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Summary

One of the strongest paleontological arguments in favor of the origin of bilaterally symmetrical animals (Bilateria) prior to their obvious and explosive appearance in the fossil record in the early Cambrian, 542 million years ago, is the occurrence of trace fossils shaped like elongated sinuous grooves or furrows in the Precambrian [1–5]. Being restricted to the seafloor surface, these traces are relatively rare and of limited diversity, and they do not show any evidence of the use of hard appendages [2, 6]. They are commonly attributed to the activity of the early nonskeletonized bilaterians or, alternatively, large cnidarians such as sea anemones or sea pens. Here we describe macroscopic groove-like traces produced by a living giant protist and show that these traces bear a remarkable resemblance to the Precambrian trace fossils, including those as old as 1.8 billion years. This is the first evidence that organisms other than multicellular animals can produce such traces, and it prompts re-evaluation of the significance of Precambrian trace fossils as evidence of the early diversification of Bilateria. Our observations also render indirect support to the highly controversial interpretation of the enigmatic Ediacaran biota of the late Precambrian as giant protists [7, 8].

Results

On four research dives at 750–780 m near Little San Salvador Island (Bahamas) in the *Johnson-Sea-Link* submersible, we observed a multitude of grape-like objects associated with tracks up to 50 cm long (Figure 1A; also [Movie S1 in the Supplemental Data](#)) on the seafloor. On sloped regions of the

seafloor, tracks were often aligned as if the objects were moving uphill (Figure 1B). However, we found tracks in all orientations, including tracks running in opposite directions in the same region (Figure 1A). The tracks were commonly sinuous grooves bordered by two low lateral ridges with a central ridge that was especially well defined near the objects themselves (Figures 1C–1F).

Examination of the collected specimens identified them as testate amoebas of the genus *Gromia*, which is a sister group of Foraminifera within the supergroup Rhizaria [9, 10]. We sequenced a fragment of the small-subunit ribosomal RNA from one of the specimens. Phylogenetic analysis comprising previously reported sequences from a variety of deep-sea gromiids [11] indicated that our organisms should be classified as *Gromia sphaerica*, a species previously known only from the Arabian Sea [12] (Figure 2).

Bahamian *Gromia* looks very much like a small dark-green grape or ball (Figures 1 and 3) up to 30 mm in diameter. A thin layer of protoplasm containing fine greenish grains of sediment underlies its membranous transparent wall (test), whereas its fluid-filled center appears to be devoid of living or sediment matter. This bubble-like organization of Bahamian *G. sphaerica* represents a sharp contrast to other known macroscopic deep-sea protists (Xenophyophores, Allogromiids, and Komokiaceans [13, 14]), all of which are filled with agglutinated sediment feces (stercomata). Our Bahamian specimens demonstrate an important diagnostic feature of the Arabian *G. sphaerica*: Unlike all other gromiids, their tests have numerous evenly scattered apertures rather than just one or a few terminal apertures [11, 12] (Figure 3A). Projections with poorly defined shapes associated with some of the apertures can sometimes be seen in freshly collected specimens (Figure 3B), ostensibly representing collapsed pseudopodia. However, Bahamian and Arabian *G. sphaerica* are notably different in body shape and lifestyle. Arabian *G. sphaerica* is nearly perfectly round (as the species name implies) and sedentary: these organisms were observed in situ with only a narrow area of lighter sediment all around the naked tests as evidence of their activity [12]. In contrast, Bahamian *G. sphaerica* is usually grape shaped rather than round (although almost round individuals can also be found: Figures 3A and 3B), is commonly fully covered in situ by a thin layer of sediment (Figures 1A–1F and 3C), and is associated with tracks that suggest motility (Figure 1). Interestingly, despite the overall morphological similarity, Arabian *G. sphaerica* was reported to have a stercomata-filled rather than a bubble-like body [12], which may reflect the difference between the sedentary and motile lifestyles of the Arabian and Bahamian ecomorphs. Still, there is a possibility that the original description of the Arabian *G. sphaerica* was not entirely accurate in this respect, and it might have a bubble-like organization after all (A.J. Gooday, personal communication).

