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# The First Observation on Plant Cell Fossils in China

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**Abstract: For a long time, paleontologists have been focusing on hard parts of organisms during different geological periods while soft parts are rarely reported. Well-preserved plant cells, if found in fossils, are treated only as a rarity. Recent progress in research on fossil cytoplasm indicates that plant cytoplasm not only has excellent ultrastructures preserved but also may be a quite commonly seen fossil in strata. However, up to now there is no report of plant cell fossils in China yet. Here plant cell fossils are reported from Huolinhe Coal Mine (the early Cretaceous), Inner Mongolia, China. The presence of plant cytoplasm fossils in two cones on the same specimen not only provides further support for the recently proposed hypothesis on plant cytoplasm fossilization but also marks the first record of plant cytoplasm fossils in China, which suggests a great research potential in this new area.**

**Key words: fossil, cells, plant, China, Cretaceous**

## 1 Introduction

People have learned much about the evolution of organisms through the geological history during the past hundreds of years. Our understanding on the organisms of the geological history is mainly based on the research on fossils of their hard parts. The fossils of soft parts of organisms, because of their rarity, more or less raise curiosity of the public when reported. The recent examples include chloroplasts in *Metasequoia* from the Eocene in Canada (Schönhut et al., 2004), plant cytoplasm fossils and their ultrastructures from the Cretaceous in USA (Wang, 2004, 2006, 2007), soft tissue in dinosaur bone from the Cretaceous in USA (Schweitzer et al., 2005), and DNA preserved in fossil nuclei from Russia (Ozerov et al., 2006). The reason behind this curiosity is that people believe that cells and their contents are easy to decay and cannot be preserved in fossils. However, the research on the plant fossils from the Cretaceous in Kansas, USA (Wang, 2004, 2006, 2007) indicates that plant cytoplasm fossils not only can be preserved almost perfectly but also should be a common type of fossils. This superficially absurd statement is still in need of more evidence to confirm. Even though China has yielded many magnificent fossil plants (Sun et al., 2002; Ji et al., 2004), there is no report on plant cytoplasm fossils in China yet. In this paper, based on the study on two female gymnospermous cones collected from the Huolinhe Coal Mine, Inner Mongolia, China, the authors report for the first time the presence of plant cytoplasm fossil in China. This result not only marks the first report of plant cytoplasm fossils and their ultrastructures in China, provides badly needed evidence for the recently proposed hypothesis on plant cytoplasm fossilization, but also implies a great potential for further research in this new frontier of science.

## 2 Materials and Methods

The studied materials were collected from the strata exposed in the stripping mine Huolinhe Coal Mine (the lower Cretaceous) in Inner Mongolia. The specimen is about 65 mm long, 40 mm wide and 15 mm thick. There are two female cones, one of Pinaceae and the other of Bennettitales, on the same specimen. These two cones are embedded in gray siltstone. The specimen was photographed using the Nikon D100 and the pictures are saved in tiff format. Small coaly pieces of the specimen are removed from the specimen for analysis. The samples are processed with 20% HCl for 2 hours, 40% HF for 48 hours and 20% HCl for 0.5 hour to remove inorganic contaminations. After this processing, the samples contain only organic material derived from the original plant material. The samples are then divided into two portions: one for scanning electron microscope (SEM) observation

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and the other for embedding and sectioning. The SEM sample is coated with platinum, analyzed using energy dispersive X-ray microanalysis (EDXMA) and observed under the LEO 1530VP SEM in the Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences. The SEM electron micrographs are saved in tiff format. The sample embedding and sectioning are done in Nanjing Normal University. The samples are first put in acetate for 3 hours, then in Epon resin solution in acetate of concentration 50% and 67% each for one hour, 100% resin solution for 15 hours. Then the samples are cured in pure Epon resin in an oven set at 30°C, 45°C and 60°C, each for 24 hours. Then the cured samples are sectioned with a Reichert-Jung Ultracut E ultramicrotome or Leica Ultracut R ultramicrotome at an interval of 2 μm for light microscope or 70 nm for transmission electron microscope (TEM). The thin sections are photographed with an Olympus polarizing microscope (model BX51-75J21PO standard set). The ultrathin sections are stained with lead citrate for 5 seconds, and observed under Jeol JEM-1230 electron microscope at the Nanjing Institute of Geology and Palaeontology. All micrographs are saved in tiff format and pieced together for publication in Photoshop 7.0.

