Predatorial Borings in Late Precambrian Mineralized Exoskeletons

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The late Precambrian tube-forming *Cloudina*, the earliest known animal to produce a mineralized exoskeleton, shows evidence of having been attacked by shell-boring organisms. Of more than 500 tubes from Shaanxi Province, China, 2.7% have rounded holes 40 to 400 micrometers in diameter. The relation between the size of the holes and the width of the bored tubes suggests that the attacking organism was a predator, selecting its prey for size. If true, this would be the oldest case of predation in the fossil record and would support the hypothesis that selection pressures from predation was a significant factor in the evolution of animal skeletons around the Precambrian-Cambrian boundary.

The evolutionary mechanisms behind the origin of mineralized skeletons in animals at the Precambrian-Cambrian transition about 550 million years ago have been vigorously debated. One school holds that skeletal biomineralization began as a detoxification process (*1–4*), another that it evolved mainly to promote biomechanically efficient constructions for locomotion, feeding, and so on (*5, 6*), yet another that it arose as a response to predation (*7–11*). Each of these mechanisms is likely to have played a role in the history of the various skeleton-forming lineages at one time or another. Yet, a crucial question is whether any of them served as a trigger for the abrupt mass appearance of mineralized animal skeletons in the fossil record. This event was part of a major radiation referred to as the "Cambrian explosion" (*12, 13*). We report that the earliest known animal with a mineralized skeleton, the late Precambrian tube-dweller *Cloudina*, was under attack from organisms seeking to penetrate the tube wall. Such attacks could well have provided selection pressures in favor of mineralized exoskeletons. The presence of shell-borers in the late Precambrian is consistent with hypotheses that the rise of herbivory and carnivory was a major factor behind the "Cambrian explosion" (*14, 15*).

First discovered in the Nama Group of Namibia (*16*), *Cloudina* is now known to be a widespread fossil in latest Precambrian deposits in various parts of the world (*17–19*). The tubes are normally preserved as thin calcareous lamellae in limestones or dolomites; hence they have hitherto been studied only in petrographic thin sections or, occasionally, as reliefs on weathered rock surfaces.

We have studied phosphatized specimens of *Cloudina hartmannae* Germs 1972 from the Lijiagou section, about 3 km south of the village of Kuanchuanpu, Ningqiang County, Shaanxi Province, China (Fig. 1). The fossils occur in light-gray dolomites of the Dengying Formation [Upper Member, Bed 8; belonging to the Gaojiashan Formation of Chen *et al.* (*20*)], overlain by about 35 m of interbedded cherts and limestones. The upper part of the latter unit [Upper Member, Bed 13; within the Kuanchuanpu Formation of Chen *et al.* (*20*)] contains the first assemblage of Cambrian skeletal fossils (*Anabarites trisulcatus* assemblage), occurring about 30 m above *Cloudina* in the section (Fig. 1). About 55 m below the level with *Cloudina* there have been found compressions in shale of what may be organic metazoan tubes, described as *Shaanxilithes ningqiangensis* Xing, Yue, and Zhang (*21*).

Indeterminable *Cloudina*-like fossils have previously been reported from Lijiagou and a neighboring locality (*18, 21*). The new material is well preserved through secondary phosphatization and can be chemically extracted from the rock. This makes it possible to combine information from three dimensionally preserved specimens with that derived from petrographic thin sections of *Cloudina* (Fig. 2). The material confirms and improves earlier reconstructions (*19*), giving significant new information on morphology and fine structures. Of particular importance is the observation that the tube walls are sometimes penetrated by borings.

The tubes consist of a series of excentrically stacked conical segments, each slightly flaring aperturally and open or closed basally. The thin (about 5 μm) lamellae that make up the cones were originally mineralized with $CaCO_3$ to varying degrees and show evidence of both flexible and brittle properties (*19*). Typically a new cone formed as a growth increment inside an older one, attaching itself to one side (Fig. 2). The initial parts of the tubes are closed and slightly bulbous (Fig. 3, A and C). The tubes are thus made up of individual thin lamellae separated by what was probably empty spaces during the life of the animal (*19*). Larger tubes may show more complicated patterns of wrinkled and folded lamellae reinforced by what appears to be secondary shell material.

