FONDAZIONE EDMUND MACH

CLUB ALPINO ITALIANO

Corso ONTAM,
Hotel Terme di Frasassi, Genga (AN)
11 settembre 2022

Conservazione della biodiversità: la rilevanza della componente genetica, 4

Applicazioni della genetica per lo studio della biodiversità

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Fondazione Edmund Mach
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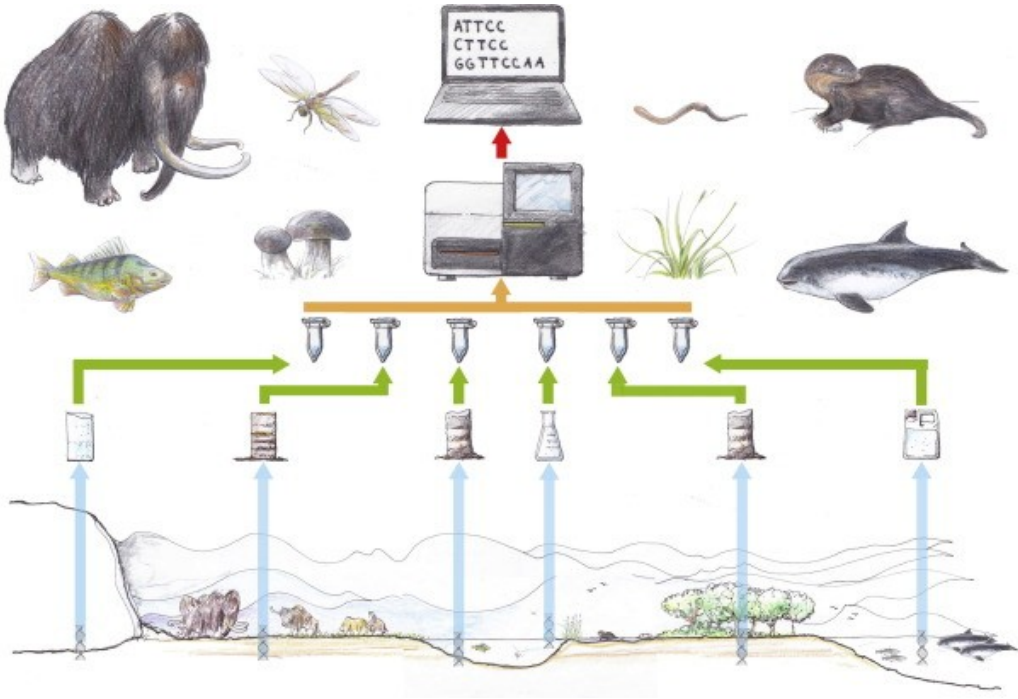
What?

eDNA (environmental DNA) is defined as DNA that is collected from a variety of environmental samples such as soil, seawater, or even air rather than directly sampled from an individual organism.



Where?

Where does mainly eDNA come from?

Several different sources such as faeces, urine, pollen grains, skin, carcasses, larvae, eggs, leaves and hair.

What eDNA is mainly used for?

Taxonomic identification

Taxonomic identification from eDNA can be accomplished by means of two – mutually non exclusive- approaches:

