

Fossilized embryos are widespread but the record is temporally and taxonomically biased

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SUMMARY We report new discoveries of embryos and egg capsules from the Lower Cambrian of Siberia, Middle Cambrian of Australia and Lower Ordovician of North America. Together with existing records, embryos have now been recorded from four of the seven continents. However, the new discoveries highlight secular and systematic biases in the fossil record of embryonic stages. The temporal window within which the embryos and egg capsules are found is of relatively short duration; it ends in the Early Ordovician and is roughly coincident with that of typical “Orsten”-type faunas. The reduced occurrence of such fossils has been attributed to reducing levels of phosphate in marine waters during the early Paleozoic, but may also be owing to the increasing depth of sediment mixing by infaunal metazoans. Furthermore, most records younger than the earliest Cambrian are of a single

kind—large eggs and embryos of the priapulid-like scalidophoran *Markuelia*. We explore alternative explanations for the low taxonomic diversity of embryos recovered thus far, including sampling, size, anatomy, ecology, and environment, concluding that the preponderance of *Markuelia* embryos is due to its precocious development of cuticle at an embryonic stage, predisposing it to preservation through action as a substrate on which microbially mediated precipitation of authigenic calcium phosphate may occur. The fossil record of embryos may be limited to a late Neoproterozoic to early Ordovician snapshot that is subject to dramatic systematic bias. Together, these biases must be considered seriously in attempts to use the fossil record to arbitrate between hypotheses of developmental and life history evolution implicated in the origin of metazoan clades.

INTRODUCTION

Despite the perceived vanishing probability of embryo fossilization, embryos of early metazoans have begun to be recovered from rocks of Cambrian (Zhang and Pratt 1994; Bengtson and Yue 1997; Kouchinsky et al. 1999; Yue and Bengtson 1999; Chen et al. 2004; Dong et al. 2004; Hua et al. 2004; Steiner et al. 2004a, b; Dong et al. 2005) and late Neoproterozoic age (Li et al. 1998; Xiao et al. 1998, 2000; Xiao and Knoll 1999, 2000; Zhang et al. 1998; Yin et al. 2001, 2004; Xiao 2002; Chi et al. 2003; Yuan et al. 2002) (for a review see Donoghue and Dong 2005). These discoveries, including both “lower” invertebrates (*Tianzhushania*, *Megaclonophycus*, *Olivoides*) and bilaterians (*Markuelia*, *Pseudoooides*), open up wide avenues toward the investigation of development in

fossil animals (Bengtson 1998). Indeed, these discoveries, all of direct-developing metazoans, have already fuelled speculation that cherished assumptions, such as the plesiomorphy of indirect development among Bilateria (Haeckel 1874; Jägersten 1972; Nielsen 2001) are founded on artifacts of convergence and extinction (Bengtson and Yue 1997; Conway Morris 1998, 2004).

However, the scope of this new research program is limited by the prevalence of fossilized embryos, both in terms of their temporal distribution and the taxonomic diversity of organisms represented. Here we report new discoveries and verify hitherto unsupported records of fossilized embryos, greatly extending the number, geographic distribution, and stratigraphic extent of the known embryo fossil record. Nevertheless, the diversity of embryos recovered is extremely low,

and the forms from the new localities are in fact all attributable to the direct developing embryos of the priapulid-like scalidophoran *Markuelia*. Despite our extension of the stratigraphic range of sites from which fossilized embryos have been recovered, the record appears to be restricted to an interval that does not extend beyond the earliest Ordovician. Furthermore, only embryos of *Markuelia* are known beyond the early Cambrian. This suggests that there are systematic and secular biases in the nature of the embryo fossil record. We argue that these arise because of a number of factors: (1) diminishing levels of phosphate within the world's oceans during the early Phanerozoic; (2) an increase in the depth of sediment mixing by marine fauna during the early Ordovician that led to a dramatic drop in fossilization potential through the maintenance of oxygenated pore waters (and, thus, aerobic bacteria) deep within to the sediment; and, (3) the precocious development in *Markuelia* of a cuticle within the embryo, a factor identified empirically as integral in the authigenic replication of soft tissue structure in deposits such as the Chengjiang, Burgess Shale and Orsten. By implication, paleontological insights into the embryo fossil record may be limited to a late Neoproterozoic to early Ordovician snapshot that is itself highly biased by factors such as taxonomy and developmental mode. Thus, direct testing of hypotheses implicating or denying life history evolution as an important factor in the origin of metazoan clades, may never be possible.

