Inorganic Cretaceous Dinosaur Remains are Colonized by Specialized Communities of Fungi that Thrive within Bones and Support Fungivore Nematodes

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Inorganic Cretaceous Dinosaur Remains are Colonized by Specialized Communities of Fungi that Thrive within Bones and Support Fungivore Nematodes

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Fungi are known to be abundant in terrestrial sediments and are specialized to colonize various substrates including rock and buried fossils particularly skeletons and bones, and often in the presence of bacterial infestations [1-8]. Although an extremely diverse group (some estimates are upwards of 2 to 4 million species), only about 900 living species have been described and of those only about 90 are found routinely in human habitations [1-3]. Highly effective fungi and other microorganisms that colonize buried bones are also few in number and reports of bones (especially dinosaur bones) colonized by such fungi are scarce [4-9], see Armitage [9] which illustrates an expansive crustose fungal hyphae invading dinosaur bone known to be infested with nematodes, (Figure 5a). The diagnostic features of fungus are difficult to resolve microscopically in fossils thus it is considered a difficult line of research [2].

Terrestrial fungi are extremely hardy and can live in inhospitable environments, even within hydrothermal vents and acid mines [10]. Fossil fungi have been known from the middle 1800s, although historically they have been considered extremely delicate with poor preservation potential yet they show stunning preservation in amber [2]. They are known to produce and secrete metabolites that can break down rock and bone and once there can produce reproductive structures [4,5,7]. Fossil fungi have also been described from within buried dinosaur bones, whether mineralized within intrusive calcite [4], or invading subterranean partially buried bone. Most often they are within actively decomposing skeletons or isolated bone [5-8]. Even museum specimens of inorganic bone are susceptible to bioerosion from living fungal hyphae [8].

As mentioned large infestations of nematodes have been reported in freshly excavated *Nanotyrannus* bones from Jordan, Montana [9]. In 2024, the authors performed modified Baermann funnel experiments on soils and freshly excavated bone shards of *Triceratops* and *Edmontosaurus* in Glendive Montana. One living nematode was collected from each of three methods, the fruit trap method, the soil immersion method, and the bone shard immersion method. We experienced difficulty in fixing these three captured wild nematodes however the nematode extracted from the bone shard was properly fixed and sent to a nematologist for examination. This resulted in an identification of it as a fungivore, or fungus eating nematode (Figure 1), [11 personal communication].

We also made 40 µ ground thin sections from *Triceratops* and *Edmontosaurus* bone shards collected at the same site. We found examples of living fungal hyphae tunneling through compact bone (Figure 2) as well as isolated fungal structures within our decalcification solutions (Figure 3). The tunneling hyphae were observed to emanate from within the organic material adhering to the inorganic walls of bone vessel canals, which we interpret to be endogenous clotted blood, and spread into the bone surrounding vessel canals (not shown here). In many cases the bone tunneling hyphae seem to be aligned to intersect with osteocytes still embedded in the inorganic bone. These hyphae are rugouse and very robust. They exhibit a completely rough and red, crusty border with no visible septae and did not resemble *Aspergillus* or *Acaulium* as reported in underground bat skeletons (SEM Figure 4) [5]. Light brown, semi translucent fungal spores were also found in our demineralization solutions, resembling *Sporochisma* (Figure 3). No hyphae were found within vessel canals as reported elsewhere [4]. We also report here population density of nematodes found in *Triceratops* and *Edmontosaurus* thin sections. More work is required to characterize the varieties of living fungus and living nematodes co-inhabiting inorganic dinosaur bone in Jordan and Glendive, MT.

