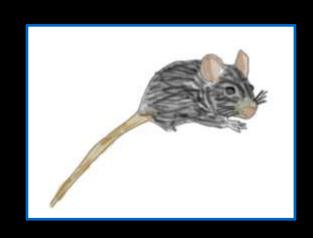# Paleo-Metagenomics of Late Quaternary Packrat Middens

Robert Harbert,
Stonehill College
Grace Moore*,
Smith College
Seth Cunningham,
AMNH
Michael Tessler,
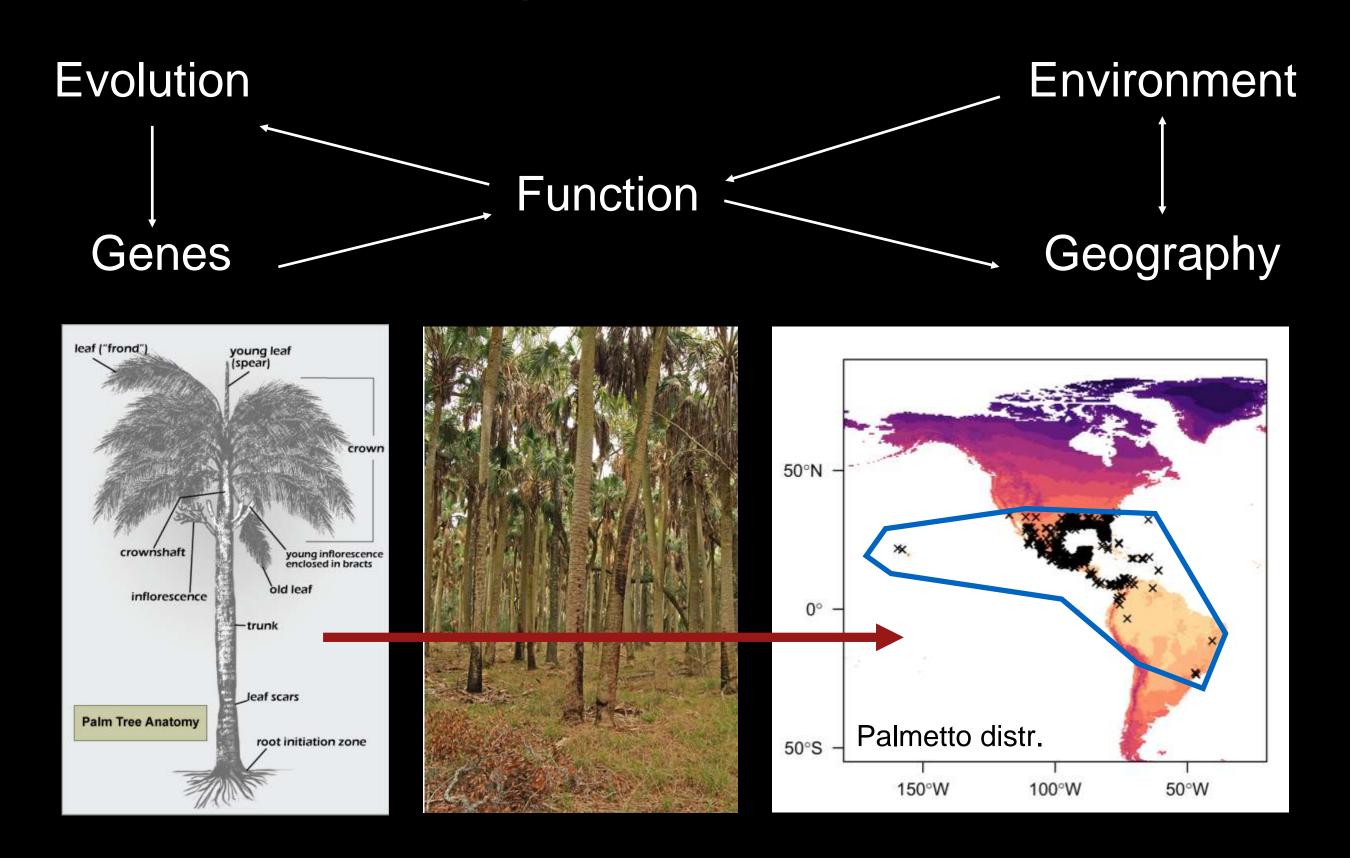*AMNH*
Julio Betancourt,
USGS

