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Method and Application of Technology in the Study of Neoproterozoic Ediacaran Microfossils: Taking the Study of the Weng'an Biota as an Example

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Abstract

In the Neoproterozoic Ediacaran Doushantuo Formation in the Yangzi area of South China, a large number of multicellular organisms, algae and suspected fossils are preserved. With the study of the origin and evolution of life in the early Precambrian, These fossils are of great significance to the division and comparison of the Ediacaran biostratigraphy. This article takes the Weng'an Biota as an example to summarize the methods and applications in the fossil study of the Weng'an Biota. For dolomitic phosphate rock, it can be dissolved by soaking in 7% concentration of acetic acid to separate the fossil from carbonate rock and then observe the three-dimensional structure of the fossil. However, it is more difficult to obtain fossils from siliceous phosphate rock and can only be obtained by Section to observe the planar structure of the fossil. However, with the development of science and technology, many new technologies and new methods have emerged in the research of fossils.

Keywords

Weng'an Biota; Microfossils; Research Methods; Doushantuo Formation; Ediacaran.

1. Introduction

The Ediacaran period represents a key turning point in animal evolution, climate change, and ocean redox history. The large thorny suspected source is an important fossil component of the Doushantuo Formation. It is widely distributed around the world and has been reported on the Eastern European platform, Norway, Siberian platform, Yangtze plate, India and Australia. The large thorny source class began to appear in the lower part of the Doushantuo Formation shortly after the end of the "Snowball Earth" event, and the number and type of the upper Doushantuo Formation decreased significantly. A large suspected source species was found in the upper part, extending its distribution range to the entire Ediacaran.

The geological survey of the Weng'an area began in the 1860s, and until the 1980s, Zhu Shixing and Chen Meng'e, etc. respectively reported on the multicellular algae and large thorny source species in the phosphorites in the Weng'an area. Since then, Zhang Yun, Yuan Xunlai, Xue Yaosong and others have all studied the fossils in the area. In 1998, Xiao Shuhai and others reinterpreted the spherical fossils found in Weng'an area as animal embryos, which aroused more controversy afterwards. The Weng'an Biota has become the focus of early life research in the world, attracting a large number of scholars at home and abroad, and more opinions have emerged in the debate on the biological properties of spherical fossils. In 2014, Chen et al. discovered spherical fossils of cell differentiation from rock slices, and believed that these fossils may belong to the dry group of animals.

Although the fossils in the Weng'an Biota are exquisitely preserved and numerous in number, the controversy is huge because the preserved fossil structure is very simple and lacks the characteristics

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that can clearly distinguish the species of organisms. Especially after the birth of the "animal embryo" hypothesis, scholars around the world have never stopped questioning it, because fossil materials with cellular differentiation and complex morphological structures have been missing, which has caused scholars to apply various new technologies and methods to increase their concerns about urns. On the exploration of new materials for fossils in the Anan biota, this article will summarize the methods and technical means for the study of fossils in the Wengan biota.

2. Traditional methods

The fossil reserves in the Weng'an Biota are very large and well-preserved, but the individuals are relatively small, generally 200μm-500μm in diameter. The fossils of the Weng'an Biota are basically preserved in two types of lithology, one is gray-white dolomitic phosphorite, and the other is black siliceous phosphorite. The types of fossils preserved in these two rocks are not significantly different, except that in the siliceous phosphorite, because the fossils are silicified, more organic matter structures are preserved.

For dolomitic phosphate rock, acetic acid etching is usually carried out. The sample is processed into blocks of moderate size in a beaker, and 7% concentration of acetic acid is added for acid etching. The acetic acid solution is replaced every 1-2 days. During the second replacement, pour the liquid in the beaker into the sieve and filter and then discard it. A 1000-mesh sieve is selected to ensure that the fossils that are etched by each acid will not be poured into the waste liquid, and the fossils that are retained on the sieve are not poured into the waste liquid. After drying, we can obtain a large number of three-dimensional Weng'an biota fossils. Although these fossils are three-dimensionally produced, the morphology and structure are too simple. Some small organic matter structures such as spines on the surface of some large fossils with suspected sources may be destroyed during burial or acid etching. These embryonic fossils undergo hydrodynamic conditions and then undergo sedimentation after phosphate. In this process, some structural features will be lost. The processing in our laboratory will also cause certain damage to the fossils.

