First Report of Fossil Insect Mandibles, Cadavers and Larval Molt Casts from Decalcified Bones of Cretaceous Dinosaurs (Edmontosaurus, Triceratops and Nanotyrannus): Scavengers Feeding on Organic Sources within Inorganic Bones

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First Report of Fossil Insect Mandibles, Cadavers and Larval Molt Casts from Decalcified Bones of Cretaceous Dinosaurs (*Edmontosaurus, Triceratops* and *Nanotyrannus)*: Scavengers Feeding on Organic Sources within Inorganic Bones

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Insect activity (scratches, pits and borings) on dinosaur bones, including traces made during feeding (osteophagy) and reproduction (pupal chamber digging) have been reported globally for nearly six decades [1-6]. The University of Kansas maintains a website of such ichnofossils [7] profiling many reports of ancient insect activity on fossil wood, plants and bones.

Most studies report only trace evidences of insects scavenging, burrowing and feeding but few reports provide examples of actual fossil insect remains. Only mollusks and insects are known to damage bones within sedimentary layers. The insects normally implicated in such ichnofossils are generally termites, moths and dermestid (or other kinds of) beetles [2].

Trace cutting marks are common in most Cretaceous bones and frass (insect feces) within bone is often accompanied by fine bone shards left in burrows and pits created by the mandibles of osteophageous (bone eating) beetles [1-3,5,6]. Reports clearly establish that beetles are in search of nutritional elements in these bones, including nitrogen, lipids and other soft tissue carrion, which might be present within dinosaur bones [1,2,4], although it is not completely understood how any nutrients can exist within the hydroxyapatite of such ancient bones. We have observed lipid droplets associated with many fibers and other elements in decalcified bone (not shown here). Because these beetles (and some termites which leave ichnofossils) have very robust mouthparts [2,3], it is not unusual to expect them to be in close proximity of dinosaur bones, or, in the case of juveniles, within the dinosaur bones themselves. This is because buried ancient bones do harbor microorganism communities (bacteria and fungi) [8,9], which could be attracting dermestid beetles. Few bone ichnofossil reports include examples of actual insect fossil mandibles, appendages or other insect remains salvaged from within dinosaur bones.

We collected bone specimens of *Nanotyrannus lancensis*, *Triceratops horridus* and *Edmontosaurus annectens* from established dinosaur digs in Glendive and Jordan Montana. Freshly excavated bones were immersed in fixative (10% neutral buffered formalin) at the dig site and were transported to the lab. After robust washing to remove sediment, and air-drying, specimens were placed in 14% EDTA for bone decalcification. Decalcified bone debris was removed from the bottom of dishes by pipette at regular intervals, air dried on glass slides and examined under brightfield and polarized light microscopy. Our goal was to characterize any soft tissues that were still present in bone but we were surprised to find fossil insect cadavers and mandibles in our decalcification solutions.

A beetle thorax (missing the head and some limb segments, Figure 1a) was recovered from the decalcification of a *Triceratops* buried frill shard collected from Glendive, MT. The thorax exhibited minimal decay/weathering although some limb segments were missing. It was unscratched, uniformly light green in color and measured 0.02mm in width by 0.038mm length. An intact beetle mandible (probably dermestid) was recovered from deep bone decalcification of *Nanotyrannus* vertebra and measured .004mm length and .002mm width (Figure 1b). Two distinct teeth on each side of the paired and symmetrical mandibles are visible as are thin outlines of soft structural elements inside of the robust and scleritized structure.

Additionally, insect pupal molts (Figure 2a-2d) were found within decal solutions from all three dinosaur bones. A paired set of seeds, (Figure 3) were also discovered in decal solutions of *Nanotyrannus* measuring 2u in width and 45u long.