MATERIALS AND METHODS

New sites were discovered either by targeted sampling for soft-tissue preservation or else discovered through routine sampling for microfossil biostratigraphy. The embryos were recovered by dissolution of the host carbonate rocks using 6–15% acetic acid, in some instances buffered (Müller 1985; Jeppsson et al. 1999). The recovered residue was washed through a series of mesh screens to sort size fractions and remove particles that are too large or small. The residue was then sorted manually with the aid of a binocular microscope to recover the embryos; in some instances the calcium phosphate fraction was concentrated using a heavy liquid (Bromoform, Tetrabromoethane, or Sodium polytungstate) at an interim stage. Specimens were coated with a conductive medium and examined using a scanning electron microscope.

NEW LOCALITIES

Siberia

Elevation 1291 m, at the right side of the Gonam River (left tributary of the Uchur River), 30 km from the mouth, from the lowermost 0.65 m of the Pestrotsvet Formation; left bank of the Aldan River, 7 km upstream from the mouth of the

Ulakhan–Sulugur River, from the lowermost 0.3 m of the Pestrotsvet Formation; same locality, from the lowermost 10 cm of the Pestrotsvet Formation; left bank of the Aldan River, 4–5 km upstream the mouth of the Dyalkhakh River, outcrop “Dvortsy,” from the lowermost 0.5 m of the Pestrotsvet Formation.

Australia

Middle Cambrian of the Georgina Basin, northern Australia, North Rogers Ridge, Inca Shale; North Rogers Ridge, 250 m south of telegraph line, Inca Shale (late Templetonian—? early Floran); Mount Murray, north Phosphate Hill, top of Beetle Creek Formation; Mount Murray, Monastery Creek Formation (late Templetonian) (Walossek et al. 1993).

United States

US Geological Survey sample 9446-CO, Vinini Formation, Tremadocian, Early Ordovician, Battle Mountain, Carlin, Nevada.