The specimens of pinaceous cone (PB20773) and bennettialean cone (PB20774) are deposited in the Nanjing Institute of Geology and Palaeontology, Nanjing, China.

## 3 Results

The pinaceous female cone is about 1.7 cm in diameter (Plate I-1). Cone axis is about 4.4 mm in diameter (Plate I-1). Scales and bracts are spirally arranged around the cone axis (Plate I-1). Scale is about 13.6 mm long, 8.2 mm wide, with a notch at its tip (Plate I-1, white arrow). Bract is triangular-shaped, about 8 mm long and 2 mm wide (Plate I-1, black arrow). Observation under microscope indicates that the scale tissue is mainly composed of epidermis and parenchymatous cells (Plate I-2−7). The parenchyma takes up most portion of the scale, its cells about 8−15 × 16−22 μm in cross section, with cytoplasm filling up the lumina (Plate I-3−7). The fossil tissue is composed of brown blocks or stripes under optical microscope (Plate I-7). No distinct difference between cells and their cell walls is seen under SEM (Plate I-3, 4). The profiles of the cell outlines can be traced by following the intercellular space and texture on material surface (Plate I-4−5). The distinction between cells and cell walls is enhanced by lead citrate staining under TEM (Plate I-6, 8). Cytoplasm is heterogeneous, with a central elliptical region different from the surrounding regions in structure and morphology (Plate I-5). Membrane is distinct from other components under TEM (Plate I-8, arrow). Membrane composed of sac-like structures in the cell is very similar to endoplasmic reticulum (ER) (Plate I-8, white arrow). Subcellular structure with two layers of enclosing membranes and the inner membrane with inward foldings is very similar to mitochondrion (Plate I-11). Other subcellular structures have complicated intermembrane and are enclosed by membranes (Plate I-9−10). EDXMA indicates that the fossil material is of 61.32%−62.82% carbon (C) and 29.64%−30% oxygen (O) besides platinum (Pt) coating (6.37%−7.46%) (hydrogen is undetectable in EDXMA).

The bennettialean female cone is about 1.16 cm in diameter, with more than 6 whorls of seeds, each half whorl including up to 11 seeds (Plate II-1). Seed is more than 2.3 mm long, about 0.85 mm wide (Plate II-1). Seeds with smooth surface are of charcoal material, composed of parenchyma and epidermis (Plate II-2−5). Under optical microscope, fossil material appears as brown blocks with cracks (probably due to drying during fossilization) in between (Plate II-3−6). Parenchymatous cells are about 5−15 × 8−25 μm in cross section (Plate II-3, 5, 6), with cell walls in between (Plate II-7). Cytoplasmic membranes are distinct from other parts under TEM thanks to staining (Plate II-7−8). Some elliptical subcellular structures outlined by membranes are present in cytoplasm (Plate II-8). EDXMA indicates that the fossil material is of 56.75% −58.22% C and 24.09%−25.77% O besides Pt coating (15.8%−16.51%) (hydrogen is undetectable in EDXMA).

## 4 Discussions

Cleaning sample with HF-HCl is an effective routine frequently used by geologists and chemists to remove inorganic minerals (Poirier et al., 2000, 2002; Eusterhues et al., 2003). After this processing, inorganic minerals in fossil material can be completely removed and only material of organic source remains. In our case, the only organic source for the fossil material is plants themselves and their contents. This estimation is favored by the EXDMA results: the elemental composition mainly of C and O, comparable to those of extant charcoal (Poirier et al., 2000, 2002; Eusterhues et al., 2003), suggests that the fossil material is derived from the original plant material. If the plant cells and their contents had completely decayed before fossilization, what are seen in these fossil materials should be empty cellular lumina, just like what Hooke saw of plant tissue under the microscope for the first time (Fig. 1-1b, Ambrose and Easty, 1979). However, the electron micrographs of this fossil material indicate that the fossil material is composed of solid blocks (Plate I-2−6; Plate II-3−6) rather than empty lumina. TEM observation suggests that there are some subcellular structures similar to those in extant plant cells preserved in these fossil materials (Plate

I-8−11; Plate II-7−8). These cells and subcellular structures are heterogeneous, with surrounding and internal membranes. Although it is hard to make a positive identification of these subcellular structures at this time, the subcellular structure with two enclosing membranes and the inner one with inward foldings does imply mitochondrion (Plate I-11), and the sac-like membranes connecting the cytoplasmic membrane resemble ER very much (Plate I-8, white arrow). In short, plant cells and their structures indeed are preserved in these fossils, and their preservation is comparable to their extant counterparts to certain extent.