Topics

- 1) Packrat middens as paleoecological and paleoclimatological record

- 2) Ancient DNA profiling of fossil packrat middens

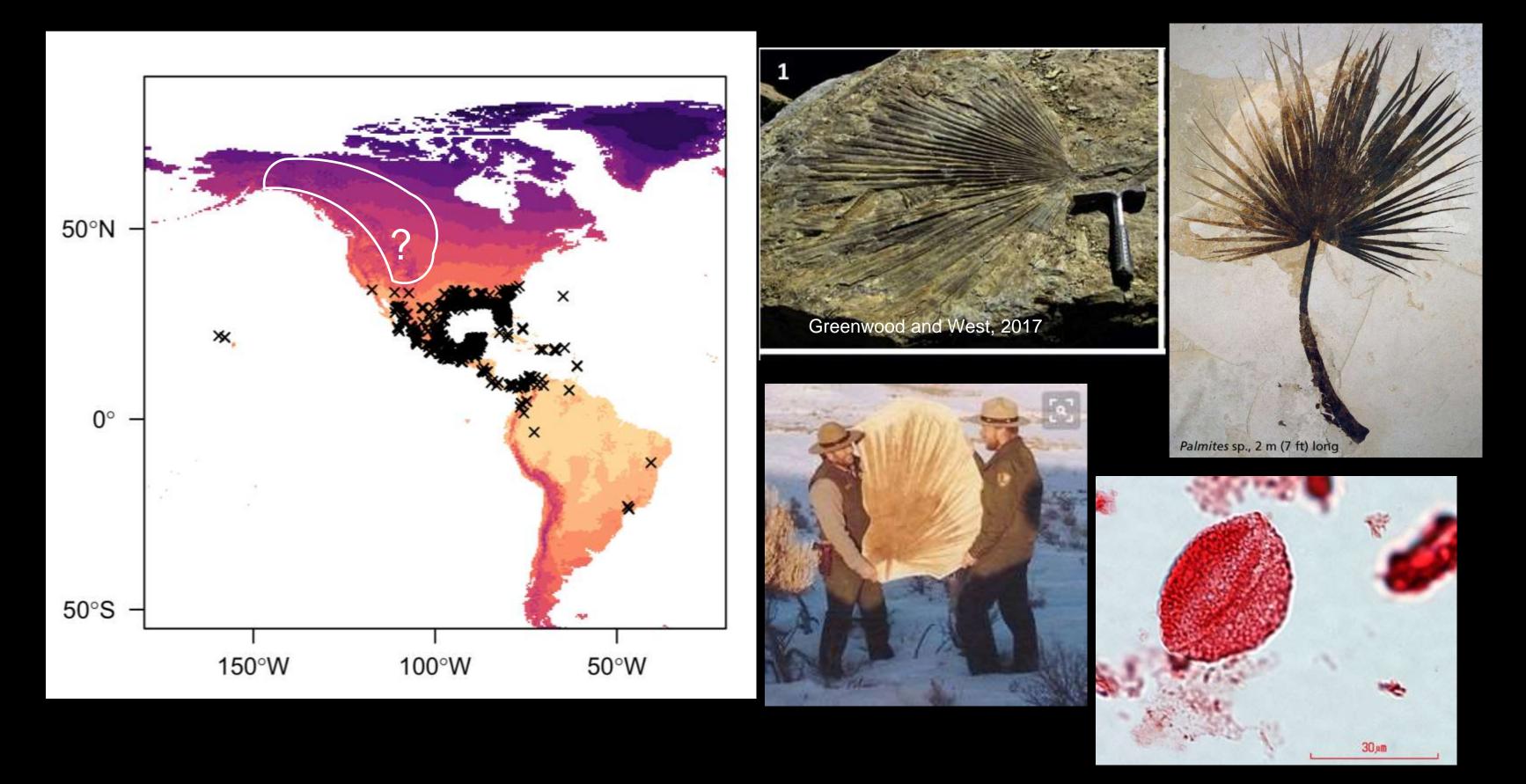- 3) Current teaching/research topics

What can plant communities tell us about climate?
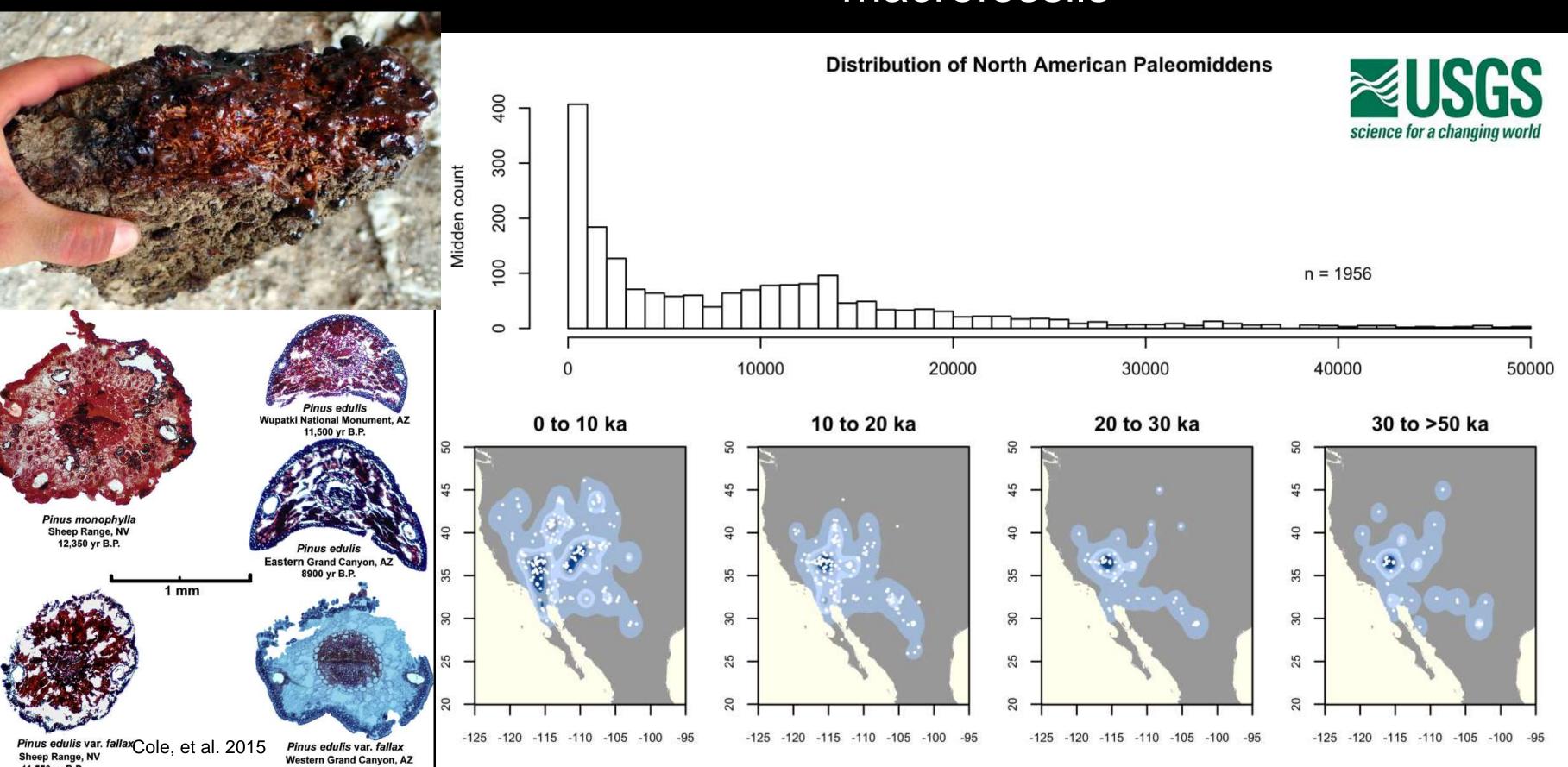
Ecological Feedback:

Evolution

Environment

Function

Genes

Geography

Palmetto distr.

Extrapolation in the Fossil Record

Greenwood and West, 2017

Palmites sp., 2 m (7 ft) long

Packrats

Google image search: "packrat nest"
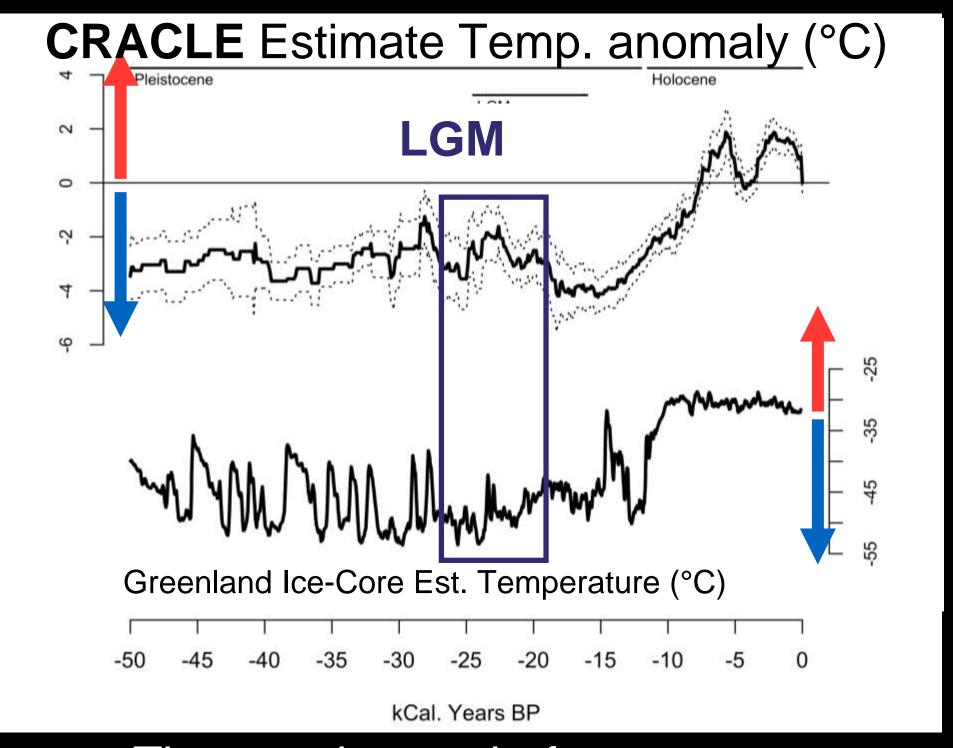
Late Quaternary Packrat (*Neotoma* spp.) midden macrofossils

Distribution of North American Paleomiddens

n = 1956

Midden count

Pinus monophylla
Sheep Range, NV
12,350 yr B.P.

Pinus edulis
Wupatki National Monument, AZ
11,500 yr B.P.

Pinus edulis
Eastern Grand Canyon, AZ
8900 yr B.P.

