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How to exploit Alpine glaciers as biological archives: new DNA metabarcoding approaches for biodiversity analyses on ice cores extracted from the largest and deepest southern Alps glacier, Adamello, Italy

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Within the framework of the three-years CALICE (CALibrating Biodiversity from ICE cores) project, funded by Euregio on 2017, we aim at estimating plant biodiversity changes through time in the Adamello glacier catchment area. Adamello is the largest, 16.4 km2, and deepest, 270 m, Italian glacier whose catchment area is mainly lying in the Po valley, that is an area that witnessed dramatic land use and climatic changes in the last decades. The good preservation of environmental DNA (eDNA) from ice cores and the possibility to retrieve a fine-grained stratigraphy -years and seasons- offer the unique opportunity of fully exploiting the promise of metabarcoding for assessing how plant biodiversity has changed over time. In particular CALICE is focussing on a 45 m depth ice core. At minus 30 m from the surface we found a strong tritium (H3) signal, most likely the signature of 1963 thermonuclear explosions. This means that the overall 45 m would reasonably correspond to about 70-80 years before present.

We previously conducted some preliminary experiments, especially for setting up the eDNA metabarcoding procedures. We adopted a classical approach, targeting with PCR a 150 base pair (bp) region of the plastidial genome (cpDNA), the trnL intron. Though promising, the results highlighted major caveats related to the following issues: co-amplification of untargeted organisms such as bacteria, taxonomic resolution seldom down to the species level, preferential amplification of some organisms and, last but not least, difficult identification of contamination.

We present here the results of a different approach for taxonomic identification of plant species through eDNA metabarcording of the first 10 m (from the bottom) of the entire CALICE ice core (45 m). A so-called bait has been synthesised: the about 10 kb (kilobases) of this bait represent the most variable regions across cpDNA of a wide range of taxa, allowing in principle the ease identification of these taxa at the species level. The approach is based on 'fishing' out of the total eDNA extracted from ice core only the part that hybridize with the bait, without any PCR amplification. As a result, cpDNA from eDNA is enriched in the most variable regions contained in the bait. What derives from this sequence 'capture' step, is then assembled in libraries whose massive sequencing produces hundreds of thousand reads for each ice core sample. These reads will be then compared against a reference database, PhyloAlps, containing records for all the plant taxa of the entire Alpine chain.

We show how this 'sequence capture' metabarcoding approach can significantly improve our ability to infer plant biodiversity from ice cores, really making ice cores a very informative archive for addressing the impact of global change on biodiversity.