Exploring diversity of marine eukaryotes across 385 ka old gravity core using sedaDNA

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Context

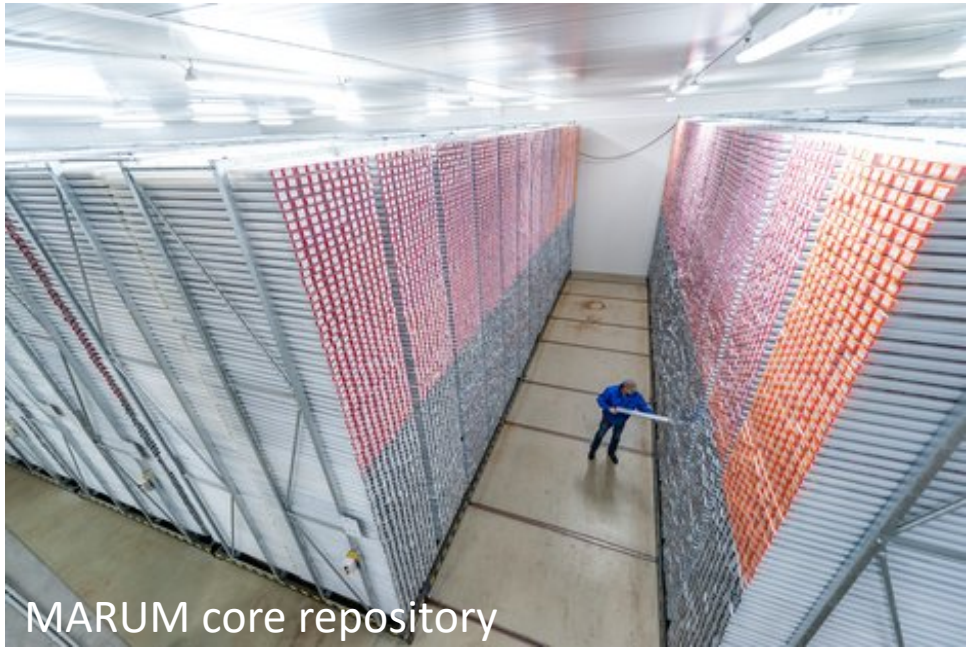
Cores repositories store archive half cores not used for geological analysis.

They are stored at 4°C, the temperature of the ocean floor from where they were recovered.

Working with sedaDNA requires rigour and precautions from sampling to sequencing to avoid contaminants.

In most studies, the cores are freshly recovered and processed immediately.

Here we test the potential of cores stored during several years.



MARUM core repository

ELSEVIER

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Ancient DNA from marine sediments: Precautions and considerations for seafloor coring, sample handling and data generation

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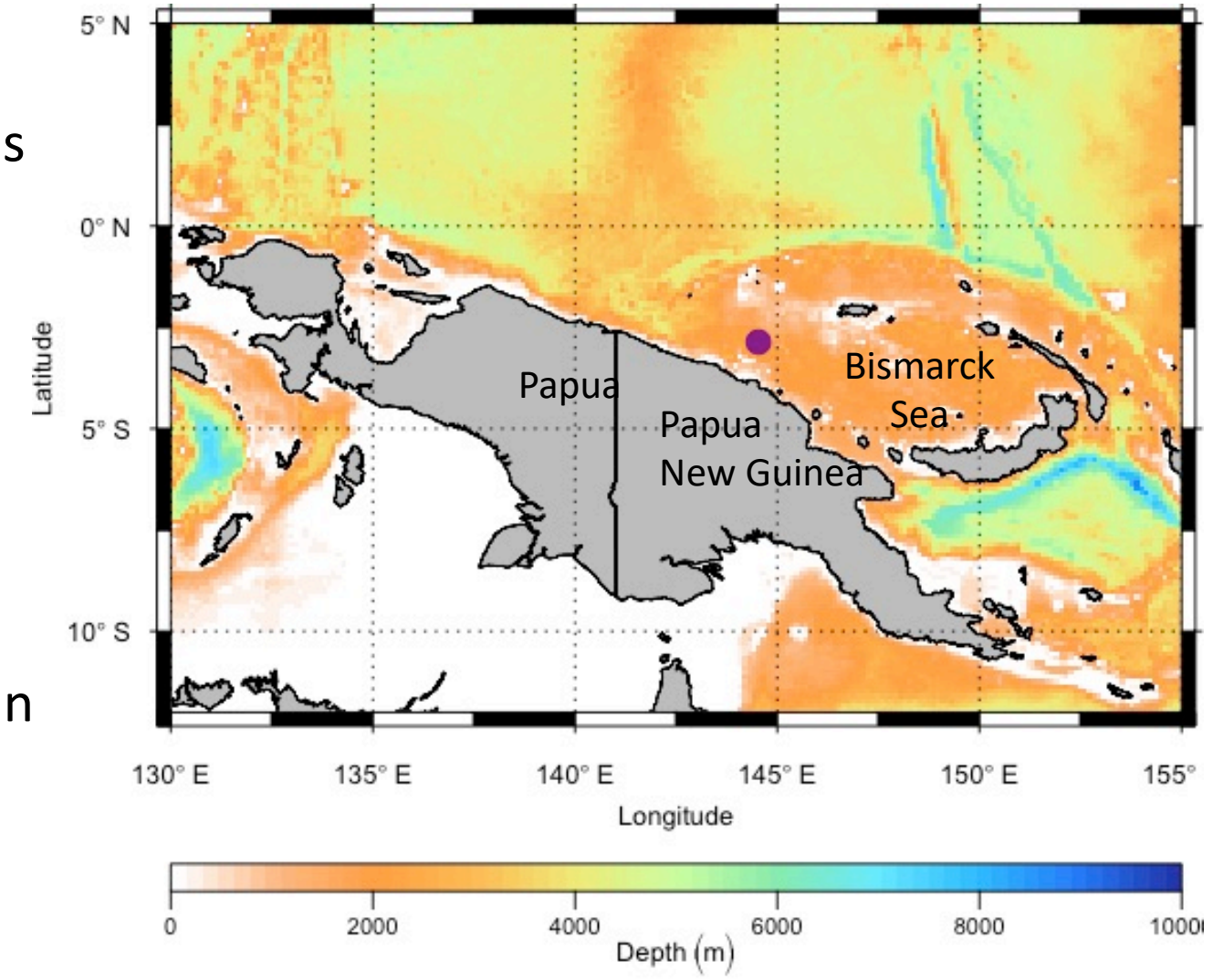
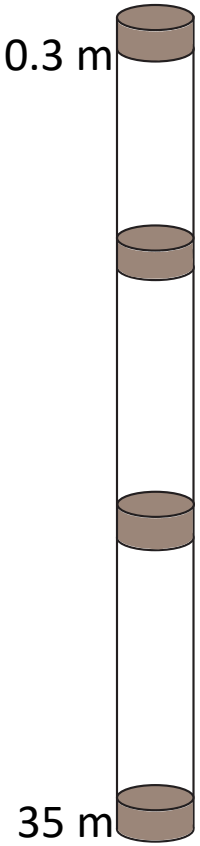
Core MD05-2920

