Comparative topology and structure of *Thiomargarita* and *Parapandorina* cell clusters: Both Doushantuo and *Thiomargarita* diads exhibit an undisturbed division plane (Fig. 1b, b’). Three-cell clusters, thought to result from the incomplete division of a two-cell stage are present in both *Parapandorina* and *Thiomargarita* (Fig. 1c, c’). Whether or not the undivided larger cell of the *Thiomargarita* triads later undergoes reductive division to produce a tetrad is presently unknown. Both *Thiomargarita* and *Parapandorina* tetrads exhibit a variety of cell configurations. Four-radiate cross-junctions of division planes in tetragonal Doushantuo tetrads are shown in Figure 1d’, a configuration common in extant non-mammalian embryos and in *Thiomargarita* tetrads (Fig. 1d). Some Doushantuo octads are also observed to exhibit radiate cross-junctions (Fig. 1c in 1), which undoubtedly resulted from division of tetragonal tetrads. Rhomboidal tetrads, which are characterized by two opposite bodies in contact, and two opposite bodies separated by a gap, are also observed in *Parapandorina* (Fig. 7.5 from 2, reproduced in Figure 1e’), and in *Thiomargarita* tetrads (Fig. 1e). Perhaps a more common topology observed in Doushantuo tetrads are decussate or tetrahedral configurations, which result from deformation of preexisting cell-division planes 3. Such deformation is thought to require non-rigid cell walls/membranes and produces Y-shaped triple junctions of division planes (Supplementary Fig. 1). Although bacterial cell walls were once thought to be rigid structures, they are now known to be quite elastic 4. Deformation of division planes resulting in Y-shaped triple junctions are observed in multi-planar *Thiomargarita* tetrads, including partially-deformed tetragonal tetrads (Supplementary Fig. 2a), decussate tetrads (Supplementary Fig. 2), and tetrahedral tetrads.
(Supplementary Fig. 3). Y-shaped triple junctions are also observed in 8-cell *Thiomargarita* and multi-cell *Parapandorina* specimens (Supplementary Figure 4, 5c, 5d). Although *Thiomargarita* from off the coast of Namibia are known to form chains\(^5\), *Thiomargarita* from the Gulf of Mexico, the focus of this study, have not been observed to form chains.

*Thiomargarita* cell clusters have yet to be observed containing hundreds of internal bodies, such as *Megaclonophycus*, another globular microfossil from the Doushantuo Formation. However, these microfossils have never been demonstrated to be part of a developmental continuum with *Parapandorina*\(^2\). *Megaclonophycus* are loosely-packed clusters that contain rounded, rather than polyganol, internal bodies\(^2\), and do not exhibit a blastocoel, as would be expected in embryos with similar numbers of cells\(^6\), thus calling into question their metazoan affinities.

**Internal bodies:** Some *Parapandorina* specimens include smaller subcellular structures of non-diagnostic shape\(^6\). Such bodies are consistent with diagenetically-altered inclusions of the type that are common in *Thiomargarita*, but as with other hypotheses, their origin is ultimately ambiguous. A few *Parapandorina* specimens (n=10) include larger spherical-to-reniform internal structures\(^6\). Hagadorn et al.\(^6\) suggest that these bodies might be organelles, however they also allow for the possibility that the larger internal bodies resulted from inorganic mineral precipitation or shrunken cytoplasm as observed in other microfossils\(^5\)\(^6\)\(^7\), including algae from the Doushantuo Formation (Fig. 3H in \(^6\)). As a sheathed organism with vacuole contents that are chemically-distinct from surrounding waters, *Thiomargarita* cells could also have undergone cytoplasmic shrinkage or internal diagenetic mineral precipitation resulting in
intracellular structures. Inclusions in *Thiomargarita* also occasionally form aggregates (Supplementary Fig. 6), likely as a result of cytoplasmic degradation, that are similar in size and shape to the large intracellular structures observed in a small number of *Parapandorina* specimens. These aggregates often exhibit approximate symmetry across division planes (Supplementary Fig. 6b), and can sometimes be found as pairs in each cell of a multi-cell cluster, as is also observed in at least one Doushantuo tetrad\(^6\), though these aggregates likely result from degradational, rather than physiological, processes.

**Supplementary Figure 1:** *Thiomargarita* tetrad exhibiting deformation of the preceding division planes with Y-shaped triple junctions; (see arrows) as observed in a cartoon modified after \(^3\) showing an intermediate stage in the deformation thought to result in many Doushantuo *Parapandorina* tetrads. Scale bar = 100 \(\mu m\).
Supplementary Figure 2: *Thiomargarita* tetrad in an approximately decussate geometric configuration, with two cell pairs approximately at right angles to one another, similar to the geometry observed in some *Parapandorina* tetrads (cartoon modified after 3). The dark material surrounding the cluster is composed primarily of small filamentous and spherical cells, with sizes and morphologies similar to the filamentous and spherical structures commonly found on surfaces of Doushantuo microfossils. Scale bar = 100 μm.