The obtained fossils can be used with scanning electron microscopy, allowing us to see the fine structure of the fossil, especially the surface decoration or internal cell morphology of the "animal embryo" fossil. In an article published by Xiao et al. in 2014, the suspected sources such as Megasphaera and Tianzhushania can be seen. Fossils are divided into genus and species based on the difference of fossil surface decoration and the number of internal cleavage cells. But there are also some disadvantages. Firstly, some structures on the surface of the fossil may be destroyed, and secondly, the cleavage cells inside the fossil may also be destroyed.

In order to obtain better fossil materials, siliceous phosphorite can also be selected for rock slicing. Grinding black siliceous phosphorite into 30-50μm thick rock flakes for observation under a microscope, it is also possible to observe a large number of exquisitely preserved fossil materials. Fossils in siliceous phosphorite are less affected by later redeposition. Although only two-dimensional planar fossils can be obtained, they can preserve more exquisite microstructures, especially the internal structure of fossil materials and the shape of thorns.

3. New technology

3.1 X-ray imaging techniques

With the continuous deepening of research by scholars around the world, the information obtained by traditional technical methods is far from sufficient. Scholars have begun to try new technologies and new methods, including X-ray three-dimensional non-destructive imaging, laser confocal technology and other methods. Bold innovation in traditional methods.

In recent years, the application of X-ray three-dimensional non-destructive imaging technology in the field of paleontology has become more and more extensive. Fossil X-ray imaging technology has been applied to paleontological research since 1896, but due to the lack of science and technology, the resolution of the obtained image is not high, and in order to obtain the three-dimensional structure

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image of the fossil, the fossil needs to be cut into thin slices or ground into pieces. Powder, which destroys the integrity of the fossil and loses the possibility of re-researching the fossil by other methods, so it cannot meet the needs of scientific research. According to the different imaging modes that can be provided, CT technology can be divided into phase contrast CT imaging and absorption contrast CT imaging. Conventional medical and industrial CT equipment can provide absorption contrast CT imaging. This technique uses the heterogeneity of the internal material composition of the research object to cause the differential absorption (attenuation) of X-rays as the basis to invert the internal structure of the object for imaging. The CT imaging technology based on X-rays with high brilliance (high light flux) generated by synchrotron radiation devices can not only collect information on the differential absorption of X-rays by different parts of the research object, but also analyze the phase of X-rays after penetrating the research object. Based on the changed information, it is possible to obtain the three-dimensional non-destructive imaging of the fossil based on the inversion of the three-dimensional structure of the research object. In 2014, Yin Zongjun compared the application of the two technologies in the fossil study of the Weng'an Biota. The synchrotron radiation X-ray phase-contrast microscopy imaging technology whether in morphology, anatomy or in fossil burial It has excellent performance in scientific research. This technology can reconstruct the three-dimensional structure without destroying the fossil material. As early as 2011, researcher Yin Zongjun applied synchrotron radiation phase-contrast microscopy imaging technology to the "invaginated animal gastrulation fossils" of the Weng'an Biota, which is of great significance in the study of fossil morphology.

Yin have reconstructed the three-dimensional structure of a four-cell animal embryo fossil. Not only can the three-dimensional appearance structure of the fossil be observed from any angle, but also the spatial geometric relationship between the blastomeres can be intuitively understood, and the Observe any interesting research area inside the fossil at sub-micron resolution. The article analyzes and compares the invaginated structure of gastrulation through this technology. Thanks to the three-dimensional structure reconstruction, the image data obtained is compared with the invaginated animal gastrulation. At the same time, synchrotron radiation phase contrast micro-CT technology can provide fossils. Internal virtual section, you can observe whether there is any cavity structure similar to the blastocyst cavity and the gastrointestinal cavity inside the fossil. Through comparison, it is found that the fossils with obvious invagination structure found in the Weng'an Biota are not convincing to explain as invagination animal gastrulation. This is also a typical application of three-dimensional non-destructive stereo imaging in paleontology. It can be obtained that the virtualization of fossil materials is a cross-section, high resolution, and does not need to destroy fossils, and can be used for other research. Especially for the research of micro-fossils, the small volume of fossil materials, the internal structure of the sedimentation, the preservation of more ambiguous, so that many early fossils can not get a better biological explanation, the application of this technology for the systematic paleontology of the Weng'an biota The evolution of early life is of great significance.