EMBRYOS

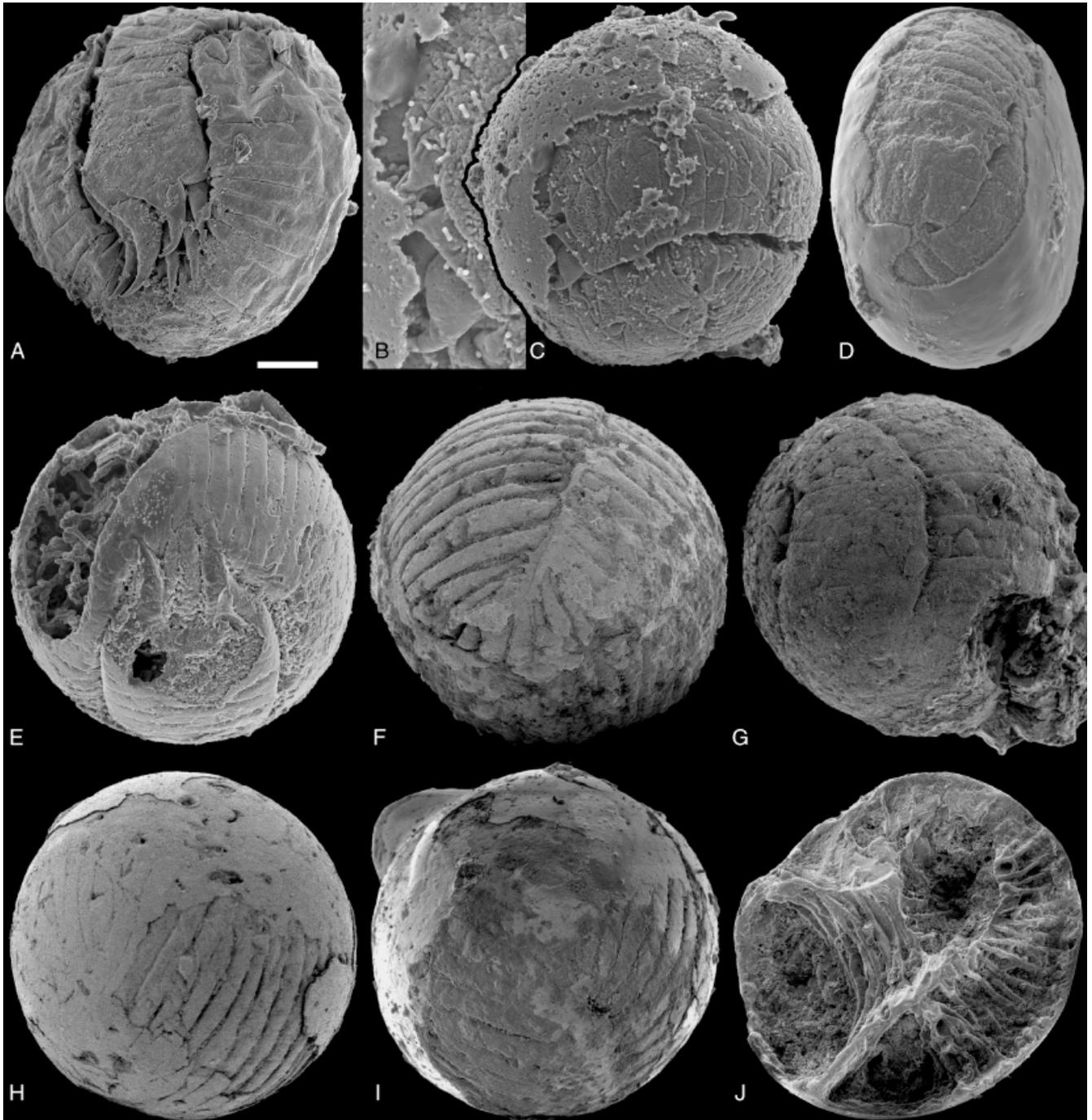
All of the embryos recovered from the new localities (Fig. 1) are closely comparable, preserving an elongate and profusely annulated vermiform organism tightly coiled in an “S”-shaped or inverted “S”-shaped loop into a sphere, with anterior and posterior poles juxtaposed laterally. However, some specimens exhibit differences. The embryos from the middle Cambrian of Australia (Fig. 1A–D) are typically smaller than those from the other sites, while those from the early Ordovician of North America (Fig. 1E) lack the posteriorly directed conical protuberances emerging from the annulated trunk seen in embryos from other sites. Where the posterior extremity of the embryo is preserved, the tail spines are usually curved (Fig. 1A and C), although they are approximately straight in the embryo from the early Ordovician of Nevada (Fig. 1E). The anterior region is well-preserved only in specimens from the middle Cambrian of Australia where it exhibits a presumed terminal orifice preceded by a subtly tapering region that is not annulated, the outer rim of which is adorned with a row of posteriorly directed conical spines that appear to intercalate with another such row that is situated just within the orifice (Fig. 1A). The embryos also exhibit differences in the orientation of coiling, with some exhibiting inverted (Fig. 1A), and others normal “S” coiling (Fig. 1C and E).