The borings (Fig. 3, A to K) are conspicuous rounded holes, 40 to 400 μm in diameter, penetrating otherwise smooth and well-preserved tube walls. Examination of 524 tubes revealed 17 such holes distributed on 14 specimens, that is, 2.7% of the tubes had been bored. The shape of the holes varies from almost perfectly circular with smooth edges (Fig. 3, B, F, and K) to more irregularly rounded (Fig. 3E). They frequently penetrate more than one lamella (Fig. 3E); the outer lamellae may then suffer considerably more damage than the inner ones, which have a smaller, round hole (Fig. 3I). In two cases, only the outer lamellae have been penetrated (Fig. 3, G and H) (we could not determine whether the inner, unbroken lamellae were formed later than the hole or whether the hole never penetrated to the inside of the tube). Because the penetrated walls are so thin, no further features of the borings, such as bevelling or angle of penetration, can be

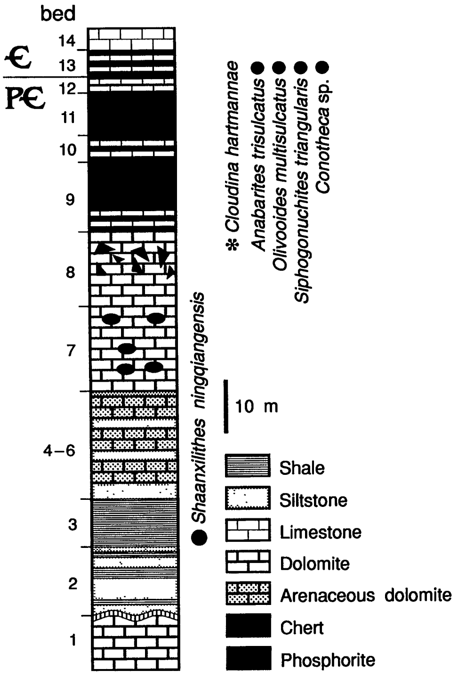

Fig. 1. Stratigraphical column through part of the Dengying Formation, Lijiagou section, Ningqiang County, Shaanxi Province, China, after Xing and Yue (*20*), with levels of metazoan fossils (circles are at stratigraphic position); level of *Cloudina hartmannae* is marked with an asterisk. Top and bottom of formation not exposed; sedimentary discontinuity between beds 1 and 2 marks boundary between Lower and Upper members. Precambrian-Cambrian boundary drawn at base of bed with first Cambrian assemblage (*Anabarites trisulcatus* assemblage in Bed 13).

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observed. No scratch marks are visible around the holes.

Smaller rounded holes (~10 μm and less) also occur (Fig. 3L). These grade into endolithic-type borings that occur on both inner (Fig. 3, M and N) and outer (Fig. 3, O to Q) sides of the tubes, often forming a corrosion-like pattern (Fig. 3P). We interpret these holes as having been formed by endolithic cyanobacteria or fungi and therefore unrelated to the 40- to 400-μm borings.

The size distribution of the tubes with the larger holes is not significantly different from that of the whole sample (Kolmogorov-Smirnov $D = 0.2625$), and there is a positive correlation between the diameter of the hole and the width of the tube at the level of the boring ($r = 0.8822$). If the hole diameter reflects the size of the boring organism, as is the case with Recent naticid gastropods (*22*), this suggests that the holes were made by an animal of the same size order as *Cloudina*, selecting tubes of approximately its own size. Together with the fact that some holes have an almost perfect circular shape and occur in tubes showing no other sign of damage, the size correlation also strongly speaks against the holes having been formed by taphonomic processes such as corrosion, wear, or postmortem boring by endoliths.

We do not regard it likely that the holes were produced by budding individuals, as there is no sign of wall buildup around the holes, and some holes do not penetrate the innermost lamellae. It is also less likely that the holes are attachment scars of epizoans, as such scars would not be expected to be correlated with tube width (small specimens would be as well off living on large tubes). Some Cambrian tubicolous torellellids grew gregariously on each other to produce circular attachment scars (*23*), but this is not likely for *Cloudina*, as its tubes have a bulbous end unlike the expanded disk of the torellellids.

Thus the evidence suggests that the larger holes were drilled by a metazoan roughly the size order of *Cloudina* itself. Although the purpose could conceivably have been parasitism or commensalism, the relation between hole and tube size is easier to explain assuming a predator that devoured a substantial part of the prey. With naticid gastropod predators, there is usually a good correlation between predator and prey size (*22, 24*). Predation on modern tubicolous organisms by shell borers is less well known, although the nudibranch *Okadaia*, a predator of serpulid tube worms, is reported to be strongly selective for boring sites but not for prey size (*25*). The multiple borings in two of the *Cloudina* tubes (Fig. 3C) would seem to speak against predation, but this evidence is hardly conclusive. Multiple completed borings occur, though rarely, with both fossil and Recent shell-boring predators (*24*). The repeated penetration of the two *Cloudina* tubes could be due to interruption of the attack after the boring was completed or to poor ability to determine the fruitful sites of attack.