Ichnofossil beetle markings have been well established for decades in the literature [7], therefore it would not be unusual to expect to find the remains of such beetles deeply inside buried fossil bone. The dimensions of the beetle mandible and cadaver shown here are approximately 10-20 times smaller than adult beetles described in the ichnofossil literature [2]. This might be consistent with juvenile offspring borne within the bone canals as a result of beetle activity. We have observed juvenile nematodes in dinosaur bone canals indicating that enough nutrients exist within the inorganic hydroxyapatite of bones to support worm populations including smaller juveniles [10]. Moreover, workers have discovered colonies of bacteria within dinosaur bones, which are not found in the soils surrounding the bone or above ground [8]. Thus the inorganic bone material of dinosaur remains harbors enough nutrients to serve as a food resource for biological scavengers (even unique ones), which have left their fossil traces, cadavers and biofilms within the bones. It is unlikely that the seeds observed were forced into the bone by environmental factors as they would have been bent, fractured or modified under such pressures, but the seeds appear unaffected. It could be that they clung to dermestid beetles entering bone canals to scavenge and thus were dragged into deep bone. We intend to pursue examination of decalcification solutions for more examples of communities of microorganisms inhabiting the inorganic vestiges of dinosaur remains.

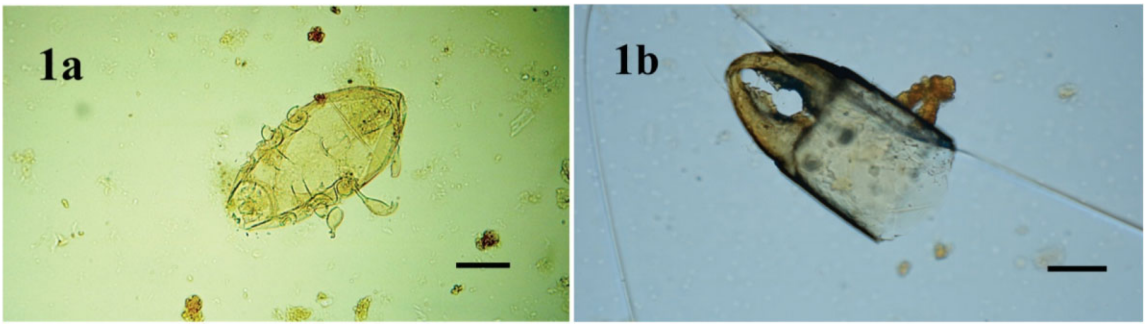

Fig.1. Insect cadaver parts from decalcified dinosaur bone, **1a.** Beetle cadaver from *Triceratops* frill, scale bar = .012mm. **1b.** Beetle mandible from *Nanotyrannus* vertebra, scale bar = .005mm.

