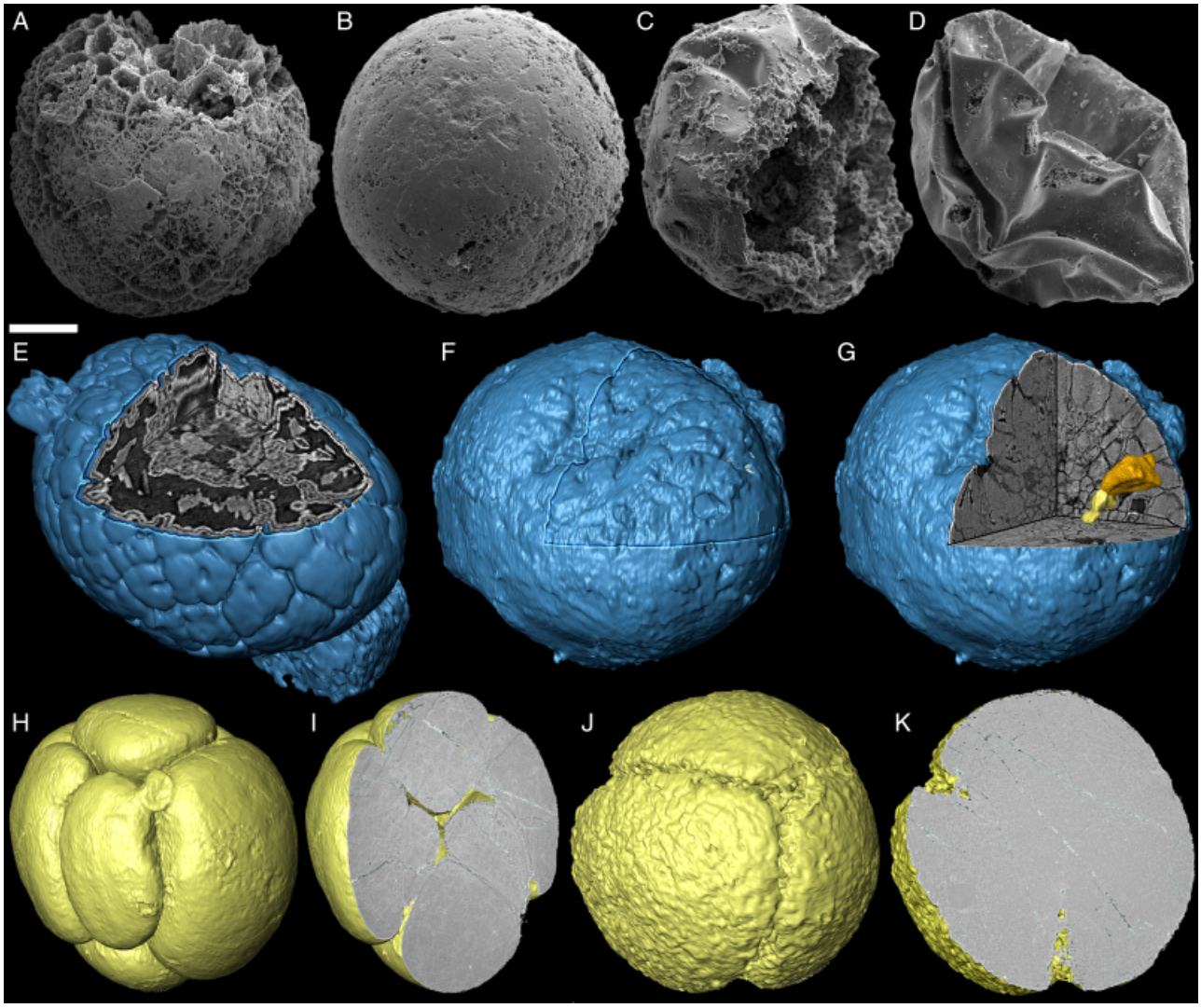

Fig. 4. Exemplar specimens from the Lower Cambrian Kuanchuanpu fauna (A–G) and Ediacaran Doushantuo fauna (H–K) outline the spectrum of preservation quality. (A) Partial specimen displaying preserved blastomere boundaries (GMPKU2300). Internal cavities have been filled with autolithified microbial structures, showing extensive microbial decay of cells. (B) Whole specimen with authigenically replicated chorion (GMPKU2301). (C) Partial specimen showing degraded chorion (GMPKU2302). (D) Embryo displaying physical degradation of the chorion (GMPKU2303). (E) Tomographic section through cleavage embryo showing no preservation of blastomere boundaries internally (GMPKU 2204). (F–G) Probable gastrula showing preservation of blastomere boundaries throughout (MESIG 20062). (H–I) Rendered and tomographically sectioned seven-cell embryo showing preservation of blastomere boundaries throughout (SMNH X3853). (J–K) Rendered and tomographically section four-cell embryo showing distinction between adjacent blastomeres on the surface of the embryo (SMNH X3855). F–G previously illustrated in Donoghue et al. (2006a). Relative scale bar: A 133 μm; B–C 155 μm; D 140 μm; E 70 μm; F–I 100 μm; J–K 130 μm.

et al. 2006a). Indeed, the preponderance of suggested affinities for fossil embryos has been with the ecdysozoans, cnidarians, and sponges (Donoghue and Dong 2005).

