Mycorrhizae in fossilized roots from the Early Cretaceous of Mongolia

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Introduction

Conifers form important symbiotic relationships with fungi. This association is hosted in the plant roots where mycorrhizae fungi provide water and nutrient capabilities. Two types of mycorrhizae include:

- Endomycorrhizae: where the hyphae penetrate the root cell walls
- Ectomycorrhizae: the hyphae grow around the cells of the root.

Little is known about mycorrhizae from the Early Cretaceous, and particularly in conifer- dominated and swamp-like environments. The new lignified fossil material from the Early Cretaceous of Mongolia (~100-120 million years old) preserves abundant roots (Figs 1 & 2). The flora is dominated by members of the Pinaceae family such us *Picea, Pityostrobus*[4], (spruce genus) and *Schizolepidopsis*[2] and other Cupressaceae[1,4] and extinct conifers[5].

Is there any evidence of mycorrhizae in fossilized roots from the Early Cretaceous of Mongolia? What kind of mycorrhizal association was the most dominant type in the swamp-like flora? Here, we report exquisitely preserved mycorrhizal fungi, ~100-120 million years old, found in the fossil roots of coniferous plants. This is the first time that fossil fungi are reported in lignified roots. Given the abundance of fossil roots in the Mongolian flora, this material provides an important and unique opportunity to discover fossil mycorrhizae.

Materials & Methods

Samples from Mongolia of lignified plant litter were disaggregated in H_2O and cleaned with HCl

Plant locality, (Leslie et al, 2013)

Roots were sorted and rehydrated in distilled water for one day and then cleared with hydrogen peroxide for ~24 hours

Root Staining Process

1. Specimen placed on silver mesh and submerged in KOH

2. Root was heated for ~2-3 minutes at 62°C, following a washing process with distilled water for 3 rounds

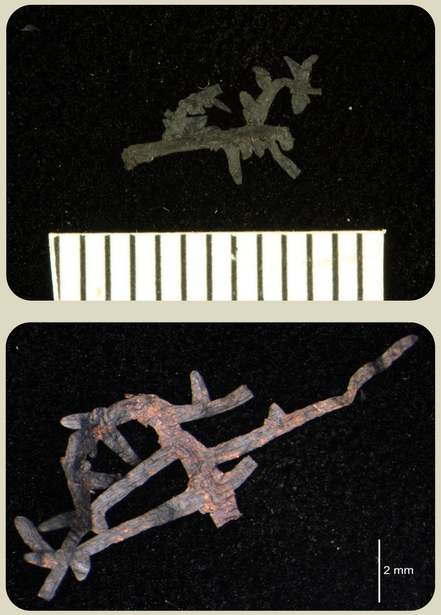
3. Roots were immersed in HCl for ~ 5 minutes, then treated with a blue trypan dye for 20 minutes to stain

4. Drops of glycerol were added on the root to wash remaining blue dye stain

Roots were mounted with glycerol on glass slides, analyzed and photographed using light and fluorescence microscopes

Results : This is the first time that fossil fungi are reported in lignified roots

Figure 1 and 2: Two types of general morphology of roots sorted found in lignified plant litter from Early Cretaceous in Mongolia



