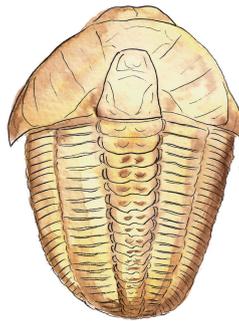


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Chapter vignette artwork by Brigitte Baldrian.
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INTRODUCTION

For anyone who has cared for animal embryos, it beggars belief that these squishy cellular aggregates could be fossilised. Hence, with hindsight, it is possible to empathise with palaeontologists who found such fossils and, in their naming of *Olivoooides*, *Pseudoooides*, etc., drew attention to their likeness to animal eggs and embryos but without going so far as to propose such an interpretation. However, in 1994, Zhang Xi-guang and Brian Pratt described microscopic balls of calcium phosphate from Cambrian rocks of China, one or two of which preserved polygonal borders that resembled blastomeres on the surface of an early cleaving animal embryo (Zhang and Pratt 1994). In retrospect, these fossils are far from remarkable, some of them may not be fossils at all, and it is not as if anyone ever conceived Cambrian animals as having lacked an embryology. But Zhang Xi-guang and Brian Pratt dared the scientific world, not least their fellow palaeontologists, to believe that the fragile embryonic stages of invertebrate animals could be fossilised, that there was a fossil record of animal embryology, that this record hailed from the interval of time in which animal body plans were first established, and that it had been awaiting discovery in the rocks, for want of looking. The proof of this concept came a few years later, when phosphatised Cambrian fossils from China and Siberia were shown to display indisputable features of animal embryonic morphologies (Bengtson and Yue 1997). In the case of *Olivoooides*, a series of developmental stages from cleavage to morphogenesis through hatching and juvenile growth could be tentatively identified; in *Markuelia*, the coiled-up body of an annulated worm-like animal could be clearly seen within its fertilisation envelope.

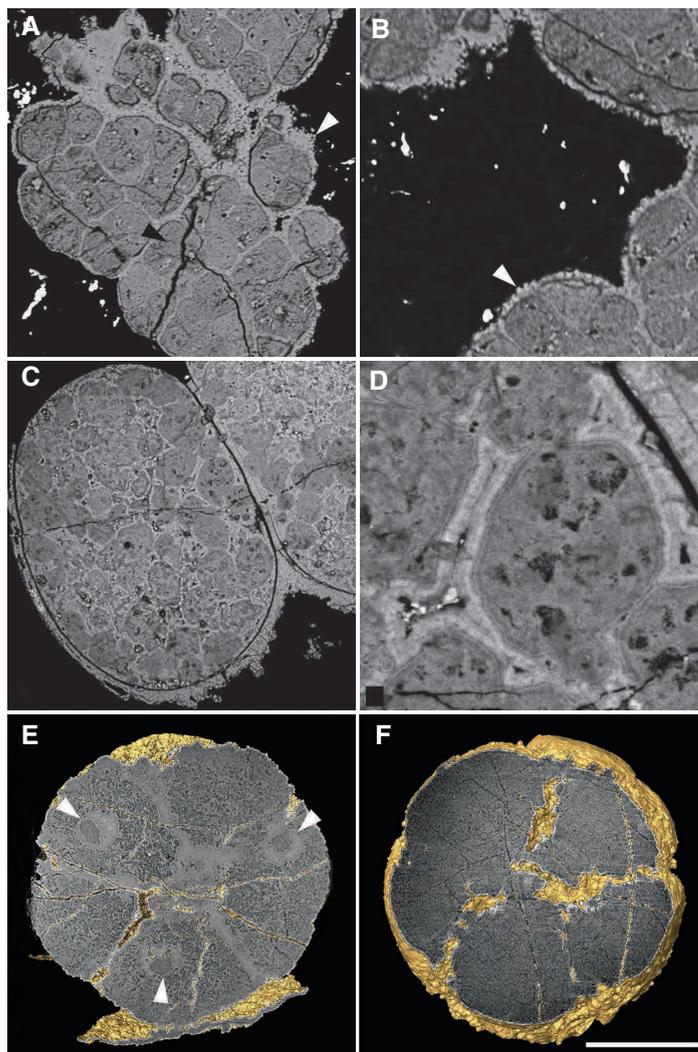
It is not as if palaeontologists had been sitting on their hands until then. There has long been a strong tradition of assaying rocks of all ages, including these, for microscopic phosphatic fossils, principally conodonts (Donoghue et al. 2000) and elements of the enigmatic small shelly faunas (Bengtson 2005), driven principally by attempts to establish a global stratigraphy as a basis for establishing a relative timescale for Earth history. Indeed, the majority of discoveries of fossil embryos made subsequently have been

based on the redescription, reinterpretation, and augmentation of knowledge of fossils that had been described long before or the discovery of new fossils from the deposits that had, on re-examination, previously yielded embryonic remains. There was palpable excitement in these early days that an extra dimension to the fossil record had been revealed and evolutionary biologists would soon be integrating the embryology of trilobites, ammonites, and anomalocaridids, with that of their living kin, effecting tests of developmental evolution that would be as direct as possible without the aid of a time machine, settling centuries-old debates over the plesiomorphy of gastrulation modes and the like (Donoghue and Dong 2005). Indeed, embryos and larvae of a great diversity of animals have been reported, including stem-metazoans (Hagadorn et al. 2006), sponges (Chen et al. 2000, 2009a), cnidarians (Bengtson and Yue 1997; Kouchinsky et al. 1999; Yue and Bengtson 1999; Chen et al. 2000, 2002, 2009a; Chen and Chi 2005; Dong et al. 2013), ctenophores (Chen et al. 2007), bilaterians generally (Chen et al. 2000, 2006, 2009a, b), or, more specifically, arthropods (Chen et al. 2004) and scalidophorans (Dong et al. 2004, 2005, 2010; Donoghue et al. 2006a; Steiner et al. 2014), the majority of which are from the Ediacaran Doushantuo Formation and the Early Cambrian Kuanchuanpu Formation, both of South China. Not all of these interpretations have withstood scrutiny, principally because palaeobiologists and embryologists have been unprepared in interpreting these most remarkable of fossil remains.

DISINTERRING THE BIOLOGY OF FOSSIL EMBRYOS

Given that the preservation of purported Ediacaran and Cambrian fossil embryos extends beyond the cellular to the subcellular and organelle level (Hagadorn et al. 2006; Hultgren et al. 2011), it seems that there might be a compelling case to make direct comparisons to the embryos of living animals. However, fossils are not merely the decayed remains of once living organisms, and, somewhat ironically, exceptionally preserved

Fig. 3.1 Biological features and diagenetic artefacts in the Ediacaran Doushantuo biota. (A–D) are scanning electron micrographs, (E–F) are synchrotron radiation X-ray tomographic microscopy-based reconstructions. (A) An alga from Doushantuo with algal anatomy preserved in a low atomic number phase (*black arrowhead*); high atomic number material encrusts the algal cells and fills spaces between cells (*white arrowhead*). (B) Detail of the same specimen as (A) showing that the high atomic number phase consists of elongate crystals with their long axes normal to the surface of the alga (*arrowhead*). (C) An embryo-like fossil with structures interpreted as lipid vesicles or yolk droplets within the cells. (D) Detail of the same specimen as (C) showing that the spaces between these structures are filled by layered diagenetic cements. (E) Embryo-like specimen that preserves subcellular anatomy including possible nuclei (*arrowheads*). (F) Embryo-like specimen that preserves only surface anatomy. Parts (A–D) also figured by Cunningham et al. (2012a); parts (E–F) also figured by Cunningham et al. (2012b). Relative scale bar: (A) 50 μm , (B) 30 μm , (C) 145 μm , (D) 27 μm , (E) 200 μm , (F) 125 μm



fossils are among the most difficult to interpret. This is because decay is an essential prerequisite to the mineralisation of labile biological tissues, which is invariably mediated microbially (Briggs 2003). Thus, the biological substrates that are available for mineralisation will not reflect perfectly the *in vivo* condition, which will have been defiled by the heinous processes of death, autolysis, and microbial decay, at the very least. What is more, organic structures decay at different rates and may be more or less predisposed to mineral replication by fossilisation. Hence, most exceptionally preserved fossils constitute a mineralogical *mélange* of crystal growth on or within original biological structures that will have undergone a spectrum of decay across different structures

