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Mineralogical controls on cave sedimentary ancient DNA preservation and extraction

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Sedimentary ancient DNA (sedaDNA) is a powerful tool for reconstructing past ecosystems by providing important paleoecological insights independent of fossil presence. However, sedaDNA extraction can be unpredictable because only some samples yield sufficiently preserved ancient DNA and there is still no clear explanation why that is the case. One possibility is the adsorption to mineral surfaces. Swelling clay minerals such as smectites might exceptionally contribute to the preservation of sedaDNA by intercalating DNA molecules into their structural nanopores, *i.e.* interlayers, where spatial confinement minimizes degradation.

To investigate the role of smectites in sedaDNA preservation, we targeted cave sediments from El Mirón (Spain) and Satsurblia (Georgia). Cave sediments are relatively protected from weathering but are heterogeneous, often reworked, comprising firing residues, coprolites and secondary phosphate minerals formed through the alteration of bone and organic matter. We used X-ray diffraction and scanning electron microscopy coupled to electron dispersive spectroscopy to characterise and quantify mineral and amorphous phases, of both bulk samples and clay fraction (<2 μm). We reacted the clay fraction with 30 bp pure salmon sperm DNA to determine whether intercalation into identified swelling clays is possible. These data were compared with sedaDNA yields obtained from the same samples. We found limited correlation between apatite content and total DNA reads. This suggests that sedaDNA is likely extracted from bones (and coprolites), particularly since applied extraction protocol targeted ancient DNA from bones. Swelling clays did not intercalate DNA, suggesting limited contribution to sedaDNA preservation in these sediments. This was supported by the absence of correlation between smectite abundance and extracted sedaDNA yields.

The relationships and interactions between minerals and sedaDNA in sediments require further investigation to unravel the sedaDNA survival, and mechanisms that govern DNA preservation. These interactions also affect extraction efficiency because binding strength determines the physicochemical conditions necessary to release DNA from mineral surfaces. A mechanistic understanding of mineral controls on sedaDNA preservation will enable more targeted sampling strategies and optimized extraction protocols, ultimately improving the reliability and scope of sedaDNA-based paleoreconstructions.