Plants are composed of cells. Cells have nuclei and cytoplasm, which is enclosed by cytoplasmic membranes. All these concepts frequently used in neobiology are rarely mentioned in palaeontology mainly because everyday experience tells that soft parts of organisms cannot be preserved for a long time in natural environment. This generalized experience somehow hinders our healthy scientific thinking. Actually, the decay of cells and their contents is a very complex organic reaction. Not as unconditionally as assumed, this reaction cannot occur without the involvement of enzymes. Enzymes, as special proteins, are very sensitive to environmental changes (such as temperature) and require certain temperature scope to function. Geological studies (Scott and Jones, 1991; Jones and Lim, 2000) indicate that wild fires are very common in the geological history. It is not hard to imagine that, besides destroying part of the vegetation, wild fires must have arrested the enzymatic reactions in plant cells and therefore stopped the decay processes of plant cells and their contents. Furthermore, high temperature of wild fires can convert perishable plant bodies to nutritionless, inert and stable charcoal through the Millard reaction (Poirier et al., 2000, 2002; Baldock and Smernik, 2002; Gonzalez-Perez et al., 2004) and produce permanent fossil record of plants in strata (Scott, 2001; Wang, 2004, 2006, 2007). Thus, at least theoretically, plant cells and their contents not only can be preserved in fossil record but also should be a quite common object for scientific research. Actually, there have been some reports of fossilized cells and their contents already (Darrah, 1938; Bradley, 1946, 1962, 1967, 1970; Mamay, 1957; Baxter, 1964; Eisenack, 1965; Schopf, 1968; Gould, 1971; Satterthwait and Schopf, 1972; Millay and Eggert, 1974; Oehler, 1977; Taylor and Millay, 1977; Niklas et al., 1978, 1985; Niklas, 1983; Schönhut et al., 2004; Wang, 2004, 2006, 2007; Schweitzer et al., 2005; Ozerov et al., 2006). But little is known about the fossilizing mechanisms behind these fossils, and the progress of research on fossilized cytoplasm is very slow. Wang (2004, 2007) reexamines and reanalyzes the fossilizing process of plant cells and cellular contents, relates it to some frequently seen natural phenomena (such as lightning and wild fires), and predicts the common occurrence of plant cytoplasm fossils in strata. In this way, the theoretical roadblock for fossil cytoplasm research is removed. The presence of cytoplasm fossils in two cones in the same piece of rock that is just a few centimeters long in this report strongly supports the common occurrence of plant cytoplasm fossils, favors the recently proposed cytoplasm fossilizing mechanism (Wang, 2004, 2007), and implies the great potential in this new frontier.

Besides Wang's (2004, 2007) hypothesis, works by Niklas et al. (1978), Schönhut et al. (2004) and Ozerov et al. (2006) suggest that some ergastic substances (such as tannin) may be released after death and self-fix the cell contents. Although this mechanism is not universal, it definitely increases the chance of plant cytoplasm fossilization. It is apparent that plant cytoplasm fossilization is a complex process and has many factors involved in, and that no clear picture can be developed before much more efforts are invested.

Human being has accumulated much experience on biological sample preparation in the past centuries. The quality of the sample preparation directly influences the corresponding research. For example, atomic force microscope (AFM) is a power observation tool with resolution up to atom scale. However, its resolving power is much compromised when it encounters soft and watery biological samples. Any technique that can improve the resolution of AFM on biological sample will benefit biological and medical researches very much (Hogan, 2006). One example of the good trials is Muys et al.'s (2006) Bioimprint, a technique that faithfully makes a hard replica of the fine and delicate structures on cell surface using polymers. This technique improves the AFM resolution by increasing the hardness of the target. Ultrastructural studies on fossil cytoplasm (Wang, 2004, 2006) indicate that not only fossil cytoplasm is hard enough for a good AFM observation but also its preservation fidelity is comparable to its extant counterpart to certain extent. If research on cytoplasm fossils can provide some hints on how to make a hard and lasting biological sample of high preservation fidelity, it will improve the quality of biological research, at least when AFM is applied. How to make a lasting biological sample with high fidelity preservation has been a challenge for biologists in the past centuries. Considering the ancient age of the fossils reported here and before (Wang, 2004, 2006, 2007), apparently there are much more to learn from nature. In short, study on fossil plant cytoplasm not only points to a new palaeontological research field with a great potential but also may contribute to improving the technology in processing biological samples for other scientific research.

