

Embryos and Ancestors

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ABSTRACT

The role of paleontology in evolutionary developmental biology has been limited to constraining models of developmental evolution by providing a more complete understanding of anatomical evolution than is possible based on extant fauna alone. However, with the discovery of fossilized embryos, paleontology has an opportunity to make a more direct input into models of developmental evolution, providing a direct test of embryologies reconstructed for long extinct organisms based on vicarious genes and gene networks in extant representatives of descendent lineages. We review the paleoembryological record and consider its implications for the evolution of life history strategies in metazoan evolution. Conventionally, metazoans are thought primitively to have a planktotrophic larval stage and undergo metamorphosis to the adult. The record of Neoproterozoic and Cambrian embryos is incompatible with this view. Although the preponderance of direct developers at this time is likely to be a preservational artifact, their very existence then challenges some theories of developmental evolution that seek to explain the "Cambrian Explosion" phenomenon. Finally, we consider the likely extent of the paleoembryological record. Although more diverse assemblages probably remain to be discovered in the Ediacaran and early Cambrian, the likelihood of a temporally more extensive record is low.

KEYWORDS

Evolution, development, embryology, metazoa, fossil, Cambrian, Ediacaran.

Introduction

With the rise of evolutionary developmental biology there has been a resurgence of interest in the fossil record as a guide to anatomical evolution intermediate of extant lineages and, therefore, as a constraint on models of developmental evolution. The fossil record has made no greater material contribution because it yields pitifully little developmental data even on those organisms that have left a record. Indeed, it has been argued that gaps in the phylogenetic sequence of adults in the fossil record are an artifact of "clandestine" developmental evolution (De Beer 1958). What little we know of development from fossils is limited largely to a skeletal record of late ontogeny, such as that preserved in the bones of vertebrates, the shells of brachiopods and molluscs, and the molts of arthropods (Speyer and Chatterton 1989; Chatterton and Speyer 1997). Development at slightly earlier stages can be interpreted from ossification patterns (Caldwell 2002), but there are few examples where embryological processes can be in-

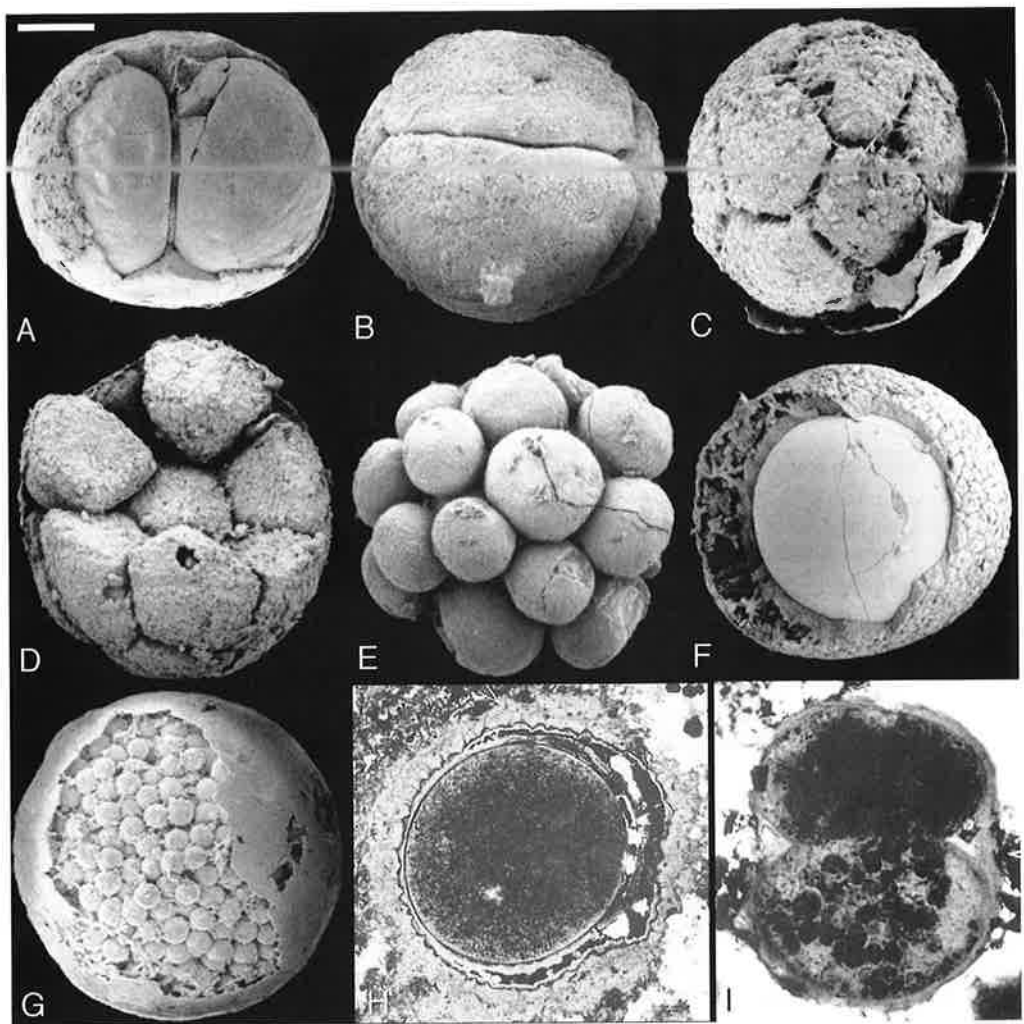


FIGURE 1. Phosphatized embryos from Ediacaran (late Neoproterozoic) of Weng'an, Guizhou Province, China. A–E, *Parapandorina*. A, Two-cell embryo. B, Four-cell embryo. C, ?16-cell embryo partially enclosed in the remains of an enveloping membrane. D, Incompletely preserved ?16-cell embryo revealing the stereoblastic arrangement of cells. E, Later stage embryo with differently shaped blastomeres possibly betraying an affinity distinct from the other developmental stages figured. F, *Tianzhushania*, interpreted as a zygotic resting stage. G, *Meganclonophycus* still partially enveloped by its zygotic membrane. H–I, Thin sections of *Tianzhushania* embedded in chert. Hm Showing the structure of the outer wall not preserved in acid maceration preparations. I, Possible two-cell cleavage stage. Relative scale bar: 117 μ m (A), 165 μ m (B), 126 μ m (C), 145 μ m (D), 112 μ m (E), 130 μ m (F), 142 μ m (G), 178 μ m (H), 130 μ m (I). A–G reproduced from Xiao and others (1998); H–I reproduced from Yin and others (2004). Used with permission of the authors and the publisher.

ferred. Exceptions include the bony embryos of fish (Grande 1989), pterosaurs (Ji and others 2004; Wang and Zhou 2004), dinosaurs (Varricchio and others 2002) and birds (Schweitzer and others 2002) but these, although invaluable, still represent very late stages of development. Thus, aside from the remarkable larval stages of Cambrian crustaceans (Waloszek 1995), knowledge of early development in ancient organisms has been largely inferential, such as through the larval shells of molluscs (Dzik 1978, 1983; Jablonski and Lutz 1983; Chaffee and Lindberg 1986; Jablonski 1986; Nutzel and Mapes 2001; Nutzel and Frýda 2003), brachiopods (Rowell 1986) and the record of life history strategy preserved in the crystallography of the gen-

ital plates of indirect developing echinoids as a vestige of the spicular arms of the larva (Emlet 1985, 1989; Jeffery 1997; Smith and Jeffery 1998).