Although we did not see the protists' movement directly, there are several observations that virtually exclude the possibility that the tracks are due to currents or sediment slides moving the organisms around rather than the organisms' own activity. The bubble-like body organization makes the Bahamian *Gromia* nearly neutrally buoyant. A current dragging

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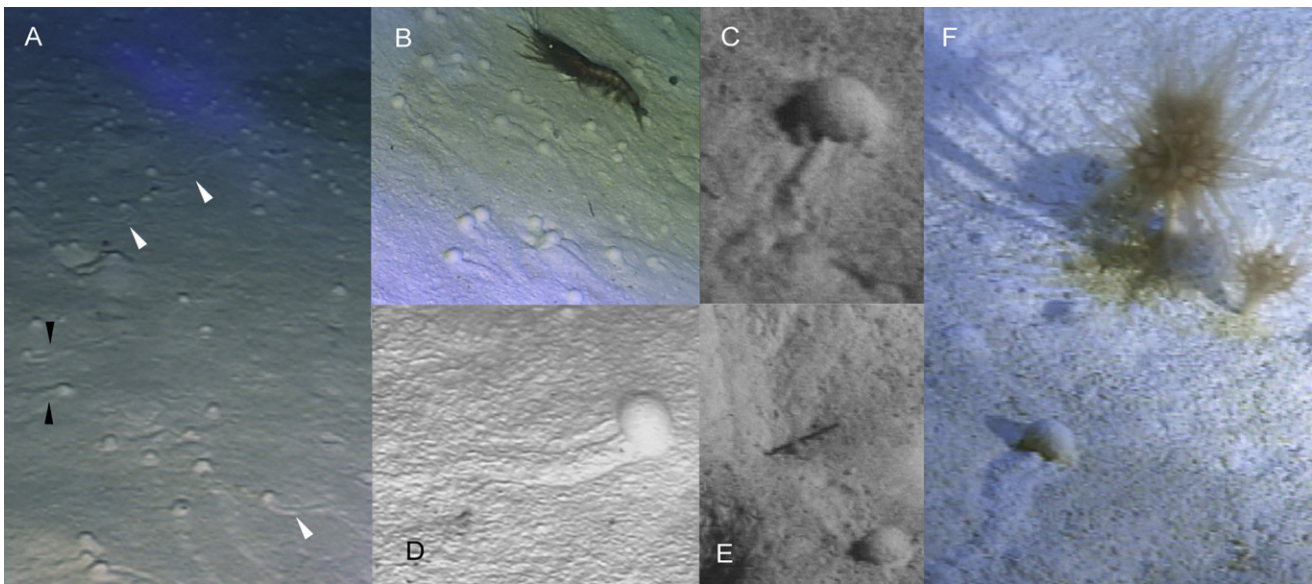


Figure 1. Tracks of the Bahamian *Gromia sphaerica*

(A) Gently sloping seafloor with numerous *G. sphaerica* visible. White arrowheads indicate notably curved tracks; black arrowheads indicate adjacent tracks running in opposite directions.

(B) Alignment of the tracks on a steeper slope.

(C–F) Details of the tracks, demonstrating the characteristic bilobed profile with the central ridge that is especially prominent near the organism. In panel (E), note that the track proceeds through a dip in the terrain, suggesting active locomotion. In panel (F), a group of three large cup corals growing on a half-buried sea urchin test indicate a remarkable sediment stability that may facilitate track persistence.

such an object across the seafloor would not produce a groove similar to the typical track; in fact, we frequently observed these organisms being carried by currents produced by the submersible without leaving any imprint on the sediment (Movie S2). Shifting sediment would carry the organisms along with it rather than generate tracks because the organisms don't seem to be anchored in the deeper (unmoving) sediment layers. The obvious uphill movement observed on the slopes (Figure 1B) excludes the possibility of passive rolling down the slope. The tracks successfully negotiate dips (Figure 1E), which can be another indication of active locomotion. The variability seen between tracks of different individuals further supports the conclusion that these tracks were left by these organisms: The tracks often curve and run in different directions (Figure 1A). Such patterns would be difficult to explain if the tracks were due to external causes because such causes should affect all the organisms in a given locality in the same way.