Aim:

- Could aDNA preserved in cores stored for several years at 4°C storage?
- Could obtained aDNA data be usable?

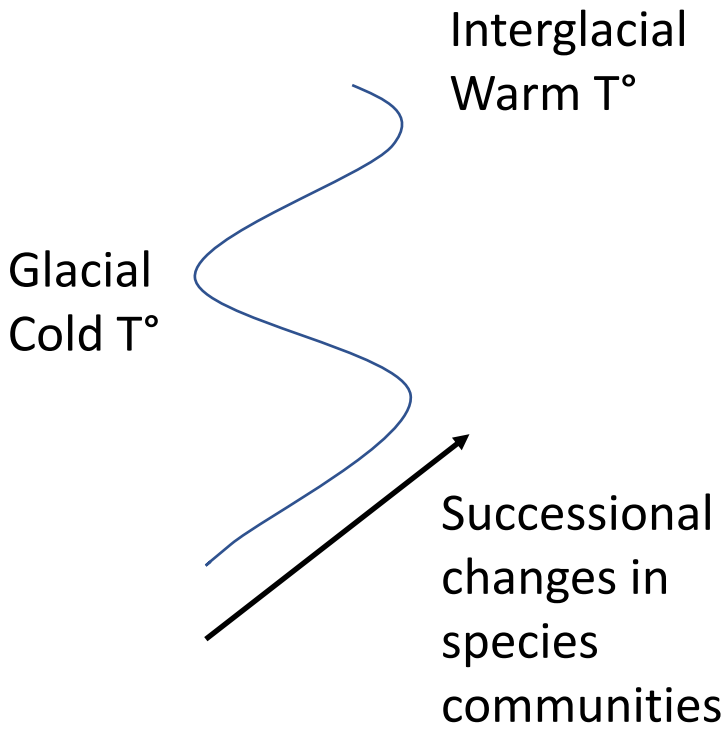
How: subsampling an “archive half “ core and sequencing the specific regions of 18S rRNA gene



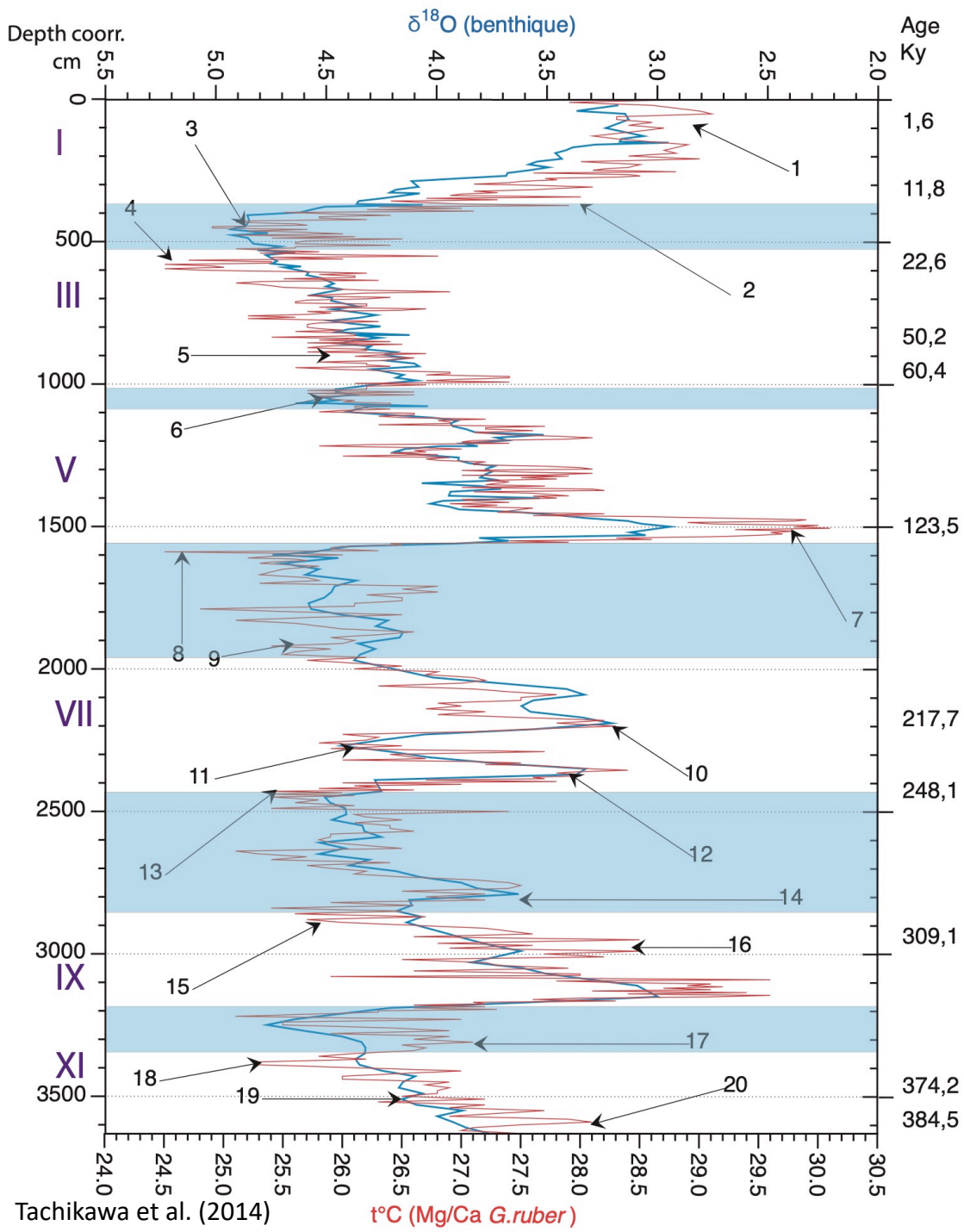


Recovered: 2005
DNA extraction: 2019
Water depth: 1843m
Sedimentation rate: 10cm/ky