(Fig. 3.1A–F), both within and between individual carcasses. While some biological structures are preserved by mineral impregnation or templating, residual structures decay away to unrecognisable clumps of organic matter that serve as templates for mineralisation or leave voids that are filled much later by percolating fluids rich in mineral ions during the process of sedimentary diagenesis. The resulting complex geode-like diagenetic mineralisation patterns can be readily mistaken for original biological structure (Bengtson and Budd 2004; Donoghue and Purnell 2009). Fossilisation history can be further complicated by later phases of mineral growth that obliterate original biological and intervening diagenetic structure. All of this may be confused further by the laboratory

processes that palaeontologists use to recover the fossils from the rock, which invariably employ acids that exploit differences in the solubility of the mineral comprising the fossil versus the mineral cement that binds the sedimentary rock, such that the fossils may be recovered from the disaggregated matrix. However, it can be difficult to control the pH and chemistry of these experiments, leading to artefacts introduced into the fossils by etching or through removal or one or more of the phases of mineralisation introduced during their fossilisation history (Jeppsson et al. 1985).

Fossil embryos are far from immune from the introduction of artefacts as a consequence of these processes of fossilisation and fossil recovery (Xiao and Knoll 2000; Cunningham et al. 2012a). Indeed, fossils interpreted to reflect the earliest stages of embryonic development are simple geometric arrangements of spheroids that can themselves be difficult to discriminate from inorganic structures. However, in interpreting these fossils, it can be difficult to move beyond gainsaying and to obtain an objective approach to discriminating mineral phases that preserve biological structure versus later phases associated with the mineralisation of decayed remains, void filling, or still later phases of mineral growth.

The interpretation of purported embryo fossils from the Ediacaran Doushantuo Formation have proven particularly contentious, with claims of exceptional preservation of labile structures matched by counterclaims that the critical structures on which these interpretation are based merely represent void-filling cement fabrics (Xiao et al. 2000; Bengtson 2003; Cunningham et al. 2012a). Cunningham et al. (2012a) discriminate these phases on the basis of their mineralogical fabric and elemental chemistry. Generally, the earliest mineral phases that preserve biological structure grow within organic substrates, and so the crystals are irregularly arranged and comparatively small (typically at most only a few micrometres in length; Fig. 3.1A–F). Void-filling phases of mineralisation nucleate on the existing mineral substrates and grow approximately perpendicularly to these substrates, yielding an aligned and centrifugally and centripetally layered mineral fabric charac-

terised by comparatively large crystals (typically tens of microns in length), and commonly a botryoidal texture (Fig. 3.1A–F). These two phases of mineralisation are also invariably correlated to differences in elemental chemistry. By demonstrating these characteristics in fossils or features from the same deposit whose biology can be interpreted uncontroversially, it is possible to discriminate mineral phases that preserve original biological structure in more controversial fossils. In this manner, it has been possible to reject many claims for the presence of derived embryonic animals in the Ediacaran Doushantuo Formation (Cunningham et al. 2012a).

ONTOGENY AND TAPHONOMY

Discriminating the biology of preserved fossil embryos is just the beginning of the process of obtaining material insights into the embryology of fossil organisms. The embryology of living animals is difficult enough to study in itself, but at least it is possible to observe the development of a single organism within a Petri dish. The study of fossil embryos requires that developmental stages are correctly identified as such within a fossil assemblage, and there is no guarantee that all stages are preserved. The only insights we have into the relative preservation potential of different developmental stages is based on experimental studies of the decay of *Artemia salina*, which showed quite surprisingly that the rate of decay increases with development, from the encysted diapause stages through postembryonic larval stages through to adults (Gostling et al. 2009). When maintained under reducing conditions, the dead encysted embryos remained stable as physical substrates available for mineral replication, for a period of more than a year (Gostling et al. 2009), a timescale that is readily compatible with the establishment of conditions required for microbially mediated mineralisation of those substrates (Briggs et al. 1993). Indeed, the long-term physical stability of embryonic structure post-mortem appears to be a general phenomenon for animal embryos in reducing conditions (Raff et al. 2006; Gostling et al. 2008),

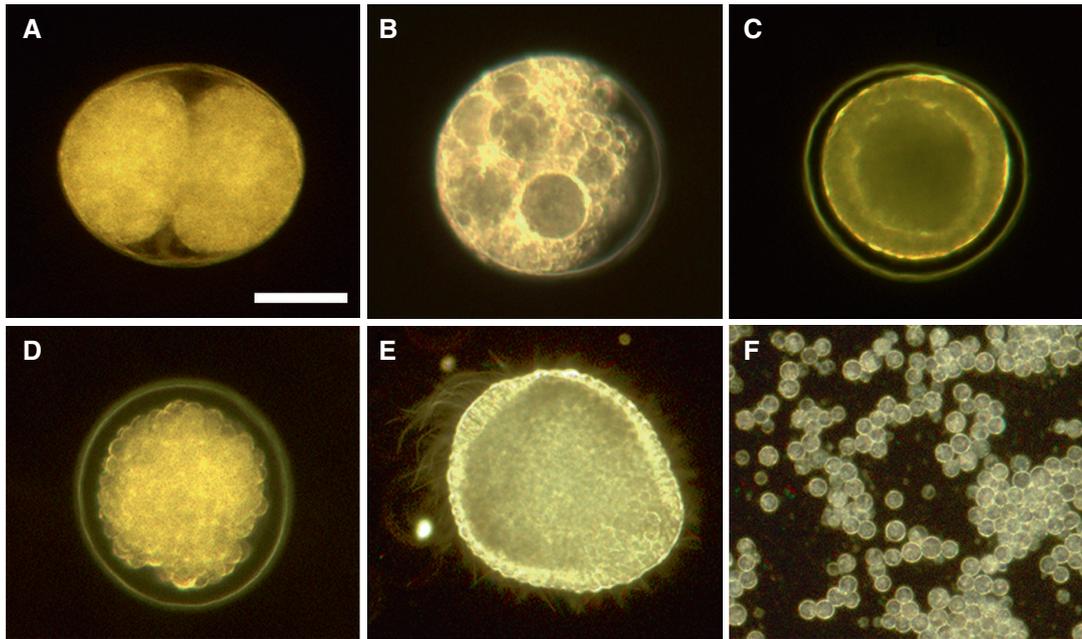


Fig. 3.2 Experimental taphonomy of embryos of the echinoid *Lytechinus pictus*. (A) The physical structure of the 2-cell embryo is intact after 26 days post-mortem in a medium of seawater and beta-mercapto ethanol to simulate the reducing conditions necessary for authigenic mineralisation. (B) The physical structure of the 2-cell embryo has deteriorated as a consequence of autolysis and consequent lipid coalescence after just 5 h post-mortem in normal seawater. (C) Live unhatched blastula showing the columnar cells of the wall of blastula and

the blastocoel within. (D) Unhatched blastula as in (C) but euthanised in seawater containing beta-mercapto ethanol; the component cells are intact, but they have lost adhesion and the blastocoel has collapsed. (E) Live hatched blastula. (F) Hatched blastula as in (E) but euthanised in seawater containing beta-mercapto ethanol; the component cells are intact but they have lost adhesion and, in the absence of a fertilisation envelope, they have disaggregated (From Raff et al. (2006)) Scale bar: (A–F) 48 μ m

perhaps in large part a consequence of the consumption and replication of the original tissue, cellular, and/or subcellular structure by microbial biofilms (Raff et al. 2008). Regardless, the insights afforded by *Artemia* into the relative preservation potential of developmental stages may go a long way to explain why deposits such as the Doushantuo and Kuanchuanpu Formations preserve some but not all developmental stages (Gostling et al. 2009).