On the basis of the observations that in situ, the grape-shaped Bahamian *G. sphaerica* were oriented with their axes perpendicular to the tracks (Figures 1C–1F), were completely covered with a thin layer of sediment, and had pseudopodia that could issue from any part of the body, we believe that they move by rolling, unlike smaller gromiids that crawl by pulling themselves along with pseudopodia issuing from a terminal aperture [15]. The rolling mode of locomotion is not uncommon in smaller protists with thin pseudopodia (filopodia) emanating from all around the body [16]. We further hypothesize that the Bahamian *Gromia* feeds as it rolls by picking up the top layer of sediment in front of the test and discharging the processed sediment behind. The central ridge of the trace (Figures 1C–1E) might represent the discharged sediment and, if so, might be viewed as a fecal trail. The extensive perturbation of the sediment associated with such a feeding process is likely to

be the primary cause of the track production because pure locomotion (irrespective of its mode) by such a nearly neutrally buoyant organism would hardly disturb the sediment at all. It is important to note that the significance of *Gromia* tracks for a re-evaluation of the trace fossil record does not depend on any particular mode of locomotion, as long as there is no doubt concerning causal association of the protists with the tracks.

In the future, it will be important to document the movement of these protists. However, this might turn out to be problematic because at this particular site, the movement might be extremely slow and still leave prominent tracks. The near-bottom current was commonly 0.1 knot and never exceeded 0.2 knots on any of the dives over 3 days. These low-current conditions, combined with a fine, soft, but nonflowing consistency of the sediment, seem to facilitate the retention of a great number of tracks of various deep-sea organisms (Movie S1). A good indication of sediment stability is the presence of three solitary corals that were up to 45 mm tall and were growing on a deteriorated sea urchin test that was half-buried in the sediment (Figure 1F). Growth of these corals would have taken several years [17], during which the test must have remained in the same position without ever getting turned over or buried. It is therefore possible that the observed *Gromia* tracks may have been produced over a course of weeks if not months.

Discussion

Molecular clock estimates unanimously place the origin of Bilateria before the appearance of their body fossils 542 million years ago in the Cambrian; these estimates have sometimes been as early as a billion years ago [18] but have recently converged on 50 million to 80 million years before the Cambrian explosion [19, 20]. However, the fossil evidence of bilaterian animals in the Precambrian is scarce. There is only

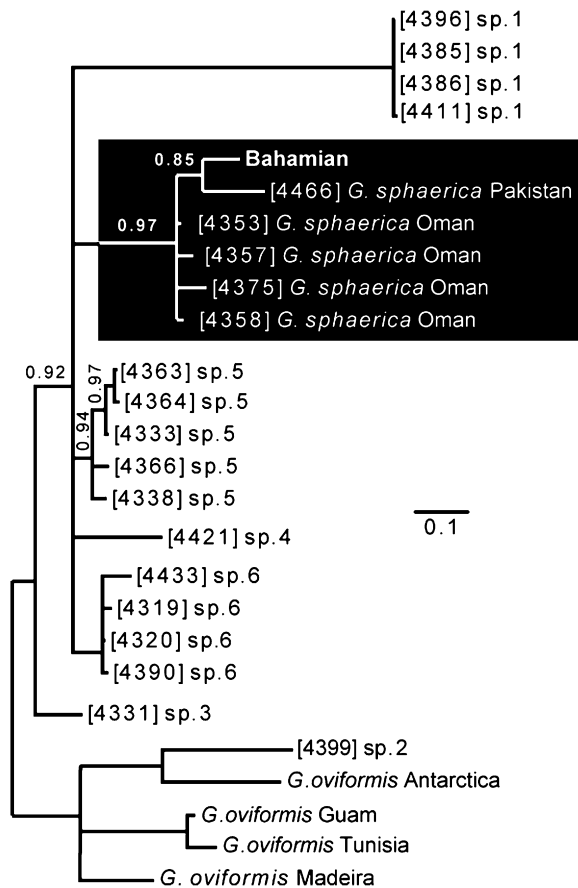


Figure 2. Phylogenetic Position of the Bahamian *Gromia* with Respect to Other Characterized Gromiids, According to the Partial Sequence of the Small-Subunit Ribosomal RNA

Six as-yet-unnamed deep-sea species are represented (sp.1 to sp.6 [11]), along with several isolates of shallow-water *Gromia oviformis* [41] and *Gromia sphaerica* from the Arabian sea [11]. The *G. sphaerica* clade, to which the Bahamian specimen belongs with high posterior probability (0.97), is highlighted. The edges with posterior probability less than 0.9 are collapsed, with the exception of the one rendering additional support to the placement of the Bahamian isolate within the *G. sphaerica* clade.

