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Correlative Structure-Property Characterisation of the Leafcutter Ant (*Atta cepholotes*) Mandible

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Materials formed in nature, often driven by evolution, perform challenging mechanical functions. These can provide inspiration for better engineered materials requiring similar functions. This study looks at the leafcutter ant, *Atta cephalotes,* which as their name suggests, cut and collect leaves. They use leaves to fuel their colony through a symbiotic mutualism relationship with fungus. The leaves are used to grow fungus gardens, the ants provide materials and favourable conditions for the fungi to grow, for subsequent consumption.

The mandibles of the leafcutter ant seen in Figure 1, are used as cutting tools. To power and control these mandibles, roughly 50% of the weight of the ant's head is muscle. Logically, durability of the cutting surface is important over the lifespan; ants with worn mandibles have shown to conform to carrying instead of cutting [1]. Previous studies have shown the mandibles have a zinc (Zn) enriched layer at the cutting surface, with around 16% Zn compared to 0.08% in the rest of the mandible [2, 3]. The impact of Zn incorporation and its concentration within the teeth has been linked to a variation in hardness (an increase in most cases) at the cutting surface in contrast to the rest of the mandible structure (4). Metals such as zinc, manganese, iron & calcium have also been found in high concentrations within cuticles of other arthropod taxa in more than just mandibles e.g. claws [2, 5-7]. Metal incorporation in biological structures is known to occur in the form of biomineralization, however, Zn incorporation in many of the examples provided does not. Studies on similar metal incorporation suggest the form involves binding with proteins during the sclerotization process.

In this study, multiple correlated characterisation techniques are connected to analyse the surface and potential sub-surface layers within the zinc-rich region of the *Atta cepholotes* mandible. Initial X-ray microscopy/microtomography (XRM/μCT) using a lab-based Zeiss Xradia Versa520 XRM identified discrete layering in the zinc-rich region. These presented as defined layers of different greyscale/X-ray attenuation, which is a function of the material density or atomic/Z-number, suggesting discrete chemical banding. XRM served as a 'bioprospecting' tool, identifying previously hidden but potentially noteworthy and functional layered microstructures, warranting further investigation of possible structure-property/form-function relationships. This method has been previously shown for cuttlebone dorsal shield [10]. From the 3D XRM data, a sub-surface region was identified, and targeted mechanical grinding and polishing [11] was used to reveal this region of interest (ROI). The prepared surface was imaged using optical and electron microscopy with chemical analysis provided by energy dispersive X-ray spectroscopy (EDS) on a Zeiss Evo LS25 SEM, to compare to the corresponding XRM data. Structure-property relationships in biological materials often require mechanical data to supplement the morphological, chemical, and microstructural information. Nanoindentation was carried out on a Bruker Hysitron Ti950 using a cube corner tip geometry, 800 μN load at 5 μm spacing generating XPM (accelerated property mapping) hardness and reduced Modulus maps (432 indents) of the same region of interest spanning the outer and intermediate layers of the mandibular teeth, and the mandibular cuticle beneath these.

The optical, SEM, EDS, and nanoindentation data was aligned to the specific plane within the original XRM dataset to provide a correlative multimodal view of the same feature. Figure 2 shows the data from each of the methods used in the correlative workflow, putting each in context with the other techniques. The XRM dataset shows three distinct phases of different greyscale – the surrounding mandibular cuticle, the zinc-rich teeth tips, and also an intermediate region between them. The SEM backscatter image of the same area also identifies three distinct regions. The EDS mapping confirmed highest Zn content in the outer layer, with lowest Zn in the mandibular cuticle below the teeth, and an intermediate level of Zn in the intermediate layer. The Zn content correlated to the nanoindentation XPM maps, with higher zinc content in the outer layer providing greater hardness and Modulus, lowest in the surrounding cuticle, with intermediate mechanical properties for the intermediate layer. The chemical and mechanical property differences are distinct between the layers, rather than gradual. This could infer potential functional mechanical significance of the discrete layers. Mechanical layering in biological materials has been shown to influence toughness and crack arresting behaviour previously.

The combination of techniques in a correlative workflow enables us to probe the potential for materials science and biology crossover. Understanding structure-property relationships can help us understand possible form and function in nature. Learning from this structure could inform new composite material design for challenging applications [12].

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Figure 1. (a) 3D X-ray microscopy render of a single leafcutter ant mandible. Mandibular teeth are false coloured grey. (b) X-ray image of resin-mounted and partially ground single mandible. Sample is ground from the top surface in this view. The mandibular teeth are brighter due to the increased X-ray attenuation of the denser Zn-rich layers.