Even accepting that assemblages of fossil embryos preserve only snapshots into the development of the component organisms, it is important to consider whether or not the fossilised developmental stages faithfully reflect the organisation of the embryo in vivo. Raff et al. (2006, 2013) have shown that through the process of autolysis, cytological structure is disrupted

through the condensation of lipids (Fig. 3.2A, B). This process is halted or diminished under the reducing conditions required for fossilisation via authigenic mineralisation, when the gross physical integrity of early cleavage stage embryos can be maintained for weeks to months (Fig. 3.2A; Raff et al. 2006). However, within experiments, the component cells in later embryonic and larval developmental stages can lose adhesion and reorganise relative to their original in vivo arrangement (Fig. 3.2C, F). Thus, though the integrity of component cells is maintained, much of their biological context is lost such that evidence of a blastocoel, gastrulation, an archenteron, etc., can be lost as a consequence of the loss of cell adhesion (Fig. 3.2C, F; Raff et al. 2006). Furthermore, while during embryonic stages the component cells remain associated

because they are enclosed within the fertilisation envelope (Fig. 3.2C, D), postembryonic stages are readily disaggregated, and evidence of the origin of the component cells (Fig. 3.2E, F), if fossilised, is lost entirely (Raff et al. 2006).

Evidently, fossil remains of animal embryos must be interpreted with great caution. The fidelity of their preservation can be beguiling, but careful analysis of their mineralisation history, discriminating biology from geology and interpreting that biology in light of knowledge of biases in the preservation of developmental stages and the faithfulness with which such fossils reflect their original embryology, can yield material insights into developmental evolution. Since some of the fossils are among the very oldest fossil evidence for the existence of animals in evolutionary history, the stakes could not be higher in our aim of uncovering the role of developmental evolution in effecting the origin and early diversification of animal biodiversity. We now cast a critical eye upon fossil embryos themselves and evaluate competing interpretations of their biological affinity and, consequently, their evolutionary significance.

FOSSIL INVERTEBRATE EMBRYOS

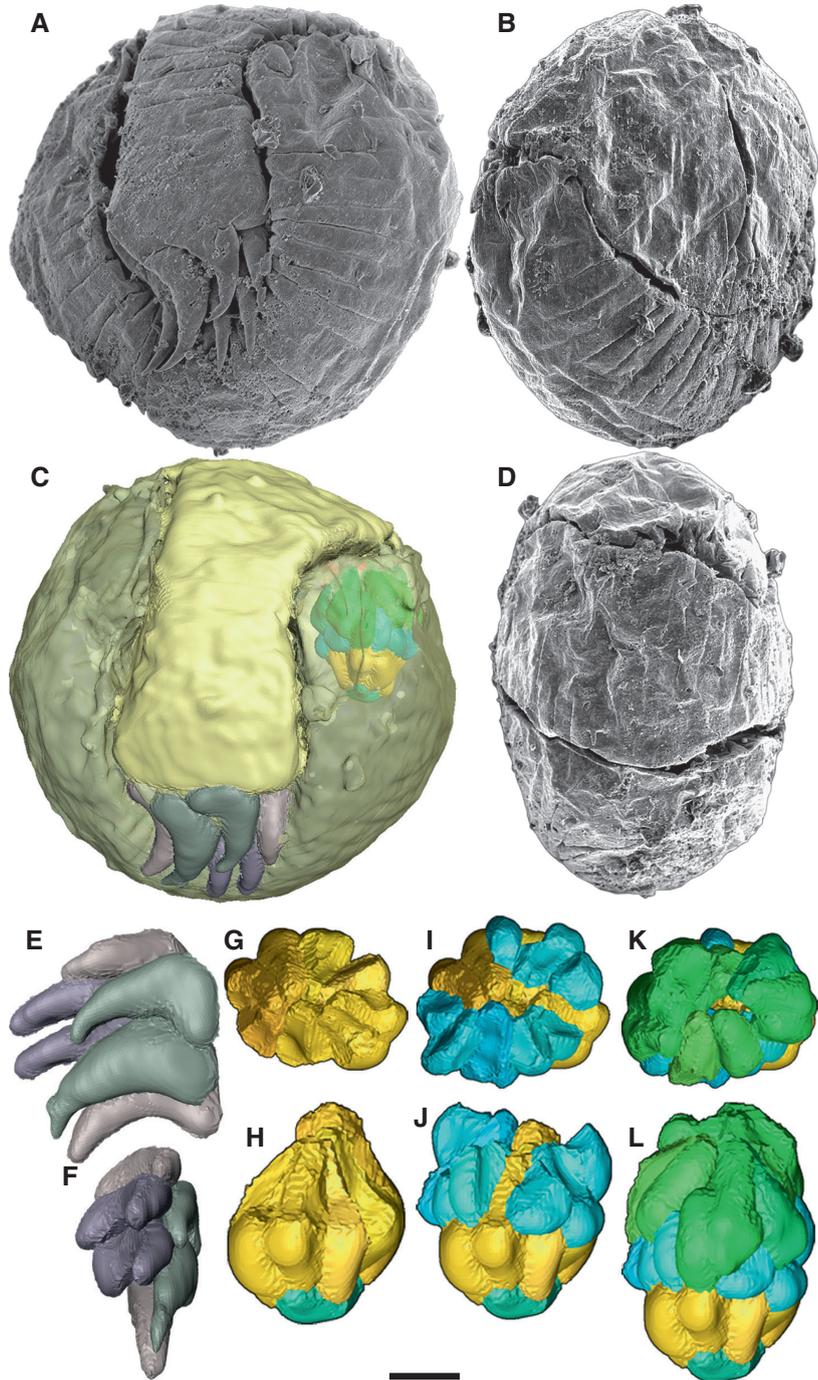
The sum total of fossil remains of embryonic stages of animal development does not extend far beyond the initial deposits from which they were reported, the Early Cambrian Kuanchuanpu and Pestrosvet Formations, Middle Cambrian Gaotai Formation, and the Ediacaran Doushantuo Formation, though possible eggs and embryos have been recovered from a small number of other deposits in the Middle Cambrian through Early Ordovician. Some of these reports are tenuous and constitute little more than spheroids comprised of calcium phosphate or silica that are more or less filled with diagenetic cement (Lin et al. 2006; Pyle et al. 2006; Broce et al. 2014; Mathur et al. 2014). Whether or not these fossils represent embryos and some are more convincing than others (Broce et al. 2014), they constitute little more than curios of fossilisation until they can be joined with other developmental stages and their phylogenetic affinity constrained (Donoghue and Dong 2005). These criteria are,

as yet, met by precious few species known from fossilised embryonic remains, described below.