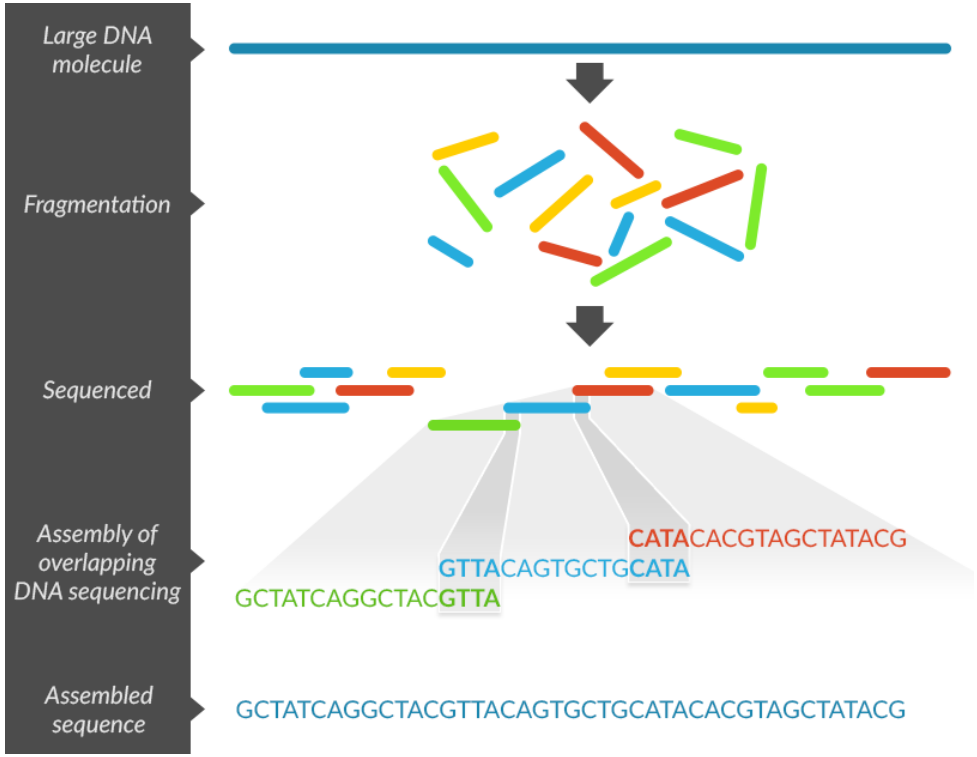
Metagenomics

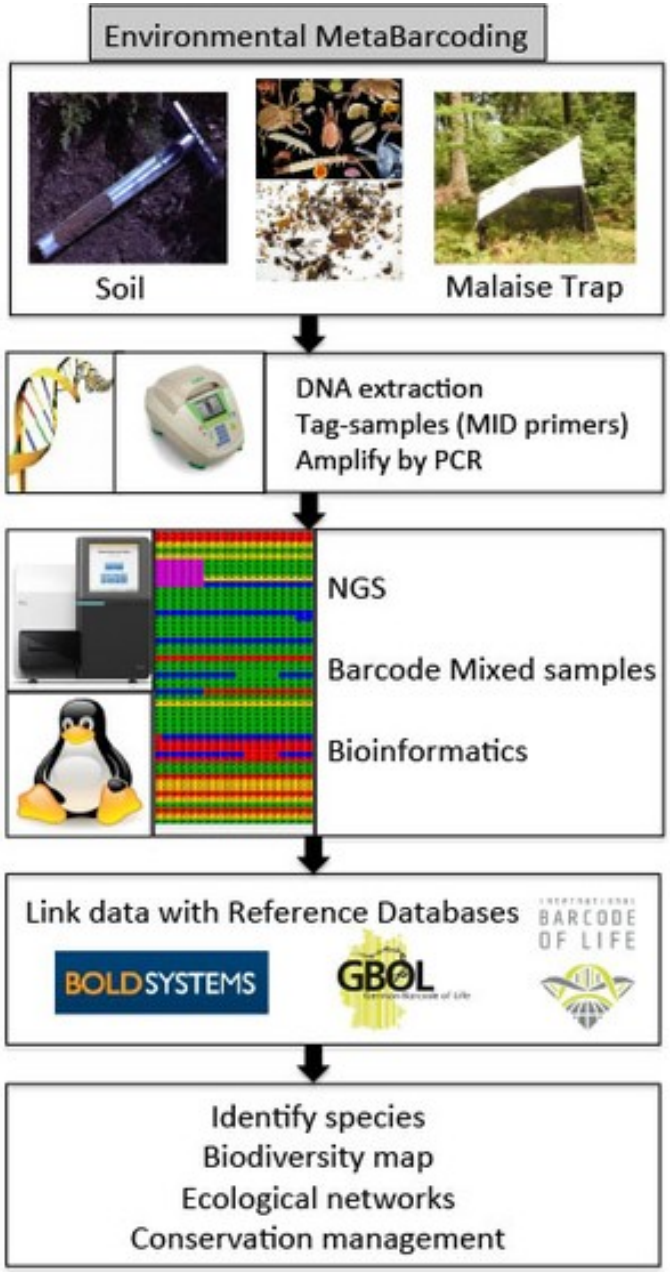
Metabarcoding

Enviromental sample

Total genomic DNA extraction

Shotgun genome sequencing



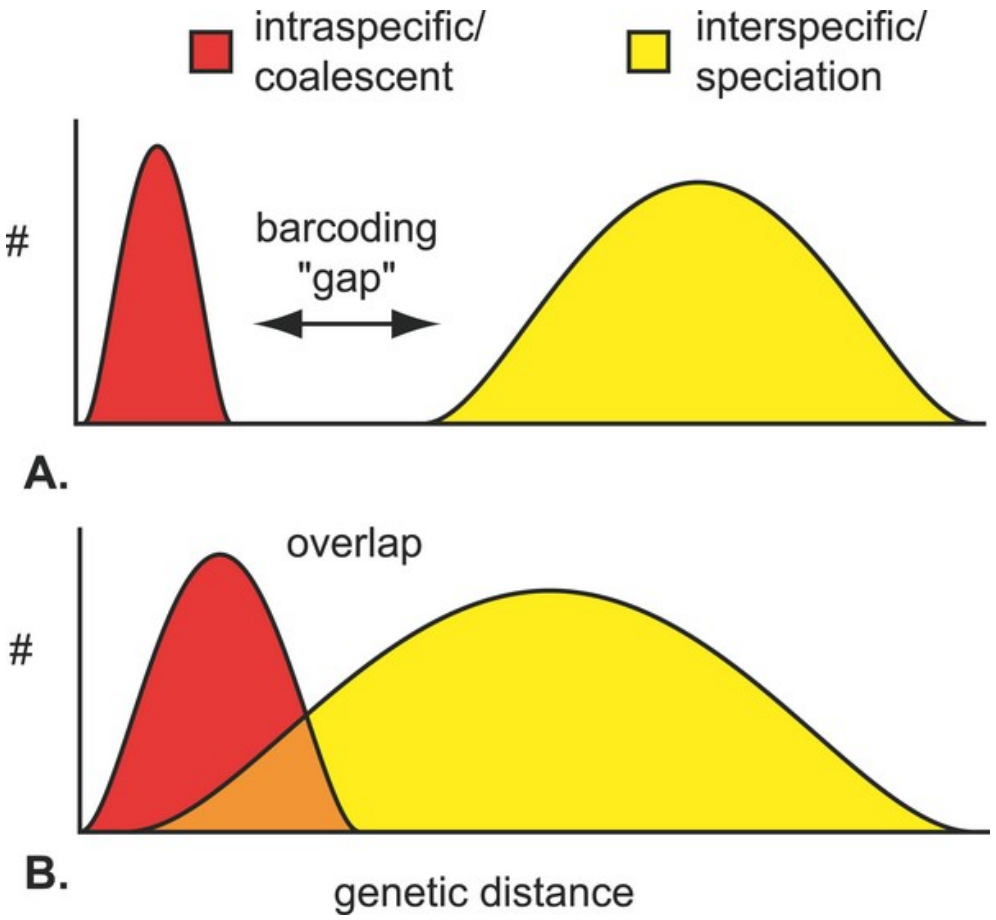


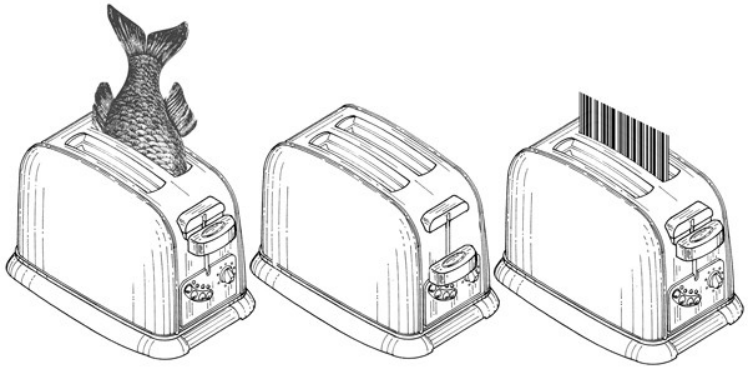
PCR amplification: selection of target sequences

Retrieved sequences are compared to a specific reference database

What a DNA barcode marker is?

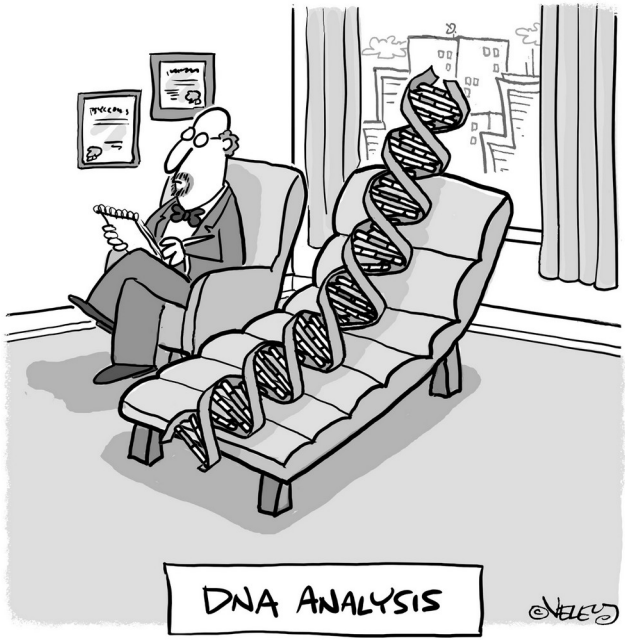
How does a DNA barcode marker work?





Pros:
Barcode marker specificity
Reference database restricts bioinformatics burden - especially with custom-made reference database
Even very low DNA quantity can be barcoded

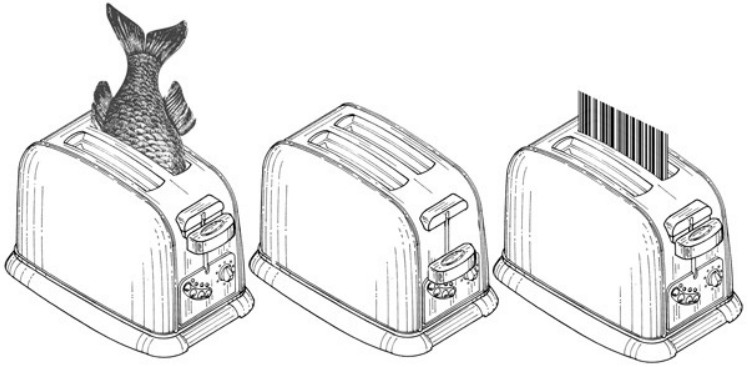
Cons
PCR can introduce significant biases
For some organisms (e.g. plants) a single barcode marker is not sufficient. Mind the gap!
Curation of reference database



The reference database

The wet lab analyses:

from an environmental sample to a bunch of barcodes



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The *in silico* analyses

eDNA samples: unknown

Bioinformatics analyses

Reference Database: generic

The *in silico* analyses: results

eDNA samples: unknown

there's no a good match : unknown samples identified to large taxonomic categories such as Families, Orders or even Classes

Reference Database: generic

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The *in silico* analyses: results

eDNA samples: unknown

100 % match: unknown samples identified to Species level

Reference Database:
very well curated,
usually custom made
with some additional efforts
in terms of wet lab analyses

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Applications to some relevant Conservation Biology issues

Mammals biodiversity survey

Terrestrial habitats

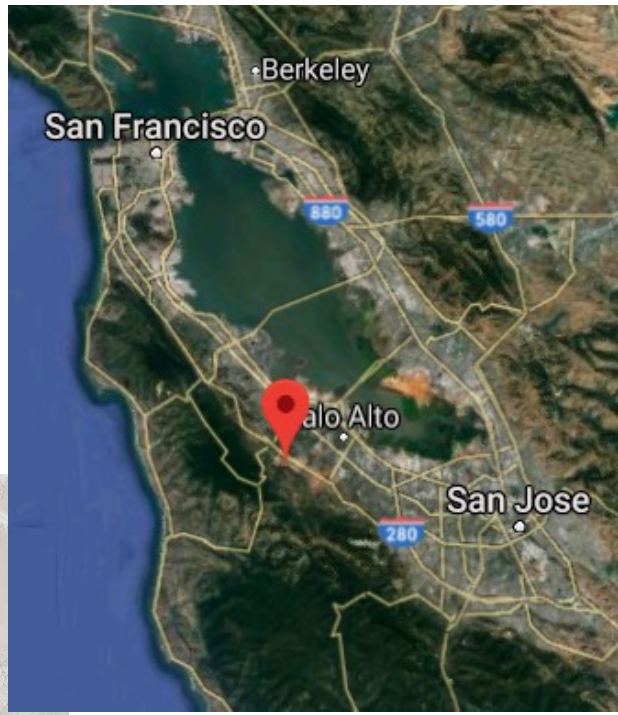
A comparison of eDNA to camera trapping for assessment of terrestrial mammal diversity

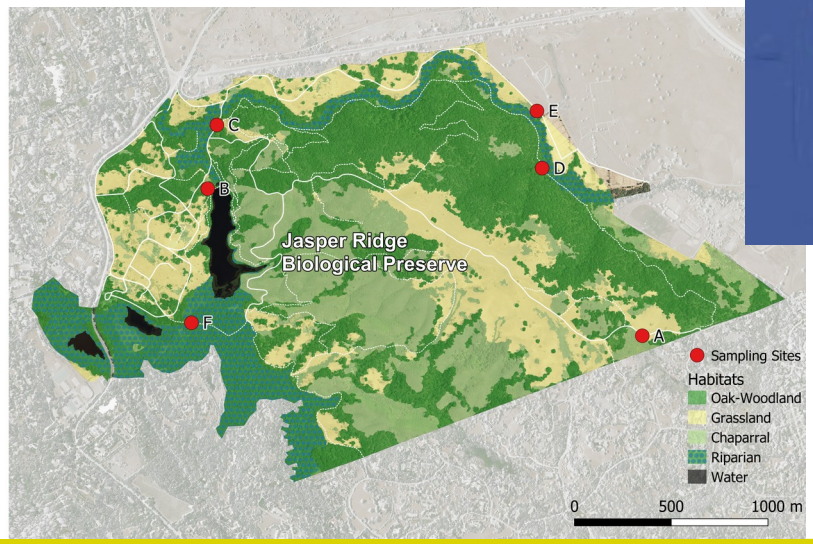
Kevin Leempoel[1,2], Trevor Hebert[2] and Elizabeth A. Hadly[1,2,3]

[1]Department of Biology, [2]Jasper Ridge Biological Preserve, and [3]Woods Institute for the Environment, Stanford University, Stanford, CA, USA

THE ROYAL SOCIETY PUBLISHING

Proc. R. Soc. B **287**: 20192353.