These rare exceptions aside, knowledge of embryological processes and mechanisms in extinct organisms has been restricted to phylogenetic inference – comparative analysis of extant representatives of lineages as a means of inferring the nature of the embryology of their most recent common ancestor. However, as comparisons are made between progressively more distantly related organisms, the reliability of the inferences becomes increasingly tenuous, often indicating correlations between traits that differ from the correlations exhibited by the universe of living organisms. Either these inferences are spurious or they indicate that the life histories of living organisms are a poor analogue for their long extinct relatives (Strathmann 1993). Our ability to test between these alternatives and, therefore, to determine the causal relationship between developmental and anatomical evolution is seriously handicapped by the virtual absence of a fossil record of early development. This is perhaps no more obvious than in the ongoing debate over the timing and mechanisms underlying the divergence of metazoan phyla, and particularly crown group bilaterians (Smith 1999). Most paleontological estimates require that the bilaterians diverged within a very short period, leading to the suggestion that development was more plastic at this time (Riedl 1978; Wimsatt 1986; Valentine and Erwin 1987; Gould 1989; Kauffman 1993; Valentine 2004); molecular clock estimates posit that a protracted period of evolutionary history precedes the bilaterian fossil record and that this discrepancy could be because Precambrian adults were more comparable to the larval stages of living representatives (Davidson and others 1995; Peterson and others 1997; Peterson and Davidson 2000). Testing between these alternative explanations of the same pattern is difficult. In the words of Rudy Raff, “We need to be able to run the Cambrian over again, with developmental biologists present this time” (Raff 1994: 500).

Luckily, embryos of animals began to be discovered steadily from late Neoproterozoic and Cambrian strata from about the time that Raff was penning his summary. Although the earliest discoveries of embryos date back several decades (Qian 1977), the first embryos to be recognized as such were poorly preserved cleavage embryos, attributed to co-occurring trilobites, from the Middle Cambrian (Zhang and Pratt 1994; Pratt and Zhang 1995). More convincing finds appeared quickly, including the complete life cycle of an early Cambrian scyphozoan cnidarian, and late stage embryos of bilaterian metazoans (Bengtson and Yue 1997; Yue and Bengtson 1999a, 1999b; Dong and others 2004, 2005; Chen and others 2004; Steiner, Li and others 2004; Steiner, Zhu and others 2004). Cleavage stage embryos of sponges and, possibly, bilaterian metazoans have been recovered from the late Neoproterozoic (Xiao and others 1998; Zhang, Yin and others 1998; Xiao and Knoll 1999, 2000; Xiao 2002; Chi and others 2003; Yin and others 2004), including some rather more dubious records (Chen and others 2000; Xiao and others 2000; Yin, Yue and others 2001; Chen and others 2002). Lower Cambrian cnidarian-like embryos have also been described (Kouchinsky and others 1999). To date, however, they have been little more than curios of remarkable preservation, and their significance for understanding the evolution of development has been the subject of little more than speculation, which is not surprising given the paucity of data.

Paleoembryology: Quo Vadis?

The main limitation on the known paleoembryological record is that there is little constraint on phylogenetic affinity, a prerequisite to the resolution of the significance of these fossils. Obviously this reflects von Baer's Law, which observes that organisms are more similar at earlier than at later developmental stages. Thus, cleavage embryos could represent any number of candidates and more than one taxon could well be represented in individual deposits. Although these data potentially provide a powerful test of the evolutionary significance of cleavage patterns, to assign affinity based on cleavage pattern alone would be to exclude the possibility that patterns of cleavage have evolved in clades that now have a particular uniform cleavage pattern.