Despite their differences, the entire suite of embryos can be attributed to *Markuelia*, based on comparative material described previously from a number of sites in the Early Cambrian of Siberia, but interpreted first as fossilized embryos by Bengtson and Yue (1997). Only two of these records have

been verified hitherto; we provide illustrative support for five additional localities (Fig. 1F–J). Three species of *Markuelia* are known, *M. prima*, *M. secunda* (*M. prima* is probably a junior synonym of *M. secunda*) from the Lower Cambrian of Siberia (Bengtson and Yue 1997), and *M. hunanensis* from the Middle Cambrian Huaqiao and Chefu formations, and the Late Cambrian Bitiao Formation, at Wangcun, Hunan, south China (Dong et al. 2004, 2005). The key similarities to specimens hitherto assigned to *Markuelia* are the profusely annu-

lated trunk coiled in an “S”-shaped (or inverted “S”-shaped) loop into a sphere, and the paired, bilaterally arranged, terminal appendages.

The embryos from the Middle Cambrian of Australia exhibit greatest similarity to *M. secunda* from the Lower Cambrian of Siberia (Fig. 1F–J), which also possesses conical protuberances that alternate in position along the trunk (Fig. 1G). However, the Australian specimens are significantly smaller (average diameter 300 vs 400–500 μm),



the protuberances are more slender and less numerous, and there is some variation in the morphology of the terminal spines among specimens (Fig. 1A and C), possibly indicating that these collections include more than one new species.

The embryo from the Lower Ordovician of Nevada (Fig. 1E) exhibits greatest similarity to *M. humanensis*, falling within the same size range, and exhibiting simple annulae lacking protuberances. The tail, though incompletely preserved, is composed of simple spines including two straight central spines and two pairs of larger curved spines toward the margins.

DISCUSSION

TAXONOMIC AND TEMPORAL BIAS IN EMBRYO FOSSILIZATION

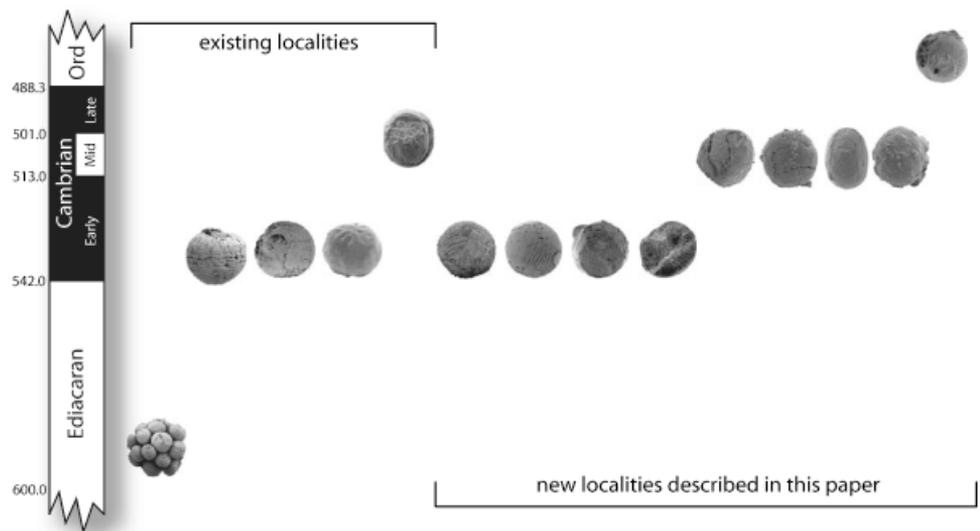
Our verification of old records and description of new records significantly extends the stratigraphic and geographic ranges, both of *Markuelia* and of the known embryo fossil record (see summary Figure 2). If nothing else, these records reveal that the conditions necessary for the fossilization of embryos must have been much more common than has been considered previously, and on a variety of spatial and temporal scales. Although all of the *Markuelia* records from the Lower Cambrian of Siberia are from the same (Aldan) river basin and from approximately the same stratigraphic level, they are geographically distinct from one another. Furthermore, we have documented that the conditions necessary for embryo fossilization were reproduced on continents that were at least as distinct in the Cambrian and Early Ordovician as they are today. However, the diversity of embryos recovered remains low; the vast majority of sites preserve only embryos referable to *Markuelia*. Furthermore, the stratigraphic extent of embryo records remains confined to an interval that ends in the lowermost Ordovician. Thus, on the basis of the available evidence, it appears that the fossilization of embryos may be a taxonomic and, possibly, a temporal phenomenon.