royalsocietypublishing.org/journal/rspb Proc. R. Soc. B 287: 20192353

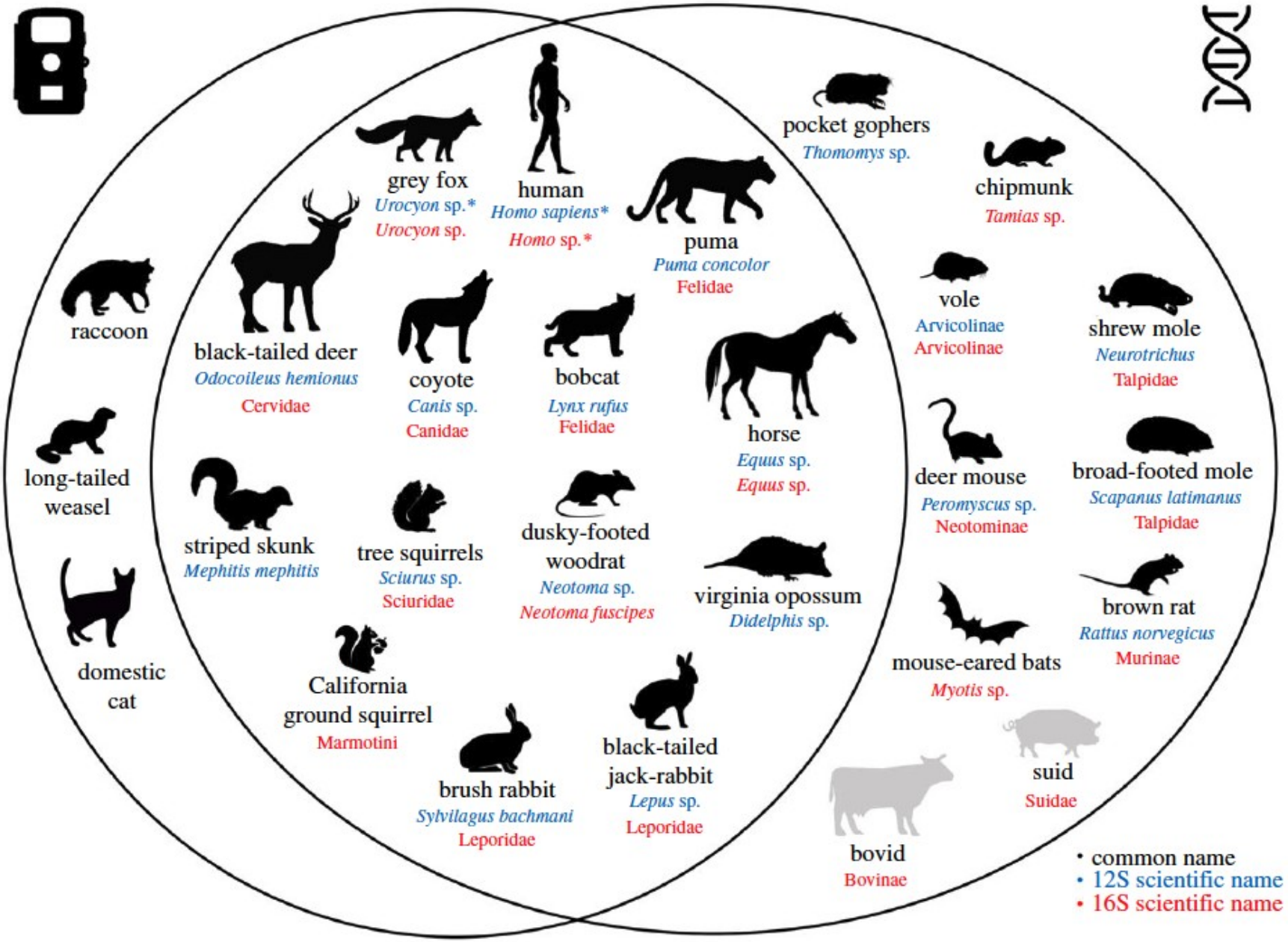


Figure 2. Venn diagram between species recorded by camera traps and species detected with eDNA from both metabarcodes. Species detected with the 16S are marked in red and those detected with the 12S are in blue. Scientific names are given at the maximum rank reached with each metabarcode. Species known to be present in the study area are in black. Species absent from the study area but detected with eDNA are in grey. Species considered as contaminant are indicated with asterisks. Credits for illustration are in electronic supplementary material. (Online version in colour.)

Our study demonstrates once more that eDNA is a remarkably promising approach for ecosystem assessment, and opens new possibilities for managers and researchers to reveal the distribution and interaction of species in a single survey. From soil surface samples, we detected most species present in the study area, including those which are generally too small to trigger camera traps. As such, eDNA alone was enough to obtain a reasonable picture of species diversity without requiring previous knowledge of the study area. In terms of sampling design, neither the number of

well as the strategy to adopt for technical replication. While promising, eDNA remains currently time-consuming and cannot yet be scaled up to a landscape level.

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Fungi biodiversity survey

Airborne samples

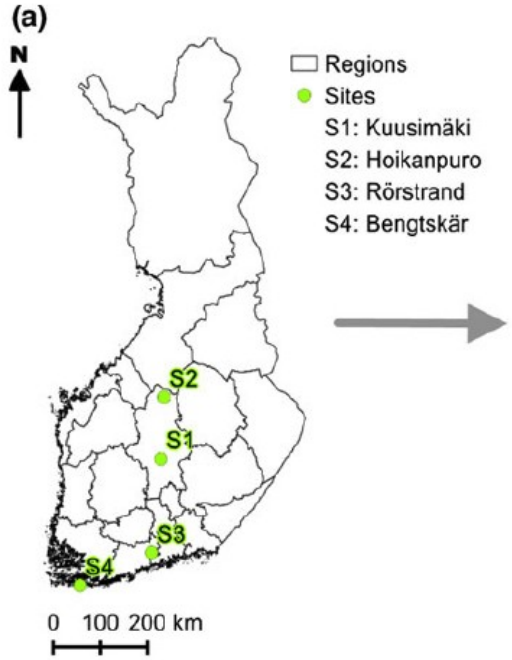
RESOURCE ARTICLE

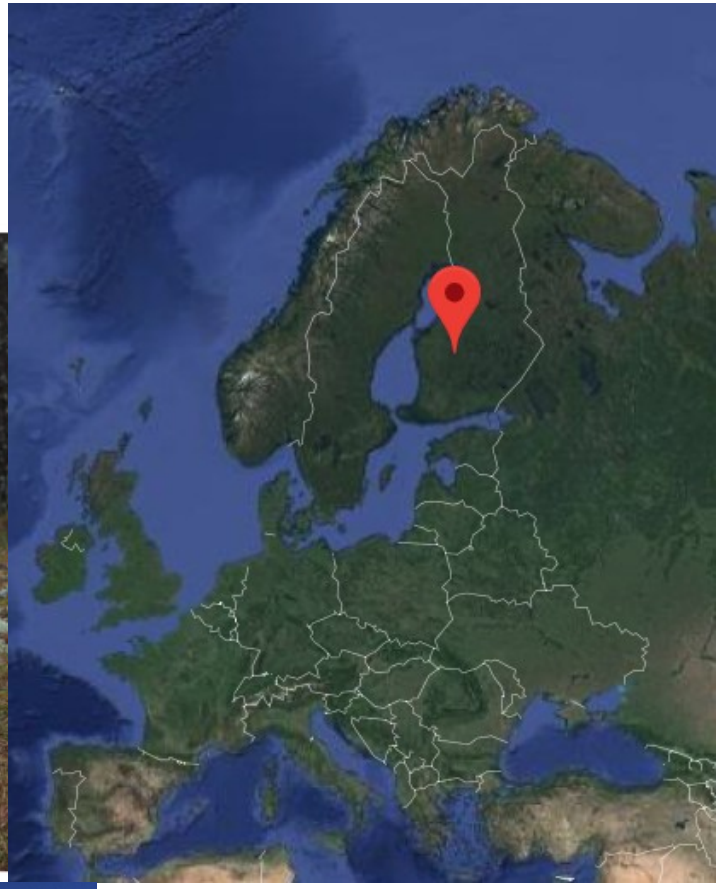
WILEY MOLECULAR ECOLOGY RESOURCES

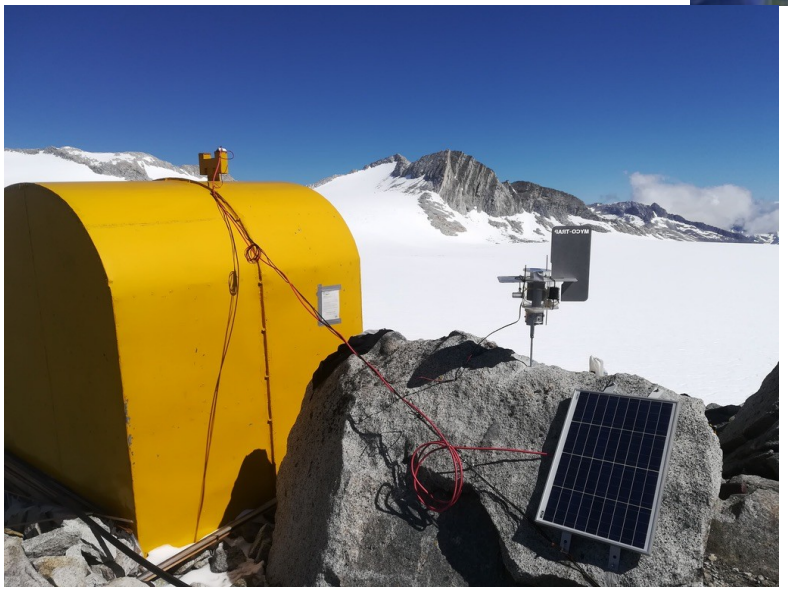
Give me a sample of air and I will tell which species are found from your region: Molecular identification of fungi from airborne spore samples

Nerea Abrego[1] | Veera Norros[2,3] | Panu Halme[4] | Panu Somervuo[2] | Heini Ali-Kovero[2] | Otso Ovaskainen[2,5]

Mol Ecol Resour. 2018;18:511–524.