Markuelia

The first fossilised embryos to be described as such are attributable to *Markuelia* (Fig. 3.3A–C), though they were then known only from cleavage stages and were interpreted as arthropod embryos (Zhang and Pratt 1994). Recovery of further material from the original site revealed the cleavage embryos to be associated with *Markuelia*, an annulated vermiform organism, coiled into an approximation of a sphere, enclosed within a fertilisation envelope (Fig. 3.3A–D; Zhang et al. 2011). However, *Markuelia* was originally described much earlier as a globular fossil (<1 mm diameter) of unknown affinity with parallel double-walled septa, from the Early Cambrian of Siberia (Val'kov 1983, 1987). It was later shown that these were fossilised embryos, with spines associated with their transverse annulae and a series of paired and variably recurved spines associated with their posterior end (Fig. 3.3A, C, E, F; Bengtson and Yue 1997). *Markuelia hunanensis*, *M. qianensis*, and *M. spinulifera* are known species from the Middle and Late Cambrian of South China, *M. secunda* from the Lower Cambrian of Siberia, *M. lauriei* and *M. waloszeki* from the Middle Cambrian of Australia, as well as undetermined species from the Lower Ordovician of the USA (Donoghue et al. 2006b; Dong et al. 2010; Zhang et al. 2011). Affinities to lobopods (extinct onychophoran-like panarthropods; Bengtson and Yue 1997), annelids (Bengtson and Yue 1997), and halkieriids (armoured worms currently interpreted as stem-mollusks; Conway Morris 1998) were considered until discovery of specimens preserving the anterior end of the organism revealed a terminal mouth surrounded by rings of teeth-like scalids. This narrowed phylogenetic debate to the clade Scalidophora, which is comprised of the phyla Kinorhyncha, Loricifera, and Priapulida (see Vol. 3, Chapter 1), all characterised by the presence of circumoral rings of scalids associated with their introvert (Dong et al. 2004). A more precise affinity can be established for *Markuelia* based principally on details of the number of

Fig. 3.3 *Markuelia*, a scalidophoran from the Cambrian of Australia, China, and Siberia and the Ordovician of the USA, known only from embryonic stages of development. (A–L) *Markuelia waloszeki* (Dong et al. 2010) from the Cambrian of Australia. (A, B, D) are scanning electron micrographs; (C, E–L) are synchrotron radiation X-ray tomographic microscopy-based reconstructions. (A) Embryo with tail (centre) and head (upper right) juxtaposed. (B, D) Same embryo rotated to show the opposing sides, revealing the annulated trunk coiled in a broad S-shaped loop. (C) Virtual model of the same embryo (in broadly the same orientation as in (A)) derived from synchrotron tomography characterisation of the fossil, showing the tooth-like scalids within the head. (E, F) Three pairs of tail spines, recurved ventrally, oriented about anal opening. (G–L) The assemblage of scalids that comprise the introvert, viewed from rostrum (G, I, K) and lateral (H, J, L). These specimens were figured by Dong et al. (2010). Relative scale bar: (A–B) 47 μm , (C–D) 50 μm , (E–F) 24 μm , (G–L) 25 μm



scalids arranged in the first three rings around the mouth cone (8-8-9), comprising 25 longitudinal rows (Fig. 3.3G–L). Such characters, along with a terminal anus surrounded by three pairs of bilaterally arranged spines (Fig. 3.3A, C, E, F) and the apparent absence of an armoured pharynx, resolve *Markuelia* as a stem-group scali-

dophoran (Dong et al. 2004, 2005, 2010; Donoghue et al. 2006a; Harvey et al. 2010; Duan et al. 2012). It is not known whether the extensive annulation of the trunk of *Markuelia* reflects segmentation, but it suggests that annulation may be a shared primitive feature of scalidophorans and, indeed, cycloneuralians.

Though *Markuelia* is known from cleavage (Zhang and Pratt 1994; Dong et al. 2004; Zhang et al. 2011) and late embryonic stages (Bengtson and Yue 1997; Dong 2007; Dong et al. 2004, 2005, 2010; Donoghue et al. 2006a, b; Haug et al. 2009; Zhang et al. 2011), little can be gleaned concerning its development, save that because the late embryonic stages are readily reconcilable with adult scalidophorans, and cyclo-neurals more generally, that *Markuelia* was a direct developer. This contrasts with living loriciferans and the majority of living priapulids, which are indirect developers.

Olivoooides* and *Quadrapyrgites

The olivoooids, *Olivoooides* and *Quadrapyrgites*, are known only from the Early Cambrian Kuanchuanpu Formation of South China (Fig. 3.4A–H). Two principal components of the life cycle were originally described independently, viz., the cone-shaped postembryonic theca stage hitherto named ‘Punctatus’ (Fig. 3.4F–H) and the embryonic stage *Olivoooides* (Fig. 3.4D, E), which has taxonomic seniority. Both the embryonic and postembryonic stages of development in *Olivoooides* exhibit pentaradial symmetry, manifested through the apex and the single terminal orifice which are folded in five principal rays (Fig. 3.4D, F). The anatomy of the embryo is known mainly from features of its integument, which is ornamented by stellae that resemble twisted bundles of fibres, approximately 10 µm in length (Fig. 3.4D, E). The embryo increases in size through the episodic release of striated integument from the orifice, ultimately developing the tube-shaped characteristic of the postembryonic theca (Fig. 3.4G, H). Indeed, the principal evidence supporting the link between the embryonic and postembryonic stages of development is the stellate and striate integument that envelops the embryo and the base of the hatched theca (Fig. 3.4D, E, G, H; Bengtson and Yue 1997; Yue and Bengtson 1999). The theca expanded in length throughout life through the episodic release of striate integument from the otherwise closed aperture, reflected in a series of circumferential growth rings

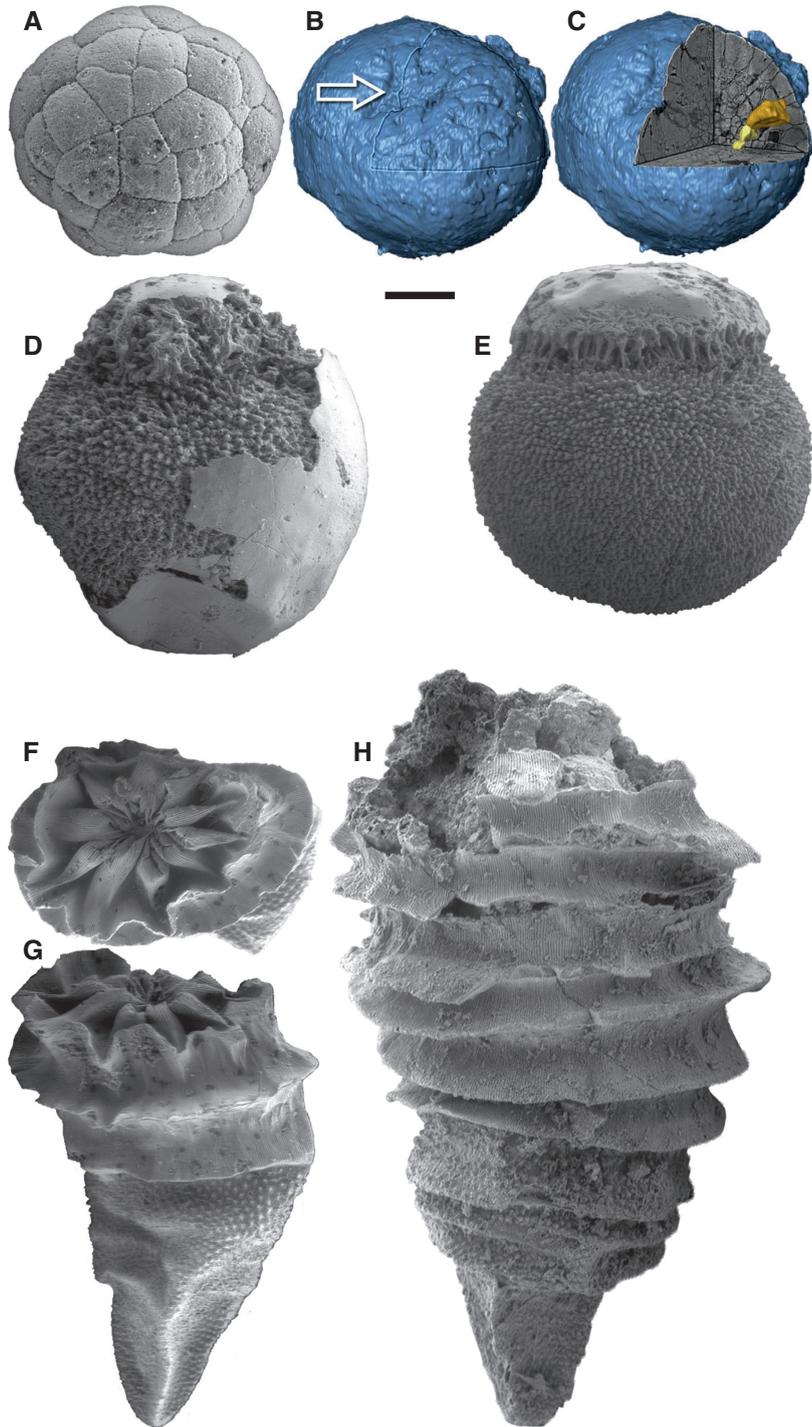
(Fig. 3.4G, H). The nature of the internal anatomy of the adult has only been inferred based on an assumed hypothesis of affinity.