3.2 Atomic Force Microscope, AFM

Generally speaking, there are two ways to observe fossil materials: one is to observe directly with eyes, such as ordinary optical microscopes, TEM, SEM and other instruments, which essentially belong to this category, and the other is "blind people touching the elephant". , Using the "tactile" corresponding to the surface of the sample to reproduce the shape of the food, the latter is the basic working principle of the scanning probe microscope (scanning probe microscope, SPM). SPM is the collective name of scanning tunneling microscope (STM) and atomic force microscope (AFM). The two most critical components of AFM are the probe and the scanner. By approaching the probe at a certain distance from the sample surface, a signal related to the distance of the probe surface is generated on the sample surface, and the scanner is moved in the X and Y directions. , So as to obtain the signal of the entire surface, and then according to the publicity of the signal and distance, the undulation degree of the sample surface is removed, and the topography of the sample surface is obtained.

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The fossils in the Weng'an biota have preserved micron-scale cellular structures and even subcellular structures. There are very few reports of submicron cell structure. For traditional optical microscopes, the resolution is on the order of microns. If we want to obtain a more detailed structure at the sub-micron level, we will choose a scanning electron microscope to obtain the desired result. However, scanning electron microscopy requires the sample to be conductive. Under normal circumstances, if we want to observe the results, non-conductive samples need to be coated with a certain conductive film. Bradley et al. (1997) found that after coating, from the SEM, some small structures in the sample are lost, and AFM does not require conductive objects. It can scan samples at the sub-micron scale and has been widely used in biological sciences and materials science.

In 2007, Chi et al. used atomic force microscopy to find some sub-micron-scale ultrastructures on the surface of embryo-like fossils, and found four types of structures on the surface of selected fossil embryos. Scanning electron microscope and atomic force microscope were used to observe 20 fossil embryos. On average, about 78% of embryos can see ultrafine structures, which indicates that ultrafine structures are present in most fossil embryos. Among the four structures discovered are diamond-like structures, which are believed to be mineral crystals that grow outward during the formation of fossils, or are the product of secondary mineralization. The second structure is tightly coupled large subunits with sizes ranging from 250 nanometers to 645 nanometers. It was also found that there is a super layer on the surface with a thickness of about 10 nanometers. These large subunits may be single organisms, because the smallest limit diameter of an organism cannot be less than 200 nanometers; otherwise, the organism will not have enough space to accommodate cells, organelles, and genetic material. There are also two structures that are small particles without any corners and small subunits arranged closely parallel to each other. There is no convincing evidence to explain these two structures. They may be some parts of the fossil surface, submicron membranes. ingredient. The application of this technology will provide new evidence for the burial science of Doushantuo Formation fossils and the evolution of early life. It also provides a new method for observing the ultrastructure of the fossils of the Weng'an Biota on a sub-microscopic scale.

3.3 Confocal Laser Scanning Microscope

Fossils of algae in the Weng'an Biota can emit fluorescence, and laser scanning confocal microscopy (CLSM) was conceptualized in 1953 and later used for biological research, chemical analysis, and material testing. The laser is used as the scanning light source to quickly scan and image point-by-point, line-by-line, and side-by-side. The scanned laser and fluorescent collection share an objective lens. The focal point of the objective lens is the focal point of the scanning laser and is also the object point for instantaneous imaging. The system is focused once, and the scan is limited to one plane of the sample. When the focusing depth is different, images of different depth levels of the sample can be obtained. The image information is stored in the computer, and the three-dimensional structure of the cell sample can be displayed through computer analysis and simulation.

The two kinds of algae in Chi et al.'s 2006 article have been arguing and there is no exact definition. Through the comparison of the transmitted light image and the fluorescence image, it is understood that the fluorescence image almost expresses all the information captured by the transmitted light microscope, so the algae fossils It can be completely represented by a three-dimensional fluorescent image and has more structure. Therefore, the three-dimensional images of the algae fossils scanned by CLSM provide more information than the images taken by the optical microscope. Based on the comparison with the algae fossils reported by other researchers, we believe that the two algae fossils in this study are red algae with similar morphology.

The research on early algae mostly stays in the biological description, but the traditional optical microscope and scanning electron microscope can not get more structure and details, and the laser scanning confocal microscope can provide us with more evidence for the study of algae fossils.