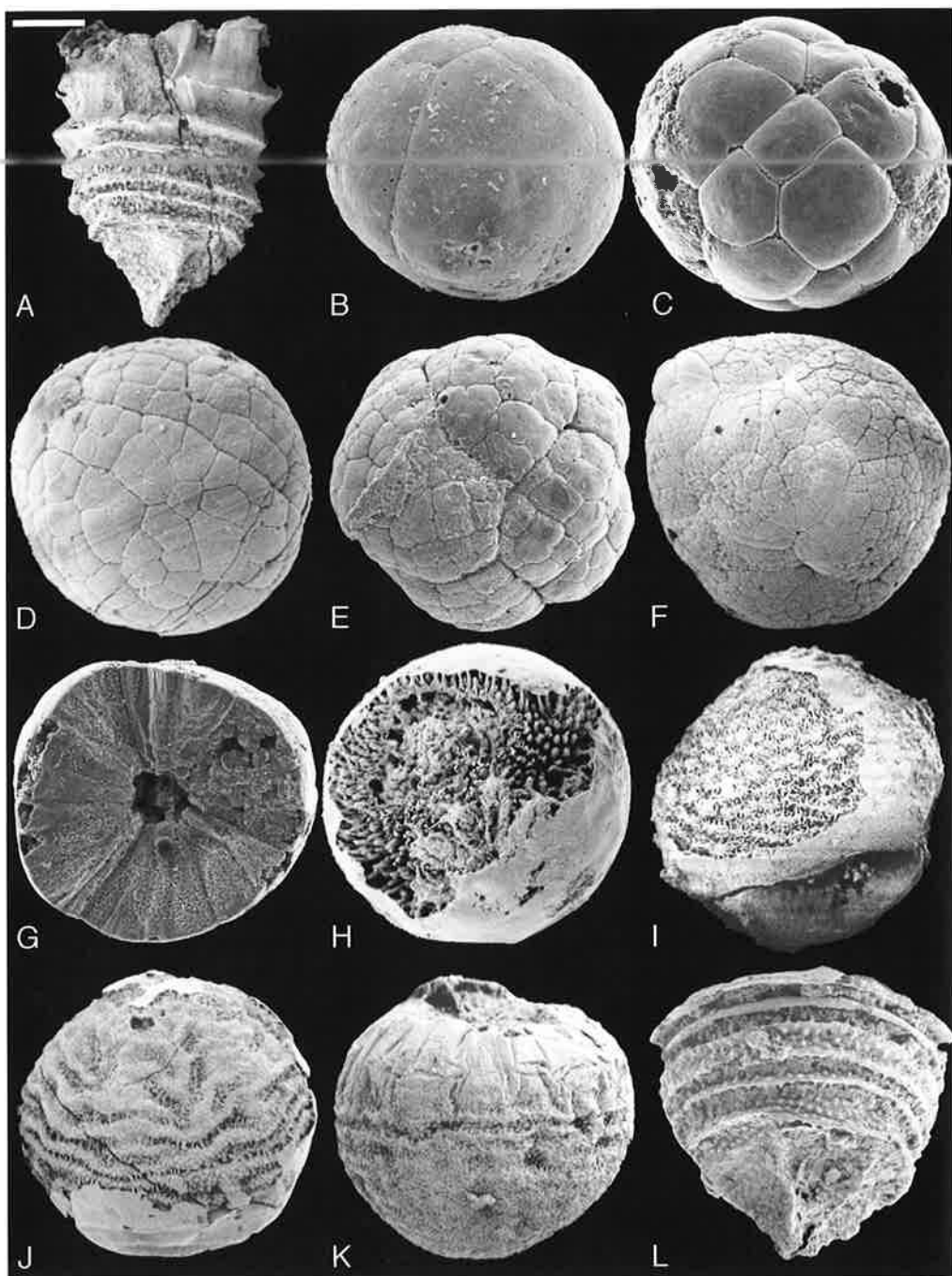


FIGURE 2. A–L, Embryonic and adult stages attributed to *Olivoooides* from the early Cambrian Kuanchuanpu Formation of southern Shaanxi, central China. A, Adult *Punctatus/Pyrgites* stage. B, Eight-cell stage blastula. C, ?32-cell stage blastula. D–F, Later stage blastulae and possible gastrulae. G, Fractured embryo revealing putative coeloblastic arrangement of the blastomeres (Yue and Bengtson 1999a) or yolk pyramids (Chen and others 2004). H–K, Successive stages of development of the stellate embryos of *Olivoooides* showing the progressive development of the parallel folds, the unornamented oral area, and the stellae that suggest an identity with the early hatchlings. L, Early hatchling of *Olivoooides*. Relative scale bar: 424 μ m (A), 45 μ m (B), 66 μ m (C), 91 μ m (D), 83 μ m (E), 153 μ m (F), 117 μ m (G), 158 μ m (H), 170 μ m (I), 170 μ m (J), 169 μ m (K), 284 μ m (L). A, D, E, F, H–L reproduced from Yue and Bengtson (1999a); B, C, E, G reproduced from Steiner, Zhu and others (2004). Used with permission of the authors and publishers.

Continued surveying of the faunal composition of these and other deposits has been accompanied by increasingly rigorous analyses of, and justification for, reconstructed paleoembryological sequences and phylogenetic affinity (Dong and others 2004, 2005; Steiner, Zhu and others 2004; Xiao, Zhou and others 2004). Below we review the paleoembryological record and consider the evidence for phylogenetic affinity. We summarize the taxonomic spread of knowledge in the last decade and discuss its implications for understanding the controls on preservation and the significance of fossil embryos for unravelling the evolution of development.

Neoproterozoic Doushantuo Formation

The Doushantuo Formation is of Neoproterozoic age and outcrops across the Yangtze Platform. Its precise age remains equivocal. Barford and co-workers (2002) dated it at 584 ± 26 Ma using Lu–Hf, and at 599.3 ± 4.2 Ma using Pb–Pb, indicating that it predates much of the Ediacaran biota. More recently, Condon and others (2005) provided a more detailed geochronology for the formation, also using Pb–Pb, indicating that it ranges in age from 635.5 ± 1.3 Ma to 550.55 ± 0.75 Ma; that is, over 90% of the Ediacaran. The embryos are recovered from phosphorites within the middle part of the Doushantuo Formation at Weng'an, Guizhou Province, China. These phosphorites are nearshore, shallow marine deposits that are often so rich in secondarily phosphatized acritarchs, algal thalli and animal embryos that the host rock has the appearance of an oolite. The embryos have also been encountered in another, deeper water lithofacies of cherts and dolomites elsewhere in the South China Platform, including the type area of the Yangtze Gorge (Yin and others 2004). The two lithofacies require distinct approaches to their study. The cherts are studied directly as rock thin-sections, and the dolomites by acid-dissolution and scanning electron microscopy analysis of the insoluble phosphatic residue. This difference of approach has led to some confusion over the identification of biotic elements common to both lithofacies (Yin and others 2004).

The Doushantuo Formation has mainly been known for its acritarchs and algal thalli, the latter preserved to a cellular level of detail (Zhang 1989; Zhang and Yuan 1992, 1996; Yuan and Hofmann 1998; Zhang, Yin and others 1998; Zhang, Yuan and others 1998; Xiao, Knoll and others 2004), although it has become more famous in recent years for reports of animal embryos (Li and others 1998; Xiao and others 1998; Chen and others 2000; Xiao and Knoll 2000; Xiao and others 2000; Chen and others 2002; Xiao 2002). Two main grades of animal embryo fossils have been described, along with several less well-documented forms. However, because many represent very early cleavage stages, the possibility that they encompass the embryos of a variety of organisms is quite high (Xiao and Knoll 2000). Therefore, they should be considered little more than form taxa until a more complete survey of the faunal composition of the Doushantuo Formation has been completed.

PARAPANDORINA. One of the two most completely described animal embryos from the Doushantuo Formation is *Parapandorina* Xue and others 1995 (Figure 1A–E). Described as a volvocacean colonial green alga by the original authors (Xue and others 1995, 1999), Xiao and colleagues have reinterpreted it as a sequence of animal eggs and cleavage embryos on the grounds of size difference, organization, comparative taphonomy and cleavage patterns, and because they fail to show reproductive habits expected of volvocaceans (Xiao and Knoll 1999; Xiao and others 1998; Xiao and Knoll 2000; Xiao 2002). The embryos have clear metazoan characteristics, not least in the maintenance of size and, therefore, cytoplasmic volume through progressive cleavage (Xiao and Knoll 2000). Cleavage is equal (holoblastic) and essentially radial, but does not progress beyond relatively few cleavages, and the embryos seem to be stereoblastic. Although the tetrahedral arrangement of the blastomeres is unusual (but not unknown) for metazoans, Xiao and colleagues (Xiao and others 1998; Xiao and Knoll 2000; Xiao and others 2000) interpreted these embryos as metazoan, including a range of possibilities ranging from stem Metazoa to crown Bilateria. The precise affinity of *Parapandorina* remains unclear, not least because of uncertainty concerning its relationship to co-occurring embryo form taxa

