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Helga C. Lichtenegger, *et al.*

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TECHNICAL COMMENT

Response to Comment on "High Abrasion Resistance with Sparse Mineralization: Copper Biomineral in Worm Jaws"

Schofield and Nesson (*1*) express concern that the correlation between the element concentration in *Glycera* jaws and the mechanical properties of the jaw (*2*) may not reflect the correlation between atacamite mineral content and mechanical properties. They argue that there may be "unidentified nondiffracting copper and/or chlorine compounds that are predominantly responsible for mechanical properties" and criticize the use of chlorine as an indicator for the occurrence of the mineral.

Although determining the exact amount of copper and chlorine bound to the mineral is indeed difficult and small amounts of further, unidentified copper-chlorine compounds (UCCCs) may well exist in the sample, our results do not yield any indicators for their occurrence. Despite careful reevaluation, we could not see the supposed significant differences between the copper, chlorine, and electron density maps in our original manuscript (*3*). We have, however, reexamined our transmission electron microscopy (TEM) samples with energy dispersive x-ray analysis (Fig. 1). Clearly, the regions containing mineralized fibers are rich in copper and chlorine (Fig. 1A), with the copper peak greatly reduced in height in the matrix material (Fig. 1B). The remaining amount of copper is comparable to the amount in the fiber region that exceeds the Cu:Cl ratio of 2:1 in atacamite. No chlorine at all was detected in matrix regions devoid of mineralized fibers. Hence, we conclude that chlorine constitutes a reliable indicator for the occurrence of the mineral.

Notwithstanding that, we acknowledge that UCCCs could be part of the mineralized fibers. Dark-field TEM in Bragg contrast showed that the major part of the fibers consists of diffracting atacamite crystallites, but this does not exclude the existence of small amounts of UCCCs (or other, possibly biopolymeric substances) in the spaces in-between. This would lead to some overestimation of the amount of atacamite when calculated from element analysis, but would not influence the correlation of the occurrence of mineralized fibers and an increased local hardness and stiffness.

We concur with Schofield and Nesson that zinc occurs in a noncrystalline amorphous form in *Nereis* jaws, mostly together with chlorine. Recent data obtained by x-ray absorption spectroscopy suggest that zinc and chlorine in *Nereis* jaw are directly bound to the protein, rather than within an amorphous inorganic compound (*4*). Excess copper in the *Glycera* jaw matrix may play a similar role, but different ways of metal and halogen coordination in each case cannot be excluded, particularly in view of the somewhat different amino acid compositions of *Glycera* and *Nereis* jaws.

For nanoindentation experiments, we agree that samples should be homogenous and well supported for any such experiment, not just ones using the Oliver and Pharr method (*5*). Biological specimens, unfortunately, are hardly ever ideal in that sense. Nevertheless, reproducible results have been obtained for embedded samples of dentin and even bone (*6*) that contains a much larger fraction of voids than the very compact *Glycera* jaw. In contrast to the situation with metals and alloys, elastic recovery during the unloading sequence and after indentation is a major concern in the case of biological specimens (*7*). In this situation, the Oliver and Pharr method (*5*) remains the best compromise and has been shown to yield reasonable results for biological materials (*7*). It takes into account the depression around the indenter and uses the load-displacement curve to determine the mean pressure at which plastic flow occurs, yielding physically meaningful hardness values (Meyer hardness). Using the depth of the remaining imprint as an indicator for hardness, as Schofield and Nesson suggest (*1*), seems hardly advisable in view of the elastic recovery of the sample once the indenter is removed. Imaging the imprints with the same tip used for indentation (imaging of an object with threefold geometry using a three-sided pyramid) induces further errors depending on the scanning direction.

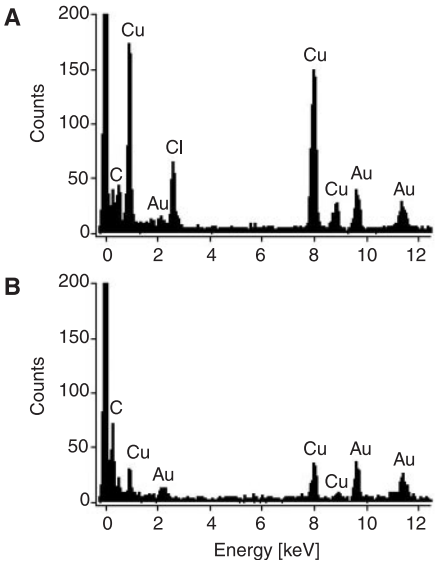


Fig. 1. Energy dispersive x-ray analysis recorded in TEM of thin sections of a *Glycera* jaw tip. (**A**) Element concentration in region containing atacamite fibers. (**B**) Element concentration in matrix. The Au peaks in both analyses are due to background scattering coming from the gold sample grid.

Like Schofield and Nesson (*1*), we found that the mechanical properties of *Glycera* jaws change considerably upon hydration. Our study (*2*) focused on a dry sample for several reasons. (i) An almost perfectly smooth cross section is essential for obtaining precise and reproducible results and could be achieved with dry samples only. (ii) Measuring wet samples considerably complicates the experiment (sample mounting, storage, fluid cell), making the results highly dependent on external parameters and reducing consistency. (iii) A reproducibly "dry" state of biological samples is much easier to achieve, and less dependent on individual interpretation, than a reproducibly "wet" or "close to original" state. Additional experiments on *Glycera* jaws with different degrees of hydration showed that the relative variation of hardness and modulus within the sample slightly decreases with increasing water content (dry: H = 0.4 to 1.46 GPa, E = 7.5 to 21 GPa; wet: H = 0.24 to 0.7 GPa, E = 4.5 to 14 GPa), but the distribution and thus the H- and E-maps are not changed. Similar results have been reported for bone (*8*).

The significance of the ratio $H^{3/2}/E$ as a measure for the abrasion or wear resistance has been discussed, for example, in (*9*). The ratio has been used for the characterization of technical hard coatings, although some researchers use the simple ratio H/E (*10*); we used it in (*2*) as a crude estimate for comparing the properties of *Glycera* jaws with those of other tooth materials. We have not yet found a reproducible method for measuring the true resistance to wear or abrasion of biological materials, but we certainly agree that a direct determination and comparison of the abrasion resistance would be preferable.

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