Climate setting

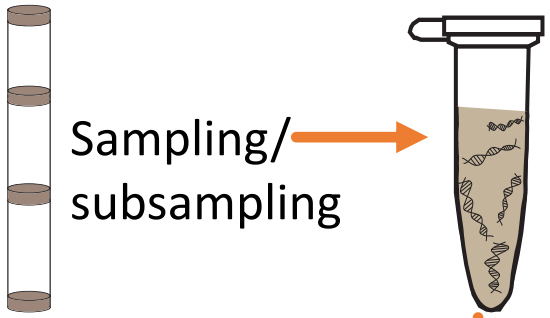


- Samples were selected from inter- and glacial events
- Blue zone = glacial periods
- Numbers in purple (y-axis) = marine isotopic stage (MIS)



Tachikawa et al. (2014)

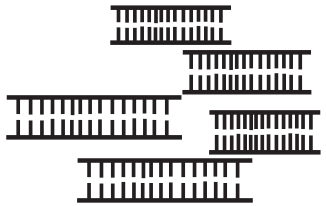
Methods - Metabarcoding



Sampling/
subsampling

DNA
extraction

PCR
Amplification

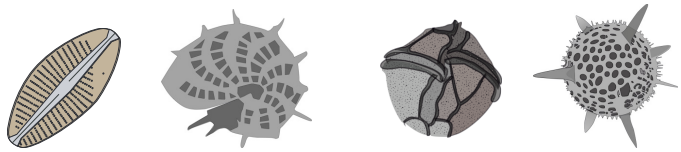


Sequencing

Bioinformatics

\+ Reference
database

Species identification



Precautions:

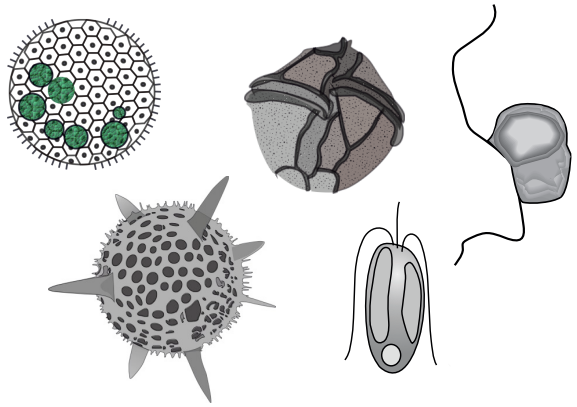
- Subsampling an intact “ archive half core”
- Clean working place
- Negative controls

Methods – regions from SSU ribosomal RNA gene

To target planktonic DNA, we use several specific regions of 18S rRNA gene.

V9

130 - 200 bp

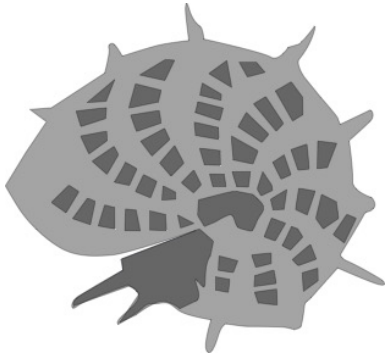


eukaryotes

Amaral- settler et al. 2009

37f - 41f

320-400 bp

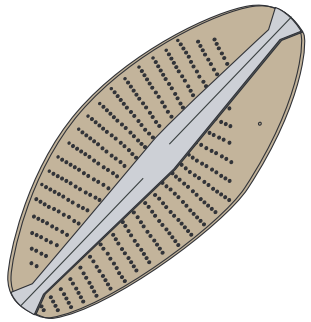


foraminifera

Pawlowski et Lecroq 2010

V4 diatoms (DIV4)