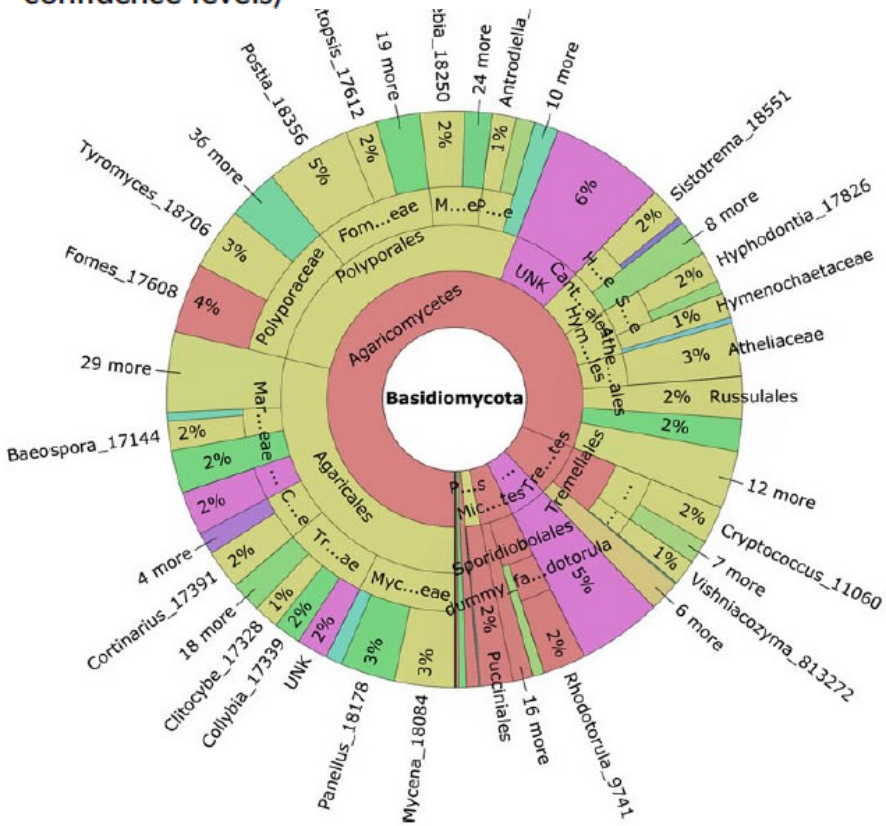


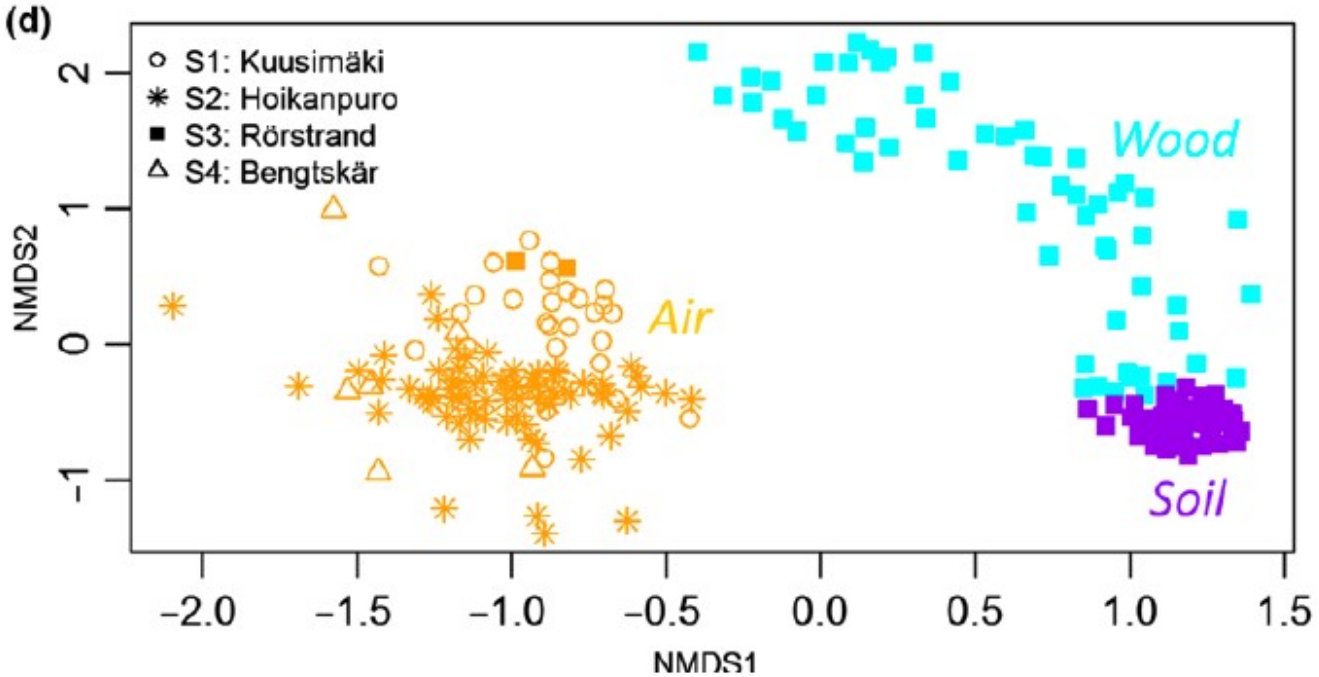


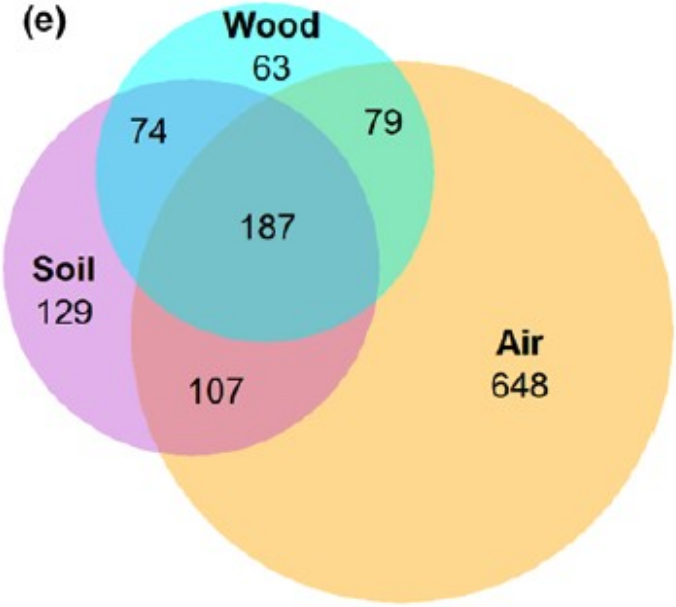


Pian di Neve, Adamello
Giannantonj, 3200 m

FIGURE 2 The taxonomic distribution of the samples for the pooled data, either for Ascomycota (a) or Basidiomycota (b). The pie charts are snapshots from interactive web page (provided as Appendix S2), in which the taxonomic compositions can be studied in detail. The colours corresponding to confidence level 1–2 highlight >50% reliable classifications to well-identified taxa (see Somervuo et al., 2017 for explanations of the other confidence levels)







Most relevant conclusions:

-dispersal limitation at intermediate to large scales: aerial fungal composition was clearly different between sites (>100 km apart) but not within sites (<10 km apart)

-eDNA on aerial samples can simultaneously characterize composition for fungi growing on many kinds of substrates (wood and soil)

-opportunity for early detection of pathogenic species (agriculture, forestry or house construction) already upon their arrival as spores, e.g. *Heterobasidion annosum* and *Claviceps purpurea*

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Pollination: ecosystem service

South Africa: National Insects Collection

ORIGINAL ARTICLE

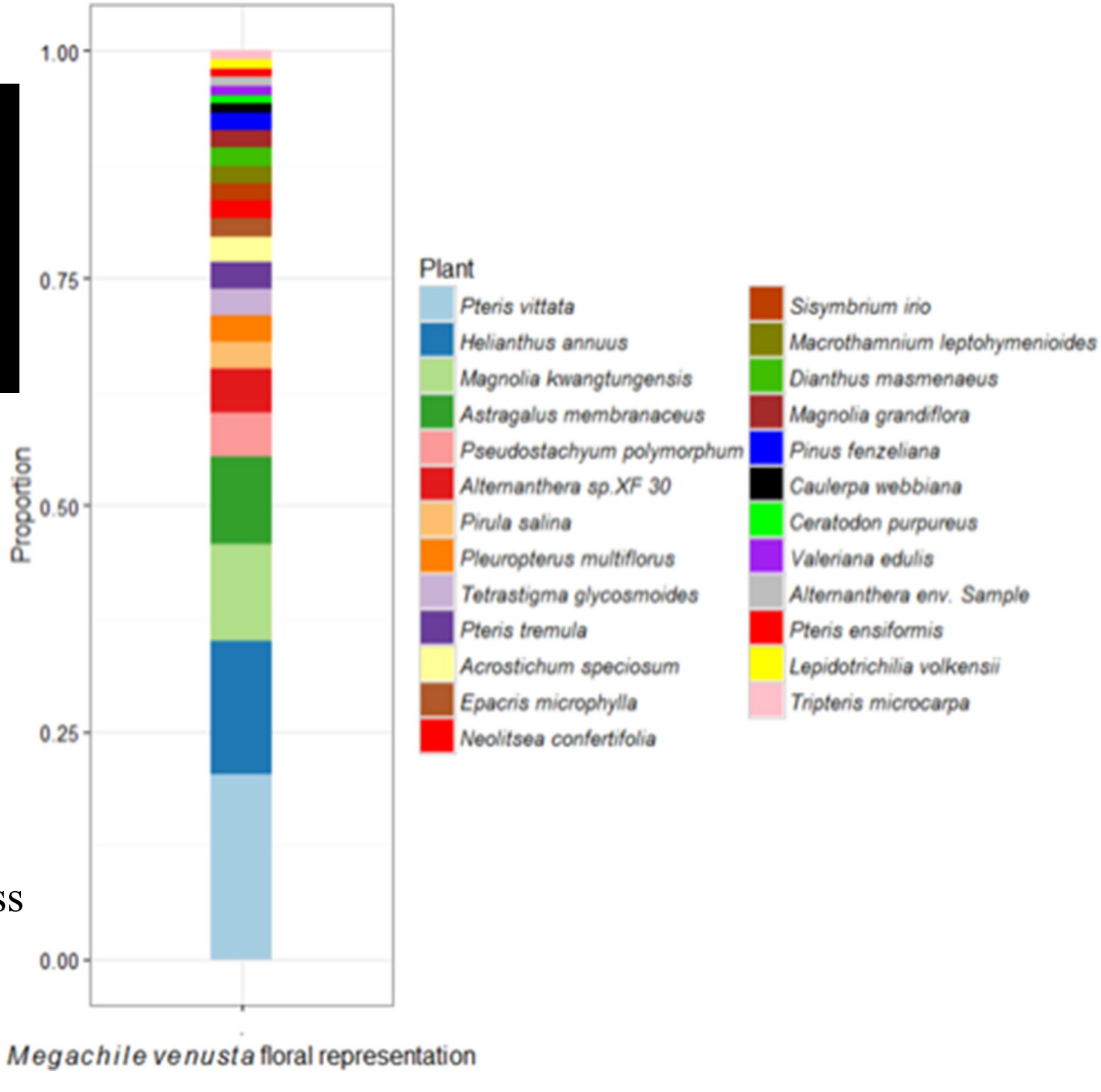
WILEY Evolutionary Applications Open Access