one common Precambrian body fossil—that of *Kimberella quadrata*—whose interpretation as a primitive mollusk has stood up to scrutiny thus far [21]. Some microscopic fossils [22] from the Doushantuo formation in China, dating back to 580 million years ago, have been described as bilaterians, but this interpretation is considered highly controversial [23, 24]. The discovery of what appear to be fossilized bilaterian embryos in the Doushantuo [25, 26] generated a lot of excitement [27] but was later contested by the reinterpretation of these structures as giant sulfur bacteria [28]. In the absence of unequivocal body fossils, arguably the most convincing evidence of the earliest bilaterians is traces shaped like elongated sinuous grooves or furrows [1–5]. It is puzzling, however, that some such traces date back to 1.5 billion to 1.8 billion years ago [29–31], which outdates even the boldest claims of the time of origin of animal multicellularity and forces researchers to contemplate the possibility of an inorganic or bacterial origin [32, 33].

The apparent need for two planes of asymmetry for trace-generating directional locomotion over the water-sediment interface is precisely why it was consistently viewed as

a prerogative of bilaterally symmetrical animals. Some benthic protozoans such as foraminiferans are able to displace sediment as they move [34, 35], but because of the submillimeter size of most of these motile forms and their tendency to move within the sediment rather than on top of it, this activity is unlikely to produce fossilizable traces. Some small epiphytic Foraminifera grazing on seagrass leaves embankment-like organic trails [36], but these are even less likely to be preserved as fossils. The possibility of extended surface rolling by larger protists, or any other extinct organisms, has thus far not been considered as a possible mechanism for the production of fossilized traces; a few exceptions include large fusiform foraminiferans and some bryozoans. Our observations make it plausible that certain Precambrian protists, similar to Bahamian *Gromia sphaerica*, could have reached macroscopic size while retaining the inherently protist-like rolling locomotion [16] and thus may have been responsible for at least some of the groove-like trace fossils currently attributed to bilaterians. The fact that the Precambrian traces are restricted to the sediment surface [2, 6, 30] corroborates the possibility of their production by rolling protozoans. The feeding activity associated with locomotion might explain how such traces could have been produced across the dense bacterial mats that covered the seafloor in the late Precambrian [37, 38], and it opens the possibility that the protists might have fed directly on the mats. There is good evidence for the existence of diverse amoeboid protists in the Precambrian. A variety of fossils of testate amoebas are known from at least as early as 742 million years ago [39, 40]. Molecular phylogenies suggest that gromiids in particular represent one of the ancient lineages of amoeboid eukaryotes with filopodia (i.e., long and thin pseudopodia) [10, 41]. This group of organisms underwent a major radiation around one billion years ago, resulting in the rise of Foraminifera from a putative *Gromia*-like ancestor [42], which implies that *Gromia*-like protists existed before that event. Among the Precambrian trace fossils that resemble the Bahamian *G. sphaerica* tracks are bilobed traces such as those of *Aulichnites*, *Nereites*, *Bilinichnus*, and *Archaeonassa* [6, 43–45] (Figure 4). Most remarkable, however, is the similarity to the enigmatic *Myxomitodes* traces from the Stirling formation [30, 31, 46], the origins of which are controversial [2, 33] primarily because of their extreme age of 1.8 billion years (Figure 4E). Notably, the Stirling formation also contains discoidal imprints 3–12 mm in diameter [47] that were interpreted as remains of “globular or bulbous collapsible bodies” [31], a description that fits *Gromia* quite well.