~ 400 bp



diatoms

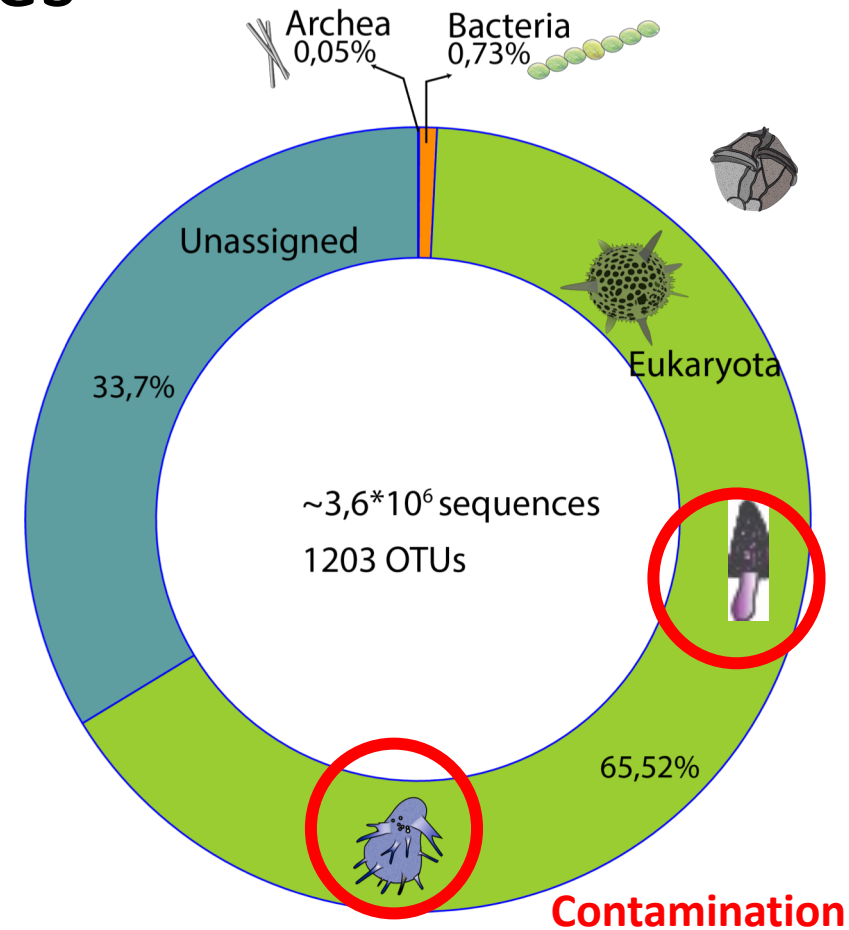
Zimmermann et al. 2011

Advantage of short regions -> aDNA is already degraded and fragmented

Results: V9 - eukaroytes

From which phylum/species aDNA can be obtained?

- Eukaryotes:
  - Alveolata
  - Rhizaria
  - Stramenopiles
  - Amoebozoa
  - Opisthokonta

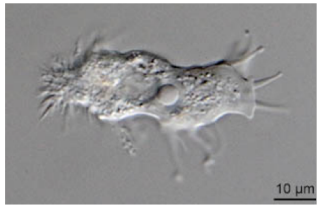


The first 3 are mostly composed of planktonics species and cannot come from an ex-situ (storage) contamination

Contamination!
In situ during the storage

Negative
Controls -> ok

Acanthamoeba sp.+ funghi dominates the assemblage (80 %)