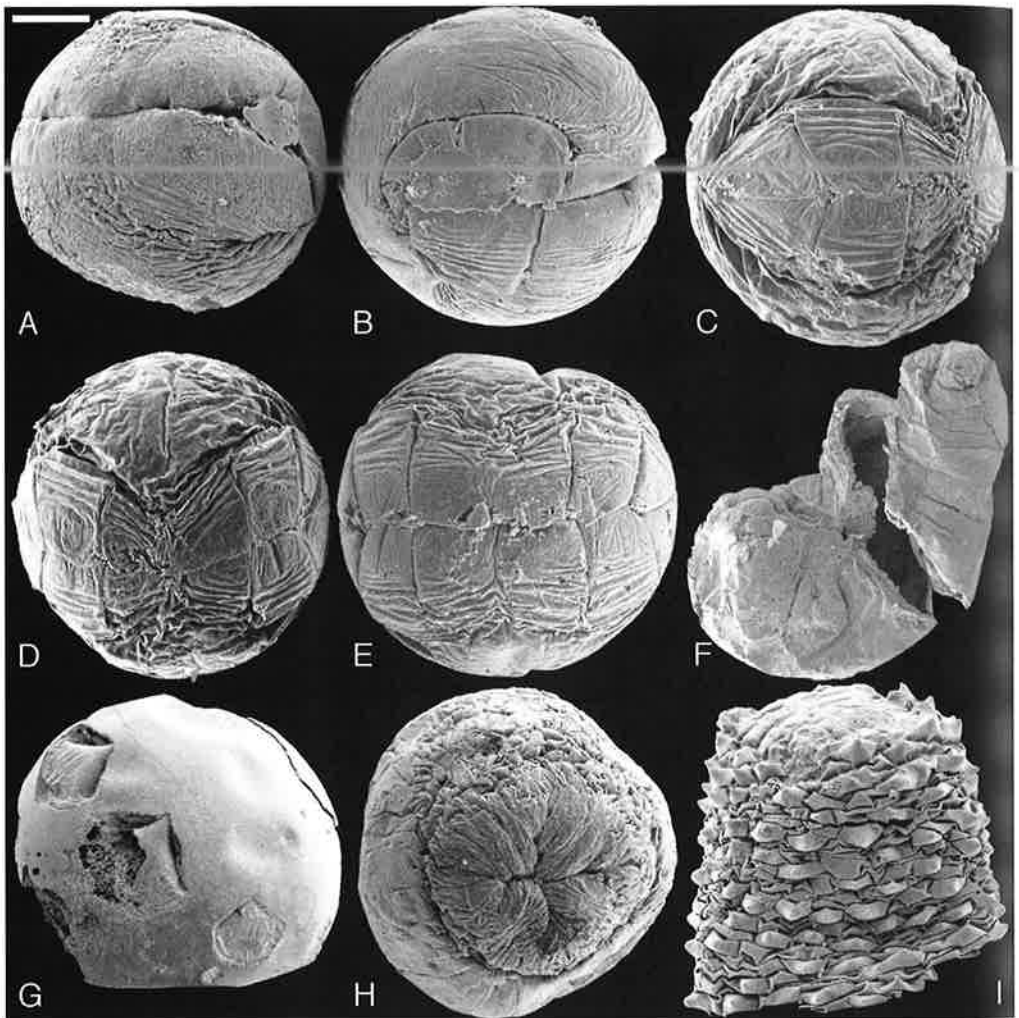


FIGURE 3. A–F, Embryonic and putative larval stages of *Pseudoooides* from the early Cambrian Kuanchuanpu Formation of southern Shaanxi, central China. A, Developing germ band with its median furrow. B, Terminal furrows rostral and caudal of the developing germ band. C–D, Embryo with pinched and annulated germ band. E, Annulated germ band at a later stage of development. F, Putative postembryonic larval stage showing serial divisions distinguishing head (left) trunk and tail (right) as well as divisions within these regions. G–H, Other elements of the Kuanchuanpu Fauna. G, Embryo with broad-based and laterally compressed spines emerging from the enveloping membrane. H, Animal pole of developing embryo with tetramerous symmetry. I, Putative hatchling of H. Relative scale bar: 95 μ m (A), 78 μ m (B), 80 μ m (C), 80 μ m (D), 81 μ m (E), 274 μ m (F), 246 μ m (G), 119 μ m (H), 180 μ m (I). All figures reproduced from Steiner, Zhu and others (2004). Used with permission of the authors and publisher.

(such as *Megaclonophycus*) and the absence of adult stages. Most recently, Xiao, Zhou and co-workers (2004) attempted to link stereoblastulae such as *Parapandorina* to co-occurring spiral embryos and to tubular, tabulated adults such as those described previously by Xiao and co-workers (2000).

TIANZHUSHANIA. *Tianzhushania* was originally described as an acanthomorph acritarch. More recently, Yin and co-workers (2004) recognized it as a taphonomic variant (and senior synonym) of the better characterized *Megasphaera*, which is interpreted as a resting egg of animal origin based on its size, rigid and ornamented outer membrane and an absence of algal repro-

ductive patterns (Xiao and Knoll 2000). Although the affinity of *Tianzhushania* remains far from clear, its ornamented outer membrane has been compared to the eggs of branchiopods (Xiao and Knoll 2000). However, this spiny membrane suggests a pelagic resting stage and based on this Yin and others (2004) have suggested an affinity to copepods.

OTHER RECORDS. Other records of embryos from the Doushantuo Formation include *Megacolonophycus*, *Spirallicellula* and *Caveasphaera* (Xiao and Knoll 2000), although their veracity is questionable. Even more dubious are the records of embryos from thin sections of the chert lithofacies described by Chen and colleagues (Chen and others 2000, 2002). Interpretation of remains from the chert lithofacies has a checkered history arising, at least in part, from the difficulty of interpreting a three-dimensional structure from thin sections (Zhang, Yuan and others 1998; Xiao and others 2000). While there can be no doubt that the structures described resemble sections through embryos of living cnidarian and echinoid larvae, the authors take no account of the biostratigraphic and diagenetic history of these remains. The boundaries between the putative "cells" represent no more than junctions between polarized crystal bundles resulting from space constraints. Evidence of gastrulation is more likely due to the plane of section cutting through a cavitated, but otherwise featureless, spheroidal membrane. There is no evidence of a cnidarian or echinoid identity and the remains are more likely to represent algal cysts and sphaeromorph acritarchs, which are common in the deposit (Yin 1999; Xiao and others 2000; Yin 2001; Yin, Gao and others 2001; Zhou and others 2001).

