Lower Cambrian yolk-pyramid embryos from Southern Shaanxi, China*

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Abstract Phosphatized globules with radially arranged pillars were recently recorded from the Lower Cambrian phosphate deposit, Ningqiang, Shaanxi by Yue and Bengtson. These authors interpreted the globules as blastula stage of embryos and the pillars as blastomeres. On the basis of new additional material, we reinterpret these structures as yolk-pyramid stages of possible arthropod eggs. The 20 embryos under present study range from 380 μm to 600 μm in diameter and contain about 120 pyramids. Some embryos having a higher number of pyramids are tentatively interpreted as slightly later developmental stages of the same animal. These 543-million-year-old embryos may push back the evolutionary history of the arthropods to a deeper time and also suggest that one important pattern of arthropod development was already present at the beginning of the Cambrian.

Keywords: superficial cleavage, fossil yolk-pyramid egg, Cambrian explosion, ground pattern of arthropod cleavage, ningqian fauna.

The Cambrian explosion is widely accepted as the sudden appearance of numerous bilaterian animal phyla at or near the beginning of Cambrian time[1, 2]. The 530-million-year-old Maotianshan Shale fauna contains the oldest good whole-body fossils of bilaterians, documenting an increasing number of present-day animal phyla[3, 4] or subphyla[5] (even including vertebrates[6~8] as well) known from Lower Cambrian. The beginning of the Cambrian period is dated at 543 million years ago, when the first large and elaborate fossil burrows together with the microscopic plate-like and spine-like mineralized fossils known as "small shelly fossils" appeared, and these became increasingly abundant and diverse in the following 13 million years (Ma) leading to the main burst of the Cambrian explosion recorded in the Maotianshan Shale. However, the "small shelly fossils" and trace fossils both only provide coarse information and the Early Cambrian world in its first 13 million years remains a great mystery.

In southern China, the entire duration of the first 13 Ma in Cambrian is represented by two lithological units, the early Meishucunian phosphates in its lower part and the Shiyantuo silt shale (represented by Shitantuo Formation in central Yunnan) in its upper part. The early Meishucunian phosphates preserve not only abundant "small shelly fossils", but also metazoan eggs[9, 10].

The diverse metazoan eggs and embryos of early Meishucunian called Ningqian fauna occur in black bituminous and sparite phosphate limestone beds of the Lower Cambrian Kuanchuanpu Formation, in Ningqian, southern Shaanxi[11]. Some of the black bituminous phosphate beds bear extremely rich accumulations of tiny globules. Most of these are various developing eggs apparently from a great diversity of marine organisms. Studies of the affinities of these eggs could make a significant contribution to understanding the mysterious world of the first 13 Ma of the Cambrian Explosion.

Although arthropods were already highly abundant and diversified in the 530-million-year-old Maotianshan Shale fauna[12~14] and trace fossils suggest that they likely evolved at the start of the Cambrian[15] or earlier, no arthropod body fossils have been recorded in strata older than this.

Small globular fossils, known as *Olivooides* and

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Pseudooides since 1977[16], have been widely recorded from basal Cambrian phosphate deposits in southern China. As implied by their names, these fossils are egg-like and many contain cellular subparts that indicate that they are eggs and embryos. The first detailed morphological analysis of these eggs and their possible affinities with modern animals were carried out by Bengtson and Yue[17]. In 1987, these authors described a developmental sequence of *Olivooides* ranging from various developing stages of embryo to hatched animals. Although the authors assigned a cnidarian origin to *Olivooides*, our studies indicate that the eggs were diverse and of polyphyletic origin. In addition, Bengtson and Yue illustrated a few spherical structures that they called cleavage embryos of unknown affinity. The surface of these globules bears polygonal structures and one of them (Figs. 1B and 1B' in Ref. [15]) displays an internal structure with radially arranged pillars. In the present paper, we report the discovery of more such globules, with radially arranged pillars. These structures show similarities to yolk pyramid stages found in a number of extant arthropods[18,19].