Pinus edulis var. *fallax*
Sheep Range, NV

Pinus edulis var. *fallax*
Western Grand Canyon, AZ

1 mm

0 to 10 ka   10 to 20 ka   20 to 30 ka   30 to >50 ka

Cole, et al. 2015

Harbert & Nixon. 2018. **Quantitative Late Quaternary Climate Reconstruction from Plant Macrofossil Communities in Western North America**

Thousand years before present
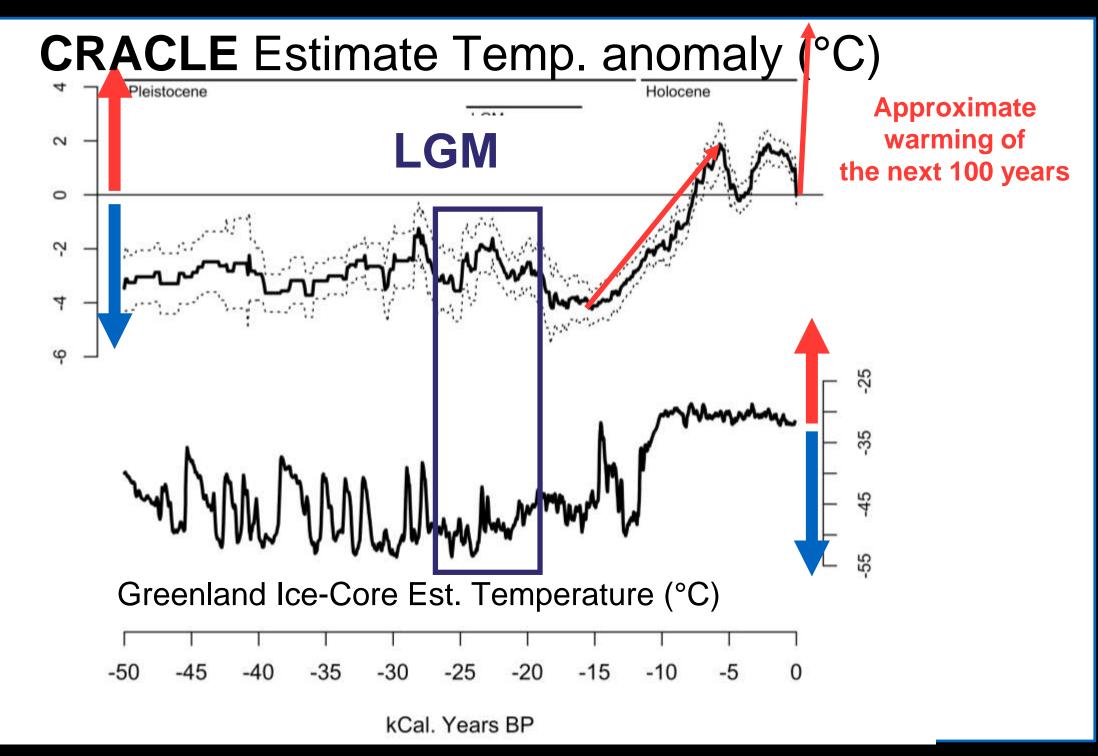
Harbert & Nixon. 2018. **Quantitative Late Quaternary Climate Reconstruction from Plant Macrofossil Communities in Western North America**

Coming Soon:

- ## CRACLE R package:

  - ### cRacle (https://github.com/rsh249/cRacle.git)

    - R implementation of the CRACLE paleoclimate estimation algorithm and associated functions for data access and visualization.

Ancient DNA

Ancient DNA from Ice-Cores

- DNA from plants and insects dating to >500,000 years ago!
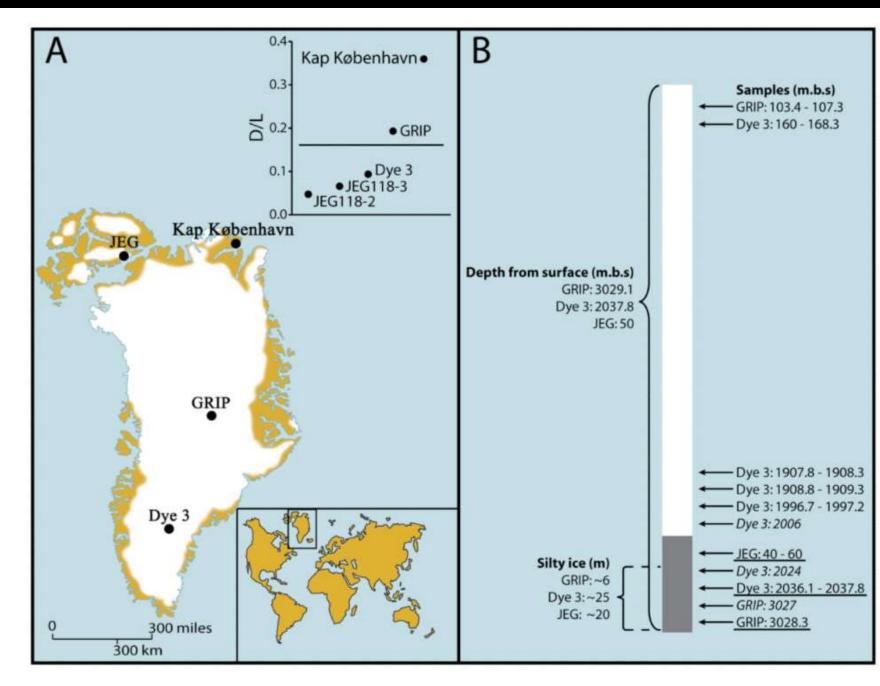
Ancient Biomolecules from Deep Ice Cores Reveal a Forested Southern Greenland

Eske Willerslev[1,*], Enrico Cappellini[2], Wouter Boomsma[3], Rasmus Nielsen[4], Martin B. Hebsgaard[1], Tina B. Brand[1], Michael Hofreiter[5], Michael Bunce[6,7], Hendrik N. Poinar[7], Dorthe Dahl-Jensen[8], Sigfus Johnsen[8], Jørgen Peder Steffensen[8], Ole Bennike[9], Jean-Luc Schwenninger[10], Roger Nathan[10], Simon Armitage[11], Cees-Jan de Hoog[12], Vasily Alfimov[13], Marcus Christl[13], Juerg Beer[14], Raimund Muscheler[15], Joel Barker[16], Martin Sharp[16], Kirsty E.H. Penkman[2], James Haile[17], Pierre Taberlet[18], M. Thomas P. Gilbert[1], Antonella Casoli[19], Elisa Campani[19], and Matthew J. Collins[2]

[1]Centre for Ancient Genetics, University of Copenhagen, Denmark [2]BioArch, Departments of

Packrat midden aDNA

Midden collected in field

Lab Processing:

Pollen sample removed

Voucher sample removed

Midden weighed & disassociated

wet sieved & dried

dry sieved & sorted

pellets measured

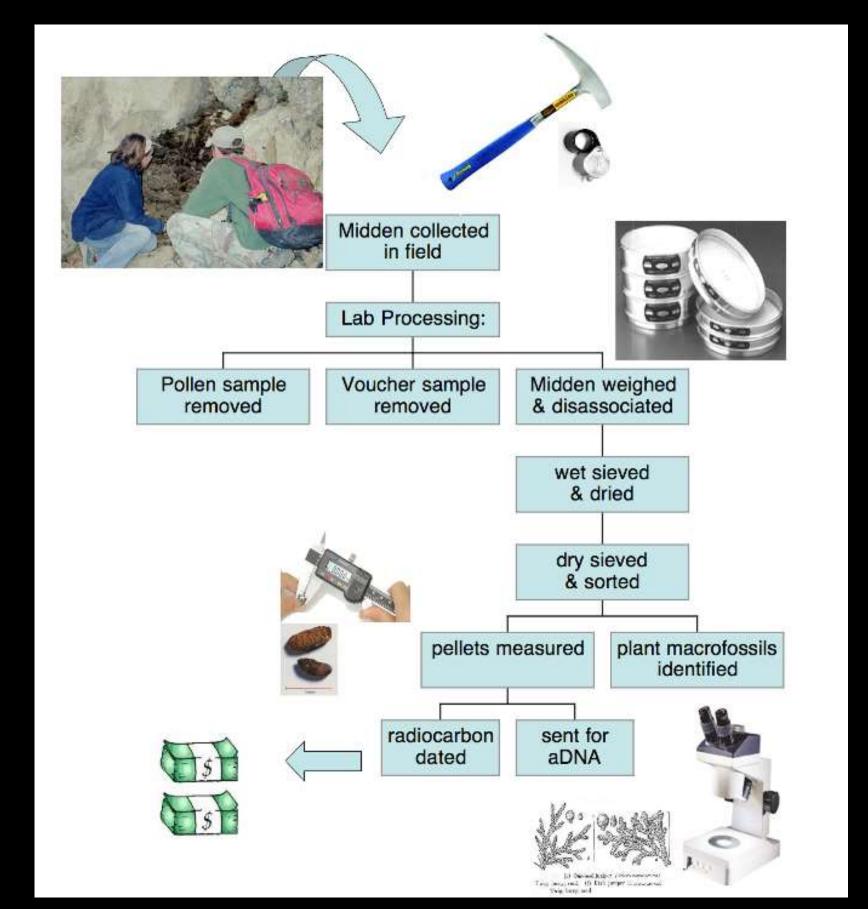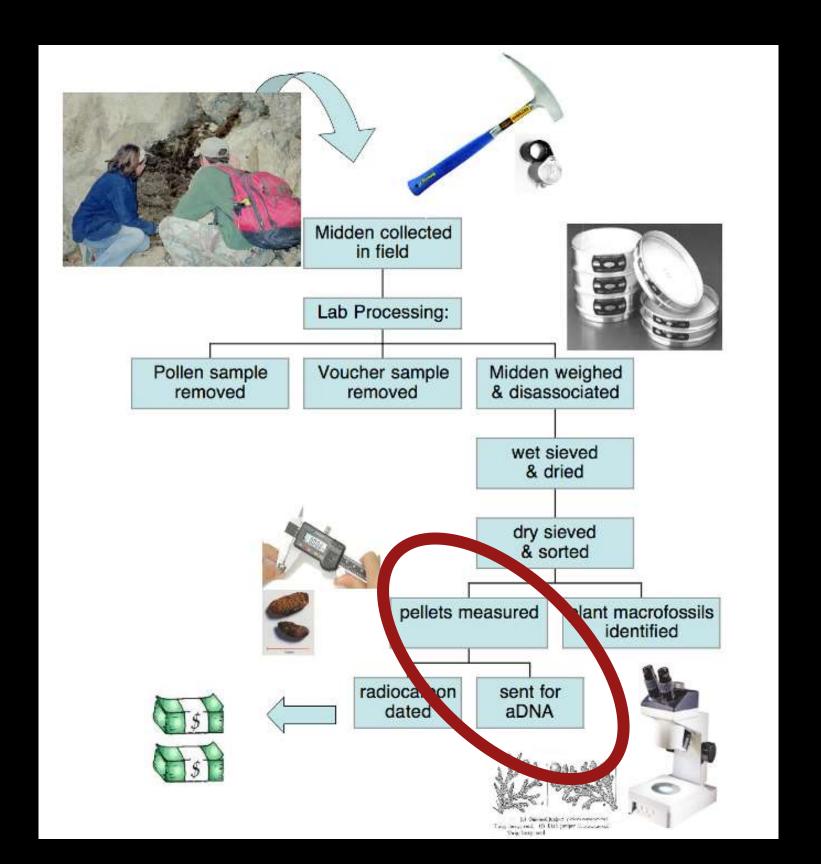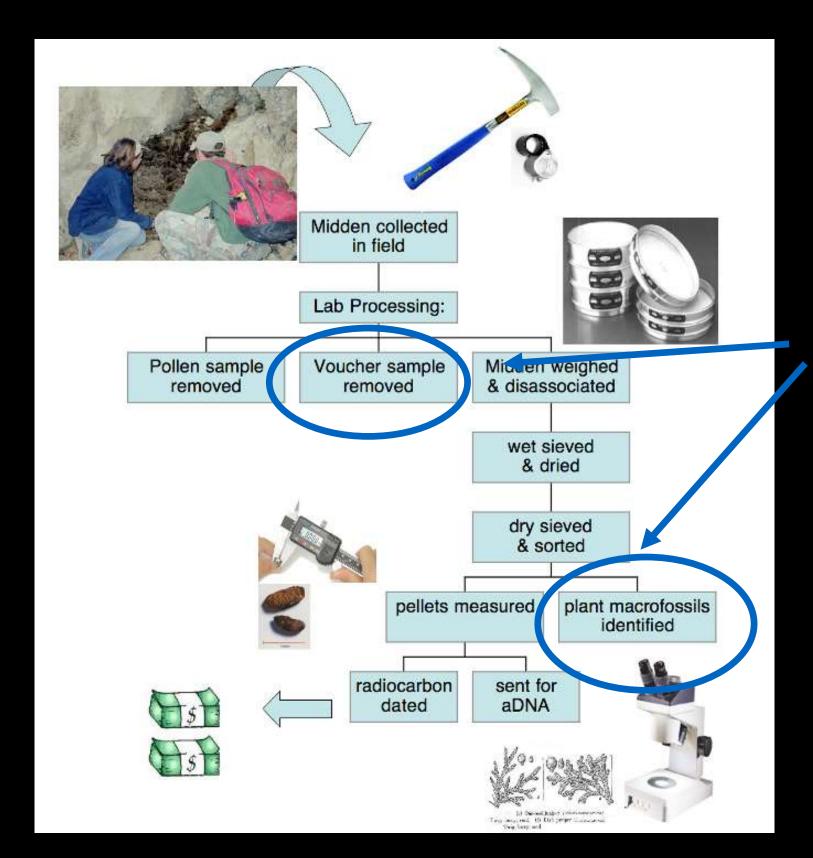plant macrofossils identified

radiocarbon dated

sent for aDNA

Photo Credit: J. Betancourt, K. Rylander (USGS)
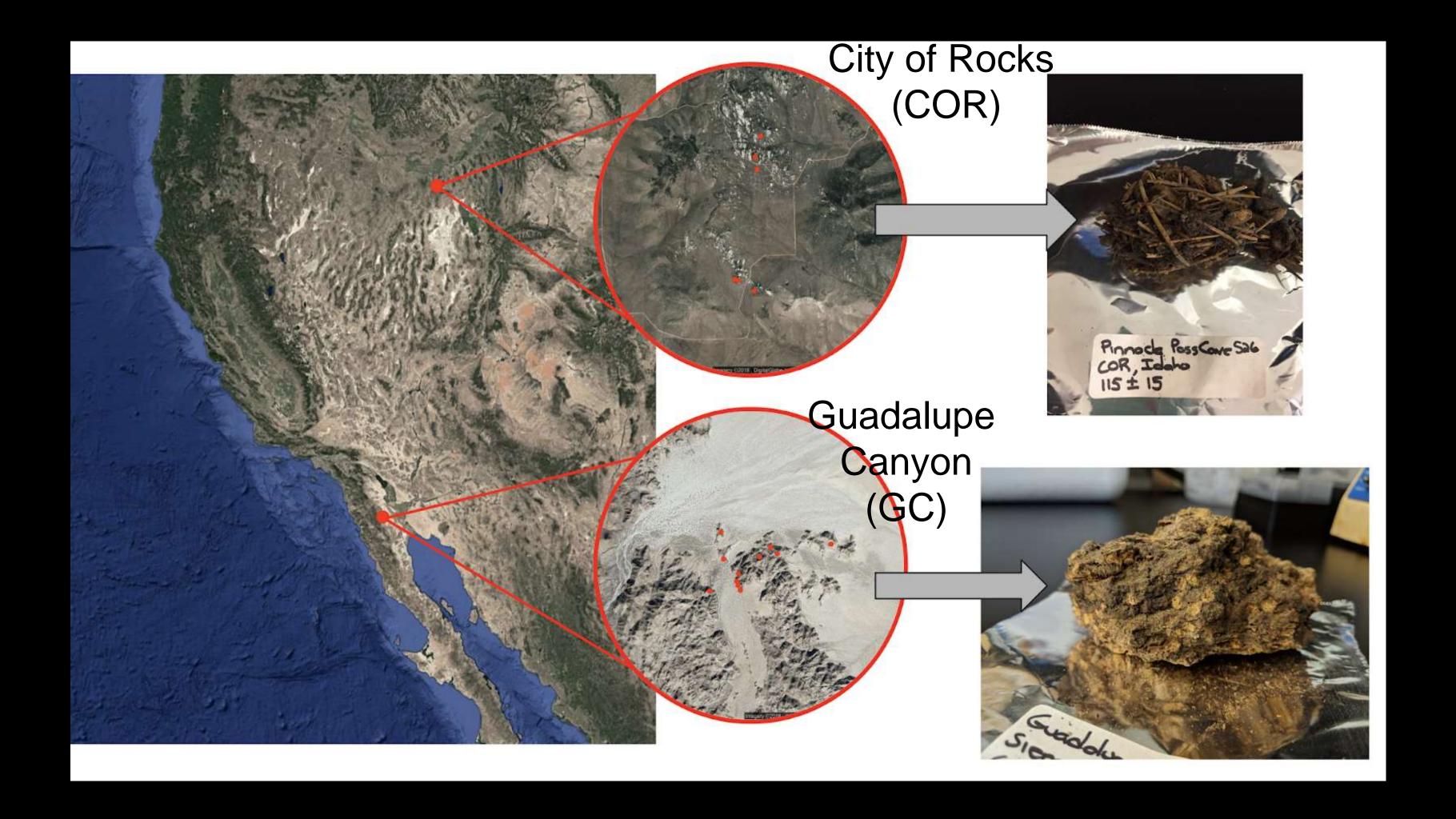
Packrat midden aDNA

