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

Alexis Marchesini[1], Matteo Girardi[1], Antonella Cristofori[1], Valter Maggi[2], Daniela Festi[3], Camilla Wellstein[3], Stefan Zerbe[3], Klaus Oeggl[4], and Cristiano Vernesi[1]

[1] Dept. of Biodiversity and Molecular Ecology, Research and Innovation Centre – Fondazione Edmund Mach, S. Michele all'Adige (TN), Italy; [2] Earth and Environmental Sciences Dept., University of Milano Bicocca, Italy; [3] Faculty of Science and Technology, Free University of Bozen-Bolzano, Italy; [4] Institute of Botany, University of Innsbruck, Austria; Email: **alexis.marchesini@fmach.it**

INTRODUCTION: Pollen eDNA metabarcoding of ice cores

Recent advances in Next Generation Sequencing DNA sequencing technologies (NGS), coupled with a metabarcoding approach (for multispecies detection) now permit to retrieve past environmental DNA (eDNA) sequences from many different sediment sources. Ice cores offer a twofold advantage for inferring environmental changes through time: good preservation of eDNA and a detailed timescale, possibly at year and season level.
Within the framework of the CALICE (CALibrating Plant Biodiversity in Glacier ICE) project, we aim at reconstructing plant biodiversity and its trends archived in the Adamello glacier (Eastern Italian Alps) by pollen and environmental DNA (eDNA). A 46 m ice core was retrieved on April 2016, encompassing the last 70-80 years, according to preliminary tritium radioactivity results.
Different eDNA metabarcoding approaches will be presented and discussed, together with their pros and cons. The results should serve as a calibration data set for future studies in other glaciated areas, aimed at investigating biodiversity changes for large regions.

Adamello glacier:

deepest and largest Italian glacier

Altitude: 2586-3406 m a.s.l.
Max thickness: 270 m
Area: 16.4 km
Ice core to be analyzed:
46 m = ~70 years

METHODS/1 – Standard eDNA metabarcoding

Environmental DNA sample → Ice core section

DNA extraction

DNA amplification (**PCR**) → *Target DNA*: **pollens**; *Barcode region*: 150 bp chloroplast **trnL** fragment

DNA sequencing of amplicons (Next Generation Sequencing, NGS) → ILLUMINA MySEQ

Sequence analysis (bioinformatics) → quality filter, sequence merging, chimera removal, OTU clustering,…

Taxonomy assignment
-> identification of multiple taxa → Reference sequence database: **Alpine flora**

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>Pinus sylvestris trnL gene
GGACTTGAACCCTCACGGTCTA..

>Pinus nigra trnL gene
GGACTTGAACCCTCACGGTCTA...

>Pinus mugo trnL gene
GGACTTGATCGAATTGAGCCTT...
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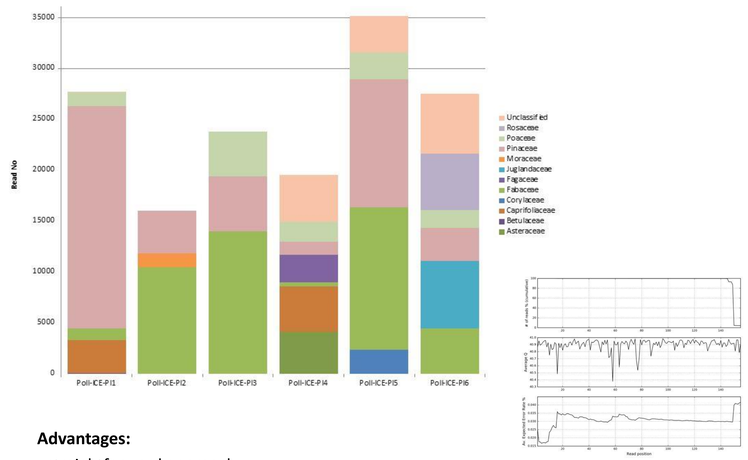
PRELIMINARY RESULTS – Standard eDNA metabarcoding (trnL gene)



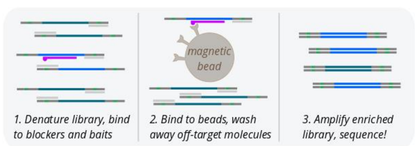
Advantages:
- straightforward approach
- high recovery of DNA sequences
- high universality (ability to recover sequences from different taxa/plant groups)

Drawbacks:
- **sub-optimal discriminatory power** (taxonomic resolution seldom down to the species level)
- **preferential amplification** of some taxa
- PCR-based: **increases the risk of PCR bias** (contamination, cross-amplification and sequences errors)

METHODS /2– Targeted sequence-capture metabarcoding (under development)

Principle: PCR-free approach; selective enrichment of target regions using probes (baits)

Proposed target regions:
standard barcode markers
- rbcL
- matK



Advantages:
- PCR-free: **reduces the risk of PCR bias**
- improved coverage and sequencing depth
- **optimal approach for degraded DNA** (e.g. ancient eDNA)

Drawbacks:
- requires an expert-based experimental design:
  (a) selecting the target and capture baits is a crucial step! (bioinformatics)
  (b) need for a complete and accurate database of input sequences (used to design the baits)
- [there may still the risk of preferential amplification, when the above conditions are not met]

CONCLUSIONS

Our study demonstrated that ice cores from Alpine glaciers are a valuable source of eDNA.

Though promising, our preliminary results from pollen eDNA metabarcoding highlighted major caveats related to the following issues: taxonomic resolution seldom down to the species level, preferential amplification of some organisms, potential PCR bias and, last but not least, difficult identification of contamination.

In order to improve the accuracy and reliability of our results, we are developing a targeted sequence-capture metabarcoding approach, which aims at overcoming the limitations of standard metabarcoding procedures.

The opportunity to gain accurate information on plant biodiversity (at the species level) from different ice core depths, coupled with (a) palynological data derived by morphological identification, and (b) standard chronological proxies (e.g. stable isotopes, ^{210}Pb), can really make ice cores a very informative archive for addressing the impact of global change on biodiversity.

The biodiversity estimates gained by pollen analysis and eDNA will be validated by current and historical biodiversity assessments in the catchment area of the glacier (Trentino-Südtirol, Lombardia- Italy). Our goal is to evaluate vegetation/biodiversity changes, detect the factors that triggered them (e.g. climatic, socio-economic, land use) and the consequences.

FUTURE PERSPECTIVES

Preliminary metabarcoding experiments, using the standard COI barcode gene, highlighted the possibility to extend the potential of ice cores as biological archives also for arthropods diversity.

Focusing on (a) indicators of specific environments and (b) functional groups, the opportunity to use eDNA also for insect identification would reveal:

(a) how glacial and peri-glacial invertebrate fauna reacted to climate changes;

(b) how plant and insect ecological interaction (e.g. pollination) responded to environmental and anthropogenic pressures, thus shedding light on functional biodiversity modifications.

Insect diversity

Butterfly wing scale, found in ice sample