1 Locality, preservation and methods

All the embryos are collected from the phosphate deposits of the Lower Cambrian Kunchuanpu Formation near Xuanjiangping village, Kuanchuanpu, Ningqiang County, southern Shaanxi (Fig. 1). The Kuanchuanpu Formation crops out repeatedly along gullies on the north side of the Shizhonggou valley near Xuanjianping village. Where the Kuanchuanpu Formation represents a sequence of about 80 m phosphatic carbonate deposits, lying continuously above the Precambrian carbonate rocks, called Dengying Formation. The *Cloudina* fossil assemblage occurs near the top of the Dengying Formation and this assemblage is accepted as defining the terminal stage of the Precambrian[20].

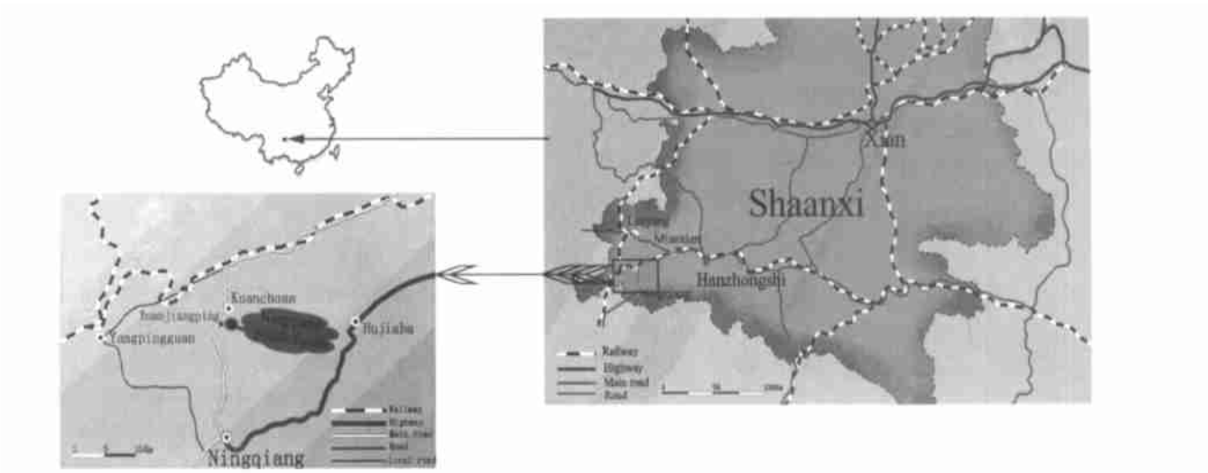

Fig. 1. Index map of the fossil locality, the right side showing the general location of the fossil site within southern Shaanxi Province; and the left-bottom side showing the fossil site of the Lower Cambrian Ningqiang fauna within the Ningqian area.

Our samples were collected mainly from a bituminous black, phosphatic sparite limestone bed in the lower part of the Kuanchuanpu Formation, where the rocks were mined for phosphate several years before. Because the rocks are bituminous and have a lower concentration of phosphates, they break easily and loose slabs of the globule-rich bed had fallen a short way down the slope of the quarry.

The selected samples were smashed into pieces a few cm in size and treated in 10% ~15% acetic acid. The rock samples were then sieved through a set of three nylon sieves, with openings of 1200 μm on top; 600 μm in the middle; and 300 μm below. The residues of different sizes were washed separately and dried. The fossils were picked by hand under a dissecting microscope. Scanning electron microscopy (SEM) images were taken using a Hitachi-S2600N in the NASA Project Lab in the Early Life Research Center, Chengjiang, Yunnan.

Other fossiliferous rock samples from the same bed were ground into thin slices about 50 μm thick, allowing some three-dimensional visualization at different planes of focus. Images of these were obtained by light microscopy under directly transmitted light at 100 ~ 1000X magnifications. All the images are recorded with a digital camera and stored within a computer.

2 Fossilized embryos resembling yolk pyramid stages of some modern arthropods

Comparative studies are important for understanding the origins and phylogeny of the fossil eggs[21]. Among the globules from the Kuanchuanpu phosphates, we have identified a number of developing eggs that are comparable to the yolk-pyramid stages of extant arthropods.