Packrat midden aDNA

Two types of samples that may be used for DNA analysis

Photo Credit: J. Betancourt, K. Rylander (USGS)

City of Rocks (COR)

Guadalupe Canyon (GC)

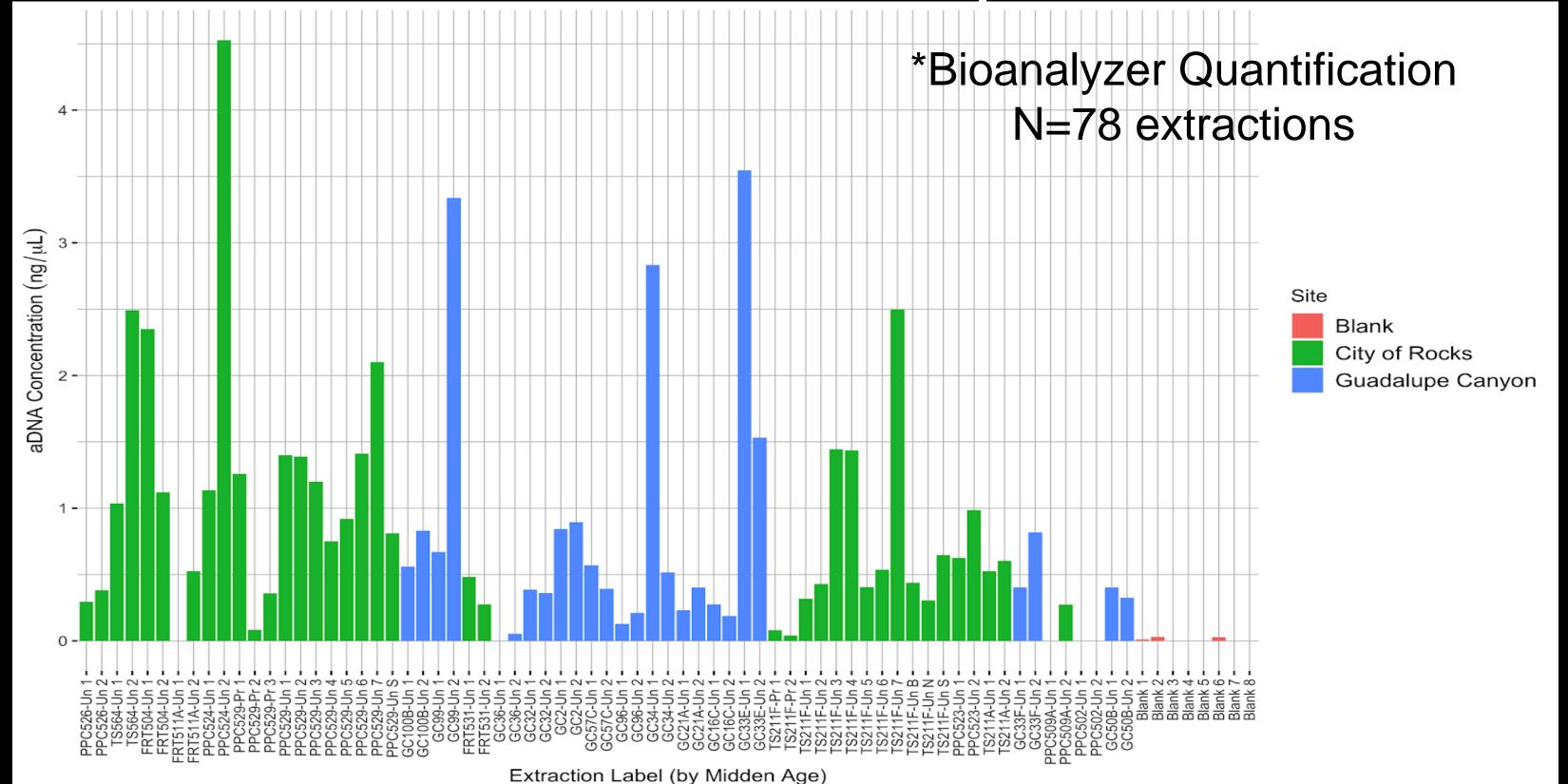Pinnacle Pass Cave S26
COR, Idaho
115 ± 15

Guadalu
Sie

aDNA Extraction Methods

- DNeasy PowerSoil® Kit protocol
- Measured resulting DNA concentration
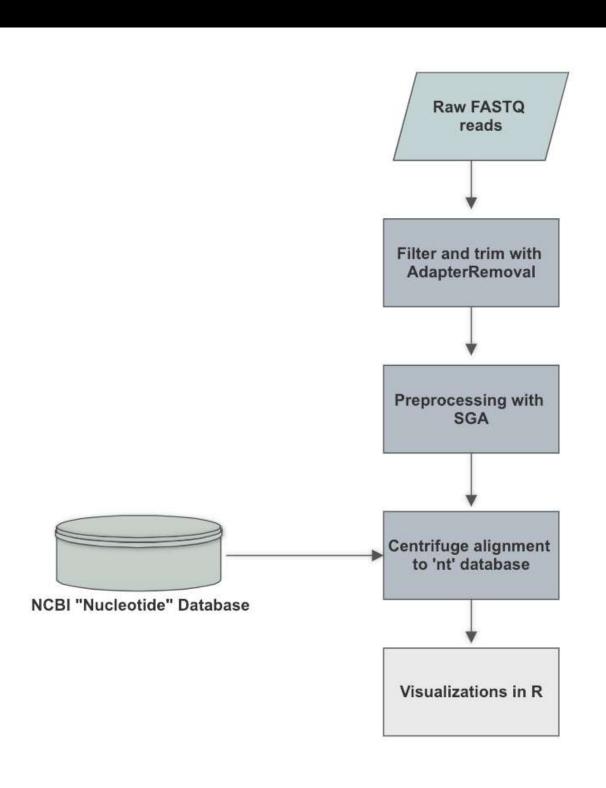 - Qubit® 2.0
  - Agilent 2100 Bioanalyzer

