Giant Deep-Sea Protist Produces Bilaterian-like Traces

Mikhail V. Matz,1,* Tamara M. Frank,2 N. Justin Marshall,3 Edith A. Widder,4 and Sönke Johnsen5

1Section of Integrative Biology
University of Texas at Austin
Austin, TX 78712
USA

2School of Biomedical Sciences
The University of Queensland
Brisbane, Queensland 4072
Australia

3Ocean Research and Conservation Association
Fort Pierce, FL 34949
USA

4Biology Department
Duke University
Durham, NC 27708
USA

5Biology Department
University of Texas at Austin
Austin, TX 78712
USA

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
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 (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...
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 Doushantu 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 Doushantu [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 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 foraminifers 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 foraminifers 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 or-
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 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 from 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
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.

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achieved in the cockroach escape response. Variability for a set of windsensory inputs seems not to be achieved by producing a widely spread unimodal distribution of turns, or a truly random distribution of turns. Rather, there seem to be ‘preferred’ angles of escape with respect to an incoming stimulus signaling predatory strike. It is as if the strategy of Proteus was to elude pursuers by shifting unpredictably through a defined repertoire of shapes, rather than assuming an infinite variety of shapes at random.

The work of Domenici et al. [4] raises some questions that may stimulate additional research. Is this mechanism for generating protean behavior a general strategy used in other escape systems? It is unclear, for example, if and how it would be incorporated into a system like the teleost tail-flip escape, where there is often a stereotyped C-start followed by a more variable swim [8]. What happens when escape networks are used for other behaviors, as when fish use tail flips during the sequence for capturing prey [9]? Most fundamental of all: how, at the neural circuit level, is a coordinate system for the spatial organization of preferred trajectories established? This might be influenced by such features as presence or absence of a motor planning phase [10]. Finally, why use a mechanism with constrained variability in the first place? Perhaps it leads to responses favoring the most appropriate vectors for effective escape. Only additional work will reveal the shape of the answers.

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Division of Biological Sciences, University of Montana, Missoula, MT 59812, USA.
E-mail: christopher.comer@umontana.edu
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Precambrian Biota: Protistan Origin of Trace Fossils?

Some Precambrian trace fossils have been presented as evidence for the early origin of bilaterians; the recent finding that large amoeboid protists leave macroscopic traces at the bottom of the deep ocean questions the metazoan nature of early trace fossils, stressing the importance of single-cell organisms in Precambrian biota.

Jan Pawlowski¹ and Andrew J. Gooday²

Most modern protists (single-celled eukaryotes) are microscopic and only few, like giant kelps and deep-sea xenophyophores, reach a much larger size. These giant protists are usually immobile and have never been considered as potential makers of macroscopic trace fossils, almost all of which are attributed to metazoans [1,2]. In a recent issue of Current Biology, however, Matz et al. [3] argue that some traces may have been produced by large, amoeboid protists resembling those they observed from a subsurface at 700 meters depth on the ocean floor off the Bahamas.

In their paper, Matz et al. [3] report large tracks on the seafloor associated with Gromia sphaerica, a deep-sea testate amoeboid protist distantly related to Foraminifera that grows up to several centimetres in size. Although they did not observe Gromia moving, the position of tracks and their shape clearly indicate that they were produced by gromiids. The authors suggest that the tracks were produced by the rolling movement of the spherical or grape-like gromiids. Whatever form of locomotion produced these tracks, their protistan origin seems beyond doubt.

By showing that not all modern deep-sea traces are produced by animals, Matz et al. [3] add a new level of uncertainty to the interpretation of trace fossils. These ‘ichnofossils’ are classified based on morphology into ichnogenera or ichnospecies usually without any reference to the identity of the trace maker [1]. Yet it is generally assumed that they are all produced by invertebrates. Based on this assumption, some very old (more than a billion years) ichnofossils have been interpreted as evidence for an early origin of metazoans [4,5]. Although it is generally accepted that these traces were made by living organisms, their metazoan origin is highly questionable [6,7]. For example, it has been proposed that they represent disrupted microbial mats [6]. The study of Matz et al. [3] raises the new possibility that protists might have played a part in the formation of these and other early fossil traces.

Several lines of evidence suggest that protists formed a well diversified assemblage long before the appearance of the first metazoans. The Proterozoic fossil record includes representatives of almost all supergroups of eukaryotes currently recognized [8]. Although the taxonomic identification of these fossils is sometimes controversial [9], there is little doubt about their eukaryotic origin. An additional argument for a deep eukaryote radiation predating the Cambrian explosion is provided by
molecular data. This radiation may not be as old as some authors have proposed [10], but there is a relatively good consensus of genetic data that the radiation of extant eukaryotes occurred between 950 and 1350 million years ago [9,11].

Modern gromiids like those found by Matz et al. [3] have an organic theca with limited fossilization potential (Figure 1A). Although the oral capsule (Figure 1B) seems more resistant to decay than the rest of the theca, and might fossilise in a recognisable form [12], there are no reports of these structures being preserved in the fossil record. Nevertheless, the molecular timescale suggests that the lineage leading to modern gromiids diverged more than 600 million years ago [9]. In molecular phylogenies, gromiids represent an old lineage, deeply branching within the supergroup Rhizaria [13]. It has been suggested that they form a sister group to Foraminifera [14]. Although the earliest fossil foraminifera are reported from the Cambrian, the molecular phylogenies suggest that a large radiation of non-fossilized single-chamber (monothalamous) foraminifera occurred in the Neoproterozoic [15].