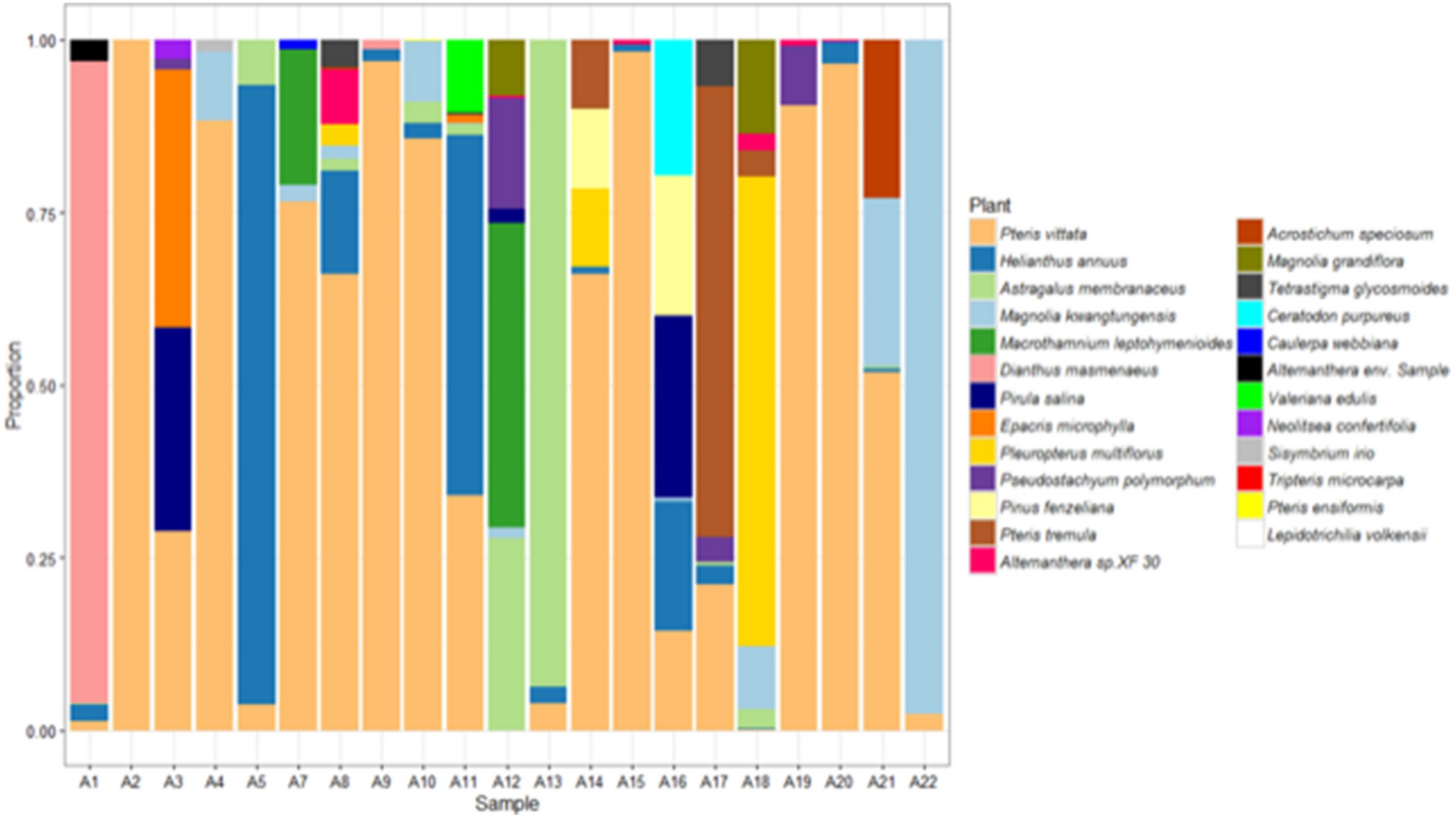
Plant–pollinator interactions over time: Pollen metabarcoding from bees in a historic collection

Annemarie Gous[1,2] | Dirk Z. H. Swanevelder[1,3] | Connal D. Eardley[2,4] | Sandi Willows-Munro[2]

Evolutionary Applications. 2019;12:187–197.

Different biomes across South Africa over a period of 93 years (1914–2007)





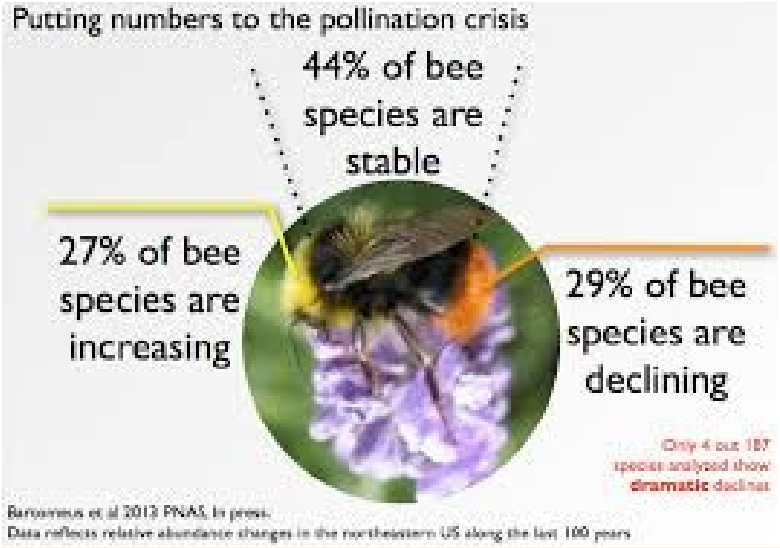
Bar graph representing 22 samples classified with the ITS2 database. The different colours indicate different plant species identified with the sequence database, and in which percentage it was detected in each sample

Most relevant conclusions:

-Pollen eDNA metabarcoding of ***historic collections*** opens up the possibility to reconstruct the plant communities that pollinators visited in the past and their associated changes through time.

-understanding the ***influences*** of factors such as ***climate change and land use change*** on plant–pollinator interactions could prove vital in the conservation of vulnerable species, both plant and animal

-Species-level plant classification is possible with ITS2, but ***without a comprehensive*** local plant sequence ***reference database***, ***family based interpretations are more reliable.***



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INVITED REVIEWS AND SYNTHESES

Molecular Ecology. 2017;26:5872–5895.

Environmental DNA metabarcoding: Transforming how we survey animal and plant communities

Kristy Deiner[1] | Holly M. Bik[2] | Elvira Mächler[3,4] | Mathew Seymour[5] | Anaïs Lacoursière-Roussel[6] | Florian Altermatt[3,4] | Simon Creer[5] | Iliana Bista[5,7] | David M. Lodge[1] | Natasha de Vere[8,9] | Michael E. Pfrender[10] | Louis Bernatchez[6]

WORKFLOW

Study design

Basic science or applied? (e.g., environmental biomonitoring)

What is your study goal?
- presence/absence
- diversity assessment
- absolute quantification

What taxa will you target?

Is the scale of inference for your sample type appropriate to your question?

Can you compare complementary data types? (e.g. traditional vs. eDNA)

Does your sampling/ replication scheme provide good statistical power?

In the field

What type of sample is needed? (water, soil, air)

What metadata should you collect?

How many replicates will you collect?

Does your sampling protocol minimize/ control for :
- **contamination** (e.g., positive and negative controls)
- **any known biases** (e.g., inhibitors, sample volume)

In the laboratory

Sample Handling Phase

What extraction method? (physical vs. chemical)

How much sample?

What locus and primers?

Do you need to generate reference sequence data?

Are technical replicates needed?

What library preparation method will you use?

How many samples will you index and pool?

What sequence depth is needed per sample ?

What read length will you use?

DNA Processing Phase

What sequencing platform will you use?

Do you need paired-end sequencing?

Have you included appropriate quality assurances? (e.g., mock community, qPCR, bioanalyser traces)

Does your laboratory protocol minimize/ control for:
- **contamination** (e.g., positive and negative controls)
- **any known biases** (e.g., primer bias, coverage, taxonomic resolution)