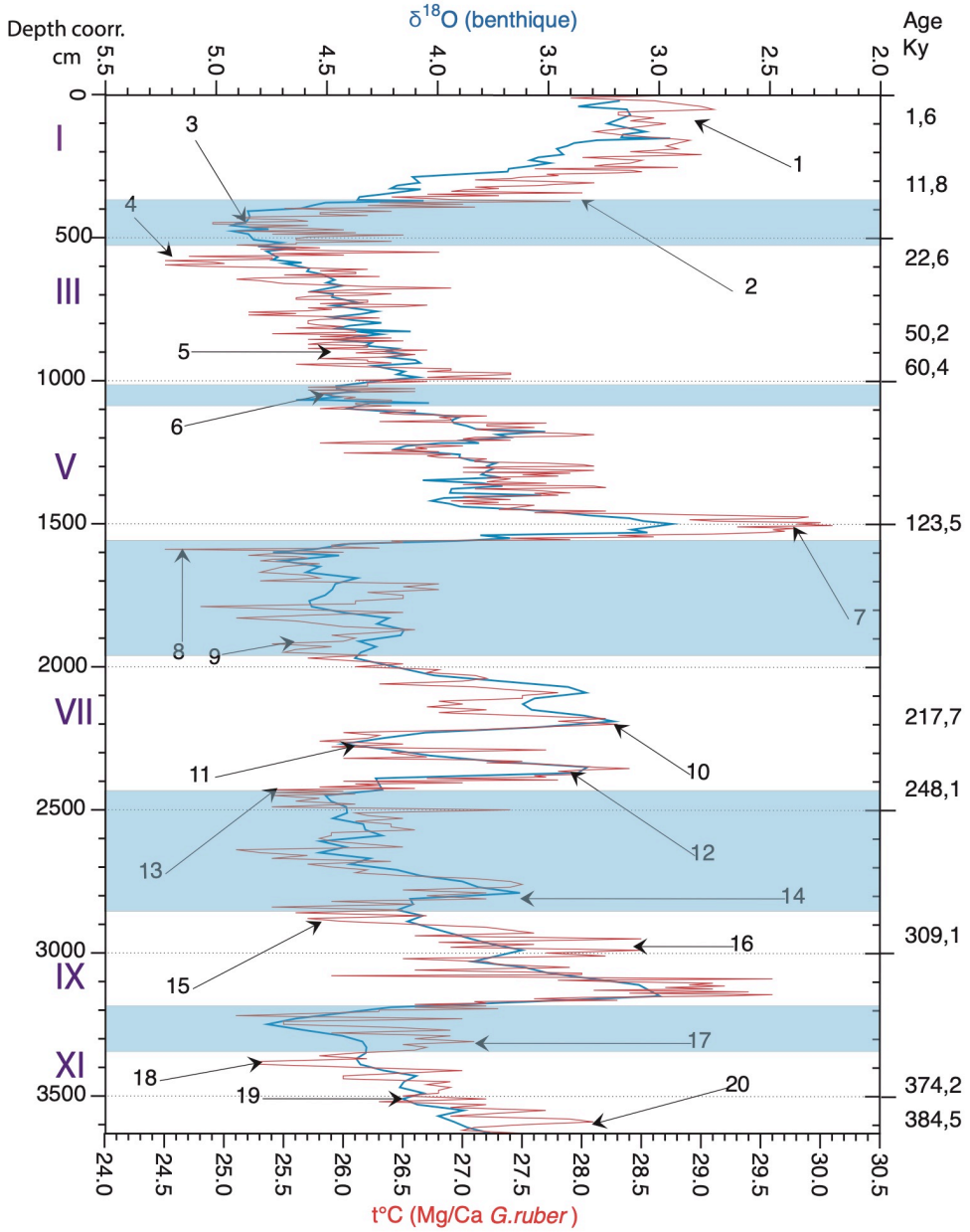


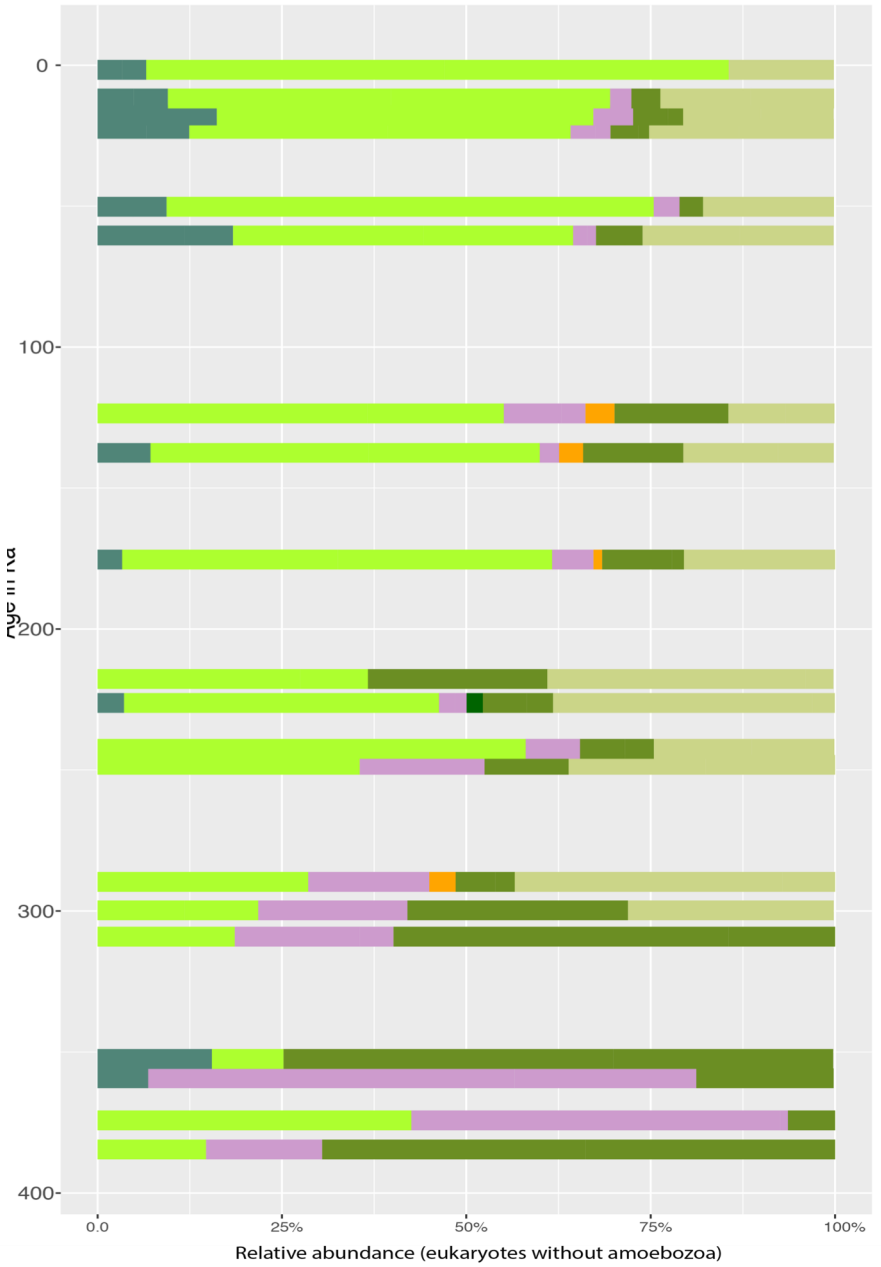
Results: V9 – Planktonic species

Planktonic sequences were found at each layer (in green colours)

Main planktonic phylum:

- Rhizaria (foraminifera + radiolaria)
- Alveolata
- Archeaplastida
- Stramenopiles