Our observations of the Bahamian *Gromia sphaerica* make it tempting to revisit the controversy surrounding the enigmatic Ediacaran biota that dominated the shallow-water marine megafauna of the late Precambrian, 580 million to 543 million years ago [48]. Although most researchers consider Ediacarans to be multicellular, Seilacher and coworkers proposed that they be interpreted as giant rhizopods with flexible organic walls, subdivided into hydrostatically supported chambers [7, 8]. Most paleontologists were unwilling to accept the possibility of giant turgid protist bodies with flexible walls, so much so that they deemed the affinity of the Ediacarans with fungi or lichens more plausible [49]. In our view, the multilayered flexible organic test of *Gromia sphaerica* [12], which is typical of all gromiids [50], fits the expectations of Seilacher’s hypothesis very well. The test of *G. sphaerica* is compatible with substantial growth along with maintenance of the function of hydrostatic support, and it also seems to have sufficient strength to support the bodies of even the largest Ediacarans,

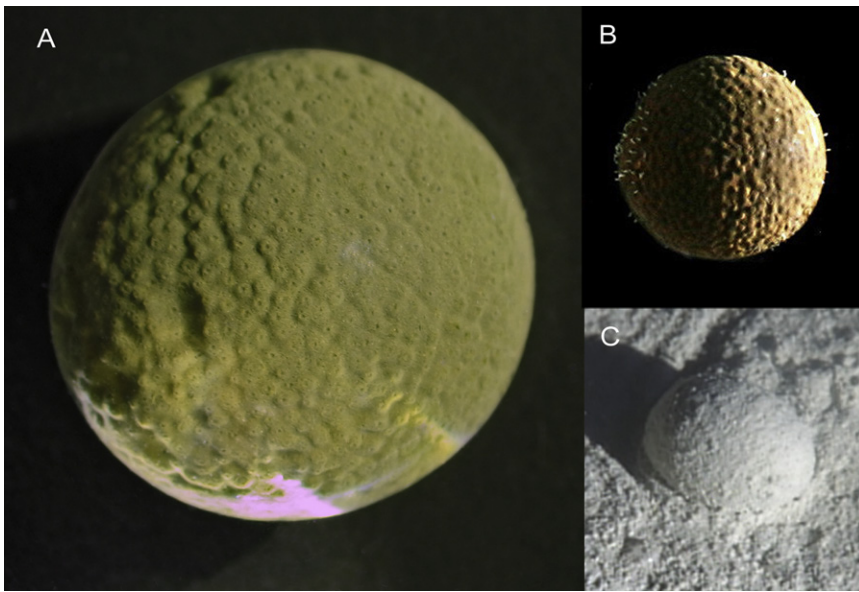


Figure 3. External Appearance of the Bahamian *G. sphaerica*

(A) Collected specimen demonstrating transparent membranous test, multiple evenly scattered apertures, and dark-green sediment contained in the protoplasm under the test's surface. (B) Freshly collected specimen with collapsed pseudopodia still visible (white projections). (C) Typical grape-shaped specimen in situ, fully covered with sediment. The track of this one is toward the top right corner of the picture.

G. sphaerica lacks is the chambered organization [51]. It seems plausible, however, that many of the small non-chambered discoidal Ediacaran fossils [52] represent organisms similar to *G. sphaerica*. Another major argument that has been put forward against the protozoan nature of the Ediacarans is the evidence of motility in some large

although properly addressing this issue will require a dedicated study. The only Ediacaran-like body characteristic that

forms [4, 53, 54], which has never seen in protists of comparable size. Our observations clearly demonstrate that an amoeboid protozoan can combine a large hydrostatically supported body and a motile lifestyle, lending indirect support to the interpretation of Ediacarans as giant protists.

In conclusion, our observations of the giant deep-sea amoeboid protist of the genus *Gromia* and its peculiar roving behavior provide fresh fuel for the debate on the history of both multicellular and unicellular animals. The example of *G. sphaerica* demonstrates that protists can be large, motile, and capable of producing macroscopic traces. This adds an important general option for interpretation of the trace fossil record and, in particular, makes it plausible to suggest that many trace fossils currently attributed to early bilaterian animals are in fact tracks of giant motile protozoans. It is also tempting to speculate that extant gromiids might be close relatives of the Ediacaran organisms, or even their direct descendants still roaming the deep ocean floor. Finally, there is a tantalizing possibility that *Gromia*-like protists might have been responsible for the tracks and fossils of the Stirling formation, and hence their extant representatives may be the ultimate macroscopic "living fossils," morphologically unchanged since 1.8 billion years ago. Further research into the ecology, biomechanics, and phylogeny of these bizarre mega-protists might bring substantial insight into the earliest chapters of evolution of macroscopic life on Earth.