Kuanchuanpu fauna

In the South China Platform, the Doushantuo Formation is overlain by the Dengying Formation, the upper part of which (referred to as the Kuanchuanpu Formation in southwestern Shaanxi and northern Sichuan) is attributed to the Meishucunian, a regional equivalent of the Nemakit–Daldynian, which is early Cambrian in age. Phosphorites are abundant in the sequence and have yielded a diversity of embryos and larvae (Figures 2A–L and 3A–I) co-occurring with the earliest skeletal fossil assemblages (Bengtson and Yue 1997; Yue and Bengtson 1999a, 1999b; Chen and others 2004; Steiner, Li and others 2004; Steiner, Zhu and others 2004).

OLIVOOIDES. Originally described as a globular fossil of uncertain affinity, *Olivoooides* (see Figure 2G–K) has subsequently been allied with co-occurring *Punctatus* and *Pyrgites*, and the three interpreted as a complete life cycle, from adult (*Punctatus/Pyrgites*; Figure 2A) through blastula (Figure 2B–G) and stellate embryo (Figure 2H–K) to juvenile (Figure 2L) (Bengtson and Yue 1997; Yue and Bengtson 1999a). The adult stages (see Figure 2A) are represented by small (approximately 3 mm in length), strongly annulated cylinders that are open aperturally and taper apically into a shallow cone. The walls of the test are thin and invariably are folded and compressed consistent with an original unmineralized condition. Thus, their preservation and mineralization is a secondary phenomenon. Longitudinal striae dominate much of the outer surface of the test, except where stellate structures, which Yue and Bengtson (1999a) described as having the appearance of a soft sheet draped in radiating folds around protruding spikes, dominate the adapical region. The sculpture links the adults to the embryonic stages. Later embryos bear the same stellate ornament, as well as circumferential rings comparable to the annulae of the adult (Figure 2H–K). In addition to these features, late embryos have a pentaradial aperture defined by the surrounding folded cuticle that lacks other ornamentation (Figure 2K). Still earlier developmental stages are represented by irregularly cleaved blastulae (Figure 2B–G). However, these stages range broadly in size (260 to 850 μm) and it is likely that they represent blastulae of several different organisms. Steiner, Zhu and others (2004) even argued that the blastulae described by Yue and Bengtson (1999a) belong to *Pseudoooides* (see Figure 3A–F), a possible arthropod that occurs at the same site.

Debate over the affinity of *Olivoooides* has centered on the adult stages that broadly resemble the loricae of priapulid larvae (for example, Lemburg 1999). However, Bengtson and Yue (1997)

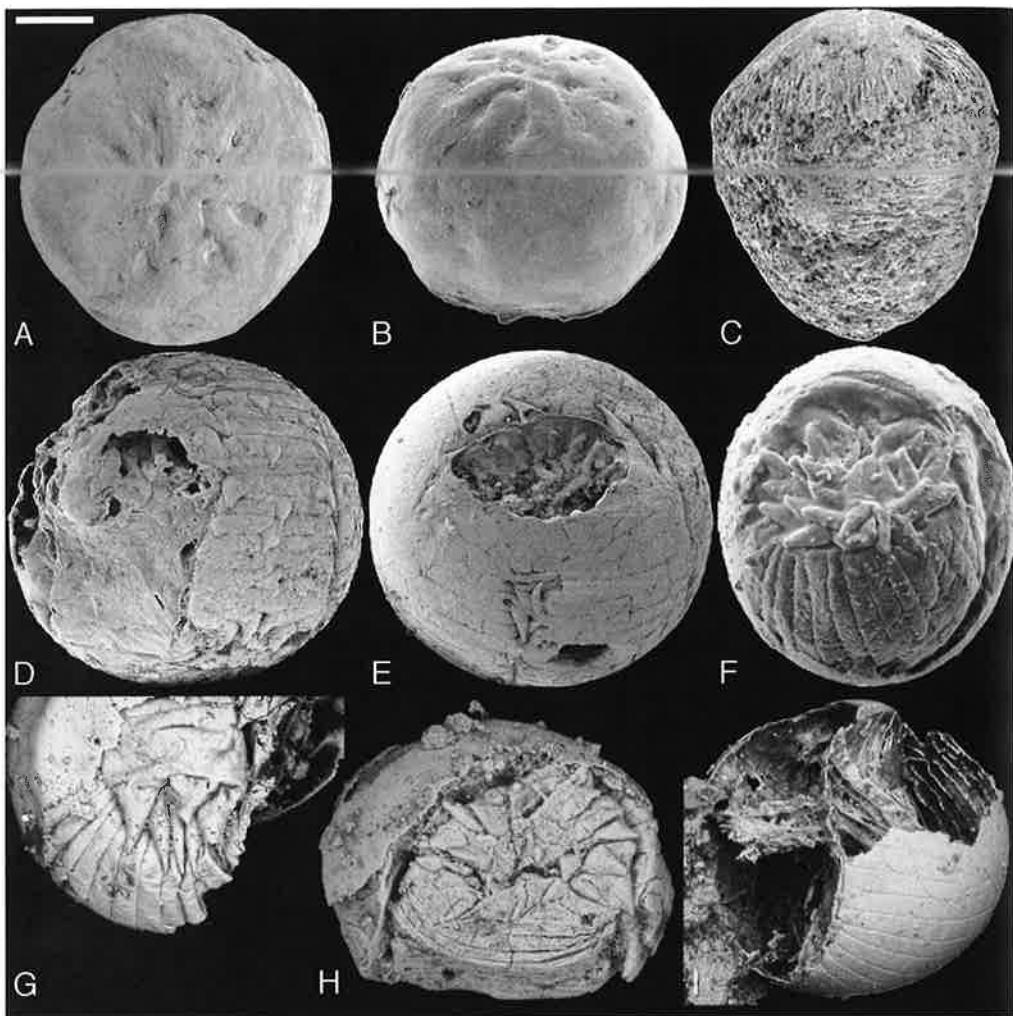


FIGURE 4. A–C, Embryos from the early Cambrian of Yakutia of possible cnidarian or lophotrochozoan affinity. A–B, Oral and oblique-oral view of the tetradial structure. C, Other ?larvae found in the same deposit showing a complex filamentous structure. F, Embryos of *Markuelia*. D–E, *Markuelia secunda* from the early Cambrian Petrosvet Formation of Siberia. D, Embryo showing head (left) and tail (right) juxtaposed and directed up and down, respectively. Note the arrangement and number of the tail spines and the alternating trunk spines that emerge from the annulae. E, Fractured embryo revealing internal organs that are in register with the surface annulae. F–I, *Markuelia hunanensis* from the late Cambrian Bitiao Formation of Wangcun, Hunan Province, China. F, View of the oral region armed with a series of radially arranged spines. G, Detail of the tail region and the terminal spines. H, Oral region with circumoral spines in center with tail spines to the right. I, Fractured embryo revealing preservation extending internally and including extension of annual divisions internally though not defining distinct compartments. Relative scale bar: 122 μ m (A), 122 μ m (B), 96 μ m (C), 118 μ m (D), 123 μ m (E), 28 μ m (F), 33 μ m (G), 85 μ m (H), 86 μ m (I). A–E reproduced from Kouchinsky and others (1999); D, E reproduced from Bengtson and Yue (1997); F–H reproduced from Dong and others (in press); I reproduced from Dong and others (2004). Used with permission of the authors and publishers.