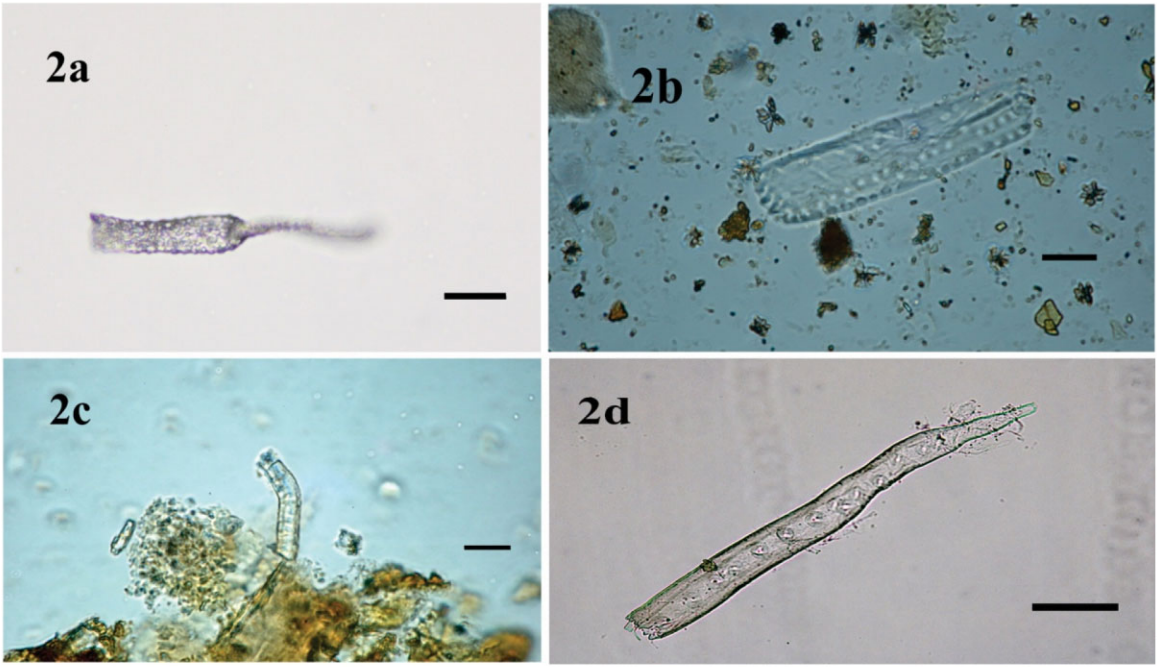

Fig. 2. Insect pupal molt casts from decalcified dinosaur bone, **2a.** molt cast from *Triceratops* horn, scale bar = .012mm. **2b.** molt cast from *Edmontosaurus* rib, scale bar = .003mm. **2c.** molt cast from *Nanotyrannus* vertebra, scale bar = .003mm. **2d.** molt cast from *Triceratops* horn, scale bar = .012mm.

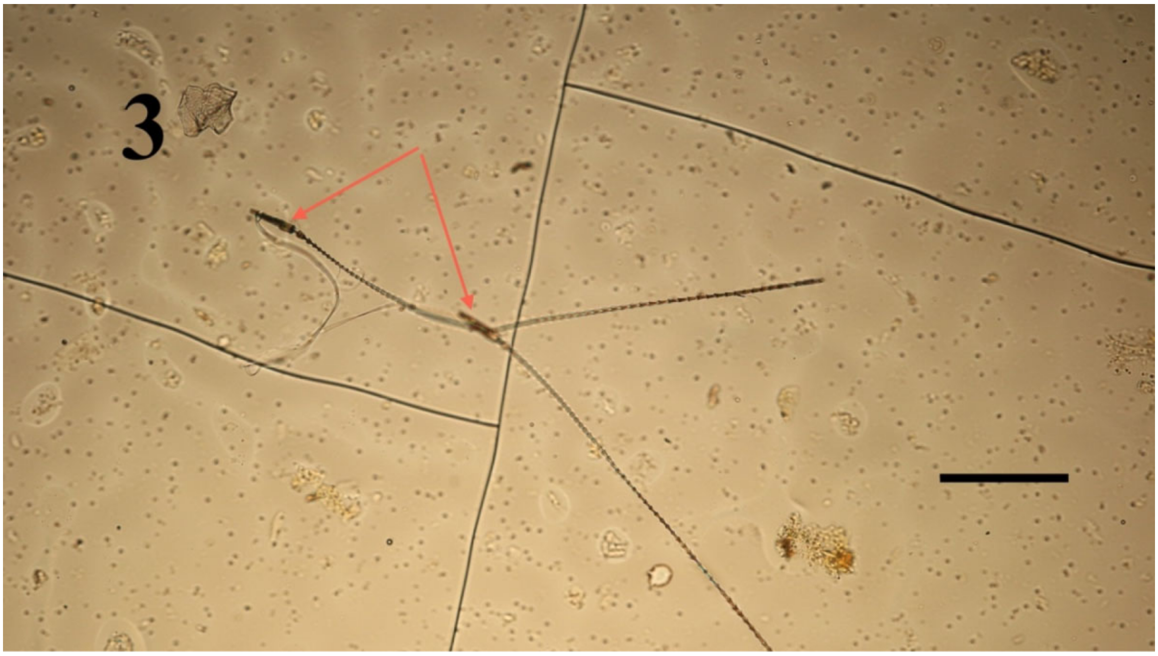

Fig. 3. Paired seeds (red arrows) from *Nanotyrannus* vertebra, scale bar = .012mm.

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