Ancient midden DNA quantification

*Bioanalyzer Quantification
N=78 extractions

Sequencing

- Shotgun/Whole Genome

- Illumina HiSeq 2500, 2x125bp reads

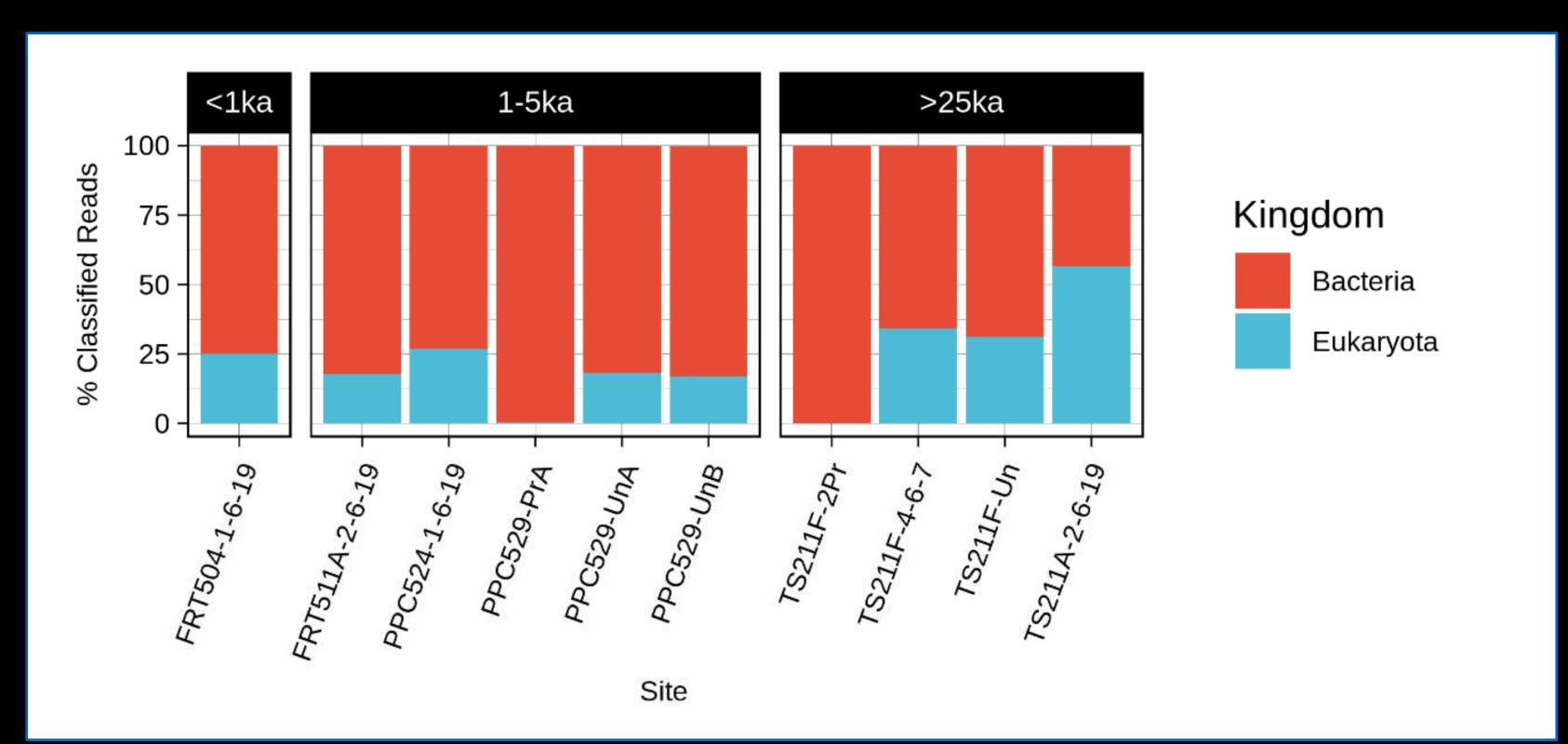- 22 samples submitted → 11 successful libraries
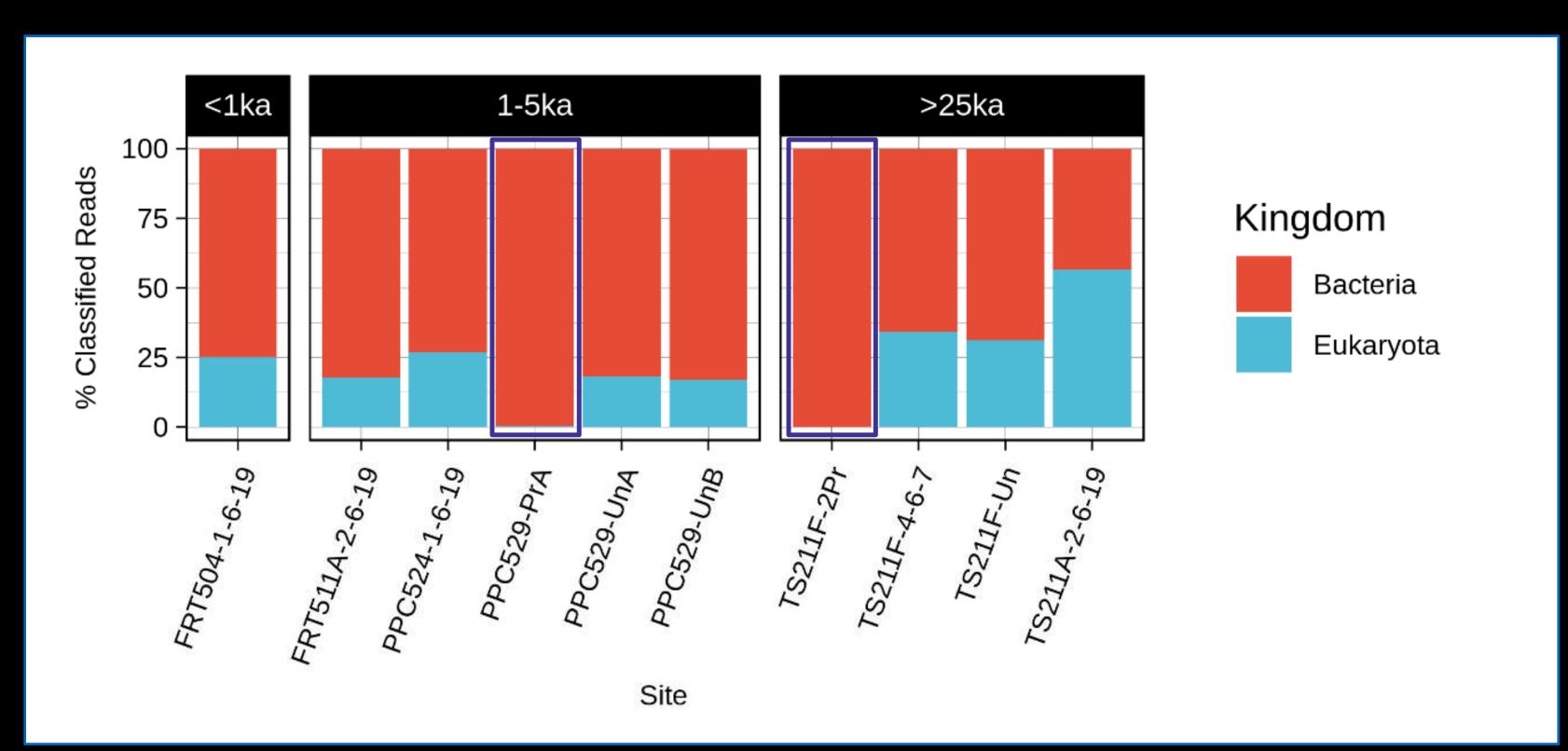
- ~30 – 60 million reads per sample