At the keyboard

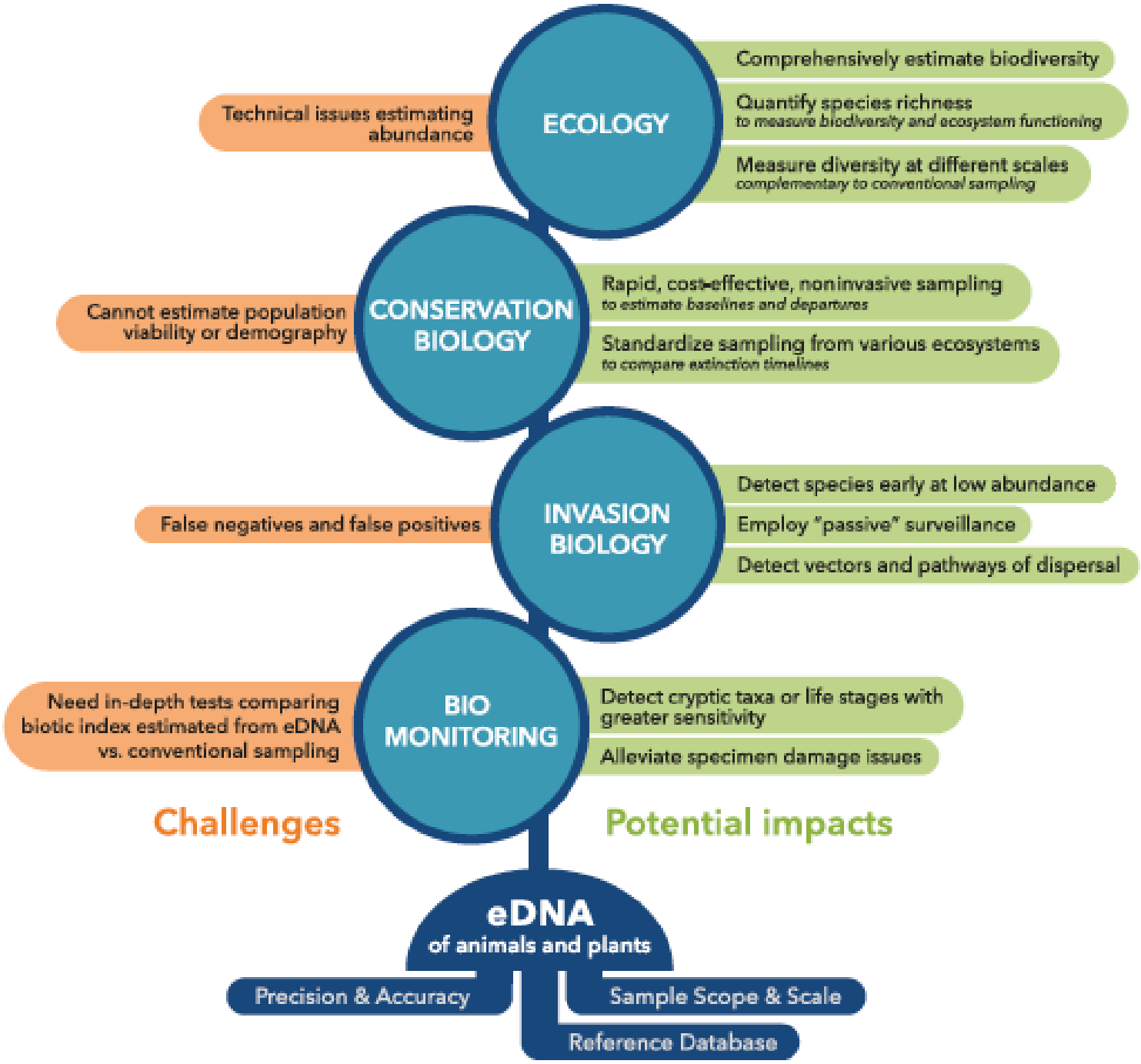
How complete is the reference database?

Do you have adequate sequencing coverage across samples?

Are you using appropriate choices for software tools, parameters?

Are your biological conclusions upheld using alternative parameters and workflows?

Are you including appropriate quality filtering of your data? (see Box 2)


ECOLOGY
Technical issues estimating abundance
Comprehensively estimate biodiversity
Quantify species richness
to measure biodiversity and ecosystem functioning
Measure diversity at different scales
complementary to conventional sampling
CONSERVATION BIOLOGY
Cannot estimate population viability or demography
Rapid, cost-effective, noninvasive sampling
to estimate baselines and departures
Standardize sampling from various ecosystems
to compare extinction timelines
INVASION BIOLOGY
False negatives and false positives
Detect species early at low abundance
Employ "passive" surveillance
Detect vectors and pathways of dispersal
BIO MONITORING
Need in-depth tests comparing biotic index estimated from eDNA vs. conventional sampling
Detect cryptic taxa or life stages with greater sensitivity
Alleviate specimen damage issues
Challenges
Potential impacts
eDNA
of animals and plants
Precision & Accuracy
Sample Scope & Scale
Reference Database

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Where:

Adamello glacier, Italy

Largest – 16 km²

Deepest – 270 m (estimate)

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What

Biological particles embedded in the ice

We extracted a 45 m ice core from Adamello in April 2016

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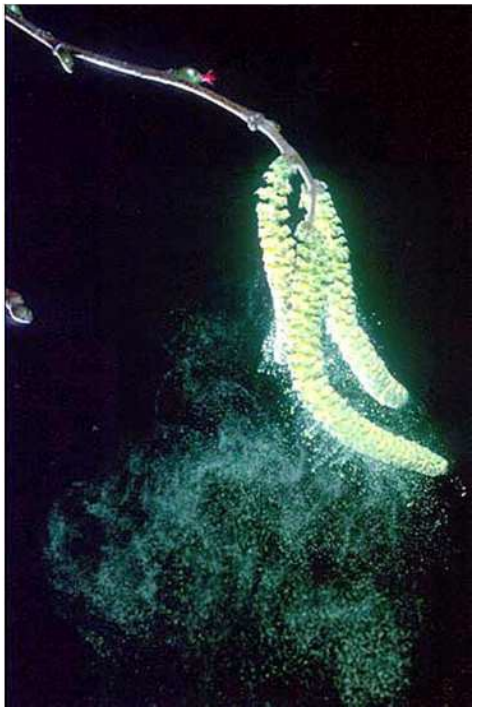

The Cryosphere Discuss., doi:10.5194/tc-2016-221, 2016
Manuscript under review for journal The Cryosphere
Published: 22 November 2016

The Cryosphere Discussions
Open Access
EGU

Linking pollen deposition, snow accumulation and isotopic composition on the Alto dell'Ortles glacier (South Tyrol, Italy) for sub-seasonal dating of a firn temperate core

Daniela Festi[1], Luca Carturan[2], Werner Kofler[1], Giancarlo dalla Fontana[2], Fabrizio de Blasi[2], Federico Cazorzi[3], Edith Bucher[4], Volkmar Mair[5], Paolo Gabrielli[6,7], Klaus Oeggl[1]

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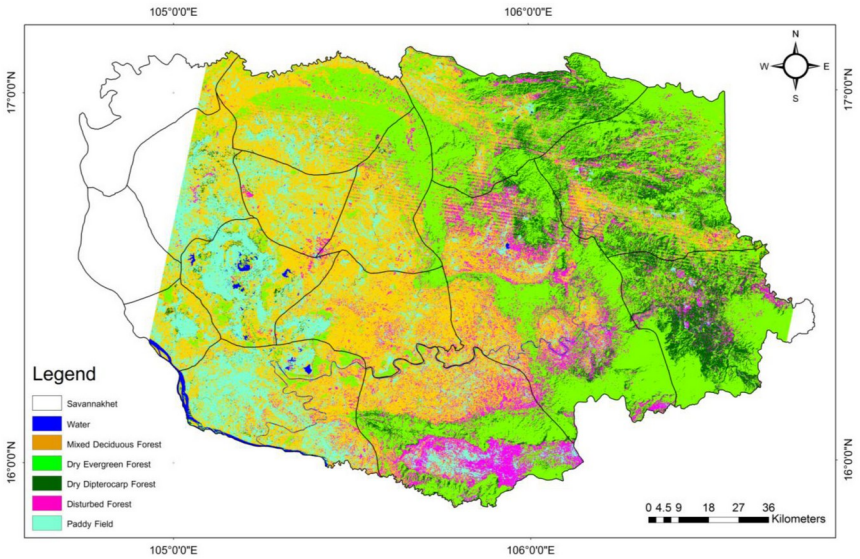

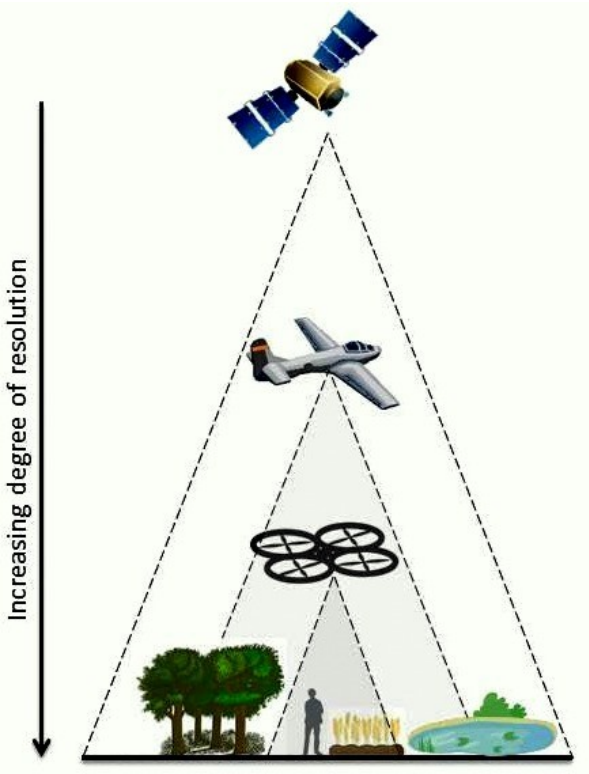

Why

Testing the hypothesis that the biodiversity preserved in ice cores reflects that in the catchment area of the glacier