These eggs are either spherical or oval, ranging from 380 to 600 μm in size, and are tightly packed within a thin, smooth egg shell. Because the shell is sometimes detached, the polygonal structures below it are exposed to the surface in a number of specimens (Fig. 2A and B). These polygons are tightly packed, mainly pentagons, and of variable size (Fig. 2A ~ D). Some of the eggs are broken into two parts and reveal that these polygons represent the distal ends of radially arranged pillars (Fig. 2C ~ F). The same structures are also recognized from the transmitted-light images of the thin sections (Fig. 3). These structures are tightly packed and converge toward the center of the embryo. They resemble strikingly the yolk-pyramid stages of the early developing eggs of separate groups of extant arthropods including *Scolopendra*[22] and *Pauropus* in myriapods; a river crayfish *Astacus* in crustaceans[23]; and a cellar spider *Agelena* in cheliceratids[18, 19].

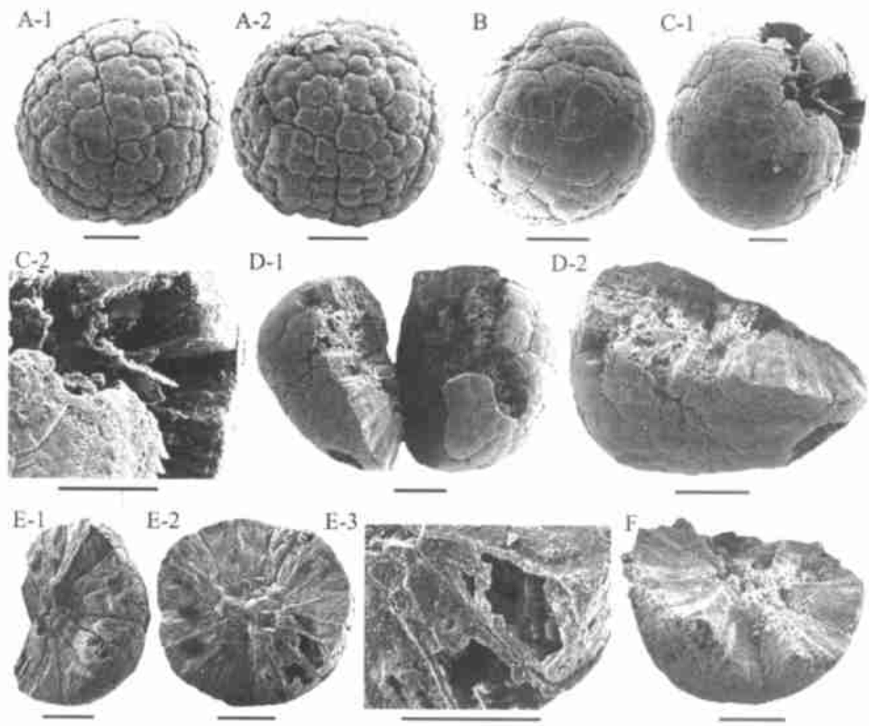

Fig. 2. SEM images of yolk-pyramid eggs from Lower Cambrian Kuanchuanpu, Xuanjiangping, Kuanchuanpu of Ningqiang, southern Shaanxi: A1-2, showing polygonal surface structures of an advanced yolk-pyramid stage of the eggs (collection no. KXSB009, B002-S022); B, showing polygonal structure of an egg with ca. 120 pyramids (collection no. KXSB009, B002-S058); C1-2, showing polygonal structures of an egg with ca. 240 pyramids (C-1), and the yolk pyramids (C-2) (collection no. KXSB009, B002-S057); D1-2, showing polygonal and pyramid structures of an eggs at the advanced yolk-pyramid stage (collection no. KXSB009, B002-S022); E1-3, showing yolk-pyramids, blastocoele and possible cleavage energids within blastocoele at 120-yolk-pyramid stage (collection no. KXSB009, B002-S058); F, showing yolk-pyramids and blastocoele of an egg (collection no. KXSB009, B002-S058). Scale bar= 100 μm; all the material (including Fig. 3) stored in Early Life Research Center at Chengjiang.

Different eggs have different numbers of pyramids, which presumably represents different successive yolk-pyramid stages of the developing eggs. As estimated from the specimens with well-defined polygons and from the average sizes of the pyramids or polygons, the eggs contain three different numbers of the pyramids. They are about 120 (Fig. 2B, E; Fig. 3(a) ~ (d); Fig. 4(a), 240 (Fig. 2C, F; Fig. 3(e) ~ (g); Fig. 4(b)) and 360 (Fig. 2A, D; Fig. 3(h); Fig. 4(c)) in number. The size of pyramids is smaller when the pyramids are more abundant (Fig. 4).