Why assemblages of fossilized embryos exhibit such low diversity is enigmatic in that there is nothing obviously special

about the character of the organisms preserved. Indeed, many of the deposits exhibit distinct biases to earlier or later developmental stages (Conway Morris 2003). Bengtson and Yue (1997) suggested that their database “may represent a biased sample of early metazoan development, because eggs of direct developers are generally larger (and yolzier) than those of indirect developers and thus may be more readily preserved as fossils and easier to identify” (p. 1648). Thus, the absence of other embryos in the record may still be consistent with the hypothesis that indirect development is the primitive condition for metazoans and the vast majority of metazoan phyla (Jägersten 1972; Davidson et al. 1995; Nielsen 2001), and the assumption that primary larvae implicitly have a nonexistent preservation potential (Kerr 1995). However, even indirect developers pass through cleavage and gastrulation as embryos, and such stages are preserved in the Neoproterozoic (Xiao et al. 1998; Xiao and Knoll 2000) and earliest Cambrian, in association with *Olivoooides* and *Pseudoooides* (Bengtson and Yue 1997; Yue and Bengtson 1999; Steiner et al. 2004b), and with *Markuelia* (Dong et al. 2004; Dong et al. 2005). Nevertheless, the embryos of *Markuelia*, *Olivoooides* and *Pseudoooides* are large, even by the standards of direct developers. For instance, nematodes are direct developers, but their embryos are typically a mere 40 μm in maximum dimensions (Lee 2002). Even if they were preserved they would not be recovered by standard micropaleontological techniques not specifically designed for the recovery of minute fossils; mesh sizes of less than 63 μm are rarely utilized during the recovery of acid insoluble residues. Although our database has been constructed on collections representing a variety of processing techniques, it is still possible that there is a diverse fossil record of embryos that is being recovered but subsequently washed down laboratory waste pipes. In analysis of the early Cambrian Kuanchuanpu Formation, the most diverse of all fossil embryo assemblages, we have tested for this possibility using recovery processes in which all sediment size fractions are recovered, but smaller embryos are not present.

Fig. 1. Scanning electron micrographs of embryos of *Markuelia* (A–J). A, *Markuelia* n. sp. from the Middle Cambrian Monastery Formation of N. Rogers Ridge, Georgina Basin, N. Australia; this specimen shows the oral (upper right) and aboral (center) regions juxtaposed; UB W 132. B and C, *Markuelia* n. sp. from the Middle Cambrian Beetle Creek Formation of Mt Murray, Georgina Basin, N. Australia; B is a detail of C showing the morphology and arrangement of the tail spines which are partially obscured by the preserved chorion overlying; UB W 133. D, *Markuelia* n. sp. from the Middle Cambrian of the Georgina Basin, N. Australia; E, *Markuelia* sp. from the earliest Ordovician Vinini Formation exposed on the southern flanks of Battle Mountain, near Carlin, northern Nevada; the tail is only partially preserved (center) and the trunk wall broken through to reveal preserved hyphae within; USNM 530283. (F–J). *Markuelia secunda* from the early Cambrian of Siberia. F and G, from the lowermost 65 cm of the Pestrotsvet Formation at a height of 1291 m, right side of the Gonam river, 30 km from the mouth; F, SMNH X3801; G, SMNH X3802; H, From the lowermost 0.5 m of the Pestrotsvet Formation on the left bank of the Aldan river, 4–5 km upstream the mouth of the Dyalkhakh river, outcrop “Dvortsy”; SMNH X3803, I. From the left bank of the Aldan river, 7 km upstream the mouth of the Ulakhan–Sulugur river, from the lowermost 30 cm of the Pestrotsvet Formation; SMNH X3804. J, Same locality, lowermost 10 cm of the Pestrotsvet Formation; SMNH X3805. Repository abbreviations: UB, University of Bonn Palaeontological Museum; USNM, National Museum of Natural History, Smithsonian Institution; SMNH, Swedish Museum of Natural History. Relative scale bar: A, 59 μm; B, 23 μm; C, 50 μm; D, 55 μm; E, 82 μm; F, 86 μm; G, 73 μm; H, 87 μm; I, 84 μm; and J, 86 μm.