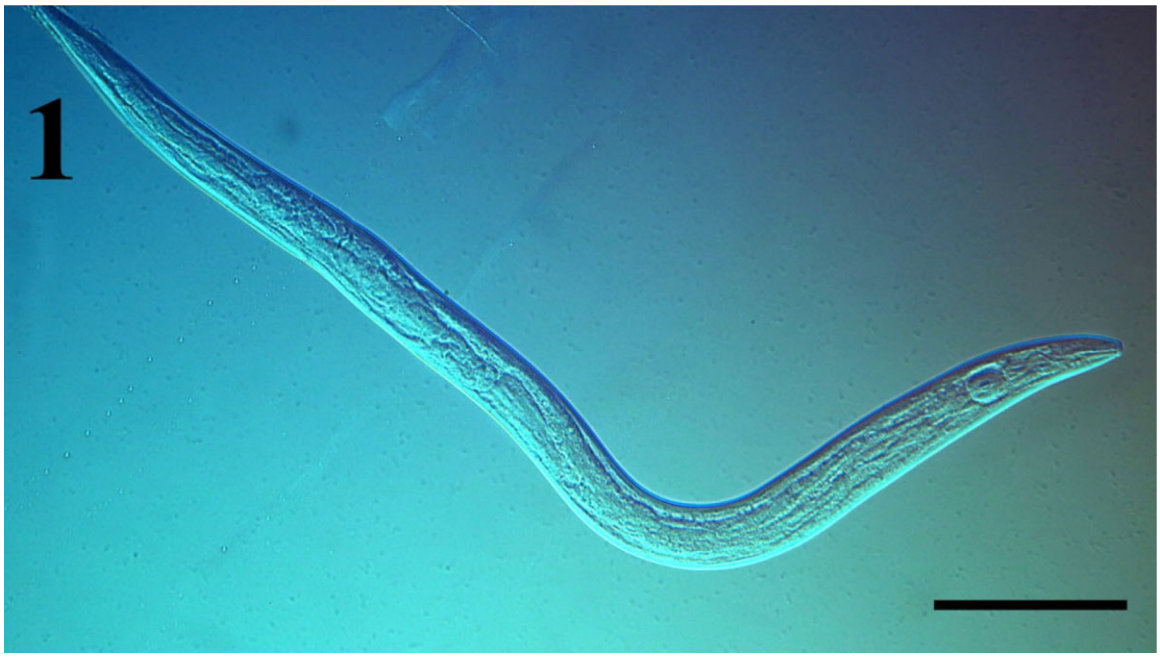

Fig. 1. Wild fungivore nematode from *Triceratops* bone shard, Glendive, MT. Scale bar = 12u.

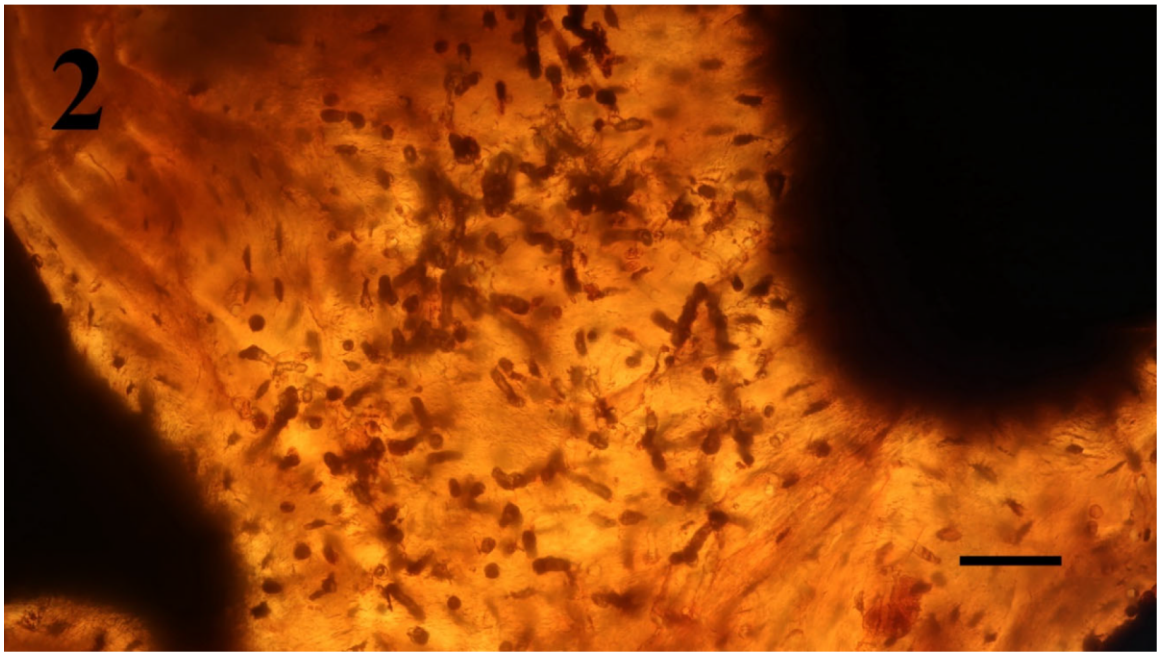

Fig. 2. Ground Section (40u), *Edmontosaurus* jaw bone, Glendive MT. Note fungal hyphae within compact bone. Scale Bar= 100u.