We therefore conclude that the holes

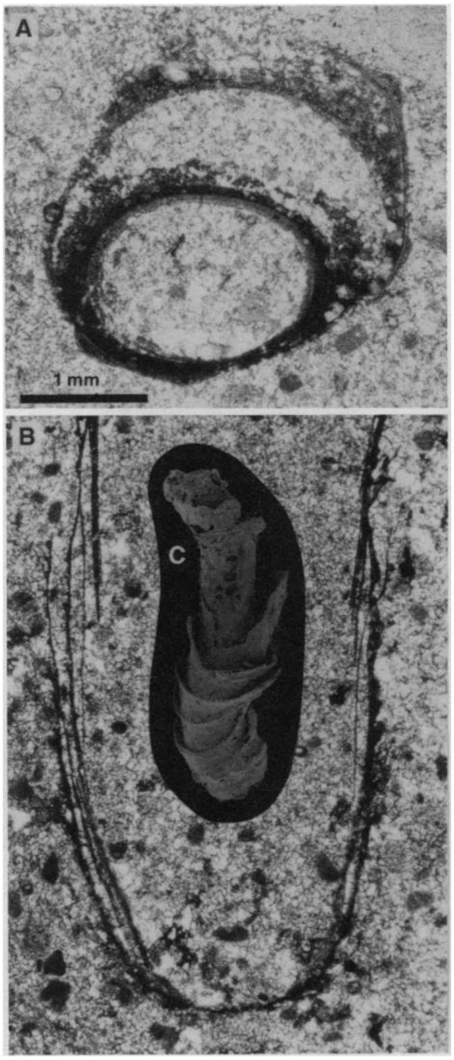

Fig. 2. *Cloudina hartmannae* from Lijiagou; all figured specimens deposited in the Institute of Geology, Chinese Academy of Geological Sciences, Beijing. (**A** and **B**) Transverse and longitudinal thin sections of specimens in dolomite, showing "traditional" view of *Cloudina* as excentrically stacked rings or half-rings. Specimen isolated from the rock matrix (C).

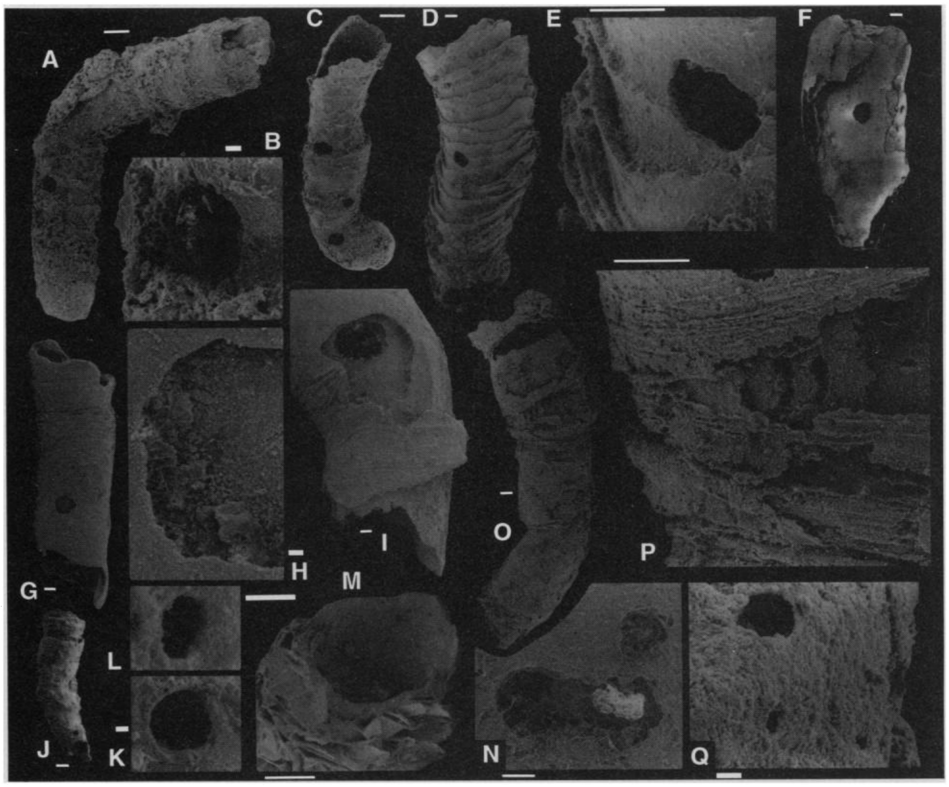

Fig. 3. *Cloudina hartmannae* from Lijiagou, with predatorial (**A** to **K**) and endolithic (**L** to **Q**) borings. Thin scale bars represent 100 μm; thick ones, 10 μm. (**A** and **B**) Specimen with rounded end and single, circular hole. (**C**) Specimen with bulbous end and three rounded holes. (**D** and **E**) Specimen with rounded hole penetrating at least two lamellae. (**F**) Specimen with circular hole. (**G** and **H**) Specimen with hole penetrating outer but not inner lamellae. (**I**) Specimen with hole penetrating both outer and inner lamellae. (**J** to **L**) Specimen with predatory (K, lower part of tube, and endolithic, L, upper part of tube) borings. (**M** and **N**) Specimen with endolithic borings on inner surface of tube. (**O** to **Q**) Specimen with endolithic borings and general corrosion-like pattern.

were most probably made by predators. It should be recognized, however, that there is an intergradation between parasitism and predation that is usually not resolvable in the fossil record, and also that a mineralized shell is useful to hold off any attacking organism, whether parasite or predator.