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**Explanation of Plates**

**Plate I A female pinaceous cone with its internal structure. The lower Cretaceous, Huolinhe Coal Mine, Inner Mongolia. Deposited in the Nanjing Institute of Geology and Palaeontology. Specimen No.: PB20773.**

1. An oblique view of the cone. Note the axis of the cone (A), the notch at the apex of the scale (white arrow), and elongate triangular-shaped bract (black arrow). Bar = 1 cm.
2. A cross view of a scale tissue. The three rectangular regions from right to left are enlarged in Figs. 3, 5 and 4, respectively. SEM 9494. Bar = 50 μm.
3. Cells in the scale tissue. Enlarged from the right rectangle in Fig. 2. Note the coalified cells and their contents. The cuticle (C) is to the lower of the electrograph. SEM 9412. Bar = 10 μm.
4. Epidermal cells in the scale tissue. Enlarged from the left rectangle in Fig. 2. Note the outline of the cell (dotted line) and its contents. The cuticle (C) is to the lower of the electrograph. SEM 9403. Bar = 5 μm.
5. Cells in the scale tissue. Enlarged from the middle rectangle in Fig. 2. Note the outlines of the cells (dotted line), their contents and the central nucleus-like region. SEM 9414. Bar = 5 μm.
6. Parenchymatous cells in the scale tissue. Note the cell wall (dark portion, arrows) surrounding the cell (central gray portion). TEM MCone-12000X-31, section thickness 70 nm, bar = 1 μm.
7. A cross section of the scale. Note that all the materials (including the cell wall and cells) are all coalified, therefore the outlines of the cells are not always distinct. Optical light microscope section PB20773-S1, section thickness 2 μm, bar = 0.1 mm.
8. A detailed view of the rectangular region in Fig. 6. Note the spatial relationship among the cytoplasmic membranes of two adjacent cells (black arrows), the cell wall (CW) between them, ER-like membrane structures (white arrow). TEM MCone-100000X-33, section thickness 70 nm, bar = 200 nm.
9. A subcircular subcellular structure in the cell. Note the two enclosing membranes and the inner membrane (arrows). TEM MCone-150000X-88, section thickness 70 nm, bar = 100 nm.
10. A subcellular structure in the cell. Note the relationship between the outer and the inner (arrow) membranes. TEM MCone-120000X-67, section thickness 70 nm, bar = 100 nm.
11. A subcellular structure in the cell. Note that the two enclosing membranes and the foldings (arrows) connected with the inner one imply mitochondrion. TEM MCone-200000X-122, section thickness 70 nm, bar = 50 nm.

**Plate II A bennettialean female cone and its internal structure. The lower Cretaceous, Huolinhe Coal Mine, Inner Mongolia. Deposited in the Nanjing Institute of Geology and Palaeontology. Specimen No.: PB20774.**

1. A general view of the cone. Note the general morphology and whorled seeds. Bar = 0.5 cm.
2. A fragment of the cone tissue. Note the epidermis and exposed tissue within. SEM 9423, bar = 0.1 mm.
3. A cross section of the cone tissue. Note blocks of cells and cracks between them probably due to drying. Optical microscope PB20774-S1, section thickness 2 μm, bar = 0.1 mm.
4. A detailed view of the tissue, enlarged from the rectangular region in Fig. 2. Note that there is no distinct framework of cell wall but rather spongy materials. SEM 9429, bar = 50 μm.
5. A detailed view of the tissue. Note the blocks of cells. SEM 9421, bar = 50 μm.
6. A cross section of the cone tissue. Note the cellular blocks and cracks in between similar to those in Fig. 3. TEM MBerryU-1500X-1, section thickness 70 nm, bar = 10 μm.
7. A detailed view of a cell in the cone tissue. Note that there is light line (cytoplasmic membrane, white arrow) separating the cell and the cell wall (black arrow), and that the bright portion is the crack between tissue blocks. TEM Mberry-6000X-3, section thickness 70 nm, bar = 2 μm.
8. A detailed view of the rectangular region in Fig. 7. Note the sub-circular subcellular structure and the cytoplasmic membrane (arrow). TEM Mberry-100000X-4, section thickness 70 nm, bar = 100 nm.