Fig. 2. Summary representation of the stratigraphic distribution of new embryo-bearing deposits and localities. From left to right, previously described deposits late Neoproterozoic (Ediacaran) Doushantuo Formation (preservation known from a number of sites in the South China platform); early Cambrian (Manykaian) Kuan-chuanpu Formation (preservation known from a number of localities, though few have been well documented); early Cambrian (Tommotian) locality in the Dvortsy Section of the Aldan Basin, Siberia (Bengtson and Yue 1997); early Cambrian (Tommotian) Siberian locality described in Kouchinsky et al. (1999); middle and late Cambrian of Hunan as described by Dong et al. (2004); new localities described in the text: four early Cambrian (Tommotian) localities in the Aldan River Basin, Siberia; four localities in the middle Cambrian of the Georgina Basin, northern Australia; one locality in the early Ordovician of Nevada, USA.



Systematic bias notwithstanding, there remains an apparent secular bias in the known fossil record of embryos, although it is possible that the apparent pattern is artifactual. For instance, it could be argued that fossilized embryos are a recently discovered phenomenon and insufficient time has elapsed to sample the stratigraphic record to maturity. However, the Phanerozoic record has been densely sampled for phosphatic microfossils for biostratigraphic purposes over a period of many decades. Indeed, the interpretation of *Markuelia*, *Olivoides* and *Pseudoides* as fossilized embryonic stages (Bengtson and Yue 1997; Yue and Bengtson 1999; Steiner et al. 2004b) was based, in most instances, on existing collections and came many years after their initial description (Val'kov 1983; Qian 1977). Restudy of geographically and stratigraphically wide-ranging biostratigraphic collections of phosphatic microfossils (USGS conodont biostratigraphic collections cover the Palaeozoic of North America and many European sites; Peking University conodont collection covers much of the Cambrian and Ordovician of China; Leicester University conodont collection covers the Lower Palaeozoic of the UK and the Silurian of much of Europe) has identified sites yielding fossilized embryos but, so far, all records younger than the earliest Cambrian have been assignable to *Markuelia* (bar the unassigned blastulae of Zhang and Pratt 1994). Many tens of further sites have also been identified throughout North America, Europe, Australia and China, that yield fossils comparable with the chorions of *Markuelia* (e.g., Ethington 1981), but do not preserve embryos within. These are all confined to an interval that spans the Middle Cambrian to early Ordovician.

Long-standing hypotheses of metazoan life history evolution (Haeckel 1874; Jägersten 1972; Davidson et al. 1995; Nielsen 2001) argue in favor of the late emergence of direct-developers (though see Ax 1996; Jenner 2000; Peterson 2005). This is because in marine metazoans, most clades that exhibit direct development appear, by comparison with living outgroups, to have been derived from indirect-developing ancestors (Strathmann 1978; Emler 1991; Wray 1995; Ax 1996; Jeffery et al. 2003; Sly et al. 2003). Thus, if anything the record should improve, not disappear. Conflation of preparation biases, introduced during the recovery of the fossils, and the size of embryos, may well introduce a secular bias but we would anticipate a spectrum of embryo sizes across and within metazoan groups, as there is today. These expectations are not met, and a dearth of records post-Tremadoc still points to a secular bias in the record unless it is accepted that large embryos are a phenomenon of the Cambrian and the Recent alone, which can be rejected on phylogenetic grounds.