Predator pressure may induce a variety of responsive strategies with great evolutionary potential for differentiation and speciation (*10*). With regard to shell-boring, the best investigated cases concern gastropod predation on bivalves. The main factors in prey selection appear to be the ratio between prey biomass and shell thickness, and the relative size of predator and prey (*22*). Responses by nonmotile prey would thus involve reduction in shell penetrability and modifications of ontogenetic growth (*26, 27*).

The low percentage of bored *Cloudina* is comparable to the generally low levels of shell-boring predation through the Palaeozoic, before the Mesozoic evolution of shell-boring gastropods (*10, 24, 28, 29*). The apparent predator on *Cloudina* selected its prey for size, as do shell-boring gastropods. Due to the secondarily phosphatized preservation of the *Cloudina* walls in our material, the original shell thickness is difficult to measure in specimens isolated from the rock. Thus there are currently no data indicating whether the predator also preferred individuals with a high ratio of biomass to shell thickness. Furthermore, as *Cloudina* is nowhere known to span a substantial stratigraphic interval and no evolutionary ancestors or descendants have been positively identified, there is currently no time frame available in which to study the possible evolutionary effects of early predation.

Nevertheless, the apparent predation on *Cloudina* is highly significant for our understanding of the dramatic biotic events around the Precambrian-Cambrian boundary. First, it would mean that the marine food web already extended to macrophagous predation in the late Precambrian ecosystems. Second, the occurrence of borings, in particular the unsuccessful ones, suggests that at least one function of the first mineralized skeletons was to thwart predators. Third, the possibility of active selection for size by this Precambrian predator indicates a level of neural complexity usually associated with bilaterian animals. Fourth, the evidence fits the prediction of those models of Precambrian-Cambrian evolution that affix a high significance to the appearance of carnivory (and herbivory) both for the origin of mineralized skeletons and for the general ecological diversification. Fifth, we have a possible way to obtain the data necessary for more rigorous testing of such hypotheses in the future.

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Crystal Structure of Transforming Growth Factor–β2: An Unusual Fold for the Superfamily

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The transforming growth factors–β (TGF-β1 through -β5) are a family of homodimeric cytokines that regulate proliferation and function in many cell types. Family members have 66 to 80% sequence identity and nine strictly conserved cysteines. A crystal structure of a member of this family, TGF-β2, has been determined at 2.1 angstrom (Å) resolution and refined to an *R* factor of 0.172. The monomer lacks a well-defined hydrophobic core and displays an unusual elongated nonglobular fold with dimensions of approximately 60 Å by 20 Å by 15 Å. Eight cysteines form four intrachain disulfide bonds, which are clustered in a core region forming a network complementary to the network of hydrogen bonds. The dimer is stabilized by the ninth cysteine, which forms an interchain disulfide bond, and by two identical hydrophobic interfaces. Sequence profile analysis of other members of the TGF-β superfamily, including the activins, inhibins, and several developmental factors, imply that they also adopt the TGF-β fold.

The transforming growth factors–β (TGF-βs) are a family of multifunctional growth and differentiation factors that act on most cell types with activities dependent upon the cell type, stage of proliferation, and environment [for reviews, see (*1–5*)]. Of particular interest are their abilities to stimulate connective tissue synthesis, to suppress proliferation and function of immune cells, to inhibit proliferation of endothelial and other epithelial cells, to stimulate osteogenic activity, and to be chemotactic for monocytes and fibroblasts. Five isoforms that have similar but not identical activities (TGF-β1 to -β5) have been discovered and have 66 to 80% sequence identity (*6–17*).

A larger group of proteins, referred to as the TGF-β superfamily, have ~30% sequence identity to TGF-β1 and seven invariant cysteines [see (*2*)]. They include the activins and inhibins, Mullerian inhibiting substance (MIS), the bone morphogenetic proteins (BMPs), the decapentaplegic (DPP-C) gene complex of *Drosophila*, and the closely homologous *Vg1* and *Vgr-1* genes of *Xenopus* and mouse, respectively.

The TGF-βs are homodimers with molecular masses of ~25,000 daltons. Each

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