considered an affinity to a scyphozoan cnidarian more likely, perhaps more specifically to the extinct conulariids. Further sampling of the same site has yielded polyps of comparable preservational style and size to the apical region of the *Punctatus/Pyrgites* adult (Steiner, Li and others 2004). However, the link between pentaradial embryos to tetradial conulariid adults is compromised by the discovery of tetradial embryos (see Figure 3H) among material from the Kuanchuanpu Formation (Steiner, Zhu and others 2004).

PSEUDOVIDES. The other abundant element of the Kuanchuanpu fauna is *Pseudoooides* Qian 1977 (Figure 3A–F). Originally described as a globular fossil of uncertain affinity, *Pseudoooides* has more recently been reported in association with the coeloblastic cleavage embryos (see Figure 2B–G) previously allied to *Olivoooides* (Bengtson and Yue 1997; Yue and Bengtson 1999a). The evidence to link these early and late cleavage embryos is comparable size, based on the observation that the cytoplasmic volume of embryos does not change before hatching (Steiner, Zhu and others 2004). The early blastulae have a pattern of cleavage comparable to the irregular spiral cleavage of living arthropods (Scholtz 1998; Steiner, Zhu and others 2004). Gastrula stage embryos exhibit a central furrow and central pore that may be a vestige of gastrulation (Figure 3A), and develop into later stages that have a characteristic pinched germ band that eventually divides into 12 serial divisions that cross-cut the longitudinal line of bilateral symmetry represented by a median furrow (see Figure 3B–E). The serial divisions are imposed onto the established germ band, rather than by terminal addition, as in the short germ development of most living arthropods. This developmental sequence is reminiscent of the long germ band mode of development in dipterans (Schwalm 1997) and onychophorans (Walker 1995; Scholtz 1998; but see Mayer 2005). A few incompletely preserved appendage-bearing unciliated larvae have been recovered in association with the embryos (see Figure 3F) and Steiner, Zhu and co-workers (2004) considered these to be the post-hatchling. They noted a strong distinction between head and trunk in the larva, composed of two and four divisions, respectively, believed to correspond to the 12 divisions in the germ band of the embryo. Appendages are clearly present in fragments of later stage embryos and these are unsegmented and multilobate with terminal setae. They do not resemble the jointed appendages of arthropods and Steiner, Zhu and co-workers (2004) compared them to annelids, onychophorans and tardigrades.

The evidence for the affinity of *Pseudoooides* is contradictory: irregular spiral cleavage, long germ band development and the overall form of the larva are suggestive of primitive arthropod or panarthropod affinity, while appendage morphology is not characteristically arthropodan. This may reflect a classic stem taxon combination of primitive and derived characteristics (Donoghue, in press). However, the evidence linking embryos to larvae, and one larva to another, amounts to little more than co-occurrence. Further surveys of the Kuanchuanpu Fauna will provide greater insight into the embryology of *Pseudoooides* and test the currently hypothesized sequence of development stages (Steiner, Zhu and co-workers 2004).

OTHER ELEMENTS OF THE FAUNA. Spine-bearing embryos (see Figure 3G) have also been recovered from the Kuanchuanpu Formation at Kuanchuanpu (Steiner, Zhu and co-workers 2004). The published material amounts to little more than an effaced sphere, presumably representing the preserved chorion, from which emerge a series of broad-based and laterally compressed spines. These have been compared to the sclerites of the Cambrian lobopod *Onychodictyon* (see, for example, Ramsköld and Chen 1998) and the Cambrian conodont-like fossil *Fomitcella* (see Bengtson 1983). However, any constraint over the affinity of this embryo type is illusory.

The tetradial embryos mentioned above (see Figure 3H) manifest symmetry through ingressive folding of their apertural region. After arguments presented in favor of a conulariid affinity for *Olivoooides*, these tetradial embryos are far more likely candidates. However, a scaliophoran-like larva (see Figure 3I) has also been recovered from the same samples and Steiner, Zhu and co-workers (2004) suggested, based on common surface ornamentation, that it represents the post-hatchling of the tetradial embryo. Given that the Kuanchuanpu Fauna continues to yield new elements, attempts to associate embryos with adults may be premature.

Putative cnidarians from Yakutia

Kouchinsky and others (1999) described embryos from the early Cambrian of Siberia that show tetradial or octoradial symmetry in the form of two sets of four interdigitating arms (Figure 4A–C). They suggested an affinity to cnidarians based largely on the co-occurrence of anabartids, a group of conical microfossils that generally have triradial symmetry and which, after

some debate, have been allied to scyphozoan cnidarians (Kouchinsky and Bengtson 2002). The link to anabaritids is tenuous and rests largely on the phenomenon of varying symmetry seen in living scyphozoans (Gershwin 1999). Kouchinsky and others also compared the octo- or tetradially arranged arms in these embryos (see Figure 4A, B) with the “annelid cross” and “molluscan cross” seen in the spirally cleaving blastulae of annelids, echiurans, molluscs and sipunculans. The affinity of these embryos is unknown, but they are unlikely to be cnidarians.