A small number of specimens have shown that the external pentaradial symmetry is imposed more fundamentally upon the internal anatomy which has been preserved in only the most exceptional of circumstances, revealing a series of circumference parallel walls with pentaradial divisions and diverticulations, leading to an open apertural space otherwise occupied by an axial pentaradial process (Dong et al. 2013; Han et al. 2013). Additionally, a set of two juxtaposed pentaradial objects has been described in association with *Olivoooides* and interpreted as ephyrae (juvenile medusae) in the process of strobilation (budding in scyphozoan cnidarians; see Chapter 6) (Dong et al. 2013).

Quadrapyrgites occurs in association with *Olivoooides*, and in general terms its embryonic and postembryonic growth and developmental stages are identical to those described for *Olivoooides*, with the principal distinction that *Quadrapyrgites* is tetradial (Steiner et al. 2014).

Debate over the affinity of the olivoooids has been wide-ranging, including echinoderms (Chen 2004), scalidophorans (Steiner et al. 2014), cnidarians (Bengtson and Yue 1997; Yue and Bengtson 1999; Han et al. 2013), and diploblastic stem-eumetazoans (Yasui et al. 2013). The hypothesis of echinoderm affinity is based on little more than pentaradial symmetry and can be rejected since the olivoooids lack key echinoderm apomorphies, not least a mineralised skeleton comprised of calcite stereom (Dong et al. 2013; Han et al. 2013; Steiner et al. 2014). The scalidophoran interpretation is based principally on the general similarity between the theca of olivoooids and the lorica of loriciferans and larval priapulids, together with similarities in general symmetry and the requirement of the aperture to open and close akin to a scalidophoran introvert (Steiner et al. 2014). However, these similarities are vague, and the demonstrable absence of a through gut and a scalid-bearing introvert are incompatible with a scalidophoran interpretation of the olivoooids. The diploblast stem-eumetazoan interpretation is based principally on the assumption that a single specimen of theca,

Fig. 3.4 *Olivoooides multisulcatus* from the Early Cambrian Kuanchuanpu Formation of South China, known from embryonic and postembryonic stages of development. (A–C) Associated cleavage and gastrulation stages; (A, D–H) are scanning electron micrographs, and (B, C) are synchrotron radiation X-ray tomographic microscopy-based reconstructions. (A) Cleavage embryo. (B, C) Surface model (blue) of putative gastrula from synchrotron tomography characterisation of the fossil, showing the interpreted blastopore as a deep sulcus (arrowed; B) and cells (orange, yellow) within, some of which have been reconstructed in 3D (C). (D, E) Embryonic stages of *Olivoooides* with the characteristic stellate integument and remains of the fertilisation envelope obscuring the pentaradial aperture. (F–H) Postembryonic developmental stages with the adapertural stellate ornament retained from the embryo and the characteristic pentaradial aperture through which the additional striate integumentary tissue is released to increase the length of the theca. Specimens figured by Dong et al. (2013) except for B and C (figured by Donoghue et al. 2006a, b). Relative scale bar: (A) 98.5 μm , (B–C) 145 μm , (D) 119 μm , (E) 144 μm , (F) 197 μm , (G) 282 μm , (H) 254 μm



which bears a diagenetic mineral plug beneath the aperture, reflects a miniature gut and infers that the remaining theca constituted a vast body cavity (Yasui et al. 2013). However, this is

a misinterpretation of mineralised decayed remains as reflecting *in vivo* anatomy.

The cnidarian interpretation of the olivoooids was based originally on comparison that had

been drawn between the theca and conulariids, an extinct group of cnidarians that have been interpreted as coronate scyphozoans (Conway Morris and Chen 1992; Yue and Bengtson 1999), with the theca interpreted as the sclerotised integument of the adult polyp (Chapter 6). The pattern of direct development, from embryo to adult theca, is unusual for scyphozoans, specifically, and cnidarians generally. The pattern of pentaradial symmetry seen in *Olivoooides* is also unusual for cnidarians, as is the aperture which is difficult to rationalise with the presence of a polyp. Many of these concerns are diminished if not dismissed based on recent description of internal anatomy, which Han et al. (2013) interpret (and likely grossly over-interpret) in the image of a cubozoan polyp. Details of this comparison are problematic, not least the evident fact that the embryo of *Olivoooides* develops into a sessile polyp (if it is indeed a cnidarian), not a medusa, and what is more, the polyp stage of living cubozoans is grossly reduced. Indeed, the principal points of similarity between the olivoooids and cubozoans, as interpreted by Han and colleagues (2013), are cnidarian or at least medusozoan symplesiomorphies (cf. Chapter 6). Thus, the internal anatomy of olivoooids, as evidenced by *Olivoooides*, supports a cnidarian affinity at the least. Concerns over differences in the pentaradial symmetry of *Olivoooides* and the generally (but far from exclusively) tetradiality of cnidarians may certainly be dismissed on the description of *Quadrapyrgites*, which differs materially from *Olivoooides* only in terms of its tetradial symmetry. Finally, the description of minute pentaradial strobilating medusae in association with *Olivoooides* would appear to settle debate over its affinity (though this is disputed by Steiner et al. 2014). In sum, the available evidence supports the interpretation of the olivoooids as medusozoan cnidarians, and their similarity to scyphozoans must represent either shared derived or shared primitive characteristics; only a better understanding of the interrelationships of extant cnidarians and morphological character evolution among them will aid a more precise classification for the olivoooids. Either way, the olivoooids evidence the fact that although indirect development is the norm

among extant cnidarians, known lineages in the Cambrian underwent direct development.

Pseudoooides

Pseudoooides prima has also been described from the Early Cambrian Kuanchuanpu Formation from a number of localities in South China (Fig. 3.5A–C; Qian 1977; Steiner et al. 2004a, b; Donoghue et al. 2006a). After the initial description as a globular microfossil of unknown affinity (Qian 1977), Steiner and colleagues recognised it as an embryo, typically 250–500 µm in diameter, characterised by a segmented ‘germband’ that can extend around the majority of the diameter of the fossil (Fig. 3.5A–C). The remainder of the surface is undifferentiated, and no internal anatomy appears to have been preserved in any of the material described to date (Donoghue et al. 2006a). The segmented band pinches out at its extremity (Fig. 3.5A) and is divided longitudinally along the midline and transversely into up to twelve compartments (Fig. 3.5C). The centre of the band may also be pinched before the central compartments develop (Fig. 3.5B), leading to the inference that the compartments are added from the centre (Donoghue et al. 2006a). Steiner et al. (2004b) present specimens that indicate that the compartments develop through progressive division of a band that is initially undifferentiated save for the longitudinal furrow, without which it would be difficult to attribute the embryos to *Pseudoooides*. A number of cleavage and gastrulation stage embryos have been attributed to *Pseudoooides* as opposed to co-occurring olivoooids (Fig. 3.4A–C), on the basis of their size (Donoghue et al. 2006a; Steiner et al. 2004b, 2014). None of these data are particularly phylogenetically informative, although Steiner et al. (2004b) associated these embryos with fragmentary remains of an arthropod or arthropod-like organism. The apparent pattern of germband development, if that is what it represents, is extremely unusual for an arthropod or, indeed, any bilaterian (Donoghue et al. 2006a). *Pseudoooides* requires further study before its embryology, phylogenetic affinity, and evolutionary significance can be determined.

