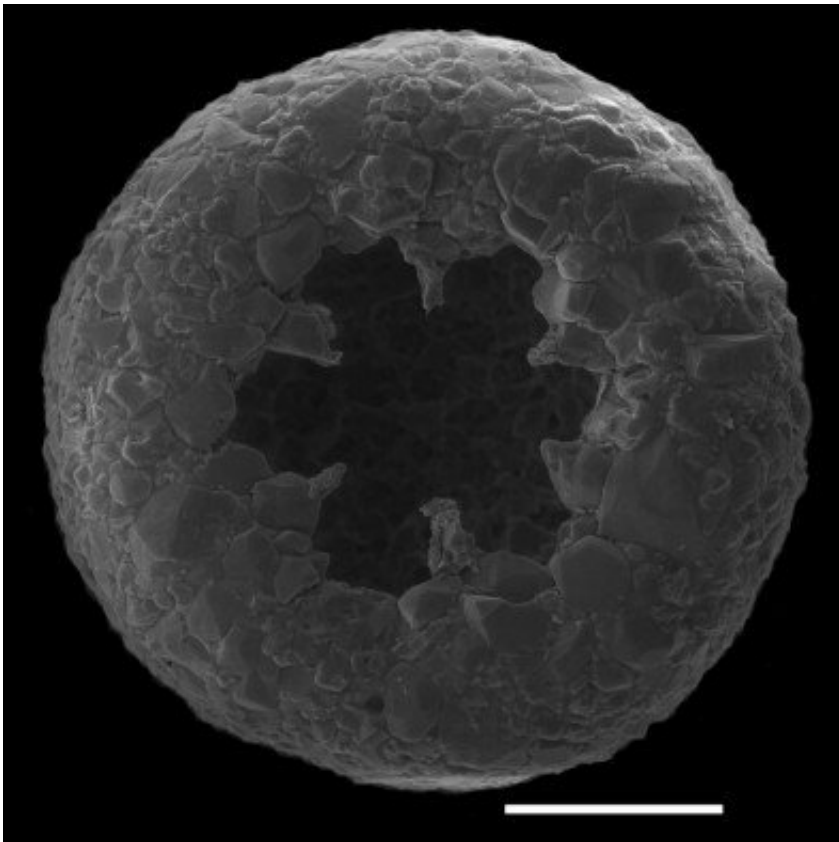


Amoebae diversified at least 750 million years ago, far earlier than expected

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Reconstitution of Amoebozoa's evolution shows significant Precambrian species diversity. This study changes the view of how life evolved in the very remote past and deepens the understanding of current climate change (a Thecamoebian protist of genus *Cyclopixys*) Credit: Daniel Lahr (IBUSP)

Brazilian researchers have reconstructed the evolutionary history of

amoebae and demonstrated that at the end of the Precambrian period, at least 750 million years ago, life on Earth was much more diverse than suggested by classic theory.

The study, which was supported by São Paulo Research Foundation—FAPESP, revealed eight new ancestral lineages of Thecamoebae, the largest group in Amoebozoa. Thecamoebians are known as testates because of their hard outer carapace or shell.

Interpretations of the evolution of Earth's atmosphere and [climate change](#) are also affected by the discovery that amoebae are more diverse than previously thought.

In this study, published in the journal *Current Biology*, researchers affiliated with the University of São Paulo's Bioscience Institute (IB-USP) in Brazil, in partnership with colleagues at the University of Mississippi in the United States, used innovative techniques to reconstruct the phylogenetic (evolutionary) tree of Thecamoeba, which belongs to the order Arcellinida.

The new phylogenetic tree was created using mathematical algorithms and the transcriptomes of 19 arcellinids found in nature today. The researchers also established the morphology and composition of the hypothetical ancestors of this group of amoebae and compared them with the [fossil record](#).

The results showed that at least 750 million years ago, ancestors of the thecamoebians were already evolving. This finding indicates that the late Precambrian was more diverse than previously thought.

"We reached our conclusions using a combination of two major scientific areas—paleontology and phylogenetic systematics, the field within biology that reconstructs [evolutionary history](#) and studies the

patterns of relationships among organisms. In this way, we were able to untangle one of the knots in evolutionary theory about life on the planet," said Daniel Lahr, a professor at IB-USP and lead author of the article.

Reclassification of Amoebozoa

The researchers completely dismantled the previous classification of thecamoebians. "We succeeded in developing a robust structure and for the first time, discovered eight deep lineages [from 750 million years ago] of arcellinids about which nothing was known," Lahr told.

The old thecamoebian classification was based on shell composition. "They were divided into agglutinate and organic. However, from our molecular reconstruction, we discovered that the classification is actually determined by shell shape rather than composition," Lahr said.