Markuelia

Originally described as an annulated sphere, *Markuelia secunda* was identified as an embryo by Bengtson and Yue (1997), who revealed its annulated vermiform outline, coiled in an S loop to assume a sphere, with head and spine-bearing tail juxtaposed but oriented in opposing directions (see Figure 4D–I). Although *Markuelia* is clearly annulated, there is some evidence that it may be segmented, based on the preservation of meristically arranged internal organs in register with surface annulations in *Markuelia secunda* (see Figure 4E). Based on its bilateral symmetry, among other characters, Bengtson and Yue (1997) identified *Markuelia* as the embryo of a bilaterian metazoan, considering its affinity to annelids, arthropods and lobopods. Subsequently, Conway Morris (1998a, 1998b, 1998c) argued for an affinity to halkieriids, the cuticular remains of which occur in association. Halkieriids have been considered members of the stem lineage of Annelida, Brachiopoda and Mollusca (Conway Morris and Peel 1995) although it has also been argued that they are more closely allied to Mollusca (Conway Morris and Peel 1990; Vendrasco and others 2004; Vinther and Nielsen 2005). More recently, Dong and others (2004) described more completely preserved examples of *Markuelia hunanensis* from the middle and late Cambrian of Hunan Province, south China (Figure 4F–I), in which the head is surrounded by multiple, broadly radially arranged, rows of spines (see Figure 4F, H) reminiscent of the scalids of cycloneuralians (phyla Nematoda, Nematomorpha, Kinorhyncha, Loricifera and Priapulida). However, while the overall form of *Markuelia* compares favorably to adult priapulids, it contrasts sharply with their larvae, which are neither annulated nor segmented and are almost entirely enveloped by a cuticular lorica from which the introvert emerges. *Markuelia* also compares favorably to the larvae (but not the adults) of nematomorphs (see, for example, Bohall and others 1997). A cladistic analysis by Dong and others (2004) indicated a stem Scalidophora (Kinorhyncha, Loricifera and Priapulida) affinity for *Markuelia*, although this plesiomorphic position may be an artifact of missing data (Dong and others 2005) and *Markuelia* could turn out to be more closely related to priapulids. A better constrained interpretation of the affinity of *Markuelia* awaits elucidation of the precise organization and in particular the symmetry of the circumoral scalids, which are hexaradial in nematodes and nematomorphs, and pentaradial in kinorhynchs, loriciferans and priapulids (Nielsen 2001).

Markuelia is the most widely distributed fossil embryo taxon known, with records extending from the Lower Cambrian to Lower Ordovician, and from Siberia, North America, China and Australia (Donoghue and others, in a manuscript in review). This may reflect something about the nature or ecology of the animal that predisposes the embryos to preservation. However, most examples of *Markuelia* yield no anatomy beyond the annulated trunk. The most completely known records are *Markuelia secunda* from the Lower Cambrian of Siberia and *Markuelia hunanensis* from the Late Cambrian of Hunan. *M. secunda* bears posteriorly directed, broad-based but compressed cuticular projections that emerge from the annulae and are organized into a few longitudinal files offset one from another (see Figure 4D). These spines are absent in *M. hunanensis*, which bears only infrequent conical narrow-based elongate spines that are more than 10 times smaller than the cuticular projections in *M. secunda*. The tails of both species are also different (compare Figure 4E and G). Both bear two pairs of curved posterior spines, but a third pair of smaller, almost straight spines occurs between the other two pairs in *M. hunanensis*. While the head of *M. hunanensis* is relatively well characterized, that in *M. secunda* is completely unknown. The trunk is overtly bilateral in *M. secunda*, but apparently radial in *M. hunanensis*. Given these differences it is possible that the relatively rich *Markuelia*

record is not a peculiar preservational artifact, but rather a grade of developmental stages common to a diversity of organisms.

Significance of the Known Record

A fossil record of embryonic development holds out the possibility of uncovering the modes of development of long-extinct common ancestors of extant lineages. Embryonic sequences of extinct organisms would provide a better understanding of developmental evolution and the significance of shared developmental stages and expression patterns of regulatory genes. These are being used to infer not only features of development in the common ancestors of distantly related model organisms, such as *Drosophila* and the mouse, but also the nature of the ancestral adult and the homology of phenotypic characters (De Robertis 1997; Tabin and others 1999; Balavoine and Adoutte 2003). This approach may be questioned because of its reliance on model animals—appropriate genomic data may not be available for phylogenetic intermediates—but data on model animals impact on patterns of anatomical character evolution and underpin hypotheses of homology. Furthermore, molecular-based hypotheses of anatomical homology are often based on simplistic datasets, such as the expression domains of individual pleiotropic regulatory genes (Jenner 1999; Davidson 2001; Erwin and Davidson 2002; Minelli 2003). Paleontological data provide the only conceivable test of such hypotheses, because it is possible to recognize taxa that are closer to ancestors than are their extant representatives (Donoghue, in press). A fossil record of embryonic development allows us to take this approach a step further by constraining the embryology of ancestral organisms.

A fossil record of development from the late Neoproterozoic through Cambrian could be expected to provide evidence of the gamut of metazoan phyla with a biphasic life history strategy involving a feeding (planktotrophic) larval stage. This is because metazoans have been interpreted conventionally to have evolved from a pelagic ancestor whose descendants adopted a benthic adult phase while maintaining a pelagic larva. This is believed to have resulted in specialization of the adult (benthic adaptation) and the larva (ciliation for swimming and feeding) and, inevitably, to metamorphosis (Haeckel 1874; Jägersten 1972; Nielsen and Nørrevang 1985; Nielsen 1987, 1994; Rieger 1994; Nielsen 1998, 2001). This view is supported by the occurrence of planktotrophic larvae in virtually all phyla (Strathmann 1987) and by the homology of ciliary bands in these larvae (Nielsen 1987). A development of this hypothesis is the “set aside theory,” which posits that most metazoan phyla diverged deep within the Proterozoic and that the pelagobenthic transition evolved convergently in the early Cambrian, within these already long distinct lineages (Davidson and others 1995; Peterson and others 1997; Cameron and others 1998; Peterson and others 2000). Does the evidence meet these expectations?

The known record of fossilized embryos has grown rapidly after the seminal work of Bengtson and Yue (1997), but the sample remains low, both in numeric diversity and phylogenetic disparity. Only the Phanerozoic examples can be assigned a phylogenetic affinity with any degree of certainty, and not in every case. At present we have evidence of the embryology of a scalidophoran ecdysozoan (*Markuelia*), a possible primitive arthropod (*Pseudoooides*), a possible lophotrochozoan (the “cnidarian” of Kouchinsky and others 1999), and a scyphozoan cnidarian (*Olivoooides*). While there is breadth to the phylogenetic sampling, it is hardly representative. Nevertheless, it could be enough to test the hypothesis that late Neoproterozoic and early Cambrian metazoans underwent a biphasic life history strategy, including a planktotrophic larva.

One clear feature of the known embryo fossil record is that, where data are available, the life history strategy is direct development. *Markuelia* and *Olivoooides* are direct developers. *Pseudoooides* is interpreted as developing from blastula, through gastrula to germ band embryo, and eventually to a larva, but this is not a larva in the strict sense, with a body plan distinct from the adult. Thus, *Pseudoooides* is also a case of direct development among early Cambrian metazoans. Doushantuo embryos are peculiar in that they are not associated with adults, although at least one of the taxa present underwent direct development (Xiao, Zhou and others 2004).

This incongruence between the expectation of indirect developers and the paleontological record dominated by direct developers could be a taphonomic artifact. While fossilization of embryos may be unlikely, the preservation of larvae may be even more so. Although some might contend with the plesiomorphy of indirect development, the evidence of larval shells of brachiopods and molluscs indicates that larvae were present, but there are no records of the remains of the soft tissues of primary larvae in the fossil record.

