Characterisation of East Siberian Paleodiversity based on ancient DNA analyses of the Batagay megaslump exposure

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Paleodiversity studied from DNA?

**Metabarcoding sequencing**: established method

**Shotgun sequencing**: new method (few studies from environmental ancient DNA\(^1\))

Sample → DNA extraction

- **Use of primers** (trnl g/h\(^2\)) + PCR amplification (P6 loop)
- **Library** preparation ssDNA2.0\(^3\)
- **Sequencing amplified DNA** (Plants here)
- **Sequencing all DNA extracted** (All taxa)
- **Bioinformatic and statistical analyses**
  - Taxa assignment with **OBITools to EMBL/Arctborbryyo databases**\(^4\);
  - with **kraken2 to full genome chloroplast database**\(^5\)

Can shotgun sequencing be used as a new robust proxy to investigate paleodiversity?
The study site: Batagay megaslump

- **World largest known thaw slump**: 840m wide and ~55m deep in 2016

- Exposes Holocene and Pleisocene permafrost formations **from MIS16 to MIS 1**

Expedition in 2017, **5 samples for preliminary DNA investigation:**

<table>
<thead>
<tr>
<th>Depth (m)</th>
<th>2.15</th>
<th>2.3</th>
<th>25.8</th>
<th>47.6</th>
<th>49.5</th>
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</thead>
<tbody>
<tr>
<td>MIS</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>7-6</td>
<td>16</td>
</tr>
</tbody>
</table>

More samples recovered on a second expedition in 2019
Past vegetation direct comparison

Preliminary results

- **Metabarcoding**: more reads assigned to plants and reads counts more consistent

- **Shotgun**: decrease in reads count with age and huge drop for MIS 16

- More families detected with shotgun in all samples

- In the shotgun: many plant taxa detected by *less than 10 reads*

**Shotgun detect more plant taxa with less reads**
Past vegetation direct comparison

Comparison between samples of relative proportion for the 12 most represented plant families present in both methods.

- **Similar signal** between methods: same overrepresented families (e.g. Asteraceae or Poaceae) and similar trends between samples.

- **Some differences** though: e.g. Fabaceae, always more represented with shotgun and some signal only present in metabarcoding (e.g. Pinaceae).

Shotgun signal is as relevant as metabarcoding one: similar ecological conclusions can be drawn with both methods.
Shotgun provides more

**Preliminary results**

**Shotgun provides also relevant information on other kingdoms**

**Zoom in Bacteria**: typical phylum for permafrost samples

**Zoom in Mammals**: typical pleistocene taxa such as Mammuthus or Mylodon

- Plot without primates

- **Bacterial reads assigned vs NCBI nt database**
  - Actinobacteria
  - Alphaproteobacteria
  - Betaproteobacteria
  - Gammaproteobacteria
  - Delta- and epsilon-proteobacteria
  - Firmicutes
  - Planctomycetes
  - Bacteroidetes/Chlorobi
  - Others

- **Mammals reads assigned vs Metazoan mitochondria genome database**
  - Rodentia
  - Other Artiodactyla
  - Bovidae
  - Suidae
  - Cervidae
  - Ursidae
  - Mustelidae
  - Canidae
  - Felidae
  - Chiroptera
  - Equidae
  - Eulipotyphla
  - Mammuthus primigenius
  - Mylodon darwini
  - Bradypus variegatus
References


6. **Opel, T. et al.** (2018) „Middle and Late Pleistocene climate and continentality inferred from ice wedges at Batagay megaslump in the Northern Hemisphere’s most continental region, Yana Highlands, interior Yakutia“, *Climate of the Past Discussion*, 1-32.

7. **Murton, J. B. et al.** in preparation