Supplementary Figure 3: *Thiomargarita* tetrad exhibiting an approximately tetrahedral geometry akin to another geometry commonly observed in microfossil tetrads from the Doushantuo Formation. Scale bar = 100 μm.
Supplementary Figure 4: *Thiomargarita* octads exhibit Y-shaped junctions (white arrows), similar to those observed in multi-cell *Parapandorina* clusters (after \(^2\), Fig. 8.8). Scale bar = 100 μm.

Supplementary Figure 5: *Thiomargarita* cell clusters (a: 3-cell cluster, b: 4-cell cluster, c, d: probable 8-cell clusters) show a variety of geometries that indicate deformation of the previous division plane, as observed in *Parapandorina* clusters. All scale bars = 100 μm.

Supplementary Figure 6: Aggregates of internal inclusions, presumably resulting from cytoplasmic degradation, appear as large spheroidal to reniform intracellular bodies in a small number of a) solitary *Thiomargarita* cells, and b,c) multi-cell clusters. All scale bars = 70 μm.

Comparative morphology of *Tianzhushiana, Megasphaera inornata*, and *Thiomargarita*: The Doushantuo Formation contains abundant large spherical
microfossils that exhibit a range of morphological features. Abundant indistinct smooth spheres of uncertain affinities, generally categorized as sphaeromorphic acritarchs, are often thought to represent algal resting cysts. Some Doushantuo spherical bodies are encased in an envelope (*Megasphaera*). It has been recognized that some of these envelopes possess external surface ornamentation (*Megasphaera ornata*), while others lack ornamentation (*Megasphaera inornata*). This ornamentation has been central to the argument for a metazoan egg interpretation of *Megasphaera*². Recently, it was proposed that *Megasphaera* results from taphonomic alteration of the acanthomorphic acritarch *Tianzhushiana tuberifera*¹⁰, which is characterized by external processes that penetrate a multi-lamellate outer wall¹¹,¹². Similarities between the middle wall of *T. tuberifera* and the outer wall of *M. ornata*, and the finding of an additional outer wall on a few *M. ornata* specimens lead to the hypothesis that they represent the same species. Based on the hypothesis that unornamented globular fossils may have once had an ornamented outer wall that was not preserved, the unornamented *M. inornata* was also proposed to be synonymous with *Tianzhushania*¹⁰. Given the great abundance of unornamented globular microfossils in the Doushantuo phosphorites, we find this explanation insufficient to explain most unornamented specimens. Therefore, we retain the use of *M. inornata*, which we interpret as possible fossilized giant sulfur bacteria based on its abundance in phosphorites. Deflated envelopes lacking internal bodies are also observed in the Doushantuo microbiota² (Supplementary Fig. 7a'), and similar deflation is commonly exhibited in *Thiomargarita* cells with ruptured internal vacuoles (Supplementary Fig. 7a). However, modern animal eggs and phosphatized Doushantuo acritarchs are also observed
to exhibit deflation and collapse. Ultimately, unornamented globular fossils are taxonomically ambiguous and could represent more than one organism.

Supplementary Figure 7: Deflated *Megasphaera* (a’) (after 2) may indicate an initially hollow interior, such as the large vacuole in *Thiomargarita*, which is occasionally observed to rupture resulting in a deflated cell (a). Scale bar = 100 μm.

**Abundance of Doushantuo microfossils:** The unusual abundance of globular microfossils in the Doushantuo Formation has long been considered problematic for the animal embryo interpretation; concentration via sedimentary processes has been proposed as a possible solution. Such a circumstance does fall within the realm of possibility. However, the globular microfossils generally occur as grains within larger reworked clasts. Literature on this topic suggests that the fossils were phosphatized, size sorted by currents, cemented together, ripped-up, and re-deposited elsewhere within the larger clasts. Each stage of cementation followed by reworking and re-deposition loses the memory of the former clast size. Such multiple reworking would more likely dilute, not concentrate, the microfossils in the deposit. Thus, we do not find concentration by sedimentary processes to be the most likely solution.

The interpretation of the microfossils as bacteria more easily explains their abundance, as *Thiomargarita* is known to occur in great abundance (up to 200 g m⁻²),
which would translate to approximately $10^7$ cells per m$^2$. In the Gulf of Mexico, abundance is somewhat lower at ~ $10^5$ cells per m$^2$ (see Supplementary Fig. 8).

Supplementary Figure 8: Hydrocarbon-seep sediments covered with very abundant Thiomargarita cells. The width of the pH microelectrode tip at center is ~500 μM.

3. Xiao, S., Mitotic topologies and mechanics of Neoproterozoic algae and animal embryos. Paleobiology 28, 244-250 (2002).