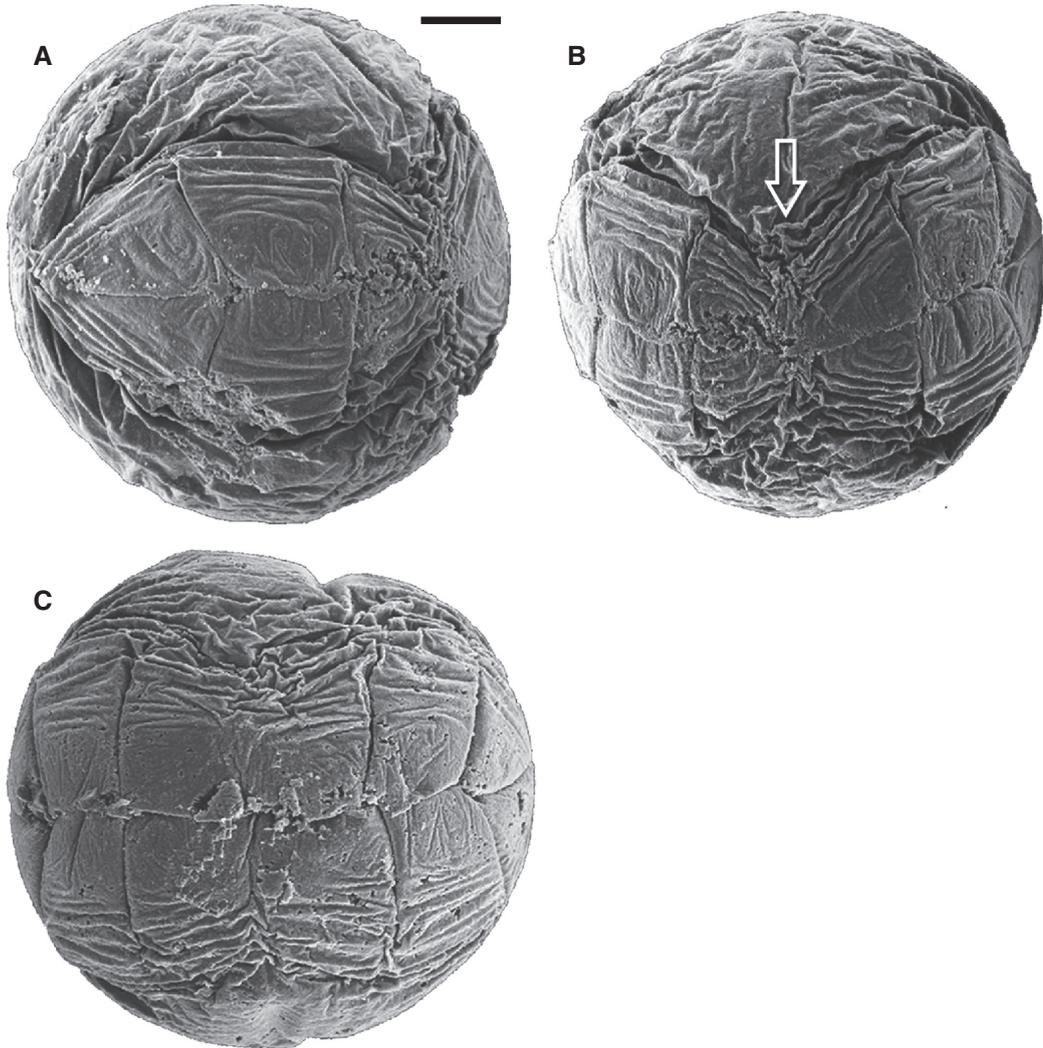


Fig. 3.5 Scanning electron micrographs of *Pseudoides prima* from the Early Cambrian Kuanchuanpu Formation of South China, known only from embryonic stages of development. (A, B) The segmented 'germband' with a central pinch (arrowed), interpreted to the point from

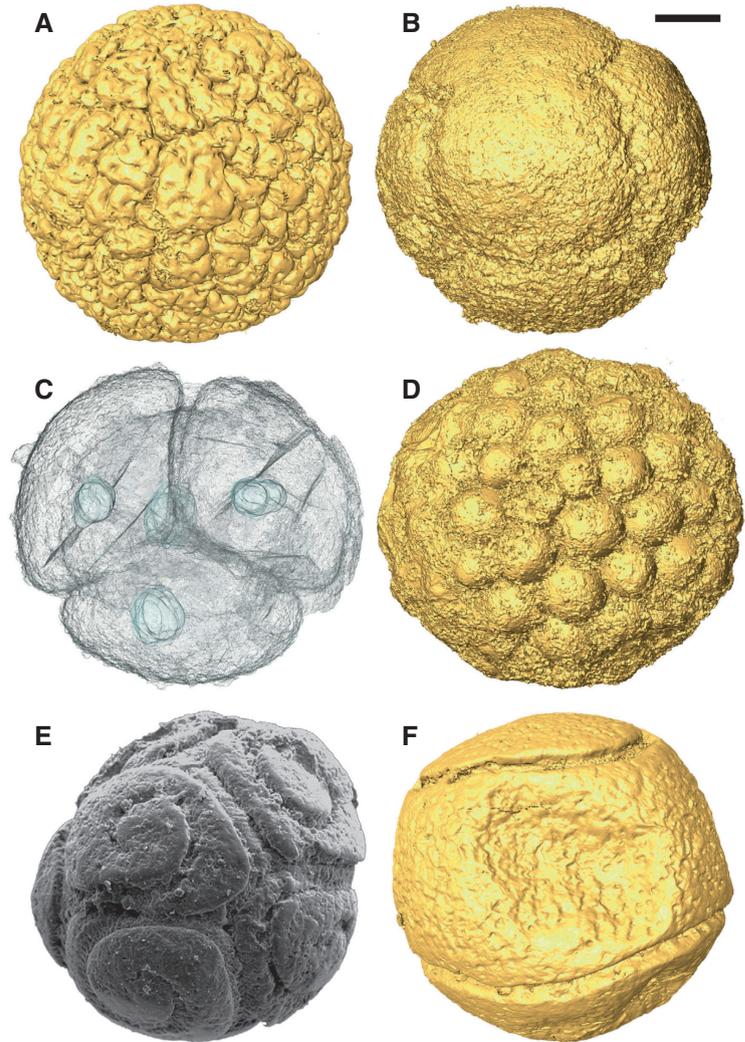
which new segments are developed. (C) Twelve-segment germband without central pinch (These figures are reproduced with permission from the publishers from Steiner et al. (2004b)). Relative scale bar: (A, B) 53 μm , (C) 56 μm

Tianzhushania

In addition to the well-accepted embryos from the Cambrian and lowermost Ordovician, there have also been more contentious reports of animal cleavage embryos (Fig. 3.6A–D; Xiao et al. 1998) from the Ediacaran (i.e., the final period of the Precambrian). The fossils in question are from the Doushantuo biota of southern China and are approximately 570 million years old

(Xiao et al. 1998). The Doushantuo Formation varies in its composition throughout its broad occurrence in South China, ranging from black shales through cherts and phosphorites, although the purported fossils of animal embryos are preserved in calcium phosphate in both chert and phosphorite. In the phosphorite at least, the fossilisation occurred elsewhere, and the fossils were resedimented and size sorted (Xiao et al. 2007).