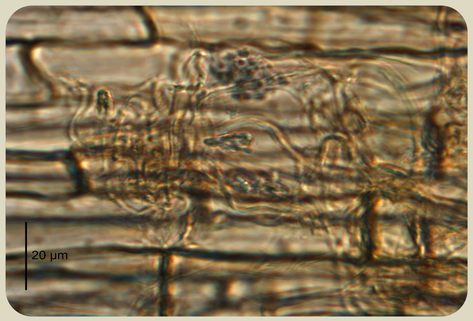


***Figure 3*:** Coils located in the cell wall of the root

***Figure 5*:** Spores within the cell wall

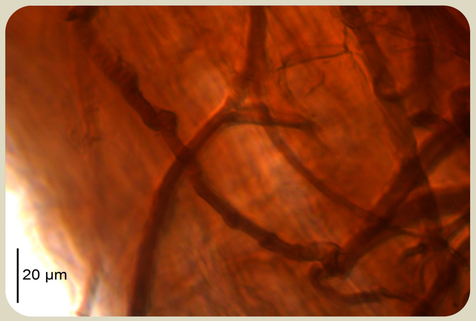


***Figure 7*:** Septate in hyphae penetrating cell wall

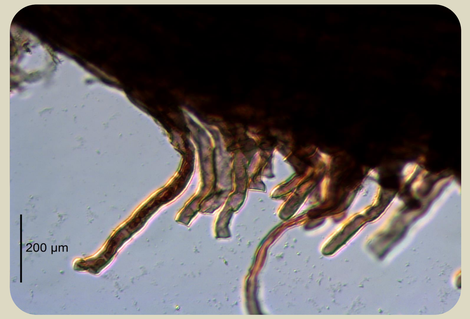


Figure 9: Pathogens present in the root hair

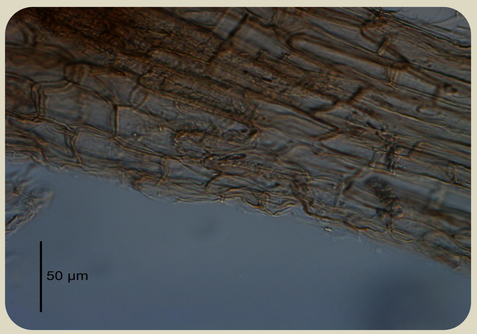


***Figure 4*:** Coils found in the cell wall of the root

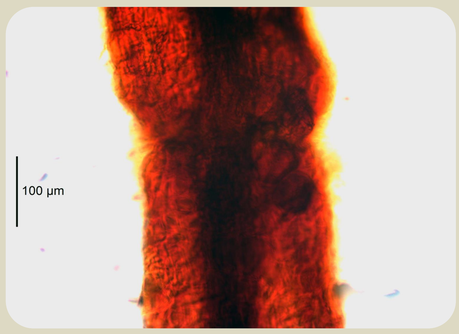


Figure 6: Vesicles within the cell wall of fossilized roots

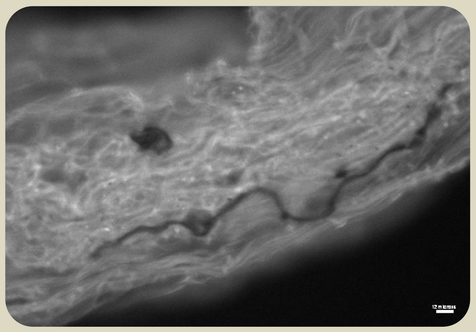


***Figure 8*:** Hyphae observed under inflorescence microscope

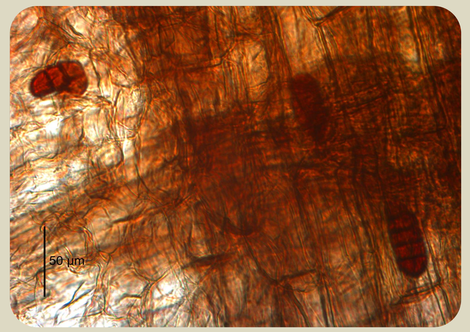


***Figure 10*:** *Alternaria* pathogen spotted within the cell wall

Discussion

The presence of abundant endomycorrhizae shows that a gymnosperm-dominated swamp forest from the Early Cretaceous of Mongolia formed similar symbiotic associations to what it is seen today. This novel and modified root staining methodology used here can be useful for other studies.

We have not found any evidence of ectomycorrhizae, this is unexpected given that spruce and pine members, present in the Mongolian flora form this kind of symbiotic relationship today. How we can explain the absence of ectomycorrhizae? Some potential explanations why ectomycorrhizae may be absent within the roots collected include:

- Ectomycorrhizae may have not evolved during the Early Cretaceous period
- Ectomycorrhizae did not preserve well in the root samples
- Ectomycorrhizae may not have been present in swamp environments in Mongolia. We have not sampled enough fossil material yet.

Future Directions

- More exhaustive sampling of fossil roots and epifluorescence to verify the absence or presence of ectomycorrhizae
- Clear understanding between the morphology of mycorrhizae in fossilized roots and living examples of mycorrhizae
- Knowledge of pathogens within the fossilized root samples

References

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