Although there is no definitive evidence of the presence of lophotrochozoans in the Ediacaran, there is no shortage of evidence of the existence of lophotrochozoans and deuterostomes in the Cambrian and, indeed, of adult lophotrochozoans in the same fossil assemblages that the embryos are encountered (Bengtson and Yue 1997; Conway Morris 1998; Steiner et al. 2004a). There are clearly differences in the life history stategies that are generally adopted by ecdysozoans versus lophotrochozoans and deuterostomes. The absence from the fossil record of the primary larva stages characteristic of lophotrochozoans and deuterostomes can be explained by the fact that they decay within hours to days even in conditions that slow the rate of decay and promote authigenic replication of soft tissues (Raff et al. 2006). However, there is no evidence of different preservation potentials of the

embryonic stages of ecdysozoans, lophotrochozoans, and deuterostomes, respectively.

Furthermore, the absence of fossil embryos of lophotrochozoans and deuterostomes is not readily explained by biases in identification or interpretation. Both clades are dominated by animals that undergo unequal cleavage, producing embryos composed of micromeres and macromeres, and these are readily distinguished from the equally cleaving embryos that dominate the fossil record. It is possible that unequal, spiral cleavage arose convergently among lophotrochozoans and deuterostomes. It has been shown that the transition from unequal to equal cleavage (Freeman and Lundelius 1992), and the complete loss of the spiral pattern (Boyer and Henry 1998), has occurred many times in the evolutionary history of lophotrochozoans. However, given the preponderance of unequal spiral cleavage among bilaterians (Valentine 1997), it seems unlikely that this mode of cleavage went unrepresented among Ediacaran and early Paleozoic lophotrochozoans. Neither is small size an adequate explanation because many indirect developers possess eggs and embryos of a size comparable to those recovered in fossil assemblages (Steiner et al. 2004b).

Although the dearth of fossil embryos attributable to lophotrochozoans and deuterostomes cannot be fully explained by their developing from larvae, larval development is invariably associated with free or broadcast spawning. Eggs and embryos distributed in this manner are unlikely to be entrained within the interstitial pore waters below the sediment–water interface. This decreases substantially their chances of fossilization, compared with ecdysozoan taxa such as nematodes, scalidophorans, and many arthropods that deposit directly into the sediment. This is because the mechanism of mineralization implicated in fossilization is predicated upon reducing conditions (Briggs and Kear 1993, 1994; Briggs et al. 1993) that are more readily established and maintained within sediment pore waters.

However, such factors must be marginal in determining the preservation of embryos of one organism or another, because although fossilized embryos are now known to be geographically widespread, the majority of records are accounted for by a single taxon, *Markuelia*, which is probably preserved preferentially because of its precocious cuticular development (Donoghue et al. 2006b). Fossil embryos are otherwise very rare fossils, and the majority of known diversity can be accounted for by just two assemblages, the Ediacaran Doushantuo Formation, and the Lower Cambrian Kuanchuanpu Formation, in the Yangtze Platform. Thus, the main controlling factor on the preservation of fossil assemblages may be the unique conspiracy of environmental conditions at these sites rather than factors intrinsic to the organisms or their embryos.

The dearth of larvae and embryos that can be attributed to metazoans that undergo larval development has been used as indication against the long-standing hypothesis that larval development is primitive for metazoans (Bengtson and Yue 1997; Conway Morris 1998, 2004; Donoghue and Dong 2005). The conspicuous occurrences in the Early Cambrian of direct developers interpreted to represent taxa that today typically undergo larval development (Kouchinsky et al. 1999; Yue and Bengtson 1999) would seem to point in the same direction. Our experimental results do not identify any taphonomic bias against the fossilization of embryos of metazoans that undergo larval development. Nonetheless, other fossilization biases against indirect developers may exist, as discussed above. As spectacular as instances of fossil preservation of embryonic stages of animal development may be, the fossil record cannot currently be called upon to support the idea that indirect development was absent in the Ediacaran and Early Cambrian.

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