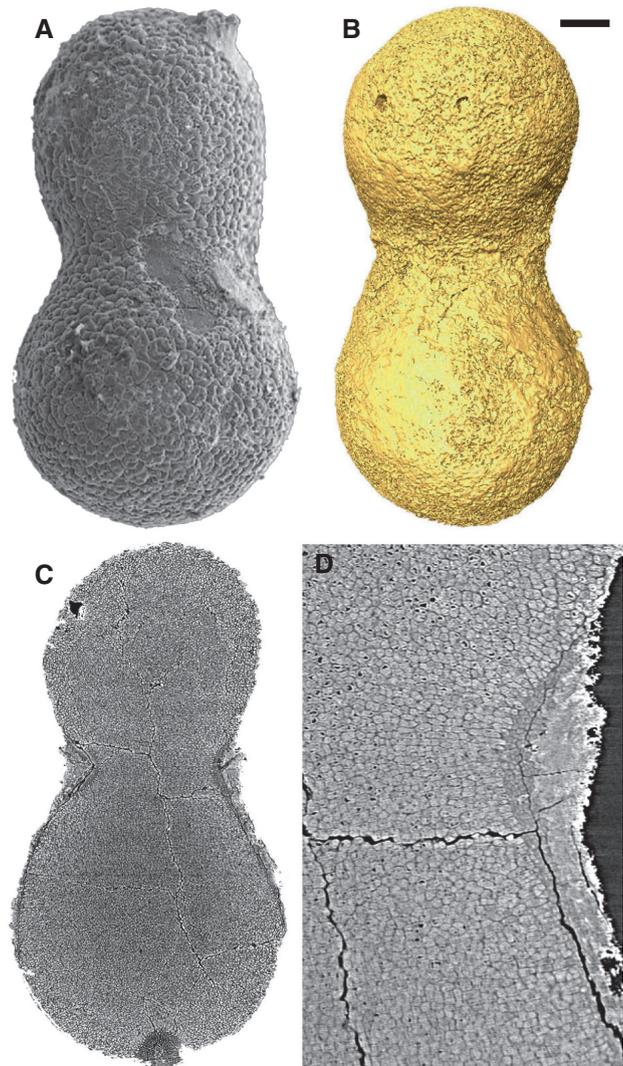
Fig. 3.6 Embryo-like fossils from the Ediacaran Doushantuo biota assigned to the genera *Tianzhushania* (A–D), *Spirallicellula* (E), and *Helicoformamina* (F); (A–D, F) are synchrotron radiation X-ray tomographic microscopy-based reconstructions, while (E) is a scanning electron micrograph. (A) *Tianzhushania* specimen with an ornamented outer envelope. (B) Early cleavage stage of *Tianzhushania*. (C) Early cleavage stage of *Tianzhushania* showing possible nuclei. (D) Later cleavage stage of *Tianzhushania*. (E) *Spirallicellula* specimen showing cells coiled helicospirally. (F) *Helicoformamina* specimen showing a single helicospiral body (Parts A, B, D, and F also figured by Cunningham (2012); part C was also figured by Cunningham et al. (2014); part E was previously figured by Tang et al. (2008)) Relative scale bar: (A) 140 μm , (B, C) 105 μm , (D) 115 μm , (E) 200 μm , (F) 120 μm



The vast majority of putative embryos can be assigned to the genus *Tianzhushania* (senior synonym of *Megasphaera*, *Parapandorina*, and *Megaclonophycus*; see Yin et al. 2004). They would originally have been encased within a spinose cyst, but this has been lost in most specimens recovered through acid dissolution of the surrounding matrix. Specimens range from single cells through early cleavage stages (Fig. 3.6B, C) to those with thousands of cells. *Tianzhushania* shares a number of features with animal embryos including reductive division, Y-shaped junctions between cells, and a multi-layered ornate envelope surrounding the cells (Fig. 3.6A; Xiao 2002). These microfossils are preserved with remarkable fidelity with cellular

and even subcellular details including possible nuclei having been fossilised (Figs. 3.1E and 3.6C; Hagadorn et al. 2006). They have attracted much attention, not only because they could represent the oldest animal fossils in the entire record but also because they might potentially allow palaeontologists to study embryology at the time when animal body plans were first starting to become established. However, other workers have challenged the animal affinities of these fossils. Firstly, Bailey et al. (2007a, b) suggested that the embryo-like fossils might be better interpreted as giant sulphur bacteria comparable to the extant *Thiomargarita*, which can reach 750 μm in diameter and undergoes reductive division. This provided an explanation for the

Fig. 3.7 A peanut-shaped fossil from the Ediacaran Doushantuo biota. **(A)** Scanning electron micrograph of a peanut-shaped specimen preserving the ornamented outer envelope. **(B)** Synchrotron radiation X-ray tomographic microscopy-based surface rendering of a peanut-shaped specimen. **(C)** SRXTM section through the specimen in **(B)**. **(D)** Detail of an SRXTM section through the specimen in **(B)** showing many cells. These specimens were figured by Hultgren et al. (2011). Relative scale bar: **(A–C)** 100 μm , **(D)** 36 μm



lack of associated later developmental stages. Subsequent work has revealed a number of problems with this model. Bacteria do not produce structures with complexity of the ornamented envelope that surrounds the fossils (Xiao et al. 2007), they do not have nuclei (Hultgren et al. 2011), and they can only achieve this giant size by means of a vacuole that takes up around 98 % of the volume, which is absent in the fossils (Donoghue 2007). Moreover, decay experiments suggest that, unlike animal embryos, giant sulphur bacteria would be unlikely to be preserved three-dimensionally because of collapse of the vacuole (Cunningham et al. 2012b). Secondly, Hultgren et al. (2011, 2012) argued that the

embryo-like fossils were in fact cyst-forming protists. They showed that none of the characters that had been used to identify the fossils as animals are metazoan synapomorphies. Although these features are compatible with an animal interpretation, they are at best animal symplesiomorphies, found in more universal clades. Thus, the characters identified in the fossils and used to evidence an animal interpretation may be necessary to identify *Tianzhushania* as an animal, but they are not sufficient. Hultgren et al. (2011) also reported fossils interpreted as later stages in the life cycle of *Tianzhushania*, on the basis that they possess an identical ornamented envelope (Fig. 3.7A–D). These fossils have features that

cannot be reconciled with metazoan development. In particular, the specimens have hundreds of thousands of cells and yet show no sign of tissue differentiation – something that is present in all extant animals by this stage of development. While the authors could not definitively rule out a placement within the stem group of animals, they found that there was no evidence to place them here either. Others have suggested that the new fossils are not part of the life cycle of the same organism (Xiao et al. 2012) and instead present the same metazoan symplesiomorphies as evidence of animal affinity. Additional work to establish the variability of forms in the deposit is needed to assess whether or not intermediate developmental stages between the embryo-like forms and the new specimens exist.

A third possibility is that the fossils are multicellular green algae. Before being interpreted as animals, the fossils were initially compared to the alga *Pandorina* by Xue et al. (1995), and as a result the cleavage stages were named *Parapandorina*. This interpretation has been resurrected in a recent comment by Butterfield (2011). This possibility requires further investigation but seems unlikely given that extant green algae like *Pandorina* maintain cell adhesion in the cleavage stages by means of cytoplasmic bridges that are absent from the fossils.

Spirallicellula* and *Helicoforamina

Associated with *Tianzhushania*, but much rarer, are similar forms that differ in having each cell coiled into a spiral. These enigmatic forms are assigned to the genus *Spirallicellula* (Fig. 3.6E) and also contain nucleus-like structures (Hultgren et al. 2011) and have also been considered to be embryos. In addition, *Helicoforamina* (Fig. 3.6F), a form with a helical groove running around a spherical body, is also known from the Doushantuo biota (Xiao et al. 2007). One suggestion is that *Helicoforamina* is an elusive later developmental stage of *Tianzhushania*, perhaps representing a coiled embryo of a vermiform or tubular organism (Xiao et al. 2007). On the other hand, *Spirallicellula* and *Helicoforamina*

have been associated together by various authors (Tang et al. 2008; Hultgren et al. 2011; Zhang and Pratt 2014). These have been considered as embryos (with *Helicoforamina* being the single-celled stage), possibly representing the embryonic stages of the enigmatic ctenophore-like fossil *Eoandromeda*, which has eight spiral arms (Tang et al. 2008). Alternatively, they have also been interpreted as cyst (*Helicoforamina*) and dividing stages (*Spirallicellula*) of protists (Hultgren et al. 2011) or green algae (Zhang and Pratt 2014).