Experimental Procedures

In Situ Observations and Specimen Collections

We made our observations on the *Johnson-Sea-Link* submersible at 720–780 m depth off Little San Salvador island, Bahamas (24° 34.5 N; 076° 00.1 W), JSL dive numbers 3614, 3615, 3619, and 3620. The specimens for on-board study and molecular analysis were collected with the suction sampler mounted on the front of the submersible, as well as with the benthic grab tool. The images were obtained with a digital video camera (Panasonic AW-E600 with a Canon J8xKRS lens) mounted on the movable arm in front of the submersible.

Obtaining the Sequence of the Small-Subunit RNA

One collected specimen was collapsed (so that excess water was removed), immersed in approximately 10 volumes of RNAlater solution (Ambion), stored overnight at 4°C, and transferred for longer-term storage at –20°C. The total RNA from approximately 1/10 of the specimen was isolated with

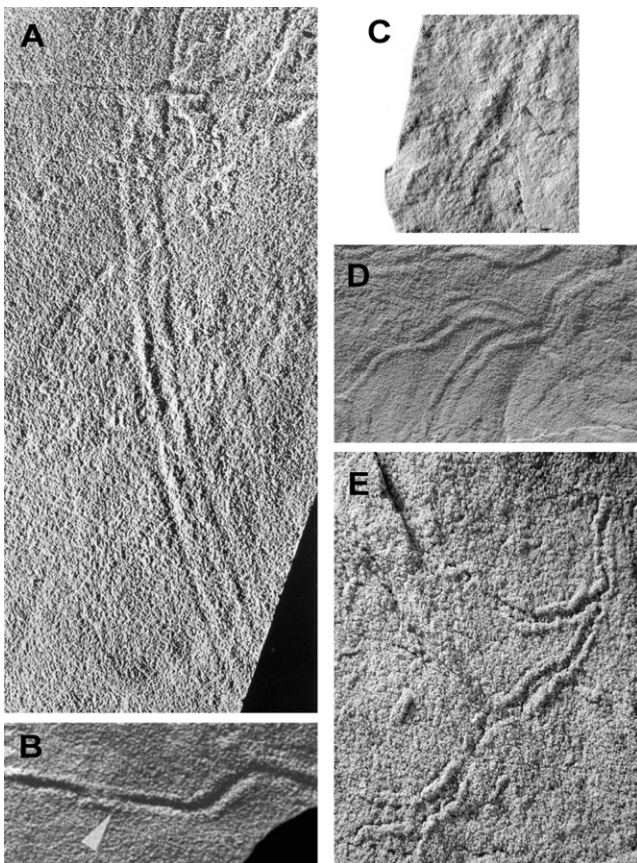


Figure 4. Precambrian Bilobed Trace Fossils Resembling Tracks of the Bahamian *G. sphaerica*

- (A) *Aulichnites* [5].
- (B) *Nereites* [45]; arrow indicates a lateral ridge.
- (C) *Bilinichnus* [61].
- (D) *Archaeonassa* [6].
- (E) *Myxomitodes* [31], the trace fossil from 1.8 billion years ago.

the RNAqueous kit (Ambion), and cDNA was amplified as described earlier [55]. The amplification product was ligated into pGEM-T vector (Promega) and transformed into *E. coli* Top10 strain (Invitrogen) according to the manufacturer's protocols. The sequence of the small-subunit ribosomal RNA was obtained upon the sequencing of 96 randomly picked clones.

Phylogenetic Analysis

The sequence was added to the previously published alignment [11] with ClustalW software (v 1.83.1) [56]. The alignment was then manually edited in the GeneDoc program [57]. The phylogeny was reconstructed with MrBayes v. 3.1 [58] under the GTR model of evolution [59] and with the assumptions of two different gamma-distributions of rate variation and proportions of invariable sites for the variable V7 region [60] and the rest of the alignment. We ran the MCMCMC chain for 2.5 million steps and collected 25,000 trees, of which we discarded ("burned") the first 24,000 to give statistical support to the nodes. We ran the analysis three times to ensure convergence.

Supplemental Data

Supplemental Data include two movies and are available with this article online at [http://www.current-biology.com/supplemental/S0960-9822\(08\)01397-3](http://www.current-biology.com/supplemental/S0960-9822(08)01397-3).

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