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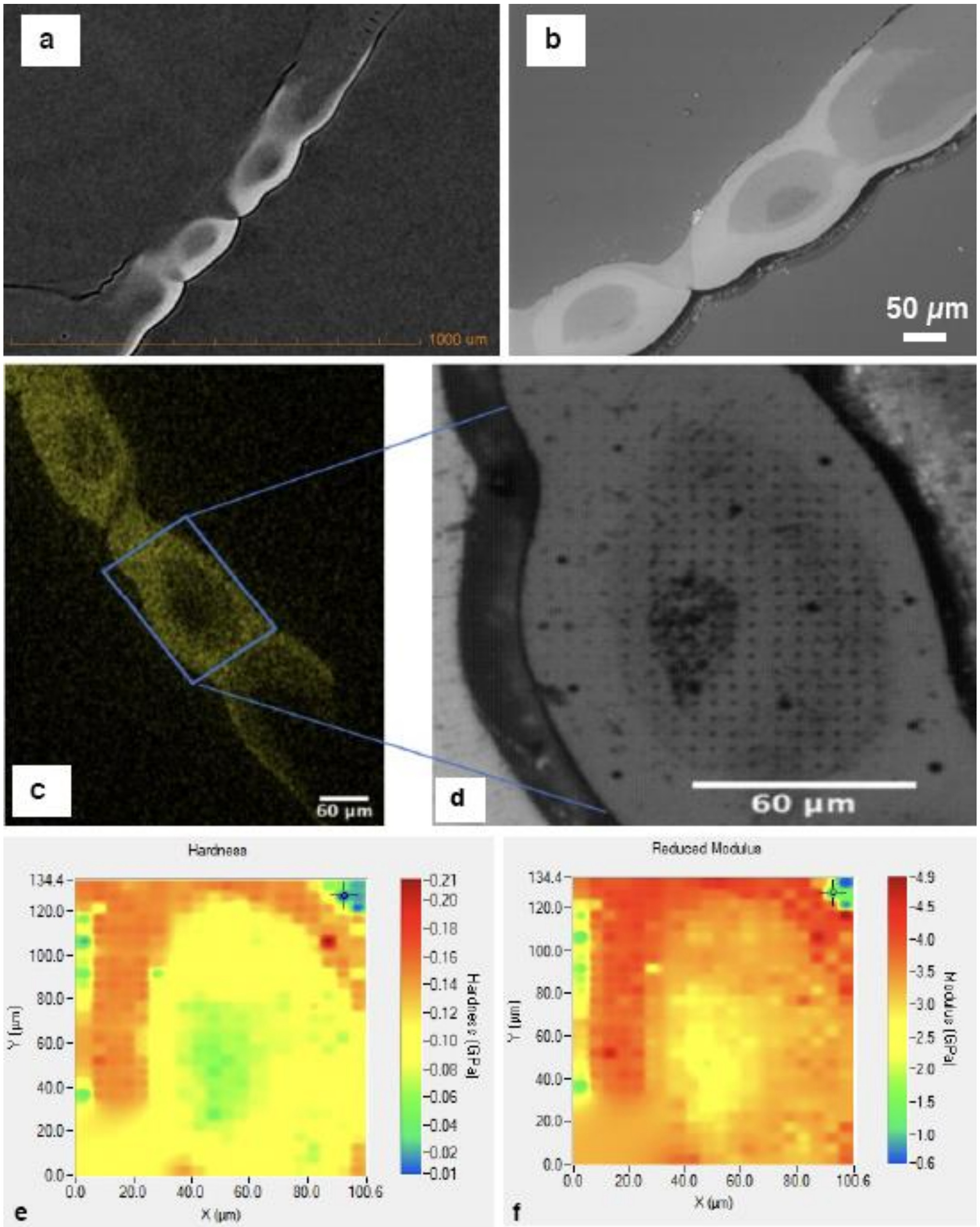

Figure 2. Correlative imaging workflow and region of interest tracked between characterisation techniques (a) digital slice through XRM tomogram identifying the three distinct layers based on differing greyscale (density). (b) backscatter SEM also indicating three distinct 'phases'. (c) EDS map of calcium with three levels of Ca content mapped to the three phases. (d) Optical image of region of interest with nanoindentation XPM indent array visible. (e) Nanomechanical characterisation XPM map of indentation hardness across the region of interest, revealing three distinct tiers of hardness. (f) XPM map of reduced Modulus, showing three distinct tiers of Modulus across the phases.

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