In this case, we could have a record of biodiversity changes through time

We could link these changes to precisely dated man-related events

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Results

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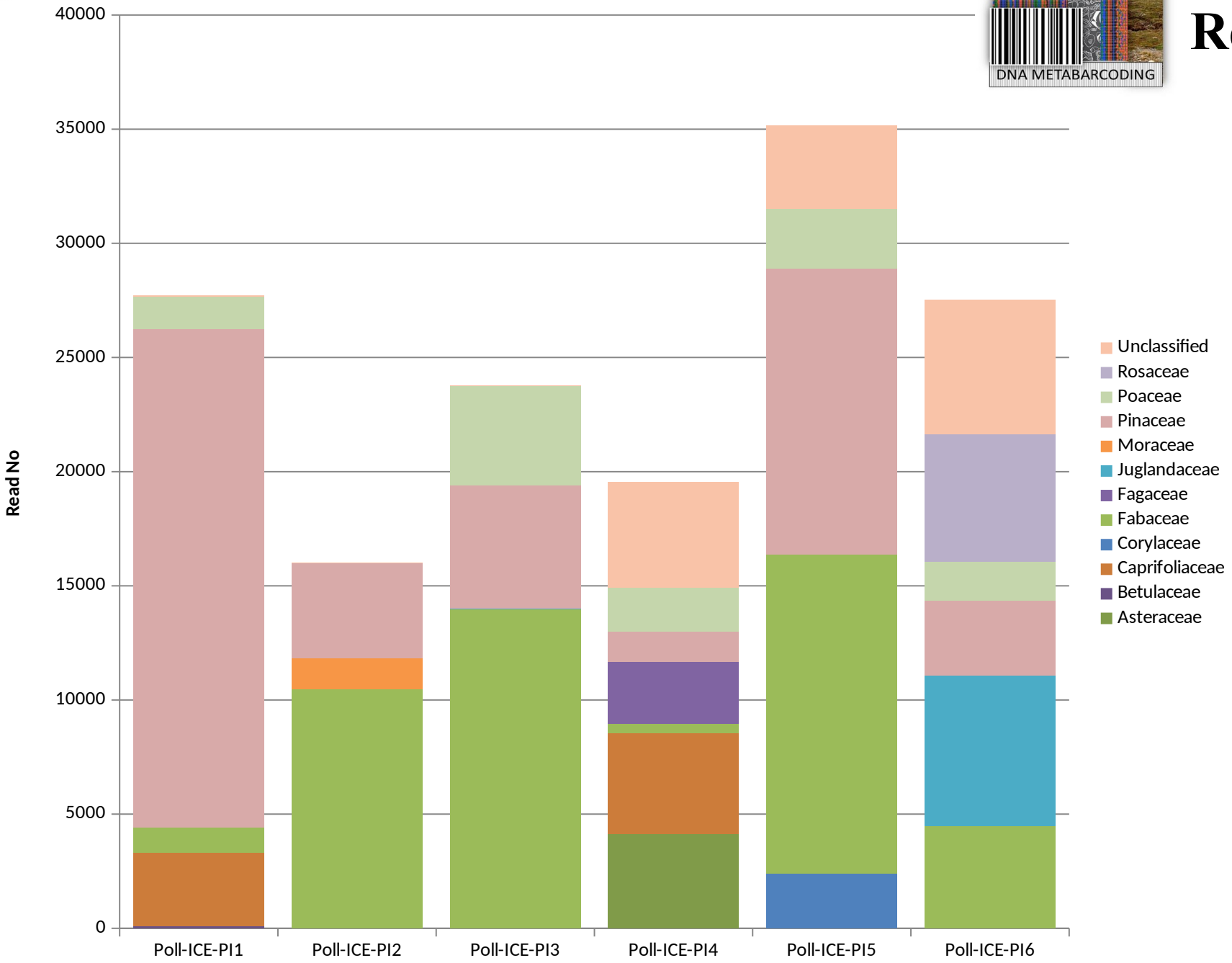
Results



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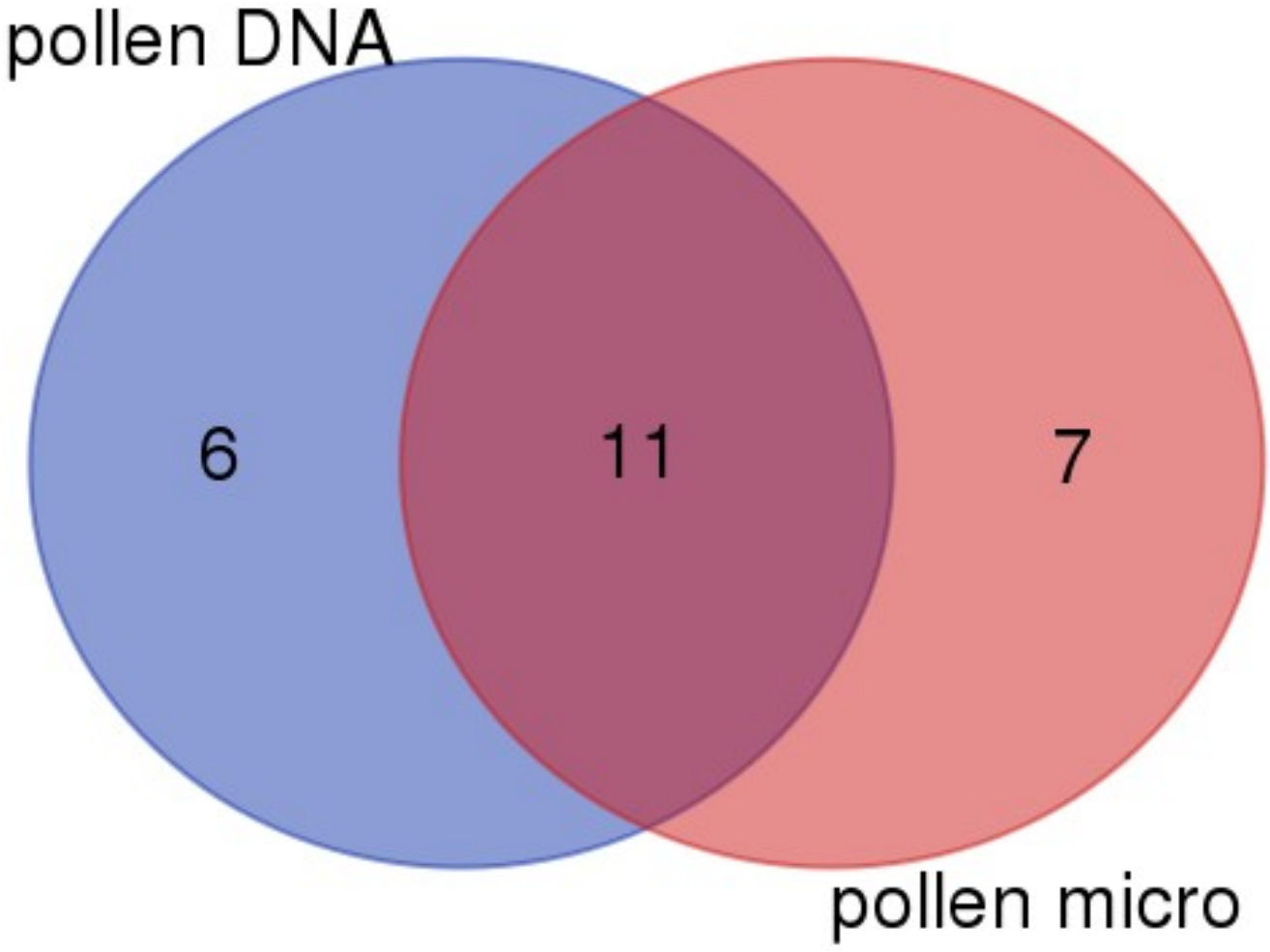
Results

Venn diagram
Number of plant families



Investigating unconventional archives

Earth and Planetary Science Letters 302 (2011) 95–106

Contents lists available at ScienceDirect

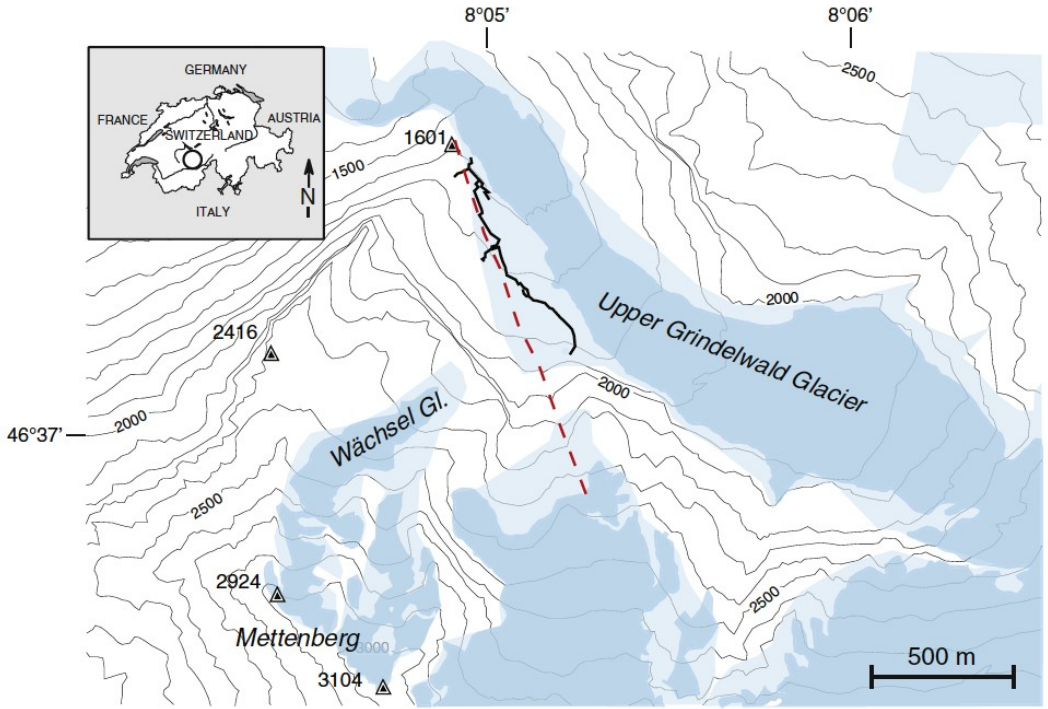
Earth and Planetary Science Letters

journal homepage: www.elsevier.com/locate/epsl

Holocene glacier history from alpine speleothems, Milchbach cave, Switzerland

M. Luetscher [a,b,*], D.L. Hoffmann [c], S. Frisia [d], C. Spötl [a]

[a] Institute of Geology and Paleontology, University of Innsbruck, Austria
[b] Swiss Institute for Speleology and Kars
[c] CENIEH, Burgos, Spain
[d] Earth Sciences, University of Newcastle