Thus, there appears to be a temporal window within which embryo fossilization was relatively widespread. This coincides with the window of "Orsten"-type preservation that has been celebrated for its exquisitely preserved micro-arthropods, faithful to the smallest bristled seta (Waloszek 2003). This is obviously not a coincidence; the embryos exhibit the same surface textures indicative of microbially mediated replacement of soft tissues by calcium phosphate (Bengtson and Yue 1997). Calcium phosphate soft-tissue replacement is by no means restricted to the early Paleozoic, with records from as recent as the Holocene (McCobb et al. 2004) but most famously from the Cretaceous Santana Formation of Brazil where the structure of vertebrate soft tissues is preserved to a

subcellular level (Martill 1990). However, Orsten-style preservation is quite distinct from the majority of these later records, being limited to replacement of surface structure (Müller et al. 1995), although rare records of more extensive replacement are known (Andres 1989), and with an apparent upper limit on the size of organisms preserved of approximately 2 mm. Furthermore, the preponderance of sites of this style of preservation remains a late Neoproterozoic and early Paleozoic phenomenon. This is usually attributed to higher levels of phosphorous or phosphate solubility in the world's oceans at this time, which are thought to have declined sharply after the Cambrian (Cook 1990; Porter 2004). Curiously, however, the diminution of the Orsten preservational window coincides with the disappearance of Burgess Shale-type preservation (Butterfield 2003). This has been correlated to emergence of bioturbators in low-oxygen environments (Droser and Bottjer 1989; Allison and Briggs 1993; Bottjer et al. 2000), precisely those in which soft-tissue replication by either mode, is implicated (Orr et al. 2003). Indeed, the youngest record of a fossilized embryo that we describe is from early Ordovician flat-pebble conglomerate facies, another early Paleozoic phenomenon the disappearance of which has been explained by the expansion of bioturbators (Sepkoski 1982). Thus, the temporal window of embryo preservation appears to result from a conflation of high phosphate availability and an absence of bioturbators.

The peculiar exclusivity of *Markuelia*-type embryos beyond the early Cambrian remains, however. Cambrian and Early Ordovician diversity of marine animals was rapidly rising (Sepkoski 1981; Adrain et al. 1998), but taken at face value the record of embryos would seem to suggest the opposite. This of course is unrealistic, but so is the interpretation that *Markuelia* is the only direct developer with large eggs in an early Paleozoic sea teeming with animals representing most living phyla. Which aspects of the embryology of this organism's anatomy, physiology or ecology predisposes its fossilization? The diverse record of embryos of similar large size in the late Neoproterozoic to earliest Cambrian confirms that this type of egg was not unique to *Markuelia*, among early animals. It should be kept in mind, however, that whereas the earlier embryo assemblages in South China are extreme in their abundance (the phosphoritic carbonates of the Doushantuo and Kuanchuanpu formations commonly have the appearance of oolites), *Markuelia* embryos are seldom common fossils. If *Markuelia* embryos had structural or biochemical properties that even slightly increased their propensity for fossilization in a phosphate-rich environment, their exclusive representation in the small number of finds hitherto reported may reflect a taphonomic bias. Chief among these has to be the precocious presence of cuticle in the embryonic *Markuelia*, apparently an important substrate in bacterially mediated authigenic mineralization (Briggs 2003; Butterfield 2003). Cuticle has also been implicated in slowing

decay (Wilby and Briggs 1997) and in controlling pH (Briggs and Wilby 1996). Thus, *Markuelia* may be the only organism whose embryos could pass the taphonomic threshold of Orsten-type preservation before the opportunity finally closed in the early Ordovician. Thus, as with the Burgess Shale-type taphonomic windows on early life, the embryo fossil record provides a brief but no less direct glimpse into the embryology of animals during their early diversification. It has the potential to test competing hypotheses of the interplay between development and evolution in the establishment of extant lineages. However, the record is biased, and knowledge of these biases is integral to the veracity of conclusions that can be derived from it, although the biases we identify may be so limiting as to severely compromise tests for such over-arching hypotheses as the role of life history evolution in the establishment of metazoan clades.

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