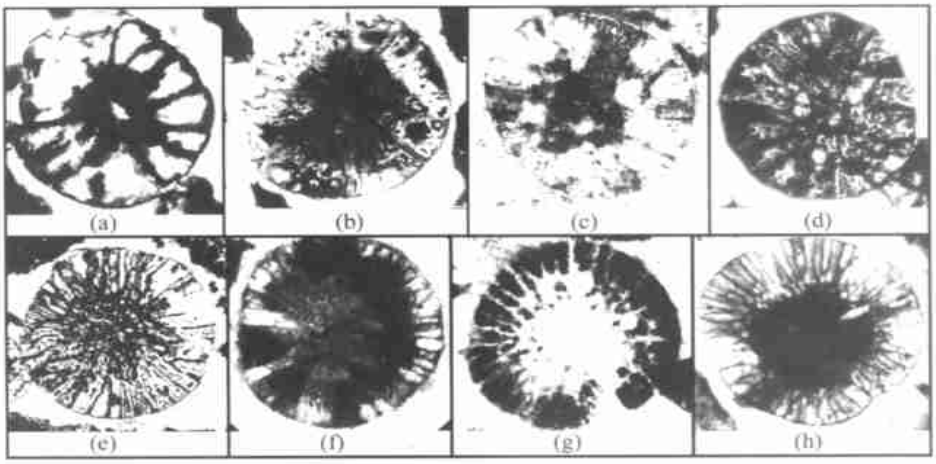

Fig. 3. Sections displaying yolk pyramids of the fossil eggs from Lower Cambrian Kuanchuanpu, Xuanjiangping, Kuanchuanpu of Ningqiang, southern Shaanxi. (a) ~ (d) Eggs with ca. 120 yolk-pyramids, showing pyramids, a small blastocoele and possible cleavage energids within the blastocoele (collection nos. (a) SB12-T-705 (37. 17-106. 7); (b) SB 12 T-690 (39. 8-105); (c) SB 12-T 11 (47. 2-100. 2); (d) SB 6 #-Z-440 (30. 8-111. 5). (e) ~ (g) Eggs with 240 yolk-pyramids, showing pyramids, blastocoele and possible cleavage energids within the blastocoele; (e) SB 12-T-889 (41. 7-120. 5); (f) SB 12-T-647 (39-115); (g) SB 12-T-892 (30. 1-119. 6); (h) an egg with ca. 360 pyramids, showing pyramids and a large blastocoele which is lacking cleavage energids; Coll. No., KXSB009 G-588 (23. 4-115. 4). Scale bar= 100 μm.

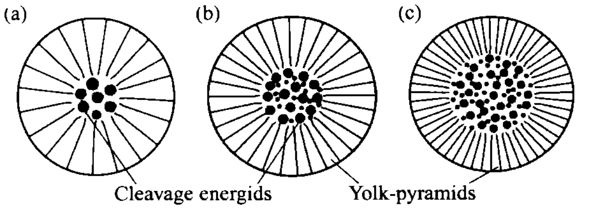

Fig. 4. Explanatory of a developing sequence of the three yolk-pyramid stages of a unknown arthropod from Ningqian fauna, Lower Cambrian, Kuanchuanpu, Ningqian of southern Shaaxi. (a) early yolk-pyramid stage with about 120 yolk-pyramids; (b) middle yolk-pyramid stage with about 240 yolk-pyramids; and (c) late yolk-pyramid stage with about 360 yolk pyramids.

The yolk pyramids do not reach the center of the embryo. Based on arthropods, we interpret the cavity lying interior to the yolk-pyramids to be a blastocoele or primary body cavity. The blastocoele at the earliest yolk-pyramid stage (with 120 pyramids) is small, ca 50 μm in diameter and bears numerous pelleted structures, which may represent the cleavage energids before migration toward the surface of the embryo (Fig. 2E; Fig. 3(a) ~ (d)).