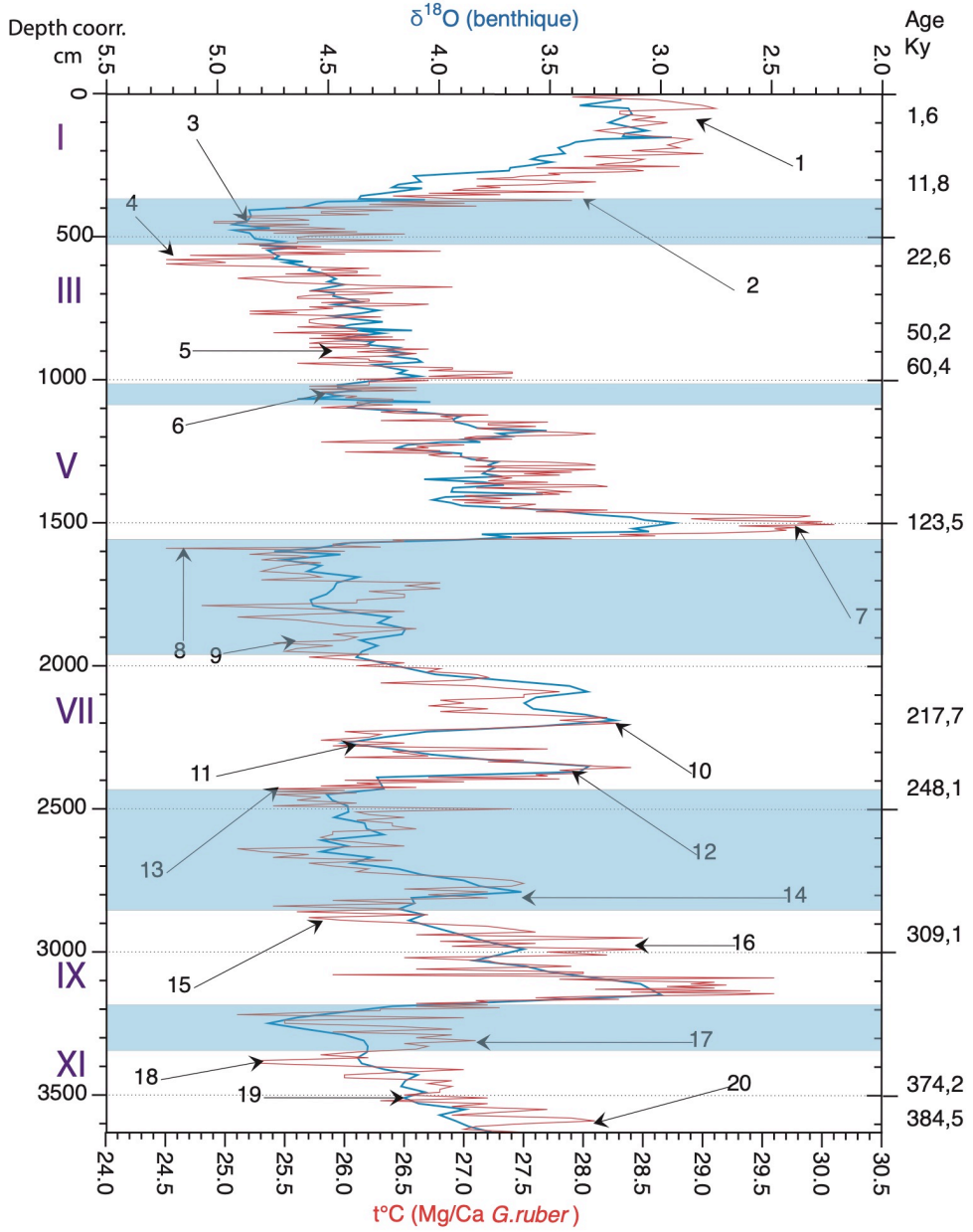


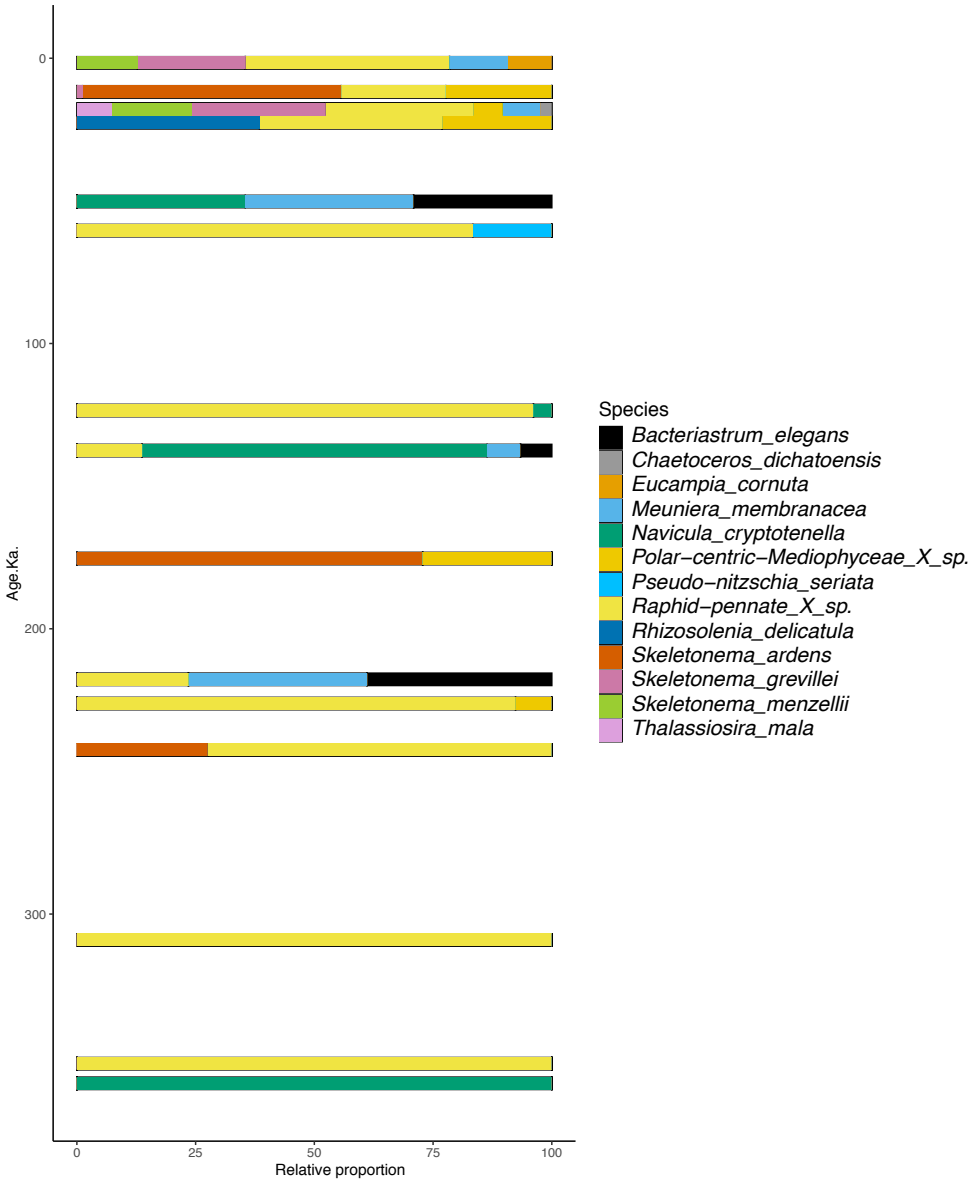
Results: V4 - diatoms

5/20 layers do not amplified.

Species: Raphid pennate is present throughout the core excepts around glacial Periods