Quaternary Research 86 (2016) 45–53

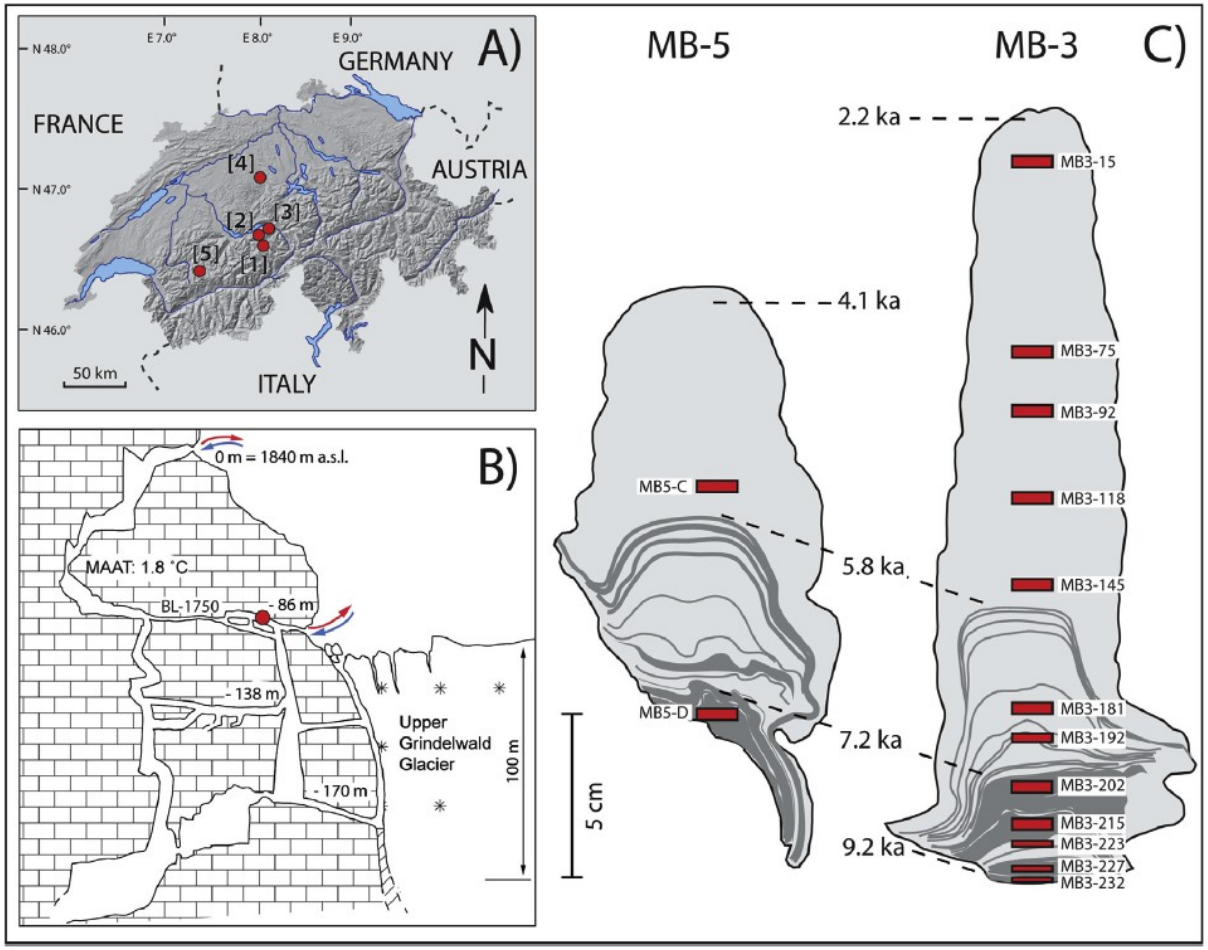
Contents lists available at ScienceDirect

Quaternary Research

journal homepage: http://www.journals.elsevier.com/quaternary-research

Pollen from accurately dated speleothems supports alpine glacier low-stands during the early Holocene

Daniela Festi [a, *], Dirk L. Hoffmann [b], Marc Luetscher [c, d]



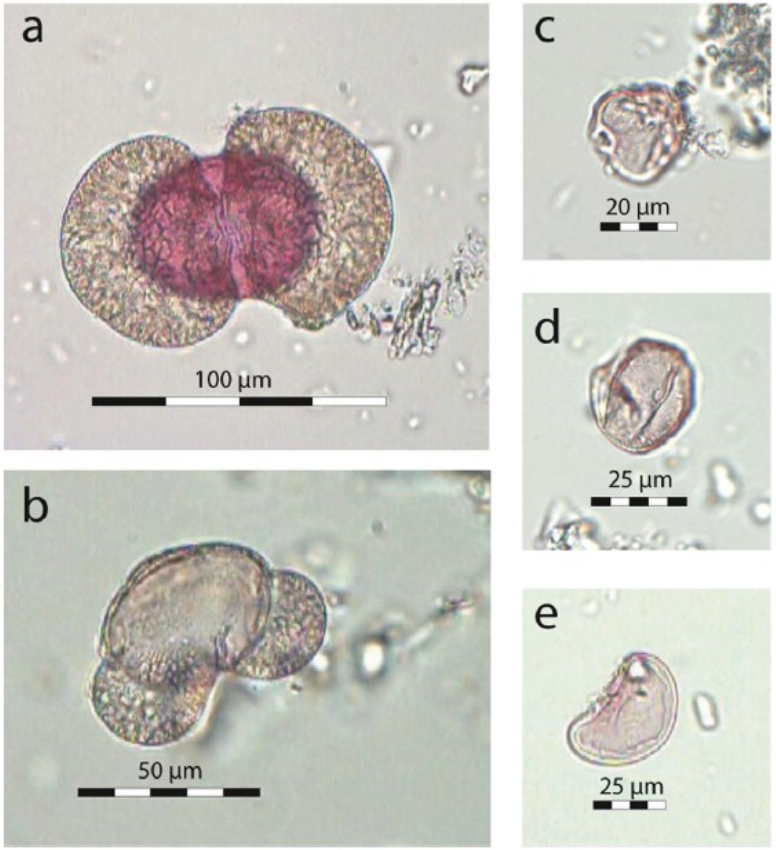
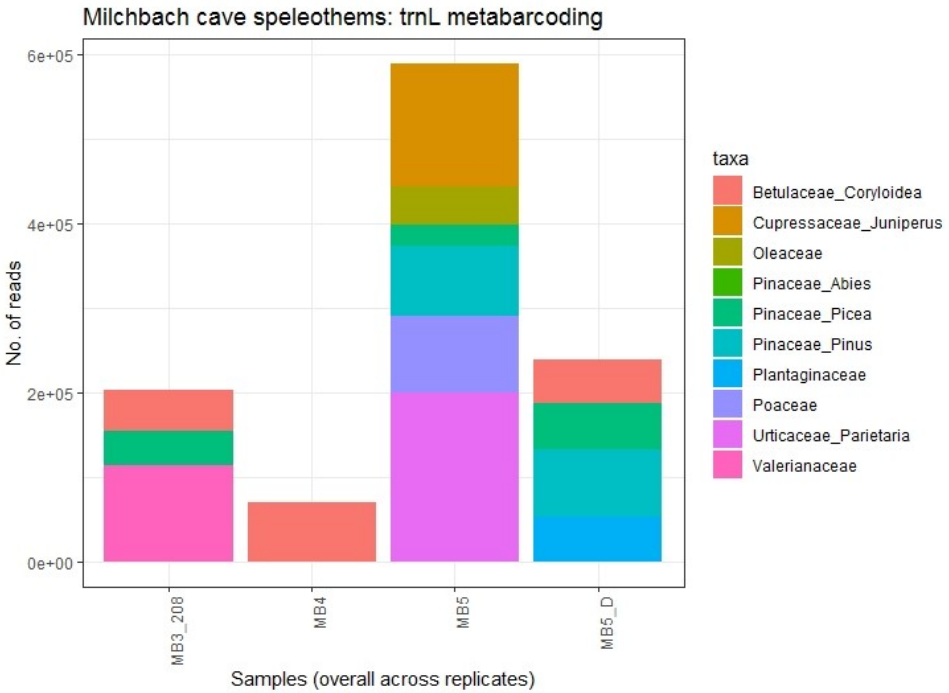
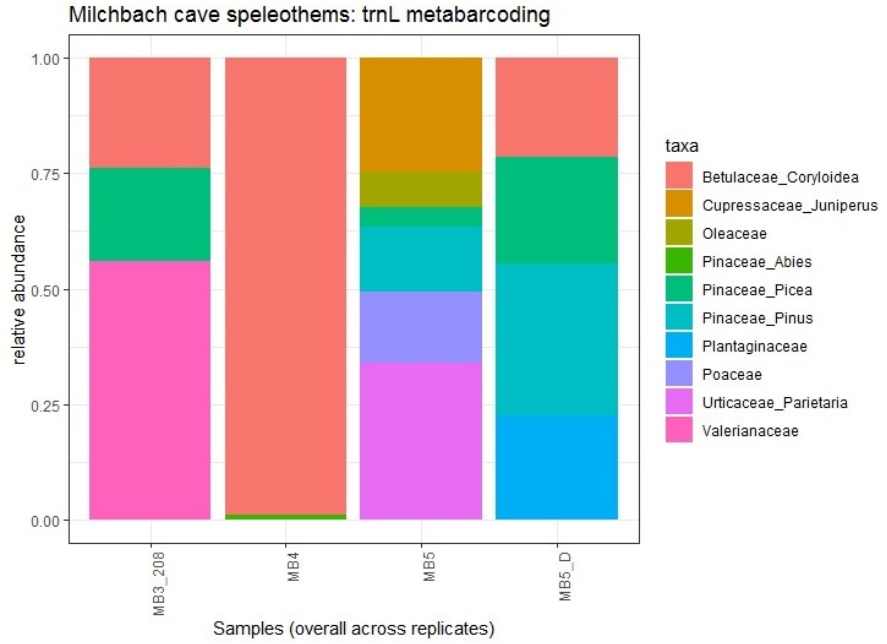


Figure 5. Palynomorphs recorded in Milchbach cave samples. **a.** *Abies*; **b.** *Pinus* sp.; **c.** *Tilia*; **d.** *Quercus robur*-type; **e.** monolete spore.





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- Alexis Marchesini , CNR
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- Daniela Festi, Naturhistorisches Museum Wien