The old classification, he added, had been questioned for several years, but more evidence was needed to demolish it. Previous [genetic research](#) has shown that the classification was unsustainable, but not enough data were available to justify a new classification.

"The scientific community suspected that the arcellinid testate amoebae had emerged and evolved sufficiently to diversify some 750 million years ago. We've now succeeded in demonstrating this hypothesis," he said.

Past and future

According to Lahr, the study presents a different view of how microorganisms evolved on the planet. The late Precambrian was considered a period of low biotic diversity, with only a few species of

bacteria and some protists.

"It was in this period 800 million years ago that the oceans became oxygenated. For a long time, oxygenation was assumed to have led to diversification of the eukaryotes, unicellular and multicellular organisms in which the cell's nucleus is isolated by a membrane, culminating in the diversification of macroorganisms millions of years later in the Cambrian," Lahr said.

The study, he added, focuses on a detail of this question. "We show that diversification apparently already existed in the Precambrian and that it probably occurred at the same time as ocean oxygenation. What's more, geophysicists are discovering that this process was slow and may have lasted 100 million years or so," he said.

However, scientists do not know what pressure triggered this oxygenation. "Regardless of the cause, oxygenation eventually led to more niches, the eukaryotes diversified, and there was more competition for niches. One way to resolve the competition was for some lineages to become larger and hence multicellular," Lahr said.

The study has also contributed to a better understanding of today's climate change. "We began to understand in more depth how this microbial life affected the planet in several ways," Lahr said. "The climate changed in fundamental ways during the period, which saw the occurrence of the Sturtian glaciation some 717 million years ago. This was one of the largest glaciation events ever."

According to Lahr, these changes may have had biological origins. "By increasing the resolution of how life evolved in the very remote past, we can understand a little better how life affects the planet's climate and even its geology. That will help us understand the climate changes we're currently experiencing," he said.

In rock

In addition to the discovery of greater diversity in the Precambrian, the study also innovates by reconstructing the morphology of the ancestors of thecamoebians to establish that the vase-shaped microfossils (VSMs) found in various parts of the world already existed in the Precambrian and even in the major ice ages that occurred during this era.

VSMs are presumed to be fossils of testate amoebae. They are unicellular and eukaryotic and have an external skeleton. Significant diversity of VSMs has been documented for the Neoproterozoic Era, which spanned between 1 billion and 541 million years ago, and was the terminal era of the Precambrian.

"The study constitutes a very different vision of how microorganisms evolved on the planet. Although the fossils do not contain genetic information, it is possible to obtain morphological and compositional information and to verify whether they are organic or silica-based. So it's possible to compare their shape and chemical composition, which in this case are especially well preserved, with those of current thecamoebians reconstituted by big data," said Luana Morais, a postdoctoral researcher with a scholarship from FAPESP and coauthor of the article.

Innovative techniques

In addition to the lack of DNA-containing fossils, the researchers faced another obstacle in reconstructing the phylogenetic tree: thecamoebians cannot be cultured in the laboratory, and genetic sequencing by conventional means is therefore ruled out.

The solution to this problem was to use the single-cell transcriptome technique to analyze phylogenetics (instead of gene expression, its

normal application). "We sequenced whole transcriptomes of arcellinid [amoebae](#) using live samples," Lahr explained. "This yielded several thousand genes and some 100,000 amino acid sites, or 100,000 datapoints giving us the phylogenetic tree, which had never been seen before."

The researchers used transcriptome-based methodology to capture all messenger RNAs from each individual cell and convert them into a sequenceable complementary DNA library.

"Our research drew fundamentally on single-cell transcriptomics, in which our lab is one of the worldwide pioneers," Lahr said. "It's a revolutionary technique in this field because it enables us to find a single [unicellular] amoeba, isolate and clean it, and perform all the laboratory procedures to sequence the whole [transcriptome](#)."

In this study, the researchers selected 250 genes to construct the phylogenetic tree. "It's no good looking at only one cell when you're studying gene expression, because the resolution will be insufficient," Lahr said. "In an evolutionary study, however, this doesn't matter. You need to obtain the sequence, not the number of times a gene is expressed. So it's possible to use this technique, which was originally developed for tumor cells, and adapt it, with the advantage that an amoeba cell is much larger than a tumor cell."

Before the technique was developed, only organisms grown in the laboratory could be sequenced. "It extends the range of my research in this field by enabling me to obtain [genetic information](#) from organisms I've only found once. It's estimated that only 1 percent or less of all biodiversity is cultivable," Lahr said.

More information: "Phylogenomics and morphological reconstruction of Arcellinida testate amoebae highlight diversity of microbial

eukaryotes in the Neoproterozoic", *Current Biology* (2019).
[dx.doi.org/10.1016/j.cub.2019.01.078](https://doi.org/10.1016/j.cub.2019.01.078)

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