N. cryptotenella is freshwater diatoms, probably inputs from rivers. The site is 100 km off Sepik river.



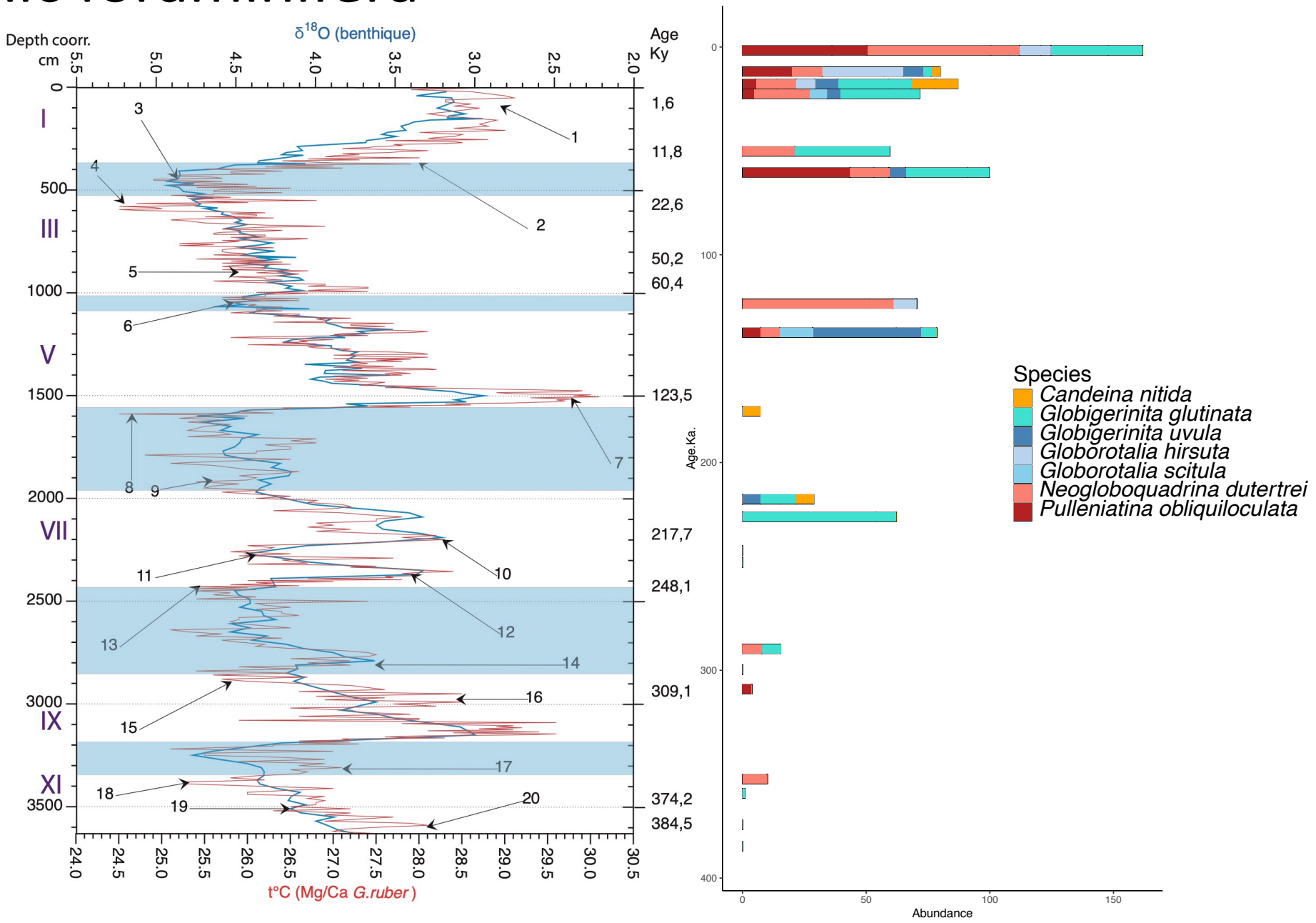


Results: planktonic foraminifera

5/20 layers do not amplified.

Typical assemblages of tropical sites:
- warm waters (in red/orange colours)
- temperate waters (blue colours)

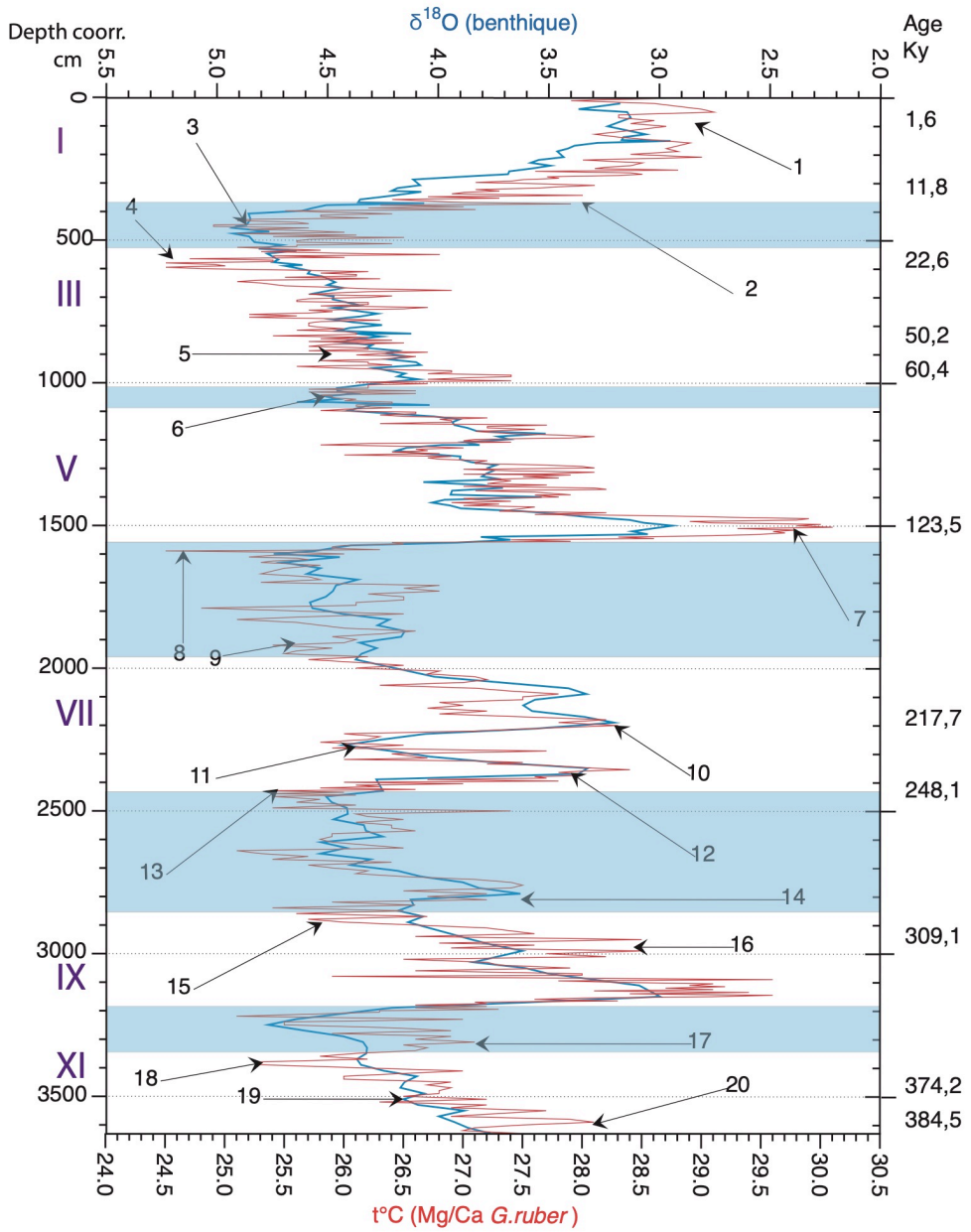
Some samples are dominated by temperate assemblages, those samples correspond to less warm periods