Pipeline

- Input: Raw Illumina 'fastq' files

- Data: HiSeq 2500, 2x125bp

- Output: Taxonomic classification of reads

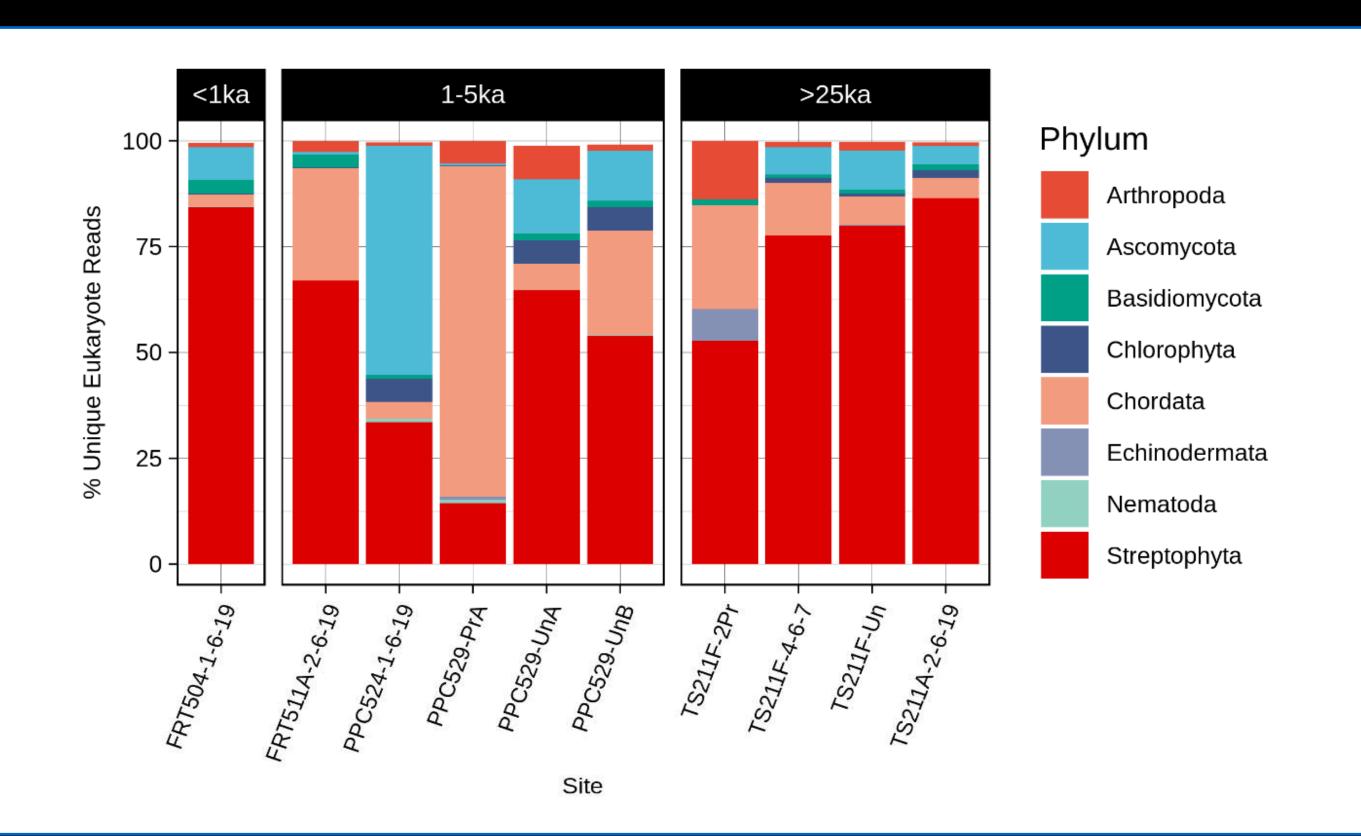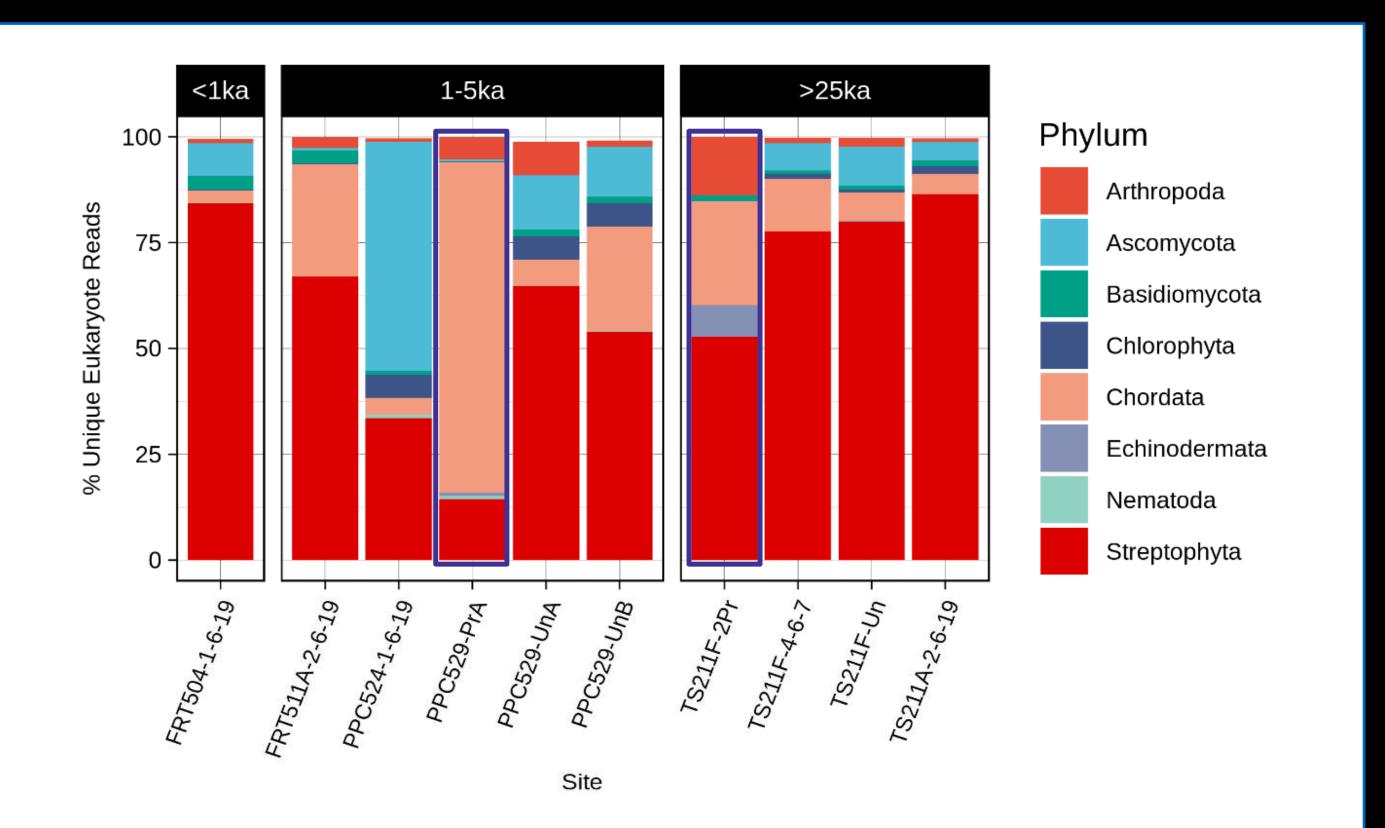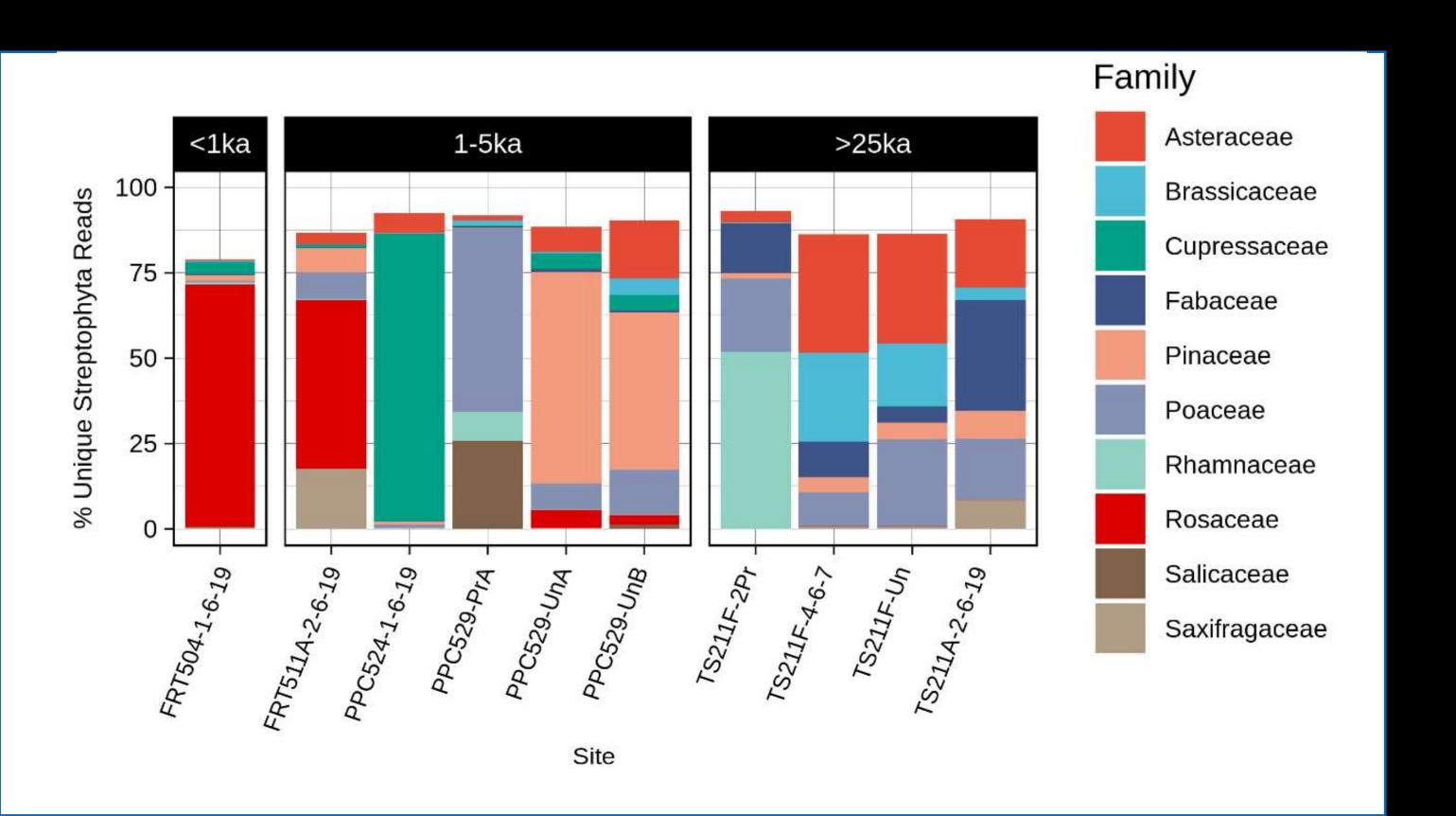# Taxonomic Classification