4. Experimental simulation- Buried Experiment

For the spherical fossils of the Weng'an Biota, their size and shape are important criteria for judging the properties of the fossils. Researchers usually make interpretations of phosphate burial experiments

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based on the comparison of some characteristics of spherical fossils (including size, division mode, overall division mode, etc.) with modern organisms. Xiao et al. identified some phosphated spherical fossils as metazoan embryos. They conducted extremely detailed statistical studies on the characteristics of the fossils in this form (such as the lack of late development stage, early stage preservation), and provided a series of explanations for the fossil phenomenon. One of the important judging characteristics is that the diameters of the spheres at different splitting stages are basically the same. Xue Yaosong and others raised objections to the above-mentioned view of embryo fossils. They believed that the diameter of these spherical fossils changed greatly, beyond the range of basically the same diameter. Some researchers have interpreted the Doushantuo stage phosphated spherical fossils as bacteria. Again, these conclusions are based on the comparison of the size of biological tissues and fossil forms. The main basis for this conclusion is: in terms of morphology, volume, cell lysis speed, etc., Thiomargarita and phosphated spherical fossils have certain similarities. The size of the fossils is very similar to the size of the individual bacteria, and most researchers agree The view that the diameter of the fossils of the same species does not change much, and the diameters of the spheres in different division stages are basically the same. The huge difference in the size of the spheres may reflect the respective developmental stages of different types of embryos. In the rough classification of spherical fossils, researchers have also widely adopted shape and size as the criteria for determining attributes.

Zhang et al. simulated the phosphating of spherical fossils in the laboratory in 2014. The experiment used the eggs of Megalobrama amblycephala. The treated eggs were put into a jar and poured into the calcium phosphate mixture. At the same time, different experimental conditions were set up: (1) Oxygen-enriched conditions, Xiang Guang Fill the mouth bottle with oxygen until the solution is saturated with oxygen; (2) add microorganisms; (3) change its pH to 6 by adding HCL solution; (4) change its pH to 8 by adding NaOH solution, and place it in a 15°C water bath. under. After collecting the sample, rinsing with phosphate buffer, soak the sample in 2.5% glutaraldehyde solution for 3 hours, then rinse with 0.1 mol/L-1 phosphate buffer 3 times for 45 minutes, and finally fix with 1% osmium acid 2 Hours, then gradient dehydration, and finally drying.

Finally, the samples were collected, and it was found that the most individuals were preserved under acidic conditions, followed by oxygen-rich conditions, and then microbial conditions. Under alkaline conditions, the samples were almost completely decomposed without being preserved. However, under oxygen-enriched conditions, the shells of fish eggs are mostly preserved and show a rough outline, but the nucleus disappears. Under acidic conditions, the core is preserved, but the outer shell disappears. Percentage statistics of the preservation state show that this morphological difference is not accidental, but caused by environmental differences. The experimental results confirm the morphology of bream bream eggs under different environmental conditions The difference is significant. Some researchers believe that the preservation of fossils of the same species does not change much, and it is used as a criterion for judging individuals of the same species. Obviously, the premise of this change rule is that individuals at various developmental stages do not have the strong preservation that appears in this experiment Morphological differentiation. But our data shows that even if the individuals are in the same early state, the morphology will be greatly differentiated under different conditions. If the early preservation experiments are similar to those under acidic conditions, the diameters of individuals of the same species are likely to be inconsistent Therefore, the fossils in the Weng'an Biota may also have such a situation. There are certain differences in the environment of life after death, which may also cause the different forms of fossils we find now.

The simulation of modern experiments also provides us with new ideas and new understandings for the study of early life, and can also provide new evidence for the study of fossils in the Weng'an Biota.

5. Conclusion

The fossil research of the Weng'an Biota has been going on for decades, and the discovered fossil materials are also very rich. In the past, everyone relied on traditional technical means, and the new

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materials that could be discovered became less and less. With the development of science and technology , We can discover more details from past fossil materials and provide new evidence for early life evolution, especially X-ray non-destructive imaging and laser confocal microscopy, allowing scholars to discover tiny structures and gain more evidence At the same time, the emergence of simulation experiments can better compare fossils with modern organisms, allowing us to more intuitively understand the death and burial of early life. The research on the fossils of the Weng'an biota is far from over. With the increasing replacement of science and technology, we can also discover more and better new fossil materials, and more new technologies and new methods will emerge.

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