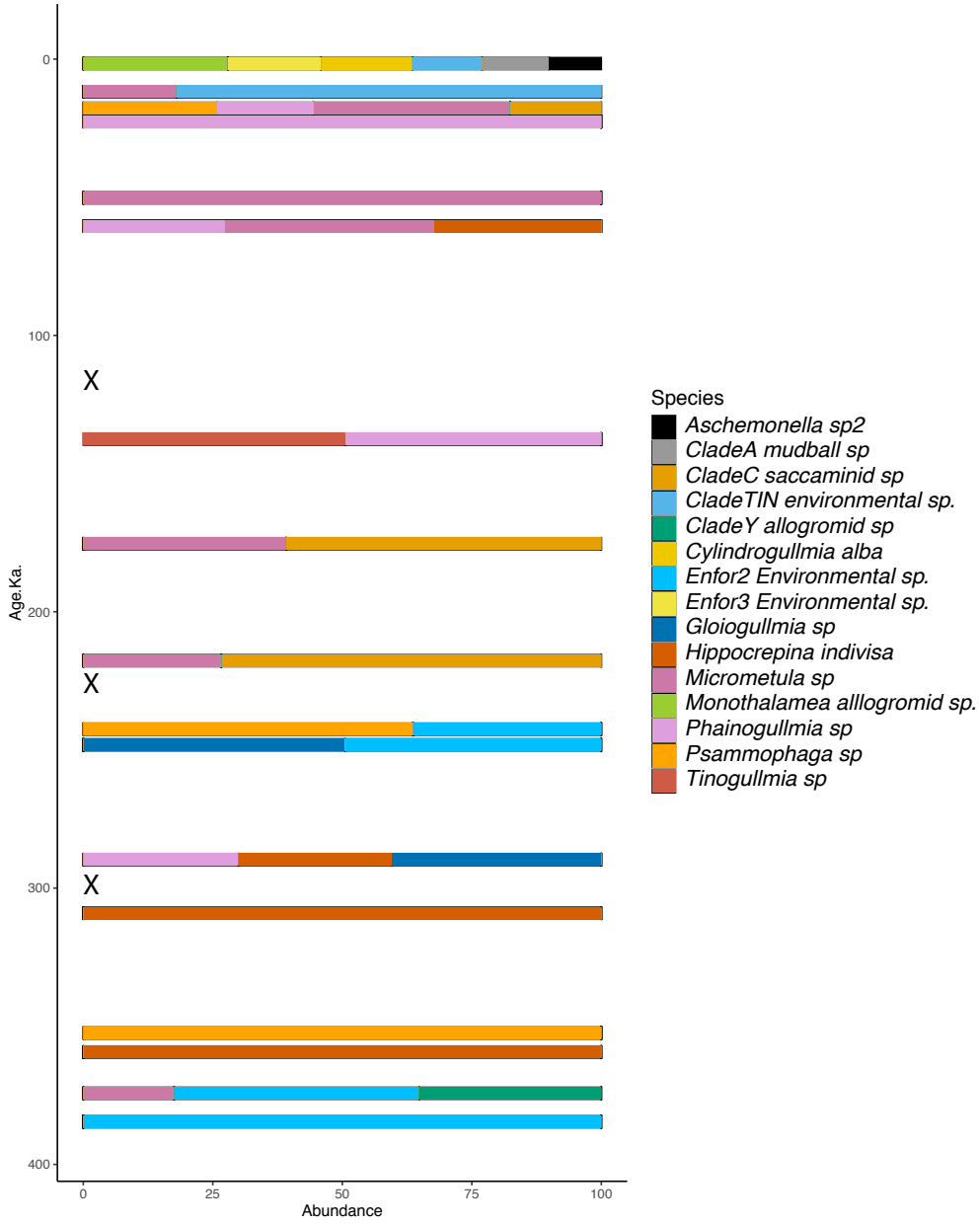


Results: soft-shell foraminifera

3/20 layers do not amplified.

Non - fossilized foraminifera metabarcodes could be also obtained.





Conclusion

- Could aDNA preserved in cores stored for several years at 4°C storage? and the obtained aDNA is usable?

  Marine species aDNA was still preserved and could be extracted at almost every layer.
  Eukaryotes metabarcodes were mostly composed of amoeba.
  Specific primers for diatoms and foraminifera provide more sequences and assignments until species level.

- Core repositories brim with sedimentary cores which if they are intact could be used depending on targeted species.

- To target bacteria, funghi, … better use freshly recovered core.

Thanks!

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In the last ten years, sedimentary ancient DNA (sedaDNA) becomes a new proxy for paleoceanographic analyses that provide information about large range of non-fossilized taxa. Usually, the sediment samples destinated for sedaDNA study are immediately frozen after collection or stored in special buffer to preserve the DNA. However, there are many cores that have been collected long time before the advent of paleogenomics and that are commonly refrigerated and stored at 4°C. Here, we test whether such cores can be used as a source of ancient DNA, by analysing the sedaDNA samples from 36 meters long marine gravity core that was stored during 14 years at 4 °C. The core MD05-2920 was retrieved during the MD148/PECTEN – Images XII cruise, in Bismarck Sea, off New Papua Guinea, and records the past 385 ka. We analysed samples from 20 layers spanning the interval from 1.6 ka to 384 ka, where isotopic measures of $\partial^{18}O$ showed significant paleoceanographic changes. We started by analysing a universal eukaryotic marker, the V9 (170 bp) region of the 18S rRNA. However, the obtained datasets were dominated by sequences belonging to species of fungi and amoebae that probably originated from post-collection storage. More data were obtained by using markers specific to selected marine taxa, such as foraminifera, radiolaria, and diatoms. The analysis of these data show clearly that the DNA is preserved in marine sediment down to 385 ka old layers. Our study also shows a possibility to exploit the sedaDNA from refrigerated material stored in cores repositories.