Plate I

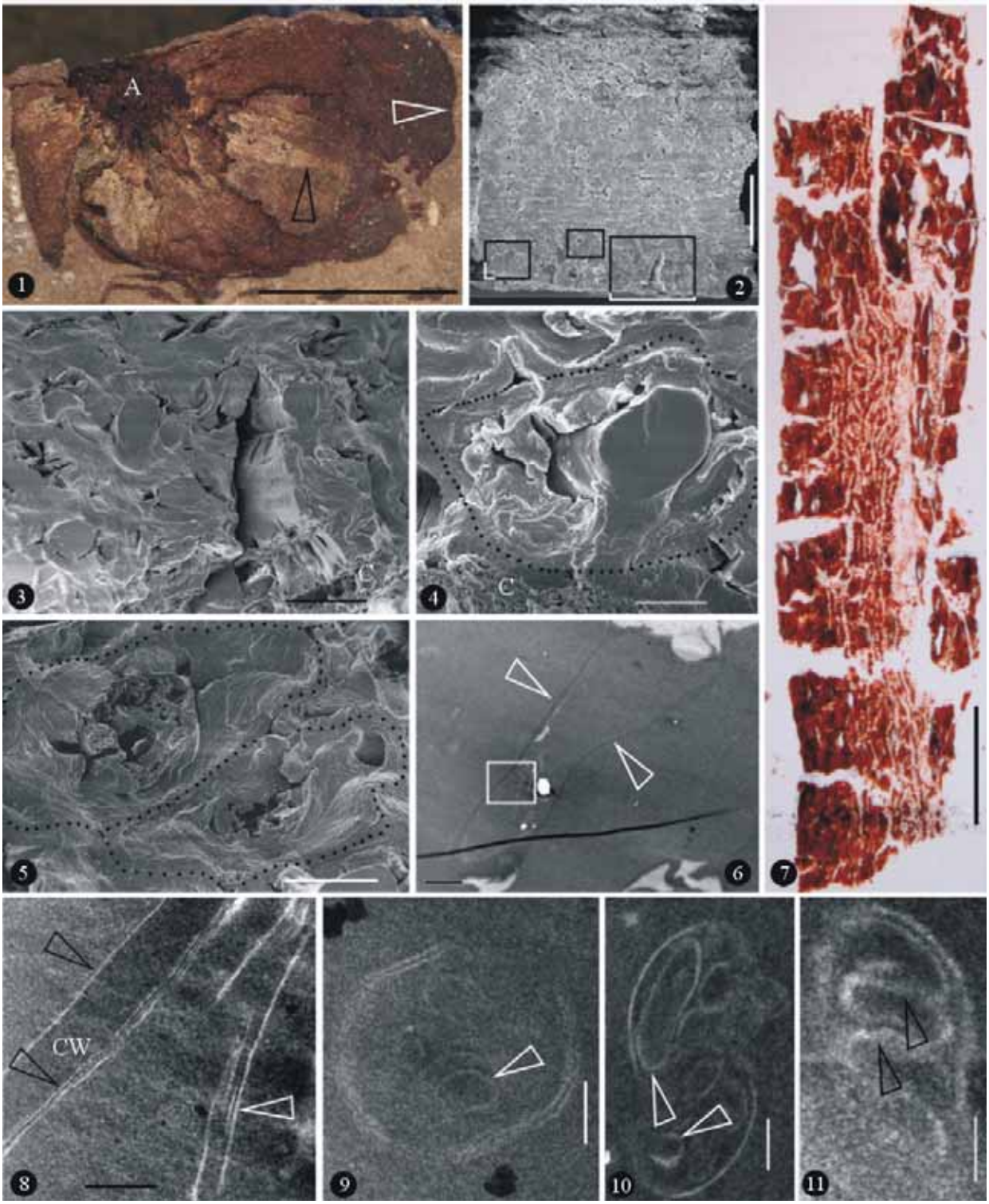

Plate II