In fact, monothalamous foraminifera are other potential makers of early fossil traces. Today, the muddy ocean floor is inhabited by a diverse and abundant assemblage of naked, organic- or agglutinated-walled monothalamids [16]. Some of them superficially resemble Gromia, as indicated by their name (Allogromiida). Most are small, but macroscopic species are also known. Their capacity to move is well documented; for example, the spoon-sized cells of *Toxosarcon alba* from Scottish fjords ‘rapidly’ climb aquarium walls [17]. While crawling across the mud, they could potentially produce tracks similar to those observed by Matz et al. [3].

Both gromiids and monothalamous foraminifera are relatively poorly known because their simple forms (often resembling fecal pellets; Figure 1A) rarely catch the attention of marine biologists. Moreover, their naked, organic or loosely agglutinated tests are poorly represented in the fossil record, and are of little interest to the micropaleontologists who normally study foraminifera. Yet several recent studies have shown that gromiids and monothalamids are a dominant component of the benthos in deep-sea and high-latitude settings, and sometimes reach macrofaunal sizes [16,18]. Genetic studies suggest that their simple morphologies conceal a plethora of diverse, sometimes very distantly related lineages. Some deep-sea species show worldwide distribution. This is well illustrated by the remarkable genetic similarity of the Bahaman specimens of *G. sphaerica* and those from the Arabian Sea where this species was first discovered [18]. These geographically widely separated populations raise important questions regarding biogeographic patterns and gene flow in the deep sea, in addition to stimulating ideas about the nature of the Precambrian biota.

As well as being abundant and diverse in modern oceans, gromiids and early foraminifera could have been an important component of the Neoproterozoic biota. Seilacher et al. [19] proposed that amoeboid protists constituted the major part of the Ediacaran biomass and compared the enigmatic Vendobionta to large multinucleate xenophyophores. Although revised molecular clock studies [20] suggest that bilaterally symmetrical animals were already present in the Neoproterozoic, their ecological impact was probably limited until the Cambrian explosion. Large amoeboid protists such as gromiids are common in modern deep-sea settings and some groups, including the xenophyophores, are confined to bathyal and abyssal depths. As illustrated by Matz et al. [3], the study of giant protists in these remote environments can yield new insights into the history of life before the animals take the stage.

**References**


![Figure 1. Gromiid protists.](image)

(A) Undescribed gromiid species photographed on the undisturbed surface of a sediment core from the Arabian Sea (23°22.10’N, 59°05.60’E, 1390 m water depth). Two morphotypes are visible, grape-shaped and sausage-shaped. The grape-like specimens are about 1 cm long. Photograph: Ana Aranda da Silva. (B) Undescribed gromiid, about 2 mm diameter, from the deep Weddell Sea (70°39’S, 14°43’W, 3100 m water depth). The oral capsule is at the top. (Photograph: Nina Rothe.)
Visual Perception: Tracking the Elusive Footprints of Awareness

Subjective visual experience leaves two distinct, overlapping ‘footprints’ within visual cortex: a small ‘footprint’ evident in multi-unit activity, and a much larger ‘footprint’ that dominates activity indexed by haemodynamic responses.

Randolph Blake¹ and Jochen Braun²

At a professional meeting in 1999 an overwhelmingly popular presentation was a poster manned by Yoram Bonneh from Israel’s Weizmann Institute. Throng of people crowded around his video monitor to experience what can only be characterized as visual magic: a small cluster of stationary yellow dots disappeared surrounded by a swarm of coherently moving blue dots [1]. You can experience a version of this compelling phenomenon by navigating to: http://www.michaelbach.de/o/erot_mot_mib/and by non-specific modulations (see below), the authors found that only

In their monkey study, Wilke et al. [3] recorded target-evoked multi-unit activity and local-field potentials in visual areas V1, V2, and V4. They found that fluctuations in the perceptual presence of the target was reflected only in the multi-unit activity of area V4; in areas V1 and V2, neither multi-unit activity nor high frequency local-field potentials reflected the perceptual state reported by the monkey. Interestingly, however, the lower frequency bands of the local-field potential presented a completely different picture: here, the power of the target response, which was reduced by the onset of the moving dots, was reduced in all three areas (V1, V2 and V4), more so when the target disappeared from perception than when it remained visible. The latency of these perception-related reductions in the low frequency local-field potential components increased from V1 to V2 to V4, suggesting a feed-forward signal.

A tantalizing parallel to these results emerges in the recent study by Donner et al. [5], who used functional magnetic resonance imaging (fMRI) to measure blood oxygen level dependent (BOLD) signals in multiple visual cortical areas in the human brain. Evaluating the BOLD activity that accompanies perceptual target disappearance and reappearance during motion induced blindness, the authors focused on the retinotopic representation of the target in ventral visual areas V1, V2, V3 and V4. After discounting contaminations to the target response by attention (which is likely drawn to a perceptual transient) and by non-specific modulations (see below), the authors found that only


¹Department of Zoology and Animal Biology, University of Geneva, Sciences III, 1211 Geneva 4, Switzerland. ²National Oceanography Centre, Southampton, Ocean Biogeochecmistry and Ecosystems, European Way, Southampton S014 2ZH, UK. E-mail: Jan.Pawlowski@unige.ch; ang@noc.soton.ac.uk

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