The blastocoele in the later two yolk-pyramid stages is larger and its diameter is about 100 μm and 250 μm, respectively. In the 240-pyramid stage, the presence of pelleted structures suggests that the cleavage energids have not yet migrated toward the surface (Fig. 2F; Fig. 3(e) ~ (g)). In the 360-pyramid stage, however, we cannot find any trace of the pellets (Fig. 3(h)). Presumably the cleavage energids at this stage have migrated to the yolk surface to form blastoderm nuclei, although the nuclei are not visible in the fossils.

In arthropods, as the blastoderm matures, the yolk-pyramids fuse once more to form a unitary, nucleated yolk mass. All the embryos illustrated in the present study bear yolk pyramids and therefore must represent the yolk-pyramid stages of cleavage eggs before the maturity of the blastoderm.

3 Affinities

The morphological resemblance of the fossils to the yolk-pyramid stages of extant arthropod cleavage eggs is striking. The resemblance suggests an arthropod identity of these eggs, all of which may have been laid by the same animal species. We have not documented the entire developing sequence for this animal, because the later stages are lacking. However, we have identified many co-occurring globules, which bear either an oval, superficial smooth area with an elongated extension, or a superficial segmented germ band, known as *Pseudooides*[16].

Zhang and Pratt in 1994 described five embryos from the Middle Cambrian limestone bed in Duyun, Guizhou[24]. The embryos are ovoid, 0. 30 ~ 0. 35 mm long and 0. 24 ~ 0. 27 mm wide, and enveloped with a thin egg shell. They were interpreted by authors to be produced by a co-occurring eodiscoid trilobite. Beneath the shell were about 60 tightly packed polygons, which were previously interpreted as blastomeres comprising the blastula. They resemble our material here, however, so an alternate interpretation of these polygonal structures could be yolk-pyramids.

A number of different metazoans have a blastula with thick cellular columns. However the lack of evidence for an initial process of cell division of the fossil eggs suggests that our embryos with pyramids are unlikely to be blastulas of non-arthropod metazoans.

4 Discussion

Understanding ground patterns or ancestral states of the organisms is essential for phylogenetic reconstruction[25]. Crown-lineage arthropods commonly known as euarthropods[26] (cheliceratids, crustaceans, hexapods and myriapods) have a great variety of the cleavage patterns. The ground pattern of early cleavage in euarthropods remains a great puzzle. The widespread occurrence of superficial cleavage could reflect the evolutionary starting point of early cleavage pattern in euarthropods, as proposed by a number of authors. It is characterized by yolky eggs with intralecithal cleavage division and by cleavage energids, which are not separated by membranes. Later, the energids migrate to the embryo's surface to form the blastoderm with a central yolk mass. Weygoldt in 1986 proposed that superficial cleavage with a yolky egg is synapomorphic condition of the onychophora+arthropodan clade[27]. Other authors, by contrast, believe that the ground pattern is either holoblastic cleavage with a coeloblastula or a mixed cleavage type. (The latter has an early holoblastic stage, then shifts to superficial cleavage in the blastoderm stage.). Therefore, holoblastic cleavage with little yolk in the eggs was suggested as a plesiomorphic condition of the arthropods by Siewing in 1969[28].

The 543-million-year-old eggs with yolk-pyramid stages not only provide new fossil evidence pushing back the evolutionary history of the arthropods into a deeper time, but they also add some support for superficial cleavage with intralecithal division as the ground pattern of arthropod development (including Onychophora) as proposed by Ax[29]. Alternatively, if it is advanced rather than primitive, then the yolk-pyramids condition of our fossils shows that this advanced state appeared quite early, by the base of the Cambrian.

The 580-million-year-old Wengan fauna has yielded a great abundance and variety of developing eggs. Not only do these provide evidence that crown-lineage cnidarians and bilaterians evolved in Precambrian time[30, 31], but they also suggest that a number of bilateria phyla including nematodes, flatworms and molluscs have a Precambrian history[11]. These fossil findings would challenge a traditional thought that all the animal phyla are suddenly present in Cambrian. Despite intensive study, no embryos with superficial cleavage or yolk pyramids have been recognized from the Wengan fauna. This, along with our present findings, implies a later, earliest-Cambrian appearance for this mode of arthropod development.

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