Taxonomic Classification

Eukaryotes

Eukaryotes

Plants

Top Plant Genera: Hints of A Changing Ecosystem

3,260 year-old midden

28,460 year old midden

Pinus

Triticum

Diplostephium

Juniperus

Cercocarpus

Triticum

Diplostephium

Lupinus

Poa

Artemisia

Still working on:

- Amplicon Sequencing comparison

- Analysis of DNA damage patterns → Attempt to confirm ancient origins
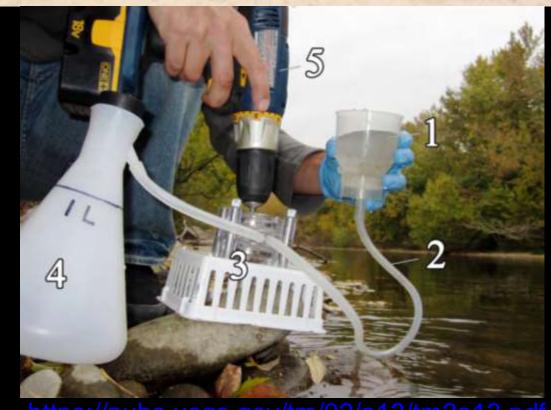
- Evaluation of non-plant data

Current teaching/research topics

Teaching & Research
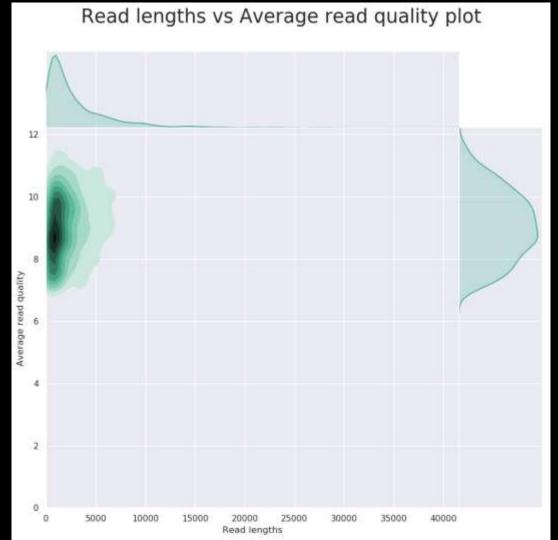
- Developing UG Bioinformatics courses

  - https://rsh249.github.io/bioinformatics

  - https://rsh249.github.io/applied_bioinformatics

- Oxford Nanopore MinION

- eDNA – Detecting plant communities from aquatic environmental DNA

https://pubs.usgs.gov/tm/02/a13/tm2a13.pdf

MinION: *E. coli*

Read lengths vs Average read quality plot

Histogram of read lengths

Reads up to 40kb
Avg. = 3kb
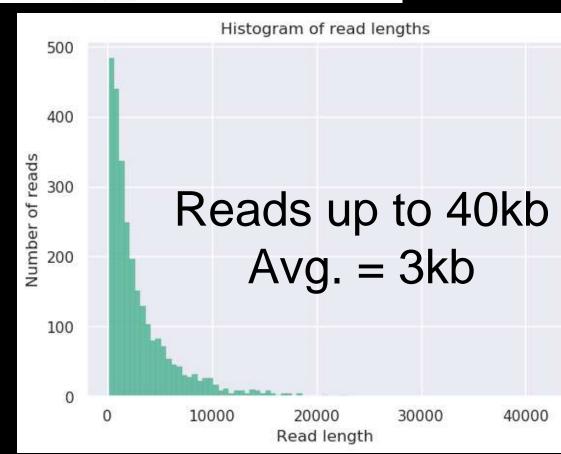
~5000 reads in 6hrs = ~95% E. coli

Acknowledgements
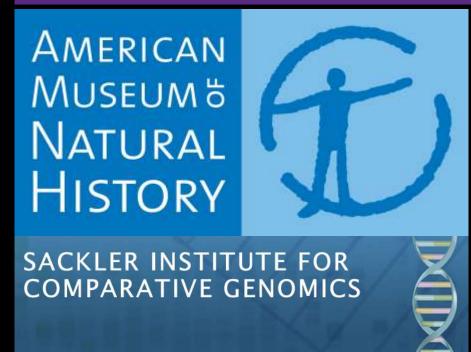
Links

- These Slides: https://rsh249.github.io/files/harbert_seminar_UMassD_3_20_19.pdf

- Papers:

  - CRACLE https://bsapubs.onlinelibrary.wiley.com/doi/full/10.3732/ajb.1400500

  - Packrat Paleoclimate https://www.openquaternary.com/articles/10.5334/oq.46

  - Packrat aDNA **Coming soon to bioRxiv!**

- Courses:

  - Introduction to Bioinformatics – https://rsh249.github.io/bioinformatics

  - Applied Bioinformatics (Nanopore) – https://rsh249.github.io/applied_bioinformatics