Clearly, the existence of organisms with a planktotrophic larval stage should not be discounted, but the key point is that at least some organisms were undergoing direct development in the late Neoproterozoic and early Cambrian. This is incompatible with the orthodox view that metazoan phyla diverged in the Cambrian and that they primitively had a planktotrophic larval stage (Bengtson and Yue 1997; Conway Morris 1998a, 1998b, 1998c, 2004). Of course, it is possible to sidestep the data by arguing for an earlier divergence of metazoans and the early evolution of their developmental modes in some lineages but not others. However, the hypothesis of planktotrophic plesiomorphy is already being challenged with the evidence on which it is formulated: the homology of larvae and their component ciliary bands used in feeding and locomotion.

Over the last couple of decades, histological investigation, functional analysis and more rigorous phylogenetic solutions have combined to shift the balance of evidence in many critical clades, leading to the conclusion that lecithotrophic (nonfeeding), rather than planktotrophic, larvae represent the primitive condition for Metazoa, if not all metazoan phyla (Haszprunar and others 1995; Jenner 2000). Furthermore, similarities in the topology of ciliary bands in planktotrophic larvae have been shown to be superficial and the result of physical rather than phylogenetic constraints; that is, the effect of viscous forces on the swimming and feeding of small organisms in a low Reynolds environment. It is likely that larval similarities are convergent (Emlet 1991; Strathmann 1993; Rouse 1999; Strathmann 2000). Given this, Jägersten's theory and its derivatives (the Trochaea theory of Nielsen and the set aside theory of Davidson and his colleagues), all of which are predicated on plesiomorphy of a planktotrophic larval stage, must be rejected.

Even under this analysis (Haszprunar and others 1995), plesiomorphy of the biphasic life cycle (Rieger 1994) stands firm, although many authors maintain that direct development is primitive for bilaterian metazoans (Wolpert 1994; Conway Morris 1998b; Knoll and Carroll 1999; Wolpert 1999; Rouse 2000; Sly and others 2003; Conway Morris 2004). Indeed, most primitive metazoans, as identified in the "new animal phylogeny" (Adoutte and others 2000; Halanych 2004), are direct developers (Haszprunar and others 1995; Conway Morris 2004) and the only Cambrian evidence is of direct developers. Because of the strict selective pressure imposed on early developmental stages by their physical environment, convergence is likely to have been a very important factor in developmental evolution (Conway Morris 1998b; 2004). Further sampling of the paleoembryological record, together with experimental analyses of biases in preservation potential (Martin and others 2003, 2004), are likely to prove integral in teasing apart the evolution of life history during and after the divergence of complex animals.

The Extent of the Paleoembryological Record

Based on the taxonomic spread of fossil embryos, their preservation does not reflect a taxonomic bias. Bengtson and Yue (1997) considered, using *Olivoooides* and *Markuelia*, that direct developers could dominate the record because their larger and yolzier eggs facilitate their preservation and identification. Larger eggs, however, do not necessarily have a higher yolk content (Villinski and others 2002) and the role that yolk plays in fossilization, if any, is probably minor given that few embryos of any kind are preserved in spite of the likely presence of many other direct developers. Peculiarities of the ecology of the adult stages of these organisms may predispose their embryos to fossilization, but without more data on the adults this is difficult to test. Preservational biases are most likely to be revealed by experimental attempts to simulate the decay and preservation of embryos and other developmental stages. The controls on the mineralization of embryos are likely to be similar to those on authigenic replacement of muscle

tissue and cuticle with calcium phosphate (Briggs and Kears 1993; Briggs and others 1993; Briggs and Kears 1994; Briggs and Wilby 1996). Although authigenic mineral replication of egg membranes has been achieved in the laboratory (Martin and others 2003, 2004), replication of embryos has yet to occur in experiments. The fossilization of embryos may involve a mode of authigenic mineral replacement distinct from the more widely encountered phenomenon of “Orsten-type” preservation (Waloszek 2003), which seems to rely on a cuticular substrate (Butterfield 2003). Either way, there is a vacuum of data on decay rates for developmental stages, and no understanding of how life history strategy and other variables affect preservation potential.

Preliminary data suggest that the conditions that promote the preservation of embryos were more prevalent during certain intervals of geologic time. The early Phanerozoic has already been densely sampled for phosphatic microfossils for biostratigraphic purposes, and reanalysis of these collections should provide some insight into the potential wealth of the paleoembryological record. Donoghue and co-workers (in a manuscript in review) have examined collections representing decades of biostratigraphic research into the early Phanerozoic of North America, Europe, Russia, Australia and China. This has revealed several new fossil embryo-bearing localities, extending the record into the late Cambrian and earliest Ordovician, and to North America and Australia. However, all these new records yield *Markuelia*-grade embryos. Thus, the record of embryos seems to be concentrated in a late Neoproterozoic to early Ordovician interval. Within this temporal window are two parallel trends. First, there is a diminution from the diversity of the Doushantuo and Kuanchuanpu faunas to *Markuelia* alone in the middle and late Cambrian and early Ordovician. In parallel, there is a reduction in the degree of phosphatization from the earliest embryos (often so heavily phosphatized that they are sometimes solid balls of phosphate) to the extremely lightly phosphatized examples from the middle and late Cambrian of Hunan and the early Ordovician of Nevada (in which often only the body margin is preserved in phosphate and the remaining cavity is in-filled with calcium carbonate). This trend is evident even in the record of *Markuelia* alone. Donoghue and co-workers (in a manuscript in review) argue that the restricted stratigraphic distribution and the decreasing phosphatization of the embryos reflect higher levels of phosphorous or phosphate solubility in the world's oceans in the late Neoproterozoic and Cambrian, which are thought to have declined sharply after the Cambrian (Brasier 1990; Cook 1990; Mackenzie and Guidry 2000; Butterfield 2003; Porter 2004).

Conclusion

The imperfections in the fossil record recognized by De Beer (1958) as representing episodes of clandestine developmental evolution can no longer be so easily explained away. The paleoembryological record has the potential to provide crucial insights into the developmental biology of metazoans (for instance, during diversification), but is likely largely confined to the Neoproterozoic and early Paleozoic. Continued sampling will yield a wealth of developmental data. Together with experimental investigation of the relative preservation potential of embryos and larvae representative of different developmental stages, life history strategies, ecologies and affinities, these data will provide a basis for more directly inferring embryology and developmental evolution in the late Neoproterozoic and early Phanerozoic. Developmental biologists need not revisit the Cambrian or Neoproterozoic to gain insight into the embryology of early metazoans, but direct evidence of developmental evolution throughout the remainder of the Phanerozoic seems as intangible as ever.

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