Other Candidate Embryos from the Doushantuo Biota

There are different perspectives on the diversity of the organisms represented by the embryonic and larval stages from the Doushantuo Formation, with some arguing for a diverse assemblage of animals, including derived bilaterians (Chen et al. 2000, 2002, 2004, 2006, 2009b; Chen and Chi 2005), while at another extreme, others rationalise the majority of remains as representing one or a few species that may represent only stem-metazoans (Hagadorn et al. 2006; Xiao et al. 2012), or else that all or a majority of such fossils may not represent animals or embryos at all (Bailey et al. 2007a, b; Hultgren et al. 2011, 2012; Zhang and Pratt 2014). Much of this equivocation will be resolved with debate over the phylogenetic affinity of *Tianzhushania*; however, the interpretation of a diverse biota is based principally on the spurious interpretation of diagenetic mineral fabrics as preserving original biological structures (Bengtson 2003; Xiao and Knoll 2000; Xiao et al. 2000; Cunningham et al. 2012a). For instance, Li et al. (1998) described sponge embryos and larvae of sponges based on effectively two-dimensional thin sections of rock; however, the critical structures interpreted as amoebocytes, blastomeres, flagellae, mesohyl, a plasma membrane, porocytes, sclerocytes, spongocoel, and spicules are indistinguishable from layered and clotted void-filling diagenetic mineralisation, unrelated to the replication of biological structure, that is common in

phosphatised Doushantuo fossils (Xiao and Knoll 2000; Hagadorn et al. 2006; Cunningham et al. 2012a). Similarly, Chen et al. (2000, 2002) describe anthozoan gastrulae, larvae and polyps, as well as hydrozoan gastrulae, although these fossils preserve no more biological structure than what may constitute a fertilisation envelope infilled with an anastomosing diagenetic mineral cement. None of these records withstand scrutiny (Xiao and Knoll 2000; Xiao et al. 2000; Bengtson 2003; Cunningham et al. 2012a).

Other Records of Fossil Embryos

Phosphatised embryos have been described from the Early Cambrian of Yakutia, Siberia (Fig. 3.8A–C), that preserve a cross-like structure (Fig. 3.8A, B) that has been compared to the appearance of micromeres on spiralian blastula-stage embryos and to incipient tentacles in cnidarian actinula larvae, substantiating a tentative link to co-occurring anabaritids, long considered cnidarians (Kouchinsky et al. 1999). Silicified eggs and embryos have been described from the Middle Cambrian Kaili Biota of Ghizou, China (Lin et al. 2006); however, their phylogenetic affinity is unknown, and they provide no material insights into embryology.

THE NATURE OF THE FOSSIL RECORD OF EMBRYOS

The fossil record of embryos could hardly be considered representative. Precious few organisms are represented by fossilised embryonic stages, certainly not even those organisms known from fossil remains in the same deposits, and those that are preserved represent only a small proportion of their embryological development. It is not clear what, if anything, unites these organisms to justify the preservation of their embryological stages. Indeed, it may merely be a conspiracy of environmental circumstances rather than anything more intrinsically biological. Ultimately, however, there appear to be two principal classes of structures preserved: (i) dividing cells in early stages of palintomy (Doushantuo embryo-like

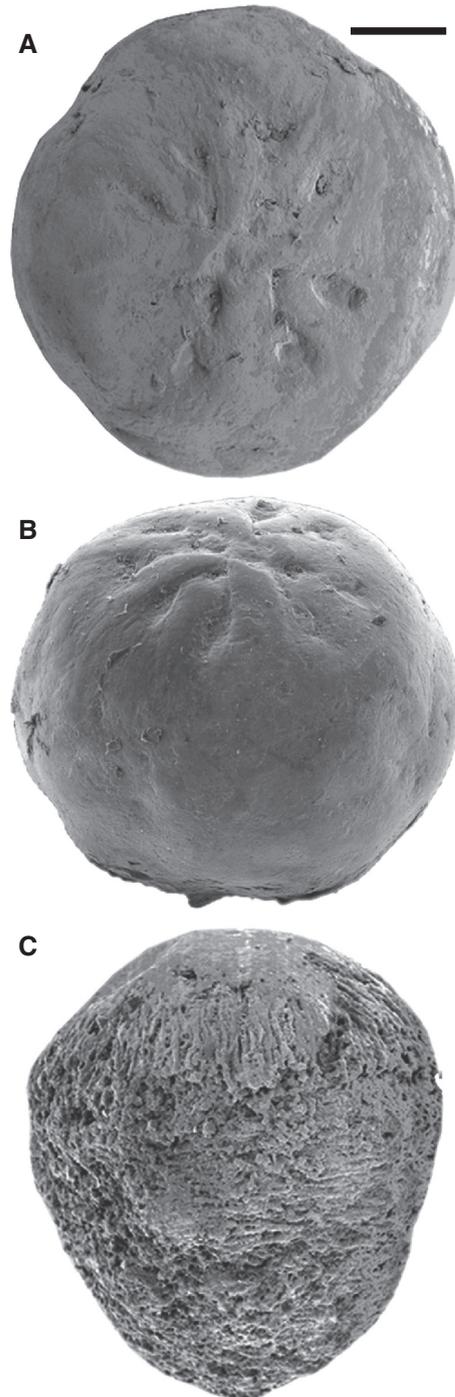


Fig. 3.8 Scanning electron micrographs of putative cnidarian embryos from the Early Cambrian of Siberia, known only from these embryonic stages of development. (A, B) Embryonic stage with cross-like structure. (C) Embryonic stage with spicule-like structures on its surface. These specimens were figured by Kouchinsky et al. (1999). Relative scale bar: (A) 100 μm , (B) 103 μm , (C) 89 μm

fossils), cleavage or gastrulation (Kuanchuanpu and *Markuelia* embryos), and (ii) cuticle and/or integument, as in the case of *Markuelia*, *Olivoooides*, *Quadrapyrgites*, and possibly also *Pseudoooides*. Primary larvae (as opposed the arthropod larvae which dominate the Orsten biota) are rarely preserved, and the record is dominated by large and, therefore, presumably yolky embryos. These taxonomic and developmental biases may be explained by the inherent biases in the pattern of decay seen in taphonomy experiments (Raff et al. 2006; Gostling et al. 2008, 2009). The preponderance of large embryos may not be a sampling bias for size since attempts to control for this have failed to yield further discoveries in sites where embryo fossils are already known (Donoghue et al. 2006b). The absence of fossilised primary larvae in the embryo-bearing deposits may be a taphonomic artefact, and so it does not follow that their absence from fossil assemblages is evidence of their absence during life. However, the mere presence of large marine invertebrate embryos in the Cambrian suggests that direct development may have evolved early among animal lineages and may be a primitive feature of metazoan development (Donoghue and Dong 2005).

Palaeontologists have dared to believe that there was a hitherto undiscovered fossil record of embryos and, without doubt, there will be further discoveries. However, it appears that the broad extent of this fossil record has been plumbed. Thus, the fossil record of marine invertebrate embryos is very clearly biased to the Ediacaran and/or the earliest Phanerozoic interval. This may reflect a combination of factors (Donoghue et al. 2006b), including the widespread deposition of marine phosphates at this time. Another factor must be the paucity of deposit feeders which, in later times, more effectively recycled organic remains directly and, indirectly, served to expand the depth of sediment oxygenation and, with it, aerobic microbial activity, the principal vector of decay. Nevertheless, would we wish for a fossil record of embryology from any interval of Earth history, it would be this one, and so we should make the most of what we have. Thus, future research

should focus on better resolving the biological nature of fossils known to preserve embryological stages and to prospect for new remains to better understand embryology in deep time.

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