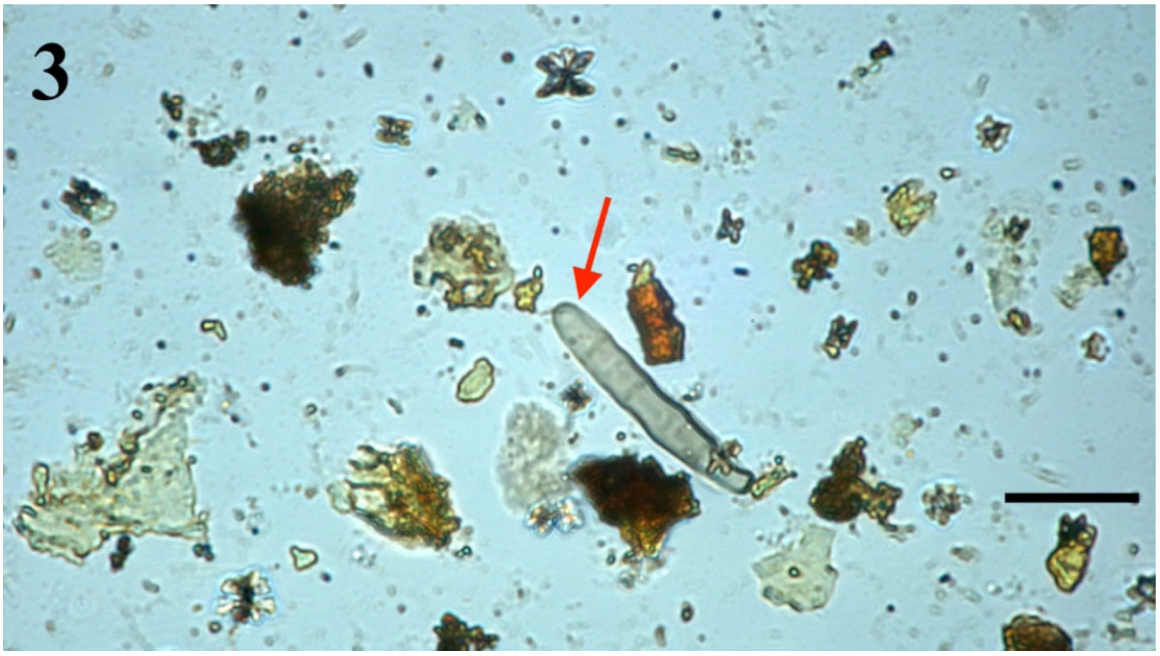

Fig. 3. Demineralized *Edmontosaurus* jaw bone (unwashed), unidentified fungal element (red arrow). Scale Bar = 35u.

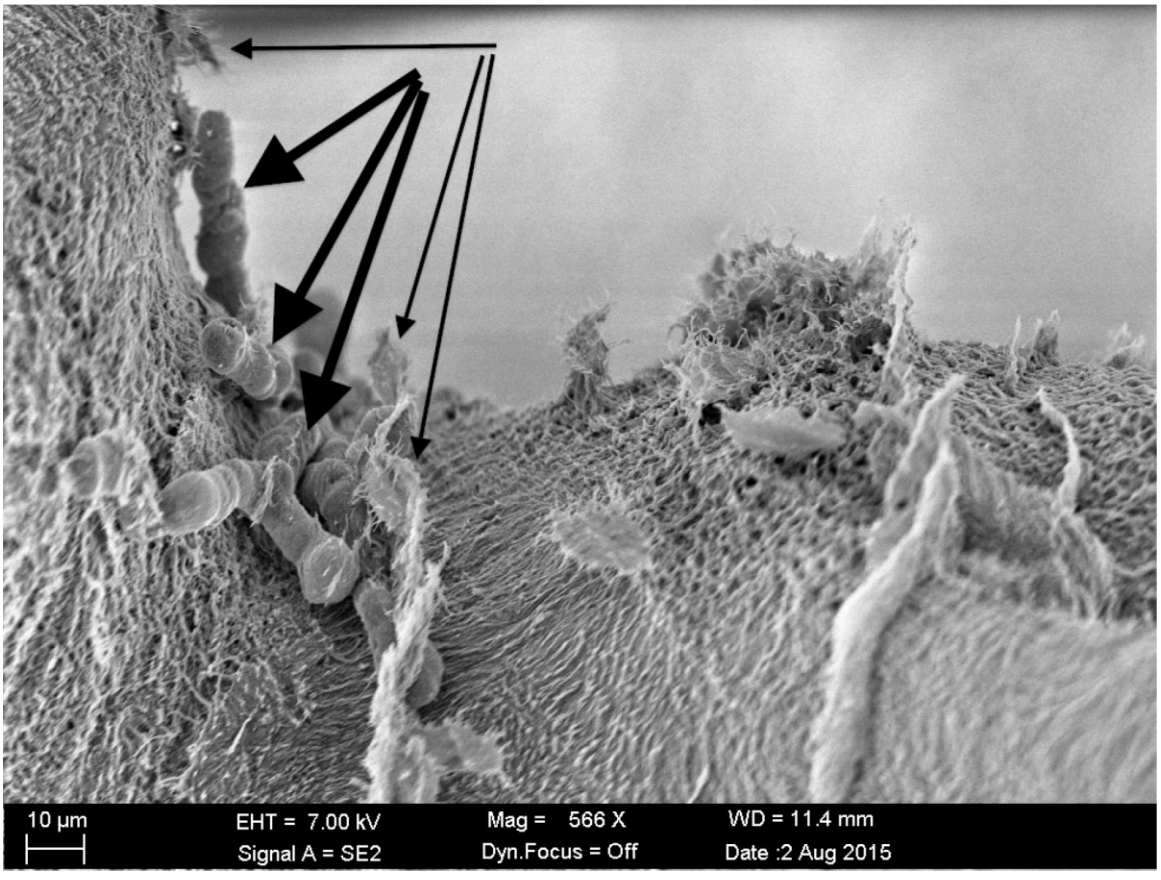

Fig. 4. SEM variable pressure, demineralized *Triceratops* horn bone shard. Note that osteocytes (thin arrows) and unidentified fungal hyphae (thick arrows) were exposed during decalcification. Scale Bar = 10u.

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