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ZooMS analysis of two Châtelperronian faunal assemblages

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Recently, ZooMS has been introduced as a cost-effective taxonomic identification method for bone specimens using MALDI-TOF-MS analysis of collagen type I (COL1; [1]). Assemblage-wide applications of this technique have been few, however, and no data is available on success rates for Pleistocene bone assemblages. Further, there have been no comparisons between the two main extraction protocols used, acid demineralization and non-destructive buffer extraction using ammonium-bicarbonate [2, 3].

Here, we present ZooMS results on Châtelperronian faunal assemblages from Les Cottés, France (including the Châtelperronian unit 06) and Quinçay, France (Châtelperronian units Ej, Em and En). For Les Cottés, 145 bone samples were demineralized while for Quinçay 448 bone and tooth samples were incubated in ammonium bicarbonate buffer to extract soluble COL1. Previously available data on faunal assemblage composition for both sites indicate differences in the number of identified specimens (NISP) as well as species richness.

Our results indicate that both extraction procedures result in similar percentages of successful identifications ($\approx 90\%$). The remaining specimens ($\approx 10\%$) yielded limited taxonomic information, while a single specimen for Les Cottés and two specimens for Quinçay remained unidentified. Despite overall similarity in success rates, there are striking differences in peptide marker presence between acid demineralization and buffer extraction. This observation is especially relevant for peptide marker C ($\alpha 2(I)$ 512–529), and should guide future extraction strategies.

ZooMS adds previously unrepresented species to both sites and all studied units. These include carnivores, leporids, and herbivores from a range of size classes. For Les Cottés, our results indicate a significantly higher species richness for unit 06 compared to the similarly-sized morphologically identified assemblage [2].

The success rate observed is promising for the application of ZooMS to other faunal assemblages covering the Middle to Upper Palaeolithic transition and characterized by a low number of identified specimens, providing a better informed picture of environmental conditions during this time period and more accurate data for discussing the subsistence strategies developed by the involved hominin populations. Finally, we will highlight recent advancements in *de novo* COL1 sequencing and its potential to improve taxonomic identifications using ZooMS [4].

References: [1] Buckley, M., Collins, M., Thomas-Oates, J., Wilson, J.C., 2009. Species identification by analysis of bone collagen using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Rapid Commun. Mass Sp.* 23, 3843-3854. [2] Welker, F., Soressi, M., Rendu, W., Hublin, J.-J., Collins, M., 2015. Using ZooMS to identify fragmentary bone from the Late Middle/Early Upper Palaeolithic sequence of Les Cottés, France. *J. Archaeol. Sci.* 54, 279-286. [3] Van Doorn, N.L., Hollund, H., Collins, M.J., 2011. A novel and non-destructive approach for ZooMS analysis: ammonium-bicarbonate buffer extraction. *Archaeol. Anthropol. Sci.* 3, 281-289. [4] Welker, F., Collins, M.J., Thomas, J.A., Wadsley, M., Brace, S., Cappellini, E., Turvey, S.T., Reguero, M., Gelfo, J.N., Kramarz, A., Burger, J., Thomas-Oates, J., Ashford, D.A., Ashton, P.D., Rowsell, K., Porter, D.M., Kessler, B., Fischer, R., Baessmann, C., Kasper, S., Olsen, J.V., Kiley, P., Elliott, J.A., Kelstrup, C.D., Mullin, V., Hofreiter, M., Willerslev, E., Hublin, J.-J., Orlando, L., Barnes, I., MacPhee, R.D.E., 2015. Ancient proteins resolve the evolutionary history of Darwin's South American ungulates. *Nature* DOI: 10.1038/nature14249.